The Epidemiology of the Soybean/Diaporthe Phaseolorum Var. Caulivora Pathosystem in Louisiana.

Guy Boyd Padgett

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The epidemiology of the soybean/Diaporthe phaseolorum var. caulivora pathosystem in Louisiana

Padgett, Guy Boyd, Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1992
THE EPIDEMIOLOGY OF
THE SOYBEAN / DIAPORTHE PHASEOLORUM
VAR. CAULIVORA PATHOSYSTEM IN LOUISIANA

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Plant Pathology and Crop Physiology

by

Guy Boyd Padgett
B.S., Louisiana Tech University, 1984
M.S., University of Georgia, 1987
December 1992
TO MARILYN
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Finally, the one person who has stood by my side through it all, my wife. This surely would not have been possible if not for her untiring support and encouragement. I express my thanks and love for her persistence.
TABLE OF CONTENTS

DEDICATION ........................................................................................................ ii

ACKNOWLEDGEMENTS ...................................................................................... iii

LIST OF TABLES .................................................................................................... vii

ABSTRACT .............................................................................................................. ix

CHAPTER

I Introduction / Literature Review ................................................................. 1
   References Cited ................................................................................................. 7

II Relationship Between Plant Growth Stage at Time of Inoculation with Diaporthe phaseolorum var. caulivora, Environmental Parameters and Soybean Yield .................................................................................................................. 10
   Introduction ..................................................................................................... 11
   Materials and methods .................................................................................... 13
   Results ............................................................................................................. 16
   Discussion ....................................................................................................... 22
   References Cited .............................................................................................. 26

III The Relationship Between Soybean Growth Stage and Infection by Diaporthe phaseolorum var. caulivora ................................................................. 30
   Introduction ..................................................................................................... 31
   Materials and methods .................................................................................... 33
   Results ............................................................................................................. 36
   Discussion ....................................................................................................... 44
   References Cited .............................................................................................. 47

IV Relationship of Stem Canker Severity and Threecornered Alfalfa Hopper Injury ................................................................. 51
   Introduction ..................................................................................................... 52
   Materials and methods .................................................................................... 54
   Results ............................................................................................................. 55
   Discussion ....................................................................................................... 59
   References Cited .............................................................................................. 61
LIST OF TABLES

Table 2.1: Stem canker disease severity and yields of 'Bedford' and 'Wilstar 550' inoculated at planting, V3, V6, or R2 at Burden Research Plantation, Baton Rouge, LA, 1989 (Study 1) ....17

Table 2.2: Rainfall and temperature records for 1989, Burden Research Plantation, Baton Rouge, LA (Study 1) ............................................. 17

Table 2.3: Stem canker disease severity and yields of 'Bedford' and 'Wilstar 550' inoculated at Vc, V3, V7, R1, or R3 at Burden Research Plantation, Baton Rouge, LA, 1990 (Study 1) .... 19

Table 2.4: Rainfall and temperature records for 1990, Burden Research Plantation, Baton Rouge, LA (Study 1) ............................................. 19

Table 2.5: Stem canker disease severity and yields of 'Bedford', 'Wilstar 550', and 'Bay' inoculated at Vc, V3, V7, R1, or R3 at Ben Hur Research Farm, Baton Rouge, LA, 1991 (Study 1) ..................................... 20

Table 2.6: Rainfall and temperature records for 1991, Ben Hur Research Farm, Baton Rouge, LA (Study 1) ............................................. 21

Table 2.7: Perithecia on 'Bedford' and 'Wilstar 550' stem debris taken from plots inoculated at Vc, V3, V7, R1, or R3, at Burden Research Plantation, Baton Rouge, LA, 1990 (Study 1) ............................. 22

Table 3.1: Stem canker incidence in plant sets placed at weekly intervals in a soybean field with a history of stem canker epidemics, Burden Research Plantation, Baton Rouge, 1989 (Study 2) ............................................. 38

Table 3.2: Pearson correlation coefficients for stem canker, rainfall, temperature and relative humidity, Burden Research Plantation, Baton Rouge, LA, 1989 (Study 2) ................................. 38

Table 3.3: Stem canker incidence in plant sets placed at weekly intervals in a soybean field with a history of stem canker epidemics, Burden Research Plantation, Baton Rouge, 1990 (Study 2) ............................................. 40
Table 3.4: Pearson correlation coefficients for stem canker, temperature, rainfall, and relative humidity, Burden Research Plantation, Baton Rouge, LA, 1990 (Study 2) ....................... 40

Table 3.5: Stem canker incidence in plant sets placed at weekly intervals in a soybean field with a history of stem canker epidemics, Burden Research Plantation, Baton Rouge, 1991 (Study 2) ................................. 42

Table 3.6: Pearson correlation coefficients for stem canker, temperature, rainfall, and relative humidity, Burden Research Plantation, Baton Rouge, LA, 1991 (Study 2) ....................... 42

Table 4.1: Plant growth parameters and stem canker disease on 'Bragg' soybean girdled or not girdled at stem bases by threecornered alfalfa hopper (Baton Rouge, LA, 1988) (Study 3) ........................................... 57

Table 4.2: Plant growth parameters and stem canker disease severity on 'Bedford' soybean girdled or not girdled at stem bases by threecornered alfalfa hopper (Baton Rouge, LA) (Study 3) ........................................... 58

Table 5.1: Percentage of inoculated plants from which Diaporthe phaseolorum var. caulivora was isolated in a greenhouse experiment, Louisiana State University, Baton Rouge, LA, 1990 (Study 4) ........................................... 73

Table 5.2: Percentage of plants with pycnidia, number of perithecia, and number of pycnidia for weeds inoculated with Diaporthe phaseolorum var. caulivora. Louisiana State University, Baton Rouge, LA, 1991 (Study 4) ................. 75
ABSTRACT

Field and greenhouse experiments were conducted during 1988-1991 to investigate the epidemiology of the soybean / Diaporthe phaseolorum var. caulivora (Dpc) pathosystem. The following points were addressed: (i) the effect of inoculation timing on soybean yield and disease severity, (ii) the efficacy of Dpc inoculum during the growing season, (iii) the possibility of alternative hosts for Dpc, and (iv) the relationship between threecornered alfalfa hopper injury and stem canker severity.

Inoculation timing had a significant effect on the yield of Dpc susceptible or moderately susceptible soybean cultivars. Soybean yield was reduced most (83 to 93%) and disease severity was greatest (84 to 99%) when cultivars were inoculated at the Vc or V3 growth stage. Yield reduction was not as severe when cultivars were inoculated at the late vegetative or early reproductive growth stages. Significant positive correlations were recorded between rainfall and stem canker severity and relative humidity and stem canker severity.

The ability of Dpc to infect soybean during the growing season was investigated. Stem canker incidence was greatest 4-7 weeks after planting. While infection of soybean by Dpc decreased 7 weeks after planting, Dpc inoculum was still infecting soybean 11 weeks after planting.
Results from Dpc host range experiments indicated weeds commonly found in south Louisiana soybean fields can serve as alternative hosts for Dpc. Dpc colonized and reproduced in several morningglory species, several leguminous weeds, and wild poinsettia.

Threecornered alfalfa hopper injury resulted in increased stem canker severity compared to soybean not injured by this insect. Stem diameter and length and seed and pod yields were reduced compared to noninjured plants infected with Dpc.
CHAPTER I

INTRODUCTION/LITERATURE REVIEW
Soybean (Glycine max (L.) Merrill), an important crop worldwide, is grown primarily for vegetable oil and as a protein source (1.2). Soybean oil accounts for one-half of the world total for oil produced from oilseed crops (1.2). Understandably, a crop that contributes this much to total oilseed production should be managed as efficiently as possible. Therefore, to develop management strategies which lead to economic and efficient production of soybean is imperative.

Soybean is affected by more than 100 pathogens, insects, weeds, and abiotic factors that interfere with growth and development (1.1, 1.2). Stem canker disease of soybean, caused by the fungus Diaporthe phaseolorum (Cke. & Ell.) Sacc. var. caulivora Athrow & Caldwell (Dpc), is a destructive disease that can limit yields of susceptible cultivars up to 80% (1.3, 1.6). The disease has been reported in Europe, the United States, and Canada (1.2). Stem canker was first identified in the United States during the early 1940's in Iowa (1.24). Yields of soybean were reduced up to 50%. By eliminating the cultivars Hawkeye and Blackhawk, the disease was reduced to an economically acceptable level (1.3). Today, this disease has little impact on soybean yields in the midwestern United States (1.3).

Stem canker was reported in the southern United States in the mid-1950's, but was of little importance until 1973 (1.22). Stem canker spread slowly throughout the southern
United States and was not reported in Louisiana until 1981. In the southern United States, management of soybean stem canker is more complex because asymptomatic, resistant cultivars allow Dpc to produce inoculum for the next growing season (1.3). This eliminates the use of disease history as an indicator for potential stem canker epidemics because Dpc inoculum might be underestimated (1.3). Therefore, to better manage this disease, it is important to understand the epidemiology of this pathogen.

Currently, stem canker is thought to be a monocyclic disease. Inoculum (ascospores and conidia) for the current growing season epidemic is produced on soybean stem debris infected from the previous growing season (1.3). During the early spring, ascospores and conidia are disseminated by rain splash to surrounding soybean plants. The fungus infects and colonizes the plant, and if disease continues to develop, the plant dies prematurely.

While Dpc may infect plants when they are young, symptoms usually are not evident until the early to mid reproductive stages of the crop (1.3). Foliar symptoms initiate as an interveinal chlorosis. If conditions favor disease development, this chlorosis progresses to a necrosis, and the dead leaves remain attached to the plant. While the foliar symptoms are similar to other soybean diseases (ie. red crown rot and sudden death syndrome), the stem symptoms are very diagnostic. Stem cankers begin as pinpoint,
reddish-brown lesions, usually at the base of a petiole on the lower stem. If the disease progresses, these cankers elongate up and down the stem.

Keeling and McGee (1.7,1.9) have shown that differences in pathogenicity exist between southern and northern isolates of Dpc, suggesting control measures effective in the northern United States may not be effective in the southern United States. Lee (1.8) provided further evidence of a difference between southern and northern United States isolates of Dpc by showing that they differ genetically. Surveys conducted throughout the southern United States have indicated that "southern" stem canker epidemics can be devastating to soybean yields and difficult to predict in contrast to epidemics in the northern United States (1.10,1.11,1.12,1.18,1.22). Therefore, research has been conducted to better understand the epidemiology of this disease (1.3,1.4,1.14,1.15,1.19,1.20). Backman et al. (1.3) suggested there is limited potential for long range spread because stem canker is monocyclic. High percentages of seed transmission (>25%) occur only in the northern United States (1.3). Backman (1.3) reported ascospores cause primary infections from April through June, but there is no mention of role of the conidia. In greenhouse studies, Ploetz and Shokes (1.13) demonstrated ascospores and conidia cause infection in soybean, indicating the possible role of conidia in stem canker epidemiology. Although stem canker
is thought to be monocyclic, the presence of pycnidia during the growing season and evidence of plant to plant spread might indicate otherwise (1.21). The existence of asymptomatic hosts and the missing information concerning the role of conidia in stem canker epidemiology may explain how stem canker epidemics develop in areas with no apparent source of inoculum.

Other studies concerning the epidemiology of stem canker have shown soybean to be most susceptible to Dpc at early vegetative stages (V3-V6) (1.3,1.5,1.19,1.20). Rothrock et al. found that conventional tillage and a soybean/fallow cropping system in combination with resistance resulted in lower disease incidence compared to no-till and a double cropping system (1.14,1.15). Another effective management practice is to delay planting (1.20). Work conducted by Damicone et al. (1.4) indicated the importance of free moisture as it related to successful infection. Subbarao et al. (1.23) demonstrated the importance of soil moisture in the development and sporulation of perithecia. Other research has demonstrated the importance insect/pathogen interactions and how these relationships affect stem canker severity (1.16,1.17). All of the above information has added to the understanding of stem canker, but there are still questions to be answered concerning this disease.
Knowledge is lacking concerning the longevity of initial inoculum during the growing season. Studies should be conducted to determine if infection by Dpc can occur at certain soybean growth stages and not affect yield. Finally, it is important to realize the relationships between Dpc and other pests (i.e. weeds, nematodes, and insects). Most of the interaction research has been conducted in microplots or the greenhouse. The results from these experiments must be confirmed under field conditions. This information could be of significance when using decision making models or applying fungicides. Therefore, the following objectives are addressed in this dissertation:

1. To determine how inoculation with Dpc at a specific soybean growth stage affects stem canker severity and soybean yield, and to determine how environmental parameters affect stem canker development.
2. To quantify infection by Dpc during the growing season.
3. To evaluate the relationship between stem canker severity and injury caused by the threecornered alfalfa hopper, Spississtilus festinus (Say).
4. To evaluate common weeds found in south Louisiana fields as hosts for Dpc.
References Cited


CHAPTER II

RELATIONSHIP BETWEEN PLANT GROWTH STAGE
AT TIME OF INOCULATION
WITH *Diaporthe phaseolorum* var. *caulivora*,
ENVIRONMENTAL PARAMETERS, AND SOYBEAN YIELD
Introduction

Stem canker, caused by Diaporthe phaseolorum (Cke. & Ell.) Sacc. var. caulivora Athow & Caldwell (Dpc), is an important fungal pathogen affecting soybean (Glycine max (L.) Merrill. Stem canker was reported in the midwestern United States in the 1940's and was managed by eliminating cultivars 'Blackhawk' and 'Hawkeye' from production (2.2). Today, stem canker is not a problem in the midwestern United States, but it is a problem in the southern United States. In years when epidemics are severe, soybean yields can be reduced 80% in susceptible cultivars (2.10). This is due in part to the fact that strains of Dpc in the northern United States differ in pathogenicity (2.11), morphology (2.13) and genetics (2.12) from those in the southern United States. Stem canker resistant cultivars used in the northern United States may not be resistant to Dpc isolates in the southern United States. Therefore, the development of techniques and strategies to manage "southern" stem canker is important.

To reduce stem canker, an understanding of the factors affecting its epidemiology is crucial. Research has been conducted to ascertain information about "southern" stem canker epidemiology. The majority of the work has been directed at evaluating the effect of environmental factors, resistance, and cultural practices on disease development. Moisture and temperature are key factors for infection and reproduction by Dpc (2.7,2.15,2.16,2.24). Epidemics also are
affected by cropping systems and cultural practices (2.18,2.19). Understanding how these factors affect stem canker epidemics has contributed to the management of this disease, but other research areas still need attention.

Currently, the use of host plant resistance is the most economical strategy for managing stem canker. Variety evaluations and stem canker nurseries are routinely used to identify resistance to Dpc (2.4,2.5,2.23). Inoculum of Dpc is introduced at an early vegetative stage or present as "natural inoculum" on infected stem debris from the previous growing season. Because plants are inoculated at an early vegetative stage or infection is allowed to occur "naturally" (unknown time), subsequent yield loss in varieties inoculated late in the growing season has not been evaluated.

The relationship between inoculation timing and subsequent disease severity has been evaluated (2.22). However, the relationship between soybean growth stage at the time of inoculation with Dpc and yield loss has not been determine. Knowledge of the relationship between growth stage at the time of inoculation and subsequent yield loss is critical for developing yield loss and disease management models. How crop growth stage at the time of inoculation affects subsequent production of perithecia and pycnidia by Dpc also is important. The objectives were to determine how growth stage at time of inoculation affects yield, stem canker severity, and inoculum load for the next season.
Materials and Methods

Experimental unit and design. Experiments were conducted during 1989-1991 at Burden Research Plantation (1989 & 1990) and Ben Hur Research Farm (1991), Baton Rouge, LA. These experiments were conducted where stem canker epidemics had not occurred for several years. Soybean cultivars 'Bedford' (Dpc susceptible) and 'Wilstar 550' (Dpc moderately susceptible) were used in 1989 and 1990, and 'Bay' (Dpc resistant) was added in 1991. Cultivars were planted on 5 May, 1989, 16 May, 1990; and 1 July, 1991. Seeds were inoculated with the labelled rate of commercial inoculant (Nitragin, *Rhizobium japonicum*, LiphaTech, Inc., Milwaukee, WI) prior to planting. Row spacings were 0.76 m in 1989 and 1991, and 0.91 m in 1990. Experimental units consisted of 4 row plots 6.09 m long. The experiments were arranged as a randomized complete block design with four replications. Replications were separated by 6.09 m of fallow ground and plots within reps were separated by 3 m of fallow ground to reduce interplot interference.

Metolachlor plus metribuzin herbicide (1.752 l/ha) was applied preemergence in 1989. Metolachlor plus metribuzin herbicide (2.337 l/ha) plus glyphosate herbicide (4.674 l/ha) was applied preemergence in 1990 and 1991. Imazaquin (0.803 l/ha) was applied preemergence in 1991. Tralomethrin (0.241 l/ha) insecticide was applied once in 1990 and 1991. Bentazon plus salt of acifluorfen (1.752 l/ha), sethoxydim
(1.168 l/ha), fluazifop-P-butyl (1.752 l/ha), and bentazon (2.337 l/ha) were applied postemergence as needed for general weed control. Nonionic surfactants were applied at the labelled rate with the postemergence herbicides.

**Inoculum production and application.** Inoculum of Dpc was produced on oats. Prior to transfer to oats, Dpc was maintained on potato dextrose agar acidified with lactic acid 2 ml acid / 1 agar (pH 4.5). Jars (0.95 l) (Ball Corp. Muncie, Indiana) were filled with oats, and enough tap water was added to fill the jar. Oats were allowed to imbibe water for 24 hr, after which the excess water was decanted. Lids with a cotton-filled hole (9.6 mm) were placed on each jar. Oats were autoclaved three times (one hour/autoclaving on three consecutive days). Mycelial plugs (8 mm) of Dpc (isolate: Opelousas-3, Burden) were transferred from the petri dishes to the oats and allowed to grow. Inoculum was stored for 8 to 10 weeks in the lab (approximately 23°C) prior to use. Prior to each inoculation, inoculum was removed from jars and mixed in a common container to ensure homogeneity. Each plot received a single application of Dpc inoculum at a predetermined plant growth stage (2.8): planting, V3, V6, V8, or R2 in 1989; Vc, V3, V7, R1, or R3 in 1990; and Vc, V3, V7, or R1 in 1991. A noninoculated control was included for each cultivar in each experiment. The two center rows of each plot were inoculated by spreading 0.95 l of Dpc infested oats over each row. Immediately after inoculation, screens
(3.04 m x 25 cm) were placed or soil banks (15 cm in height) were constructed perpendicular to the four rows of the plot to contain inoculum within plots. Inoculations were conducted in the evening between 1800 and 2000 hr.

**Disease rating.** Plants were monitored weekly throughout the growing season for symptoms of stem canker. When symptoms were evident, isolations were made to confirm the presence of Dpc. Isolations were performed by excising a 3 mm x 3 mm section of the margin of a stem lesion, washing the tissue in a 1:80% solution of sodium hypochlorite/water for 1 minute followed by a 3 minute washing in sterile water. The tissue was blotted dry and placed onto acidified potato dextrose agar (p.H. 4.5).

When soybean plants were at growth stage R6, ratings for stem canker severity were made. Both foliar and stem disease ratings were made when present using a 0 - 100% scale (0 = no disease, 100 = all of the rated stem or foliage exhibiting symptoms of stem canker). Stem ratings were made on the lower half of the stem of plants on one m of each inoculated row. Foliar ratings were conducted by estimating the percent of foliage exhibiting stem canker symptoms on each plant.

The two center rows of each plot were harvested for yield comparisons. Soybean was harvested at R8 using a small plot combine (Almaco SPC 20). Soybean seed from each plot was weighed and moisture content was determined. The yields for each plot were adjusted to kg/ha at 13% moisture.
For the experiment initiated in 1990, two stems from each plot were collected 10 February 1991 and perithecia were quantified. The lower three nodes of each stem were labelled by plot number and taken to the laboratory. The number of perithecia in a 0.5 X 0.5 cm section of each stem was counted and converted to number perithecia / cm².

Data were analyzed using the general linear models procedure in SAS version 5.18 (2.21).

Results

In 1989, stem canker severity was greatest and yields were lowest when 'Bedford' and 'Wilstar 550' were inoculated at V3 (Table 2.1). Compared with the control, yields were reduced 93 and 83% in 'Bedford' and 'Wilstar 550', respectively, when inoculated at V3. Cumulative rainfall of 12.1 cm and an average relative humidity of 85% occurred during the 16 day period following the V3 inoculation (Table 2.2). Compared to the control, significant (P <= 0.05) reductions in yield also occurred when 'Bedford' was inoculated at planting and V6, but stem canker severity was significantly (P <= 0.05) different only in plots inoculated at planting. Stem canker epidemics did not develop in 'Wilstar 550' when inoculated at any growth stage other than V3. (Table 2.1).
Table 2.1. Stem canker disease severity and yields of 'Bedford' and 'Wilstar 550' inoculated at planting, V3, V6, or R2 at Burden Research Plantation, Baton Rouge, LA, 1989

<table>
<thead>
<tr>
<th>Cultivar at Inoculation</th>
<th>Growth Stage</th>
<th>Stem canker Severity</th>
<th>Yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bedford</td>
<td>Planting</td>
<td>51*</td>
<td>449*</td>
</tr>
<tr>
<td>Bedford</td>
<td>V3</td>
<td>97*</td>
<td>65*</td>
</tr>
<tr>
<td>Bedford</td>
<td>V6</td>
<td>30</td>
<td>430*</td>
</tr>
<tr>
<td>Bedford</td>
<td>R2</td>
<td>24</td>
<td>719</td>
</tr>
<tr>
<td>Bedford Control</td>
<td></td>
<td>13</td>
<td>941</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td>24</td>
<td>243</td>
</tr>
</tbody>
</table>

| Wilstar 550            | Planting     | 1                    | 1249         |
| Wilstar 550            | V3           | 84*                  | 257*         |
| Wilstar 550            | V6           | 4                    | 1646         |
| Wilstar 550            | R2           | 3                    | 1333         |
| Wilstar 550 Control    |              | 1                    | 1560         |
| LSD (0.05)             |              | 8                    | 728          |

1 Foliar severity = Percent of foliage / plant exhibiting symptoms at growth stage R6.
2 * = number in the same column differ significantly from the noninoculated control, alpha = 0.05.
3 Controls were not inoculated with Dpc.

Table 2.2. Rainfall and temperature records for 1989, Burden Research Plantation, Baton Rouge, LA

<table>
<thead>
<tr>
<th>Growth Stage</th>
<th>Period Parameters were Measured</th>
<th>Rainfall (cm)</th>
<th>Average Temperature (C)</th>
<th>Average Relative Humidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planting-V3</td>
<td>6/2 - 6/19</td>
<td>10.8</td>
<td>25</td>
<td>81</td>
</tr>
<tr>
<td>V3-V6</td>
<td>6/20 - 7/6</td>
<td>12.1</td>
<td>26</td>
<td>85</td>
</tr>
<tr>
<td>V6-R1</td>
<td>7/7 - 8/1</td>
<td>4.5</td>
<td>26</td>
<td>81</td>
</tr>
<tr>
<td>R1-R3</td>
<td>8/2 - 8/15</td>
<td>0.4</td>
<td>25</td>
<td>75</td>
</tr>
</tbody>
</table>

1 Average daily temperature.
2 Average daily relative humidity.
During 1990, greatest stem canker severity and lowest yields occurred when cultivars were inoculated at Vc (Table 2.3). Compared with the control, yields from plots inoculated at Vc were reduced 91 and 90% in 'Bedford' and 'Wilstar 550', respectively. Stem canker severity and yields differed significantly (P <= 0.05) from the control when 'Bedford' was inoculated at V7 and when 'Wilstar 550' was inoculated at V3 and R3. Stem canker severity and yield were not affected when 'Bedford' was inoculated at V3 or the reproductive stages. Yield of 'Wilstar 550' was not affected when inoculated at V7 or R1, even though foliar disease symptoms were evident following inoculations at V7. High rainfall amounts were recorded for the periods following the Vc and V7 inoculations (Table 2.4).
Table 2.3. Stem canker disease severity and yields of 'Bedford' and 'Wilstar 550' inoculated at Vc, V3, V7, R1, or R3 at Burden Research Plantation, Baton Rouge, LA, 1990

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Growth Stage at Inoculation</th>
<th>Stem Canker Severity</th>
<th>Foliar Yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bedford</td>
<td>Vc</td>
<td>99*</td>
<td>232*</td>
</tr>
<tr>
<td>Bedford</td>
<td>V3</td>
<td>3</td>
<td>2189</td>
</tr>
<tr>
<td>Bedford</td>
<td>V7</td>
<td>16*</td>
<td>1318*</td>
</tr>
<tr>
<td>Bedford</td>
<td>R1</td>
<td>0</td>
<td>2628</td>
</tr>
<tr>
<td>Bedford</td>
<td>R3</td>
<td>1</td>
<td>2143</td>
</tr>
<tr>
<td>Bedford</td>
<td>Control</td>
<td>0</td>
<td>2692</td>
</tr>
<tr>
<td></td>
<td>LSD (0.05)</td>
<td>7</td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Growth Stage at Inoculation</th>
<th>Stem Canker Severity</th>
<th>Foliar Yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilstar 550</td>
<td>Vc</td>
<td>98*</td>
<td>195*</td>
</tr>
<tr>
<td>Wilstar 550</td>
<td>V3</td>
<td>11*</td>
<td>1352*</td>
</tr>
<tr>
<td>Wilstar 550</td>
<td>V7</td>
<td>2</td>
<td>1699</td>
</tr>
<tr>
<td>Wilstar 550</td>
<td>R1</td>
<td>6</td>
<td>2303</td>
</tr>
<tr>
<td>Wilstar 550</td>
<td>R3</td>
<td>25*</td>
<td>1259*</td>
</tr>
<tr>
<td>Wilstar 550</td>
<td>Control</td>
<td>0</td>
<td>1953</td>
</tr>
<tr>
<td></td>
<td>LSD (0.05)</td>
<td>8</td>
<td>28</td>
</tr>
</tbody>
</table>

1 Stem severity = Percent of lower stem covered with lesions at growth stage R6. Foliar severity = Percent of foliage exhibiting symptoms.
2 * = number in the same column differ significantly from the noninoculated control, alpha = 0.05.
3 Controls were not inoculated with Dpc.

Table 2.4. Rainfall and temperature records for 1990, Burden Research Plantation, Baton Rouge, LA

<table>
<thead>
<tr>
<th>Period</th>
<th>Average Rainfall (cm)</th>
<th>Average Temperature (°C)</th>
<th>Average Relative Humidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Stage</td>
<td>Parameters Measured</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vc</td>
<td>5/25 - 6/6</td>
<td>12.6</td>
<td>25</td>
</tr>
<tr>
<td>V3</td>
<td>6/7 - 6/20</td>
<td>2.7</td>
<td>28</td>
</tr>
<tr>
<td>V7</td>
<td>6/21 - 7/5</td>
<td>9.6</td>
<td>26</td>
</tr>
<tr>
<td>R1</td>
<td>7/6 - 7/25</td>
<td>3.6</td>
<td>26</td>
</tr>
<tr>
<td>R3</td>
<td>7/26 - 8/9</td>
<td>1.2</td>
<td>27</td>
</tr>
</tbody>
</table>

1 Average daily temperature.
2 Average daily relative humidity.
In 1991, no significant differences were noted in stem canker severity and yield in any of the treatments (Table 2.5). Stem canker epidemics did not develop until late in the season. In addition, the greatest stem canker severity (38%) occurred in the noninoculated 'Bedford'. Rainfall and average relative humidity were low in 1991 compared to 1989 and 1990 (Table 2.6).

Table 2.5. Stem canker disease severity and yields of 'Bedford', 'Wilstar 550', and 'Bay' inoculated at Vc, V3, V7, R1, or R3, at Ben Hur Research Farm, Baton Rouge, LA, 1991

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Growth Stage at Inoculation</th>
<th>Stem canker Severity¹</th>
<th>Yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bedford</td>
<td>Vc</td>
<td>22</td>
<td>1935</td>
</tr>
<tr>
<td>Bedford</td>
<td>V3</td>
<td>30</td>
<td>1861</td>
</tr>
<tr>
<td>Bedford</td>
<td>V7</td>
<td>35</td>
<td>2088</td>
</tr>
<tr>
<td>Bedford</td>
<td>R1</td>
<td>23</td>
<td>2293</td>
</tr>
<tr>
<td>Bedford Control ²</td>
<td>38</td>
<td>1999</td>
<td></td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td>41</td>
<td>499</td>
</tr>
<tr>
<td>Wilstar 550</td>
<td>Vc</td>
<td>33</td>
<td>2012</td>
</tr>
<tr>
<td>Wilstar 550</td>
<td>V3</td>
<td>22</td>
<td>2137</td>
</tr>
<tr>
<td>Wilstar 550</td>
<td>V7</td>
<td>24</td>
<td>2125</td>
</tr>
<tr>
<td>Wilstar 550</td>
<td>R1</td>
<td>9</td>
<td>2280</td>
</tr>
<tr>
<td>Wilstar 550 Control ²</td>
<td>6</td>
<td>2485</td>
<td></td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td>33</td>
<td>876</td>
</tr>
<tr>
<td>Bay</td>
<td>V3</td>
<td>20</td>
<td>1203</td>
</tr>
<tr>
<td>Bay</td>
<td>R1</td>
<td>5</td>
<td>1534</td>
</tr>
<tr>
<td>Bay Control ²</td>
<td>2</td>
<td>1599</td>
<td></td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>29</td>
<td>1097</td>
<td></td>
</tr>
</tbody>
</table>

¹ Percent of lower stem covered with lesions at growth stage R6.
² Controls were not inoculated with Dpc.
Table 2.6. Rainfall and temperature records for 1991, Ben Hur Research Farm, Baton Rouge, LA

<table>
<thead>
<tr>
<th>Growth Stage</th>
<th>Period</th>
<th>Rainfall (cm)</th>
<th>Average Temperature (°C)(^1)</th>
<th>Average Relative Humidity(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vc</td>
<td>7/12 - 7/21</td>
<td>0.0</td>
<td>28</td>
<td>43</td>
</tr>
<tr>
<td>V3</td>
<td>7/22 - 8/1</td>
<td>0.5</td>
<td>28</td>
<td>42</td>
</tr>
<tr>
<td>V7</td>
<td>8/2 - 8/11</td>
<td>0.7</td>
<td>28</td>
<td>46</td>
</tr>
<tr>
<td>R1</td>
<td>8/12 - 8/21</td>
<td>0.0</td>
<td>26</td>
<td>46</td>
</tr>
</tbody>
</table>

\(^1\) Average daily temperature.
\(^2\) Average daily relative humidity.

Significant correlations (P<= 0.005) were noted between yield and stem canker severity (-0.674), rainfall (-0.707), temperature (0.555), and relative humidity (-0.591); between stem canker severity and rainfall (0.544). Stem canker severity and average relative humidity were correlated (P<= 0.1066, -0.591).

Perithecia were produced on stem pieces collected in 1991 from plants inoculated in 1990 regardless of inoculation timing. Numbers of perithecia were not significantly different (P <= 0.05) between any treatments, except when 'Bedford' was inoculated at Vc (Table 2.7). Stems of plants inoculated at Vc supported more perithecia than plants inoculated at other growth stages.
Table 2.7. Perithecia on 'Bedford' and 'Wilstar 550' stem debris taken from plots inoculated at Vc, V3, V7, R1, or R3, at Burden Research Plantation, Baton Rouge, LA, 1990

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Inoculation Timing</th>
<th>Perithecia (cm²)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bedford</td>
<td>Vc</td>
<td>46</td>
</tr>
<tr>
<td>Bedford</td>
<td>V3</td>
<td>18</td>
</tr>
<tr>
<td>Bedford</td>
<td>V7</td>
<td>27</td>
</tr>
<tr>
<td>Bedford</td>
<td>R1</td>
<td>21</td>
</tr>
<tr>
<td>Bedford</td>
<td>R3</td>
<td>22</td>
</tr>
<tr>
<td>Bedford</td>
<td>Control²</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>LSD (0.05)</td>
<td>33</td>
</tr>
<tr>
<td>Wilstar 550</td>
<td>Vc</td>
<td>50</td>
</tr>
<tr>
<td>Wilstar 550</td>
<td>V3</td>
<td>39</td>
</tr>
<tr>
<td>Wilstar 550</td>
<td>V7</td>
<td>31</td>
</tr>
<tr>
<td>Wilstar 550</td>
<td>R1</td>
<td>19</td>
</tr>
<tr>
<td>Wilstar 550</td>
<td>R3</td>
<td>37</td>
</tr>
<tr>
<td>Wilstar 550</td>
<td>Control</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>LSD (0.05)</td>
<td>43</td>
</tr>
</tbody>
</table>

¹ Average number of perithecia covering a cm² area on the lower three nodes of the stem. Collected February 10, 1991.
² Controls were not inoculated with Dpc.

Discussion

Yields and stem canker severity were affected differently depending on the growth stage at which plants were inoculated. In 1989 and 1990, yield reductions and stem canker severity were most severe in both cultivars when inoculated at an early vegetative stage (Vc-V3). Stem canker severity was greater in 'Wilstar 550' in 1990 than in 1989. In 1990, when cultivars were inoculated at Vc and V7, stem canker foliar severity was similar for 'Wilstar 550' and
'Bedford'. Results from previous research have demonstrated that reaction to Dpc is inconsistent with 'Wilstar 550' and other cultivars (2.4, 2.5, 2.6, 2.14).

Moderate stem canker severity (43%) was noted in 1990 when 'Wilstar 550' was inoculated at R3. Although measures were taken to prevent interplot interference, Dpc inoculum may have been introduced from adjacent plots. The 'Wilstar 550' plots inoculated at R3 were adjacent to plots inoculated at Vc or V3 in every replication. A rainfall amount of 12.6 cm occurred the week after the Vc inoculations were conducted. It is possible that the rainfall caused the dispersal of inoculum from one plot to adjacent plots.

In 1991, stem canker epidemics developed late in the year. Small cankers were seen early in the 1989 and 1990 growing season on the stems of plants inoculated at early vegetative stages. Cankers were not evident on plants inoculated at early vegetative stages until late in the 1991 growing season. Therefore, yield probably was not affected by Dpc in 1991. The reason why epidemics developed late in the year in 1991 was due in part to dry weather during the time inoculations were conducted. Rainfall was not present which probably reduced infection by Dpc (Table 2.6). The fact that stem canker epidemics developed in the 'Bedford' control plots may be due to the introduction of Dpc inoculum from an adjacent field where soybean varieties were screened for resistance to Dpc the previous year. Stem canker
epidemics occurred in the adjacent field the previous year. Infested soybean stem debris may have been moved from field to field by discing during seedbed preparation.

While growth stage at time of inoculation experiments can provide valuable information on the epidemiology of disease, environmental factors that vary from inoculation period to inoculation period can confound results. The effects of environment on these types of experiments are mentioned briefly by Sah and MacKenzie (2.20). The correlation of rainfall (moisture) and stem canker severity further supports the importance of free moisture for infection by Dpc demonstrated previously in the greenhouse (2.7). Inferences about the effects of temperature and relative humidity on disease development are limited because within year variation was minimal, yet year to year average relative humidity varied considerably. In 1991, average relative humidity varied between 42 and 46% compared to 1989 and 1990 when average relative humidity varied between 72 and 85%. Stem canker was more severe in years when relative humidity was high (72-85%) implicating a possible role of relative humidity in stem canker epidemiology. During periods of high relative humidity, available free moisture (dew) persist longer permitting a longer wetness period for spore germination.

Perithecia were present on stems the following year regardless of growth stage at inoculation. Stem canker is a
monocyclic disease, so inoculum must be produced on infected stem debris from the previous year for present seasons epidemics. No research has been conducted to determined the amount of inoculum needed to initiate and sustain an epidemic. Therefore, the production of inoculum on cultivars inoculated at early reproductive stages may be important for stem canker epidemics in subsequent years. Cultivars inoculated at some early reproductive stages did not exhibit symptoms during the season. The occurrence of asymptomatic plants further complicates disease management because growers would not know that inoculum is being produced.

Growth stage at time of inoculation and disease severity have been evaluated by Smith et al. (2.22), but the relationship between yield loss and growth stage at time of inoculation was not evaluated. Backman et al. (2.3) correlated disease severity with yield loss, however, growth stage was not evaluated. The relationship between the growth stage at which plants become infected and resulting loss in yield is critical to developing yield loss models. Similar studies have been used in other crops to evaluate the effect of inoculation timing on yield (2.1,2.9,2.17). Agrios and Walker (2.1) found disease severity and yield reductions were most severe when pepper was inoculated with cucumber mosaic virus early in the growing season. Other research (2.9,2.17) showed yields of rice and tobacco were reduced most when plants were inoculated with Xanthomonas campestris pv. oryzae
(Ishiyama) and tobacco mosaic virus, respectively, early in the growing season. Our results demonstrated a similar relationship between growth stage at time of inoculation, disease severity, and relative yield loss.

With monocyclic diseases the severity of epidemics in previous years can affect the severity of current season epidemics. Although more research needs to be conducted to relate disease severity with subsequent production of inoculum (perithecia and pycnidia), these results provide preliminary evidence that Dpc can reproduce on apparently healthy plants.

This research provides information on the interrelationships between yield loss, growth stage at which plants are inoculated, and environmental conditions with Dpc. Although no fungicides are recommended for control of stem canker in Louisiana, this information can be incorporated into yield loss models and used in making decisions in the future when fungicides might be recommended.

References Cited


CHAPTER III

DETECTION OF INOCULUM AND INFECTION BY \textit{Diaporthe phaseolorum} var. \textit{caulivora} ON SOYBEAN DURING THE GROWING SEASON
Introduction

Stem canker, caused by *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* Ahow and Caldwell (Dpc), is a destructive disease of soybean (*Glycine max* (L.) Merrill). This disease was first reported in the United States in Iowa during the 1940's (3.1). Initially, stem canker devastated soybean in the midwestern United States, but eliminating Dpc susceptible varieties 'Hawkeye' and 'Blackhawk' from production reduced this disease to acceptable levels. Today, stem canker is of little importance in the midwestern United States.

Stem canker was not recognized as a serious problem in Louisiana until 1981 (3.30). Stem canker epidemics in the southern and northern United States differ. Southern United States Dpc isolates are more aggressive than northern United States Dpc isolates (3.28). Research indicates that cultivars resistant to Dpc isolates from the northern United States may not be resistant to Dpc isolates from the southern United States (3.14,3.17). Therefore, a source of Dpc resistance used for soybean varieties in the northern United States might or might not provide Dpc resistance for soybean varieties grown in the southern United States. Differences in pathogenicity, morphology, and genetics of northern and southern Dpc isolates have been reported (3.14,3.16,3.17). Because differences exist between "northern" and "southern"
stem canker epidemiology, different management strategies and sources of resistance must be developed.

Managing southern stem canker requires an understanding of the disease epidemiology. The majority of research concerning southern stem canker has been conducted to evaluate the effect of environmental factors, resistance, and cropping practices on epidemics. Environmental parameters, such as moisture and temperature, are key factors affecting stem canker epidemiology (3.10,3.15,3.19,3.22,3.31). Moisture and specific temperatures are necessary for optimum production of perithecia and pycnidia by Dpc (3.19,3.31). Moisture also is a requisite for infection by Dpc (3.10).

Management practices can affect stem canker epidemics. Stem canker can be reduced by discing to bury inoculum and by avoiding soybean/wheat double cropping (3.23,3.24). Other research has been conducted to compare stem canker epidemics in varieties differing in resistance to Dpc (3.9). Currently, resistant varieties and delayed planting are effective in managing southern stem canker. Varieties are routinely screened for resistance to Dpc (3.4,3.8,3.12,3.13,3.18,3.25,3.28,3.29,3.33). While considerable research has been conducted to gain a better understanding of stem canker epidemiology, more research is needed to characterize the role of initial inoculum (inoculum produced on plants infected the previous season) in stem canker epidemics.
Currently, stem canker is thought to function primarily as a monocyclic disease, with the production of secondary inoculum late in the growing season (3.3, 3.32). The presence of initial inoculum, produced on soybean plants infected during the previous growing season, is critical for initiating stem canker epidemics (3.3). Infection by Dpc at predetermined soybean growth stages during the growing season and resultant disease severity has been evaluated (3.27) but infection by Dpc during the growing season has received little attention (3.20). To determine if initial inoculum infects soybean throughout the growing season would be beneficial to incorporate information into disease forecasting models and used for making management decisions. The objective of this research was to determine the availability of infectious Dpc inoculum during the growing season.

**Materials and Methods**

**Field preparation.** Experiments were conducted to monitor stem canker incidence in soybean during the 1989-1991 growing season at the Burden Research Plantation, Baton Rouge, LA. Experiments were located in an area where stem canker epidemics had occurred in previous years. Prior to planting, the field (30 X 60 m) was disced and smoothed with a conditioner. In 1989 and 1990, stem debris (six, 170 liter bags) with Dpc perithecia was spread uniformly over the surface of the soil immediately after planting. The soybean
cultivar 'Bedford' was planted (25 rows x 60 m) 31 May, 1989, 1 May, 1990, and 2 July, 1991. Row spacings were 0.76 m in 1989 and 1991, and 0.91 m in 1990.

Bait plants. The presence of Dpc inoculum was determined weekly using bait plants (soybean plants in pots). Plastic pots (7.6 l) ('Classic 600', Nursery supplies Inc. Fairless Hills, PA) were filled with a soil mix (2 parts soil to 1 part sand). Soil was sterilized in 1990 and 1991 by drenching soil with a water/metham (Hi-Yield, Voluntary Purchasing Groups, Inc., Bonham, Texas 75418) solution (5 ml metham/pot). Pots were then covered with a plastic sheet. Fourteen days later, the plastic was removed, and the soil surface was broken to release any residual metham. Pots then were seeded with 'Bedford'. Seedlings were allowed to emerge and thinned to two plants per pot in 1989 and one plant per pot in 1990 and 1991.

One type of bait plant set was utilized in 1989-1991 and a second type was used in addition to the first type in 1990. The first type of set, "temporal sets", was planted simultaneous to planting the experimental field. Soybean plants in these sets were the temporal age of the plants in the experimental field. In 1990, additional sets, "V3 sets", were planted at time intervals to obtain plants at a desired growth stage (V3) (3.11) for placement in the experimental field on weekly intervals. Each bait plant set consisted of 25 pots. Before being taken to the experimental field, bait
35 plants were maintained at a location (location 1) remote from the experimental field.

**Experimental design and procedures.** After planting, the field was divided into five sections (25 rows, 9.1 m long), and five locations were randomly chosen in each section. At each location, a hole was dug in the center of each row to accept a 7.6 l pot with the top flush to the surface of the soil. Beginning the day the field was planted, bait plants (potted soybean) were placed in holes at each location in the field and left for 7 days (location 2). After 7 days, the bait plants were labelled by location (row number) and taken to a location (location 3) remote from the field. Immediately after a bait plant set was removed from the field, it was replaced with a new bait plant set from location 1.

After bait plant sets were removed from the field and placed at location 3, these sets were monitored daily for symptoms of stem canker. When sets were at growth stage R6-7, stem canker incidence was calculated for each set (plants exhibiting stem canker symptoms / plants in set). Data were analyzed using the general linear models procedure in SAS version 5.18 (3.26). Data were analyzed by year and over years.

To confirm the presence of Dpc in 1989, isolations were made from plants exhibiting symptoms of stem canker. A portion (approximately 5 x 5 mm) of stem tissue from the
margin of a lesion was surface-sterilized and placed on Phillip's selective medium (3.21). Surface sterilization was conducted as follows: (i) 1 minute washing in a 1:80% sodium hypochlorite/water solution, (ii) 3 minute washing in sterilized water, and (iii) placing the tissue in petri dishes containing Phillip's medium. The plates were observed for Dpc 3-7 days after isolations were made. Plants infected during 1990 and 1991 were retained during the winter. In the summer of 1992, observations were made for the presence of perithecia or pycnidia on stems.

Control bait plant sets (sets never placed in the experimental field) were placed at locations 1 and 3 to detect any Dpc that might be endemic in these areas.

Temperature, rainfall, and relative humidity were recorded using the Louisiana AgriClimatic Information System for each period a set remained in the field. Correlation analysis was conducted using the "Proc Corr" procedure in SAS to relate stem canker incidence and environmental parameters (3.26).

**Results**

In 1989, stem canker incidence was greatest in the temporal set placed in the field 4-7 weeks after planting, with an incidence of 50% in the set placed in the field seven weeks after planting (Table 3.1). Plants in these set ranged from growth stage V4-7. Cumulative rainfall of 6.8 cm, an average temperature of 27C, and an average relative humidity
of 82% were recorded during the seventh week (Table 3.1). Stem canker incidence was 30% in the set placed in the experimental field the 1st week after planting, then decreased in sets placed in the experimental field the 2nd and 3rd weeks. Stem canker incidence then increased in sets through the 7th week (Table 3.1). Compared to the 7th week, stem canker incidence was lower in plant sets for the remainder of the experiment. Stem canker incidence was 2% in the set placed in the experimental field 10 weeks after planting, when soybean plants were at growth stage R1.

Forty percent of the control plants at location 3 (where plants were taken after removal from the field) were infected with Dpc. Control plants at location 1 (location of sets prior to being placed in the experimental field) did not exhibit symptoms of stem canker.

Cumulative rainfall varied between weeks, but limited variation occurred in weekly temperature and relative humidity averages (Table 3.2). No significant correlations (P <= 0.05) occurred between stem canker incidence and temperature and rainfall, but there was a significant correlation between stem canker incidence and average relative humidity.
Table 3.1. Stem canker incidence in plants placed at weekly intervals in a soybean field with a history of stem canker epidemics, Burden Research Plantation, Baton Rouge, LA, 1989

<table>
<thead>
<tr>
<th>Week</th>
<th>Stem canker Incidence (%)</th>
<th>Total Rain (cm)</th>
<th>Average Temp (°C)</th>
<th>Average Rel Hum (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 (Ve)</td>
<td>11.2</td>
<td>25</td>
<td>88</td>
</tr>
<tr>
<td>2</td>
<td>12 (V2)</td>
<td>8.5</td>
<td>26</td>
<td>79</td>
</tr>
<tr>
<td>3</td>
<td>12 (V3)</td>
<td>8.5</td>
<td>24</td>
<td>78</td>
</tr>
<tr>
<td>4</td>
<td>32 (V4)</td>
<td>24.4</td>
<td>25</td>
<td>85</td>
</tr>
<tr>
<td>5</td>
<td>48 (V5)</td>
<td>4.4</td>
<td>26</td>
<td>86</td>
</tr>
<tr>
<td>6</td>
<td>38 (V6)</td>
<td>2.7</td>
<td>27</td>
<td>81</td>
</tr>
<tr>
<td>7</td>
<td>50 (V7)</td>
<td>6.8</td>
<td>27</td>
<td>82</td>
</tr>
<tr>
<td>8</td>
<td>16 (V10)</td>
<td>2.1</td>
<td>25</td>
<td>82</td>
</tr>
<tr>
<td>9</td>
<td>10 (V12)</td>
<td>1.3</td>
<td>27</td>
<td>80</td>
</tr>
<tr>
<td>10</td>
<td>2 (R1)</td>
<td>1.0</td>
<td>26</td>
<td>76</td>
</tr>
<tr>
<td>11</td>
<td>16 (R2)</td>
<td>0.1</td>
<td>24</td>
<td>74</td>
</tr>
</tbody>
</table>

LSD(0.05) 17

1 Weeks after planting.
2 Numbers in parenthesis under temporal column indicate the growth stage of the plant set when in the field.
3 Cumulative rainfall for the week the temporal set was in the field.
4 Average temperature for the week the temporal set was in the field.
5 Average relative humidity for the week the temporal set was in the field.

Table 3.2. Pearson correlation coefficients for stem canker, rainfall, temperature and relative humidity, Burden Research Plantation, Baton Rouge, LA, 1989

<table>
<thead>
<tr>
<th></th>
<th>Rain¹</th>
<th>Temp²</th>
<th>RH³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem⁴</td>
<td>0.2700</td>
<td>0.2278</td>
<td>0.6467</td>
</tr>
<tr>
<td></td>
<td>(0.4220)</td>
<td>(0.5005)</td>
<td>(0.0315)</td>
</tr>
</tbody>
</table>

¹ Rainfall.
² Temperature.
³ Relative humidity.
⁴ Stem canker incidence.
In 1990, regardless of plant set type, maximum stem canker incidence was recorded in the plant sets placed in the experimental field 4 weeks after planting, when plants in temporal sets were at growth stage V3 (Table 3.3). Stem canker incidence in the V3 plant set placed in the field 4 weeks after planting was similar to stem canker incidence in the plant set placed in the field 5 weeks after planting (P <= 0.05). Compared to stem canker incidence in the temporal set at 4 weeks after planting, stem canker incidence was similar to incidence in temporal sets placed in the field 5-8 weeks after planting. Stem canker incidence ranged from 12-24% in V3 sets and 12-20% in temporal sets placed in the field the first 3 weeks after planting (Table 3.3). Stem canker incidence was less than 37% in plant sets placed in the experimental field 6-10 weeks after planting. Stem canker incidence was lowest in the set placed in the field 9 weeks after planting, when plants were at growth stage V9. Trends in stem canker incidence were similar for both types of bait plant sets.

Maximum rainfall occurred five weeks after planting (Table 3.3). Amounts of weekly rainfall varied, but limited variation occurred in average temperature and average relative humidity. No significant correlations (P <= 0.05) occurred between stem canker incidence and recorded environmental parameters (Table 3.4).
Table 3.3. Stem canker incidence in plants placed at weekly intervals in a soybean field with a history of stem canker epidemics, Burden Research Plantation, Baton Rouge, 1990

<table>
<thead>
<tr>
<th>Week</th>
<th>Stem canker Incidence (%)(^1)</th>
<th>Total Rain (cm)(^5)</th>
<th>Average Temp (C)(^6)</th>
<th>Average Rel Hum (%)(^7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16 (Vc)</td>
<td>3.9</td>
<td>21</td>
<td>69</td>
</tr>
<tr>
<td>2</td>
<td>12 (V1)</td>
<td>0.3</td>
<td>24</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>24 (V2)</td>
<td>1.6</td>
<td>24</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>76 (V3)</td>
<td>4.0</td>
<td>24</td>
<td>71</td>
</tr>
<tr>
<td>5</td>
<td>52 (V5)</td>
<td>10.5</td>
<td>27</td>
<td>72</td>
</tr>
<tr>
<td>6</td>
<td>20 (V6)</td>
<td>1.9</td>
<td>27</td>
<td>73</td>
</tr>
<tr>
<td>7</td>
<td>20 (V7)</td>
<td>0.5</td>
<td>29</td>
<td>68</td>
</tr>
<tr>
<td>8</td>
<td>20 (V8)</td>
<td>5.1</td>
<td>26</td>
<td>66</td>
</tr>
<tr>
<td>9</td>
<td>4 (V9)</td>
<td>5.5</td>
<td>27</td>
<td>72</td>
</tr>
<tr>
<td>10</td>
<td>4 (V10)</td>
<td>0.2</td>
<td>28</td>
<td>72</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td>21</td>
<td>24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Numbers in parenthesis under temporal column indicate the growth stage of the plant set when in the field.
2 Weeks after planting.
3 V3 bait plant set.
4 Temporal bait plant set = same age as plants in the field.
5 Cumulative rainfall for the week the temporal set was in the field.
6 Average temperature for the week the temporal set was in the field.
7 Average relative humidity for the week the temporal set was in the field.

Table 3.4. Pearson correlation coefficients for stem canker, temperature, rainfall, and relative humidity, Burden Research Plantation, Baton Rouge, LA, 1990

<table>
<thead>
<tr>
<th></th>
<th>Rain(^1)</th>
<th>Temp(^2)</th>
<th>RH(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem(^4)</td>
<td>0.31105</td>
<td>0.05284</td>
<td>-0.26451</td>
</tr>
<tr>
<td></td>
<td>(0.3817)</td>
<td>(0.8847)</td>
<td>(0.4602)</td>
</tr>
</tbody>
</table>

1 Rainfall.
2 Temperature.
3 Relative humidity.
4 Stem canker incidence.
In 1991, stem canker incidence was greatest (68%) in the temporal set placed in the experimental field 5 weeks after planting, when soybean plants in sets were at growth stage V4, but similar to sets placed in the field 3, 6, and 7 weeks after planting (Table 3.5). Stem canker incidence ranged from 24-48% in sets placed in the field 5-8 weeks after planting. Lowest incidence (16%) was recorded for the set placed in the experimental field the 2nd week after the field was planted.

Compared to 1989 and 1990, stem canker incidence was similar, but rainfall amounts were low for the periods measured in 1991 (Table 3.5). Temperature ranged between 26 and 28 C. Relative humidity for the periods varied from 32-50%. No significant correlations (P <= 0.05) occurred between stem canker incidence and environmental parameters measured (Table 3.6)
Table 3.5. Stem canker incidence in plants placed at weekly intervals in a soybean field with a history of stem canker epidemics, Burden Research Plantation, Baton Rouge, 1991

<table>
<thead>
<tr>
<th>Week</th>
<th>Stem canker Incidence (%)</th>
<th>Total Rain (cm)</th>
<th>Average Temp (°C)</th>
<th>Average Rel Hum (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>9.6</td>
<td>28</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>16 (pre)</td>
<td>0.6</td>
<td>28</td>
<td>39</td>
</tr>
<tr>
<td>3</td>
<td>44 (Vc)</td>
<td>0.0</td>
<td>28</td>
<td>34</td>
</tr>
<tr>
<td>4</td>
<td>36 (V2)</td>
<td>0.4</td>
<td>28</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>68 (V3)</td>
<td>0.0</td>
<td>28</td>
<td>34</td>
</tr>
<tr>
<td>6</td>
<td>40 (V4)</td>
<td>1.8</td>
<td>27</td>
<td>44</td>
</tr>
<tr>
<td>7</td>
<td>48 (V5)</td>
<td>0.2</td>
<td>28</td>
<td>41</td>
</tr>
<tr>
<td>8</td>
<td>24 (R1)</td>
<td>0.2</td>
<td>26</td>
<td>42</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td></td>
<td>29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Weeks after planting.
2 Numbers in parenthesis under temporal column indicate the growth stage of the plant set when in the field.
3 Cumulative rainfall for the week the temporal set was in the field.
4 Average temperature for the week the temporal set was in the field.
5 Average relative humidity for the week the temporal set was in the field.


<table>
<thead>
<tr>
<th></th>
<th>Rain¹</th>
<th>Temp²</th>
<th>RH³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem⁴</td>
<td>-0.11311</td>
<td>0.26629</td>
<td>-0.19518</td>
</tr>
<tr>
<td></td>
<td>(0.8092)</td>
<td>(0.5638)</td>
<td>(0.6749)</td>
</tr>
</tbody>
</table>

¹ Rainfall.
² Temperature.
³ Relative humidity.
⁴ Stem canker incidence.
When data were pooled for the temporal sets the 1st 8 weeks of the 1989-1991 experiments, stem canker incidence in plant sets increased to a maximum 5 weeks after planting and decreased, thereafter (Figure 3.1). Although stem canker incidence varied in plant sets between years, similar trends in stem canker incidence were noted. The pooled data were fit with a 3rd order polynomial ($R^2 = 0.47$).

Figure 3.1. Stem canker incidence in plants placed in a field with a history of stem canker, Burden Research Plantation, Baton Rouge, LA, 1989-1991.
Discussion

Stem canker incidence varied among plant sets from week to week in all three experiments, indicating infection by Dpc is not uniform over time. This variability could be due to several reasons, such as age or availability of Dpc inoculum, plant growth stage at time of infection, and environmental conditions at time of infection.

Stem canker incidence in plant sets was highest in soybean plants placed in the experimental field at growth stages V2-8. These results were similar to work conducted by Smith et al. (3.28), but previous work did not rely on infection by naturally occurring Dpc inoculum already present in the field. Therefore, any conclusions concerning initiation and severity of epidemics caused by natural inoculum cannot be made (3.20,3.27,3.28). The present study relied solely on natural inoculum, and these results represent what actually occurs in grower fields.

Stem canker incidence in plant sets was greatest when sets were placed in the field 4-7 weeks after planting, but the fungus continued to infect soybean plants placed in the field 11 weeks after planting. This indicates natural inoculum of Dpc is available and capable of infecting soybean plants at any growth stage including early reproductive stages (R1-2). While soybean plants infected at early reproductive stages may not result in significant yield reductions, these plants probably contribute to initial
inoculum for the next season (3.20, 3.27). Soybean plants infected at reproductive stages generally do not exhibit severe symptoms of stem canker (3.28). Therefore, the potential inoculum load for the next season may be underestimated. This may explain why stem canker epidemics occur when there is apparently no source of inoculum. The amount of inoculum needed to initiate stem canker epidemics has not been determined, therefore inoculum produced on plants infected at late reproductive stages may be important and should be evaluated.

The results of the present research may explain the effectiveness of delayed planting, as well as the ineffectiveness of fungicide sprays made on soybean at early to mid-reproductive stages (3.3, 3.6). By delaying planting, soybean plants are not exposed to Dpc spores released early in the season, and therefore the source of inoculum is probably being depleted in the absence of soybean. Soybean planted later may not be subjected to the amount of Dpc inoculum earlier planted soybean would be. If fungicides are to be effective in managing stem canker, treatments should be applied before V7, based on the results from this study and other research (3.2, 3.3, 3.7). Applications of protectant fungicides made during R3 and R5 would probably not be effective in managing stem canker since the majority of infections would have occurred before the mid-reproductive stages.
In 1989, 40% of the control plants at location 3 exhibited symptoms of stem canker. The source of the Dpc inoculum in this set is not known. Low incidence in other plant sets at the same location indicates that infection by Dpc was not uniform across plant sets (Table 3.1). Seed transmission of Dpc is not likely because high disease incidence was limited to a few sets. Wind dispersal of inoculum also is unlikely because ascospores and conidia of Dpc are produced in a gelatinous matrix. There was an infestation of insects early in the season in these sets at this location, but any conclusions about insect vectors at this point would be premature. Even though controls tested positive for Dpc in 1989, trends in stem canker incidence in plant sets placed at the field location were similar to those in 1990 and 1991. No stem canker incidence was detected in controls during 1990 and 1991.

Initial inoculum is essential for disease epidemics where additional inoculum is not produced to any degree during the present season (3.34). Initial or primary Dpc inoculum, produced on stem debris infected during the prior growing season, is an important inoculum source for stem canker epidemics (3.3). Therefore, to determine if this inoculum is exhausted, or ineffective at some point in the growing season would be beneficial for developing forecasting models and for making management decisions. Forecasting models that rely on initial inoculum have been developed
Therefore, monitoring weekly Dpc incidence during the growing season would indicate the relative efficacy of initial Dpc inoculum. Results from this study have demonstrated that initial inoculum of Dpc is infectious at least 11 weeks after soybean are planted, but the degree of stem canker incidence decreases significantly 5-7 weeks after planting.

References Cited


CHAPTER IV
RELATIONSHIP OF SOYBEAN STEM CANKER SEVERITY AND
THREECORNERED
ALFALFA HOPPER INJURY
Introduction

Over 50 insects and pathogens attack soybean (Glycine max (L.) Merrill) grown in the southern United States (4.1). The simultaneous existence of these pests in soybean makes it necessary to determine the effect of multiple pest interactions on soybean production. While the effects conferred by an individual pest have been studied in great detail, the effects of multiple pests have received limited attention. The threecornered alfalfa hopper (Spissistilus festinus (Say)) is an economically important insect pest of soybean. Injury by threecornered alfalfa hopper is characterized by girdles around the soybean stem and petioles. Girdles disrupt vascular bundle organization (4.8) and reduce plant vigor and yield by causing stem breakage and lodging.

Stem canker, caused by Diaporthe phaseolorum (Cke. & Ell.) Sacc. var. caulivora Athow & Caldwell (Dpc), can devastate soybean, reducing yields up to 80% in years when epidemics are severe (4.2). The fungus overwinters on infested soybean debris, and inoculum for the next season epidemic is produced on this debris. Research has been conducted to ascertain the effects of moisture (4.4), temperature (4.16), time of infection (4.14), and cropping systems (4.10) on stem canker. While these are important considerations, an area deserving attention is the effects of other pests on stem canker epidemics.
Russin et al. (4.12) studied the effect of threecornered alfalfa hopper injury on stem canker severity and found stem canker severity was greater on plants with basal stem girdles than on plants without girdles. Soybean plants were inoculated with Dpc using the toothpick method described by Crall (4.3) and lesion length was compared for girdled and nongirdled plants.

The simultaneous presence of the threecornered alfalfa hopper and Dpc in soybean is common, allowing for the possibility of interactions to occur. While the detrimental effects of this insect and pathogen alone are significant, the determination of the combined effects of these organisms on soybean and other organisms would be beneficial.

Insect injury that does not reduce soybean yield is generally not economically important, but if this injury predisposes the plant to infection by plant pathogens, then this injury could have significant effects on soybean yield. Therefore, the interaction of these pests could be very important.

The objectives of this work were to determine the effects of basal stem girdles caused by the threecornered alfalfa hopper on stem canker severity, development of Dpc and soybean growth and yield in fields where stem canker epidemics were naturally occurring.
Materials and Methods

General procedures. Studies using stem canker susceptible soybean cultivars 'Bragg' and 'Bedford' were conducted during 1988, 1989, and 1990 at Burden Research Plantation, Baton Rouge, LA. Cultivars were planted on rows 0.76 m wide in 1988 and 0.91 m wide in 1989-90. Seeds were inoculated with the labelled rate of commercial inoculant (Nitragin, *Rhizobium japonicum*, LiphaTech, Inc., Milwaukee, WI) prior to planting. Studies were conducted in fields where stem canker epidemics had occurred for the past several years. Statistical analysis was conducted using the general linear models procedure in SAS and means were separated using T-tests (4.13).

Threecornered alfalfa hopper / stem canker severity. 'Bragg' was planted on 7 June, 1988. When plants were at the R6 growth stage (4.5), 100 soybean plants infected with Dpc (50 without girdles and 50 with girdles caused by threecornered alfalfa hopper) were severed at the soil line and taken to the laboratory. For each plant, stem length and diameter (at stem midpoint) were measured (cm), pods were shelled by hand and seed were dried for 72 h at 60°C. Yields were expressed as dry weight of seed per plant. Cankers per stem were counted and areas of individual cankers were calculated using the formula for the area of an ellipse \((\pi/4 \text{ length} \times \text{diameter})\). These values were used to calculate mean canker area and total cankered area per stem. For
comparison to quantitative stem canker data, stem canker severity (%) was rated visually using a scale of 0-100%: (0 = no cankers on the stem and 100 = stem surface completely covered by cankers caused by Dpc).

'Bedford' soybean was planted on 31 May, 1989 and 30 May, 1990. In both years, 100 soybean plants at R6 (50 without stem girdles and 50 with stem girdles caused by threecornered alfalfa hopper) infected with Dpc were collected. Stem length, stem diameter, and stem canker severity were determined.

In 1990, 10 of the 50 pairs of plants collected from the field were taken and pods were counted, yields were measured (dry weight of pods containing seed), and perithecia were quantified for the girdled and nongirdled stems. To quantify perithecia produced by Dpc, stems (10 without stem girdles and 10 with stem girdles) were placed on moist paper towels in covered clear plastic containers (34x26x10 cm) and kept in the laboratory at approximately 23°C. Stems were monitored daily for the presence of perithecia. After two weeks, perithecia were counted on one 0.5 X 0.5 cm section of each stem using a micrometer. Data were converted to perithecia/cm² of stem surface.

Results

In 1988, diseased 'Bragg' plants with basal stem girdles by the threecornered alfalfa hopper exhibited reductions in stem diameter and seed yield, but not stem
height (Table 4.1). Compared to plants without girdles, plants with basal girdles supported greater numbers of cankers, which resulted in increased total cankered area and increased stem canker per stem. Canker size, however, was not different between girdled and nongirdled stems. Because results taken in 1988 from visual ratings mirrored those from actual canker measurements, only visual ratings were recorded in the subsequent experiments.

Effects of basal girdles on diseased 'Bedford' soybean plants were similar to those on 'Bragg' (Table 4.2). In 1989, pods per plant and dry weights were lower on girdled stems than on nongirdled (Table 4.2). Severity of stem canker disease, however, was reduced only in 1989 (Table 4.2).

In 1990, stems with basal girdles had smaller stem diameters and fewer pods compared to stems without basal girdles (Table 4.2). Compared to nongirdled plants, stem diameter and pod number were reduced 31.9 and 53.2%, respectively, in girdled plants. Stem canker severity and production of perithecia did not differ significantly between girdled and nongirdled plants (Table 4.2).
Table 4.1. Plant growth parameters and stem canker disease on 'Bragg' soybean girdled or not girdled at stem bases by threecornered alfalfa hopper, Baton Rouge, LA, 1988

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stem Length (cm)</th>
<th>Stem Diameter (mm)</th>
<th>Yield (g)²</th>
<th>Disease Severity³</th>
<th>Number/Severity</th>
<th>Mean Canker Area (cm²)</th>
<th>Total Canker Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not girdled</td>
<td>95.4</td>
<td>7.4</td>
<td>9.0</td>
<td>5.1</td>
<td>5.2</td>
<td>1.4</td>
<td>6.2</td>
</tr>
<tr>
<td>Girdled</td>
<td>90.7</td>
<td>5.6</td>
<td>5.6</td>
<td>8.4</td>
<td>6.6</td>
<td>1.7</td>
<td>9.4</td>
</tr>
</tbody>
</table>

1 At stem midpoint.
2 Average dry weight of seeds from single plant.
3 Visual approximation of the percentage of the stem surface that was cankered.
4 ns = not significant, * = p=(0.05), ** = p=(0.01), *** = p=(0.001), **** = p=(0.0001).
Table 4.2. Plant growth parameters and stem canker disease severity on 'Bedford' soybean girdled or not girdled at stem bases by threecornered alfalfa hopper, Baton Rouge, LA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stem Length (cm)</th>
<th>Stem Diameter (mm)</th>
<th>Disease Severity</th>
<th>Pod Number (g)</th>
<th>Perithecia (#/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nongirdled</td>
<td>65.0</td>
<td>5.0</td>
<td>19.4</td>
<td>70.1</td>
<td>17.1</td>
</tr>
<tr>
<td>Girdled</td>
<td>64.7</td>
<td>4.0</td>
<td>34.6</td>
<td>32.8</td>
<td>8.7</td>
</tr>
</tbody>
</table>

1989

| Nongirdled  | 87.4             | 9.63               | 8.7             | 180            |
| Girdled     | 89.9             | 6.55               | 10.4            | 197            |

1990

- At stem midpoint.
- Visual approximation of the percentage of the stem surface that was cankered.
- Mean perithecia of ten girdled and ten nongirdled stems.
- Data not recorded.
- ns = not significant, * = p=(0.05), *** = p=(0.001), **** = p=(0.0001).
Discussion

This research indicates that insect injury can effect stem canker severity. Stem canker severity increased in conjunction with threecornered alfalfa hopper injury compared to severity in the absence of threecornered alfalfa hopper injury.

Injury by the threecornered alfalfa hopper also has been implicated in increased incidence of southern stem blight (Sclerotium rolfsii Sacc.) (4.6), and increased severity of pod and stem blight (D. phaseolorum (Cke. & Ell.) var. sojae (Lehman)), and anthracnose (Colletotrichum truncatum (Schw.) Andrus & W.D. Moore) (4.11). Herzog determined that girdles provided entry for S. rolfsii, but the mechanism of how the girdles affected stem canker severity in the present experiment was not determined.

Our results support work conducted by Russin et al. (4.12) that demonstrated disease severity was greater (increased canker length) in girdled plants compared to nongirdled plants. In Russin's experiment, however, plants were inoculated using Dpc infested toothpicks which eliminated the possibility of girdles providing entry for the fungus. Russin concluded that the increase in stem canker severity was probably physiological because entry was bypassed. Physiological changes to soybean plants caused by the threecornered alfalfa hopper girdles have been documented. Hicks et al. (4.7) demonstrated that girdles
alter the physiology of soybean by causing \(^{14}\)C-labelled glucose to accumulate above the girdle as well as reducing root dry weight and number of \textit{Rhizobium} nodules. In the present experiment, all evaluated plants were infected with \textit{Dpc} regardless of insect injury, indicating insect injury probably did not provide entry for \textit{Dpc}. Therefore, a physiological change due to girdling may have created a more conducive environment for disease development after penetration occurred.

The effects of girdling may only be temporary as demonstrated by Spurgeon and Mueller (4.15), who demonstrated that translocation of glyphosate was blocked temporarily by petiole girdles on soybean. Therefore the interaction of girdling and stem canker may only be temporary as well. Mueller and Jones (4.9) categorized soybean response to girdling into five classes, ranging from death of the plant (class 1) to complete recovery (class 5). The plants evaluated in the present experiment sustained a minimal amount of insect injury and would be placed in class 4 or 5, but this injury resulted in a significant increase in stem canker severity.

The plants in our study sustained minimal insect injury, yet significant increases in stem canker severity occurred in two of the three experiments. Over 50\% of girdled soybean plants in a field fully recover or are only slightly weakened by threecornered alfalfa hopper (4.9). The plants evaluated
in the present experiment (class 4 or 5 plants) probably would have appeared healthy most of the growing season. This is important because growers might not be conscious of injury by the threecornered alfalfa hopper, but this injury may increase the severity of stem canker epidemics. Therefore, early monitoring for threecornered alfalfa hopper injury in fields with a history of stem canker may be important.

Knowledge of the individual effects of a single pest on a plant is important, but information about the effects conferred by multiple pests on the host and other pests would be more realistic of field occurrence. The results presented in this study would add diversity to disease forecasting models. By implementing the effects of pest/pest interactions, disease forecasting models could compensate for negative or positive effects due to these pest interactions.

References Cited


CHAPTER V

ALTERNATIVE HOSTS OF THE

SOUTHERN SOYBEAN STEM CANKER PATHOGEN

Diaporthe phaseolorum var. caulivora
Introduction

Soybean (*Glycine max* L. (Merrill)) is an important crop grown worldwide. This crop is affected by more than 100 weeds, pathogens, and insects. Therefore, to understand how these pests affect soybean is important to maximize production. Competition from weeds results in reduced soybean yield and quality. Weeds affect soybean directly through competition for light, nutrients, water, and space, and indirectly by serving as hosts for pathogens and insects.

Stem canker of soybean, caused by *Diaporthe phaseolorum* var. (Cke. & Ell.) Sacc. *caulivora* Athow and Caldwell (Dpc), is a destructive disease affecting soybean. In years when epidemics are severe, yields can be reduced 80% in susceptible cultivars (5.2). This disease was first reported in the United States in the 1940's in Iowa (5.1). Stem canker was not recognized as a problem in soybean grown in Louisiana until 1981 (5.18).

An understanding of the factors affecting epidemiology is critical for managing soybean stem canker. Considerable research has been conducted to ascertain information concerning the role of environmental factors and cropping practices in stem canker epidemiology (5.4, 5.6, 5.8, 5.10, 5.12, 5.13, 5.20). Moisture and temperature are key factors for infection by Dpc and development of stem canker (5.4, 5.8, 5.10, 5.20). Cultural practices and cropping systems also can affect the development of stem canker epidemics (5.12, 5.13).
While the role of environmental factors and cultural practices in stem canker epidemiology has been evaluated, the existence and role of alternative hosts of Dpc in stem canker epidemiology has received little attention (5.7).

Primary inoculum (inoculum produced on debris from plants infected the previous growing season) is considered to be an important inoculum source for stem canker epidemics (5.2, 5.17). If alternative hosts serve as inoculum sources for plant disease epidemics, these hosts must be considered as factors affecting disease development. Weeds may be significant sources of Dpc inoculum. Research has been conducted to evaluate weeds and other crops as hosts for soybean pathogens (5.3, 5.5, 5.14, 5.15). Hepperly et al. (5.5) isolated Phomopsis sojae Leh., Colletotrichum dematium (Pers. ex Fr.) Grove var. truncata (Schw.) Arx, and C. gleosporioides (Penz.) Sacc. from velvetleaf (Abutilon theophrasti (Medik.)). Other research revealed that Diaporthe spp. will infect cotton (Gossypium hirsutum L.) and Dpc will infect lima bean (Phaseolus limensis L.) (5.3, 5.14).

To determine if Dpc inoculum sources other than soybean exist would add to the understanding of stem canker epidemiology. Therefore, to survey weeds endemic to Louisiana soybean fields as hosts for Dpc is imperative. The objectives of this research were to evaluate weeds as hosts for Dpc and quantify the reproductive capability of Dpc in these weed hosts.
Materials and Methods

Greenhouse experiment. A greenhouse experiment was conducted to evaluate predominant weeds found in south Louisiana soybean fields as hosts for Dpc and potential stem canker inoculum sources. Plant species evaluated were tall morningglory (*Ipomoea purpurea* L. (Roth.)), smallflower morningglory (*Jacquemontia tamnifolia* (L.) Griseb.), curly dock (*Rumex crispus* L.), hemp sesbania (*Sesbania exaltata* (Raf.) Rydb. ex A.W. Hill), sicklepod (*Cassia obtusiifolia* L.), 'Coker 983' wheat (*Triticum aestivum* (L.)) nightshade (*Solanum nigrum* L.), and spiny amaranth (*Amaranthus spinosa* L.). 'Centennial' soybean (Dpc resistant), and 'Bedford' soybean (Dpc susceptible) were included as controls.

Clay pots, 10 cm in diameter, were filled with sterilized soil (Convent sandy loam, sterilized with methyl bromide). Seeds were planted 4 January 1990. After emergence, plants were thinned to one plant per pot. Each species was replicated nine times and arranged in a completely randomized design on a greenhouse bench. To facilitate a near 100% relative humidity environment during and following inoculations, a wooden frame (3.6 X 1.2 X 1.3 m), covered with clear plastic sheeting on the top and sides, was constructed over the pots. Supplemental lighting, LU400 sodium vapor lights (Energy Technics, Conservation Specialist, 3925 Ridgewood Rd., P.O. Box 3424, York, PA 17402) (3 per 7.3 meters of bench length) and fluorescent
banks containing gro-lux (40 watts) or fluorescent super-saver (40 watts) (Sylvania, Danvers, MA 01923) (2 per 7.3 meters of bench length), were placed above the plants. Day/night regime consisted of 14 hours light and 10 hours of darkness.

Dpc inoculum (Opelousas 3 isolate, Opelousas, LA) was produced on mature "Bedford" soybean stem pieces collected from a soybean field. Stem pieces (5.5 cm in length) were autoclaved on 3 consecutive days (1 hr/autoclaving). Immediately after the third autoclaving, stem pieces were transferred to petri dishes containing acidified (2 ml of lactic acid/liter of agar) potato dextrose agar (p.H. 4.5). One agar plug (8 mm in diameter) containing mycelium of Dpc was placed in the center of each dish and allowed to grow over the stem pieces. Petri dishes were maintained in the laboratory where temperatures ranged from 20-25C. Approximately 8 weeks after incubation (when mature perithecia were evident on stem pieces), ascospores were harvested by flooding dishes with tap water and scraping perithecia from stem pieces with a metal spatula into the water. The mixture was filtered through 2 layers of cheesecloth to remove agar and stem debris. Ascospores were quantified with a hemocytometer. To enhance coverage of inoculum on the plant surface, polyoxyethylene-sorbitan monolaurate (Tween 20, Sigma Chemical Co., P.O. Box 14508,
St. Louis, MO, 63178) was added at the rate of 2 drops per liter of inoculum suspension.

Inoculations were conducted by spraying individual plants with an ascospore/water suspension 53 (3 \( \times \) 10^6 ascospores/ml of inoculum) and 56 (1.5 \( \times \) 10^6 ascospores/ml of inoculum) days after planting. Inoculum (3 ml per plant) was applied using a devilbliss glass atomizer. Immediately after inoculation, clear plastic sheeting was placed over the wooden frame and two humidifiers were operated under the plastic to create a near 100% relative humidity environment for a period of 3 days following each inoculation. Noninoculated controls (inoculated with water and polyoxyethylene-sorbitan monolaurate, 2 drops per liter of water) also were included for comparisons to the inoculated plants.

Three days after the last inoculation, the plastic was removed and plants were monitored daily for stem lesions or foliar symptoms. Stem canker incidence per species (number of plants infected per 10 plants inoculated per species) was calculated 58 days after the last inoculation. Isolations for Dpc were conducted on 29 April, 1990, by excising a small portion of stem tissue (0.3 mm x 0.3 mm) from the margin of a stem lesion (if present) from each plant. Each sample was placed in a separate envelope labelled for that species. Samples were grouped by species prior to surface sterilization. Tissue samples were surface sterilized by
washing samples for 2 minutes in a 1:80% sodium hypochlorite/water solution. Samples were then rinsed (each species separately) for 1.5 minutes in sterile distilled water, blotted dry, and placed in petri dishes (100 x 15 mm) containing Phillip's selective medium (5.9). Petri dishes containing the tissue samples (four samples of the same species per dish), were observed daily for the presence of Dpc. Three days after isolation, the percentage of the samples from which Dpc was isolated was calculated for each species. Data were analyzed using the general linear models procedure in SAS and means were separated using protected least squared means (5.18).

Outdoor experiment. Plant species evaluated in the outdoor study were wild poinsettia (*Euphorbia heterophylla* L.), prickly sida (*Sida spinosa* L.), redweed (*Melochia corchorifolia* L.), pitted morningglory (*Ipomoea lacunosa* L.), northern jointvetch (*Aeschynomene virginica* (L.) B.S.P.), hemp sesbania (*Sesbania exaltata* (Raf.) Rydb. ex A.W. Hill), entireleaf/ivyleaf morningglory (*Ipomoea hederacea* var. *integriculus* Gray / *Ipomoea hederacea* (L.) Jacq.), smallflower morningglory (*Jacquemontia tammifolia* (L.) Griseb.), indigo (*Indigofera hirsuta* L.), barnyardgrass (*Echinochloa crus-galli* (Griseb.) Nash.), johnsongrass (*Sorghum halpense* (L.) Pers.), and two soybean cultivars 'Asgrow 7985' (Dpc resistant) and 'Hartz 7126' (Dpc susceptible). Pots (7.4 l) were filled with a soil mix
(3:1:2) of soil (Convent sandy loam), vermiculite, and peat moss. Seeds were planted on 4 August, 1991, in the 7.4 l pots. Plants (single species / pot) were allowed to germinate and thinned to three plants per pot. Plant species were arranged in a randomized complete block design at a location with no history of stem canker epidemics. Plant species were replicated 3 times with 2 pots / replication.

Dpc inoculum (Opelousas 3 (Opelousas, LA)) was produced on soybean stem pieces as described for the greenhouse experiment. Inoculations were conducted 23 and 54 days after planting by spraying an ascospore and alpha conidia/water suspension on the plants using a compressed air sprayer (1 l of inoculum / inoculation) at a rate of 336 ml/minute. The inoculum suspension contained approximately a 1:1 ascospores/alpha conidia mixture (1.93 X 10⁶ ascospores and 2.55 X 10⁶ alpha conidia / ml of inoculum for the 1st and 2nd inoculation, respectively) with two drops of polyoxyethylene-sorbitan monolaurate added per l of water/spore inoculum. Noninoculated controls (six plants per species evaluated) were sprayed with distilled water. Plants were wet with water for 15 min prior to each inoculation using an oscillating sprinkler. To create an environment with high relative humidity for 12 hr after the first inoculation, plants were covered with plastic sheeting supported by a wooden frame. Plants were not covered with clear plastic sheeting after the 2nd inoculation.
Plants were monitored weekly until plant senescence for stem lesions or foliar symptoms of stem canker. Isolations for Dpc were conducted 163 days after planting as described for the greenhouse experiment. The percentage of plants in each species with perithecia or pycnidia produced by Dpc was calculated 171 days after planting (5.16). Perithecia and pycnidia were quantified for each evaluated species 360 days after planting. Quantification of fruiting structures was conducted on 3 plants of each species per replication. Perithecia and pycnidia were quantified on one 0.2 X 0.2 cm stem section/plant with the aid of a micrometer. Data were then converted to perithecia or pycnidia per square centimeter of stem. Data were analyzed using the general linear models procedure in SAS and means were separated using protected least squared means (5.18).

Results

In the greenhouse and field experiments, Dpc infected, colonized, and reproduced on plants of several weed species (Tables 5.1 and 5.2). Indigo and sesbania were the only weed species to exhibit stem canker symptoms (stem lesions). Therefore, visual ratings were not a reliable means of discerning Dpc colonization in weed species. In the greenhouse experiment, Dpc was isolated from 100 and 70% of 'Bedford' and 'Centennial' plants, respectively (Table 5.1). Dpc was isolated from significantly fewer weed plants than from 'Bedford' (P <= 0.05). However, 60% of the inoculated
tall morningglory and sicklepod plants were infected with Dpc. Dpc also was isolated from sesbania, smallflower morningglory, spiny pigweed, and black nightshade, but not from 'Coker 983' wheat or curly dock plants. Dpc was not isolated from the noninoculated control plants.

Table 5.1. Percentage of inoculated plants from which Diaporthe phaseolorum var. cauliavora was isolated in a greenhouse experiment, Louisiana State University, Baton Rouge, LA, 1990

<table>
<thead>
<tr>
<th>Weed Species</th>
<th>Percent Dpc¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bedford</td>
<td>100</td>
</tr>
<tr>
<td>Centennial</td>
<td>70</td>
</tr>
<tr>
<td>Tall morningglory</td>
<td>60</td>
</tr>
<tr>
<td>Sicklepod</td>
<td>60</td>
</tr>
<tr>
<td>Hemp sesbania</td>
<td>50</td>
</tr>
<tr>
<td>Smallflower morningglory</td>
<td>40</td>
</tr>
<tr>
<td>Spiny pigweed</td>
<td>10</td>
</tr>
<tr>
<td>Black nightshade</td>
<td>10</td>
</tr>
<tr>
<td>Coker 983</td>
<td>0</td>
</tr>
<tr>
<td>Curly dock</td>
<td>0</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>40</td>
</tr>
</tbody>
</table>

¹ Number of plants in the species evaluated from which Diaporthe phaseolorum var. cauliavora was isolated divided by the total number of plants inoculated for that species.

In the field experiment, pycnidia were observed on 70% of 'Asgrow 7986' soybean and 83% of inoculated 'Hartz 7126' soybean plants (Table 5.2). Of the inoculated weeds, 100% of
entireleaf and pitted morningglory plants had pycnidia on the stem surface. High percentages of northern jointvetch (95%) and wild poinsettia (91%) also had pycnidia on stems. Pycnidia also were present on smallflower morningglory, hemp sesbania, and indigo. Pycnidia were not produced on prickly sida, redweed, johnsongrass, or barnyardgrass. The percentage of plants with pycnidia did not differ significantly (P <= 0.05) compared to the soybean controls except in indigo, where a lower percentage of indigo plants had pycnidia (Table 5.2).

More perithecia (P <= 0.05) were produced on plants of the soybean cultivar 'Asgrow 7986' than on the weed species or soybean cultivar 'Hartz 7126' colonized by Dpc (Table 5.2). Of the inoculated weed species, northern jointvetch, indigo, hemp sesbania, wild poinsettia, and entireleaf morningglory supported the most perithecia (Table 5.2). Fewest perithecia were observed on smallflower and pitted morningglory. Perithecia and pycnidia were observed on stems of soybean plants and weeds of the noninoculated controls.

Number of pycnidia was greatest on hemp sesbania and 'Hartz 7126'. Number of pycnidia differed significantly (P <= 0.05) between 'Hartz 7126' and 'Asgrow 7986'. While significantly (P <= 0.05) more pycnidia were produced on hemp sesbania, numbers of pycnidia did not differ between the other weed species with pycnidia; although, no pycnidia were observed on redweed, prickly sida, johnsongrass, or barnyardgrass (Table 5.2).
Table 5.2. Percentage of plants with pycnidia, number of perithecia, and number of pycnidia for weeds inoculated with *Diaporthe phaseolorum* var. *caulivora*. Louisiana State University, Baton Rouge, LA, 1991

<table>
<thead>
<tr>
<th>Weed Species</th>
<th>Plants with Pycnidia (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Number of Perithecia&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Number of Pycnidia&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asgrow 7986</td>
<td>70</td>
<td>368</td>
<td>81</td>
</tr>
<tr>
<td>Hartz 7126</td>
<td>83</td>
<td>3</td>
<td>222</td>
</tr>
<tr>
<td>Entireleaf MG&lt;sup&gt;4&lt;/sup&gt;</td>
<td>100</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>Pitted MG</td>
<td>100</td>
<td>31</td>
<td>116</td>
</tr>
<tr>
<td>Northern jointvetch</td>
<td>95</td>
<td>210</td>
<td>185</td>
</tr>
<tr>
<td>Wild poinsettia</td>
<td>91</td>
<td>128</td>
<td>117</td>
</tr>
<tr>
<td>Smallflower MG</td>
<td>90</td>
<td>60</td>
<td>129</td>
</tr>
<tr>
<td>Hemp sesbania</td>
<td>66</td>
<td>181</td>
<td>335</td>
</tr>
<tr>
<td>Indigo</td>
<td>37</td>
<td>195</td>
<td>75</td>
</tr>
<tr>
<td>Redweed</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prickly sida</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Johnsongrass</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Barnyardgrass</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>30</td>
<td>115</td>
<td>133</td>
</tr>
</tbody>
</table>

<sup>1</sup> Percent plants with pycnidia, 171 days after planting.

<sup>2</sup> Mature perithecia per square centimeter of stem surface, 360 days after planting.

<sup>3</sup> Mature pycnidia per square centimeter of stem surface, 360 days after planting.

<sup>4</sup> Morningglory.

**Discussion**

While research has been conducted to determine the host range of many pathogens (5.21), the host range of Dpc has received little attention (5.3, 5.7, 5.14, 5.15). Dpc infected, colonized, and reproduced in several plant species, indicating soybean is not the only host of Dpc. Most of the weed species infected with Dpc were asymptomatic in contrast to symptomatic infected soybean. The fact that
most of the weed hosts were asymptomatic is significant because Dpc inoculum could increase undetected, resulting in an underestimation of inoculum for epidemics the following year. Asymptomatic hosts would enable stem canker epidemics to develop in areas where there is no apparent source of Dpc inoculum.

The role of weed hosts in stem canker epidemiology has not been fully addressed. Roy and Miller (5.14) determined that Dpc could utilize cotton as a host, but the role of cotton in stem canker epidemiology was not determined. Other research indicated Phomopsis was capable of colonizing five weed species and Dpc induced a reaction in lima bean, but inoculum production was not mentioned (5.3,5.15). The research in the present study supports other research evaluating the host range of Dpc, and provides additional information concerning the relative ability of Dpc to reproduce on weeds compared to the reproductive ability on soybean (5.3,5.15). While perithecial production was significantly (P <= 0.05) less on the weed hosts compared to soybean, pycnidia were produced equally well on most weed hosts and 'Hartz 7126' soybean. Alpha conidia of Dpc can infect soybean (5.11). Therefore, the pycnidia can be considered as a viable component of Dpc inoculum.

The role of weed hosts in stem canker epidemiology could be significant. Dpc produces spores in a gelatinous matrix, therefore long range spread by wind is unlikely. High
percentages of seed transmission of "southern" Dpc has not been reported (5.2). Therefore, inoculum for stem canker epidemics is probably produced on stem debris at the location where the epidemics occur. Because Dpc can infect and reproduce in weed hosts, these hosts must be considered as potential sources of inoculum for stem canker epidemics.

The present study provides information to demonstrate Dpc can infect, colonize, and reproduce in weed hosts.

The presence of Dpc perithecia on several of the control plants was probably due to inoculum drift during inoculations.

References Cited


CHAPTER VI
CONCLUSIONS
To effectively manage any disease, knowledge of epidemiology is important. Stem canker, caused by the fungus *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* Athew and Caldwell (Dpc), is devastating to soybean yield in years when epidemics are severe. The results from this investigation address several pertinent aspects of stem canker epidemiology.

The role of initial or primary inoculum is important in stem canker epidemiology. This inoculum is required for initiation of stem canker epidemics. Because initial inoculum is produced on plants infected from the previous season, the development of epidemics during the previous season probably affects the development of epidemics during the current season. Therefore, any factors affecting the build-up or depletion of Dpc initial inoculum should be considered.

The growth stage at which soybean was inoculated had significant effects on soybean yield and stem canker severity. Yields were lowest and disease severity was greatest when cultivars were inoculated at growth stages Vc or V3. Yields of soybean plants inoculated at late vegetative or early reproductive stages were either not affected or differed only slightly from yields of the noninoculated controls. Stem canker severity of soybean plants inoculated at late vegetative or early reproductive stages was less than soybean plants inoculated at earlier
growth stages. While stem canker symptoms did not develop when soybean plants were inoculated at the later growth stages, perithecia were produced indicating these plants could be inoculum sources for an epidemic the following season.

Stem canker incidence was greatest in plant sets placed in the field 4-7 weeks after planting compared to incidence in plant sets placed in the field earlier or later in the season. The results were similar to the growth stage at inoculation experiment. The ability of Dpc to infect soybean 11 weeks after soybean were planted is significant. Stem canker incidence in plant sets placed in the field 11 weeks after planting did not develop to the magnitude in other sets, but these plants could serve as an inoculum source.

Weeds commonly found in Louisiana's soybean fields were demonstrated to be alternative hosts for Dpc. Most of these hosts were asymptomatic, indicating that Dpc inoculum could be increased in hosts that were apparently healthy. Dpc produced pycnidia as well on some of these hosts as a Dpc susceptible soybean cultivar. Therefore, when considering the inoculum potential for stem canker epidemics, these hosts must be considered.

In reality, pests exist simultaneously in soybean. Pests are detrimental individually to soybean. The effects of one pest are affected by interactions with another pests. Girdling caused by the threecornered alfalfa hopper resulted
in increased stem canker severity compared to diseased plants without girdles. The plants evaluated in our study had not sustained severe insect injury, but minimal injury caused a significant increase in stem canker severity.

This research provides new information about the effects of growth stage at time of inoculation on stem canker severity and soybean yield, availability of Dpc inoculum in a "natural field situation", alternative hosts, and insect/fungus interactions. All of these factors affect the development of stem canker epidemics and resultant production of inoculum (especially initial inoculum). These results support previous conclusions that infections occurring soon after planting will result in the greatest yield reductions and stem canker severity. Infections that occur soon after planting will probably translate into a heavier inoculum load for the next season. Infections that occur to soybean at early reproductive stages, while not yield limiting, may also contribute to inoculum for the next season.

Producers also should be aware that soybean is not the only host for Dpc and controlling the weed hosts should help reduce Dpc inoculum. Other factors that affect stem canker severity also should be considered when developing a stem canker management program. Insect injury by the threecornered alfalfa hopper has been implicated in increased stem canker severity.
In conclusion stem canker epidemiology is affected by many factors. This research provides new information concerning stem canker epidemiology that can be implemented into management programs and perhaps eventually forecasting models.
VITA

Guy Boyd Padgett was born March 30, 1962, in Monroe, LA. He attended high school at River Oaks Academy and graduated in May 1980. He was married in 1983 to Marilyn. He received his Bachelor of Science degree in Agricultural-Business at Louisiana Tech University in 1984. Upon completing his B.S. degree, he enrolled at the University of Georgia and subsequently was awarded an M.S. degree in plant pathology in 1987. In 1988, he enrolled at Louisiana State University in the Department of Plant Pathology and Crop Physiology to obtain a Ph.D. While at Louisiana State University he not only worked for a Ph.D. degree, he also was hired as a research associate in this department.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Guy Boyd Padgett

Major Field: Plant Health

Title of Dissertation: The Epidemiology of the Soybean/Diaporthe Phaseolorum Var. Caulivora Pathosystem in Louisiana

Approved:

[Signatures]

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination:

October 19, 1992