Pathways Analyses for the Introduction to the U.S. of Plant Pathogens of Economic Importance

Prepared by the

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Methodology for Pathway Analysis

of an Intentionally Introduced Plant Pathogen

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The terrorist events of September 11, 2001 and subsequent bioterrorist anthrax attacks have resulted in the legitimate concern over U.S. vulnerability to agricultural bioterrorism. Agricultural targets offer terrorists a virtually open area to assault, often with little cost or expertise required and with a potential for high probability of inflicting significant economic impact on U.S. agriculture.

Analysis of potential pathways for exotic disease entry and establishment, and assessment of U.S. capacity to minimize the impact of such an introduction can form a basis for developing safeguards to ensure rapid detection, containment and mitigation in the event of an attack. Pathway analyses provide a tool for assessing the potential threat of an introduced plant pathogen to the U.S. and thereby help policy makers allocate resources wisely. A basic conceptual framework for pathway analysis of an introduced plant pathogen can be applied as new disease risks arise or when changes occur that affect the risks associated with known pathways or diseases. The plant pathways methodology developed and applied herein has two primary components, a) a disease introduction and development pathway and, b) an associated response strategy pathway to minimize disease impact and enhance preparedness (Fig. 1).

All areas of the pathway are founded on a thorough review of the biology and epidemiology of the pathogen. This includes pathogen identity, hosts, geographic distribution and impact,

disease cycle and epidemiology, symptoms, and methods of detection. If vectors are involved in secondary spread of a pathogen, the vector's life cycle, alternate host distribution, transmission ability, etc. must be incorporated in the analysis. Published and unpublished literature, along with personal communication with experts, develops the most comprehensive base possible. Knowledge gaps in pathogen epidemiology, establishment in the U.S. and control/mitigation strategies are then identified. A list of knowledgeable scientists with contact information should be included for each pathogen.

A disease introduction and development pathway initiated by covert action (Fig. 1a), reviews means and materials for intentional introduction. The probable route of terrorist entry, propagule(s) for initiation, ease of propagation and dissemination, and quantity of propagule(s) required, all contribute to understanding the probability of a successful introduction.

On introduction, rapid detection can modify the likelihood of successful establishment, potential disease spread, and mitigation. The pathogen was assigned a qualitative risk (high, moderate, or low) of successful introduction and establishment based on 29 different criteria (Appendix 1). The likelihood of successful introduction was broken down into 5 categories. These included: quantity of inoculum required to introduce and establish damage, likelihood of surviving initial introduction, likelihood of dissemination beyond the point of introduction, likelihood of alternate host infection and likelihood of early detection.

Risk establishment was rated with respect to six elements: climate, host range, dispersal, persistence, economic impact, and environmental impact. The first four risk elements provided a basis for prediction of the pathogen's survival and disease distribution ranges. When available, predictive models for the pathogen or disease distribution patterns of taxonomically related pathogens with similar biology were employed. The site of introduction within the U.S. influences outcome and may result in more than one disease development pathway scenario. Finally the pathogen was assigned an over-all risk rating.

The response strategy pathway (Fig. 1b) indicates where and how intervention can curtail impact. The initial step considers how an attempted introduction can be recognized ("Initiating event") through observation/diagnosis of presence, interception, and "Intelligence" information. The diagnostic and action pathway recently developed by NPDN (National Plant Diagnostic Network) coordinates the movement of samples and data, as well as communication between various responders (Fig. 2). The following questions complete the

basic structure of the response pathway. How can rapid diagnosis/detection be improved? Is containment and eradication of the pathogen possible? If so, what is the best containment response? After a thorough inventory of control and mitigation methods, what will be the best control strategy in the U.S.? Are any regulatory or legal changes required in order to reduce potential entry of the pathogen or implement post-introduction strategies? Optimum immediate response options to minimize impact of an introduced exotic pathogen complete the pathway.

Figure 1. Generic plant pathogen pathway analysis and response summary

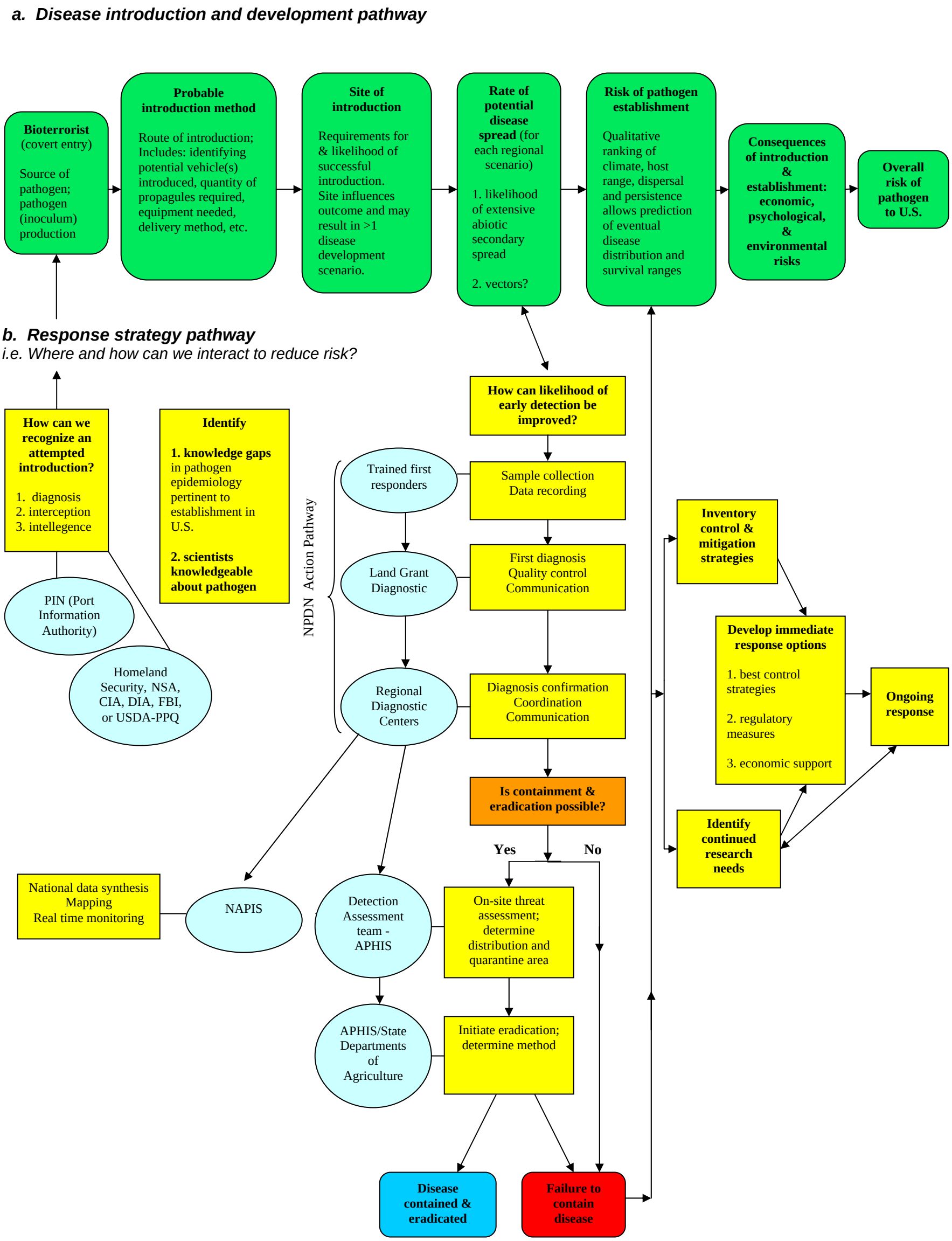
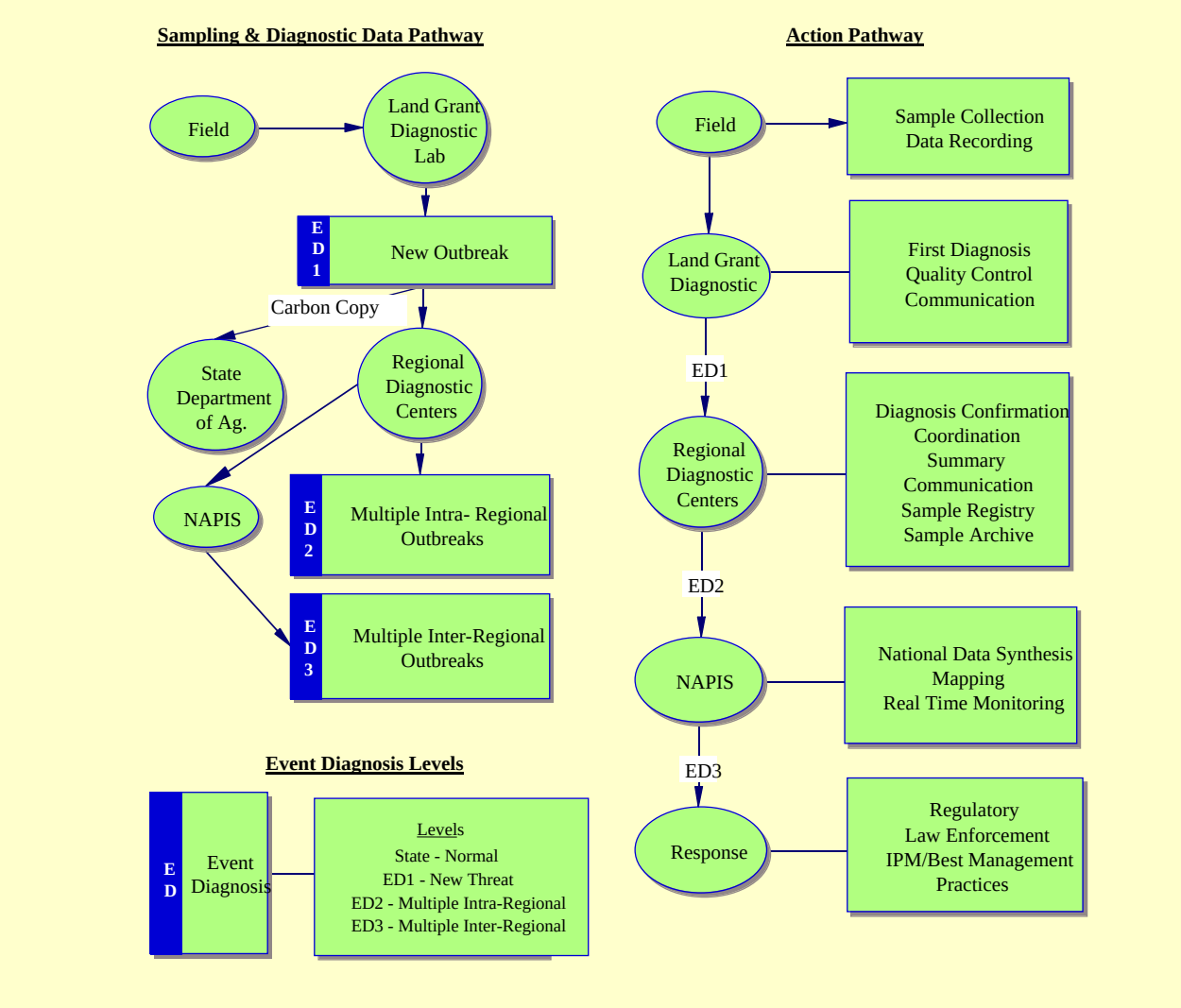


Figure 2. Sampling + diagnostic data and action pathways for NPDN



Cardwell, K. 2004. Project description: National Plant Disease and Pest Diagnostic Network. http://npdn.ppath.cornell.edu/

Appendix 1. Criteria For Bioterrorism Agent And Rating

A. Host-pathogen compatibility	**Level of risk =**	**Low**		**Medium**		**High**	
1. Range of pathogen versus range of host	**(limited)**	1	2	3	4	5	**(all)**
2. Percentage of crop infected	**(low)**	1	2	3	4	5	**(high)**
3. Percentage of susceptible cultivars grown	**(few)**	1	2	3	4	5	**(all)**
4. Degree of crop loss	**(limited)**	1	2	3	4	5	**(all)**
5. Value of the crop	**(low)**	1	2	3	4	5	**(high)**
6. Virulence enhancement (genetic)	**(unfeasible)**	1	2	3	4	5	**(easy)**
7. Persistence under field conditions	**(short)**	1	2	3	4	5	**(long)**
8. Pathogen of quarantine significance	**(no)**	1	2	3	4	5	**(yes)**
9. Toxins of human or animal significance	**(no)**	1	2	3	4	5	**(yes)**
B. Epidemiology							
10. Infectivity period	**(short)**	1	2	3	4	5	**(long)**
11. Environmental constraints for crop area	**(limited)**	1	2	3	4	5	**(none)**
12. Ease of establishment in the crop area	**(limited)**	1	2	3	4	5	**(easy)**
13. Spread of organism by natural means	**(slow)**	1	2	3	4	5	**(fast)**
14. Degree of infection	**(limited)**	1	2	3	4	5	**(fully)**
15. Repeating cycles during crop season	**(one)**	1	2	3	4	5	**(many)**
C. Logistics							
16. Ease in obtaining a culture	**(limited)**	1	2	3	4	5	**(easy)**
17. Ease in producing needed quantity	**(hard)**	1	2	3	4	5	**(easy)**
18. Ease in handling and delivery	**(limited)**	1	2	3	4	5	**(easy)**
19. Retention of viability in storage	**(short)**	1	2	3	4	5	**(long)**
20. Knowledge and resources required	**(little)**	1	2	3	4	5	**(much)**
D. Detection							
21. Ease of detection and identification	**(hard)**	1	2	3	4	5	**(easy)**
22. Symptom expression	**(few)**	1	2	3	4	5	**(clear)**
23. Diversity of the pathogen (forms)	**(one)**	1	2	3	4	5	**(many)**
24. Reliability of symptoms/identification	**(good)**	1	2	3	4	5	**(poor)**
25. Time required for identification	**(hours)**	1	2	3	4	5	**(days)**
E. Control							
26. Availability of plant resistance	**(multiple)**	1	2	3	4	5	**(none)**
27. Availability of chemical controls	**(none)**	1	2	3	4	5	**(many)**
28. Persistence over time	**(limited)**	1	2	3	4	5	**(persists)**
29. Alternate hosts	**(none)**	1	2	3	4	5	**(many)**

A Conceptual Framework for the Analyses of Pathways

for the Introduction of Plant Pathogens

K. A. Garrett, with input from other participants from
KSU, Purdue, and TAMU

Executive Summary

A conceptual framework for the study of pathways for introduction of plant pathogens is under development. Such a conceptual framework benefits policy makers by helping to direct efficient evaluation of a potentially invasive pathogen and benefits the scientific community through contributions to a general comparative epidemiological theory. At each stage or step of the pathway for introduction, we ask the following questions.

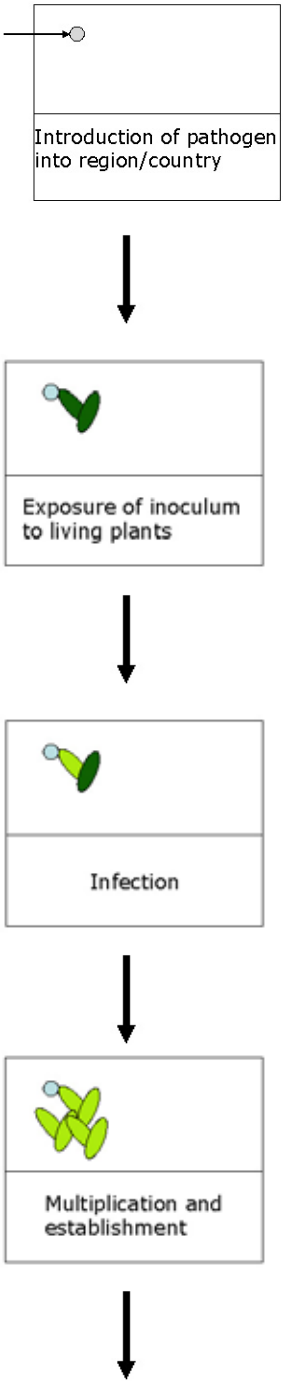
1. What actions can be taken to reduce risk?

2. What parameters describing the host-pathogen system need to be known for evaluating strategies and predicting the probability of further movement along the pathway?

3. What parameters describing the current epidemic need to be estimated for decision-making?

A schematic of a pathway is outlined here with more detail given below. A publication summarizing these ideas and with additional input from other analysts will be published in a peer-reviewed journal.

Diagram of pathway

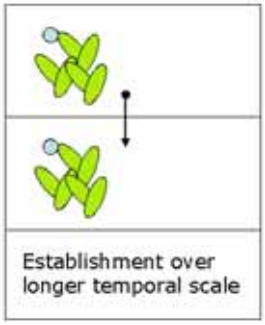


Consideration can be given to how to reduce the risk of pathogen introduction, both at U.S. borders and in countries of origin.

In the case of intentional introductions, progress to this stage is not a limiting step since access to living crop plants is generally readily available. For accidental introductions, however, progress to this stage is probably often limiting.

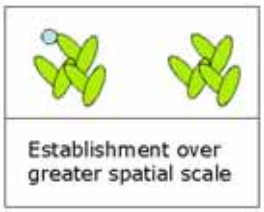
Environmental conditions, host status, and pathogen propagule status will be key in determining whether infection occurs at any given time.

Environment, host, and pathogen interactions must be even more conducive for enough reproduction to occur for at least short-term establishment and potential detection.



Establishment for longer periods of time will depend on overwintering and/or oversummering capabilities of pathogens in the current environment.

AND/OR. . .



Spread of the pathogen will depend on pathogen dispersal characteristics in combination with the environmental characteristics of the potential new infection sites.

Introduction

What we might want from a pathways conceptual framework

- For policy makers
  - Framework to direct efficient evaluation of a potentially invasive pathogen
    - When the threat is new and time for study is limited
    - When the threat is known and research priorities need to be defined
- For the scientific community
  - Framework for better development of general epidemiological theory
    - Comparative epidemiology is not well-developed since most plant pathologists specialize in a particular crop or even a particular pathogen

Some key policy questions

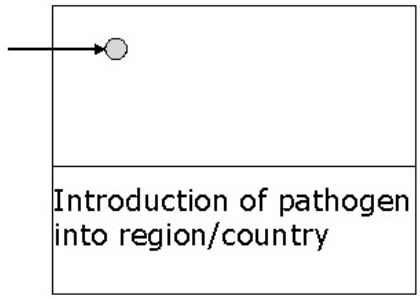
- What is the environmental impact of action and inaction in response to a potential new introduction?
- How will responses differ if an introduction is intentional or unintentional?
- Will it be feasible to prevent introduction?
- Will it be feasible to eradicate a particular pathogen?
- Will it be feasible to limit the geographic distributation?
- Is it worth attempting to limit the geographic distribution if this can be achieved only for a limited time?

Important characteristics of pathogens as invasive species

- A pathogen generally becomes important economically because its host is abundant – but an abundant host makes density-dependent reproduction easier for the pathogen
- Pathogens cannot effectively invade without encountering living host tissue
- Most pathogens may go unrecognized for long periods of time while increasing in abundance

Questions for each step and each stage in the pathway

- What actions can be taken to reduce risk?
  - What are the possibilities for eradicating or spatially limiting the pathogen?
  - What are the costs and benefits of action (and inaction) in response to detection of pathogen at each step?
- What parameters describing the host-pathogen characteristics need to be known…
  - … for predicting the probability of further movement along the pathway
  - …for evaluating strategies for limiting or eradicating the pathogen?
- What parameters describing the current epidemic need to be estimated?



0. Prior to introduction of pathogen

- What actions can be taken to reduce risk?
  - Modify number and timing of samples tested at ports of entry
- What parameters describing the host-pathogen characteristics need to be known?
  - Probability of introduction through all relevant ports of entry
    - Known for Karnal bunt for some ports of entry
  - Potential risk reductions from negotiations to modify crop management in exporting countries
  - Threshold values for establishment

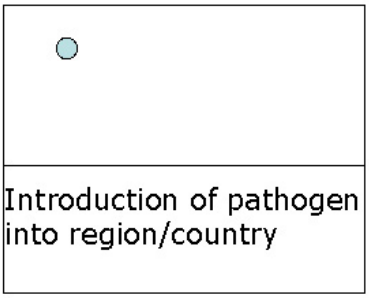
PRIOR TO INTRODUCTION

System	Notable characteristics at this stage or step
Karnal bunt of wheat	Negotiations are being made to try to reduce trade barriers
Slime disease of wheat	Seed processing methods have already been developed to manage introductions
Sorghum ergot	Already present in U.S.
Brown strip downy mildew (sorghum)	Information needed about possible host range extension
Soybean rust	Work is being done to develop fungicides and resistant varieties since introduction is considered nearly inevitable
Soybean mosaic virus	This pathogen is already present in the US, but there is concern that a more damaging strain might be introduced
Philippine downy mildew of corn	More information about likely introduction routes needed

Late wilt of corn	More information about likely introduction routes needed
Bacterial leaf streak of rice	More information about likely introduction routes needed
Bacterial leaf blight of rice	More information about likely introduction routes needed

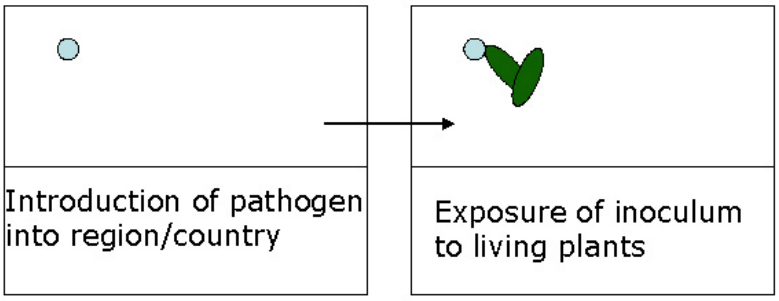
AT INTRODUCTION

System	**Notable characteristics at this stage or step**
Karnal bunt of wheat	Teliospores would probably be the propagule, though secondary sporidia could be windblown to new region
Slime disease of wheat	Infected seed would be the likely vehicle
Sorghum ergot	Infected seed would be the most likely vehicle
Brown strip downy mildew (sorghum)	
Soybean rust	Urediniospores would be the propagule form
Soybean mosaic virus	Infected seed would likely be the vehicle of natural introduction; viruliferous aphids could be reared for intentional introduction
Philippine downy mildew of corn	
Late wilt of corn	Infected seed would be the most likely form of introduction; intentional introduction could also be accomplished by rearing the fungus on bran or grain
Bacterial leaf streak of rice	
Bacterial leaf blight of rice	



1. Introduction of pathogen

- What parameters describing the current epidemic need to be estimated?
  - To what locations was the inoculum distributed?
    - For intentional introductions, this information may be supplied (though its reliability may be suspect)
  - What was the quantity and form of inoculum?

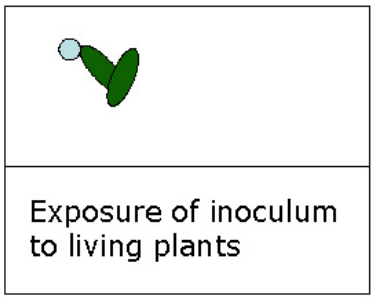


- What actions can be taken to reduce risk?
  - Effectiveness of screening of people entering the US may be increased
- What parameters describing the host-pathogen characteristics need to be known?
  - Does the pathogen have a vector, such as an insect, that will try to reach a host plant?
  - How long can the pathogen survive without host tissue?
  - Can the pathogen survive in food products?

For these example systems, exposure to any of these crop plants could easily be brought about if terrorists desired to introduce the pathogens. Notes below refer to accidental introductions.

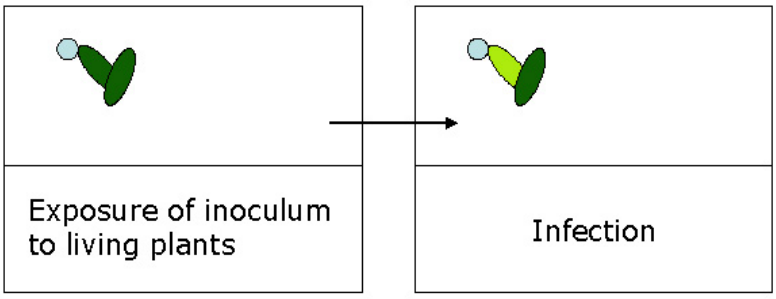
EXPOSURE

System	Notable characteristics at this stage or step
Karnal bunt of wheat	Teliospores and sporidia may be windblown
Slime disease of wheat	Infected seed would need to be in a wheat field for infection
Sorghum ergot	Limited movement through wind and water are possible
Brown strip downy mildew (sorghum)	
Soybean rust	Urediniospores are readily airborne, so the question would be whether there are enough to contact host tissue when diluted over space
Soybean mosaic virus	Seedborne virus is automatically in contact; aphids transmitting the virus can rapidly move over short distances
Philippine downy mildew of corn	
Late wilt of corn	Seedborne pathogen is automatically in contact
Bacterial leaf streak of rice	
Bacterial leaf blight of rice	



2. Exposure of inoculum to living plants

- What parameters describing the current epidemic need to be estimated?
  - A critical step, but challenging to study!
  - What were the environmental conditions when contact occurred?
  - How greatly diluted was inoculum by the time it reached host plants?
  - Is there evidence that exposure was intentional?

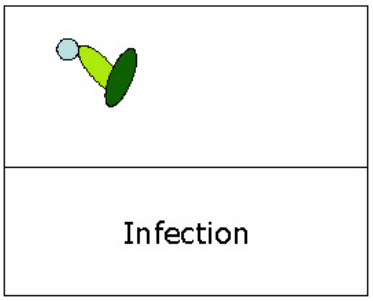


- What actions can be taken to reduce risk?
  - Are resistant plants in use?
  - Is a useful pesticide available?
  - Are biocontrol agents available?
- What parameters describing the host-pathogen characteristics need to be known?
  - What climatic conditions are necessary for infection?

- What threshold level of propagules is necessary for infection?
- How variable might this threshold be?
- Is there an Allee effect?

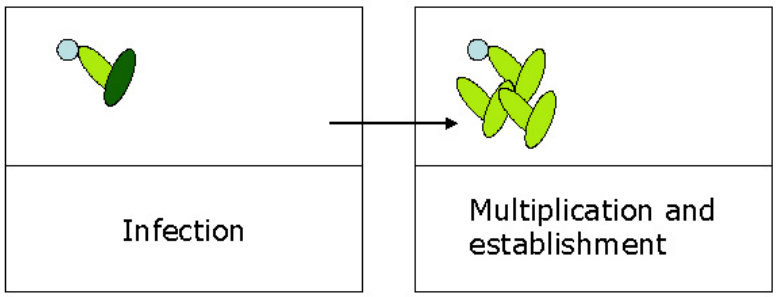
INFECTION

System	**Notable characteristics at this stage or step**
Karnal bunt of wheat	An Allee effect makes infection through a small number of windblown sporidia unlikely; bunted kernels would increase the chance of infection through concentration of inoculum
Slime disease of wheat	
Sorghum ergot	Environmental conditions during a small window for potential infection are critical
Brown strip downy mildew (sorghum)	
Soybean rust	Dew and temperature ranges common in soybean production are necessary for infection
Soybean mosaic virus	Seedborne infection “automatic”; aphid transmission may be increased through introduction of the soybean aphid
Philippine downy mildew of corn	
Late wilt of corn	Seedborne infection “automatic”; inoculum in stubble/debris could easily contact new seedlings and environmental conditions common in corn production are conducive
Bacterial leaf streak of rice	
Bacterial leaf blight of rice	



3. Infection

- What parameters describing the current epidemic need to be estimated?
  - What is the current level of infection?
  - What is the inoculum production for this level of infection given current climatic conditions?
  - What is the spatial extent of infection?



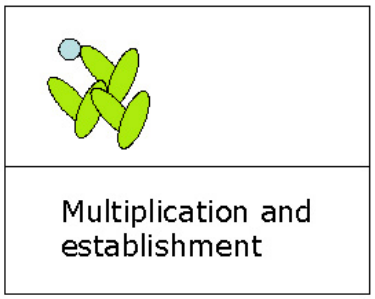
- What actions can be taken to reduce risk?
  - Is a useful pesticide available?
  - Is quarantine useful?
  - Is a biocontrol agent available?
- What parameters describing the host-pathogen characteristics need to be known?
  - Parameters describing reproductive rates and dispersal of the pathogen
  - Parameters describing host abundance and connectivity

- Host range of pathogen

- What are the latent period and infectious period and how are these affected by environmental conditions?

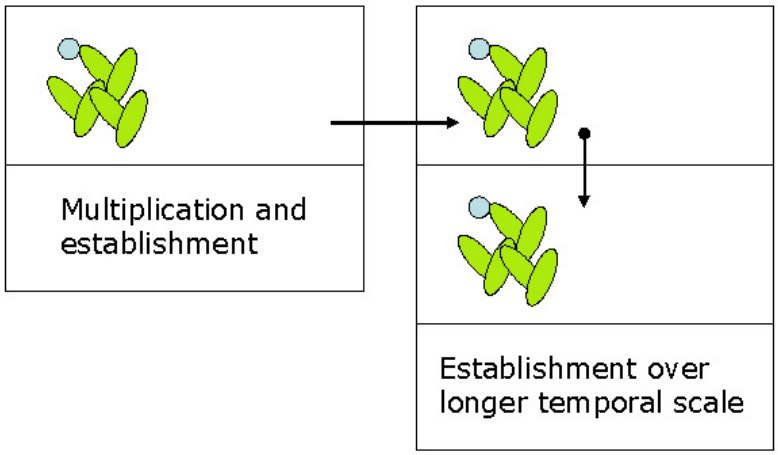
MULTIPLICATION AND ESTABLISHMENT

System	Notable characteristics at this stage or step
Karnal bunt of wheat	An Allee effect will slow multiplication if the infection rate is low
Slime disease of wheat	Secondary spread may be limited
Sorghum ergot	Local multiplication may be limited unless the environment is very conducive
Brown strip downy mildew (sorghum)	
Soybean rust	Pathogen generation time is around 10 days
Soybean mosaic virus	Aphid vectors provide secondary dispersal; the introduction of the soybean aphid may increase the risk of establishment
Philippine downy mildew of corn	
Late wilt of corn	Conducive conditions are common in corn production areas
Bacterial leaf streak of rice	
Bacterial leaf blight of rice	



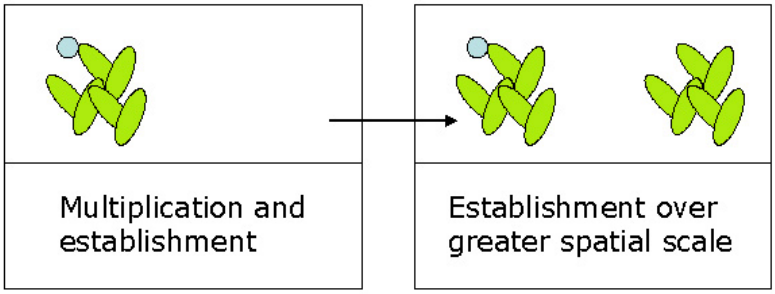
4. Multiplication and establishment

- What parameters describing the current epidemic need to be estimated?
  - What is the mean level of infection?
  - What is the spatial extent of infection?
  - How many plants of what species are infected?
  - What level of inoculum production is occurring?

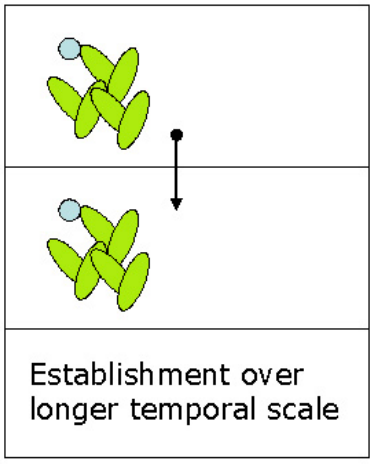


- What actions can be taken to reduce risk?
  - Can crop rotation be used?
  - Are there useful pesticides?
  - Are there useful biocontrol agents?

- What parameters describing the host-pathogen characteristics need to be known?
  - What climatic conditions are necessary for overwintering or oversummering?
  - Does the pathogen have long-lived propagules that can survive multiple unfavorable seasons?
  - What threshold level of propagules is necessary for infection to occur in the coming seasons?



- What actions can be taken to reduce risk?
  - Would quarantine of an area such as a county be useful?
  - Would eradication of the pathogen at this point in time stop spread?
- What parameters describing the host-pathogen characteristics need to be known?
  - What are its long-distance transport characteristics?
  - Does an Allee effect limit new establishment?
  - What is the level of host abundance and connectivity?



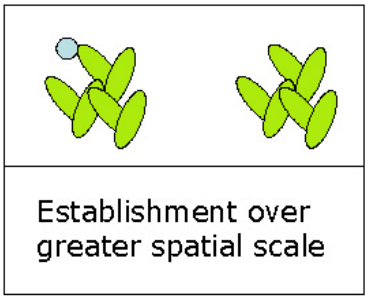
5A. Establishment over longer temporal scale

☐ What parameters describing the current epidemic need to be estimated?

- Is the population increasing or decreasing over time?
- Are there genetic changes within the population that could lead to increased reproduction?

LONGER TEMPORAL SCALE

System	**Notable characteristics at this stage or step**
Karnal bunt of wheat	Teliospores are long-lived and can potentially maintain population through non-conducive years
Slime disease of wheat	Seed processing techniques and rotation can effectively eliminate the pathogen
Sorghum ergot	Environmental variability may limit long-term establishment
Brown strip downy mildew (sorghum)	
Soybean rust	Overwintering may not currently be possible in most of the northern US, but the pathogen might adapt to colder climates; the abundant southeastern US host Kudzu could help to maintain pathogen populations
Soybean mosaic virus	The presence of overwintering hosts for the aphid vectors may be important for long-term establishment
Philippine downy mildew of corn	
Late wilt of corn	The pathogen can persist in stubble and corn debris; no-till systems may help to maintain it
Bacterial leaf streak of rice	
Bacterial leaf blight of rice	



5B. Establishment over greater spatial scale

☐ What parameters describing the current epidemic need to be estimated?

- Are most susceptible hosts between the infection sites infected?
- Are the populations at different infection sites genetically similar?
- Did spread occur naturally or intentionally?

GREATER SPATIAL SCALE

System	**Notable characteristics at this stage or step**
Karnal bunt of wheat	An Allee effect may limit long-distance transport via sporidia, but teliospores may be carried in combines
Slime disease of wheat	Long-distant transport would rely on movement of infected seed or soil
Sorghum ergot	
Brown strip downy mildew (sorghum)	
Soybean rust	Rust spores are well-adapted for long-distance wind dispersal
Soybean mosaic virus	Aphids tend to disperse over relatively short distances; infected seed transport could spread the disease
Philippine downy mildew of corn	
Late wilt of corn	Infected seed can spread the disease, but careful seed management can limit this
Bacterial leaf streak of rice	Central and western U.S. rice production may be distant enough to minimize long-distant transport between populations
Bacterial leaf blight of rice	Central and western U.S. rice production may be distant enough to minimize long-distant transport between populations

*Additional comparative analyses could include response possibilities and how these possibilities differ for different host-pathogen systems.

Soybean Mosaic Virus

Pathway Analysis:

Intentional Introduction of a

Highly Virulent Strain of Soybean Mosaic Virus

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Soybean Mosaic Virus Pathway Analysis

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Executive Summary: Soybean Mosaic Virus Pathway Analysis

- The intentional introduction of a highly virulent strain of soybean mosaic virus (SMV) poses a low to moderate threat to U.S. soybean production.

- Soybean is the second most economically important field crop in the U.S., with a value of $14.8 billion in 2002. The economic impact of a new SMV strain is expected to be moderate if it persists for more than one year. SMV incidence and severity is expected to be greatest in double-cropped or late-planted soybean in the southern U.S. Added control costs could range from 5-10%.

- SMV is the most widespread and economically important virus of soybean. Significant losses have been reported in Korea, Japan, Indonesia, China and Ecuador. Although SMV is present in the U.S., SMV incidence has remained low primarily because little primary inoculum is available in seed. SMV is prevalent in the upper southern production area of the U.S. in late-planted and double-cropped soybean.

- SMV belongs to the potyvirus group. Alternate hosts are deemed unimportant in the U.S. and seed is the primary inoculum source responsible for seasonal virus carry-over. Current inoculum levels in commercial certified seedlots are typically very low (<0.01% in Illinois, Dr. J. Hill, personal communications) and subsequent virus spread occurs too late to have a significant impact on seed transmission levels and yield.

- SMV spread in the Midwest could be significant if sufficient primary inoculum is available (Irwin and Goodman, 1981); thus, the intentional introduction of a highly virulent strain of SMV such as strain G7H recently identified in Korea, could increase primary inoculum and over-all severity.

- Over 30 aphid species (Appendix 2) transmit SMV in a non-persistent stylet-borne manner. Secondary spread is rapid but occurs over short distances (<50 m). Each species has a separate probability of SMV transmission dependent on its innate ability to transmit SMV, probing response, landing rate, and timing in relation to soybean growth.

- Yield losses, seed transmission and secondary spread are greatest when infection is initiated prior to growth stage R_2 (*i.e.* $\geq$ 2 flowers).

- The recent introduction and rapid spread of the soybean aphid (*Aphis glycines*) across the north-central soybean production region may increase the potential for SMV epidemics. The role that *A. glycines* will play in SMV transmission in the U.S. will be determined largely by the numbers and timing of migrating alatae in relation to soybean growth stage.

- A schematic **SMV disease and response pathway is presented in Appendix 6**.

- Seed would be the easiest vehicle for covert SMV introduction. Single gene resistance employed in SMV-resistant cultivars in the U.S. are likely ineffective against exotic strains. Minimal expense and equipment would be required for on-site production and single-season storage of moderate quantities of infected seed.

- Viruliferous aphid vectors could be used to introduce SMV into an area although considerable equipment, knowledge and planning would be required.

- *A. glycines* is predicted to be distributed over approximately 90% of the total U.S. soybean production area (Fig. 6), and widespread transmission of an introduced SMV strain could be expected if alate vectors fly in the early soybean growth stages.

- The level of risk would be greatest if soybean seed nurseries were targeted, increasing the potential for infected seed to be dispersed through commercial seedlots.

- A virulent strain of SMV has a moderate likelihood of permanent establishment. Since SMV must be triggered each year by infected seed, incidence will depend upon the ability of seed companies to recognize and eliminate infected seed from commercial seedlots. Early detection is unlikely; some strains, like G5H and G7H, can cause atypical SMV symptoms (severe necrosis) in the U.S.

- The best measures to preclude losses are using SMV-free seed and avoiding late planting. Quarantine and containment of SMV will not be possible.

- Resistant varieties will be necessary if low levels of SMV in seed cannot be maintained.

Immediate Response Options

- The introduction of a new virulent strain of SMV presents a particular threat to soybean seed nurseries. Seed production fields should be isolated from commercial soybean areas. Phytosanitary seed certification using reliable screening techniques would be required to maintain virus-free seed. Additional training of seed producers, scouts and seed certifiers will increase the likelihood of early detection.

- Although, chemical vector control is not fully effective in eliminating plant viruses, it may be justified to reduce the spread of a newly introduced strain if detected early. Late applications (soybean stage $>R_1$) are unlikely to have a significant impact on SMV incidence.

- The development of SMV-rate-reducing transgenic soybeans and identification of SMV-resistant genotypes in maturity groups appropriate for the North Central region could reduce the impact of exotic and SMV strains already present in the U.S. In the event of an outbreak of a new virulent strain of SMV, resistant cultivars should be introduced as rapidly as possible to minimize damage.

Soybean Mosaic Virus

Pathway Analysis for the Intentional Introduction of a

Highly Virulent Strain of

Soybean Mosaic Virus

Soybean mosaic virus (SMV) is the most widespread and economically important virus of soybean (Irwin and Goodman, 1981; Ruesink and Irwin, 1986). SMV does not have quarantine status because it is present everywhere soybeans are grown. In the U.S., SMV is generally not recognized as a serious problem, but it is prevalent in the upper southern production area in late-planted and double-cropped soybean (Ghabrial *et al.*, 1977; Ren *et al.*, 1997b), and can be especially damaging when it occurs with other soybean diseases such as *Phomopsis* spp. and bean pod mosaic virus (Gu *et al.*, 2002).

SMV incidence has remained low primarily because very little primary inoculum is available from infected seed in the U.S. (Dr. J. Hill, personal communication), but the intentional introduction of a highly virulent strain of SMV, such as G7H recently identified in Korea (Kim *et al*., 2003), could increase primary inoculum and over-all severity. Aphids are responsible for all known secondary spread of SMV. There is growing concern that the recent introduction and rapid spread of another SMV vector, *Aphis glycines*, in the north-central soybean growing area of the U.S., increases the potential for SMV epidemics. This report is a pathway analysis for the intentional introduction of a highly virulent strain of SMV into the U.S. A disease pathway and response schematic is presented in Appendix 6.

I. Biology and life/disease cycle of the pathogen

1. Identity

a. Preferred Name: Soybean mosaic virus Gardner and Kendrick (1921)

Taxonomic Position:

Kingdom: Virus

Family: Potyviridae

Other Names of the Pathogen:

Soybean mosaic potyvirus

Soja virus 1

Soybean virus 1

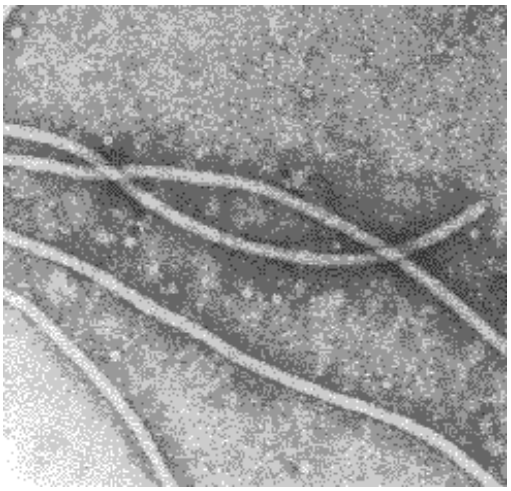


Fig. 1. Flexuous rods of SMV. Photo from Rothamsted Exp. Stn., 1994

b. Causal Agent Description

SMV particles are flexuous rods averaging 15-18 x 750 nm (Bos, 1972) (Fig. 1). They have a helical symmetry with a pitch of 3.4 nm and contain 5.3% single-stranded RNA with a molecular weight of 3,250,000 (Hill and Brenner, 1980). Two of the viral strains (G2 and G7) have been completely sequenced and are 9,588 nucleotides long. A genetic map has been constructed and nine virus-encoded proteins have been predicted (Jayaram *et al*., 1992).

c. SMV Variability and Nomenclature

The blister strain (SMV-B) was identified in the U.S. in the 1970s (Ross, 1975) and the necrotic strain (SMV-N) in Korea (Cho *et al*., 1977). SMV has been classified into nine strains in the U.S. by differential reactions on eight soybean cultivars (Buss *et al*., 1989). In 1979, 98 isolates from the USDA germplasm collection were classified into seven strains, G1-G7 (Cho and Goodman, 1979). Strains G7a and C14 (Lim, 1985) were later identified. Takahashi *et al*. (1980) differentiated five SMV strains in Japan based on reactions on four cultivars, but their relationship to the U.S. identified strains is unknown (Buss *et al.*, 1989). Still other strains have been reported in China (Gai *et al*., 1989) and Brazil (Almeida, 1981). Most recently, G5H and G7H, are reported to cause necrosis in previously unchallenged

resistant soybean cultivars (Kim *et al*., 2003). G7H causes the most severe necrotic symptoms of any strain identified to date. Taxonomic rules for naming variants do not exist, but the cultivars used by Cho and Goodman (1979) and Lim (1985) are generally used to identify strains reported in the literature.

SMV belongs to the large and important potato virus Y (potyvirus) group. SMV, cowpea aphid-borne mosaic virus, passionfruit woodiness potyvirus and zucchini yellow mosaic potyvirus are all considered to be distinct species closely related to bean common mosaic; however, Taiwan isolates of SMV, peanut stripe virus, blackeyed cowpea mosaic virus and azuki bean mosaic virus are considered to be isolates of bean common mosaic virus (McKern *et al.*, 1992). Based on genome sequence comparison, peanut stripe potyvirus is most closely related to SMV (Gunasinghe *et al.*, 1994).

2. Geographic Distribution

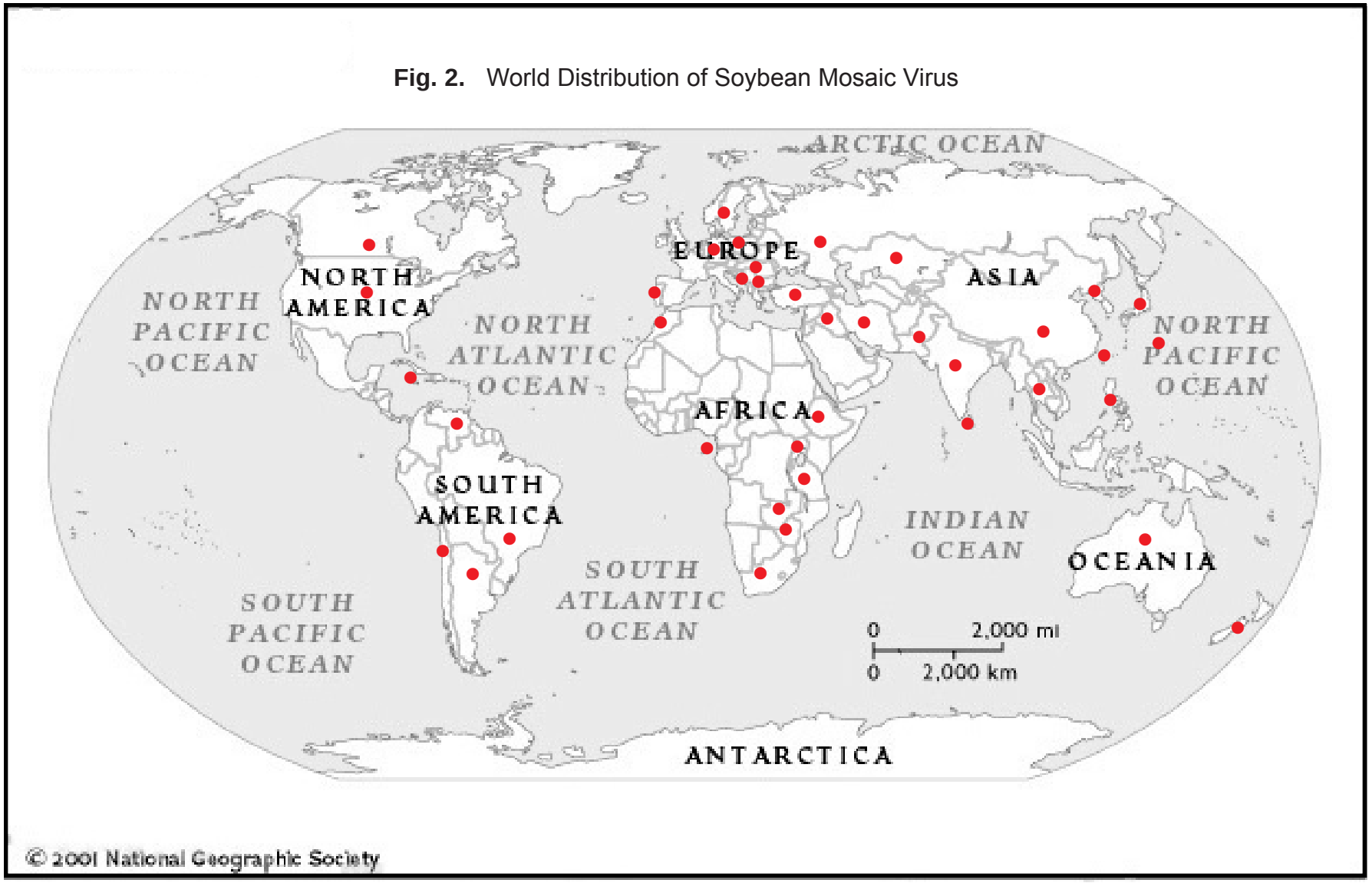
Soybean mosaic virus (SMV) occurs in all soybean-production areas of the world (Table 1 and Fig. 2) and probably reflects the prevalence of seed transmission.

Table 1. Countries reporting the presence of soybean mosaic virus.

Continent	Countries
Africa	Ethiopia, Morocco, South Africa, Tanzania, Uganda, Zambia, Zimbabwe
Asia	China, Japan, Taiwan, India, Iran, Iraq, Kazakhstan, Korea, Malaysia, Pakistan, Philippines, Sri Lanka, Thailand, Turkey
Europe	Bulgaria, Former Yugoslavia, Germany, Italy, Moldova, Poland, Portugal, Romania, Russian Federation, Sweden, Ukraine, Yugoslavia
Western Hemisphere	Argentina, Brazil, Canada, Chile, Jamaica, USA, Venezuela
Oceania	Australia, New Zealand

From CABI (1999)

Fig. 2. World Distribution of Soybean Mosaic Virus



Adopted from CABI, 1999.

3. Host Range of the Pathogen

Soybean is the most economically important host of SMV. Although SMV is often cited as having a narrow host range limited almost entirely to Fabaceae (CABI, 1999), it is capable of infecting a few species in five other plant families when artificially inoculated (Appendix 1). Local lesion and latent infection in some hosts are known. Cultivated soybean is practically the only known natural host of the virus although *Phaseolus vulgaris* and a few other plants have been reported naturally infected (CABI, 1999).

The virus can be artificially transmitted to a number of Fabaceae species. The symptoms of SMV in experimental hosts are similar to those of other potyviruses and suggest that natural SMV infection in such species may be mistaken for infection by other viruses. Symptom expression is varied and dependent upon species, cultivar, temperature, and SMV strain (Walters, 1963; Hill, 1999). SMV recovered from symptomless plants indicated systemic infection in *P. vulgaris, P. speciosus, Vigna sinensis* (Galvez, 1963) and *Macroptilium lathroides* (Porto and Hagedorn, 1974), suggesting that these species may be a natural source of infection (CABI, 1999). Aphids successfully transmitted SMV from artificially infected *M. lathroides* to soybean plants in Brazil, but SMV was not seed-borne in *M. lathroides* (Porto and Hagedorn, 1974).

Of the potential nonlegume hosts, only *Amaranthus* sp., *Chenopodium album*, *Setaria sp.*, *Physalis virginiana, P. longifolia,* and *Solanum carolinense* are important to the North Central soybean producing area (Hill *et al*., 1980). The most prevalent of these (*Amaranthus* sp., *C. album* and *Setaria* sp.) are annual plants and, barring transmission through seed, are unlikely to be overwintering hosts for the virus (Hill *et al.*, 1980).

4. Disease Impact

a. Effect on yield and seed transmission

SMV is generally not recognized as a serious problem in the U.S. or Brazil, the two major soybean-producing countries. Reports on the natural incidence of the disease range from less than 2% of plants infected in fields in Ontario, Canada (Tu, 1986) to 50% in Korea (Cho *et al*., 1977) and 45-65% in Georgia, Asia (Lekveishvili, 1976). Serious epidemics and yield losses have been reported in Japan, Indonesia, Ecuador, and China (Irwin and Goodman,

1981). In field experiments, yield reductions usually range from 7-35 % (Hartwig and Keeling, 1982; Hill *et al*., 1987; Ross, 1969, 1977) with some losses reported as high as 94-95 % (Chen *et al*., 1988; Goodman and Oard, 1980) depending on time of infection, dissemination, and cultivar. Eighty cultivars field inoculated with 7 strains of the virus, clearly demonstrate that losses due to SMV are highly dependent on cultivar and virus strain (Tu, 1989).

Yield losses (Irwin and Goodman, 1981; Ren *et al.*, 1997; Ross, 1969; Tu, 1992) and seed transmission (Bowers and Goodman, 1979) are more serious when SMV infection occurs at early compared to late growth stages. Ren *et al.* (1997) found no reduction in yield when plants were infected after flowering (after growth stage R_2) and proposed potential yield losses due to natural SMV infection could be calculated with Large's critical stage model. Early infection is usually accompanied by more secondary spread and thus a higher incidence of SMV and yield loss (CABI, 1999).

Irwin and Goodman (1981) speculate that SMV has not been a major problem in Iowa because most virus spread occurs too late to have a significant impact on seed transmission levels and yield. Crop losses at low disease incidence are disproportionately low because surrounding healthy plants compensate for yield losses from diseased plants (Ross, 1983). Based on field studies using artificial SMV inoculation, Hartwig and Keeling (1982) concluded that planting seed from virus infected cultivars having 10-15% virus transmission to seedlings has little effect on yield or maintaining SMV in the area. In their study, SMV infected seedlings died within three weeks of emergence. Virus infected seedlings that remain relatively vigorous may result in greater losses because they continue to serve as an inoculum source for secondary spread and occupy space in the canopy with less potential for seed production (Irwin and Goodman, 1981). Inoculum levels in commercial certified seedlots in Illinois are typically very low (Dr. J. Hill, personal communications) (<0.01%), but SMV will spread in Midwestern soybean fields if sufficient primary inoculum is provided (Irwin and Goodman, 1981).

SMV is prevalent in the upper southern soybean production area of the U.S. (Ghabrial *et al.*, 1977; Ren *et al.*, 1997b) where double-cropping of soybean is an important production system. In these areas, the most rapid field spread of SMV occurs in late July and early August (Ren *et al.*, 1997). Soybean may be infected by SMV at earlier developmental stages when planted late compared to early, making them more susceptible to yield

reductions. A SMV infection rate as low as 18% can produce a yield loss in Kentucky in the late crop, presumably because of reduced vegetative growth and the greater impact of SMV infection in late plantings (Ren *et al.*, 1997b). Irwin and Goodman (1981) predicted that the incidence of seedborne SMV could build up over several seasons in second crop soybean and recommended that seedlots from late-planted soybean not be used for planting.

SMV predisposes soybean to other disease organisms. SMV-susceptible cultivars inoculated with SMV exhibited a 3- to 8-fold increase in *Phomopsis* spp. seed decay (Koning *et al.*, 2002). Bean pod mottle, cowpea mosaic virus, or peanut mottle virus are synergistic with SMV and cause much greater losses than SMV alone (Anjos *et al.*, 1992; Quiniones *et al*., 1971; Ross, 1968, 1969).

b. Effect on seed quality

SMV infected seeds are generally smaller and may be mottled brown to black (Fig. 3) which reduces commercial value. Mottled seedcoats showed higher accumulations of anthocyanin, chlorophyll a and b, and ferritin than non-mottled seed (Tu, 1975). It is well documented that SMV can induce seedcoat mottling, but the relationship between mottling and virus transmission is inconsistent (Bryant *et al.*, 1982; Hartwig and Keeling, 1982; Tu, 1989) and can depend on environment, plant growth stage at infection, cultivar and genotype. Mottling does not indicate that the virus is present in the seed, since not all mottled seeds contain virus and some infected seeds are not mottled. Non-SMV-infected plants may produce low percentages of mottled seeds. The correlation between the percent of infected mottled seeds and the severity of mottling varies from year to year (Hill *et al.*, 1980). Seedcoat mottling decreased in inoculated plants as temperature increased from 15-20°C to 30°C (Ross, 1970; Tu, 1992). Seedcoat mottling was most pronounced when plants were infected with SMV at early growth stages (Tu, 1992) and little mottling resulted from plants inoculated after growth stage R_1 (Ren *et al*., 1997). One cultivar yielding 92% and another 11% mottled seed, both had 10% yield depression (Hartwig and Keeling, 1982). More seedcoat mottling was induced by the Japanese SMV strain that caused the least amount of seed infection (Iwai *et al.*,1985). Mottling can also be controlled by a single gene *Im*, in the absence of SMV (Cooper, 1966).

Seed size was reduced 10 to 20% in SMV infected seed (Irwin and Goodman, 1980) and yield by 12% (Hartwig and Keeling, 1982). Seed germination was reduced 7% in a

susceptible cultivar in the U.S. (Hartwig and Keeling, 1982) and 17% in cultivars in Egypt (El-Amrety *et al.*, 1985), but no reduction in germination was reported in other studies (Irwin and Goodman, 1981). Oil content is generally reduced and protein increased in infected seed (Suteri, 1980). SMV predisposes plants to infection by other pathogens such as *Phomopsis* spp. that reduce seed quality and vigor (Koning *et al.,* 2002, 2003).

5. Symptoms

SMV produces variable symptoms depending on cultivar, plant growth stage at time of infection, strain of the virus, and environmental conditions. Most infected cultivars are slightly stunted and have fewer pods that may be flattened, glabrous, and empty. Seeds from infected plants are generally smaller and may be mottled brown to black (Fig. 3).

The first symptom on trifoliate leaves is a transient vein clearing, followed by characteristic light and dark green mosaic areas that may later become raised or blistered (Fig. 4). Wavy leaf margins or downward curling and veins that fail to grow together also may develop and resemble herbicide injury. Local leaf and veinal lesions develop on some cultivars that may be followed by yellowing and leaf abscission. When SMV infection occurs early in soybean growth, symptoms are more pronounced (Tu, 1992). Some strain-cultivar mixtures result in severe symptoms, e.g. progressive necrosis of the petioles and stems, bud necrosis, defoliation, terminal necrosis, and death (Cho *et al.*, 1977). Severe symptoms are similar to tobacco ring spot and tobacco streak virus infections. Different SMV strains often produce different symptoms on the same cultivar. Most symptoms are more severe at 20°C than at 25-30°C (Tu, 1992; Walters, 1963). Rugosity is most pronounced in plants grown at 18°C and symptoms are largely masked at temperatures above 30°C.

6. Detection and Diagnostic Methods

Symptoms are not fully diagnostic because of differences at various stages of plant growth, cultivar differences and environmental interactions. SMV can be detected in all parts of a systemically infected plant by techniques that detect the positive-sense, single-stranded RNA or the single polyprotein precursor it encodes for. The length of time between infection and symptom expression is longer at cooler temperatures (14 days at 18.5°C versus 4 days at 29.5°C) and masked at temperatures above 30°C. SMV can be distinguished from non-

Fig. 3. Seed mottling caused by SMV
Photo by Craig Grau

Fig. 4. Blistering of SMV infected soybean leaves.
Photo by Laura Sweets

potyviruses in soybean by the presence of granular pinwheel (cross section) or bundle (longitudinal section) type intracellular inclusion bodies in epidermal strips observed under light microscopy (Christie and Edwardson, 1977) or the presence of 750 nm-long flexuous particles in crude sap by electron microscopy (Boss, 1972). Sophisticated detection techniques are needed for definitive confirmation. SMV can also be detected by reaction with broad-spectrum potyvirus monoclonal antiserum.

Mottled seeds are an indication of probable infection, but poor indicator of virus transmission since the pathogen can also be transmitted by nonmottled seed (Hill *et al*., 1980). A number of seed "grow-out" methods have been developed (Bowers and Goodman, 1982; Iwai *et al.*, 1985; Porto and Hagedorn, 1975; Tu, 1975) where greenhouse-grown seedlings are examined for SMV symptoms, and confirmed by inoculation of indicator plants or by ELISA.

SMV can be detected by: enzyme-linked immunosorbent assays (ELISA) (Lister, 1978; Ma *et al.*, 1982; Maury, 1984; Maury *et al.*, 1985), solid-phase radioimmunoassay (SPRIA) (Bryant *et al.*, 1983; Hill *et al.*, 1984), and serologically specific electron microscopy (SSEM). SSEM is sensitive enough to detect one infected seed from 1000 healthy seeds (Brlansky and Derrick, 1979). Nucleic acid hybridization analysis is a sensitive and specific technique to determine the amount of SMV-RNA in seeds (Koning and TeKrony, 2003).

Since noninfectious SMV antigen in the seed testa can result in overestimates of virus transmission to seedlings, Maury (1985) developed a method to remove seedcoats before testing seedlots for infection in order to avoid false positive reactions. Seedlots can be evaluated reliably for percentage of SMV transmission by using a statistical method using groups of 30 seeds, where the number of groups depends on the precision required (Maury *et al.*, 1985).

SMV strains are typically identified by inoculating differential cultivars, which is a time consuming procedure. SMV strains also can be compared using the nucleotide sequence of the cylindrical inclusion (CI) region. Nucleotide sequences of SMV strains G2, G7 (Jayaram *et al.*, 1992), G5H and G7H (Kim *et al*., 2003) have been determined. These sequence variations can be exploited to distinguish different strains using PCR or DNA hybridization techniques.

7. SMV Biology and Dissemination

a. Virus properties

The virus remains infective in expressed plant sap for 2-5 days and has a thermal inactivation point of 55-70°C (CABI, 1999). The virus moves both acropetally and basipetally for systemic infection of plants. Plants can be a source for SMV 5-6 days after infection and for as long as four months (Schultz *et al.*, 1983). Upper and middle fully expanded leaves are the best sources of inoculum.

b. Pathogen transmission

SMV is sap (mechanical and vector) and graft transmissible, but is not transmitted by dodder (*Cuscuta* spp.). Seed is considered the primary inoculum source for the pathogen and is responsible for dissemination of the virus from one geographical region to another (Irwin and Goodman, 1981). SMV continues to be spread through germplasm used in breeding programs (Cho and Goodman, 1979), and breeders' materials used in multilocational testing. The virus is introduced via commercial seed into crops each growing season. Secondary spread of SMV is due primarily to transmission by a large number of aphid species in a non-persistent manner.

i. Effects of transmission timing on soybean disease impact

Plants in all stages of growth are susceptible to SMV but yield losses (Irwin and Goodman, 1981; Ren *et al.*, 1997; Ross, 1969; Tu, 1992) and seed transmission (Bowers and Goodman, 1979) are more serious when SMV infection occurs at early compared to late growth stages. No significant reduction in yield and low seed transmission were found when plants were infected after flowering (after growth stage R_2) (Ren *et al.*, 1997; Tu 1992; Wilcox and Laviolette, 1968). Early infection is usually accompanied by more secondary spread, leading to higher disease incidence and yield loss (CABI, 1999).

ii. Seed transmission (primary inoculum)

The pathogen is seed-borne and plants grown from infected seed play an important role in the epidemiology of SMV as primary inoculum. Hill *et al.* (1980), using uncaged and caged plots to protect from insect-borne inoculum, verified this hypothesis where an aggregate SMV spatial distribution suggested plant-to-plant spread (presumably by aphids) from

primary random foci within the field. Irwin and Goodman (1981) showed that SMV spread outward from point sources of inoculum in an initially SMV-free field. These findings, along with the suspected lack of overwintering hosts in the northern soybean producing area of the U.S., imply that infected soybean seed is the primary source of virus inoculum (Hill *et al.*, 1980).

The incidence of seed transmission from parent plants is influenced by stage of plant growth, temperature, cultivar and SMV strain. SMV was transmitted at rates ranging from 0 to 75.6% (in 80 cultivars inoculated with 7 SMV strains) in the greenhouse (Tu, 1989). In most cultivars, seed transmission is less than 5% (Hill, 1999). In fields with 100% incidence of infection, seed transmission of SMV averaged 10% in plants inoculated prior to flowering, compared to 3.5% for plants inoculated after flowering (Ren *et al.*, 1997). Maximum seed transmission occurred at 20°C, followed by 15 and 25°C and the lowest transmission at 30°C (Tu, 1992).

Both mottled and non-mottled seed from SMV infected plants can transmit SMV (Kennedy and Cooper, 1967). Some research shows SMV transmission is consistently higher in mottled seed (Koning *et al.*, 2003; Tu, 1992), while other cases show no correlation (Bryant *et al.*, 1982; Hartwig and Keeling, 1982; Tu, 1989). SMV accumulation in seedcoats was not directly related to the degree of seedcoat mottling (Koning *et al.*, 2003).

A positive correlation exists between virus titer in the parent plant and transmission to seed (Tu, 1992) but not all seed produced by a SMV-infected plant are infected (Bowers and Goodman, 1979; Hill *et al.*, 1980). Although SMV antigen can be detected serologically in the seed testa, the particles may not be infectious (Bowers and Goodman, 1979; Tu, 1975). Removing the coat of infected seed did not affect seed transmission (Tu, 1975). As seeds mature and dry, SMV is inactivated in the testa and in SMV resistant soybean cultivars, inactivation also occurs in the embryo (Bowers and Goodman, 1979). Virus is found only in the embryo of dormant seed. Thus embryo infection is a prerequisite for SMV transmission by seed (Irwin and Goodman, 1981). SMV transmission has been demonstrated by two-year-old seeds (Kendrick and Gardner, 1924) and virus infectivity can exceed the germinability of the seed.

iii. Aphid transmission (secondary spread)

Until the year 2000, U.S. soybean was thought to be free of colonizing aphids and SMV transmission was attributed solely to winged transient aphid species. In July 2000, the Asian soybean aphid (*Aphis glycines* Matsumura*)* was identified in southeastern Minnesota. By 2001, *A. glycines* was reported from Missouri to Manitoba and Virginia to North Dakota. The rapid spread of *A. glycines* across the northern soybean growing areas of the U.S. is cause for concern because of both the potential for direct insect damage and transmission of viruses such as SMV. Researchers anticipate SMV will become more widespread in the U.S. soybean crop due to the presence of the soybean aphid (Dr. G. Hartman, personal communication). Because of the potential importance of this newly arrived soybean-colonizing aphid in spreading SMV, *A. glycines* is considered in detail in section I-7iv.

SMV causes the most damage when vector intensity values (vector's innate ability to transmit SMV + probing response and vector landing rate) are high during the first 4-5 weeks after seedling emergence (Irwin *et al.,* 2000). Thus, the timing of aphid flights and its correspondence to soybean development is critical to SMV incidence, yield loss and seed transmission.

Non-colonizing Aphids

SMV is transmitted non-specifically by aphids in a non-persistent, stylet-borne manner. Secondary spread of SMV by aphids occurs at a relatively fast rate from a point source of inoculum (Hill, 1999) but over relatively short distances. Infection declined exponentially with distance from foci of inoculated soybean plants, with 95% of the spread within 17 m of the foci at the end of the season and the most distant infected plant was 45m away (Irwin and Goodman, 1981). Aphid-borne, long-distance movement of SMV has never been demonstrated (Irwin *et al.*, 2000); however, long distance transport (12 km) by aphids has been reported for other non-persistently transmitted legume viruses (CABI, 1999). Wind speed and direction influence flight and, therefore, SMV spread is most pronounced downwind from virus-infected source plants (Irwin and Goodman, 1981).

Secondary spread of SMV depends on cultivar susceptibility, time of crop development, vector availability, population density, behavior and transmission efficiency. The later factors may vary considerably between aphid species. At least 32 species of aphid belonging to 15 different genera transmit SMV (Appendix 2). Each species has a separate

probability of successfully transmitting SMV based on the vector's innate ability to transmit the virus and its behavioral probing response after landing. Two isolates of SMV were transmitted 2-3 times more efficiently by the green peach aphid (*Myzus persicae*) than the corn leaf aphid *(Rhapalosiphun maidis)* (Lucas and Hill, 1980). Most aphid vectors can transmit the virus only once per acquisition; however, *M. persicae* could occasionally transfer SMV (2.94%) to a second plant (Guo and Zhang, 1989). In Illinois, 60 aphid species were collected in soybean fields, but only 5 species were responsible for 93% of all SMV transmissions, *i.e. Aphis craccivora, Macrosiphum euphorbiae, M. persicae, R. maidis and Rhapalosiphun padi* (Irwin and Goodman, 1981). Although *R. maidis* was a relatively inefficient transmitter, it had the greatest impact on virus transmission in that study because of its large population size. Virus strains show some vector specificity; SMV-O isolate is transmitted by *M. persicae* but not by *R. maidis* (Lucas and Hill, 1980).

Extensive studies have been conducted on SMV epidemiology in relation to aphid behavior. Aphid probing and landing rates, and flight patterns influence the field spread of SMV. Aphid vectors transmit the virus most efficiently after acquisition probes of 30-60 seconds, and significantly less efficiently after access times in excess of 15 minutes (Irwin and Goodwin, 1981; Schultz *et al*., 1983). More densely pubescent soybean cultivars retarded the spread of SMV by inhibiting the probing activity of aphids (Gunasinghe *et al.*, 1988; Ren *et al.,* 2000). The effect of trichome density was species dependent; the probing behavior of *M. persicae* being less affected by trichome density than that of *R. maidis* (Gunasinghe *et al.*, 1988). The incidence level of SMV at R_1 was 25%, 18% and 5% for normal, dense and extra-dense pubescent isolines respectively. Extra dense isolines also delayed the time at which maximum SMV spread occurred (Ren *et al.,* 2000)

The strong correlation between alatae landing rate and the spread of SMV has been demonstrated in several experiments (Halbert and Irwin, 1981; Irwin and Goodman, 1981; Schultz *et al.*, 1985)**.** Landing rates of aphids depend on the amount of canopy cover, weather, canopy color, and other environmental variables (Halbert and Irwin, 1981). Most studies report fewer landings/unit area by most aphid vectors in denser soybean cover (Bottenberg and Irwin, 1992a, 1992b; Halbert and Irwin, 1981; Irwin and Kampmeier, 1989). A preference for landing in an open canopy may not be true of all species since *Aphis citricola* and *Myzocallis punctatus* prefer a closed canopy (Halbert and Irwin, 1981). Field experiments using two isolines of soybean that differed only in their chlorophyll content showed aphid alighting ratios of 2:1 in favor of the normal green isoline over the chlorophyll

deficient, with a proportional reduction in SMV spread in the deficient isoline (Irwin and Kampmeier, 1989).

iv. Soybean Aphid

The recent introduction of *A. glycines* into North America raises serious questions in regard to the spread of viruses in soybean fields. The soybean aphid is capable of transmitting a number of viruses known to naturally infect soybean in the U.S. including SMV, bean yellow mosaic virus, peanut mottle virus, alfalfa mosaic virus and peanut stunt virus (Wang and Ghabrial, 2002). *A. glycines* is a colonizing aphid in Asia where SMV can have devastating effects on soybean yield.

U.S. Distribution

Since July 2000 when *A. glycines* was first identified in the U.S. it has spread across the U.S. Midwest at an alarming rate (Fig. 5; Appendix 3). By autumn of 2000, *A. glycines* was observed in Illinois, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, Ohio, West Virginia, and Wisconsin. The heaviest infestations were reported in southeastern Michigan, southern Wisconsin, southeastern Minnesota, northern Illinois, and northern Indiana (DiFonzo and Hines, 2001).

The average rate of spread in Minnesota was 3.1- 6.3 miles per day, but this rate was not constant throughout the season (Patterson, 2003). For two periods (June 27-July 3 and August 1-7), aphids spread at a considerably faster rate of 6-8 miles/day, presumably parallel to the time of alate (winged) soybean aphid flight. A soybean aphid watch website (http://www.pmcenter.org/Northcentral/saphid/) has been created to supply information on the weekly seasonal appearance of *A. glycines* on soybean across the U.S. and Canada. The extensive distributions of soybean aphid populations since 2000 indicate significant physiological and/or behavioral adaptations that permit successful reproduction and growth over a wide range of temperature and moisture regimes.

Kansas State University Departments of Geography and Entomology used GIS-related (Geographic Information Systems) research to perform a **pathway analysis for the soybean aphid** (Appendix 3; Hutchinson *et al.*, 2003). Genetic algorithms for rule-based prediction (GARP) were employed to characterize and quantify the spread of *A. glycines*. An asymmetric spread (E + W > N + S) was revealed with a geographic center near

Chicago, Illinois, to identify the suspected point of introduction (Appendix 3- Figure 4). Annual SBA rate of spread declined from 468 km in 2000 to 82 km in 2002 (Appendix 3- Table 1). Apparent changes in dispersal rate may simply reflect a delay in identifying the SBA outbreak and insufficient field observations. Assuming a range increase comparable to that calculated for 2002, an additional 18 states in the Great Plains, southeast, and northeast may report soybean aphid establishment this year. States into which *A. glycines* will most likely expand include North Dakota in the north and Oklahoma, Arkansas, Tennessee, and Mississippi in the south. Thus, *A. glycines* is forecast across the Midwestern and Eastern soybean-growing regions to encompass approximately 90% of the total U.S. soybean production area (Fig. 6).

Economic impact due to *A. glycines*

The most apparent economic impact of the soybean aphid reported thus far in the U.S. is due to direct insect feeding damage. In strip trials in Minnesota and Wisconsin, soybean aphid reduced yields as much as 16 bu/acre, with average yield reductions in the 3.7 to 7.4 bu/acre range (Ostlie, 2001; Ragsdale and Patterson, 2002; Appendix 3). Populations of several thousand aphids/plant can reduce yields by more than 50% with losses attributed primarily to reduced pod set and lesser effects on the number of nodes and seeds/pod (Ostlie, 2003). During the summer of 2003, soybean aphid populations ranged from hundreds to thousands per plant in some upper Midwestern counties, and insecticides were applied to a considerable acreage (Dr. R. O'Neil, personal communication).

In a preliminary survey of soybean fields in 23 Kentucky counties (Aug., 2001), SMV was detected in only 0.8% of leaf samples collected, even though *A. glycines* was widely present in those fields (Ghabrial and Hershman, unpublished data). After extensive sampling, no increase in SMV has been noted in Illinois (2001- August, 2003), implying that the incidence of seedborne SMV in commercial seed must be extremely low (Dr. L. Domier, personal communication). The full economic impact related to *A. glycines* transmission of SMV in the U.S. may not become apparent for a number of years. An increased incidence of mixed infections of SMV and BPMV is presently a major concern in the north-central and southeastern states (Gu *et al.*, 2002), and could be further aggravated by the simultaneous transmission of both viruses by *A. glycines*.

Identification and Life Cycle

A. glycines is a small yellow aphid with distinct dark cornicles and a pale cauda (Fig. 7). The soybean aphid has a complex life cycle with 15 to 18 generations annually (Fig. 8). The aphid survives the winter as eggs on woody shrubs called buckthorn (*Rhamnus* spp.) (Fig 9). Nymphs hatch in the spring and, after two generations of wingless females, a generation of winged females is produced that migrates to soybean. Aphid population growth is influenced by rainfall (heavy rainfall may cause a significant reduction in soybean aphid populations, especially when first migrating to early vegetative soybean (Ostlie, 2003). Only females that bear live young without sexual reproduction are present in the summer. The wingless form (apterae) predominates. Overcrowding or reduction in soybean quality triggers production of the winged form (alatae). Alatae disperse to deposit live nymphs on other soybean plants within the field or in other fields. Females are capable of bearing their own young within 7 days. Populations may double in as little as 2 to 3 days. In the fall, winged males and females are produced that seek out buckthorn, where sexual reproduction occurs. For an extensive review of soybean aphid biology in North America see Ragsdale *et al.* (2004).

The relative distribution of primary and secondary hosts appears to influence aphid dynamics in soybeans (Takahashi *et al.*, 1993). Aphids appear to infest soybeans earlier and at higher densities in fields near overwintering sites than in soybeans located farther from overwintering sites (Ostlie, 2003). The spatial relationship between *Rhamnus* populations and soybean fields may therefore play a critical role in the timing and intensity of aphid infestations. The distribution of *R. frangula* and *R. cathartica* in the upper Midwest has recently been surveyed but information on some counties is incomplete (Fig. 10). Although *R. cathartica* (Fig. 9)*, R. frangula, R. davurica, R. alnifolia, and R. lanceolata* have been reported as winter hosts (Appendix 3), only *R. cathartica* and *R. alnifolia* had overwintering eggs deposited on them by *A. glycines* in a recent study (Voegtlin *et al*., 2004).

SMV transmission by *A. glycines*

Hill *et al.* (2001) demonstrated that the soybean aphid in the North Central region transmitted an endemic strain of SMV. The potential for spread of SMV was confirmed when *A. glycines* transmitted six field isolates of SMV that originated from naturally infected

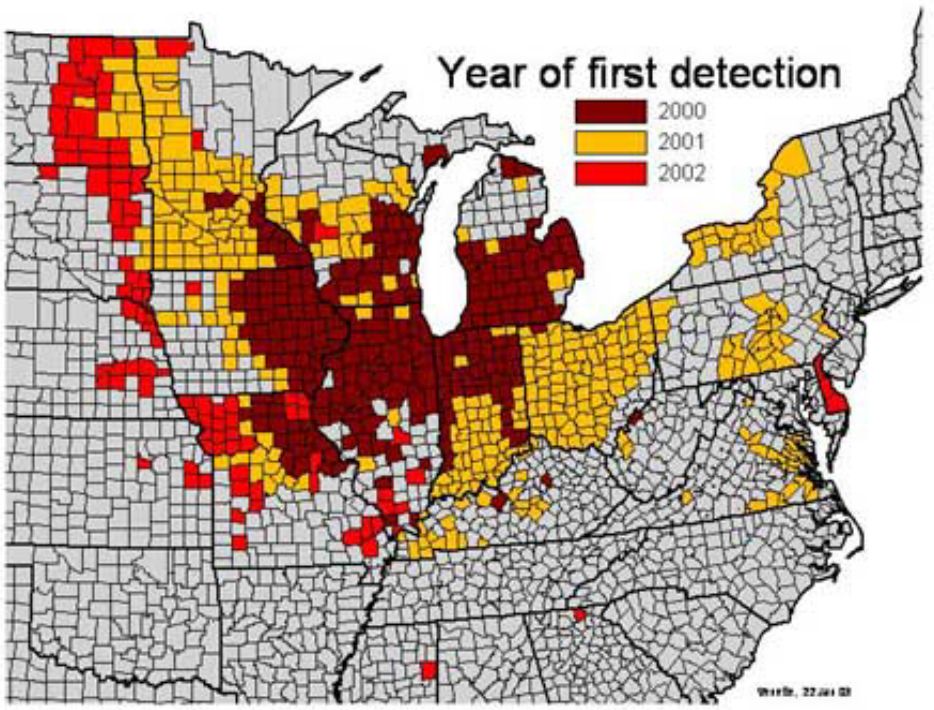


Fig. 5. Distribution of soybean aphid by county as of February of 2003 (after Patterson, 2003)

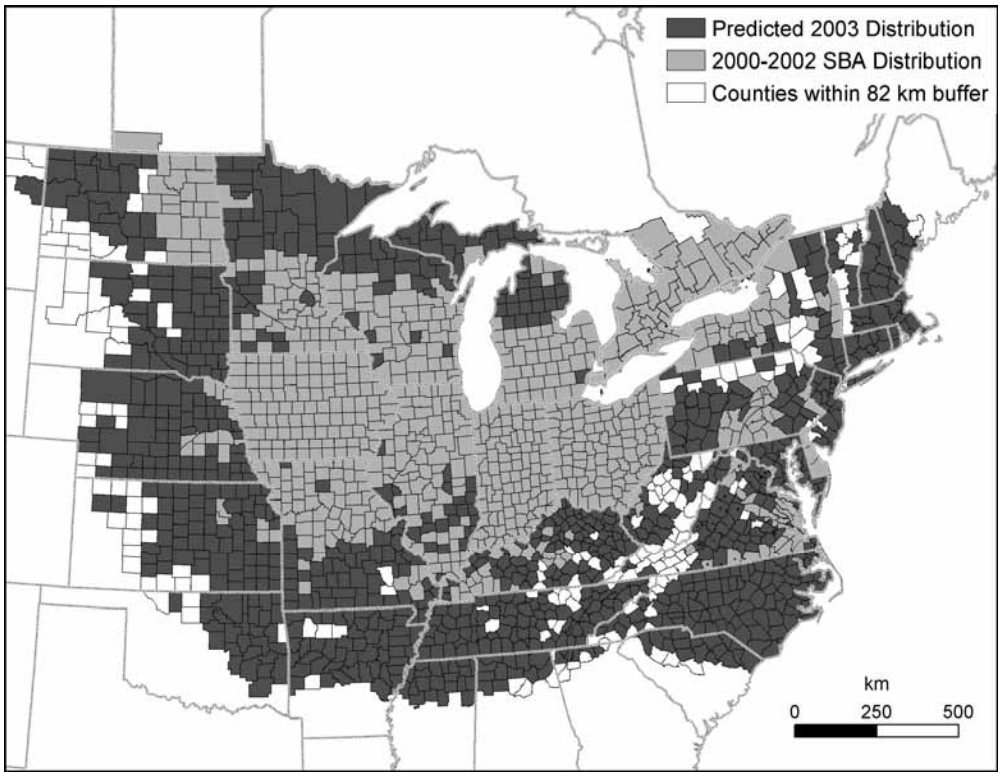


Fig. 6. Predicted soybean aphid distribution in the U.S. for 2003 (after Hutchinson *et al.*, 2003)

Fig. 7. Aphis glycines adults and nymphs
Photo by David Voegtlin

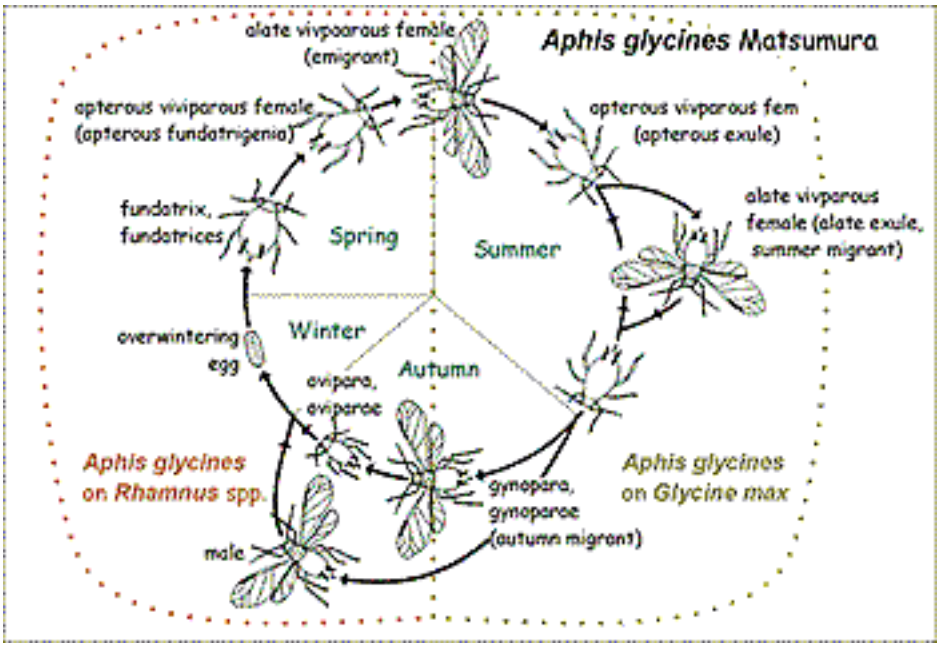


Fig. 8. Life cycle of *Aphis glycines*

Fig. 9. Buckthorn, *Rhamnus cathartica*, overwintering host of the soybean aphid.
Photo by Phillip Glogoza

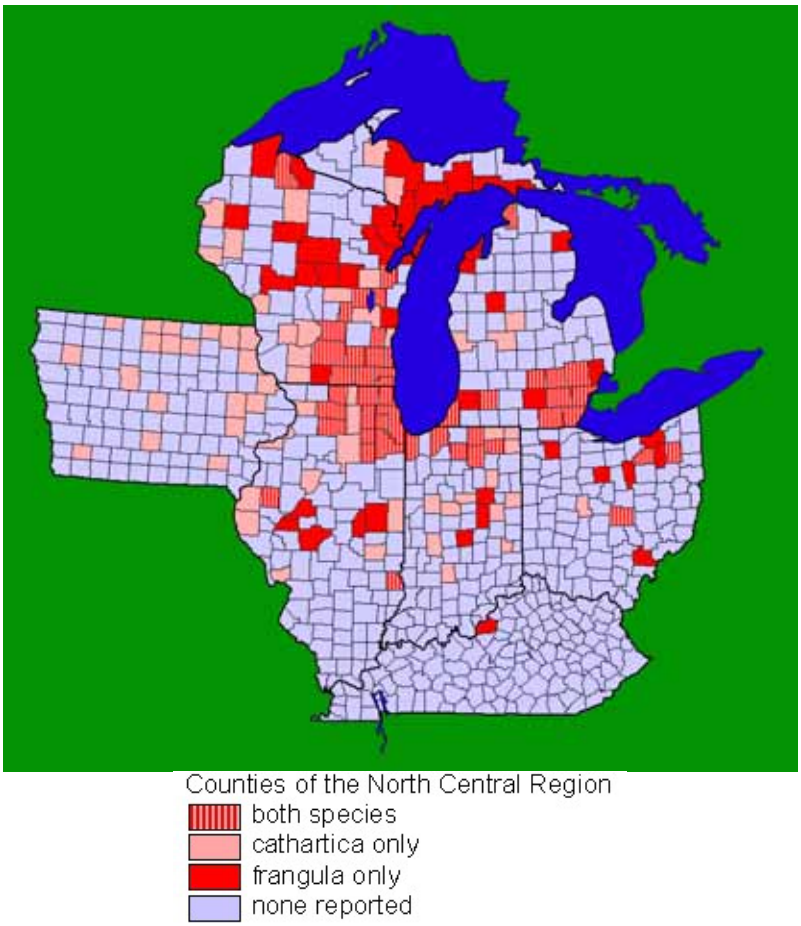


Fig. 10. Estimated distribution of *R. cathartica* and *R. frangula* in the North Central Region.
(after Gibson, 2003)

soybean plants in Indiana and Kentucky (Clark and Perry, 2002). Since *A. glycines* prefers to feed on the phloem and SMV is acquired from the epidermis, it is likely to be a less important SMV transmitter than more transient alatae aphids that visit the crop, probe briefly and move on (Dr. G. Kampmeier, personal communication). Soybean aphids proved poor vectors (0.83% transmission) when allowed to acquisition feed overnight on SMV leaves, but were very efficient vectors (34.7% transmission) when allowed only a 1-minute acquisition probe (Wang and Ghabrial, 2002). Thus, conditions that allow sustained feeding by *A. glycines* make them inefficient vectors but the initial flight of winged aphids that involves movement from plant-to-plant and brief probing results in more efficient virus transmission.

The time of appearance and abundance of migrating alatae in relation to soybean development are critical factors determining the incidence of SMV in the field. SMV incidence has not been correlated to apterous A. glycines populations (Chen *et al*., 1988). In northeastern China, epidemics of SMV were closely correlated to peak flights of alatae aphids where populations were primarily composed of A. glycines (Gau and Zhang, 1989). When aphids had their migration peak after soybean bloom, there was no correlation with SMV (Li and Pu, 1991). Although A. glycines is often the most prevalent species on soybean in China, its alatae population may peak later than other aphid species and important SMV vectors are usually “pass-by-aphids” (Chen *et al*., 1988).

The transmission efficiency of *A. glycines* (30-40%) after a 3-minute acquisition probe is not significantly different from that of *M. persicae* (Hill *et al.*, 2001), an important transient SMV-vector in the Midwest. The role that *A. glycines* will play in SMV transmission in U.S. soybean fields will be determined largely by the initial distribution of infected plants and the number and timing of migrating alatae. Any vector, even a poor one, can effectively spread the virus if winged forms are numerous. This concept was previously demonstrated when *R. maidis*, a relatively inefficient vector, had the greatest impact on SMV spread in Illinois because of its abundance (Irwin and Goodman,1981). During the summer of 2003, soybean aphid populations of over a thousand per plant were reported in parts of the upper Midwest (Dr. R. O’Neil, personal communication). With such high numbers of aphids, an increase in SMV can be expected, particularly if you have lots of alatae prior to soybean bloom (Dr. S. Halbert, personal communications). The impact will be greatest if significant numbers of alatae fly early when plants are the most susceptible to SMV, while transmission will be

minimized if alatae populations migrate after soybean flowering stage R_2 when no reduction in yield and low seed transmission occur (Ren *et al.*, 1997).

Alates were first reported on soybean July 16 in central Indiana (Tippecanoe county) in 2003, with peak proportions of plants (up to 100%) harboring *A. glycines* alates Aug 13-20 (Appendix 4A). Where vegetative stage V_0 (unifolate expanding) occurred June 4 (seeds planted in late May), early alate presence corresponded to plant stage R_2 which is unfavorable for SMV transmission (Appendix 4B). When planting was delayed until mid-June, alatae were present on plants for at least 2 weeks prior to R_2, a condition potentially favoring SMV transmission (Appendix 4C). Monitoring of alatae populations and corresponding soybean growth stages can indicate an increased risk of SMV spread and damage.

II. Initiating event (recognizing an attempted introduction of a more virulent SMV strain)

1. Observation/diagnosis of presence

SMV has been present at endemic levels in the U.S. for several years so most diagnosticians are familiar with symptoms and diagnostic procedures (see VI-5). Although some SMV strains may be differentiated based on nucleotide sequences (see I-6), it will be extremely difficult to demonstrate that an exotic strain of SMV has been intentionally introduced.

2. Interception: individual/ pathogen

Interception of an individual carrying infected soybean plant material or seeds at a port of entry should be responded to immediately. SMV infected seed are often mottled and therefore should be confiscated. Interception through routine traffic stops, although somewhat improbable, should not be discounted and confirmatory procedures initiated. Because of confidentiality of mail deliveries, the probability of interception of shipped SMV contaminated seed to an in-country location is much lower than personal interception.

3. "Intelligence" information

Intelligence information from Homeland Security, NSA, CIA, FBI, or USDA-PPQ about an overt agroterrorism intent is another potential initiating event. This information should be provided to personnel at the county level to enhance the probability of early detection.

III. Probable route of terrorist entry/dissemination

Pathogen – seed and aphid vectors

As an obligate, vectored pathogen, the production of large quantities of inoculum required for rapid, widespread damage on covert introduction is not feasible. SMV remains infective in expressed plant sap for only 2-5 days (CABI, 1999), in desiccated leaves for 7 days and for long periods if stored as desiccated leaves at –80°C (Hill, 1999). Field infections have been easily initiated by artificial inoculation (Cho and Goodman, 1979, 1982; Koning and TeKrony, 2003; Ren *et al.*, 1997; Tu, 1989). Although there are minor variations in technique, inoculum is basically prepared by grinding SMV-infected soybean leaves in sodium phosphate or potassium phosphate buffer in a blender, adding a small amount of powdered carborundum and rubbing the mixture onto young leaflets with cheesecloth.

Using aphid vectors as a source of introduction from an established "in-country" location is a possibility since stylet-borne viruses may persist for some hours after uptake (CABI, 1999), but individual aphids are generally capable of only a single transmission (Guo and Zhang, 1989). If this method were employed, SMV infected plants (virus source) and aphid-rearing equipment would be required. Although this method would require considerable knowledge and planning, it could result in the spread of SMV if large populations of SMV-carrying alatae were released into pre-flowering fields of soybeans.

Seed would be the easiest, most logical vehicle for SMV introduction. Production of infected seed would be inexpensive and provide the largest quantity of inoculum with minimal equipment. Storage of infected seed for one season would not require specialized equipment other than preventing desiccation or very high temperature exposure. Longer-term storage would require refrigeration to delay loss of germination potential.

In the simplest scenario, SMV infected seed could be acquired by simply collecting infected seed from natural infections. Many U.S. soybean cultivars contain no resistance to SMV and those that do are controlled by a single dominant gene that is not effective against all SMV strains (see VII-3). An example of such a scenario would be the collection of 'Suweon 97' seed infected with SMV strain C14 or G7H in Korea. This cultivar is known to be resistant to all SMV strains identified in the U.S. Even the collection of infected seed from late-planted soybean in the U.S. would be a possible vehicle of introduction to other production areas.

IV. Probable distribution: Spread

1. Point Introduction

The most likely route of intentional introduction would be infected seed, followed by viruliferous vectors. Infected seed, distributed early throughout the soybean production areas could initiate severe localized epidemics the initial year with extensive losses in subsequent years.

Inoculum levels in commercial certified seedlots are typically low but SMV will spread in Midwestern soybean fields if sufficient primary inoculum is provided (Irwin and Goodman, 1981). Thus, one means of creating an epidemic would be to distribute the primary inoculum in seed to create as many foci of infection as possible (see VI-1).

2. Secondary Dissemination

Secondary spread of SMV depends on cultivar susceptibility (*i.e*. the efficiency at which an infected plant can act as a source of further spread), availability of vectors, the stage of crop development when alatae are present, vector population density, and vector activity and efficiency (which varies with aphid species). SMV has not been a major problem in the Midwest because most virus spread occurs too late to have a significant impact on seed transmission levels and yield (Irwin and Goodman, 1981). With the soybean aphid producing higher populations of alatae aphids in soybean fields, SMV severity is likely to increase, particularly if additional primary inoculum is provided. *A. glycines* is expected to be distributed over approximately 90% of the total U.S. soybean production area in 2003

(Fig. 6) (Hutchinson *et al.*, 2003), so that widespread transmission of an introduced SMV strain could be expected.

V. Consequences of introduction and establishment

The consequences of introduction of a new strain of SMV and subsequent risk of establishment in the U.S. were rated with respect to six risk elements: climate, host range, dispersal/vector, economic impact, environmental impact, and persistence. The pathogen was ranked for 29 different criteria encompassed within the six risk element categories.

1. Establishment

a. Climate *Risk = High*

At least 8 strains of SMV are known to occur in the U.S. Therefore, it seems likely that other SMV strains could readily establish under U.S. climatic conditions.

b. Host Range *Risk = Low*

Cultivated soybean is practically the only known natural host of the virus, although *Phaseolus vulgaris* and a few other plants have been reported naturally infected (CABI, 1999). A small number of potential non-legume hosts occur in the Midwest, but the most prevalent (*Amaranthus* sp., *C. album* and *Setaria* sp.) are annual plants and barring transmission through seed of these hosts, are considered unlikely to be overwintering hosts for the virus (Hill *et al.*, 1980).

c. Dispersal/vector *Risk = High*

Since alternate hosts are deemed unimportant in the U.S., seed transmission currently accounts for virus carry-over from one season to the next. Secondary spread of SMV by aphids occurs at a relatively fast rate from a point source of inoculum (Hill, 1999), but over relatively short distances of less than 50 m (Irwin and Goodman, 1981). More initial infection foci (seedlings from infected seed) will provide more inoculum for aphid transmission and increase the chance of SMV spread. Irwin *et al.* (2000) calculated that an initial inoculum level of 0.1% (1 infected seed /1000), could produce yield losses >8% when

aphids flew early. These predictions were made prior to knowledge of *A. glycines* in the U.S.

The rapid expansion of soybean aphid populations indicate that these potentially important vectors of SMV can survive and reproduce effectively over a wide range of temperature and moisture regimes to provide extended distribution and persistence to a new SMV strain.

The ecological range of *A. glycines,* the only colonizing aphid of soybean and vector of SMV is predicted to overlap approximately 90% of the 73 million acres of soybeans in the U.S. in 2003 (Fig. 6). Widespread transmission of an introduced SMV strain can be expected if alate vectors fly in the early soybean growth stages.

d. Economics *Risk = Moderate*

Soybean is the second most economically important field crop grown in the U.S. In 2002, soybean was grown over 73 million acres in 29 states producing 2.652 billion bushels, with a value of $14.84 billion (Appendix 5A, 5B). The top six producing states and associated percent of total production in 2002 were Iowa (18.5%), Illinois (16.0%), Minnesota (11.2%), Indiana (8.8%), Nebraska (6.4%), and Ohio (5.7%).

A new SMV strain introduced into the area could potentially have a significant economic impact if it was able to persist for more than one year. Where continuous soybeans or extensive soybean in the rotation is practiced, a new strain of SMV has the greatest chance of becoming established and causing damage annually. Double-cropped soybean is an important production system in the upper south and southeastern U.S. Soybean may be infected by SMV at earlier developmental stages when planted late than when planted early, making them more susceptible to yield reductions. A SMV infection rate as low as 18% can produce a yield loss in Kentucky in the late crop, presumably because of reduced vegetative growth and the greater impact of SMV infection in late plantings (Ren *et al.*, 1997b). SMV incidence and severity is exaggerated in double cropped or late planted soybean in the southern U.S., thus SMV introductions under these conditions are likely to have the greatest impact. Added control costs if a more virulent strain was introduced could range from 5-10% (Dr. Huber, personal communication).

e. Environmental Impact *Risk = Low*

The environmental impact resulting from SMV is likely to be low. Although *P. vulgaris* has been reported naturally infected with SMV and kidney beans and lima beans can be artificially infected (CABI, 1999), symptoms were mild (local lesions) or infections were latent. It therefore seems unlikely that SMV would have a significant impact on commercial crops other than soybean.

f. Persistence *Risk = Moderate*

A virulent strain of SMV has a moderate likelihood of permanent establishment even though an overwintering host is lacking in the Midwestern U.S. and epidemic development must be triggered each year by infected seedlots. Whether SMV could persist will therefore be dependent upon the ability of seed companies to recognize and eliminate SMV infected seed from commercial seedlots.

2. Over-all risk rating for establishment of a new SMV strain

Area in Question	Climate	Host Range	Dispersal	Economics	Environmental Impact	Persistence
U.S. soybean producing areas	**High**	**Low**	**High**	**Moderate**	**Low**	**Moderate**

VI. Likelihood of successful introduction

1. Quantity of inoculum required to introduce and establish damage

Large enough quantities of inoculum (whether produced or collected) to produce wide-spread damage in a single growing season are unlikely. However, an overt introduction of a highly virulent strain in seed or vector could lead to significant losses over a 3-5 year time period since ample vectors are already present in the major soybean production areas.

An SMV epidemic can be described as a sigmoid slope. Crop losses at low disease incidence are disproportionately low because soybean has considerable potential to compensate for stand loss. At 5% SMV incidence, the steep slope of the sigmoid curve commences and is therefore defined as epidemic onset (Steinlage *et al.*, 2002). The number of seeds required to cause significant loss is dependent upon the many factors affecting secondary spread, especially the presence of alate aphid populations when soybean plants are young. Using a SMV simulation model, Ruesink and Irwin (1986) calculated that 1% seed transmission is the upper limit of acceptable seed infection for farmers who replant their own seed, even when vector intensity is low.

An estimate of the number of SMV infected seed/acre needed to create widespread infection can be calculated from information provided by Irwin and Goodman (1981) where 25% of SMV spread occurred within 2 m of the source and 95% of all infection was within 17 m of the source by the end of the growing season. A modest average estimate of spread from each foci would therefore be approximately 5 m.

Area of potential disease spread from each foci (assumes aphids can move virus 5 m in every direction)	$(5\ m)^2$ = 269 sq. ft. = .0061 acres
In 1 acre, the number of foci required (i.e. SMV-seed)	.0061acre/1acre = 164 seed/acre
Number of seed typically planted/acre = 200,000	
Percent of SMV-seed required	164 /200,000 = .082%

Thus only a handful of SMV-infected seed (0.1% or 0.1 lb/acre) would be required to produce notable disease within a field. This is in agreement with Irwin *et al.* (2002) who used an initial inoculum level of 0.1% (1 infected seed /1000), to calculate yield losses >8% and >0.5% seed transmission when aphids flew early. More serious SMV losses would be expected in fields planted with 1% SMV-infected seed (1 lb/acre), the severity being dependent upon cultivar and vector flight timing in relation to soybean development stage. Thus, the introduction of a new stain of SMV into the United States may require only a few pounds of contaminating, infected seed distributed over a few well-chosen soybean fields.

2. Likelihood of surviving initial introduction

SMV would survive an initial introduction via seed. SMV survival in seeds can exceed the germinability of the seed.

3. Likelihood of dissemination beyond the point of introduction

Some strains of SMV result in seedling death of some cultivars, and seedlings must survive long enough to be sources of SMV for vectors. Secondary spread of SMV by aphids occurs at a relatively fast rate from a point source of inoculum (Hill, 1999) but over relatively short distances (Irwin and Goodman, 1981). Once introduced, there is a high likelihood that SMV would be disseminated by alatae beyond the point of introduction with the extent of damage dependent upon the timing of alatae flight in relation to soybean development. If SMV-infected seed were distributed over a total of 20 acres, small-localized epidemics could be produced the first year. This would be particularly important if seed nurseries were targeted, since some infected seed might remain undetected and be passed onto commercial growers to perpetuate the disease for a second season. Secondary spread would likely follow the expanding soybean aphid's distribution, predicted in 2003 to cover 90% of the current U.S. soybean production area.

4. Likelihood of alternate host infection

A few potential alternate hosts occur in the Midwest, but the most prevalent (*Amaranthus* sp., *C. album* and *Setaria* sp.) are annual plants where overwintering could be accomplished through seed transmission, although this is currently considered unlikely (Hill *et al.*, 1980). For practical purposes, the host range of SMV is considered confined to the family Fabaceae and species of the genus *Glycines* and some close relatives (Irwin and Schultz, 1981). Among the potential alternate hosts, seed transmission has been demonstrated only in *Phaseolus vulgaris* (Castano and Morales, 1983). Further investigation is required to eliminate the possibility of seed or overwintering transmission in other alternate hosts that occur in the U.S.

5. Likelihood of early detection

Since SMV already occurs in the U.S., strains causing typical symptoms are likely to be detected early by plant diagnostic clinics. Symptoms are not fully diagnostic however, because they may vary with stage of plant growth, cultivar interaction and temperature. Strains like those isolated in Korea (SMV-N)(Cho *et al.*, 1977), C14, and G7H can cause a severe necrosis not typical of SMV symptoms known in the U.S., making an early diagnosis less likely. Mottled seed are a useful indicator of the presence of the virus, but a poor

indicator of the extent of infection (see I-7bii). Various serological techniques are available for positive SMV diagnosis.

6. Overall risk

The most significant route of introduction of a new strain is through seed. The inadvertent risk of introduction of SMV with a vector is low, but the ease of colonization is high. Interplanting infected seed in recently planted fields, or contamination of seedlots with infected seed are possible routes of covert introduction. The level of risk would be greatest if soybean seed nurseries were targeted, increasing the potential for infected seed to be dispersed through commercial seedlots. Increased levels of SMV in commercial fields would force seed nurseries to relocate to disease-free regions.

7. Likelihood of an agroterrorist trying using SMV as a biological weapon = low.

There are too many variable factors influencing SMV epidemics to make the success of an overt introduction predictable. There are other pathogens that can be easily mass-produced, and have a higher probability of devastating U.S. agriculture.

VII. Control/Mitigation strategies after establishment

SMV does not have quarantine status because it already exists everywhere soybeans are grown. At present the best measures to preclude losses are using SMV-free seed and avoiding late planting of soybean (Hill, 1999). Other important control methods include the use of SMV-resistant varieties and combinations of cultural techniques. When new SMV infections occur after R_2 (mid-flowering), they have no impact on seed infection rates and little impact on yield (Ren *et al.*, 1997; Irwin *et al.*, 2000) so tactics that effectively delay infection are of value. An epidemiological model for SMV has been used to predict the benefits of potential control methods and combinations of those methods (Irwin *et al.*, 2000).

1. Seed certification

Certification of commercial seed to ensure seed with low SMV incidence is an important control measure. Fortunately, U.S. seed companies have maintained very low levels of SMV diseased seed (Dr. J. Hill, personal communication). Production of low SMV-infected soybean seed may become increasingly difficult with the presence of *A. glycines* throughout the production area.

2. Seed Treatment

Hot water seed treatment (70°C) will kill the virus but significantly reduces seed germination (CABI, 1999). Microwave treatment of seed at 8.5% moisture content reduced seed transmission from 45% to 7%, but germination was significantly reduced at 16% moisture (Jolicoeur *et al.*, 1982). Dry heat was less effective than hot water in reducing infection but not as damaging to germination (CABI, 1999).

3. Host Resistance

Resistant soybean cultivars have been used to successfully control SMV and numerous soybean cultivars exist with varying degrees of resistance. Resistance is largely strain specific so breeding for resistance is a continuous activity. In the southeastern U.S., SMV has been a problem in late-planted soybean. Resistant genotypes used in late planting of double-cropped soybean resulted in a 12% yield benefit, and greatly reduced SMV seed transmission in Kentucky (Ren *et al.*, 1997b). Incorporating resistance to new SMV strains into soybean cultivars necessitates a search for additional germplasm and development of varieties with acceptable agronomic characters.

In 1979, 98 isolates of SMV from seeds in the USDA soybean germplasm collection were classified (based on differential reactions on soybean cultivars) into seven strains, G1-G7, where G1 was considered the least virulent and G7 the most virulent (Cho and Goodman, 1979). Strains G7a and C14 (Lim, 1985) were later identified. The differential cultivars used in the U.S. formed the foundation for genetic studies of SMV resistance in soybean. Differentiation of SMV isolates using soybean cultivar reactions has also been reported in Japan (Takahashi *et al*., 1980), China (Chen *et al*., 1986), Korea (Cho *et al*., 1977; Cho *et al*., 1983), and Brazil (Almeida, 1981); but host genotypes and virus isolates are seldom

similar in these studies. G5 and G5H were the major strains causing significant damage in Korea until 1999 when a new isolate, G7H, that is virulent against previously unchallenged resistant cultivars, was reported (Kim *et al*., 2003).

Resistance to SMV in soybean is generally conditioned by single dominant (R) genes known to map to at least three distinct genetic loci, *Rsv1*, *Rsv3*, and *Rsv4*. *Rsv1* is the resistance locus most commonly found in commercially available cultivars (Chen *et al.,* 1994). Eight alleles with different recognition specificities have been identified to date at this locus: *Rsv1* (PI 96983), *Rsv1-t* (Ogden), *Rsv1-y* (York), *Rsv1-m* (Marshall), *Rsv1-k* (Kwanggyo), *Rsv1-r* (Raiden), *Rsv1-h* (Suweon 97), and *Rsv1-s* (PI 486355) (Chen *et al*., 1991, 1994, 2002; Kiihl and Hartwig, 1979; Ma *et al*., 1995). *Rsv1* genes are partially dominant in that they confer systemic necrosis in the heterozygous condition (Chen *et al*., 1994). Most of the *Rsv1* genes confer resistance to lower-numbered strains of SMV (Cho and Goodman, 1979) (Table 2), with the exception of *Rsv1-h,* which confers resistance to all strain groups identified in the U.S. and is therefore considered a valuable source of genetic resistance (Chen *et al.*, 2002). Unfortunately, *Rsv1-h* (Suweon 97) does not confer resistance to SMV isolates G5H or G7H. Three Korean cultivars (Suweon 172, 179 and Miryang 41), out of 42 tested proved resistant to strains G5H, G7H, G7, G5 and G2 (Kim *et al*., 2003). Further investigation into inheritance of resistance of these strains is warranted.

Resistance conferred by some *Rsv1* genes can be overcome at low temperatures (<10°C) (Mansky *et al*., 1991), and infection by combinations of strains can produce a severe systemic necrosis (Mansky *et al*., 1995) rather than cross-protection.

The *Rsv3* locus appears to be characterized by two functional alleles: one conditions resistance to G7 and necrosis to G1 (Buzzell and Tu, 1989), and another that confers resistance to G5-G7 (Buss *et al*., 1999). Thus, genes at *Rsv3* condition resistance to higher-numbered strains. Another independent locus, *Rsv4* (Ma *et al.*, 1995) contains a gene that confers resistance to all SMV strains G1-G7 (Buss *et al.*, 1997; Hayes *et al*., 2000).

Table 2. Comparison of differential reactions of soybean genotypes to identified SMV strains.

		Reaction to SMV strains[a]									
Cultivar Source	**Genotype**	G1	G2	G3	G4	G5	G5H	G6	G7	G7a	G7H
Lee68/Essex	*rsv*	S	S	S	S	S	-	S	S	S	-
York	*Rsv1-y*	R	R	R	N	S	S	S	S	S	S
Marshall	*Rsv1-m*	R	N	N	R	R	R	N	N	S	N
Ogden	*Rsv1-t*	R	R	N	R	R	R	R	N	S	N
Kwanggyo	*Rsv1-k*	R	R	R	R	N	N	N	N	N	N
PI 96983	*Rsv1*	R	R	R	R	R	-	R	N	S	-
Raiden/L88-8431	*Rsv1-r*	R	R	R	R	N	-	N	R	N	-
Suweon 97	*Rsv1-h*	R	R	R	R	R	N	R	R	R	N

[a]R = resistant (symptomless), N = necrotic (systemic necrosis), S = susceptible (mosaic). Data from Chen *et al.* (2002), Cho and Goodman (1979), Gunduz *et al.* (2002) and Kim *et al.* (2003).

The resistance that this gene provides is completely dominant, unlike *Rsv1* alleles, and is not associated with necrosis. A fourth locus also may exist that provides a novel type of resistance to G1 and G7 where plants are completely resistant at the seedling stage but develop a mild mosaic (late susceptibility) three weeks later (Ma *et al*., 2002). Like *Rsv1*, resistance provided by this gene is partially dominant.

The introduction of cultivars with resistance conferred by major (R) genes leads to strong directional selection pressure on the virus to develop new strains that can overcome resistance. In Korea, production of leading soybean cultivars thought to be resistant to SMV was hampered when a necrotic-inducing strain of the virus emerged (Cho *et al.*, 1977). Interestingly, resistance to that strain was conferred by a single recessive gene (Kwon and Oh, 1980). Another Korean soybean line, Suweon 97, reported resistant to all known SMV strains (Cho and Goodman, 1982), contracted necrosis when isolate C14 was differentiated on it (Lim, 1985). A new isolate, G7H, now accounts for 50% of all SMV damage in Suweon cultivars planted in Korean fields (Kim *et al*., 2003). G7H causes more severe symptoms than any other strain identified to date. Since soybean originated in the Korean peninsula and Manchuria, there is always the possibility of new SMV strains developing, and resistant varieties may continuously be needed in that region (Kim *et al.*, 2003). To create cultivars with the most complete resistance, a range of SMV strains differing in virulence should be used in soybean breeding programs (Cho and Goodman, 1979). Further, care must be taken when using alien germplasm to avoid introducing new strains of SMV (CABI, 1999).

A number of cultivars that display resistance to a wide range of SMV strains have resistance genes at two different loci (Table 3). The broad-spectrum resistance offered by these

cultivars is less likely to breakdown because two different loci are involved which may act by different mechanisms. This supports the concept of "pyramiding" genes to achieve broad resistance to SMV (Liao *et al*., 2002). The possibility of pyramiding SMV resistance genes into commercial cultivars can be facilitated by the extensive molecular research being done to map disease resistance genes. Map positions for *Rsv1* (Yu *et al*., 1994) and *Rsv4* (Hayes *et al.*, 2002) loci in the soybean genome have been determined.

Alternative approaches to using dominant gene resistance have been proposed in order to reduce selection pressure on the virus. Since infected seed is a major inoculum source for SMV, breeding for resistance to seed transmission has been proposed (Irwin and Goodman, 1981) and some resistant lines have been identified (Bowers and Goodman, 1982).

Table 3. Some cultivars known to contain SMV resistance genes at two different loci.

Cultivar	Loci	Resistance reaction to SMV strains	Reference
V94-5152 (derived from PI486355)	*Rsv1* *Rsv4*	G1-G7	Ma *et al*. (1995)
Zao18	*Rsv1* *Rsv3*	G1-G7	Liao *et al*. (2002)
Tousen 140	*Rsv1* *Rsv3*	G1-G7	Gunduz *et al*. (2002)
Hourei	*Rsv1* *Rsv3*	G1-G7	Gunduz *et al*. (2002)
Columbia	*Rsv3* R4 (loci not identified)	G1-G3 & G5-G7	Ma *et al*. (2002)

Another strategy is to use "rate-reducing resistance" that is effective against all strains of SMV. This strategy allows some infection within the plant population, but reduces the rate of plant-to-plant disease spread and minimizes adverse effects on yield and quality. Rate reducing lines that delay the onset of SMV epidemics until well after flowering can significantly reduce virus transmission to seed (Steinlage *et al.*, 2002).

Using mixtures of resistant and susceptible isolines to minimize yield loss was suggested by the finding that yields of resistant lines increased as their ratio declined within a blend with susceptible lines, while the yield of the susceptible isoline was unaffected by ratio (Ross, 1983). When SMV-susceptible plants are scattered among other plants, vectors are less likely to encounter them than in pure stands; this "dilution" might delay epidemics sufficiently long to reduce both yield loss and seed transmission. According to the Irwin *et al.* (2000) model, incorporating only 25% resistant plants among susceptible ones, will cut yield losses

by nearly 50% and seed transmission rates by over 50%, even with early season aphid flights.

Transgenic resistance achieved by inserting genes from the genome of the pathogen, typically viral coat protein (or CP genes), can provide complete immunity; however, Steinlage *et al*. (2002) generated transgenic SMV-CP soybeans to significantly lower SMV infection rates. Two transgenic soybean lines with the SMV-CP insertion did not confer total immunity but most infections occurred after flowering and did not reduce yield or quality. This rate-reducing transgenic strategy is expected to remain durable (stable).

Most U.S. commercial soybean varieties are susceptible to SMV. Hutchinson is the most commonly used group V cultivar with SMV resistance, but some SMV strains can break its resistance (Dr. S. Tolin, personal communication). Isolines of Essex have been developed by Dr. Glenn Buss's group that contain *Rsv*1, *Rsv*3 and *Rsv*4 resistance genes (Dr. S. Tolin, personal communication). Other promising genotypes with SMV resistance have been identified in maturity groups appropriate for the North Central region and recommendations for SMV resistant soybean varieties will be available to growers in the near future (Hill and Grau, 2004). Among these are Colfax, which has expressed resistance to all field isolates tested in Wisconsin (Dr. C. Grau, personal communication). SMV resistance in Colfax has not been characterized. Other accessions, such as Parker, are essentially asymptomatic when SMV-infected. Investigations are underway to determine whether accessions that express mild symptoms have agronomic relevance.

4. Vector Control

Vector control is not generally economical or fully effective because insecticides do not kill quickly enough to control non-persistently transmitted viruses and winged aphids quickly recolonize treated fields, and resume virus transmission. Slow-acting insecticides can potentially increase the spread of viruses because they temporarily increase aphid activity. SMV can be significantly reduced by applications of vegetable (Lu, 1970) or machine oil followed by synthetic pyrethroids (Nakano *et al.*, 1987), but are not practical because of mechanical damage from the applications and expense.

Synthetic pyrethroids may act rapidly enough to prevent epidemics caused by non-persistent viruses. Cyhalothrin (Karate®) decreased SMV incidence in Illinois (Irwin and

Kampmeier, 1989) but did not act quickly enough to prevent virus infection. This approach could reduce virus spread by preventing acquisition or by incapacitating viruliferous aphids before they could move to another plant. The SMV epidemiological model suggests that weekly applications of cyhalothrin would be required to delay epidemics and reduce yield losses by 75% and seed transmission rates by 80% (Irwin *et al.*, 2000). Multiple pyrethroid applications to control SMV on soybean are not justified because it is labor intensive, economically impractical, detrimental to beneficial insects and may increase selection pressure for insecticide resistance.

Damage caused by direct feeding of the soybean aphid may change some of the dynamics of soybean management in North America. University and government extension offices are creating control recommendations for heavy infestations. In the North-Central soybean producing areas, soybean aphids normally reach peak numbers during flowering so chemical treatment is recommended at growth stage R_1 - R_2 at a threshold of ≥ 25 aphids sampled per leaflet (Glogoza, 2002; Baute, 2002). Since SMV yield losses and seed infection are greatly reduced when plants are infected after soybean growth stage R_1, such late control measures will be unlikely to have an impact on SMV. Spraying soybean for aphids before flowering is not recommended (Hammond, 2002; Ostlie, 2003) since it may intensify the direct damage caused by *A. glycines* on soybean by killing beneficial insects that keep aphid populations in check.

During the summers of 2000-2001, lacewings (Chrysopidae), the multicolored Asian lady beetle (*Harmonia axyridis*), and fungal pathogens (*Pandora neioaphidis* and *Conidiobolus thromboides*) were active in fields colonized by the soybean aphid (Anonymous, 2002). *Orius insidiosus* and *Harmonis axyridis* have been identified as potentially key predators (Rutledge *et al*., 2004). The impact that biological control agents will have on North American soybean aphid populations, and in turn on SMV incidence, is yet to be determined. APHIS-PPQ is funding a soybean aphid biological control project in cooperation with state coordinators at the University of Minnesota, Purdue University, Michigan State University and Kansas State University. The project will explore the impact of native natural enemies, translate foreign literature, and screen for potential effectiveness exotic natural enemies.

5. Cultural Control Methods

Infections that occur during the first weeks after soybean seedling emergence are the most damaging and open canopies are most attractive to alatae. Thus, cultural control methods that delay infection may reduce damage. **Sowing dates** can be manipulated to avoid peak aphid flights during optimal crop susceptibility. Early planting, around May 10th in the Midwest, avoid the greatest SMV losses in yield and seed transmission according to model forecasts (Irwin *et al.*, 2002). Early planting may not be practical in all areas and its benefits need to be weighed against any agronomic disadvantages.

Crop density can be modified by seeding rate or row spacing. Dense soybean ground cover generally reduces aphid-landing rates (Bottenberg and Irwin, 1992a, 1992b; Halbert and Irwin, 1981; Irwin and Kampmeier, 1989), but this is aphid species dependent (Halbert and Irwin, 1981). SMV incidence and seed transmission is predicted to increase by 1.5 times (50 days after planting) as row spacing increases from 64 to 80 cm and from 80 to 96cm (Irwin *et al.,* 2000) suggesting that growers should use narrow rows if practical.

More densely pubescent cultivars of near-isogenic soybean lines retarded the spread of SMV by inhibiting the probing activity of aphids (Gunasinghe *et al.*, 1988). Ren *et al.* (2000), confirmed a negative correlation between leaf **trichome density** and SMV, reporting incidence levels of SMV at R_1 as 25%, 18% and 5% for normal, dense and extra-dense pubescent isolines respectively. Extra dense isolines also delayed the time at which maximum SMV spread occurred. Consideration of leaf pubescence in Irwin *et al*.'s (2000) model suggest that isolines with sparse pubescence are subject to early epidemics, even when aphid flights occur late in the season. Plant breeders should maintain at least 200 trichomes/cm^2 on soybean leaves and farmers should use varieties having high trichome densities.

Mixed cropping of soybean with corn (Bottenberg and Irwin, 1992b) or sorghum (Bottenberg and Irwin, 1992a) to intercept vector virus transfer has been proposed. Intercropping should be done with cereals of similar height in order to avoid soybean yield losses (Bottenberg and Irwin, 1992a). Incorporating intercropping into Irwin *et al.*'s (2000) model predicts as little as 25% non-soybean may reduce SMV incidence (50 days after planting) by nearly 50% and seed transmission by over 50%. This suggests that intercropping can be a powerful tool for reducing or delaying SMV epidemics, but may not

be practical in intense soybean production common in the U.S. Continuous cropping or crop overlap should be avoided where possible since rouging of infected plants is generally impractical and may be ineffective because symptoms tend to be masked at temperatures above 30°C.

Modeling the potential impact of control method combinations (Irwin *et al.*, 2002) suggests that increasing seeding rates and reducing row spacing have an additive effect, resulting in seed transmission 1/7 of controls (Fig. 11a). Increased seeding rates combined with dense leaf pubescence could reduce SMV incidence by as much as 10 times (Fig. 11b) and planting date combined with intercropping could reduce SMV incidence and seed transmission dramatically (Fig. 11c). The model shows the greatest benefit by combining seeding density with leaf pubescence. This would be a relatively cost effective method for soybean growers to adapt.

6. SMV Epidemiology: Forecasting Models

Disease progress curves for SMV are generally sigmoid, steep and often approach 100% incidence during the growing season. At 5% SMV, the steep slope of the sigmoid curve commences and is defined as epidemic onset (Steinlage *et al.*, 2002). The steepness of the curve is highly correlated with the quality and timing of activity of the principal vector species (Schultz *et al.*, 1985). Large's critical stage model assesses soybean losses due to SMV infection (Ren *et al.*, 1997) and SMV disease progress curves can be compared using Gilligan's nonlinear method (Ren *et al.*, 2000). An SMV forecasting model have been developed and refined (Irwin and Kampmeier, 1989; Irwin *et al.,* 2002; Ruesink and Irwin, 1986). A flow diagram presenting important factors influencing SMV epidemics that are incorporated into the model is shown in Fig. 12. The number of initial inoculum sources (infected seed) is an important base number in generating model output. Aphid vectors are responsible for almost all interplant spread that leads to SMV epidemics so factors that influence their behavioral responses influence disease incidence and impact. "Vector propensity" varies with aphid species and is dependent on the vectors innate ability to transmit SMV and probing response once the aphid has landed. Vector intensity is dependent on vector landing rates and vector propensity. Over 20 years of data on species composition and landing rates were used to generate probabilities in the models.

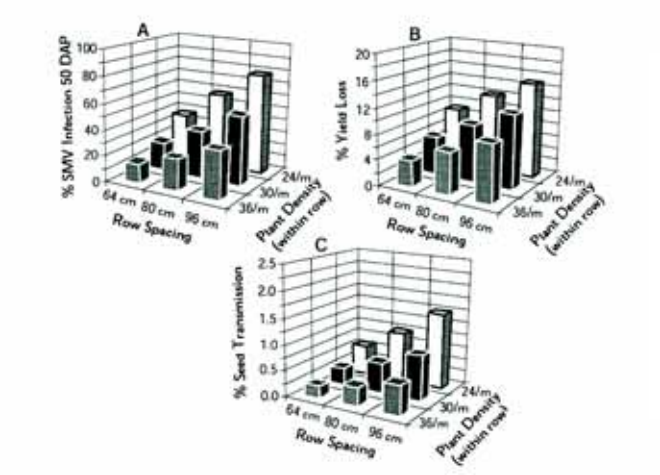


Fig. 11a . Three-D diagrams of SMV model outputs combining two pliant factors, within-row planting density and inter-row spacing of soybean; both factors affect plant density. The y axes present outputs of the model: (a) SMV incidence at 50 days after planting, (b) yield loss and (c) percentage of SMV-infected seed at harvest.

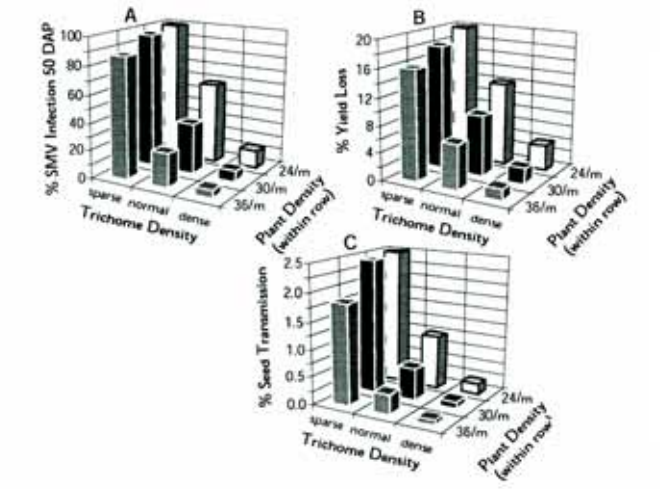


Fig. 11b . Three-D diagrams of SMV model outputs combining two pliant factors, within-row planting density and soybean isolines that differ in leaf pubescence (trichomes per unit leaf area). The y axes present outputs of the model: (a) SMV incidence at 50 days after planting, (b) yield loss and (c) percentage of SMV-infected seed at harvest.

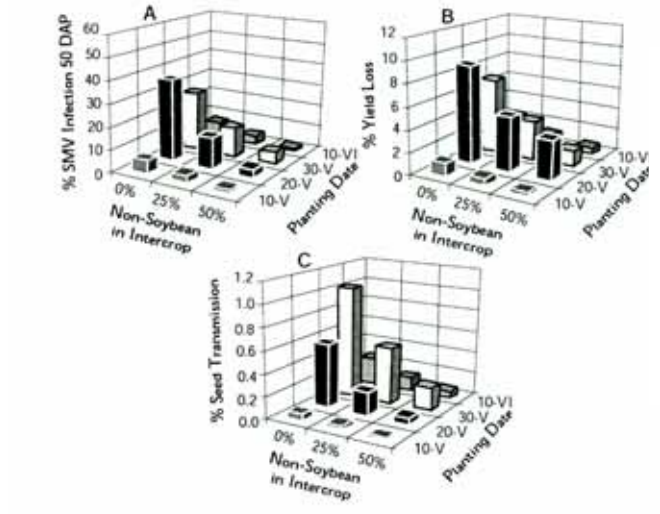


Fig. 11c. Three-D diagrams of SMV model outputs combining two pliant factors, within-row planting density and different rates of intercropping rows of soybean with rows of another crop. The y axes present outputs of the model: (a) SMV incidence at 50 days after planting, (b) yield loss and (c) percentage of SMV-infected seed at harvest.

(after Irwin *et al*., 2000)

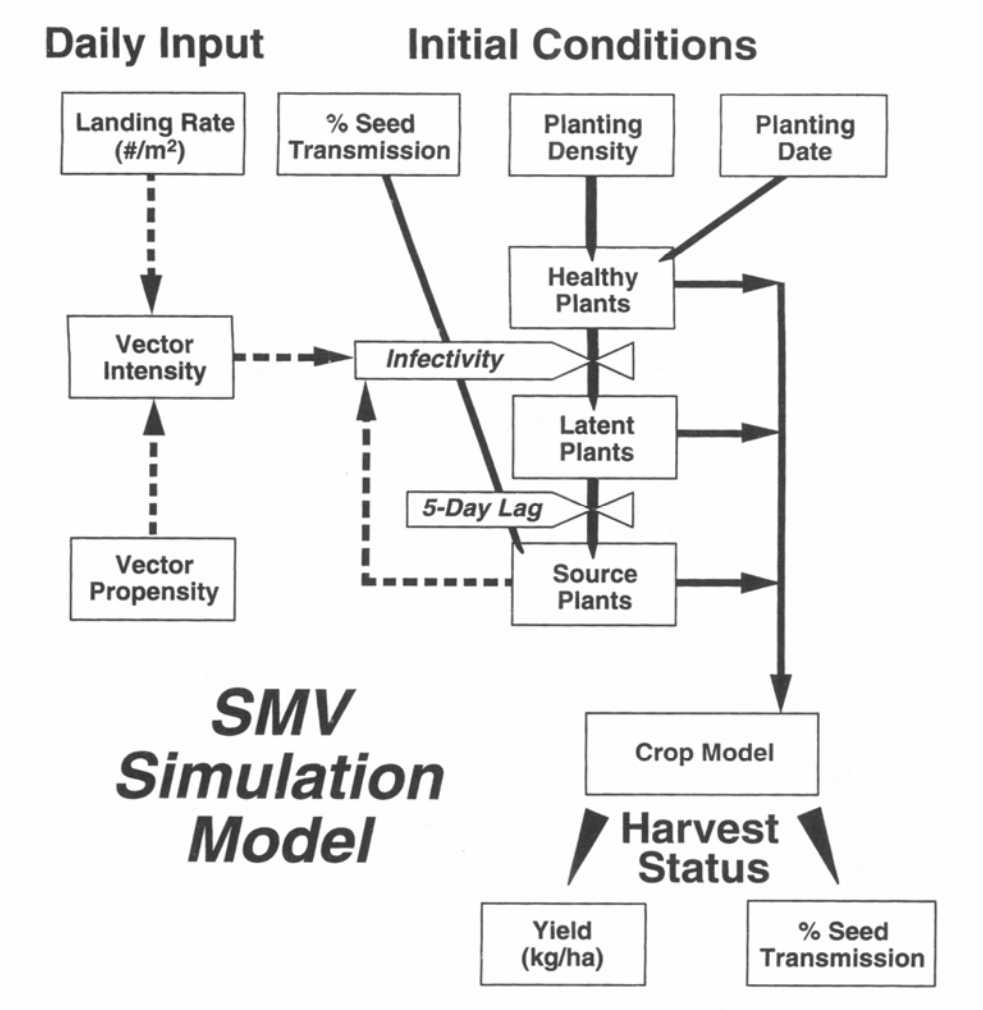


Fig. 12. Flow diagram of the SMV simulation model of Ruesink and Irwin (1986) and modified by Irwin *et al.* (2000). The model predicts disease progress during the growing season and estimates yield loss and percentage seed transmission at harvest.

The rate, magnitude, and timing of SMV epidemics under seven “pliant factors” (factors that influence insect behavior and can be altered) and five pairs of mitigating field conditions were examined using the model to predict their effect on an epidemic (Irwin *et al.*, 2002). The model predicted that SMV would cause little damage except when seed transmission rates and vector intensity values are high during the first 4-5 weeks after seedling emergence. The impact of early aphid flights usually over shadows pliant factors to cause overwhelmingly high SMV. Several pliant factors were found to have great potential for SMV control (see VII-4). Seeding density and leaf pubescence are predicted to have strong beneficial effects in reducing SMV, even with early aphid flights. The recent introduction and almost universal distribution of the soybean aphid throughout the U.S. soybean production area may require major reevaluation model predictions since,

a) vector propensity and landing rates (important components of the model) are aphid species specific and do not include data on the soybean aphid and

b) the model assumes alatae aphids are non-colonizers and originate outside of the field, alight and probe plants as they “fly through” with neighboring fields having similar SMV incidences.

VIII. Knowledge gaps

Important gaps in our present knowledge include:

1. The impact of *A. glycines* on SMV incidence and damage in U.S. soybean production regions.

2. Will significant numbers of *A. glycines* alatae migrate early enough in soybean development to increase SMV?

3. Identification of cultivar-SMV-strain relationships.

IX. Immediate response options

A pathway and response summary for the intentional introduction of a highly virulent strain of SMV is presented in Appendix 6.

1. Virus-free seed

SMV already exists in the U.S. in commercial seedlots at very low levels. The introduction of a new virulent strain of SMV would present a particular threat to soybean seed producing nurseries. In the event of localized SMV epidemics, seed production should be isolated from commercial soybean areas to assure SMV-free seed. Phytosanitary seed certification using reliable screening techniques would be required to maintain virus-free seed at current low levels. Additional training of seed producers, scouts and seed certifiers will help in early detection of SMV.

2. Vector control

Although chemical vector control is not fully effective in eliminating plant viruses, it may reduce the incidence of SMV by temporarily reducing aphid populations and delaying the alatae form. However, insecticide applications to control soybean aphid damage in 2003 were recommended no earlier than growth stage R_1 – R_2 in order to preserve beneficial insect populations that keep aphid populations in check. Such late applications are unlikely to have a significant direct impact in reducing SMV incidence.

3. Cultural control

Modification of planting practices such as increasing plant density, early sowing, using cultivars with high-density pubescence (> 200 trichomes/cm^2), and combinations of these techniques could significantly reduce SMV incidence. Further research on field effectiveness of these methods could provide a solid basis for comprehensive IPM recommendations.

4. Resistant Varieties

Resistant soybean cultivars have successfully controlled SMV in many countries. The development of SMV-rate-reducing transgenic soybeans and identification of SMV-resistant genotypes in maturity groups appropriate for the North Central region look promising to provide resistance to SMV strains already present in the U.S. Soybean lines presently deemed useful against SMV damage in the U.S. may be ineffective against a new virulent strain of SMV. The impact of an outbreak of a new strain of SMV can be most effectively

minimized by the rapid introduction of resistant cultivars. The time required for the development of such cultivars can be minimized if virulent exotic SMV strains are introduced into current U.S. germplasm screening programs.

Appendix 1. Reported plant hosts of SMV

Family	Species
Fabaceae	*Astragalus monspessulanus, Canavalia ensiformis, Cassia laevigata, C. occidentalis, Crotalaria spectabilis, Cyamopsis tetragonoloba,• Dolichos biflorus,• D. falcatus, Galactia sp., Glycine max, G. soja, G. ussuriensis, Hippocrepis multisiliquosa, # Indigofera hirsuta, Kummerowia stipulacea, K. striata, Lablab purpureus,• Lotus tetragonolobus, # Lourea vestertilionis, Lupinis albus, L. angustifolius,* L. luteus,* Macroptilum lathyriodes, Macrotyloma uniflorum, Mucuna pruriens* var. *utilis, Neonotonia wightii, Phaseolus acutifolius, P. lunatus, P. nigricans, P. speciosus, # P. vulgaris,* P. vulgaris* cv. Top Crop•, *P. vulgaris* (some cultivars) # *Pisum sativum,* Scorpiurus sulcata, # Senna occidentalis, Sesbania exaltata, Stizolobium deeringianum, Trigonella carulea, T. foenum-graecun, Vicia faba,* V. narboensis, Vigna mungo,* V. unguiculata, V. unguiculata* subsp. *cylindrical* *
Amaranthaceae	Gomphrena globosa,* Amaranthus sp.
Chenopodiaceae	Chenopodium album•, C. amaranticolor,* C. quinoa•
Passifloraceae	*Passiflora edulis* var *flavicarpa,* P. lingularis,* P. quadrangularis,* P. edulis**
Poaceae	*Setaria* sp.
Schropulariaceae	Antirrhinum majus
Solanaceae	*Nicandra physalodes, Nicotiana benthamiana, N. tabacum,* Petunia x hybrida,* Physalis virginiana, P. longifolia, Solanum carolinensis*

* hosts that have also been reported nonsusceptible to SMV, suggesting differences in cultivar and SMV strain.

• hosts that display local lesions

hosts that display latent infections

Adapted from Galvez, 1963; Hartman *et al.*, 1999; Hill *et al.*, 1980.

Appendix 2. Aphids known to transmit SMV

Acryrthosiphon pisum
Aphis armoraciae
Aphis citricola
Aphis craccivora
Aphis fabae
Aphis glycines
Aphis gossypii
Aphis laburni
Aphis nasturtii
Aphis nerii
Aphis rumicis
Aulacorthum circumflexum
Aulacorthum solani
Capitophorus elaeagni
Hysteroneura setariae
Lipaphis erysimi
Macrosiphum euphorbiae
Macrosiphum rosae
Megoura viciae
Melanaphis sacchari forma indosacchari
Myzus ornatus
Myzus persicae
Rhopalosiphum insertum
Rhopalosiphum maidis
Rhopalosiphum padi
Schizaphis graminum
Therioaphis trifolii
Uroleucon ambrosiae
Uroleucon ? nigrotibium
Uroleucon ? nigrotuberculatum
Uroleucon sonchi

(After Irwin and Goodman, 1981)

Appendix 3. Pathway Analysis for the Soybean Aphid: Predicting the Dispersal of an Introduced Aphid Species

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Introduction

The introduction of non-native pathogens and insect pests into the domestic agricultural production system pose significant threats to the United States. In the event of exotic agricultural disease or insect outbreaks, rapid detection and containment is key in minimizing economic and public health impacts. The soybean aphid (*Aphis glycines* Matsumura) is a native of Asia. The soybean aphid was positively confirmed in 11 states within the U.S. in August 2000; the fact that it was confirmed simultaneously in such a wide area suggests that it may have been present for some time before being detected. While several migratory aphid species may briefly feed on soybeans, the soybean aphid is the only aphid in North America to develop large colonies on soybeans (Sloderbeck *et al.,* 2003). A serious threat to soybean production, the soybean aphid is a known vector for several soybean diseases (*e.g.,* soybean mosaic virus) and has the potential to serve as a bioterrorism agent through genetic modification; it reproduces parthenogenetically and thus gives rise to genetically identical clones. The soybean aphid is a small (approx. 1.6 mm), yellowish-green aphid with black "tailpipes", or cornicles, near the tip of its abdomen (Figure 1).

Figure 1. Wingless and winged soybean aphid adults, and soybean aphid nymphs, on soybean leaves.
Photo by Gregory Zolnerowich.

As a very recent invasive species, the soybean aphid offers researchers the opportunity to monitor its dispersal characteristics and evaluate strategies for the rapid collection and dissemination of soybean aphid status information as a proxy for an actual agroterrorism event.

Soybean aphid populations can build up at any time from early vegetative through the bloom stages of the plant. Initially, most colonies will be found in the outer canopy on new leaves. As the plants reach maturity, the aphids may move deeper into the foliage, most

commonly on the undersides of leaves, and many may be found on stems and pods. Some reports indicate that a second population increase may occur from late August through early September (Sloderbeck *et al.,* 2003).

The soybean aphid exhibits a complex life cycle, passing through 15-18 generations annually; such rapid generation times explain in part the huge populations that can occur. It overwinters in the egg stage on buckthorn (*Rhamnus* species), making it incredibly difficult to detect at that stage. The North Central Pest Management Center (2002) reports that nymphs hatch in spring and, after two generations of wingless females, a generation of winged females is produced. The winged form then migrates from buckthorn in search of soybean plants. Once established on soybean, the summer season begins a series of wingless generations followed by a winged generation that, depending on population density, may disperse to other soybean plants. These generations are parthenogenic, and as mentioned above, give rise to genetically identical clones of each female. In the fall, winged females move back to buckthorn to produce a generation of egg-laying wingless females. Male aphids mature on soybean and then search for buckthorn in the fall to mate with wingless females who lay eggs on buckthorn twigs.

The soybean aphid, which has quickly established itself as an important soybean pest in the Midwest (Higgins, 2001; Higgins *et al.,* 2001), can reduce soybean yields substantially. Wang *et al.* (1996) found yield reductions of 27.8% in infested plots compared to uninfested controls. Therefore, the soybean aphid has the potential of causing losses of $61.44/acre in infested areas (27.8% of 34 bushels/acre and $6.50/bushel average yield; so that 27.8% X 34 bushels/acre X $6.50/bushel = $61.44). More recent soybean prices have been close to twice these levels, so that far greater losses could occur depending upon price. If only one tenth of Kansas' soybean acreage became infested, annual losses would approach $14.7 million in direct yield loss alone. In addition, the soybean aphid can transmit at least six plant viruses that can severely affect soybean productivity. Thus, the potential for this insect to seriously damage soybean crops in Kansas, and the Great Plains region is significant. The effect of the soybean aphid on soybean crop production has been significant. In some areas of high infestation, yields have been reduced to below detectable levels (Sloderbeck *et al.,* 2003). In addition to direct damage caused by feeding, SBA is capable of transmitting a number of viruses that can impact growth and production. Relevant viruses that naturally infect soybean include alfalfa mosaic, soybean mosaic, bean yellow mosaic, peanut mottle, peanut stunt, and peanut stripe viruses. These pathogens cause many symptoms such as

leaf mottling and distortion, reduced pod numbers, deformed pods, and discolored seed. The combination of feeding damage and effective virus transmission make SBA an especially troublesome, and potentially dangerous insect pest.

Buckthorn (*Rhamnus* spp.) is the only confirmed overwintering host of the soybean aphid (Grau *et al.,* 2002). Although numerous species of buckthorn are found in North America, only five have been identified as acceptable winter hosts: *R. davurica, R. frangula, R. alnifolia, R. lanceolata* and *R. cathartica*. All species are exotics and current research is examining the suitability of other introduced or native species as winter hosts. The only confirmed summer host in North America is the cultivated soybean (*Glycine max*); however, literature from Asian countries indicates legumes such as tick clover (*Desmodium* spp.) and kudzu (*Pueraria* spp.) also may be acceptable summer hosts (North Central Pest Management Center, 2003).

Approach

The Geographic Information Systems Spatial Analysis Laboratory (GISSAL) and the Remote Sensing Research Laboratory (RSRL), KSU Department of Geography, used GIS-related research to perform the pathway analysis for the soybean aphid. *Genetic algorithms for rule-based prediction* (GARP) (Hutchinson *et al.,* 2003) was used to quantify the spread of the soybean aphid. Lessons learned from this research include; (1) significant lag times (*e.g.,* 1-2 years) likely exist between time of introduction and first detection and (2) the importance of accurate and real-time regional and national reporting systems as a means to alert stakeholders and provide invaluable occurrence data to researchers.

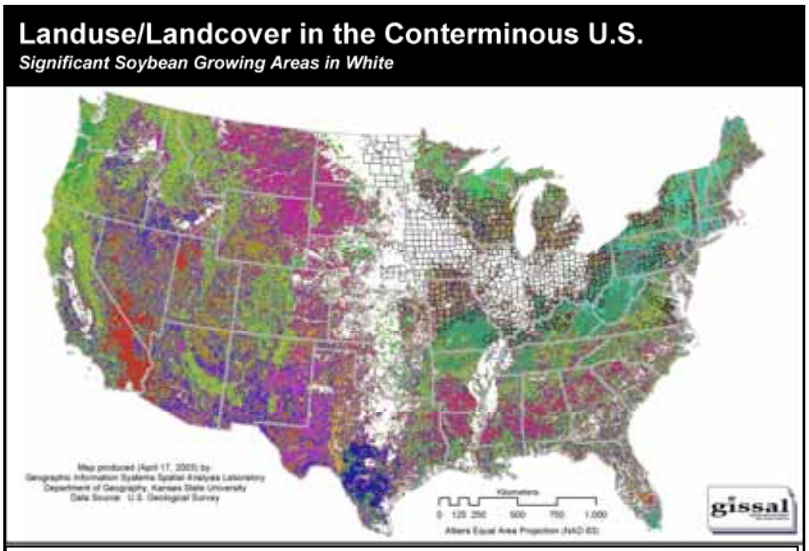

Figure 2. Significant soybean growing regions in the U. S., shown here in white.

Data concerning the presence of soybean

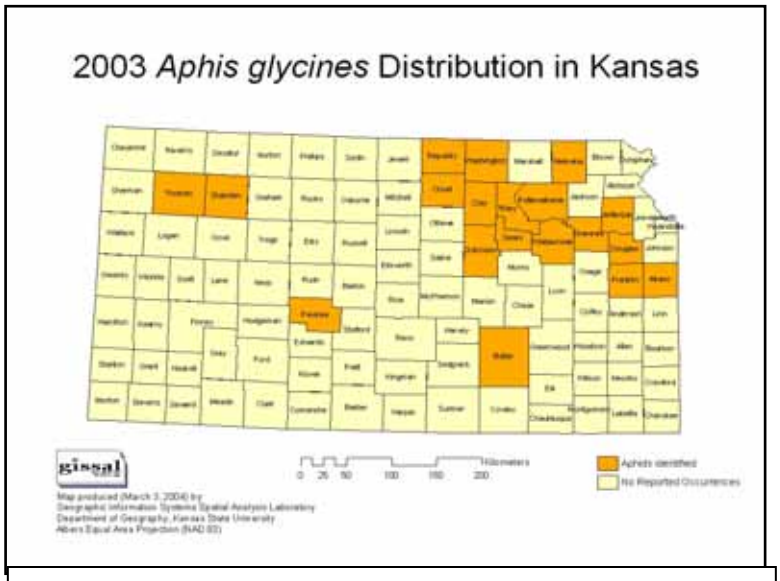

Figure 3. Occurrence of soybean aphids in Kansas counties in 2003.

aphids was collected from the North Central Pest Management Center's Soybean Aphid Watch Project (www.pmcenters.org/Northcentral/Saphid/aphidindex.htm), entered into a geographic information system (GIS) database, and cross-referenced for accuracy with several researchers and extension agents throughout the study area. Obviously, groundcover for primary and secondary hosts is critical. Buckthorn is quite ubiquitous, but soybean growing areas (Figure 2) were included in our analysis.

Occurrence data were used to construct three annual population distribution maps (2000, 2001, and 2002) (data for Kansas in 2003 shown in Figure 3) for subsequent analysis of dispersal rates. Common techniques for quantifying range distances include the square root of the area occupied, linear distance, and neighborhood measurements (Shigesada and Kawasaki, 1997). Technique selection is dependent on the pattern of range expansion. For example, range distances for species exhibiting a radial spread from a point of introduction is approximated by computing the square root of the area occupied by the expanding population. However, organisms with asymmetric dispersal patterns, caused by geographic barriers or lack of observation data, are better described by neighborhood

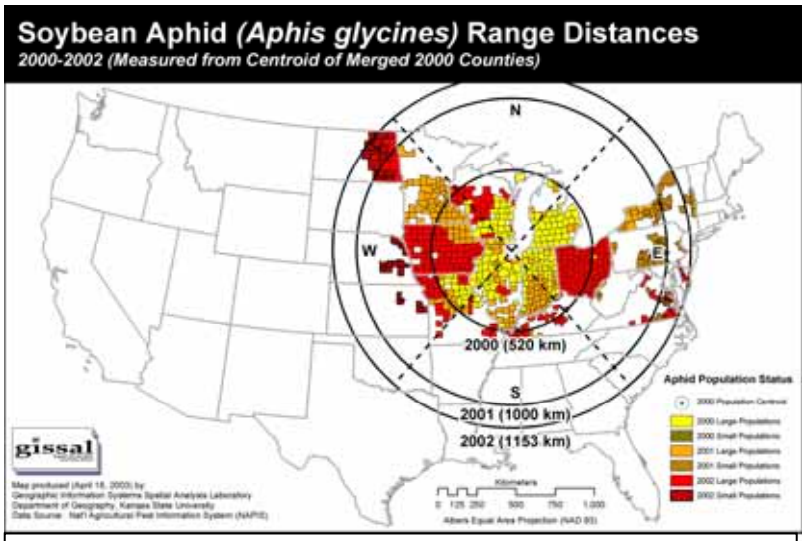

Figure 4. Calculation of point of origin of the soybean aphid in the U. S.

methods.

The characteristic pattern of soybean aphid dispersal was determined using a three-year (2000, 2001, and 2002) neighborhood assessment of maximum annual range distances measured from the 2000 SBA distribution geographic centroid (near Chicago, Illinois) (Figure 4).

Maximum distances, operationally defined as the straight-line distance from the 2000 centroid to the most distant county in which soybean aphids were observed, were determined in each of four cardinal directions using measurement tools within a GIS.

Because the pattern of expanding aphid populations appears to be asymmetric (east + west > north + south), and is influenced by the non-radial spatial distribution of host plant species, the neighborhood measurement of range expansion was selected (Figure 5). Neighborhood measures determine the rate of spread as the average of minimum and maximum range increments in local areas throughout a geographic boundary unit (Andow *et al.,* 1993):

[1] $.r = [(.r_{max}^2 + .r_{min}^2) / 2]^{1/2}$

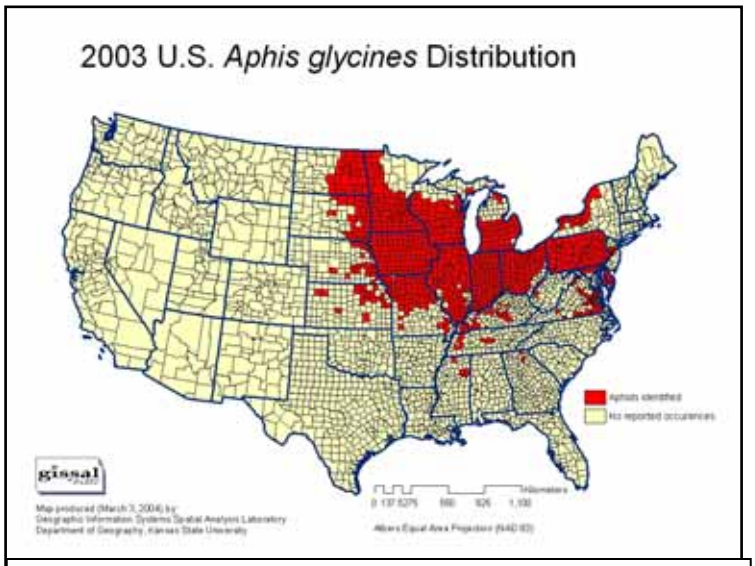

Figure 5. Occurrence of the soybean aphid in the U. S. in 2003.

Local neighborhoods were not incorporated during this stage; rather, minimum and maximum extents for each of the annual SBA distributions were measured and used to estimate annual rates of spread.

Within a GIS, a buffer layer was generated extending a distance, determined by the estimated 2002 range distance (82 km yr-1), from the boundary of the 2002 soybean aphid distribution. Counties located within that buffer (full or partial containment) were identified as preliminary candidates for SBA establishment in 2003. These counties were then compared to a raster GIS layer of major soybean production areas extracted from a national dataset of dominant landcover/landuse. Counties meeting

the criteria of being within the buffer distance and containing soybean acreage > 200 ha comprised the predicted locations for SBA expansion.

Results and Discussion

Annual SBA rates of spread have declined 82 percent from a high of 468 km yr^{-1} in 2000 to 82 km yr^{-1} in 2002 (Table 1). Minimum and maximum dispersal has declined each year of the study period, with a significant decrease in mean rate of spread in 2002. However, these values may simply reflect a delay in identifying the SBA outbreak and insufficient field observations. The SBA may have been present in the U.S. prior to 2000, going undetected for as many as three to four years (North Central Pest Management Center, 2003); thus, the high spread rates in 2000 and 2001 may be a result of increased vigilance by crop scientists and extension agents in the field.

Table 1. Soybean aphid rates of spread for 2000-2002.

ESTIMATED SBA RATES OF SPREAD (KM YR^{-1}).

Year	r_{max}	r_{min}	Δr
2000	640	168	468
2001	592	21	419
2002	116	7	82

The pattern of SBA expansion has been asymmetric, with somewhat more movement in the eastward and westward directions. The apparent eastward movement is caused, in large part, by the presence of SBA in counties of Virginia and Delaware that are non-contiguous to those within the 2000 distribution and separated by the Appalachian Mountains. Expansion has been much more continuous in the west and closely followed areas of significant soybean production. From the initial 2000 distribution, SBA has dispersed to the northeast through the Western Corn Belt Plains ecoregion of Iowa and southern Minnesota, to the Northern Glaciated Plains and Lake Agassiz Plain of the Dakotas and Western Minnesota, and into Southern Manitoba (Omernik, 1987). In the west and south, SBA is moving down the Interior River Lowlands of Missouri and Illinois and across the Central Irregular Plains in Missouri and into Kansas.

Counties located within 82 km of the 2002 SBA distribution “frontier” were labeled as candidate areas for SBA expansion. Those counties containing 200 hectares or more of soybean were identified as prime expansion locations. Assuming a range increase in 2003 comparable to that calculated for 2002, an additional 18 states in the Great Plains, southeast, and northeast may report SBA establishment in 2003. States into which SBA appears most likely to expand, if not already present, include North Dakota in the north and Oklahoma, Arkansas, Tennessee, and Mississippi in the south (Figure 5).

Missing in this analysis are detailed distribution maps of buckthorn, the plant species on which most SBA mating and egg-laying/overwintering occurs. While a common introduced plant across the United States and Canada, the presence of buckthorn appears to be critical for completion/initiation of the SBA life cycle. Readily available information concerning the distribution of buckthorn is limited to state level presence/absence data. More detailed information on the location and density of buckthorn would greatly improve predictions of future movement.

Concurrent SBA research is examining climatic factors that influence seasonal migration and survival. Most seasonal insect adaptations are directed towards the timing of life cycle events such as reproduction, migration, and dormancy (Tauber *et al.*, 1986). Phenomena such as photoperiod and temperature are the primary stimuli regulating these seasonal cycles. However, data on food availability (derived from growing-degree day estimates), insect population density, and location and density of predator species may prove more useful for SBA in North America.

Perhaps even more important is identification of suitable climatic ranges for insect survival. Ongoing content analysis of past entomological research from Asia is providing evidence of unfavorable temperature and atmospheric moisture conditions that disrupt all or portions of the SBA life cycle. In contrast, distributions of SBA populations in the U.S. and Canada since 2000 appear to indicate significant physiological and/or behavioral adaptations that permit successful reproduction and growth over a wide range of temperature and moisture regimes, especially in cooler and drier regions.

Conclusion

The pathway below (Figure 6), quantifies the spread of an invasive species, makes predictions of its spread, and increases the efficiency of databases.

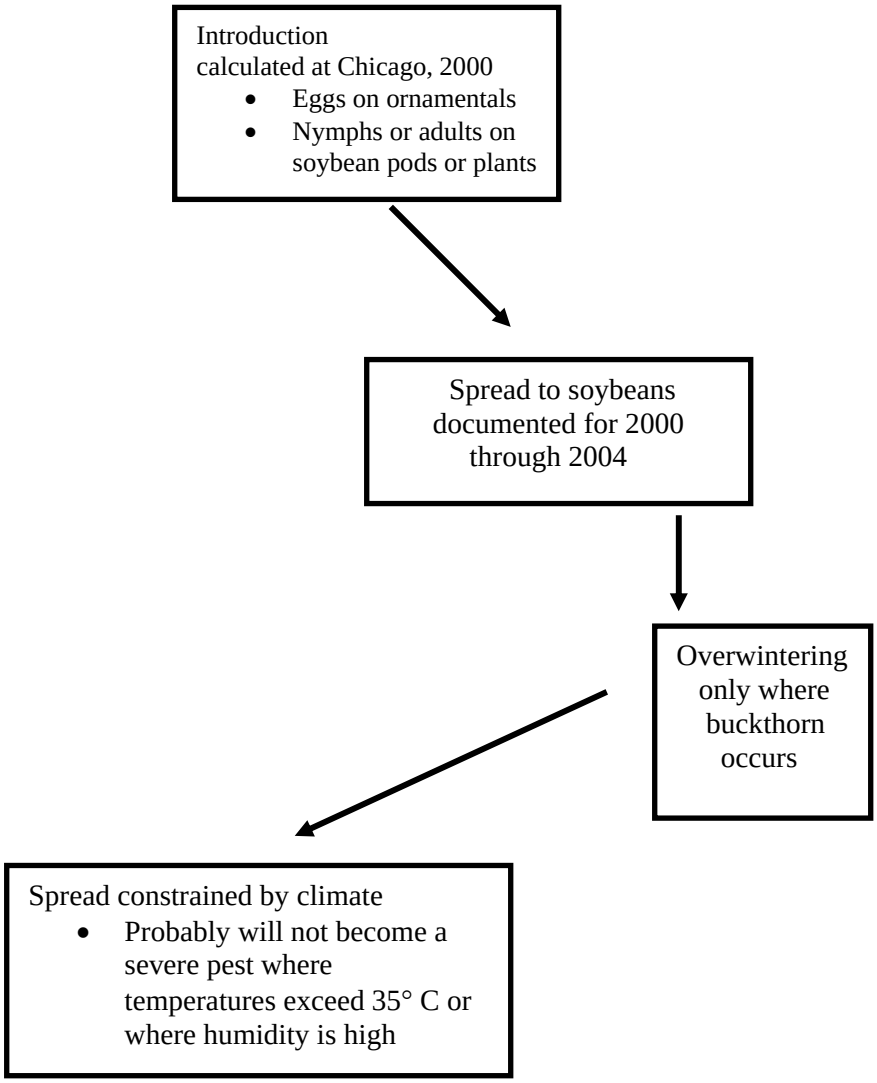

Figure 6. Pathway analysis of the introduction and spread of the soybean aphid in the U. S.

Future Work

Leaf pigments in the palisade mesophyll and epidermal cells in the visible portion of the electromagnetic spectrum (400-700 nm) (e.g., chlorophyll *a* and *b*, carotenes, xanthophylls, and anthocyanins) exert primary control over reflectance. In the near infrared spectrum (700-1300 nm), cell wall structure of the palisade perinkium contributes to high percentages of incident energy being reflected or transmitted rather than absorbed (Gausmann *et al.* 1969, Tucker 1978, Peterson and Running 1989). In the middle-infrared (MIR) region, leaf reflectance is inversely related to the water content of spongy mesophyll cells (Carter 1991), with the magnitude of MIR absorptance being a function of both leaf water content and leaf thickness (Jenson 2000).

The combination of these factors yields characteristic spectral reflectance curves for healthy green vegetation. Deviations from these "spectral signatures" generally indicate the onset of plant stress, originating either in the environment (biotic and abiotic) or due to anthropogenic causes. These patterns of variability can, therefore, be considered diagnostics for canopy condition. Though the potential exists to use spectral reflectance to monitor and quantify various forms of vegetation stress, Carter and Knapp (2001) point out that it is not known how: (1) different stressors within a species may produce distinguishable spectral reflectance response curves and (2) variance of the spectral reflectance of the same stressor among species. While the initial hope of satellite-based observations of insects and plant diseases have remained largely unfulfilled (Riley 1989), the application of hyper-resolution remote sensing techniques may help answer such questions by allowing researchers to identify very fine spectral responses caused by particular stressors.

Acknowledgements

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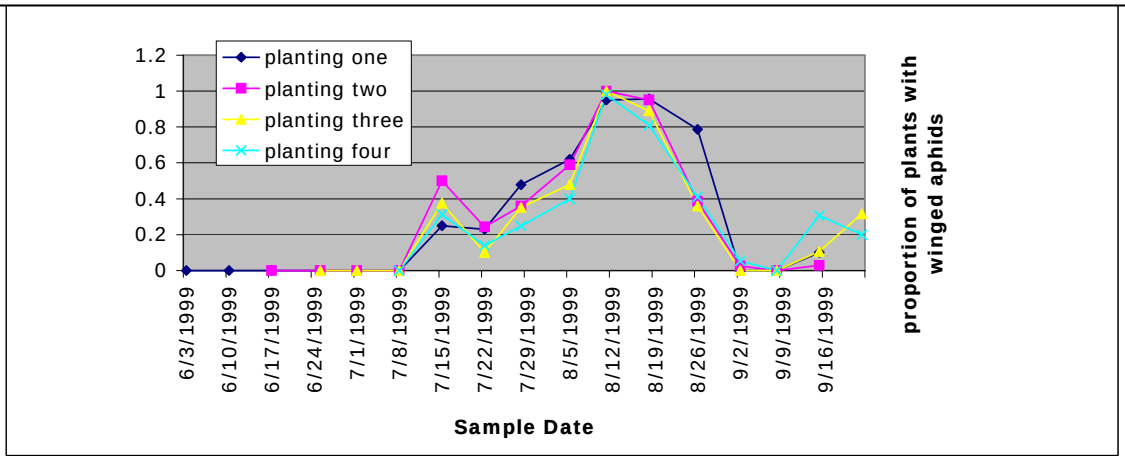
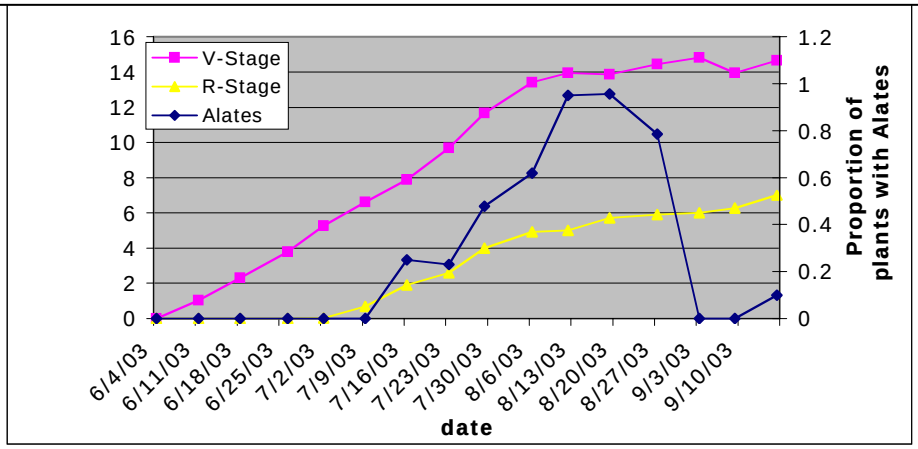
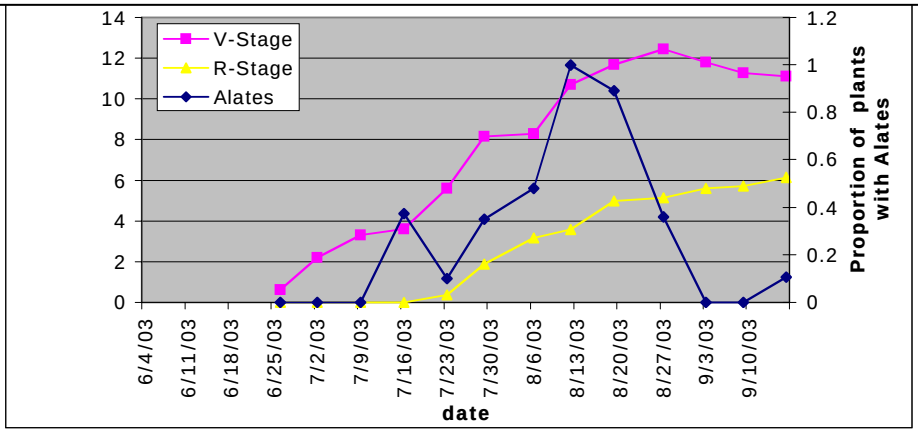
Appendix 4A. Effect of soybean planting date on winged soybean aphids inTippecanoe County, IN in 2003



Appendix 4B. Proportion of soybean plants with winged soybean aphids at various soybean growth stages (site #1) -- Tippecanoe County, IN on sample dates in 2003

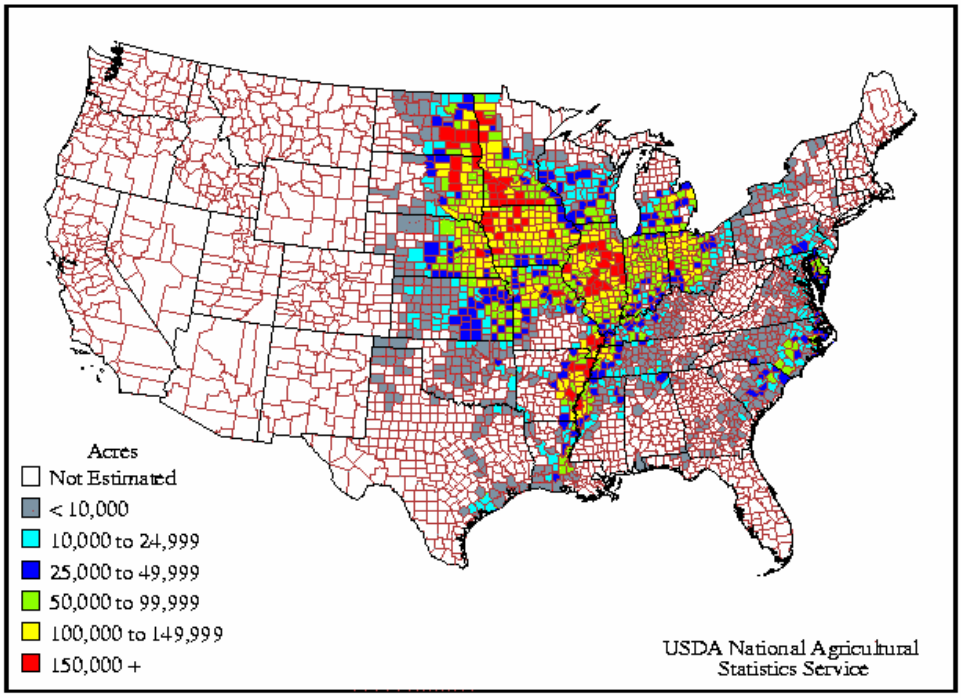


Appendix 4C. Proportion of soybean plants with winged soybean aphids at various soybean growth stages (site #3) Tippecanoe County, IN on sample dates in 2003

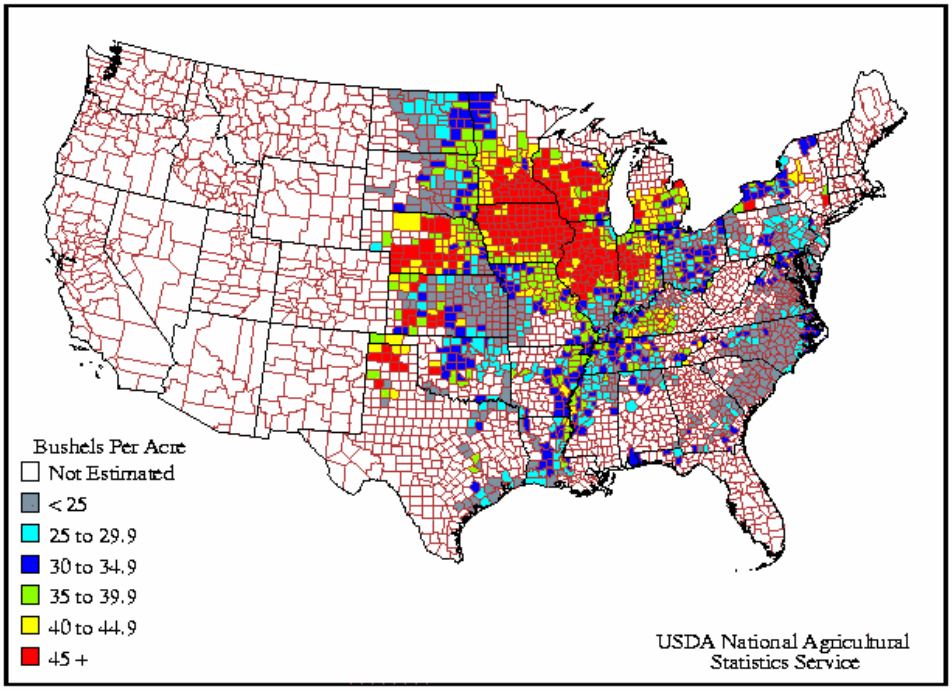


(C. Rutledge, personal communication)

Appendix 5A. Harvested soybean acres in 2002

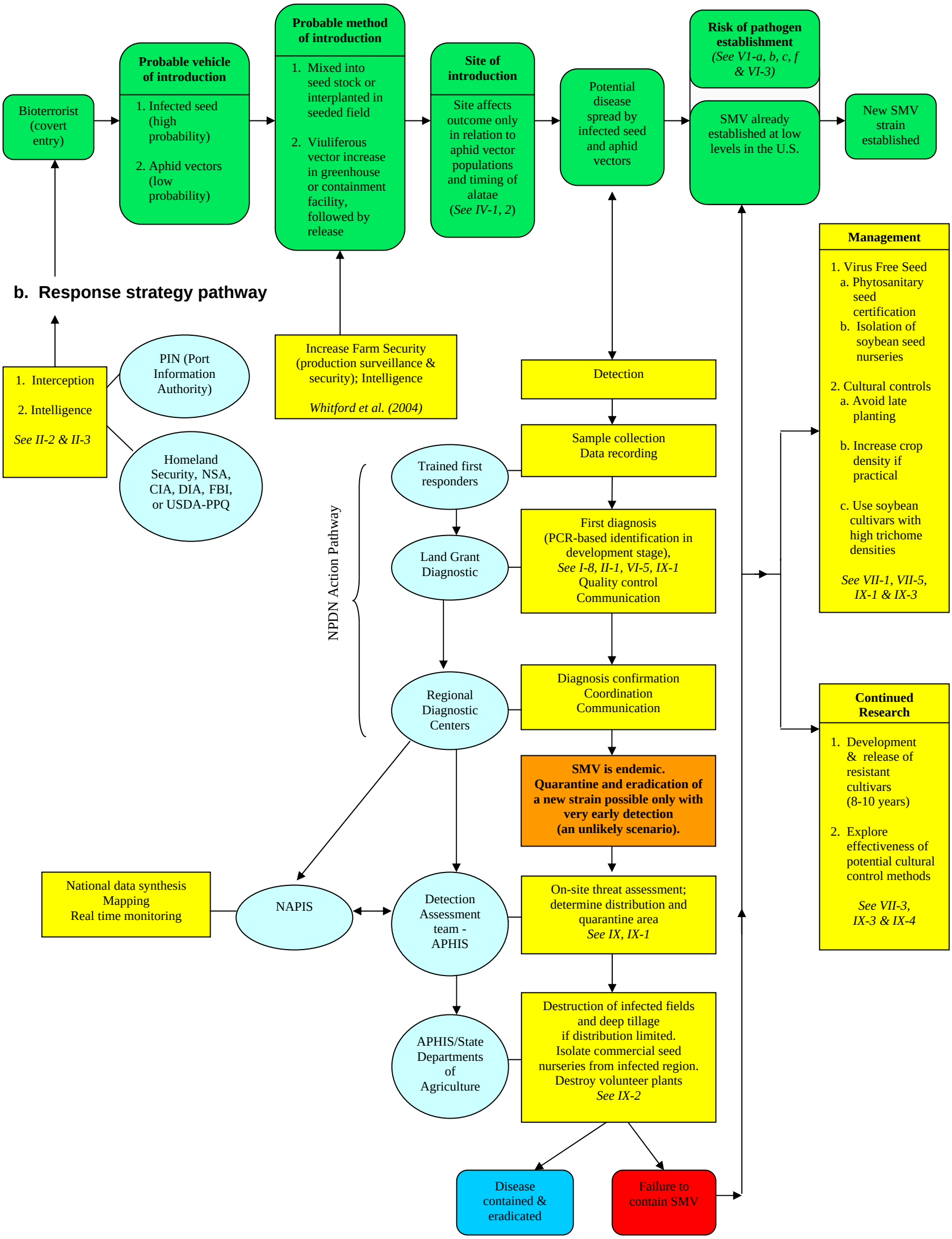


Appendix 5B. Yield/acre of soybeans in 2002



Appendix 6. Pathway and response to the intentional introduction of a highly virulent strain of soybean mosaic virus

a. SMV introduction and development pathway



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Soybean Rust

Pathway Analysis:

Intentional Introduction of

Phakopsora pachyrhizi

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Soybean Rust Pathway Analysis

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Executive Summary: Soybean Rust Pathway Analysis

- Introduction of *Phakopsora pachyrhizi*, causal agent of Australasian soybean rust (SBR), poses a severe threat to soybeans in the U.S. and is currently identified in the Agricultural Bioterrorism Protection Act (USDA-APHIS, 2002).

- SBR occurs in all major soybean-growing regions of the world except North America. It has quickly spread across much of Africa and South America since 2000.

- SBR had a $1.3 billion impact to Brazil's soybean industry in 2002-2003.

- Soybean is the second most economically important field crop in the U.S., with a value of $14.8 billion in 2002. Estimated net negative impacts of SBR (natural wind-borne entry) to the U.S. economy ranged from $640 million to $1.3 billion the year of entry and $240 million to 2.0 billion annually after SBR is established (Livingston *et al*., 2004). These predictions may be greatly exceeded the year of introduction if significant quantities of SBR urediniospores are intentionally introduced into major soybean production regions.

- Although potential total losses on an absolute scale are very large, they represent a loss of $<$1% of net benefits derived from the U.S. agriculture sector. Required fungicide treatments could make soybean production unprofitable in some states and greatly reduce U.S. global competitive advantage, but the U.S. agricultural sector is anticipated to be resilient enough to withstand the shock of SBR establishment (Livingston *et al*., 2004).

- A schematic **SBR disease and response pathway is presented in Appendix 10**.

- A covert bioterrorist introduction could be accomplished with as little as 0.2g of urediniospores (the probable propagule of introduction), a quantity easily produced on just a few infected leaves.

- Urediniospores can be easily collected in large quantities from host leaf surfaces and disseminated by hand, from a pickup truck or by small plane to initiate a large scale epidemic the year of introduction. The small quantities of urediniospores required for introduction make interception by intelligence or PPQ personnel unlikely.

- Once introduced, rapid disease spread, establishment, and persistence are predicted based on current knowledge of SBR epidemiology.

- Although a natural introduction of SBR is considered imminent, an overt introduction of *P. pachyrhizi* will seriously challenge any effective response to its introduction.

- The small quantity of spores required, rapid secondary dispersal, numerous alternate hosts, and potential for rapid and significant economic impact makes *P. pachyrhizi* a primary candidate for agroterrorism.

- There are numerous collateral hosts in the U.S. including kudzu (*Pueraria montana* var. *lobata*), a highly invasive weed that may produce year-round inoculum in Florida, southern Texas, the coastal Gulf States, Mexico and the Gulf islands.

- Prevailing northern wind currents will allow long distance dispersal from southern inoculum sources for annual reestablishment of SBR in the Corn Belt, similar to wheat rusts (*Puccinia graminis* f. sp. *graminis* and *Puccinia recondita*) (Appendix 4B).

- Annual SBR frequency extrapolated from China, (geo-climate similar to the U.S.) indicate SBR can be reintroduced annually to cause severe epidemics between 20-30N, frequent occurrences between 30-35N and occasional SBR as far north as 35-48N (Appendix 4C). Comparable latitudes in the U.S. are more favorable for the disease and damage is expected to surpass that in China.

- The site and timing of an intentional introduction of SBR will determine the dynamics of pathogen spread and persistence. An introduction of *P. pachyrhizi* into a southern soybean producing area presents the highest risk. SBR introduced into the northern U.S. is expected to be less damaging the year of introduction and unable to persist unless urediniospores reach southern regions where SBR overwintering is expected (Appendix 4F).

Immediate Response Options

- Containment and eradication of SBR in the U.S. will not be possible (USDA, 2002a); however, its impact can be minimized by rapid detection and timely implementation of chemical control measures.

- Immediate response will require fungicide applications to minimize the impact of SBR in the U.S. Registered products plus those requested in a preliminary Section 18 Quarantine Exemption Request to the EPA could only treat 7% of the 73 million U.S. soybean acres. Seven additional fungicides have been added to the Section 18 request. Quantities required are expected to fall far short of needs. World stockpiles of effective chemicals are being depleted by extensive usage in South America and Africa. It could be prudent for the U.S. government to subsidize the production and storage of effective chemicals.

- To date, no soybean line has been identified as totally resistant to SBR. Commercial varieties of resistant soybeans will not be available for at least 5 years.

Soybean Rust

Pathway Analysis for the Intentional Introduction of *Phakopsora pachyrhizi*

Soybean rust (SBR), caused by *Phakopsora pachyrhizi,* is the most devastating foliar disease of soybean worldwide (Miles *et al.*, 2003). SBR is present in most areas where soybeans are grown, except the U.S. and Canada. Since first reported in South America in 2001, SBR has spread across over 95% of the soybean-producing regions of Brazil with consequent yield losses of 3.4 million tons (~ 6% of total production) in the 2002/03 season (Livingston *et al.*, 2004). *P. pachyrhizi* is capable of rapid spread due to its enormous inoculum production, long-range spore transport, and multiple hosts. In accordance with the 2002 Agricultural Bioterrorism Protection Act (USDA-APHIS, 2002), *Phakopsora pachyrhizi* has been identified as a biological agent with the potential to pose a severe threat to plant health in the U.S. Because the natural introduction of SBR into the U.S. is perceived as imminent, great efforts are being made to plan response options. As a result, the U.S. is more prepared to deal with an intentional introduction of SBR than any other exotic plant pathogen.

This report is a pathway analysis for the intentional introduction of *Phakopsora pachyrhizi* into the U.S; a summary, in the form of a disease pathway and response schematic, is presented in Appendix 10.

I. Biology and life/disease cycle of the pathogen

SBR is caused by two fungal species, *Phakopsora pachyrhizi* and *Phakopsora meibomiae*. *P. pachyrhizi,* often referred to as the Asian or Australasian SBR, is the more aggressive pathogen. *P. meibomiae*, referred to as the New World type, is a much weaker pathogen and is the pathogen that has been previously reported infrequently in Central and South

America. This paper's discussion will be limited to *P. pachyrhizi*, which has not been reported on the U.S. mainland.

1. Identity

Pathogen Name: *Phakopsora pachyrhizi* H. Sydow & Sydow

Synonyms: *Phakopsora sojae* Fujikuro
Phakopsora calothea H. Sydow
Malupa sojae Ono, Britica, & Henen comb. Nov.
Uredo sojae P. Hennings

Taxonomic position: Phylum: Basidiomycota
Class: Urediniomycetes
Order: Uredinales
Family: Melampsoraceae

Common name: Soybean rust

2. Hosts

Because of confusion over the taxonomy of the pathogens causing SBR, *P. meibomiae* and *P. pachyrhizi*, and differential reactions within host species, the list of hosts of *P. pachyrhizi* may be incomplete. *P. pachyrhizi* is reported to naturally infect 31 species in 17 different genera within the Papilionoideae subfamily of legumes (Sinclair and Hartman, 1996) and 60 species in 26 genera have been successfully inoculated in the lab (Minnesota and South Dakota Departments of Agriculture, 2003). *Glycine max, G. soja, Pachyrhizus eronus, Pueraria montana* var. *lobata and Vigna unguicualta* are the principle hosts (CABI, 2001). A list of species that develop rust symptoms when inoculated with *P. pachyrhizi* are in Appendix 1. Weed hosts present in the U.S. include: Alyce clover, yellow sweet clover, black medic, Colorado river hemp, narrow-leaved lupine, yellow lupine, vetch, and kudzu. *Lupinus angustifolius* is cultivated as a winter forage crops in the southern U.S.

Weed hosts are of significance because they may aid the movement, establishment and survival of the rust in the U.S. Although the pathogen will probably *not* persist from season-to-season as overwintering urediniospores and uredinia on soybean in most areas of the

U.S., the fungus will probably persist in the Deep South or southern Florida on collateral host weeds or crops. Subsequently, urediniospores will be transported northward for long distances on wind currents.

In Paraguay and Brazil, kudzu (mainly *Pueraria phaseoloides*) growing along roadsides and ditches is severely infected with rust but this host displays no apparent loss of vigor (Dr. M. Miles, personal communication). A number of kudzu species exist in South America that display variable susceptibility to *P. pachyrhizi* and SBR has not been observed on the species of kudzu inhabiting Hawaii (Palm, 2004). In the U.S., *Pueraria montana* var. *lobata*, is the highly invasive kudzu species, and has been demonstrated susceptible to *P. pachyrhizi* in quarantine greenhouse studies (Dr. M. Palm, personal communication). Kudzu has become common in the southern U.S., growing as far north as central Illinois. Since kudzu remains green year-round in southern Florida, it is anticipated to be a significant source of overwintering inoculum once *P. pachyrhizi* is established in the U.S.

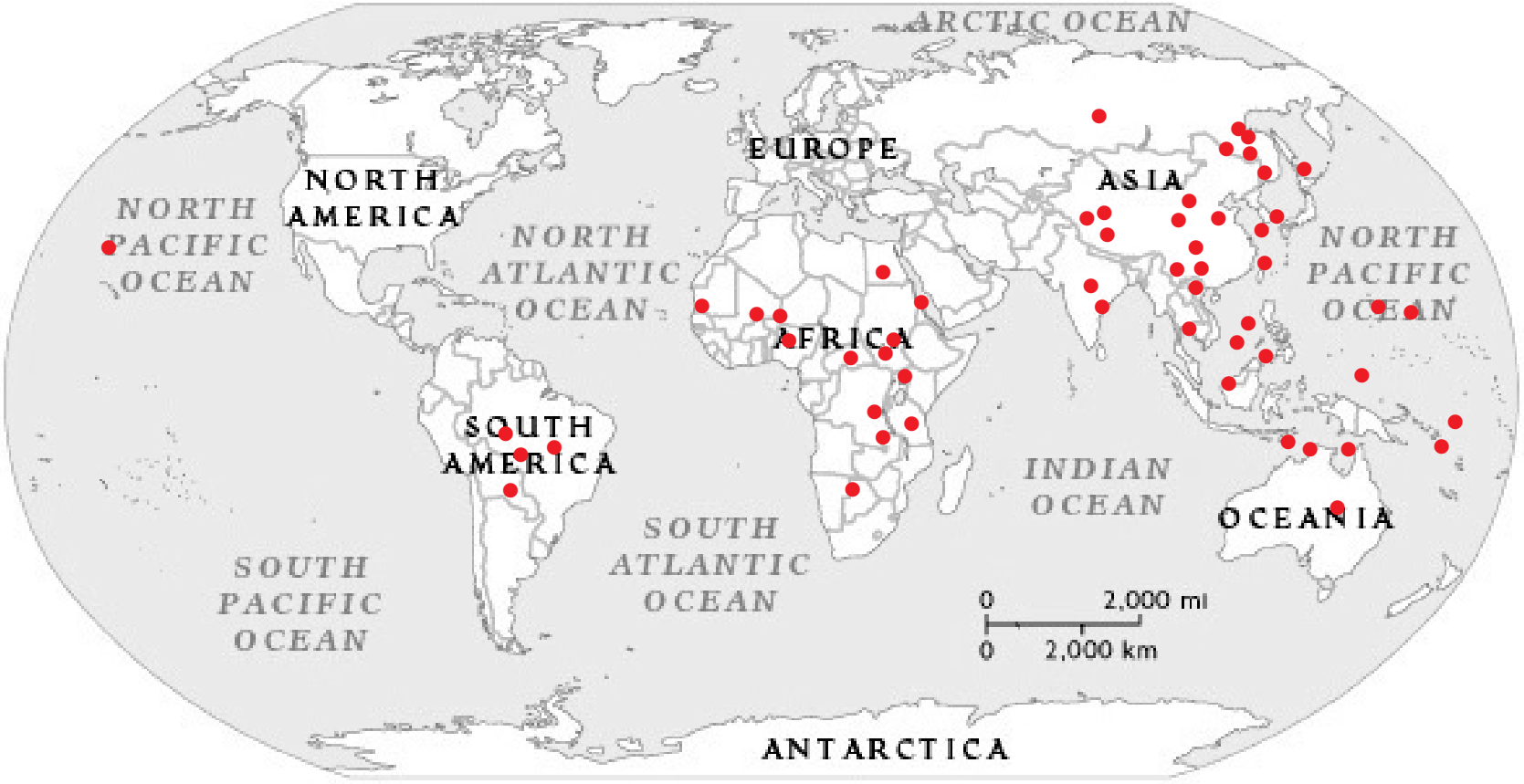
3. Geographic Distribution and Impact

SBR is widely distributed and has been reported in most major soybean growing areas of the world, except the United States and Canada (Fig. 1). It is especially destructive on soybean in tropical and warm temperate climates where temperatures remain between 15-28°C with high humidity and frequent rainfall. Yield losses are primarily due to premature leaf drop, which reduces photosynthetic area, pod and seed production, and seed weight (Yang *et al.*, 1991b).

SBR was first detected in Japan in 1902 and has caused extensive damage in Asia and Australia. India and Asia experienced epidemics in the 1970s and 1990s. Yield losses of 40% in Japan (Bromfield, 1984), 10-50% in southern China, 10-40% in Thailand, and 18-57% in Taiwan (Chen, 1989) have been reported. In Australia, SBR epidemics tend to occur on a four-year cycle, likely due to local weather cycles (Syngenta, 2003).

The “restricted distribution” of *P. pachyrhizi* reported by CABI (citing EPPO, 1999) in Mexico in 1999 was almost certainly the mistaken identification of *P. meibomiae* (Dr. G. Hartman, personal communication). It is generally accepted that *P. pachyrhizi* does not yet exist in the northern hemisphere of the New World, with the exception of Hawaii.

Fig. 1. World Distribution of Soybean Rust caused by *Phakopsora pachyrhizi*



Adopted from CABI, 1999. updated with reports from years 2000-2003.

P. pachyrhizi was reported in Hawaii in 1994 (Killgore *et al.*, 1994). Many Hawaiians consider green soybeans a local delicacy so soybean is grown primarily as a vegetable crop in Hawaii. There is speculation that immigrant farmers brought rust into Hawaii from Southeast Asia on soybean plants or planting material, although this has not been fully substantiated (Killgore, 1996). SBR has not become a yield-limiting disease in Hawaii, perhaps because soybeans are grown under relatively dry conditions there (Smith, 2003). Isolates collected from Hawaii were demonstrated as virulent as Asian, South American and African isolates in containment greenhouse tests (Dr. R. Frederick, personal communication).

The recent rapid spread of SBR in Africa has caused serious losses. It was first observed in Uganda in 1996 (Kawuki *et al.*, 2003), Rwanda and Zimbabwe in 1998 (Kloppers, 2002); Nigeria (Akinsanmi *et al.*, 2001), Mozambique, and Zambia in 1999; and South Africa in 2001 (Kloppers, 2002). In Nigeria, seed weights were reduced 28-52% with disease severity higher on the medium-maturing cultivars than those planted late (Akinsanmi *et al.*, 2001). Yield of commercial soybean varieties in Uganda were reduced 27-37% (Kawuki *et al.*, 2003) and 60-80% in Zimbabwe (Dr. C. Levy, personal communication). In South Africa, yield losses ranged from 10-80% in 2001. Losses of up to 100% have been reported where monocropping (with no rotation) is practiced (Caldwell & Laing, 2002).

SBR in South America is reported in Paraguay (2001), Argentina and Brazil (2002) (Frederick, 2003) and Bolivia (2003) (Dr. A. Ivancovich, personal communication). Disease spread in South America appears to be as rapid as in Africa because of abundant hosts and similar climate (USDA, 2002b). Yield losses as high as 80% have been reported in experimental plots in Paraguay (Miles *et al.*, 2003b). In 2002, an exceptionally hot dry season, SBR was estimated to cause 1% yield reduction in southern Brazil. Rains late in the 2003 season allowed SBR to extend its range to 95% of Brazil's soybean producing areas. The top soybean producing state (Mato Grosso) in Brazil lost approximately 7% of its projected 2002/03 crop due to rust, even with fungicides, and those who didn't spray lost up to 50% yield (Stewart, 2003). The total impact on Brazil's soybean industry was estimated at $1.3 billion (yield losses plus control costs), of which $544 million was spent on fungicides (Yorinori, 2003). These losses coincided with hot dry conditions in southern Brazil that were not conducive to disease development until late in the growing season. Since Brazil is a major soybean producer, with a production area similar in size to the soybean area of the U.S., the Brazil experience illustrates what may happen in the U.S. (Smith, 2003). It should,

however, be noted that soybean production in Brazil occurs in frost-free regions where vegetation lives year-round and multiple crops are possible.

A virulent race of *P. pachyrhizi* introduced into United States soybean production areas could cause significant crop and economic losses to growers and associated industries. In addition, alternate hosts of economic value (green, kidney, lima bean, and cowpea) could also experience losses. Costs of chemical control could significantly reduce U.S. production efficiency and global competitiveness. Models predicting disease spread, yield losses, and economic consequences for the U.S. are discussed in sections V-1d and VII-4.

4. Disease Cycle and Epidemiology

P. pachyrhizi is an obligate pathogen that infects leaves primarily (Fig. 2, 3 and 4), although lesions can appear on petioles, pods and stems. Uredinia are most abundant on the abaxal leaf surface but are also found on the upper leaf surface. Soybean plants are susceptible to *P. pachyrhizi* at any stage of development, but symptoms usually appear from flowering to late in the season (Fig. 6). The pathogen progresses rapidly from lower to upper leaves. The pathogen is not seedborne (Sinclair and Hartman, 1999); however, urediniospores may occur in contaminated soybean residue with the seed.

The disease cycle (Fig. 5) is initiated when a wind-blown urediniospore is deposited on a soybean leaf, germinates and infects the leaf. The cycle ends when the fungus sporulates and disperses an abundance of new urediniospores. Urediniospores require free water on leaf surfaces for germination and penetration. An appressorium develops in 2-3 hours at the end of the germ tube, followed by direct penetration into the epidermal cell. This rust has the unique ability to directly penetrate the epidermis; most rusts enter the leaf through stomatal openings and penetrate cells once inside the leaf. The direct penetration of epidermal cells and nonspecific induction of appressoria (Koch and Hoppe, 1988) may facilitate infecting the broad host range of *P. pachyrhizi* and may slow the development of resistant cultivars (Miles *et al.*, 2003a).

Infected plants show chlorotic or tan-colored flecks 5-7 days after penetration; uredinia are differentiated in 5-8 days, and urediniospore liberation commences in 9-10 days (Fig. 7 and 8). A single uredium may produce urediniospores for 3 weeks. Uredinia develop for up to 4

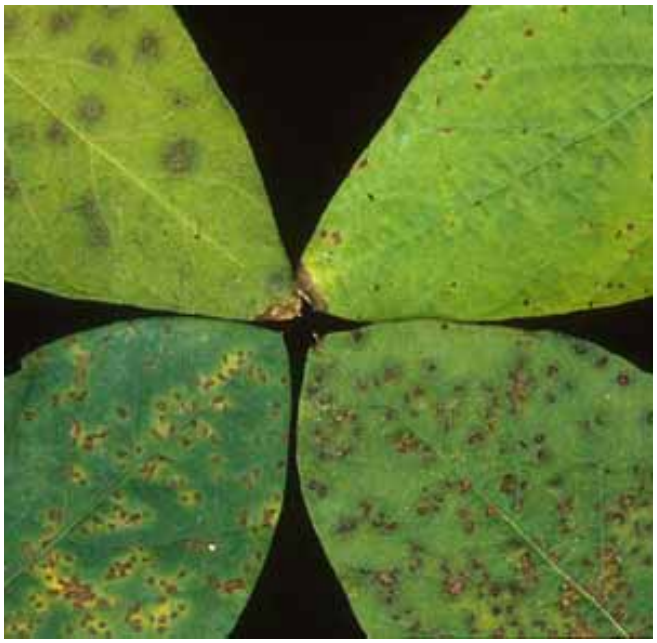


Fig. 2. Soybean varieties differ in their response to rust infection.

(Dr. G. Hartman)

Fig. 3. Tan colored lesion (TAN) on a soybean leaf caused by *P. pachyrhizi*

(Dr. R. Frederick)

Fig. 4. Reddish-brown lesions (RB type) on a soybean leaf caused by *P. pachyrhizi*

(Dr. R. Frederick)

Fig. 5. Disease Cycle of Soybean Rust caused by *Phakopsora pachyrhizi*

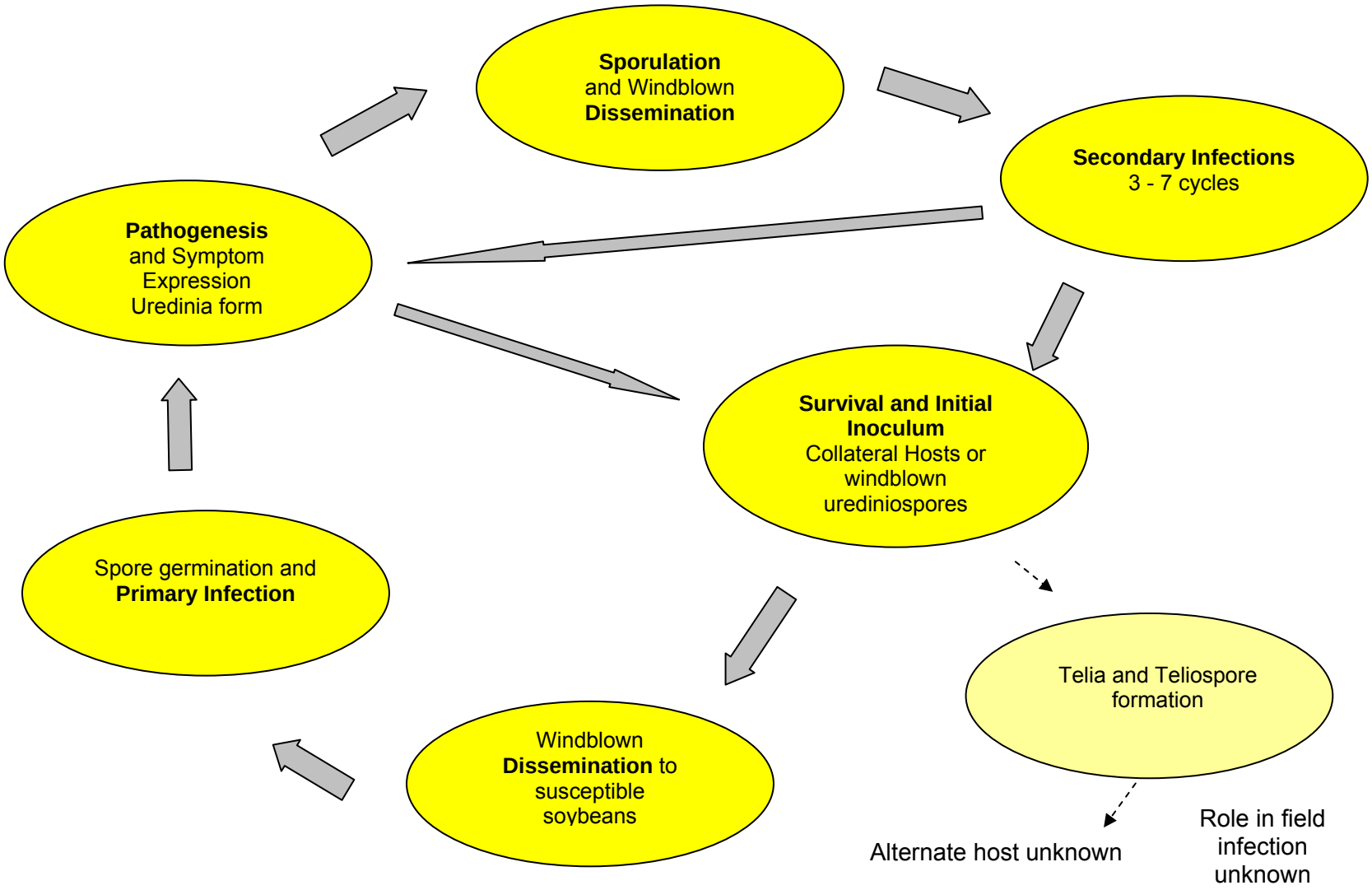


Fig. 6. Soybean field with typical soybean rust symptoms (left). Soybean treated with a protective fungicide (right).

(Dr. G. Hartman)

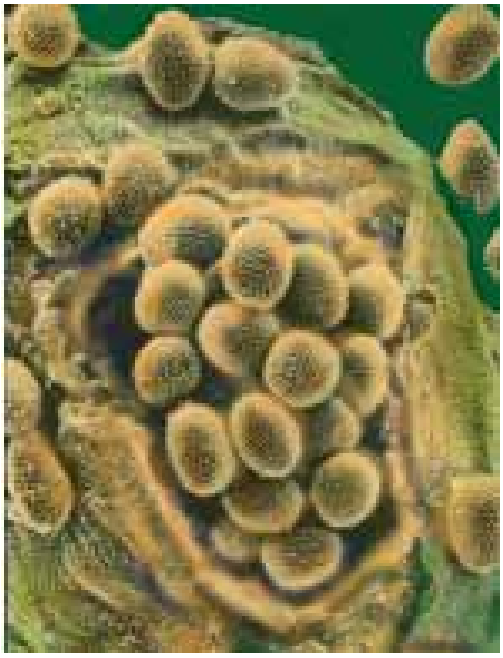


Fig. 7. Urediniospores of *P. pachyrhizi* erupting from ostiole.

(Dr. G. Hartman)

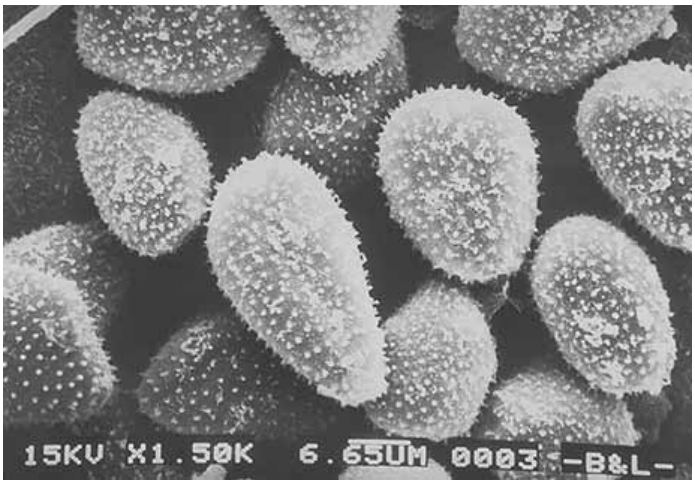


Fig. 8. Urediniospores of *Phakopsora pachyrhizi.*

(Dr. G. Hartman)

weeks after a single inoculation, and secondary uredinia continue to arise on the margins of the initial infection for an additional 7-8 weeks. The mean number of urediniospores produced per lesion during a 5-week sporulation period range from 2,000 to 6,600 depending on the isolate, host genotype, and environmental conditions. At the height of infection, 3 billion spores/plant can be released, or approximately 1000 trillion spores/ha/day (http://soybeanrust.zedxinc.com). Prolific sporulation from an initial infection could continue for up to 15 weeks. This extended sporulation capability allows the pathogen to be broadly disseminated and persist during unfavorable periods when dry conditions, excessive precipitation, or mean daily temperatures below 15°C (or greater than 30°C) inhibit rust development. Even if conditions for secondary infection are sporadic, significant inoculum potential exists from the initial infection to reestablish an epidemic. Anywhere from 3-7 infection cycles can occur during a growing season. In the tropics and subtropics, rust may persist throughout the year on any of its collateral hosts to provide a ready source of inoculum for a subsequent soybean crop.

The sexual stage of *P. pachyrhizi* is known to exist but the role of teliospores in the epidemiology of the disease is uncertain. Dark blackish-brown telia are occasionally produced late in the season both among uredinia within old lesions and at the periphery of lesions. Teliospores have germinated consistently in the lab after exposure to 10-12 wetting and drying cycles, and high germination rates were observed when telia were stored at 5°C for 5-6 months before breaking dormancy (Saksirirat and Hoppe, 1991). Although teliospores can be found in the field late in the growing season, basidiospores have been reported only in the greenhouse and an alternate host is unknown. It is considered unlikely that an alternate host for *P. pachyrhizi* exists in the U.S. (Dr. M. Miles, personal communication). Further evidence that the sexual stage probably does not play a role in epidemiology is that isoenzymes among isolates of *P. pachyrhizi* are identical, so there does not appear to be sexual recombination (Bonde *et al.*, 1980). The question of sexual recombination will be further elucidated, once the genome of *P. pachyrhizi* is sequenced.

Conditions favoring infection: Conditions that promote good growth and canopy development of the soybean crop also favor the development of SBR. *P. pachyrhizi* urediniospores germinate between 10-28.5°C, with a broad optimum range of 15-25°C (Marchetti *et al*., 1976). A minimum of 6 hours and optimum of 12 hours dew is required for infection between 16-26.5°C (Melching *et al.*, 1989). A relative humidity of 75-80% is

optimal for infection and spread of the pathogen (Palm, 2004). The relationship between infection, temperature and dew period is effectively depicted in a three dimensional graph (Fig. 9). Typical dew events in the U.S. Midwest provide enough leaf wetness for infection. Overhead irrigation increases rust severity, as does precipitation, which aids its spread and provides leaf moisture for infection. As demonstrated in Brazil in 2002, hot dry weather can limit the development of the disease (Smith, 2003).

Spore viability: Lab experiments revealed urediniospore viability was favored by storage temperatures between 15-25°C, with spore viability ≥68% after 30 days and 0-17% after 50 days, but no survival at 4-5°C after 5 days (Patil *et al.*, 1997). Greenhouse experiments where urediniospore inoculated plants were exposed to bright light revealed a dramatic decrease in infection if dew was delayed for 72 hours and no rust occurred when dew was delayed for more than 7 days, even with cloud cover (Melching *et al.*, 1989). Other estimates of urediniospore viability when exposed to the elements are in the range from 1-2.5 weeks (Isard, 2004), to as long as 2 months if humidity is high and ultraviolet radiation is low (http://soybeanrust.zedxinc.com). Not much data is available on upper level atmospheric spore survival. The viability of spores under natural conditions needs to be further investigated to determine the effect of humidity, temperature and ultraviolet light. The longevity of viability can help determine the potential for long-range atmospheric transport and how long seed needs to be stored before planting in order to eliminate the potential for SBR initiation via urediniospores in seed contaminants.

Long distance dispersal: The primary means of infection is via urediniospores spread by wind and storms. The long distance dispersal of rust fungi is an accepted fact. Nagarajan and Singh (1990) mention several instances of trans-Atlantic spread of pathogens. The SBR pathogen was thought to be wind-borne from Asia to Africa (Caldwell and Laing, 2002).

A natural introduction of SBR to the U.S. would likely not occur via direct movement of spores from South America to the U.S. because equatorial winds move from east to west rather than south to north. An aerobiological spore dispersal model for the western hemisphere is being developed by an APHIS-SBR Research Group (see V-1c and VII-4) (Dr. S. Isard, 2004). A more likely means of natural introduction would be a progressive movement of *P. pachyrhizi* via the Central American landbridge, and eventual arrival in Texas or other southern state (Yang, 2004). Barley yellow rust is an example of a pathogen that spread along this pathway. It was first reported in Ecuador in 1975, in Mexico in 1987

and spread throughout the U.S. from 1991 to 1994. Barley yellow rust is a cool weather disease and the spread of SBR may be more rapid (Yang, 2004).

SBR could also be unintentionally introduced to the U.S. mainland via seed residue or by Asian gardeners, as may have been the case in Hawaii. The high probability of *P. pachyrhizi*'s natural arrival to the U.S. has prompted a USDA (2002) plan to minimize the impact of its introduction. This "Strategic Plan to Minimize the Impact of the Introduction and Establishment of SBR on Soybean Production in the United States" includes early detection survey procedures using sentinel plots with susceptible (early maturing) soybean cultivars. Plant Protection and Quarantine (PPQ), in cooperation with the Agricultural Research Service (ARS) and the Cooperative State Research, Education and Extension Service (CSREES), is reviewing existing air current data in an effort to correlate potential dispersal pathways of SBR. U.S. and international collaborators are also developing remote sensing techniques for monitoring the spread of SBR in fields (USDA, 2002a). The land bridge north, the extensive movement of plant material, and the history of other pathogens that have moved from South America to the U.S., indicate that it's just a matter of time before SBR arrives on the mainland (Dr. M. Miles, personal communication).

Pathogenic Races: Nine races of *P. pachyrhizi* have been identified in Asia on a set of differential plants; however, the predominant race was compatible with 10 of the 11 differentials, and all races were compatible with three of more of the differentials (AVRDC, 1990). Some races of *P. pachyrhizi* in the field possess multiple virulence factors to known or suspected genes for resistance (Sinclair and Hartman, 1999). *P. pachyrhizi* strains recently isolated from South America display an apparent heightened virulence (Dr. M. Bonde, personal communication) and would likely result in severe destruction of U.S. soybean if introduced. In South America, *P. pachyrhizi* is already a mixed population composed of many strains, even though it was only introduced a few years ago. It is not known if this diversity is due to multiple trans-Atlantic introductions or frequent mutation leading to the development of variants (Minnesota and South Dakota Departments of Agriculture, 2003). There is a plan to sequence the entire genome of *P. pachyrhizi* and develop methods for the molecular identification of rust isolates (Miles *et al.*, 2003a).

5. Symptoms

The early stages of SBR are difficult to detect because early symptoms appear as chlorotic mosaic discoloration on the upper surface of leaves in the lower-middle canopy. As the disease progresses, infected leaves turn yellow and signs and symptoms move upwards in the canopy. Lesions are initially small with a water-soaked appearance but gradually increase in size and turn from gray-green to tan or reddish-brown. One to many uredinia form within a lesion 5 to 10 days after infection and are most abundant on the abaxal leaf surface. The color of the lesion depends on its age and the genotype interaction with the race of pathogen (Fig. 2). Lesions are usually angular, restricted by leaf veins, and 2 to 5 mm in diameter. Tan lesions (TAN) indicate a susceptible reaction (Fig. 3 and 10) and when mature, consist of small pustules with masses of tan colored urediniospores on the surface.

Reddish-brown lesions (RB type) (Fig. 4 and 11) represent a hypersensitive reaction, have reddish brown polygonal necrotic areas surrounding pustules, and produce fewer urediniospores than TAN. The TAN reaction is likely in the U.S. because cultivars grown here are susceptible to SBR (Bonde, 2004). Lesions can also appear on petioles, pods and stems. Infected plants lose vigor. Pods are reduced in number, size and frequently unfilled, resulting in seed reduction. High lesion densities result in premature defoliation and early maturity (Fig. 6). Heavily infected plants may mature up to two weeks earlier than noninfected plants (Sinclair and Hartman, 1996) and plants can defoliate rapidly – within a week after symptoms are first observed (Streit and McNeill, 2003). Symptoms caused by *P. pachyrhizi* on kudzu, *Pueraria montana* var. *lobata,* are similar to reddish-brown lesions on soybean (Fig. 12a and 12b).

Early stages of infection may be confused with bacterial pustule (*Xanthomonas axonopodis* pv *glycines*), bacterial blight (*Psuedomonas savastanoi* pv. *glycinea*) and brown spot (*Septoria glycines*). Early stages of these diseases are difficult to distinguish if bacteria or spores are not visible. Bacterial pustule appears on the underside of soybean leaves as a

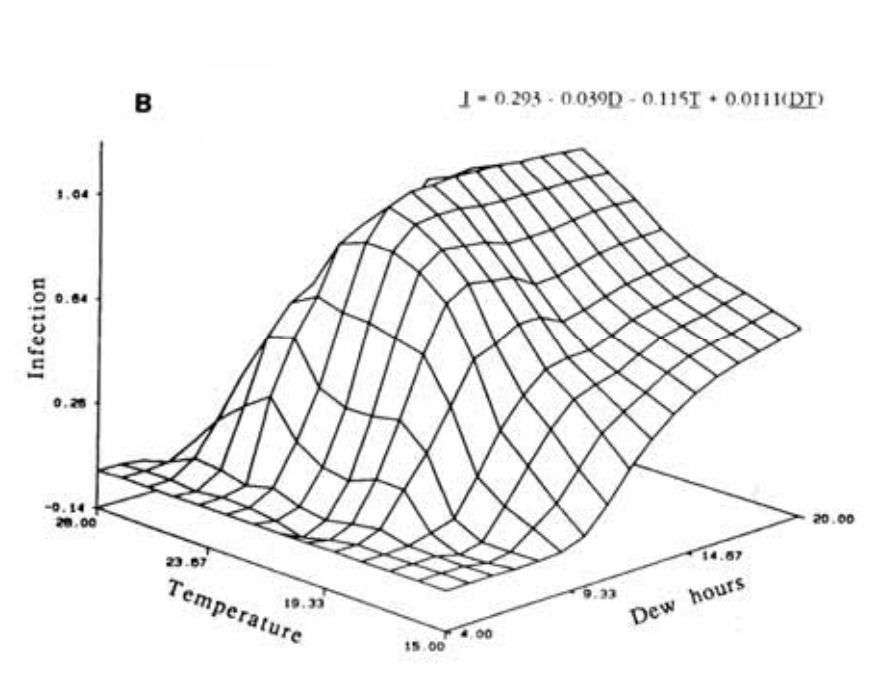


Fig. 9. Influence of dew period and temperature on infection (infection index = no. of pustules/cm^2 at each temperature – dew period divided by the no. of pustules/cm^2 at 20°C and 12 hr dew period)

(Yang *et al.*, 1991)

Fig. 10. Close up of tan colored lesions caused by *P. pachyrhizi.*

(Dr. A. Tschanz)

Fig. 11. Close up of reddish-brown lesions caused by *P. pachyrhizi.*

(Dr. A. Tschanz)

Fig. 12a. Soybean rust symptoms on kudzu, *Pueraria montana* var. *lobata*.

(Dr. W. Morel)

Fig. 12b. Soybean lesions on the underside of a kudzu leaf.

(Dr. W. Morel)

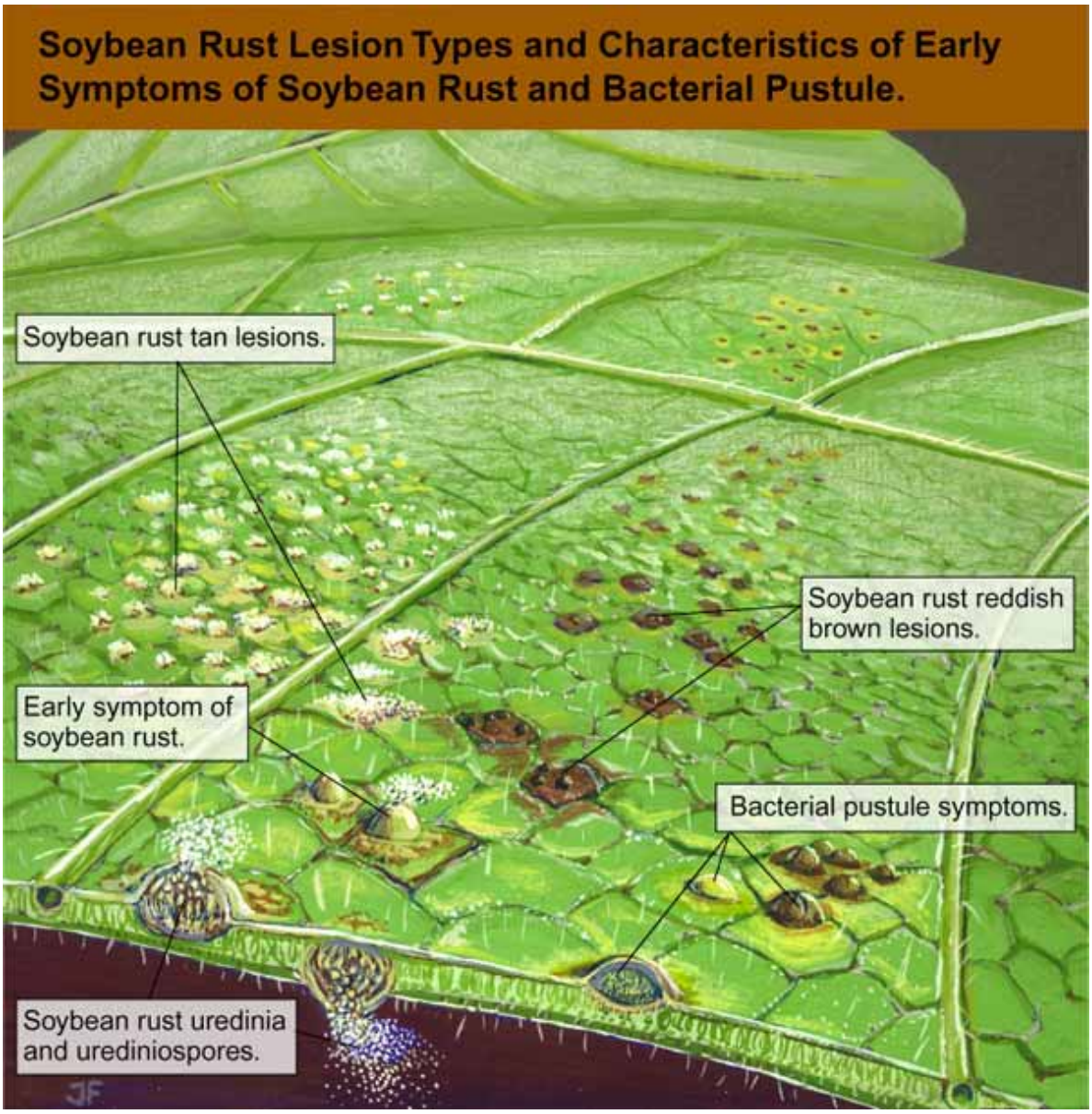


Illustration by Joel Floyd, USDA, APHIS, PPQ

Fig. 13. Illustration of soybean rust and bacterial pustule symptoms.

From *http://www.aphis.usda.gov/ppq/ep/soybean_rust/illustration.html*

raised, light brown blister within a lesion that is frequently surrounded by a yellow halo not present with SBR. At a more advanced stage, these diseases can be easily differentiated with a hand lens or dissecting microscope by the presence of the ostiole and uredinia within the rust lesion, in contrast to the irregular crack that usually appears in the bacterial pustule lesion (Fig. 13). The buff-colored mounds of urediniospores extruded from underlying uredinia can be rubbed off the leaf surface. Placing a *P. pachyrhizi* infected leaf in a plastic bag with a damp paper towel overnight will stimulate sporulation (Syngenta, 2003).

6. Identification: Distinguishing between *P. pachyrhizi* and *P. meibomiae*

Two species of *Phakopsora* were identified as causal agents of SBR in 1992. They were distinguished by morphological differences between their anamorphs and teliomorphs (Ono *et al.*, 1992). Most of the distinguishing features focus on telia and teliospores from the sexual life cycle and are not readily found in the field. Sequencing the genomes of *P. pachyrhizi* and *P. meibomiae* is a current research priority of the USDA-ARS soybean rust project, in collaboration with the DOE Joint Genome Institute.

Classical and real-time florescence PCR (polymerase chain reaction) assays have recently been developed to identify and differentiate between *P. pachyrhizi* and *P. meibomiae* (Frederick *et al.*, 2002). The technique was developed using an ABI Prism 7700 Sequence Detection System and specific primers, Ppm1 and Ppa 2 (Fig. 14). These assays have since been adapted for use with portable analytical thermal cycling instruments, the Smart Cycle® (Cepheid, Inc., Sunnyvale, CA) and R.A.P.I.D.® (Ruggedized Advanced Pathogen Identification Device) (Dr. R. Frederick, unpublished). These portable instruments cost under $30,000. Identification of the aggressive *P. pachyrhizi* from soybean leaves using real-time PCR permits positive diagnoses in about 5 hours. No isolation or purification of suspect organisms from infected tissue is necessary and diagnosis can be done in the field, eliminating the lag time required to send samples to the lab for analysis. People with no specialized mycological training can perform this procedure rapidly and accurately.

Although technical means of identifying *P. pachyrhizi* exist, the application of these techniques is restricted in the U.S. Control DNA is required for PCR identification of *P. pachyrhizi.* SBR is on the USDA-APHIS Select Pathogen List, that limits the organism and its DNA to BL3 containment facilities or state university diagnostic labs that have been

granted waiver permits. This limitation is a roadblock to the rapid diagnosis required for effective mitigation and control of SBR.

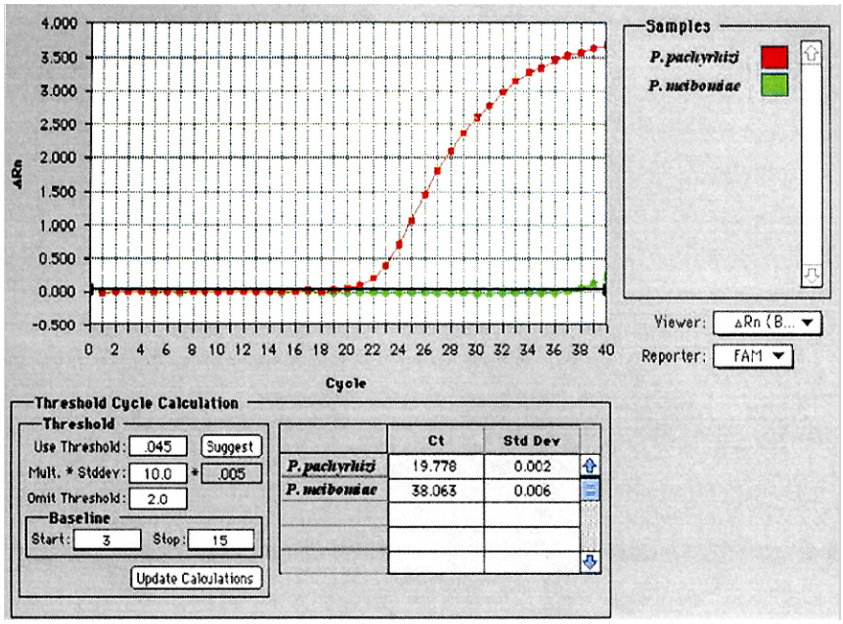


Fig. 14. Real-time PCR amplification of DNA from *P. pachyrhizi* and *P. meibomiae* by TaqMan PCR using an ABI Prism 7700 Sequence Detection System. Ppm1 and Ppa2 were used with a 5'-FAM-labeled internal probe. The left axis (RQ) is the change in florescence that measures probe cleavage efficiency, and the bottom axis is the PCR cycling stage.

II. Initiating event (recognizing an attempted introduction)

1. Observation/diagnosis of presence

Diagnosing an exotic pest in the field early is critical and should be possible with SBR (see VI-5). The recent development of real-time PCR offers an accurate tool for rapid identification of *P. pachyrhizi* if current restrictions on DNA possession can be resolved.

Since the natural spread of SBR has been a concern in recent years, USDA and university extension personnel have been educating growers, scouts, crop specialists, and plant pathologists to recognize symptoms in the field. The Crop Advisor Institute at Iowa State University has created a SBR learning module CD-rom (Yang and Brueland, 2003; www.iastate.edu) that allows Certified Crop Advisors and other agricultural professionals to earn continuing education units (CEUs) online.

The recent establishment by the USDA of the National Plant Diagnostic Network (NPDN) is intended to provide a cohesive information system to quickly detect pests and pathogens

that have been deliberately introduced and report to appropriate responders and decision makers. NPDN, made up of experts at land-grant universities, is a key part of the Homeland Security effort. NPDN is divided into five regions, each with a regional hub (Appendix 9A). Web-based diagnostic and reporting systems are being developed and an effective communication network between diagnostic labs and regulatory agencies has been established (Appendix 9B). Modules to train first detectors are being developed by NPDN. This system should facilitate the detection of anomalies, such as simultaneous outbreaks at many locations, and thereby help identify a bioterrorist attack. Select data collected from the NPDN regions will be archived at the National Agricultural Pest Information System (NAPIS) located at Purdue University.

2. Interception: individual/ pathogen

Interception of an individual carrying some quantity of the pathogen or infected plant material at a port of entry should be responded to immediately. Isolation and containment of the material should prevent escape into the environment. Interception through routine traffic stops, although somewhat improbable, should not be discounted and confirmatory procedures initiated. The probability of interception of shipped inoculum (urediniospores) to an in-country location is much lower than personal interception and confidentiality of mail deliveries could avoid detection.

3. "Intelligence" information

Intelligence information from Homeland Security, NSA, CIA, FBI, or USDA-PPQ about an overt agroterrorism intent is another potential initiating event. This information should be provided to personnel at the county level to enhance the probability of early detection.

III. Probable route of terrorist entry/dissemination

Pathogen – culture, Urediniospores

P. pachyrhizi is an obligate pathogen and, as such, is not amenable to mass production on artificial media in the lab. Spores are abundant on soybean plants or in crop residue so that a small number of plants could provide an effective inoculum source. Since there is a fair

probability that plant material would be intercepted at a port of entry, terrorists are likely to use a less detectable method, such as the collection of urediniospores from infected plant material in the field. Large quantities of urediniospores can be easily collected from infected soybean leaves with very simple hand-held or equipment mounted vacuum devices.

Quantities of contaminated soybean leaves or pure urediniospores could enter the U.S. via parcel post, in a traveler's luggage or backpack, or in a small container on a traveler's person. The small quantities of spores required by terrorists for introduction (see section VI-1) could be easily concealed, making it unlikely for intelligence or PPQ personnel to intercept the entry of SBR inoculum.

Once inside the U.S., rust urediniospores disseminated by hand at key locations during early stages of soybean growth could produce small-localized epidemics, which would rapidly spread. Among the many thousands of continuous acres of soybean, an individual making small-scale introductions from a pickup truck would likely go unnoticed. Dissemination of a virulent strain during favorable weather conditions early in the growing season would produce the greatest disease intensity and severe yield loss.

Another simpler scenario, leading to either the intentional or unintentional introduction of SBR into the U.S., is via urediniospore contamination on the clothing and boots of persons traveling from infected areas (Dr. M. Miles, personal communication). A person could walk through a rust infected field in Brazil, get on a plane to a U.S. destination, and walk through a domestic soybean field to potentially initiate infection. This risk is somewhat reduced by the fact that our soybean growing season is opposite that of South America's. When the soybean crop in South America is maturing, U.S. growers are just beginning to plant.

If the intention of an overt introduction was to produce a wide-spread epidemic that same year, larger quantities of rust inoculum could be aerially disseminated rapidly over large soybean growing areas using a small plane or a pickup truck.

IV. Probable distribution: Spread with three scenarios

1. Point Introduction

a. Southern Soybean Area

Inoculum introduction at a single or a few multiple sites could potentially produce localized SBR epidemics during the year of introduction. The pathogen will probably be capable of persisting in the Deep South or in southern Florida (see V1-a, d) on weed or crop hosts year-round to provide a constant source of rust inoculum. Spread of SBR in the south is likely to be rapid following introduction, as has recently occurred in Africa and South America where climatic conditions are favorable and non-crop hosts are numerous. Of particular concern is the host kudzu, an invasive weed, which is now distributed across much of the southern U.S. soybean area.

b. Corn Belt

Point introduction(s) in the Corn Belt (north central states) will likely result in a single season SBR episode. The pathogen is unlikely to persist from season-to-season by means of overwintering urediniospores in the Corn Belt, but infection of southern soybeans by *P. pachyrhizi* could permit it to overwinter, and then be reintroduced to initiate subsequent year's infection (see V-1c).

c. Mexico

An alternative means of overt SBR introduction would be to disseminate *P. pachyrhizi* urediniospores to soybean in northeastern Mexico, adjacent to the U.S. border. The natural northward spread from Mexican soybean to U.S. soybean near the border can be expected to be rapid if weather conditions favorable for infection prevail. A climate-based model predicts conditions for SBR survival are good in central-eastern and northeastern Mexico, and south Texas (Pivonia, 2003; Pivonia and Yang, 2004). In this scenario, neither the perpetrators nor the disease-initiating agent need to be overtly introduced into the U. S. The chance of intercepting such a clandestine operation is highly unlikely. This scenario would probably result in permanent establishment of the pathogen in the U.S.

2. Secondary Dissemination

a. Windborne

The most probable mode of secondary dissemination is windborne urediniospores. Rust spores can travel thousands of miles on wind currents. If SBR was introduced and established in the southern U.S. or Mexico, it could potentially spread throughout the soybean producing areas via northward air currents from southern host species (Appendix 3A and 3B). Whether these northbound urediniospores would arrive early enough in the growing season to cause significant soybean yield losses in the Corn Belt is unknown at this time because of the relatively short seasonal differential from the southern to northern U.S. production area. Such annual reintroductions of SBR are common in China, which has a similar landmass as the U.S. (Appendix 4C).

If the pathogen were introduced only into the Corn Belt, its natural spread southward would be slower than northward due to prevailing northward wind currents. Some southward spread can be expected, especially in the central and eastern states where prevailing winds from May to August move in a cyclic pattern (Appendix 3A and 3B). Dispersal is discussed further in section V-1c.

In order to accurately develop the aforementioned scenarios, more needs to be learned about the potential dispersal pathways of SBR. A review of existing air current data, prevailing winds, and widespread acreage of continuous soybeans susceptible to rust indicate potential crop saturation within the growing season regardless of route of early entry (Appendix 3A and 3B).

b. Seed-borne

Although the pathogen is not seed-borne, spores may contaminate soybean residue that is present with seed. This has prompted seed companies to vigorously clean seed destined for the U.S. (Sprangler, 2003). A recent USDA-APHIS-PPQ (2004) report consider clean soybean seed, grain and soybean meal not to be pathways for the introduction of SBR; however, leaf debris associated with “foreign material” found in soybean grain is a theoretical, but unlikely, pathway for SBR introduction. Spore survival under different environmental conditions needs to be determined so recommendations can be made on how long urediniospore contaminated seed needs to be stored before planting.

V. Consequences of introduction and establishment

The consequences of introduction of *P. pachyrhizi* and the risk of SBR establishment in the U.S. were rated with respect to six risk elements: climate, host range, dispersal, economic impact, environmental impact, and persistence. The pathogen was ranked for 29 different criteria encompassed within the six risk element categories. The disease distribution range of an airborne pathogen is frequently much larger than its survival range and dependent on factors such as climate, distribution of collateral hosts and the pathogen's capacity for dispersal. The specific *P. pachyrhizi* isolate that enters the U.S. is expected to have a large impact on the severity of SBR.

1. Establishment

a. Climate *Risk = High*

The disease distribution range of SBR in the U.S. will be dependent on regional climatic pattern. SBR is established in a number of climatic zones throughout the world that correspond to climatic zones of the major soybean-growing areas in the U.S. (USDA, 2002b). In Asia, the intensity of the disease is dependent on seasonal air currents, temperature, and moisture. In Australia, SBR epidemics tend to occur on a four-year cycle, likely due to local weather cycles (Syngenta, 2003). The U.S. geo-climatic situation is most similar to that of Argentina and China (Pivonia, 2003; Pivonia and Yang, 2004).

Major soybean-producing areas in the U.S. (Appendix 2A) usually receive 3-5 inches of precipitation (Appendix 3A) and 7.5-10.4 days with measurable rainfall (Appendix 3B) each month between April and July. This frequency and quantity of rainfall provides adequate free moisture for urediniospore germination and perpetuation of *P. pachyrhizi*'s 9-10 day disease cycle. Rainfall also plays an important role in washing spores that are traveling in air currents down to a susceptible crop canopy. All states east of the Mississippi and most states in the Great Plains are very suitable for SBR epidemics based on the frequency of 15 or more favorable days during the growing season over the past 30 years (Magarey *et al*., 2003) (Appendix 4D). Favorable days were defined by the pathogen's requirements for temperature and moisture for infection.

Cold stress is probably the most important factor limiting *P. pachyrhizi*'s survival range in temperate regions. A map displaying regional frequency of temperatures below 0°C (Magarey, 2003) implies *P. pachyrhizi*'s overwintering ability is likely to be limited to southern Florida, southern Texas, southern Louisiana, and southern California (Appendix 4E). A more complex analysis to determine year-round SBR survival has been developed by Pivonia and Yang (2004). Their model integrates temperature-stress (CLIMEX software) with dry-stress. The analysis incorporates the maximum time permitted between two infection events to maintain a *P. pachyrhizi* population (*i.e.* 70-90 days) and environmental conditions required for urediniospore production, survival, and germination. The resulting "stress-free index" indicates a moderate to high chance of survival in the southern tip of Texas and central-southern Florida, as well as regions of Mexico and the Caribbean (Appendix 4F). Because information on the effects of short periods of low temperature exposure on urediniospore viability is not available, it remains uncertain if SBR can survive in Florida where occasional short periods of freezing temperature occur. A less conservative approach (using 4°C instead of 7°C as the temperature preventing *P. pachyrhizi* survival) would indicate coastal regions of Mississippi and Louisiana could be part of the survival range of SBR.

Climatologists and soybean breeders continue to work together to map the high and low risk areas for SBR within the U.S. (Wynstra, 2003).

b. Host Range *Risk = High*

The broad host range of *P. pachyrhizi* will be a primary factor contributing to establishment of SBR in the U.S. Besides various cultivated legume species, many weed hosts such as: Alyce clover, yellow sweet clover, black medic, Colorado river hemp, lupine, vetch, and kudzu are present in the U.S.

c. Dispersal *Risk = High*

The rapid establishment of *P. pachyrhizi* over large areas of U.S. soybean production will be possible because the disease is effectively wind dispersed. Rust urediniospores can be carried by storms and air currents over hundreds of miles.

The ability of rust fungi to spread rapidly was illustrated by sugarcane rust (*Puccinia melanocephala),* which was first reported in the Americas in the Dominican Republic in July

1978. Transatlantic air currents probably introduced urediniospores of sugarcane rust into that region from West Africa (Purdy *et al.*, 1985). Sugarcane rust was subsequently reported in Jamaica in September 1978, Puerto Rico in October 1978, and Florida by March 1979.

A number of possible "models" have been suggested for the spread of SBR in the U.S. (Minnesota and South Dakota Departments of Agriculture, 2003). In 1970, southern corn leaf blight (*Cochliobolus heterostrophus*) was first reported in the southern Gulf States. It moved northward along the Mississippi river valley, then spread in all directions to encompass the entire east half of the U.S. by September (Appendix 4A). The annual dispersal of urediniospores of wheat stem rust (*Puccinia graminis* f. sp. *graminis*) and wheat leaf rust (*Puccinia recondite*) in North America is attributed to the pathogen's survival on southern hosts that remain green through the winter (Appendix 4B). Perhaps even more indicative, is the annual spread of SBR in China, which has a similar landmass to the U.S. (Yang, 2003b). The pathogen overwinters between latitude 20-30N, where it can cause severe epidemics (Appendix 4C). This latitude corresponds to the Gulf Coast of North America (Appendix 2B). SBR is reintroduced annually above 30N with frequent reoccurrence between 30-35N and occasional SBR north of 35N.

Whether SBR is initiated by a natural or intentional introduction, existing air current data is sufficient to predict potential SBR dispersal throughout the U.S. soybean producing areas. General predictions can be made based on maps of prevailing wind direction and mean monthly precipitation (Appendix 3A and 3B).

Soybean is planted in March and early April in the southern soybean producing area. By May, plants are large enough and typical spring precipitation patterns favorable to support initial infection patterns; thus, inoculum introductions at that time would produce the greatest impact. With a disease cycle of only 9-10 days and up to 7-infection cycles/season, significant quantities of secondary inoculum would be produced. Prevailing winds would carry SBR urediniospores northward primarily, with cyclic distribution in the eastern soybean producing areas becoming more pronounced from June to August. Ample rainfall episodes occur across the soybean growing area to wash urediniospores from prevailing air currents and provide free moisture periods for infection. June would be the most important month for widespread disease distribution. If spores arrive and initiate infection in the central soybean belt by mid-June, SBR is likely to be dispersed throughout the entire U.S. soybean

producing area, similar to southern corn leaf blight (Appendix 4A). Although the rate of disease spread in the U.S. is speculative, the rapid movement of SBR in South America and Africa implies a similar scenario in the U.S.

The delayed soybean-growing season in the northern Corn Belt would mean effective *P. pachyrhizi* introduction would have to be made in June or July. Prevailing winds would carry spores northward primarily, but cyclic summer prevailing winds east of Illinois could also carry inoculum east and southward. This southward movement may impact the second crop of double-cropped soybeans in the southern Corn Belt.

The introduction of SBR into U.S. soybean growing areas earlier than July will likely result in the rapid distribution of SBR throughout the soybean growing region. SBR outbreaks from a late introduction may be somewhat localized the first year, but it will likely become widespread the second year if the disease becomes established in the south. Since *P. pachyrhizi* is unlikely to overwinter successfully in the northern U.S., long distance dispersal via prevailing wind currents from southern inoculum sources will probably be the means for annual reestablishment of the pathogen in the Corn Belt. The extent of annual regional reinfestation remains speculative but if the Chinese experience is considered indicative, frequent SBR may be expected in regions between 30-35N (see Appendix 2B for latitudes in U.S.). Regions in China between latitudes 35-48N experiencing occasional SBR (Appendix 4C) are much drier and have fewer soybean acres than comparable latitudes in the U.S.; therefore, disease occurrence and impact in comparable U.S. regions are expected to surpass those in China. Furthering understanding of the factors responsible for *P. pachyrhizi* urediniospore dispersal patterns in China and Japan will help predict the risk and timing for long distance transport of urediniospores in the U.S. (Pivonia and Yang, 2004).

d. Economics *Risk = High*

Soybean is the second most economically important field crop grown in the U.S. In 2002, soybean was grown over 73.0 million acres in 29 states (Appendix 2A) to produce 2.65 billion bushels (Table 1), with a value of $14.84 billion. The top six producing states and associated percent of total production in 2002 were Iowa (18.5%), Illinois (16.0%), Minnesota (11.2%), Indiana (8.8%), Nebraska (6.4%), and Ohio (5.7%). Yield/acre by county in 2002 is displayed in Appendix 2B.

In 1984, the USDA Economic Research Service (ERS) and Agricultural Research Service (ARS) conducted comprehensive economic analyses of the potential impact of SBR on the U.S. (Kuchler and Duffy, 1984; Kuchler *et al.*, 1984). The econometric-simulation model took into account regional scenarios, changes in market forces including commodity price elasticity, and losses to consumers, processors, and livestock producers. The predicted net negative impact to the U.S. economy ranged from $47 million/year in the case of 1-3% SBR loss in southern states (with fungicide sprays) to $4.5 billion/year in the case of a massive epidemic causing 25% yield loss over all U.S. soybean growing regions (with no fungicides available).

A recent simplified economic analysis by Smith (2003) uses the yield loss assumptions in the ERS reports (Kuchler and Duffy, 1984; Kuchler *et al.*, 1984) with 2001 production and market prices but market and ancillary effects are not considered (Table 1). Yield impact the year of introduction is expected to be in the single digits nationally, but impacts will increase annually until the pathogen spreads throughout the entire range of U.S. soybean production where it can survive. Annual yield impact could be as high as 10-15%, or higher, with associated losses of $1.2-3.2 billion (Smith, 2003). Another economic estimate based on Yang *et al.* (1991a)'s regional yield loss predictions generated similar results (Table 2). Yield loss of >10% were predicted in nearly all U.S. soybean growing areas, with losses of up to 50% in the Mississippi Delta and southeastern coastal areas (Yang *et al.*, 1991a) (Fig. 15). A hypothetical SBR infection in the U.S. (based on 2001 crop values and modest loss estimates) might result in direct crop losses of over $225 million for each of four states: Iowa, Illinois, Missouri and Minnesota, and $1.4 billion for the entire country. Values in table 1 and 2 do not include indirect losses, such as the impact on secondary industries dependent on soybean, attempted control measures, losses to alternate hosts of economic value, or price response to reduced yields caused by SBR.

The most recent systemic modeling analysis of the economic implications of natural wind-borne entry of SBR into the U.S. was released in April, 2004 by the USDA-ERS (Livingston *et al.*, 2004). Factors considered include varying regional susceptibilities to rust establishment, SBR control costs, price elasticity, shifts in land use to other crops, impacts on the livestock sector, trade flow, and consumer effects. Changes in the structure of the domestic and international soybean industry since the earlier 1984 study were also accounted for. The Southern States comprised only 16% of U.S. soybean acreage between 1998 and 2002 compared to 35% between 1980 and 1984. Production geography is relevant

because SBR is most likely to become established in the south. Acreage expansion following U.S. crop losses are considered modest compared to those of the 1970s and 80s due to increased South American soybean production.

The analysis estimates the first year net economic losses due to natural wind-borne SBR between $640 million to $1.34 billion depending on the severity of yield impact. The model assumes producers have sufficient advance warning and chemical availability to make fungicide applications (a scenario not likely until the seconds or third year after introduction because this is not a current practice). In the worst-case scenario (9.5% reduction in yield) yield losses plus fungicide costs were estimated to result in soybean producers incurring 75% of the total societal cost. In the best-case scenario, soybean yields were projected to actually increase due to fungicides controlling other diseases presently not profitable to manage, although some loss is incurred due to application expenditures and price declines for soybeans.

The potential impact 3 years post entry ("medium-term") with permanent establishment of SBR estimates losses are predicted from $240 million to $2.0 billion annually (Table 3). Regions experience different yield shocks and producers respond by adjusting the number of acres planted to soybean as they shift to alternative crops for increased return to those crops. Less production increases soybean prices so that producers in unaffected regions will profit from SBR infestations elsewhere. Under a severe SBR infestation, a 21% decrease in returns to soybean producers and a decline in U.S. soybean exports between 0.6 and 5.6% are expected. Higher soybean prices elevate feed costs and lower poultry and livestock profits. Corn Belt producers incur the greatest losses with producers shouldering 60-70% of the cost of adjusting to SBR, and livestock producers and consumers paying the remainder (Table 3).

Table 1. Expected yield impact of SBR in the United States based on 2001 crop data*

Yield impact (%)	Soybean acreage (x 1000)	Yield per acre (bushels)	Production impact (x 1000 bu)	Value per bushel ($)	Total value of yield loss (x 1000 $)
-1	74,105	39.6	-29,346	4.30	-126,187
-3	74,105	39.6	-88,037	4.30	-378,559
-7	74,105	39.6	-205,419	4.30	-883,302
-10	74,105	39.6	-293,456	4.30	-1,261,861
-15	74,105	39.6	-440,184	4.30	-1,892,279
-25	74,105	39.6	-733,639	4.30	-3,154,650

* Crop data taken from 2002 Agricultural Statistics (USDA, 2002a)

(Smith, 2003)

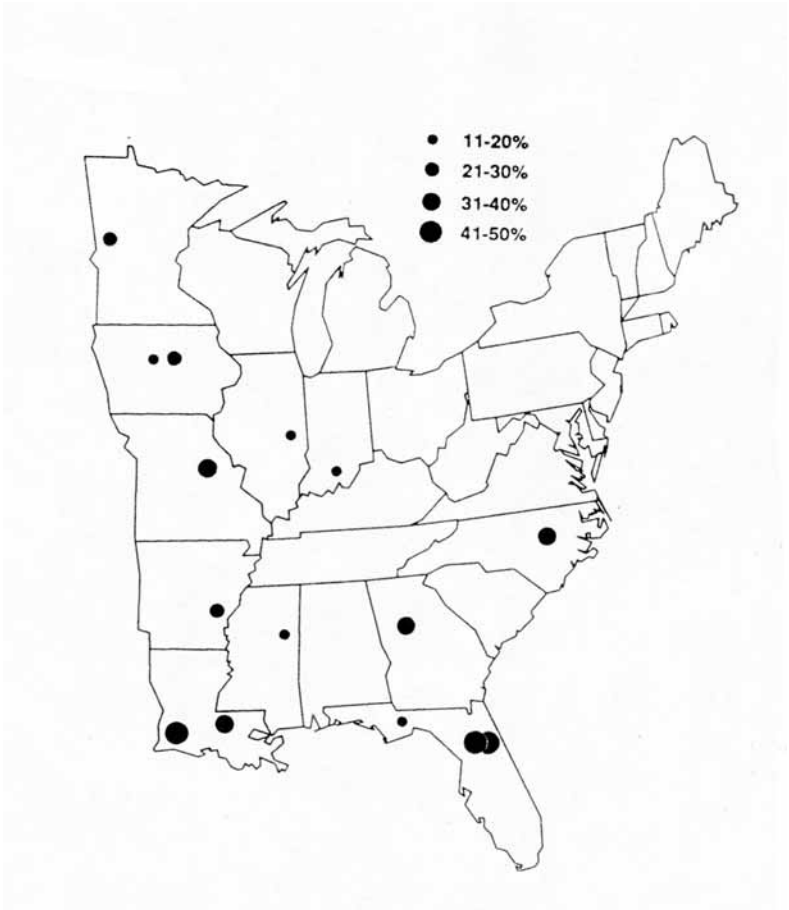


Fig. 15. Percentage of potential yield losses at 15 locations in the U.S., with loss = 100X (potential yield – disease yield)/potential yield.

(Yang *et al*., 1991a)

Table 2. Soybean production for 2002, crop value for 2001 and estimated direct economic impact of a potential SBR infection by state.

State	Bushels/acre in 2002	Million Bushels in 2002	$ million 2001	Projected yield loss (Yang *et al.*, 1991a)	Projected economic loss ($ million)
AL	28	4	21		
AR	34	99	401	21-30%	84.2
DE	20	4	32		
FL			1	41-50%	0.4
GA	24	3	18	31-40%	5.6
IL	41	424	2,151	11-20%	236.5
IN	41	233	1,205	11-20%	132.6
IA	46	490	2,066	11-30%	227.3
KS	23	60	363		
KY	30	36	215		
LA	33	25	90	31-50%	27.9
MD	21	11	82		
MI	36	70	268		
MN	43	297	1,106	21-30%	232.3
MS	33	47	165	11-20%	18.3
MO	32	149	801	31-40%	248.3
NE	36	169	936		
NJ	25	2	13		
NY	32	5	23		
NC	24	31	181		
ND	35	84	287		
OH	33	152	826		
OK	25	7	21		
PA	28	11	59		
SC	18	8	40		
SD	31	129	561		
TN	30	34	159		
TX	28	7	24		
VA	23	11	70		
WV			2		
WI	39	55	254		
US	**37**	**2,656**	**12,440**		**1,400.4**

*Calculations = 2001 soybean crop values in $ millions (NASS, Crop Values, 2001 Summary) X % minimum potential yield loss (Yang *et al.*, 1991a). Source for yield and production; NASS (2002b) - Crop production report, September 12, 2002. Source for cash values: NASS (2002a) - Crop Values, 2001 Summary, February 2002

Table 3. Medium-term economic impacts of soybean rust outbreaks, as changes from baseline projections

	U.S. agriculture				
	Soybean producers	Livestock producers	Other crop producers	Consumers	Total net change[a]
2008 baseline ($million)[b]	5,776	33,000	18,904	340,233	397,913
Scenarios[c]					
High Spread ($million)	-1,213	-137	22	-675	-2,004
Percent change from baseline	-21.01	-0.41	0.11	-0.20	-0.50
Medium Spread ($million)	-828	-57	5	-287	-1,168
Percent change from baseline	-14.34	-0.17	0.03	-0.08	-0.29
Low Spread ($million)	-164	-9	18	-84	-240
Percent change from baseline	-2.84	-0.03	0.09 -	-0.02	-0.06

[a]Total net change is the sum of changes experienced by all crop producers, all livestock producers, and all consumers of affected commodities.
[b]Economic impacts are compared to 2008 levels derived from baseline projections (USDA, 2001).
[c]High Spread = All Soybean Regions; -9.5% yield shock and $25/acre treatment on a portion of the regional acreage that reflects the regional suitability index;
Medium Spread = AP, SE, DL, CB, and NE Regions; -4.3% yield shock and $25/acre treatment on a portion of the regional acreage that reflects the regional suitability index; and
Low Spread = AP, SE, and DL Regions; positive 0.9% yield shock and $25/acre treatment on a portion of the regional acreage that reflects the regional suitability index; where
NE (Northeast) = CT, DE, MA, MD, ME, NH, NJ, NY, PA, RI, VT; LS (Lake) = MI, MN, WI; CB (Corn Belt) = IA, IL, IN, MO, OH;
NP (Northern Plains) = KS, ND, NE, SD; AP (Appalachia) = KY, NC, TN, VA, WV; SE (Southeast) = AL, FL, GA, SC;
DL (Delta) = AR, LA, MS; SP (Southern Plains) = OK, TX.

(Livingston *et al.,* 2004)

The ERS analysis (Livingston *et al.*, 2004) estimates impact based on the “wind-borne” entry of SBR into the U.S., *i.e*. it incorporates climatic transport probabilities for spores from South America. Spores are most likely to reach southern U.S. regions the year of entry. A large covert introduction of SBR into the high production areas of the Corn Belt may have a considerably greater economic impact the year of introduction. In Brazil, yield losses due to SBR averaged approximately 6 - 7% in 2002, with yield losses in individual fields of up to 50%. There is the potential for comparable serious losses in the U.S., if sufficient inoculum was introduced in May or June and typical weather conditions prevailed favoring infection and dispersal. Inoculum introductions delayed until July would likely cut losses by half during the year of introduction. In addition, news of an intentional introduction is likely to

result in public alarm and an erosion of confidence in U.S. food security in general. A 10% loss in major production areas would have a destabilizing effect on soybean prices and world markets (Smith, 2003), especially with carryover and world soybean stocks very low at the present time.

Fungicide sprays will have product purchase and application costs (see VII-3, Appendix 7) that further reduce efficiency and profits. Even a highly effective fungicide may be too expensive or require too much effort for small-scale and marginal growers of legumes. Additional costs associated with controlling SBR could make the production of soybean in the southern-most states unprofitable (USDA, 2002a).

Although total losses estimated on an absolute scale appear large, they represent a loss of <1% of net benefits derived from the U.S. agriculture sector. The ERS report concludes, "the small relative impact of rust establishment is an indication of the resilience of the agricultural sector to withstand unanticipated shocks" (Livingston *et al*., 2004). This resiliency can be attributed to the availability of substitute crops in areas where SBR is expected to be most severe, alternatives to soybean and its derivative products for consumption as soybean prices rise, and inputs and technology available to limit economic losses.

The new ERS analysis has implications for public policies and programs such as crop insurance and disaster assistance. Producers and consumers could benefit by as much as $67 million for each 1% of soybean yield loss that could be prevented (Livingston *et al*., 2004). This quantifies the benefit from funding research on the development of more tolerant soybean cultivars and improving the effectiveness of fungicide applications.

e. Environmental Impact Risk = High

The availability of alternate crops and wide host range for *P. pachyrhizi* could result in extensive direct and indirect environmental impacts. The negative impact of this pathogen may be partially offset if rust infection were to reduce the competitive advantage of some invasive weeds; however, South American kudzu (*Pueraria phaseoloides*) displays no apparent loss of vigor when severely infected with *P. pachyrhizi* (Dr. M. Miles, personal communication).

Collateral host crops (green bean, kidney bean, lima bean and cowpea) would also experience losses. If *P. pachyrhizi* spores were able to reach Michigan and Wisconsin early enough in the growing season, green beans and lima beans production would be negatively impacted.

Potential changes in acreage, regional cropping patterns and input use associated with SBR outbreaks in the U.S. have been examined recently by the USDA-ERS (Livingston *et al.*, 2004). Discharge of fungicides into the environment is expected to increase by 0.1 – 0.2 lb (measured in active ingredient) per treated acre of soybean. As producers shift to alternative crops, such as corn and cotton, there will be an overall increase in pesticide use per cultivated acre in the U.S. Further, regions cultivating fewer soybean acres are expected to experience more leaching and runoff of nitrogen and phosphorus.

f. Persistence ***Risk = High (Southern U.S.)***

Risk = Low (Corn Belt)

A virulent strain introduced into the Southern U.S. has a high likelihood of establishment and persistence. In contrast, an intentional introduction of *P. pachyrhizi* into the Corn Belt may result in only a single year episode of the disease unless an overwintering mechanism is established. It may be possible that cyclic summer prevailing winds in the eastern Corn Belt (Appendix 3A and 3B) could move urediniospores southward to areas that *P. pachyrhizi* could overwinter on collateral hosts.

The U.S. geo-climatic situation is most similar to Argentina or China, according to a climate-matching simulation model CLIMEX (Pivonia, 2003). Overwintering ability is predicted in southern Texas, central-southern Florida, and perhaps southern Louisiana, as well as many regions of Mexico and the Caribbean (Appendix 4F). The occurrence of rust epidemics in the U.S. soybean belt will be dependent on the south to north dispersal of urediniospores from regions of persistence.

2. Over-all risk rating for establishment of *P. pachyrhizi*

Area in Question	Climate	Host Range	Dispersal	Economics	Environmental Impact	Persistence
Southern U.S.	**High**	**High**	**High**	**High**	**High**	**High**
Corn Belt	**High**	**High**	**High**	**High**	**High**	**Low**

VI. Likelihood of successful introduction

1. Quantity of inoculum required to introduce and establish damage

A terrorist-type event with the long-term goal of soybean yield reduction would require very small quantities (0.2 g) of viable urediniospores. Two grams of spores, about the quantity produced on 30-40 plants, could spot inoculate 10 fields to create 10 widely dispersed initial loci of infection (Dr. D. Huber, personal communication). Secondary spread with favorable environmental conditions early in the season could cover extensive areas. This quantity of inoculum can easily be collected from a small number of infected soybean leaves. In order to produce a widespread, inundating epidemic the initial year of introduction, larger quantities (in the order of 1 ton) of urediniospores would be needed (Dr. D. Huber, personal communication).

2. Likelihood of surviving initial introduction

There is a high likelihood that urediniospores would survive an initial introduction during the growing season into any U.S. soybean production area because of favorable climate and collateral hosts.

3. Likelihood of dissemination beyond the point of introduction

There is a high likelihood that urediniospores would be disseminated beyond the point of introduction. Short-range secondary dissemination could occur from rain splash and wind. Long-range dissemination, many hundreds of miles, is expected to occur via storms or prevailing wind currents. Although it has been suggested that the progressive extension for SBR in the U.S. will occur over a three to five year period (Smith, 2003), the rapid dissemination of SBR in Brazil implies that all soybean production areas in the U.S. may be affected in as little as one to two years after an introduction into any U.S. soybean production area.

4. Likelihood of alternate host infection

Unlike most rust fungi, *P. pachyrhizi* has numerous hosts present in the U.S. and is likely to infect those hosts. Of particular concern is the prolific weed kudzu, which remains green year-round in the southern tip of Florida, starts growing in late February in Central Florida, early April in the Gulf states and early May in Tennessee (Yang, 2003a). Thus *P. pachyrhizi* could overwinter in southern Florida or Texas and serve as a continuous source of inoculum to reinfect southern, as well as northern soybean growing areas annually.

5. Likelihood of early detection

There is a moderate likelihood of early detection especially with sentinel plots planted for the early detection of SBR using susceptible (early maturing) soybean (USDA, 2002a). A SBR surveillance program was set up in Florida early in 2003 by the Florida Department of Agriculture in collaboration with Dr. Yang of Iowa State University. The program uses 25 plots of soybean and kudzu across four Florida zones. While this is a refined program and other efforts are underway for early season surveillance in the southern U.S., little is being done to use trap or sentinel crops as an early warning system in most of the U.S because natural introduction is most likely from the south.

Since SBR is an exotic pathogen, most agricultural workers and plant pathologists are not familiar with its identification in the field. Because early symptoms are extremely difficult for the untrained eye to detect, wide spread distribution and losses are probable by the time symptoms are detected (Smith, 2003). SBR symptoms may be confused with bacterial

pustule caused by *Xanthomonas axonopodis* pv *glycines*. The most likely first responders will be county agents, field agronomists (scouts), elevator operators, or other practitioners not trained as professional plant disease diagnosticians. Fortunately, in this regard, the USDA and University cooperative extension departments throughout the country are alerting growers to the potential of a natural introduction of SBR. Many recent articles in agricultural newsletters, web-based communications, and teaching modules discuss symptom recognition and provide photographs of SBR on hosts (Sprangler, 2003; Sweets, 2002; USDA, 2003; Yang and Brueland, 2003).

To assist in rapid detection, an APHIS sponsored "Train the Trainers" session on the identification and biology of *P. pachyrhizi* was held in April 2003. Participants from NPDN hubs and related diagnostic clinics observed *P. pachyrhizi* and real-time PCR detection techniques at Ft. Detrick, Maryland. This preparation should allow trainers to provide continuing education to first responders through short courses and workshops at land grant universities.

Molecular identification of *P. pachyrhizi* from spores or soybean leaves can be made in 5 hours using real-time PCR. This PCR method is currently being validated for accuracy by USDA-APHIS. Once validated, PCR assays will be passed on to the 5 regional NPDN centers. Because SBR is on the USDA-APHIS "Select Pathogen List", the organism and it's DNA (required for PCR analysis) are restricted to BL3 containment facilities and select state university diagnostic labs that have been granted waiver permits. A number of universities in each of the five NPDN regions currently hold permits (there are 3 in the North-central region: Purdue, University of Illinois and Michigan State). Two PCR training sessions for diagnosticians from universities holding permits were held in February 2004. Rapid diagnosis and response would be enhanced if restrictions on the possession of *P. pachyrhizi* DNA were removed so more labs could participate in SBR diagnosis.

In two recent pilot tests, NPDN demonstrated a response time of 36 hours from initial SBR (virtual sample) submission to final sample determination (Palm, 2003). In this process, samples were moved from a state diagnostic clinic, referred to the NPDN hub, and sent for final microscopic and molecular identification to the APHIS National Mycologist in Beltsville, Maryland. Similar test runs will continue throughout 2004 until all states have participated.

APHIS is initiating “Detection Assessment Teams” in order to quickly respond to the discovery of new infestations of select agents, including SBR, and to determine the threat of each occurrence (Spaide, 2003). A team of USDA and university experts is scheduled to arrive at the site of infection within 24 hours following APHIS confirmation of pathogen identification.

6. Overall risk = High

The evidence presented suggests that most of the soybean acres in the U.S. could be compromised by soybean rust, with corresponding economic repercussions. With the current level of knowledge on SBR, there is no way of knowing with any degree of scientific certainty, what the risk of the disease will be at specific locations in advance of an epidemic (Minnesota and South Dakota Departments of Agriculture, 2003). The site and timing of the initial introduction will play a role in the epidemiological dynamics of pathogen spread and determine the ability of SBR to persist. An SBR introduction in a southern soybean producing area presents a high risk of *P. pachyrhizi* survival on collateral hosts, while SBR introduced in the north may not be able to persist beyond the year of introduction, particularly if made late in the growing season.

7. Risk of an agroterrorist trying to use *P. pachyrhizi* as a biological weapon = High.

The small quantity of urediniospores required to initiate infection, rapid secondary dispersal, presence of alternate hosts, and potential for rapid and significant economic impact make SBR a weapon of choice (primary candidate) for agroterrorism.

VII. Control/Mitigation strategies after establishment

Because natural introductions of SBR into the U.S. will likely occur in the not too distant future, response options are already being planned. Thus, the U.S. is more prepared to deal with an intentional introduction of SBR than many other exotic pathogens. Current management practices that would minimize the long-term effects of SBR in the U.S. focus

primarily on the development of commercial resistant soybean varieties, registration of effective fungicides, and use of predictive models (USDA, 2002a).

The USDA-ARS, in collaboration with state universities and support from the United Soybean Board, have embarked on a large scale project focused on identifying resistant germplasm, evaluating fungicide efficacy, and sequencing the genomes of *P. pachyrhizi* and *P. meibomiae*. State and regional response efforts should employ preemptive as well as post-introduction strategies. The SBR scenario in Brazil provides important insights to develop effective control strategies in the continental U.S.

1. Cultural Control

Crop rotation, tillage and early planting of soybean are ineffective strategies for SBR control (Minnesota and South Dakota Departments of Agriculture, 2003). Although early planting has been reported to help soybean escape the most serious disease buildup, this would not be practical in the northern U.S. where planting dates are strictly limited by weather factors. In Brazil, growers are favoring early-maturing cultivars in an attempt to avoid SBR (Todd, 2004); however, this strategy tends to speed up the progress of SBR epidemics (Levy, 2003b). Growers should consider removing non-cultivated SBR hosts from field borders prior to the establishment of this disease in order to reduce available host material and sites for inoculum buildup (USDA, 2002a). Since weed hosts are numerous and widespread (like kudzu), and the pathogen is able to disperse long distances, only limited cultural control is likely in this manner, but it could serve as a delaying tactic until more effective options are available. Other cultural practices reported to reduce disease severity include optimum phosphorus fertility levels for soybean and delaying irrigation until mid-day or at night (Caldwell and Laing, 2002).

2. Resistance

Any long-term strategy for minimizing the effect of SBR in the U.S. must include the development of resistant varieties. No “immune” germplasm has been identified but moderate levels of resistance or tolerance to *P. pachyrhizi* exist. Resistant germplasm is rare. In India, only 6 resistant cultivars were found from 7,300 screened between 1971 and 1974. Research by the Asian Vegetable Research and Development Center in Taiwan

(AVRDC) revealed 20 resistant varieties from 9,000 candidates. A recent survey of resistant soybean varieties originating in various countries is reported in Appendix 8.

The four major dominant genes for resistance to *P. pachyrhizi* have been identified as *Rpp1* (McLean and Byth, 1976), *Rpp2, Rpp3* (Bromfield and Hartwig, 1980), and *Rpp4* (Hartwig, 1986) although other lines are suspected to contain additional genes for resistance (Sinclair and Hartman, 1999). Most single gene resistance has been quickly overcome after the establishment of rust in an area. Komata (*Rpp1*), identified in 1961-1963, was no longer considered a useful source of resistance by the mid 1970's (Kochman, 1977); resistance in accession PI230970 (*Rpp2*) broke down in 5-6 years, and resistance in Ankur (*Rpp3*), identified in the early 1970's, was overcome in the early 1980's (Bromfield, 1984). Only Bing Nang, the source of *Rpp4*, remains resistant in the field, but some *P. pachyrhizi* isolates are virulent on it in greenhouse trials (Frederick *et al.*, 2003, unpublished). Other single genes for resistance likely exist (Hartman, 2004). Soybean lines demonstrated resistant at one location often prove susceptible at another location (Hartman, 2004). Even in South America where SBR has only recently invaded, *P. pachyrhizi* populations exist as mixed races limiting the efficacy of deploying a single resistance gene (Minnesota and South Dakota Departments of Agriculture, 2003).

Partial or "rate reducing" resistance, where the rate of rust progression is slow, exists in soybean (Wang and Hartman, 1992) but has not been fully exploited because evaluation methods are time consuming and difficult to incorporate into breeding programs. Because of these difficulties, a strategy to select genotypes with "tolerance" or "yield stability", *i.e.* the ability to maintain yield potential in the presence of disease, was developed for SBR. Rust severity was not correlated with yield loss in tolerant materials. Using fungicide protected plots as controls, tolerant lines from breeding populations have been identified as early as the F5 generation without having to take detailed notes on rust severity (Hartman, 1995). Tolerant cultivars have been used successfully in Taiwan and Indonesia (Hartman, 2004).

No commercial U.S. soybean cultivars were found to be resistant in early studies (Bromfield, 1980; Green, 1984). Three lines developed at the University of Illinois by Dr. R. L. Bernard in the 1980s were said to be resistant but these are no longer in commercial use (Yang and Brueland, 2003). The USDA-ARS, in collaboration with state universities and United Soybean Board support, are in the process of identifying resistant germplasm. Core sets of soybean varieties currently grown in the southern and northern areas of the U.S., as well as

exotic germplasm, are being evaluated for resistance at different locations worldwide where the disease already exists (Wynstra, 2002). Of 174 pedigrees of international commercial varieties and those deemed previously to be resistant, none proved resistant at all 6 test locations (Brazil, Paraguay, South Africa, Zimbabwe, China and Thailand) (Hartman, 2004). At the USDA's Foreign Disease and Weed Science Research Unit a mixture of *P. pachyrhizi* isolates from Africa, Asia and South America are being employed for resistance screening on seedlings to evaluate germplasm. None of the 940 commercial cultivars tested were resistant to mixed inoculum and were classified as moderately susceptible, susceptible or super susceptible (Hartman, 2004). Over 12,000 out of 16,724 accessions of commercial and public germplasm have been screened, with less than 200 candidates identified with some level of resistance (Dr. M. Miles, personal communication). For the top 40 performers, adult plant (quantitative) resistance will be evaluated in six countries in 2004. Candidates will also be evaluated for tolerance.

Novel genes for resistance also occur in wild perennial *Glycine* species (Hartman *et al.,* 1992) and efforts are being made to transfer this resistance to *Glycine max* (Sinclair and Hartman, 1999). Over 1,000 *G. soja* accessions will be screened in the USDA-ARS project, along with perennial *Glycine* spp. previously reported resistant. Once sources of resistance are identified, crosses will be made to incorporate resistance into adapted backgrounds (Miles *et al.,* 2003a).

The development of adapted cultivars with effective rust resistance and acceptable agronomic characteristics for commercial soybean production are still 5 to 7 years away (USDA, 2002a).

3. Chemical Control

Fungicides have been effective in controlling SBR in Asia (Sinclair, 1977), Zimbabwe, South Africa, Brazil (USDA, 2000) and Paraguay. Fungicide applications can prevent significant soybean losses, with estimates of as little as 1-to 3-bu/acre-yield loss when two or more fungicide applications are used (Dr. D. Huber's communications with farmers in Paraguay and Brazil, 2003).

Dithane® M45 (zineb) was one of the earliest fungicides reported to control SBR (Sinclair, 1977), but requires frequent applications. A number of other fungicides are effective against

P. pachyrhizi (Appendix 5; Minnesota and South Dakota Departments of Agriculture, 2003). Two to four foliar fungicide applications are generally required and protect plants for 7 to 20 days, depending on the chemical. By the time symptoms of SBR are detected, losses have often already been sustained. The key to successful control is careful disease monitoring, early application of fungicides, and full coverage of the lower canopy. Fungicides must be applied at or before 5% infestation of the crop for greatest efficacy (Syngenta, 2003).

Fungicide recommendations for SBR from field trials conducted by Dr. Clive Levy in Zimbabwe and South Africa (NPAG, 2002; Miles *et al.*, 2003a) state first application should be made at first flowering (R1 stage) or 50 days after planting, followed by second and third applications at 20-day intervals in order to protect during pod fill. In low severity areas, only 2 applications may be needed. Shavit® (tridimenol) and Punch® (flusilazole/carbendazim) were the most effective compounds to control SBR in Africa (Appendix 5).

Recommendations to Brazilian farmers in 2003 were to apply a preventative spray at R3 (early pod formation) and R5 (pod filing) to combat the late-season disease complex common there (Syngenta, 2003). An increase in second soybean plantings with irrigation last year in Brazil produced ample inoculum for the early appearance of SBR in the 2003-2004 crop. Fungicides are applied at first detection, as early as growth stage V3, followed by subsequent applications at 10-25 day intervals, depending on the fungicide selected. Under conditions favorable for SBR development, the presence of the pathogen in the vicinity is adequate incentive to commence prophylactic sprays (Smith, 2003). If a first application is required before flowering (F1), a total of four applications will likely be needed (Dr. M. Miles, personal communication) which will significantly raise production costs. Indeterminate soybean are the most difficult to protect because of their continuous new growth.

It is imperative that effective chemicals be available and accessible to growers prior to SBR establishment. Three fungicides have been registered for foliar application on soybean in the U.S. These are azoxystrobin (Quadris®), chlorothalonil (Bravo Weather Stik® and Echo®) and thiabendazole (Benlate and Topsin® M). Efficacy data on all three classes of fungicides is limited. Research data indicates thiabendazole is ineffective against *Phakopsora* rusts (Hartman *et al.*, 1992; Minnesota and South Dakota Departments of Agriculture, 2003). Chlorothalonil, which acts only as a protectant, has been reported

moderately effective (Appendix 5), but offers the advantage of a multi-site mode of action and therefore low resistance development potential.

Limited trials on Quadris® (azoxystrobin) demonstrate its effectiveness against SBR, but like all strobilurin fungicides, manifests a single point of activity (QoI mode of action) which could lead to the rapid development of fungal resistance (Dr. M. Miles, personal communication). Although no cases of resistance have been reported in *Puccinia recondita* (wheat leaf rust), a close relative to *Phakopsora*, resistance to this mode of action has shown up in many other pathogens including: *Alternaria solani*, *Venturia inequalis*, *Plasmopara viticola*, *Septoria tritici*, and *Erysiphe tritici* f. sp. *tritici* (Minnesota and South Dakota Departments of Agriculture, 2003). Azoxystrobin is more costly than other fungicides, even at its lowest recommended rate, and there is concern that expense may encourage growers to apply reduced application rates leading to increased selection pressure for resistant strains. Fungicide Resistance Action Committee (FRAC) guidelines recommend a 1:>1 rotation of QoI chemistry with fungicides having other modes of action; thus, only one application could be used out of the 2-3 that may be needed per season (Minnesota and South Dakota Departments of Agriculture, 2003). To minimize resistance development it is important to have a number of effective fungicides available with differing modes of action for SBR control (USDA, 2002a).

Inadequate availability of fungicides is also a concern. Brazil's supply of effective fungicides was depleted during their 2002 outbreak of soybean rust (Dr. G. Hartman, personal communication). The U.S. supply of Quadris® early in 2003 would cover only 2 million acres of soybean. Estimates of currently registered products plus products requested by the Emergency Exemption for SBR would treat approximately 5 million acres, which is totally inadequate to protect the 75 million U.S. acres of soybean (Paul, 2003), particularly if two or more applications were required.

Recognition that SBR poses an imminent threat to the U.S. soybean production has resulted in preparations to assure adequate, effective fungicides be available with two or more modes of action to reduce the probability of SBR resistance development. USDA and individual registrants have been conducting comparative efficacy trials to provide a basis for section 3 and section 18 registrations. In 2002-2003, 19 compounds were evaluated for efficacy and yield benefits in Paraguay in cooperation with the Centro Regional Investigación Agricola (Miles *et al.*, 2003b). Three fungicide applications were generally

more effective than two. Disease severity was reduced most by BASF 500 (Headline®, pyraclostrobin), Stratego® (trifloxystrobin + propiconazole), Echo® (chlorothalonil), Eminent® (tetraconozole), and Quadris® (azoxystrobin), but only Folicur® (tebuconozole) and BAS 516 (pyraclostrobin + boscalid) provided significant yield protection. Results on the relative effectiveness of fungicides in preventing yield loss, however, were inconclusive due to low disease severity caused by a hot, dry season. The authors recommend future trials employ irrigation and inoculation to increase disease severity. Phytotoxicity of fungicides to soybean flowers can reduce yields and requires further investigation (Dr. M. Miles, personal communication).

Chemical companies that currently have fungicides active against SBR and already registered for use on other crops in the U.S., are requesting label changes through the EPA. The South Dakota and Minnesota Departments of Agriculture, in cooperation with the USDA Office of Pest Management Policy, the Soybean Rust Technical Science Working Group, and registrants submitted a Section 18 Quarantine Exemption Request to the EPA in November, 2003 (Minnesota and South Dakota Departments of Agriculture, 2003). Fungicides requested have triazole or stobilurin chemistry and include: propiconazole (Tilt®, PropiMax™ EC, Bumper®), tebuconazole (Folicur®), myclobutanil (Laredo ™ EC), propiconazole + trifloxystrobin (Stratego®), tetraconazole (Eminent®), pyraclostrobin (Headline®), and pyraclostrobin + boscalid (Pristine®). A compilation of detailed efficacy data for these fungicides can be found in the SBR Section 18 Quarantine Exemption Application at http://plantsci.sdstate.edu/draperm/soybeanrustsection18/ As of April 23, 2004, the EPA had granted this quarantine exemption for myclobutanil and propiconazole. Quarantine exemptions for other requested section 18 chemicals are expected to follow.

Triazole fungicides can be used as curative systemic eradicants in the early phases of disease development or as protectants, and have antisporulant activity. Triazoles have been most consistent in providing curative control in Brazil with a minimal risk of fungicide resistance and low cost. The triazole metabolite, 1,2,4-triazole, is currently under review by the EPA due to developmental toxicity concerns. This has resulted in a moratorium on all new uses for triazoles. Risk assessments by chemical companies are currently in progress. An industry Triazole Task Force has submitted worst-case studies to the EPA demonstrating a reasonable certainty of no harm for 1,2,4-triazole stemming from triazole-derivative fungicides for food and water. The most recent toxicity data available will be used to try to get the Section 18 request approved.

Stobilurin fungicides (azoxystrobin, trifloxystrobin, pyraclostrobin) are strong penetrants that can be used as a systemic and protectant with curative activity but are recommended for use only as protectants due to concerns over resistance development. Suggested product use regimens are described in Appendix 6. Once Section 18 use is granted to the requesting states, other states will be able to submit piggyback Quarantine Exemption Requests, which carry a 3-year approval. When Quilt, a new combination product of Quadris®+Tilt® (azoxystrobin+propiconazole) is registered, it may become another option for rust control.

Many questions remain concerning fungicide application. Since infection moves upward from the lower leaves, full coverage of the lower canopy from flowering through pod fill is essential to achieve effective control. At this growth stage, the dense canopy acts as a barrier to chemical penetration. Ground application of fungicides was more efficacious and inexpensive than air application in Africa (Levy, 2003a). Aerial applications in Brazil in 2003 proved disappointing, presumably because fungicides did not effectively penetrate the canopy (Topp, 2004). Application methods need to be developed that will uniformly deliver ample fungicide into the lower portion of the soybean canopy (Miles *et al.*, 2003a). A multi-state project is in progress, funded by the Critical Issues Program of the USDA-CSREES, to test ground and aerial application methods.

Detection early in the season (<5% of crop infected) with properly timed applications of fungicides, presents the best approach for controlling an initial outbreak of SBR in the U.S. The need for chemical control of rust will reduce soybean production profit margins and reduce U.S. global competitiveness. The bottom line will be cost effectiveness of treatments against SBR. In 2002/03 in Mato Grosso, Brazil, most producers applied a minimum of 2 fungicide applications at a cost of about $50/hectare (Livingston *et al*., 2004), raising total production costs by ~15% (Reuters, 2004). At an estimated $7-35/fungicide application acre, U.S. growers producing less than 40 bu soybean/acre would have to look closely at the economic cost-benefit ratio of 2-4 applications. An economic analysis of chemical control costs (single application) and projected return in the U.S. (based on Minnesota cost of production and national yield and price figures) is presented in Appendix 7. The appendix allows a comparison of labeled chemicals, azoxystrobin and chlorothalonil, with those requested in the Section 18 exemption and shows the comparative economic advantage of using triazole products over azoxystrobin. Using myclobutanil, the most expensive of the triazoles versus azoxystrobin (the best labeled product) yielded an estimated revenue

benefit of $2.4 million/1 million soybean acres treated (Minnesota and South Dakota Departments of Agriculture, 2003). More precise estimates will be possible as more data on efficacy of products under different disease pressures and production environments is available. Total fungicide cost increases to the U.S. after SBR establishment are predicted between $246 million to $961 million annually, depending on spread severity (Livingston *et al*., 2004). Treatment costs would increase if multiple fungicide applications were required.

4. Modeling Disease Incidence, Spread and Economic Impact

Predictive models that forecast the spread and distribution of rust in the nation's soybean crop are potentially powerful disease management tools. Information generated by models could be valuable for rust surveillance, monitoring and timing of fungicide applications. Comprehensive models designed to determine the potential impact of SBR on the U.S. economy serve as useful policy making tools.

Efforts to understand SBR epidemiology and crop loss have been based on data from containment facility research and fields studies in regions that experience the disease. The potential effects of the disease on soybean yield and economic impact in the U.S. has been quantified through computer modeling. Yang *et al.* (1991a) integrated a rust disease model (SOYRUST) (Yang *et al.*, 1991b) with a soybean growth simulation model (SOYGRO) to predict disease progress and potential yield losses on a regional basis in the U.S. (Fig. 15). SOYRUST consists of four rate variables driven by dew period and temperature, and of six state variables that predict disease development in daily increments. SOYGRO then determines how disease development affects photosynthesis and plant growth. Yield losses of up to 50% were predicted for the Mississippi delta area and southeastern coastal regions where moisture levels are high but acreage is low. Significant yield losses of >10% were forecast for Corn Belt states and other major production areas.

Rising concerns over the natural arrival of SBR has led to enhance research efforts in the development of new models to predict SBR spread. Dr. Z. Pan and Dr. X. B. Yang are using climate forecasting to predict when conditions are favorable for SBR infection in the hope that favorable conditions for disease can be predicted 3 to 4 months in advance of SBR reaching the U.S. Pivonia and Yang (2004) have predicted the global year-round survival range of SBR using a model that integrates temperature-stress (CLIMEX software) with dry-stress. The analysis incorporates the maximum time permitted between two

infection events to maintain a *P. pachyrhizi* population (*i.e.* 70-90 days) and environmental conditions required for urediniospore production, survival, and germination. The resulting "stress-free index" indicates a moderate to high chance of survival in the southern tip of Texas and central-southern Florida, as well as regions of Mexico and the Caribbean (Appendix 4F). The model predicts the U.S. geo-climatic situation is most similar to Argentina or China. In China SBR survives year-round only below 25N, and is annually reintroduced into a broader disease distribution zone. Furthering understanding of the factors responsible for *P. pachyrhizi* urediniospore dispersal patterns in China and Japan will help predict the risk and timing for long distance transport of urediniospores in the U.S. (Pivonia and Yang, 2004).

Aerobiological models to predict SBR spore movement pathways in the western hemisphere are also being developed (Dr. S. Isard, 2004). The basic conceptual model includes: spore production (preconditioning) → escape from canopy (takeoff and ascent) → turbulent transport and dilution (horizontal transport) → survival while airborne → deposition (decent and landing) → infection (impact). The first 3 components in the model have been run to predict spore movement originating in South America for periods from 1979 to 2001 (http://aries.zedxinc.com/sbrust.php). NOAA HYSPLIT trajectory models are being used to validate the models used by Dr. Isard's group. The next phase is to incorporate spore survival and spore deposition into the model. Such a model may serve as an early warning device for the natural introduction of *P. pachyrhizi* into the U.S. Accurately predicting the survivability of urediniospores will require data on the effects of UV light, moisture and heat on spore longevity. It is hoped this model can eventually be used as a forecasting system on a real time basis to help growers make SBR control decisions (Dr. S. Isard, 2004). Combining evaluations for urediniospore arrival with soybean rust risk models (Yang *et al.*, 1991b) will allow improved risk assessments for different regions of the U.S. soybean production area (Pivonia and Yang, 2004).

Econometric-simulation models were developed in 1984 by the ERS and ARS to analyze the potential impact of SBR on the U.S. (Kuchler and Duffy, 1984; Kuchler *et al.*, 1984). The model took into account scenarios of regional yield loss, producer responses, and changes in market forces. The predicted net negative impact to the U.S. economy ranges from $47 million to $4.5 billion annually.

The most recent systemic analysis of the economic implications of wind-borne entry of SBR on the U.S. was released in April, 2004 by the USDA-ERS (Livingston *et al*., 2004). Biological models based on updated information were used to determine varying regional susceptibilities to rust establishment (climate suitability index) and estimate transport probabilities from South America. Modern SBR control costs, price elasticity, shifts in land use to other crops, impacts on the livestock sector, trade flow, and consumer effects are considered. Changes in the structure of the domestic and international soybean industry since the 1984 studies are accounted for. Potential impacts of SBR establishment and subsequent outbreaks were simulated using a spatial equilibrium, mathematical programming model (USMP) of the U.S. agricultural sector. The analysis estimates the first year net economic losses due to wind-borne SBR between $640 million to $1.34 billion depending on the severity of yield impact and assumes advance warning and fungicide availability (conditions unlikely to be met at present). The potential impact 3 years post entry with permanent establishment of SBR, predicts losses from $240 million to $2.0 billion annually depending on severity of spread (Table 3).

Although all models have limitations because of incomplete data, unforeseen events, and uncertainties in risk assessment, there is adequate information generated to date that establishes a scientific need for allocating essential resources to minimize the economic impact on soybean production in the U.S. Producers and consumers could benefit by as much as $67 million for each 1% of soybean yield loss that could be prevented (Livingston *et al.*, 2004). This need becomes more critical with the potential use of SBR as a bioterrorist agent.

VIII. Knowledge gaps

Important gaps in our present knowledge include:

1. The extent to which *P. pachyrhizi* will spread throughout the U.S. Current information indicates spread will be rapid and extensive, however, experience with other diseases (*i.e.* Pierce's disease) indicates there may be unique characteristics that may limit or enhance this disease.

2. The anticipated rate of spread of SBR.

Are there unique characteristics indicated in Africa and South America that impact dissemination and establishment?

3. The northern most boundary of overwintering.
   Although current information indicates urediniospores will likely survive year-round in the U.S. only in southern Texas, central-southern Florida and perhaps the gulf coast of Mississippi and Louisiana, can the pathogen adapt to climates further north, or persist there, in perennial weed hosts? More detailed information on the effects of short periods of freezing on *P. pachyrhizi* urediniospores is required before overwintering sites can be accurately predicted.

4. Are there currently unknown opportunities for *P. pachyrhizi* survival in the Corn Belt? Little is known about the potential survival in northern perennial hosts. The role and production of teliospores in rust epidemiology is still not fully understood.

5. Will urediniospores on overwintering hosts in the south move northward early enough to impact soybean production in the Corn Belt? Available information from Brazil and Africa, and prevailing wind and precipitation patterns in the U.S. (Appendix 3A and 3B) indicate a probable rapid and extensive spread; however, the extent of annual reinfestation from overwintering sites remains speculative. A clearer understanding of the factors responsible for *P. pachyrhizi* urediniospore dispersal patterns in China and Japan will help predict the risk and timing for long distance transport of urediniospores in the U.S. Further, it is unknown if the inoculum potential of urediniospores from kudzu and other alternate hosts is similar to that from the soybean host.

6. How long can *P. pachyrhizi* urediniospores remain viable with respect to environmental variables such as ultraviolet radiation, temperature, moisture, etc.? Viability studies can shed further light on long-range transport potential and survivability as seed contaminants.

7. The potential role of Hawaii in SBR movement to the U.S. mainland is still not fully understood. Extensive research on rust has been proposed for Hawaii since SBR has been present there since 1994. Does this increased activity pose a greater threat for introduction to the mainland through movement of seed and personnel?

IX. Immediate response options

Containment and eradication of a generalized introduction of SBR, the traditional response to an introduction of a serious exotic disease of a major crop, will not be technically possible (USDA, 2002a). Even an intentional introduction at a single or a few sites would likely not be detected early enough to allow for eradication of the disease (Dr. M. Miles and Dr. G. Hartman, personal communication). By the time rust symptoms are noticed in the field, millions of urediniospores would have traveled considerable distances to create secondary infection sites. Fortunately, response plans are already being formulated for SBR because of the potential for its natural introduction. A primary purpose of the Technical Science Working Group on Soybean Rust (http://www.ipmcenters.org/NewsAlerts/soybeanrust/) is to better prepare state departments of agriculture and extension specialists to effectively respond in the event of an introduction. The potential impact of SBR from agroterrorism can be minimized by education to facilitate rapid detection leading to timely implementation of control measures.

A SBR pathway and response summary for the intentional introduction of *P. pachyrhizi* is presented in Appendix 10.

1. Rapid Detection

Sentinel plantings of susceptible soybean lines strategically placed in soybean producing areas will be important for early detection of SBR (USDA, 2002a). Since SBR manifests primarily on maturing plants, the sentinel plantings should mature about three weeks before the commercial crop. This early warning system should allow time to implement chemical control measures in commercial plantings from a natural (accidental) introduction. Although a SBR surveillance program has been initiated in Florida, most of the U.S. lacks an early warning system. A broader distribution of sentinel plantings in soybean producing areas is essential to rapid detection, particularly if introduction is initiated by agroterrorists in a central or northern soybean growing area.

The creation of the NPDN represents a major step to improve the potential for rapid detection. A key objective of this cohesive information system is to facilitate the detection of anomalies, such as simultaneous outbreaks at multiple sites, thereby identifying a

bioterrorist attack (Appendix 9B). Trial runs using virtual samples are a key preparatory step to improve system efficiency and minimize SBR detection time.

Although rapid diagnostic techniques are available to confirm SBR, current regulations requiring containment levels ≥BL3 for DNA of select pathogens limits the application of this readily available technology. Although a select group of state university diagnostic labs and NPDN centers have been granted waiver permits, many other diagnostic clinics and responders responsible for early detection are prohibited from possessing the *P. pachyrhizi* DNA required for PCR identification. These restrictions do not appear to be scientifically sound or appropriate, considering the threat posed by *P. pachyrhizi*, and should be removed as quickly as possible.

Efforts by APHIS such as "Train the Trainer", "Detection Assessment Teams" and response time tests conducted by NPDN are useful preparatory steps to minimize the time required for *P. pachyrhizi* identification and on site threat assessment. Further efforts need to focus on the education of first detectors to encourage the recognition of SBR symptoms, increase awareness of the potential for agroterrorism, and heighten farm biosecurity.

2. Fungicide Availability

The most immediate control response should be fungicide applications (see VII-3). Timely fungicide applications will be especially important if the introduction occurs in southern soybean growing areas where the pathogen might successfully overwinter on weed hosts.

It is imperative that effective chemicals be available and accessible to growers. Seven million gallons of Quadris® would be needed for a single application to all U.S. soybean acreage (Draper, 2004). In mid-2003, it was estimated that registered products plus products requested by the preliminary Emergency Exemption would treat approximately 5 million acres of soybean (Paul, 2003). This quantity is grossly inadequate to protect the 73 million acres of U.S. soybeans from SBR, particularly since more than a single application is required. Only 48 million acres of fungicide product is currently used on all food crops combined in the U.S. annually (Tally, 2004). The EPA requested a more extensive prioritized list of unregistered, but efficacious chemicals, in order that several additional fungicides can be chosen to increase the availability of fungicides for the control of SBR. In

November 2003, a Section 18 Quarantine Exemption Request was submitted to the EPA for five compounds and two combination fungicides (Minnesota and South Dakota Departments of Agriculture, 2003). To minimize resistance development and maximize availability, two classes of effective fungicides were included in that Section 18 request.

Additional fungicide classes should be tested for efficacy so that the broadest fungicide chemistry base possible is available to battle SBR. Even with additional fungicides registered, quantities will fall far short. Unfortunately, it is not in the economic interest of chemical companies to stockpile (for an indeterminate time) the massive quantities of fungicides that will be required. Since SBR in the U.S. seems eminent, it would make sense for the U.S. government to subsidize the production and storage of some of these chemicals. The fungicide availability issue needs to be further addressed.

3. Resistance Breeding

Since commercial varieties of resistant soybean will not be available for at least 5 years, the potential overall economic impact of an earlier introduction of this disease is expected to be great. Partial resistance or tolerance may be the most effective resistance strategy, especially if it can be combined with stacked single genes or genes with broad resistance (Miles *et al.,* 2003a). Breeding programs need to be fully implemented with ready sources of resistance developed beyond the germplasm evaluation stage. In order to produce commercial cultivars, SBR resistance genes need to be incorporated into herbicide resistant soybean varieties and would appear to be an obligation of “proprietary” breeding programs.

Appendix 1. Hosts of Australasian Soybean Rust

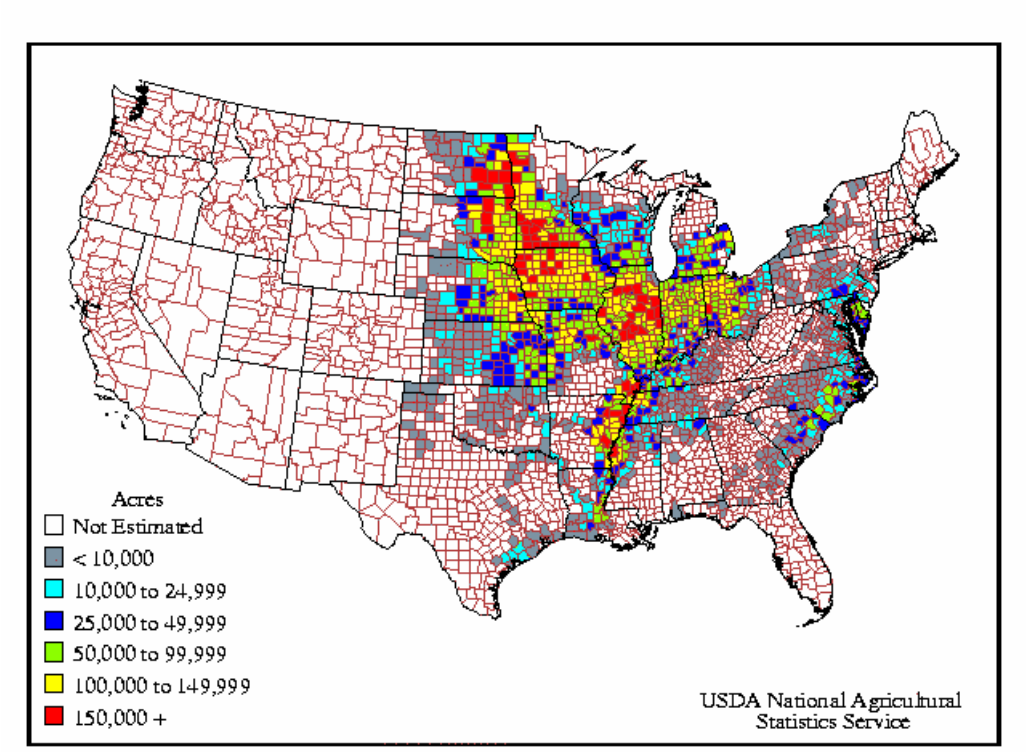
The following plants have been reported as hosts for *P. pachyrhizi*.

Scientific Name	Common Name	Distribution/ Comments
*Alysicarpus glumaceus**	Alyce clover	Naturalized in West Indies, in Florida
Alysicarpus nummularifolius		Introduced to tropical America (weed)
Alysicarpus vaginalis	Alyce clover	Tropical America; in Florida (forage)
A. rugosus	Alyce clover	West Indies (weed)
*Cajanus cajan**	Cajan; pigeon pea	Widely cultivated in tropics
*Centrosema pubescens**	Butterfly pea	Frequent in fields in W. Indies, Mexico, Brazil, Columbia, Paraguay (forage and weed)
Clitoria ternatea	Kordofan pea	Widespread in tropics (forage)
*Crotalaria anagyroides**	Rattlebox	Native to South America (forage)
Crotalaria saltiana	Rattlebox	Cosmopolitan in tropics (weed)
*Delonix regia**	Royal Poinciana	Wide-branching tree (forage)
Desmodium triflorum	3-flower beggarweed	Tropics throughout world (forage)
*Glycine canescens**	Soybean relative	
*G. clandestine**	Soybean relative	
*G. falcata**	Soybean relative	
*G. max**	Soybean	Major agricultural crop in U.S. & other countries
*G. tabacina**	Soybean relative	
*Lablab purureus**	Lablab; hyacinth bean	Used for hay and silage (forage)
*Lotus americana**		
Lupinus angustifolius	Narrow-leaved lupine	North America (weed)
*L. hirsutus**	Blue lupine	Annual in southern Europe
L. luteus	Yellow lupine	North America (weed)
*Macroptilium atropurpureum**	Siratro; purple bean	Grows wild in Central and S. America (forage)
*Medicago arborea**	Medic	Shrub; southern Europe
Medicago lupulina	Black medic	Widespread in North America (weed)
*Melilotus officinalis**	Yellow sweet clover	Eurasia, naturalized in N. America
*M. speciosus**		
*Mucuna cochinchinesis**	Velvetbean relative	
*Neonotonia (Glycine) wrightii**	Glycine	Old World (forage)
*Pachyrhizus erosus**	Yam bean; jicama	Central America; naturalized in Florida
*Phaseolus lunatus**	Butter bean, lima bean	Tropical S. America; important edible bean
*P. vulgaris**	Kidney bean; green bean	Widely cultivated in tropical America
Pueraria montana var. *lobata**	Kudzu	Southeastern U.S. (weed)
*Pueraria phaseoloides**	Tropical kudzu	Common host in tropical S. America (forage)
*Rhynchosia minima**		Creeping tropical weed
*Sesbania exaltata**	Colorado River Hemp	N.Y. to Florida, west to southern California
*S. vescaria**		
*Trigonella foenum-gracecum**	Fenugreek	Asia and southern Europe; forage
Vicia angustifolia	Narrow-leaf vetch	Throughout U.S. (weed)
V. narbonensis	Broad-leaf vetch	Sparse in U.S. (weed)
*Vigna unguiculata**	Cowpea, black-eyed pea	Widely planted in warm regions of the world

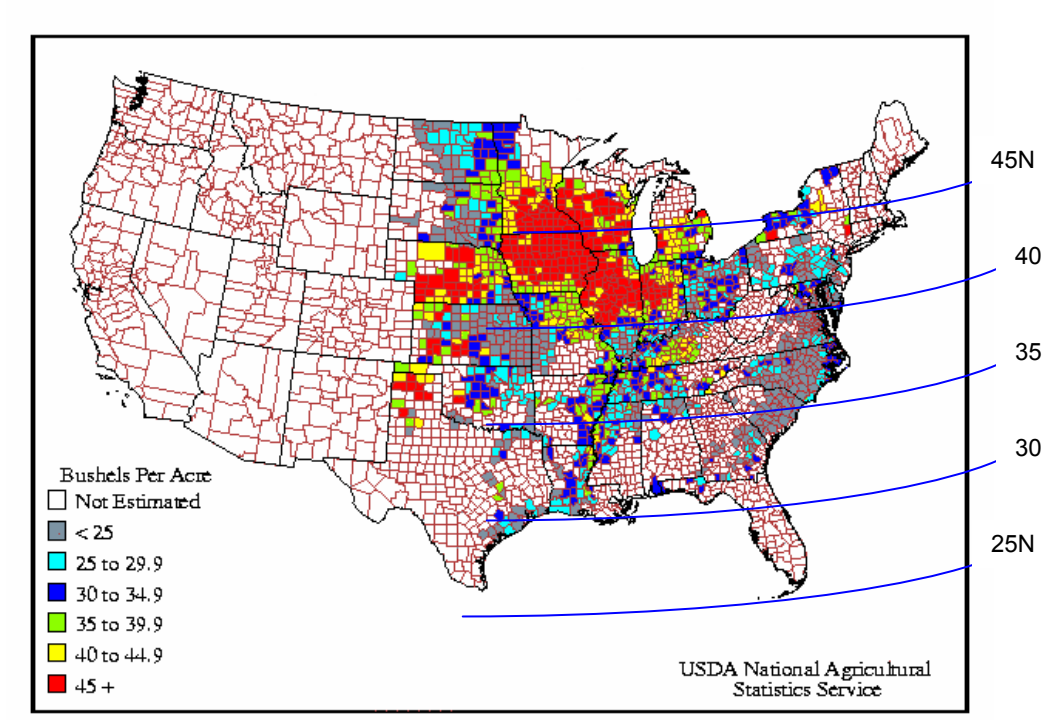
Adapted from USDA, 2002 and USDA, 2002b (compiled from Bailey & Bailey, 1976; Duke, 1981; Gohl, 1981; Hartman, per. communication; Ono *et al.*, 1992; Reed and Hughes, 1977; Tschanz, 1985, 1982; USDA-ARS, 1970).

* Species reported to develop rust symptoms, uredinia and urediniospores.

Appendix 2A. Harvested soybean acres in 2002

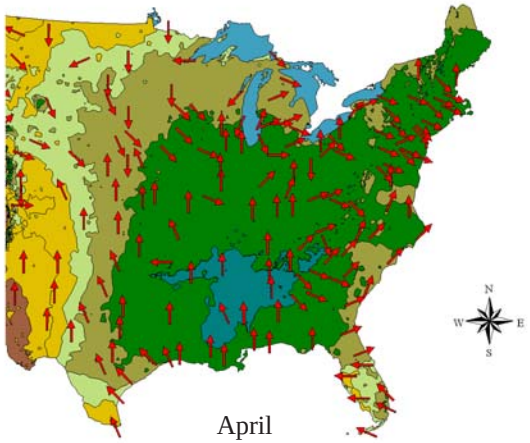


Appendix 2B. Yield/acre of soybeans in 2002



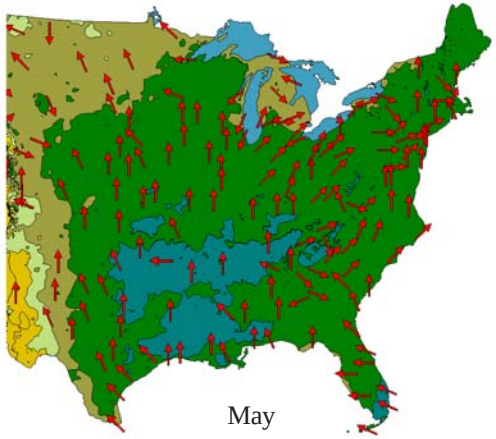
Appendix 3A. Mean Total Precipitation and Prevailing Wind Direction, April to August

a. Mean Total Precipitation and Prevailing Wind Direction for April



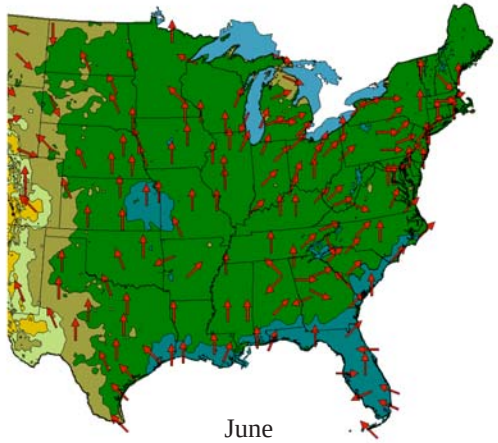
April

b. Mean Total Precipitation and Prevailing Wind Direction for May



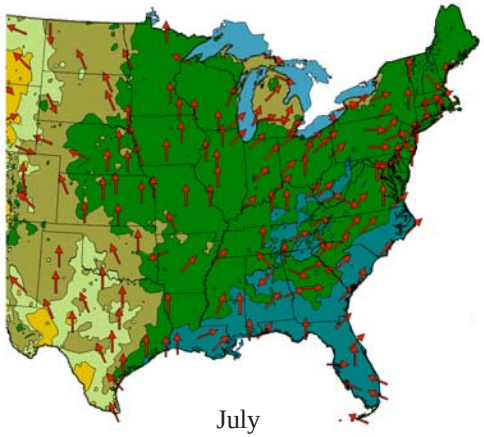
May

c. Mean Total Precipitation and Prevailing Wind Direction for June



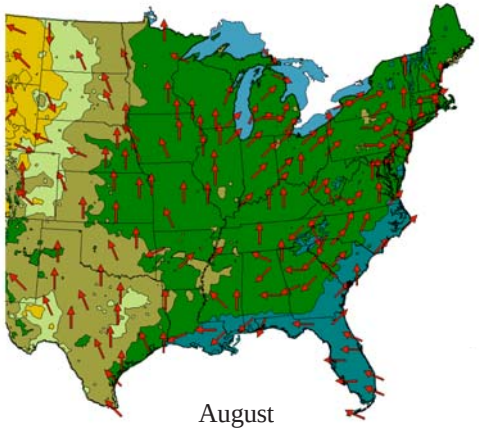
June

d. Mean Total Precipitation and Prevailing Wind Direction for July



July

e. Mean Total Precipitation and Prevailing Wind Direction for August



August

Prevailing Wind Direction

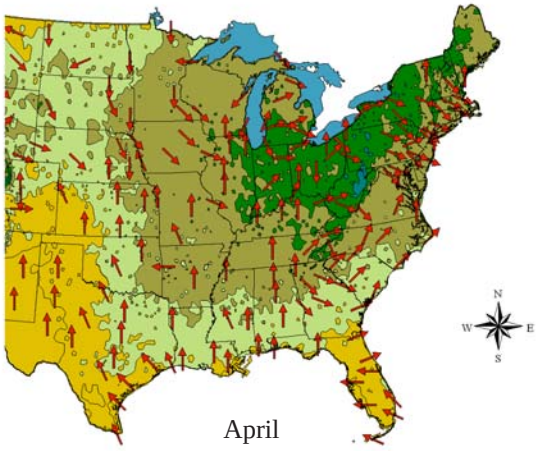
Mean Total Precipitation (inches)

- A < 0.51
- B 0.51 - 1.00
- C 1.01 - 1.50
- D 1.51 - 2.00
- E 2.01 - 3.00
- F 3.01 - 5.00
- G 5.01 - 10.00
- H 10.01 - 20.00

NOAA (2000)

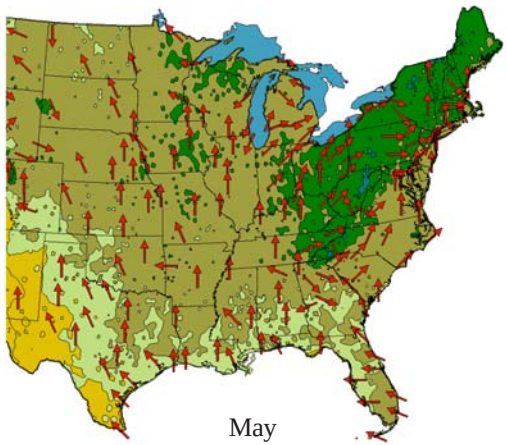
Appendix 3B. Mean Number of Days with Measurable Rainfall and Prevailing Wind Direction, April to August

a. Mean Number of Days with Measurable Rainfall and Prevailing Wind Direction for April



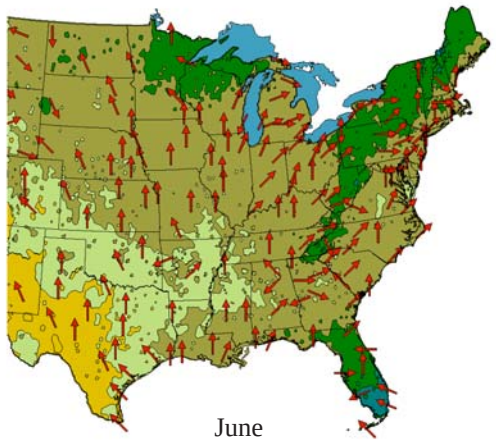
April

b. Mean Number of Days with Measurable Rainfall and Prevailing Wind Direction for May



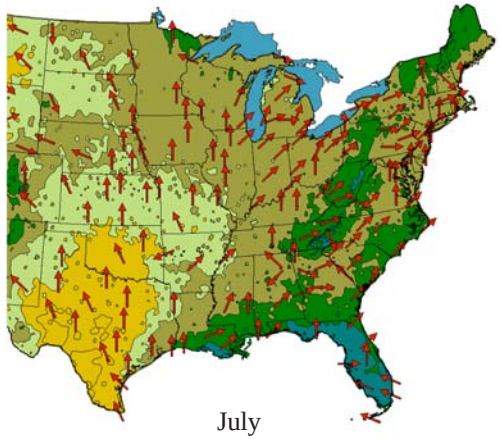
May

c. Mean Number of Days with Measurable Rainfall and Prevailing Wind Direction for June



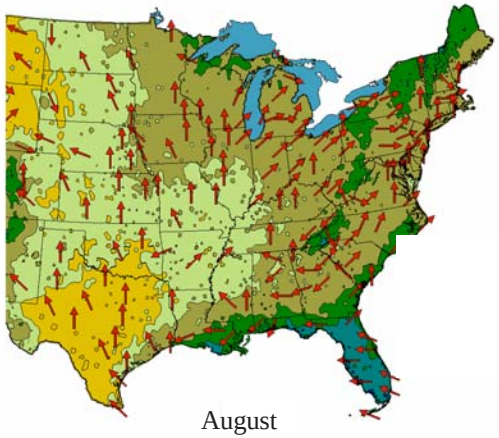
June

d. Mean Number of Days with Measurable Rainfall and Prevailing Wind Direction for July



July

e. Mean Number of Days with Measurable Rainfall and Prevailing Wind Direction for August



August

Prevailing Wind Direction

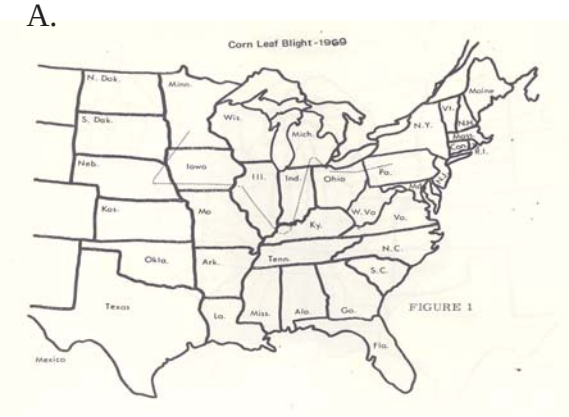
Mean Number of Days with Measurable Rainfall

- A < 0.5
- B 0.5 - 3.4
- C 3.5 - 5.4
- D 5.5 - 7.4
- E 7.5 - 10.4
- F 10.5 - 13.4
- G 13.5 - 16.4
- H 16.5 - 20.4
- I > 20.4

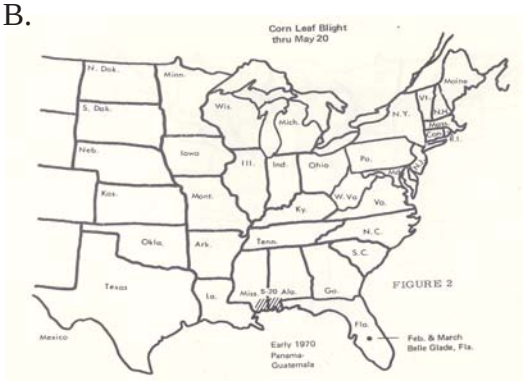
NOAA (2000)

Appendix 4A. Spread of Southern corn leaf blight during the 1970 US epidemic.

A.



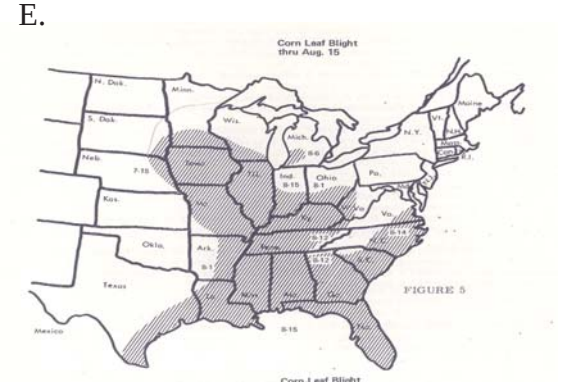
B.



C.

D.

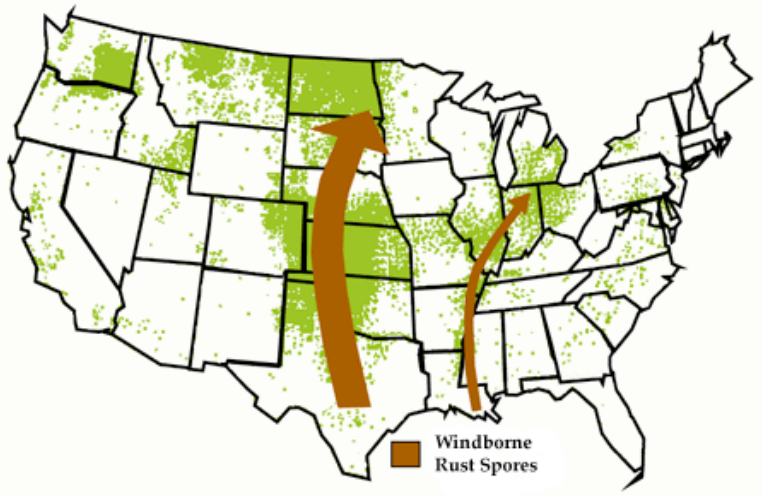
E.



F.

Shaded areas indicate infection in 1969 (A), May 20, 1970 (B), June 18, 1970 (C), July 15, 1970 (D), August 15, 1970 (E), and September 1, 1970 (F). A similar pattern of spread to the US may be expected with Australasian soybean rust. Moore, W. F. (1970).

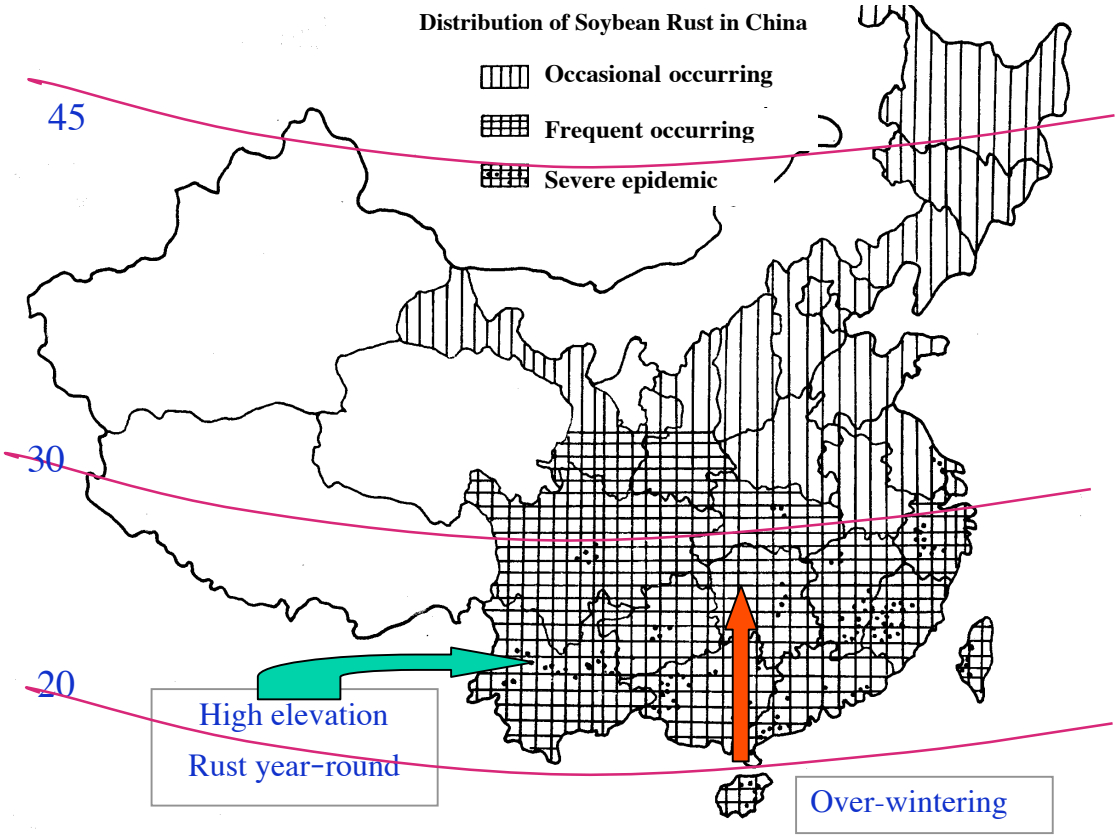
Appendix 4B. Puccinia Pathway: Wheat Production (green) and the Annual Spread of Rust Epidemics



Annual dispersal of urediniospores of the wheat stem rust (*Puccinia graminis* f. sp. *graminis*) and wheat leaf rust (*Puccinia recondita*) pathogens. The pathogens survive on hosts that remain green through the winter months.

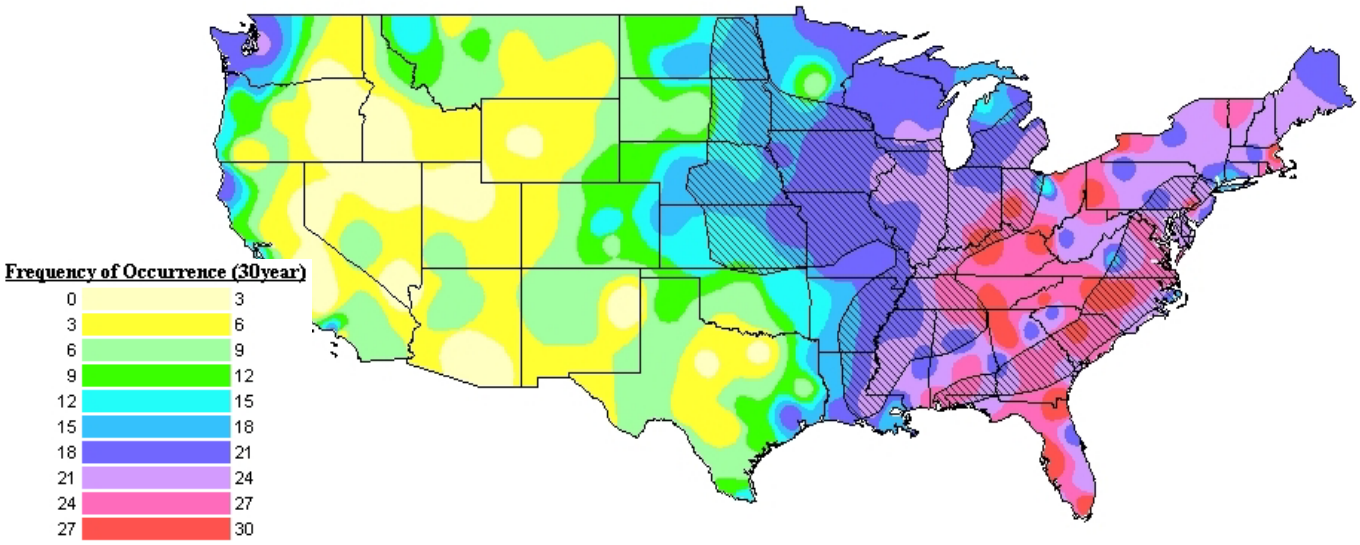
(http://www.cdl.umn.edu/introduction/pathway.html)

Appendix 4C. Occurrence of SBR in China. Overwintering areas are in the south with movement northward on winds during the growing season. Coastal area where the pathogen overwinters are 20-30N latitude, approximately the same as the Gulf Coast of N.A.



Tan *et al.* (1996) modified by Dr. X.B. Yang (2003, Section 18 Exemption Request)

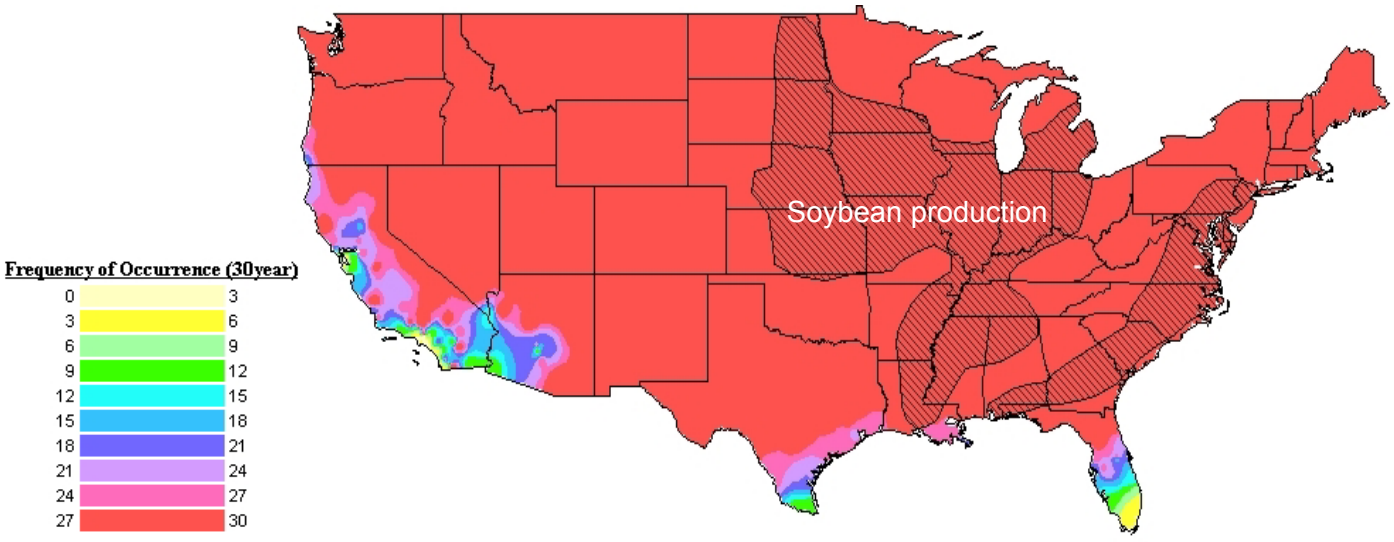
Appendix 4D. Suitability of temperature and moisture for SBR epidemics



Colors represent frequency of >15 favorable days for SBR infection per growing season over a 30 year period. The map is based on an infection model using temperature response function scaled to a wetness duration requirement.

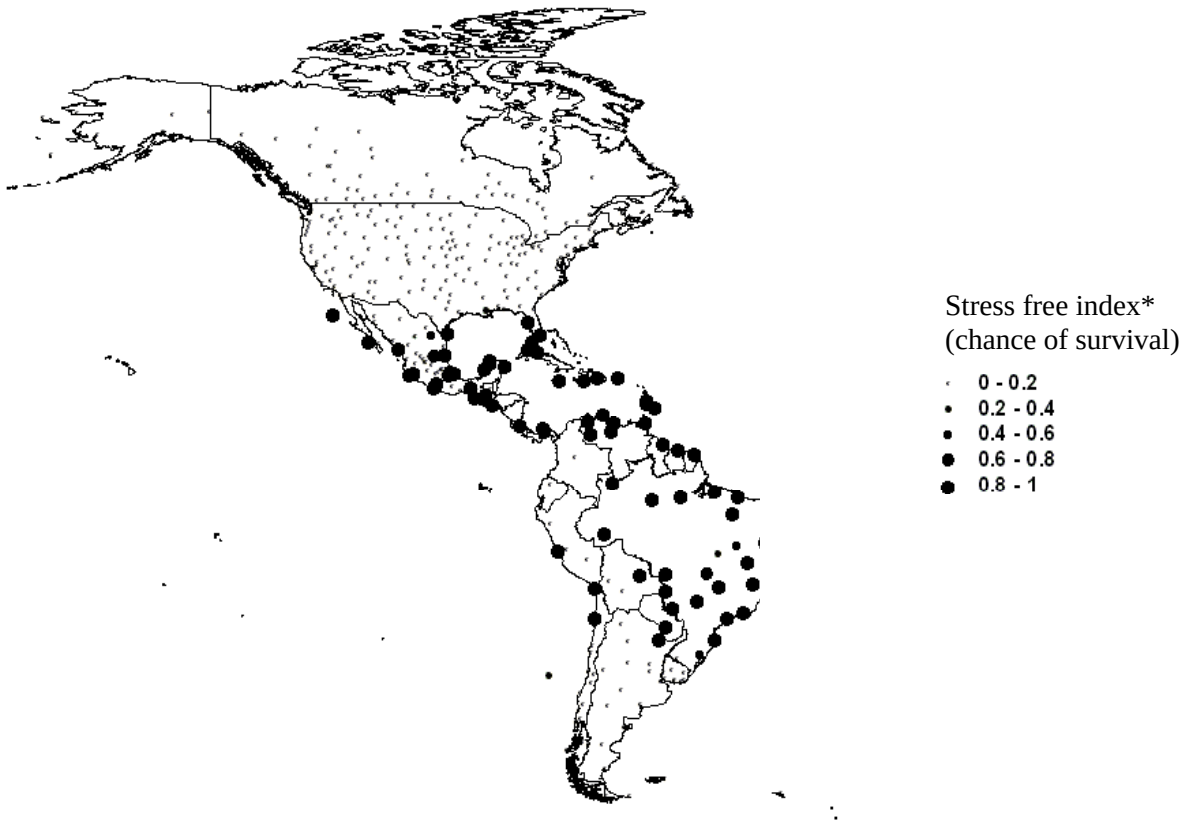
From Magarey *et al.* (2003)

Appendix 4E. Probability of temperatures ≤ 0°C in January and February



From Magarey *et al.* (2003)

Appendix 4F. Prediction of year-round SBR survival in North and South America



* Stress free index = (1- cold stress) X (1- heat stress) X (1- dry stress). Index values are between 0 and 1. Higher index values represent higher survival chances and values <0.25 represent low survival chances.

Pivonia and Yang (2004)

Appendix 5. Partial list of fungicides used to control SBR caused by Phakopsora pachyrhizi, with a summary of effectiveness and recommendations.

Active ingredient	Compound	Study Country	Summary of application trials and recommendations	References
Triadimefon[a]	Bayleton	Thailand, India, Taiwan, Japan,	Protection was inconsistent when compared to Dithane M45, although it was Philippines used as a control in yield loss studies. EBDC fungicides appear to be more effective and in limited testing increased yield up to 33%. First application at flowering, then at 10-20 day intervals.	(Hartman *et al.*, 1992; Patil & Anahosur, 1998; Vyas, *et al.* 1997)
Thiabendazole	Benlate Topsin	Thailand Zimbabwe South Africa	Off registration in the U.S., not as effective as Dithane M45, was effective only when used with Plantvax. Phytotoxic as a seed treatment. Topsin M is registered for use on soybean in the U.S.	(Hartman *et al.*, 1992; C. Levy, pers. comm.)
Chlorothalonil	Bravo Equus	South America India	Limited data available. Yield protection similar to or less than Mancozeb. Not as effective as other compounds in some studies. Registered for use on soybeans in the U.S.	(Patil & Anahosur, 1998; Vyas *et al.*, 1997)
Ethlenebisdithio-carbamates (EBDC)[a]	Dithane M45 Mancozeb Manzate D Zineb Maneb	Australia China India Philippines Taiwan	The EBDC products have been effective in controlling soybean rust when applied 7 to 21 days apart, with the first applications as early as three weeks after planting and as late as flowering. Not all studies showed control or yield increases.	(Anon., 1983; Anon., 1992; Hartman *et al.*, 1992; Ogle *et al.*, 1979; Sinclair, 1977)
Oxycarboxin[a]	Plantvax	India Taiwan Thailand	Not as effective as Dithane M45 or Manzate D, did not always control rust, yield protection varied by study. Apply when lesions first appear, then at 7-day intervals.	(Bonde *et al.*, 1976)
Hexaconazole[a]	Contof	India	Effective in reducing disease and protecting yield; 25% yield increase in limited study.	(Patil & Anahosur, 1998)

Appendix 5. continued

Active ingredient	Compound	Country	Summary of application trials and recommendations	References
Propiconazole[a]	Tilt	India Brazil	Effective in reducing disease and protecting yield; 33% yield increase in limited studies. Two applications, 15 days apart, starting at flowering.	(Patil & Anahosur, 1998)
Difenconazole	Score	Zimbabwe South Africa India	Yield protection varied by study, more effective than Mancozeb. Two or three applications needed starting at flowering.	(Patil & Anahosur, 1998; C. Levy, pers. comm.)
Tridimefon[a]	Shavit	Zimbabwe South Africa India	Extremely effective in reducing disease incidence. Highest yielding treatment. Two or three applications needed, starting at flowering.	(Patil & Anahosur, 1998)
Flusilazole/ Carbendazim[a]	Punch Extra	Zimbabwe South Africa	One of most effective fungicides in Africa. Two or three applications needed, starting at flowering.	(C. Levy, pers. comm.)
Tebuconazole[a]	Folicur	Zimbabwe South Africa	Not effective in limited testing in Africa.	(C. Levy, pers. comm.)
Azoxystrobin	Quadris	Brazil	Limited data. Good control but single, late application did not control rust or protect yield. Registered for use on soybean in the U.S.	(A. Talley, pers. comm.)

[a] Not registered on soybean in U.S.

Table from Miles *et al.* (2003a)

Appendix 6. Suggested fungicide product use regimen against SBR

1. If disease is established on site (curative treatment):

♦ Treat with a Section 18 triazole product (propiconazole, tebuconazole, myclobutanil, or tetraconazole).

♦ If a second application is needed, treat with azoxystrobin if disease is at a minimal level, otherwise retreat with a Section 18 product.

♦ If a third application is needed, treat with chlorothalonil.

2. If disease is expected, but not yet present (preventative):

♦ Treat with azoxystrobin or pyraclostrobin.

♦ If a second application is needed, treat with a Section 18 triazole product (propiconazole, tebuconazole, myclobutanil, or tetraconazole).

♦ If a third application is needed, treat with chlorothalonil or a Section 18 product.

3. If disease is expected, but not yet present (preventative), and develops after initial treatment:

♦ Treat with azoxystrobin or pyraclostrobin.

♦ If a second application is needed, treat with a Section 18 triazole product (propiconazole, tebuconazole, myclobutanil, or tetraconazole).

♦ If a third application is needed, treat with chlorothalonil or a Section 18 product.

4. If disease is expected, but not yet present (preventative):

♦ Treat with propiconazole + trifloxystrobin (Stratego) or pyraclostrobin + boscalid (Pristine).

♦ If a second application is needed, treat with chlorothalonil or a Section 18 triazole product (propiconazole, tebuconazole, myclobutanil, or tetraconazole).

- or -

♦ Treat with azoxystrobin or pyraclostrobin.

♦ If a second application is needed, treat with a Section 18 triazole product (propiconazole, tebuconazole, myclobutanil, or tetraconazole).

From Section 18 Quarantine Emergency Exemption Request on soybean for Australasian soybean rust (Minnesota and South Dakota Departments of Agriculture, 2003)

Appendix 7. Projected return from fungicide use against SBR

	A	B	C (= A X B)	D	E = C- D
Option	**Expected Yield* /Increase (bu/A)**	**Soybean Price**	**Gross Revenue/A from fungicide (best case)**	**Fungicide Cost + Applic. Cost/A**	**Net Revenue/A**
azoxystrobin (labeled)	38 / 12.6	$ 4.78	$ 60.23	$ 16.79 – 35.92 $ 18.36 – 37.49	$ 43.44 – $ 22.74
chlorothalonil (labeled)	38 / 2	$ 4.78	$ 9.56	$ 10.70 – 15.31 $ 12.67 – 17.20	($ 1.14 – 5.75) ($ 3.11 – 7.64)
tebuconazole	38 / 12.6	$ 4.78	$ 60.23	$ 13.97 $ 15.94	$ 46.26 – $ 44.29
propiconazole	38 / 12.6	$ 4.78	$ 60.23	$ 13.66 $ 15.63	$ 46.57 – $ 44.60
myclobutanil	38 / 12.6	$ 4.78	$ 60.23	$ 14.40 $ 15.97	$ 45.83 – $ 44.26
propiconazole + trifloxystrobin	38 / 12.6	$ 4.78	$ 60.23	$ 15.66 $ 17.47	$ 44.57 – $ 42.76
tetraconazole	38 / 1.2	$ 4.78	$ 5.74	$ 19.90 $ 21.47	($14.16) ($16.00)
pyraclostrobin + boscalid	38 / 4.6	$ 4.78	$ 21.99	$ 15.90 – 26.90 $ 17.90 – 28.47	$ 6.09 – (4.91) $ 4.09 - (6.48)
pyraclostrobin	38 / 2.7	$ 4.78	$ 12.91	$ 14.73 – 25.55 $ 16.30 – 27.12	($ 1.82 – 12.64) ($ 3.39 – 14.21)
Untreated control	25.4	$ 4.78	N/A	N/A	($ 60.23)

* Assuming the protection of a 38 bu/A soybean yield.

From Section 18 Quarantine Emergency Exemption Request on soybean for Australasian soybean rust (Minnesota and South Dakota Departments of Agriculture, 2003)

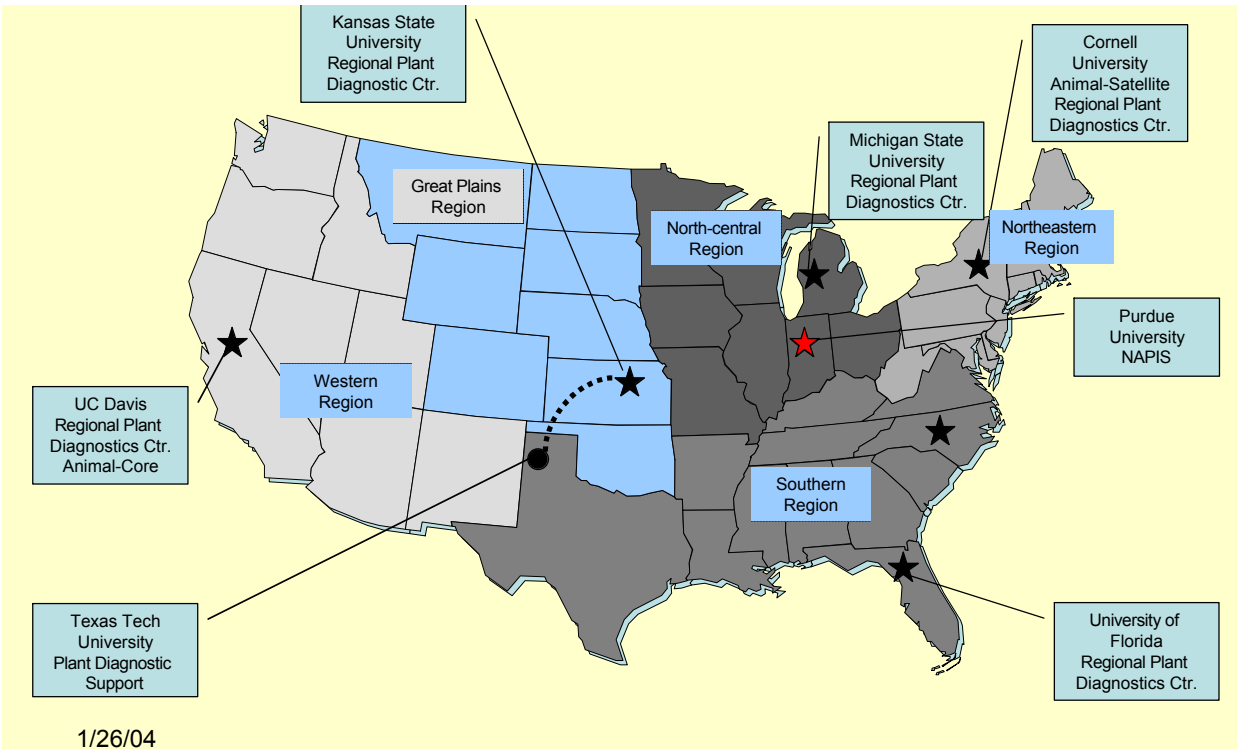
Appendix 8. Soybean varieties resistant to P. pachyrhizi (latest survey known to exist).

Location	Number of varieties	Latest screening date
Japan	3	1978
AVRDC	9	1987
India	9	1987
Indonesia	4	1977
Australia	4	1977
Thailand	3	1996
Taiwan	4	1986
China	8	1997
U.S.*	3	1996

* Developed by Dr. R.L. Bernard in the 1980s, no longer in commercial use.

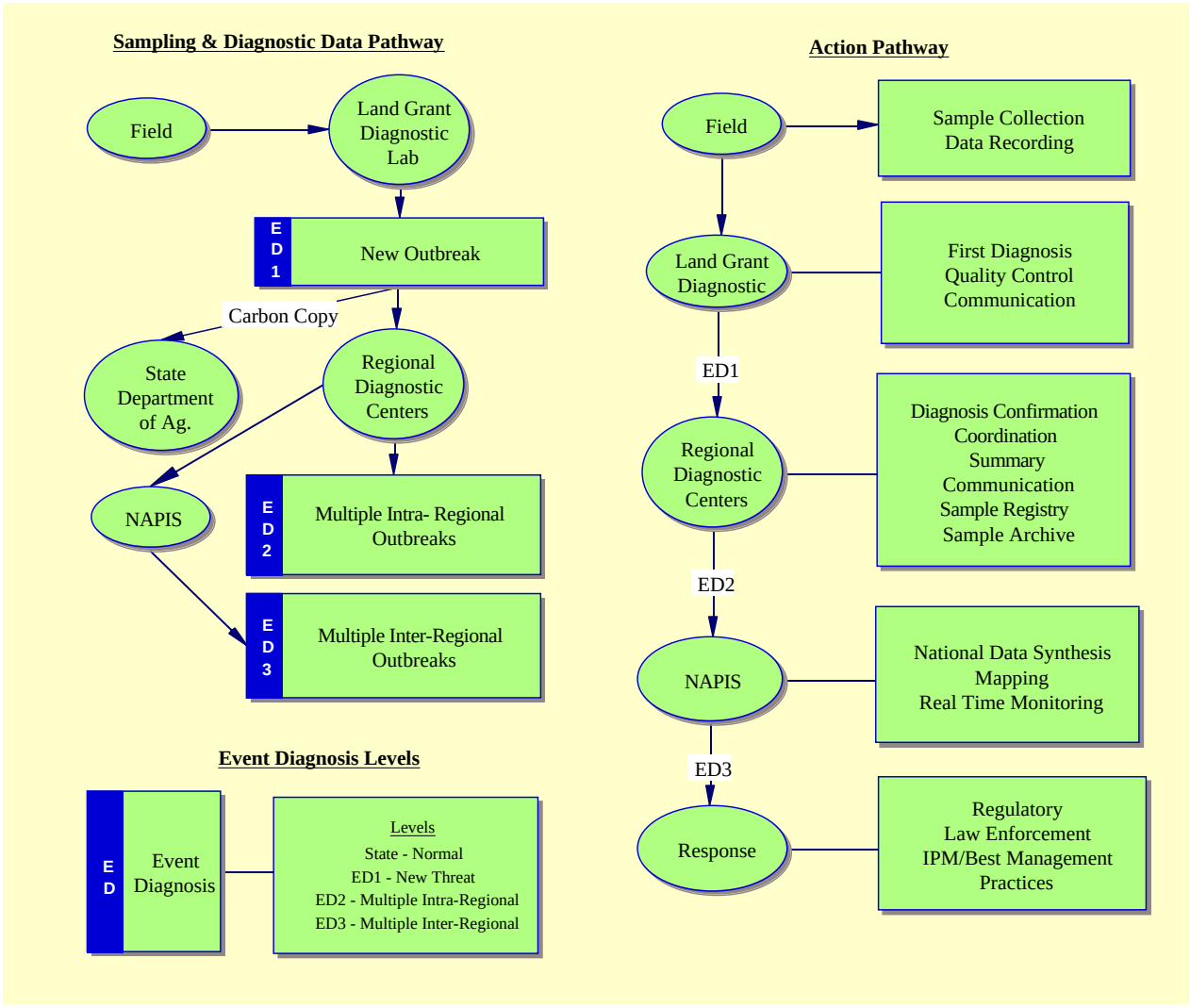
From Yang and Brueland (2003)

Appendix 9A. National Plant Diagnostic Network (NPDN) regions and regional centers



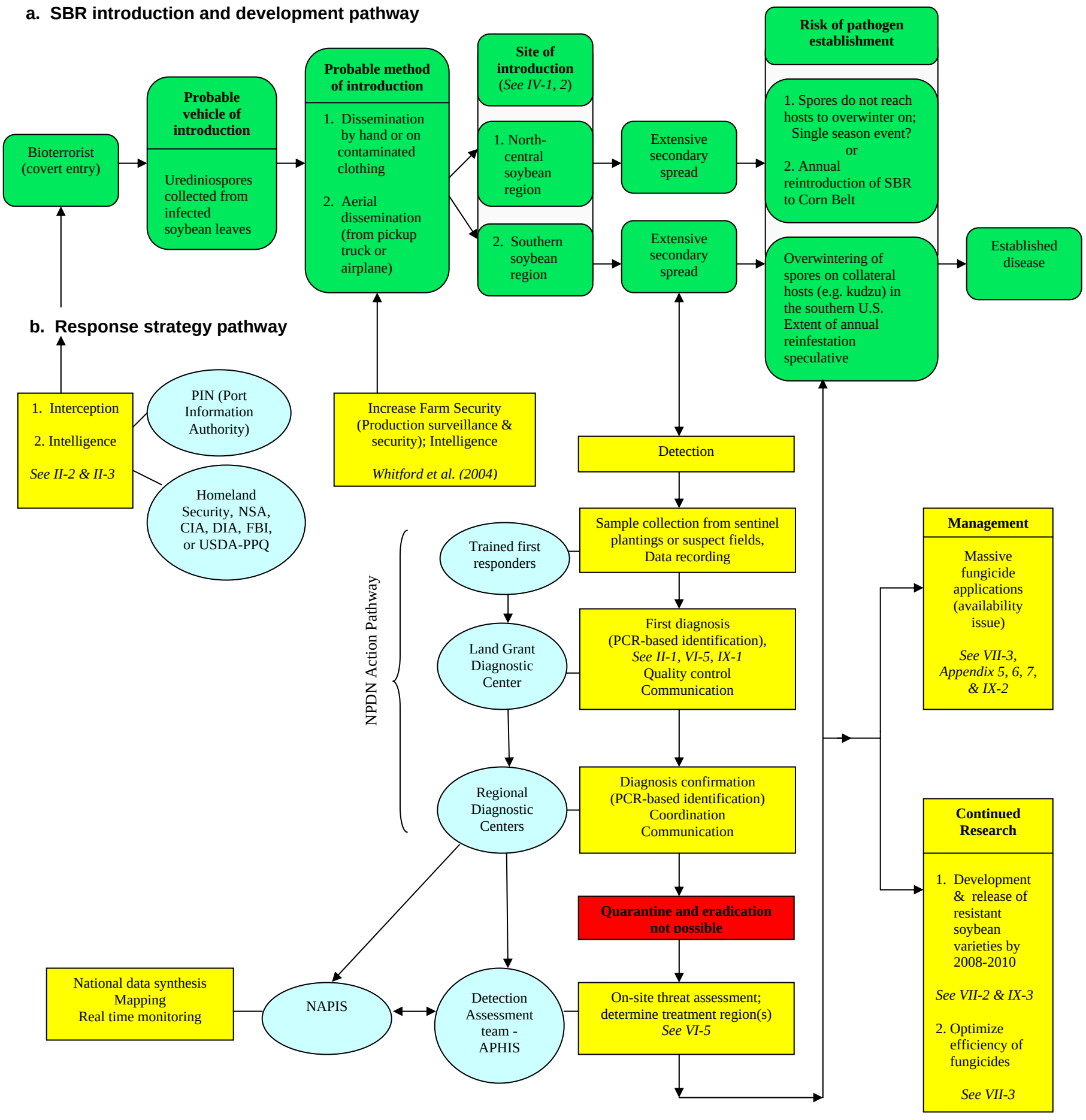
From Cardwell (2004)

Appendix 9B. Sampling + diagnostic data and action pathways for NPDN



From Cardwell (2004)

Appendix 10. Pathway and response to the intentional introduction of P. pachyrhizi, the cause of soybean rust.



Appendix 11. Scientists Knowledgeable of Soybean Rust

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Late Wilt of Corn (Maize)

Pathway Analysis:

Intentional Introduction of

Cephalosporium maydis

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Late Wilt Pathway Analysis

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Executive Summary: Late Wilt Pathway Analysis

- Introduction of *Cephalosporium maydis,* causal agent of late wilt of maize, poses a moderate threat to U.S. corn production.

- Maize yield losses from late wilt approached 40% in Egypt before the introduction of resistant cultivars (Samra *et al.*, 1971) and 70% in India, with associated economic losses up to 51% (Payak and Sharma, 1978).

- *C. maydis* has also been reported in Egypt, India, Israel, and Hungary; with unconfirmed reports in Romania, Kenya and Portugal, to imply that some strain(s) of the pathogen are capable of surviving climates similar to U.S. corn production regions.

- *C. maydis* is a soilborne vascular wilt pathogen that also is seed-borne. The pathogen overseasons primarily as sclerotia or mycelia on host debris. *Lupinus termis* is the only reported alternate host of *C. maydis*; however, it is unknown if *Lupinus* spp. in the U.S. are collateral hosts.

- Disruption of export markets could be achieved by a covert bioterrorist action through introduction of a relatively small volume of infected seed, culture (mycelia + conidia and/or sclerotia), or plant material from which natural increase and subsequent dissemination could occur over a period of years.

- Production of *C. maydis* inoculum, short-term storage, and distribution would be simple, inexpensive and require only basic household equipment. A substantial initial impact on yield would require distribution of a large volume of inoculum over an extensive area; however, tons of solid substrate inoculum could be produced in 4 - 6 weeks.

- The most probable methods of dissemination are aerial distribution of solid substrate inoculum or mixing inoculum (or infected seed) into seed stocks for inadvertent dissemination by growers.

- Optimum conditions for maize growth also are optimum for late wilt infection. Cold tolerance data for *C. maydis* have not been published, but reports of *C. maydis* in Hungary suggest the pathogen can overwinter in temperate areas (section VIII).

- Persistence of *C. maydis* in southern U.S. corn producing areas is likely, with inoculum increase especially where double-cropping and no-till systems are practiced. Secondary dissemination would be through the movement of agricultural equipment and contaminated seed. Natural increase and secondary spread of *C. maydis* in the Southern Corn Belt, could infest up to 75% of the corn production area within 8-10 years if contiguous frequent corn crops continue to be grown under no-till production.

- Corn is the most economically important crop in the U.S., with a value of $21.2 billion in 2002. Direct yield losses attributable to a late wilt are difficult to predict; however, any report of *C. maydis* in the U.S. could result in a long-time quarantine, crop embargo, and restricted movement of agricultural equipment to and subsequently cause serious economic impact. Added control costs could range from 10-15%, with the over-all economic consequence of *C. maydis* establishment in the U.S. considered moderate.

- A **late wilt disease pathway and response schematic (Appendix 4)** summarizes findings.

Immediate Response Options

- Secondary dissemination of *C. maydis* will be moderately slow and permit containment and eradication if the pathogen was introduced into a localized area. Effectiveness of containment and quarantine actions are dependent on rapid identification and timely implementation of control measures.

- Rapid detection of late wilt in the U.S. at the present time is unlikely. Symptoms may not be readily distinguished from abiotic stresses or indigenous pathogens that produce wilt. Symptoms appear late in the season, infected seed display no external symptoms, and the myriad of fungi that inhabit dying maize tissue make *C. maydis* isolation and diagnosis difficult. Recently developed molecular techniques may make rapid identification possible in the near future (Saleh and Leslie, unpublished).

- Assembling a “Detection Assessment Team” with expertise on late wilt, as has been done by APHIS for soybean rust, is a preparatory step that can minimize the time required for on-site threat assessment.

- The introduction of late wilt into no-till U.S. corn systems may require a return to tillage in order to minimize inoculum build-up in affected areas.

- Although some fungicides were effective against *C. maydis* in India, chemicals provided unsatisfactory control in Egypt. Large-scale use of fungicides in the U.S. may not be economically practical.

- The only economically feasible means to control late wilt is the development of resistant maize lines. The incorporation of late wilt resistance into commercial hybrids should be a long-range response plan to minimize damage from *C. maydis*.

Late Wilt of Corn (Maize)

Pathway Analysis for the Intentional Introduction of *Cephalosporium maydis*

Late wilt is a vascular wilt disease of corn first reported in Egypt in 1960 (Sabet *et al.*, 1961) caused by *Cephalosporium maydis* (Samra *et al.*, 1963). "Late wilt" symptoms appear late in corn development, usually during or after flowering. Significant economic losses due to late wilt have been reported in Egypt and India. Although the disease does not occur in the U.S., it is considered a potentially important pathogen (Warren, 1983). This report is a pathway analysis for the intentional introduction of *C. maydis* into the U.S; a summary, in the form of a disease pathway and response schematic, is presented in Appendix 4.

I. Biology and life/disease cycle of the pathogen

1. Identity

Pathogen Name: *Cephalosporium maydis* Samra, Sabet, & Hingorani (most common name used).

Synonyms: *Acremonium maydis* (preferred name in CABI, *Cephalosporium* is considered an obsolete synonym for the genus *Acremonium*)
Harpophora maydis syn.

Taxonomic position:		
	Kingdom:	Fungi
	Phylum:	Mitosporic fungi
	Class:	Hyphomycetes
	Order:	Hypocreales
	Family:	Hypocreaceae

The taxonomic position of the causal agent of late wilt is presently under review (Saleh and Leslie, unpublished) and is being transferred to a newly created genus, *Harpophora* (Gams, 2000), from a portion of the *Phialophora* genus. *C. maydis* now goes by the name *Harpophora maydis* (Samra *et al.*) W. Gams. Since the pathogen causing late wilt of maize is consistently referred to as *Cephalosporium maydis* in the literature, that nomenclature will be used in this report.

Common name: Late wilt of corn, cephalosporiosis del maiz (Spanish), cephalosporiose du mais (French), Gefaessbuendelkrankheit: mais, Welke: mais (German).

2. Hosts

Zea mays (maize) and *Lupinus* (lupine) are considered the only known hosts of *C. maydis* based on a limited study of only 11 plant species (Sabet *et al.*, 1966a). *C. maydis* causes significant damping-off and stunting of *Lupinus termis*, a species cultivated in Egypt (Sahab *et al.*, 1985). Cotton (Bahteem 185 cultivar) displays local lesions on hypocotyls, but these disappear as plants mature and *C. maydis* could not be recovered from these lesions (Sabet *et al.*, 1966a).

3. Geographic Distribution

Late wilt was first reported as a vascular wilt disease of corn in Egypt in 1960 (Sabet *et al.*, 1961) and is currently distributed throughout Egypt. Late wilt has also been reported in Andra Pradesh (Payak *et al.*, 1970), Uttar Pradesh, Bihar and Rajasthan provinces of India (Payak and Sharma, 1985), with unconfirmed reports in Kenya (www.agron.missouri.edu/cgi-bin/) and Romania (Dr. A. Ellingboe, personal communication). Identification of the causal agent of a wilting maize plant is challenging and a correct diagnosis is difficult. A *Cephalosporium* sp. has been isolated from maize plants displaying late wilt-like symptoms in Portugal (Dr. A. Ellingboe, personal communication). Recently, *C. maydis* was reported in Israel (Sharon *et al.*, unpublished), Italy (Dr. D. Smith, personal communication), and was isolated and characterized from maize plants with wilt symptoms in Hungary (Pecsi and Nemeth, 1998) (Fig. 1). The appearance and activity of the pathogen in Hungary has been attributed to global warming and dry early summers (Pecsi and Nemeth, 1998). The Egyptian, Indian and Hungarian isolates of *C. maydis* differ in morphology, pathogenicity, and route of infection.

4. Disease Impact

Late wilt is economically the most important fungal disease of maize in Egypt (Samra and Sabet, 1966; Samra *et al.*, 1963; 1971) where 100% infection occurs in some fields (Galal *et al.*, 1979) and yield losses approached 40% before the introduction of resistant cultivars (Samra *et al.*, 1971). Late wilt is also destructive in India, with incidence as high as 70% and economic losses up to 51% (Payak and Sharma, 1978). Although the disease does not occur in the U.S., it is considered a potentially important pathogen (Warren, 1983).

Cob formation is reduced, kernels are poorly developed and seed quality is adversely affected by late wilt. The pathogen can be seed-borne.

5. Symptoms

Root tips of infected plants are stained red during early stages of infection but above ground parts generally remain symptomless until tasseling when a rapid wilting of lower leaves progresses upward. Leaves appear streaked as tissue between veins becomes dull green and then chlorotic (Fig. 2) before eventually rolling inward and appearing scorched. Yellow to reddish brown streaks appear on the basal internodes of the stalk (Fig. 3). Pith and vascular bundles become dark yellow to brownish (Fig. 4). Lower parts of the stalk become dry, shrunken and hollow. Fewer cobs are produced and kernels are poorly developed. Young seedlings may have stunted roots (Fig. 5).

Late-wilt infection is often associated with infection by secondary invaders such as *C. acremonium*, *Sclerotium bataticola*, *Fusarium moniliforme*, and various bacterial rots (Samra and Sabet, 1966), collectively referred to as the “stock rot complex”. Symptoms may be modified by these secondary invaders, making the identification of late wilt in the field difficult. Some of these pathogens produce “wet rots” (tissue decay in the lower internodes with different degrees of wetness) or soft rot symptoms (basal internodes turn into a soft mass of disintegrating brown tissue).

Fig. 1. World Distribution of Late Wilt of Corn caused by *Cephalosporium maydis*

- Confirmed reports
- Unconfirmed reports (or in the process of confimation)

Adopted from CABI, 1999 and updated from Saleh *et al.*, 2003.

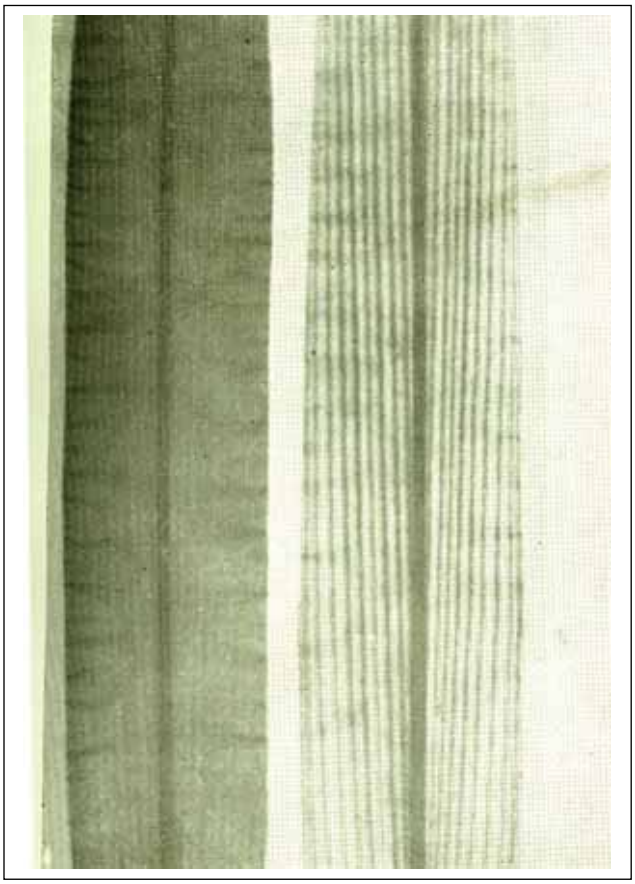


Fig. 2. Leaf streaking due to late wilt (R), compared to healthy leaf (L).

(Sabet *et al.,* 1966a)

Fig. 3. Discolored and necrotic tissue observed with late wilt on roots and lower stem nodes.

(L.E. Claflin)

Fig. 4. Vascular discoloration caused by *Cephalosporium maydis.*
(A.J. Ullstrup and B.L. Renfro)

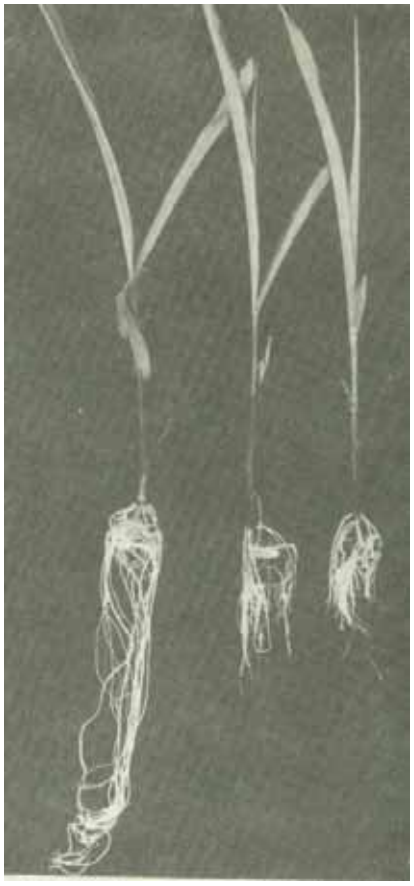


Fig. 5. Reduced root development of inoculated maize seedlings (R) compared to uninoculated control (L).

(Samra *et al.*, 1962)

6. Disease Cycle and Epidemiology

a. Initial inoculum and infection

C. maydis is a soil-borne vascular wilt pathogen that is also seed-borne (Michail *et al.*, 1999). The pathogen survives as sclerotia on corn debris (Dawood *et al.*, 1979) and infects seedlings through the roots or mesocotyl. Indian strains are capable of infecting stalk tissue through wounds (Payak *et al.*, 1970; Singh and Siradhana, 1988b).

The disease cycle of *C. maydis* is summarized in Fig. 8. Sabet *et al.* (1970b) has described progress of the pathogen in maize. *C. maydis* initially grows epiphytically on roots and produces short, thick-walled hyphae with swollen cells. Penetration can occur anywhere on the root system or mesocotyl (except root tips) but is most common where lateral roots originate or the zone of root elongation. Appressorium-like structures are produced and epidermal cells beneath them eventually collapse. The fungus penetrates directly through collapsed epidermal cells, and grows intra- and intercellularly to the xylem. Root injury predisposes plants to the disease and insect damage provides additional avenues for entry. Late wilt of maize increased when the nematode *Heterodera zeae* was present (Singh and Siradhana, 1988a).

After penetration, the fungus colonizes xylem tissue where it spreads slowly the first five weeks before growing rapidly upward throughout the plant (Fig. 6 and 7). At flowering (9-10 weeks), the fungus is distributed throughout the stalk and many vessels are blocked with hyphae and a dark gum-like substance (Sabet *et al.*, 1970b). Vascular occlusion appears to be the principle cause of symptom development in late wilt (Abdel-Rahim *et al.*, 1998). By 12-13 weeks the fungus is in the cob (Sabet *et al.*, 1970b), from which it moves through the pedicels to seed embryos (Michail *et al.*, 1999).

Seed rot or preemergence damping-off have been demonstrated when soils are artificially inoculated with high concentrations of inoculum (Payak *et al.*, 1970) but have not been demonstrated with natural seedborne inoculum (CABI, 1999). Heavy soil inoculum may delay emergence and reduce seedling vigor (Payak *et al.*, 1970).

No sexual stage of *C. maydis* is known (Zeller *et al.*, 2000).

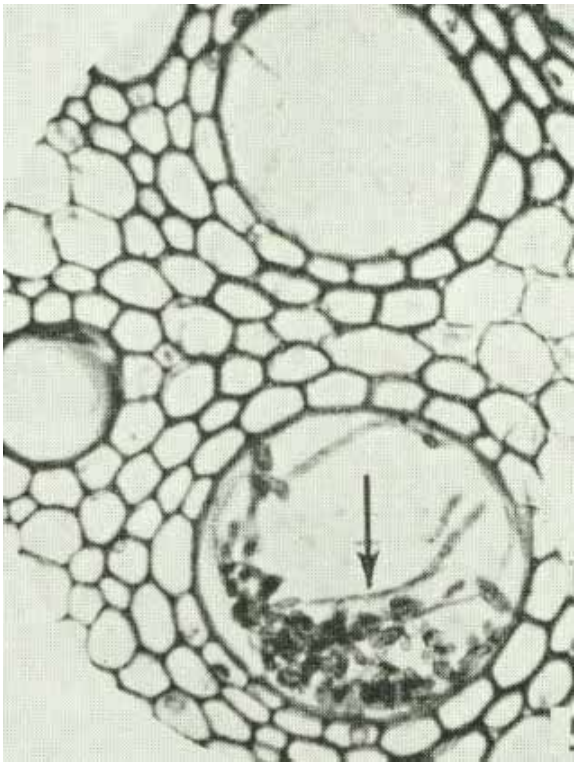


Fig. 6. Cross-section of a root of a 35-day-old plant, showing *C. maydis* hyphae and conidia in xylem vessels (X600).
(Sabet *et al.*, 1970b)

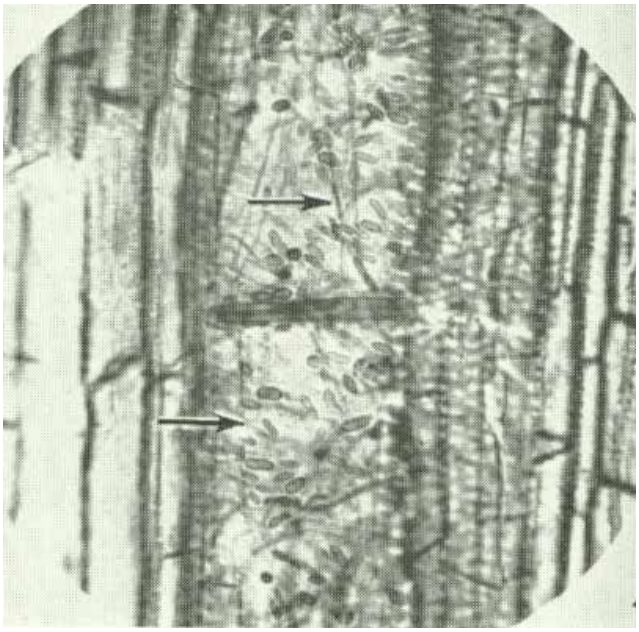
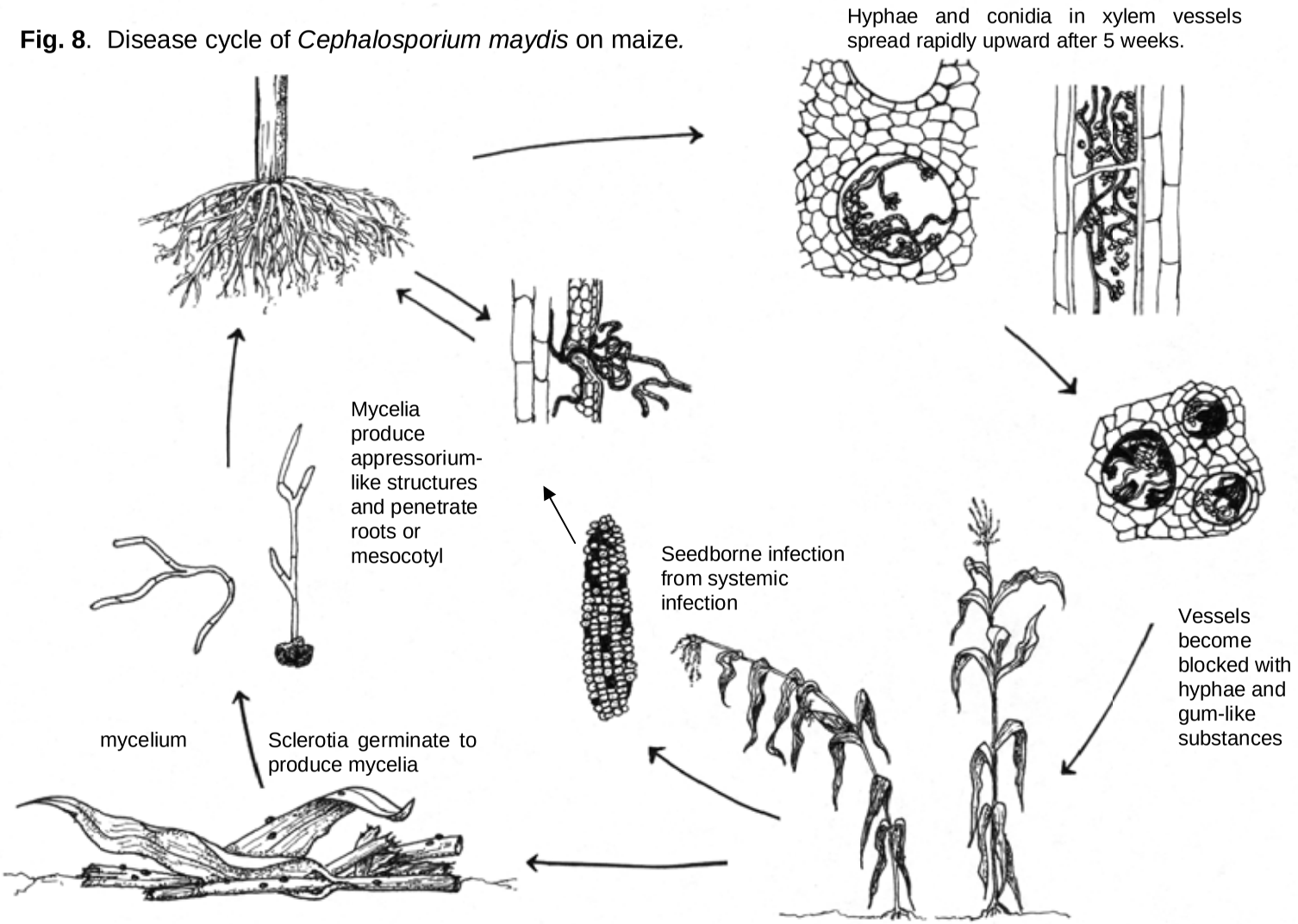


Fig. 7. Longitudinal section of a root of a 35-day-old plant showing *C. maydis* hyphae and conidia in xylem vessels (X600).
(Sabet *et al.*, 1970b)

Fig. 8. Disease cycle of *Cephalosporium maydis* on maize.



C. maydis survives as sclerotia or mycelia on decaying plant material or in soil.

Corn plants show progressive wilting, first on lower leaves, followed by wilting of the entire plant and collapse of the stalk.

b. Growth stage vulnerability

As plants mature they become more resistant to infection and 50-day-old plants grown in inoculated soil were no longer susceptible to systemic infection, unless roots were injured (Sabet *et al.*, 1970b). Infection routinely occurs after inoculating *C. maydis* (Indian and Hungarian isolates) into the 1st or 2nd stem node of 50-60 day old plants (Payak *et al.*, 1970; Pecsi and Nemeth, 1998; Singh and Siradhana, 1988b), but this bypasses the most likely avenue of resistance in natural infection through the roots.

c. Conditions that favor disease

Late wilt develops readily at temperatures between 20-32°C, with optimum disease at 21-27°C (Singh and Siradhana, 1987a). *C. maydis* growth in soil is sharply inhibited above 35°C (Sadik, 1974). *C. maydis* can grow over a wide range of soil pH from 4.5-10.0, with an optimum at pH 6.5 (Singh and Siradhana, 1987a). Late wilt epidemics have occurred only in arid climates and reports of optimum moisture for disease vary. In India, the natural incidence of late wilt is highest when rainfall is above average or irrigation frequent (Singh and Siradhana, 1987a), but frequent watering decreased infection in Egypt (Samra *et al.*, 1966). Late wilt incidence can be reduced by irrigation intervals of 9 (Samra *et al.*, 1966) or 10 days (Satyanarayana, 1996). Excessive soil moisture also reduces late wilt. *C. maydis* is generally sensitive to anaerobic conditions in water-saturated soils (Samra *et al*., 1966) and survival of sclerotia is favored by low soil moisture (25% saturation) (Dawood *et al.*, 1979). Optimum moisture conditions for maize growth are also optimal for disease development (Warren, 1983).

d. Inoculum persistence and dissemination

Dissemination is primarily through movement of infested soil, crop residue, or seed-borne inoculum.

i. In soil/on debris

C. maydis is reported to persist on corn stubble for 12 to 45 months (Sabet *et al.*, 1970a; Samra *et al.*, 1966; Singh and Siradhana, 1987b); however, inoculum survival in soil is generally poor and restricted to the top 20 cm of soil (Sabet *et al.*, 1970a). A low competitive saprophytic ability and poor saprophytic colonization of fresh substrate indicates

the survival of *C. maydis* depends primarily on the persistence of parasitically infected host remains (Sabet *et al.*, 1970a). Non-tillage production practices tend to favor persistence and dissemination of this disease. The correlation between saprophytic ability in soil, survival in surface residue common with no-till production, and pathogenicity to the plant remains an important unanswered question (Dr. J. Leslie, personal communication).

Sclerotia in infected host debris ensures long-term survival of the pathogen in no-till systems, but low viability of sclerotia on naturally infected stalk pieces buried in the soil has been reported (Dawood *et al*., 1979). Low atmospheric humidity (70% RH) encourages sclerotial production (Dawood *et al*., 1979). Sclerotia can survive for 15 months under lab conditions (Sabet, 1984). Survival of sclerotia in soil is favored by low temperatures (optimum at 8-10°C), low soil moisture (25% saturation), and a high C/N ratio (Dawood *et al.*, 1979).

Lupine, the only known alternate host (Sabet *et al.*, 1966a), facilitates parasitic survival of the pathogen.

ii. Seed transmission

Much of the literature implies seed-borne infection plays a limited role in epidemics. Severely infected seeds rot or seedlings damp-off (Payak *et al.*, 1970). Emergence may be delayed and seedling vigor is poor in *C. maydis* infected soils. *C. maydis* has been isolated from freshly harvested and stored seed corn (Mohamed *et al*, 1967; Sabet *et al.*, 1966b; Fathi, 1971) and the seed coat, endosperm and embryo of naturally infected, dissected seeds (Michail *et al.*, 1999). Infected seed that were surface sterilized produced plants with late wilt symptoms when sown in autoclaved soil, resulted in the infestation of soil, and the subsequent development of late wilt in healthy seeds grown in that soil (El-Shafey *et al.*, 1976). *C. maydis* survived in seed for 10 months under unfavorable conditions (high temperatures and low humidity) in India, but longer survival is predicted at low temperatures (Singh and Siradhana, 1987b).

Seed are more likely to be infected if disease onset in the parent plant is early. *C. maydis* within seeds decreased when disease onset was delayed from 80 to 100 days after sowing and no diseased seeds were produced when symptom onset was 110 days (El-Shafey *et al.*, 1976). The pathogen was detected in 42/43 naturally infected seed samples from various regions of Egypt at infection levels between 1 and 11% (Mohamed *et al.*, 1967) and

in 12/13 samples at infection levels of up to 9% for white corn varieties and 1-3% for yellow corn cultivars (Michail *et al.*, 1999). This implies that seed infection is common and although the percentage of infected seed is low, it represents a potential means of disease spread. The role of seed-borne infection in epidemic initiation remains speculative.

7. Causal organism

a. C. maydis in culture

C. maydis grows well in culture on potato dextrose agar (PDA) (Pecsi and Nemeth, 1998; Samra *et al.*, 1962), especially when supplemented with 0.2% yeast extract (Michail *et al.*, 1999; Sabet *et al.*, 1966). Colonies have a felt-like appearance and are initially white but become gray or black with age with a characteristic "rhizoid" margin (Samra *et al.*, 1963). Conidiophores are terminal or lateral with five to eight hyaline, single celled, oblong to oval conidia in heads (Fig. 9), that vary in length depending on isolate from 3.6-14 x 3-3.6 µm (Egypt, Samra *et al.*, 1963), 6-19 x 3-3.6 µm (Hungary, Pecsi and Nemeth, 1998), 7-11 x 3.0-4.0 µm (India, Payak *et al.*, 1970), and 2.3-13.8 x 1.2-3.4 µm (El-Shafey and Claflin, 1999). Conidia usually germinate by 1-2 polar germ tubes, and rarely, 3 germ tubes may form. Anastomosis of germ tubes is common. In cultures more than 10 days old, conidia production ceases and sclerotia-like bodies (Fig. 10) predominate (Payak *et al.*, 1970).

The optimum temperature for growth and germination is 28-30°C (on PDA) with no growth observed below 10°C or above 36°C (Pecsi and Nemeth, 1998; Samra *et al.*, 1963), and no germination below 20°C or 74% RH (Sabet *et al.*, 1966a). Sclerotia production is enhanced and regularized when the fungus is grown on Farlene-glucose-agar at 30°C (Sabet, 1984).

b. Pathogen variability

There are differences between the Egyptian and Indian isolates of *C. maydis* in morphology (see 1-7a.), route of infection and pathogenicity. Stem inoculation of maize plants with 7 isolates from Egypt produced no infection (Sabet *et al.*, 1966a; 1970b), whereas stem inoculations (1^{st} – 2^{nd} node) routinely caused infection with Indian and Hungarian isolates (Payak *et al.*, 1970; Singh and Siradhana, 1988b; Pecsi an Nemeth, 1998). Further, seed treatment with fungicides is reported to significantly reduce late wilt in India but not in Egypt (see VII-3). The pathogenic capabilities and the relationship of these populations of *C. maydis* to one another are presently unknown (Saleh *et al.*, 2003).

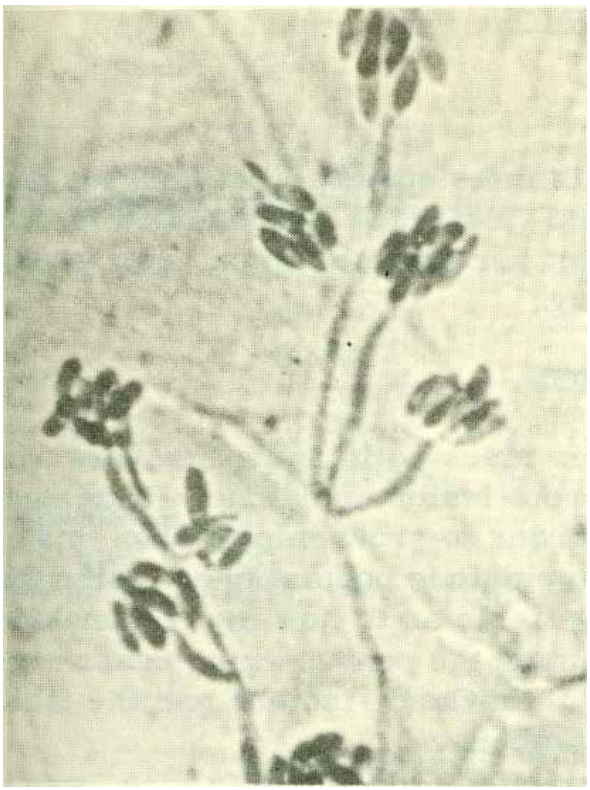


Fig. 9. Formation of clusters of spores on conidiophores of *C. maydis*.
(Samra *et al.*, 1963)

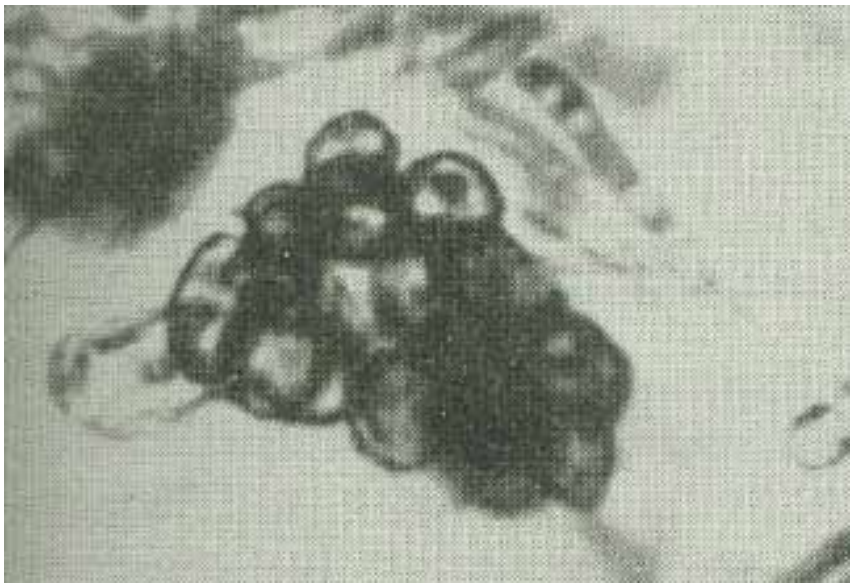


Fig. 10. Botroyoform sclerotia-like bodies formed in old cultures of *C. maydis* on PDA.
(Samra *et al.*, 1963)

Early work demonstrated considerable pathogenic variability of 15 isolates from different regions of Egypt on maize (Sabet *et al.*, 1966a) suggesting high levels genetic variation; however, molecular markers (AFLP) used to characterize 48 Egyptian isolates clustered them into four phylogenic lineages (I-IV) implying limited genetic variation (Zeller *et al.*, 2000). More recently, 866 isolates of *C. maydis* from Egypt were characterized into four lineages (Saleh *et al.*, 2003). Lineages I-III were found throughout Egypt, but lineage IV was recovered only from the Nile River Delta and seems to be evolving more rapidly than other lineages (Dr. J. Leslie, personal communication). The number of isolates per haplotype was nonuniform, supporting the hypothesis that *C. maydis* populations are reproducing clonally rather than sexually (Saleh *et al.*, 2003).

Lineages vary in their pathogenicity (Zeller *et al.*, 2002) and colonization ability (El-Assiuty *et al.*, 1999). Lineage IV was most virulent alone but least competitive on susceptible maize accessions when mixed inoculated with other lineages. Lineage II was the least virulent when tested alone but the most competitive (dominating 70% of infections) in mixed lineage inoculations (Zeller *et al.*, 2002). These results imply that virulence and competitive ability are not the same in this host-pathogen system.

8. Diagnostic Methods

Late wilt does not occur in the United States and would not be readily recognized or distinguished from abiotic stresses initially. Symptom recognition is based on the dull green, desiccated (scorched) leaves, “collapsed” stalk and discolored pith tissues. Symptoms are not definitive and morphological and microscopic characteristics described by Samra *et al.* (1963) are still used to identify *C. maydis* (Zeller *et al.*, 2002); thus isolation, culture, direct microscopic evaluation, pathogenicity tests, or PCR are required for positive identification. Species-specific PCR primers have been developed and can be used for organism identification (Saleh and Leslie, unpublished).

Isolation of *C. maydis* from plants is difficult because of its slow growth in culture and the relative abundance of other more rapidly growing fungi such as *Fusarium* spp. in the stalk rot complex (Saleh *et al*., 2003). A successful recovery technique is outlined by Zeller *et al.* (2002) where the internode of symptomatic plants is sterilized (5%NaHCl) and split with a sterile knife. A small piece of discolored vascular bundle is placed on PDYA (PDA + 0.2% yeast extract). Single-spore isolates can be obtained by dilution plating.

Seed health tests

Infected seeds display no discernable external symptoms and cannot be identified visually. The fungus can be cultured by disinfecting seed in mercuric chloride (0.1%) for 2 minutes, washing several times with distilled water, plating on PDA and incubating at 27°C for 10 days (Mohamed *et al.*, 1967). Identification is completed using spore morphology and pathogenicity tests.

PDYA is reported to be a more efficient medium than blotter tests for detecting *C. maydis* in seed (Michail *et al.*, 1999). In this method, seeds are soaked in 1% hypochlorite for 3 minutes, plated on PDYA, incubated at 20°C under 12 hour cycles of alternating near-ultra-violet light and darkness, and examined after 24 hours.

II. Initiating event (recognizing an attempted introduction)

1. Observation/diagnosis of presence

Diagnosing an exotic pest in the field early is critical for containment and eradication. University extension personnel, growers, scouts, crop specialists and plant pathologists may not identify late wilt readily in the field. Species-specific PCR primers can be used for organism identification (Saleh and Leslie, unpublished) when commercially available.

The recent establishment by the USDA of the National Plant Diagnostic Network (NPDN) is intended to provide a cohesive information system to quickly detect pests and pathogens that have been deliberately introduced and report to appropriate responders and decision makers. NPDN, made up of experts at land-grant universities, is a key part of the Homeland Security effort. NPDN is divided into five regions, each with a regional hub (Appendix 1A). Web-based diagnostic and reporting systems are being developed and an effective communication network between diagnostic labs and regulatory agencies has been established (Appendix 1B). This system should facilitate the detection of anomalies, such as simultaneous outbreaks at many locations, and thereby help identify a bioterrorist attack. Select data collected from the NPDN regions will be archived at the National Agricultural Pest Information System (NAPIS) located at Purdue University.

2. Interception: individual/ pathogen

Interception of an individual carrying the pathogen or infected plant material at a port of entry should be responded to immediately. Isolation and containment of the material should prevent escape into the environment. Each year the Port Information Authority (PIN), maintained by APHIS, makes 53,000 interceptions in arriving cargo or baggage with exotic plant pathogens comprising 25% of the interceptions (National Research Council, 2002). Interception through routine traffic stops is somewhat improbable since very small amounts of the pathogen would be required to initiate larger scale inoculum production within the U.S. (see III and VI-1). Interception of contaminated seed should not be discounted and confirmatory procedures initiated. The probability of interception of shipped inoculum to an in-country location is much lower than personal interception, and confidentiality of mail deliveries could avoid detection.

3. "Intelligence" information

Intelligence information from Homeland Security, NSA, CIA, DIA, FBI, or USDA-PPQ about overt agroterrorism intent is another potential initiating event. This information should be provided to personnel at the county level to enhance the probability of early detection.

III. Probable route of terrorist entry/dissemination

Infected seed, culture (mycelia + conidia and/or sclerotia), or infested soil could be used to introduce the pathogen into a new area. The difficulty in moving the volume of infected soil required to initiate a localized late wilt epidemic make soil an unlikely candidate for initiating a bioterrorist event. If disruption of export markets was the goal of the covert action, pathogen introduction could be accomplished through a relatively small volume of infected seed or plant material from which natural increase and subsequent dissemination could take place over a period of years.

1. Cultures of *C. maydis*

Production, short-term storage, and distribution of *C. maydis* would be simple and require only basic equipment common in many households, albeit a considerable volume of material would be required to create a widespread epidemic.

As a saprophytic pathogen, inoculum can be easily produced on supplemented wheat bran (Sabet *et al.*, 1970a) held at 29°C for 3 weeks (Sabet *et al.*, 1972b). More recently, inoculum has been raised on autoclaved sorghum grain, moistened with water in milk bottles (El-Shafey *et al.*, 1988) and held at 26-30°C for 3-4 weeks (Zeller *et al.*, 2002).

The most reliable technique for viable sclerotial production uses Farlene-glucose agar (manufactured by Farley Health Products, Ltd., UK) incubated at 30°C for five weeks (Sabet, 1984). Lids are then removed from cultures for 2 days to promote drying so sclerotia can easily be harvested.

Liquid or agar culture production would be costly, require specialized equipment and produce a “less robust” inoculum for aerial distribution. Modern solid substrate methods allow large quantities of inoculum to be produced in any facility (including a kitchen or garage) with minimum equipment and little expense. “Spawn bags” filled with supplemental wheat bran or sorghum grain could potentially produce a ton or more of solid substrate inoculum (mycelium + conidia/sclerotia) in 4-6 weeks. Equipment would be required to sterilize the solid growth medium selected since *C. maydis* grows more slowly and is less competitive than many other soil-borne saprophytes. A substantial initial impact on grain yield would require distribution of a large volume of inoculum over an extensive area. Aerosolization of solid substrate inoculum may be a potential means of widespread distribution without requiring the large volume associated with conidia. Inoculum could potentially be effectively mixed into seed stocks so a grower would inadvertently disseminate it.

Serious market damage would result from any occurrence of this exotic disease within a region and result in long-time quarantine, embargo of crop produced, movement of equipment, etc.

2. Seed

Contaminated seed would be the easiest and most likely vehicle of introduction for late wilt. Seed-borne inoculum could be harvested from infected crops, or seed could be contaminated with either conidial or sclerotial inoculum of the pathogen. Storage of infected kernels for one season would not require specialized equipment other than preventing desiccation or very high temperatures. Long-term storage would require refrigeration to inhibit secondary, saprophytic decay by other organisms. The inadvertent introduction of *C. maydis* on lupine used for erosion control or forage may also be possible.

IV. Probable distribution

1. Point Introduction: Midwestern versus Southern corn production areas

The most likely route of intentional introduction would be infected seed, distributed at one or multiple sites to produce localized late wilt epidemics during the year of introduction. Infected seeds cannot be visually discerned by external symptoms from healthy ones in a seed lot and the U.S. seed process does not include seed health tests for late wilt. The pathogen will probably persist year round in southern corn growing regions but may overwinter less well in cold climates (see V-1a). Since late wilt occurs in Hungary, it seems likely that at least the Hungarian strain could overwinter in the Midwestern Corn Belt. Thus, infected seed distributed early throughout the southern Corn Belt, could initiate a severe localized epidemic and provide adequate sequential inoculum through movement of agricultural commodities and equipment, for gradual contamination of much of the Corn Belt. “No-till” and “eco-fallow” management practices generally employed throughout the Corn Belt could facilitate establishment and persistence of this disease.

2. Secondary Dissemination

Even if an introduced strain of *C. maydis* failed to successfully overwinter in northern corn growing areas, it could be annually reintroduced from the southern Corn Belt. Secondary dissemination of *C. maydis* would primarily be due to the movement of agricultural commodities and equipment and contamination of commercial seed lots. The vast

distribution of lupine species in the U.S. (see V-1b) may make them important alternate perennial sources of inoculum.

V. Consequences of introduction and establishment

The consequences of introduction of *C. maydis* and risk of late wilt establishment in the U.S. were rated with respect to six risk elements: climate, host range, dispersal, economic impact, environmental impact, and persistence. The pathogen was ranked for 29 different criteria encompassed within the six risk element categories.

1. Establishment

a. Climate *Risk = High*

The potential for the disease to spread in the U.S. will be dependent on whether *C. maydis* can thrive in the Corn Belt's temperate climate. Maximum disease incidence occurs at 21-27°C when rainfall is above average or irrigation frequent in India (Singh and Siradhana, 1987a). Optimum moisture conditions for maize growth are also optimal for disease development (Warren, 1983) so growing season conditions in the U.S. Corn Belt may be ideal.

The potential range of *C. maydis* on introduction to the U.S., generated by the climate-matching simulation model CLIMEX, is presented in Appendix 2A and 2B. Based on the present distribution of *C. maydis* (five locations: Cairo, Alexandria, Hyderabad, Budapest and Jerusalem), matching meteorological conditions occurred only in the southwestern area of the U.S. (Appendix 2A). Incorporating conditions the *C. maydis* isolate in Hungary encounters (i.e. using only the one location of Budapest), a much broader climate match with many corn-growing areas in the U.S. is found (Appendix 2B). Approximately 90% of the ecological range of the 79 million acres of corn in the United States is the same as the ecological range of the *C. maydis* isolate from Hungary. Our use of CLIMEX did not consider irrigation, which is used in some corn growing regions of Egypt and India. Additional water supplied by irrigation may skew results and create more climate matches in the U.S. Another major limitation of climate-matching models is the underlying assumption

that climate is the only determinant of species distribution, ignoring biotic components of the environment.

Although overwintering of *C. maydis* is best documented in warmer areas, *C. maydis* survives in Hungary (Pecsi and Nemeth, 1998) and is reported in areas of Israel that get relatively cool (Dr. J. Leslie, personal communication), which suggests it can successfully overwinter in temperate conditions. A related species, *Gaeumannomyces graminis*, survives very well in the U.S. Corn Belt and into Canada. A simple but important investigation is to determine the survivability of *C. maydis* (especially sclerotia) when exposed to extended periods below 0°C.

b. Host Range *Risk = High*

Lupinus termis, a cultivated species in Egypt is a known host of *C. maydis* (Sahab *et al.*, 1985), but it is not known how many of the over 600 *Lupinus* spp. are also hosts. Lupines are widespread in the U.S.; however, it is unknown if any of these species can serve as collateral hosts. Some species grown in the U.S. are native to North America, while many nonnative species have become common perennial ornamentals. There is a potentially high risk of a geographically widespread alternate host of *C. maydis* in the U.S.

c. Dispersal *Risk = Moderate*

Dissemination of the pathogen is primarily through the movement of infested soil, crop residue, or seed-borne inoculum. The distribution of infected seed via commercial seed lots would be the quickest means of dispersal and it is unlikely that infected seed would be detected early since notable symptoms are absent. The vigorous seed production and processing procedures practiced by seed producers should minimize secondary dissemination via this route. Movement with contaminated equipment will be limited to local areas since there is little custom harvesting in most areas of the Corn Belt.

d. Economics *Risk = Moderate*

Corn, grown for both grain and silage (forage), is the most economically important crop produced in the U.S. In 2002, corn was a grown on 79.1 million acres in 48 states (Appendix 3A) to produce 9 billion bushels of grain with a value of $21.2 billion. The top five corn producing states and associated percent of total production in 2002 were IA with

21.8%, IL with 16.6%, MN with 11.7%, NE with 10.4%, and IN with 7.0% of the U.S. total production (USDA, 2003). Yield/acre by county in 2002 is displayed in Appendix 3B.

The disease is of phytosanitary importance since it is of restricted distribution and economically damaging where found indigenously. Serious market damage would result from any occurrence of this disease within a region and result in long-time quarantine, embargo of crop produced, restricted movement of equipment, etc. Since *C. maydis* is seedborne, commercial seed nurseries would need to be isolated from late wilt infected regions.

Added control costs if *C. maydis* is introduced could range from 10-15%, depending on the effects of no-tillage production practices. Where continuous corn or extensive corn in the rotation is practiced, *C. maydis* could become established and damage occur annually. In the absence of infected seed, persistence is anticipated because the pathogen is soilborne, produces sclerotia survival structures and the potential alternate host, lupine, is extensively distributed throughout the U.S. Extensive "no-till" agriculture that leaves infected residue on the soil surface removed from competition by soil organisms should enhance severity of this disease. Over-all economic importance is considered moderate.

e. Environmental Impact *Risk = High*

Late wilt infections in *Lupinus termis*, a long-time cultivated plant in Egypt, resulted in 38% of seedling damping-off and 50% reduction in biomass in surviving plants (Sahab *et al.*, 1985). Other *Lupinus* spp. may be susceptible to late wilt. Some native lupines are considered endangered or threatened, such as *Lupinus perennis* (sundial lupine) which is the only known plant food of the endangered Karner blue butterfly larva (*Lycaedis melissa samuelis*) (IDNR, 1994). If *L. perennis* can serve as a host for late wilt, additional stress might be put on dependent natural populations. Ornamental lupine, often used as a ground cover, may also be adversely affected. Thus, late wilt could potentially have an economic impact on commercial ornamental nurseries that supply popular species, such as *L. angustifolius*, *L. albus* and *L. perennis.*

In Egypt, most maize fields are plowed at least annually and are double- or triple-cropped (Dr. J. Leslie, personal communication). Another potential environmental impact of *C. maydis* is the build up of inoculum in the residue of "no-till" corn systems commonly used in

the U.S. Such a build up may result in the necessity for returning to tillage as a sanitation measure.

f. Persistence *Risk = High*

Infection of young (seedling) plants could be very damaging in the first season if inoculum distribution was extensive and environmental conditions conducive at the time of introduction. *C. maydis* persists in soil and on corn debris for over 1 year (Samra *et al.,* 1966) between susceptible crops in Egypt. A possible limiting factor in this pathogen's establishment in the U.S. Corn Belt is cold tolerance (see V-1a); however, it is established in Hungary with similar temperature extremes and related species are common on other crops throughout the Corn Belt. Persistence in southern corn producing areas seems a likely scenario with inoculum build up especially where double-cropping and no-till systems are practiced. The southern survival of *C. maydis* would allow for annual reintroduction into the U.S. Corn Belt.

2. Over-all risk rating for establishment of *C. maydis*

Climate	Host Range	Dispersal	Economics	Environmental Impact	Persistence
High	**High**	**Moderate**	**Moderate**	**High**	**High**

VI. Likelihood of successful introduction

1. Quantity of inoculum required to introduce and establish damage

If disruption of export markets was the goal of the covert action, pathogen introduction could be accomplished with a relatively small volume of infected seed, *C. maydis* culture, or even infected plant material. If introduced into the Southern Corn Belt, natural increase and secondary spread of the pathogen could contaminate up to 75% of the current corn production area within 8-10 years if contiguous frequent corn crops continue to be grown under no-till production.

A substantial initial impact on grain yield would require distribution of a large volume of inoculum over an extensive area. "Spawn bags" filled with supplemental wheat bran or sorghum grain could potentially produce a ton or more of solid substrate inoculum (mycelium + conidia/sclerotia) in 4-6 weeks (see III-1). Distribution of large quantities of solid substrate inoculum could be effectively distributed by air or ground to produce widespread infection. Large quantities of *C. maydis* contaminated seeds distributed at a number of sites may also produce a substantial impact on exports in the year of introduction.

2. Likelihood of surviving initial introduction

It is highly probable that *C. maydis* would become permanently established and survive in soil and on debris. *C. maydis* inhabits only the upper 20 cm of the soil and no-till systems may favor inoculum buildup. Although there may be some question that this pathogen will successfully overwinter the cold intercontinental climate of much of the U.S. corn production area (see V-1a), its persistence in Hungary, extensive sclerotial production, and near-optimum summer climate suggest the pathogen will be capable of persisting year-round in southern corn growing regions.

3. Likelihood of dissemination beyond the point of introduction

Secondary dissemination of *C. maydis* would primarily be due to the movement of agricultural equipment and contamination of commercial seed lots. Thus dissemination would occur at a moderately slow rate. Although seed companies vigorously clean seed destined for the commercial market, the certification process in the U.S. does not include

seed health tests for late wilt. Once introduced, it seems likely that a low level of *C. maydis* seed infection could persist in commercial seed.

4. Likelihood of alternate host infection

Over 150 *Lupinus* species occur in the U.S. (distributed over at least 47 states). Some or all of these species may serve as hosts of *C. maydis*.

5. Likelihood of early detection

There is a low likelihood of early detection because most agricultural workers, county agents, and field agronomists are not familiar with symptoms of this exotic pathogen. There are several indigenous pathogens of corn that produce wilt symptoms, so an exotic pathogen is not likely to be suspected initially. The absence of external symptoms until flowering or later further complicates diagnosis. Even if diseased maize samples are submitted to professional plant disease diagnosticians at the onset of symptoms, identification of the causal agent is likely to take weeks rather than days. Many different fungi can be isolated from dying maize plants (some which grow more aggressively in culture than *C. maydis*), which makes an accurate diagnosis of the causal agent difficult (Dr. A. Ellingboe, personal communication).

Molecular techniques (PCR-based) may make rapid identification possible (Saleh and Leslie, unpublished). At present, late wilt is unlikely to be diagnosed rapidly and infected seed could make their way into commercial seed lots because discernable external symptoms are absent.

6. Overall risk = Moderate

Although the potential exists for *C. maydis* to cause problems in the U.S., the overall risk is not high (Dr. H. Warren, personal communication).

7. Likelihood of an agroterrorist trying to use *C. maydis* as a biological weapon = Low.

There are many unknowns related to the ability of *C. maydis* to survive and produce significant levels of disease under U.S. climatic conditions. Dissemination of *C. maydis* would be moderately slow, particularly if *C. maydis* infected seed are able to be detected readily in seed lots. Other exotic pathogens exist which could cause more significant damage in a shorter period of time. The primary potential in terrorist's hands would be economic restriction of export of corn or grain products.

VII. Control/Mitigation strategies after establishment

The most effective way to control late wilt is with resistant germplasm (El-Shafey *et al.*, 1988). Some cultural and chemical control methods moderately reduce the impact of late wilt.

1. Resistance

The development of resistant maize lines is the only economically feasible control for late wilt (Zeller *et al.*, 2000). Because *C. maydis* is an exotic pathogen, there has been little evaluation of late wilt resistance in commercial breeding programs in the U.S. Since classic symptoms are not always produced upon inoculation with *C. maydis*, the selection of resistance in breeding programs is more difficult than with other diseases and resistant plants cannot always be separated from escapes (Dr. Ellingboe, personal communication). Intensive efforts to test germplasm against late wilt were initiated by the National Maize Program, Agricultural Research Center, Giza, Egypt in 1963. Thousands of local and exotic materials were screened and many sources of resistance identified. The wide release of resistant varieties that began in 1980 has considerably reduced late wilt in Egyptian farmer's fields (El-Shafey *et al*., 1988).

Breeding for resistance is a continuous process and becomes especially important when new virulent isolates of *C. maydis* are recognized. Lineage IV of *C. maydis*, seems to be evolving faster than other lineages and may be responding to the extensive use of resistant

varieties in the Nile River Delta (Dr. J. Leslie, personal communication). New sources of resistance continue to be identified and commercial cultivars developed (El-Shafey *et al.*, 1988; Soliman and Sadek, 1998). Inbred lines Gm.4, Gm.5, Gm.6, Gm.13 and Gm.26 exhibited late wilt resistance and high yield characteristics, offering the potential for developing new lines, while (Gm.26 x Gm.30) was the most superior cross with a resistance rating of 99% (Soliman and Sadek, 1998). New resistant lines also have been developed in India, including X102, CM111, CM202, and (CM104 x WL) (Satyanarayana, 1995).

Maize germplasm in Egyptian resistance breeding programs has been challenged primarily with isolates from two (II and IV) of the four genetic lineages (Zeller *et al.*, 2000). *C. maydis* lineages differ in their ability to colonize maize plants and in their relative aggressiveness in single culture inoculations (El-Assiuty *et al*., 1998; 1999; Zeller *et al*., 2002). While lineage IV is highly virulent when inoculated alone on some cultivars resistant to lineages I-III, it is ineffective when applied as mixed inocula containing all lineages. All four lineages of the pathogen should be used to challenge host material during the development of resistant germplasm (Saleh *et al.*, 2003). Standard breeding protocols that screen for resistance to late wilt using mixed inocula may need to be modified. Separate screening tests need to be conducted using single lineages, at least with strains of lineage IV, and perhaps with II, in order to accurately select maize resistant lines (Zeller *et al.*, 2002).

Limited information is available on the inheritance of resistance. Most studies were conducted in the 1970s using traditional quantitative genetic approaches. With the exception of one study (Shehata, 1976b), which claimed resistance to be controlled by a single dominant gene, the interaction of maize with *C. maydis* appears to be under polygenic control. Labib (1972) indicated that resistance was partially dominant and estimated five loci were controlling resistance to late wilt. El-Morshidy (1974) demonstrated resistance to late wilt was additive with at least three loci controlling resistance. Shehata and Salem (1972) and El-Iriby *et al.* (1984) concluded that at least 3 major genes contributed to resistance. Dominance and epistasis have been cited as major contributors to resistance, with additive effects of lesser importance (Shehata and Salem, 1972; Shehata, 1976). However, Galal *et al.* (1979) concluded that resistance was primarily the result of additive gene action. Given the information available, it may be concluded that all of the above mechanisms probably play a role in the inheritance of late wilt resistance.

Physiological mechanisms of resistance and susceptibility to late wilt in maize have been examined (Kamal *et al.*, 1972). Roots of resistant varieties were unaffected by fungal metabolites, their root sap impaired *C. maydis* growth, and their root zones encouraged the growth of antagonistic bacteria and fungi. In contrast to susceptible roots, resistant roots had a more moderate elongation rate, more lateral roots and closely arranged exodermal and endodermal cells.

2. Cultural Control

Effectiveness of containment and quarantine regulations in place to contain the pathogen are dependent on rapid identification and eradication. Soil solarization, balanced soil fertility, and flood fallowing have been partially effective.

Inoculum survival is restricted to the top 20 cm of soil and survival depends primarily on persistence of parasitically infected host remains (Sabet *et al*., 1970a). Sanitation measures such as deep tilling may have a significant impact on disease. In Egypt, double- and triple-cropped maize fields are plowed at least annually (Dr. J. Leslie, personal communication). The use of no-till corn systems in the U.S. could potentially result in the build up of inoculum in the soil.

Hot water dips (60°C) for 10-15 minutes suppressed the development of late wilt from infected seed (Sabet *et al.*, 1966b) but are not generally practical except for breeding stock. The growth of *C. maydis* in soil is sharply inhibited by temperatures above 35°C (Sadik, 1974). Soil solarization, using transparent polyethylene sheets to raise soil temperatures, was effective in reducing late wilt over three seasons in Egypt (Fayzalla *et al.*, 1994), but there are few areas in the U.S. Corn Belt where this would be practical.

Early sowing of maize in Egypt reduced late wilt (El-Shafey *et al.*, 1988), but planting in late summer reduced disease incidence in India (Singh and Siradhana, 1988). Reduced late wilt in relation to planting time might be attributable to unfavorable environmental conditions such as low rainfall (Singh and Siradhana, 1988).

Balanced soil fertility can reduce the severity of late wilt but does not provide complete control. In the field, nitrogen fertilization (60kg N/ha) increased late wilt (Singh and Siradhana, 1990), but resulted in overall higher maize yields (Samra *et al.*, 1972, Abdel-Rahim *et al.*, 1984). However, higher doses of nitrogen reduced late wilt infection (120kg

N/ha) in the field (Singh and Siradhana, 1990). Late wilt was also reduced by potassium in the greenhouse (Abdel-Rahim *et al.*, 1984) and field in India (Singh and Siradhana, 1990), but not in Egyptian fields (Samra *et al*., 1966; Samra *et al.*, 1972) where soils already contain high levels of potassium. Phosphorus applied alone or in combination with potassium (Singh and Siradhana, 1990), organic amendments such as straw, cotton cakes and brodrett (Abdel-Rahim *et al.*, 1984) and micronutrients (Cu, Fe, Mn, and Zn at 10-20kg/ha) also reduced disease severity (Singh and Siradhana, 1990).

Intercropping soybean or cowpea with maize reduced stalk rot (a disease complex in which *C. maydis* is an important component) in the field (Samra *et al.*, 1972), but increased stalk rot caused by *Fusarium moniliforme* (Botros *et al.*, 1990). Reductions of late wilt were inconsistent from one year to the next where cowpea (with or without rhizobium) and maize were intercropped (Abdel-Rahim *et al.,* 1984).

Maize did not develop late wilt when preceded by a paddy-cultivated rice crop (Samra *et al.*, 1966). The results were attributed more to paddy-cultivation than properties of the rice. *C. maydis* is sensitive to lack of oxygen (Samra *et al.*, 1966). Thus crop rotation with rice or flood-fallowing, where possible, may be a useful cultural control method.

Contradictions exist in the literature concerning the effect of soil moisture on late wilt. Moisture stress is a major predisposing factor to late wilt (Abdel-Rahim *et al.*, 1998). In Egypt, frequent watering reduced late wilt infection in the greenhouse and saturated soils lessen the incidence of *C. maydis* (Samra *et al.*, 1966). In arid areas of India, the natural incidence of late wilt is highest when rainfall is above average or irrigation frequent (Singh and Siradhana, 1988). A 10-day irrigation schedule significantly reduced late wilt in India and improved yields over 12% compared to a 7-day irrigation schedule (Satyanarayana, 1996); a 9-day-irrigation interval was beneficial in Egypt (Samra *et al.*, 1966).

3. Chemical Control

In India, captan, carbendazim, carboxin and thiram seed treatments significantly reduced late wilt severity and increased yields 22-91% in the field (Begum *et al.*, 1989; Satyanarayana and Begum, 1996). Captan (Captaf® at 1g/kg seed) was most effective and provided the best economic return to growers (Begum *et al.*, 1989). Seed treatments consistently failed to control late wilt in Egyptian trials (Abdel-Rahim *et al.,* 1982; El-Assiuty,

1976; Sabet *et al.*, 1972; Shata *et al.*, 1984) perhaps due to differences in the virulence or chemical sensitivity of *C. maydis* isolates or the complexity of the stalk-rot disease complex in Egyptian soils. Systemic fungicides and their fungitoxic products translocate to maize leaves within 2 days and can persist in maize roots for 90 days (Abdel-Rahim *et al.*, 1982), so *C. maydis* is suppressed by these chemicals within the root, as well as in the soil (Shata *et al.*, 1984). Soil treatments with systemic fungicides, such as benomyl, carbendazim, and thiophanate-methyl successfully controlled late wilt in experiments conducted in pots but results were generally disappointing in the field (Abdel-Rahim *et al.*, 1982; Sabet *et al.*, 1972; Shata *et al.*, 1984; Singh and Siradhana, 1989). Lack of success has been attributed to reduced absorption of systemic fungicides by maize in the field compared to experiments in pots (Sabet *et al.*, 1972). Vitavax plus thiram decreased stalk rot incidence when applied simultaneously as a seed and soil treatment but yields were not significantly improved (Shata *et al.*, 1984). Benlate (5-10kg/acre) applied at 30 days post sowing resulted in increased yields without reducing disease incidence (Abdel-Rahim *et al.*, 1982). The best control was achieved with four applications of Benlate (2.5 kg/acre) at 15-day intervals commencing at sowing (Abdel-Rahim *et al.*, 1982; El-Assiuty, 1976). The cost and labor required for frequent fungicide applications make this control method prohibitive in the U.S.

4. Biological Control

Inoculations with mixtures of stalk rot pathogens reduced late wilt incidence compared to inoculations with *C. maydis* alone (Singh and Siradhana, 1988). *C. maydis* is known to be a poor saprophytic competitor (Sabet *et al.*, 1970a). Disease decreased significantly when *C. maydis* was mix inoculated in pots with *M. phaseolina* (Singh and Siradhana, 1988) and *Trichurus spiralis* (or its filtrate) (Abdel-Hamid *et al.*, 1981). *Bacillus subtilis, Pseudomonas florescens* and *Verticillium tricorpus* have also been evaluated as biological control agents (El-Assiuty *et al.,* 1991). The success of biological control on a large scale has not been reported.

5. Modeling Disease Incidence and Spread

No predictive models are known that forecast the spread and distribution of late wilt of corn.

VIII. Knowledge gaps

Important gaps in our present knowledge include:

1. Is the climate of the U.S. Corn Belt able to support *C. maydis*? Our temperate climate with cold winters differ considerably from the climates of Egypt (arid, hot) and India (arid, hot or savannah), the only regions reported to experience serious epidemics of late wilt. Although late wilt has been reported in Hungary, its persistence and the extent of damage caused are unknown.

2. Which *Lupinus* spp. present in the U.S. can act as hosts for *C. maydis* and is the pathogen seed-borne in lupine?

3. What role do soil structure and other organisms play in disease development and the "stalk-rot complex"? Will the soil ecosystem in the U.S. favor late wilt?

4. What importing countries would be likely to embargo *C. maydis* contaminated corn?

5. To what extent are U.S. hybrids presently in use susceptible to *C. maydis*?

IX. Immediate response options

Because *C. maydis* dissemination would be moderately slow, containment and eradication may be possible if the pathogen was introduced into a localized area. Effectiveness of containment and quarantine actions are dependent on rapid identification of the pathogen and eradication by timely implementation of control measures. Because of the seed-borne potential of late wilt, seed nurseries could not operate within the containment quarantine area.

A late wilt pathway and response summary for the intentional introduction of *C. maydis* is presented in Appendix 4.

1. Rapid Detection

At present, there is a low likelihood of early detection because first responders are not familiar with late wilt symptoms and symptoms only appear late in crop growth. The odds of

early detection may be improved by incorporating late wilt symptom recognition into continuing education courses and workshops for first responders. Increasing awareness of the potential for agroterrorism and farm biosecurity through classes, newsletters, and internet will also be useful.

Once samples are submitted, diagnosis is complicated by difficulties in isolating *C. maydis* in dying maize tissue. Molecular techniques (PCR-based) may make more rapid identification possible (Saleh and Leslie, unpublished) when commercially available. A test run to determine the time required for *C. maydis* identification by the National Plant Disease network (NPDN) would illuminate potential difficulties.

Assembling a "Detection Assessment Team" with expertise on late wilt, as has been done by APHIS for soybean rust, is a preparatory step that can minimize the time required for on-site threat assessment. A team of USDA and university experts should arrive at the site of infection within 24 hours following APHIS confirmation of pathogen identification.

2. Cultural Control

As a sanitation measure, no-till corn systems may need to return to tillage to prevent *C. maydis* inoculum from building up in affected areas. Flood fallowing to destroy sclerotia, may be effective in eliminating late wilt over a limited acreage, but is not practical for most of the Corn Belt.

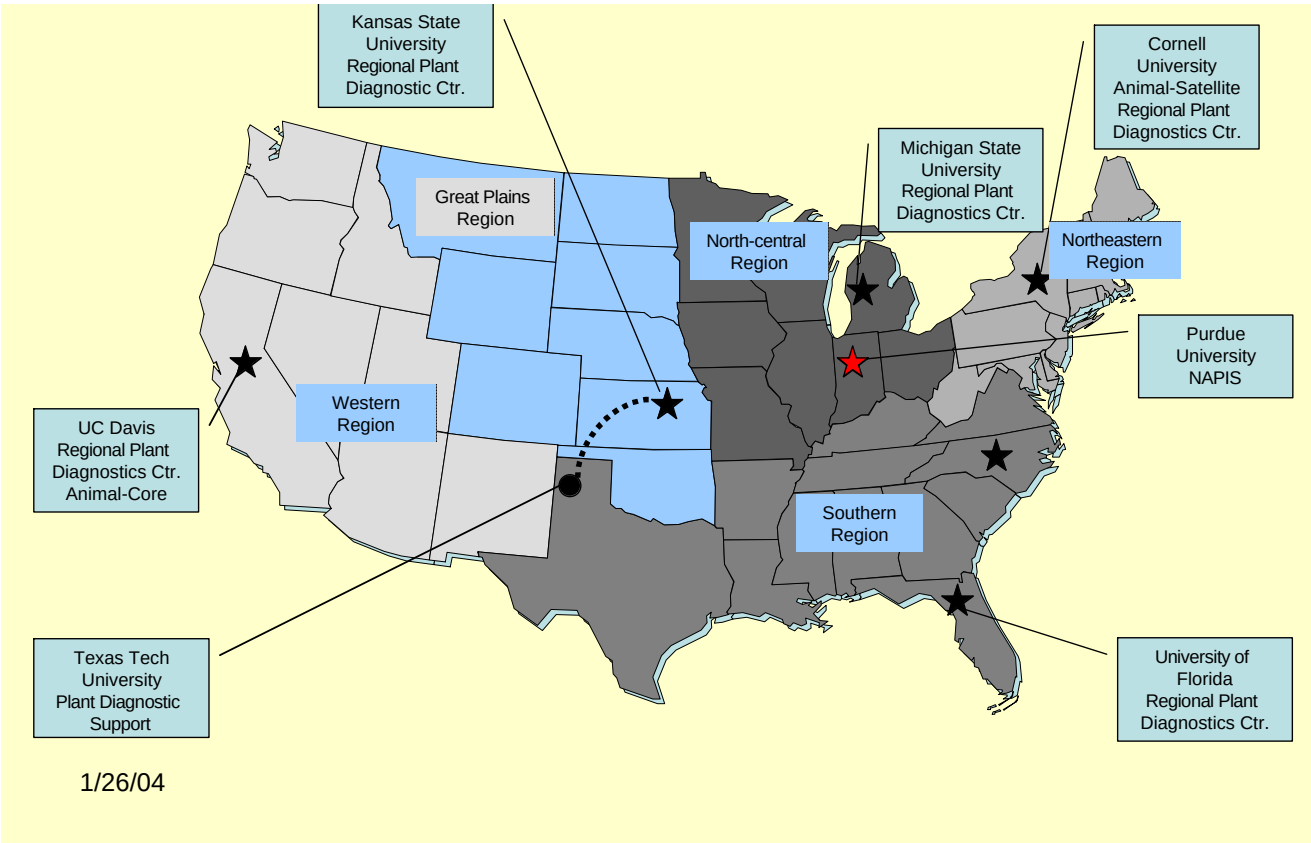
3. Fungicides

Some fungicides were effective under Indian conditions against the India strain of *C. maydis* but failed to provide satisfactory late wilt control in Egypt. Efficacy testing in the U.S. would be required to determine the most effective chemicals and application methods. Fungicides (e.g. Benlate) may prove useful to contain late wilt within an area. The economic practicality of fungicide use against late wilt in the U.S. is questionable on a large scale.

4. Resistance Breeding

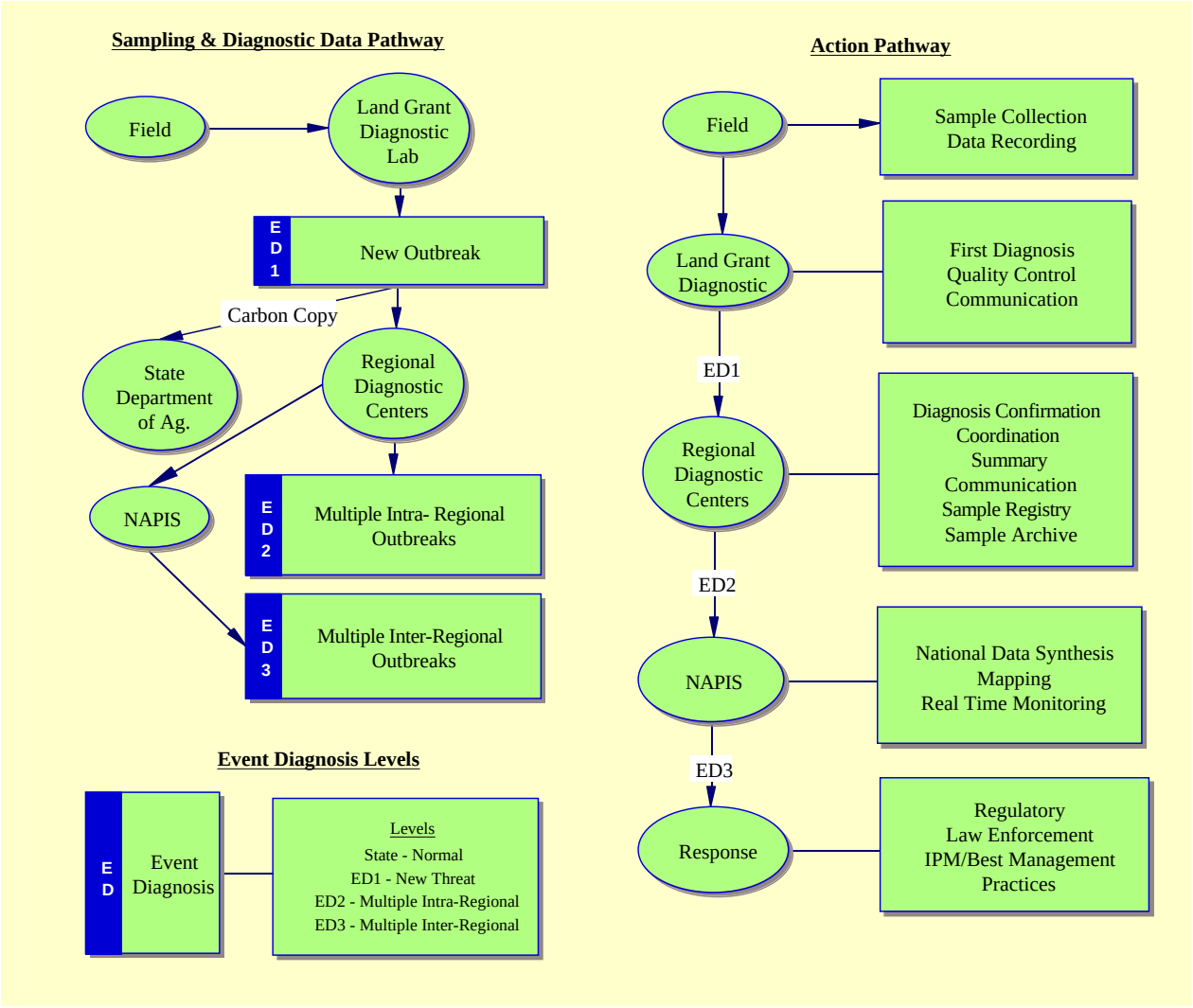
Ultimately, the only economically feasible means to control widespread late wilt is the development and use of resistant maize lines (Zeller *et al.*, 2000). The release of resistant maize varieties and hybrids has significantly reduced late wilt in Egypt (El-Shafey *et al*., 1988). In the U.S., there has been little evaluation of late wilt resistance in commercial breeding programs. The evaluation of U.S. germplasm for late wilt resistance and incorporation into public and private breeding programs should be considered as a long-range response plan because resistant cultivars should be introduced as rapidly as possible to minimize damage after introduction of late wilt to the U.S.

Appendix 1A. National Plant Diagnostic Network (NPDN) regions and regional centers



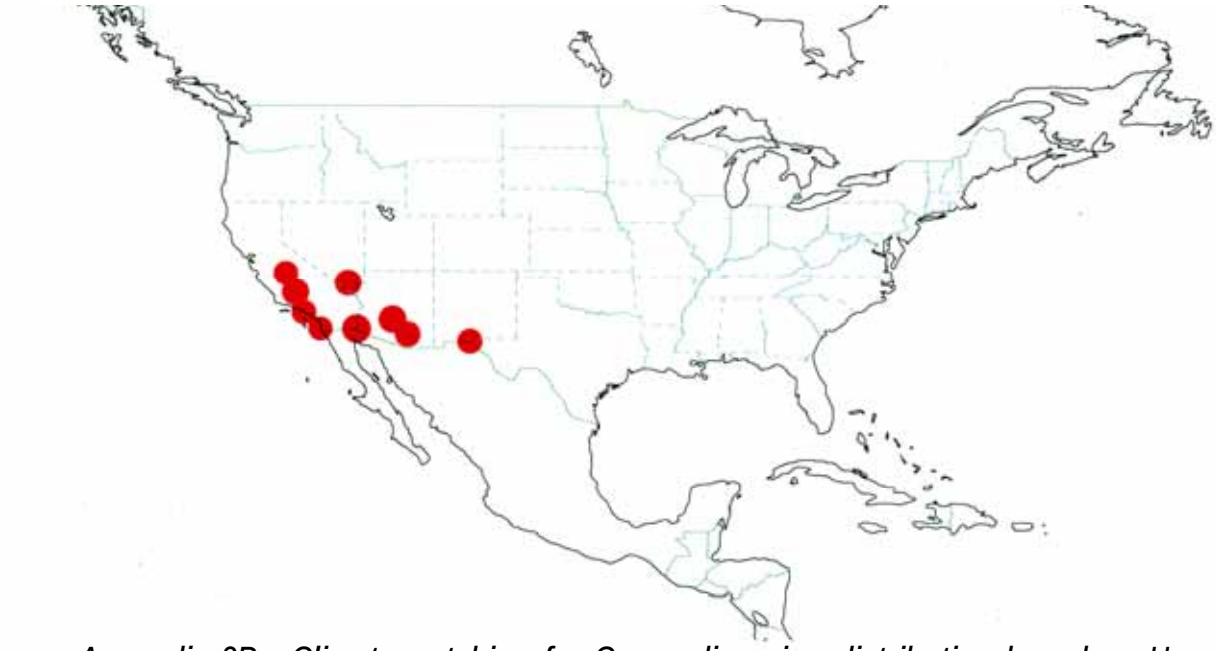
From Cardwell (2004)

Appendix 1B. Sampling + diagnostic data and action pathways for NPDN



From Cardwell (2004)

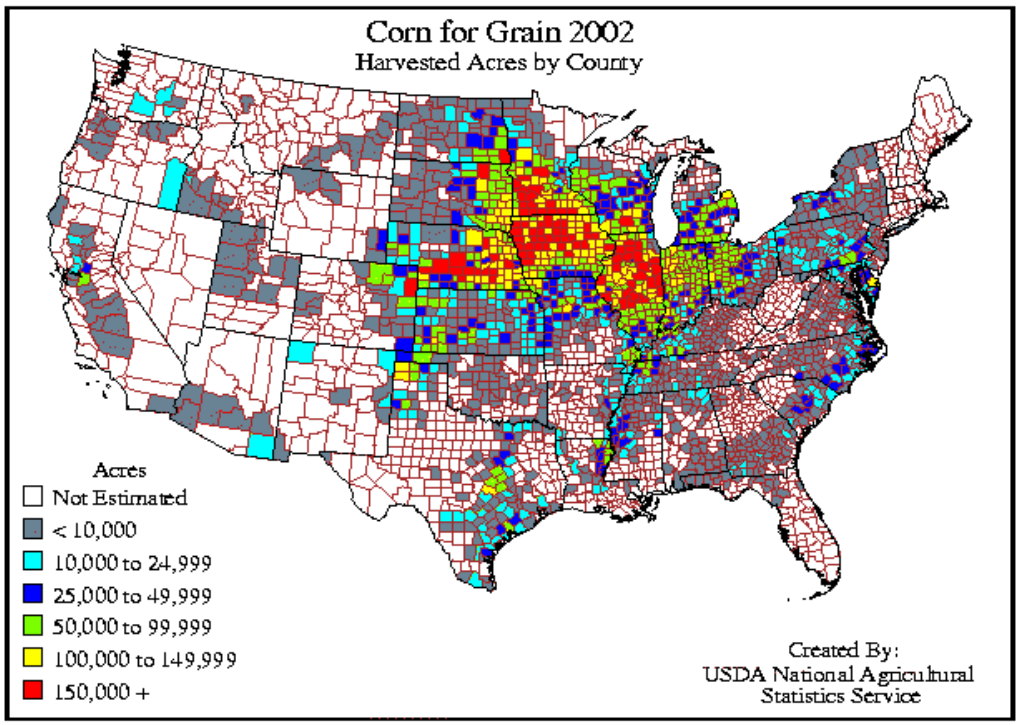
*Appendix 2A. Climate-matching for **C. maydis** using present species distribution of 5 locations (represented by Cairo, Alexandria, Hyderabad, Budapest and Jerusalem).*



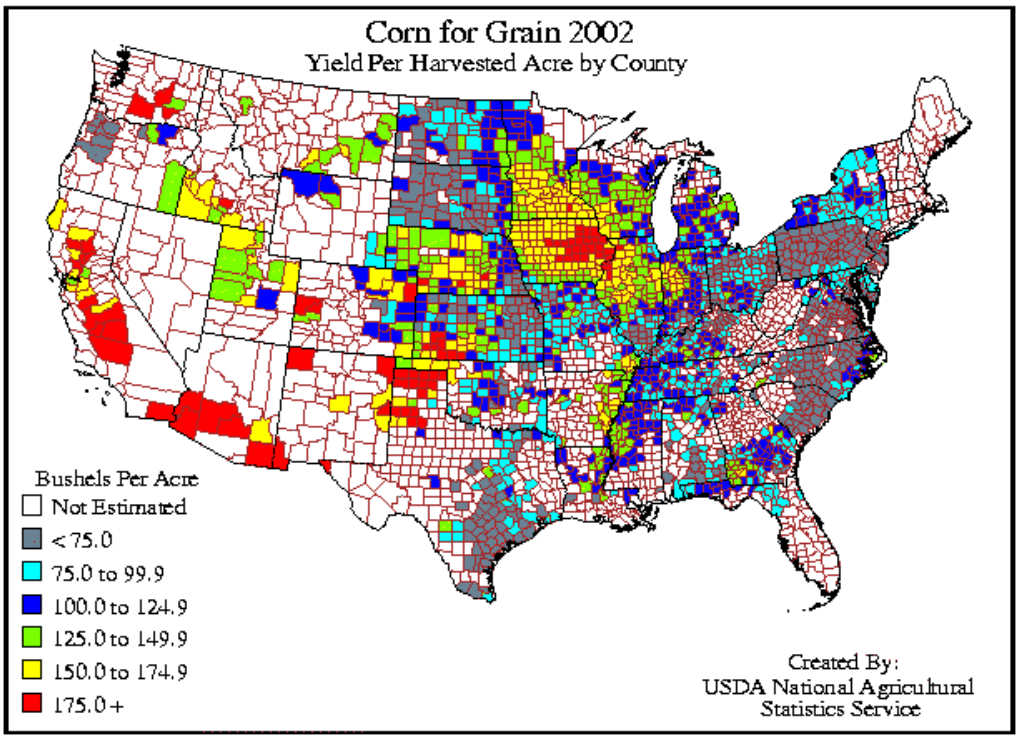
Appendix 2B. Climate-matching for C. maydis using distribution based on Hungary (Budapest location).

Maps were generated using the simulation model software CLIMEX (Sutherst and Maywald, 1999). Dots represent locations in the U.S. that match 75% of climatic parameters in the locations of origin specified.

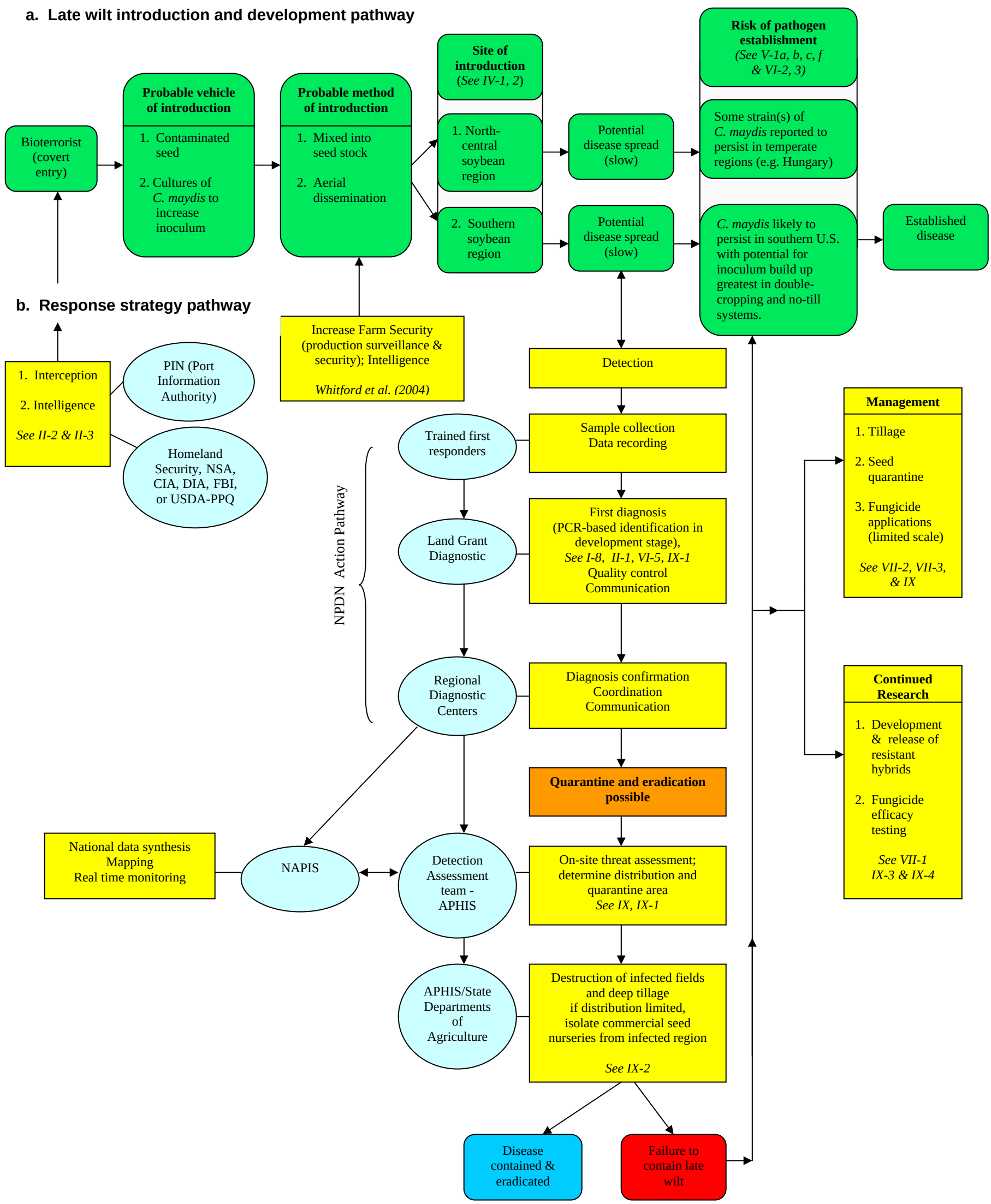
Appendix 3A. Harvested corn for grain acres in 2002



Appendix 3B. Yield/acre of corn in 2002



Appendix 4. Pathway and response to the intentional introduction of C. maydis, cause of late wilt of corn



Appendix 5. Scientists Knowledgeable of Late Wilt of Corn

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Philippine Downy Mildew of Corn (Maize)

Pathway Analysis:

Intentional Introduction of

Peronosclerospora philippinensis

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Philippine Downy Mildew Pathway Analysis

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Executive Summary: Philippine Downy Mildew Pathway Analysis

- Introduction of *Peronosclerospora philippinensis,* causal agent of Philippine downy mildew (PDM), poses a low to moderate threat to the U.S. The pathogen is currently identified in the Agricultural Bioterrorism Protection Act of 2002 (APHIS, 2002).

- PDM is one of the most damaging of ten species causing downy mildews on corn. PDM's economic impact has been greatest in the Philippines, with yield losses in individual fields ranging from 40 – 100%.

- Conidia are the primary propagule for PDM initiation and dispersal. Spores disseminate only short distances (a few meters) and oospore (long-term survival propagule) production is limited. Substantial evidence (see I-1b) indicates that *P. philippinensis* and *Peronosclerospora sacchari* are conspecific.

- A number of alternate hosts of *P. philippinensis* exist in the U.S. that may serve as overwintering reservoirs (Appendix 1), and include *Sorghum halepense* (johnsongrass), a perennial weed prevalent in the southern two thirds of the U.S. Overwintering of *P. philippinensis* in systemically infected perennial host tissue, such as johnsongrass rhizomes, is speculated but has not been demonstrated.

- A **PDM disease pathway and response schematic (Appendix 8)** summarizes findings.

- Disruption of export markets could be accomplished with a relatively small volume of conidia, infected plant material, or seed used to introduce the pathogen. Natural increase and dissemination occurring over a period of years could affect substantial areas. A small vial could contain enough conidia to inoculate several hundred-corn seedlings.

- A substantial initial impact on yield would require large volumes of inoculum distributed over an extensive area. This would necessitate inoculum production in living maize, sugarcane, or weed host plants and an isolated production area. Production, storage

and widespread distribution during the narrow infection window would require considerable knowledge and timing. A single source plant can produce sufficient inoculum to infect over 4 acres.

- *P. philippinensis* could likely survive an initial introduction in the near-optimum summer climate of the U.S. Corn Belt. Persistence of this obligate pathogen will depend on the availability of alternate hosts.

- Natural increase and secondary spread of the pathogen could infest up to 30% of the 79 million U.S. corn producing acres within 3-6 years. Southern areas with high humidity are at greatest risk for economic damage, with subsequent spread along the Mississippi and Tennessee River valleys. Late spring flooding along rivers could carry conidia or oospores considerable distances across "river bottom" farmland.

- Corn is the most economically important crop produced in the U.S., with a grain value of $21.2 billion in 2002. Economic impacts comparable to those in the Philippines are not expected in the U.S. because continuous cropping produces large inoculum loads year round in the Philippines.

- The impact of potential long-term quarantines and an embargo imposed by other nations on U.S. corn seed could be substantial and require phytosanitary certification of U.S. seed with its associated costs.

Immediate Response Options

- PDM introduction into the southern Corn Belt would require rapid action. The slow to moderate dissemination rate of *P. philippinensis*, could permit containment and eradication if it is introduced into a localized area, rapidly detected and control measures were quickly implemented.

- Since symptoms are not definitive, first responders may misidentify PDM as an indigenous maize downy mildew, such as *Peronosclerospora sorghi* (sorghum downy mildew). DNA hybridization and PCR techniques are in the development stage (see VI-5). A "Detection Assessment Team", with expertise on PDM similar to that created by APHIS for soybean rust, could minimize on-site assessment time. Once a containment

area is identified, eradication by plowing, chemical desiccation, or burning may be required.

- Fungicides are effective against PDM and virtually all commercial corn seed in the U.S. is treated with metalaxyl or mefenoxam; however, the low rates currently applied (1 - 4 g a.i./100 kg seed) will not effectively curtail PDM. Much higher rates of metalaxyl or mefenoxam seed treatments may successfully control PDM the year after introduction. Additional research is required to determine optimum fungicide concentrations and strategies to minimize the development of fungicide resistance.

- Development of resistant maize lines is the most economically feasible means to control PDM. U.S. hybrids are moderately to severely susceptible to PDM. The incorporation of PDM resistance into U.S. germplasm should be considered in the long-range response plan.

Philippine Downy Mildew of Corn (Maize)

Pathway Analysis for the Intentional Introduction of *Peronosclerospora philippinensis*

Philippine downy mildew (PDM), caused by *Peronosclerospora philippinensis*, is considered one of the most damaging of ten species causing downy mildews on corn. Its economic impact has been greatest in the Philippines with yield losses in individual fields ranging from 40 – 100% (Exconde and Raymundo, 1974). Although PDM does not occur in the U.S., there has been long standing concern over its introduction and potential threat to U.S. corn. As a result, USDA scientists have significantly contributed to our understanding of PDM and its potential threat, from the classic synthesis by A.H. Weston (1920) to the further elucidation of epidemiological requirements and host range by the Bonde-Peterson-Melching group. In 2002, *P. philippinensis* was placed on the USDA-APHIS select agents list (APHIS, 2002). This report is a pathway analysis for the intentional introduction of *Peronosclerospora philippinensis* into the U.S. A summary, in the form of a disease pathway and response schematic, is presented in Appendix 8.

I. Biology and life/disease cycle of the pathogen

1. Identity

a. Preferred name: *Peronosclerospora philippinensis* (*W. Weston) C.G. Shaw (most authors)*

Peronosclerospora sacchari (T. Miyake) Shirai and Hara (see I-1b)

Sclerospora philippinensis (CABI, 1999)

Taxonomic position:

Kingdom:	Chromista
Phylum:	Oomycota
Order:	Sclerosporales
Family:	Sclerosporaceae

Common names: Philippine downy mildew

b. Taxonomy and Nomenclature: relationship to Peronosclerospora sacchari

In most of the literature, the causal agent of PDM is referred to as *Peronosclerospora philippinensis* and will be referred to as such in this report. Substantial evidence suggests that *P. sacchari* and *P. philippinensis* are conspecific.

P. philippinensis and *P. sacchari* (causal agent of sugarcane downy mildew) are indistinguishable in morphology of conidia and conidiophores (Bonde *et al*., 1984a), environmental requirements for sporulation, germination, and infection (Bonde and Melching, 1979), by isozyme analysis (Bonde *et al*., 1984b; Micales *et al*., 1988), and by DNA hybridization (Yao, 1991; Yao *et al*., 1991a). Two cultures of *P. philippinensis* from the Philippines, and one of *P. sacchari* from Taiwan, had all 16 alleles in common indicating that they are probably the same species (Bonde *et al*., 1984b). This was confirmed by electrophoresis when six *P. sacchari* and eight *P. philippinensis* isolates exhibited identical phenotypes for 22/26 enzymes (Micales *et al*., 1988). In four cultures of the *P. sacchari* - *P. philippinensis* complex, apparent polymorphisms (allelic variations) were evident in six of 13 loci examined and thus considerable potential for variation exists (Bonde *et al*., 1984b). Identical RFLP patterns on Southern blots of *P. philippinensis* and *P. sacchari* isolates also support this conclusion (Yao *et al*., 1991a; Wang *et al.*, 1994).

Originally, *P. sacchari* and *P. philippinensis* were classified as separate species based primarily on the inability of *P. philippinensis* to infect sugarcane and produce oospores (Weston, 1920). A more recent study (Bonde and Peterson, 1983) revealed that *P. philippinensis* can infect sugarcane and these two pathogenic species have a remarkably similar host range. Therefore, the classification of *P. sacchari* and *P. philippinensis* as separate species, should be reevaluated (Duck *et al*., 1987; Micales *et al*., 1988). Since *P. sacchari* is the type species in the genus *Peronosclerospora*, it has been suggested this name be maintained and *P. philippinensis* discarded (Yao, 1991; Wang *et al.*, 1994).

2. Host Range

Although corn, sorghum, and cane are primary hosts, *P. philippinensis* has a very broad host range within the Gramineae (Appendix 1). The potential host range of *P. philippinensis* was investigated in two extensive inoculation studies. In the Philippines, 8 species out of 76 tested displayed systemic symptoms: *Avena sativa*, *Euchlaena mexicana, E. mexicana* X *Z. mays* hybrid, *Saccharum spontaneum*, *Saccharum officinarum*, *Sorghum bicolor*, *Sorghum halepense*, and *Sorghum propinguum* (Exconde *et al*., 1968a). In a second investigation of 72 gramineous species, 23 species were susceptible *i.e.*, 19 species in 6 genera in the tribe Andropogoneae and 4 species in the tribe Maydeae (Bonde and Peterson, 1983). Some accessions susceptible to one isolate of *P. philippinensis* were not susceptible to another (Bonde and Peterson, 1983).

Collateral hosts can play a major role in the epidemiology of PDM as sources of primary inoculum and reservoirs to maintain the pathogen during unfavorable periods (Bonde, 1982). Sources of infection in the Philippines are diverse. *Saccarum officinarum*, *S. spontaneum*, *Sorghum bicolor*, *S. halepense* and *S. propinquum* could serve as an important reservoir of *P. philippinensis* because of their abundance in and around cornfields (Exconde *et al*., 1968a; 1968b). In India, the main source of infection for maize plants is *Saccharum spontaneum* (kans grass or wild sugarcane) that exhibit the disease 7-10 days before adjacent maize crops (Chona and Suryanarayana, 1955; Payak, 1975). The absence of susceptible hosts during the dry season in Nepal is credited with limiting the disease to a few districts (Shah, 1976).

A number of host species of *Andropogon*, *Bothriochloa* and *Schizachyrium* are common wild grasses or perennial forage in the U.S. *Andropogon gerardii* (big bluestem) and

Schizachyrium scoparium (little bluestem) are particularly important components of wild hay. *Sorghum halepense* (johnsongrass) is a common perennial weed, prevalent in the southern two thirds of the U.S. Perennial grasses could potentially be reservoir hosts of *P. philippinensis* in the U.S. if infected plants survive the winter season (Bonde and Peterson, 1983). In Israel, *Peronosclerospora sorghi* (agent of sorghum downy mildew) can overwinter in the rhizomes of some johnsongrass lines (Kenneth and Klein, 1970) and johnsongrass is considered an endemic source of *P. sorghi* in the U.S. In the central and southern U.S., johnsongrass spreads by rhizomes, but in northern areas, spread occurs only via seed because rhizomes do not tolerate freezing temperatures well. The ability of *P. philippinensis* to overwinter in systemically infected perennial weed host tissue, such as johnsongrass rhizomes, is speculated but has not been demonstrated.

3. Geographic Distribution and Economic Impact

PDM was initially reported in India in 1912 (Payak, 1975). The disease has been present in the Philippines since 1916 (Weston, 1920), where it can be extremely damaging. According to CABI (1999), PDM is currently reported in China, Japan, India, Pakistan, Indonesia, Nepal, Philippines, Thailand, Mauritius and South Africa (Fig. 1). Reports of its presence in the U.S. by CABI (1999) are erroneous (Dr. H. D. Thurston, personal communication).

PDM is considered the most virulent of the downy mildew pathogens on corn. The disease is usually not severe in India, but losses of up to 60% have been reported (Bains and Jhooty, 1982; Bonde, 1982; Payak, 1975). In the Philippines, crop losses for 1974-1975 were 8%, or 205,470 metric tons valued at U.S. $23,000,000 (Exconde, 1976), with losses in individual fields ranging from 40–100% (Exconde and Raymundo, 1974). Yield losses are directly proportional to the percentage of plants infected (Exconde and Raymundo, 1974). PDM is more damaging in wet years than dry, but serious epidemics can occur in all seasons. Continuous cropping of maize in both wet and dry seasons likely contributes to epidemics in the Philippines (Payak, 1975). Even with the advent of resistant varieties and effective fungicides, substantial losses to this downy mildew were experienced in the Philippines during the 1990s (Raymundo *et al*., 1993; Raymundo, 2000). Downy mildews continue to be the top biotic constraint limiting maize productivity in Asia (Pingali, 2001)

In sugarcane, *P. philippinensis* has caused yield losses of 36% (Husmillo, 1982).

4. Symptoms

Symptom expression induced by *P. philippinensis* varies according to plant age at time of infection, inoculum concentration, isolate, host genotype and environmental conditions (Weston, 1920). General symptoms include chlorotic areas or stripes on leaves covered with grayish-white mycelia and deformed maize parts. Downy sporulation is a key identification characteristic of the disease (Weston, 1920) and is most abundant on the underside of leaves (Fig. 2).

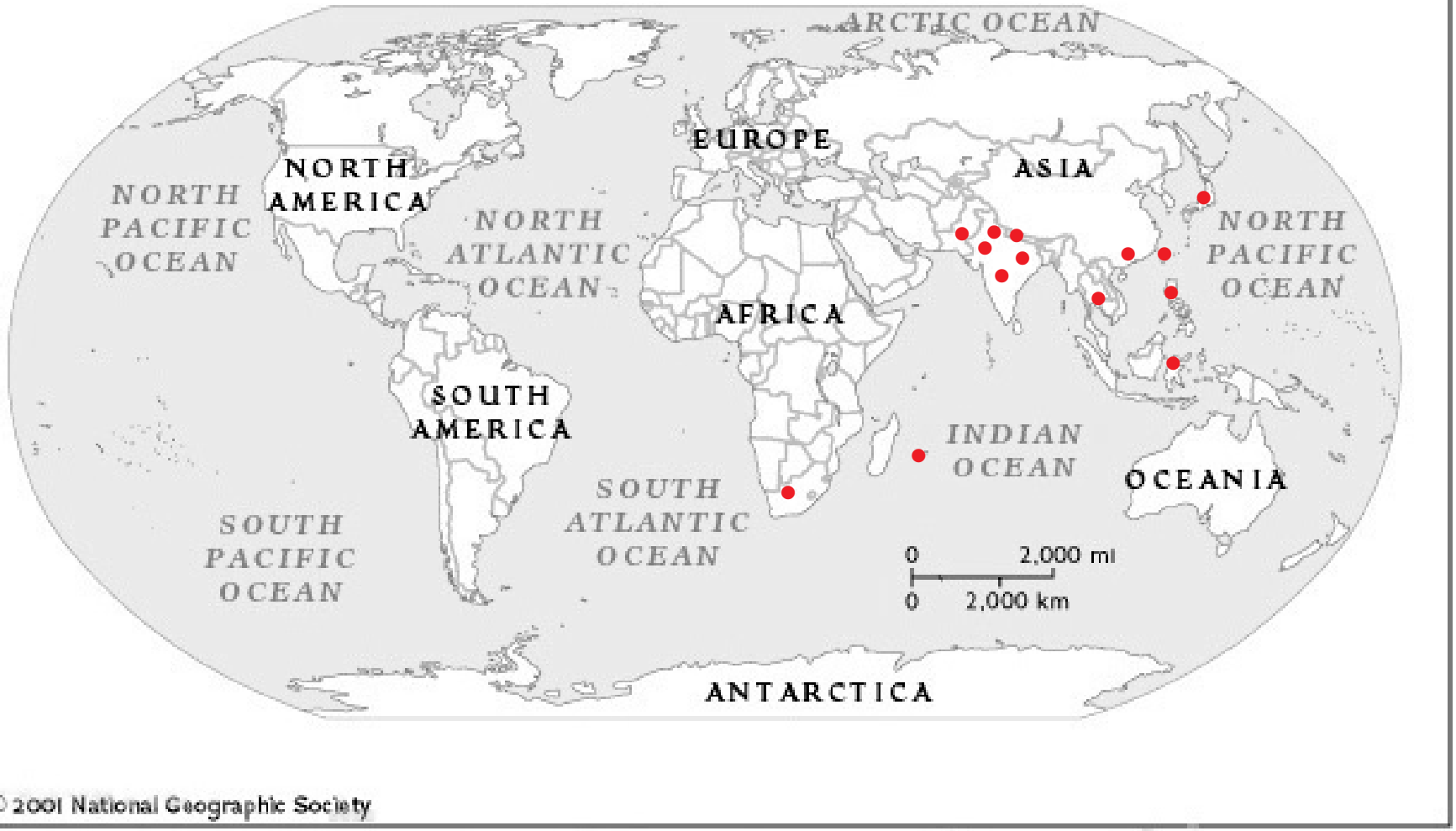
Systemic symptoms appear on the first true leaf as chlorotic streaks or a pale yellow color throughout the entire leaf as early as 3 days after seedling inoculation (Exconde, 1976). Seedlings inoculated at 8-10 days of age, first display systemic symptoms 7-8 days later (Dalmacio and Exconde, 1969). Once the fungus is established in the shoot apex, it produces chlorotic areas that are initially confined to the base of the lower leaf but increase in size in each succeeding leaf until the youngest leaf emerging from the whorl is completely chlorotic (Exconde, 1976). Local symptoms of long narrow chlorotic stripes with a grayish-white downy growth of conidia and conidiophores appear between the two-leaf stage and tasseling.

Early infected seedlings may die or are stunted with narrow, stiff, leaves (Fig. 3). In later infected seedlings, growth is relatively unaffected, but chlorotic striping intensifies on succeeding leaves (Fig. 4 & 5). As late infected plants mature, sporulation ceases and chlorotic areas become less pronounced.

Localized and systemic symptoms can develop from seedborne infection (Advincula and Exconde, 1976). Localized symptoms on the second or third leaf occur as early as 12 days post sowing (Fig. 6). Systemic symptoms appear as chlorotic stripes in the first true leaf as early as 9 days after planting with subsequent unfurling leaves emerging partially or completely white to pale yellow (Fig. 7).

Various patterns of systemic symptoms chlorotic streaking develop among inbred maize lines (Ebron and Raymundo, 1987b). Susceptible lines tend to display long, broad chlorotic stripes on leaves, whereas chlorotic stripes on resistant lines tend to be narrow and/or broken.

Fig. 1. World Distribution of Philippine downy mildew caused by *Peronosclerospora philippinensis*



Adopted from CABI, 1999.

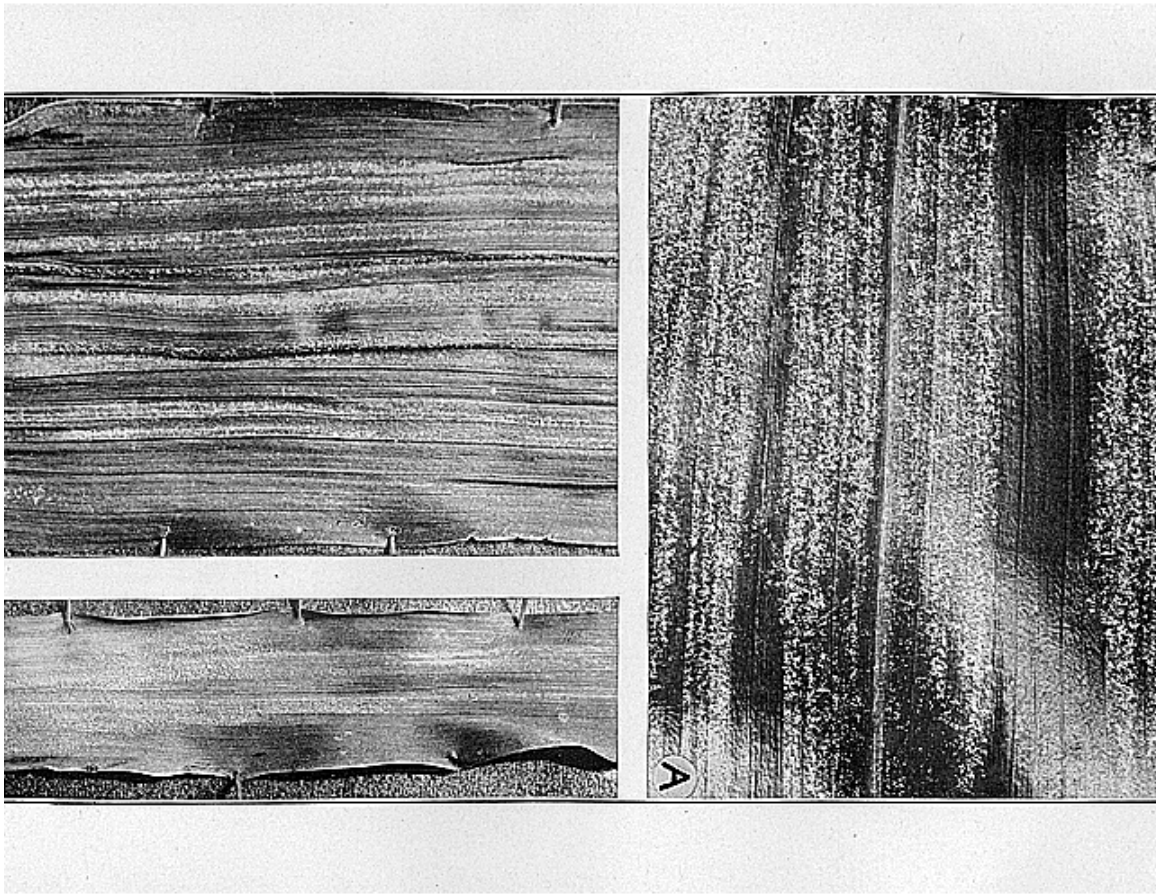


Fig. 2. Conidiophore production on the surface of three maize leaves, displaying different degrees of infection.

(Weston, 1920)

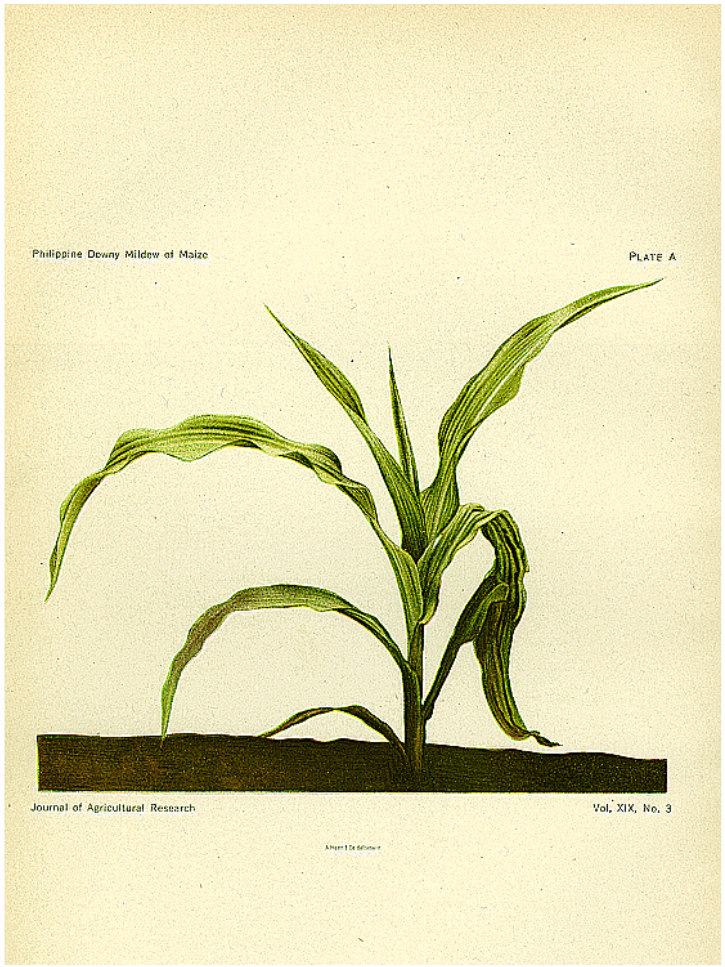


Fig. 3. Symptoms of very early attack of Philippine downy mildew on a 32-day-old corn plant. Notice the dwarfing, narrow, stiff leaves with striping throughout length. Symptoms appeared at 2 weeks.

(Weston, 1920)

Fig. 4. Symptoms of a later attack of Philippine downymildew on two right hand plants (31 days old). Symptoms appeared after 25 days. Although leaves display extensive chlorosis at of leaf base, leaves are broad and flexible and growth rate is normal.

(Weston, 1920)

Fig. 5. Typical chlorotic striping of leaves caused by *P. philippinensis* on corn.

(CIMMYT)

Fig 6. Local symptoms resulting from seedborne infection on the second or third leaf appear as long narrow chlorotic stripes on 12-day-old seedlings.

(Advincula and Exconde, 1976)

Fig. 7. Systemic symptoms resulting from seedborne infection in the form of chlorotic streaks or complete chlorosis appear in the first true leaf as early as 9 days.

(Advincula and Exconde, 1976)

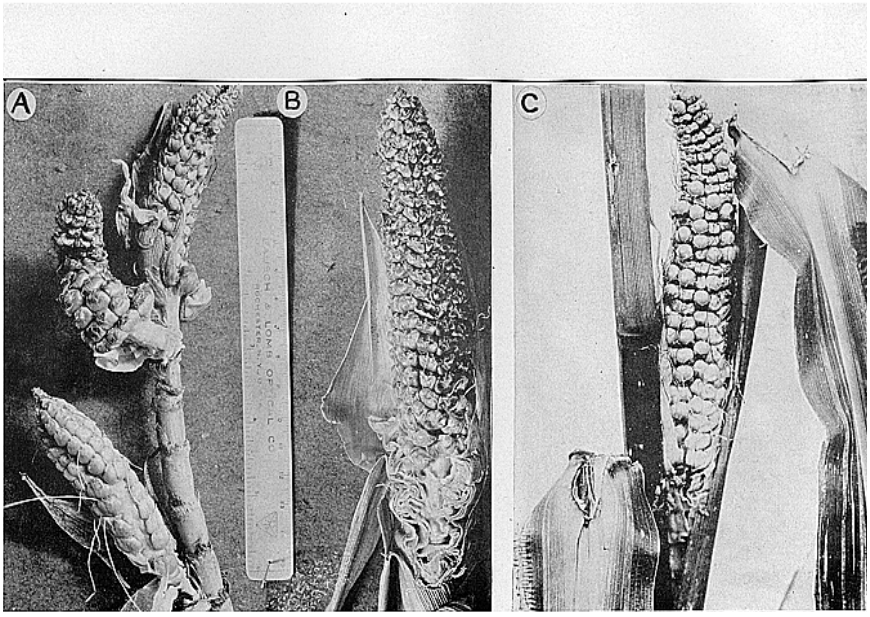


Fig. 8. Deformed and partly sterile maize ears caused by *P. philippinensis*. Many florets are sterile; only a few viable seeds may be produced.

(Weston, 1920)

Tassels on infected plants produce less pollen and ears are often aborted or small. Various malformations of tassels and ears have been reported (Weston, 1920) (Fig. 8). There are no external symptoms on infected seeds or stems. The fungus invades the stem, moving upward and downward to become established in the shoot apex, where it can be found throughout the life of the infected plant.

When new tillers on mature plants become infected, thin lower leaves display interrupted, narrow, pale yellow-green or rust-green stripes along their length; middle leaves display these markings only distally, and only the tip or none of the upper leaves display symptoms (Weston, 1920).

5. Causal organism

a. Morphology

The morphological characteristics of *P. philippinensis* vary with environmental conditions (Weston, 1920; Kimigafukuro, 1979), isolate (Josue and Exconde, 1979a) and host (Exconde *et al*., 1968a). Mycelia are branched, slender (8 µm in diameter) and irregularly constricted. Erect conidiophores grow out of the stomata (Fig. 9 & 10), are dichotomously branched 2-4 times and 15-26 x 150-400 µm (Smith and Renfro, 1999; CABI, 1999; Weston, 1920), 18-28 x 360-520 µm (Visarathanonth and Exconde, 1976), and 9-20 x 313-552 µm (Josue and Exconde, 1979a). Sterigmata are ovoid to subulate, slightly curved, and 10 µm long. Conidia (Fig. 9 & 10) are ellipsoid to round-cylindrical, hyaline, slightly rounded at the apex and 17-21 x 27-39 µm (Smith and Renfro, 1999; CABI, 1999; Weston, 1920), 11-17 x 29-39 µm (Visarathanonth and Exconde, 1976), and 10-27 x 21-52 µm (Josue and Exconde, 1979a). A rise in temperature increases the length of *P. philippinensis* conidia (Kimigafukuro, 1979).

Oospores of other downy mildew species can survive 6 -10 years (Smith and Renfro, 1999) and play an important role in dispersal and overseasoning. Oospores of *P. philippinensis* have been reported in the literature on disintegrating leaves kept in a moist chamber or buried in the soil for 6 days (Acedo and Exconde, 1967). Oospores are spherical, smooth walled, 15.3-22.6 µm in diameter, and germinate with a side germ tube (Fig. 11 and 12).

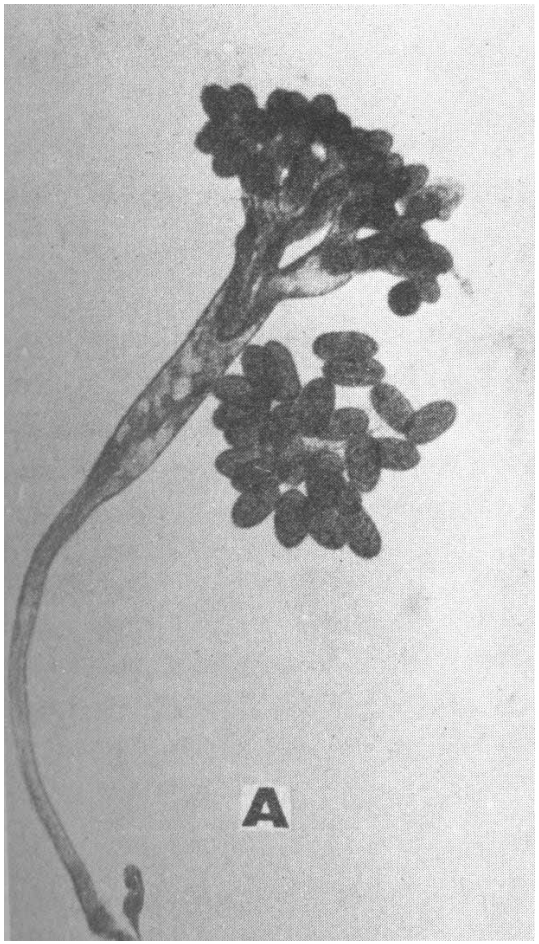


Fig. 9. Conidiophore and conidia of *Peronsclerospora philippinensis* on corn (x 211)

(Exconde *et al*., 1968a)

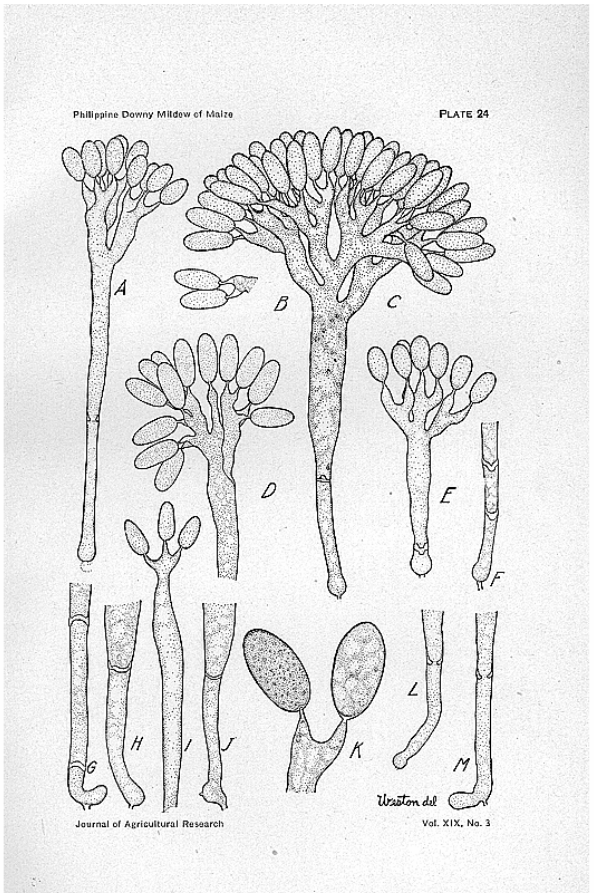


Fig. 10. *Peronosclerospora philippinensis* (x 375). A. Partially matured conidiophore from corn during heavy dew. B. Tip of branch with two conidia. C-D. Mature conidiophore from corn during heavy dew. E. Stunted conidiophore during light dew in hot, dry season. F-H, J, L. Basal cells of conidiophores. K. Sterigmata bearing conidia. M. basal cell of conidiophore from teosinte.

(Weston, 1920)

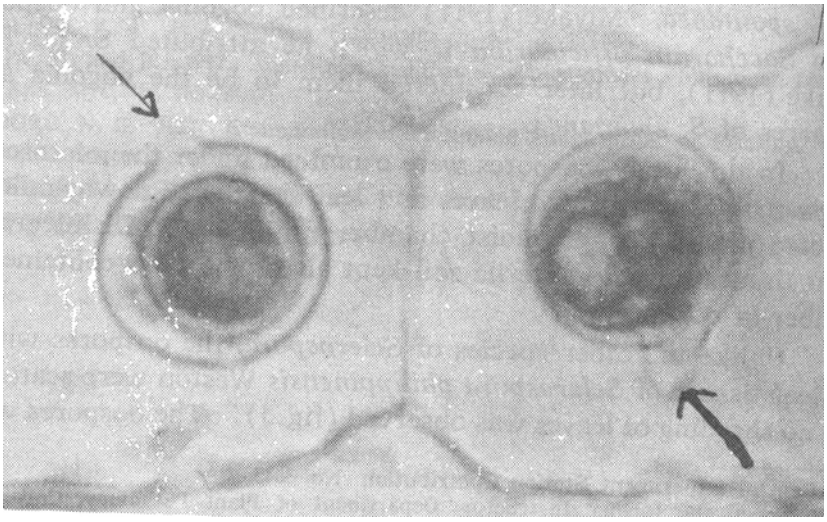


Fig. 11. Mature oospores of *P. philippinensis* (x1315). Arrow notes presence of oogonial stalk.

Acedo and Exconde (1967)

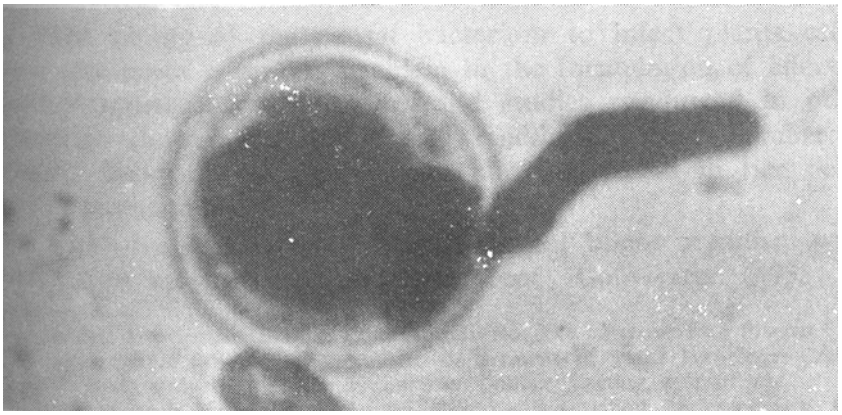


Fig. 12. Germinated oospore of *P. philippinensis* (x2105). Only a single germ tube is formed.

Acedo and Exconde (1967)

Pathogenicity tests with oospores produced infection (24-33%) on sweet corn (Exconde, 1970). In nature, oospores of *P. philippinensis* are considered "rare or nonexistent" (Smith and Renfro, 1999) and their role in epidemiology has not been elucidated (Bains and Jhooty, 1982; Bonde and Peterson, 1983; Bonde *et al*., 1984b; Frederiksen and Renfro, 1977; Weston, 1920). *P. sacchari*, which has been proposed conspecific with *P. philippinensis*, is reported to produce oospores on sugarcane (Sun *et al*., 1970; Bonde *et al*., 1984b) and less readily on maize (Payak, 1975). Thurston (1973) alluded to the potential danger *P. philippinensis* oospores may pose in long distance dissemination and persistence. Oospores have not been detected in the mature leaf tissue of any collateral host species systemically affected with *P. philippinensis* (Bonde and Peterson, 1983).

Comparative characteristics of *P. philippinensis* and downy mildew pathogens that currently infect corn in the U.S. are in Appendix 2.

b. Isolate Variability

Differences in virulence and aggressiveness were observed among 7 (Tititan and Exconde, 1974) and 15 isolates of *P. philippinensis* (Josue and Exconde, 1979b), but distinct physiological races could not be determined because differential host inbreds (each possessing a single, unique gene) were not available. Considerable isolate variability has been confirmed (Ebron and Raymundo, 1987a), indicating physiological specialization. In one study, the most virulent isolate resulted in susceptible reactions on 6/9 inbred lines and intermediate susceptibility on the other 3 (Josue and Exconde, 1979b). *P. philippinensis* isolates can readily adapt to other hosts within their host range and they generally sporulate more vigorously on their new host with the passage of time (Duck *et al*., 1987); however, sporulation did not significantly differ between 15 isolates of *P. philippinensis* maintained on maize (Josue and Exconde, 1979b).

6. Detection and Diagnosis

Diagnosis is based on the long, white to yellow streaks on leaves and "fluffy" signs of the pathogen developing on leaf streaks. The latter is only apparent in the a.m. hours until shortly after sunrise. Symptoms are not definitive for the various downy mildews. The primary characteristics used to define *Peronosclerospora* spp. are shape and size of conidia and conidiophores, host range, and presence or lack of oospores (Kimigafukuro, 1979).

Comparative characteristics with indigenous corn downy mildews are presented in Appendix 2. Morphological identification is difficult because the size and appearance of conidia and conidiophores of these species overlap and vary considerably within a pathogenic species depending on host species, cultivar, environmental conditions, time of collection, and the mounting fluid used for microscopic examination (Exconde *et al*., 1968a; Kimigafukuro, 1979; Pupipat, 1975; Weston, 1920; Williams, 1984). Seed health tests have been described (Appendix 3) (Singh *et al*., 1967; 1968), but the use of staining methods to distinguish between the identities of fungal mycelia is exceedingly difficult (Yao *et al*., 1991a).

Ideally, classic taxonomic methods would be confirmed by reliable molecular techniques. Isozyme analysis can successfully identify *Peronosclerospora* spp. (Bonde *et al*., 1984b; Micales *et al*., 1988), but is not practical for routine identification because it requires a pure culture maintained on living plants and production of a sufficient quantity of spores for enzyme analysis (Yao *et al*., 1991a). Molecular techniques based on DNA hybridization, such as RFLP (Restriction Fragment Length Polymorphism) analysis (Yao, 1991; Yao *et al*., 1991a; 1991b) or PCR (Polymerase Chain Reaction), with species-specific primers utilizing polymorphisms ITS (Internal Transcribed Spacers) of rRNA genes (Yao, 1991; Yao *et al*., 1992) are reported to differentiate maize downy mildews. The later has been used to differentiate *P. sorghi, P. maydis, P. sacchari* and *P. zeae* (Yao *et al*., 1992). CIMMYT studies in Asia, using PCR-based *Peronosclerospora*-genus specific and ITS markers, were unable to reveal differences in pathogen populations. RFLP probe pCLY83 was able to differentiate pathogen populations between countries (India, Philippines and Thailand), but not within countries (Dr. M. George, personal communication). Downy mildew fingerprinting research continues in Indonesia, the Philippines and Thailand (AMBIONET, 2003).

Molecular diagnostics allow DNA to be extracted directly from infected leaves, with no need for pathogen isolation. Samples of severely infected leaves are simply detached and air-dried for 2 days at room temperature prior to analysis. Tissue can be stored for several months at –70°C (Yao, 1991; Yao *et al*., 1991a). This greatly simplifies identification, which previously required the shipping of live infected seedlings. PCR techniques are the easiest to use for diagnostics because they do not require the radioactive probes needed for DNA hybridization.

7. Disease cycle and Epidemiology

a. Initial inoculum and infection

Infected volunteer plants, collateral hosts, or plants growing during an alternate season are the primary natural means of survival and source of initial inoculum (conidia). The fungus may survive as mycelium in kernels (pericarp) but transmission does not occur when seeds are air-dried before planting. On dewy nights, heavy sporulation may occur and conidia are disseminated to other plants by wind or splashing rain. The role of oospores in PDM epidemiology is unclear, but oospore formation is considered rare (see I-5a).

b. Sporulation and dissemination

Inoculum production has been extensively studied. Field experiments indicate that dew or a thin film of moisture over the surface of infected leaves is the determining factor in spore production. Conidiophores and conidia are produced on both sides of wet leaves in the dark, but are most prominent on the undersurface of leaves (Bonde, 1982). Weston (1923b) gives the following nocturnal cycle of development on maize in the Philippines:

7-8 p.m.	dew deposits begin on leaves
10-11 p.m.	leaf surface wet and conidiophore initials appear
11-12 p.m.	conidiophores develop
12-1 a.m.	conidia develop
1-2 a.m.	discharge and dispersal begin
2-3 a.m.	maximum conidia discharge
3-4 a.m.	discharge lessens, conidia germinate
4-5 a.m.	little discharge, extensive germination
5-6 a.m.	conidiophores dry

Maximal conidia production occurred between 2-4 a.m. in Nepal (Bonde, 1982) and 4-8 a.m. in India (Bains and Jhooty, 1982). Sporulation always occurs at or above 90% RH (Dalmacio & Raymundo, 1972) and a film of water must be maintained for 4-5 hrs for optimum production (Exconde, 1970). Sporulation continues until moisture disappears. Optimum temperatures reported for sporulation are 20-28°C, with 3 hrs of free moisture (Shah, 1976), 23-24°C with 6-8 hrs of free moisture (Exconde, 1976; Fuentes, 1976) and 18-23°C with 5-6 hrs dew (Bonde *et al*., 1992). Sporulation has also been correlated with

the maximum temperature from the previous day, with maximum sporulation following days >30°C. (Kimigafukuro, 1988).

High sporulation capacity increases the threat of a disease epidemic. *P. philippinensis* often produces 4,000-10,000 conidia/cm^2 of infected leaf (Duck *et al*., 1987), but as many as 20,000 conidia/cm^2 can develop within 12 hrs of continuous leaf wetness (Exconde *et al*., 1967). It is estimated that one infected maize plant can produce 758-5,946 million conidia/night (Weston, 1923b)! Under favorable conditions, conidial production can continue on individual maize plants for more than two months (Weston, 1923a; 1923b). Sporulation on collateral hosts, such as wild sugar cane (*Saccharum spontaneum*) may continue up to 8 months and contribute to year-long infection in the Philippines (Thurston, 1998).

Sporulation did not differ between 15 isolates of *P. philippinensis* from maize (Josue and Exconde, 1979b). *P. philippinensis* isolates that originated from sugarcane initially sporulated poorly on maize, but sporulation on maize increased with the passage of time to suggest that *P. philippinensis* can adapt to its new host by selection, mutation or activation of previously inactive portions of its genome (Duck *et al*., 1987).

Conidia, spread by air currents and splashing rain, act as primary inoculum. Mycelia cannot initiate infection (Dr. M. Bonde, personal communication). Conidia on each conidiophore are synchronously discharged from sterigmata a distance of 1-2 mm, allowing spores to be carried away by air currents (Weston, 1923a), primarily in the downwind direction (Bains and Jhooty, 1982). Conidia are fragile and unlikely to remain viable for more than a few hundred meters; thus, wind plays only a limited role in long-distance dispersal. In Indian experiments, over 40% of infected seedlings were within 2 m of an inoculum foci and all infected plants within 8m (Bains and Jhooty, 1982). Spore dispersal for *P. sacchari* has been reported up to 1/4 mile; however, maize varieties more that 1/2 mile from an inoculum source rarely become infected (Sun, 1970). Dissemination and secondary spread of the disease are increased in areas where plants are in various stages of growth or where there is an abundance of collateral hosts.

Spores shrivel and become non-viable when exposed to drying conditions such as sun, wind, or low humidity for 1-2 hr (Weston, 1920). Even under optimum conditions, conidia are short-lived (Smith and Renfro, 1999). *P. sacchari* is reported to remain viable for 3 hours at 100% humidity and 10ºC (Yang *et al*., 1962). Isolated spores lose their ability to

infect after 10 hr dry storage, but some conidia will remain viable up to 20 hr in saturated air on young maize leaves.

c. Spore germination and germ tube growth

The optimum temperatures for spore germination have been reported as 15-33°C (Bonde, 1982), 10-30°C (Bonde *et al*., 1992) and 19-20°C (Exconde, 1970). Conidia are capable of germinating at temperatures as low as 6.5°C (Weston, 1920). Viable conidia germinate in less than one hour if conditions are favorable (Dalmacio and Exconde, 1969). Dew is a better medium for conidial germination than water in leaf whorls (Exconde, 1970). The optimum temperatures for germ tube growth are 22-28°C with 2 hrs incubation or 18-26°C with 5 hrs incubation (Bonde, 1982).

d. Penetration and establishment

P. philippinensis penetrates leaves through stomates as germ tubes from germinating conidia or by hypha produced by the spore (Dalmacio and Exconde, 1969; Weston, 1920). In most cases an appresorium is formed over the stomata (Exconde, 1976). Penetration is followed by intercellular invasion of mesophyll cells from which it grows through the leaf sheath into the stem to become established in the apical meristem where it persists and grows systemically with newly developing tissue. The fungus spreads throughout the plant, but is confined to chlorotic tissue and sporulates when conditions are favorable. Leaf blades and sheaths, tassels, glumes and ears are all infected (Dalmacio and Exconde, 1969). According to Weston (1920) roots are not extensively invaded, however; mycelia have been observed in roots, primarily in the cortex (Dalmacio and Exconde, 1969).

Penetration begins within 2 hrs of inoculation (Dalmacio and Exconde, 1969) and requires a minimum of 2 hr free moisture and 2 hr darkness to cause high disease incidence (Barredo and Exconde, 1973; Bonde, 1982). A 4-hr dew period (post inoculation) is as effective as 18 hrs in inducing systemic infection of *P. sacchari* and *P. philippinensis* on maize (Bonde, 1981; Bonde and Melching, 1979). Optimum systemic infection occurs over a wide range of temperatures from 16-30°C, with lower levels (20 to 40% infection) between 10-16°C (Bonde *et al*., 1992). Spore concentrations of 10,000-50,000 conidia/ml sprayed onto plants produced a high incidence of infection (Bonde *et al*., 1992; Bonde and Peterson, 1983;

Kimigafukuro, 1988); however, 6,500 spore/ml or 650 spores/ml produced only a 45% and 2.5% infection rate respectively (Barredo and Exconde, 1973).

Temperature and relative humidity, but not rainfall or solar radiation, had a significant impact on disease severity on sweet corn (Exconde *et al.*, 1968b). Systemic infection is positively correlated with night relative humidity, spore production, day relative humidity, and rainfall, but negatively correlated with night temperature, day temperature, and duration of sunshine (Exconde, 1976). The high temperature (30-33°C) of the previous day, followed by cooler nights (23-26°C) were correlated with higher sporulation and disease incidence (Kimigafukuro, 1988). Disease development is limited by conidial maturity, inoculum density, age of host, and time of inoculation (Barredo and Exconde, 1973).

8. Host plant vulnerability

The fungus can infect corn plants at all stages of growth, but plants are most susceptible to systemic infection between seedling emergence and one-month of age. Symptom intensity is determined by the age of the plant at infection (Fig. 3 & 4). Susceptible maize plants (Philippine hybrid 801) displayed 95, 40 and 6% systemic infection when inoculated 1-3, 5 or 7 days after emergence respectively (Barredo and Exconde, 1973). In field tests, reductions in yield of 85, 75 and 72% were reported for infections that occurred at 2, 3 and 4 weeks after planting, respectively (Exconde and Advincula, 1970). Susceptible plants become immune to systemic infection after 4 weeks from germination, but local symptoms (minute pale green spots) form on some plants inoculated at 5-6 weeks of age (Dalmacio and Exconde, 1969; Weston, 1920). New tillers on mature plants are susceptible and infection can spread into the main stalk but conidiophores are not produced (Weston, 1920).

Conidial suspensions sprayed onto whole plants or inoculated at the remnant of the coleoptile produced over 97% systemic infection in young seedlings, while inoculation into the whorl and to localized areas of the leaf surface produced 50 and 0% systemic infection, respectively (Barredo and Exconde, 1973).

Concurrent systemic infections of maize streak virus and PDM reduced maize biomass and masked symptoms of PDM, but sporulation of PDM /unit leaf area was unaffected (Damsteegt and Bonde, 1993).

9. Seed transmission

P. philippinensis mycelium has been detected in the pericarp, endosperm, embryo, and endosperm of maize seeds (Bains and Jhooty, 1982; Dalmacio and Exconde, 1969; Exconde, 1976; Weston, 1920). Maize kernels are infected through parenchymous tissue along the vascular strands of the plant to the ovary wall of the developing caryopsis. Later in kernel development, *P. philippinensis* becomes established as mycelium in the pericarp, leaving the embryo and endosperm uninvaded (Exconde, 1976).

Seeds from systemically infected maize plants often do not produce infected plants (Bains and Jhooty, 1982). Although 70% of seed embryos originating from systemically infected plants contained mycelia, only 4.7% of seedlings raised from newly harvested seed developed typical localized PDM symptoms but not systemic infection (Bains and Jhooty, 1982). Transmission (3-11% depending on maize hybrid or variety) from seed to seedlings is possible only if hard dough kernels containing 36-38% moisture are sown immediately after harvest (Advincula and Exconde, 1976). Drying seed to 14-30% moisture destroyed the fungus and prevented transmission (Advincula and Exconde, 1976; Dalmacio and Exconde, 1969; Exconde, 1976). Thus, seed-borne transmission will not occur in commercially dried and stored seed and seed quarantine regulations appear unnecessary. Since infected plants mature more slowly than healthy ones, diseased ears are often left in the field post harvest, where they may serve as a new inoculum source (Advincula and Exconde, 1976; Chang, 1986).

II. Initiating event (recognizing an attempted introduction)

1. Observation/diagnosis of presence

Diagnosing an exotic pest in the field early is critical for containment and eradication. Since symptoms of various downy mildews are similar, first responders (university extension personnel, growers, scouts, crop specialists) may misidentify PDM as an indigenous maize downy mildew, such as *P. sorghi*. *Peronosclerospora* spp. are differentiated morphologically by only small variations in conidia, conidiophore, oospores and host range (Appendix 2). Fortunately, DNA hybridization and PCR techniques are being developed

which may differentiate the maize downy mildews (see I-6) and allow the definitive identification of *P. philippinensis*.

The recent establishment by the USDA of the National Plant Diagnostic Network (NPDN) is intended to provide a cohesive information system to quickly detect pests and pathogens that have been deliberately introduced, and report to appropriate responders and decision makers. NPDN is made up of experts at land-grant universities and is a key part of the Homeland Security effort. NPDN is divided into five regions, each with a regional hub (Appendix 7A). Web-based diagnostic and reporting systems are being developed and an effective communication network between diagnostic labs and regulatory agencies has been established (Appendix 7B). Modules to train first detectors are being developed by NPDN. This system should facilitate the detection of anomalies, such as simultaneous outbreaks at many locations, and thereby help identify a bioterrorist attack. Select data collected from the NPDN regions will be archived at the National Agricultural Pest Information System (NAPIS) located at Purdue University.

2. Interception: individual/ pathogen

The probable means of introduction would be infected plant material (tissue or seed). Besides maize tissue, PDM infected sugarcane sets are a potential means of entry. If the vehicle of entry was *P. philippinensis* conidia, packaging in dry ice would be required and the number of hours conidia could survive under these conditions is limited.

Interception of an individual carrying the pathogen or infected plant material at a port of entry should be responded to immediately. Isolation and containment of the material should prevent escape into the environment. Interception through other routes is improbable since very small amounts of the pathogen would be required to initiate larger scale inoculum production within the U.S. (see III and VI-1). Interception of contaminated seed should not be discounted and confirmatory procedures initiated. The probability of interception of shipped inoculum to an in-country location is very low and confidentiality of mail deliveries could avoid detection.

3. “Intelligence” information

Intelligence information from Homeland Security, NSA, CIA, DIA, FBI, or USDA-PPQ about an overt agroterrorism intent is another potential initiating event. This information should be provided to personnel at the county level to enhance the probability of early detection.

III. Probable route of terrorist entry/dissemination

Three types of inoculum could be used to introduce *P. philippinensis* into an area: infected plant material, culture (conidia), and infected seed. As an obligate pathogen, all techniques would require production in a living susceptible host and an isolated production area (greenhouse or field) unless obtained from a commercial field where it already causes extensive damage. Inoculum would be most effectively propagated on corn plants originating from untreated seed since metalaxyl and mefenoxam seed treatments (at rates of 200g /kg seed) are known to protect young seedlings against PDM. If disruption of export markets was the goal of the covert action, introduction of PDM could be accomplished through plant material, or possibly infected seed, from which natural increase and dissemination could take place over a period of years. Conidia (from inoculum suspensions, infected sugarcane, weed hosts, or maize transplants) have the potential to create the most substantial initial disease impact, but production, storage and distribution would require considerable knowledge.

1. Conidia

Conidia are the primary inoculum of PDM. Since no *in vitro* culture technique has been developed, conidial inoculum must be collected from diseased plants. The ephemeral nature of conidia means contact with the host must be made within a short time to cause infection and production would need to be in reasonable proximity to target areas. Conidial production, storage, and widespread aerial distribution would require considerable knowledge, equipment, support and a considerable volume of water. Greenhouse or isolated field conditions would be needed to produce adequate inoculum for a substantial initial impact on grain yield.

A long-term **storage technique** has been developed for conidia of *Peronosclerospora* spp. (Gale *et al*., 1975; Long *et al*., 1978). Success is dependent on conidia in 10-15% dimethyl sulfoxide (DMSO) being slowly frozen (1°C/min) to –30°C before storage and then quick thawed in a 40°C water bath on retrieval. Maize seedlings were successfully infected with *P. sorghi* and *P. sacchari* after being stored for 780 and 28 days respectively. *P. philippinensis* produced high levels of infection after 5 days of cryogenic storage, but long-term storage was not reported (Long *et al*., 1978). *P. philippinensis* probably can be stored cryogenically for extended periods of time. Thus, *P. philippinensis* could be introduced into the U.S. via a small vial held at –30°C (in liquid nitrogen or on dry ice), in the same way that restriction enzymes are packaged and shipped. Whether conidia would remain viable after the many hours of transport that may be required is speculative. The successful entry of a single vial of viable conidia, could initiate large-scale production of *P. philippinensis* on susceptible maize varieties within the U.S.

Mature **conidia collection** is optimum on 3-5 week old infected plants incubated in the dark with 5-6 hrs dew at 18-23°C (Bonde *et al*., 1992) or 6-8 hrs at ≥ 90% RH and 23-24°C (Exconde, 1976; Fuentes, 1976). Conidia can be washed from leaf surfaces with cold (5°C) deionized water delivered by an atomizer at approximately 34.5 (5 lb/in^2) air-line pressure (Bonde *et al*., 1992). The resulting spore suspension can be filtered through a 150μm screen and the concentration adjusted with cold DH_2O. Optimum infection rates are obtained with 10,000-50,000 conidia/ml (Barredo and Exconde, 1973). The resulting suspension can be maintained for 8-10 hrs in cold water. Germination of conidia can be delayed up to 24 hrs (after setting) without a loss of pathogenicity when infected leaves are placed on a 2% agar medium containing 1/8M KNO_3 (Appendix 4) (Yamada *et al*., 1976).

Timing of **inoculation** would be paramount in a covert operation. Maximum infection (>95%) has been reported when seedlings are sprayed with conidial suspensions within 3 days of emergence (Barredo and Exconde, 1973), although seedlings remain relatively susceptible the first week and progressively develop resistance up to 4 weeks of age. Free moisture in the form of dew and temperatures of 16-30°C are required for optimum infection (Bonde *et al*., 1992). Infection occurs after a minimum of 2 hrs (Dalmacio and Exconde, 1969; Bonde, 1982) and optimum 4 hrs post inoculation (Bonde and Melching, 1979). Field inoculations would need to be done early in the morning, no later than 4 a.m., since conidia shrivel once dew has evaporated.

The most effective method of **inoculum application** is spraying of whole plants. Addition of a particulating moisture agent, may greatly extend the time available for penetration and infection by delaying desiccation and exposure to other environmental constraints (Montecillo *et al*., 1982). Aerosolization of conidial suspensions is a potential means of widespread distribution but is limited by the amount of inoculum that could be produced, rapidly harvested and distributed. Smaller operations could employ backpack or truck mounted sprayers, but conidia are fragile and unlikely to withstand high pressure. Conidial suspensions can produce infection when dispersed with a backpack sprayer at 5- psi pressure (Kenneth *et al*., 1970).

2. Infected plant material

Inoculum prepared from ground, infected leaves could also be distributed over a wide area but the effectiveness of such a technique is unknown. Mycelia alone are not capable of initiating infection (Dr. M. Bonde, personal communication) and conidia sporulate only on live plant tissue, are short-lived, and shrivel quickly at low humidity. Preparation of large quantities of pulverized infected green leaves may be used but would be cumbersome to store and disperse.

P. sacchari has been widely disseminated in infected sugarcane sets (Bonde, 1982). *P. philippinensis* has been routinely maintained on sugarcane for experimental purposes in the Philippines and ample sporulation can continue for at least 18 months (Kenneth *et al*., 1970). PDM could be initiated from spores produced on sugarcane, then inoculated onto a susceptible variety of sweet or popcorn to multiply inoculum. Systemically infected sugarcane sets are less fragile than maize plants or vials of conidia and could be easily packaged and shipped into the U.S.

The most effective means of employing infected plant material would be transplanting infected source plants into a few widely dispersed fields early in the season. This method is the basis of the “spreader row” technique used extensively to provide inoculum for infection in maize breeding programs (Kaneko and Aday, 1980b). It would require the potentially conspicuous transplanting of infected plants in close proximity (within 2 m) to young seedlings. However, the method offers advantages; it does not require the labor of preparation and distribution of large inoculum suspensions and infected transplants remain

a source of inoculum for weeks unless destroyed (in contrast to a one-time conidial suspension application).

3. Seed

Theoretically, infected seed could be distributed over a fairly wide area and would provide an initial susceptible host from which secondary spread could occur. Hard dough kernels containing 36-38% moisture have produced up to 11% PDM infected seedlings, but only when sown immediately after harvest into sterile soil under controlled growth chamber conditions (Advincula and Exconde, 1976). No transmission was found from seeds with moisture contents between 43-47% or 14-30% and no transmission from seed to seedling was obtained under natural field conditions. Although refrigeration can preserve wet, infected kernels by inhibiting secondary, saprophytic decay by other organisms, a narrow range of seed moisture content would need to be maintained and it is unclear how long *P. philippinensis* would remain viable in refrigerated seed. Thus, the potential impact of planting infected kernels is speculative. Such an operation would need to be done at least 3 weeks prior to field planting, since source plants would need to be producing inoculum during the first weeks of crop emergence. Unfavorable spring weather conditions may make such early planting of corn seed dubious. Infected kernels remain the simplest way of getting *P. philippinensis* into the U.S., and could be used to initiate conidia production on live maize plants at facilities within the country. PDM infected seedlings do not develop from kernels mixed with infected fresh leaf sections or when directly inoculated with conidial suspensions (Advincula and Exconde, 1976).

IV. Probable distribution

1. Point Introduction: Midwestern versus Southern corn production areas

Although infected seed would probably be the easiest method of introduction into a localized area, the impact of such an operation is speculative. The most probable method of successful disease initiation (and therefore most likely route of intentional introduction) is via conidial suspension or spreader plants (as a source of conidia), distributed at one or multiple

sites. Conidia distributed in fields of young maize seedlings throughout the Corn Belt could initiate a severe localized epidemic and provide adequate sequential inoculum for substantial spread in the year of introduction but would require massive amounts of inoculum and optimum meteorological conditions.

Although virtually all U.S. commercial corn seed is treated with fungicides containing metalaxyl or mefenoxam for pythium control, present U.S. application rates are 1-2 orders of magnitude lower than those reported effective against PDM in other countries and 4-8 times lower than current rates recommended in the U.S. for the control of other downy mildews on corn (see VII-3). It is therefore unlikely that PDM initiation or geographic spread would be significantly hampered by current seed treatment practices.

This pathogen exists primarily in tropical regions where hosts harbor the pathogen in green tissue year round. PDM is not expected to overwinter in the northern Corn Belt. PDM introduced into southern corn producing regions, where alternate hosts are common and available year round, is much more likely to successfully persist and could lead to the gradual infestation of the southern one-third of the Corn Belt (see V-1a, b, c).

2. Secondary Dissemination

Secondary dissemination of *P. philippinensis* would be primarily via natural dispersal of conidia in air currents and rain. Limited secondary dissemination may occur via infested seed from PDM plants left in the field post harvest. The vast distribution of collateral host species in the U.S. may make them important alternate sources of inoculum and a reservoir for *P. philippinensis* when maize plants are unavailable. In the absence of collateral hosts or infected seed, little persistence is anticipated because oospores production is considered “rare to nonexistent” (Smith and Renfro, 1999).

V. Consequences of introduction and establishment

The consequences of introduction of *P. philippinensis* and risk of PDM establishment in the U.S. were rated with respect to six risk elements: climate, host range, dispersal, economic impact, environmental impact, and persistence. The pathogen was ranked for 29 different criteria encompassed within the six risk element categories.

1. Establishment

a. Climate *Risk = High (during corn production season)*

= Low (winter in most of U.S.)

Infection requires environmental conditions that favor sporulation, germination and systemic infection by *P. philippinensis* during the first month of maize seedling growth. Two to four hrs of free moisture is required for infection after inoculation (Bonde, 1982; Bonde and Melching, 1979; Dalmacio and Exconde, 1969), but under natural conditions, an additional 4 hrs of dew is required for optimum sporulation and dispersal. Infection is restricted by dew period temperatures below 18°C, although low levels of infection (20-30%) can occur between 10-14°C (Bonde *et al*., 1992). By June 1, temperature and moisture requirements favorable for infection by *P. sacchari* and *P. philippinensis* are common in much of the U.S. corn belt (Bonde and Melching, 1979; Bonde, 1981) and therefore the introduction of these species poses a threat to the U.S. (Bonde *et al*., 1992). River valleys and other high humidity regions with abundant weed hosts are at greatest risk.

Theoretically, the potential disease distribution range for PDM epidemics can be estimated by identifying regions frequently experiencing ≥6 hr-long dew periods and minimum temperatures ≥18°C concurrent with young corn seedling growth. The maximum potential disease distribution range, extending to areas where PDM might occasionally occur with low severity, encompasses a much larger region where minimum dew period temperatures fall in the 10-17°C range. Unfortunately, dew formation and duration are not monitored by the National Weather Service or its cooperative observers so dew period information over large regions of the U.S. is unavailable. Maps of mean dew point temperature (Appendix 5A) and mean daily minimum temperature (Appendix 5B) for the months of May and June (periods when susceptible corn seedlings ≤ 4 weeks old would be present in much of the U.S. corn producing areas) allow an approximation of the PDM distribution range. In May, regions with a mean daily minimum temperature of 60–70°F (16–21°C) could experience a high incidence of disease (see I-7d; Bonde *et al*., 1992), assuming a ≥8 hr-long dew period had occurred when inoculum and susceptible seedlings were available. This includes areas of eastern Texas, Louisiana, Florida and southern Georgia. By June, mean daily minimum temperatures of 60-70°F extend northward to encompass Missouri, southern Indiana and Illinois (Appendix 5B).

The largest potential limiting factor is the ability of *P. philippinensis* to overwinter and thereby affect the pathogen's survival range in the U.S. *P. sorghi* isolates from Indiana have lower temperature requirements than isolates from the tropics so the fungus was able to adapt to other environments (Bonde, 1980). However, *P. sorghi* produces oospores able to survive in Indiana soils. In nature, oospores of *P. philippinensis* are considered "rare or nonexistent" (Smith and Renfro, 1999) and their role in epidemiology has not been elucidated (Bains and Jhooty, 1982; Bonde and Peterson, 1983; Bonde *et al*., 1984b; Frederiksen and Renfro, 1977; Weston, 1920). In the absence of oospore production, *P. philippinensis* might be capable of overwintering only on any of its perennial Gramineae hosts in southern areas where green tissue persists. In the northern Corn Belt, it is anticipated that the PDM disease cycle will be abruptly interrupted by a lack of green host material for conidia production unless it can survive in systemically infected perennial hosts such as johnsongrass.

The disease distribution range of an airborne pathogen is frequently much larger than its survival range and dependent on many factors besides favorable climate. Annual reintroduction into regions outside of *P. philippinensis*'s survival range will also depend on the distribution of collateral hosts and the pathogen's capacity for dispersal. It is estimated that ≤30% of the corn production area of the U.S. could be annually infected with *P. philippinensis*.

b. Host Range *Risk = High*

P. philippinensis has a broad host range within Gramineae and can infect grasses that are common in the U.S. (Appendix 2). Among the most widespread are *Andropogon gerardii* (big bluestem), *Schizachyrium scoparium* (little bluestem), and *Sorghum halepense* (johnsongrass). Perennial grasses could potentially be reservoir hosts of *P. philippinensis* in the U.S. if infected plants survive the winter season (Bonde and Peterson, 1983). The potential survival range of PDM will, in part, depend on the geographic distribution of perennial collateral hosts or volunteer corn that remains green over winter months. The prevalence of johnsongrass in the U.S. makes it a primary suspect as an overwintering host for PDM; however, the total range of johnsongrass distribution is not indicative of that of PDM. In the northern U.S., johnsongrass reestablishes annually via seed but johnsongrass exists as a true perennial in many regions of the central and southern U.S., south of 40°N latitude (e.g. Tipton County, IN) (Dr. T. Bauman, personal communication).

c. Dispersal *Risk = Low*

It is believed that oospore production by *P. philippinensis* is “rare to non-existent” therefore conidia are the only sources of disease spread and intensification (Bonde, 1982). Millions of conidia may be produced on a single maize plant/night for a period of up to two months (see I-7b). Although conidia are not dispersed long-range (usually within a few meters of origin), significant dispersal can occur if contiguous acres of susceptible maize or other Gramineae hosts are available. Since long-range dispersal is unlikely, except through spring flooding, the distribution range of PDM will likely extend only a few meters beyond *P. philippinensis*’s survival range in the U.S. Although conidia are short lived, it is possible that they could be dispersed a number of miles via flooding episodes. Survival and dispersal are important factors limiting the estimated disease distribution range of PDM to the central and south to constitute ≤30% of total U.S. corn producing areas.

Seed-borne dissemination of the pathogen is not a concern in commercial seed, because the pathogen is not viable at seed moistures below 30% (see I-9); however, diseased ears left in the field post harvest may grow into infected seedlings and provide a new inoculum source (Advincula and Exconde, 1976; Chang, 1986).

d. Economic Impact *Risk = Low - Moderate*

Corn, grown for both grain and silage (forage), is the most economically important crop produced in the U.S. In 2002, corn was grown on 79.1 million acres in 48 states (Appendix 6A) to produce 9 billion bushels with a grain value of $21.2 billion. The top five corn producing states and associated percent of total production in 2002 were IA (21.8%), IL (16.6%), MN (11.7%), NE (10.4%), and IN (7.0%) of the U.S. total production (USDA, 2003). Yield/acre by county in 2002 is displayed in Appendix 6B.

Although much of the U.S. corn growing season climate can support *P. philippinensis*, winter climate and a slow dispersal rate will likely limit the pathogen to ≤ 30% of the 79 million corn producing acres. Economic impacts like those experienced in the Philippines (I-3) are not expected in the U.S. because PDM’s survival range in the Philippines encompasses the entire maize production area, which is often continuously cropped and producing a large inoculum load year round.

Of perhaps greater economic importance than yield losses would be the impact of potential long-term quarantines and embargo imposed by other nations on U.S. corn seed. Since *P. philippinensis* is easily controlled by drying seed to commercial specifications for storage, this pathogen should be considered a low phytosanitary risk (CABI, 1999). However, political concerns abroad may necessitate phytosanitary certification of U.S. seed with its associated costs. Over-all economic importance is considered low to moderate.

e. Environmental Impact *Risk = Low (in northern Corn Belt)*
= Moderate (in southern Corn Belt)

Collateral host populations growing in the U.S. could potentially be impacted. Of economic importance are sugarcane, oats and sorghum. Sugarcane production (for sugar and seed) in 2001 was valued at $1.003 billion, but was grown only in Florida, Louisiana, Hawaii and Texas. Of these, only Texas is a corn producing state. In 2002, oats were harvested on 2.1 million acres at a value of $211 million, while sorghum (grain + silage) was harvested on 7.6 million acres with a crop value of $887 million. Oats are primarily grown in the northern Corn Belt, while sorghum is grown in the central and southern Corn Belt.

Wild host populations such as wild sorghum, wild sugar cane, and johnsongrass are also susceptible to PDM.

f. Persistence *Risk = Moderate (in southern Corn Belt)*
= Low (in northern Corn Belt)

The introduction, spread and persistence of *P. sorghi*, causal agent of sorghum downy mildew, may shed some light on the ability of the related PDM pathogen to spread and persist. *P. sorghi* was first discovered on sorghum in Texas in 1961 (Reyes *et al.*, 1964), and was reported as far as Kentucky in 1972 (Williams and Herron, 1974) and Indiana (Warren *et al*., 1974) and Illinois (Dr. H. L. Warren, personal communication) in 1973. The disease in these areas was associated with feral sorghum ("shattercane"). Bonde (1980) found lower temperature requirements for isolates from Indiana than isolates from the tropics and concluded that the fungus could adapt to other environments. However, *P. sorghi* produces oospores able to survive in Indiana soils. In the absence of abundant oospore production by *P. philippinensis*, PDM could theoretically persist in infected seed from maize plants left behind after harvest and produce systemically infected plants as a

new source of inoculum to infect collateral hosts. More likely, the pathogen could overwintering on any of its perennial Gramineae hosts or volunteer plants. Johnsongrass rhizomes survive the winter as far north as Tipton County, IN (40°N latitude). Although oospores were not produced on mature tissue of collateral hosts in the green house (Bonde and Peterson, 1983), much remains to be learned about the potential role of such a wide range of alternate hosts in PDM persistence.

2. Over-all risk rating for establishment of *P. philippinensis*

Climate	Host Range	Dispersal	Economics	Environmental Impact	Persistence
High (**spring/summer**) Low **(winter in much of US)**	**High**	**Low**	**Low-Moderate**	**Low (most of US)** **Moderate (southern Corn Belt)**	**Low (much of US)** **Moderate (southern 1/3 of Corn Belt)**

VI. Likelihood of successful introduction

1. Quantity of inoculum required to introduce and establish damage

If disruption of export markets was the goal of the covert action, *P. philippinensis* introduction could be accomplished through a relatively small volume of conidia, infected live plant material, or perhaps infected seed. Since conidia are delicate and remain viable for only a few hours, the most successful medium of entry is in cryogenic storage (-30°C). A

single vial could contain tens of thousands of conidia, enough to inoculate several hundred corn seedlings for the purpose of inoculum multiplication.

An alternative approach would be to bring PDM into the U.S. as infected seed. This method may be less efficient than entry with conidia, since only freshly harvested seed with moisture contents > 30% contain viable mycelia, and such seeds tend to rot rather quickly. Further, only 2-11% of infected seeds produce plants with infection. Because of these and other issues (see I-9, III-3) the large-scale use of infected seed is not likely, but seed could serve as a means of *P. philippinensis* entry, followed by multiplication of conidia on corn seedlings.

A substantial initial impact on grain yield would require distribution of a large volume of inoculum over an extensive area. Since *P. philippinensis* can produce upwards of 20,000 conidia/cm^2 of maize leaf (Exconde *et al*., 1967) and a single plant is estimated to produce up to 5.9 billion conidia/night (Weston, 1923b), the collection of large volumes of inoculum is possible but coordination of production with the window of infection for seedlings (less than 3-4 weeks) may be difficult.

If a mature plant produces 1 billion conidia and 10,000 conidia/ml produce optimum infection, 100,000 seedlings could be inoculated/source plant. If a volume of 1ml/seedling is sufficient (0.5 ml containing 5,000 spores is required when dropped within the whorl (Bonde, personal communication), and 25,000 seedlings are grown/acre, 25L of conidial solution would be required/acre. A single source plant would be sufficient to initiate infection over 4 acres.

2. Likelihood of surviving initial introduction

The near-optimum summer climate (V-1a) suggest that *P. philippinensis* would survive an initial introduction in the U.S. Long-term survival and spread of this obligate pathogen primarily depends on the production of conidia and therefore the continuous availability of live susceptible maize or collateral host tissue. This likely limits the pathogen's capability to persist year-round to southern corn growing regions (V-1f). Perpetuation is possible via freshly harvested seed with high moisture content, but not in commercially dried seed.

U.S. hybrid corn seed is routinely treated for seedling blights, with compounds containing mefenoxam (1- 2 g /100kg seed) or metalaxyl (2- 4 g/100 kg seed). These fungicides are effective against downy mildews only at significantly higher rates (see VII-3) and present

U.S. rates are likely insufficient to protect seedlings from PDM over the 4 week susceptibility period. The impact current low fungicide rates could have on reducing disease severity in the U.S. is unknown, but suspected to be ineffective.

3. Likelihood of dissemination beyond the point of introduction

Unlike many other downy mildews, *P. philippinensis* does not disseminate long distances or survive for long periods via oospores. Contamination of commercial seed lots is not an issue because *P. philippinensis* cannot withstand drying.

The ability of PDM to spread rapidly was demonstrated in Nepal where the first localized epidemic occurred in 1967 (Shah, 1976). Epidemics occurred in three adjoining districts in 1971 and 1973. In 1973, PDM was also found in a district not contiguous with other infested areas. A temporal break during the dry season in susceptible host availability is credited with limiting the disease to a few districts in Nepal (Shah, 1976).

The geographic distribution of potential collateral hosts capable of harboring the pathogen year round will play an important role in determining the eventual distribution of PDM in the U.S.

Dissemination could occur at a slow to moderate rate. If introduced into the Southern Corn Belt, natural increase and secondary spread of the pathogen could contaminate up to 30% of the current corn production area within 3-6 years. Southern areas with high humidity would be at greatest risk for PDM damage, with the potential for spread up the Mississippi and Tennessee River valleys. Late spring flooding along these and other rivers (Wabash, Missouri, Ohio, etc.) is common when corn plants are in a susceptible growth stage. Inoculum from perennial systemically infected hosts, such as johnsongrass, could spread considerable distances from extensive coverage of “river bottom” farmland by conidia.

4. Likelihood of alternate host infection

Collateral weed and forage hosts are common in the U.S. (Appendix 1). There is a high likelihood that collateral hosts would become infected if adjacent to an inoculum source.

5. Likelihood of early detection

Ten species of downy mildew have been reported world wide on maize (Pupipat, 1975), but only three exist in the U.S.: *Peronosclerospora sorghi* (Sorghum downy mildew of sorghum or maize), *Sclerospora graminicola* (green ear downy mildew of pearl millet or maize) and *Sclerospora macrospora* (crazy top). Since symptoms are not definitive for many of the downy mildews, first responders (agricultural workers, scouts, university extension personnel, crop specialists) may initially not suspect an exotic pathogen and misidentify PDM as an indigenous maize downy mildew, such as *P. sorghi*. This would be a logical deduction because *P. sorghi* has previously been reported on shattercane in the Midwest. Lack of oospore production cannot be used as a definitive identifying characteristic for *P. philippinensis*, since *P. sorghi* is less likely to be produce oospores on maize than on sorghum (Yao, 1991). This emphasizes the need for first responders to promptly forward suspect downy mildew samples to plant diagnostic clinics for evaluation by traditional and molecular techniques.

Techniques based on DNA hybridization and PCR techniques are still in the development stage. Yao *et al*. (1991a; 199b; 1992) reported the ability to differentiate *Peronosclerospora* species, however, CIMMYT researchers have been unable to repeat this success using samples collected recently from various Asian locations (Dr. M. George, personal communication). RFLP probe pCLY83 was able to differentiate pathogen populations between countries (India, Philippines and Thailand), but not within countries. It is unknown if available PCR-based techniques would successfully differentiate an introduced *P. philippinensis* from *P. sorghi* in the U.S. Continued research on downy mildew fingerprinting will hopefully reveal molecular techniques that consistently differentiate *Peronosclerospora* species.

6. Overall risk = Moderate

A preliminary APS ad hoc Committee survey conducted in 2001 (Dr. L. Madden, personal communication) ranked the threat of *P. philippinensis* to the U.S as a “small to moderate” threat. APS ad hoc Committee activities are outlined by Madden (2001). If *P. sacchari* and *P. philippinensis* are synonymous, indicating a greater ability to produce oospores, the risk should be considered “moderate”.

7. Likelihood of an agroterrorist using *P. philippinensis* as a biological weapon = Low

The intent of an overt introduction of *P. philippinensis* would be to produce a long-term impact and initiate quarantine (embargo) action against U.S. corn.

VII. Control/Mitigation strategies after establishment

Despite the introduction of downy mildew resistant cultivars and use of metalaxyl seed treatment, severe PDM incidence continues to occur in localized areas in the Philippines (Dalmacio, 2000). In light of the cost of fungicidal seed treatments and the emerging problem of chemical resistance of the pathogen (Raymundo, 2000), resistant varieties offer the most cost effective and environmentally friendly alternative for controlling PDM.

1. Cultural Control

Little research is reported in the literature on cultural practices to control PDM. High levels of nitrogen (≥ 350 kg/ha) increased susceptibility (Yamada and Aday, 1977). Additions of phosphorus and potassium did not influence PDM.

Cultural practices such as early rouging and destruction of infected plants may be of value to reduced PDM in seed production fields. Rouging plants is labor intensive and therefore not practical over large acreages. Since infected plants mature more slowly than healthy ones, diseased ears are often left in the field post harvest, allowing them to serve as a new inoculum source. Fields should be sanitized by removing and burning corn plant refuse, especially where corn is continuously cropped (Advincula and Exconde, 1976; Chang, 1986). Such measures are essential to contain and eradicate the pathogen.

The following cultural practices have been suggested to reduce potential losses (Thurston, 1998):

a) Remove weed hosts and volunteer maize in and near maize fields.

b) Manipulate planting dates to avoid the disease and regulate plant density by wider spacing or interplanting with other crops to allow better air circulation and promote rapid drying to reduce the time favorable for infection.

c) Remove infected plants to reduce inoculum levels.

d) Regional crop rotation.

2. Resistance

Control of downy mildews is most effective with resistant cultivars (Ajala *et al*., 2003; Frederickson and Renfro, 1977; Raymundo and Calilung, 1994); however, there has been little evaluation of resistance to *P. philippinensis* in U.S. commercial breeding programs since this is an exotic pathogen. Sweet corn is particularly susceptible to PDM (Exconde, 1970). U.S. maize varieties and breeding lines resistant to *P. sorghi* are highly susceptible to *P. sacchari* (Bonde and Melching, 1979), so susceptibility to *P. philippinensis* is speculated.

Successful disease resistance breeding programs against maize downy mildews have been conducted in the Philippines, India, Indonesia and Thailand. In the Philippines, a breeding program for PDM resistance initiated in 1953 screened local and introduced cultivars, selected resistant maize lines, and developed composite maize populations and varietal hybrids with PDM resistance and other desirable agronomic traits (Aday, 1975). Only locally originating germplasm proved a useful source of resistance (Thurston, 1998). Resistant cultivars and hybrids became commercially available in 1982 and were widely planted across the Philippines. Philippine developed varieties, such as Ph DMR 1 and Ph DMR 5, have been successfully adopted in India (Sharma and Payak, 1985).

Early resistant materials such as Aroman white and Aroman 206 reduced disease incidence (from >90% to 21-42%) and reduced sporulation significantly (Exconde *et al*, 1967, 1968b; Barredo and Exconde, 1973). Immunity is unknown (Frederickson and Renfro, 1977) and resistance can be overwhelmed if inoculum pressure is high (Bonde, 1982). Improved varieties and hybrids impart “moderate resistance” to PDM and the disease is far from being contained (Ebron and Raymundo, 1987b). As a result, metalaxyl seed treatment (see VII-3) is often integrated with resistant varieties.

Problems arose in the 1980s and 90s when Philipino farmers began planting F_2 seeds of commercial hybrids due to the increasing costs of F_1 seed. The result was severe PDM epidemics, since hybrids from which the F_2 originated are predominantly of susceptible foreign parentage. Also, modern corn cultivars with PDM resistance genes are normally treated with metalaxyl, but F_2s are not. Tests evaluating the F_2 generation of 29 hybrids, many originating from U.S. based companies, revealed most hybrids were susceptible (Raymundo *et al*., 1993).

In screening for resistance, two inoculation methods have been used. In the first method, seedlings are either whorl- or spray-inoculated with a suspension of mature conidia in a controlled environment (Barredo and Exconde, 1973; Ebron and Raymundo, 1987b; Exconde, 1970). This system offers the advantage of providing optimum conditions for disease and regulation of inoculum quantity. The second method uses spreader rows as a source of inoculum and results in significantly higher infection rates than whorl inoculations (Kaneko and Aday, 1980b). In this technique, nursery boxes full of test seedlings are placed in between rows of infected sporulating maize plants. Thousands of seedlings can be screened without the labor of inoculation, and a high and uniform incidence of infection is achieved (Kaneko and Aday, 1980a; Williams, 1984). The technique is optimized when 6-day-old seedlings are exposed to spreader rows 3.75 m apart for a minimum of 2 days (Kaneko and Aday, 1980b).

Systemic symptoms (chlorotic streaking patterns) vary among inbred maize lines and can be used to categorize host varieties as resistant or susceptible (Ebron and Raymundo, 1987b). Susceptible lines display long broad chlorotic stripes on leaves, whereas resistant lines tend to have narrow and or discontinuous chlorotic streaks; moderately resistant lines display a combination. Local symptoms are not reliable indicators of host susceptibility.

Resistance is inherited polygenically and controlled mainly by additive gene effects (Capuno and Carpena, 1982; Ebron and Raymundo, 1987b; Leon *et al*., 1993), but with a threshold that is dependent on the level of infection (Kaneko and Aday, 1980a). Quantitative resistance was demonstrated in PDM resistant inbred lines that displayed extended duration of local infection, delay in onset of systemic infection, a slow rate of disease development, and small areas under the disease progress curve (Ebron and Raymundo, 1987b).

Simple phenotypic recurrent selection was used successfully in the development of two corn populations (CPRP1 and CPRP3) with strong resistance to *P. philippinensis* and good agronomic characteristics (Raymundo and Calilung, 1994). CPRP1 and CPRP3 were base populations from resistant plants of the F_2 generation derived from commercially available hybrids; after 2-3 cycles of selection under high inoculum pressure, PDM was reduced by 86-87% in both populations. The CIMMYT (International Maize and Wheat Improvement Center) Asian Regional Maize Program continue to improve downy mildew resistant lines; notable are populations 100, 145, 300, 345 and MDR-DMR (in which ≤ 10% of plants are infected) and a number of inbred-hybrid lines with high levels of resistance and good combining abilities (CIMMYT, 2002).

How stable resistant lines will remain is unknown but quantitative inheritance typically results in relative stability (Thurston, 1998). Nine inbred lines deemed resistant, proved susceptible (*i.e.* > 50% infection) to 3 or more of 16 *P. philippinensis* isolates tested (Josue and Exconde, 1979b). These authors conclude that breeding for resistance should be done in light of the prevalence of virulent races in a particular location.

A high correlation of resistance to several downy mildews of maize appear to exist (Fuji, 1975). Maize lines resistant to PDM also give resistance to *P. sacchari* in Taiwan and *P. sorghi* (now *P. zeae*) in Thailand (Thurston, 1973). Disease resistant inbred lines developed by CIMMYT's Asian Regional Maize Program have recently been evaluated for cross-resistance. Several sources of resistance to *P. sorghi* in southern India were found to also impart resistance to other downy mildew pathogens (Dr. D. Jeffers, personal communication). The possibility of developing maize lines resistant to several downy mildews simultaneously would be ideal for Asia where at least 5 downy mildews impact production.

The nature of resistance remains only partially understood. Using QTL (quantitative trait loci) mapping, AMBIONET (Asian Maize Biotechnology Network) has identified 6 genomic regions (on chromosomes 1, 2, 6, 7 and 10) in disease resistant inbred maize lines that confer resistance to 5 downy mildew pathogens, including *P. philippinensis*, at five locations in four Asian countries (George *et al*., 2003). A key finding of this study was a strong QTL on chromosome 6 that was important for resistance to all 5 downy mildew pathogens. Also, SSR (Simple Sequence Repeat) markers tightly linked to this QTL were identified that can

be used in marker-assisted selection. Such research is a first step towards the development of high-yielding maize varieties with durable resistance to multiple downy mildews.

3. Chemical Control

Fungicides have been extensively tested against PDM. None of the 63 conventional fungicides tested in the Philippines before 1965 provided effective economic control (Exconde, 1975; Payak, 1975; Fuji, 1975). Intensive efforts in the 1970's yielded more favorable results.

Since seedlings are most susceptible between emergence and 3 weeks of age, sprays must be applied 2-3 days after emergence. Alternate sprays of Duther (20% triphenyl tin hydroxide) and Dithane M-45 (80% zinc, iron, and manganese ethylene bisdithiocarbamate) significantly reduced downy mildew infection (Dalmacio and Exconde, 1971); however, four combination applications were necessary to provide incomplete (12-30% infection) protection (Exconde *et al*., 1976). In India, two applications of Dithane M-45 or Dithane Z-78 were as effective as four (Sharma *et al.*, 1981). Economic analysis revealed both combinations of Duther + Dithane M-45 and F-1243 (mineral oil) + Dithane M-45 yielded additional income, but resistant varieties remained the most economical means of control (Estrada and Exconde, 1976). Seed treatment with chloroneb followed by applications of dithiocarbamate (Shultz, 1971) also proved effective but cost prohibitive for large hectarages (Payak, 1975).

The first effective, economically acceptable protection was demonstrated in 1978 in the Philippines with the systemic fungicide metalaxyl (Ridomil 25 WP) as a seed dressing (Exconde and Molina, 1978). It was hoped that the small amount of chemical required (2 g a.i./kg seed) to obtain 100% control and ease of a single application would allow farmers to adopt this method (Molina and Exconde, 1981a) however, commercial fungicide treated seed remained beyond the financial reach of many resource-poor farmers (George *et al*., 2003). Six grams of Apron 35 SD (metalaxyl)/kg of seed (*i.e.* 0.6%) is more effective when mixed with seed as a slurry than mixed as a dust, and provided complete control regardless of rainfall frequency (Molina and Exconde, 1981a). Slightly higher slurry concentrations (0.7% - 0.8% Apron 35 SD) were required for complete protection in India (Dey *et al*., 1983; Sharma *et al*., 1981). Metalaxyl-treated-seed may be stored (5°C or 28-32°C) for at least 5 months without loss of fungicidal activity or significant reduction in germination (Molina and

Exconde, 1981b). The amount of water used in slurry preparation is a critical factor for storage, with 10 ml/kg of seed being optimum, but increasing amounts of water did not affect metalaxyl performance when seeds were planted immediately after seed-dressing (Molina and Exconde, 1981b).

Metalaxyl (Ridomil MZ 58) also gave excellent control when applied as a foliar spray 10 days after planting at the same rate recommended for seed dressing (40 g a.i./ha) (Cordero and Tangonan, 1983). Foliar sprays might be most useful in breeding and disease screening programs where thousands of accessions are handled in small quantities making seed treatment tedious.

As a systemic fungicide, metalaxyl is absorbed and translocated via the transpiration system. The success of metalaxyl can be attributed to its persistence in plants for 30-35 days, long enough for plants to acquire natural resistance to *P. philippinensis* (Dey *et al*., 1983; Sharma *et al*., 1981). Unfortunately, reports of resistance to metalaxyl became frequent in the Philippines by the late 1990s and concern is growing over the buildup of chemical resistance (Raymundo, 2000). Isolates of various oomycete pathogens (e.g. potato late blight) that quickly developed resistance to metalaxyl also showed resistance to mefenoxam (Gallian *et al*., 2002). In Texas in 2002, *Peronosclerospora sorghi*, the causal agent of sorghum downy mildew, appeared to be developing resistance to Apron/Allegiance seed treatment, which led to recommendations for higher doses (Isakeit and Odvody, 2003). Long-term use of metalaxyl and mefenoxam need to be integrated into programs that minimize the buildup of resistant pathogen strains.

Since 1996, mefenoxam has replaced some metalaxyl products and is the R-enantiomer of metalaxyl. Mefenoxam provides the same level of efficacy as metalaxyl at half the application rate (EPA, 1996). A number of products with the a.i. mefenoxam (Apron XL, Maxim XL) or metalaxyl (Allegiance FL) are presently available in the U.S. Commercial corn seed is routinely fungicide-dressed in the U.S. to protect against a complex of damping-off and seedling pathogens. In recent years, approximately 80% has been treated with MaximXL® (fludioxonil + mefenoxam) and most of the remainder with a combination of Captan® and Allegiance® (metalaxyl) (Giesler, 2003). For 2004, approximately 80% of U.S. corn seed will be treated with the equivalent of 2g mefenoxam or 4 g metalaxyl per 100 kg seed, and the remaining 20% at half of that a.i. rate (M. Jirak of Syngenta, personal communication). These rates are significantly lower than those effective against downy

mildews. Fungicides are registered for domestic use against downy mildews at 15 g metalaxyl/100 kg of seed and up to 30 g/100 kg of seed for export corn (M. Jirak of Syngenta, personal communication). In Texas, recommended treatment rates/100 lb corn seed for sorghum downy mildew are 1 oz. dry wt metalaxyl (i.e. ~62.5 g/100 kg) or 0.5 oz. dry wt mefenoxam (i.e. ~31.3 g/100 kg). Effective seed treatment rates against PDM in Asian research were in the order of 100 - 200 g/100 kg of seed (Dey *et al*., 1983; Exconde and Molina, 1978, Molina and Exconde, 1981a; 1981b; Sharma *et al*., 1981), because 50 g/100 kg seed gave incomplete control (Molina and Exconde, 1981a). Thus, present U.S. seed treatment rates are grossly insufficient to protect seedlings from PDM over the 4-week susceptibility period.

4. Modeling Disease Incidence and Spread

No predictive models are known that forecast the spread and distribution of PDM of corn.

Tobacco blue mold and Cucurbit downy mildew epidemics are predicted using meteorological models developed by the North American Plant Disease Forecasting Center (NAPDFC). Forecasting allows growers to make timely decisions about economical applications of fungicides. The HY-SPLIT model can calculate 3-D atmospheric spore concentrations, movement, and estimate ground deposition of conidia allowing risk levels to be assigned in real time to regions across North America. This type of model cannot be applied to PDM epidemics because *P. philippinensis* conidia are fragile, short-lived and disperse only a few meters from their source. Also, foliar fungicide treatment of large acreages of corn is impractical.

VIII. Knowledge gaps

Important gaps in our present knowledge include:

1. Will *P. philippinensis* be able to persist in the U.S.? In what regions?
2. Under what conditions does *P. philippinensis* produce oospores?
3. Are collateral hosts capable of producing *P. philippinensis* oospores that contribute to overwintering and dispersal in the U.S.?

4. Can *P. philippinensis* survive in the apical meristem of perennial collateral hosts overwinter and initiate inoculum (conidia) production the following spring? Of particular interest is johnsongrass.

5. How far will *P. philippinensis* be able to disperse annually from its overwinter survival range; *i.e. w*hat is PDM's maximum disease distribution range?

6. What importing countries would be likely to embargo *P. philippinensis* contaminated corn?

7. What seed treatment rates of metalaxyl or mefenoxam will provide optimum protection for young corn plants against *P. philippinensis*, while minimizing the potential for pathogen resistance development? Are any recently developed fungicides effective against PDM?

IX. Immediate response options

Although virtually all U.S. commercial corn seed is treated with metalaxyl or mefenoxam, the low rates currently applied (1 - 4 g a.i./100 kg seed) are unlikely to effectively curtail the development of PDM in this country. *P. philippinensis* dissemination would proceed at a slow to moderate pace. Since PDM infects only young seedlings and a single crop is grown simultaneously over much of the U.S. corn producing area, containment and eradication may be possible if the pathogen was introduced into a localized area. If introduced into the northern Corn Belt, the event may be limited to a single season since the pathogen appears to have only limited means of overwintering there and no action may be required. If introduced into the southern or central Corn Belt, the pathogen may be able to overwinter on alternate hosts, and rapid action is required. Containment and quarantine should be possible if *P. philippinensis* is rapidly identified and timely control measures are implemented. A PDM pathway and response summary for the intentional introduction of *P. philippinensis* is presented in Appendix 8.

1. Rapid Detection

The creation of the NPDN represents a major step to improve the potential for rapid detection. A key objective of this cohesive distribution system is to facilitate the detection of anomalies such as simultaneous outbreaks at multiple sites, thereby identifying a bioterrorist attack (Appendix 7A & 7B). Symptoms are not definitive for many of the downy mildews so that first responders may misidentify PDM as an indigenous maize downy mildew, such as *P. sorghi*. This might be considered a logical deduction because *P. sorghi* has been reported on shattercane in the Midwest. This suggests a low probability of early detection of PDM in the U.S. First responders must be encouraged to promptly forward suspect downy mildew samples to state university diagnostic clinics for thorough evaluation. Samples will be passed on to NPDN regional centers where diagnosis will be confirmed and information communicated with NAPIS and regulatory agencies.

The odds of early detection may be improved by incorporating PDM symptom recognition into continuing education courses and workshops for first responders. Increasing awareness of the potential for agroterrorism and farm biosecurity through classes, newsletters, and Internet will be useful.

DNA hybridization and PCR techniques are still being developed. Yao *et al*. (1991a; 199b; 1992) reported the ability to differentiate *Peronosclerospora* species, however, CIMMYT researchers have been unable to repeat this success using samples recently collected from various Asian locations (Dr. M. George, personal communication). These inconsistencies indicate molecular techniques cannot be used with a high degree of certainty to differentiate an introduced *P. philippinensis* from *P. sorghi* in the U.S. Continued downy mildew fingerprinting research is required. An analysis of available molecular techniques, using samples of *P. sorghi* collected from various U.S. locations and samples of *P. philippinensis* from a number of countries in Asia, could illuminate potential identification difficulties and the value of present DNA hybridization and PCR-based techniques to distinguish these pathogens in the U.S. Once a molecular identification technique is deemed reliable, NPDN could run trials to determine the time required for *P. philippinensis* identification.

Molecular techniques (PCR-based) should allow rapid identification once reliable primers are identified and made commercially available. Since *P. philippinensis* is on the select pathogen list, the organism and its DNA are limited to ≥BL3 containment facilities.

Prohibition on the possession of *P. philippinensis* DNA will hamper diagnosis and delay identification. These restrictions do not appear to be scientifically sound and should be removed as quickly as possible to ensure rapid identification.

Assembling a "Detection Assessment Team" with expertise on PDM, as has been done by APHIS for soybean rust, is a preparatory step that can minimize the time required for on-site threat assessment. A team of USDA and university experts should arrive at the site of infection within 24 hours following APHIS confirmation of pathogen identification.

2. Cultural Control

If identified early, containment might be possible if the pathogen was introduced into a localized area. The extent of spread would need to be identified and corn crops destroyed within the specified area. Eradication by plowing, chemical desiccation, or burning may be required. An additional precaution would be the removal of collateral host weeds (Appendix 1) in the containment area.

3. Fungicides

If the suspected area of infection was of limited acreage, fungicide sprays would be useful in the containment process. Foliar sprays of metalaxyl (Ridomil MZ 58 at 40 g a.i./ha) applied 10 days after planting provide excellent control (see VII-3).

Although most commercial corn seed in the U.S. is treated with metalaxyl or mefenoxam, routine dosages are an order of magnitude lower than those recommended for downy mildew control on corn seed in the U.S. Still higher rates are recommended for use against *P. philippinensis* in the Philippines than for *P. sorghi* in the U.S. In the event of a PDM introduction, a simple increase in the concentration of metalaxyl or mefenoxam seed treatments may successfully control the disease the year after introduction. Additional research is required to determine the optimum concentrations of modern formulated chemicals capable of protecting against PDM. Although rates as high as 200 g of metalaxyl /100 kg seed have been demonstrated nonphytotoxic, seeds treated with 400 g/100 kg seed produced seedlings with depressed growth for 2 weeks post-emergence (Exconde and Molina, 1978). Such high application rates may not be acceptable in the U.S. because of residue concerns.

Between 0.24 and 1.5 million kg of the a.i. metalaxyl (at rates of 30 g/kg or 200 g/kg respectively) would be required to treat seed for the entire U.S. corn production area for a single year. Fungicides containing metalaxyl or mefenoxam are used on a variety of crops worldwide and sufficient quantities could be quickly made available for large-scale seed treatment at rates presently recommended (15 – 30 g/100 kg seed) for downy mildews (M. Jirak of Syngenta, personal communication) in the event of a PDM outbreak in the U.S.

Mefenoxam use may be preferable from an environmental viewpoint because only half the quantity of a.i. is required compared to metalaxyl. As resistance of *P. sorghi* in the U.S. and *P. philippinensis* in the Philippines to metalaxyl based compounds has been reported (see VII-3), strategies to minimize the build up of resistance would need to be considered if long-term use was required. Investigation into the efficacy of recently developed fungicides against PDM may uncover new candidates for control.

4. Resistance Breeding

Ultimately, the only economically feasible means to control widespread PDM is the development and use of resistant maize lines. In the U.S., there has been little evaluation of PDM resistance in commercial breeding programs. U.S. varieties and breeding lines resistant to *P. sorghi* are highly susceptible to *P. sacchari*, considered conspecific with *P. philippinensis* (Bonde and Melching, 1979). A number of U.S. hybrids, used as parentage for hybrids developed in the Philippines, are moderately to severely susceptible to PDM (Raymundo *et al.*, 1993).

The evaluation of U.S. germplasm for PDM resistance and incorporation into public and private breeding programs should be considered as a long-range response plan because resistant cultivars should be introduced as rapidly as possible to minimize damage after introduction of PDM to the U.S.

Appendix 1. Reported hosts of Peronosclerospora philippinensis

Scientific Name	Common Name	Place of Plant Origin	Source
Tribe: Andropogoneae			
Andropogon gerardii	big bluestem	U.S.	Bonde and Peterson (1983)
Andropogon sorghum	sorghum		Weston (1920)
Avena sativa	oats	Philippines	Exconde *et al.* (1968a)
Bothriochloa ambigua		Australia	Bonde and Peterson (1983)
Bothriochloa bardinodis	cane beardgrass	U.S.	Bonde and Peterson (1983)
Bothriochloa decipiens	crown beardgrass	Australia	Bonde and Peterson (1983)
Bothriochloa edwardsiana		Argentina	Bonde and Peterson (1983)
Bothriochloa ischaemum	Turkistan bluegrass	Hungary	Bonde and Peterson (1983)
Bothriochloa laguroides	silver bluegrass	Brazil	Bonde and Peterson (1983)
Bothriochloa perforata		U.S.	Bonde and Peterson (1983)
Bothriochloa springfieldii		U.S.	Bonde and Peterson (1983)
Bothriochloa woodrowii		India	Bonde and Peterson (1983)
Euchlaena luxurians			Weston (1920)
Euchlaena mexicana	teosinte	Philippines	Exconde *et al.* (1968a) Weston (1920)
Eulalia fulva		Australia	Bonde and Peterson (1983)
Miscanthus japonicus	Japanese silvergrass		Weston (1921)
Saccharum officinarum	sugarcane	U.S. Philippines	Bonde and Peterson (1983) Exconde *et al.* (1968a)*
Saccharum spontaneum	wild sugarcane	India Philippines	Chona & Suryanarayana (1955) Exconde *et al.* (1968a)* Weston (1921)
Schizachyrium hirtiflorum		U.S.	Bonde and Peterson (1983)
Schizachyrium microstachyum	hierba colorada	Argentina	Bonde and Peterson (1983)

Schizachyrium scoparium	little bluestem	U.S.	Bonde and Peterson (1983)
Sorghum bicolor	grain sorghum	Philippines	Exconde *et al*. (1968a)* Weston (1920)
S. bicolor [drummmondii]	shattercane	Australia	Bonde and Peterson (1983)
S. bicolor [gambicum]		Chad	Bonde and Peterson (1983)
S. bicolor [hewisonii]		Sudan	Bonde and Peterson (1983)
S. bicolor [japonicum]		Portugal	Bonde and Peterson (1983)
S. bicolor [melaleucum]		Algeria	Bonde and Peterson (1983)
S. bicolor [miliiforme]		Argentina	Bonde and Peterson (1983)
S. bicolor [nigricans]		Portugal	Bonde and Peterson (1983)
S. bicolor [niloticum]		Ethiopia	Bonde and Peterson (1983)
S. bicolor [saccharatum]		Argentina	Bonde and Peterson (1983)
S. bicolor [sudanense]		U.S.	Bonde and Peterson (1983)
S. bicolor [technicum]		U.S.	Bonde and Peterson (1983)
Sorghum halepense	johnsongrass	U.S. Philippines	Bonde and Peterson (1983) Exconde *et al.* (1968a)*
Sorghum plumosum	perennial canegrass	Australia	Bonde and Peterson (1983)
Sorghum propinquum	sorghum	Philippines	Exconde *et al*. (1968a)*
Tribe: Maydeae			
Tripaticum dactyloides		U.S.	Bonde and Peterson (1983)
Zea diploperennis		Mexico	Bonde and Peterson (1983)
Zea mays subsp *mexicana*	teosinte	Mexico Philippines	Bonde and Peterson (1983) Exconde *et al.* (1968a)
Zea perennis	perennial teosinte	Mexico	Bonde and Peterson (1983)

* Infection initiated by stem injection, no infection when inoculum dropped into plant whorl.

Appendix 2. Comparison of P. philippinensis with domestic corn downy mildew pathogens

	Peronosclerospora philippinensis (Philippine DM)	*Peronosclerospora sorghi* (Sorghum DM)	*Sclerospora graminicola* (Green Ear DM)	*Sclerophthora macrospora* (Crazy Top)
Conidiophores	Hyaline, length 150-400 µm, bloated, widening abruptly, dichotomously branched 2-4 times, ephemeral	Hyaline, length 180-300 µm, bloated often dichotomously branched 2-3 times, septate near base, ephemeral	Hyaline, average length 268 µm, bloated, nonseptate, irregularly dichotomously branched, ephemeral	Hyaline, average length 13.8 µm, simple, hypoid, determinate
Asexual spores	Conidia hyaline, elongate-ovoid to round-cylindrical, apex slightly rounded, 27-39 x 17-21 µm	Conidia hyaline, oval to almost spherical, 15-26.9 x 15-28.9 µm	Sporangia hyaline, broadly elliptical, operculate, papilate, 14-23 x 11-17 µm	Sporangia hyaline, lemon-shaped, operculate, 60-100 x 30-60 µm
Germinate by	Germ tube	Germ tube	Zoospores	Many zoospores
Oospores	Rare, Hyaline to straw colored, smooth walled, spherical, 15.3-22.6 µm diameter (Acedo & Exconde, 1967)	Usually brown to subhyaline spherical, 25-42.9 µm diameter	Pale brown, spherical, usually smooth walled, 35 µm diameter	Hyaline to pale yellow, mainly in vascular bundles, 45-75 µm diameter
Germinate by	Side germ tube	Wide germ tube	Germ tube	Sporangium
Hosts	*Zea mays* *Bothriochloa* *Saccharum* *Schizachyrium* *Sorghum* (see appendix 1)	*Zea mays* *Panicum trypheron* *Sorghum*	*Zea mays* *Echinacloa* *Panicum* *Pennisetum americanum* *Setaria*	*Zea mays* *Avena sativa* *Setaria* *Echinacloa* *Eleusine* *Horteum vulgare* *Miscanthus* *Paspalum* *Saccharum* *Sorghum* etc.

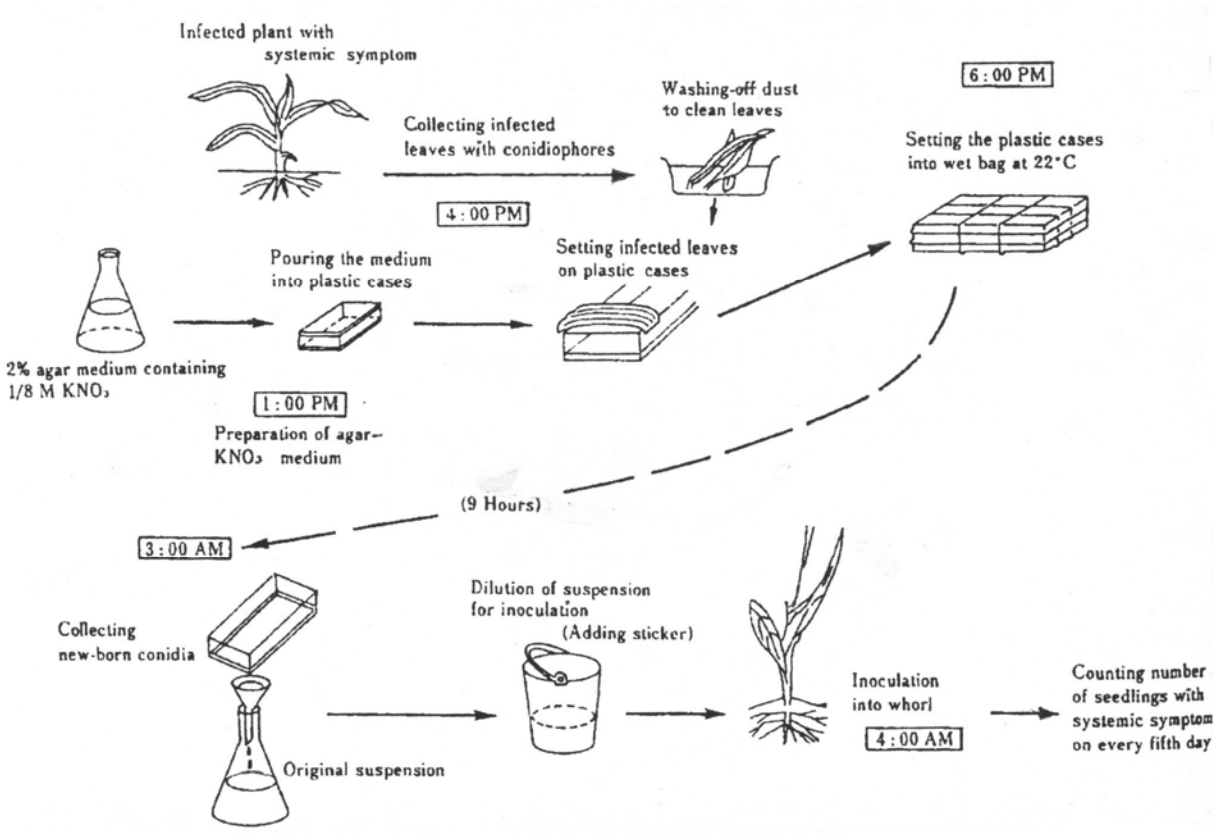
(adapted from Chang, 1986)

Appendix 3. Seed Health Tests for P. philippinensis

a) Embryo examination (Singh *et al*., 1967)
- collect cobs from symptomatic plants 20-30 days after pollination
- remove 25 kernels from each cob
- remove pericarp and aleurone layers around embryo
- separate embryo with or without scutellum
- boil embryo with scutellum in 20%KOH solution
- mount in lactophenol cotton blue and press with cover slip.

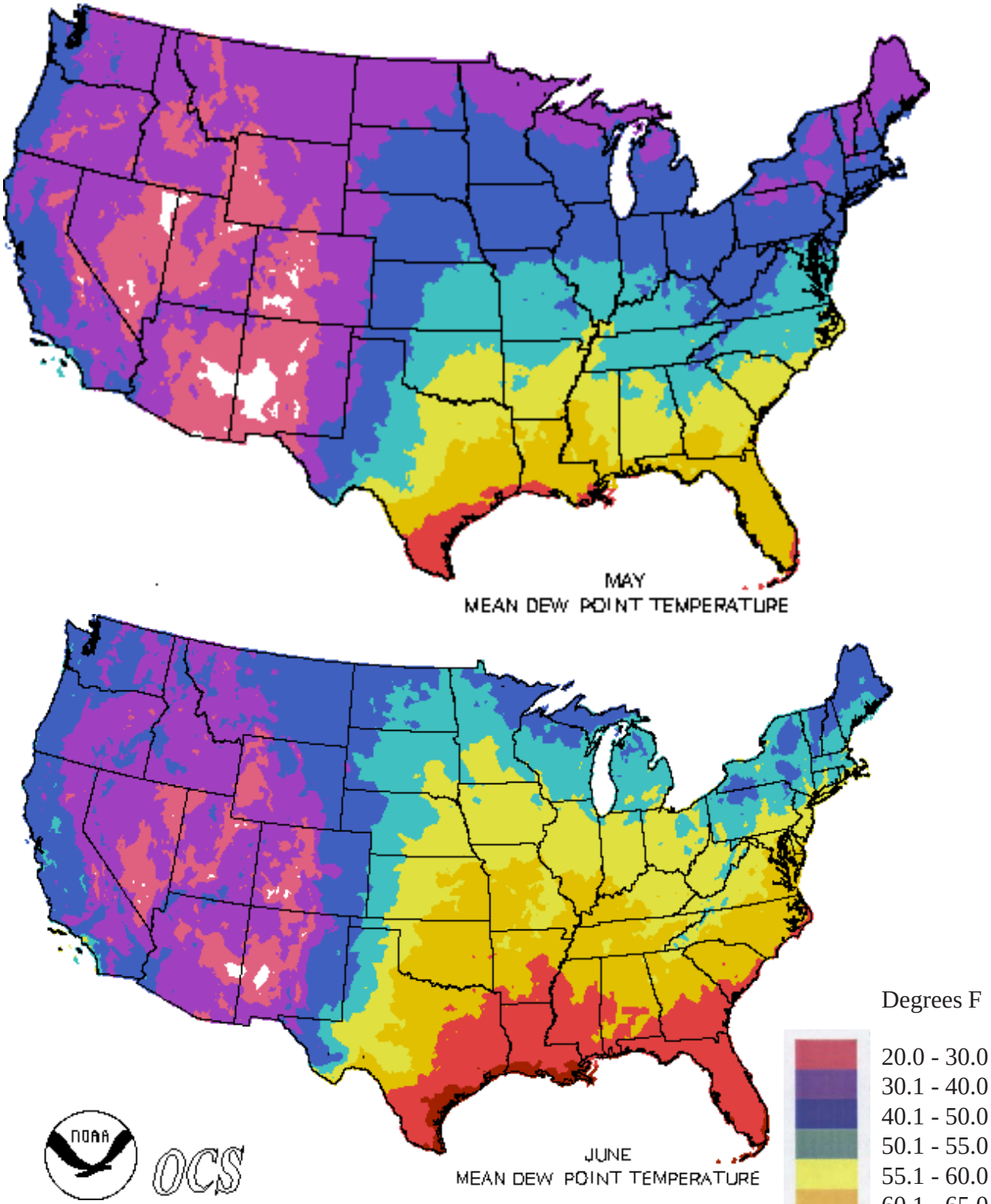
b) Direct Embryo examination (Singh *et al*., 1968)
- place samples (cut into small pieces) in 2% NaOH or KOH for 0.25-12 hrs (depending upon the hardness of the sample) at 45-50°C.
- rinse repeatedly in distilled H_2O
- keep samples in 0.1% cotton blue in lactic acid or 50% glycerin for 15-20 min at the same temperature
- examine mounted slides

Appendix 4. Procedure for artificial inoculation of downy mildew, P. philippinensis, to maize for screening at the seedling stage.



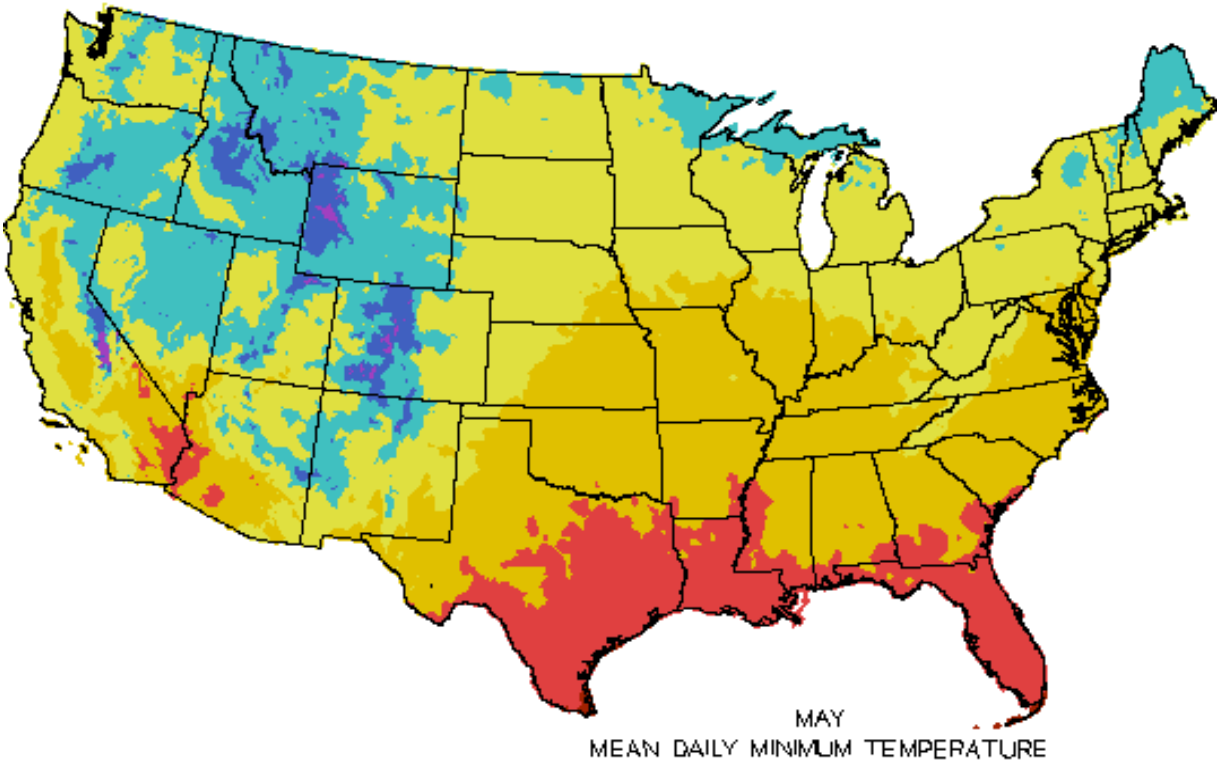
(Yamada *et al*., 1976)

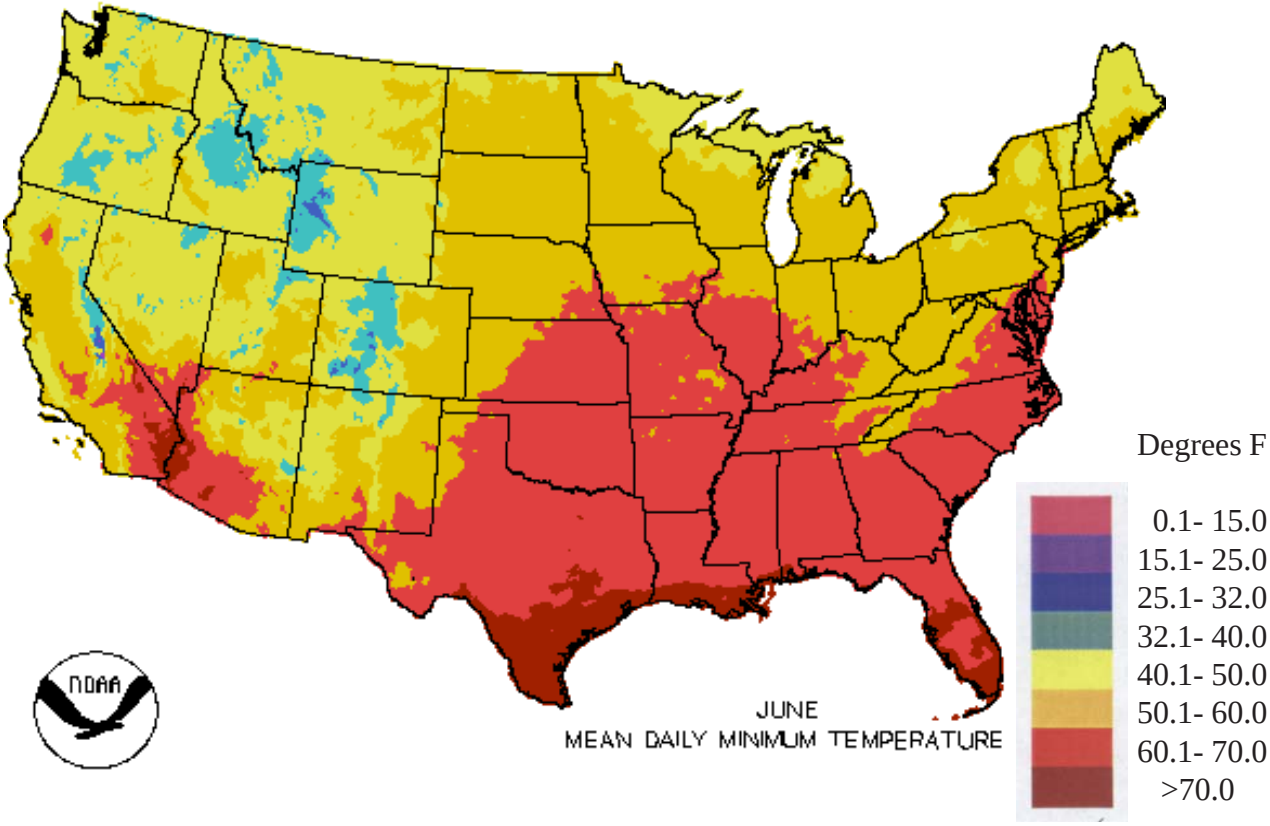
Appendix 5A. Mean dew point temperature for May and June



(NOAA, 2000)

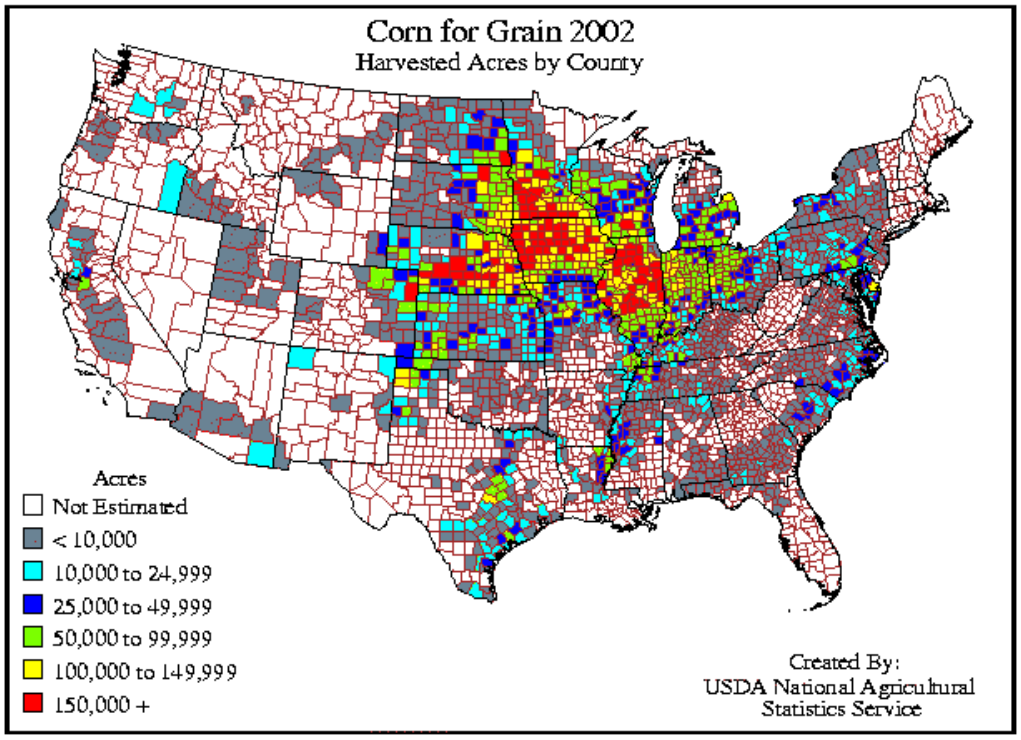
Appendix 5B. Mean daily minimum temperature for May and June



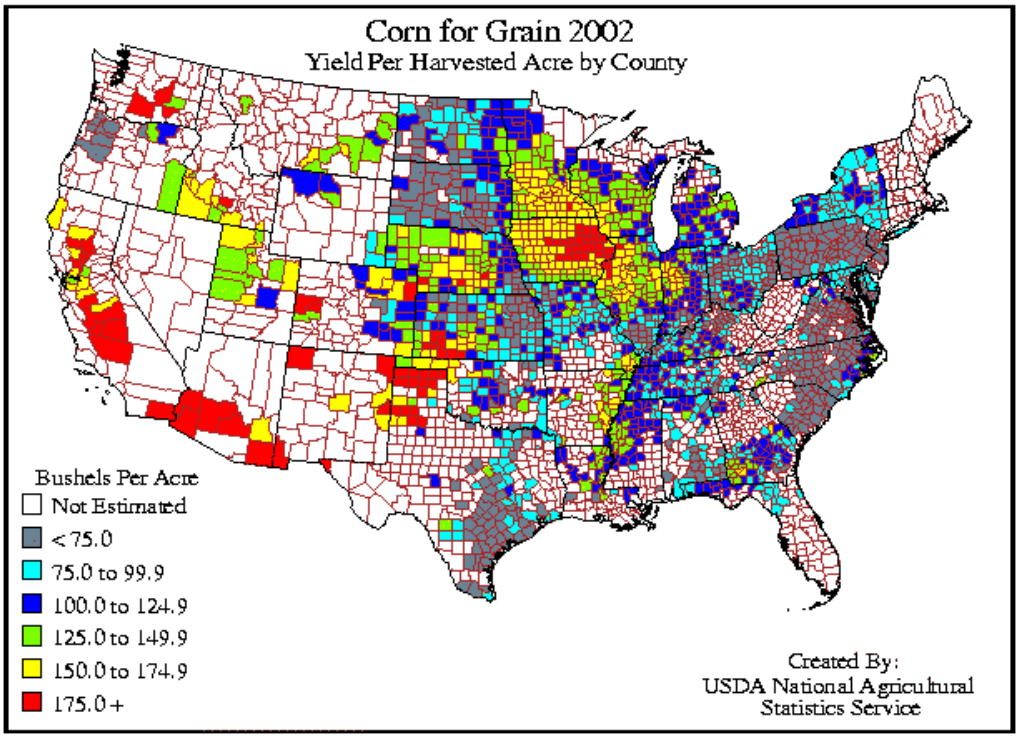


(NOAA, 2000)

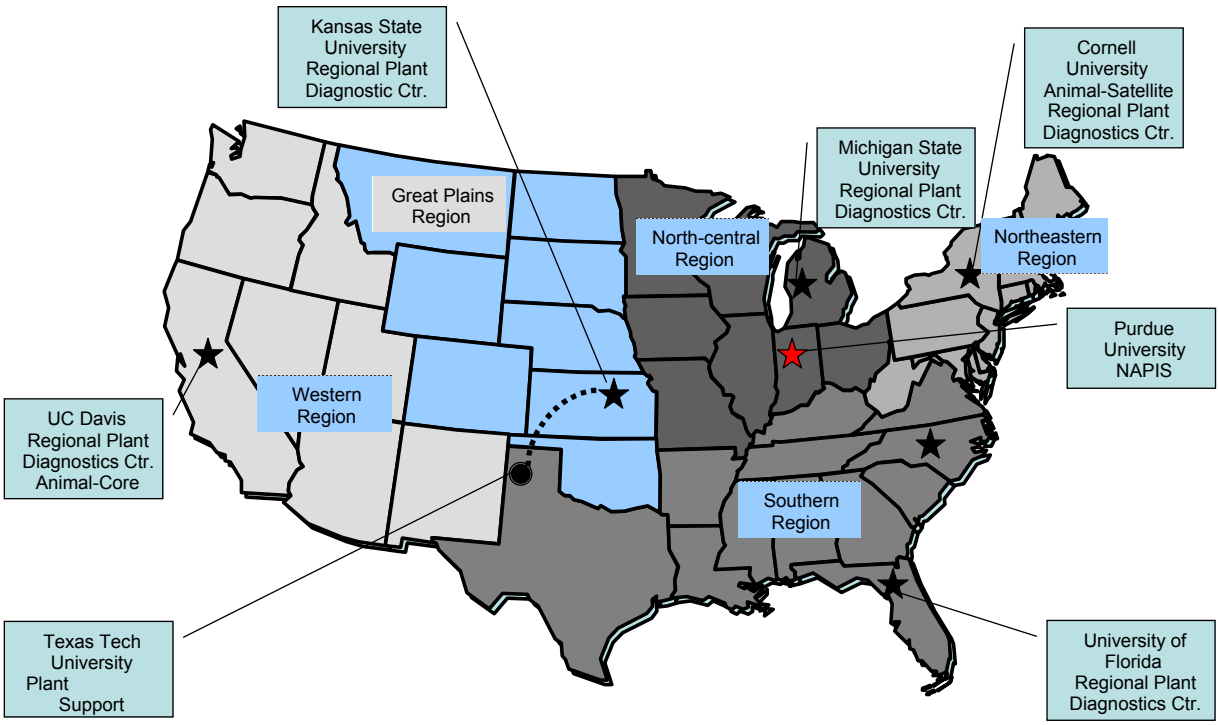
Appendix 6A. Harvested corn for grain acres in 2002



Appendix 6B. Yield/acre of corn in 2002

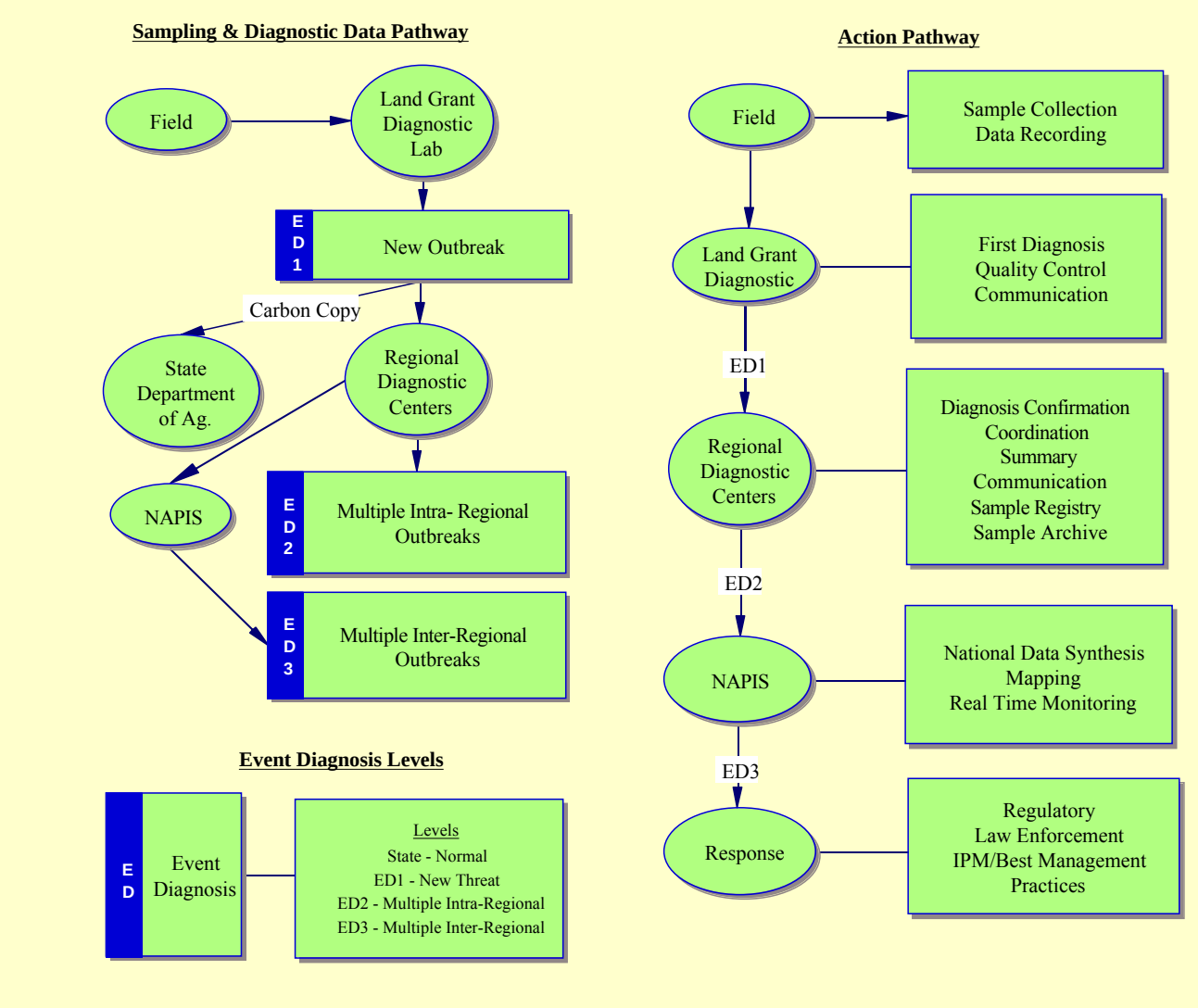


Appendix 7A. National Plant Diagnostic Network (NPDN) regions and regional centers



From Cardwell (2004)

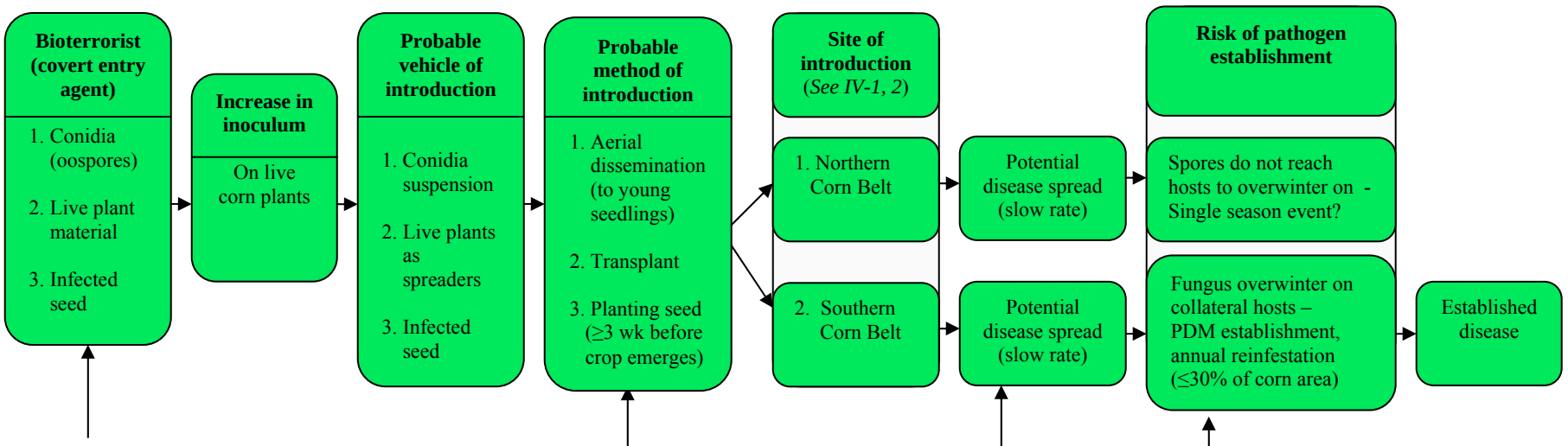
Appendix 7B. Sampling + diagnostic data and action pathways for NPDN



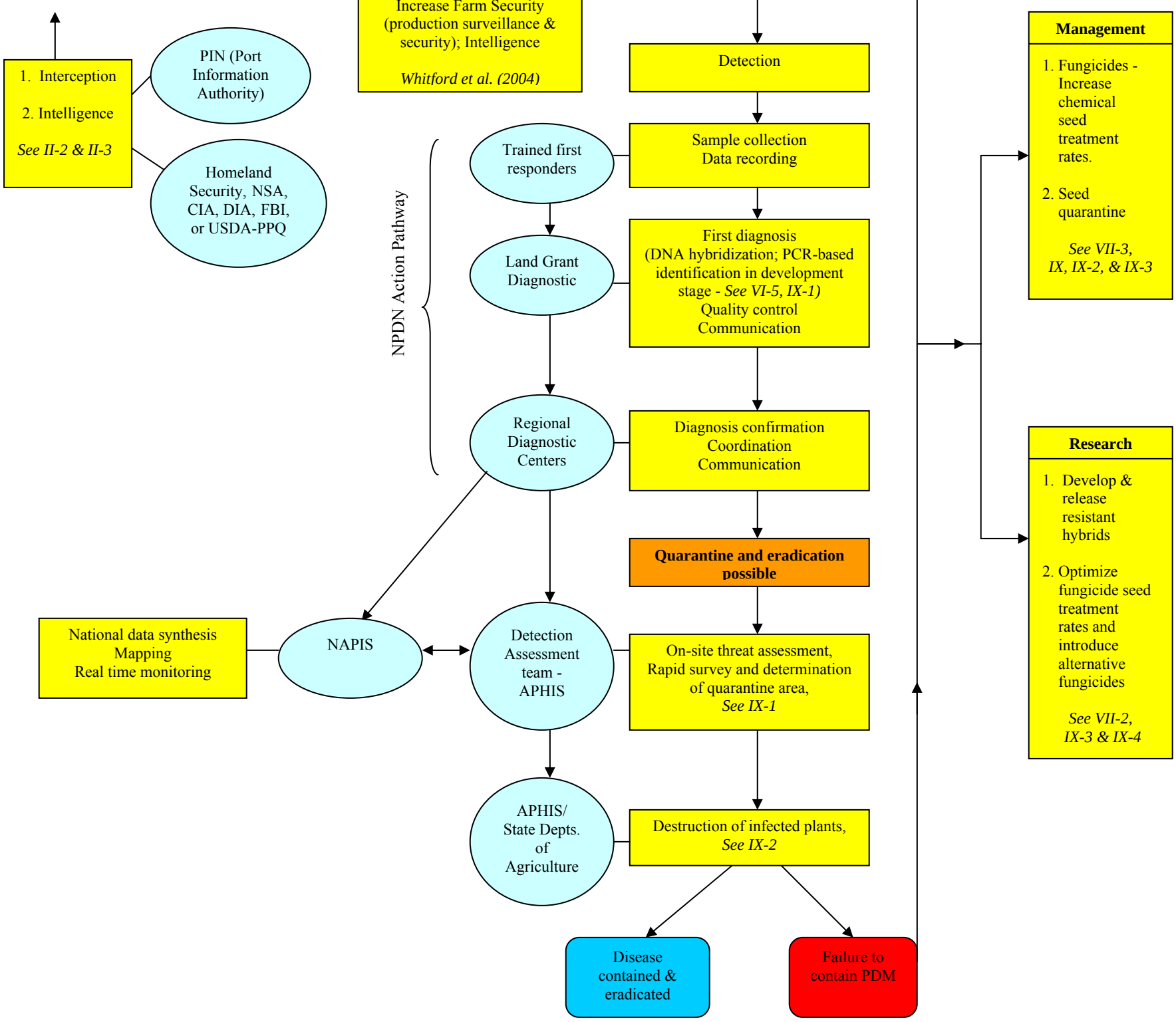
From Cardwell (2004)

Appendix 8. Pathway and response to the intentional introduction of P. philippinensis, the cause of Philippine downy mildew of corn.

a. PDM introduction and development pathway



b. Response strategy pathway



Appendix 9. Scientists Knowledgeable of Philippine Downy Mildew

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Bacterial Leaf Blight of Rice

Pathway Analysis:

Introduction of

Xanthomonas oryzae

pv. *oryzae*

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Bacterial Leaf Blight Pathway Analysis

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Executive Summary: Bacterial Leaf Blight

- A comprehensive search for available information on bacterial leaf blight and the causal organism, *Xanthomonas oryzae* pv *oryzae*, its potential for causing crop damage, host range, known geographical distribution, disease cycle (life cycle), and epidemiology was fundamental to understand the nature of the threat of this disease on rice production in the United States. With this information, the potential for introduction, establishment and dissemination of bacterial leaf blight within the United States was addressed. Two principle scenarios of possible introduction were considered, 1) inadvertent introduction on imported, infested rice seed and 2) deliberate introduction with malicious intent.

- The principle natural means of long-distance dissemination of *X. o. oryzae* appears to be on infested rough rice (not de-hulled). The rice is transported to rice mills and dehulled. The hulls are then discarded or enter a trade that is unrelated to rice production, such as compost development. The *X. o. oryzae* that may be contaminating the rough rice would be removed with the hulls. Very limited quantities of rough rice are imported into the United States. Perhaps, the greatest threat of inadvertent introduction the bacterium into the rice crop of the United States is on imported rice seed for rice breeding purposes. Rice breeding programs need to be very cautious and vigilant in their acceptance of and handling of newly imported rice germplasm, especially if originating from areas of the world where bacterial blight is known to occur.

- In the situation of deliberate introduction of *X. o. oryzae* with malicious intent, the use of infested seed as a medium of introduction would be a very non-efficient method. Seed transmission of the bacterium does occur, but the efficiency of this method is limited. Bio-terrorists most likely would directly introduce the bacterium into the production fields. However, to effectively do this on such a scale as to create a widespread epidemic the same year or following year of the introduction would require a sizeable amount of bacterial inoculum freshly prepared within 2 to 4 days prior to inoculation. Such a requirement would suggest that inoculum increase probably would have to occur within the United States. If large quantities of inoculum were prepared and disseminated in rice

irrigation systems, the risk of establishment of bacterial blight would be “moderate to high” in the southern rice belt, but “low” in the California rice production area. The climate for bacterial blight development is favorable in the southern rice belt, but much less favorable in the relatively dry California area.

- The host range of *X. o. oryzae* includes several common weeds native to the southern rice belt. Over winter survival of the pathogen would depend largely on weed hosts, since the bacterium does not survive well in soil, has limited longevity in irrigation water, and is not easily disseminated in infested seed. The pathogen’s survival on infected rice stubble is limited because of the common crop rotation and crop stubble destruction practices in the southern rice production area of the United States.

- If the bacterial blight pathogen were to contaminate an irrigation canal system, numerous fields could become infested subsequently. Weeds hosts along the banks of the irrigation canals could serve as a source of overwintering bacterium.

- If infection of rice by bacterial blight is detected early, before it becomes widespread, there is reasonable opportunity for disease containment and possible eradication of the disease. This would involve:

    a) Planting of rice seed from fields free of bacterial blight.

    b) Non-harvest of infested fields or, if harvested, very careful grain transport to a mill and disposal of the rice hulls far from any rice field.

    c) Disking of infested fields after harvest followed by weed management through periodic disking to maintain a low weed environment for one year.

    d) Follow the year of disking with at least one additional year of fallow before planting rice seed from a field evaluated to be free of symptoms of bacterial blight.

Bacterial Leaf Blight Of Rice

Pathways Analysis of the Introduction of

Xanthomonas oryzae pv. *oryzae*

I. Biology and life / disease cycle analysis of the pathogen

The biology and life / disease cycle analysis will emphasize those aspects relevant to the potential for the pathogen's introduction, establishment, and spread within the United States.

A. Taxonomy

Taxonomic Position:	Kingdom:	Proteobacteria
	Class:	Zymobacteria
	Order:	Xanthomonadales
	Family:	Xanthomonadaceae
	Genus:	Xanthomonas

Proper preferred name:

Xanthomonas oryzae pv. *oryzae* (Ishiyama 1922) Swings et al. 1990

Synonyms or superseded names:

Xanthomonas campestris pv. *oryzae* (Ishiyama 1922) Dye 1978

Xanthomonas oryzae (Uyeda & Ishiyama) Dowson 1943

Bacterium oryzae (Uyeda & Ishiyama) Nakata 1927

Pseudomonas oryzae Uyeda & Ishiyama 1922

Bacillus oryzae Hori & Bokua 1911

NOTE: Prior to 1957, the rice bacterial leaf streak pathogen (now classified as *Xanthomonas oryzae* pv. *oryzicola*) had not been distinguished from the rice bacterial blight pathogen (*Xanthomonas oryzae* pv. *oryzae*). Therefore, literature references to bacterial blight of rice prior to 1957 may actually have refereed to both pathogens.

Common names of the disease include:

English

Bacterial leaf blight of rice

Rice leaf blight

Rice bacterial blight

Rice kresek disease

French

Maladie bacterienne des feuilles du riz

German

Bakterielle weissenfleckenkranheit

Bacterieller Blattbrand

Spanish

Enfermedad bacteriana de las hojas del arroz

B. Symptoms

The bacterium primarily enters the plant leaf tissue through the hydrathodes or through wounds and enters the vascular system. Foliar symptoms initially appear as water soaked and yellowish lesions along the leaf veins, often beginning near the leaf tips, then increasing in length and width and spreading down the leaf. The lesions often have a wavy or curvy margin and not limited by the leaf veins. In the early morning, bacterial ooze often can be observed on the surface of leaf lesions as milky to opaque droplets. The leaf lesions turn from yellow to grayish-white as the disease advances. Disease incidence and severity increases with plant growth, peaking at the flowering stage. The term “kresek” refers to a severe form of the disease which can develop when roots and leaves are damaged and infected at the seedling stage during transplanting. Infection at this early stage often results in seedling death after transplanting. Fortunately, in the United States, rice is direct seeded

into the fields and no seedling transplanting occurs. The “kresek” expression of the disease would be rare if the disease becomes established in U.S. rice production.

C. Potential Yield Losses

Bacterial blight is particularly destructive in Asia during the heavy rains of the monsoon season. Severe infection causes poor grain development, broken grains, and deterioration in chemical composition. In many Asian countries where rice is in continuous cultivation, bacterial blight is endemic. Yield reductions as high as 50% have been reported in severely infected fields where infection was established early in the tillering stage of the rice. However, more commonly, plants become infected later during the tillering stage, and yield reductions are reduced approximately 10% to 20% in such situations (Elings et al. 1997; Ou, 1985). In Africa, yield losses of 3% to 40% have been reported (Awoderu et al. 1991).

D. Host Range

Plants reported to be hosts of the bacterial blight bacterium observed either through natural infection or deliberate inoculation include (Li et al., 1985; Bradbury, 1970; Bradbury, 1986; Gonzalez et al., 1991; Valluvapardasan and Mariappan, 1989):

Poaceae (grasses):

Bracharia mitica
Cynodon dactylon
Echinocloa crus-galli
Leersia hexandra
Leersia sayanuka
Leersia oryzoides
Leersia japonica
Leptochloa chinensis
Zizania aquatica
Leptochloa filiformis
Leptochloa panacea
Oryzae australiensis
Oryza perennis
Oryza sativa
Oryza sativa f. spontanea
Phalaris arundinacea
Paspalum scrobiculatum

Cyperaceae (sedges):

Cyperus difformis
Cyperus rotundus

E. Geographical Distribution

Bacterial blight of rice occurs widespread globally. It is found in most rice-growing areas of Asia, the Sahel region and western portions of Africa, northern Australia, Central America, and South America. *Xanthomonas campestris* pv. *oryzae* was reported in the United States in Texas and Louisiana in 1989 (Jones et al., 1989; Up and Gonzalez, 1991). However, the virulence on rice of this reported bacterium was low and yield losses to the disease were insignificant. Dr. Jan Leach, phytobacteriologist and *Xanthomonas* expert of the Department of Plant Pathology at Kansas State University, who had been involved in the initial identification of the United States strain of the bacterium, continued to work with this organism. In personal communication in June, 2003, she has indicated, "The putative *X. oryzae* pv. *oryzae* from Texas and Louisiana is a *Xanthomonas.* We are fairly convinced it is not *Xanthomonas oryzae* pv. *oryzae,* but may be another species or a related, non-described pathovar. We base this on further RFLP and rep-PCR, etc."

A repetitive DNA element cloned from *X. o. oryzae* has been used as a probe in RFLP analysis to differentiate *X. o. oryzae* from *X. o. oryzicola* and pathovars of *X. campestris* (Leach et al. 1990). The strains of the putative X*. o. oryzae* from the United States are exceptional compared with all strains tested from Asia, Australia, and South America in that they contain fewer copies of the repetitive element. Also, a set on monoclonal antibodies of *X. o. oryzae* distinguishes between the United States and Asian isolates (Benedict et al. 1989). One monoclonal antibody (*Xco-5),* generated to the putative strain of *X. o.* oryzae isolated from the outbreak of "bacterial blight" in Texas and Louisiana in 1987, reacts to all strains from the United States but not to Asian, Australian, or South American strains. The mild symptoms evoked by the strains from the United States on U.S. rice cultivars and the inability of the strains to induce symptoms on the IRRI differential rice cultivars further indicate significant differences between Asian and U.S. strains (Benedict et al. 1989). The above should be taken into account when interpreting distribution lists for *X*. *oryzae* pv. *oryzae* that include the United States. The following list includes records of the reports of

bacterial blight in the United States, but based on the above information, it is suggested that they may be erroneous reports.

World Distribution of *Xanthomonas oryzae* pv. *oryzae*

Asia		
Bangladesh	widespread	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Cambodia	restricted distribution	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
China	widespread	Bradbury, 1986; Zhang & Huang, 1990; EPPO, 2003
Taiwan	widespread	Bradbury, 1986; Zhang & Wang, 1990; CABI/EPPO, 1997; EPPO, 2003
India	widespread	CABI/EPPO, 1997; EPPO, 2003
Indonesia	restricted distribution	CABI/EPPO, 1997
Japan	restricted distribution	Bradbury, 1986; CABI/EPPO, 1997
Korea, DPR	restricted distribution	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Korea, Republic of	restricted distribution	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Laos	widespread	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Malaysia	widespread	CABI/EPPO, 1997; EPPO, 2003
Myanmar	restricted distribution	Singh et al., 1983; CABI/EPPO, 1997; EPPO, 2003
Nepal	restricted distribution	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Pakistan	restricted distribution	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Philippines	restricted distribution	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Sri Lanka	restricted distribution	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Thailand	restricted distribution	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Vietnam	restricted distribution	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Africa		
Burkina Faso	present, no further details	CABI/EPPO, 1997; EPPO, 2003
Cameroon	present, no further details	Jones et al., 1991; CABI/EPPO, 1997; EPPO, 2003
Gabon	present, few occurrences	CABI/EPPO, 1997; EPPO, 2003
Mali	present, no further details	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Niger	present, no further	Bradbury, 1986; CABI/EPPO, 1997;

	details	EPPO, 2003
Senegal	present, few occurrences	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Togo	present, no further details	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003 North America
North America		
Mexico	present, no further details	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
USA *	restricted distribution	Jones et al., 1989; CABI/EPPO, 1997
Louisiana *	present, no further details	Jones et al., 1989; CABI/EPPO, 1997; EPPO, 2003
Texas *	present, no further details	Jones et al., 1989; CABI/EPPO, 1997; EPPO, 2003
Central America		
Costa Rica	present, no further details	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
El Salvador	present, no further details	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Honduras	present, no further details	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Panama	present, few occurrences	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
South America		
Bolivia	present, no further details	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Colombia	present, no further details	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Ecuador	present, few occurrences	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Venezuela	present, no further details	Bradbury, 1986; CABI/EPPO, 1997; EPPO, 2003
Oceania		
Australia	restricted distribution	Bradbury, 1986; CABI/EPPO, 1997

* See section above under “Geographical Distribution” for a discussion on the bacterial strain of *X. o. oryzae* found in the United States, which may be significantly different than exotic strains.

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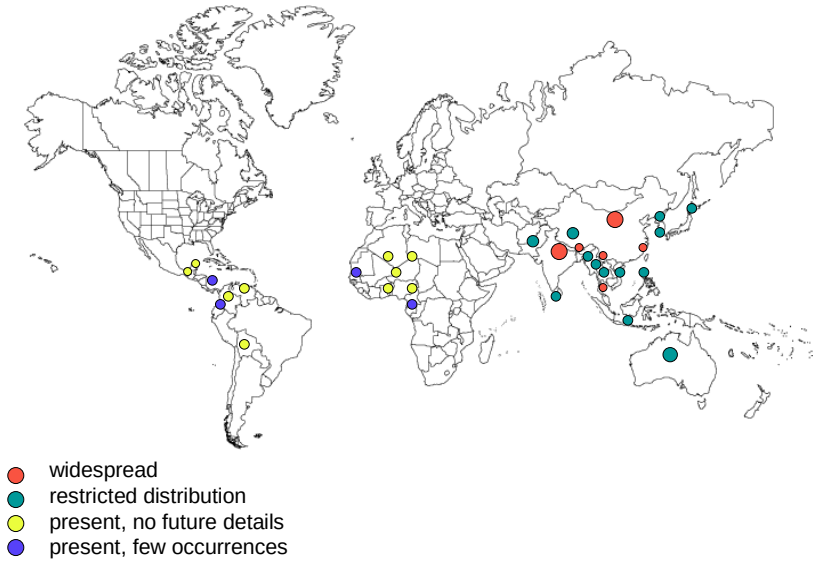
World Distribution of *Xanthomonas oryzae* pv. *oryzae*



F. Life Cycle or Disease Cycle

Bacterial blight of rice is favored by warm temperatures, high humidity, and frequent rainfall. *X. o. oryzae* primarily enters the rice plant through the hydrathodes on leaves, wounded tissue, and growth cracks at the base of leaf sheaths caused by emerging roots. The hydrathodes of rice consist of 10 to 20 water pores each and are densely distributed along the edge of the upper surface of the leaves, predominantly near the leaf apex. Hydrathodes exude water droplets during periods of high humidity, such as during early morning hours. Leaf-surface bacteria can become ensnared in these water droplets and be drawn into the leaf as the water droplet is absorbed into the leaf when lower relative humidity occurs.

Once inside the leaf of a susceptible rice host plant, the bacterium multiplies and enters the vascular system, eventually restricting water movement and transpiration. Normal exudation of water from the hydrathodes in the form of guttation droplets facilitates the ingress and egress of the pathogen. As the pathogen population increases, masses of bacterial cells ooze from the hydrathodes. Subsequently, they can be disseminated by splashing rain and wind to other infection sites or drop to the irrigation water where further spread occurs. Transmission through irrigation water is important, but the pathogen's survival in irrigation

water apparently is limited to about two weeks (Singh 1971; Hsieh and Buddenhagen 1975). By means of the irrigation flood water, the bacterium moves, encounters, and infects susceptible weed hosts.

Overseasoning of *X. o. oryzae*, or pathogen survival from one crop season to the next crop season, has been reported to occur on rice stubble, rice straw, volunteer rice, weed hosts, and infected seed, although some of the reports are often contradictory or controversial (Reddy and Yinshang-Zhi 1989).

1. Rice Straw and Stubble

Annual sequential production of rice on the same land increases the possibility of X*. o. oryzae* persisting in infected rice straw and stubble and serving as a source of inoculum for the following rice crop. The probability of this would increase if a second or ratoon rice crop was grown, thereby extending the growing season, followed by another main-crop rice planting the following spring.

Several investigators demonstrated the pathogen on post-harvest rice straw and stubble, but the reported longevity the survival on these sources vary considerably.

Goto et al. (1953) reported that the bacterium overwintered in rice straw. Inoue et al. (1957) indicated that the pathogen could survive 5 months on infected rice straw. Using a bacteriophage multiplication technique, it was demonstrated that the bacterium survived 3 to 4 months in straw and stubble (Reddy 1972). In Japan, rice stubble which survives the winter has been found to harbor the organism in the base of the stem and the roots until the following spring (Ou 1985).

Survival of *X. o. oryzae* in rice straw and stubble is influenced by prevailing temperatures and relative humidity. In general, the bacterium appears to survive longer under reduced temperature and relative humidity. Thus the pathogen might survive longer in infected rice stubble and straw in temperate zones compared with humid tropical regions. If infected rice straw and stubble is disked thoroughly into moist soil, survival time for the pathogen is reduced significantly (Tagami 1958).

In summary, *X. o. oryzae* does not appear to have a significant saprophytic existence on dead rice tissue, but survives and multiplies on live rice plant tissue. Therefore, thorough disking of rice straw and stubble into the soil between rice crops or planting rice on a

rotation where rice does not follow rice in sequential years should minimize any significant role infested rice straw and stubble may have in serving as an overseasoning source of inoculum for bacterial blight. Diseased rice straw would not appear to be a significant means of survival and perpetuation of *X. o. oryzae* in U.S. rice production, unless non-rotated, sequential rice crops becomes common and thorough disking of the rice fields following harvest becomes less common.

2. Weed Hosts

In the United States, *Leersia hexandra* was reported to be an alternate host of *X. o. oryzae* by natural infection (Gonzalez et al. 1991). However, subsequent research suggests that the pathogen reported as *X. o. oryzae (X. campestris* pv. *oryzae)* in the apparent bacterial blight in Texas and Louisiana is probably different from the bacterial blight bacterium of Asia. Dr. Jan Leach, phytobacteriologist at Kansas State University and world recognized expert in Xanthomonad research, states that there is significant evidence that the isolates of the bacterium from the United States are significantly different from the Asian isolates of the pathogen and may more appropriately be a different species or pathovar based on further RFLP and rep-PCR studies (see section 1.5 Geographic Distribution) . Nevertheless,

L. hexandra was also reported as a host of the bacterial blight pathogen (Rao and Kauffman 1970; Reddy and Nayak 1974). *Leersia hexandra* commonly grows in the irrigation ditch banks of rice irrigation systems in Texas, Mississippi, and Louisiana.

Plants reported to be hosts of the bacterial blight pathogen observed either through natural infection or artificial inoculation are listed in this report in section 1.4 Host Range. Of those listed, the species known to occur in the rice production areas of the Southern United States include at least:

Scientific name	**Common name**
Cynodon dactylon	bermudagrass
Cyperus rotundus	purple nutsedge
Echinocloa crus-galli	barnyard grass
Leptochloa filiformis	red sprangletop
Leersia hexandra	southern cutgrass
Leersia oryzoides	rice cut grass
Oryzae sativa	red rice

The confirmation of certain weeds as hosts of *X. o. oryzae* does not prove their role as sources of overwintering inoculum. However, in Japan, the overwintering of the bacterium was indicated on roots and rhizomes of *Leersia oryzoides* var. *japonica* and *L. sayanuka* confirmed by a phage technique. Also, natural bacterial blight lesions develop in nature much earlier on *L. sayanuka* than on rice, suggesting that *L. sayanuka* could function as a source of inoculum in Japan. (Goto et al. 1953; Inoue et al. 1957; Yohimura et al. 1959). In Japan, weed hosts are considered one of the most important sources of primary inoculum of bacterial blight (Ou 1985). Although further studies are needed to determine the role of alternate weed hosts as sources of primary inoculum for bacterial blight of rice, the possibility of weed hosts of *X. o. oryzae* providing such as role appears very significant.

3. Infested Soil

There appears to be no evidence of significant longevity or overwintering of *X. o. oryzae* in soils of infested rice fields. The bacterium may survive in the soil for 1 to 3 months depending on soil moisture, pH, and the antagonistic activity of soil microorganisms (Mizukami and Wakimoto 1969). Therefore, the soil is not considered to be a significant source of inoculum of bacterial blight (Ou 1985).

4. Irrigation Water

Rice leaves infected with bacterial blight will often ooze masses of bacteria, which can be washed from the leaves into the irrigation water or can dry into small beads which can fall into the water. This activity can spread the pathogen within a field or from field to field along with irrigation water in a canal system (Reddy and Yin Shang-zhi 1989). Te bacterial blight organism has been reported to survive for only 15 days in rice field water (Singh 1971), and for less than 6 days at 30° C, 12 days at 20° C, 37 days at 10° C, and 60 days at 1° to 4° C (Hsieh and Buddenhagen 1975). Therefore, irrigation flood water appears to be a likely means by which the bacterium can be spread within a field from an infection focus, be it an infected rice plant or infected weed host. This pathogen spread could also be from field to field where a common irrigation water source is used.

5. Seed Transmission

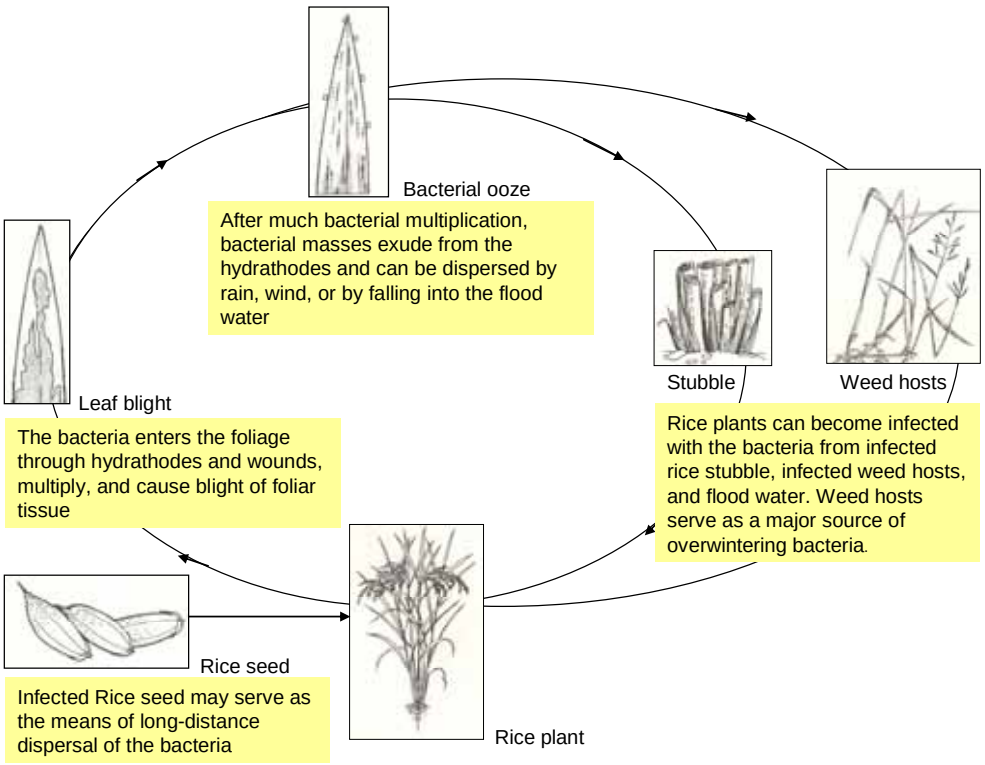
There is considerable controversy concerning the seed-borne nature of the bacterial blight pathogen. It is common for rice seed from fields infested with bacterial blight to harbor *X. o.*

oryzae, and a number of researchers have demonstrated seed infection by the bacterium for several months after rice harvest and even from harvest to the next planting season (Reddy and Yiu 1989). However, the actual transmission of *X. o. oryzae* from infected seed to rice seedlings either in nurseries or fields has not been demonstrated satisfactorily despite extensive efforts to do so in Japan (Mizukami and Wakimoto 1969; Murtz and Devadath 1984; Tagami et al. 1963; Mew et al. 1993).

The controversial reports concerning seed transmission of *X. o. oryzae* from seed to seedling may be due to the detection methods used. Mew et al. (1989) address this issue and state that the methods used had inherent weakness and often were not sufficiently sensitive or reliable enough to detect low numbers of the bacterium. With the advent of highly sensitive molecular detection techniques, more convincing data has evolved. Sakthivel et al. (2001), using a polymerase chain reaction (PCR) technique was able to detect the bacterium in naturally infected seed at a level of 55 fg DNA of *X. o. oryzae,* which is roughly equivalent to seven bacterial cells. The bacterium was detected from seed washes and DNA extracted from the seed washes seed. When the rice was stored at 4° C, the pathogen was detected up to 4 months and 9 months from naturally infected seed of cultivars Java and TN1, respectively.

The bacterium was also detected in rice seedlings, mature rice plants, and seed collected from plants developed from naturally infected seeds. Therefore, it appears that *X. o. oryzae* can be seed borne from one planting season to another, however, the bacterial population often seems to be very low. Seed-borne inoculum of *X. o. oryzae* probably plays an important role as the primary means of dissemination of the bacterial blight into regions where bacterial blight has not yet occurred. However, in areas where the disease is already established, its role as a source of inoculum probably is insignificant compared to other sources of inoculum.

Life cycle of *Xanthomonas oryzae* pv. *oryzae*



II. Possible Introduction, Establishment and Spread in the United States

A. Introduction of Xanthomonas oryzae pv oryzae

Two principle scenarios of possible introduction of the rice bacterial blight organism into rice production areas in the United States involve 1) **inadvertent introduction** on imported infested rice seed or 2) **deliberate introduction** with malicious intent.

1. Inadvertent Introduction

Infested rice seed appears to be the most likely means of inadvertent introduction. There is a significant difference between imported rough rice (not dehulled) and milled rice. Most microorganisms associated with rice seeds are located on or in the hulls. Removing the hulls

removes most of these organisms, including the bacterial blight pathogen. Hull disposal becomes a problem, since they remain a source of inoculum even when removed from the grain. This is especially important, since rice milling is a very specialized process and all of the rice mills are located in rice production areas. Any rough rice brought into the United States must be delivered to a rice production area where grain dispersal during transport can occur, and the dispersed grain could serve as a source of pathogen inoculum. Rice hulls remaining following milling must be disposed of without serving as a possible source of inoculum for rice planted in the area.

The greatest risk of unintentional introduction of the bacterial blight pathogen is through the importation of rice seed for direct planting. Germplasm exchange as part of rice breeding programs would be the only activity where imported rice is used for planting purposes.

Imported milled rice imposes a negligible threat as a source bacterial blight inoculum.

After the hulls are removed, no or negligible traces of *X. o. oryzae* would be left behind on the milled rice. Furthermore, milled rice would most likely be shipped in containers or bags and be shipped to areas of the country where rice production is non-existent, thus posing no reasonable threat as a source of bacterial blight inoculum.

Although significant quantities of milled rice are imported into the United States annually (421,917 metric tons in 2001/2002), much less rough rice enters the U.S. from abroad. The United States Department of Agriculture, Foreign Agricultural Service, reports rough rice imports listed as “Rice in Husk, Paddy” as follows:

U.S. Imports of Rice In Husk, Paddy, (MT)

Country of Origin	1998	1999	2000	2001	2002	2003
Canada	0.0	0.0	0.0	0.0	4.0	0.0
India	0.0	0.0	0.0	0.0	0.0	1.3
Italy	2.0	0.0	0.0	0.4	0.0	4.2
Pakistan	0.0	0.0	0.0	0.0	0.0	9.1
Thailand	10.3	0.0	0.0	0.0	0.0	0.0
Total	12.3	0.0	0.0	0.4	4.0	14.7

(Data Source: Department of Commerce, U.S. Census Bureau, Foreign Trade Statistics).

U.S. rough rice imports over the six-year period of 1998 – 2003 totaled only 31.4 metric tons. However, bacterial blight is present in three of the countries listed, India, Pakistan, and Thailand. The rice imported from Canada must have originated from a rice-producing country and been transferred through Canada. Thus the origin of that rice is uncertain. The imported rough rice for milling and rice imported for seed purposes are two possible sources of inadvertent introduction of *X .o. oryzae*. Data on the importation of rice for seed use was not found, however, the several rice breeding programs in the United States would be the most likely recipients.

2. Deliberate Introduction

Anyone involved in the deliberate introduction of *X. o. oryzae* with malicious intent would probably not seek to introduce the pathogen on infested seed. Such an attempt probably would involve importing a sizeable amount of infected rice seed and distributing it into many rice fields across a wide area. A more likely method would be to introduce the pathogen into the rice ecosystem directly by arranging for the spraying of rice fields during crop development with a suspension of bacterial spores or by introducing the bacterium into the flood water in rice fields.

The bacterium could be introduced into the country in a small vial and increased on appropriate growth media. A relatively large quantity of the bacterium would have to be cultured and dispersed in order to induce a rapid and widespread epidemic the first year. If numerous, smaller infection foci were the goal with the intention of establishing numerous initial infection sites for eventual natural spread over subsequent years, this could probably be accomplished with smaller amounts inoculum, but still substantial enough quantities where inoculum increase would have to performed in the United States. With adequate vigilance, a relatively small number of restricted infection foci (fields) could be noticed and identified early and the pathogen possibly contained and managed, perhaps even eradicated. This potential will be discussed below.

B. Establishment of *Xanthomonas oryzae* pv. *oryzae* in the U.S. Rice Ecosystem

1. Climate *Risk = High*

During the growing season in the U.S. southern rice belt, environmental conditions should prove favorable for development of bacterial blight of rice. The disease is favored by warm temperatures (25 – 30^{o} C), high humidity, rainfall, and a deep flood. These conditions are typical of what usually prevails in the south-central United States rice production areas. The rice production area of California would have less favorable conditions due to lower humidity and little rainfall during the rice-production season.

2. Host Range *Risk = High*

The occurrence of reported weed hosts in the rice production areas of the U.S. would also favor the potential establishment of the pathogen. Reported weed hosts that are present in the U.S. rice production areas include:

Cynodon dactylon - bermudagrass
Cyperus rotundus - purple nutsedge
Echinochloa crus-galli - barnyardgrass
Leersia hexandra - cutgrass (not California rice area)
Leersia oryzoides - rice cut grass
Leptochloa filiformis - red spangletop (not California rice area)
Oryza sativa - red rice (not California area)

There is a tendency in the southern rice belt toward shorter crop rotations, which could favor pathogen survival from one rice crop to the subsequent rice crop in weed hosts.

3. Persistence *Risk = Moderate*

As addressed above in section **I.F. Life Cycle or Disease Cycle**, the bacterial pathogen does not survive well in the soil, has limited longevity in irrigation water, and is not readily distributed in infected seed. These factors should make long term survival dependent on infection of weed hosts, and to a lesser extent infected rice stubble. With most rice acreage rotated, the importance of the latter diminishes. Therefore, long-term survival of X*. o. oryzae* in U.S. rice production areas would depend significantly on weed hosts. Common rice

rotation patterns and an increase in conservation tillage should lessen the role of in-field weed hosts in long-term survival of the pathogen.

4. Potential Spread of the Pathogen Risk = Moderate

Rice is irrigated either by water from wells on individual farms or by a canal system in which a number of farms utilize the common water source. If the bacterial blight pathogen were to contaminate a canal system, numerous fields and farms could subsequently become infested from the same source. Also, weeds along the banks of the canal could harbor the pathogen between rice irrigation seasons. If the bacterium should infest a field or fields irrigated from a well, it would be possible to contain the infestation within those fields and eliminate it by maintaining a clean fallow for an entire season. Many rice farmers commonly leave a field fallow after a rice crop, so a clean fallow should not be unreasonable.

A conservation tillage practice called “stale seed bed” has been gaining popularity in the southern rice belt. This practice involves preparing the soil during favorable opportunities in the autumn prior to the spring seeding and controlling weeds with one or more applications of herbicide, often with glyphosate used as the “burn down” product. Rice seeding is accomplished in the spring with a drill designed for conservation tillage. This method should be favorable for management of bacterial blight and for hindering its establishment in a field. The stale seed bed system would destroy possible bacterial blight infected weed hosts, volunteer rice, and ratoon rice plants 5 to 7 months prior to seeding. Since the bacterium does not survive well in soil in the absence of an infected host plant, the stale seed bed system would make it more difficult for the bacterium to become well established in such fields.

Localized spread of the bacterial blight pathogen within a field after plants become infected could occur by bacterial exudate coming in contact with adjacent plants as the foliage is moved by wind, rain splashing of bacterial exudate, and movement of the bacterium from infected rice plants or infected weed hosts through irrigation water. Violent rain storms often cause rapid spread within infested fields and to neighboring fields. More distant spread is facilitated through irrigation water and infested seed.

Risk Assessment Summary for Establishment of *X. oryzae* pv. *oryzae*

Rice Area	Climate	Host Range	Spread	Persistence
Southern U.S.	**High**	**High**	**Moderate**	**Moderate**
California (Sacramento Valley)	**Low**	**Moderate**	**Low**	**Low**

Overall risk: Moderate to High (southern U.S.); Low (California)

Risk of deliberate introduction and establishment by a bioterrorist: Moderate to High.

The perpetrator would have to overcome some considerable challenges to get the disease established such as developing large quantities of freshly developed inoculum and widely disseminating it.

III. Potential Management or Mitigation Strategies in the Event of Disease Initiation

Rapid detection and accurate confirmation of *X. o. oryzae* will be critical in the initial management strategy of a bacterial blight initiation event in the United States. If confirmed early and before the disease becomes widespread, attempts to contain and/or eradicate the disease may have reasonable chance of success. It is very important that any identification of a bacterial blight incident in the United States be able to differentiate the weakly virulent *X. c. oryzae* that previously has been reported in the United States from the highly virulent Asian strain of the pathogen. See the section **I.E.** above concerning Geographic Distribution for a discussion on this issue and how some experts believe the strain of the pathogen previously reported in the United States actually is not *X. o. oryzae* but perhaps a yet non-described pathovar.

A. Current Diagnostic Tools

1. Isolation Using Semi-Selective Media

Xanthomonas species tend to grow relatively slowly on artificial media compared with numerous potential contaminant bacteria found on plant material and seed. A semi-selective media (XOS) was developed which is reported to be quantitatively and qualitatively superior to other available media for the isolation of *Xanthomonas* species from seed or plant tissue. Pathogenicity tests are required after *Xanthomonas* species are isolated to confirm that they are *X. o. oryzae* or *X. o. oryzicola* (Di et al. 1991) A modification of the XOS media by removal of the phosphate component was shown to promote more rapid colony development (Alvarez et al. 1997).

2. Serological Analysis with Monoclonal Antibodies

Serological methods can serve as sensitive tools for detection of the pathogen. Serological methods include the enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies and/or polyclonal antibodies (Benedict et al. 1989), miniplate enrichment / ELISA, immunofluorescence and immunofluorescence colony-staining technique (IFC). Gnanamanickam et al. (1994) successfully demonstrated the detection of *X. o. oryzae* in rice seed inoculated with the bacterium using ELISA, subsequently also assaying the colonies that tested positive to monoclonal antibodies specific to the pathogen by a direct immunofluorescent technique (IF). Van Vuurde and Van der Wolf (1995) reported on an improved immunofluorescent colony staining technique (IFC). The advantage of IFC over ELISA and immunofluorescence is a reported greater sensitivity at lower bacterial populations and an ability to distinguish *X. o. oryzae* from nonpathogenic xanthomonads in rice seed extracts (Van Vuunde and Van der Wolf, 1995; Alvarez et al. 1997; Gnananamickam et al. 1994).

3. Molecular Methods

Restriction fragment length polymorphism (RFLP) analysis with selected probes was used to differentiate. *X. o. oryzae* from other xanthomonads (Leach et al. 1990).

Molecular probes are capable of detecting very low numbers of the pathogen through amplification of DNA by polymerase chain reaction (PCR). Vera Cruz et al. (1996) compared

Rep-PCR with RFLPs produced by the hybridization with IS1112, an insertion element isolated from *X. o. oryzae*. The genetic groups detected by Rep-PCR were consistent with those found by RFLP analysis. Sakthivel et al. (2003) developed a PCR technique to detect *X. o. oryzae* in rice seed and to study the pathogen's transmission from seed to plant. Primers TXT and TXT4R from the insertion sequence IS1113 of the pathogen were used to amplify a 964-bp DNA fragment. A combined biological and enzymatic amplification (BIO-PCR) technique was used. The level of detection for these primers was 55 fg DNA, which is roughly equivalent to seven bacterial cells of *X. o. oryzae*. The recent development of real-time PCR should provide opportunity for rapid diagnosis of *X. o. oryzae*.

4. Fatty Acid Analysis

Stead (1989) used fatty acid analysis to distinguish *Xanthomonas campestris* pathovars of cereals and grasses. Fatty acid composition of bacterial has been used extensively to aid in microbial characterization. This technique requires 1) a pure bacterial culture, 2) a release of the fatty acids from the cell surface by saponification, 30 methylation of the fatty acids to increase volatility, 4) analysis by gas chromatography, and 5) examination of the fatty acid profile for classification or identification. The identification is often made by comparison of the profile from the unknown bacterium with those profiles of known bacteria. This comparison is often done automatically by computer software that scans a library of profiles. Classification can involve statistical analysis using fatty acid profiles from related or similar bacteria. (Stead et al. 1992).

B. Responses after Pathogen Confirmation

Immediately upon the first confirmation of bacterial blight, an extensive survey of rice fields is suggested to determine the geographic distribution and extent of the disease. If the number of infested fields is limited, it might be decided that these fields not be harvested. However, if harvested, great care should be taken to move the grain to the mill without spillage of grain. The hulls of this grain should be disposed of far from rice fields. The infested fields should be disked during the following autumn with subsequent periodic disking for weed management for one year. It is preferable that the field be left fallow a second year, with rice planting permitted on the third year. All rice for seed should come from fields where the fields has been scouted and found to be free of symptoms of bacterial blight. The bacterial blight management or mitigation proposal involves:

a) Planting of rice seed from fields free of bacterial blight.

b) Non-harvest of infested fields or, if harvested, very careful grain transport to a mill and disposal of the rice hulls far from any rice fields.

c) Disking of infested fields after harvest followed by weed management through periodic disking to maintain a low weed environment for one year.

d) Follow the year of disking with at least one additional year of fallow before planting rice seed from a field evaluated to be free of symptoms of bacterial blight.

The potential for successful execution of a bacterial blight containment, suppression, and possible eradication effort with depend significantly on rapid and accurate identification of the

newly introduced disease. The Southern Plant Diagnostic Network (SPDN) system could play a major role in the rapid diagnosis of the problem, appropriate dissemination of the information, and in the prior training of “first responders” including rice consultants, appropriate county agents and Extension specialists, rice farmers, and others in the rice production side of the rice industry.

Several characteristics of the pathogen make this disease management protocol feasible. The bacterium does not survive well in the soil for more than a few months. Therefore, the destruction and decompositions of any infested rice debris, rice volunteer plants, and infected weed hosts should subject the bacterium to a short longevity in the soil. Avoiding new introduction of the pathogen by use of seed free of the bacterium and by irrigation with water free of the bacterium should permit the pathogen population to be eradicated from that field. However, if the field is irrigated with water from a canal system, it would be necessary to insure that additional sources of pathogen inoculum do not exist up-stream in the form of other infested rice fields or infected weed hosts on the banks of the canal system. If an irrigation canal system is found to be infested with the pathogen, consideration might be given to eliminating weeds along the canal banks with glyphosate herbicide when the canals are drained in the autumn and before re-flooding in the spring. Should bacterial blight be found in the southern rice production areas, in most of the inclusive states there is presently considerable opportunity for temporary rotation of rice production to other fields in attempts to eradicate or mitigate the impact of the disease.

If the above mentioned management strategy proves inadequate and bacterial blight becomes a well entrenched disease in the United States rice-production area, it might become necessary to incorporate bacterial blight resistance into cultivars adapted to the United States rice production areas. The use of resistant cultivars is the most effective and most common management practice adopted by rice farmers in most countries of Asia where the disease is significant. In Asia, where various strains of the pathogen are present, it is recommended to grow resistant cultivars possessing field resistance (dilatory resistance) genes. However, breeding resistance in this process could take many years, and effective screening of breeding material for field resistance often requires significant disease pressure in a field setting.

IV. Knowledge Gaps

A. There is uncertainty concerning how persistent *X. o. oryzae* would be in the rice production areas of the United States. It is suspected that the prevalent low humidity conditions during the growing season in the Sacramento Valley of California would be unfavorable for the pathogen. In the southern United States, most crop acreage is in a crop rotation system. The level of impact this will have on suppressing the population of *X. o. oryzae* is uncertain. Weed hosts and infected rice stubble have been shown to be a significant source of inoculum survival between rice crops in Asia, but rotation with clean fallow could have a significant negative impact on pathogen survival. There is insufficient information on this issue at this time. Also, it is interesting that the putative *X. o. oryzae* that was reported in the United States in 1989, but later considered not to be *X. o. oryzae* but a closely related pathovar or species (see section **I.E. Geographical Distribution**), has virtually disappeared or not been reported for many years.

B. It is uncertain if a diagnostic tool presently exists which would quickly and accurately separate the putative strain of *X. o. oryzae* that was reported in the United States in 1989 from the virulent Asian strain of *X. o. oryzae.* The putative U.S. strain is presently considered to be different from the Asia strain and perhaps should be considered a different pathovar or even a different species. To confuse these different stains in any attempt to determine if the Asian strain has entered the United

States or in surveying the rice belt if the Asian strain is initially found, could cause great confusion with regrettable results.

C. Much of the information from the literature concerning weed hosts should be perceived with caution. Artificial inoculation and subsequent induction of disease does not indicate the role a weed is capable of playing as a source of over-seasoning inoculum. It also does not adequately indicate the relative incidence of the disease that might be expected on the host in nature.

D. It is uncertain how damaging the disease would be to southern United States rice production if it were introduced.

Appendix 1. Select Experts on Bacterial Blight or Xanthomonas oryzae pv oryzae

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Bacterial Leaf Streak of Rice

Pathway Analysis:

Introduction of

Xanthomonas oryzae

pv. *oryzicola*

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Bacterial Leaf Streak Pathway Analysis

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Executive Summary: Bacterial Leaf Streak Pathway Analysis

- A comprehensive search was made for available information on bacterial leaf streak of rice and the causal organism, *Xanthomonas oryzae* pv *oryzicola.* Information on the disease, the causal bacterium, its potential for causing crop losses, host range, geographical distribution, disease cycle (life cycle) and epidemiology was fundamental to addressing the nature of the threat of this disease should it enter the rice production areas of the United States. With this information, the potential for introduction, establishment and dissemination of bacterial leaf blight within the United States was addressed. Two principle scenarios of possible introduction of bacterial leaf streak were addressed; 1) **inadvertent introduction** on imported, infested rice seed and 2) **deliberate introduction** with malicious intent.

- The principle natural means of long-distance dissemination of *Xanthomonas oryzae* pv *oryzicola* is on infested rough rice (not de-hulled). Very limited quantities of rough rice are imported into the United States. Perhaps the greatest threat of inadvertent introduction of *X. o. oryzicola* is on imported rice for seed in rice breeding programs. Breeding programs need to be cautious and vigilant in their handling of imported rice germplasm from areas of the world where bacterial leaf streak is known to occur.

- Use of infested seed for the deliberate introduction of the bacterium into the United States with malicious intent would not be a very efficient method. Very little or no imported rice is used in the United States for planting, except in rice breeding programs. Bio-terrorists would more likely directly introduce the pathogen into rice production fields during the growing season. To do this effectively in such a manner as to cause a significant epidemic the same year as the introduction would require a large quantity of bacterial inoculum freshly prepared within 2 to 5 days prior to inoculation. Such a requirement would suggest that the inoculum increase would probably have to be performed locally in the United States. This would require access to sterilization facilities, suitable containers, and large quantities of bacterial growth medium. If large quantities of inoculum were prepared and disseminated, the risk of establishment, spread, and persistence appears moderate for the southern U.S. rice belt and low for California. Although the climate in the southern rice belt is favorable for infection during the rice growing season, the disease has never been reported in temperate Asia or Japan, although it is widespread in tropical Asia. Thus, the pathogen's longevity in the

temperate climate of the southern rice belt is in question. The reported list of weed hosts appears limited and often natural infection is uncertain. The bacterium does not survive well in the soil, and common rice crop rotation and crop debris destruction practices would limit the role that infected rice stubble might have in overwintering the pathogen in the southern United States.

- If bacterial leaf streak was detected early, before it became widespread, there is reasonable opportunity for containment and possible eradication of the disease. This would involve:

  a) Non-harvest of infested fields or, if harvested, very careful grain transport to a rice mill and disposal of the contaminated rice hulls far from any rice fields.

  b) Disking of infested fields after harvest followed by weed management through periodic disking to maintain a low weed environment of one year.

  c) Follow the year of disking with one additional year of fallow before planting with a rice crop.

  d) Plant with rice seed from fields free of bacterial leaf streak

Bacterial Leaf Streak of Rice

Pathways Analysis of the Introduction of

Xanthomonas oryzae pv. *oryzicola*

I. Biology and life / disease cycle analysis of the pathogen

The biology and life / disease cycle analysis will emphasize those aspects relevant to the potential for the pathogen's introduction, establishment, and spread within the United States.

A. Taxonomy

Taxonomic Position:	Kingdom:	Proteobacteria
	Class:	Zymobacteria
	Order:	Xanthomonadales
	Family:	Xanthomonadaceae
	Genus:	Xanthomonas

Proper preferred name:

Xanthomonas oryzae pv. *oryzicola* (Fang et al.) Swings et al. 1990

Synonyms or superseded names:

Xanthomonas campestris pv. *oryzicola* (Fang et al.) Dye 1978

Xanthomonas oryzicola Fang et al.

Xanthomonas translucens f. sp *oryzicola* (Fang et al.) Bradbury

NOTE: Prior to 1957, the rice bacterial leaf streak pathogen (now classified as *Xanthomonas oryzae* pv. *oryzicola*) had not been distinguished from the rice bacterial blight pathogen (*Xanthomonas oryzae* pv. *oryzae*). Therefore, literature references to bacterial blight of rice prior to 1957 may actually have refereed to either or both pathogens.

Common names of the disease include:

English

Bacterial leaf streak (of rice)

French

Brulure bacterienne (du riz)

Stries bacteriennes (du riz)

Spanish

Quemaduras bacterianas (del arroz)

Estrias bacterianas (del arroz)

B. Symptoms

Early symptoms appear as narrow, water-soaked, dark-green, translucent, interveinal leaf streaks from 0.5 mm to 1.0 mm wide and 3 mm to 5 mm long. In this early stage, the lesions tend to be confined between the major leaf veins. The lesions lengthen and sometimes advance laterally across the major leaf veins. The enlarged lesions turn yellowish-orange to light brown and eventually coalesce. On susceptible varieties, a yellow halo may form around the edge of the lesion. Tiny, amber droplets of bacterial ooze may appear on the surface of lesions during humid conditions. The droplets desiccate during dry periods and form tiny, yellow beads which may be numerous on the elongated lesions. As the disease progresses, entire leaves may turn brown, become necrotic, and may be colonized by many saprophytic microbes. In the advanced stages of the disease, the disease often is difficult to distinguish from bacterial leaf blight, caused by *X. oryzae* pv *oryzae*, however, with bacterial leaf streak, the margins of the leaf lesions tend to remain more linear while the margins of lesions caused by bacterial leaf blight tend to be wavy.

C. Potential Yield Losses

Mew (1992) reports yield losses caused by bacterial leaf streak ranging from 1% to 17% depending on varietal susceptibility, growth stage of the rice during infection, and climatic conditions. Losses of 5% to 30% have been reported from India, while in the Philippines losses were not considered significant in either the wet or dry season (Opina and Exconde 1971).

Bacterial leaf streak appears generally to be a less damaging rice disease than is bacterial leaf blight.

D. Host Range

There is considerable uncertainty about potential alternate hosts of *X. o. oryzicola.* Species of *Oryza* were infected by artificial inoculation. Mew (1992) reports that no other hosts of the bacterium are known. But other reports suggest that the bacterium "may also infect" other listed weed species. Plants reported to be hosts or potential hosts of the bacterium causing bacterial leaf streak include the following (Mew 1992; Ou 1985; EPPO/CABI 1992; Ranga Reddy and Nayak 1975):

Leersia hexandra (southern cut grass)
Leptochloa filiformis (red sprangletop)
Oryza perennis
Oryza sativa (rice)
Oryzae sp.
Paspalum orbiculare
Zizania aquatica (annual wild rice)

E. Geographical Distribution

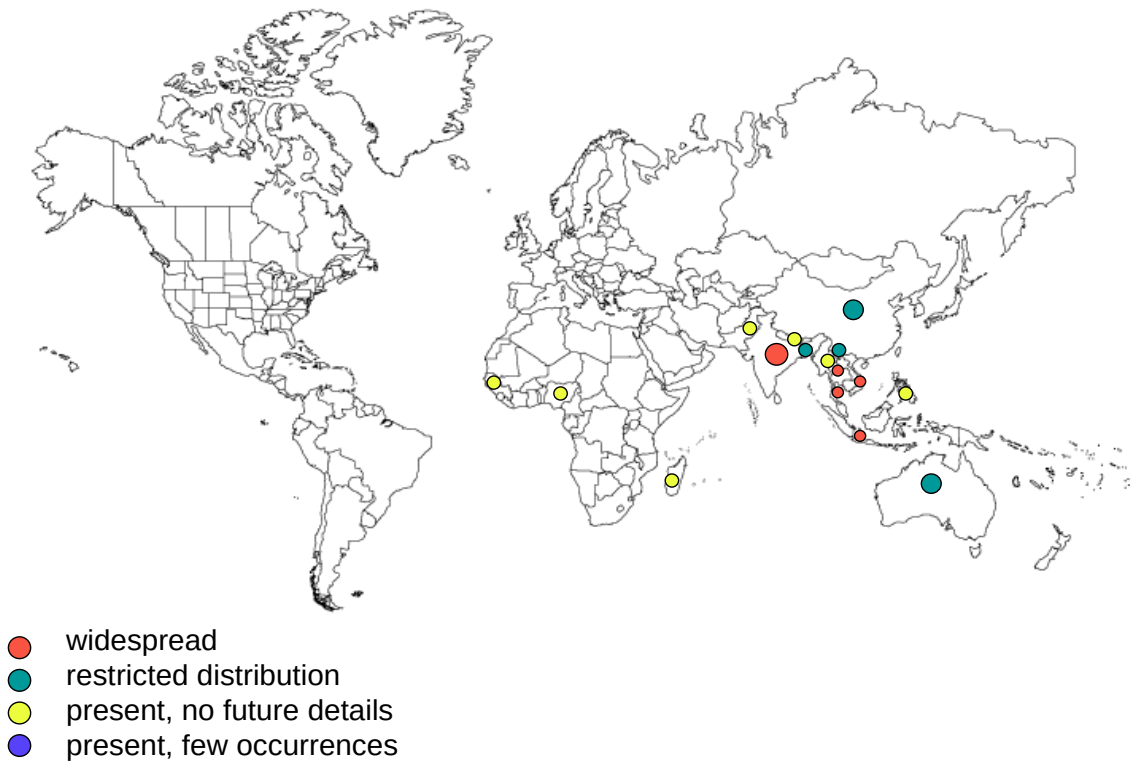
Bacterial leaf streak of rice occurs widely distributed in tropical and sub-tropical Asia and parts of West Africa in both lowland and upland rice-production areas. It is also reported from the Northern Territory, Queensland, Australia (Bradbury 1986; Buddenhagen 1985; CABI/EPPO 1997; CABI/EPPO 1998). Bacterial leaf streak has not been reported from temperate regions of the world including Japan (Ou 1985).

World Distribution of *Xanthomonas oryzae* pv. *oryzicola*

Asia		
Bangladesh	restricted distribution	CABI/EPPO, 1997; EPPO, 2003
Cambodia	widespread	CABI/EPPO, 1997; EPPO, 2003
China	restricted distribution	CABI/EPPO, 1997
India	widespread	CABI/EPPO, 1997; EPPO, 2003
Indonesia	widespread	CABI/EPPO, 1997; EPPO, 2003
Laos	restricted distribution	CABI/EPPO, 1997; EPPO, 2003
Malaysia	widespread	CABI/EPPO, 1997; EPPO, 2003
Myanmar	present, no further details	CABI/EPPO, 1997; EPPO, 2003
Nepal	present, no further details	CABI/EPPO, 1997; EPPO, 2003
Pakistan	present, no further details	CABI/EPPO, 1997; EPPO, 2003
Philippines	present, no further details	CABI/EPPO, 1997; EPPO, 2003
Thailand	present, no further details	CABI/EPPO, 1997; EPPO, 2003
Vietnam	widespread	CABI/EPPO, 1997; EPPO, 2003
Africa		
Madagascar	present, no further details	Buddenhagen, 1985; CABI/EPPO, 1997; EPPO, 2003
Nigeria	present, no further details	CABI/EPPO, 1997; EPPO, 2003
Senegal	present, no further details	CABI/EPPO, 1997; EPPO, 2003
Oceania		
Australia	restricted distribution	CABI/EPPO, 1997

World distribution map for Bacterial Leaf Streak disease induced by

Xanthomonas oryzae*. pv. *oryzicola



F. Life Cycle or Disease Cycle

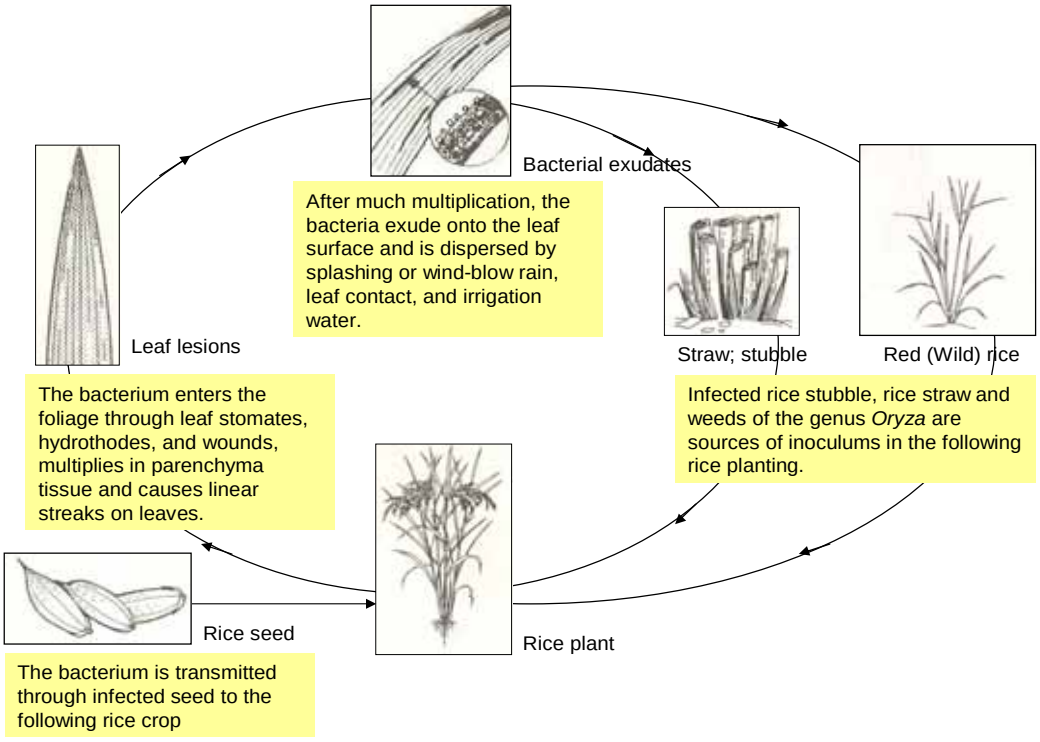
Bacterial leaf streak of rice is favored by warm temperatures (28 – 30° C), high humidity, and frequent rainfall. *X. o. oryzicola* primarily enters the rice plant through the leaf stomata and wounded tissue, including wounds caused by insects. Once inside the leaf of a susceptible rice host plant, the bacterium multiplies within the sub-stomatal cavity and progresses intercellularly in the parenchyma tissue. The leaf veins act as barriers to lateral spread of the bacterium within the leaf. During humid conditions, the bacteria may ooze from the leaf lesions. During dry conditions, the bacterial exudate forms tiny, yellow beads. The bacterial exudate can be disseminated by splashing rain and wind to other infection sites or drop to the irrigation water where further spread occurs. Field-to-field transmission of the bacterium through irrigation water is an important means of dissemination (Ou 1985; Mew 1992).

The survival of *X. o. oryzicola* from one crop season to the next crop season has been reported to occur on rice stubble, rice straw, and infected seed, but not in soil (Devadath and Dath 1970b). It has been established that transmission of the bacterium by infested seed serves as a source of primary inoculum (Shekhawat et al. 1969; Faan and Wu 1965: Rao 1987; Devath and Dath 1970b). The role of weed host in the bacterium's survival is uncertain, perhaps due to a lack of definitive studies. Wild and domesticated species of *Oryza* can be infected by artificial inoculation (Ou 1985) and *O. perenne* was found naturally infected in India (Ranga Reddy and Nayak 1975). Mew (1992) reported that no other hosts of the bacterium are known, but another report suggests that the pathogen "may also infect" other species including (EPPO/CABI 1992):

Leersia sp.
Leptochloa filiformis (red sprangletop)
Paspalum orbiculare
Zizania palustris (annual wild rice)
Zizania aquatica (annual wild rice)
Zoysia japonica (zoysia grass)

Prior to 1957, the bacterium that causes bacterial leaf streak of rice (*Xanthomonas* oryzae pv *oryzicola*) had not been distinguished taxonomically from the bacterium that causes bacterial blight of rice (*Xanthomonas oryzae* pv *oryzae*). Therefore, there is less published information on the disease cycle and epidemiology of bacterial leaf streak than for bacterial leaf blight. Also, some of the information published for bacterial leaf blight prior to 1957, may actually refer to one or both diseases while referring only to bacterial leaf blight.

Life cycle of *Xanthomonas oryzae* pv. *oryzicola*



II. Possible Introduction, Establishment and Spread in the United States

A. Introduction of Xanthomonas oryzae pv oryzicola

Two principle scenarios of possible introduction of the rice bacterial blight organism into rice production areas in the United States involve 1) **inadvertent introduction** on imported infested rice seed or 2) **deliberate introduction** with malicious intent.

1. Inadvertent Introduction

Infested rice seed appears to be the most likely means of inadvertent introduction. There is a significant difference between imported rough rice (not dehulled) and milled rice. Most microorganisms associated with rice seeds are located on or in the hulls. Removing the hulls removes most of these organisms, including the bacterial blight pathogen. Hull disposal

becomes a problem, since they remain a source of inoculum even when removed from the grain. This is especially important, since rice milling is a very specialized process and all of the rice mills are located in rice production areas. Any rough rice brought into the United States must be delivered to a rice production area where grain dispersal during transport can occur, and the dispersed grain could serve as a source of pathogen inoculum. Rice hulls remaining following milling must be disposed of without serving as a possible source of inoculum for rice planted in the area.

The greatest risk of unintentional introduction of the bacterial blight pathogen is through the importation of rice seed for direct planting. Germplasm exchange as part of rice breeding programs would be the only activity where imported rice is used for planting purposes.

Imported milled rice imposes a negligible threat as a source bacterial leaf streak inoculum.

After the hulls are removed, no or negligible traces of *X. o. oryzicola* would be left behind on the milled rice. Furthermore, milled rice would most likely be shipped in containers or bags and be shipped to areas of the country where rice production is non-existent, thus posing no reasonable threat as a source of bacterial blight inoculum.

Although significant quantities of milled rice are imported into the United States annually (421,917 metric tons in 2001/2002), much less rough rice enters the U.S. from abroad. The United States Department of Agriculture, Foreign Agricultural Service, reports rough rice imports listed as “Rice in Husk, Paddy” as follows:

U.S. Imports of Rice In Husk, Paddy, (MT)

Country of Origin	1998	1999	2000	2001	2002	2003
Canada	0.0	0.0	0.0	0.0	4.0	0.0
India	0.0	0.0	0.0	0.0	0.0	1.3
Italy	2.0	0.0	0.0	0.4	0.0	4.2
Pakistan	0.0	0.0	0.0	0.0	0.0	9.1
Thailand	10.3	0.0	0.0	0.0	0.0	0.0
Total	12.3	0.0	0.0	0.4	4.0	14.7

(Data Source: Department of Commerce, U.S. Census Bureau, Foreign Trade Statistics).

U.S. rough rice imports over the six-year period of 1998 – 2003 totaled only 31.4 metric tons. However, bacterial leaf streak is reported as present in three of the countries listed, India, Pakistan, and Thailand. The rice imported from Canada must have originated from a rice-producing country and been transferred through Canada. Thus the origin of that rice is uncertain. The imported rough rice for milling and rice imported for seed purposes are two possible sources of inadvertent introduction of *X .o. oryzicola*. Data on the importation of rice for seed use was not found, however, the several rice breeding programs in the United States would be the most likely recipients. Rice for seed is not imported into the United States for direct commercial production but is imported as sources of germplasm for rice breeding purposes.

2. Deliberate Introduction

Anyone involved in the deliberate introduction of *X. o. oryzicola* with malicious intent would probably not seek to introduce the pathogen on infested seed. Such an attempt probably would involve importing a sizeable amount of infected rice seed and distributing it into many rice fields across a wide area. This would be highly unlikely and impractical. A more likely method would be to introduce the pathogen into the rice ecosystem directly by arranging for the spraying of rice fields during crop development with a bacterial suspension or by introducing the bacterium into the flood water in rice fields. The bacterium could be introduced into the country in a small vial and increased on appropriate growth media. A large quantity of the bacterium would have to be cultured and dispersed in order to induce a rapid and significantly widespread epidemic the first year. If numerous, smaller infection foci were the goal with the intention of establishing numerous initial infection sites for eventual natural spread over subsequent years, this could probably be accomplished with smaller amounts inoculum. In either situation, substantial quantities of bacterial inoculum would have to be produced in a relatively short period (24 – 72 hours) prior to inoculation. Therefore, inoculum increase would most likely have to performed in the United States. With adequate vigilance, a relatively small number of restricted infection foci (fields) could be noticed and identified early and the pathogen possibly contained and managed, perhaps even eradicated. This potential will be discussed below.

B. Establishment of *Xanthomonas oryzae* pv. *oryzicola* in the U.S. Rice Ecosystem

1. Climate *Risk = Moderate*

During the growing season in the U.S. southern rice belt, environmental conditions should prove favorable for development of bacterial blight of rice. The disease is favored by warm temperatures (28 – 30^{o} C), high humidity, rainfall, and a deep flood. These conditions are typical of what usually prevails in the south-central United States rice production areas. The rice production area of California would have less favorable conditions due to lower humidity and little rainfall during the rice-production season. Although the bacterium is widely distributed in tropical Asia and tropical West Africa, it has not been reported from areas of the world with temperate climates, including Japan (Ou 1985). Although the southern rice belt in the United States may be considered to have tropical to sub-tropical climatic conditions during much of the rice-growing season, especially in the Gulf Coast regions of Texas and Louisiana, the winter months could not be considered tropical with common freezing temperatures. It is interesting that the disease has not been found in temperate Asia, including Japan and Korea. Therefore, it is questionable how well the pathogen could survive long-term in the southern rice-production areas in the United States.

2. Host Range *Risk = Moderate*

The status of the importance of weed hosts as a source of primary inoculum for bacterial leaf streak is questionable. Ou (1995) reported that wild and domesticated species of *Oryzae* were successfully artificially inoculated with the pathogen and that *O. perenne* was found to be naturally infected in India. Mew (1992) reported that no other weed hosts are known in addition to those species of the genus *Oryza,* however, another report lists several weed species that the bacterium “may also infect” (EPPO/CABI 1992). This list is printed above in section entitled Life Cycle or Disease Cycle. Of the potential weed hosts listed, domestic and wild *Oryza sativa* are certainly present in the southern rice production areas of the United States. The wild version of *O. sativa* is called red rice, and is a common weed problem. Two species of *Leersia* (*L. hexandra* and *L. oryzoides*) are present in the southern U.S. rice-production area. *Leptochloa filiformis* (red sprangletop) is a common weed in the area. *Zizania aquatica* (syn. *Z. palustris*) has, at least, limited distribution in the southern U.S. rice belt. A number of species of

Paspalum are common in the area, but *P. orbiculare* does not appear to be listed in the as a species in the United States or is improperly named (Hitchcock 1950).

3. Persistence *Risk = Moderate*

As addressed above in the section entitled Life Cycle or Disease Cycle, the bacterial pathogen does not survive well in the soil and has a limited, questionable host range. Since most of the rice acreage in the southern United States is on crop rotation system, these two characteristics of the epidemiology of the bacterium should significantly restrict its longevity in many parts of the rice production area. Crop rotation with thorough destruction of crop debris through timely disking of rice stubble and straw and the use of seed rice only from non-infested production fields should seriously challenge the survival of the bacterium if it were to become established. Also, the fact that the disease is widespread in tropical Asia, but has not been reported from temperate, rice-production areas of Asia, such a Japan and South Korea, raises a question as to how well it could survive in the southern U.S. rice belt.

C. Potential Spread of the Pathogen: *Risk = Moderate*

Rice is irrigated either by water from wells on individual farms or by a canal system in which a number of farms utilize the common water source. If the bacterial leaf streak pathogen were to contaminate a canal system, numerous fields and farms could subsequently become infested from the same source. Also, weeds along the banks of the canal could possibly harbor the pathogen between rice irrigation seasons. If the bacterium should infest a field or fields irrigated from a well, it should be possible to contain the infestation within those fields and eliminate it by maintaining a clean fallow for an entire season. Many rice farmers commonly leave a field fallow after a rice crop, so a clean fallow should not be unreasonable.

A conservation tillage practice called “stale seed bed” has been gaining popularity in the southern rice belt. This practice involves preparing the soil during favorable opportunities in the autumn prior to the spring seeding and controlling weeds with one or more applications of herbicide, often with glyphosate used as the “burn down” product. Rice seeding is accomplished in the spring with a drill designed for conservation tillage. This method should be favorable for management of bacterial leaf streak and for hindering its establishment in a field. The stale seed bed system would destroy possible bacterial blight infected weed hosts, volunteer rice, and ratoon rice plants 5 to 7 months prior to seeding. Since the bacterium does not survive well in

soil in the absence of an infected host plant, the stale seed bed system would make it more difficult for the bacterium to become well established in such fields.

Localized spread of the bacterial blight pathogen within a field after plants become infected could occur by bacterial exudate coming in contact with adjacent plants as the foliage is moved by wind, rain splashing of bacterial exudate, and movement of the bacterium from infected rice plants or infected weed hosts through irrigation water. Violent rain storms often cause rapid spread within infested fields and to neighboring fields. More distant spread is facilitated through irrigation water and infested seed.

Risk Assessment Summary for Establishment of *X. oryzae* pv. *oryzicola*

Rice Area	Climate	Host Range	Spread	Persistence
Southern U.S.	**Moderate**	**Moderate**	**Moderate**	**Moderate**
California (Sacramento Valley)	**Low**	**Low**	**Low**	**Low**

Overall risk: Moderate

Risk of bioterrorist use of *Xanthomonas oryzae* pv *oryzicola* as a bio-weapon: Low to

Moderate. In order to cause a widespread epidemic in the first year or two, large quantities of bacterial inoculum would have to be produced. To overcome this logistics problem the inoculum increase most likely would have to be done locally, probably in a laboratory with appropriate facilities in the United States. Unlike a wind-borne pathogen, *X. o. oryzicola* would largely have to be spread over a wide area intentionally. If the disease should become established, but only in localized areas, a properly executed disease containment and eradication program could very well be successful. Furthermore, bacterial leaf streak has not been reported from temperate Asia, so its ability to survive in the temperate southern rice-production area of the United States is questionable.

III. Potential Management or Mitigation Strategies in the Event of Disease Initiation

Rapid detection and accurate confirmation of *X. o. oryzicola* will be important in the initial management strategy of a bacterial leaf streak initiation event in the United States. If confirmed early and before the disease becomes widespread, attempts to contain and/or eradicate the disease may have reasonable chance of success.

A. Current Diagnostic Tools

1. Isolation Using Semi-Selective Media

Xanthomonas species tend to grow relatively slowly on artificial media compared with numerous potential contaminant bacteria found on plant material and seed. A semi-selective media (XOS) was developed which is reported to be quantitatively and qualitatively superior to other available media for the isolation of *Xanthomonas* species from seed or plant tissue. Pathogenicity tests are required after *Xanthomonas* species are isolated to confirm that they are *X. o. oryzae* or *X. o. oryzicola* (Di et al. 1991) A modification of the XOS media by removal of the phosphate component was shown to promote more rapid colony development (Alvarez et al. 1997).

2. Serological Analysis with Monoclonal Antibodies

Serological methods can serve as sensitive tools for detection of the pathogen. Serological methods include the enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies and/or polyclonal antibodies. Benedict et al. (1989) developed pathovar-specific monoclonal antibodies that distinguished between *X. o. oryzae* and *X. o. oryzicola*. ELISA has been used to rapidly detect rice seeds infected with *X. o. oryzicola* (Wang et al. 1993).

3. Molecular Methods

Diagnostic primers based on a cloned repetitive element (Raymundo et al. 1999) were developed and used in a PCR-based technique for the detection of *X. o. oryzicola* (Perez et al. 2001). With this technique, *X. o. oryzicola* could be differentiated from *X. o. oryzae*. DNA from other *Xanthomonas* and other bacterial genera were also amplified but with a much different banding pattern. Molecular tools were also used to analyze the genetic diversity and population structure of *X. o. oryzicola* in the Philippines (Raymundo et al. 1999). A repetitive DNA element

was selected as a probe for restriction fragment length polymorphism (RFLP) analysis and sequenced. Ochiai and Kaku (1998) were able to differentiate *X. o. oryzicola* and *X. o. oryzae* using PCR-RFLP with restriction enzyme *Alu*I, and the results of RFLP analysis using EPS-related gene as probe and four restriction enzymes showed that strains of *X. o. oryzae* and *X. o oryzicola* were clearly differentiated.

4. Fatty Acid Analysis

Stead (1989) used fatty acid analysis to distinguish *Xanthomonas campestris* pathovars of cereals and grasses. Fatty acid composition of bacterial has been used extensively to aid in microbial characterization. This technique requires 1) a pure bacterial culture, 2) a release of the fatty acids from the cell surface by saponification, 30 methylation of the fatty acids to increase volatility, 4) analysis by gas chromatography, and 5) examination of the fatty acid profile for classification or identification. The identification is often made by comparison of the profile from the unknown bacterium with those profiles of known bacteria. This comparison is often done automatically by computer software that scans a library of profiles. Classification can involve statistical analysis using fatty acid profiles from related or similar bacteria. (Stead et al. 1992).

B. Responses after Pathogen Confirmation

Immediately upon the first confirmation of bacterial leaf steak an extensive survey of rice fields is suggested to determine the geographic distribution and extent of the disease. If the number of infested fields is limited, it might be decided that these fields not be harvested. However, if harvested, great care should be taken to move the grain to the mill without spillage of grain. The hulls of this grain should be disposed of far from rice fields. The infested fields should be disked during the following autumn with subsequent periodic disking for weed management for one year. It is preferable that the field be left fallow a second year, with rice planting permitted on the third year. All rice for seed should come from fields where the fields has been scouted and found to be free of symptoms of bacterial leaf streak. The bacterial leaf streak management or mitigation proposal involves:

a) Planting of rice seed from fields free of bacterial leaf streak.

b) Non-harvest of infested fields or, if harvested, very careful grain transport to a mill and disposal of the rice hulls far from any rice fields.

c) Disking of infested fields after harvest followed by weed management through periodic disking to maintain a low weed environment for one year.

d) Follow the year of disking with at one additional year of fallow before planting rice seed from a field evaluated to be free of symptoms of bacterial leaf streak.

The potential for successful execution of a bacterial leaf streak containment, suppression, and possible eradication effort with depend significantly on rapid and accurate identification of the

newly introduced disease. The Southern Plant Diagnostic Network (SPDN) system could play a major role in the rapid diagnosis of the problem, appropriate dissemination of the information, and in the prior training of “first responders” including rice consultants, appropriate county agents and Extension specialists, rice farmers, and others in the rice production side of the rice industry.

Several characteristics of the pathogen make this disease management protocol feasible. The bacterium does not survive well in the soil for more than a few months. Therefore, the destruction and decompositions of any infested rice debris, rice volunteer plants, and infected weed hosts should subject the bacterium to a short longevity in the soil. Avoiding new introduction of the pathogen by use of seed free of the bacterium and by irrigation with water free of the bacterium should permit the pathogen population to be eradicated from that field. However, if the field is irrigated with water from a canal system, it would be necessary to insure that additional sources of pathogen inoculum do not exist up-stream in the form of other infested rice fields or infected weed hosts on the banks of the canal system. If an irrigation canal system is found to be infested with the pathogen, consideration might be given to eliminating weeds along the canal banks with glyphosate herbicide when the canals are drained in the autumn and before re-flooding in the spring. Should bacterial leaf streak be found in the southern rice production areas, in most of the inclusive states there is presently considerable opportunity for temporary rotation of rice production to other fields in attempts to eradicate or mitigate the impact of the disease.

If the above mentioned management strategy proves inadequate and bacterial leaf streak becomes a well entrenched disease in the United States rice-production area, it might become necessary to incorporate bacterial leaf streak resistance into cultivars adapted to the United States rice production areas. The use of resistant cultivars is the most effective and most common management practice adopted by rice farmers in most countries of Asia where the

disease occurs. However, breeding resistance to bacterial leaf streak into rice cultivars suitable to U.S. production could take many years, and effective screening of breeding material for field resistance often requires significant disease pressure in a field setting or a reliable, feasible technique to screen plants in a controlled greenhouse or laboratory setting.

IV. Knowledge Gaps

A. There is uncertainty concerning how persistent *X. o. oryzicola* would be in the rice production areas of the United States. The disease it causes, bacterial leaf blight, is not known to occur in temperate Asia and has not been reported from Japan. The climate of the southern rice-production area of the United States is certainly more warm-temperate than tropical. It is suspected that the prevalent low humidity conditions during the growing season in the Sacramento Valley of California would be unfavorable for the pathogen. In the southern United States, most crop acreage is in a crop rotation system. The level of impact this will have on suppressing the population of *X. o. oryzicola* is uncertain. The literature suggests that weed hosts outside of the genus *Oryza* seem very limited or uncertain. Infected rice stubble has been shown to be a source of inoculum survival between rice crops in Asia. The system of crop rotation practiced in rice production in the United States in conjunction with clean fallow could have a significant negative impact on pathogen survival.

B. Much of the information from the literature concerning weed hosts should be perceived with caution. Artificial inoculation and subsequent induction of disease does not indicate the role a weed is capable of playing as a source of over-seasoning inoculum. It also does not adequately indicate the relative incidence of the disease that might be expected on the host in nature.

C. It is uncertain how damaging the disease would be to southern United States rice production if it were introduced.

Appendix 1. Select Experts on Bacterial Blight or Xanthomonas oryzae pv oryzae

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Huanglongbing of Citrus

Pathway Analysis:

Intentional Introduction of

Candidatus Liberobacter africanus and

Candidatus Liberobacter asiaticus

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Huanglongbing Pathway Analysis

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Executive Summary: Huanglongbing Pathway Analysis

This summary is a brief description of a pathways analysis for the intentional introduction into the United States of the causal agents of citrus greening, *Candidatus* Liberobacter africanus and *C*. Liberobacter asiaticus. Citrus greening, or huanglongbing (HLB) of citrus, causes a serious disease of all citrus cultivars and citrus relatives throughout Asia and parts of Africa.

HLB infection of citrus trees leads to a dramatic reduction in tree health, eventually rendering the trees useless for production. Due to the potential damage to U.S. citrus and worldwide production, the HLB pathogens are on the USDA APHIS Ag Bioterrorism Agent and Toxin list and are on the European Plant Protection Organization's A1 list of regulated quarantine agents.

The HLB pathogens are unculturable bacteria restricted to living in the phloem of their host plants. Each of the two HLB pathogens has distinct ranges due to their climatic preferences. *C*. Liberobacter africanus is heat sensitive and is more prevalent in Africa. *C.* Liberobacter asiaticus is more prevalent in Asia and is capable of causing disease in both cool and warm conditions. Both of these species have similar host ranges and symptomology. Each does, however, have different but related vectors responsible for spreading them through citrus groves. Both of the vectors are plant psyllid, which alone are not considered to be particularly limiting go citrus production. *Trioza erytreae* and *Diaphorina citri* are the psyllid vectors known to vector the African and Asian pathogens, respectively. *D. citri* has recently migrated naturally or accidentally into the citrus producing areas in Florida and Texas. This migration has great implications for the threat of the Asian pathogen to the U.S. citrus industry.

This pathways analysis is a risk assessment based on seven processes believed to be instrumental in the act of intentionally inflicting a plant pathogen on a target crop. The seven steps and the risk rating for each in the case of HLB and the U.S. citrus crop are:

- Likelihood of acquisition of pathogen and vectors at point of origin – MODERATE
- Entry potential – LOW

- Establishment potential - MODERATE
- Spread potential – HIGH
- Economic potential – MODERATE
- Environmental damage potential – LOW
- Social and political considerations – LOW

The summary conclusion is that there is a low to moderate risk of an intentional introduction of the HLB pathogens with the intent of harming the U.S. citrus industry. There is a fairly good possibility that HLB will be introduced naturally or accidentally into the U.S. citrus crop, particularly in light of the migration and establishment of one of the major vectors of the pathogens. It has been noted that elsewhere in the world when the vector appears it is a matter of only a few years until HLB also appears. The possibility of such an event obscures the impact of any attempt to intentionally introduce these pathogens.

The HLB pathogens exhibit many biological traits that decrease their attractiveness as biological weapons threatening agricultural crops. They are impossible to manipulate in the laboratory, they will not survive outside of the host or vector, and they appear to be erratically distributed in their respective hosts. Infected budwood used for propagation of nursery stock appears to be the primary mode of accidental introduction of HLB into orchards, with subsequent movement within the orchards via the infected psyllids. Acquisition and movement of the infected budwood or vectors needed to insure the success of an introduction through ports of entry in the U.S. would be a difficult venture. Should such steps be successful, it would also be difficult to establish the pathogen into the U.S. citrus production system. In the unlikely event that a successful establishment was achieved, there is a fairly good possibility that the pathogen would spread and become another disease with which citrus producers would have to contend. There are, however, a number of control measures that appear to reduce the threat of losses and would limit the damage should the pathogen become dispersed. Citrus, however, is an extremely valuable crop and production costs would undoubtedly increase should HLB reach the U.S.

Huanglongbing of Citrus

Pathway Analysis for the Intentional Introduction of

Candidatus Liberobacter asiaticus
and
Candidatus Liberobacter africanus

I. Introduction

A. Justification and charge

The following report describes how to predict a complex series of events that could culminate with the introduction of a known, exotic plant pathogen into a susceptible agricultural crop growing in the United States. This series of events can be summarized as a pathway for invasion by a nonindigenous plant pathogen. The United States of America National Agricultural Biosecurity Center Consortium (NABC) commissioned this exercise. Based on this report, recommendations will be made to the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) on how to better protect the nation's food and fiber production systems from the threat of intentionally introduced plant pathogens. This analysis specifically concerns the causal agents of **citrus greening (Huanglongbing), *Candidatus* Liberobacter spp.**, and the threat they pose to the U.S. citrus industry.

Numerous recent events have proven that agricultural terrorism must be considered as a "legitimate and immediate concern". The results of introducing a plant pathogen into a susceptible ecosystem can be devastating. There are several well-known cases of just how damaging such introductions can be, even when accidental. For example, the North American chestnut blight epidemic, caused by the fungal tree pathogen *Cryphonectria parasitica*, is an excellent lesson on the circumstances that might generate an extremely

successful introduction of an alien plant pathogen. This tree disease is certain to be mentioned in discussions of the potential devastations caused by introductions of nonindigenous pathogens (Grossblat, 2002). *C. parasitica* causes a relatively innocuous canker on chestnuts of Chinese origin and is considered to be native in Asia where it probably evolved. However, when introduced into the United States accidentally, it took on all of the characteristics of a "super" pathogen. As the cause of rapidly expanding killing cankers on the ill-fated American chestnut (*Castanea dentata*), the pathogen is able to reproduce at rapid rates over long periods of time, spread rapidly by means of an efficient vector (wind), survives well in the North American climate, and makes use of readily accessible infection courts. These traits, combined with the lack of genetic resistance in *C. dentata*, resulted in one of the worst ecological disasters in North America. The economic cost and impact on the ecosystem were tremendous.

There are hundreds of examples of annual introductions of exotic, alien species into the United States annually. Although none results in the devastation of Chestnut blight, many must still be contended with in order to limit the impact. One example is a recent introduction of Giant Asian Dodder (*Cuscuta japonica*) into Houston, TX. This weedy plant parasite is on the USDA Resource Conservation Service Federal Noxious Weed List (http://plants.usda.gov/). Although very different then the fungal pathogen *C. parasitica*, Giant Asian Dodder is extremely aggressive on a wide host range of economically important plants and could possibly be a huge threat to southern field crops. It has been introduced on three other occasions in the U.S, and has been successfully eradicated on each. The eradication of the most recent introduction in Texas is nearly complete (*personal communication*, Ms. Kim Camilli, Texas Forest Service). The reason that this dangerous weed is readily eliminated has to do with a significant limitation in the life cycle. Although the plant produces flowers, there has been no viable seed produced in Houston so that the dispersion is completely dependent on spread of vegetative vine segments. This introduced noxious weed is an example of an invasive pest with low potential for causing significant damage to U.S. agriculture. These two contrasting examples illustrate just a few of the factors that must be considered in assessing the potential for an alien exotic species to cause damage to U.S. agricultural production.

The following report has two comprehensive goals. The first is to study potential pathways for a foreign, exotic plant pathogen to enter into the domestic agricultural system. The second is to assess America's capacity to contain such a disease were it to occur. Although

there are hundreds of published journal articles, reviews and descriptions of citrus greening, the following treatise is limited to the review of literature pertinent to the potential for the pathogen to be introduced and affect the U.S. citrus crop. The pathways analysis will consist of determining where the agent would originate, the medium in which it would move or the vectors on which it would move, the point of contact (crop) and potential recognition (responders), the mechanisms of dispersion, and impact the introduction might have. This analysis will be carried out in the context of current import operations and the production of the susceptible crop throughout the U.S. Prevention of such an introduction is the most effective means of managing the threat, so that recommendations will be made on how to close the pathways. Further recommendations will describe the most effective response options in the event of an introduction, and also gaps in our knowledge and recommendations for future actions to address those gaps will be made.

There are 10 sections to the following document. Following the Introduction (Section I), the Biology and Life/Disease Cycle of the Pathogen (Section II) are discussed. Section III concerns current state of the Detection and Recognition/Diagnosis of greening. Section IV describes the Likelihood of Natural/Accidental Introductions. Models describing the acquisition, introduction, establishment and spread scenarios are contained in Section V (Likelihood of Intentional Introduction/Risk Assessment). Section V also contains a risk rating for the processes necessary to intentionally release a greening epidemic in the U.S. citrus industry, as well as a risk rating for the potential economic, political and environmental impact of greening. Control/Mitigation Strategies after establishment are discussed in Section VI. Section VII (Knowledge Gaps) discusses limitations in our understanding of greening disease relevant to the potential bioterrorist threat it poses to our citrus industry. Steps that can be taken to immediately prepare for the greening threat are given in Section XIII (Immediate Response Action). The final two sections contain the Literature Cited and Appendices (Sections IX and X, respectively).

II. Biology and life/disease cycle of the pathogen

A. Description of the pathogen

Huanglongbing (HLB) is one of the most serious diseases of citrus, caused by a phloem-limited, uncultured bacterium belonging to the alpha-proteobacterial subdivision (phloem

restricted, gram negative bacteria with cell walls) (Bove and Garnier, 2003). The causal agent was originally considered to be a virus, but in the 1970s the nature of the bacterium-like-organism (BLO) was determined. The genus name of the bacterium is "*Candidatus* Liberobacter" (da Graca, 1991). Original reports of HLB were from China in the early 1900s and it is now known as a serious disease in Asia, Southeast Asia, the Arabian Peninsula, and Africa. HLB is known by many other names throughout its range. In South Africa, it is known as greening, in Taiwan as likubin, in the Philippines as leaf mottling, and in Indonesia as vein-phloem degeneration. Officially, the disease is now known by the Chinese name, huanglongbing (yellow shoot disease), based on the appearance of the disease in affected trees. In the literature, the organisms are variously referred to as greening fastidious bacteria (GFB) or greening organisms (GO). The abbreviations HLB and GFB will be used in this report to refer to the disease and pathogen type, respectively.

There are three "species" in the genus *Candidatus* Liberobacter. They are all restricted to living in the seive tubes of plant phloem tissue, and none have ever been cultured outside of plants. Two of the known species are citrus pathogens. One, *Candidatus* Liberobacter africanus, causes a heat-sensitive African form of HLB. Symptoms are only expressed under relatively cool conditions (20 – 25^{o}C). *Candidatus* Liberobacter asiaticus is the second citrus pathogen causing a heat-tolerant Asian form of HLB under both cool and warm conditions (up to 35^{o}C). Both of these species have similar host ranges and symptomology. The third species (*Candidatus* Liberobacter africanus subsp. capensis) is a pathogen on Cape chestnut (*Calodendrum capense*) in the Western Cape Province of South Africa (Bove and Garnier, 2002), but it is not yet known whether it infects citrus.

B. Host range

All citrus (Family *Rutaceae*, Genus *Citrus*) cultivars, species and hybrids appear to be susceptible to infection by *C*. Liberobacter spp., as well as some citrus relatives (Xue-Yuan, 1981, see the website for CABI International, http:www.cabicompendium.org) (Appendix Table 1). Sweet oranges, mandarin hybrids and tangelos are extremely susceptible (da Graca, 1991). Grapefruit, rangpur lime, lemons, calamondins and pummelos are less severely affected and Mexican lime, trifoliate orange, and trifoliate orange hybrids are the most tolerant showing only minor symptoms of infection (Garnier and Bove, 2000). The host range of the *C*. Liberobacter spp. also includes some rutaceous plants used as ornamentals as well as some wild, non-cultivated species (see the website of the Food and

Agriculture Organization of the United Nations, http://www.fao.org/documents/). The common non-rutaceous ornamental *Catharanthus roseus* (also *Vinca rosea*) has been experimentally infected with GFB by transmission through dodder (*Cuscuta* spp.) from infected *Citrus* spp (da Graca, 1991). A few plant species are useful for indexing, and some non-rutaceous hosts have been shown to be affective supplemental hosts in ornamental plantings and/or volunteer situations in orchards (Hung et al., 2000) (see Appendix Table 1).

The relative susceptibility of citrus species to GFB is also influenced by the suitability of prospective hosts to the insect vectors. For example, the tolerant Mexican lime (*Citrus aurantifolia*) is a preferred host of the *Diaphorina citri*. Vector relationships must therefore be considered in relation to disease development and will be discussed in the **Section II.C.** below. In addition, the vectors themselves serve as hosts to the bacterium, an important factor in epidemiology of the disease.

C. Vector relationships

Two species of psyllids (also known as jumping plant lice) play prominent roles in the epidemiology of citrus greening by vectoring the HLB pathogen. The ability to transmit the pathogens derives from an adaptation for sucking plant vessels by means of ventral elongated mouthparts. Each of these vectors is closely associated with outbreaks of the disease, but there are differences in the ecology and life cycles of these related psyllids. The “African vector”, *Trioza erytreae* (Del Guercio), occurs in Africa, Yemen, Madagascar, and Reunion and Mauritius islands in the Indian Ocean. *T. erytreae* is responsible for the spread of *C.* Liberobacter africanus, and breeds only on citrus and related species. *Clausena anisata* (horsewood), *Vepris lanceolata* (white iron wood), and *Toddalia lanceloata* (lopez root) are preferred indigenous hosts. Mexican lime, lemon and citron are the best hosts, but most common citrus species are acceptable. The African vector does not survive well in hot, dry climates. Nymphs of *T. erytreae* undergo five instars, but after the second moult, they generally stop crawling and settle into pits on the undersides of young leaves (Aubert and Quilici, 1983). Under artificial conditions, *T. erytreae* nymphs were unable to transmit HLB (Aubert, 1987). Affected leaves are galled and curled from the feeding activities of the nymphs. Leaves and branches on trees affected by this and other psyllids may be covered with sooty mold (Knapp, et la., 1998). Adults emerge after completion of the fifth instar.

The second vector is known as the Asian citrus psylla, *Diaphorina citri* Kuwayama. This vector has been shown to transmit both GFB species. *D. citri* is widespread throughout southern Asia and is capable of developing in hotter, drier climates than the African vector *T. erytreae*. The range extends from the southern Japanese islands in the west through southern China and into Pakistan to the west (French, Kahlke and da Graca, 2001). Other reports are from eastern Iran, Saudi Arabia, the Indian Ocean islands of Reunion and Mauritius, and New Guinea. All of these areas are within the ranges of the GFB pathogens, where the psyllids feed on many species of rutaceous plants. *D. citri* has also been reported in several locations beyond the range of HLB, including Brazil, St. Helena, and recently in Florida and Texas (Knapp et al., 1998). *D. citri* also undergoes five moults, but the nymphs actively crawl and feed until arising as adults after the fifth instar. During feeding, they excrete white pellets or threads lending a dusted appearance to the shoots and lower leaves. Artificially reared 4^{th} and 5^{th} instar nymphs of *D. citri* have been shown to transmit HLB, but younger stages were unable to vector the pathogen (Aubert, 1987).

The psylla feed primarily on younger shoots and leaves, but during tree dormancy adults are able to feed on mature tissues. With life spans of 80 – 90 days, they may acquire the bacteria and emerge in the spring able to initiate new infections. Spread of the pathogen depends on the inoculum density and the population density of the vectors. These insects are particularly suited to transmission of GFB because the pathogen is persistent and able to multiply in the vector. These vectors also have extremely high reproductive rates, are able to fly, and can increase population sizes on wild alternate rutaceous hosts (Aubert, 1987). However, they are considered to be weak fliers and only spread long distances with the assistance of wind and man (Knapp, 1998).

The expansion in the ranges of either of the citrus psyllids is of critical importance. It has been noted that within a few years of the arrival of the vectors an outbreak of the disease generally occurs (Anonymous, 2003). The original discovery of *D. citri* in Texas was in the form of nymphs on nursery citrus seedling (Knapp et al., 2001). During the same year, all life stages of the psylla were eventually found on commercial groves throughout 2 counties in South Texas. Similar observations were made in Florida during 1998 - 2000, but the disease has not been found there. It is possible that these introductions are the result of natural movement of the insect from the Caribbean and Central America, as well as subsequent movement in the nursery trade within the U.S. (Michaud, 2004).

D. Disease cycle

The disease cycle for HLB can be found in Figure 1.

III. Detection and Recognition/Diagnosis:

A. Detection by symptoms (see Figures 2 – 7 for illustrations)

The initial symptom of HLB on an infected tree is the appearance of a yellow shoot. The whole tree may not be necessarily affected so that only individual branches are symptomatic (Garnier and Bove, 1993). This symptom will slowly spread throughout the entire canopy. Thin canopies and twig dieback characterize chronic infections (Garnier and Bove, 2000). Foliar symptoms include reduced size, pale yellowing, and a blotchy mottling. The leaf mottle is the most characteristic symptom of HLB. Chronically infected trees also exhibit symptoms of zinc and manganese deficiencies (Kiozumi, 1994). The yellow leaves may have veins remaining green, or, alternatively green leaves with chlorotic veins. The fruits on infected trees are typically small, lopsided, and poorly colored. The seeds of affected fruits often abort (Zhao, 1981), juice is bitter, and they are often low in soluble solids and high in acids.

B. Detection by Clinical Procedures

C. liberobacter spp. have been detected by electron microscopy of ultra-thin sections of diseased tissues, or transmission to indicator species by grafting (Su, 2001), insect vectors, or via parasitic dodder. Common indicator species include sweet tangor, Duncan grapefruit and sweet orange seedlings (Knapp et al., 1998). Indexing by means of tissue-grafts is done by budding at least four buds from suspect trees onto the indicator seedling (2 buds/seedling). The seedlings are then held in warm conditions (Su, 2001). These methods have many disadvantages. They require a great deal of time, and are sometimes difficult to conduct on a routine basis. Another limiting factor relates to sampling, in that the bacterium is present in very low concentrations in host tissues and is unevenly distributed throughout the host plants (Hung et al., 2000). In order to increase the efficiency and sensitivity of detection protocols, DNA probes for the specific detection of GFB have been

Fig. 1. Disease cycle for Huanglongbing disease of citrus cause by *Candidatus* Liberobacter spp.

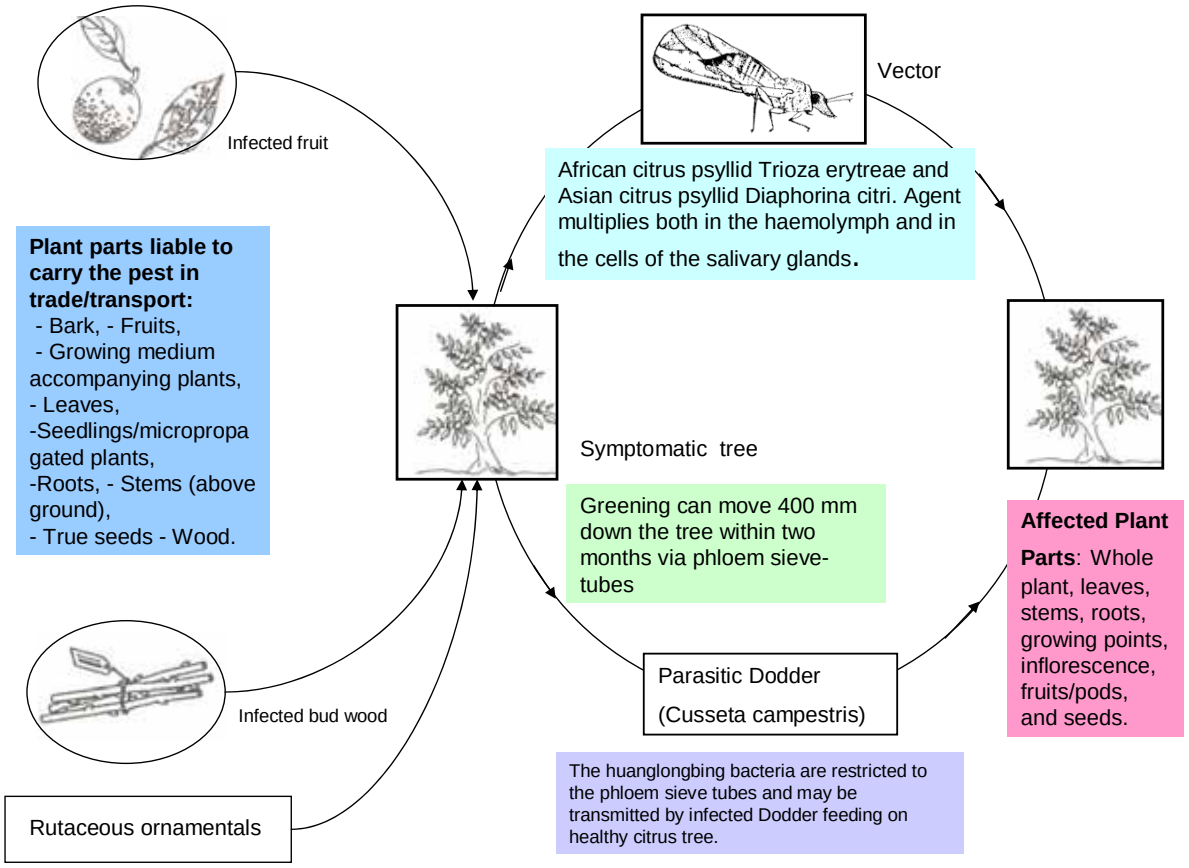


Fig. 2. Citrus Greening symptoms on plant leafs. (Photographs courtesy of Dr Patricia Barkley, Elizabeth Macarthur Agricultural Institute, NSW Agriculture)

Fig. 3 and 4. Citrus Greening symptoms on plant fruits and leaves. (Photos courtesy of Institute of Tropical and Subtropical Crops)

Fig. 5. Citrus Greening (likubin) symptoms on plant leafs of Wentan pummelo, showing yellowing and mottling of leaves and vein corking, compared to healthy leaf (right)(Photos courtesy of Food and Fertilizer Technology Center)

Fig. 6. Citrus Greening (likubin) symptoms on diseased twig with yellow leaves and diseased fruit of Tankan tangor. (left): the fruit is small and pale green in color. A healthy green leaf and normal large fruit are shown to the right. (Photos courtesy of Food and Fertilizer Technology Center)

Fig. 7. Citrus Greening (likubin). The Asian citrus psylla *Diaphorina citri* Kuwayama. which transmits the Asian strain of the greening organism. (Photograph by: Douglas L. Caldwell, University of Florida.)

Fig. 2.

Fig. 3.

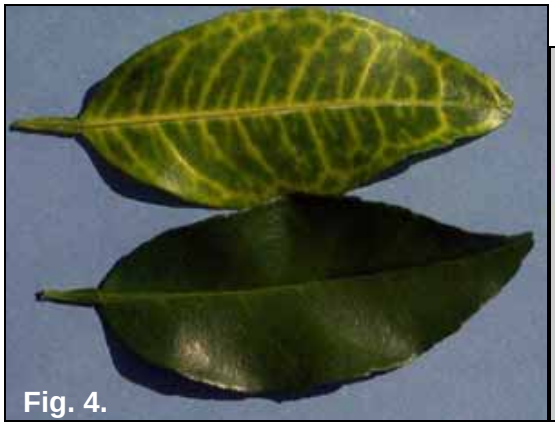


Fig. 4.

Fig. 5.

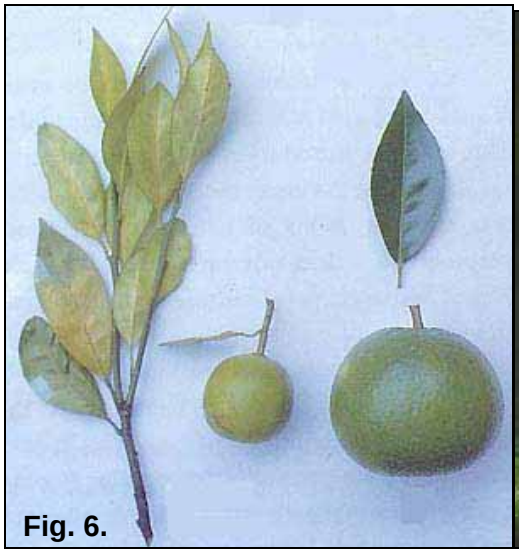


Fig. 6.

Fig. 7.

developed. The first of these efforts was a PCR protocol based on amplification of an 1160 bp fragment of 16S rDNA of the two *C*. Liberobacter spp. (Jagoueix, Bove, and Garnier, 1996). Amplification of 16S rDNA by PCR does not allow for distinguishing among the GFB species. However, this technique was useful in confirming the presence of GFB in the leaves of mandarin (*Citrus reticulate* Blanco) with seven kinds of symptoms from Thailand, as well as in an insect vector *D. citri* (Nakashima, Ohtsu and Prommintara 1998).

A different PCR approach was developed that allows for direct identification of the two main GFB species. This method is based on the amplification of part of the β operon of the two strains (Hocquellet, Toorawa, Bove and Garnier, 1999, Hocquellet, Bove and Garnier 2000). The primer in the ribosomal protein genes rplAa and rplJ, named AS and J5, respectively, allows specific amplification of a 669bp fragment from *C.* Liberobacter africanum and a 703 bp fragment from *C.* Liberobacter asiaticum*.*

A standard PCR method for the routine detection of GFB in Taiwan used a pair of primers chosen from the sequences of a cloned GFB-specific DNA fragment (0.24kb). The total DNA extracted from a citrus host could be used in the PCR-based assay without interference and successful detection of GFB could be made within 6 h with as little as 0.25 of citrus leaf midrib. This method is used for the quarantine and management of pathogen-free nurseries in Taiwan (Hung, Wu and Su 1999).

There has also been a need for detection of GFB in or on the bodies of psyllid parasitoids imported for biological control of citrus greening (Hoy et al., 2001). Preliminary tests with standard PCR on the parasitoids produced an abnormally high number of false positives. In order to improve reliability, a Long PCR protocol incorporating two DNA polymerases was tested and proven to be more sensitive and consistent then Standard DNA, both in parasitoid and plant tissues.

C. Similar Conditions and Diseases

The worldwide range of citrus production is extremely extensive. Citrus fruits are grown both in the Northern and Southern hemispheres between 40^{o} North and 40^{o} south. Therefore, management problems relating to biotic and abiotic diseases are numerous and varied (Giudice 2002). The symptoms of HLB are rather non-specific; yellowing, mottling, reduced growth of leaves and branches, twig and branch dieback could be easily confused with many other problems. The leaf mottle is recorded to be similar to symptoms of citrus

stubborn-affected trees (Garnier and Bove, 1993). Nutrient deficiencies, nematodes, viruses, and certain problems of unknown etiology could easily be confused with GFB when the diagnosis is based on limited numbers of small samples. Similar conditions may be caused by the citrus nematode (*Tylenchulus semipenetrans*), citrus stubborn, Australian citrus dieback, and tristeza stem-pitting and some Phytophthora diseases. Two symptoms, the yellowing of leaf midrib and veins accompanied by slight swelling, and the aborted seeds in bitter, small fruits are considered to be unique and useful in distinguishing greening from other citrus declines (Knapp et al., 1998)

IV. Likelihood of Natural/Accidental Introductions

A. Pathogen introduction/natural or accidental

The possibility exists that the greening pathogen may be introduced into the U.S. citrus crop via natural pathways. These means of introduction would consist of the immigration of contaminated vectors (psyllids) on air currents or infested nursery stock. The likelihood of such an event increases only if the pathogen migrates to regions closer to the U.S. The possibility of such an event should be considered, due to the repeated discovery of the vectors in important citrus growing regions in the U.S. (see **Section IV.B.** below). The information on recognition of the pathogen and the potential for spread in this report would be useful in this regard.

The potential for accidental introduction of the greening agent already has been considered due to the extreme threat the pathogen poses to the U.S. citrus industry (Hoy et la., 2001). Budwood, along with ornamentals, for example, is probably the most likely source of introduction and there are several state programs in place to prevent such an occurrence (Davis et al., 2000). The Florida Citrus Budwood Protection Program (see website of the Bureau of Citrus Budwood Registration http://www.doacs.state.fl.us/pi/budwood/iocv.html) is typical of the state programs in place precisely for the purpose of preventing introduction of a disease like HLB on propagating material (Knapp et al., 1998). These programs are guided by advisory groups consisting of growers, nurserymen, researchers and regulators who advise on testing strategies and procedures for handling imported budwood material. Similar programs for Texas and California are described respectively on the internet websites of the Texas Citrus Budwood Certification Program and the National Clonal

Germplasm Repository (NCGR) at Riverside, California. The latter facility is administered by USDA-ARS and has the mission of providing safe germplasm to provide genetic diversity needed in the citrus industry and by researchers to deal with pest and disease problems as well as the discovery of new citrus products. Through acquisitions with other countries, a variety of methods are used in these various programs to test for a variety of diseases.

B. Vector introduction/natural or accidental

Generally, it is believed that if the psyllid vector of HLB, *D. citri*, is introduced into an area, then the disease will follow in a few years (Hoy, 1998, Anonymous, 2003). This belief makes the recent arrival of this vector in Texas and Florida extremely important in future efforts to prevent and/or recognize the disease (French et al., 2001). It is unknown how the vector managed this significant expansion in its range. Importation on nursery stock of some other host of the insect than citrus is a definitely a possibility. According to Sailer (1983), 20% of non-indigenous insects in the U.S. were initially introduced on nonindigenous plants. The initial discovery of *D. citri* in Florida was on dooryard citrus and ornamental orange jasmine plants (*Murraya paniculata*). The vector may have also spread on wind currents from infested areas in the Caribbean or from Central America. Once introduced, the insect was able to rapidly grow and become established. Three years following the initial discovery, *D. citri* has been found throughout most citrus growing regions in Florida (Hoy et al., 2001). Insecticide sprays are now required to protect young trees (Michaud, 2004).

There are a number of parasitoids to the psyllids vectors of greening being tested for biological control. The importation of these parasitoids has been recognized as an additional pathway for the introduction of *Liberobacter* spp (Hoy et al., 2001). Recent evidence indicates this is not a credible threat.

There is a very high likelihood that HLB will eventually be introduced into the U.S. either naturally or accidentally. It is difficult to predict the timing of the event, but the potential obviously exists for additional introductions of the vector. Without a clear understanding of the pathway, then contaminated vectors may eventually arrive.

V. Likelihood and Consequences of Intentional Introduction/Risk Assessment

The likelihood of an intentional, successful introduction of a potentially damaging plant pathogen depends on a series of processes, each of which can be rated according to the risk posed by the characteristics of the pathogen, host and environment. These processes relate to the life history of an organism and the features that contribute to its fitness in a new environment. A thorough understanding of these features is important, but in the case of C. l*iberobacter* spp. there are many aspects of the pathogen that are poorly understood. They include such characteristics as association of the history of the invasiveness of the organism throughout its range, the survival of the organism in transit and ease of detection, the suitability of the environment, the dispersal potential of the organism, and its ability to grow and reproduce in the new environment. For the case of HLB, the psyllid vectors must also be considered in the analysis. However, given their relatively recent presence in two of the four major citrus growing regions in the U.S., the question of their introduction may not be entirely relevant.

The risk analysis below for *C*. Liberobacter spp. and their associated vectors is based on a qualitative pest risk-assessment for imported solid wood packing materials (Grossblat, 2002). Specific criteria have been modified to account for the unique characteristics of the agents, so that the risk rating more accurately reflects the potential for a damaging epidemic. The background and justifications for the assigned risk ratings are found in the relevant sections above, as indicated.

A. Likelihood of acquisition of pathogen and vectors at point of origin = MODERATE RISK

Pathogen acquisition

As in case of an accidental introduction, budwood removed from diseased trees would be the first logical and most accessible source of inoculum to intentionally introduce the greening agent into the U.S. Diseased materials would have to be obtained from a country within the range of the pathogen. This budwood is may be smuggled into the country by conventional means. Alternatively, it could be done so with compliance from citrus producers and shippers in the operation. However, such an operation would require the

budwood be processed through USDA Budwood Certification programs, and the chance of discovery would increase.

The successful, intentional introduction of *C*. Liberobacter africanus or *C*. Liberobacter asiaticus for the purpose of damaging the U.S. citrus industry would require an extensive understanding of the biology of the pathogen and its vectors. A person or persons intent on introducing the disease would first need to have access to a source of inoculum in the form of diseased trees. The precise volume of inoculum necessary to initiate a greening epidemic in the U.S. is unknown. Theoretically, one bacterium would be sufficient, but it is not likely to be effective. It is more reasonable to assume that dozens, and perhaps hundreds, of infected bud tissues would need to be acquired from an infected tree (or trees) and prepared for transport in some container to keep the infected plant tissue viable. There are certainly sufficient numbers of diseased trees at several, widely distributed points of origin available for collecting the proper materials.

Vector Introduction

D. citri is already present in Florida and Texas, so that the movement of psyllids within the U.S. is now more critical than the issue of introduction into the U.S. Collection of a large population of contaminated psyllids is also an option available for initiating an epidemic. Again, the precise numbers of insects sufficient to accomplish the task is unknown, but would presumably be in the thousands and tens of thousands.

B. Entry Potential = LOW RISK

There has been no history of repeated interceptions of infected budwood or contaminated psyllids for HLB disease of citrus. The technologies available for detection of the pathogen in infected tissues are in place, and the quarantine policies for the prevention of introducing any budwood for citrus propagation is already established. Careful preparation would be necessary to insure maintaining the viability of the bacterium in the tissue. Such preparation would probably consist of keeping the tissues cool and moist for an extended period during transit. The equipment needed would consist of ice chests, cold packs, and plastic wrapping. These materials are certainly readily available, but also easily detectable and would likely be discovered under current quarantine conditions.

Just as with pathogen inoculum, the insects would have to be stored properly to insure survival in transit, and would probably be relatively easy to detect due to the storage requirements.

Incorporation of the diseased budwood into the US citrus industry would require grafting of the diseased materials onto healthy rootstock and then planting the new materials in the vicinity of existing production citrus groves. The likelihood of successful invasiveness increases with multiple introductions (Grossblat, 2002). Managing such multiple introductions would be very difficult.

C. Establishment Potential = MODERATE RISK

The ultimate success of the introduction of an exotic, invasive agent will depend on a number of complex, interacting factors. In the case of virulent plant pathogens, such as the two GFB the first presumption is the presence of a susceptible host. This presumption is met throughout all major citrus growing regions in the United States.

The second presumption is that the environmental conditions in the U.S citrus growing regions are conducive to survival and spread of the pathogen. Climate matching has been a common exercise used to predict whether an exotic organism might survive and persist in a new region (Kriticos and Randall, 2001, Sutherst, 1999). Computer based - models are available to assist in predicting the potential geographic distributions of introduced plants, microbial pathogens, and arthropods under both current conditions and global climate-change scenarios. There are limitations to basing the dispersal prediction of the agent solely on climate (Grossblat, 2002). The biotic environment and chance dispersal factors are also considered to be important indicators. These influences would be particularly significant in the case of a microbial plant pathogen like the GFB, where hosts and vectors play a critical role in the life history of the organism.

Citrus is grown in a relatively narrow, uniform region throughout the globe due to the temperature and moisture requirements of the tree. The worldwide distribution of HLB is therefore coincidental with citrus production (Fig. 8 and Appendix Table 2). Citrus trees are subtropical in origin. They need warm climates with mild and nearly frost-free conditions. Therefore, citrus growing regions in the U.S. (Fig. 9) match well with the climatic regions where HLB is a problem simply because the crop requirements are so limiting.

D. Spread Potential = HIGH RISK

In the early stages of infection, *C.* Liberobacter spp. move very slowly through citrus trees. The latent period, the time before between inoculation of the host and appearance of symptoms, is relatively long (Hung et al., 2000, Su, 2001). Therefore, it could be a matter of a few years before a minor, erratic syndrome consisting of a few unnoticed symptomatic branches might develop into a recognizable epidemic. The infectious period is also long, and the distribution of the pathogen in infected citrus trees is less than complete. As a result, secondary spread by the psyllid vectors will also be a lengthy process, further delaying the potential for discovery and increasing the possibility that the pathogen will be well-established before infection. Just as with pathogen establishment, the environmental conditions throughout the U.S. citrus ranges are conducive to the processes involved with dispersion and spread. The pathogen and vectors will both survive environmental extremes and reproduce efficiently. Host composition and orchard structures will encourage secondary spread of the pathogen, making it highly likely that once established, HLB spread sufficiently to become a permanent feature of citrus production. These conclusions are based on the presumption that no control measures would be implemented to reduce the potential spread.

The best predictor of the invasiveness of an introduced, nonindigenous agent beyond its natural range is the record of dispersion in other geographic areas (Grossblat, 2002). In this regard, there is very little quantitative data on the greening agent. Most of the descriptions of spread are more of an anecdotal nature (Koizumi et al., 1997).

E. Economic Damage Potential = MODERATE RISK

U.S. Citrus production – the target crop

U.S. annual per capita citrus consumption during the 1990s exceeded 25 lbs., second only to bananas among fresh fruits (http://www.fred.ifas.ufl.edu/citrus/pubs/misc/wp2000-1.pdf). In 2000, the average American consumed 5.8 gallons of orange juice, equaling approximately 79.5 pounds of fruit. U.S. orange production is the second largest in the world. The majority of citrus production is in California, Florida, Texas and Arizona (see http://www.ultimatecitrus.com/ and Figure 9). Farm gate receipts for U.S. citrus production during the 1990s averaged $2.3 billion annually.

Fig. 8. Worldwide distribution of huanglongbing disease of citrus.

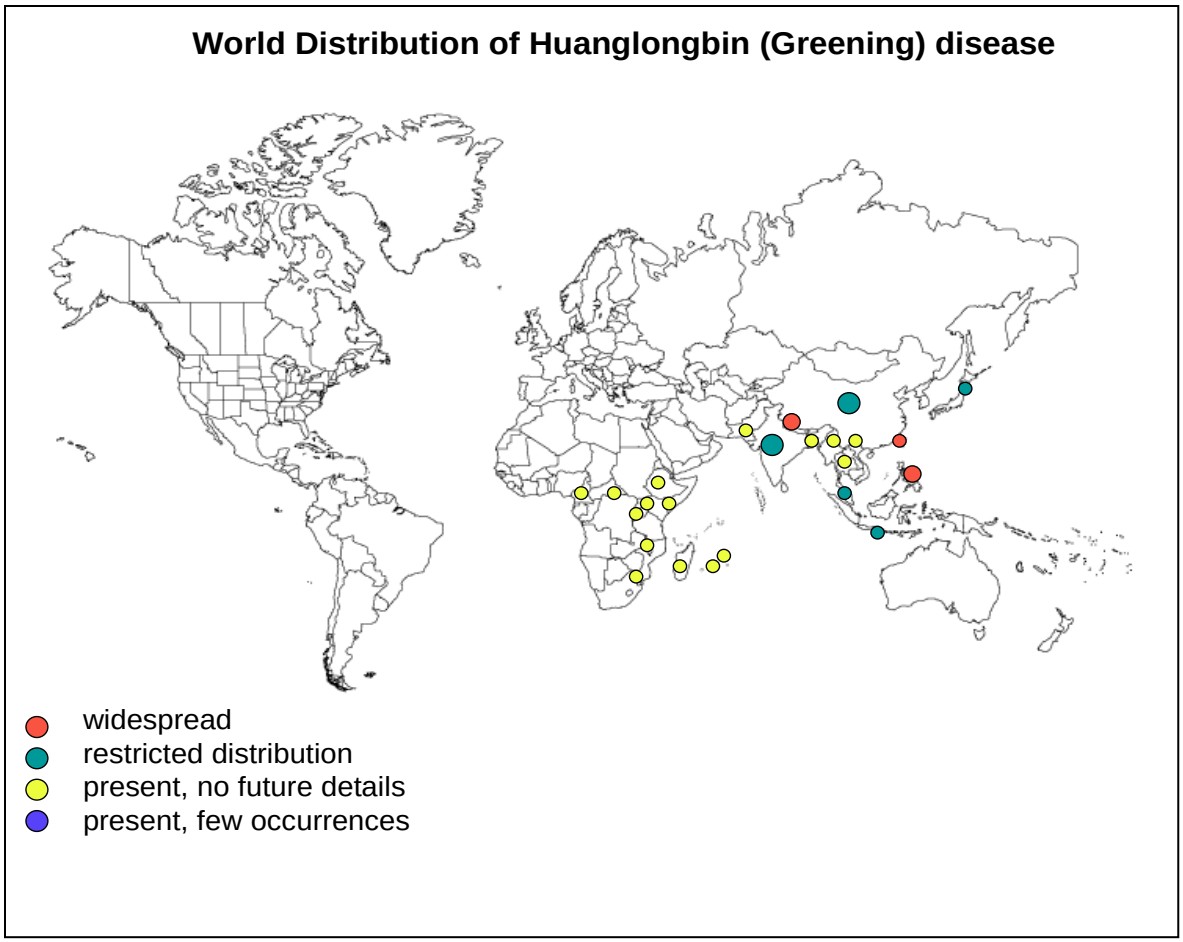
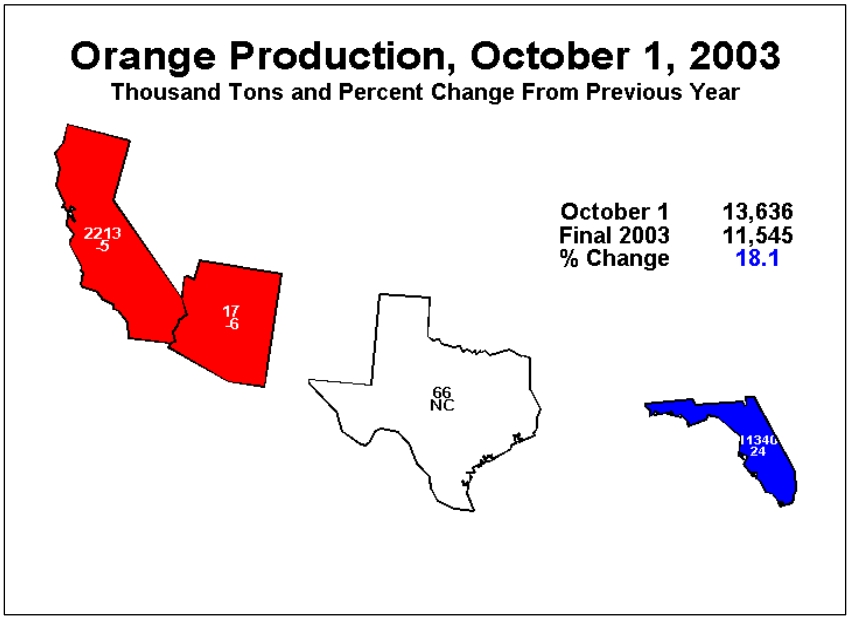


Fig. 9. Major U.S. citrus producing states.



Citrus greening is capable of causing widespread and destructive epidemics, as has been repeatedly demonstrated in numerous countries throughout Asia, the Philippines, and South Africa (da Graca 1991). Historically, orchard losses to this disease have been enormous, sometimes achieving 100% (da Graca, 1991). Many of these records were observed prior to the proper identification of the pathogen and vector. With improved methods for early detection and disease management, losses would be considerable reduced. However, this pathogen still has the ability to reduce yields and would require higher maintenance costs such as those required to spray insecticides.

The value of the citrus crop will justify the inputs needed to suppress losses should HLB become established, particularly with regard to insect vector control. These costs, however, would probably not be a persuasive justification on the part of a bioterrorist given the technical difficulties involved in insuring success of an attempted introduction.

F. Environmental Damage Potential = LOW RISK

There are a few environmental considerations that must be considered in the event of an HLB epidemic. First, one important management option consists of the use of insecticides. However, insecticides are already used in citrus production and they would not be considered a major problem in some of the citrus growing regions.

Another environmental consideration would relate to the need to change crop and production patterns that might result from a serious greening epidemic. Such decisions could temporarily disrupt ecosystem functions in and around regions already adjusted to the demands of large scale citrus production.

G. Social and Political Considerations = LOW RISK

Given the ability to aggressively control HLB, there is little potential for this disease to create a significant public response. Also, the high potential for natural introduction of the pathogen will make it difficult for and individual or group to make a convincing case for being responsible for an introduction. Appearance of this disease may increase the awareness of policymakers for the continued threat of nonindigenous, invasive agents to our agricultural and natural resources, but the successful control of HLB will reduce the political will to make major policy adjustments to prevent such threats.

H. Risk Summary = LOW to MODERATE

A summary of the individual processes in this analysis lead to the conclusion that there is only a low to moderate risk of an intentional introduction of the HLB pathogen into the U.S.

These individual risk ratings are listed in the table to the right.

Table 1. Summary of the risk of selected features involved in the process of intentionally introducing the causal agent of huanglongbing disease of citrus.

Risk Factor		Risk Rating
Likelihood of Acquisition		Moderate
Entry Potential		Low
Establishment Potential		Moderate
Spread Potential		High
Economic Damage Potential		Moderate
Environmental Damage Potential		Low
Social and Political Considerations		Low

VI. Control/Mitigation strategies after establishment

Control strategies are available for citrus greening. These specific control measures usually include quarantines, some measure to protect foundation stock and budwood production from being infected, sanitation measures in orchards, and management of insect vectors by chemical or biological means (da Graca, 1991, Su and Chen, 1991, Xue-Yuan, 1981, or website of the American Phytopathological Society http://www.apsnet.org/online/Archive/1999/IW00006.htm).

If this pathogen becomes well established, it is very difficult to eliminate. Eradication efforts in relatively small areas in the Zhejiang Province of China resulted in some success in controlling the disease (Tian-Sshang, 1991). In larger areas, however, eradication on a sporadic, uncoordinated basis failed to reduce the rate of disease progress. Aggressive eradication efforts and replanting of disease free-material and insecticides sprays for *D. citri* was effective in greatly reducing the level of disease in Guangdong Province of China (Chung and Fan, 1990). Insecticide sprays are recommended when new flushes of growth are present (Garnier and Bove, 2000, Xue-Yuan, 1981). A wide range of modern insecticides are believed to be effective in controlling the vectors (see website of the University of Florida Institute of Food and Agricultural Sciences http://creatures.ifas.ufl.edu/citrus/acpsyllid.htm).

Disease free foundation stock is probably the most important step in the control of greening. Shoot-tip grafting, heat-therapy of graftwood (48 – 50^{o}C for several minutes) and nucellar line selection are used to eliminate the pathogen from potentially infected plant materials (Garnier and Bove, 2000, Su and Chen, 1991, see website of Chemical and Biological Warfare Agents http://www.cbwinfo.com/Biological/PlantPath/CGD.html,).

Another option for control is the use of biological control agents to reduce populations of the psyllid vectors. The hymenopterous ectoparasites, *Tamarixia dryi* and *T. radiatus*, have been shown to effectively control the psyllids (Garnier and Bove, 2000). On the Reunion Island in South Africa, the parasitic wasps *T. dryi*, *T. radiatus*, and *Psyllaephagus pulvinatus* were found to eliminate the psylla from commercial orchards and consequently reduced the incidence of greening (da Graca, 1991). Steps to initiate a classical biological control programs have been established in Florida with promising results (Hoy et al., 2001, Michaud, 2004, Skelly and Hoy, 2004).

There is evidence that injection with antibiotics is a viable therapeutic treatment for obtaining remission of symptoms in diseased trees. Tetracycline treatments have been shown to temporarily alleviate symptoms for a few years, but the practice has been used on a limited basis (Xue-Yuan, 1981). Various other antibiotics have been tested with varying results (da Graca, 1991).

VII. Knowledge gaps

Prevention efforts for HLB would be greatly improved by promoting research in current diagnostic and assay methods. Recent advances in conventional Polymerase Chain Reaction (PCR) and real time PCR methods have produced excellent probes to use in detecting the pathogen in diseased tissues (Garnier and Bove, 1997, and see website of USDA ARS at http://www/ars.usda.gov/research/publications/publications.htm?SEQ_NO_115=121841). This and related work would also lead to better understanding strain relationships in the GFB populations and help in determining origins of outbreaks.

The best source of information on the number and identity of nonindigenous plant pests arriving in the U.S. is the Port Information Network (PIN) administered by USDA PPQ (see website at http://www.aphis.usda.gov/ppq/biosecurity/infomgmt.html). The database, however, is believed to have a number of limitations (Grossblat, 2002). These limitations

include inconsistent sampling protocols from one port to another, as well as sample collection processes that discourage effective statistical analyses. PIN databases are not readily accessible for inspection and analyses. A search of the PPQ website revealed no awareness of citrus greening and there was no background information concerning the potential pathways for this pathogen. Citrus greening should be treated as a credible problem for U.S. citrus production and should be accorded the attention given to other plant pathogens.

VIII. Immediate response options

HLB should be a subject of training for first responders in each of the major citrus growing regions of the U.S. A systematic effort should be made to educate County Extension Agents (CEAs), citrus producers, and nursery operators about the threat of this and other citrus diseases (e.g. citrus variegated chlorosis caused by *Xylella fastidiosa*). Easily accessible educational materials should be produced and distributed throughout these regions via workshops, individual contacts, and the internet. In addition to specific diseases, the rationale for pathways analyses should be a topic of discussion in these educational materials. The awareness of the general public should be raised concerning the potential problems accompanying a global economy and the consequences of moving plant materials into the country.

An urgent effort should be made to determine the sources of the psyllid vector *D. citri* in Florida and Texas. Further introductions of this vector are likely. Without any knowledge of how *D. citri* originally arrived in the U.S., it is also unknown whether HLB occurs at the origin of the vector. Therefore, there is no way to determine the threat of natural or accidental introduction of the pathogen.

Another factor pertinent to the question of our vulnerability to the introduction such potentially destructive pathogens as the GFB have to do with the free interchange of information and products via the internet. The genus *Citrus* and some wild relatives include a large number of plants considered to be of value for medicinal purposes and as nutritional supplements. These plants are discussed freely, the availabilities are readily accessible, and the marketing of seeds, plant parts, and propagating materials is commonplace. Without some controls, it is apparent that these activities will only increase. Some systematic effort must be made to catalogue the known plants susceptible to the GFB

organisms and whether they are going to be the subject of marketing over the internet. Prohibition of the movement of plants and plant parts that could potentially harbor GFB or their vectors should be implemented.

Appendix Table 1. Relative susceptibilities of Rutaceous and Non-rutaceous hosts to infection by Liberobactor *spp*

Common Name	Scientific Name	Use
	Extremely Susceptible	
Sweet orange	*C. sinensis*	fruit, rootstock
Tangelo (Orlando)	*C. regiculata* X *C. paradisi*	fruit
Mandarins	*C. reticulata*	fruit
	Relatively Tolerant	
Grapefruit	*C. paradisi*	fruit
sour orange	*C. aurantium*	rootstock
Kumquats	*Fortunella* spp.	fruit
Lemons	*C. limon*	fruit
Rough Lemon	*C. jambhiri*	rootstock
	Least Susceptible	
Trifoliate orange	*Poncirus trifoliata*	rootstock
Pummelo	*C. grandis*	fruit
Lime	*C. aurantifolia*	fruit
Rangpur lime	*C. limonia*	rootstock
Sweet lime	*C. limettioides*	fruit, rootstock
Citrange	*C. sinensis X P. trifoliata*	rootstock
	Non rutaceous	
Wood apple	*Limonia acidissima*	Ornamental
Chinese box orange	*Severinia buxifolia*	None
Perriwinkle	*Vinca rosea*	Indexing
tobacco	*Nicotiana xanthii*	Indexing

Appendix Table 2. World Distribution of Huanglongbin (Greening) disease (CABI).

Asia		
Bangladesh	present, no further details	Catling et al., 1978
Cambodia	present, no further details	Garnier & Bove, 1998
China	present, no further details	Lin & Lin, 1956
India	present, no further details	Bove et al., 1993
Indonesia	present, no further details	Aubert et al., 1985
Japan	present, no further details	Miyakawa & Tsuno, 1989
Laos	present, no further details	Garnier & Bove, 1998
Malaysia	present, no further details	Bove et al., 1993
Myanmar	present, no further details	Garnier & Bove, 1998
Nepal	widespread	Regmi et al., 1996
Pakistan	present, no further details	Garnier & Bove, 1996
Philippines	widespread	Garnier & Bove, 1996
Saudi Arabia	restricted distribution	Bove & Garnier, 1984
Thailand	present, no further details	Promintara, 1990
Vietnam	restricted distribution	Bove et al., 1996
Yemen	restricted distribution	Bove & Garnier, 1984
Africa		
Burundi	present, no further details	Aubert et al., 1988
Cameroon	present, no further details	Aubert et al., 1988
Central African Republic	present, no further details	Aubert et al., 1988
Ethiopia	present, no further details	Aubert et al., 1988
Kenya	present, no further details	Garnier & Bove, 1996
Madagascar	present, no further details	Bove & Garnier, 1994
Malawi	present, no further details	Aubert et al., 1988
Mauritius	present, no further details	Garnier et al., 1996
Reunion	present, no further details	Etienne & Aubert, 1980
Rwanda	present, no further details	Aubert et al., 1988
Somalia	present, no further details	Bove, 1995
South Africa	restricted distribution	Korsten et al., 1996
Swaziland	present, no further details	Bove & Garnier, 1994
Tanzania	restricted distribution	Bove & Garnier, 1994
Zimbabwe	restricted distribution	Garnier & Bove, 1996x

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Citrus Variegated Chlorosis

Pathway Analysis:

Intentional Introduction of

Xylella fastidiosa

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Citrus Variegate Chlorosis Pathway Analysis

Table of Contents

Executive Summary: Citrus Variegate Chlorosis Pathway Analysis

This summary is a brief description of a pathways analysis for the intentional introduction into the United States of the citrus variegated chlorosis (CVC) strain of *Xylella fastidiosa*. CVC causes a serious disease of sweet oranges (*Citrus sinensis*) and other *Citrus* spp. The disease is found only in Brazil, Argentina, and Paraguay. Infection of citrus trees leads to significant reduction in the health of trees and subsequent declines in the fruit production. Due to the potential damage to U.S. citrus and worldwide production, the CVC strain of *X. fastidiosa* is on the USDA APHIS Ag Bioterrorism Agent and Toxin list and is on the European Plant Protection Organization Organization's A1 list of regulated quarantine agents.

X. fastidiosa is a bacterial plant pathogen restricted to living in the xylem of host plants. This bacterium is very difficult to culture and manipulate in the laboratory, and has only relatively recently been recognized as the causal agent of dozens of scorch type plant diseases in the Americas. There is some evidence of host specialization among strains of *X. fastidiosa*, but the classification of these strains to the pathovar or subspecies level has been difficult. As a result, there is a fair degree of confusion regarding the relationship among populations of isolates from different hosts, the pathogenic potential of the various strains, and the degrees of resistance and susceptibility exhibited by many of the hosts of *X. fastidiosa*.

It is clear that sweet orange varieties in South America are highly susceptible to CVC. Due to the practice of orange propagation by budwood, the pathogen causes primary infections in orchards when it is introduced on diseased nursery stock. Subsequent spread is then facilitated by insect vectors. The insects identified as responsible for secondary spread of *X. fastidiosa* are the xylem feeding sharpshooters. Any consideration of the intentional introduction of the CVC pathogen into the U.S. must take this important group of vectors into account.

This pathways analysis is a risk assessment based on seven processes believed to be instrumental in the act of intentionally inflicting a plant pathogen on a target crop. The seven

steps and the risk rating for each in the case of CVC, *X. fastidiosa* and the U.S. citrus crop are:

- Likelihood of acquisition of pathogen and vectors at point of origin – MODERATE
- Entry potential – MODERATE
- Establishment potential - MODERATE
- Spread potential – HIGH
- Economic potential – HIGH
- Environmental damage potential – LOW
- Social and political considerations - LOW

The summary conclusion is that there is a moderate risk of an intentional introduction of the CVC strain of *X. fastidiosa* with the intent of harming the U.S. citrus industry. It should be recognized that there is a fair degree of risk that this pathogen will soon be introduced naturally or accidentally into the U.S. citrus crop, the possibility of which would obscure the impact of any attempt to intentionally introduce this pathogen.

There are a number of technical and biological factors that went into the conclusion that the CVC strain of *X. fastidiosa* would be moderately useful as a biological weapon. This organism is difficult to manipulate, has an unpredictable relationship with its many hosts, relies on a vector relationship that has yet to be fully understood, and has a confusing population structure which makes it difficult to characterize with a high degree of certainty. Each of the states where citrus is a major agricultural commodity have budwood certification programs which limit the introduction and dispersal of foreign propagating materials. There is a very good chance that, if the unlikely establishment of this pathogen in citrus orchards were successful, that the pathogen could very well spread rapidly and aggressively into and through the citrus growing regions in the U.S. Climatic conditions throughout the citrus producing areas in the U.S. are conducive to the survival and growth of *X. fastidiosa*. The potential vector populations are probably already in place, and the structure of the citrus crop would encourages the development of this and any pathogen. The tremendous value

of citrus production and the high costs involved in growing this crop make it vulnerable economically to spread by *X. fastidiosa* should it become established.

The rating of this pathogen as a moderate risk for intentional introduction should not be construed as a dismissal of CVC as a potentially damaging plant pathogen. When CVC does eventually reach the U.S., there will be an increase in the costs of citrus production in order to cope with the disease. Early detection is therefore an extremely important issue when considering CVC, and efforts should be expanded to improve our technical capacity to diagnose this disease and maintain an infrastructure that would facilitate a quick response when such a diagnosis does occur.

Citrus Variegated Chlorosis

Pathway Analysis for the Intentional Introduction of *Xylella fastidiosa*

I. Introduction

A. Justification and charge

The following report describes how to predict a complex series of events that could culminate with the introduction of a known, exotic plant pathogen into a susceptible agricultural crop growing in the United States. This series of events can be summarized as a pathway for invasion by a nonindigenous plant pathogen. The United States of America National Agricultural Biosecurity Center Consortium (NABC) commissioned this exercise. Based on these findings, recommendations will be made to the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) on how to better protect the nation's food and fiber production systems from the threat of intentionally introduced plant pathogens. This analysis specifically concerns the causal agent of citrus variegated chlorosis (CVC), *Xylella fastidiosa*, and the threat it poses to the U.S. citrus industry.

Numerous recent events have proven that agricultural terrorism must be considered as a "legitimate and immediate concern". The results of introducing a plant pathogen into a susceptible ecosystem can be devastating. There are several well-known cases of just how damaging such introductions can be, even when accidental. For example, the North American chestnut blight epidemic, caused by the fungal tree pathogen *Cryphonectria parasitica*, is an excellent lesson on the circumstances that might generate an extremely

successful introduction of an alien plant pathogen. This tree disease is certain to be mentioned in discussions of the potential devastation caused by introductions of nonindigenous pathogens. *C. parasitica* causes a relatively innocuous canker on chestnuts of Chinese origin and is considered to be native in Asia where it probably evolved. However, when introduced into the United States accidentally, it took on all of the characteristics of a "super" pathogen. As the cause of rapidly expanding killing cankers on the ill-fated American Chestnut (*Castanea dentata*), the pathogen is able to reproduce at rapid rates over long periods of time, spread rapidly by means of an efficient vector (wind), survives well in the North American climate, and makes use of readily accessible infection courts. These traits, combined with the lack of genetic resistance in *C. dentata*, resulted in one of the worst ecological disasters in North America. The economic cost and impact on the ecosystem was tremendous.

There are hundreds of examples of annual introductions of exotic, alien species into the United States. Although none results in the devastation of Chestnut blight, many must still be contended with in order to limit the impact. One example is a recent introduction of Giant Asian Dodder (*Cuscuta japonica*) into Houston, TX. This weedy plant parasite is on the USDA Resource Conservation Service Federal Noxious Weed List (http://plants.usda.gov/). Although very different than the fungal pathogen *C. parasitica*, Giant Asian Dodder is extremely aggressive on a wide host range of economically important plants and could possibly be a huge threat to southern field crops. It has been introduced on three other occasions in the U.S, and has been successfully eradicated on each. The eradication of the most recent introduction is nearly complete. The reason that this dangerous weed is readily eliminated has to do with a significant limitation in the life cycle. Although the plant produces flowers, there has been no viable seed produced in Houston, so that the dispersion is completely dependent on spread of vegetative vine segments. These two examples above illustrate just a few of the factors that must be considered in assessing the potential for an alien exotic species to cause damage to U.S. agricultural production.

There are two comprehensive goals of the following report. The first is to study potential pathways for a foreign, exotic plant pathogen to enter into the domestic agricultural system. The second is to assess America's capacity to contain such a disease were it to occur. The pathways analysis will consist of determining where the agent would originate, the medium in which it would move or the vectors on which it would move, the point of contact (crop) and potential recognition (responders), the mechanisms of dispersion, and impact the

introduction might have. This analysis will be carried out in the context of current import operations and the production of the susceptible crop throughout the U.S. Prevention of such an introduction is the most effective means of managing the threat, so that recommendations will be made on how to close the pathways. Further recommendations will describe the most effective response options in the event of an introduction, and also gaps in our knowledge and recommendations for future actions to address those gaps will be made.

The following report consists of 10 sections. Following the Introduction (Section I), the Biology and Life/Disease Cycle of the Pathogen (Section II) are discussed. Section III concerns current state of the Detection and Recognition/Diagnosis of CVC. Section IV describes the Likelihood of Natural/Accidental Introductions. Models describing the acquisition, introduction, establishment and spread scenarios are contained in Section V (Likelihood of Intentional Introduction/Risk Assessment). Section V also contains a risk rating for the processes necessary to intentionally release a CVC epidemic in the U.S. citrus industry, as well as a risk rating for the potential economic, political and environmental impact of CVC. Control/Mitigation Strategies after establishment are discussed in Section VI. Section VII (Knowledge Gaps) discusses limitations in our understanding of CVC relevant to the potential bioterrorist threat it poses to our citrus industry. Steps that can be taken to immediately prepare for the CVC threat are given in Section XIII (Immediate Response Action). The final two sections contain the Literature Cited and Appendices (Sections IX and X, respectively).

II. Biology and life/disease cycle of the pathogen

A. Description of the Pathogen

The pathogen responsible for CVC (also known as “pecosita”) is *Xylella fastidiosa* (Garnier and Bove, 1997). CVC has become one of the most important diseases of citrus production in Brazil, where the disease was first described in 1987 (Beretta and Leite, 2000). The disease has also been found in Argentina and Paraguay, but has never been reported outside of South America (current distribution includes South America: Argentina, Brazil (Goias, Minas Gerais, Parana, Rio do Janeiro, Rio Grande do Sul, Santa Catarina, Sao Paulo, Sergipe), Paraguay, Venezuela) (see Figure 1). Due to the perceived threat to the

U.S. citrus industry, *X. fastidiosa* is listed as a regulated Biological Agent under the Agricultural Bioterrorism Protection Act of 2002 (see website of the USDA APHIS http://www.aphis.usda.gov/ppq/permits/agr_bioterrorism/).

X. fastidiosa is a xylem-limited bacterium that causes many plant diseases nearly exclusively in the Americas, with the exception of a report of the pathogen in Taiwan (Purcell, 1997). The bacteria are considered to be "fastidious" because they are endophytic parasites the can only exist in the xylem of their hosts (Purcell and Hopkins 1996). The fastidious nature of the organism carries over to existence in laboratory cultures, where growth requirements are very strict and isolation from diseased tissues can be accomplished only on a complex growth medium (Raju and Wells, 1986). This difficulty in growing *X. fastidiosa* in the lab is one reason why the bacterium was difficult to identify and associate with the many diseases it causes (Hartung et al., 1994). The bacterium was first identified as the cause of CVC in the 1980s, although it had been associated with numerous other woody hosts since the early 1970s. Prior to then, diseases caused by *X. fastidiosa* were mostly attributed to viruses.

X. fastidiosa is a gram negative bacterium bounded by a cell a membrane and cell wall with no flagella. Electron microscopy reveals that the cell wall of the *X. fastidiosa* has a rippled appearance. The bacterium is 1 – 3.5 µm x 0.3 – 0.5 µm (Lee et al., 1991). This pathogen is sensitive to high temperatures and several antibiotics such as tetracyclines and penicillin (Agrios, 1997).

B. Host Range and Strain Relationships

All sweet orange varieties (*Citrus sinensis*) are considered to be susceptible to CVC. Tolerance and resistance may be found in mandarins, lemons, and some commercial hybrids (Laranjeira et al., 1998; Li et al., 1996 and see website of the Xylella fastidiosa Genome Project http://aeg.lbi.ic.unicamp.br/xf/home/mmachado.html). More specifically, Pera, Natal, Valencia, Hamokn and Bhaia-Navel varieties on Rangpur lime (*C. limonia*), Cleopatra, mandarin and Volkamer lemon rootstocks were susceptible (Koizumi, 1994). There were some varietal differences in resistance and susceptibility in artificially inoculated tangerines or mandarins (*C. reticulata*) growing on Rangpur lime rootstocks (Li et al., 2000). Two tangerine varieties were found to be serving as symptomless hosts, where the

Fig. 1. Worldwide distribution of citrus variegated chlorosis (CVC).

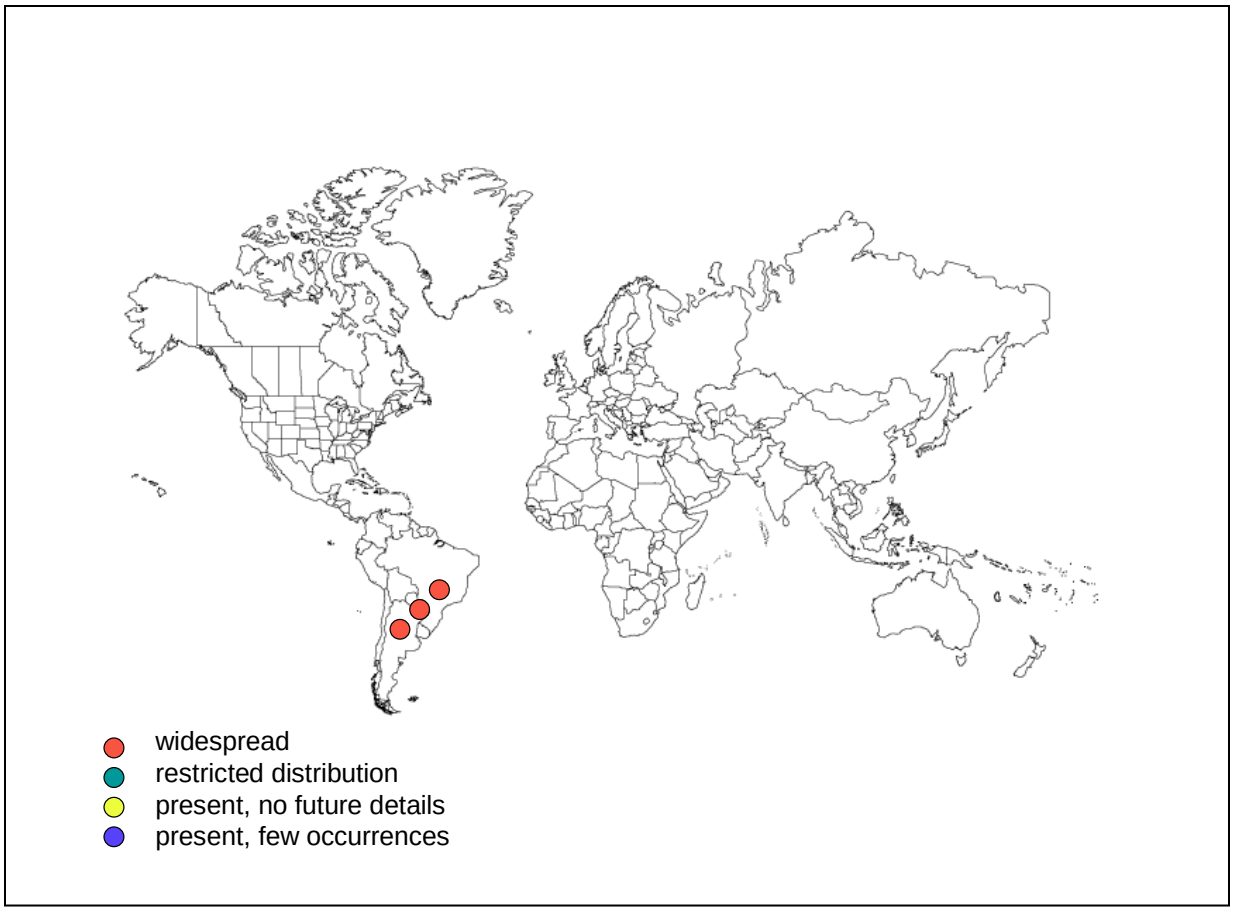
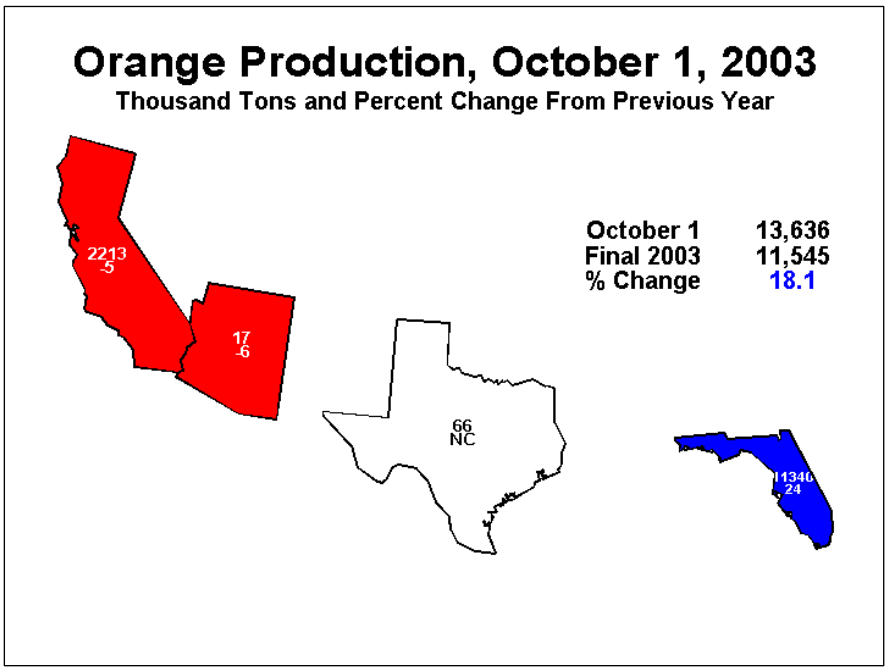


Fig. 2. Map of major citrus producing regions in the U.S.



bacterium was colonizing the trees with no plant response. Under field conditions, citron (*C. medica*) and pummelo (*C. grandis*) were also found to be symptomless hosts (Laranjeira et al., 1998). These results can have great implications for the threat of introducing the pathogen on nursery stock, particularly of non-citrus plants imported for ornamental or other purposes.

Any discussion of CVC must account for complex strain relationships involved with the pathogen, *X. fastidiosa*. The obscurities in the strain relationships can have great implications

for our ability to detect the pathogen an identify and impending epidemic. Different strains of the same bacterium are know to cause Pierce's disease (PD) of grape (*Vitis vinifera*), phony peach (*Prunus persica*), almond leaf scorch (*P. amygdalus*), oak leaf scorch (*Quercus rubra*), elm leaf scorch (*Ulmus americana*), mulberry leaf scorch (*Morus rubra*), sycamore leaf scorch (*Platanus occidentalis,*) ragweed stunt (*Ambrosia artemisiifolia*), alfalfa dwarf (*Medicago sativa*), periwinkle wilt (*Vinca minor*), and diseases in a variety of other commercial hosts (Purcell, 1997; Raju and Wells, 1986). There are also dozens of known wild hosts for this pathogen. Pathogenicity tests have revealed a very complex series of strain relationships where certain isolates can cause symptoms on some hosts and not others, with overlaps in the host groupings. For example, isolates of the PD, almond leaf scorch and alfalfa dwarf strains are reciprocal on their hosts and appear to be the same strain. Phony peach isolates and plum leaf scald isolates form another common strain grouping (Hopkins, 1989). Pathogenicity tests are therefore extremely unreliable for identifying different strains into strict pathovar taxa. One reason is because the host specificity is not strict. Strains considered to be in a distinct host grouping may successfully colonize a different host without symptom development. Inoculation trials are not sufficient to identify a pathovar or subspecies of *X. fastidiosa* with any degree of certainty.

The various strains of *X. fastidiosa* are morphologically identical, so that any taxonomic groupings beyond the species level must be discerned with more precise methods. Two distinct groups can be distinguished on the basis of growth requirements *in vitro*, but as described above the strain relationships are far more complex within those two groups. Serological tests, such as enzyme linked immunosorbent assay (ELISA) are of limited value in distinguishing strains (Hopkins, 1989). DNA-DNA hybridization, fatty acid profiles, and RNA sequence analyses have proven useful in advancing our understanding of the strain

relationships in the species. Restriction fragment length polymorphisms (RFLPs) were effective in characterizing a similarity group consisting of the PD, alfalfa dwarf, and almond leaf scorch strains (Chen et al., 1992). The most recent advances in the effort to understand the population biology of *X. fastidiosa* has been with polymerase chain reaction (PCR) and Randomly Amplified Chain Reaction (RAPDS) (Minsavage et al., 1994). For example, combinations of these technologies resulted in delineating five similarity groups, consisting of almond, citrus, plum-elm, grape-ragweed, and mulberry (Pooler and Hartung, 1995). The complexities of these strain relationships were further increased with the discovery that the Brazilian citrus strain of *X. fastidiosa* was able to cause symptoms in artificially inoculated grapevines (Li et al., 2002). Discussion of these techniques and the significance of these results for detection and diagnosis will be discussed in **Section III. B**. below.

The citrus strain of *X. fastidiosa* was first found associated with CVC in Brazil in 1987 (Lee et al., 1991). A number of studies and techniques have been conducted to determine the relationship between this new strain of the pathogen and existing groups (Beretta et al., 1997, Hartung et al., 1994, Rosato et al., 1998). Serological tests placed the citrus strain in a putative new group between previously described plum and PD serogroups (Hartung et al., 1994). Populations of citrus strains in Brazil have been described as relatively homogenous, and strains causing CVC and coffee leaf scorch in Brazil are considered to be similar based on RAPD and 16-23S spacer region analyses (Rosato et al., 1998). These results are still not sufficient to determine the origins of the Brazilian CVC outbreak or the sources of orchard infections.

C. Vector Relationships

Six species of sharpshooters, Dilobopterus costalimai, Acrogonia terminalis, Bucephalogonia xanthophis, and Homalodisca ingorata, Oncometopia facialis, Plesiommata corniculata**,** (Hemiptera: Cicadellidae: Cicadellinae) have been confirmed as vectors of X. fastidiosa in citrus (Garcia et al., 1997, Redak et al., 2004). However, there are many additional native sharpshooters in Brazilian citrus groves, and the list of potential vectors will probably increase. The sharpshooters and related spittlebugs have been proven to be vectors of numerous strains of the bacterium in their respective host crops (Hopkins, 1989). These insects feed by sucking the contents of the xylem fluid of plants. During feeding, they

are able to acquire the bacterium from infected plants and efficiently spread it to the xylem of other healthy plants during subsequent feeding activities.

Several attributes of these insects make them effective vectors of *X. fastidiosa* (Hopkins and Purcell, 2002; Purcell, 1997). The sharpshooters make up a large varying group of insects, and there are a variety of lifestyles represented among them so that different species have unique characteristics with regard to acquisition of the pathogen and introduction into new plants (Redak et al., 2004). They have extremely high rates of feeding, they retain infectivity indefinitely, and the list of woody plants on which they regularly feed is enormous (see website of the University of California, College of Natural Resources, http://nature.berkeley.edu/xylella/index.html). Depending on the species, they will undergo one to several generations per year. Having once acquired the pathogen during feeding, the adults maintain the ability to transmit the pathogen during the remainder of their lives (Purcell, 1997).

Initial observations indicated that the sharpshooters probably were most important in the spread of *X. fastidiosa* in citrus nurseries rather than the tree to tree spread in orchards (Garcia et al., 1997). However, continued survey of sharpshooter populations in tree canopies in orchards and adjacent weed populations has resulted in an expanding role for these vectors in disease epidemiology (Lopes et al., 2003; Milanez et al., 2003).

D. Disease cycle

See Figure 3 for the disease cycle of citrus variegated chlorosis.

III. Detection and Recognition/Diagnosis

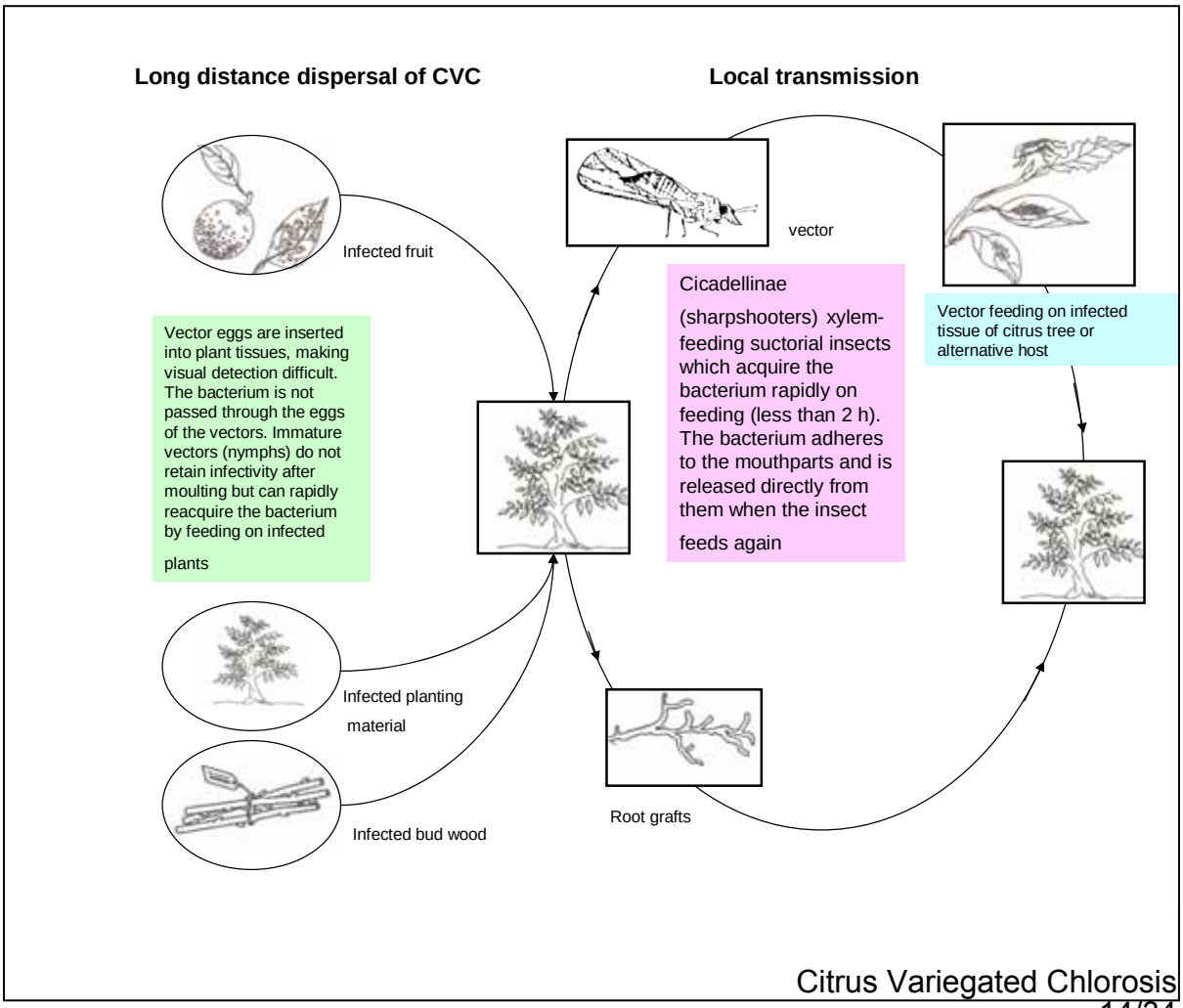
A. Detection by Symptoms

The most characteristic foliar symptoms of CVC are bright interveinal chlorosis and mottling resembling zinc deficiency (Lee et al., 1991; and the website of the Xylella fastidiosa Genome Project http://aeg.lbi.ic.unicamp.br/xf/home/mmachado.html). Symptoms appear and are more pronounced on maturing young leaves, but may also occur on older leaves. In a newly infected tree, the foliar symptoms are restricted to individual limbs. As the

condition becomes chronic, they spread throughout the entire canopy (Figures 3 - 7). With maturity, the area on the underside of the leaf corresponding to the chlorotic area on the upper side becomes light to dark brown. These lesions may become necrotic and raised due to gum formation (Lee et al., 1991). The canopy also is affected by reduced growth, dieback of twigs and branches, and thinning. Affected trees usually do not die. Trees of all ages, nursery stage to maturity, are susceptible to CVC. However, older trees, more than 15 years of age, are usually affected less by an infection, only develop symptoms in a few scaffold branches.

Orange fruits on infected trees are small, higher in sugar content, have relatively hard rinds, ripen prematurely, and exhibit sunburn damage (Fig. 7). Normal fruit thinning does not occur on infected trees, so that total fruit production on a tree remains the same as that of unaffected

Fig. 3. Disease cycle for the citrus variegated chlorosis strain of *Xylella fastidiosa*.



trees because of the greater number on the diseased trees. Although affected trees rarely die, trees in advanced stages of disease development may become nonproductive (Beretta and Leite, 2000). Once introduced into a grove, the pathogen spreads rapidly to other trees.

B. Detection by Clinical Procedures

X. fastidiosa can be readily detected in tissues sampled from infected trees that contain populations of the pathogen (Derrick and Timmer, 2000). Three techniques are usually used for routine detection of *X. fastidiosa* in diseased tissues of any hosts. These include enzyme linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and culturing of the pathogen on complex specialized media. Each of these techniques has advantages and disadvantages.

X. fastidiosa grows very slowly in axenic culture and does not compete well with other microorganisms. Therefore, a great deal of effort has been made to improve the selectivity of laboratory media for isolation of the bacterium (Davis et al., 1980; Purcell and Hopkins, 1996). The resultant media are relatively complex. Campanharo et al. (2003) have recently presented evidence for the use of a simpler media, PYE (phosphate yeast extract). Regardless of the growth medium used, there are also a number of other steps that must be taken to increase the likelihood of successfully isolating the pathogen from diseased tissues. For example, the bacterium is unevenly distributed in the host, so that thorough sampling is necessary. Sample preparation prior to plating the tissues, particularly with regard to steps needed to avoid contamination, is also an important issue. Even under the best of conditions, isolation of *X. fastidiosa* from diseased tissues is a slow, and sometimes unpredictable, process.

Serological techniques based on ELISA for detection of *X. fastidiosa* in suspected CVC cases continue to be used, although they are not effective in distinguishing different pathogen strains nor are they as sensitive as some alternative molecular methods (see website of the Xylella fastidiosa Genome Project http://aeg.lbi.ic.unicamp.br/xf/home/mmachado.html). ELISA can detect approximately 10^4 bacteria/ml.

Fig. 4: Symptoms of CVC in leaves of sweet orange 'Pera' (Photos by Marcos A. Machado Francisco Laranjeira).

Fig. 5: Symptoms of CVC in leaves of sweet orange 'Pera' (Photos by Marcos A. Machado and Francisco Laranjeira

Fig. 6: Fruits of sweet orange 'Pera' affected by CVC (Photos by Marcos A. Machado and Francisco Laranjeira).

Fig. 7: Symptoms on orange leaves:Chlorotic lesions on leaves characteristic of variegated chlorosis in sweet orange (Brazil). (By A.H. Purcell)

Fig. 8: Fruits of sweet orange 'Pera' affected by CVC (Photos by Marcos A. Machado and Francisco Laranjeira)

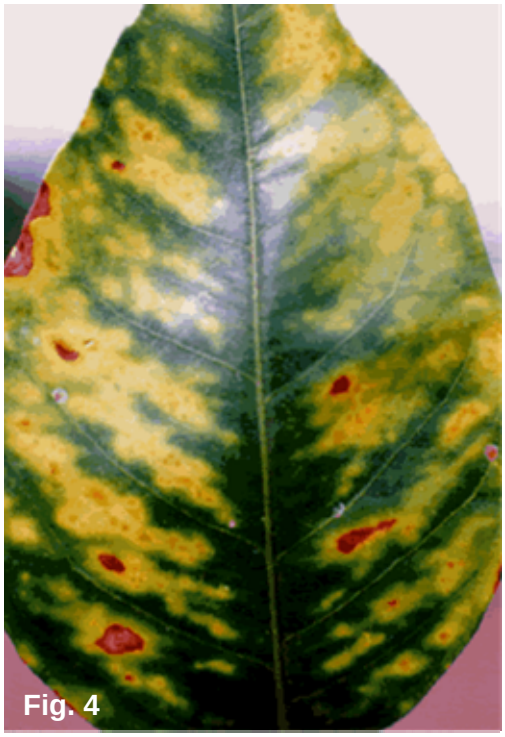


Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

The development of unique RAPID sequences and PCR primers, as described in **Section II. B.** above, has made it possible to distinguish *X. fastidiosa* strains (Banks, et al., 1999; Berretta et al., 1997; Pooler and Hartung, 1995). Numerous different PCR procedures have been developed to detect and survey for the CVC strain. Beretta et al. (1997) conducted an assay of citrus in Brazil using PCR with two tRNA consensus primers that successfully distinguished the citrus strain of the pathogen. CVC specific primers were developed by Pooler and Hartung (1995) that could differentiate between the CVC strains and grape strains, or the strains associated with citrus blight, in a single-step PCR protocol. These advances will significantly improve the ability to detect host specific strains of the pathogen. However, there remains some degree of uncertainty in the use of these methods, so that reliance on just one for diagnosis is questionable.

C. Similar Conditions and Diseases

The foliar symptoms of CVC are very similar to those of citrus blight, in that they both include symptoms of wilting and zinc deficiency (Berretta et al., 1997; Derrick and Timmer, 2000). Citrus blight (CB) is a disease of unknown etiology that has become a major limiting factor worldwide for citrus production (Derrick and Timmer, 2000). This disease is responsible for the loss of hundreds of thousands of trees annually in Florida (Timmer, 2000). There are currently no specific diagnostic tests for CB, so that the disease may only be diagnosed by exclusion, during the process of testing for other problems. The presence of non-CVC *X. fastidiosa* strains in trees with citrus blight further complicates diagnostic protocols and could obscure the successful detection of CVC (Hopkins, 1988).

Citrus is subject to a wide variety of leaf blights that could be confused with CVC when dealing with small sample sizes and various stages of disease development. There are many virus and virus-like pathogens and diseases, such as greening (*Candidatus* liberobacter spp.), citrus variegation virus, and citrus stubborn (*Spiroplasma citri*), that could be confused with CVC (Whiteside et al., 2000). The chlorotic symptoms caused by one of the most economically important disease problems in citrus worldwide, citrus tristeza virus (CTV), might be mistaken for CVC under superficial examination (Roy and Goldschmidt, 1996). In addition to zinc deficiency, the lack of iron, magnesium, boron, manganese, and molybdenum may cause the type of interveinal chlorosis exhibited by CVC affected foliage (Whiteside et al., 2000). The general nature of CVC symptoms make reliance on foliar

symptoms difficult, if not impossible, when dealing with quarantine conditions and illustrate the need for rigorous clinical analyses.

IV. Likelihood of Natural/Accidental Introductions

A. Pathogen introduction/natural or accidental

The production of citrus trees by growing seedlings has been largely replaced by budding onto rootstocks (Whiteside et al., 2000). Although increasing production efficiency and facilitating the uniform production of improved varieties, budding can be a significant mechanism for the spread of some of the most serious citrus diseases. The accidental use of infected budwood is considered to be the source and means of widespread establishment of CVC in Brazil (Lee et al., 1991). This source of pathogen introduction has stimulated several state budwood certification programs (Skaria et al., 1996; and see website of the California Citrus Clonal Protection Program http://www.ccpp.ucr.edu/about/index.html). These programs operate by being the first point of introducing budwood from external sources, often from outside the U.S. The primary tests involve the detection of graft transmissible diseases through the use of indexing onto indicator species. The California Citrus Clonal Protection Program, in cooperation with USDA APHIS, may serve as a point of introduction and eventual distribution of new and promising citrus varieties to the other citrus growing regions in the U.S.

The presence of the budwood certification programs makes the accidental introduction of CTC into the citrus industry unlikely. Another source of the pathogen could be through the importation of potential supplemental hosts in the nursery trade. Also, supplemental hosts could be harboring contaminated sharpshooters (Redak et al., 2003). It is the mission of the USDA APHIS Plant Pest Quarantine service to regulate the movement of such plant materials into the U.S., decreasing the chances that the CVC strain of *X. fastidiosa* will be accidentally introduced.

The citrus strain of *X. fastidiosa* appears to be able to exist in seed and be transmitted to seedlings of sweet orange (Li et al., 2003). The significance of this observation to the worldwide epidemiology of CVC is unknown, but it further illustrates the importance of exercising great caution in the movement of citrus plant materials.

B. Vector introduction/natural or accidental

There are native populations of sharpshooters distributed throughout all of the major U.S. citrus regions. Some of the native species have been shown to be capable of transmitting the citrus strain of *X. fastidiosa* under experimental conditions (Brlansky et al., 2002). There are no biological reasons to suspect that most of our native sharpshooters will be incapable of doing the same. There is no danger of new sharpshooter species providing a vector base to encourage a CVC epidemic. There is, however, the possibility exists that a contaminated vector from Brazil might be accidentally introduced on some transported commodity such as contaminated nursery stock or by means of natural movement on air currents. The discovery of the coffee leaf scorch pathogen in Costa Rica, which is closely related to the CVC strain of X. fastidiosa, raises the danger of a natural introduction of the pathogen in the U.S. (Brlansky et al., 2002). In summary, it is likely that over time, the CVC strain of *X. fastidiosa* will eventually be naturally or accidentally introduced into the U.S.

V. Likelihood and Consequences of Intentional Introduction/Risk Assessment

The likelihood of an intentional, successful introduction of a potentially damaging plant pathogen depends on a series of processes, each of which can be rated according to the risk posed by the characteristics of the pathogen, host and environment. These processes relate to the life history of an organism and the features that contribute to its fitness in a new environment. A thorough understanding of these features is important, but in the case of the CVC strain of *X. fastidiosa*, there are many aspects of the pathogen that are poorly understood. They include such characteristics as the strain relationships among isolates derived from different hosts, the relative abilities of the many sharpshooter species for acquisition and delivery of the pathogen, the erratic distribution of the pathogen in diseased trees, and the nature of resistance and susceptibility in the hosts.

The risk analysis below for CVC and the associated vectors is based on a qualitative pest risk-assessment for imported solid wood packing materials (Grossblat, 2002). Specific criteria have been modified to account for the unique characteristics of the agents, so that the risk rating more accurately reflects the potential for a damaging epidemic. The

background and justifications for the assigned risk ratings are found in the relevant sections above, as indicated.

A. Likelihood of acquisition of pathogen and vectors at point of origin = MODERATE RISK

Pathogen acquisition

One of the most logical methods of introducing the CVC pathogen into the U.S. citrus industry would be to acquire contaminated budwood from infected trees in Argentina or Brazil. This sort of acquisition would require a high degree of technical understanding of pathogen biology. Some complicity on the part of a knowledgeable individual or group of individuals, such as growers or scientists, would be necessary to insure that the proper plant materials were collected. The pathogen is widely distributed throughout the citrus growing regions of Brazil and Argentina, and the public awareness efforts have been extensive in those countries. Therefore, sources of infection to collect contaminated materials would be relatively easy to locate. However, problems with strain identification and the uneven distribution of the pathogen in the tree would greatly reduce the confidence in acquiring highly infective materials.

Vector acquisition

Another source of the pathogen for intentional introduction would be contaminated sharpshooters. The acquisition of large numbers of contaminated vectors would be possible, but would again require the assistance of someone with a thorough understanding of pathogen biology.

B. Entry Potential = MODERATE RISK

There has been no previous record of repeated introductions of infected budwood or contaminated sharpshooters for CVC of citrus. The technologies for detection through conventional quarantine measures and budwood certification programs are in place, so that a successful intentional introduction would be prevented unless there was complicity on the part of an individual or group employed within the relevant agencies.

The measures necessary to bring infected budwood, sharpshooters, or contaminated plants through ports of entry would probably lead to detection. Budwood would have to be stored in containers that would insure preserving freshness, and contaminated sharpshooter adults would have to be kept alive in transport. The likelihood of a successful invasiveness increases with multiple introductions (Grossblat, 2002). Large numbers of the contaminated materials and repeated attempts would therefore be necessary to insure a successful event, further increasing the chances of detection.

C. Establishment Potential = MODERATE RISK

The ultimate success of the introduction of an exotic, invasive agent will depend on a number of complex, interacting factors. In the case of a virulent plant pathogen, such as the CVC strain of *X. fastidiosa*, the first presumption is the presence of a susceptible host. This presumption is met throughout all major citrus growing regions in the United States.

The second presumption is that the environmental conditions in the U.S citrus growing regions are conducive to survival and spread of the pathogen. Climate matching has been a common exercise used to predict whether an exotic organism might survive and persist in a new region (Kriticos and Randall, 2001, Sutherst, 1999). Computer based - models are available to assist in predicting the potential geographic distributions of introduced plants, microbial pathogens, and arthropods under both current conditions and global climate-change scenarios. There are limitations to basing the dispersal prediction of the agent solely on climate (Grossblat, 2002). The biotic environment and chance dispersal factors are also considered to be important indicators. These influences would be particularly significant in the case of a microbial plant pathogen like *X. fastidiosa*, where hosts and vectors play a critical role in the life history of the organism.

Citrus is grown in a relatively narrow, uniform region throughout the globe due to the temperature and moisture requirements of the tree. The South American distribution of CVC is therefore coincidental with citrus production in Brazil and Argentina (Fig. 1). Citrus trees are subtropical in origin. They need warm climates with mild and nearly frost-free conditions. Therefore, citrus growing regions in the U.S. (Fig. 2) match well with the climatic regions where CVC is a problem simply because the crop requirements are so limiting. The only known climatic limits on *X. fastidiosa* are related to the inability of the pathogen to cause disease in cold climates (Hopkins and Purcell, 2002). Again, the requirements of

citrus production for tropical and subtropical conditions would be conducive to disease development in the citrus growing regions of the U.S.

The transmission rate of the CVC strain of *X. fastidiosa* from one citrus tree to another is low relative to sharpshooter transmission of the grape strain in vineyards (Almeida et al., 2001; Krugner et al., 2000). Such low transmission rates are believed to be due to dilute populations of the pathogen in citrus. Inefficient transmission would require high populations of contaminated vectors being smuggled into the U.S. and successfully introduced into groves of susceptible trees. Transport of thousands of living, contaminated vectors would be awkward and probably not a reasonable choice for intentional introduction of CVC.

The latent period, or period between infection and appearance of symptoms, can take up to a year to occur. Such a long latent period would probably result in ample time for the pathogen to spread beyond the initial point of introduction into a nursery or orchard before being detected.

D. Spread Potential = HIGH RISK

The best predictor of the invasiveness of an introduced, nonindigenous agent beyond its natural range is the record of dispersion in other geographic regions (Grossblat, 2002). Surveys have illustrated that the CVC strain of *X. fastidiosa* can spread from a single infected tree to 90% of the trees in a grove in 12 years (see website of the Xylella fastidiosa Genome Project http://aeg.lbi.ic.unicamp.br/xf/home/mmachado.html). Primary infections in orchards are presumed to result from the planting of diseased nursery stock. Infections were observed to only occur in the spring or summer (Laranjeira et al, 1998). The role of weeds as inoculum sources was dismissed, and neighboring groves and/or newly planted trees were implicated as primary sources of infection. Also, spread within nine orchards in Sao Paulo, Brazil, appeared to be uninfluenced by wind direction or cultural practices. In another survey in commercial sweet orange groves in Sao Paulo and Minas Gerais, Brazil, disease incidence increased from %22.36 to %36.52 over a 3 year period. There were no differences among the cultivars Pera Rio, Valenicia and Natal (Ayres et al., 2001). It is now estimated that %38 of all citrus trees in the state of Sao Paulo, approximately 68 million trees, are affected with *X. fastidiosa* (Milanez et al., 2003).

In the event of a successful establishment, the spread of the CVC pathogen would probably be significant. CVC strains in South America emerged rapidly and spread over thousands of kilometers in the period of a decade (Purcell, 1997). The climatic conditions within the range of citrus production in the U.S. that are conducive to the establishment of CVC would also facilitate the spread of the pathogen. Host composition and orchard structures will encourage secondary spread of the pathogen, making it highly likely that once established, HLB spread sufficiently to become a permanent feature of citrus production. These conclusions are based on the presumption that no control measures would be implemented to reduce the potential spread.

E. Economic Damage Potential = HIGH RISK

U.S. Citrus production – the target crop

U.S. annual per capita citrus consumption during the 1990s exceeded 25 lbs., second only to bananas among fresh fruits (http://www.fred.ifas.ufl.edu/citrus/pubs/misc/wp2000-1.pdf). In 2000, the average American consumed 5.8 gallons of orange juice, equaling approximately 79.5 pounds of fruit. U.S. orange production is the second largest in the world. The majority of citrus production is in California, Florida, Texas and Arizona (see http://www.ultimatecitrus.com/ and Figure 9). Farm gate receipts for U.S. citrus production during the 1990s averaged $2.3 billion annually.

The European and Mediterranean Plant Protection Organization (EPPO) considers the South American strain of *X. fastidiosa* to be a major risk for the citrus growing regions of the world, with the potential for greater damage than the PD strain on grapes (see EPPO website at http://www.eppo.org/QUARANTINE/lists.htm). Given the extremely wide host ranges for the pathogen, the potential for confusion in the definitive identification of strains, and the difficulties in achieving consistent control of the pervasive sharpshooter vectors, the potential for economic damage from a CVC epidemic is relatively high.

F. Environmental Damage Potential = LOW RISK

There are a few environmental considerations that must be considered in the event of a CVC epidemic. First, one important management option consists of the use of insecticides.

However, insecticides are already used in citrus production and they would not be considered a major problem in some of the citrus growing regions.

Another environmental consideration would relate to the need to change crop and production patterns that might result from a serious CVC epidemic. Such decisions could temporarily disrupt ecosystem functions in and around regions already adjusted to the demands of large scale citrus production.

G. Social and Political Considerations = LOW RISK

Given the high potential for a natural of accidental introduction of the CVC strain of *X. fastidiosa*, it would be difficult for an individual or group to make a credible case for successful introduction of this disease. If such a case was successfully made, it is possible that policymakers would respond with additional regulatory statutes and increased penalties in an attempt to prevent further acts.

H. Risk Summary = MODERATE

A summary of the individual processes in this analysis lead to the conclusion that there is only a low to moderate risk of an intentional introduction of the CVC pathogen into the U.S.

These individual risk ratings are listed in the table to the right.

Table 1. Summary of the risk of selected features involved in the process of intentionally introducing the causal agent of citrus variegated chlorosis.

Risk Factor		Risk Rating
Likelihood of Acquisition		Moderate
Entry Potential		Moderate
Establishment Potential		Moderate
Spread Potential		High
Economic Damage Potential		High
Environmental Damage Potential		Low
Social and Political Considerations		Low

VI. Control/Mitigation strategies after establishment

Prevention forms the basis for management of CVC in Brazil. The use of disease-free budwood in propagation of nursery stock is considered to be paramount to preventing further dispersal of the pathogen. This entails, among other things, using budwood for propagation with as little wood as possible attached (de Lima and de Lima, 1997, Rodas et al., 2000). Nursery management activities, e.g. locating at far distances from existing orchards, weed management, and sharpshooter management are important steps. Insecticide use to control sharpshooter populations in nurseries and orchards is recommended. Roguing of young trees, less than 4 years old, should be practiced in conjunction with regular orchard surveys for CVC symptoms. Affected branches on older trees should also be removed to reduce inoculum loads in the orchards (Rodas et al., 2000). In areas where CVC has become epidemic, the planting of susceptible cultivars should be avoided (Beretta and Leite, 2000).

VIII. Knowledge gaps

The entire genomes of the citrus and grape strains of *X. fastidiosa* were recently sequenced, providing and exceptional opportunity for a better understanding of every aspect of these complex pathogens (Bevan, 2000; Simpson et al., 2000; Van Sluys et al., 2003). For example, a great deal has been revealed about the nutritional strategies, pathogenicity factors, and responses to antibiotics in the pathogen. There are many more aspects of the pathogen and its vectors that are poorly understood. Some of these areas include studying the potential supplemental hosts for the CVC strain and vector relationships between the sharpshooter vectors and those hosts (Purcell, 1995). There are also many aspects of the seasonality of feeding habits and survival that are in need of study. Research needs also extend to a better understanding of the strain relationships in the Xylella populations. Also, the presence of X. fastidiosa in symptomless hosts needs to be studies, particularly with regard of their inoculum potential for spreading the pathogen.

Rapid response to an impending CVC epidemic is critical to the eventual containment and control of the disease, whether the pathogen is introduced intentionally or be natural or accidental means. Given the long latent periods and the questionable ability to recognize

incipient symptoms of CVC, it is important to develop additional tools for first responders to use in monitoring for an epidemic. Steps have been taken to understand the spatial dynamics of CVC (Roberto et al., 2002) using geostatistical analysis. Similar studies on plant pathogens have been valuable in understanding the underlying process involved in the development of an epidemic. Also, through the use of kriging, predictive models can be developed to characterize risk and make predictions on the dispersion of the pathogen. Further research in this area would make a valuable contribution to the effort of developing a rapid response system as a tool for first responders to detect an outbreak of CVC in a new area (see **Section IX** below).

Research is already underway to study many of the aspects of CVC necessary to prevent the introduction of the pathogen into the U.S. citrus industry (see ARS website http://www.ars.usda.gov/research/projects/projects.htm?ACCN_NO=407008&showpars=true&fy=2003).

IX. Immediate response options

Strict quarantine measures should remain in place at all ports of entry regarding the movement of citrus propagating materials, citrus related nursery stock, and any materials that might harbor the movement of sharpshooter vectors (Purcell, 1997). Technical developments in diagnosis, such as the use of PCR to detect the CVC pathogen in plant tissues, should become routine methods used to assay imported plants and plant materials.

A system of support is needed for first responders in each of the major citrus growing regions in the U.S. to coordinate and compile data concerning the outbreaks of potentially damaging diseases. This system would be similar to the one developed for Newcastle disease of poultry in Missouri (Lanclos, 2003). A geographic information system (GIS) is used to monitor, model, and facilitate the formulation of a response to the introduction of a potential foreign animal disease. Remote sensing, such as satellite imagery or aerial photography, can be used to incorporate base maps of the region-wide citrus crop in each of the states where citrus are grown. Data collected during regular surveys and as a result of responses to potential disease and insect outbreaks would be compiled in the GIS so that a permanent record could be kept of the routine problems that develop in the citrus crop. The spatial distribution and spread of these problems could then be mapped and simple models

derived to characterize existing problems and compare them to the dispersion and damage resulting from the introduction of a new, invasive pathogen. Such a system would be enhanced by incorporating the models of the spatial dynamics of CVC as described in Section **VIII** above.

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Karnal Bunt of Wheat

Pathway Analysis:

Intentional Introduction of

Tilletia indica

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Karnal Bunt Pathway Analysis

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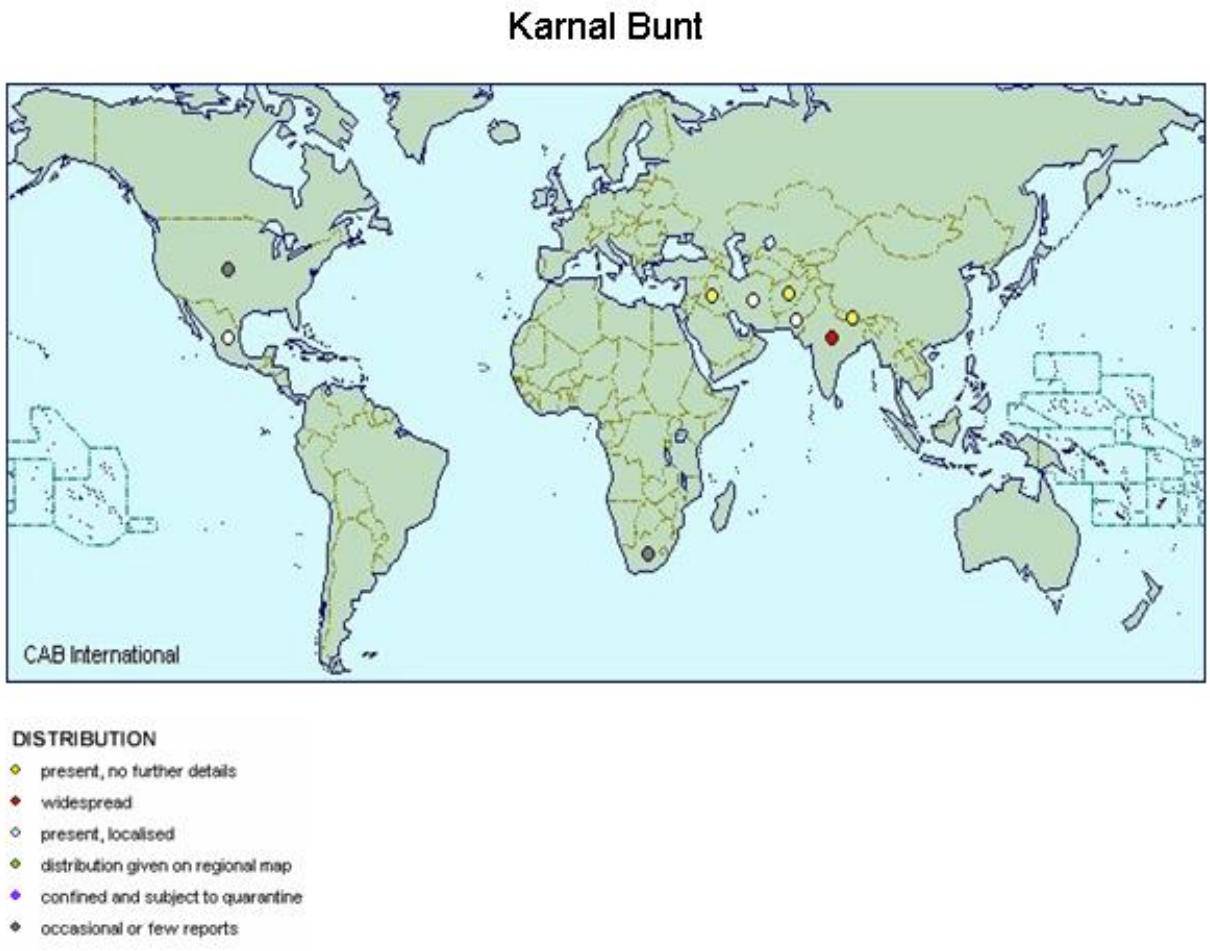
Executive summary: Karnal Bunt Pathway Analysis

Karnal bunt of wheat is caused by the fungus *Tilletia indica* Mitra. The fungus was first described in India in 1931 by M. Mitra, after being discovered at the Botanical Research Station at Karnal, Haryana, India in 1930. It is possible the disease was seen earlier, but not recognized. The disease has spread and is now found in India, Pakistan, Afghanistan, Iraq, Nepal, Mexico, and a few locations in the U.S. The disease is subject to quarantine regulation in several countries, including the U.S. Outbreaks in the U.S. have caused a few wheat producing areas in the U.S. to be subject to internal quarantine or regulation. Since some major importers of U.S. wheat have a zero-tolerance policy toward Karnal bunt, the establishment of Karnal bunt in major wheat production areas of the U.S. would have a serious economic impact on wheat exports. Karnal bunt could be introduced by spreading infected wheat kernels or teliospores in wheat production fields or by introducing inoculum into transport systems so that all sources feeding into the transport might be viewed as potentially infected.

Other names for the disease: Partial bunt

Fig. 1. Map of the distribution of Karnal bunt. (CABI)



Karnal Bunt of Wheat

Pathway Analysis for the Intentional Introduction of *Tilletia indica*

I. Biology and life/disease cycle of the pathogen

1. Identity

Taxonomic Position:

Kingdom: Fungi

Phylum: Basidiomycota

Class: Basidiomycetes

Order: Ustilaginales

Species: *Tilletia indica* Mitra (Mitra, 1931)

(synonym *Neovossia indica* (Mitra) Mundkur)

Synonyms

Most current workers use the name *Tilletia indica*. Fuentes-Dávila and Durán (1986) summarize reasons against *Neovossia* as the appropriate genus. Warham (1992) summarizes arguments for and against the use of the synonym *Neovossia indica*. Fuentes-Dávila (1996) contains references to authors who consider *Tilletia* the appropriate genus. Palm (1998)

discusses the complexities of the taxonomy of *Tilletia*. *T. horrida* was considered a synonym of the species complex *T. barclayana*, but more recent studies support the separation of *T. horrida* and *T. barclayana* (Palm 1998). In this report, *T. barclayana* and *T. horrida* are treated as synonyms for the sake of convenience, since the taxonomic situation has not been clarified. See Table 1 for synonyms of names of organisms discussed in this paper.

Table 1. Synonyms for names of organisms discussed in this paper.

Scientific names used in this document	Synonym(s)
Tilletia indica Mitra	*Neovossia indica* (Mitra) Mundkur {Mundkur 1940 #2860}
Tilletia walkeri Castlebury & Carris	*Tilletia barclayana* (Bref.) Sacc. & Syd. in Sacc. *Tilletia horrida* Tak.
Tilletia barclayana (Bref.) Sacc. & Syd. in Sacc.	*Tilletia horrida* Tak. *Neovossia horrida* (Tak.) Padwick and A. Khan *Neovossia barclayana* Brefeld *Tilletia ajrekari* Mund. *Tilletia pennisetina* H. Syd. *Tilletia pulcherrima* Ell. & Galloway *Tilletia pulcherrima var. brachiariae* Pavgi & Thirum.
Tilletia tritici (Bjerk.) Wint.	*Tilletia caries* (DeCandolle) Tulasne *Lycoperdon tritici* Bjerk. *Uredo caries* DC.
Ustilago tritici (Pers.) Rostr.	*Ustilago nuda* (Jens.) Rostr.

Diseases caused by similar organisms

Fungi in the order Ustilaginales are known as smuts or bunts. These fungi attack the kernels of cereals, replacing all or part of the kernel with a mass of spores, giving it a sooty or smutty appearance. Other bunts of wheat are dwarf bunt, caused by *Tilletia controversa*, and common bunt, caused by *Tilletia tritici* or *Tilletia laevis*. The life cycle of *T. indica* is similar to that of *T. barclayana*, which causes kernel smut of rice (Warham, 1992). Palm (1998) contains a bibliography of taxonomic and geographic references for smut and bunt fungi. See Table 2 for diseases caused by fungi in the Ustilaginales.

Table 2. Bunt and smut diseases caused by fungi in the Ustilaginales.

Disease	Pathogen
Karnal bunt of wheat	*Tilletia indica* Mitra
Common bunt of wheat Stinking smut of wheat	*Tilletia tritici* (Bjerk.) Wint. *Tilletia laevis* Kuhn
Dwarf bunt of wheat	*Tilletia controversa* Kühn
Loose smut of barley and wheat	*Ustilago tritici (Pers.) Rostr.*
Kernel smut of rice	*Tilletia barclayana* (Bref.) Sacc. & Syd. in Sacc.
Annual ryegrass bunt	*Tilletia walkeri* Castlebury & Carris

2. Hosts of *Tilletia indica* and related organisms

Commercially important hosts of *Tilletia indica* are bread wheat (*Triticum aestivum*), durum wheat (*Triticum turgidum*), and Triticale (rye X wheat, X *Tritosecale*). A bunt of ryegrass in Oregon, at first ascribed to *T. horrida* (= *T. barclayana*) (Fuentes-Dávila 1996), is now known as *T. walkeri* (Castlebury and Carris, 1999; Murray and Brennan, 1998). The teliospores of *T. indica* and *T. walkeri* are nearly identical in appearance. *Tilletia walkeri* is also found at low levels on annual ryegrass (*Lolium multiflorum*) in the southeastern U. S. (Cunfer and Castlebury, 1999). This could lead to wheat from the U.S. being mistakenly identified as contaminated by spores of *T. indica*, since ryegrass is common and may grow in or near wheat fields. According to Cunfer and Castlebury (1999), there have already been cases of teliospores of *T. walkeri* being initially misidentified in wheat samples as *T. indica*. See table 3 for hosts of *T. indica*.

Table 3. Hosts of *Tilletia indica.*

Host name	Common name(s), if any	Reference
Triticum aestivum L..	Bread wheat	Bonde et al., 1997
Triticum turgidum L.	Durum wheat	Bonde et al., 1997
X *Tritosecale* Wittm.	Triticale	Bonde et al., 1997
Triticum shareoensis *Triticum variabilis* *Triticum ovatum* *Triticum scerrit.*	Wild wheats	Aujla et al. 1985
Triticum monococcum var. *boeoticum* *Triticum timopheevi* var. *araraticum*		Royer and Rytter 1988

Oryzopsis miliacea	Smilo, smilo grass	Royer and Rytter 1988
Aegilops bicornis *Aegilops caudata* *Aegilops columnaris* *Aegilops comosa* *Aegilops cylindrica* *Aegilops searsii* *Aegilops sharonensis* *Aegilops squarrosa* *Aegilops triaristata* *Aegilops triuncialis*	Goatgrasses	Royer and Rytter 1988
Lolium perenne *Lolium multiflorum* *Lolium canariense*	Perennial ryegrass Annual ryegrass A ryegrass from the Canary Islands	Royer and Rytter 1988
Bromus tectorum *Bromus ciliatus*	Cheatgrass Fringed brome	Royer and Rytter 1988

Royer and Rytter (1988) compared the host range of *T. indica* and *T. barclayana*. They inoculated by injecting the boot stage of wheat with a water suspension of sporidia, or by spraying the flower parts with such a suspension. Inoculations of *T. indica* produced infection in species in the genera *Aegilops*, *Bromus*, and *Lolium*, and in *Oryzopsis miliacea*. They found that *T. barclayana* did not infect either annual or perennial ryegrass. They report *T. indica* infected annual and perennial ryegrass in artificial inoculations. Inoculations of *T. barclayana* produced infections in rice (*Oryza sativa)*. Aujla et al. (1985) reported *T. indica* infection in wild species of wheat, but did not state whether these were induced by artificial inoculation. See Table 3 for hosts of *T. indica* and related fungi.

Hosts that can be infected by artificial inoculations may not be susceptible to natural infection. The use of such hosts is a valuable research tool, but may not indicate a role for the host in the spread of the disease in the field. Spray inoculation more closely approximates field conditions than does boot inoculation. Boot inoculation with a water suspension tests only for physiological resistance, not morphological resistance (Warham, 1992).

3. Geographic Distribution and History

A. History

Karnal bunt was first discovered in India in 1930 (Mitra, 1931), although it may have been observed in 1909 in Pakistan (Warham, 1992). Karnal bunt is now widespread in Northwestern

India and in areas of Pakistan, Afghanistan, Iraq and Nepal (Bonde et al., 1997; Locke and Watson, 1955; Munjal, 1975; Singh and Agarwal, 1989; Warham, 1992). The affected areas of India are in the states of Punjab, Haryana, Jammu, Kashmir, Himachal Pradesh, Uttar Pradesh, Delhi, Rajasthan, and Bihar (Bonde et al., 1997; Singh, 1998).

In 1949, teliospores of *T. indica* were detected in wheat entering the U.S. from India (Warham, 1992). Infected wheat entering the U.S. from Afghanistan was intercepted in 1955 (Locke and Watson, 1955). In India, Karnal bunt has been found in interceptions of wheat from Lebanon and Mexico (Nath et al., 1981). In 1980 and 1983, India claimed to have intercepted infected wheat from Lebanon, Mexico, Sweden, Syria and Turkey, although the disease was not found in Sweden, Syria or Turkey. In 1980, the USSR instituted measures to prevent the entry of the disease into its territories (Warham, 1992).

The first report of Karnal bunt in the New World was in Mexico on material collected on February 24, 1971, in Cajeme, Sonora, Mexico (Durán, 1972). Karnal bunt was found sporadically in Sonora and Sinaloa in Northwest Mexico. In the early 1970's and up to 1982, Karnal bunt was found only in trace amounts in grain, but in 1983 it was found in research plots of The International Maize and Wheat Improvement Center (CIMMYT) plots in the Yaqui and Mayo valleys in Sonora (Warham, 1992). In 1984, the Mexican government imposed an internal quarantine in an effort to halt the spread of the disease (Bonde et al., 1997). At that time, CIMMYT also took steps to avoid disease in fields grown for seed. Babadoost (2000) concluded it is probable that teliospores of *T. indica* were carried on seed from CIMMYT to several wheat growing countries during the period from 1972 to1984. Babadoost (2000) argues that the fact that Karnal bunt is not more widespread indicates that it is not widely adapted and therefore is not a major disease problem.

B. Karnal bunt in the U.S.

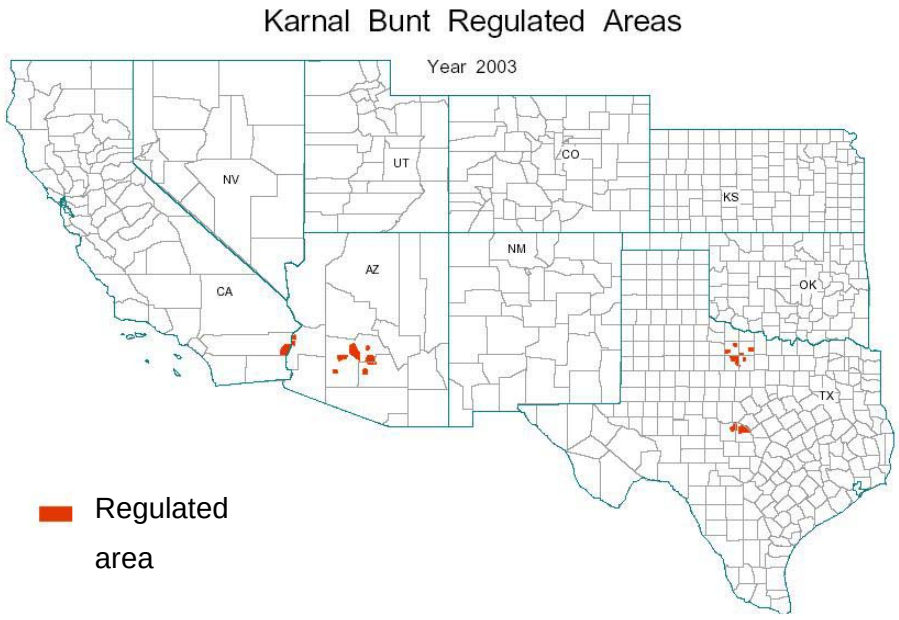
The Wheat Disease Subpart of the Foreign Quarantine Notice 7 CFR 319.59, established in 1981, prohibited the importation of wheat from any country in which Karnal bunt was found. At that time, the countries included were India, Pakistan, Afghanistan, and Iraq. In 1982, infested kernels in wheat grain from Mexico were intercepted, and USDA APHIS responded by prohibiting the importation of wheat, durum and triticale grain, straw or seed from Mexico. In 1983 Mexico was added to the list of quarantined countries (Babadoost, 2000).

On March 8, 1996, USDA release No. 0115.96 reported the discovery of Karnal bunt in durum wheat seed grown in Arizona. The fungus was detected during Arizona Department of Agriculture routine testing. Shortly after this, *T. indica* was detected in seed lots grown in Arizona that had been shipped to California, New Mexico and Texas. On March 25, 1996, an Extraordinary Emergency was declared by the Secretary of Agriculture. The entire State of Arizona, the counties Doña Ana, Hidalgo, Luna and Sierra in New Mexico, and the counties El Paso and Hudspeth in Texas were placed under quarantine. Riverside and Imperial counties in California were added to the list shortly thereafter. The quarantine restricted the interstate movement of regulated articles, including wheat, durum and triticale plants or plants parts. The quarantine regulated the movement of soil, manure, seed conditioning equipment, cultivating equipment, equipment used to store milling products and byproducts (except flour), and conveyances (railcars and the like) (Podleckis and Firko, 1998).

In October 1996, these regulated areas were divided up into restricted or surveillance areas, depending on the results of the pre-harvest survey. Each restricted area included at least one field that tested positive for Karnal bunt, while the surveillance areas did not have such a field. There were a number of specific regulations pertaining to seed, equipment, planting, and millfeed (Poe, 1998; Podleckis and Firko, 1998).

Karnal bunt appeared in 1997, in Texas in San Saba and Knox counties. It appeared in the Texas counties of Archer, Baylor, Throckmorton, and Young in 2001. In 2003, APHIS regulated areas in Imperial and Riverside counties in California, areas in La Paz, Maricopa, Pinal, and Yuma counties in Arizona, and areas in Archer, Baylor, Knox, San Saba, Throckmorton, and Young counties in Texas (APHIS, February 2003). See Figure 2.

Fig. 2. Map of regulate areas in the U.S. in 2003. (APHIS)



4. Disease Impact

Karnal bunt causes part of the wheat kernel to be replaced by teliospores of the fungus. Typically only a portion of the kernel is affected. In severe infections, the entire kernel may be affected. Infection reduces the weight of the grain, and the higher the rate of infection, the lighter the weigh so that the 1000 kernel weight is reduced. Singh (1988) produced a formula for estimating loss in grain yield calculated as actual yield X percent infected grains X 0.256/100. Infection may result in a reduction in the germination rate of the seed, and severe infection may result in the loss of germinability (Singh, 1988). Seedlings may be less vigorous or abnormal, although there is disagreement on this point (Singh, 1998).

Typically the impact on yield is not great (Fuentes-Dávila, 1998). Munjal (1975) reported a 0.2% loss in grain yield in India for 1969-1970. The most important effect of the disease is on grain quality. The fungus produces trimethylamine, which causes a rotten-fish odor. This offensive odor, along with discoloration, causes a reduction in the food quality of flour milled from the grain. (Bonde et al., 1997). One study found that only 1% bunted grain made the flour

unsuitable for making chapatis, an Indian bread product, although other studies have found 3% to be the threshold for a significant reduction in food quality (Warham, 1992). An infection rate of 5% results in grain that is not suitable for human consumption (Warham, 1992). It may be possible to reduce the detrimental effect on food quality by washing or soaking the grain in hot water, but these treatments also affect baking quality.

Because of the undesirable effects on food quality, the price of affected grain is steeply discounted in India (Bonde et al., 1997; Warham, 1992). Other economic costs may be incurred from losses due to non-certification of seed, costs of chemicals, costs of quarantine and inspection, and inability to grow susceptible varieties.

Reports of infection rates vary. Very high infection rates based on the number of grain samples containing at least one or some teliospores are reported. Most farmers in the Yaqui valley of Mexico have typically less than 3% disease in the field, but the percentage of samples containing teliospores may be much higher (Warham, 1992). Munjal (1975) found up to 10% of samples of grain in markets in India testing positive for the presence of infected kernels, but there is no reliable way to translate this figure into a disease rate in the field.

Toxins

Tilletia indica has not been reported to produce toxins. Secondary colonization of affected grain by species of *Aspergillus* is possible, and some *Aspergillus* species produce toxins (Fuentes-Dávila, 1998; Warham, 1992).

Uses of infected grain

Grain infected with Karnal bunt has been fed to rats, chickens, and monkeys without ill effect, although there was some effect on the rumen of goats fed infected grain (Warham, 1992). Liver and renal insufficiency in albino rats fed infected grain has been reported (Singh, 1998).

Since the teliospores may survive passage through the gut of livestock (Singh, 1998), infected grain must be treated before being used as animal feed in the U.S. Bonde et al. (1997) reported that steam-flake milling kills the teliospores of the fungus and produces usable animal feed. Grain is loaded by a closed conveyor into steam cabinet towers, heated to 109^{o} C for 30 minutes, then rolled into flakes. Equipment is readily available, since this method is already used to produce animal feed.

Flour milled from infected grain is believed to pose no risk of spreading Karnal bunt, since it is believed that the teliospores do not survive the milling process (Bonde et al., 1997). Untreated mill feed (by-products of the flour milling process) may pose a risk of spreading the disease if used as animal feed or if spread in fields in some fashion. Teliospores were killed by heat-treating mill feed using a Holo-Flite Thermal Processor or similar dry heat processor for 12 hours at 84^{o} C, for 5 hours at 101^{o} C, or for 2 hours at 110^{o} C (Bonde et al., 1997).

5. Symptoms

Symptoms of Karnal bunt are not easily seen in the field (Bonde et al., 1997). The infection starts at the embryo tip of the grain and usually only part of the kernel is visibly affected. Only a few kernels in each spike are infected. Sometimes the head opens up so that the infected grain can be seen, but this is rare. The grain needs to be removed from the head and examined. For this reason most discoveries of Karnal bunt are made from samples of threshed grain. Sometimes the infected plants have fewer spikes and the spikes are shorter, but not all observers report this symptom (APHIS, 2003; Warham, 1992).

Symptoms are first seen in the soft dough stage as blackened areas at the base of the grain. The blackened area may extend upwards as the fungal sorus (= spore mass) develops. Kernels may be partially or completely converted to sori. In extensive infection, the embryo is killed and the sorus fills the kernel (Nagarajan et al., 1997; Nyvall, 1999). Partially infected kernels become fragile, breaking or eroding at the basal end. If the embryo survives, germination and seedling strength are impaired (Nyvall, 1999).

6. Disease Cycle and Epidemiology

A. Life Cycle

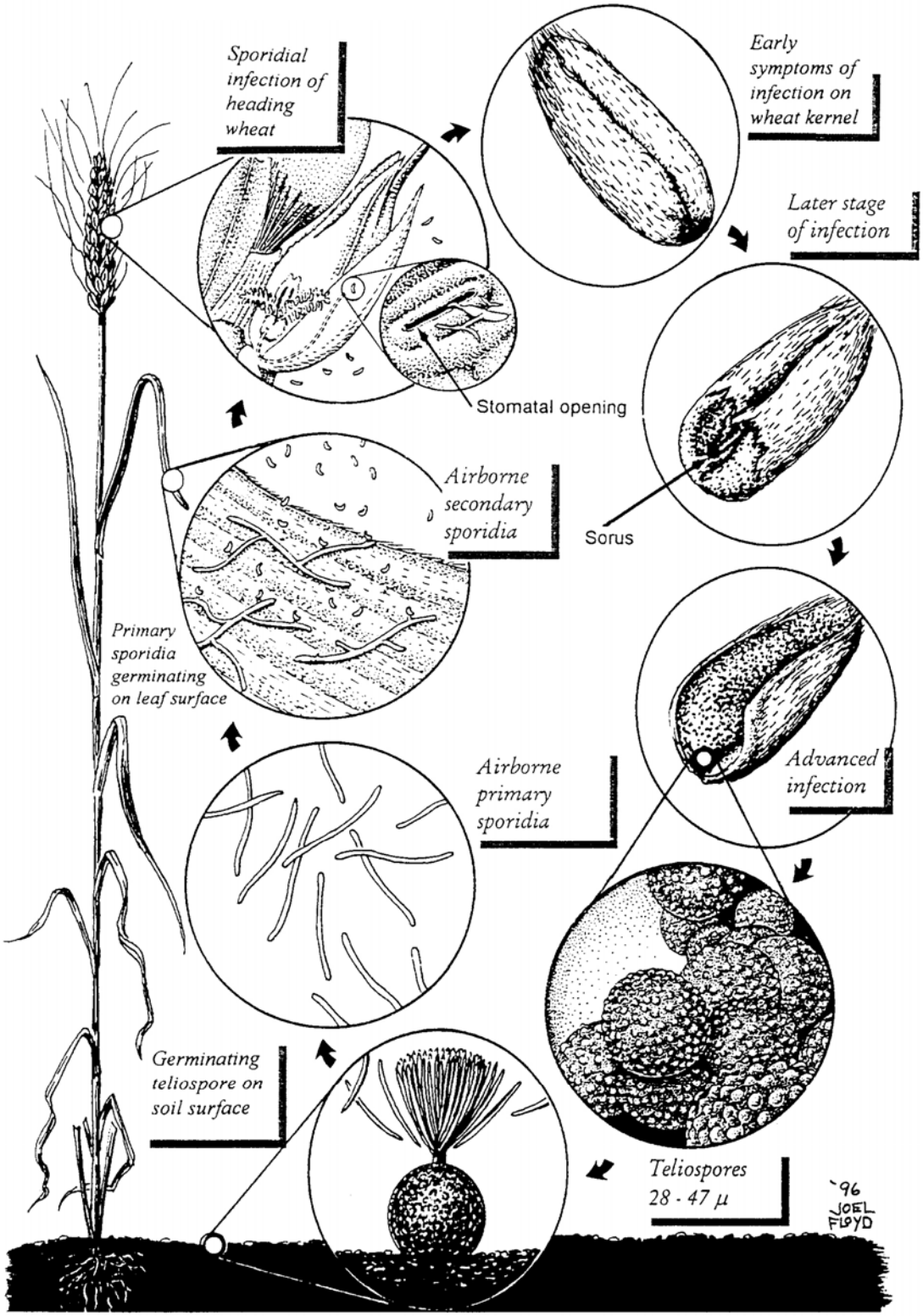
Diploid (2n) teliospores in the soil germinate to form a promycelium (=basidium). Meiosis occurs during germination, followed by several mitotic divisions. At the tip of the promycelium 32-128 or more haploid (n) primary sporidia (=basidiospores) are formed (Mitra, 1931; Holton, 1939). These sporidia are filiform (long and thin) in shape. Primary sporidia are splashed or blown up onto the leaves of the wheat plant, where they germinate, producing mycelia. The mycelia produce secondary sporidia, which most often are allantoid (slightly curved with

rounded ends, sausage-like in form) or falcate (curved), but rarely may be filiform. Goates (1988) found that all hyphae on glumes originated from secondary sporidia. The allantoid secondary sporidia, but not the rarer filiform secondary sporidia, germinate and penetrate parts of the floret, such as the glumes, lemma or palea, through the stomates. Hyphae may penetrate stomates in the rachis (Dhaliwal et al., 1983). Infection occurs only during a two to three week period during flowering. Mycelia then grow down to the base of glumes and up into developing kernel (Goates, 1988). Most observers have not seen the direct penetration of the ovary that was reported in early studies of the infection process (Munjal and Chatrath, 1976, cited in Singh, 1988). Hyphae grow intercellularly in the parenchyma and chlorenchyma. Goates (1988) found hyphae in all floral parts except stamens and anthers seven days after inoculation. The hyphae enter the pericarp of the kernel through the funiculus. The fungus is restricted to the pericarp, which swells as the fungus grows, squeezing the endosperm and embryo and causing the endosperm to shrink (Singh, 1988). Severe infection may kill the embryo (Singh, 1988). The infection is local, not systemic as in *T. tritici*. Diploid teliospores are produced in the infected kernel. Goates (1988) found that teliosporogenesis takes place at least 13 days from the initial infection at 20^{o} C. The teliospores fall to the ground and remain in the soil until the following year.

Mitra (1931) reported one promycelium in the germination of the teliospore. The promycelium may be branched. Occasionally two or three promycelia germinate from a single teliospore. (Krishna and Singh, 1981; Warham, 1988). The promycelium can be up to 1500 µm in length (Mitra, 1931; Holton, 1939). After the several mitoses that follow meiosis, haploid nuclei migrate into the sporidia. The sporidia then become septate, with two to four monokaryotic cells (Fuentes-Dávila and Durán, 1986).

It is not known when karyogamy takes place. *T. indica* is heterothallic, so mycelia of different mating types must meet in order for sexual reproduction to take place and for teliospores to be produced. There are at least 4 alleles at one locus (bipolar) in *T. indica* (Durán and Cromarty, 1977). Heterothallism results in a certain amount of genetic re-assortment in each generation. In contrast, in *T. caries* and *T. foetida*, fusion (plasmogamy) of basidiospores occurs, usually

Fig. 3. Life Cycle of *T. indica.* (USDA)



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between spores from the same basidium. This close inbreeding means intraspecific variation in *T. caries* and *T. foetida* is low (Bonde et al., 1997). In *Tilletia* species where fusion takes place between basidiospores, a characteristic H-shaped structure may be formed. Mitra (1931) did not observe H-bodies, but Holton (1949) (cited in Fuentes-Dávila, 1996) reported seeing H-bodies.

Fig. 4. Teliospores of *T. indica.* Photo coutesy of B.J. Goates.

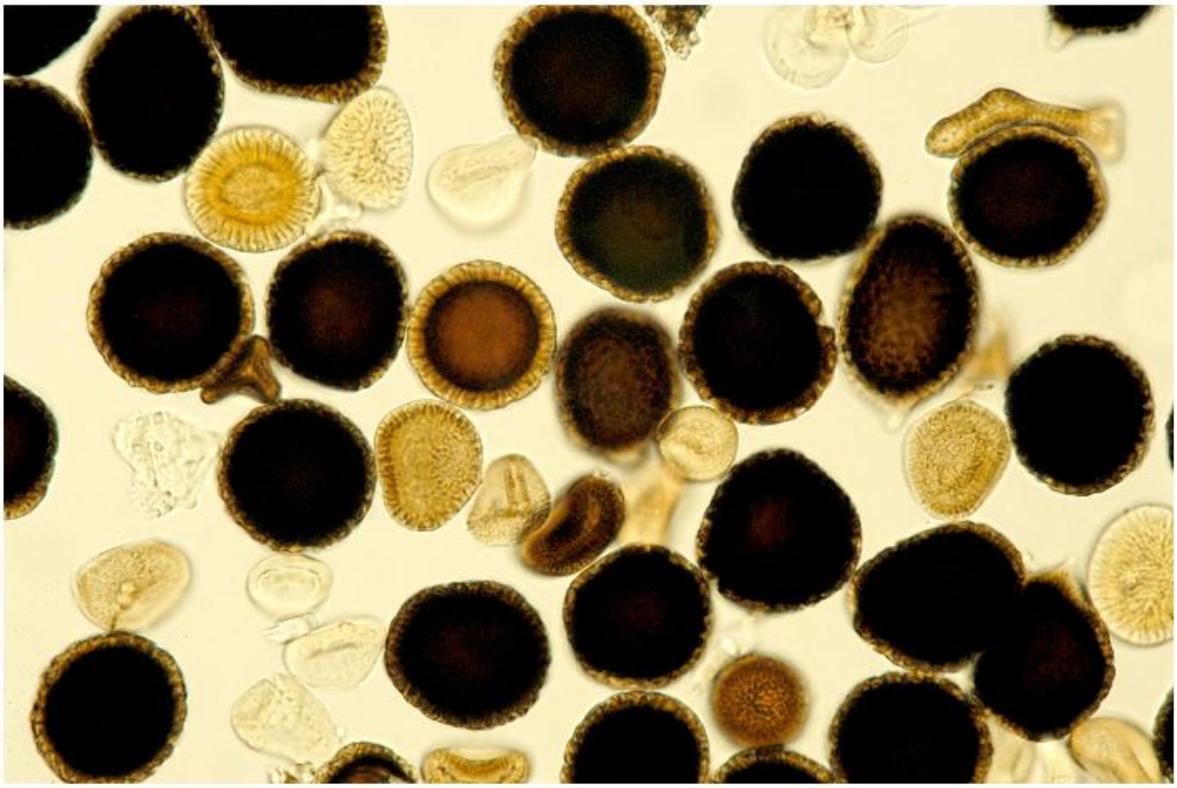

Fig. 5. Photo of bunted and healthy kernels. Photo courtesy of Tom Sim.

B. Initial inoculum and infection

Initial inoculum consists of teliospores, which germinate to form primary sporidia, which germinate and produce secondary sporidia. Infection is caused only by secondary sporidia.

Goates (1988) found that all hyphae on glumes originated from secondary sporidia. Dhaliwal et al. (1983) studied the distribution of infection within the spikelet and concluded that the pattern of infection was not random, as would be expected from the fall of air-borne spores, and that secondary infections within the spikelet probably occur. Dhaliwal et al. (1989), using detached florets and floret parts, did report rare direct penetration of the ovary. They found frequent penetration of rachis stomata, and speculated that resistance of durum wheat may be the result of the short rachis internodes being less accessible to airborne spores

C. Growth stage vulnerability

There is a limited time period during flowering during which spikelets can be infected. Environmental conditions must be favorable to disease during this short period of time. Because teliospores are so persistent, favorable weather conditions during flowering are not needed every year for the disease to persist.

D. Conditions that favor disease

Conditions that favor disease development include moderate temperatures at flowering. Air temperature below 23^{o} C, but above 10^{o} C, soil temperature between 17^{o} and 21^{o}C, and relative humidity between 54% and 89% are optimal for development of disease (Warham, 1992). Free moisture, cloudiness, high humidity, and periods of rain during anthesis all favor disease development, but high rainfall alone may not lead to disease development (Warham, 1992; Singh, 1998). A low pre-inoculation temperature of 15^{o} C, rain during flowering, and reduced sunshine favor infection, while warmer post-inoculation temperatures of 18-22^{o} C favor the spread of the disease within the spike (Singh, 1998).

Irrigation, the use of susceptible cultivars, and the addition of nitrogen favor disease development. (Note, however, that irrigation of fields prior to wheat planting may have the potential to reduce disease if teliospores germinate without an appropriate environment for sporidial development (I. Sharma, personal communication).) Singh (1998) reported that the

use of combines in harvesting helped to spread disease in India. Since in the U.S. mechanical harvesting is the rule, the spread of spores by harvest machinery must be taken into account.

The number of rainy days in the boot leaf stage is important to the establishment of the disease (Singh 1998). Weather-related forecasting models have been designed in India, based on maximum temperature and evening relative humidity at the flowering and grain filling stages. Low temperature and high humidity favor the development of disease (Jhorar et al., 1993). Models using the Humid Thermal Index have been developed (Jhorar et al., 1992; Mavi et al., 1992). These models may tend to overestimate infection rates (I. Sharma, personal communication).

E. Inoculum persistence and dissemination

Inoculum persistence

Bonde et al. (1997) found that spores buried in a container in soil survived for three years in Maryland, where winter temperatures reach -2 to -3^{o}C for a few weeks periodically. Krishna and Singh (1982) reported that teliospores buried or at soil surface survived in unbroken sori for 27-45 months. Teliospores can survive on the laboratory shelf for five to seven years (Singh, 1998), although Bonde et al. (1997) reported spores that were viable after 16 years of shelf storage at Ft. Detrick, Maryland. Teliospores germinating under more than 2 mm of soil are unable to reach the surface (Bonde et al., 1997).

Zhang et al. (1984) reported that the teliospore germination rate under dry conditions at -18^{o} C was 44.2% after one week, 1% after ten weeks, and none after 12 weeks. They reported that for teliospores embedded in soil at -18^{o} C with 20% soil moisture, the germination rate was 0.9% after 10 weeks. Singh (1998) reported that germination rate was not affected by one to three weeks at -5^{o} C, or by one to three weeks dessication. Inactivation of the spores occurred at 140^{o} C for 10 minutes (Singh, 1998).

Dispersal

The disease does not spread directly from the infected seed to the seedling, since the disease is not systemic. The teliospores from infected seed planted in the field would have to come to rest near the soil surface in order to germinate and start the disease cycle (Mathur and Cunfer, 1993). Infected kernels can shed teliospores all along the route in the grain delivery pipeline,

contaminating uninfected seed, storage areas, and transport vehicles, including trucks, railcars and ships.

Teliospores liberated during harvesting can be blown high into the air and over long distances. Singh (1998) reported “clouds” of spores at harvest in regions of Uttar Pradesh during harvest. The 1996 infections in Arizona were near the border with Mexico and may have been caused naturally by wind-borne teliospores from the adjacent state of Sonora in Mexico. Burning stubble in fields may actually help to disperse spores; teliospores have been detected 3000 m above burning fields (Bonde et al., 1997). Primary and secondary sporidia may also be wind dispersed. Infection over long distances by secondary sporidia may be limited if the sporidia are diluted over space so that the initial infections they cause do not encounter another mating type to complete the reproductive cycle (Garrett and Bowden, 2002).

Teliospores from infected grain can contaminate combines, other harvest equipment, trucks, railcars, and ships. Teliospores can be carried on the clothes, shoes and automobiles of harvest workers. Infested wheat straw clinging to machinery may harbor teliospores. Babadoost (2000) pointed out that teliospores can even be carried on the shoes of visiting scientists who travel to affected areas for research. Soil infested with teliospores has been shown to be a source of infection (Singh, 1998). Soil clinging to tires of machinery, or moved as fill or topsoil, may disperse teliospores.

Infected seed is an important source of inoculum. Seed containing even small numbers of infected kernels can introduce the disease into new areas. Babadoost (2000) states that seed from CIMMYT probably spread teliospores of *T. indica* to many wheat-producing areas of the world during the period 1972-1984. He argues that the fact that the disease is not more widely established shows that it is not much of a threat to production, in his argument against the zero-tolerance rule in the U.S. and other countries. Unregulated grain transfers between neighboring areas, even across borders in more remote areas, may be important in spreading the disease in some parts of the world (Babadoost, 2000).

Teliospores can survive the passage through the gut of chickens, cows, rats and grasshoppers (Singh, 1998). Wild birds feeding on infected grain may disperse teliospores over long distances (Bonde et al., 1997). Manure from cattle fed on infected grain, or foraging in infested stubble, may spread teliospores (Bonde et al., 1997).

7. Causal organism

A. Three types of spores

Teliospores:

Diploid teliospores are thick walled, dark, globose to subglobose with an average diameter of 35µm, range 22-49 µm (Bonde et al., 1997), but some may be 54-55µm (Castlebury, 1997; Fuentes-Dávila, 1996). Teliospores may have an apiculus (Mathur and Cunfer, 1993). The teliospore spore coat has spines and reticulations, the spines being covered with a thin hyaline membrane. Teliospores are usually intermixed with smaller sterile cells. The sterile cells are smooth, thick-walled, round to elongate, and yellow to yellow-brown in color (Mathur and Cunfer, 1993). The wall of the teliospore has three layers (Fuentes-Dávila, 1996; Fuentes-Dávila and Durán, 1986). Teliospore color may be black (Mathur and Cunfer, 1993) or range from pale orange to an opaque dark reddish brown (Castlebury, 1997).

Teliospores are initially dormant, and remain so for a period of months. Less than 10% of new teliospores may germinate (Singh, 1998). At four months, 40-60% may germinate (Smilanick et al., 1985). Bedi et al. (1990) reported that teliospores aged 4-14 months exhibited the maximum germination, although several observers report one year is best for maximum germination (references in Singh, 1998).

Germination rates are highest at between 15-25^{o} C (Bonde et al., 1987), although germination can occur between 5-30^{o} (Singh, 1998). Germination requires a relative humidity of at least 82%, but the presence of free water is more favorable to germination (Bonde et al., 1987). The optimal pH for germination is 6-9.5, with a range of pH 4-11 allowing germination (Smilanick et al., 1985). Germination is inhibited by neem oil (Singh, 1998). Plant root extracts may either enhance or inhibit germination (Smilanick et al., 1985).

Singh (1998) summarizes a number of conditions that enhance teliospore germination:

- Presoaking with water.
- Presoaking with benzaldehyde or butyric acid.
- Presoaking with eucalyptus extract or citrus juice.

- Immersion in liquid nitrogen for 15 min.
- Exposure to direct hot sun for14 days (40-43^{o} C).
- Dry heat of 100^{o} C for 10 min.
- Soil surface temperature of 10-25^{o} C.
- Addition of urea.
- Soil surface temperatures of 10-25^{o} C (although there are conflicting reports).

Primary sporidia:

Formation of primary sporidia is initiated by the promycelium 6-44 hours after promycelium extension (Warham, 1988). The average length among isolates ranges from 64.4 to 79 µm, with an average width of 1.6-1.8 µm (Peterson et al., 1984). Good germination of primary sporidia occurs between 10-25^{o} C in free water, with the optimum at 20^{o} C. Relative humidity of less than 82% prevents germination, as do temperatures below 5^{o} C (Singh, 1998).

Secondary sporidia:

Secondary sporidia are usually allantoid (Bains and Dhaliwal, 1989) or falcate (Singh, 1998). The average length among isolates is from 11.9 to 13.0 µm (Singh, 1998) and the average width among isolates is from 2.0 to 2.03 µm (Bonde et al., 1997). The secondary sporida are forcibly discharged (Fuentes-Dávila, 1996), aiding in dispersal. Good germination of secondary sporidia occurs between 20-25^{o} C in free water, and at 90% or greater. No germination was seen at RH less than 70% at 10-30^{o} C, at RH less than 96% at T 35^{o} C, or at 5^{o} C (Singh, 1998).

B. Culture

Agitation in an aqueous solution with a surfactant will release teliospores from bunted kernels. Alternately, teliospores may be obtained by punching a small hole in an intact sorus and sprinkling teliospores onto 1.5% water agar. Teliospores may be surface- sterilized with a 0.5% solution of sodium hypochlorite (Fuentes-Dávila, 1996). Water agar with a pH 4-6 is the best medium for conducting teliospore germination tests (Warham, 1992).

Warham (1992) summarized the culture from teliospores of secondary sporidia for inoculation. Secondary sporidia may be produced on potato dextrose agar (PDA), pH 4-6, at 20°C with 12 hour light/dark cycles. Cultures aged 3-6 weeks produce good germinable sporidia, while older cultures produce fewer and less germinable sporidia. Secondary sporidia can also be produced in liquid culture potato extract or potato dextrose broth. Potato dextrose broth may be supplemented with sucrose, soil extract or yeast. Sporidia produced in liquid culture are usually filiform in morphology, and are less effective in inoculations than those produced on PDA.

Durán and Cromarty (1977) produced a culture of secondary sporidia for inoculation by germinating primary sporidia on water agar, then transferring the fungus to PDA slants after three days. Mycelia and secondary sporidia from the slants were then cultured in potato extract solution for 7 days with continuous shaking at 24°C (light levels were not discussed).

Cultures of *T. indica* are usually white, although they can vary from dark to light in color, powdery, brittle or leathery, crustaceous, and umbonate with dendritic margins (Warham, 1992; Mathur and Cunfer, 1993).

C. Pathogen variability

Tilletia indica is heterothallic. Only certain paired lines are pathogenic. Durán and Cromarty (1977) tested pairs of monosporidial lines for pathogenicity by inoculating wheat. They concluded incompatibility in *T. indica* is controlled by multiple alleles. They also concluded that *T. indica* is bipolar, with four alleles at one locus.

Bonde et al. (1996) tested two isolates from Mexico and one isolate each from India and Pakistan against eight wheat cultivars boot-inoculated in the greenhouse. There were significant differences in aggressiveness among the isolates, and significant differences in susceptibility among wheat cultivars. They found that resistance genes to Karnal bunt identified in CIMMYT's breeding program were effective against isolates from India and Pakistan, and that resistance in varieties from India was effective against the Mexican isolates.

Aujla et al. (1987) identified four pathotypes in India. Another researcher has identified three pathotypes (Dhiman, 1982, cited in Fuentes-Dávila 1996). Spore morphology is not useful in differentiating pathotypes (Bansal et al 1984, Singh and Singh 1988 cited in Singh 1998). Royer and Ritter (1985) did not find differences in pathogenicity among isolates from Mexico and India in a single experiment, but this experiment was not amenable to statistical analysis.

The existence of races or pathovars of *T. indica* is not clear. More research is needed in this area. It is clear that *T. indica* is heterothallic.

8. Diagnostic Methods

A. Detection and Identification

Current sampling for Karnal bunt in the U.S. requires grain samples from every county that produces one million or more bushels of wheat. Samples are taken in alternate years. Participation is voluntary. Grain is taken from grain elevators. A 4 lb. composite sample is taken from the grain elevator and sent to an approved laboratory for testing. In 2003, over 1300 grain samples from 33 states were tested. All were negative (APHIS, 2003). In Kansas, 400-500 samples per year are tested.

Karnal bunt is not easily detected in the field, so field scouting probably will not detect it reliably. Only a few kernels in a head are affected, and usually only part of the kernel is replaced with the fungal sorus (Bonde et al., 1997). The bunted kernel may be visible in the head if glumes are spread (Mathur and Cunfer, 1993). Bunted kernels are best detected after the grain is threshed (APHIS, February 2003). Kernels are dark, either in part or entirely, are fragile, and have a fishy odor (APHIS, February 2003; Mathur and Cunfer, 1993). The fishy odor is due to the production of trimethylamine (Bonde et al., 1997; Warham 1992). The fungal sorus that replaces part of the kernel is fragile and when broken will release a powder of black spores.

Symptoms on the kernel are similar to those of black point, so it is necessary to identify teliospores of *T. indica* to diagnose the disease (APHIS, 2003). Black point is caused by a number of common fungi, including species in the genera *Alternaria*, *Stemphylium*, *Nigrospora*, *Penicillium*, *Helminthosporium*, *Fusarium*, and *Curvularia*. The spores of these fungi are easily distinguished from the teliospores of *T. indica.* Kernels affected by black point are discolored but do not have the characteristic odor of kernels affected by Karnal bunt, nor is the discolored area of the black point-affected kernel converted to black spores (Mathur and Cunfer, 1993).

In rice-growing areas of the U.S., *T. barclayana*, causing head smut of rice, may be present. The spores of *T. barclayana* are similar in appearance to those of *T. indica*, although on average smaller in size. Size ranges may overlap, so a definitive test is needed (Poe, 1998).

Other smut pathogens have spores morphologically similar to spores of *T. indica*. Annual ryegrass bunt, caused by *Tilletia walkeri*, is found in Oregon as a weed and may occur in fields where wheat is also grown. It is necessary to have methods to distinguish between *T. indica* and *T. walkeri* to avoid grain from Oregon being incorrectly diagnosed as contaminated with *T. indica*. Teliospores of *T. walkeri* are difficult to distinguish from those of *T. indica*.

Babadoost and Bonde (1998) detail a method for extracting teliospores from soil that can be used to test soils of fields suspected of being contaminated by *T. indica*.

B. Standards Used for Detection and Identification

The USDA now uses a high-speed optical sorter to inspect grain samples for bunted kernels. The sorter can process 8,800 kg/hr. It effectively removes bunted kernels and so can be used both for inspections and for removing Karnal bunt from grain intended for food or feed use Dowell et al., 2002).

The spore detection method previously used by the USDA to test for Karnal bunt has the sensitivity to recover one teliospore per 50 g of wheat, which translates into an infection level of about one infected kernel per 500,000 kernels (Poe, 1998). Because spores from one bunted kernel can contaminate so much grain, it is not possible to determine the incidence of disease in the field from the incidence of teliospores in a sample. Disease incidence in the field must be determined empirically.

Comparing the genetic profiles of suspect material with known fungal profiles using PCR (polymerase chain reaction) is a very accurate method of distinguishing the teliospores of *T. indica* from those of other *Tilletia* species (del Rocío Hernández Hernández, 2001; Smith et al., 1996; Ferreira et al., 1996), though earlier methodology may not have effectively distinguished *T. indica* from *T. walkeri*. DNA fingerprinting using amplified fragment length polymorphisms (AFLP) can also distinguish *T. indica* from grass bunt species (for example, *T. walkeri*) (Laroche, et al. 1997). Smith et al. (1996) used the primer pair TI17M1 and TI17M2 to accurately distinguish *T. indica* from other *Tilletia* species. The USDA can also compare the isozyme patterns of suspect material to various species of *Tilletia* using a fairly accurate method, but this method requires a large amount of diseased material (Poe, 1998).

C. Regulation

Current regulations base the diagnosis of the presence of Karnal bunt on the presence of bunted kernels. Since regulations change frequently, the most up-to-date version of the regulations, available on-line at http://www.aphis.usda.gov/ppq/manuals/pdf_files/KB_new.pdf, should be consulted. This document contains descriptions of the sampling plan for Karnal bunt in the US, a list of regulated articles, quarantine regulations, and a laboratory manual for the handling of samples and the identification of Karnal bunt. Regulated articles include conveyances, milling products or by-products except flour, plants, or plant parts, including grain, seed, or straw of wheat, durum wheat,and triticale, specimens of: *Tilletia indica* (Mitra) Mundkur, soil from areas where field crops are produced, manure from animals that have fed on bunted kernel positive wheat or triticale, mechanized harvesting equipment used in the production of wheat, durum wheat, or triticale, and seed conditioning equipment that has been used in the production of wheat, durum wheat, or triticale.

The following chemicals are authorized in the regulations to treat articles regulated for Karnal bunt:

Methyl bromide (15 lb/1,000 ft3 For 96 hours)
Ultra chlorine bleach (6 percent sodium hypochlorite)
Pentachloronitrobenzene (PCNB) with or without Carboxin-Thiram
Carboxin-Thiram

II. Initiating Event (Recognizing an Attempted Introduction)

1. Observation/diagnosis of presence

The sampling, detection, and response plans of the U.S. are contained in The National Karnal Bunt Wheat Grain Survey Plan 2004, prepared by Pest Detection and Management Programs, USDA, APHIS, PPQ, (4700 River Road, Unit 98, Riverdale, MD 20737). This report is dated March 19, 2004. It contains instructions for grain sampling and testing, and detailed instructions for actions to be taken in the event of a possible positive identification of Karnal bunt, whether on a weekday or on the weekend. The most current version of this document should be

consulted for information about KB sampling and testing for any given year. A putative positive sample can be confirmed very quickly, even on a weekend. The USDA could respond very rapidly to the confirmation of a positive identification. Internal quarantines and other regulations could be put into place very quickly.

2. Interception: Individual/ Pathogen

T. indica is easy to grow in culture but the sporidia are fragile and short lived (Warham, 1992). Teliospores would be the inoculum of choice. A small quantity of teliospores could be carried into the U.S. by the vial-in-pocket method. Such a quantity of teliospores could be used to make a point introduction in the U.S., or teliospores could be germinated to produce *T. indica* in culture. A small number of teliospores introduced into the field may be unlikely to produce a noticeable rate of infection given that there is only one generation of Karnal bunt a year, and that the pathogen requires favorable weather for infection. It is unlikely that a vial-in-pocket terrorist could make an immediate substantial impact on the wheat-growing regions of the U.S. through introduction of the teliospores to a field. Given the multi-year longevity of teliospores and their ability to survive cold weather, point introductions of small amounts of teliospores could, however, have a long term effect.

For larger quantities of teliospores or of bunted kernels, smuggling in a shipping container into a U.S. port might be attempted. Since legitimate grain shipments are tested, the infected kernels would have to be smuggled separately, like any contraband. For moderate quantities, a terrorist might try to bring the grain in as a normal grain shipment via railcar or truck but try to bypass the testing procedures for imports. It might be possible to bring in a quantity of a few hundred pounds of bunted kernels via the same routes that illegal aliens use to enter the U.S. from Mexico, using a number of aliens as mules carrying 20 or 30 lbs apiece for a price.

Introducing the inoculum to one area might not be an effective blow to the wheat-producing regions of the U.S. since quarantines and local regulation can potentially be set up quickly and effectively as they have been in the past. In order to have a substantial impact on the U.S., terrorists would have to target a number of areas in the wheat growing areas of the Great Plains or introduce it to a central shipping facility so that the whole source area is suspect.

A successful attack on the U.S. wheat industry would probably require a large quantity of bunted kernels. Contaminated grain could be acquired in a country where Karnal bunt is

established, such as India, Pakistan, or Afghanistan. Seed cleaning techniques could be used to separate the bunted kernels from the unaffected grain, in order to generate a large quantity of infected kernels. The same methods that are used to smuggle narcotics into the U.S. might be used to smuggle pounds or even hundreds of pounds of infected material. It may be more difficult for terrorists to distribute the inoculum than to smuggle it in.

Spreading a concentrated amount of bunted kernels in one or several areas of the wheat growing regions of the U.S. might be done in several ways. The simplest is to hand carry the inoculum to the field. The difficulty here for the terrorist is the keen eye of the rural resident, who tends to notice strangers as well as odd behavior. Spreading kernels from the air probably would be noticed. Kernels are light and would be blown about, landing in yards and on cars and people in addition to fields. Federal authorities, cognizant of the danger of aerial dissemination of pathogens via crop-dusters and small planes, have instituted precautions in rural airports. Sprinkling kernels from a vehicle would cause most of the kernels to land in the road or in a ditch. One effective way to disseminate the inoculum would be to have someone whose presence in the wheat field would be unquestioned spread some inoculum in the field at harvest (affecting future crops) or at planting. Another approach would be to drive a route distributing teliospores in fields along the edge of a road, perhaps emphasizing areas where infection might be more readily noticed. It is possible that someone could figure out a way to dribble kernels from a truck or piece of harvesting equipment, but harvesting crews know these pieces of equipment very well and could be expected to notice and be curious about any non-standard additions to the equipment. Because the symptoms of Karnal bunt in the field can be easily overlooked, especially if the level of infection is low, it might be necessary for terrorists introducing the pathogen to bring its presence to the attention of extension agents. In fact, claims that teliospores have been introduced to a wide area, even if untrue, could have an important short-term effect on local economies.

Introducing inoculum into a grain elevator might be an effective method of economic sabotage, but such a scheme would require some local knowledge of the elevator. The sample might not pick up any of the introduced teliospores if mixing was insufficient to allow the teliospores to contaminate a large quantity of unaffected grain. Adding infected kernels to a grain truck before it is unloaded at the grain elevator might be an effective way to contaminate the elevator, but this again would require some foreknowledge that the elevator was due to be sampled and would perhaps require the co-operation of the truck driver. If the infected kernels are not detected until later in transport, this might more effectively create problems.

3. Intelligence Information

The usual intelligence gathering routinely carried out by U.S. and international organizations might not notice unusual or suspicious grain-purchasing or seed-cleaning activities. Probably the usual surveillance of known unfriendly groups is all that can be expected. A person or group attempting to recruit harvest workers to drop something in fields or to operate modified harvest equipment would be suspicious.

III. Probable Route of Terrorist Entry/dissemination

The difficulties of smuggling in and disseminating the inoculum are outlined above. Perhaps the most effective method would be at harvest, by someone who would not be suspected. How the terrorists would recruit such a person is something intelligence agencies might keep in mind. Another possibility would be distribution of teliospores along roads where there is little traffic followed by a claim that contamination has taken place.

IV. Probable distribution and spread

1. Point introduction in field

Inoculum introduction at a single field site or a small number of sites would require careful timing and conducive climatic conditions to result in noticeable symptoms. Teliospores would need to be introduced in advance of wheat flowering to enable a buildup of the population of secondary sporidia, but if climatic conditions are not favorable this would not occur. This means that any given attempted introduction may not be successful. It also means that when symptoms of the disease are detected, the teliospores might have been introduced in an earlier year.

If an introduction is intentional, there may be additional locations at which teliospores have been introduced but in which climatic conditions did not support infection in the year of introduction. The detection of intentional introduction should trigger consideration of whether additional sites now have teliospores introduced that may produce infection in following years.

Interpreting the pattern of infection might be useful for determining whether introduction was intentional or not. For example, if infection tends to occur along roads or other easily accessible points, that might suggest intentional introduction by a small group of agents. However, since combines driven from south to north for custom harvesting of wheat are potential disseminators of teliospores, the path taken by a contaminated combine on its way north might show a regular pattern of movement that could be mistaken for a path of intentional introduction. The path of custom harvesters that have previously worked in an area with newly discovered infection should be carefully studied to interpret an introduction and to help predict and prevent future introductions.

Numerous accidental introductions of *T. indica* have probably occurred in the U.S. and never resulted in detected infection. Establishment of the pathogen is made difficult by its limited window for infection and by its need for encountering a mate of another mating type in wheat heads. High levels of infection are almost never reported even under the most conducive environment. However, if it does become established, overwintering would not be an apparent problem for the fungus in the U.S.

2. Secondary Dissemination

If a site of infection is detected soon after infection occurs, there may be a low risk of secondary dissemination. Sporidia from the infected field are unlikely to be important for establishing new infections in other fields unless the local infection rate was high enough to produce huge numbers that could overcome the Allee effect at smaller population sizes. The Allee effect is the reduced per capita reproductive success at low population sizes. (See section VI. Likelihood of Successful Introduction.) Teliospore dispersal might be a more important risk. Teliospores could easily be dispersed from one field to another in combines and, since combines regularly move between states, this could be an important form of dissemination across a region.

Secondary dissemination would be most important if it occurred at a level sufficient to make quarantining a small area such as a county unworkable. If larger areas become infected, foreign trade partners may become unwilling to accept that the infected area has been effectively removed from the export market.

The most likely pattern of new infections due to natural introductions would be from the southern areas of the U.S. near Mexico and near currently infected counties of the U.S. New infections distant from these sources might be indicative of intentional introduction.

3. Introduction to grain storage and transport facilities

If teliospores are detected at a grain silo, the general area from which the grain was harvested might be known but particular fields would be difficult to identify. A worst case scenario for introduction of teliospores to grain facilities might be contamination of shipping equipment that could put a whole region of the U.S. under suspicion of infection.

V. Consequences of Introduction (Risk of Pathogen Establishment)

The consequences of introduction of *T. indica* and the risk of Karnal bunt establishment in the U.S. were rated with respect to six risk elements: climate, host range, dispersal, economic impact, environmental impact, and persistence.

1. Establishment

A. Climate - Risk = High

Climatic conditions are particularly important for two stages for the life cycle of the Karnal bunt pathogen. First, climatic conditions must support the buildup of secondary sporidial populations so that infection rates are sufficiently high that the disease can be detected and/or so that enough teliospores are produced to maintain the population. Conduciveness of U.S. fields to sporidial buildup may vary greatly from season to season. Second, climatic conditions must support survival of teliospores over the winter. Limited studies suggest that teliospores can overwinter in most parts of the U.S. The multi-year survival of teliospores may make it possible for the pathogen to persist without reproduction in some unconducive years since later reproduction may compensate. See Appendix 2. Climate matching maps from model by

Fowler, Kalaris and Sequeira for the USDA: 16C.6 Exploring historical patterns of environmental suitability to Karnal bunt.

B. Host Range – Risk = Low

While *T. indica* can infect other grass species, wheat would likely be by far the most important host for initiation of epidemics and maintenance of pathogen populations. Trade embargoes in response to the presence of Karnal bunt could have an impact through limits to contaminated bulk commodity export in addition to wheat exports; for example, the same silo might be used to store both wheat and another commodity. For many regions of the country wheat is the most viable crop and there may be few alternatives if restricted trade options make wheat uneconomic to produce.

C. Dispersal – Risk = Medium

Quarantine and management of wheat production in counties in which Karnal bunt has been detected may have been fairly successful in limiting spread of the pathogen, but it may be that enough time has not passed to evaluate the program. Karnal bunt probably will not spread readily from one county to another via wind dispersal, but it could tend to move northward through the Great Plains following the well-known “Puccinia pathway” through which rust fungi move from Mexico or Texas up to northern wheat producing state. Dispersal via combines may be an important consideration. While movement of custom harvesters could theoretically be carefully managed, in practice there has not been the political will to regulate movement and potentially endanger the livelihood of these business owners and the farmers depending on them. It may be possible to regulate how combines are cleaned before movement from one county to another.

If infected grain is not detected in the field, this poses the additional risk that it will contaminate grain storage or transport facilities. This could compound trade problems by throwing doubt on the sanitation for any areas that feed into grain transport and cannot be cleared of suspicion as the source of the infection.

Karnal bunt may also potentially be spread through manure from cattle eating infected grain. There is speculation that this is the means by which it was introduced to counties in Texas.

D. Economics – Risk = High

If Karnal bunt becomes widely distributed in the U.S., U.S. trade negotiators will be motivated to try to bargain for reduced barriers in other countries to import of grain from countries with Karnal bunt. These negotiations may be successful in the long run, but may cost the U.S. in terms of current trade advantages that might need to be bargained away. And it may take several years for such negotiations to be completed so that, in the mean time, U.S. growers would be without the export market.

E. Environmental Impact – Risk = Low

Other potential hosts of *T. indica* would probably experience only minor impacts from its introduction. Its infection rates in wheat are generally low and the Allee effect will probably reduce infection rates in other less common hosts to an even greater extent. There is the possibility, however, that the pathogen will prove to be more aggressive in a native grass species than it has been in wheat to this point. If it becomes more widespread in the U.S. it would be useful to test its effects on related native grass species in controlled experiments to test for potential effects.

F. Persistence – Risk = Medium

It appears that overwintering conditions will not limit Karnal bunt establishment through most of the U.S. The longevity of teliospores makes persistence a possibility even when climatic conditions are rarely favorable for multiplication of sporidia. If a highly conducive year for sporidial multiplication occurs only rarely, it may be enough to maintain a population. On the other hand, the Allee effect for T. indica will tend to drive populations to extinction if there are many consecutive nonconducive years.

2. Over-all risk rating for establishment of *Tilletia indica*

Area in Question	Climate	Host Range	Dispersal	Economics	Environmental Impact	Persistence
Central and Northern Great	High	Low	Medium	High	Low	Medium

Plains						
Southern US	High	Low	Medium	High	Low	High
Northwestern US	High	Low	Medium	High	Low	High

Regulation

At the present time the US has a zero-tolerance policy toward the importation of wheat containing Karnal bunt. If Karnal bunt is found in the US, internal quarantine regulations are instituted. Many trading partners of the US also have a zero-tolerance policy toward Karnal bunt.

The Cost of Vigilance

Screening for Karnal bunt, both to prevent it entering the country and to detect its appearance in wheat growing areas, adds to the cost of wheat production and to the cost of government. Added to this are the costs associated with sampling necessary to issue phytosanitary certificates.

Since wheat lots destined for export are commingled with other lots as the wheat is transported to seaports for export, it is necessary to keep grain contaminated with Karnal bunt spores out of the grain transportation network. Appropriate internal quarantines are required in the U.S. to maintain U.S. export trade and avoid economic losses.

Costs of Containment

Intensive sampling in regulated areas of the US is an added cost. Compensation to farmers in the regulated areas is supported by tax dollars and increases the cost of government.

Impact of detection in the U.S.

The increased costs of monitoring in the U.S. as a result of localized detections are already in place. In counties with Karnal bunt, costs such as those from quarantine and compensation are already being incurred. The limited outbreaks of the past have not resulted in the loss of trading partners, but a more general outbreak could lead to an embargo on U.S. grain by one or more trading partners, impacting U.S. wheat exports which are currently valued at more than $5

billion a year. The costs of inspection and decontamination of export handling and shipping facilities would also be incurred.

The life cycle of *T. indica* makes reproduction at small population sizes difficult. Because individuals of different mating types must encounter each other after dispersal to a wheat head, when populations are small many individuals may fail to reproduce simply because they do not encounter another mating type (Garrett and Bowden, 2002). This reduced per capita reproductive success at low population sizes is often termed an Allee effect, after an early describer of the phenomenon in animals. For Karnal bunt, an Allee effect has several implications. First, the risk of new infection through long-distance transport of sporidia is reduced since the sporidia will become more dilute, and thus less likely to encounter another mating type, as they spread further. A single secondary sporidium cannot begin an epidemic. Second, though a single teliospore can potentially begin an epidemic, environmental conduciveness would need to be high for sufficient buildup of the sporidial population from the teliospore. Marginally conducive environments, which might be adequate for reproduction of an organism without an Allee effect, will not tend to result in maintenance of Karnal bunt. Third, bunted kernels may tend to be more reliable at initiating epidemics than a comparable number of loose teliospores because the bunted kernels will concentrate the teliospores in a limited area so that the sporidial population will also tend to be more concentrated. Garrett and Bowden (2002) considered the potential impact in a model of Karnal bunt epidemics. Sharma, Garrett, and Bowden have preliminary evidence of an Allee effect in the field from experiments in Punjab state, India.

Podleckis and Firko (1998) prepared a mathematical model based on five scenarios. They give estimates of inputs for a number of factors, based on data from the 1996 surveillance area. The model was directed only toward predicting human-mediated spread of the disease (shipping grain for milling and seed for planting from inside the regulated area to outside the regulated areas). Natural spread was not a factor in the model. They concluded that using millfeed from grain in regulated areas to feed animals creates a low risk of spreading KB. They also concluded that seed from regulated areas has a high risk of spreading KB because partially bunted seeds are not detected. The mean estimated likelihood of Karnal bunt spreading were estimated at from 1 chance in 63 to 1 chance in 37 per year.

The model of Smiley (1997) predicted that survival of spores in winter in Pacific Northwest is likely. The model is based on weather data for 13 locations, the percent of years with weather during heading favorable to infection by Karnal bunt.

Sansford (1998), using a model based on weather at sites in India, concluded that Karnal bunt could become established in UK and Europe with important negative economic consequences. Sansford (1998) stated that prevention of importation of infected seed is the most important defense, and that this would argue against importing from the U.S. if Karnal bunt were widely distributed in the US.

Economic Risks

Vocke et al., (2002) analyzed the economic effect of ending the use of phytosanitary certificates in the U.S. The model assumes that white wheat in Pacific Northwest and Northeastern areas, and durum in the Northern Plains, are not affected by Karnal bunt. Since wheat is blended, hard red wheat and soft red wheat from the Northern plains where Karnal bunt may not be able to establish would still be affected by being blended with potentially contaminated wheat. They analyzed the probable reactions of each national trading partner according to each country's regulations, predicted reaction, and the type of wheat currently used in that country. The model also reflects probable changes in wheat production by competitors to the US wheat industry. Some factors that are not taken into account in this model are costs of testing and decontamination, or the consequences of Karnal bunt contamination of facilities and ships. They conclude that in the first year there would be a 25% drop in wheat exports (7 million tons), and a $0.45 per bushel drop in price. The economic impact would be greater on Central and Southern Great Plains producers of hard red wheat and producers of soft red wheat. There would be a premium on spring wheat of about $0.50 per bushel in the first year. They predict a 35% drop in exports in the second year. For the third year, they predict a 20% drop in exports. In the long term there would be a loss of export markets for the U.S., and lower prices for US wheat, resulting in a reduction in total value of wheat produced in U.S., and a reduction of net income to U.S. agriculture.

Stansbury et al. (2002) modeled risks of and the effect of the establishment of Karnal bunt in Australia. This is a very extensive model. They conclude the likely rate of establishment is 1 establishment event every 67 years. They conclude the cost of detecting Karnal bunt would be 17% of the total value of wheat production. They estimate the time from introduction to detection as 4-11 years, depending on sampling regime, which in turn depends on the level of

funding for sampling. They conclude that the spread and containment of Karnal bunt is affected the amount of money spent on detection and containment after the initial establishment. They also conclude that the most likely routes of introduction would be imports of bulk grain or fertilizer. The total cost would be approximately $1-2 billion over 50 years if Karnal bunt were to become established in Australia.

Risk of introduction

Bunted kernels or teliospores would be the form of propagules used for introduction to the U.S. in intentional introductions. Cultures of sporidia are too perishable, while teliospores may survive a long while. Although a container of teliospores could be used if necessary, one would need a large amount of either spores or kernels to:

A) cause enough disease in the field to show up in sampling or

B) contaminate a storage/transport facility enough to show up in sampling

Introduction might be through imported grain, through importation of other commodities that could become contaminated with teliospores, through smuggling via routes similar to those used by narcotics smugglers, and, for small quantities, by the vial-in-pocket approach. Contaminated grain could be used as a vehicle through use of false phytosanitary certificates or as hidden cargo. Visitors with some ostensibly legitimate purpose, such as crop consultants, field workers, harvest workers, visiting scientists, exchange students, or university students in Agronomy and Plant Pathology, could act to introduce the pathogen.

Risk of establishment

Smiley (1997, 1998), in assessing the risk for establishment of Karnal bunt in the Pacific Northwest, concluded conditions favor establishment in irrigated and high humidity areas of the Pacific Northwest. About 20% of the wheat in Oregon and Washington is irrigated with sprinkler irrigation.

Zhang et al. (1984) concluded that teliospores of *T. indica* "...probably can survive in most of the winter wheat-growing regions of the world." They were particularly concerned about the risk of establishment in central China, which has a temperate climate. Although favorable weather is needed for the disease to infect in any given year, teliospores are long lived.

Risk of spread

A model by Fowler, Kalaris and Sequeira for the USDA (16C.6 Exploring historical patterns of environmental suitability to Karnal bunt) indicates that the Smiley (1997) algorithm is suitable for modeling risk. The maps show areas of higher risk of establishment change as the season progresses.

The October 2001 (NAPPO), Pest Risk Analysis Panel used climate matching maps to asses the risk of the establishment of Karnal bunt in Mexico, the U.S., and Canada. The maps show susceptible period, weather and host availability. There are several areas of low risk, but the risk might be greater in years when the weather deviates from the average. Maps indicate that there are several areas of low risk, but during years when weather deviates from the norm, there might be a greater risk. The model takes into account susceptible periods, weather, and host availability. Texas, Arizona and California are considered medium risk.

Babadoost et al. (2004) reported that *T. indica* teliospores can survive longer than 32 months in soil under conditions of Montana winter. *T. indica* teliospores kept in the lab in vials of soil at room temperature, and at 4 ^{o}, -5 ^{o} and -18^{o} C up to 37 months showed better survival at low temperature than at high temperature.

VI. Likelihood of Successful Introduction

1. Quantity of Inoculum Required to Introduce and Establish Damage

In the long term, a small quantity in several fields, perhaps drawing on a dozen vials of teliospores, could initiate an epidemic in a conducive environment. In the short-term, a few pounds or a few hundred pounds of infected kernels could potentially begin a detectable epidemic or be detected in grain storage or transit. New infections sites would probably not be established until the following season.

2. Likelihood of Surviving Initial Introduction

The likelihood that *T. indica* would survive an initial introduction is moderately high. Some teliospores are very likely to survive until following years, but the likelihood of establishing a detectable level of infection may be low. An advantage to terrorists in placing teliospores in grain storage or transit facilities would be that the spores would not even necessarily need to be viable for their detection to have its impact.

3. Likelihood of Dissemination Beyond the Point of Introduction

Dissemination beyond the point of introduction via sporidia is probably unlikely unless unusually large populations of the fungus are present due to highly conducive conditions for sporidial multiplication. Dissemination through combines is a primary concern and would be highly likely if a combine used to harvest an infected field is used in other fields without adequate cleaning. Quarantines have proven apparently effective in restricting dissemination of the fungus.

4. Likelihood of alternate host infection

The abundance of wheat compared to other potential hosts makes wheat likely to be by far the most important source of inoculum. Even if Karnal bunt were to become more widely distributed in the U.S., it is unlikely to become highly severe even in wheat. It is possible, however, that it will prove to be a more aggressive pathogen of another native grass species that has been little-studied in the past.

5. Likelihood of Early Detection

The extensive sampling plan for Karnal bunt in the U.S. makes it very likely that the disease would be detected if it becomes widespread. The fact that infection rates tend to be low, however, and that the symptoms are not strikingly distinctive, means that it could go undetected for some time. Identification can be performed fairly quickly, using the procedures described in the current Karnal bunt regulation (see section I.8.C Regulation).

6. Overall Risk = Moderate

Widespread infection by Karnal bunt through the U.S. could have a devastating impact, particularly for those regions such as several areas of the Great Plains that currently have few other economic options and are dependent on wheat exports for economic viability. On the other hand, *T. indica* has not proven to generally be an aggressive pathogen and its dissemination and establishment may be limited by an Allee effect. Teliospores are long-lived, but detectable epidemics may not occur the year in which the pathogen is introduced.

7. Likelihood of an Agroterrorist Trying to Use = Moderate

Karnal bunt is present in several countries in which terrorists may be active, such as Afghanistan and Iraq. If entry of large numbers of teliospores into the U.S. can be arranged, the pathogen could be distributed to production fields fairly easily by car along roadways. Distribution to grain storage and transport areas might be most effective, but harder to arrange. The economic impact could be great, but it might be necessary to wait for the impact until a later more climatically conducive year. Terrorists in search of immediate impact might look to other tools but terrorists willing to wait for a potentially very large impact could use Karnal bunt.

VII. Control/mitigation Strategies after Establishment

1. Cultural control

The most effect “cultural control” is probably quarantine. The current methods of quarantine and the costs are discussed above. Other cultural controls are not likely to be effective in preventing the spread of Karnal bunt. Crop rotation is probably not practical in many areas because the longevity of teliospores in soil may be up to three or four years (Warham, 1992). Weather is variable in the wheat-producing regions of the U.S., so changing sowing dates to reduce the chances of favorable weather during the vulnerable period may not be practicable. Burning stubble in wheat fields may actually help to disperse propagules. Increased N fertilization may increase disease, but farmers are not likely to decrease fertilization if this results in decreased yield. Soil fumigation may be effective, but is not economic for the vast

wheat-growing regions of the U.S. Irrigation may increase disease during the growing season (Bonde et al., 1997;Warham, 1992) but could potentially be used before wheat is planted to induce premature germination of teliospores (I. Sharma, personal communication).

2. Resistance

All cultivars in the Indian area were susceptible but increased disease was observed when Mexican cultivars that have uniform flowering and hi-N applications became widely cultivated (Warham,1992).

Karnal bunt infected native wheats in India but was less damaging until 1970, with the introduction of high-yielding, semi-dwarf wheats and irrigated high fertilizer input farming (Singh, 1998). A few durum and triticale may offer sources of resistance, but wheat, durum and triticale have similar degrees of susceptibility with boot inoculation. Spray inoculation more closely approximates field conditions. Boot inoculation with water suspension tests for physiological resistance, but morphological resistance may be more important and more readily available. Pubescence is probably not a factor in resistance, although only pubescent wheats were grown in India before the introduction of Mexican wheats (Warham, 1992). Bonde et al. (1997) stated that sources of resistant lines can be traced to China, India and Brazil.

Tolerant lines are available. Lines based on *Triticum tauchii* are under development (Bonde et al., 1997).

Because of the zero-tolerance standards in place for Karnal bunt and because of potential effects on quality, there is pressure to produce levels of resistance near immunity. This may change if trade negotiators are successful in arguing for reduced trade barriers.

3. Chemical control

Teliospores of *T. indica* are fairly resistant to chemical treatment. Methyl bromide kills spores in the unbroken sorus, but in general seed treatments are fungistatic, not fungicidal. Seed treatments don't last long enough to protect florets from infection (Bonde et al., 1997). . Two or more foliar sprays of propiconazole at or after spike emergence reduced incidence of infection

95% (Bonde et al., 1997). Fungicides applied to soil don=t reduce disease (Smilanicket al., 1987). The issue of fungicide residues may have to be addressed by regulation.

Seed treatment

Fungicides may reduce teliospore germination but do not prevent disease. Fungicides may prevent the germination of teliospores for a time, but this protection does not last long enough to prevent floral infection. Heat treatment may be sufficient to decontaminate seed, but this reduces seed germination (Warham, 1992).

Table 4. Fungicides tested for control of Karnal bunt.

Fungicide	**Control**	**Reference**
Benomyl	Reduced infection in the field	Singh and Prasad 1980
Bitertanol	64% control in the field	Singh et al. 1985b
Carbendazim	82-87% control in greenhouse	Krishna and Singh 1982
Carbendazim	Reduced infection in the field	Singh et al. 1985a
Carbendazim	Reduced infection in the field	Singh and Prasad 1980
Carboxin	82-87% control in greenhouse	Krishna and Singh 1982
Copper hydroxide	80% control in the field	Smilanick et al. 1987
Etaconazole	80% control in the field	Smilanick et al. 1987
Fentin hydroxide	Reduced infection in the field	Singh et al. 1985a
Mancozeb	Reduced infection in the field	Singh et al. 1985a
Mancozeb	Reduced infection in the field	Singh and Prasad 1980
Mancozeb	80% control in the field	Smilanick et al. 1987
Oxycarboxin	82-87% control in greenhouse	Krishna and Singh 1982
Propiconazole	Reduced infection in the field	Qui? ones-Leyva 1984
Propiconazole	93-98% control in the field	Salazar-Huerta et al. 1986, Salazar-Huerta and Prescott 1986, 1987
Propiconazole	80% control in the field	Smilanick et al. 1987
Triadimefon	82-87% control in greenhouse	Krishna and Singh 1982
Triadimefon	Reduced infection in the field	Singh and Singh 1985
Triadimefon	Reduced disease	Singh and Singh 1985
Triadimenol	Reduced infection in the field	Qui? ones-Leyva 1984
Triphenyltin hydroxide	Reduced infection in the field	Singh and Prasad 1980

Foliar sprays

Foliar sprays of mancozeb, copper hydroxide, or carbendazim before sporidia are released from soilborne teliospores may decrease disease. Foliar residues of contact fungicides are probably removed when grain is threshed (Warham, 1992). The use of systemic fungicides is not practical, because high rates are needed at late growth stage, which may cause unacceptable pesticide residue. See table 4 for a list if pesticides that have been tested. Limited use of fungicides may be useful for research, or to limit the spread of Karnal bunt from a point introduction, but it is not economically practical to spray large areas of the wheat-growing regions of the U.S.

Fumigants

Fumigants are too costly to be useful (Warham, 1992).

4. Biological Control

There are no known prospects for biological control of Karnal bunt. It is possible that a biological control agent could be developed to inhabit leaf surfaces and reduce sporidial reproduction.

VIII. Knowledge Gaps

1. How much variation is there in pathogenicity and aggressiveness in *T. indica*? Is there reason for concern that higher levels of infection may become common?

2. Are there races of pathogens and what is their geographic distribution?

3. How many mating types are there and do the mating types vary geographically?

4. Little is known about the ecology of the primary and secondary sporidia. What environmental factors determine the buildup of sporidial populations necessary to overcome the Allee effect?

5. For regulatory purposes, thresholds are of primary importance. What are the threshold values of teliospores necessary for initiating and maintaining populations? How does this threshold shift as a function of environmental conditions?

Much more research is needed on the genetics of the fungus and the genetics of resistance in wheat. Sources of resistance need to be identified and incorporated into new varieties in the U.S. to reduce the opportunity for the fungus to become established in the U. S. following either natural or artificial introduction.

IX. Immediate Response Options

Response of the U.S. to detection

It would be necessary to track down the source of the infection and the destination of any possibly contaminated grain that has already been transported from the grain elevator.

Where the source of infected wheat could be determined, internal quarantines would be put in place, as has been done before. It would be necessary to find the fields or areas where the grain was harvested and search for evidence of teliospores. The current regulations seem to have worked well in containing the limited outbreaks in the U.S. to date. The U.S. would have to take immediate steps to insure that contaminated wheat does not go to export facilities because of the possibility of other nations imposing a quarantine on American wheat. Poe (1998) discussed the 1991 Galveston, TX incident, in which suspected KB was found in an export elevator. This incident demonstrated that it would be difficult to trace the source of the suspect wheat, which, fortunately, turned out not to be infected with KB in this case**.**

If wheat that has already been tested at the grain elevator is later found to be contaminated, and no evidence of disease in the field can be found, then the possibility of sabotage must be entertained, perhaps leading to an investigation by law enforcement and national or international security personnel. Importers of U.S. wheat and foreign customs officials may test the grain for Karnal bunt, either at shipping or at arrival at a foreign port. A saboteur who wished to cause the U.S. economic harm could possibly contaminate an export shipment, but would have to have specialized knowledge of shipping and testing procedures, as well as access to the shipped grain, to ensure the contaminant was discovered.

1. Rapid Detection

See section II. 1. Observation/diagnosis of presence

2. Control

If the area of infection is limited, a quarantine could help reduce spread to additional areas.

3. Fungicides

Fungicides might be used to reduce infection rates and risks of spread, but probably cannot produce teliospore-free seed.

4. Resistance Breeding

Material with higher resistance is available, but not with high enough resistance to produce teliospore-free seed in an epidemic.

Appendix 1. Experts knowledgeable about Karnal bunt.

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Appendix 2. Climate matching maps from model by Fowler, Kalaris and Sequeira for the USDA: 16C.6 Exploring historical patterns of environmental suitability to Karnal bunt.

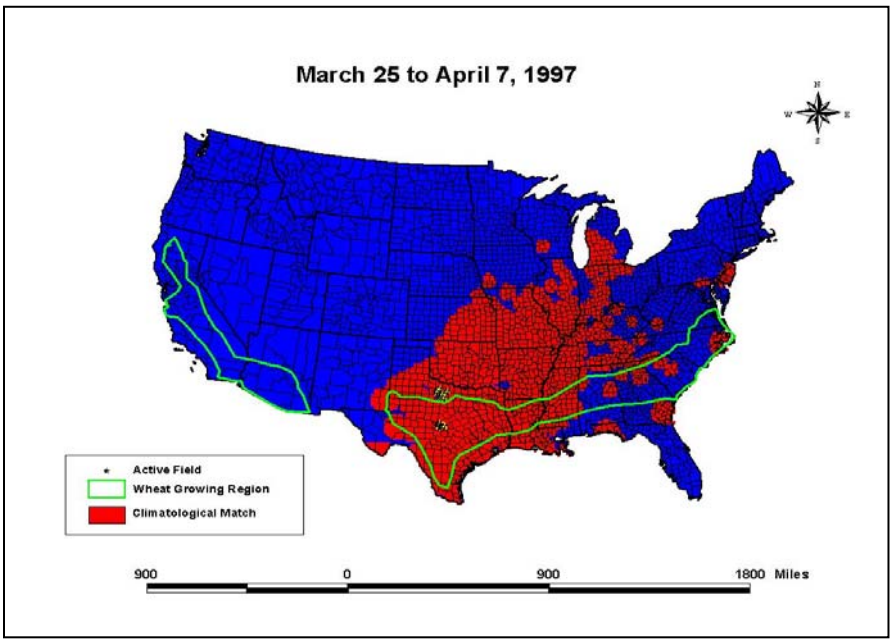


Figure 2.1. Example of a bi-weekly Karnal bunt prediction map.

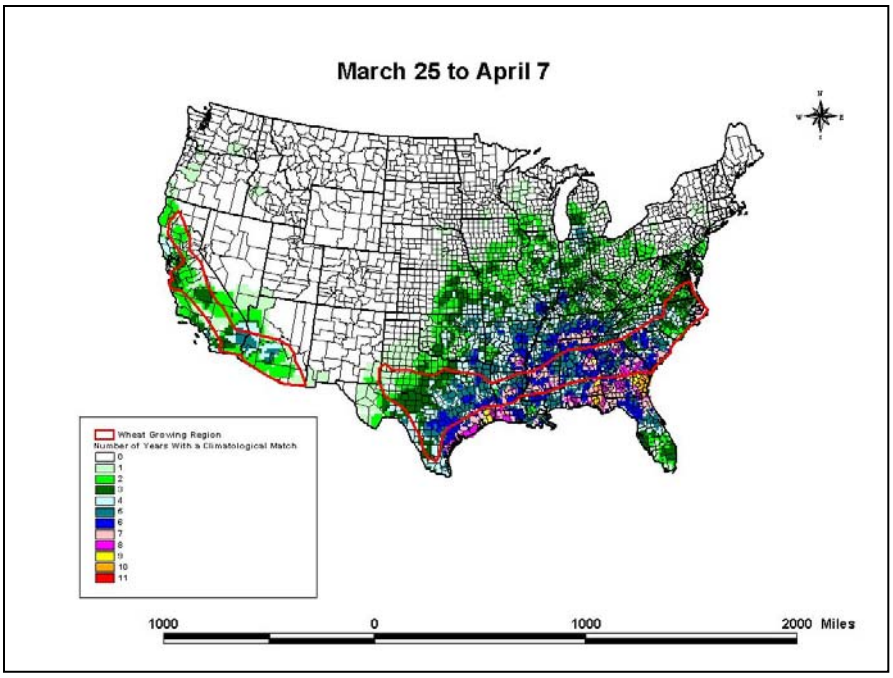


Figure 2.2. Example of a bi-weekly composite query map for 1991 to 2001.

Appendix 3. Map of susceptibility of wheat to Karnal bunt from NAPPO. October 2001. An Epidemiological Approach to Assessing the Risk of Establishment of Karnal Bunt, Tilletia indica Mita, in North America. (North Americal Plant Protection Organization (NAPPO), Pest Risk Analysis Panel)

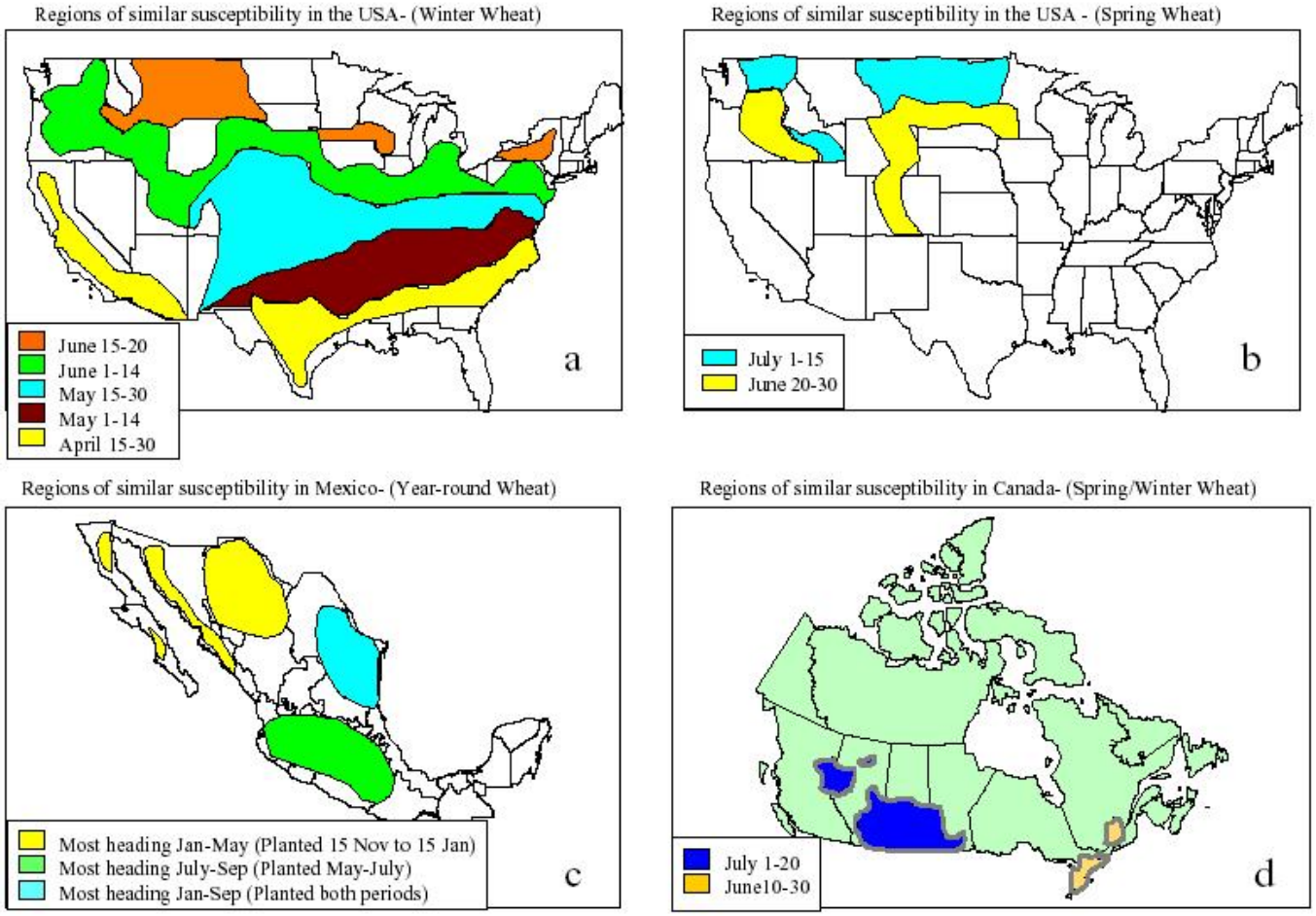


Figure 4. Regions of similar phenology (specifically the reproductive period during which wheat is susceptible to Karnal bunt) in the United States (a,b), Mexico (c), and Canada (d)

Appendix 4. Map of Karnal bunt risk from NAPPO. Octber 2001. An Epidemiological Approach to assessing the Risk of Establishment of Karnal Bunt, Tilletia indica Mitra, in north America. (North Ameican Plant Protection Organization (NAPPO), Pest Risk Analysis Panel)

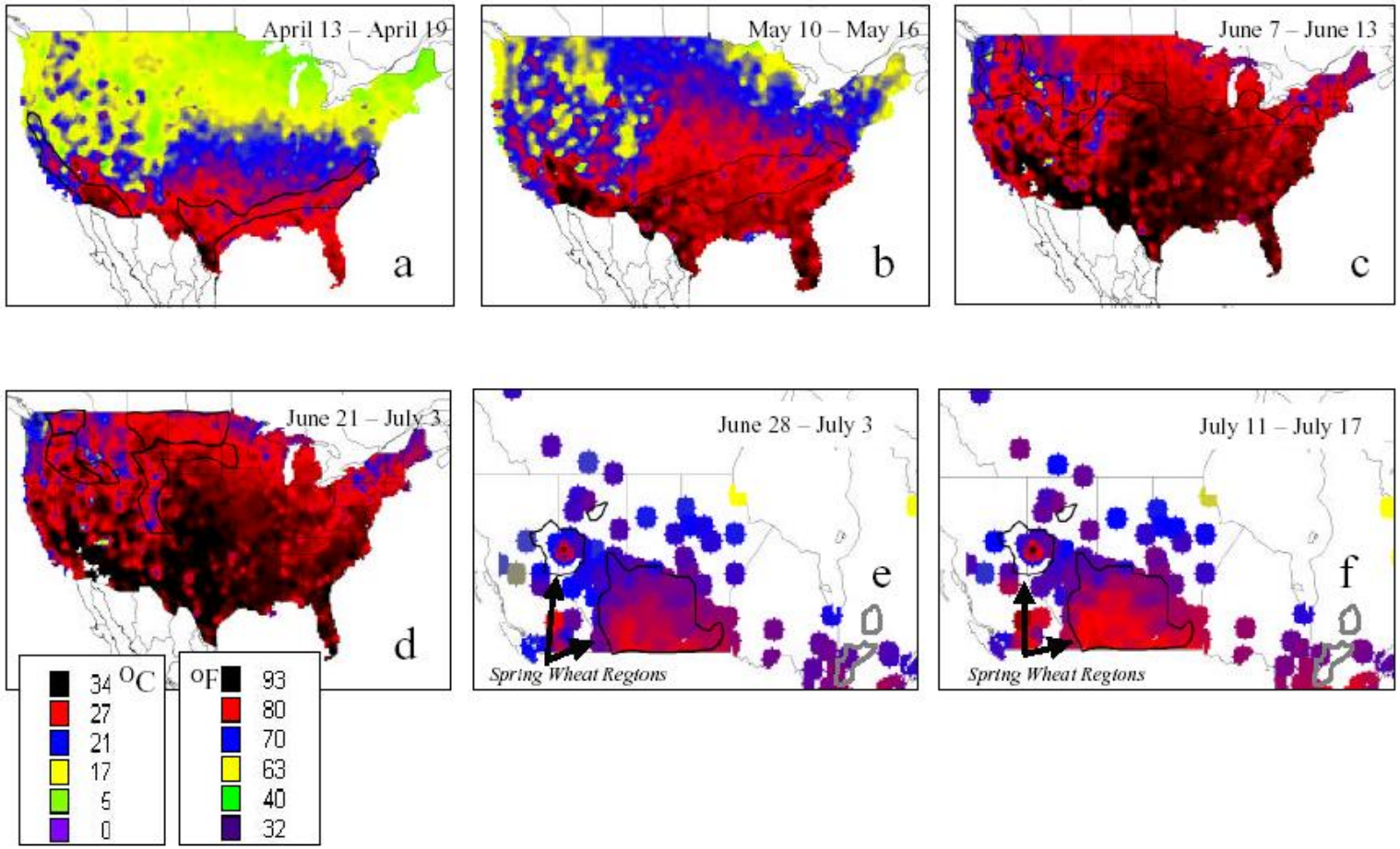
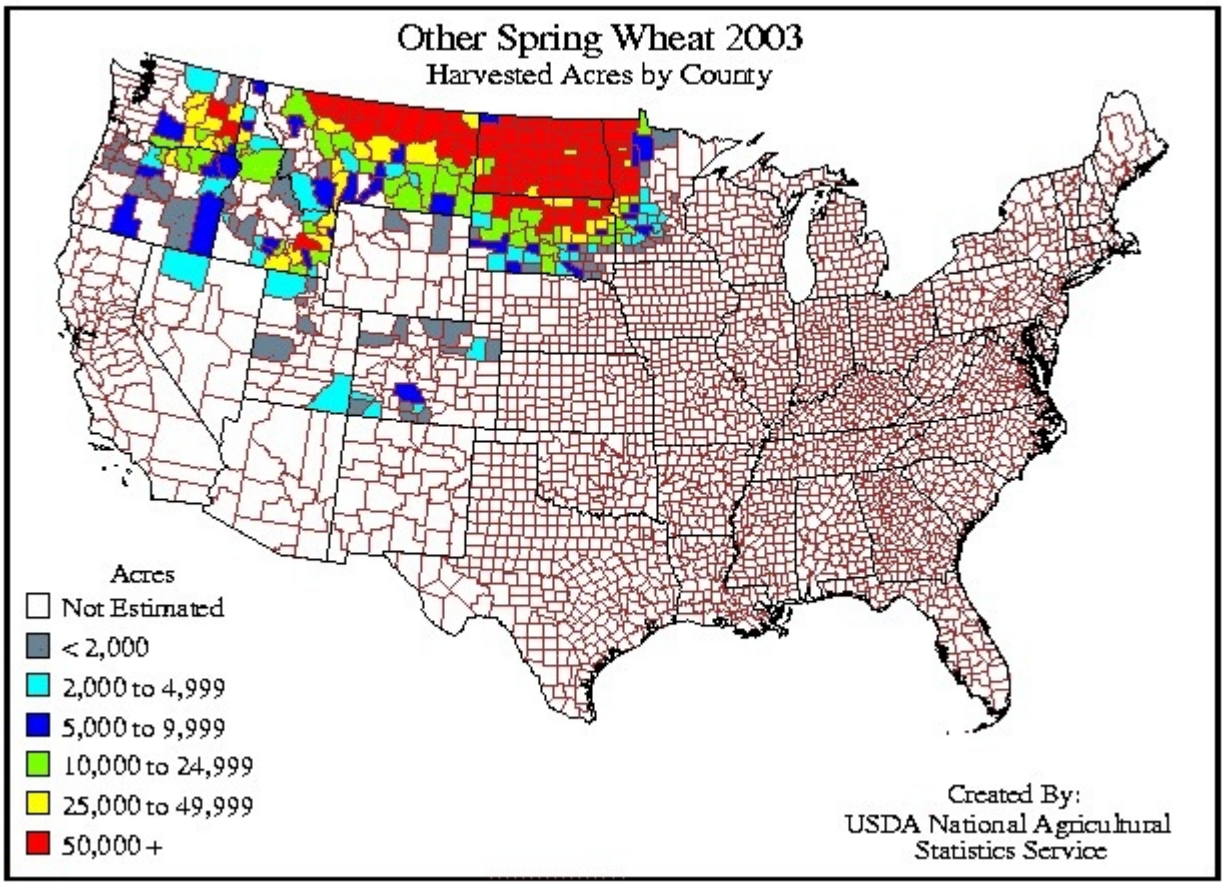
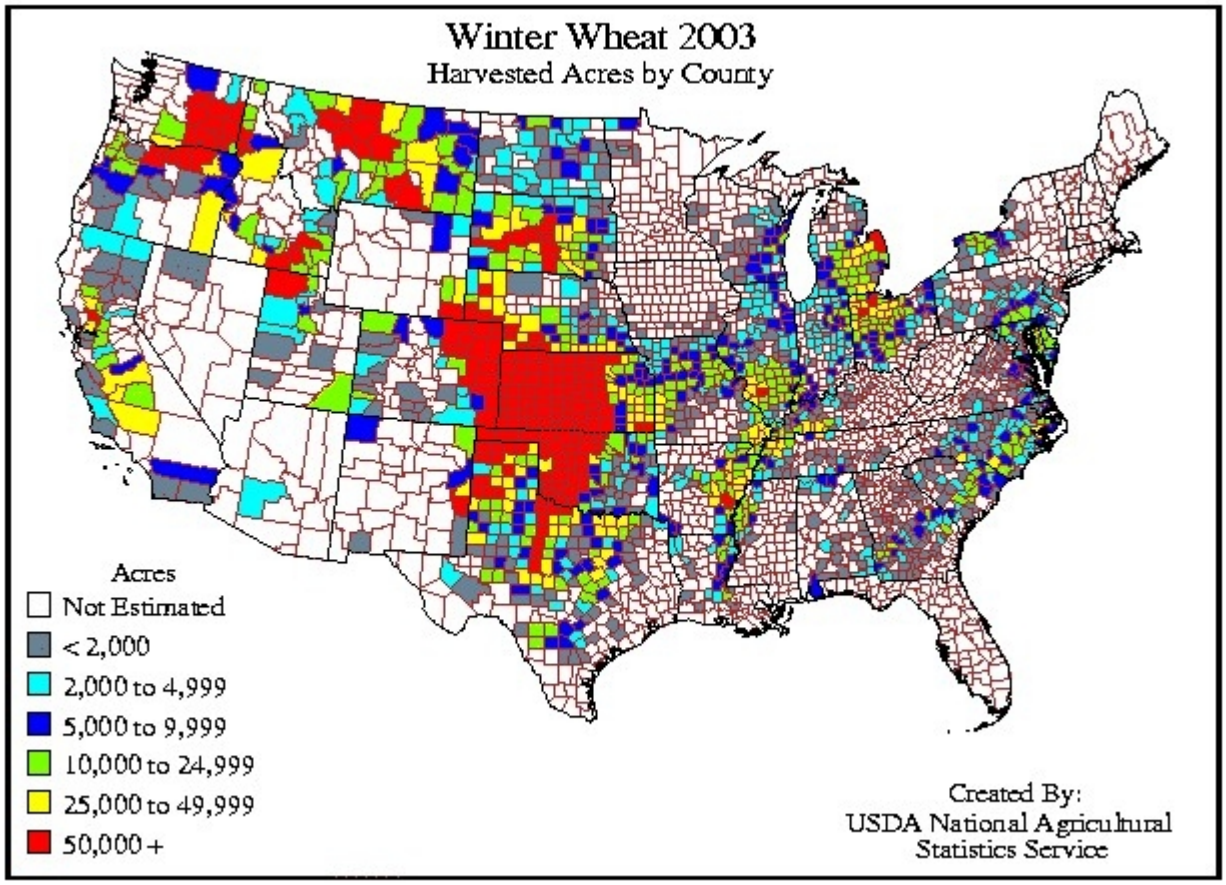


Figure 6. Wheat "Heading Period" Vs. "Iso- pathogenicity" Regions in the US (a-d); Temperature maxima regions equally conducive to pathogen development ("iso-pathogenicity") overlayed with polygons showing susceptible wheat during that period("Iso-phenology"); figure 6e,f. Regions in Canada.

Appendix 5. Map of spring wheat harvested in 2003. (USDA)



Appendix 6. Map of winter wheat harvested in 2003. (USDA)



Appendix 7. Morphology of Tilletia spp.

Source http://nt.ars-grin.gov/taxadescriptions/tilletia/ (2003)

Tilletia barclayana (Bref.) Sacc. & Syd.

Teliospores 14-36 µm, light to dark chestnut brown, semi opaque, globose to subglobose, oramentation sharply pointed spines to truncate scales, height of ornamentation 1.5-4.2 µm, sheath extending to the end of the spines, tinted.

Host Genera: Brachiaria, Digitaria, Eriochloa, Panicum, Pennisetum

Tilletia tritici (Bjerk.) Wint.

Teliospores 14-24 µm, pale yellow to gray or reddish brown, globose to subglobose, oramentation reticulate, height of ornamentation 0.5-1.5 µm, sheath inconspicuous.

Host Genera: Agropyron, Arrhenatherum, Bromus, Festuca, Secale, Triticum

Tilletia horrida Takahashi

Teliospores 20-40 µm, light to dark chestnut brown, semi opaque, globose to subglobose, oramentation sharply pointed spines to truncate scales, height of ornamentation 1.5-4 µm, sheath extending to the end of the spines, hyaline to tinted.

Host Genera: Oryza

Tilletia indica Mitra

Teliospores 28-54 µm, brown to dark reddish brown and opaque, globose to subglobose, oramentation densely echinulate to narrowly cerebriform, height of ornamentation 1.4-5 µm, sheath extending to tips of ornamenations, hyaline to tinted.

Tilletia walkeri Castlebury & Carris

Teliospores 24-44 µm, pale yellow to dark reddish brown, globose to subglobose, oramentation conical to truncate projections, appearing coarsely cerebriform to coralloid in surface view, height of ornamentation 3-6 µm, sheath extending to the tips of projections, hyaline to yellowish brown.

Host Genera: Lolium

Host Genera: Triticum

Tilletia laevis Kuhn

Teliospores 14-22 µm, light to dark olivaceous brown, globose or ovoid to elongate, oramentation smooth, height of ornamentation - µm.

Host Genera: Agropyron, Elymus, Hordeum, Secale, Triticum

Appendix 8. References for Synonymy.

Source http://nt.ars-grin.gov/taxadescriptions/tilletia/ (2003)

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Uredo caries DC. 1815. Fl. Fr. 6: 78

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Erysibe foetida Wallr. 1833. Fl. Crypt. Germ. 2: 213
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Slime Disease of Wheat

Pathway Analysis:

Intentional Introduction of

***Clavibacter tritici* or**

Clavibacter rathayi

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Karnal Bunt Pathway Analysis

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Executive summary: Slime Disease Pathway Analysis

Slime disease of wheat is caused by either of two bacteria in association with the nematode that causes seed galls in wheat. The bacteria, *Clavibacter tritici* and *Clavibacter rathayi*, require an association with the nematode, *Anguina tritici*, to cause slime disease of wheat. Inoculations with the bacteria alone result in no infection or in atypical, weak infections. The nematode may infest the wheat, causing a condition called seed gall, or earcockle, without the occurrence of slime disease. Severe outbreaks of slime disease have been reported in China, Egypt, India, and Iran (Jones, 1987). Losses from slime disease of wheat may be 40-50% with partial infection, but high infection may cause a total loss (Paruthi *et al.*, 1987). Losses may result from ear cockle alone, without the occurrence of slime disease. This disease has occurred in the U.S. before, but under normal production conditions in the U.S. it does not tend to spread; infected grain can readily be removed at the time of harvest so that it does not contaminate other fields. Terrorists might use this disease if they were able to introduce it to a wide enough area to have impact. Infected seed might be readily available in many parts of the world and could potentially be produced in the U.S. The impact might last only a few years or less, though, since keeping fields free from wheat and other grass hosts can quickly reduce nematode populations. The difficulty of producing an important impact would probably make this disease a lesser choice for use by terrorists.

Other names for Slime disease: Yellow slime disease, yellow ear rot, tundu (in India), sehar (in Pakistan), spike blight, gumming disease, yellow gum disease, gummosis of wheat. The American Phytopathological Society has used the name spike blight (Wiese, 1987).

Other names for seed gall: Earcockles, cockles, purples, peppercorns, eelworm disease, hard smut (Wiese, 1987). In Germany it is called Radenkrankheit, and in France, Blenielle. In India, it is called gegla and mamni (Suryanarayana and Mukhopadhaya, 1971).

In India, slime disease is called tundu, and in Pakistan it is called sehar. The disease is caused by the association of the nematode *Anguina tritici* and the bacteria *Clavibacter tritici* or *C. rathayi*. Infection by the bacteria alone will not cause slime disease (Cheo 1946; Gupta and Swarup, 1972; Paruthi and Bhatti, 1990; Vasudeva and Hingorani, 1952).

Slime Disease of Wheat

Pathway Analysis for the Intentional Introduction of *Clavibacter tritici* or *Clavibacter rathayi*

I. Biology and life/disease cycle

1. Identity

Taxonomic Position:

Bacterium

Kingdom: Bacteria

Subkingdom: Eubacteria

Division: Firmicutes

Genus: *Clavibacter* Davis et al. 1984, gen. nov.

Species: *Clavibacter tritici* (Carlson and Vidaver 1982) Davis et al. 1984, comb. nov.

Clavibacter rathayi (Smith 1913) Davis et al. 1984, comb. nov.

Nematode (after Paruthi and Bhatti 1990)

Kingdom: Animalia

Phylum: Nematoda

Order: Tylenchida Paruthi and Bhatti

Family: Anguinidae

Genus: *Anguina* (Scopoli, 1777)

Species: *Anguina tritici* (Steinbuch, 1799) Chitwood, 1935

Synonyms: Both species of bacteria and the species of nematode have a great many synonyms. See Table 1.

Table 1. Synonyms of names of organisms used in this report.

Names used in this report	Synonyms
Clavibacter tritici (Carlson and Vidaver) Davis, Gillaspie, Vidaver and Harris 1984	*Pseudomonas tritici* Hutchinson, 1917 [1,2, 3] *Bacterium tritici* (Hutchinson) Elliot 1930 [1, 3] *Phytomonas tritici* (Hutchinson) Bergey et al. 1930[1, 3] *Phytomonas nigrofaciens* Khodakovskit 1930, cited by Yachevskit 1935[3] *Bacterium nigrofaciens* (Khodakovskit) Yachevskit 1935[3] *Rathayibacter tritici* (ex Hutchinson 1917) Zgurskaya, Evtushenko, Akimov & Kalakoutskii 1933[2] *Agrobacterium tritici* (Hutchinson) Săvalescu 1947[1, 3] *Corynebacterium tritici* (Hutchinson, 1917) Burkholder 1948[3] *Corynebacterium michiganense* pv. *tritici* (Hutchinson 1917) Dye & Kemp 1977 *Corynebacterium tritici* (ex Hutchinson, 1917) Carlson and Vidaver 1982 *Corynebacterium michiganense pv tritici* Paruthi and Bhati 1990[3] *Rathayibacter tritici* (Carlson and Vidaver 1982) Zgurskaya et al. 1993
Clavibacter rathayi (Carlson and Vidaver) Davis, Gillaspie, Vidaver and Harris 1984[3]	*Aplanobacter rathayi* (Smith 1913) [1, 2, 3] *Bacterium rathayi* (Smith) Aujeszky 1914 [1, 2, 3] *Bacillus mucilaginosus koeleriae* Aujeszky 1914 *Pseudomonas mucilaginosus koeleriae* (Aujeszky) Moesz 1915[3] *Erwinia rathayi* (Smith) Gram and Rostrup 1923 [1,2, 3] *Phytomonas rathayi (Smith) Bergey et al.* 1923 [1, 2, 3] *Rathayibacter rathayi* (Smith) Zgurskaya, Evtushenko, Akimov & Kalakoutskii 1933 *Agrobacterium rathayi* (Hutchinson) Savalescu 1947 [1, 2, 3] *Pseudobacterium rathayi* (Smith) Krasil'nikov 1949 [1, 2, 3] *Corynebacterium rathayi* (Smith) Dowson 1942 *Clavibacter rathayi* (Carlson and Vidaver) Davis, Gillaspie, Vidaver and Harris 1984[3]
Anguina tritici (Steinbuch, 1799) Chitwood, 1935	*Vibrio tritici* Steinbuch, 1799[4, 5] *Rhabditis tritici* (Steinbuch, 1799) Dujardin 1845[4, 5] *Anguillula tritici* (Steinbuch, 1799) Grube 1849[4, 5] *Anguillula graminearum* Diesing 1851 in part[5] *Anguillulina tritici* (Steinbuch, 1799) Gervais & Van Baneden 1859[4, 5] *Anguillulina* (*Anguina*) *tritici* (Steinbuch, 1799) Gervais & Van Baneden 1859 (W. Schneider 1939)[5] *Tylenchus tritici* (Steinbuch, 1799) Bastian 1865[4, 5] *Tylenchus* (*Anguillulina*) *tritici* Bastian 1865 (Filipjev 1934)[5] *Anguina tritici* (Steinbuch, 1799) Filipjev 1936 *Anguillula scandens* Schneider 1866[4, 5] *Tylenchus scandens* (Schneider 1866) Cobb 1890[4, 5] *Anguillulina scandens* (Schneider 1866) Goodey 1932[4, 5] *Anguillulina* (*Anguina*) *scandens* (Schneider 1866) Goodey 1932 (W. Schneider 1939)[5]

1. Synonym listed in Bradbury (1973), which uses *Corynebacterium rathayi* and *Corynebacterium tritici*.
2. Synonym listed in Euzéby, 1997.
3. Synonym listed in Bradbury 1986, which uses *Clavibacter rathayi* and *Clavibacter tritici*.
4. Synonym listed in Krall (1991)
5. Synonym listed in Paruthi and Bhatti (1990)

2. Hosts

See Table 2 for a list of species, hosts, and geographical distributions of slime disease caused by *Clavibacter tritici*, *C. rathayi*, or related species, in association with the nematode *Anguina tritici*.

The most important crop affected by slime disease is wheat. Slime disease caused by *C. rathayi* or *C*. *tritici* in association with *A. tritici* occurs on some wild grasses (Dahiya and Bhatti, 1980; Paruthi et al., 1992) and on *Dactylis glomerata*. *Dactylis glomerata*, known as orchardgrass or cocksfoot grass, is grown commercially as seed for turfgrass. Earcockle caused by *Anguina tritici* has been reported in orchardgrass in the U.S. in Oregon and Virginia, and in England, Denmark, New Zealand, and Rumania (Dowson and d'Olivieri, 1935). Slime disease on orchardgrass may cause losses to seed growers. The disease is also found in emmer, spelt, rye, barley, and triticale (Paruthi and Bhatti, 1990; Paruthi and Gupta, 1987). Wheat and triticale are good hosts, but barley and kanki (*Phalaris minor*) are poor hosts, and oats are not a host, according to Paruthi and Bhatti (1990).

Table 2. Species, hosts, and geographical distributions of slime disease caused by *Clavibacter tritici, C. rathayi,* or related species in association with A*nguina tritici.*

Pathogen Species	Host	Year [1]	Location	Reference
C. rathayi	Orchardgrass or Cocksfoot grass *(Dactylis glomerata*)	1934	England	Dowson, and Oliviera, 1935
C. rathayi	Orchardgrass	1945	Oregon, USA	Hardison, 1945
C. rathayi	Orchardgrass	1951	Denmark	State Phytopathological Experiment Station, 1955
C. rathayi	Orchardgrass	1955	New Zealand	Johnston, 1956
C. rathayi	Orchardgrass	1957	Virgina, USA	Williams and Taylor, 1957
C. rathayi	Orchardgrass	1971	Rumania	Severin and Docea, 1971
C. rathayi	Wheat *(Triticum aestivum*)			Sabet 1954[2]
C. rathayi	*Triticum dicoccum*			Sabet 1954[2]

C. rathayi	*Triticum turgidum*			Sabet 1954[2]
C. tritici	Wheat	1908	Punjab, India	Hucthinson, 1917
C. tritici	Wheat	1923	Australia	Carne, 1927
C. tritici	Wheat	1936	Cyprus	Natrass, 1936
C. tritici	Wheat	1936	China	Cheo, 1936
C. tritici	Wheat	1954	Cairo, Egypt	Sabet, 1954
C. tritici	Wheat	1969	Ethiopia	Hingorani and Bekele, 1969
C. tritici	Wheat	1973	Iran	Bamdadian, 1973
C. tritici	Wheat	1977	Iraq	Al-Sabie 1981 cited in Fattah 1988
C. tritici	Wheat	1987, 1989	Pakistan	Akhtar 1987, Akhtar 1989
C. tritici	Wheat			Sabet 1954[2]
C. tritici	*Triticum turgidum*			Sabet 1954[2]
C. tritici	*Triticum dicoccum*			Sabet 1954[2]
C. tritici	Canary grass, kanki (*Phalaris minor*)	1980 1992	India	Dahiya & Bhatti 1980 Paruthi, Bhatti & Singh 1992
C. tritici	Bearded darnel (*Lolium temulentum*)	1980	India	Dahiya & Bhatti 1980
C. tritici	Annual beardgrass (*Polypogon monspeliensis*)	1980 1989	India	Dahiya & Bhatti 1980 Paruthi, Dahiya & Bhatti 1989
Aplanobacter smithii	Western wheatgrass (*Agropyron smithii*)	1916	Utah	O'Gara 1916

1. Year of the report publication (not necessarily the actual year of the incidence of the disease)
2. Artificial inoculations with *A. tritici* with *C. rathayi* or *C. tritici* resulted in wheat slime disease and earcockle.

Suryanarayana and Mukhopadhaya (1971) report that oat can host *Anguina tritici*, but rarely does. They list emmer and a number of grasses as hosts of *Anguina tritici.* Earcockle caused by *A. tritici* may occur in barley (Bhatti et al., 1978).

CMI Descriptions of Pathogenic Fungi and Bacteria, Commonwealth Agricultural Bureau (CAB) give the following descriptions of host range. No. 376: *C. rathayi* occurs naturally on *Dactylis glomerata, Cynodon dactylon* (bermudagrass) , *Secale cereale*, and by artificial inoculation with nematodes on *T. aestivum*, *T. durum*, *T. dicoccum*, and *T. pyramidale*. No 377: *C*. *tritici* on *T. aestivum*, with artificial inoculation with nematodes on *T. durum*, *T. dicoccum*, and *T. pyramidale*.

Slime disease on *Polypogon monspeliensis* (annual beardgrass) was found in five of nine wheat fields in North India in spring. There was a low incidence of disease, and the effect on the seedheads was not as severe as it was in wheat (Paruthi et al., 1989). Williams and

Taylor (1957) reported “Rathay’s disease” on *Dactylis glomerata* in Virginia, caused by *C. rathayi*. They report yellow bacterial slime and distortion of leaves, but say nothing about nematodes.

Slime disease was found on *Phalaris minor* (common names: canary grass, kanki) in Northern India in wheat fields. Almost all the wheat was infected with slime diseas in the fields where *Phalaris minor* was observed in March and April, 1987. Incidence (percent infected earheads per sample) for wheat was 60.8%, for barley was 7.3%, for kanki was 3.8%. Partially attacked earheads were counted as diseased. In wheat, partial slime disease incidence was 15%, complete slime disease incidence was 45.8%. Slime disease was not seen on wild oats *(Avena fatua)* (Paruthi and Gupta, 1987). Slime disease together with earcockle was reported on barley (Bhatti et al., 1978).

Bradbury (1973) cites Williams (1964) (Abstract in Phytopathology 54:912) successfully inoculating rye (*Secale cereale*) with slime disease but states that details of the inoculation method were not published.

O’Gara (1916) described a slime disease in Western wheatgrass, *Agropyron smithii*, in the central U.S. The disease is remarkably similar to slime disease of wheat. He described a new species, *Aplanobacter smithii*, as the bacterial pathogen.

3. Geographic Distribution

See Figure 1. Distribution map of plant diseases for slime disease, CABI 1978. This map uses the synonyms *Corynebacterium tritici* and *Corynebacterium rathayi.*

See Figure 2. Distribution map of plant diseases for slime disease, CABI 1996. This map uses the synonyms *Rathayibacter tritici* and *Rathayibacter rathayi.*

See Table 2. Species, hosts, and geographical distributions of slime disease caused by *Clavibacter tritici*, *C. rathayi*, or related species in association with *Anguina tritici*.

Slime disease

Severe outbreaks of slime disease have been reported in China, Egypt, India, and Iran (Jones, 1987; Bamdadian, 1973). Slime disease is most important in the Middle East and Far East.

A comparison of the CABI maps of 1978 and 1996 show the geographic ranges of *C. rathayi* and *C. tritici* changed little in the interval. Between 1978 and 1996, *C. tritici* spread into Pakistan and Afghanistan from India and Iran, and appeared in Zambia. The geographic distribution of *Clavibacter tritici* in 1996 was Cyprus, Egypt, Ethiopia, Zambia, India, Pakistan, Afghanistan, Iran, areas in central China, and western Australia. The geographic distribution of *C. rathayi* in 1996 was Oregon, Virginia, Quebec, Japan, New Zealand, The United Kingdom, Denmark, Germany, Norway, Romania, and Sweden.

Fig. 1. Distribution map of plant diseases for slime disease. (CABI 1978)
This map uses the synonyms for *Corynebacterium tritici* and *Corynebacterium ratayi.*

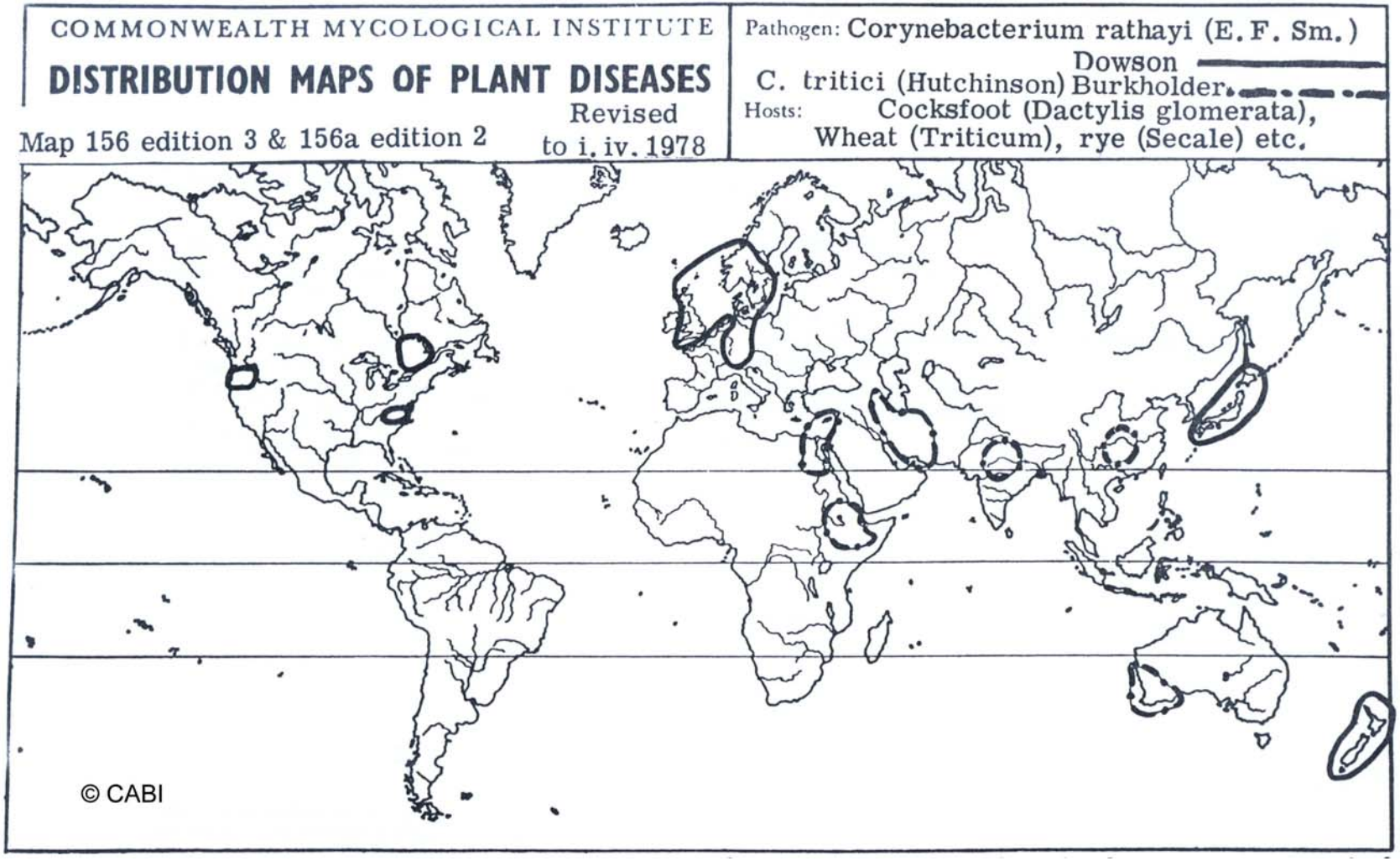
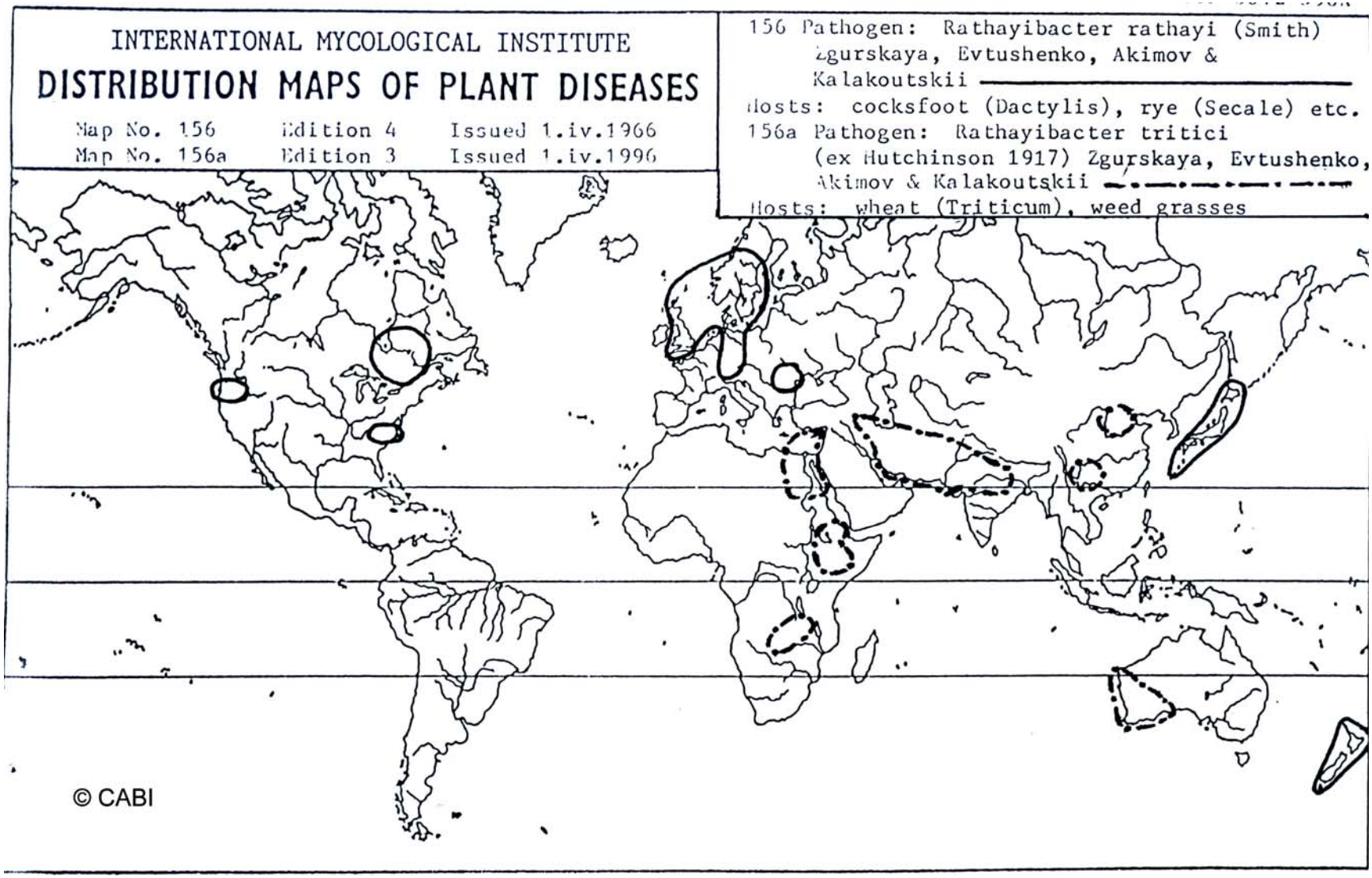


Fig. 2. Distribution map of plant diseases for slime disease (CABI 1996).
This map uses the synonyms *Rathayibacter tritici* and *Rathayibacter rathayi.*



C. rathayi occurs on cocksfoot grass (*Dactylis glomerata*) causing a yellow slime disease that is occasionally observed in Britain, and is much more common in Denmark and North Germany (Dowson, 1957). Yellow slime disease caused by *C. rathayi* occurs on cocksfoot grass in New Zealand, but is not severe (Johnston, 1956).

Seed gall

Seed gall, or earcockle, also called cockle, is the oldest reported disease of wheat (Bhatti et al., 1978; Wiese, 1987). Job 31:40 refers to cockle of barley (KJV, 1611). Shakespeare (1594) refers to cockles in *Love's Labour's Lost*.

Paruthi and Bhatti (1990) state that *A. tritici*, the cause of earcockle of wheat, is virtually extinct in the developed world due to modern seed cleaning. Earcockle was once significant world-wide, but today is a problem only in developing countries (Wiese, 1987). Modern combine harvesting blows the lighter seed galls out of the grain, preventing the nematode from being disseminated by contaminated seed (T. Todd, personal communication). Earcockle was once significant world-wide, but today is a problem in Asia, India, Yugoslavia, and southeastern Europe, parasitizing rye, emmer, spelt, and *Aegilops*, but important only on wheat (Wiese, 1987).

4. Disease Impact

Parasitization by the nematode *A. tritici* causes direct loss in yield either by seed galls alone or by slime disease. Galled wheat is discounted in the marketplace in India. Flour made from galled wheat is inferior in quality (Paruthi and Bhatti, 1990). Slime disease-affected ears fail to yield grain (Vasudeva and Hingorani, 1952).

Losses occur both from slime disease and from earcockle alone. Losses from slime disease may range from 1-2% on average in India (Delhi state) up to 50% (Vasudeva and Hingorani, 1952), range up to 50% in Ethiopia (Hingorani and Bekele, 1969), and up to 50-60% in Pakistan (Akhtar, 1987; 1989). Losses from slime disease of wheat may be 40-50% with partial infection, but high infection may cause a total loss (Paruthi et al., 1987).

Wheat ears completely infected with slime disease or completely infested by *A. tritici* produce no grain. Paruthi et al. (1987) reported that in India, in partially affected ears, grain weight loss was 51% due to *A. tritici* alone, but grain weight loss was 77% due to slime

disease. Grain from infected ears showed reduced germination. Size, shape, and color of the grain were adversely affected. Grain from infected ears was less acceptable in the market, with a 14% reduction in price.

Paruthi and Bhatti (1985) reported yield loss in the field in Haryana (N. India) due to slime disease averaged 2.3%, with a range of 0.33% to 7.06%. Incidence of seed galls (earcockles) in 11 grain markets averaged 34% of samples, with a range of 8% to 52%.

Suryanarayana and Mukhopadhaya (1971) give the following yield reduction estimates for earcockle alone:

% reduction in yield	% of galls in wheat by weight
5	0.01-0.09
30	2-2.9
54	6-6.9
69	8-8.9

5. Symptoms

Initial symptoms are basal swelling of the stem from infection by the nematode (Gupta and Swarup, 1968; Paruthi and Bhatti,1990; Wiese, 1987), and parallel yellow or white streaks along leaf veins (Wiese, 1987). Next, wrinkled, twisted, rolled, curled, or crinkled leaves emerge. This symptom is caused by the nematode. Tillering increases and plants are stunted. If severely infected, a plant may die. Diseased heads are smaller, and may lack awns. Some or all of the kernels are replaced by seed galls. The seed galls, or earcockles, are 2-3 × 3-5 mm, brownish black in color. In appearance, the seed galls are smaller, shorter, and darker than the normal kernels. Seed galls are fragile and easily crushed, and are odorless, unlike the balls formed by common bunt, which have an odor (Wiese, 1987). A seed gall may contain 800-32,000 juvenile nematodes (Suryanarayana and Mukhopadhaya. 1971). These second stage juvenile nematodes are known as infective juveniles.

A bright yellow slime or gum, composed of masses of bacteria, forms on the leaves and on the head. This exudate becomes more liquid in wet weather, but in dry weather it can become hard and dry and can appear as white flecks on the leaves and heads. The hardened exudate causes the emerging leaves and the stalk to become distorted. When more liquid, slime drips onto the lower leaves, onto the ground, and onto nearby uninfected plants (Gupta and Swarup, 1968; Paruthi and Bhatti, 1990; Wiese, 1987). The ear may not emerge, or may be distorted (Jones, 1987). Seed gall symptoms are always seen with slime disease (Wiese, 1987). Later, dried slime may be hard and brittle, and brownish in color (Gupta and Swarup, 1968). Slime may crystallize, and take on a yellow-orange color (Akhtar, 1987). If a head is partly attacked, some grains may form, but they would be of poor quality (Paruthi and Bhatti, 1990).

6. Disease Cycle and Epidemiology

Both nematode and bacterium are required to cause slime disease (Amani, 1969; Cheo 1946; Gupta and Swarup, 1972; Paruthi and Bhatti, 1990; Vasudeva and Hingorani, 1952). Suryanarayana and Mukhopadhaya (1971) cite one report of inoculation of bacteria alone causing the disease (Chaudhuri, H. 1935. Proc. Indian Acad. Sci. 1:579-585), but make the point that no later work has reproduced this result.

Life Cycle of Anguina tritici

The life cycle of *Anguina tritici* begins when infested wheat seed is sown. The seed galls come in contact with moisture in the soil. The walls of the seed gall soften and allow the infective juveniles to emerge into the soil. The juveniles climb to the growing point of the plant and are carried up into the inflorescence as the plant grows. Juveniles at this stage are found in the leaf whorl, in a film of water. There is disagreement as to whether the juveniles feed on the plant at this stage. The juveniles enter the flower primordia and stimulate the production of the seed gall. The juveniles feed endoparasitically on the flower primordia. Juveniles molt in 3-5 days and become adults after galls have completely formed. The male:female ratio is 1:2. The adults mate, and the female begins to lay eggs after 6-10 days, up to 2000 eggs per female. Adults die soon after oviposition is completed. The eggs hatch, then the nematodes molt and become second stage (infective) juveniles (Paruthi and Bhatti, 1990; Suryanarayana and Mukhopadhaya, 1971; Wiese, 1987). Eggs may hatch before the wheat head matures. If gall development is late, normal-looking

kernels may harbor nematodes (Wiese, 1987). Juveniles feeding on the leaf can stimulate the formation of a leaf gall, but this is rare. The juveniles remain in the leaf gall if this happens (Suryanarayana and Mukhopadhaya, 1971). At harvest only second stage juveniles are in galls (Paruthi and Bhatti, 1990). There is one generation per year.

Second stage juveniles resist dessication and can live for many years in a dry seed gall (Paruthi and Bhatti, 1990; Suryanarayana and Mukhopadhaya, 1971; Wiese, 1987). Paruthi and Bhatti (1990) reported that 90% of juveniles were viable after 21 years in seed galls that had been initially dried 5 minutes at 70-80˚ C and kept at low relative humidity (RH) in sealed tubes. Live juveniles have been recovered from seed galls stored for 28 years (Suryanarayana and Mukhopadhaya,1971). Juveniles survive better in dry galls than in soaked galls (Paruthi and Bhatti, 1990).

There is an antagonism between the bacteria and the nematode. Galls heavily contaminated with bacteria on the gall coat have relatively fewer nematodes than galls with little bacteria. (Paruthi et al., 1989; Amani, 1969; Gupta and Swarup, 1972). Sabet (1954) reported that spikes heavily infected with bacteria do not have earcockles.

Clavibacter tritici, Clavibacter rathayi and Slime Disease

Both the nematode and bacteria are necessary to produce disease. Gupta and Swarup (1972) demonstrated that surface-sterilized nematodes produced only earcockle. Surface sterilization was by agrimycin 1% 45 min, streptomycin sulfate 0.1% 30 min, hydrogen peroxide 8% 30 min, sodium hypochlorite 5% 30 min, or mercuric chloride 0.1% 30 min.

Cheo (1947) also earlier reported that both *A*. *tritici* and *C*. *tritici* are necessary to produce slime disease. Inoculation of wheat with *C*. *tritici* bacteria alone did not produce slime disease. Disease occurred when infected seed galls were used as inoculum, but not when pure cultures of nematode and bacteria were inoculated together. Surface sterilization of galls did not prevent disease, suggesting bacteria are carried within the gall, although others believe *C. tritici* probably is carried on the surface of the gall (Bradbury, 1973). Bacteria associated with the galls were still viable after 2.5 years. Over half of the slime-disease-infected plants failed to head.

Sabet (1954) conducted an experiment with *Triticum vulgare*, *T. pyramidale*, *T. durum*, *T. dicoccum*, *Dactylis glomerata*, *Hordeum vulgare* v. *pallidum*, *Hordeum distichum* v. *erectum*,

and *Avena sativa*. (There was no treatment using *C*. *tritici* alone because this had been shown to be ineffective in producing slime disease). Plants were grown in soil inoculated with the following five treatments. Wheat and *Dactylis glomerata* plants also inoculated with suspensions of 1, 2 or 3.

1. *C. rathayi* alone.

2. *C. rathayi* + nematode.

3. Nematodes + slime from wheat plants grown in soil infested with *C. rathayi* + nematode

4. Nematode alone.

5. *C*. *tritici* + nematode.

The results were as follows:

- *A. tritici* + *C. tritici* produced slime disease on all four *Triticum* spp.
- *A. tritici* + *C. rathayi* produced slime disease on all four *Triticum* spp.
- *C. rathayi* was less aggressive on wheat than *C. tritici.*
- Slime disease was produced in wheat grown in soil inoculated with nematodes + slime from wheat plants grown in soil infested with *C. rathayi* + nematodes.
- *C. rathayi* alone did not infect wheat.
- *Dactylis glomerata*, *Hordeum vulgare* v. *pallidum* (barley), *Hordeum distichum* v. *erectum* (2-row barley), and *Avena sativa* (oats) were not infected with either nematode or disease.

Fattah (1988) used, for nematode inoculum either seed galls or an aqueous suspension of second stage juveniles extracted from seed galls. Use of seed galls, probably with *C. tritici* adhering, caused the greatest grain loss. A low incidence of slime disease resulted from using juvenile nematodes in suspension. The lowest level of nematode inoculum produced the highest level of earcockle infection, probably due to a reduction in competition among the juvenile nematodes.

The fungus *Dilophospora alopecuri*, causing twist disease, may be found with *A. tritici* (Bamdadian,1973). Bamdadian (1973b) found that in diseased samples, sometimes *Dilophospora alopecuri* was isolated alone, but often with *A. tritici* and *C. tritici*. Inoculation with the fungus alone did not cause twist disease, but inoculation with fungus and nematode caused infection.

Fig. 3. Nematodes in a seed gall. Photo courtesy of U.C. Davis at http://plpnemweb.ucdavis.edu/nemaplex/Taxadata/G006S4.htm

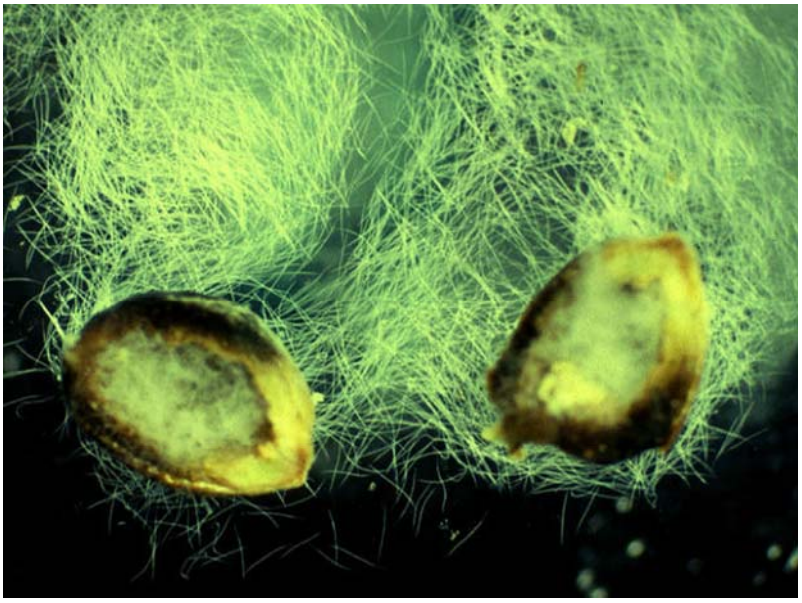


Fig. 4. Seed galls, healthy kernels and ear of wheat. (CIMMYT)

Fig. 5. Wheat plants deformed by slime disease. (CIMMYT)

A. Initial inoculum and infection

Juveniles become contaminated with *C. tritici* in soil. Seed galls and nematodes in slime-disease affected heads are contaminated with the bacterium. The bacterium parasitizes the

wheat when it enters the whorl at the plant apex, vectored by the nematode (Wiese, 1987). The disease is disseminated on seed, in soil, and by contaminated seed galls. Juveniles and cells of the bacterium can remain viable in the seed gall more than 5 years. Bacteria are carried on the surface of the nematodes into the ovaries of the plant (Davis, 2001).

Riley and McKay (1990) reported on the specificity of adhesion of the bacterium to the cuticle of the nematode. Galls from two populations of *A. tritici*/*C. tritici*, from India and Western Australia, were used. *C. rathayi* and *C. tritici* showed strong adhesion to *A. tritici*, which can act as a vector for both species of bacteria. Multiple adhesin/receptor combinations are involved in the association of microbes with the juveniles, and there is a lot of variation in adhesin receptors.

B. Growth stage vulnerability

The plant is vulnerable in early growth stages if both the nematode and the bacteria are present in the soil in which the seed is planted.

C. Conditions that favor disease

Moist soils and low-lying areas favor disease. The disease is usually not a problem on well-drained soils. Disease persists in moist soils with organic matter (Wiese, 1987). Conditions favorable to slime disease are high relative humidity, irrigation, and poor drainage (Paruthi and Bhatti, 1990).

Midha and Swarup (1972) found that the depth of sowing had a significant effect on the incidence of disease. Maximum infection occurred when seed was sown to 2 cm depth; there was less disease if seed was planted deeper. Their estimated population threshold for initiation of infection by nematodes was 10^4 juveniles per 1000 g of soil. Maximum infection of nematodes with seed galls instead of juveniles occurred at 2 galls per kg of soil, which is approximately 2 X 10^4 juveniles. At higher levels of nematodes, infection was decreased, possibly due to competition. Maximum infection with slime disease was achieved with 10^4 X 5 juveniles and a bacterial suspension.

D. Inoculum persistence and dissemination

Bacteria carried by galls can remain viable up to five years (Jones, 1973; Paruthi and Bhatti, 1990). Disease is disseminated as seed galls in seed. Nematodes may spread in soil “to a limited extent” by surface water, infested soil, or on agricultural machinery (Paruthi and Bhatti, 1990). Nematodes do not travel under their own power any significant distance. There was limited survival of nematodes passed through the gut of birds fed seed galls (Paruthi and Bhatti, 1990).

Suryanarayana and Mukhopadhaya (1971) concluded that bacteria survive a limited time in soil. Nematodes free in soil do not survive well, with only limited survival observed after living free in soil six months. Sabet (1954) found that in Egypt survival of the bacteria in soil after one year was negligible.

7. Causal Organisms

Clavibacter rathayi

Aerobic, gram positive, spore-forming rod 0.5-0.8 X 0.7-1.5 µm, mostly single or in pairs, but sometimes in V, W, or Y arrangement, non-motile, not acid-fast, capsules formed. On nutrient agar, colonies are small, lemon yellow, circular, smooth, entire and glistening. Colonies appear in 3-4 days. On PDA, colonies appear sooner and are bright yellow. Minimum temperature for growth is 3˚ C, the optimum temperature is 15- 24˚C (conflicting optima have been reported), and the maximum temperature is 29˚C. Thermal death point is 50-51˚C. *Clavibacter rathayi* differs from *C*. *tritici* in bacteriophage reactions. There is no information on races (Bradbury, 1973).

Clavibacter tritici

Clavibacter tritici is similar to *C. rathayi* except that cultures are less yellow and become orange or dirty yellow with age. Optimum temperature is 24-26.5° C. *C. tritici* differs from *C. rathayi* in not producing acid from mannitol. *C. tritici* reduces nitrate, produces acid from mannose, liquefies gelatin, does not hydrolyze starch, and uses acetate. There is no information on races (Amani 1969: Bradbury, 1973; Wiese, 1987).

A. Culture

Clavibacter rathayi

Bacteria can be isolated from diseased wheat by dilution plating on yeast-glucose-chalk or other agar (Wiese, 1987).

B. Pathogen variability

Little is known about variability in any of these organisms.

C. Toxins

Paruthi and Bhatti (1990) concluded that nothing is known about toxicity in humans and animals from long-term consumption of seed galls, but short term consumption does not appear to cause any problems.

Related nematode/bacteria associations do produce toxins. "Corynetoxins are among the most lethal toxins produced in nature, the product of a unique association between the plant pathogenic bacterium *Clavibacter toxicus* and a bacteriophage (McKay and Ophel, 1993)." Bacteria are carried to seedheads of hosts by *Anguina* spp. Hosts include *Lolium rigidum*, *Agrostis avenacea*, and *Polypogon monspeliensis*. Animal poisonings in Australia were first reported in 1956. In 1968 the bacterium was isolated and identified as *Corynebacterium rathayi* (=*Clavibacter rathayi*). However, further work demonstrated that the bacterium was not *Corynebacterium rathayi*, but a new species, *Clavibacter toxicus* Riley and Ophel 1992. The nematode vectors are *A. funesta* (=*A. agrostis*) and "another Anguina species". (McKay and Ophel, 1993)

8. Diagnostic Methods

Anguina tritici is readily identifiable to the nematologist. *Clavibacter tritici* and *C. rathayi* are readily identifiable to the bacteriologist.

II. Initiating Event (Recognizing an Attempted Introduction)

1. Observation/diagnosis of Presence

Once this disease becomes common it can be readily detected because of the yellowish slime produced by the pathogen. Because it is not aerially disseminated, if it appears in fields in which it has not previously been found, this would indicate that it has been introduced through infected seed or through intentional introduction of inoculum to the production field. Since modern seed production methods can generally remove this pathogen, new infections may be indicative of an intentional act.

2. Interception: Individual/ Pathogen

Contaminated seed galls would be the best inoculum for terrorist to use. A fairly large quantity would be needed. Since *A. tritici* is quarantined, and seed galls have distinctive

appearance, contaminated wheat would either have to be smuggled into the country, or the usual import procedures would have to be by-passed.

3. Intelligence Information

Movement of infected seed stocks into the U.S. would probably be difficult to detect, as would intentional production of infected seed within the U.S. Infections in new fields might be strongly indicative of an intentional introduction.

III. Probable Route of Terrorist Entry/dissemination

Infected seed, or possibly infested soil, would be the likely vehicle for introduction. Introduction of the nematode vector and bacteria to growing plants through an aerosol or other such method would probably result in very low rates of infection. Since the pathogen can be effectively removed from fields within five years, a wide area would need to be infested to have at least a substantial short-term effect.

IV. Probable Distribution

1. Point Introduction:

Since there will likely be essentially no aerial spread of the pathogen within a season, the pattern of infection from an intentional introduction will reflect fairly closely the original pattern of introduction, except for areas in which abiotic factors were not conducive for infection. If the pattern of infection closely follows highways, for example, this might be indicative of intentional introduction.

2. Secondary Dissemination

Secondary dissemination can only occur through movement of seed or soil. If quarantine measures are instituted, dissemination would be limited.

V. Consequences of Introduction (Risk of Pathogen Establishment)

1. Establishment

The pathogen may readily persist in the short-term, especially since inoculum can survive without the host for some time. But modern seed management practices can quickly reduce populations such that establishment in the long-term is unlikely.

A. Climate

Low temperature and high RH favor disease. More detailed information about climatic requirements is a research need.

B. Host Range

Wheat, barley, durum, cocksfoot grass are hosts that would be found in the wheat-growing regions of the U. S.

C. Dispersal

The inoculum, contaminated seed galls, has a low mobility. Human-mediated dispersal would be by the movement of seed galls in grain, or by movement of infested soils. Running water could disperse the inoculum a short distance.

D. Economics

There would be immediate economic costs to the U. S. for quarantine and perhaps compensation to farmers. In the short term, careful cleaning of grain at harvest might result in some costs. In the long term, the disease would be removed by annual combine harvesting for five years.

E. Environmental Impact

Low.

F. Persistence

Seed galls may persist for five years in the field.

2. Over-all risk rating for establishment

Medium

VI. Likelihood of Successful Introduction

1. Quantity of Inoculum Required to Introduce and Establish Damage

Intentional introduction would require both bacterium and nematode. *A. tritici* is a quarantine organism. Seed galls with bacterial contamination would be the best inoculum and a terrorist would need a fairly large quantity.

2. Likelihood of Surviving Initial Introduction

Good, if seed galls are used as inoculum, up to five years.

3. Likelihood of Dissemination Beyond the Point of Introduction

Low-The first season the disease showed up the USDA could impose a quarantine. Because there is no aerial dispersal, a quarantine would probably be quite effective.

4. Likelihood of Alternate Host Infection

The likelihood of spread from agricultural fields to natural systems is probably low unless seed or soil is transferred. Since infection in agricultural fields can be managed to eliminate the pathogen over time, it would probably also be possible to avoid movement of infected materials out of affected fields once the fields are identified.

5. Likelihood of Early Detection

The slime makes the disease easy to spot, except at very low incidence.

6. Overall Risk

Low

7. Likelihood of an Agroterrorist Trying to Use.

Low. The disease is easy to spot and thus easy to defeat using refuse destruction, non-host rotation, and seed cleaning.

VII. Control/mitigation Strategies after Establishment

1. Seed Treatment and Seed Cleaning

Removing seed galls from grain is the most effective way to prevent the spread of slime disease. The consistent use of clean seed is the most effective way to eliminate slime disease from an area.

Seed galls are smaller and lighter than the wheat kernels. Seed galls can be removed by fanning, screening, or flotation (Suryanarayana and Mukhopadhaya, 1971; Kadian and Singh, 1988; Vasudeva and Hingorani, 1952). Although fanning, screening, and flotation are not completely effective, they are cheap and may be useful in developing countries (Paruthi and Bhatti, 1990). Hot water treatment, 54-56^{o} C 10-12 minutes or 10 minutes at 54^{o} C, can be used if done carefully, and seed is checked for germinability (Jones, 1987; Paruthi and Bhatti, 1990; Suryanarayana and Mukhopadhaya, 1971; Wiese, 1987). Brine flotation with 20% brine can remove most galls, but not all (Jones, 1987; Suryanarayana and Mukhopadhaya, 1971; Vasudeva and Hingorani, 1952).

The most effective way to remove seed galls from grain is by the use of seed cleaning machines (Paruthi and Bhatti, 1990; Suryanarayana and Mukhopadhaya, 1971; Wiese,

1987). In the US, mechanical harvesting with modern combines blows the seed galls out of the grain at harvest.

2. Resistance

Paruthi and Bhatti (1990) state that although there are some wheat lines that are resistant to nematodes, “Thousands of genotypes have been screened for resistance to this nematode but successes are few”. Only a few lines of wheat of over 1500 tested were resistant to *A. tritici* (Suryanarayana and Mukhopadhaya, 1971).

3. Cultural Control

Crop rotation:

Wheat followed by rice is effective. Wheat taken out of rotation one or two years reduces nematode infestation (Suryanarayana and Mukhopadhaya, 1971). Since juveniles are released in response to moisture whether or not host crop is present, two years of nonhost cropping will usually eliminate juveniles (Wiese; 1987).

Wet soil and low-lying areas are at greater risk for infection (Wiese; 1987).

4. Chemical Control

Suryanarayana and Mukhopadhaya (1971) state that contaminated seed can’t be disinfected without injuring the wheat, because galls are very resistant to chemicals. Jain and Sehgal (1980), on the other hand, reported that aldicarb sulfone 2 kg a.i./ha as a soil treatment, and also as a soil plus seed treatment, gave good control of slime disease. Soil treatment by Furadane 3 kg a.i./ha gave good control of slime disease.

Paruthi and Bhatti (1990) reported that ethyl parathion spray 0.05% eliminated seed galls.

5. Biological Control

Paruthi and Bhatti (1990) reported that the fungus *Orthobotrytus* was found colonizing juvenile *Anguina tritici* in lab conditions. Not much is known about the possibilities for biological control of slime disease.

6. Modeling Disease Incidence and Spread

Models of disease spread have apparently not been developed for publication. This is probably at least in part because of the lack of aerial dissemination. Spread of this disease would instead depend on how seed and soil are moved.

VIII. Knowledge Gaps

There is a lack of knowledge of how edaphic factors affect severity of disease. More work needs to be done to better understand the environmental factors that influence the interaction between the nematode and the bacterium. The physiological processes in the interaction between nematode and bacterium need more study. Information on the threshold level of inoculum necessary to produce epidemics is needed.

IX. Immediate Response Options

1. Rapid Detection

Extension agents or farmers probably would notice this disease, except at very low incidence. Seed galls alone, without the slime disease, would show up at inspection at the grain elevator. *A. tritici* has been in the U.S. historically, and may be seen at low levels in the U.S. without an introduction from abroad.

2. Control

A quarantine of fields infected with slime disease, along with chemical control and crop rotation, plus the consistent use of clean seed, would probably eliminate slime disease in a few years.

3. Pesticides

On a small scale, for example in seed production fields, nematicides might be employed. For large scale production, nematicides may be too expensive.

4. Resistance Breeding

Sources of resistance are rare. More screening for sources of resistance would be useful.

Appendix 1. Experts knowledgeable about slime disease.Carlson, Randall R

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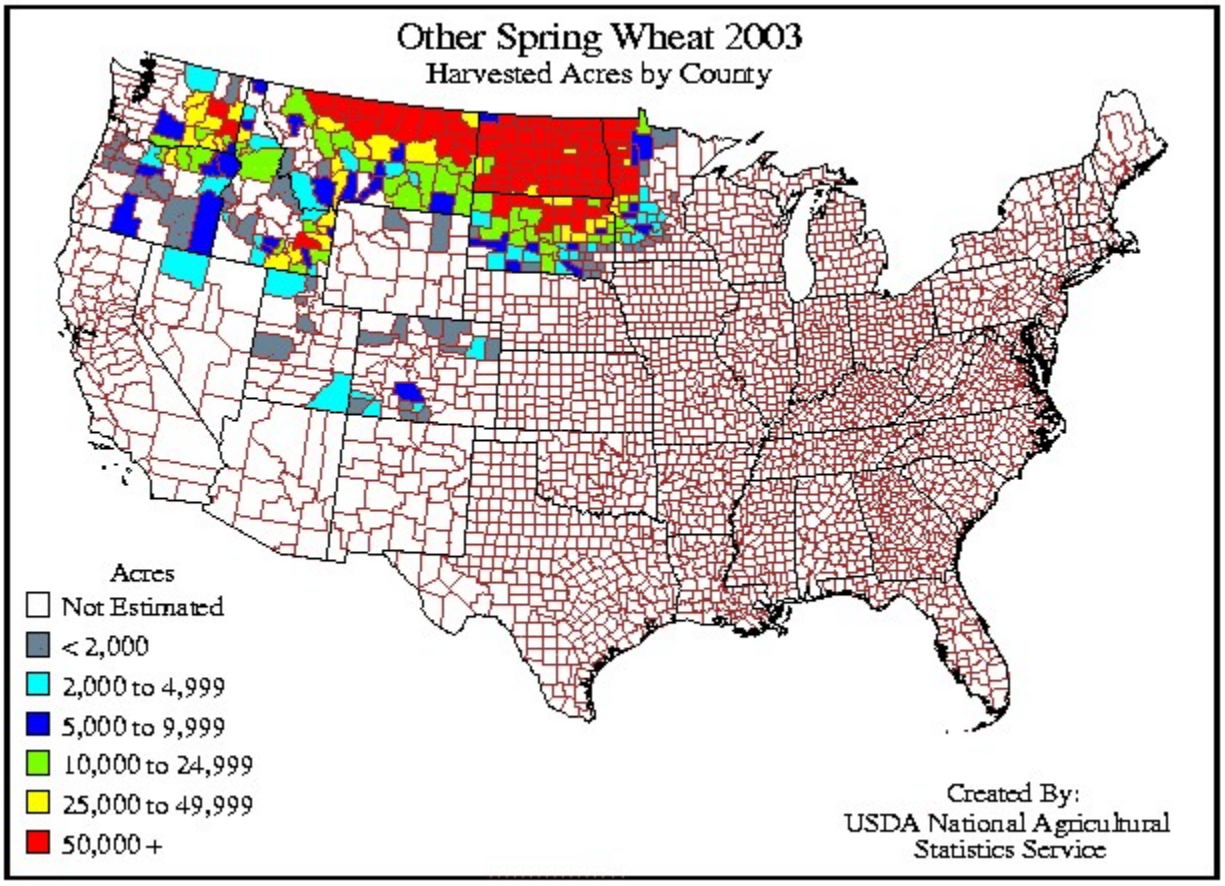
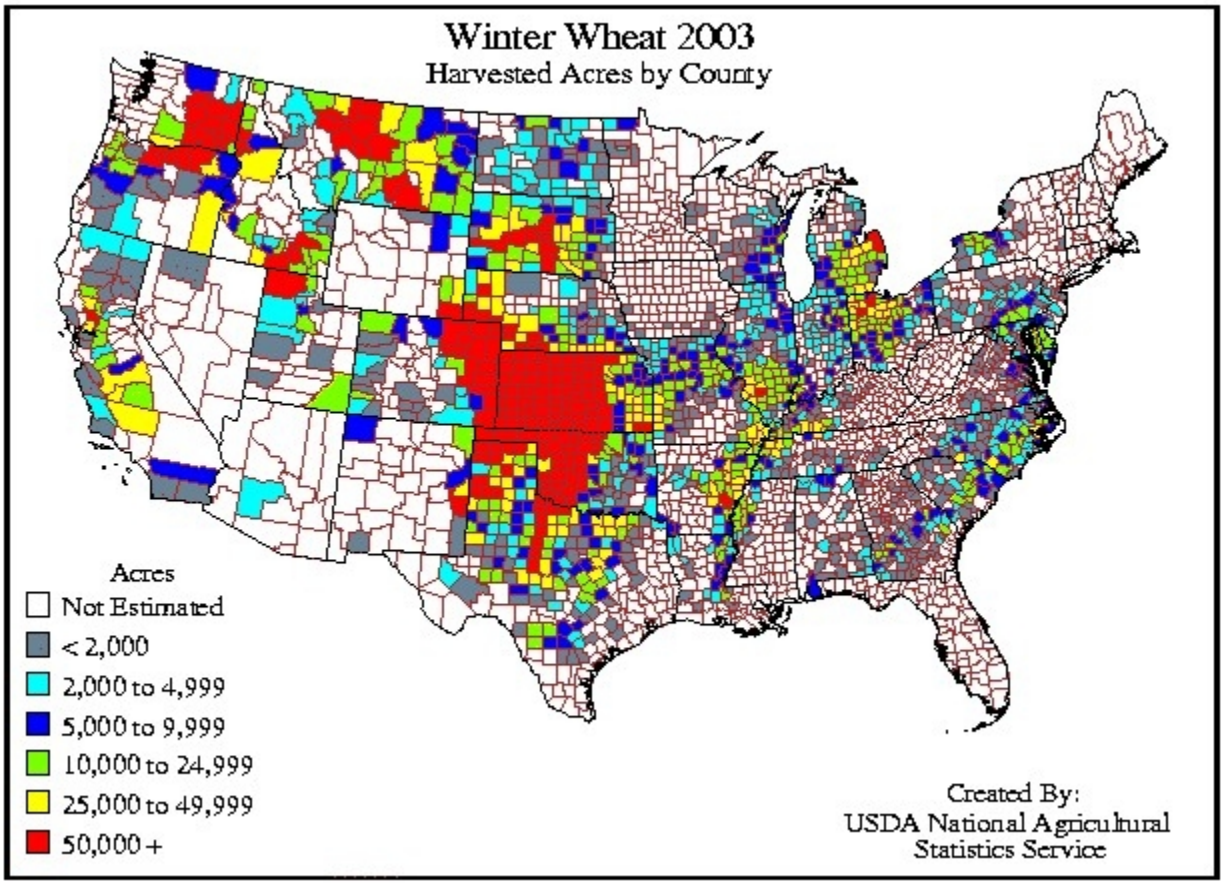
Appendix 2. Map of Spring Wheat Harvested in the U.S. in 2003. (USDA)



Appendix 3. Map of Winter Wheat Harvested in the U.S. in 2003, (USDA)



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Sorghum Ergot

Pathway Analysis:

Intentional Introduction of

Claviceps africana*: Anamorph *Sphacelia sorghi

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Sorghum Ergot Pathway Analysis

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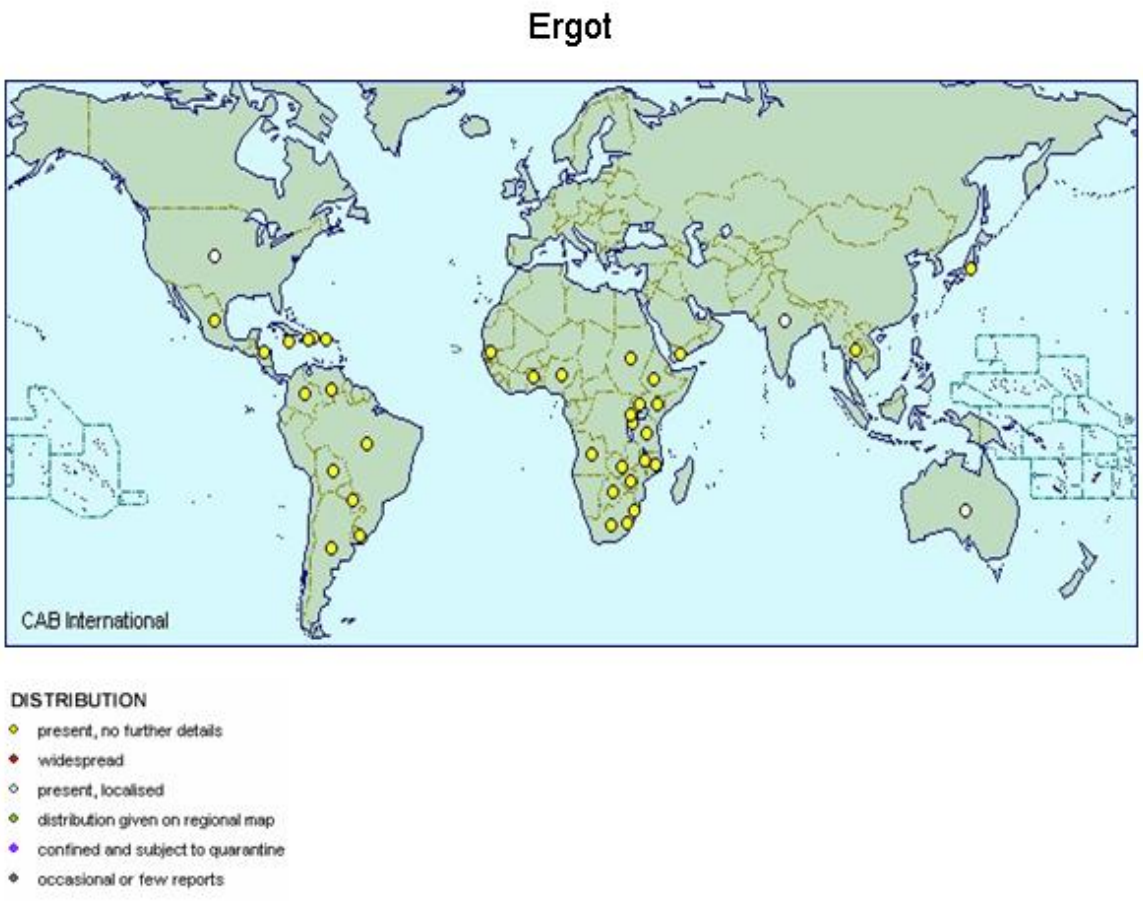
Executive Summary: Sorghum Ergot Pathway Analysis

Sorghum ergot caused by *Claviceps africana* has been detected in the U.S. before, but there is the potential for intentional introduction of this pathogen to produce damaging epidemics. The fungus causes important losses, especially in hybrid seed production, and produces a distinctive honeydew that can spread spores and lower grain quality. Infection must occur during flowering, so an intentional introduction of the pathogen would need to be timed to provide inoculum at that stage of sorghum development and its success would depend on the occurrence of conducive conditions during that time interval. For this reason, sorghum ergot could be a challenging agent for agricultural bioterrorists to develop. Still, a successful introduction over a wide area would have an important economic impact for terrorists willing to wait for a conducive year.

Fig. 1. Map of the distribution of *Claviceps africana.* (CABI)



Sorghum Ergot

Pathway Analysis for the Intentional Introduction of *Claviceps africana*: Anamorph *Sphacelia sorghi*

I. Biology and life/disease cycle of the pathogen

1. Identity

Classification

Kingdom: Fungi

Phylum: Ascomycota

Class: Pyrenomycetes

Order: Hypocreales

Genus: Claviceps

Species: Teleomorph is *Claviceps africana* Frederickson, Mantle and de Milliano 1991

Anamorph is *Sphacelia sorghi* McRae 1917

Related organisms

Four species of fungi in the genus *Claviceps* infect species of *Sorghum*, causing sorghum ergot diseases:

Claviceps africana Frederickson, Mantle and de Milliano 1991

Claviceps sorghi Kulkarni, Seshadri and Hedge 1976

Claviceps sorghicola Tsukiboshi, Shimanuki and Uematsu 1999

Claviceps pusilla Cesati 1848

In spite of some differences in conidium morphology and the fact that only *Claviceps africana* produces secondary conidia, *Sphacelia sorghi* McRae is the anamorph of both *Claviceps africana* and *Claviceps sorghi* (Pažuotová et al., 2000; Ryley et al., 2002). *C. africana*, *C. sorghi*, and *C. sorghicola* differ in teleomorph morphology. The sclerotia of *C. africana* are small and rounded, and do not protrude much, but the sclerotia of *C. sorghi* are elongated and protruding (Tsukiboshi, Shimanuki and Uematsu 1999). The imperfect stage of *C. sorghicola* is also *S. sorghi* (Bandyopadhyay et al.,1998). Conidia of the imperfect stage of *C. sorghicola* are much smaller than those of *C. africana* (Pažuotová, et al. 2000). Recent molecular studies support the taxonomic standing of *C. africana*, *C. sorghi*, and *C. sorghicola* as distinct species (Ryley et al., 2002). See Appendix 3 for descriptions of the three species.

2. Hosts

See table 1. for hosts of *Claviceps* spp. causing ergot on sorghum.b*Sorghum bicolor* (L.) Moench is the main commercially important host. There are several reports of collateral hosts of *C. africana*, but not all hosts have been verified by inoculation (Bandyopadhyay et al., 1998). More information is needed on the life cycle of *C. africana* on collateral hosts during the non-growing season of sorghum (Bandyopadhyay et al., 1998; Ryley et al., 2002).

Table 1. Hosts of *Claviceps* spp. Causing ergot on sorghum.

Host	Pathogen(s)	Reference
Sorghum bicolor Cultivated sorghum	*Claviceps africana* *Claviceps sorghi* *Claviceps sorghicola*	Ryley et al., 2002
Sorghum almum Columbus grass	*Claviceps africana*	Ryley, et al., 2002

Sorghum halpense Johnson grass	*Claviceps africana*	Torres-Montalvo and Montes-Garcia, 1999 Ryley, et al., 2002 Ramundo et al., 1999
Sorghum drummondii *Sorghum virgatum* *Sorghum arundinaceum*	*Claviceps africana*	Ramundo et al., 1999
Pennisetum glaucum Pearl millet	*Claviceps africana*	Torres-Montalvo and Montes-Garcia, 1999 Ryley et al., 2002 Ramundo et al., 1999

Inoculum of *Sphacelia sorghi* from infected sorghum produced infection in sorghum and millet (*Pennisetum typhoides*). Inoculum from *Panicum maximum* (Common guinea grass) also produced infection in sorghum and millet (Futrell and Webster, 1966). *Paspalum notatum* (Bahia grass) is also reported to be a host of *S. sorghi* (Futrell and Webster, 1966). In a greenhouse inoculation experiment, *Sorghum halpense*, *Pennisetum glaucum*, *Sorghum drummondii* (Sudan grass), *Sorghum virgatum*, and *Sorghum arundinaceum* were found to be hosts of *C. africana*, while *Eleucine coracana* (Finger millet), *Panicum miliaceum* (Proso millet), *Setaria italica* (Foxtail millet), *Zea mays* (Maize), and *Sorghum verticilliflorum* (Wild Sudan grass) were found not to be hosts of *C. africana* (Ramundo et al., 1999).

Bandyopadhyay (1992) has a long comprehensive list of hosts and non-hosts of *S. sorghi*, but this list does not list the hosts of *C. africana* separately from those of *C. sorghi*. Hosts of *Sphacelia sorghi* are: *Sorghum arundinaceum*, *S. caffrorum*, *S. halpense*, *S. membraceum*, *S. nitens*, *S. verticelliflorum*, *Pennisetum orientale*, *P. typhoides*, *Cenchrus setigerous*, and *Ischaemum pilosum*. Common names of *Cenchrus* are sandbur, birdwood grasses, and buffel grass.

Sangitrao et al. (1999) give an extensive reference list for various hosts of *S. sorghi* cited in literature at that time. They do not list the hosts of *C. africana* separately. They list the same hosts of *S. sorghi* as does Bandyopadhyay (1992) and add *Cenchrus ciliaris*, although they state there are conflicting reports. They list *Dichanthium caricosum*, which is found in India and SE Asia, as a host. Common names of *Dichanthium caricosum* are roadside bluestem, nadi bluegrass, angleton grass, and antigua haygrass. They state there are conflicting reports on *Pennisetum typhoides* as a host.

C. africana has been reported to infect *Sorghum almum, Sorghum halpense*, and *Pennisetum glaucum* (pearl millet), and at least seven native grasses in Australia (Ryley et al., 2002).

3. Geographic Distribution and History

See table 2. for the chronology of reports of occurrence of ergot on sorghum. Before the mid 1990's, sorghum ergot was found only in Africa and India, but now it is found on all continents (Ryley et al., 2002). *C. africana* is the "most widespread" of the four species of *Claviceps* causing sorghum ergot (Ryley et al., 2002). *Claviceps sorghi* has been reported only in India. (Bandyopadhyay et al., 1996; Ryley et al., 2002), but some isolates from India assumed to be *C. sorghi* were later found to be *C. africana* (Bogo and Mantle, 1999; Ryley et al., 2002). It is possible that the start of *C. africana* infections was in the mid-1980s when sclerotia of different sizes were observed. Genetic analysis of some isolates from India supports this conclusion. An analysis of the population structure of *Claviceps* is needed in India. (Pažuotová et al., 2000). *Claviceps sorghicola* has been reported only in Japan (Tsukiboshi et al., 1999; Ryley et al., 2002).

Spread of *Claviceps africana*: *C. sorghi* was first reported in India in 1915. In Africa, it was reported first in Kenya in 1924, although it is now widely distributed in Africa. *Claviceps africana* was recognized as a separate species in 1991. *C. africana* was reported in Brazil in 1995, then in Australia and in many South American countries in 1996, then in Central America, including Puerto Rico and Mexico, in 1997 (Bandyopadhyay et al., 1998).

In Mexico, in early 1997, sorghum ergot was found on rattooned plants, on volunteers, and in commercial field and seed production fields. Grain hybrids and forage sorghum were affected. Nurseries with A-lines (male-sterile) and R-lines (restorer) suffered damage. *Cerebella* was reported with the ergot. The highest rates of infection were seen in male-sterile and forage lines (Torres-Montalvo and Montes-Garcia, 1999).

The first report in the U.S. was in Texas in 1997. By the end of 1997, *C. africana* had been seen in Georgia, Kansas, Nebraska, and Mississippi. Sorghum ergot remains a threat to sorghum hybrid seed production and commercial sorghum production in Kansas, Nebraska, and South Dakota (Bandyopadhyay et al., 1998).

In April 1996, sorghum ergot was discovered in southern Queensland, Australia, and within three weeks was found in northern Australia (Ryley et al., 2002). It is possible sorghum ergot had been in Australia for a number of years before 1996 at "very low levels due to drought conditions," before becoming evident in a year with weather more conducive to the appearance of the disease (Ryley and Henzell, 1999). Most infections in Australia are caused by *C. africana* (Ryley et al., 2002).

Table 2. Chronology of reports of occurrence of ergot on sorghum.

year	species	Country or region	Reference
1915	*C. sorghi*	India	McRae, 1917 Bandyopadhyay et al., 1998
1924	*C. sorghi*	Kenya	Bandyopadhyay et al., 1998
1963-1965	*S. sorghi*	Nigeria	Futrell and Webster, 1966
1988	*C. africana*	Thailand	Frederickson et al., 1991 Bandyopadhyay et al., 1998
1991	*C. africana*	Japan	Bandyopadhyay et al., 1998
1995	*C. africana*	Brazil	Reis et al., 1996 Bandyopadhyay et al., 1998
1996	*C. africana*	Brazil, Argentina, Bolivia, Paraguay, Uruguay, Colombia, Venezuela, Honduras	Bandyopadhyay et al., 1998
1996	*C. africana*	Australia	Ryley and Henzell, 1999 Bandyopadhyay et al., 1998
1997	*C. africana*	Puerto Rico, Haiti, Dominican Republic, Jamaica, Mexico	Velasquez-Valle et al., 1998 Torres-Montalvo and Montes-Garcia, 1999 Bandyopadhyay et al., 1998
1997	*C. africana*	Texas, Georgia, Kansas, Nebraska, Mississippi	Isakait et al., 1998 Zummo et al., 1998 Bandyopadhyay et al., 1998
1998	*C. africana*	India	Bogo and Mantle, 1999

4. Disease Impact

Since the 1960s, the use of F_1 hybrids has dramatically increased sorghum productivity to about 3-5 tons per hectare, while "low-input" systems average less than 1 ton per hectare (Bandyopadhyay et al., 1998). Sorghum ergot directly affects the production of F_1 hybrid

seed by replacing the ovary with sphacelia. A sphacelium (pl. sphacelia) is a white to gray mass of spore-producing fungal mycelium.

The greatest impact of the disease is on the production of hybrid seed. The spread of sorghum ergot has had a large impact on the international seed trade (Bandyopadhyay et al., 1998). Male sterile lines used in seed production are at particularly high risk, especially if flowering is not synchronous. Forage sorghums and lines that exhibit uneven flowering are also at higher risk (Ryley et al., 2002)**.**

There have been serious economic impacts on the seed industry since sorghum ergot appeared in Australia in 1996 (Bandyopadhyay et al., 1998). In India, in hybrid seed production fields, losses of 10-80% have been reported. In Zimbabwe, losses of 12-25% have been reported (Bandyopadhyay et al., 1998).

Another negative impact, aside from the direct loss of grain, is the production of honeydew by infected panicles. Honeydew drips onto the uninfected parts of the panicle, contaminating them with spores and making them sticky. The sticky panicles make it more difficult to harvest the crop. Fungal saprophytes grow in the honeydew on the grain, lowering grain quality (Bandyopadhyay et al., 1998). Grain heavily contaminated with honeydew has a lower germinability, and dried honeydew also interferes with commercial seed dressing (Bandyopadhyay et al.(1998). Dried honeydew contaminates harvest equipment, and grain storage and transport vehicles. Increased costs of seed production, increased costs at harvest, reduced seed yields are losses associated with sorghum ergot. In addition, sorghum ergot is a disease of quarantine importance, adversely affecting international trade (Bandyopadhyay et al., 1998).

Cerebella volkesii, a fungal saprophyte, typically lives in the honeydew. It forms a characteristic black mass , which can be used to spot grain infected with *C. africana* (Bandyopadhyay et al., 1982; Bandyopadhyay et al., 1998). *Fusarium* spp. and *Cladosporium* spp. can also be found in honeydew. Since some *Fusarium* spp. produce toxins, e.g. fumonisins, this is a concern.

5. Symptoms

Usually the first sign of the disease is the appearance of honeydew on infected panicles. Honeydew may be viscous or thin, and is transparent at first. Later, honeydew becomes

pink or yellow brown. It dries to a hard mass, but in rainy conditions or under high humidity may become more liquid again. At high relative humidity (RH), a white scum-like covering develops as secondary conidia are produced on the surface of the honeydew droplet (Bandyopadhyay et al., 1998). The growth of *Cerebella* as black, convoluted globose masses may be observed on honeydew-affected panicles or in honeydew itself.

Sphacelia develop between the glumes of the infected florets. A few to all ovaries in a panicle may be colonized. Sphacelia may be found one day before honeydew is produced (Bandyopadhyay et al., 1998). Sclerotia appear between the glumes within four weeks (Bandyopadhyay et al.,1982). Sclerotia of *C. africana* do no protrude from the glumes as much as those of *C. sorghi*. *C. sorghi* have shorter sclerotia; *C. africana* have longer sclerotia. See Appendix 2 for descriptions of *C. africana*, *C. sorghi* and *C. sorghicola*.

Fig. 2. Head of sorghum with ergot and honeydew. Dumas, Texas, September 1997. Photo courtesy of Gary Odvody.

Fig. 3. Honeydew droplets. These droplets have a high water content, and are opaque due to high numbers of conidia of *C. africana.* Weslaco, Texas, May 1997. Photo courtesy of Gary Odvody.

Fig. 4. Sorghum head with ergot. The black structures are *Cerebella.* Liquid droplets of honeydew are visible, as are two white hardened crystalline honeydew droplets. Williamson Ranch near Tampico, Mexico, March 1997. Photo courtesy of Gar Odvody.

6. Disease Cycle and Epidemiology

Claviceps africana attacks only unfertilized ovaries, replacing them with fungal sphacelia, and later, fungal sclerotia. A few to all unfertilized ovaries may be colonized. A sphacelium is a spore-bearing fungal mass, a white to gray mass of fungal mycelium, taking the place of the ovary in the floret. A sclerotium is a hard, dark, fungal mass (Bandyopadhyay et al., 1998). Sclerotia appear within four weeks after infection. It appears that sclerotia do not play a role in the spread of the disease (Bandyopadhyay et al., 1982). The role of collateral hosts in the spread of the disease is not confirmed (Bandyopadhyay et al., 1992).

There are three types of spores produced by *Claviceps africana*. All three types are single-celled and hyaline. The three types are the oblong to oval macroconidia, spherical microconidia, and the pear-shaped secondary conidia.

Fig. 5. Macrocondidia of C. africana from ergot honeydew. Photo courtesy of Gary Odvody.

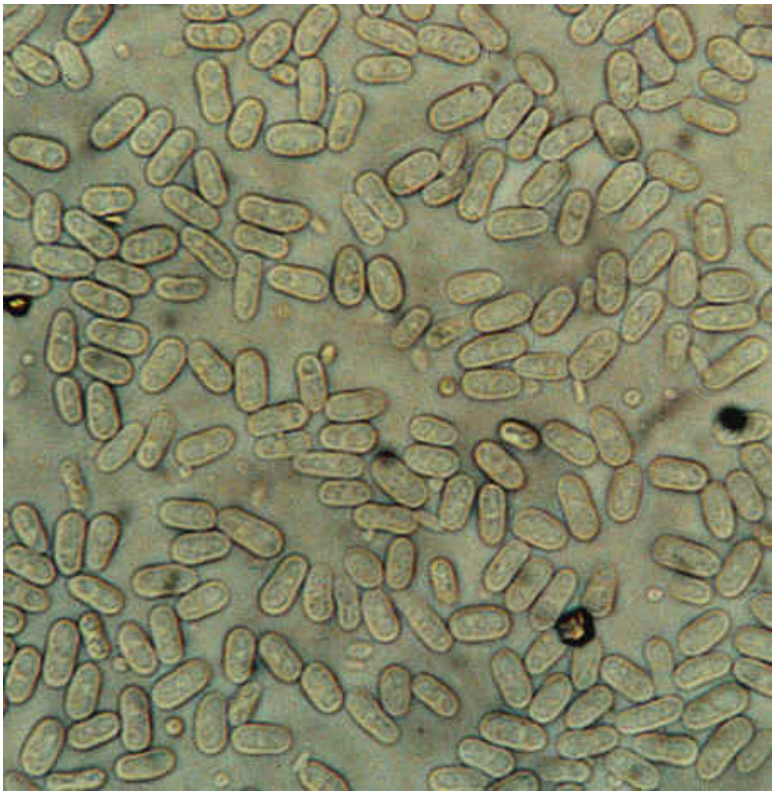
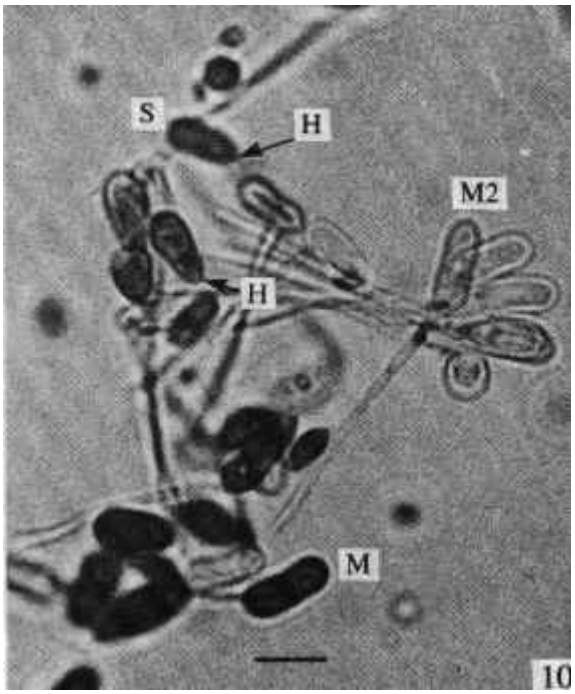


Fig. 6. Germinating macroconidia of Claviceps africana. Photo courtesy of D. Frederickson.



A. Initial Inoculum and Infection

i. Inoculum

The primary inoculum is conidia (usually macroconidia and secondary conidia) from dried honeydew. Any role in infection of ascospores of *C. africana* has not been confirmed.

ii. Infection

C. africana conidia, including secondary conidia, need a minimum 15 h for germination and 36-48 h for infection, for hyphae to reach base of ovary (Bandyopadhyay et al., 1996; Ryley et al., 2002). "The stigma is the principal site of infection although conidia ...can infect through the style and ovary wall." (Bandyopadhyay et al., 1982). The timing of highest infection (= 95%) is with inoculation four days before anthesis. Inoculation at anthesis caused 32% infection (Bandyopadhyay et al., 1998; Ryley et al., 2002). Honeydew is seen 7-11 days after infection (Ryley et al., 2002).

iii. Pollen-conidium interactions

Completed pollination leads to protection from infection in most cases (Bandyopadhyay et al., 1996; Ryley et al., 2002). Infection takes up to 96 h, but pollination takes 2.5-12 h (pollen tube growth). The mechanism of resistance after pollination is not known. There is a linear relationship pollen viability and ergot severity (McLaren and Flett, 1998; Ryley et al., 2002). There are genetic differences in pollen production amount (Ryley et al., 2002).

iv. Three types of spores

C. africana produces three types of conidiospores. All three are hyaline and mononucleate, and can infect the gynoecia, although microconidia are generally not important in infection. Macroconidia are oblong to oval. Secondary conidia are pear-shaped and are formed by iterative germination of the macroconidia when relative humidity (RH) is high. Microconidia are small and spherical. Secondary spread is by secondary conidia.

Macroconidia

Conidiophores on the surface of the sphacelium release macroconidia into the honeydew (Ryley et al., 2002). Macroconidia near the surface of the honeydew droplet send up conidiophores (sterigmata), which produce secondary conidia.

Conditions for the germination of macroconidia include a temperature range of 14-35°C, with the optimum ~19C (Ryley et al., 2002). High sugar concentrations inhibit germination of the macroconidia. (Ryley et al., (2002) reported that highest germination at 7% sucrose in the honeydew, although germination occurred at 44%

Secondary conidia

Macroconidia germinate and produce sterigmata which rise above the honeydew surface and produce secondary conidia. The mat of germinating macroconidia, sterigmata and secondary conidia can form a whitish mat on the surface of the honeydew droplet.

Secondary conidiation in *C. africana* occurs at a range of 14-30°°C when humidity is high (Ryley et al., 2002). Rainfall stimulates sporulation of secondary conidia, by reducing sugar concentration and raising humidity (Frederickson et al., 1993; Ryley et al., 2002). More secondary conidia are produced at nightfall with a rise in humidity, although the sorghum panicle may need to dry off first in order for the secondary conidia to become apparent (Frederickson and Mantle, 1989; Frederickson et al., 1993; Ryley et al., 2002)

Microconidia

Microconidia are produced by the sphacelia and are shed into the honeydew. They are spherical, hyaline, and mononucleate. Microconidia are generally not important in the spread of the disease.

v. Sclerotia

The sphacelium gradually develops into a sclerotium. Sclerotia of *C. africana* typically are dormant for a period after formation, usually 3-4 months dormancy in India. Sclerotia of *C. africana* do no protrude as much as do those of *C. sorghi*, because *C. sorghi* have shorter sclerotia than *C. africana*. See Appendix 2 for descriptions of *C. africana*, *C. sorghi* and *C. sorghicola*.

vi. Sexual stage

In *Claviceps*, the sclerotium germinates and forms stromata. The perithecia are embedded in the stromata. Ascospores are produced in the perithecium. The role of the sexual stage of *Claviceps africana* in sorghum ergot disease is not clear. *C. africana* is very difficult to germinate in the lab, and the frequency of germination in nature is not known (Ryley et al., 2002).

B. Growth stage vulnerability

The period of greatest vulnerability is before pollination. A fertilized ovary is protected from infection. Anthesis is a vulnerable stage.

C. Conditions that favor disease

Delayed pollination and favorable weather conditions

Delayed pollination increases risk of disease, as does asynchrony of flowering. Male-sterile lines, grain sorghum, and forage sorghum crops with uneven flowering are at high risk of disease if the weather is conducive (Ryley et al., 2002).

A mean temperature of 20° C during flowering is favorable for the development of disease (Frederickson et al., 1993; Ryley et al., 2002). Over 28° C, there is practically no disease (Ryley et al., 2002). Humidity near 100% for 24 h is optimal for infection (Futrell and

Webster, 1965; Ryley et al., 2002). At 100% RH for 2 h, male sterile plants can be infected using a mixture of macroconidia and secondary conidia. Infection may, however, occur at less than 100% RH (Ryley et al., 2002).

McLaren and Wehner (1990) found that cool wet weather favors disease, with 19.5° C as the optimum for disease increase. There is a sharp rise in disease as the temperature drops below 32° C. They found that temperatures below 13° C two weeks prior to flowering led to an increase in disease. McLaren (1997) concluded that genotypes vary in sensitivity to low temperatures in pollen viability reduction (cold stress).

In inoculation trials, cited in Ryley et al. (2002), infection was increased by temperatures below 28° C 1-5 days before pollen shed. There was a significant positive correlation between RH and ergot severity. There were significant correlations with maximum temperature, RH, and hours of sunshine. Cold weather with a minimum temperature less than 12° C 3-4 weeks before flowering caused male sterility by lowering pollen production. There was a correlation between the mean minimum temperature 3-4 weeks before flowering and pollen viability, and between the mean minimum temperature 3-4 weeks before flowering and ergot incidence. In inoculation trials, in male-normal lines in South Africa, the highest correlation was between disease incidence and mean minimum temperature 23-27 days before flowering, and between disease incidence and maximum temperature and maximum RH 1-5 days after flowering. In Mexico, there was a correlation between ergot severity and low temperature 13-15 days before 50% flowering and 10-12 days before flowering for early- and medium-maturing genotypes. Studies cited in Ryley et al. (2002) of the effect of temperature on ergot incidence and sorghum male sterility in South Africa, Australia, Australia, and Mexico, used inoculated, not natural infections. Differences in results of these studies may be because of differences in host or pathogen genotypes, differences in inoculation protocols, and differences in calculating the thermal units used.

Low temperatures can cause male sterility in sorghum. Ryley et al. (2002) concluded that there is a linear relationship between night temperature and pollen sterility. Wang et al. (2000), however, did not find any correlation between pollen viability, or ergot severity, and mean minimum temperature at flag leaf (= beginning of leptotene phase of pollen development). Sorghum genotypes differ in response to cold (Ryley et al., 2002).

Ryley et al. (2002), in their own trials in Australia, found that low temperature and high humidity during flowering led to the highest disease severity. The best correlation was between mean hourly temperature and ergot severity 12-18 days before 50% flowering.

D. Inoculum persistence and dissemination

i. Persistance

The role of sclerotia in the spread of disease is not clear. Locules in sphacelia may play a role in dissemination. As the tissue of the sphacelia wears away, macroconidia are exposed (Ryley et al., 2002).

The role of other hosts of in the persistence and dissemination of *C. africana* is not clear. Ryley et al. (2002) state that in Australia, *C. africana* may live on other hosts, and survive the winter in this fashion. In N. Australia, honeydew was observed on *Sorghum almum* Parodi and *Sorghum halpense* (L.) Pers., and on forage sorghums.

Macroconidia in panicles buried in soil remained viable less than 2 months. There was significant survival of macroconidia in infected heads (40% survival) stored at 1 m above soil after four months, with mean low temperature during the four months of 6.7^{o} C. Macroconidia lose viability quickly in summer. Macroconidia on seed retained some viability after 14 months at 4 ^{o} C. (Ryley et al., 2002). Survival of macroconidia under natural conditions in Mexico and Kansas showed that under winter conditions in Kansas, survival of conidia declined rapidly (Claflin and Ramundo, 1999).

ii. Dissemination and secondary spread

Natural

Macroconidia in honeydew can be dispersed by dripping or running water. Secondary conidia are air-borne, and may blow some distance locally. Frederickson et al. (1993) conducted extensive tests on the aerial dispersal of secondary conidia, and showed that airborne conidia can be an important source of inoculum. Macroconidia in honeydew can be dispersed by insects (Ryley et al., 2002).

Human-mediated

Honeydew containing macroconidia can contaminate seed, machinery, clothing, or vehicles (Ryley et al., 2002). Movement of contaminated seed, machinery and vehicles can spread the inoculum. Inoculum can be carried on the clothes and shoes of workers.

7. Causal organism

A. In culture

Macroconidia of *C. africana* will germinate on sorghum extract agar at 8-31^{o} C. Macroconidia and secondary conidia have a similar optimal temperature for germination, 19.5 ^{o} C, but after 48 h 30% of secondary conidia germinated at 37 ^{o} C. Median germination time for macroconidia was 5.9 h, secondary conidia 8.8 h in Australian isolates . Frederickson (1991) found that macroconidia germinated after 12 h at 14^{o} C and 35^{o} C , and secondary conidia after 15 h at 24^{o} C (references in Ryley et al., 2002). Asparagine-sucrose-salts medium is suitable for to culturing *S. sorghi* (Bogo and Mantle, 1999)

B. Pathogen variability

Ryley et al. (2002) speculate that since *C. africana* strains from India and Australia are molecularly similar, some genotypes may have evolved on native grasses in Australia. Sorghum ergot was probably introduced to Australia several times from different sources (Pažuotová et al., 2001; Ryley et al., 2002). Komolong et al., (2002) believe, based on genetic analysis of 65 isolates of *Claviceps* spp., *Claviceps africana* from Australia, India, Puerto Rico, Japan, U.S., *Claviceps sorghicola* from Japan, and *Claviceps pusilla* from Australia, that there were more than one or two introductions of *C. africana* to Australia.

Tooley et al. (2002) used amplified fragment length polymorphism (AFLP) comparisons among 87 *C. africana* isolates from the U.S., Mexico, Africa, Australia, India and Japan. These were single spore isolates produced from sorghum infected with ergot. They found two general geographic associations, with the U.S., Africa and Mexico in one group and India, Australia Japan in the other. Komolong et al., (2002) found 4 genotypes of *C. africana* in 50 Australian isolates. Some strains were similar to American strains.

Pažuotová, et al. (2000) analyzed 28 isolates of *C. africana* from the U.S., Mexico, Puerto Rico, Bolivia, Australia, India, and South Africa. They used random amplified polymorphic DNA (RAPD) and sequencing of internal transcribed spacer (ITS) analyses. They

condluded that *C. africana* nucleotide sequences of *C. africana* isolates differed from those of *C. sorghi* and *C. sorghicola.* Of 100 primers, 65 produced species-specific patterns. Use of 7 primers discriminated 4 groups: 1) American, some African, 2)some other African, 3)Indian , and 4) Australian. They concluded that *C. africana* is present in India. Identical ITS1 sequences were observed in some isolates from Bolivia, Australia, and India.

Scott et al. (2000) describes a method of obtaining DNA from *Claviceps*, sp., particularly *C. africana*, suitable for PCR.

C. Toxin production

Claviceps africana produces alkaloids, primarily dihydroergosine (DHES) (Ryley et al., 2002). In Australia, feed containing DHES resulted in agalactica in cows and sows (Blaney et al., 2000). Reduced weight in broiler chickens and beef cows has been reported (Bandyopadhyay, 1998; Ryley et al., 2002).

Bailey et al. (1999) conducted a feeding trial on broiler chickens. Chickens showed reduced weight gain after more than 3 weeks of feeding of 11.3 ppm total alkaloids versus controls. Usually *C. purpurea* is the problem in chicken feed with safe dietary concentration of ergot 0.3-0.8%. *C. purpurea* produces ergotomine and ergocristine. *C. africana* produces primarily dihydroergosine. Bailey et al. (1999) concluded that *C. africana* in feed is slightly toxic to chickens.

McLennan et al. (2001) conducted a feeding trial on Hereford cattle in feedlots in Australia. They concluded that even low concentrations of dihydroergosine (DHES) can impair performance of cattle in feed lots. There were two experiments, one in Summer/Autumn, and one in Winter/Spring. See Figure 7.

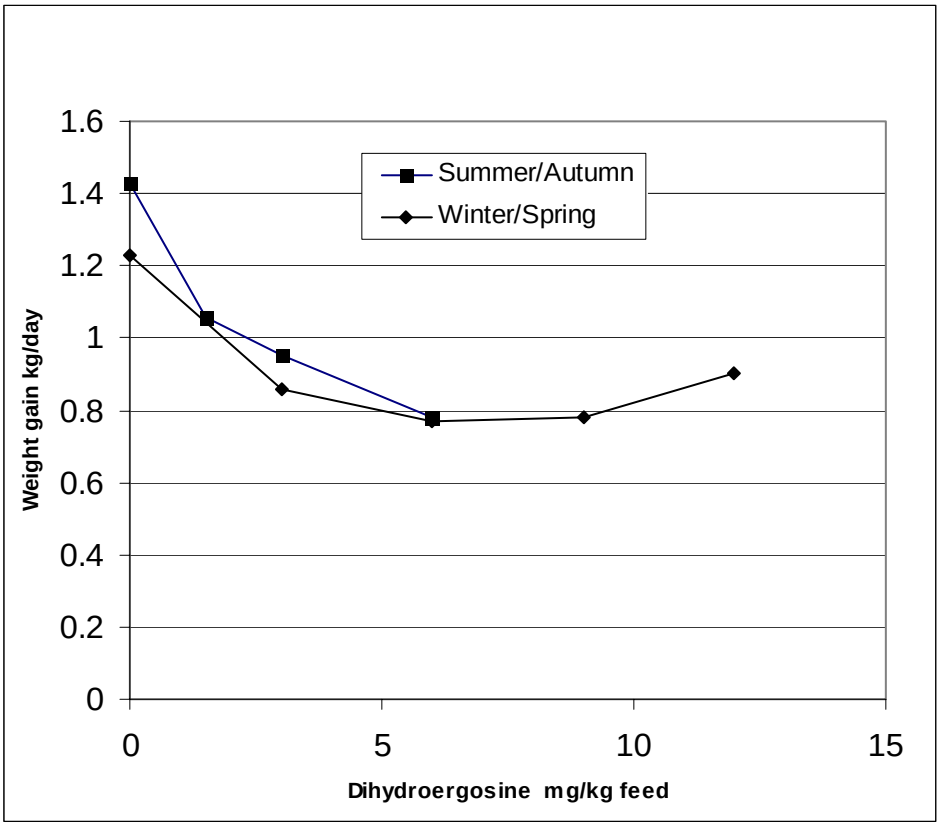
Blaney et al. (2000) concluded that sorghum ergot can cause inhibition of milk production similar to that caused by *C. purpurea*.

Differences in feeding effects in various studies suggests variability in toxic alkaloid production (Ryley et al., 2002).

Table 3. Toxins produced by *Claviceps* spp.

Species	Toxin	Reference
Claviceps africana	dihydroergosine (principal toxin) festuclavine pyroclavine dihydroelymoclavine	Baily et al., 1999
Claviceps purpurea	ergotomine ergocristine	Baily et al., 1999
Claviceps fusiformis	agroclavine	Bandyopadhyay et al., 1982

Fig. 7. Performance of cattle in feedlots. (Drawn from data in McLennan et al. 2001)



8. Diagnostic Methods

A. Seed health tests

Imported seed should be examined for contamination by honeydew.

B. Distinguishing C. africana from related organisms

Morphology of the stipe, asci, ascospores of teleomorph, maconidial size anamorph, and production of dihydroergosine in *C. africana*, and production of secondary conidia in *C. africana* are all used to distinguish *C. africana* from other *Claviceps* spp. that cause sorghum ergot (Ryley et al., 2002). For practical purposes, the production of secondary conidia is a distinguishing factor.

II. Initiating event (recognizing an attempted introduction)

1. Observation/diagnosis of presence

The disease is quite distinctive, but may not be obvious in the field if incidence is low. Once honeydew appears, a diagnosis can be made very quickly. Since the disease has already appeared in the U.S., growers and extension agents will probably recognize the disease.

2. Interception: individual/ pathogen

The fungus can be cultured, but releasing the spores alone would take quite a lot of inoculum. Contaminated seed would be the most effective way to introduce the pathogen, and then the weather would have to be conducive to disease for an outbreak to occur. Vial-in-pocket probably would not be a useful way for a terrorist to bring the pathogen into the country. Since the disease has occurred in the U.S., it might be possible to obtain a culture of the pathogen within the U.S.

Someone who could grow a quantity of sorghum and inoculate it with the pathogen, perhaps in the controlled environment of a greenhouse, could use a small culture of the pathogen to produce a quantity of contaminated seed.

Quantities of contaminated seed could be smuggled into the U.S. by the same methods used by narcotics smugglers. Since the disease has already occurred in the U. S., it probably would not be necessary for a terrorist to obtain inoculum abroad, although finding a large quantity of contaminated grain would be easier in areas where the disease is a serious problem. Then the contaminated seed would have to be introduced into the fields before flowering. If the weather did not co-operate, it is likely that no outbreak would occur.

3. "Intelligence" information

Information about attempts to bypass grain import inspections would be considered worthy of immediate follow-up.

Preparation of large quantities of inoculum of sorghum ergot might require incubation equipment that would not be commonly owned by nonscientists. Likewise, if greenhouses are used for reproduction of the pathogen on sorghum plants in a controlled environment, this might be detected. Distribution of the pathogen over a large area might be necessary to have a major impact and this might require use of an airplane.

III. Probable route of terrorist entry/dissemination

Distribution of inoculum to many fields might be the most effective approach if terrorists wished to produce an important economic impact. Introduction would be risky because of the limited time period during which infection is possible. Terrorists would need to be knowledgeable of sorghum biology to introduce inoculum at the appropriate time. It would also be possible to initiate an epidemic using infected seed, but this, too, would be somewhat risky.

IV. Probable distribution

1. Point Introduction:

Because inoculum must be present during a fairly narrow window of sorghum flowering, its introduction would need to be carefully timed and environmental conditions would need to

be conducive to infection. The pathogen has the potential to be widely distributed by wind, so evaluation of new infections to determine whether they were the result of intentional actions would be complicated. Infections that followed particular roads would be indicative of intentional introduction since this would be unlikely to occur through wind dispersal.

2. Secondary Dissemination

Secondary dissemination might not be important many years. However, in a conducive year it might be possible for infection to move northward through a region as sorghum comes into bloom. Over a number of years the pathogen could spread long distances within the U.S.

V. Consequences of introduction (risk of pathogen establishment)

1. Establishment

In any given year, establishment risk will be highly influenced by weather conditions at the time of flowering.

2. Over-all risk rating for establishment

In any given year, the probability of infection is probably low. Over time, infection in regions with conducive conditions may occur sporadically. However, when infection occurs it may be spread throughout a wide region.

VI. Likelihood of successful introduction

1. Quantity of inoculum required to introduce and establish damage

This threshold will vary greatly depending on environmental conditions. McLaren (2002) has developed models of the Ergot Breakdown Point (EBP) that describe the risk of infection based on the fact that only unfertilized ovaries are susceptible. “Weather during two critical phases of flowering usually determines ergot incidence and severity. Low night

temperatures during the 3-4 weeks pre-flowering reduce viability and predispose florets to infection. Sorghum genotypes differ in terms of critical threshold temperatures. The optimum temperature for infection and disease development is 19^{o} C, with a limited risk of infection above 28^{o} C. Humidity is also important.

2. Likelihood of surviving initial introduction

Introduction of conidia might be unlikely to result in successful infection unless the timing and environmental conditions were particularly favorable. Introduction of sclerotia might result in more long-lived inoculum and potentially a greater chance of success. More information about whether infection takes place from sclerotia is needed for effective risk assessment at that stage.

3. Likelihood of dissemination beyond the point of introduction

Conidia produced in infected panicles can be spread by wind and rain. If detected early in a small area, it might be possible to plow infected fields under.

4. Likelihood of alternate host infection

Sorghum is the most abundant host, so will be the most important source of inoculum. It is possible that the pathogen will become important for native grass species, but this remains to be seen.

5. Likelihood of early detection

Early detection is likely, since extension agents and growers in the U.S. have been warned and educated about the disease. The distinctive appearance of honeydew would tend to make the disease easy to observe.

6. Overall risk = Moderate

The pathogen has the potential for important economic effects, but it would be challenging to make a successful large-scale introduction on a first attempt. If terrorists are willing to try

several times before their efforts coincide with a conducive year, the impact could be significant.

7. Likelihood of an agroterrorist trying to use

This pathogen would probably not be the first choice for a terrorist because of the requirement that environmental conditions be conducive during a narrow window of potential infection. Terrorists would need to be knowledgeable of sorghum biology to determine the right timing for introduction of conidial suspensions or rely on distribution of infected seed in fields.

VII. Control/Mitigation strategies after establishment

1. Resistance and disease escape

Selecting for high pollen viability is one strategy for the development of resistance to *Claviceps africana* (Ryley et al., 2002). Other strategies are to incorporate fast flower opening, and a short period of receptivity to pollination. Rapid fertilization would shorten the vulnerable period. Breeding for small or short stigmas may be useful. The question is whether these traits can be incorporated into an agronomically acceptable plant (Ryley et al., 2002). Resistance relying on rapid self-pollination does no good for the male-sterile A-lines used in seed production, since they cannot self-pollinate (Frederickson and Leuschner, 1997).

Genotypes resistant in one geographic location may be susceptible elsewhere. There are few reliable sources for resistance. Resistant lines were reported from Ethiopia, but a single backcross resulted in susceptible plants (Ryley et al., 2002).

McLaren (1998b) used a model of sorghum ergot risk applied to Kansas and Texas using data from 1992-1996. The model assumes a high level of inoculum, since the model was developed using epidemics created by inoculation. With this limitation in mind, he predicts a low risk of ergot for male-normal or commercial sorghum. Higher risks may be sporadic, depending on weather. Currently escape resistance in hybrids used in S. Africa is low

(McLaren, 2000). Small variations in pre-flowering weather can have large impacts on infection and the severity of disease (McLaren, 1998a).

2. Cultural Control

In Zimbabwe and India, growers adjust the sowing date such that flowering occurs during the dry season. In Zimbabwe, farmers use crop rotation and removal of infected material to reduce disease, but epiphytotics still occur about every 5 years (Bandyopadhyay et al., 1996). Frederickson and Leuschner (1997) conclude that crop rotation, deep plowing, and changes in planting date are not very successful, and that pollen-based management is too much affected by the weather to be easily used.

3. Chemical Control

Prior to the appearance of *C. africana* in the Americas, winter nurseries outside the U.S. were used in breeding programs. Growers were concerned about importing infested seed. Contact fungicides captan and thiram were used to lower the infectivity of macroconidia without severely reducing seed viability (Dahlberg et al., 1999).

Prom and Isakeit, (2003) reported that contact fungicides were the least effective in preventing infection of panicles in the greenhouse and field. *In vitro* screening was not a reliable indicator of effectiveness in the field. They used optimal coverage of the panicle till run-off of the fungicide occurred in their fungicide tests. This may not be feasible, or may not be economic when feasible, for growers. In Texas, triadimefon and propiconazole suppressed ergot development in the field. Tebuconazole and propiconazole were found effective in Brazil. They state “complete coverage of the panicle with triazoles or strobilurins at low application rates might be an economical and effective approach....” in Texas, for seed fields, when weather does not favor disease. Multiple applications might be needed to cover the entire time of flowering. Contact fungicides plus triazoles might be useful, but more research is needed.

Frederickson and Leuschner (1997) found that control of ergot in A-lines could be obtained through judicious use of the systemic fungicide Benlate. They used a single application of 0.2% activeingredient of Benlate. Thiram was not effective in containing spread after initial outbreak. In India 2-3 sprays of Ziram, Zineb or other contact fungicides were effective.

Frederickson and Leuschner (1997) state that development of resistance to the fungicides could be a problem, since polycyclic fungi are"notorious" for rapid development of resistance.

In Mexico, seed companies sprayed propiconazole or tebuconazole 2-3 times, mostly during blooming. Seed companies had concerns about phytotoxicity in these trials (Torres-Montalvo and Montes-Garcia, 1999)

Sangitrao et al. (1999) reported that in India, a significant increase in yield in seed production plots infected with *C. sorghi* was obtained by using 2-3 sprays of 0.2% Ziram®, thiophanate methyl, Vitavax®, or captafol at 2 week intervals starting at panicle emergence. Two sprays of Bavistin® 0.1% at 50% flowering and at 2 weeks later was "also effective". Cited in a 1971 study as ineffective were Benlate®, Vitavax75®, and Tecto®.

Table 4. Fungicide categories.

Category	Example(s)
Contact fungicides	Fluazinam* Copper sulfate Captan Thiram
Ethylene bisdithiocarbamates (EBDCs)	Zineb* Mancozeb
Triazoles	Triadimefon Fenbuconazole Tebuconazole Myclobutanil Propiconazole
Strobilurins	Trifloxystrobin Azoxystrobin
Benzimidazole	Benlate* Thiophanate methyl
Diothio-carbamate	Ziram Maneb

* Not used in the U. S.

4. Seed cleaning

Soaking seed in salt solution may remove sclerotia but not all of them. This is impractical on a large scale (Bandyopadhyay et al,. 1982; Bandyopadhyay et al., 1998)

5. Biological Control

Bhuiyan et al.(2003) identified five potential biocontrol agents for C. Africana. All were fungi; they tested six bacterial isolates that were not effective. The five potential biocontrol agents were *Epicoccum nigrum,* two isolates of *Penicillium citrinum*, and two commercial formulations of *Trichoderma* spp. (Trichoflow and Trichopel). The same factors that are important in the use of chemical pesticides are important in the use of biocontrol agents. These include such factors as carrier liquid, timing of application, rate of application, and droplet size. They stated that more work is needed before potential biocontrol agents can be successfully used in the field.

6. Modeling Disease Incidence and Spread

McLaren (1998b) used a model of sorghum ergot risk applied to US Kansas and Texas using data from 1992-1996. The model assumes a high level of inoculum, since the model was developed using epidemics created by inoculation. With this limitation in mind, McLaren predicts a low risk of ergot for male-normal or commercial sorghum. Higher risks may be sporadic, depending on weather.

VIII. Knowledge gaps

Information about threshold inoculum levels required for establishment under a range of environmental conditions is needed.

Information about overwintering and oversummering requirements for sclerotia and other propagules is needed.

A method for inoculation that more closely simulates infection by secondary conidia is needed for screening purposes (Ryley et al., 2002).

More information is needed on relationships among pollen, ergot and the environment in relation to the spread of the disease and in understanding resistance.

The relationship between pollen production and environmental factors is still poorly understood (Ryley et al., 2002).

Bhuiyan et al. (2003) stated that much more work is needed before potential biocontrol agents can be successfully used in the field.

IX. Immediate response options

1. Rapid Detection

Infection that reaches the point of producing honeydew can be readily detected visually by scouts in the field. From a distance, higher levels of infection would be necessary for symptoms to be visually apparent.

2. Cultural Control

If infection is detected early and occurs only on a small scale, it might be useful to plow plants under to reduce spread of conidia from infected panicles. On larger scales, cultural controls do not seem promising for management of new infections.

3. Fungicides

Fungicides discussed above could potentially be useful for reducing a limited introduction to below threshold infection levels for establishment.

4. Resistance Breeding

Resistance varies among varieties and may be useful in the long run for managing established infections.

Appendix 1. Experts knowledgeable about Claviceps africana.

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Appendix 2. Descriptions of Claviceps species causing ergot on sorghum.

Description of *Claviceps africana*.

Federickson, D. E., P. G. Mantle, and W. A. J. de Milliano. 1991. *Claviceps africana* sp. nov.; the distinctive ergot pathogen of sorghum in Africa. Mycological Research 95:1101-1107.

"*Sclerotia* oval to spherical, 4-6 mm long, 2-3 mm wide; the pyramidal apical sphacelial portion protruding beyond the floral parts; the hard basal portion (the true sclerotium) appearing flecked red by fragments of adherent sphacelial tissue overlaying a red-brown cortex. Medulla consists of white plectenchyma. *Stromata* 1-9, arising from one or two points on the sclerotial surface. *Stipes* 8-15 mm long, 0.3-0.6 mm wide, glabrous, initially translucent whitish becoming purple, especially in the distal portion. *Capitula* sub-globose, 0.5-1.3 mm, initially opaque and light buff, becoming dark purple, papillate with maturity and enveloping the stipe insertion. *Perithecia* ovate-pyriform, 123-226 Φm long, 86-135 Φm wide. *Asci* cylindrical, up to 140 Φm long, 3.2-4.2 Φm wide within the perithecium. *Acospores* filiform, hyaline, septate, up to 45 Φm long, 0.8-1.2 Φm wide. *Conidia* (*Sphacelia sorghi* McRae) hyaline, mono-nucleate, 9-17 Φm × 5-8 Φm, oblong to oval and slightly constricted at the centre (macroconidia); 2-3 Φm diam, spherical (micrconidia)."

Description of *Claviceps sorghi*.

Kulkarni, B. G. P., V. S. Seshadri, and R. K. Hegde. 1976. The perfect stage of *Sphacelia sorghi* McRae. Mysore J. Agric. Sci. 10:286-289.

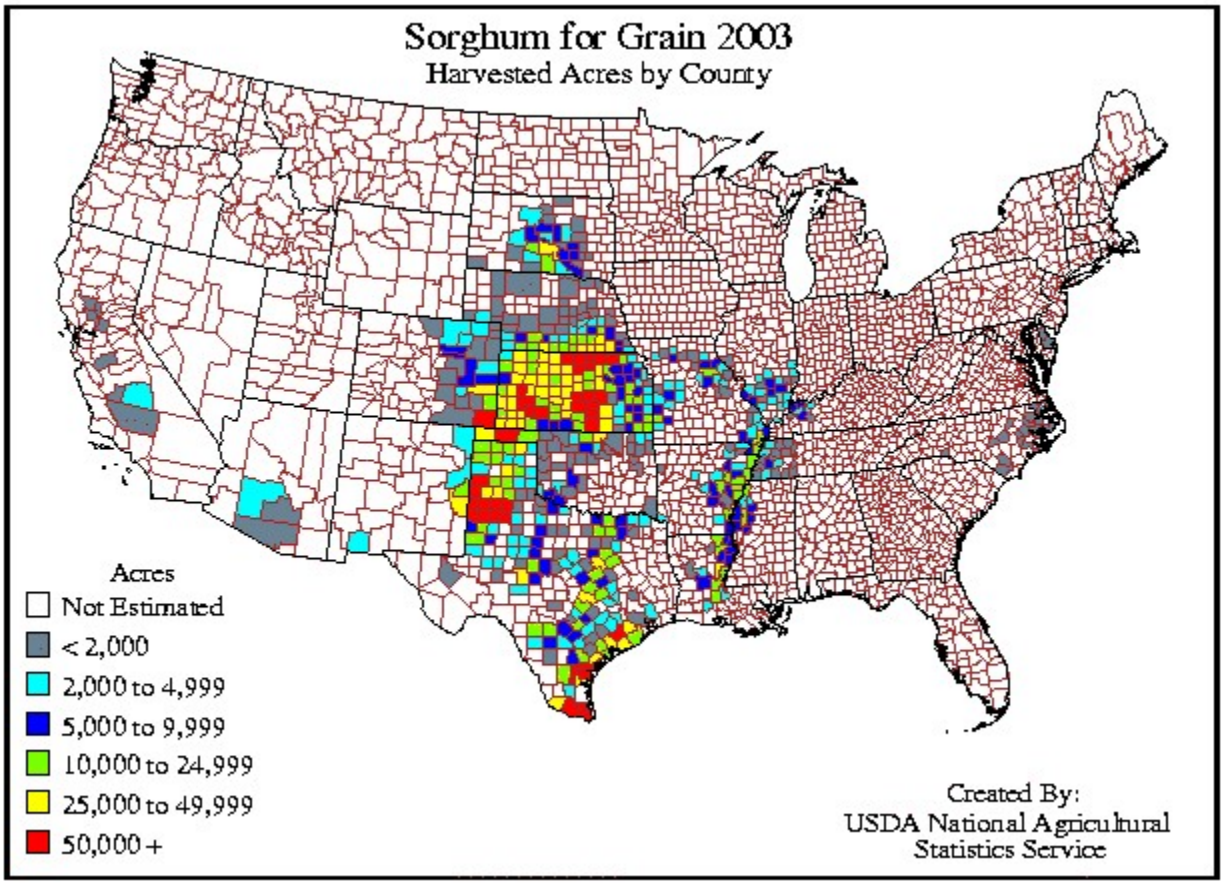
"Conidia are formed on conidiophores, closely arranged in a pallisade [sic] layer lining the folds and on the surface of the fungal mass. They are hyaline, elliptic or oblong, slightly constricted at the middle and measure 12-19 × 5.8 Φ. At a later stage sclerotia develop from the affected ovaries which have been observed to be of two types. Short and hard one are dirty white flecked with red and measure 9-20 × 1.5-2 mm while the long and soft ones are buff grey and measure 9-20 × 1.5-2 mm; pseudoparenchymatic; on germination 2-3 stromatal heads from each sclerotium develop; perithecia embedded in the stroma, flask-shaped, slightly protruding at ostiolar region, 132.8-232 × 66.4-124.5 Φ. Asci hyaline, cylindrical with somewhat tapering ands with a hyaline cap at the apex, 56-112 × 2.4-3.2 Φ. Ascospores eight per ascus, filiform, 40-85 × 0.4-0.8 Φ."

Description of *Claviceps sorghicola*.

Tsukiboshi, T., T. Shimanuki, and T. Uematsu. 1999. *Claviceps sorghicola* sp. nov., a destructive ergot pathogen of sorghum in Japan. Mycol. Res. 103:1403-1408.

"*Sclerotia* cylindrical to conical, straight or curved, 2.5-20 mm long, 1.9-3.5 mm wide; the true sclerotium purplish black to black, having longitudinal grooves on the surface, covered with white sphacelial tissues. *Stromata* 1-4, arising from one or two portions on the sclerotial surface. *Stipes* 3.5-17 mm long, brown to bronze. Capitula globose to subglobose, 0.5-1.6 mm diam., dark brown, distinctly papillate. Perithecia ovate to pyriform, 215-300 Φm long, 105-140 Φm wide, embedded in the surface of capitula, ostioles evidently erumpent. *Asci* cylindrical, hyaline, 122-215 Φm long, 2.5-3.8 Φm wide with thickened apical cap. *Ascospores* filiform, hyaline, eight per ascus, 92-205 Φm long, 0.5-1 Φm wide. *Conidia* ellipsoidal to oval, hyaline, non-septate, 5-11.3 Φm long, 2.5-3.8 Φm wide."

Appendix 3. Map of Sorghum Harvested in the U.S. in 2003. (USDA)



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Brown Stripe

Downy Mildew

Pathway Analysis:

Intentional Introduction of

Sclerophthora rayssiae* var. *zeae

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Brown Stripe Downy Mildew Pathway Analysis

Table of Contents

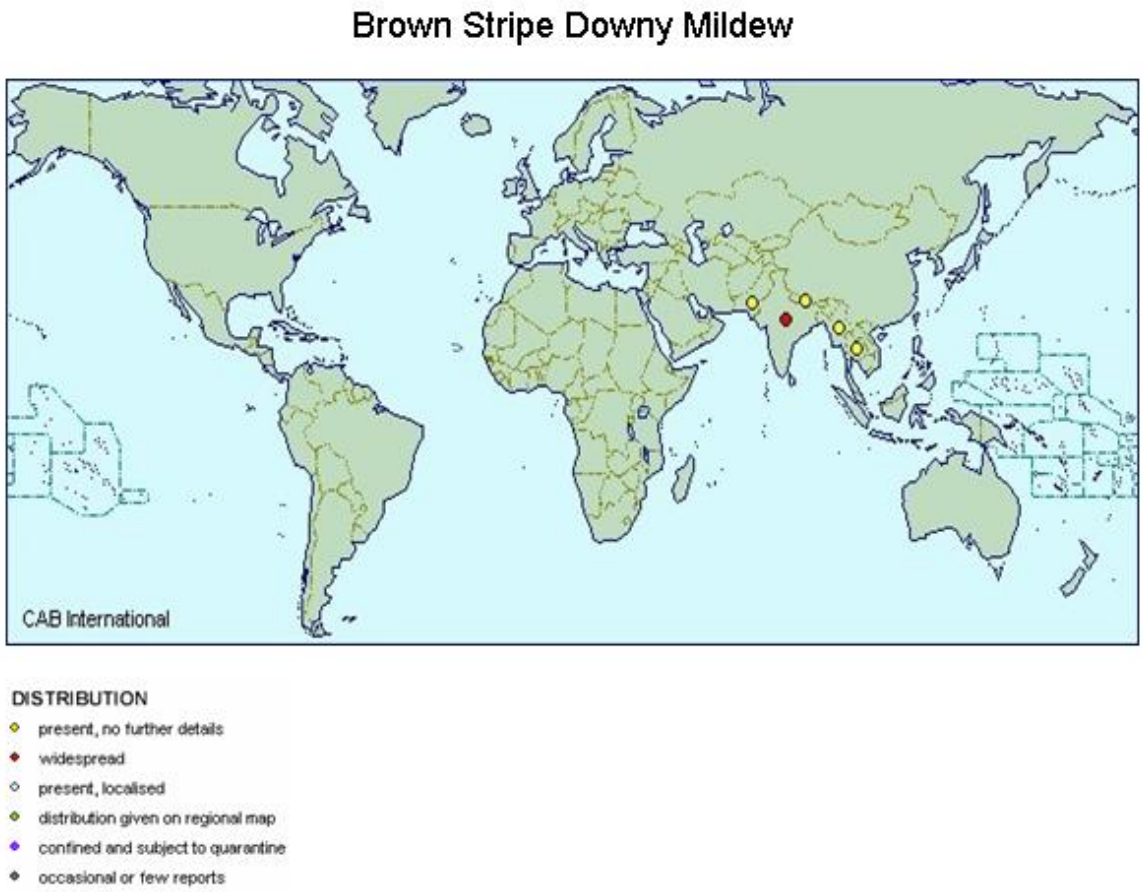
Executive summary: Brown Stripe Downy Mildew Pathway Analysis

Sclerophthora rayssiae var. *zeae* is a pathogen of maize (corn) in the tropics that could become adapted to sorghum, as well. Such movement of downy mildew pathogens from one host to another related host is not unusual. This pathogen has received little research attention, though it has been reported as an important disease of maize in India. Because little information is available about this pathogen, it is difficult to estimate the risk of introduction and establishment in the U.S. It is unknown whether conditions in the U.S. would allow overwintering of the pathogen. The pathogen could be introduced through sporangia, zoospores, or oospores. If environmental conditions were conducive, this pathogen might spread rapidly. If a localized infection occurs, it could potentially be controlled through use of pesticides developed for other downy mildews. This pathogen is very unlikely to be used by terrorists for introduction to sorghum production in the U.S. because of the perhaps insurmountable challenge of finding or producing isolates adapted to sorghum. It could potentially be introduced to maize.

Another document in this set of pathways analyses provides a discussion of the pathway of introduction for the related Philippine downy mildew of maize.

See Figure 1. Distribution map of *Sclerophthora rayssiae* var. *zeae*.

Fig. 1. Map of the distribution of Brown Stripe Downy Mildew. (CABI)



Brown Stripe Downy Mildew

Pathway Analysis for the Intentional Introduction of

Sclerophthora rayssiae var. *zeae*

I. Biology and life/disease cycle of the pathogen

1. Identity Classification

Kingdom: Chromista

Phylum: Oomycota

Class: Oomycetes

Order: Peronosporales

Family: Peronosporacea (The downy mildews)

Genus: *Sclerophthora*

Species: *Sclerophthora rayssiae* var. *zeae* (Payak and Renfro) 1967

Sclerophthora rayssiae consists of two currently known varieties: *S. rayssiae* var. *zeae* and *S. rayssiae* var. *rayssiae*. The pathogen, especially *S. rayssiae* var. *zeae*, has a wide distribution from Israel (temperate) mainly into northern states of India (temperate) (Payak *et al*., 1970; Shaw, 1984). The pathogen was reported to cause a widespread disease of maize in India (Payak and Renfro, 1967). The pathogen is currently among the agents

listed by The Animal and Plant Health Inspection Service (APHIS), USDA, as posing a severe threat to plant health.

Payak and Renfro (1967) described and named *Sclerophthora rayssiae* var. *zeae* Payak & Renfro var. n. They described it as a new variety of *S. rayssiae* on the basis of its slightly larger sporangia, lack of golden or amber brown color in both oogonia and oospores, smaller oospore size, and hyaline, glistening oospore wall. Although the type variety of *S. rayssiae* was described on barley in Israel, Payak and Renfro (1967) did not think host differences warranted erection of a new species. In contrast, Kenneth (1970), one of the authors of *Sclerophthora rayssiae*, argues that *Sclerophthora rayssiae* var. *zeae* is not closely related to *Sclerophthora rayssiae*.

Synonyms

See table 1. for synonyms for names of organisms discussed in this report.

Table 1. Synonyms for names of organisms discussed in this report.

Name	Synonym
Sclerophthora rayssiae var. *zeae* Payak & Renfro (1967)	
Sclerophthora rayssiae var. *rayssiae* Kenneth, Koltin and Wahl (1964)	
Sclerophthora macrospora (Sacc.) Thirium, Shaw & Naras.	*Sclerospora macrospora* Sacc. *Phytophthora macrospora* (Sacc.) Ito & Tanaka
Sclerospora graminicola (Sacc.) Schroet.	*Protomyces graminicola* Sacc. *Peronospora graminicola* Sacc. *Peronospora setariae* Pass. *Ustilago urbani* Magnus
Peronosclerospora maydis (Racib.) C. G. Shaw	*Peronospora maydis* Racib. *Sclerospora maydis* (Racib.) Butler *Sclerospora javanica* Palm
Peronosclerospora miscanthi (T. Miyake) C.G. Shaw	*Sclerospora miscanthi* T. Miyake
Peronosclerospora philippinensis (Weston) C.G. Shaw	*Sclerospora philippinensis* Weston
Peronosclerospora sacchari (Miyake) Shirai and K. Hara	*Sclerospora sacchari* Miyake
Peronosclerospora sorghi (Weston and Uppal) C.G. Shaw	*Sclerospora sorghi* (Kulk.) Weston and Uppal

Similar Organisms

See Table 2 for diseases caused by downy mildew fungi discussed in this report. Oomycetes are fungal-like organisms which exhibit a number of characteristics that set them apart from the true fungi. The cell walls differ from those of the true fungi in consisting mainly of β-glucans, but also containing a small amount of cellulose. Asexual reproduction takes place by means of biflagellate zoospores. Zoospores typically develop within a sporangium, borne on a sporangiophore. Zoospores may encyst under adverse conditions, and germinate when conditions are suitable. Sexual reproduction typically gives rise to an oospore, which is a thick-walled resistant structure. In the Peronospraceae, the characteristics of the sporangiophore are important in differentiating the genera. Genera in the family Peronosporacea include *Sclerospora*, *Peronosclerospora*, *Sclerophthora*, *Plasmopara*, *Bremia*, and *Basidiophora*. They are distinguished chiefly by the branching of their sporangiophores. All members of the Peronosporaceae are obligate parasites (Alexopoulos et al., 1996).

Frederiksen and Renfro (1977), discussing the global status of downy mildews in the Gramineae, stated that of 14 species of *Sclerospora*, six attack maize. Of four *Sclerophthora* species, two attack maize. Kenneth (1976) states that nine downy mildew pathogens attack maize, and that of these, eight attack other graminaceous hosts.

Payak and Renfro (1967), discussing differentiation among the *Sclerophthora*, argue that obligate parasitism separates *Sclerophthora* from *Phytophthora.* In *Sclerophthora macrospora*, the type species of *Sclerophthora*, oospores form in or around the vascular bundles of the leaf. *Sclerophthora rayssiae* oospores occur in mesophyll. *Sclerophthora rayssiae* sporangia differ from those of *Sclerophthora cryophila* in not being obpyriform. *Sclerophthora rayssiae* oospores develop eccentrically within the oogonia, unlike those of *Sclerophthora cryophila*.

2. Hosts

Maize is the only economically important host of *S. rayssiae* var. *zeae* at the present time. See Table 2 for hosts of downy mildew fungi discussed in this report. A concern with downy mildews is the possibility of host-switching to infect new related crop species. Thus there is the potential that *S. rayssiae* var. *zeae* might adapt to infect sorghum.

Table 2. Diseases caused by downy mildew fungi discussed in this report.

Downy Mildew Fungus	Crop hosts	Disease name
Sclerophthora rayssiae var. *zeae*	Maize	Brown stripe downy mildew
Sclerophthora rayssiae var. *rayssiae*	Barley	Blotch downy mildew
Sclerophthora macrospora	Maize Rice Wheat	Crazy top Yellow wilt of rice
Sclerospora graminicola	Maize Pearl millet	Graminicola downy mildew Green-ear
Peronosclerospora maydis	Maize	Java downy mildew
Peronosclerospora miscanthi	Sorghum Maize	Leaf-splitting downy mildew
Peronosclerospora philippinensis	Maize Sorghum	Philippine downy mildew
Peronosclerospora sacchari	Sugarcane Maize	Sugarcane downy mildew
Peronosclerospora sorghi	Sorghum Maize	Sorghum downy mildew

Singh (1971) inoculated 13 cereals and millets and 15 grasses belonging to 14 genera with sporangial suspensions. Of the grasses, only *Digitaria sanguinalis* was susceptible. The following were not susceptible: wheat, oat, rice, sorghum, rye, pearl millet (*Pennisetum typhoides*), finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), and barnyard grass (*Echinochloa* spp).

Chamswarng et al. (1976) found *Digitaria bicornis* infected with *Sclerophthora rayssiae* var. *zeae* in Chon Buri Province, Indonesia. Inoculations from *Digitaria bicornis* to rice and maize were unsuccessful, but inoculations back to *D. bicornis* produced infection. The symptoms on *D. bicornis* were long, parallel yellow streaks. Renfro and Pupipat (1976) reported *S. rayssiae* var*. zeae* on *Digitaria bicornis* in Thailand.

A reported wild host of *Sclerophthora rayssiae* var. *zeae* is *Digitaria sanguinalis* (Pupipat, 1976). *D. sanguinalis*, a widespread wild grass species, may be a source of primary inoculum to maize (Bains et al., 1978; Renfro and Bhat, 1981). Bains et al. (1978) found *S. rayssiae* var. *zeae* on *Digitaria sanguinalis* in a field of maize infected with brown stripe downy mildew in Punjab, India. Cross inoculations with *S. rayssiae* var. *zeae* from maize and *D. sanguinalis* were successful. They report the morphology of the fungus on *D. sanguinalis* was identical to the morphology of the fungus on maize. Singh et al. (1970)

found that *D. sanguinalis* was susceptible to *S. rayssiae* var. *zeae* in greenhouse inoculation tests using oospores from infected maize in India.

Bains (1989) inoculated *Zea* spp. with sporangia of *S. rayssiae* var. *zeae* obtained from infected *Zea mays*. Two lines of *Zea perennis* became infected. Five lines of *Zea mays* ssp *mexicana* (teosinte) became infected. *Zea mays* var. *luxuriana* was not susceptible. Age of the plant was important in one teosinte line; older plants were not susceptible.

Renfro and Bhat (1981) believe that the Asian strains of downy mildews on maize were originally pathogens of wild hosts, which later transferred to introduced crops. In most downy mildews, the important infective stage is the oospore, since the asexual spores are short-lived and disperse only a short distance. The pathogen over-seasons as oospores in plant material.

In the genus *Sclerophthora*, four species attack plants in the Gramineae. They include *Sclerophthora macrospora* (crazy top), *Sclerophthora rayssiae* var. *rayssiae* (attacking barley in Israel and two wild grasses), and *Sclerophthora rayssiae* var. *zeae* (attacking maize, *Digitaria sanguinalis*, and *Digitaria bicornis*).

Pupipat (1975) reports that in Bangalore, India, *Peronosclerospora sorghi* was well known to infect sorghum, next was found on two maize plants in 1968, and is now an important disease of maize in India.

Narro et al. (1992) state that *Peronosclerospora sorghi*, the pathogen that causes sorghum downy mildew, attacks maize but does not produce oospores in maize. *Sclerophthora macrospora* occurs in a limited area in Mexico but is not commercially important. *P. sorghi* is very effectively controlled with metalaxyl seed treatment. The most effective control is to use resistant hybrids.

Sclerophthora macrospora was found on sorghum from 1958-1963 in Texas (also earlier in 1936). In addition, *P. sorghi* was reported on sorghum, but also on one corn plant. *P. sorghi* also infected forage sorghum, and sorghum-Sudangrass hybrids (Frederiksen et al., 1970; Futrell and Frederiksen, 1970; Janke, 1983; Josephson, 1974). The first occurrence of *P. sorghi* on corn was in 1962 (Josephson, 1974). Reyes et al. (1964) speculate about an interchange of fungus on sorghum, corn and teosinte before the epidemic. They

recommend disease resistant lines worldwide, and having disease nurseries in a part of the world where disease cannot spread.

Maize downy mildews listed by Fuji (1975) are:

Sclerospora graminicola green ear downy mildew, widely distributed

Sclerospora phillipines (*S. philippinensis*)

Sclerospora sorghi leaf shredding, sorghum downy mildew, widely distributed

Sclerospora sacchari

Sclerospora spontanea limited distribution

Sclerospora miscanthi leaf-splitting downy mildew, limited distribution

Sclerospora maydis Japanese downy mildew, wilting, limited distribution

Sclerophthora rayssiae necrosis of chlorotic area

Sclerophthora macrospora often increased tillering, crazy top, widely distributed

All cause “barrenness”, or lack of seed production. Maize is not the original host of any of these fungi.

3. Geographic Distribution and History

Payak and Renfro (1967) described *Sclerophthora rayssiae* var*. zeae* in India. According to Payak et al. (1970), *Sclerophthora rayssiae* var. *zeae* was restricted to India at that time.

Pupipat (1975) reported that brown stripe downy mildew was a major disease in northern and central India, and was found in Nepal and Pakistan. It was found in Thailand but was not reported as economically important. In Thailand *S. sorghi* was more important economically than *Sclerophthora rayssiae* var. *zeae*. These are the two downy mildews reported on maize in Thailand (Pitakspraiwan and Giatgong, 1976).

Lal et al. (1980) reported *Sclerophthora rayssiae* var. *zeae* in India, Nepal, Thailand, Pakistan and Bangladesh. Kalia et al. (1994) stated that brown stripe downy mildew on

maize was an important disease in northern India. *Sclerophthora rayssiae* var.*zeae* was reported in India, Nepal, Pakistan, Sikkim, and Thailand (Williams, 1984).

Currently the majority of downy mildews parasitizing graminaceous crops are restricted to certain countries in Asia and/or Australasia (Williams, 1984). Infection of maize by *S. rayssiae* var. *zeae* is not known to occur naturally on maize in the United States.

Sclerophthora rayssiae var. *rayssiae* was reported to infect cultivated barley (*Hordeum vulgare* L.) and two wild grasses *H. spontaneum* C. Koch and *H. Murinum* L. in Israel (Kenneth et al., 1964).

4. Disease Impact

Brown stripe downy mildew is "one of the most destructive diseases of maize" in India (Singh et al., 1970). Severely affected plants do not set seed and they dry off early (Singh et al., 1970). The higher the rate of infection the lower the grain weight (Lal and Prasda, 1989). *Peronosclerospora sorghi* was reported as more important economically than *Sclerophthora rayssiae* var. *zeae* (Pitakspraiwan and Giatgong, 1976). Khehra et al. (1981) state that with severe disease, the plants die early and do not set seed.

5. Symptoms

Original report

(Payak and Renfro, 1967): Initially narrow chlorotic stripes 3-7 mm wide with well-defined margins, delimited by veins, stripes later turn reddish or purple. Severe striping and blotching due to proximity of lesions. No crazy top, no shredding. Oogonia and oospores under the stomata but not under the vascular bundles. Differs from *Sclerospora philippinensis*, which induces long chlorotic streaks of lighter color and sometimes crazy top. Differs from *Sclerophthora macrospora* which causes crazy top. There are differences in resistance and susceptibility in many varieties of maize

In Kenneth, R., Y. Koltin, and I. Wahl. 1964, the original description of *Sclerophthora rayssiae*, symptoms did not include deformation or thickening of leaves, or any leaf shredding. The fungus was first recorded and described on barley in Israel.

Fuji (1975) describes the symptoms of downy mildew as follows. One of the earliest symptoms is chlorotic striping, caused by *S. philippines* (=*S. philippinensis*), *S. sacchari, S. sorghi*, and *S. maydis*, which induces long chlorotic streaks of a lighter color on maize leaves, but this symptom is less marked in disease caused by *S. rayssiae*. Necrosis of chlorotic areas is characteristic of *S. rayssiae*.

6. Disease Cycle and Epidemiology

A. Initial inoculum and infection

Lal and Prasad (1989) found that an epiphytotic could be produced using pieces of infected fresh leaf containing sporangia inserted into the whorl of the plant. Oospores can become mixed with seed. They state that there is a small percentage of transmission by seed.

Singh et al. (1970) performed inoculation experiments using oospores, sporangia, and zoospores in India, using as a host a maize hybrid named Ganga 3. Oospore inoculum on the soil surface or in the upper 3.75 cm of soil produced over 85% infection, oospores dusted on seed or placed in soil surrounding seed produced 50% infection, oospores placed below the seed produced 12% infection. In trials using sporangia, oospores and zoospores, inoculum was sprayed, dusted or placed in the whorl. All these methods produced infection, but the highest rate of infection was obtained by using zoospores, particularly sprayed zoospores. Plants were inoculated, placed in a mist chamber 48 h, and then placed outdoors. Infection occurred at average temperatures from 24.5° C to 30° C. Zoospores remained motile at 10° C for 120 minutes, above 20° C, the time of motility dropped rapidly, with only a few minutes at temperatures above 25° C. The optimum temperature for motility appeared to be 18° C, for 150 minutes. However, 20-25° C was the optimum temperature for zoospore encystment and germination. Sporangia germinated best at 20-22° C, but germinated eventually at all temperatures between 18° and 30° C. Moisture is the most important factor in disease development. Twelve hours in a mist chamber was a sufficient period to allow infection by zoospores. Free water allowed germination of the sporangia. Sporangia were produced during the light periods of the day. Age of the plant was a significant factor and susceptibility increased as plants aged from 10 to 60 days.

Fig. 2. Maize leaf with symptoms of brown stripe downy mildew. (AGRI)

B. Growth stage vulnerability

As discussed under section I.6.A. in the context of initial infection, susceptibility of plants may increase with age (Singh et al., 1970).

C. Conditions that favor disease

As discussed under section I.6.A. in the context of initial infection, defined temperature ranges and surface moisture favor disease development (Singh et al., 1970).

D. Inoculum persistence and dissemination

Singh et al. (1970) found that 2 g of dried infected leaf material placed next to the seed at planting caused infection. They stated that disease did not appear to be seed-borne, but see Safeeulla (1975) for a contradictory report. Traps for aerially-dispersed sporangia trapped most sporangia between noon and 4 p.m. on clear days. There was no information in the report on the distance traveled by air-borne sporangia.

Singh et al. (1970) tested dispersal of disease by movement of running water. Pots with seedlings of maize were placed in running water in a ditch next to a heavily infected field for 5 minutes. This was done after a heavy rainstorm. The plants in the pots showed 10% infection. They also tested whether contact between infected and healthy leaves could spread the disease, and found that it could. They concluded that the disease could be spread by wind, rainwater, and contact. Observation of insects led them to conclude that the disease could also be spread by insects.

Oospores overseason and are infective the following year in India. Oospores remained viable for 5 years in lab under dry conditions (Singh, 1971). Disease may be spread by seed-borne inoculum (Safeeulla, 1975). Seed-borne spread of *Sclerophthora macrospora* and *Peronosclerospora sorghi* is limited (Josephson, 1974).

Lal and Prasad (1989) found that an epiphytotic could be produced using pieces of infected fresh leaf containing sporangia inserted into the whorl of the plant. They state that oospores can become mixed with seed, and that there is a small percentage of transmission by seed, although oospores can be washed off seed.

7. Causal organism

The following is taken from the Payak and Renfro (1967) original description of the pathogen:

Sexual organs numerous, scattered in leaf mesophyll or under the stomata. *Oogonia* are subglobose, thin-walled, hyaline to light straw-colored, with 1 or 2 paragynous antheridia, 33.0-44.5 µm in diameter. *Oospores* are centrally located in oogonia, spherical or subspherical, and have hyaline contents including a prominent oil globule, with a smooth, glistening, uniformly 4-µm-thick wall, which is confluent with the oogonial wall, 29.5-37.0 µm in diameter. *Sporangiophores* are short, determinate, arise from hyphae congregated in the substomatal spaces, and produce sporangia sympodially in groups of 2-6. *Sporangia* are hyaline; ovate; obclavate, or elliptic or cylindrical; smooth-walled; having a truncate or rounded apex, which is poroid; with a persistent, straight or cuneate peduncle; individually produce 4-8 zoospores: 29.0-66.5 X 18.5-26.0 µm in diameter. Encysted *zoospores* are spherical, hyaline, 7.5-11.0 µm in diameter.

Kenneth, R., Y. Koltin, and I. Wahl (1964) described and named *Sclerophthora rayssiae* sp. nov. The fungus caused a downy mildew first recorded on barley in Israel. Symptoms did not include deformation or thickening of leaves, or leaf shredding. "Oospores are globular, occasionally subglobular, always smooth and moderately thin- walled, light golden amber with the oospore wall deep golden brown: 29.6-44.4 µm (mostly 33.3 µm) in diameter. They are generally located excentrically within the oogonial walls. Oogonia enveloping oospores are unevenly thickened, usually sinuous; 44.4-59.2 (61.4) µm in diameter.... Antheridia and paragynous, closely appressed to the oogonium."

"... thin hyphoid sporangiophores" "Sporangiophores arise amphidodially either singly, in twos or more from stomata" in the lesion area. "A single sporangium may bud out perminally, or as many as 4 sporangia may form sympodially in close succession.

"Sporangia are lemon-shaped or ovate, never obpyriform, very thin-walled, hyaline, granular, bearing a persisitent wedge-shaped pedical at the base; the apex mau protrude and is poroid; 28.8-55.0 X 19.2-27.9 µm."

Cytoplasm divides into 6-10 reniform zoospores, exit single file from apical pore. Zzospores are bi-flagellate 11.0 X 7.5 µm. Secondary sporangia may germinate directly (act as conidia).

Asexual stage of *Sclerophthora rayssiae* var*. zeae*: Mycelium is plasmodia-like, intercellular and coenocytic. Sporangiophores are short, determinate, arise from an abortive hyphae congregated in substomal spaces, produce sporangia. Sporangia hyaline, elliptical with rounded apex, poroid, persistent peduncle, individually produces 10-16 zoospores. Zoospores spherical and hyaline (Singh, 1971).

Sexual stage of *Sclerophthora rayssiae* var*. zeae*: Oogonia and oospores are abundant, scaterred in leafsheath mesophyll or arranged in a linear fahion. Oogonia subglobuse, think-walled, hyaline to loght colored, with a paragynous antheridium. Oospores golden yellow in color, eccentrically located within oogonia, spherical with a uniform thick wall; having hyaline contents with a prominent oil-globule; oospores wall fused with oogonial wall (Singh, 1971).

A. Culture

Most researchers have used fresh or dried infected plant material, or fresh zoospores collected from germinating sporangia, or oospores. *Sclerophthora rayssiae* var. *zeae* is an obligate parasite, as are all the members of the Peronospraceae, and so does not lend itself to axenic culture.

B Pathogen variability

Little is know about pathogen variation in *Sclerophthora rayssiae* var. *zeae*. Pathotypes have not been reported.

8. Diagnostic Methods

Kenneth (1976) reported problems with the identification of downy mildew pathogens in Africa. *Peronosclerospora maydis* and *Peronosclerospora sorghi* are easily confused, but *Peronosclerospora sorghi* can be distinguished from *Sclerospora graminicola*. Identification of these species from areas in Africa lacking good facilities or voucher specimens should not be accepted unconditionally.

II. Initiating Event (Recognizing an Attempted Introduction)

1. Observation/diagnosis of Presence

Since brown stripe downy mildew is not well known in the U.S. and this assessment emphasizes the possibility of movement of the pathogen from corn to sorghum, initial diagnosis may be difficult. However, the symptoms may be striking enough to draw attention from farmers and extension agents. If infection is widespread, that may be indicative of intentional introduction but could also occur simply because diagnosis was difficult and thus infection spread before it was noted.

2. Interception: Individual/ Pathogen

Entry of infectious materials into the U.S. could be through any smuggling route. Infected plant materials such as leaves would probably be capable of transmitting the disease for some period of time, particularly if kept cool to reduce the development of saprophytic fungi. It might be necessary to build up populations within the U.S. before distributing inoculum over a wide area. Oospores, a resting spore adapted to longer periods of time without growth and exposure to a host, could potentially be transported for introduction. Collection or production of oospores might be more difficult if multiple mating types are not readily available.

Sporangia could be produced for aerial dispersal in corn or, if successfully selected for adaptation, in sorghum. A terrorist might take the approach of distributing the sporangia along a roadway or, possibly, from an aircraft. Environmental conditions conducive to infection would be necessary for success. Zoospores would be another possibility for introducing the pathogen, particularly in irrigated cropping systems. If zoospores could be introduced to central water storage used for irrigation, this might be a successful dissemination approach. Oospores could potentially be distributed in fields at any time of the year, but then successful infection would depend on their germination at an appropriate and conducive time.

3. Intelligence Information

Because the disease is not currently established in the U.S., it may be possible to use genetic analyses to trace the origin of a newly introduced strain. If trade routes can be ruled out as a source for the new infection, this may be evidence for intentional introduction. Selection for movement from corn to sorghum might potentially be performed by terrorists, but could also occur naturally.

III. Probable Route of Terrorist Entry/dissemination

Issues for the introduction of the pathogen are discussed under section II. The easiest approach might be to distribute oospores, if available, along highways throughout a corn-growing (or, if selection for adaptation to sorghum has occurred, sorghum-growing) region.

An approach that might be more successful would be the distribution of sporangia. Zoospores could be used if they can be produced rapidly from sporangia and distributed rapidly in moisture.

IV. Probable distribution

1. Point Introduction:

To make an impact, terrorists would probably prefer to distribute the pathogen over a wide geographic area. To make this dissemination practical, they might tend to introduce the pathogen along roadways where fields are easily accessible. Such widespread introduction along an easily traveled path could indicate an intentional introduction.

2. Secondary Dissemination

Secondary dissemination may occur through airborne sporangia or zoospores that may be dispersed through water, including irrigation water. Fields sharing irrigation sources should be considered in danger of shared infection, either from the original inoculum distribution or from the “upstream” field to the “downstream” field.

V. Consequences of Introduction (Risk of Pathogen Establishment)

1. Establishment

A. Climate

Because this pathogen has been studied little, its potential for adapting to non-tropical systems is unclear. Some observed temperature ranges are discussed above.

B. Host Range

The host range of this pathogen may or may not ever include sorghum. Movement of downy mildew pathogens from one grass species to another previously unknown grass host

is not unusual. There is also the potential that the pathogen would already be adapted to, or come to be adapted to, native U.S. grass species. Grasslands are the dominant ecosystem in the central U.S. where sorghum production is common.

C. Dispersal

Dispersal for this pathogen has received little study, but it might be expected to disperse fairly rapidly via sporangia and zoospores.

D. Economics

Potential economic damage is difficult to assess. If the pathogen is recovered from sorghum for the first time, it could be viewed as a new pathogen such that new trade barriers could be put in place against U.S. sorghum exports and potentially even exports of other commodities that share the same transport system. Likely yield loss under U.S. conditions is not known, though yield loss in India has been reported as high.

E. Environmental Impact

Adaptation of this pathogen to U.S. native grasses is a possibility. For example, several dominant tallgrass prairie grasses are in the same tribe as maize.

F. Persistence

The likelihood of persistence is an important unanswered question. Overwintering might be an important challenge to this pathogen, but information on its requirements is not available at this time.

2. Over-all risk rating for establishment

At this time the pathogen is unlikely to become established on U.S. sorghum since this would require adaptation to a new host. This may occur, but it is not likely to occur in the near future such that terrorists could become aware of it or make use of it. It is possible that it may become established on maize in the southern U. S.

VI. Likelihood of Successful Introduction

1. Quantity of Inoculum Required to Introduce and Establish Damage

This is unknown. Relatively small amounts of initial inoculum of oomycetes can initiate important epidemics. The Irish potato famine is a case in point. Epidemics of Oomycetes can become explosive if environmental conditions are conducive.

2. Likelihood of Surviving Initial Introduction

The potential for overwintering in U.S. production systems is not known.

3. Likelihood of Dissemination Beyond the Point of Introduction

If the pathogen can become established, dissemination beyond the point of introduction is likely via sporangia and/or zoospores.

4. Likelihood of Other Host Infection

If this pathogen is successfully introduced as a pathogen of sorghum, it is likely that it will also be established as a pathogen of maize. However, the reverse is not necessarily the case.

5. Likelihood of Early Detection

Taxonomic problems and unfamiliarity with this pathogen make it unlikely that epidemics would be discovered early. Particularly if the pathogen moves from corn to sorghum, it will take time for scientists to assess the situation.

6. Overall Risk

The overall risk for this species as a pathogen of sorghum is very low compared to other pathogen species already well-adapted to their potential hosts. The pathogen is likely a

greater risk to maize production in the U.S. if it can become established and is capable of overwintering.

7. Likelihood of an Agroterrorist Trying to Use.

Attempts to adapt this pathogen to sorghum might well be completely unsuccessful. The effort required to try for this adaptation would probably not be viewed as a good investment by any agroterrorist. On the other hand, the pathogen might potentially be introduced to U.S. maize production.

VII. Control/mitigation Strategies after Establishment

Control of related pathogens

Narro et al. (1992) state that *Peronosclerospora sorghi* (Sorghum Downy Mildew) attacks maize but does not produce oospores in maize. *Sclerophthora macrospora* is found in a limited area in Mexico**,** but is not commercially important. It is very effectively controlled with metalaxyl seed treatment. Narro et al. (1992) state the most effective control is to use resistant hybrids.

1. Resistance

Singh et al. (1970) maize genotypes differ in susceptibility. Of 2113 "different genetic materials scored under field conditions", 58 were highly resistant, 667 were resistant, 772 were moderately resistant, 478 were susceptible, and 138 were highly susceptible. Singh and Renfro (1971), in another screening trial, found that of 168 inbreds, 6 were highly resistant, 58 were moderately resistant, 26 were susceptible, and 24 were highly susceptible.

Dey et al., (1993) evaluated hybrids of maize for resistance to *Sclerophthora rayssiae* var. *zeae*, leaf blight (*Drechslera maydis*), and maize stalk borer (*Chilo partellus*) in Punjab. They found a few promising lines with resistance to both fungi and to the insect.

Khehra et al., (1978) state that partial dominance was important in F_1 crosses resulting from resistant or moderately resistant parents crossed with two susceptible parents.

Khehra et al. (1981) are of the opinion that breeding for resistance should exploit variations in resistance through selection. They used a land cultivar, mass selection and full sib mating to produce an improved version of the cultivar that produced a 60% higher yield than the original version. They recommend using additive resistance.

Additive effects have been reported to be important in resistance to brown stripe downy mildew in maize (Saxena et al., 1980; Singh and Asnani, 1975a), but Kalia et al. (1994) stated that resistance to BSDM is non-additive. Saxena et al. (1980) reported that 79% of variation in resistance to *S. rayssiae* var. *zeae* "appeared to be under additive control." They reported high heritability, and suggested simple mass selection as a strategy in breeding for resistance.

It appears there are multiple sources of resistance in maize that could be used to produce resistant lines for U. S. growers if the need arose. Whether these sources could be used to produce varieties that are agronomically acceptable to U. S. growers remains to be seen.

Sorghum lines are currently considered immune to the pathogen. If the pathogen can overcome this immunity, it is not known whether there will be a range of resistance types within sorghum or whether most will be susceptible.

2. Cultural Control

Singh et al. (1970) stated that the planting date in India in kharif (the rainy season in India) is important. Planting before the pre-monsoon showers resulted in less disease. Janke (1983) reported cultural controls of *P. sorghi* in Texas were deep till, roguing, and rotation, but rotation was less effective because some oospores were still present after four years. Zinc deficiency predisposes maize to brown stripe downy mildew (Safeeulla, 1975).

3. Chemical Control

Singh et al. (1970) tested germination of sporangia with 15 chemicals, finding 30 to 68% inhibition of germination. Chemicals that inhibited more than 50% were "bleaching powder", Rhizoctol, Plantvax, Fennite (9% tin hydroxide + 65% maneb), captan and copper

carbonate. Foliar sprays at 12-day intervals using Plantvax and Captan were effective. Soil drenches with Vitavax were effective.

Lal (1975) tested 14 fungicides, and found only three gave better than 50% control: Dithane M-45, (80%), Demosan 65W (65%), and Brestan (60%). Frequent foliar sprays were required for these levels of control. Lal et al. (1980) found that seed treatment with metalaxly (4g/kg seed), then foliar treatment also with metalaxyl (225 ppm active ingredient) 30 days after planting was effective. Seed treatment alone with metalaxyl was ineffective (Lal and Prasad, 1989; Lal et al., 1980).

Since current information about fungicide treatments for this pathogen is limited, fungicides developed for other downy mildews could be considered in the event of an introduction. They would be candidates for use and for quick tests of effectiveness.

4. Biological Control

No information is available about the potential for biocontrol for this pathogen on maize or sorghum.

5. Modeling Disease Incidence and Spread

Models of disease incidence and spread have not been developed. More detailed information about environmental requirements would be necessary for the development of useful models. Models developed for related pathogen species might be considered if the pathogen is introduced and risk assessments need to be performed quickly.

VIII. Knowledge Gaps

This pathogen has received little study, so almost all information required for evaluation of risks associated with it is lacking. First, it is not known how likely potential movement from maize to sorghum as a host may be. For maize or sorghum, it is not known what environmental conditions will support rapid inoculum buildup and overwintering of the pathogen under U.S. conditions. Much more such basic information would be required to develop estimates of thresholds of inoculum required for the establishment of populations in the U.S. Research is also needed to evaluate potential management responses to the

introduction or local establishment of the pathogen in either maize or sorghum to determine whether a localized epidemic has the potential for containment.

IX. Immediate Response Options

1. Rapid Detection

The symptoms of the pathogen are unusual and so might be noticed. Interpretation of the symptoms on sorghum would take time, however, since sorghum is not currently considered a host of this pathogen. Interpretation of the symptoms on maize could take time because the disease is not currently found in the U. S.

2. Control / 3. Fungicides

Fungicides used for other downy mildew species might be attempted for this pathogen, with a reasonable chance of success.

4. Resistance Breeding

Since all sorghum cultivars are currently resistant as far as we know, adaptation of this pathogen species to sorghum would require screening of representative cultivars to determine whether all were susceptible to the new pathogen strain or only some.

Appendix 1. Experts knowledgeable about Brown Stripe Downy Mildew

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Appendix 2. Description of Sclerophthora rayssiae var. zeae. (by the authors)

Payak and Renfro (1967) reported and named *Sclerophthora rayssiae* var. *zeae*.

The following taken from page p 395, Payak, M. M., and B. L. Renfro. 1967. A new downy mildew disease of maize. Phytopathology 57:394-397..

The maize downy mildew fungus reported here is presented as a new variety of *S. rayssiae* on the basis of its slightly larger sporangia, lack of golden or amber brown color in both oogonia and oospores, smaller oospore size, and hyaline, glistening oospore wall. Although the type variety was found on barley in Israel, host differences do not warrant erection of a new species.

Sclerophthora rayssiae var *zeae* Payak & Renfro **var. n.**

The fungus causes long stripes on leaves; shredding absent.

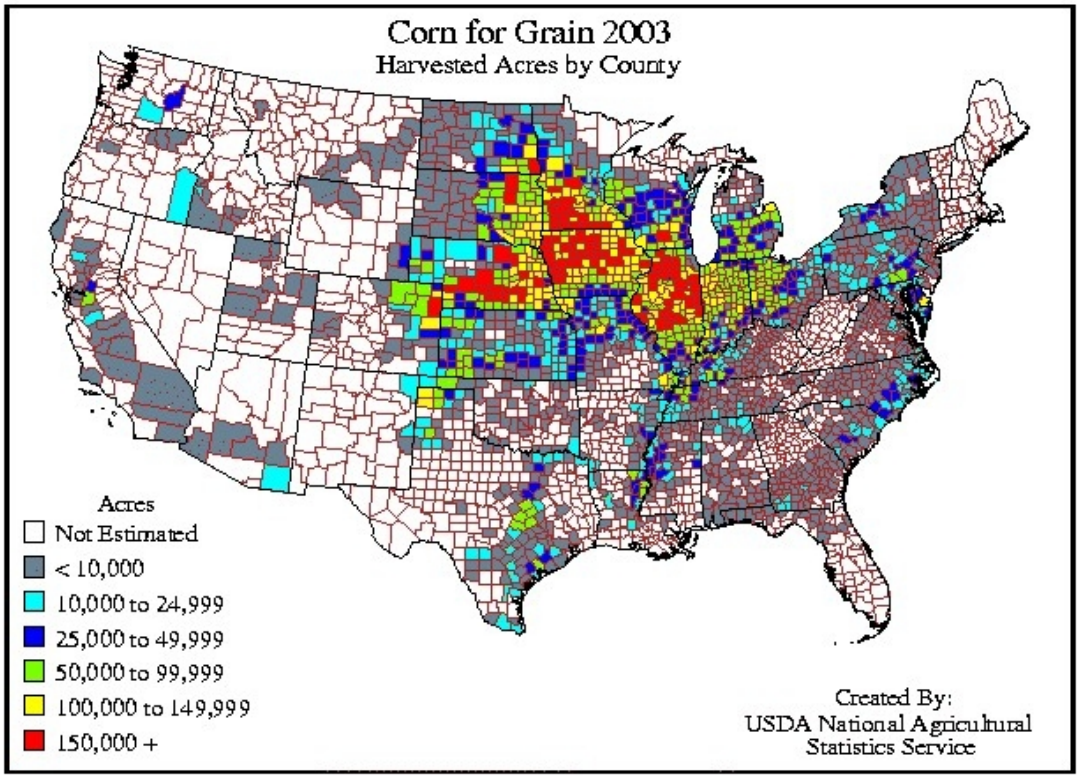
Sexual organs numerous, scattered in leaf mesophyll or under the stomata. Oogonia subglobose, thin-walled, hyaline to light straw-colored, with 1 or 2 paragynous antheridia, 33.0-44.5 Φ in diam.... Oospores centrally located in oogonia, spherical or subspherical, have hyaline contents including a prominent oil globule, with a smooth, glistening, uniformly 4-Φ-thick wall which is confluent with the oogonial wall, 29.5-37.0 W in diam....

Sporangiophores short, determinate, arise from hyphae congregated in the substomatal spaces, produce sporangia sympodially in groups of 2-6 ....

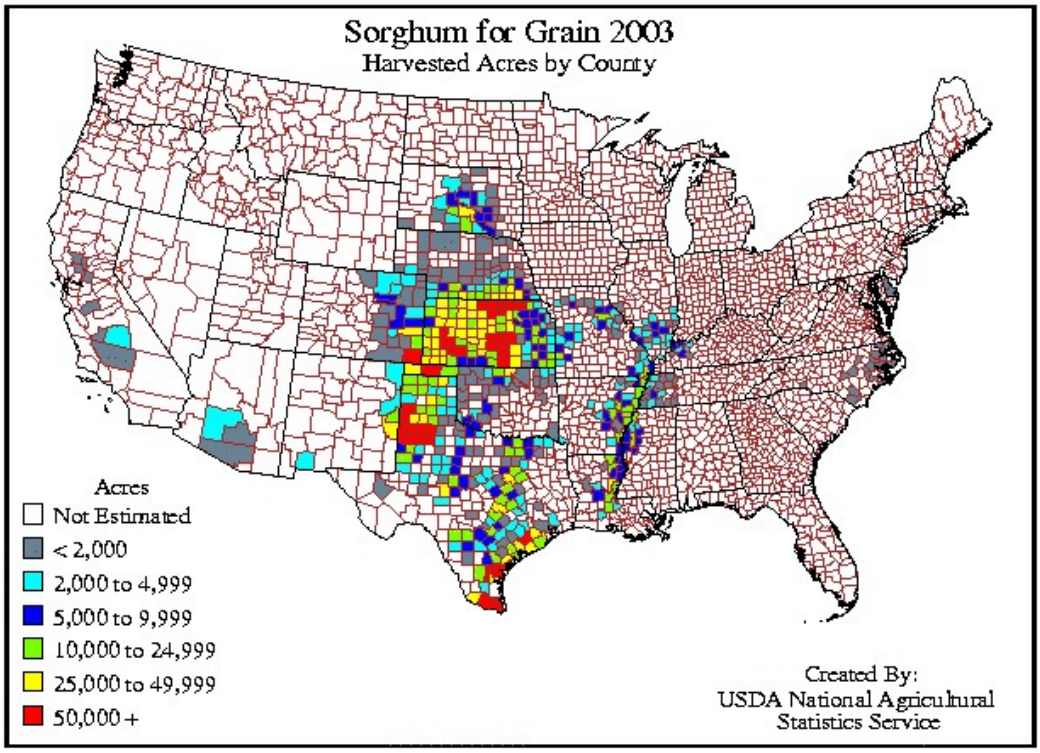
Sporangia... hyaline; ovate; obclavate, or elliptic or cylindrical; smooth-walled; having a truncate or rounded apex which is poroid; with a persistent, straight or cuneate peduncle; individually produce 4-8 zoospores...; 29.0-66.5 X 18.5-26.0 Φ in daim.

Encysted zoospores spherical, hyaline, 7.5-11.0 Φ in diam.

Appendix 3. Maize-growing regions of the U.S. (USDA)



Appendix 4. Sorghum-growing regions of the U.S. (USDA)



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