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IDENTIFICATION OF PHOMOPSIS LONGICOLLA, SP. NOV., AND ITS  
THERMAL AND BIOLOGICAL CONTROL IN SOYBEAN SEED

*The Ohio State University*

PH.D. 1984

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IDENTIFICATION OF PHOMOPSIS LONGICOLLA, SP. NOV.,  
AND ITS THERMAL AND BIOLOGICAL CONTROL  
IN SOYBEAN SEED

DISSERTATION

Presented in Partial Fulfillment of the Requirements for  
the Degree Doctor of Philosophy in the Graduate  
School of The Ohio State University

by

Thomas William Hobbs, B.S., M.S.

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The Ohio State University

1984

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To my family,  
for their support and patience.

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Several departments at the Ohio Agricultural Research and Development Center, Wooster, Ohio, were of considerable aid during these studies. The Electron Microscope and Statistics Laboratories were particularly helpful. Especial assistance was given by the staff of the Library, without whom sources for the often obscure references used to compile Appendix A might never have been found.

Finally, thanks is given to those researchers who provided Phomopsis isolates for examination, and to the curators of Herb. K and Herb. W for loaning the type materials of P. phaseoli Petch and P. glycines Petrak, respectively.

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## TABLE OF CONTENTS

	Page
Dedication.....	ii
Acknowledgments.....	iii
Vita.....	v
List of Tables.....	ix
List of Figures.....	xi
General Introduction and Literature Review.....	1
Chapter	
I. Identification of a New <u>Phomopsis</u> Species from Soybean .....	7
Introduction.....	7
Materials and Methods.....	9
Results and Discussion.....	10
II. Effect of Storage Time and Temperature on Soybean Seed Quality, and on Seed Decay Caused by <u>Phomopsis longicolla</u> .....	24
Introduction.....	24
Materials and Methods.....	25
Results.....	27
Discussion.....	38

## TABLE OF CONTENTS (cont.)

III. Potential for the Use of Resident Seedborne Fungi of Soybean to Control <u>Phomopsis longicolla</u> and Improve Seed Quality.....	42
Introduction.....	42
Materials and Methods.....	45
Culture Information.....	45
Antagonism <u>In Vitro</u> .....	45
Seed Treatments.....	46
Prophylactic Foliar Spray Treatments.....	49
Results.....	51
Antagonism <u>In Vitro</u> .....	51
Seed Treatments.....	52
Prophylactic Foliar Spray Treatments.....	56
Discussion.....	62
General Summary and Conclusions.....	66
List of References.....	68
Appendices	
A. <u>Diaporthe</u> and <u>Phomopsis</u> Species Reported on Legume Hosts, Including Anamorphs, Teleomorphs, Homonyms, Synonyms, and Unnamed States.....	89
B. Regression Equations for Storage Time and Temperature Study.....	136

## LIST OF TABLES

Table	Page
1. Chronology of the <u>Diaporthe/Phomopsis</u> complex on soybeans.....	4
2. Alpha-conidia length, width, and length-to-width ratios of two <u>Phomopsis</u> species from soybean.....	17
3. Comparison of cultural characters for two <u>Phomopsis</u> species from soybean.....	18
4. The effect of storage temperature and time on the percent of <u>Phomopsis longicolla</u> recovered from naturally-infected soybean seeds.....	28
5. The effect of storage temperature and time on the percent of normal seedlings produced by soybean seeds naturally-infected by <u>Phomopsis longicolla</u> .....	30
6. The effect of storage temperature and time on the percent of abnormal seedlings produced by soybean seeds naturally-infected by <u>Phomopsis longicolla</u> .....	33
7. The effect of storage temperature and time on the percent of dead seedlings produced by soybean seeds naturally-infected by <u>Phomopsis longicolla</u> .....	35
8. The effect of storage time and temperature on moisture content of soybean seeds.....	39
9. Zones of inhibition between test antagonists and <u>Phomopsis longicolla</u> in dual cultures.....	53

# LIST OF TABLES (cont.)

10. Percent inhibition of colony growth of <u>Phomopsis longicolla</u> by test antagonists in dual cultures.....	54
11. Effects of seed treatments on germination and vigor components of a soybean seed lot with 30% Natural <u>Phomopsis longicolla</u> infection.....	55
12. Effect of seed treatments on emergence in sand of a soybean seed lot with 30% natural <u>Phomopsis longicolla</u> infection.....	57
13. Effect of seed treatments on field emergence and yield of a soybean seed lot with 25% natural <u>Phomopsis</u> infection.....	58
14. Interaction of application time and harvest date on the incidence of <u>Phomopsis longicolla</u> in seed in 1979.....	60
15. Effect of spray treatments on germination of soybean seed in 1980.....	61
16. Effect of spray treatments on incidence of <u>Alternaria alternata</u> , <u>Chaetomium globosum</u> , and <u>Phomopsis longicolla</u> in seeds and on germination in 1981.....	63

## LIST OF FIGURES

Figure	Page
1. Reverse side of acidified potato dextrose agar cultures of <u>Phomopsis sojae</u> after 2 wk at 22-25 C under intermittent fluorescent light.....	12
2-6. <u>Phomopsis sojae</u> (Teleomorph: <u>Diaporthe phaseolorum</u> var. <u>sojae</u> ).....	13
2. Median section through a conidioma.	
3. Vertical section through a conidioma.	
4. Alpha-conidia produced on simple phialides.	
5. Beta-conidia produced on simple phialides.	
6. Asci and ascospores of teleomorph.	
7. Reverse side of acidified PDA cultures of <u>Phomopsis longicolla</u> grown as in Fig. 1.....	15
8-12. <u>Phomopsis longicolla</u> .....	16
8. Vertical section through a conidioma.	
9. Vertical section through a multi-rostrate, multi-chambered conidioma.	
10. Mediansection through a conidioma showing the globose locule and well-developed ostiole neck.	
11. Branched conidiophores and alpha-conidium.	
12. Simple and branched conidiophores.	
13. Stepwise regression of percent <u>Phomopsis longicolla</u> recovery with time at three temperatures (5, 24, 40C) for 12 seed lots with different inital levels of infection.....	29
14. Stepwise regression of percent normal seedlings produced with time at three temperatures (5, 24, 40C) for 12 seed lots with different initial levels of <u>Phomopsis longicolla</u> infection.....	31

LIST OF FIGURES (cont.)

- |                                                                                                                                                                                                          |    |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| 15. Stepwise regression of percent abnormal seedlings produced with time at three temperatures (5, 24, 40C) for 12 seed lots with different initial levels of <u>Phomopsis longicolla</u> infection..... | 34 |
| 16. Stepwise regression of percent dead seedlings produced with time at three temperatures (5, 24, 40C) for 12 seed lots with different initial levels of <u>Phomopsis longicolla</u> infection.....     | 36 |

## GENERAL INTRODUCTION AND LITERATURE REVIEW

The soybean (Glycine max [L.] Merr.), first introduced to North America in 1765 (95), has become a major grain crop and source of nutritional and industrial products (43, 214, 253). The United States annually harvests approximately 65% of the world production of soybeans for beans, and Ohio consistently ranks sixth in soybean production in this country (229).

Production and maintenance of high-quality seed (i.e., seed of high germination and vigor) should be the goals of all workers involved in soybean seed research. Soybean seed, flour, and oil qualities are adversely affected by species of Diaporthe Nits. and Phomopsis (Sacc.) Bubak (85, 86, 105, 108, 116, 123, 205, 206, 235, 236). These pathogens have been associated with seedling blights, pod and stem blight, stem canker, seed decay, and top dieback in soybeans (10, 51, 88, 89, 108, 116, 206). Pod and stem blight and seed decay at harvest are the most commonly encountered diseases of soybeans grown in Ohio that are caused by Diaporthe and Phomopsis species.



In the past, Diaporthe phaseolorum (Cooke & Ellis) Sacc. var. sojae (Lehman) Wehm. (synonym: D. sojae Lehman; anamorph: Phomopsis sojae Lehman) has been considered the primary field fungus (as opposed to storage fungus [136]) responsible for poor germination and emergence of soybean seed (11, 60, 61, 116, 119, 235, 236). A related organism D. phaseolorum var. caulivora Athow & Cald., which has no known anamorph (10), has also been found to contribute to decay and poor emergence of soybean seed (51, 106, 108, 197). Lehman (116), Luttrell (119), and Hildebrand (87) reported Phomopsis isolates from soybean which did not develop perithecia; these usually have been considered imperfect strains of D. phaseolorum var. sojae. Kmetz and co-workers (105, 106, 107, 108, 197), however, have consistently maintained that Phomopsis isolates which fail to form perithecia are morphologically and pathogenically distinct from the anamorphs of Diaporthe isolates from soybean.

Phomopsis glycines Petrak (155) and P. phaseoli Petch (143) are the only Phomopsis species other than P. sojae to have been originally described from soybean. Both of the species, however, have been overlooked in the literature concerned with Diaporthe and Phomopsis diseases on soybean. Consequently, their relationship to the Diaporthe and Phomopsis organisms already recognized as pathogenic on soybean is unknown.

The chronological history of the Diaporthe/Phomopsis complex on soybean, with references to the original papers, is summarized in Table 1.

The level of a seedborne pathogen within a seedlot may decrease as time in storage increases due to death or inactivation of the pathogen (13, 136). Several researchers have reported such a decrease in Diaporthe or Phomopsis percentages in soybean seedlots, with concomitant improvement in seed germination (68, 198, 199, 236). One study, however, has indicated that, at least in humid tropical countries, seedborne fungi, including Diaporthe and Phomopsis, do not significantly influence the rate of seed deterioration in storage (135).

Soybean seed can be protected from infection by seed decay fungi (i.e., Diaporthe and Phomopsis species) by a physical barrier (11), or by chemical (58, 59, 206) or biological (166) methods. The barrier technique suggested by Athow and Laviolette (11) may be useful as a research tool, but is too laborious and expensive to be of commercial value. Fungicide treatments may result in development of a fungicide-resistant pathogen. Strains resistant to benomyl, the fungicide of choice for soybean pod and stem blight and seed decay control, are known to exist for some fungal pathogens (27, 125). Fungicide activity of benomyl also decreases as time after application increases (62). Also, fungicide treated seed may not be used for any purpose other

Table 1. Chronology of the Diaporthe/Phomopsis complex on soybean.

Year	Researcher(s)	Organism(s)	Reference
1920	Wolf & Lehman	<u>Phoma</u> sp.	250
1922	Lehman	<u>Phomopsis sojae</u>	116
1922	Petch	<u>P. phaseoli</u>	143
1923	Lehman	<u>Diaporthe sojae</u>	117
1924	Anonymous	<u>P. sojae</u> (validation)	96
1933	Wehmeyer	<u>D. phaseolorum</u> var. <u>sojae</u>	242
1936	Petrak	<u>P. glycines</u>	155
1948	Welch & Gilman	<u>D. phaseolorum</u> var. <u>batatatis</u> & <u>D. phaseolorum</u> var. <u>sojae</u>	245
1954	Athow & Caldwell	<u>D. phaseolorum</u> var. <u>caulivora</u>	10
1974	Kmetz, Ellett, & Schmitthenner	<u>Phomopsis</u> sp.	106

than planting. For these reasons, biological control of disease is an attractive system, to either substitute for or augment present fungicide control methods.

The use of Cercospora kikuchii (Mats. & Tom.) Gardner by Roy and Abney (1966) demonstrated that seed decay fungi can be controlled by application of an antagonistic fungus. However, C. kikuchii has been shown to decrease germination and increase the number of abnormal seedlings in soybean (77, 206, 247, 252). Various studies have demonstrated the effectiveness of other biologic agents as treatments for the control of both soilborne and aerial pathogens (see [14] and [37] for examples). Among the antagonists used in these studies were species of Alternaria, Aspergillus, Chaetomium, Cladosporium, Epicoccum, and Trichoderma; these fungal genera are also represented in the seed and phylloplane mycoflora of the soybean (52, 102, 103, 104, 123, 216), and generally are not believed to be seed pathogens (123).

The purposes of the present study were to investigate:

1. if Phomopsis sp. sensu Kmetz et al (106, 197) differed in morphology from Phomopsis sojae and other Phomopsis species or anamorphs of Diaporthe species reported from soybeans;
2. the effect of seed storage at lower or higher than normal temperatures on the recovery of seed decay fungi from a seedlot and the concomitant effect on seed quality;

3. the applicability of biological control practices to control Phomopsis seed decay of soybean.

## CHAPTER I

### Identification of a New Phomopsis Species from Soybean

#### INTRODUCTION

In 1920, Wolf and Lehman reported a Phoma blight occurring on stems and pods of soybeans (Glycine max (L.) Merr.) in North Carolina (250). The disease was later renamed pod and stem blight and the pathogen assigned to the genus Phomopsis, under the binomial P. sojae Lehman (115). Lehman did not describe the fungus at that time, but later (116) provided a full description of the anamorph and teleomorph states of Diaporthe sojae, which he reported caused the disease observed in 1920. Wehmeyer later reduced D. sojae to varietal rank under D. phaseolorum (Cooke & Ellis) Sacc. as D. phaseolorum var. sojae (Lehman) Wehm. (242).

In 1948, Welch and Gilman (245) divided D. phaseolorum isolates from soybean into two varieties, which they recognized as D. phaseolorum var. sojae, the pod and stem blight pathogen, and D. phaseolorum var. batatatis (Harter & Field) Wehm., the incitant of sweet potato (Ipomoea batatas [L.] Lam.) dry rot (80, 242) and that Welch and Gilman reported also caused girdling stem cankers on soybean. Athow and

Caldwell (10) determined that the soybean stem canker organism was not the same as D. phaseolorum var. batatatis, and recognized the former as a new variety, D. phaseolorum var. caulivora Athow & Cald., in 1954.

In 1974, Kmetz et al (106) recovered a Phomopsis isolate from soybean that was morphologically and pathogenically distinct from both D. phaseolorum var. caulivora and var. sojae, which they also isolated. The fungus did not form perithecia and was more highly pathogenic to seed than either of the D. phaseolorum varieties. They later named the disease caused by this new pathogen Phomopsis seed decay (108).

Recently, Kulik (111) questioned the separation of D. phaseolorum isolates from various hosts into taxonomic varieties, as suggested by Wehmeyer (242), and also questioned the validity of recognition of Phomopsis anamorphs of these teleomorphs as distinct species. Kulik's paper is primarily based on morphological data and identifications of D. phaseolorum isolates found in the literature, and on a host range study with one isolate each of P. sojae from soybean, P. batatae Harter & Field (80) from Louisiana, and P. phaseoli (Desm.) Sacc. (187) from lima bean (Phaseolus lunatus L.). No new morphological data was presented in Kulik's paper, and Phomopsis sp. sensu Kmetz et al (106, 197) was not mentioned.

Isolates of D. phaseolorum var. caulivora rarely form an anamorph, and reports of alpha-conidial production are considered dubious (206). The current study is concerned only with Phomopsis anamorphs commonly isolated from soybean; therefore, D. phaseolorum var. caulivora will not be considered further in this paper.

Phomopsis sp. sensu Kmetz et al. and the Phomopsis state of D. phaseolorum var. sojae are the most common Phomopsis anamorphs isolated from soybean plant parts in Ohio (105, 106, 107, 108, 197). The present studies were undertaken to compare cultural morphology and other characteristics of these two anamorphs. Comparisons with the other Phomopsis species reported from soybean were also made. The results serve as a basis for the delimitation of a new Phomopsis species.

#### MATERIALS AND METHODS

Isolates used in this study were obtained as mycelial cultures from soybean seed from various Ohio counties. The type materials of P. glycines Petrak in Petrak and H. Sydow, and of P. phaseoli Petch were obtained from Herb. W and Herb. K (82), respectively.

Cultural morphology and other characteristics of the isolates were compared on plates of potato dextrose agar (acidified to pH 4.5 with 85% lactic acid) incubated under intermittent fluorescent light (about 12 hr daily) on a



laboratory bench (22-25 C). Measurements of 100 alpha-conidia were made from each of ten isolates. Conidiomata were sectioned with a razor blade under a dissecting microscope. For light microscopy, sections were mounted in 15% lactic acid and observed using Normarski Interference Contrast (NIC). Some sections were prepared for scanning electron microscopy (SEM) by washing with a 0.1 M phosphate buffer (pH 7.0) several times, agitating in 1% potassium hydroxide for 30-45 min then 1% acetic acid for 5 min and rinsing in 5-6 changes of phosphate buffer. Samples were fixed in 1% osmium tetroxide ( $\text{OsO}_4$ ) in phosphate buffer overnight at 5 C; then treated 45 min with saturated, aqueous thiocarbonylhydrazide; and then re-treated for 1 hr with  $\text{OsO}_4$  at room temperature. After 3-4 phosphate buffer rinses, samples were dehydrated in an ethanol series (20, 40, 60, 80, 95, 100, 100%; 30 min each) and dried in a Tousimis Samdri 790 critical point drier (Tousimis Research Corp., Rockville, MD 20852). Specimens were attached to aluminum stubs with silver paint and coated with platinum prior to examination with an ISI-40 Scanning Electron Microscope (International Scientific Instruments, Inc., Santa Clara, CA 95051).

#### RESULTS AND DISCUSSION

Lehman did not describe *P. sojae* when he published the binomial in 1922 (115), nor did he refer to either *P. sojae*

or the 1922 paper when he described D. sojae in 1923 (116). Therefore, prior to the study by Kmetz et al in 1974 (106), most Phomopsis isolates from soybean were recorded as the Phomopsis state of D. phaseolorum var. sojae, or just as D. phaseolorum var. sojae. In 1924, however, an anonymous reviewer validated P. sojae by connecting this name with Lehman's 1923 description of the Phomopsis state of D. sojae (96).

Isolates used in the present study could be divided into two groups based on colony appearance after two weeks. Isolates of the first group produced a floccose, ropy mycelium that was initially white, but became tan to brown as the culture aged. Isolates of this group formed pustulate stromata that seldom grew very large (Fig. 1). Conidiomata of this group had lenticular locules and seldom developed ostiole necks longer than 200  $\mu$ m (Fig. 2,3). Alpha- and beta-conidia formed within the same conidioma and were borne on simple conidiophores (Fig. 4,5). This group fit the description of P. sojae (116), and was always associated with the teleomorph D. phaseolorum var. sojae. These isolates were also similar to those described as D. phaseolorum var. sojae in several other studies (10, 100, 106, 119, 245).

Isolates of the second group produced a dense mycelium that remained mostly white, although some isolates developed greenish-yellow areas. Isolates of this group formed

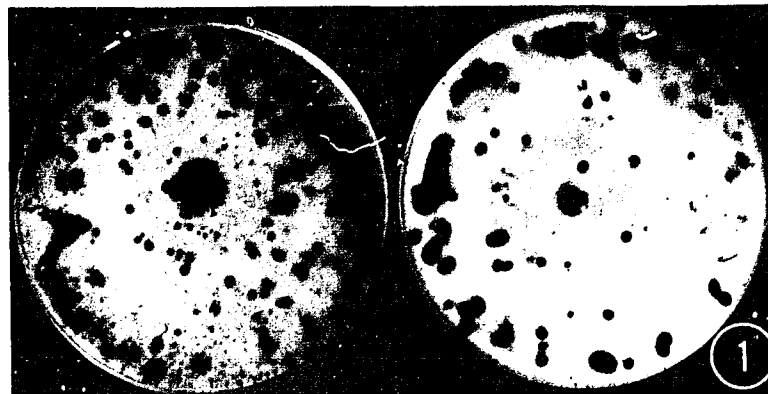


Fig. 1. Reverse side of acidified potato dextrose agar cultures of Phomopsis sojae after 2 wk at 22-25 C under intermittent fluorescent light.

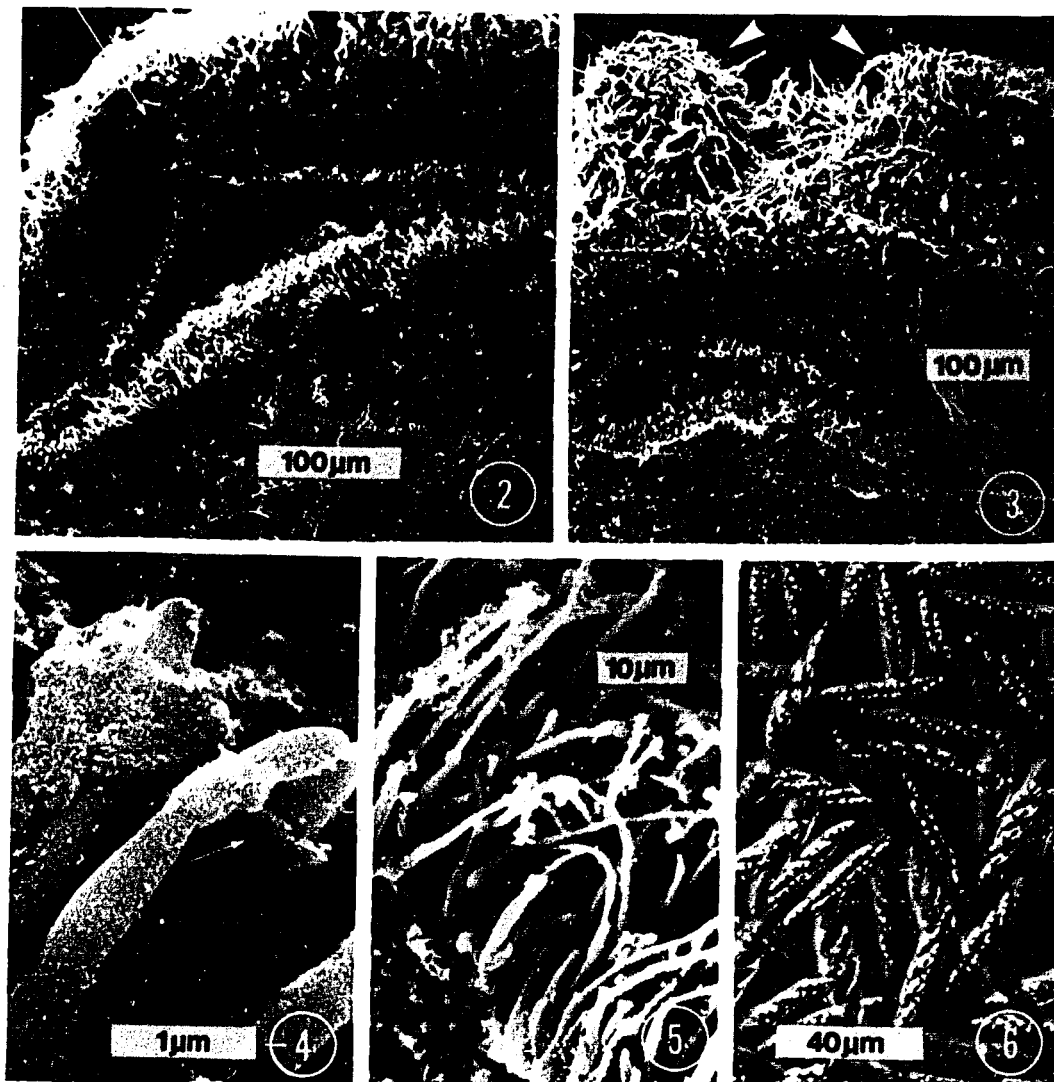


Fig. 2-6 *Phomopsis sojae* (Teleomorph: *Diaporthe phaseolorum* var. *sojae*). 2) Median section through a conidioma (SEM). Note ostiole (arrow). 3) Vertical section through a conidioma (SEM). Note lenticular locule and short ostiole necks (arrows). 4) Alpha-conidia produced on simple phialides (SEM). Note prominent collarette (arrow). 5) Beta-conidia produced on simple phialides (SEM). 6) Asci and ascospores of teleomorph (NIC).

massive, effuse stromata that often extended over the entire bottom of the culture dish (Fig. 7). Alpha-conidia were produced in conidiomata that had globose locules and long ostiole necks (Fig. 8-10). Conidiophores frequently were branched (Fig. 11,12). Beta-conidia occasionally formed in older stock cultures, but were absent from fresh cultures. These isolates never formed perithecia and were identical to Phomopsis sp. sensu Kmetz et al (196, 197).

Mean alpha-conidial length and width measurements for individual isolates of the two groups overlapped, but the overall group means were distinct (Table 2). Mean alpha-conidial length-to-width ratios were always distinct, although there was some variation among isolates within a group (Table 2) and overlap of the overall ranges for the two groups (Table 3). Distinguishing characteristics of the two groups are summarized in Table 3.

Isolates of the second group were also distinct from P. glycines and P. phaseoli Petch, the only other Phomopsis species described from soybean.

Phomopsis glycines was described by Petrak in 1936 (155). The type material consisted of two pods on which numerous conidiomata were borne. Conidiomata averaged 198 um in diameter, had little or no ostiole neck, and contained alpha-conidia borne on simple conidiophores. Thus, this species is nearly identical to P. sojae.

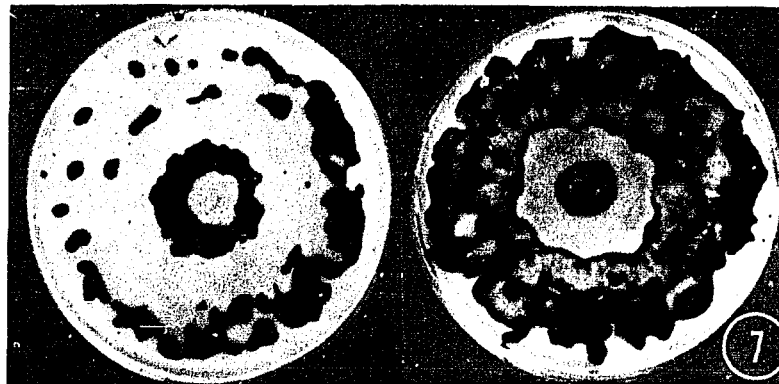


Fig. 7. Reverse side of acidified PDA cultures of Phomopsis longicolla grown as in Fig. 1.

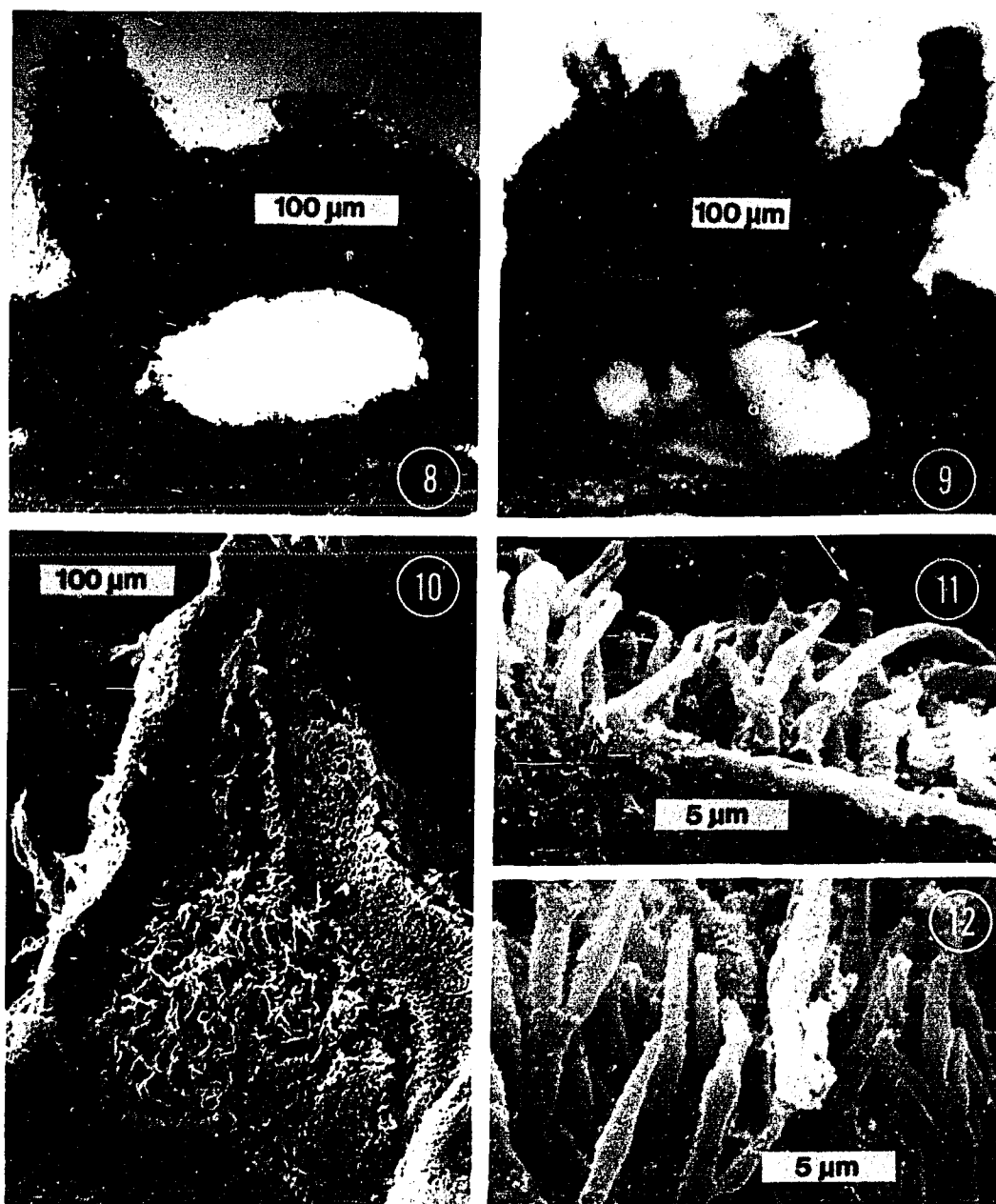


Fig. 8-12 *Phomopsis longicolla*. 8) Vertical section through a conidioma (NIC). Note globose locule and prominent ostiole neck. 9) Vertical section through a multi-rostrate, multi-chambered conidioma (NIC). 10) Median section through a conidioma showing the globose locule and well-developed ostiole neck (SEM). 11) Branched conidiophores and alpha-conidium (arrow) (SEM). 12) Simple and branched conidiophores (SEM).

Table 2. Alpha-conidia length, width, and length-to-width ratios of two *Phomopsis* species from soybean.

Species	Isolate	Length <sup>a</sup>	Width <sup>a</sup>	L/W Ratio <sup>a</sup>
<i>P. sojae</i>	D167	7.4 C	2.1 E	3.6 A
	D168	7.7 AB	2.2 D	3.5 A
	D94	7.6 B	2.3 C	3.3 B
	D18	7.3 C	2.2 D	3.3 B
	D74	7.8 A	2.4 BC	3.3 B
	Overall Mean <sup>b</sup>	7.6	2.2	3.4
<i>P. longicolla</i>	P68	6.9 D	2.4 B	2.9 CD
	P74	6.9 D	2.6 A	2.7 E
	P43	6.8 D	2.4 B	2.8 DE
	P116	7.3 C	2.4 B	3.0 C
	P32	6.8 D	2.4 BC	2.8 DE
	Overall Mean <sup>b</sup>	6.9	2.4	2.9
FLSD (0.01)		0.2	0.1	0.1

<sup>a</sup> Mean of 100 observations. Means within a column followed by the same letter do not differ significantly according to Duncan's New Multiple Range Test ( $p=0.05$ ).

<sup>b</sup> Mean of 500 observations.



Table 3. Comparison of cultural characters for two Phomopsis species from soybean.

Character	<u>Phomopsis sojae</u>	<u>Phomopsis longicolla</u>
Stromata	Pustulate	Massive, effuse
Ostiole Necks	<200 um	200-500 um or longer
Alpha-conidia		
Size		
Range	5.6-10.3 x 1.5-3.4 um	5.1-9.2 x 1.5-3.1 um
Mean <sup>a</sup>	7.6 x 2.2 um	6.9 x 2.4 um
L/W ratio		
Range	2.1-5.4	1.7-4.5
Mean <sup>a</sup>	3.4	2.9
Beta-conidia	Abundant	Rare in fresh cultures
Conidiophores	Simple, rarely branched	Simple, usually branched
Teleomorph	<u>Diaporthe phaseolorum</u> var. <u>sojae</u>	None

<sup>a</sup> Mean of 500 observations.

Phomopsis phaseoli Petch was described in 1922 (143) from soybean stem tissue collected in Ceylon and is a later homonym of P. phaseoli (Desm.) Sacc. (187), the anamorph of D. phaseolorum var. phaseolorum (111, 242). Most of the conidiomata of the type material appeared immature. Larger conidiomata bore a short ostiole neck. Conidiomata averaged only 159  $\mu$ m, although Petch reported a diameter of 250  $\mu$ m. Conidia and conidiophores of this species were not observed, but Petch's measurements of the alpha-conidia (3-6 x 1.5-2  $\mu$ m) are small in comparison to those of other Phomopsis species reported from legumes (see Appendix A). This species may be an immature specimen of a known species or represent an entirely new species. It is considered a nomen dubium in the present study.

Because isolates of the second group differ in morphology from the other Phomopsis species reported from soybean, they represent a new species, described below under the binomial Phomopsis longicolla.

Phomopsis longicolla Hobbs, sp. nov. (Fig. 7-12)  
Coloniae in agar 'potato dextrose' flocculosusae, densae, albidae cum raro viridi-flavae areae; reversum incoloratum cum magnis nigris stromatibus. Conidiomata pycnidica, nigra, stromatica, solitaria vel aggregata, unilocularia vel multilocularia, cum collis prominentibus plus quam 200  $\mu$ m longis, aperientia ostiolo apicali. Loculi uniostiolati vel multiostiolati, globosi, usque 500  $\mu$ m lati. Conidiophora

hyalina, simplicia vel vulgo ramosa, septata, 3.5-24.0 x 1.3-3.8 um. Cellulae conidiogenae hyalinae, filiformae, phialidicae. Alpha-conidia hyalina, ellipsoidia usque ad fusiforma, guttulata, 5-9.5 x 1.5-3.5 um. Beta-conidia inustiata, hyalina, filiforma, hamata. Ex seminibus, leguminibus et calamis Glycines max (L.) Merr.; TWH P74 (BPI), holotypus, Hobbs, Wooster, Ohio, 1983.

Colonies on potato dextrose agar flocculose, dense, white with occasional greenish-yellow areas; reverse colorless with large black stromata. Conidiomata pycnidial, black, stromatic, solitary to aggregated, unilocular to multilocular, with prominent necks more than 200 um long, opening by an apical ostiole. Locules uniostiolate to multioostiolate, globose, up to 500 um wide. Conidiophores hyaline, simple or usually branched, septate, 3.5-24.0 x 1.3-3.8 um. Conidiogenous cells hyaline, filiform, phialidic. Alpha-conidia hyaline, ellipsoid to fusiform, guttulate, 5-9.5 x 1.5-3.5 um. Beta-conidia rare, hyaline, filiform, hamate. Isolated from seeds, pods and stems Glycine max (L.) Merr.; TWH P74 (BPI), holotype, Hobbs, Wooster, Ohio, 1983.

Cultures studied: TWH P32, Lucas Co., Ohio, 1981; TWH P43, Franklin Co., Ohio, 1981; TWH P68, Wayne Co., Ohio, 1983; TWH P74, Wayne Co., Ohio, 1983; TWH P116, Ottawa Co., Ohio, 1984.

Additional cultures examined: Besides numerous isolates from soybean seed, pod, and stem tissues made during the course of this study, cultures from other locations were examined and identified as P. longicolla. These included: Illinois (J.B. Sinclair, 2, unnumbered), Iowa (D.C. McGee, 7, unnumbered), Maryland (ATCC #46562), and Mississippi (B.L. Keeling, Culture #PS-80-205, PS-81-26, PS-81-27, PS-81-48, PS-81-66, and PS-81-79). Cultures from other locations identified as P. sojae included: Great Britain (IMI #137167), Illinois (J.B. Sinclair, 2, unnumbered), Iowa (D.C. McGee, 2, unnumbered), and Maryland (ATCC #36295; M.M. Kulik, 6, unnumbered). Three cultures from ATCC (#12049, 12050, and 28463) designated D. phaseolorum var. sojae failed to sporulate during this study.

Isolates of P. longicolla may have been observed in the past but reported as D. phaseolorum var. sojae. Lehman first reported cultures of the latter organism that did not form perithecia (116), and others have reported non-perithecial strains of this pathogen (87, 119). The non-perithecial D. phaseolorum var. sojae isolates noted by Luttrell (119) are similar to P. longicolla in that they formed large stromata and seldom produced beta-conidia. Luttrell's isolates, however, did not form beaked pycnidia. Hildebrand described Phomopsis isolates (his category C) similar to P. longicolla and questioned their taxonomic disposition (87), and also reported numerous Phomopsis isolates that he

distinguished from the anamorphs of his Phomopsis longicolla es  
(88). Strelecki (209) reported variants of D. phaseolorum  
var. sojae that formed "sclerotia" in mats (his types 1 and  
3), but did not describe the conidiomata, nor indicate  
whether they formed perithecia.

Phomopsis longicolla differs from P. sojae not only  
morphologically, but ecologically and pathogenically as  
well. Kmetz et al (107, 108) found it to be more prevalent  
than either of the two D. phaseolorum varieties in immature  
and mature soybean seed, and also in soybean debris. They  
also reported that it readily rotted inoculated seed, while  
the other two organisms were poorly or moderately pathogenic  
to seed (106).

Differences in alpha-conidium size between P.  
longicolla and P. sojae can best be detected from isolates  
grown on artificial media. Although length and width  
measurement values overlapped, isolates of the two species  
could be readily differentiated by the mean alpha-conidium  
sizes and mean alpha-conidium length-to-width ratios. Van  
der Aa (1) and Weidemann et al (244) have found variation in  
spore size to be common among Phyllosticta species, but the  
length-to-width ratio is fairly constant and useful in dis-  
tinguishing Phyllosticta species. This appears to be valid  
for the two Phomopsis species compared in this study.  
Although there was variation of the mean ratio for isolates  
within a species, there was no overlap between species.

Further investigations should be conducted to determine if this measure can be used as a reliable tool to differentiate other Phomopsis species.

Species in the genus Phomopsis are usually associated with Diaporthe teleomorphs (242). This is the case for P. sojae. Mature perithecia were found on overwintered stems of soybeans grown in Indiana and Ohio, and also in culture. Cultures grown from either single ascospores or alpha-conidia usually produce perithecia on artificial media in four to six weeks, indicating that D. phaseolorum var. sojae is homothallic, as previously reported (100). All these cultures have also produced the anamorph, P. sojae. A teleomorph for P. longicolla has not been found.

Diaporthe phaseolorum var. sojae or its anamorph has been reported from 26 species in 17 genera of the Leguminosae (see Appendix A) and several species in other plant families (119, 206). Phomopsis longicolla, however, is known only from Glycine max. As mentioned above, some earlier identifications of Phomopsis may have been erroneously attributed to D. phaseolorum var. sojae. Further careful research will be necessary to determine the host range of this pathogen.

## CHAPTER II

### Effect of Storage Time and Temperature on Soybean Seed Quality, and on Seed Decay Caused by Phomopsis longicolla

#### INTRODUCTION

Soybean seed decay (SSD), part of the pod and stem blight disease complex (206), is the most prevalent and serious seedborne disease affecting soybeans in Ohio (108). The fungal pathogens causing SSD are Diaporthe phaseolorum (Cooke & Ellis) Sacc. var. sojae (Lehman) Wehm., D. phaseolorum var. caulivora Athow & Caldwell, and Phomopsis longicolla Hobbs (106, 108, 206). The negative effects of these seedborne fungi on germination, vigor and emergence of soybean seed are well-documented (30, 58, 60, 61, 108, 112, 113, 123, 137, 198, 199, 205, 235, 236). Although the percentage of SSD pathogens recovered from seeds decreases as time after harvest increases (68, 198, 199, 236), these fungi are viable and pathogenic following at least two years storage under cool, dry conditions (236). Two reports have shown that heating infected seed speeds death of SSD pathogens and increases germination (201, 254). The two different methods employed, exposure to radio-frequency

electric fields (201) and immersion in hot soybean oil (254), however, may not have practical value for commercial usage.

Studies concerned with soybean seed storage usually have dealt with the effects of storage fungi (such as species of Aspergillus and Penicillium) on germination and vigor, and have neglected the contribution of field fungi such as the SSD pathogens (33, 34, 216). Studies on the effects of SSD pathogens on germination and vigor have usually been made soon after harvest (112, 113) or have not evaluated the effects of storage conditions (123). The current study was undertaken to assess the effects of P. longicolla (hereafter, Phomopsis), the predominant SSD pathogen in Ohio, on germination and vigor of soybean, and the influences of storage temperature and time on these effects.

#### MATERIALS AND METHODS

Seeds of various soybean cultivars grown in 1982 were examined for the presence of Phomopsis and other seedborne fungi following harvest in the fall. Four replications of one hundred seeds of each cultivar were surface-sterilized in 1.05% sodium hypochlorite for 1 min, drained on sterile paper toweling, and plated on Difco potato dextrose agar acidified after autoclaving to pH 4.5 with 85% lactic acid.



Percentages of the various fungi isolated were determined after incubation for two weeks under intermittent fluorescent light (about 12 hr daily) on a laboratory bench (22-25 C).

Twelve cultivars were chosen for study. These cultivars represented three lots of four cultivars each, consisting of low (0-10%), medium (11-20%), and high (21-30%) initial Phomopsis infection levels. Four replications of 50 seeds of each cultivar were tested for germination (8) and vigor using a modified rolled towel test (9). Vigor components examined included speed of germination, seedling classification, and seedling growth rate, determined in this study by the average dry weight of normal seedlings.

Simultaneously, 50-gram subsamples of each cultivar were sealed in small (31x5x10 cm) polyethylene bags for later testing. Each of four replications of the 12 cultivars was then packaged into large (46x10x20 cm) polyethylene bags and these stored at 5, 24, or 40 C. At 50 day intervals, subsamples were tested for germination, vigor, and percentages of fungi present as previously described. The test was terminated after 200 days.

Moisture content (wet-weight basis) of the seeds of each of the 12 cultivars was determined at the beginning and end of the test by oven-drying whole seed at 105 C for 16 hr (33, 34).

Results were analyzed using standard analysis of variance procedures. Data were also subjected to arcsine transformation. Stepwise multiple regression was used to obtain predictive models based on the data.

## RESULTS

Cultivar interactions with the factors examined during this study were not significant after arcsine transformation of the data; however, interactions of initial Phomopsis infection percentage with the same factors were highly significant. Therefore, the results are reported as functions of the initial Phomopsis infection levels of the lot. Regression equations for the factors examined are listed in Appendix B.

Increasing storage time and temperature reduced the amount of Phomopsis recovered from the seeds in each of the three lots (Table 4; Fig. 13). The decline was greater when seed were stored at 40 C than when at 24 C, and at 24 C than at 5 C.

The effects of storage time and temperature on the percent of normal seedlings produced differed for each of the three lots (Table 5; Fig. 14). In the high Phomopsis lot, normal seedlings increased slightly with time at all three temperatures when the values were predicted by regression (Fig. 14). The observed increase appeared to plateau toward the end of the study, and even declined in

Table 4. The effect of storage temperature and time on the percent of Phomopsis longicolla recovered from naturally-infected soybean seeds.

Storage Temperature (c)	Storage Time (DA)	Lot <sup>a</sup>					
		High		Medium		Low	
		% <sup>b</sup>	Transformed <sup>b,c</sup>	%	Transformed	%	Transformed
5	0	30.6	33.4	16.1	23.3	2.6	8.3
	50	32.2	34.2	15.3	22.6	3.9	9.7
	100	29.3	32.5	15.3	22.6	2.9	8.3
	150	27.6	31.5	14.5	21.9	3.4	9.5
	200	12.8	19.7	10.9	18.7	2.3	7.6
24	0	29.3	32.4	13.6	21.1	1.9	6.9
	50	26.1	30.4	13.0	20.8	2.4	7.8
	100	27.8	31.3	15.6	22.8	2.4	8.0
	150	16.5	23.8	8.4	16.3	1.5	6.1
	200	8.8	16.8	6.4	14.2	1.2	5.0
40	0	29.1	32.4	15.3	22.8	2.6	7.7
	50	24.4	29.3	10.3	18.4	2.5	7.8
	100	15.4	22.9	8.8	16.8	2.0	7.2
	150	8.2	16.2	4.5	11.8	1.0	3.9
	200	1.3	6.5	1.3	5.1	0.4	2.3
FLSD (0.05)		5.2	3.9	3.2	3.1	NS <sup>d</sup>	2.4

<sup>a</sup> Based on initial P. longicolla levels of the seed lot. High = 21-30%; Medium = 11-20%; low = 0-10%.

<sup>b</sup> Mean of 4 replications of 4 cultivars each.

<sup>c</sup>  $\sin^{-1} (p_i)^{0.5}$ ; where  $p_i$  = proportion of seeds from which P. longicolla was recovered.

<sup>d</sup> Not significant at  $p = 0.05$ .

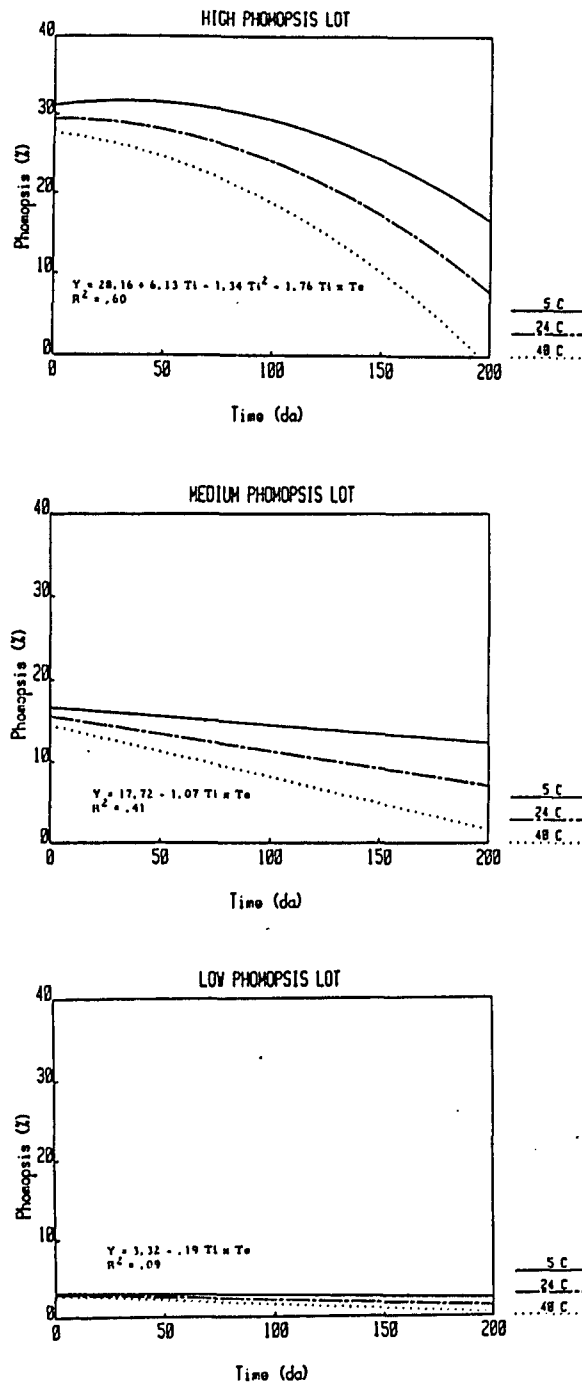


Fig. 13. Stepwise regression of percent *Phomopsis longicolla* recovery with time at three temperatures (5, 24, 40C) for 12 seed lots with different initial levels of infection. Upper: High (21%-30%) *Phomopsis* lot. Middle: Medium (11-20%) *Phomopsis* lot. Lower: Low (0-10%) *Phomopsis* lot.

Table 5. The effect of storage temperature and time on the percent of normal seedlings produced by soybean seeds naturally-infected by Phomopsis longicolla.

Storage Temperature (c)	Storage Time (DA)	Lot <sup>a</sup>					
		High		Medium		Low	
		% <sup>b</sup>	Transformed <sup>b,c</sup>	%	Transformed	%	Transformed
5	0	59.3	50.4	72.5	58.7	89.1	71.3
	50	58.8	50.3	69.3	56.5	83.0	66.4
	100	64.4	53.6	71.0	57.9	85.3	67.8
	150	68.8	55.1	70.5	57.4	83.6	66.4
	200	68.5	56.1	72.0	58.4	79.9	63.6
24	0	58.6	50.0	74.4	60.2	88.6	70.6
	50	60.5	51.2	74.4	59.8	84.9	68.1
	100	67.0	55.1	75.9	60.9	83.4	66.6
	150	73.0	58.9	78.8	62.8	83.8	66.5
	200	72.8	58.6	75.8	60.7	73.4	59.1
40	0	62.8	52.5	73.3	59.2	86.6	68.9
	50	69.4	56.6	77.9	62.7	85.6	68.2
	100	72.1	58.4	79.8	63.6	83.0	66.0
	150	69.8	56.9	71.6	58.1	71.4	58.0
	200	62.4	52.2	66.4	54.7	60.4	51.1
FLSD (0.05)		5.4	3.3	5.4	3.6	4.3	3.5

<sup>a</sup> Based on initial P. longicolla levels of the seed lot. High = 21-30%; Medium = 11-20%; low = 0-10%.

<sup>b</sup> Mean of 4 replications of 4 cultivars each.

<sup>c</sup>  $\sin^{-1} (p_i)^{0.5}$ ; where  $p_i$  = proportion of seeds from which P. longicolla was recovered.

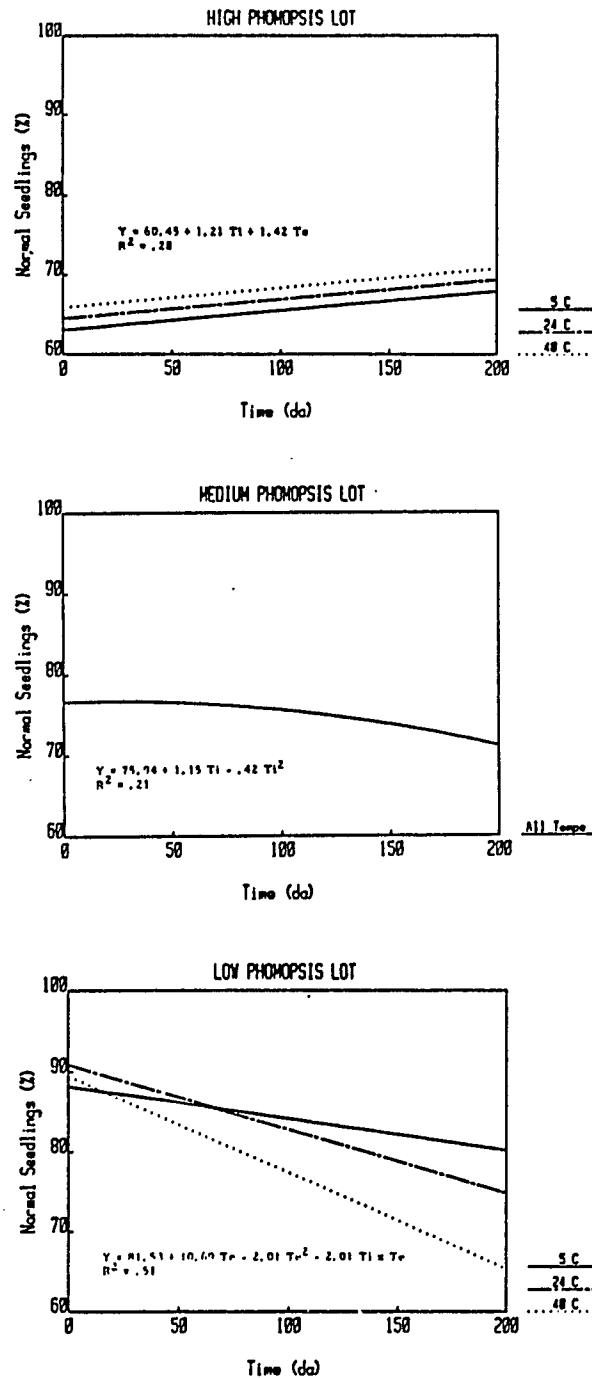


Fig. 14. Stepwise regression of percent normal seedlings produced with time at three temperatures (5, 24, 40C) for 12 seed lots with different initial levels of *Phomopsis longicolla* infection. Upper: High (21%-30%) *Phomopsis* lot. Middle: Medium (11-20%) *Phomopsis* lot. Lower: Low (0-10%) *Phomopsis* lot.

the seed stored at 40 C (Table 5).

A general decline in the percent normal seedlings was predicted by regression for the medium Phomopsis lot (Fig. 14). Storage temperature was not involved in the relationship. The observed means generally agree with the model, although, again, seed stored at 40 C appeared to pass through a plateau stage and then decline (Table 5).

Seed of the low Phomopsis lot produced fewer normal seedlings as storage time increased at all three temperatures (Table 5; Fig. 14). The decline was faster in seed stored at 40 C than in those at 24 C, and also faster in seed stored at 24 C than in seed at 5 C.

The percentage of abnormal seedlings produced increased in all three lots as storage time increased (Table 6; Fig. 15). The increase was fastest when seed were stored at 40 C and slowest when they were stored at 5 C, regardless of the initial Phomopsis infection level.

For the high Phomopsis lot, dead seedlings decreased with time at all three storage temperatures (Table 7; Fig. 16). The decline was more rapid, but ultimately not too much greater, when seed were stored at 40 C than when at either 24 or 5 C.

The percentage of dead seedlings produced by seeds of the medium Phomopsis lot declined slightly as time increased when the seed were stored at 24 or 40 C, but not when they were stored at 5 C (Table 7). The decline did not, however,

Table 6. The effect of storage temperature and time on the percent of abnormal seedlings produced by soybean seeds naturally-infected by Phomopsis longicolla.

Storage Temperature (c)	Storage Time (DA)	Lot <sup>a</sup>					
		High		Medium		Low	
		% <sup>b</sup>	Transformed <sup>b,c</sup>	%	Transformed	%	Transformed
5	0	9.1	16.4	9.6	17.7	6.8	14.1
	50	10.3	18.2	12.8	20.4	13.4	20.0
	100	11.5	19.5	12.9	20.3	10.8	18.1
	150	11.3	18.9	11.8	19.2	10.4	18.3
	200	14.8	22.2	13.0	20.8	12.9	20.7
24	0	6.8	14.3	9.0	16.2	7.4	15.4
	50	10.4	18.4	10.6	18.6	12.1	19.4
	100	11.3	19.3	8.9	17.1	11.9	19.4
	150	10.4	18.4	10.1	18.1	11.5	19.5
	200	15.4	22.8	14.9	22.3	19.1	25.7
40	0	9.4	17.5	11.1	19.2	10.5	18.5
	50	11.6	19.4	11.4	19.5	11.3	19.0
	100	14.4	21.8	12.3	20.0	13.6	21.3
	150	22.0	27.8	21.5	27.4	24.1	29.2
	200	27.5	31.4	25.8	30.4	30.4	33.4
FLSD (0.05)		4.2	3.8	3.7	3.2	3.7	3.7

<sup>a</sup> Based on initial P. longicolla levels in the seed lot. High = 21-30%; Medium = 11-20%; low = 0-10%.

<sup>b</sup> Mean of 4 replications of 4 cultivars each.

<sup>c</sup>  $\sin^{-1} (p_i)^{0.5}$ ; where  $p_i$  = proportion of seeds from which P. longicolla was recovered.



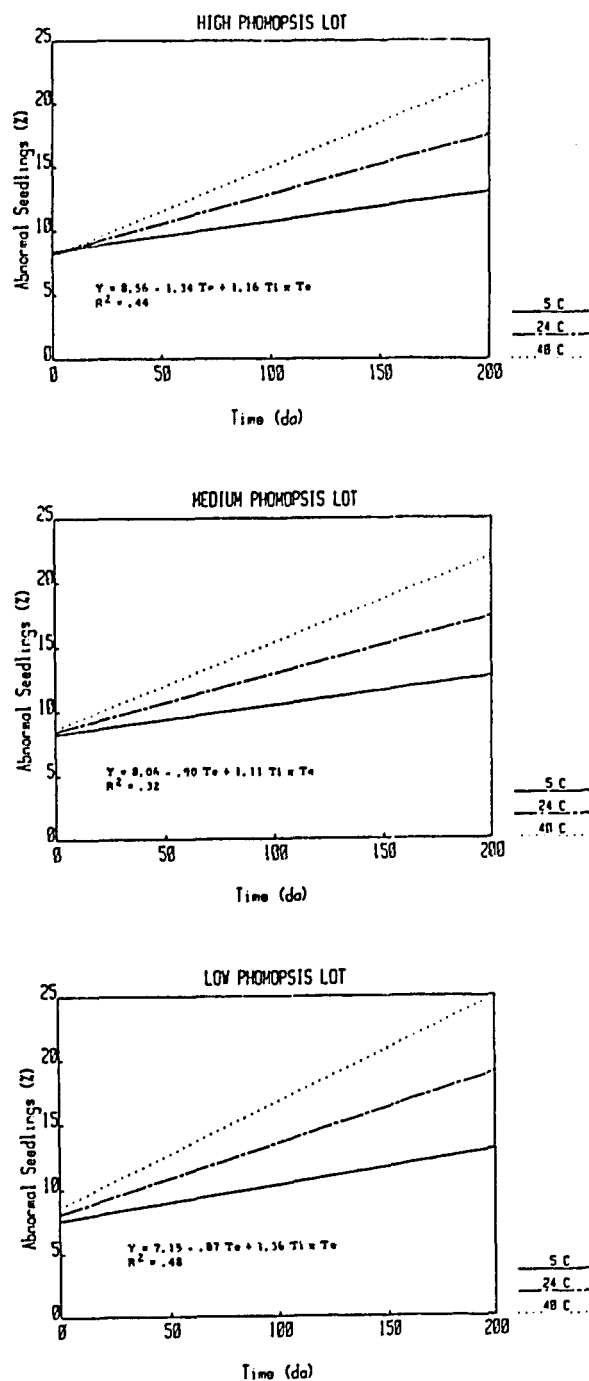


Fig. 15. Stepwise regression of percent abnormal seedlings produced with time at three temperatures (5, 24, 40°C) for 12 seed lots with different initial levels of *Phomopsis longicolla* infection. Upper: High (21%-30%) *Phomopsis* lot. Middle: Medium (11-20%) *Phomopsis* lot. Lower: Low (0-10%) *Phomopsis* lot.

Table 7. The effect of storage temperature and time on the percent of dead seedlings produced by soybean seeds naturally-infected by *Phomopsis longicolla*.

Storage Temperature(c)	Storage Time(DA)	Lot <sup>a</sup>					
		High		Medium		Low	
		% <sup>b</sup>	Transformed <sup>b,c</sup>	%	Transformed	%	Transformed
5	0	31.5	34.0	17.6	24.2	4.1	9.7
	50	31.0	33.3	18.0	24.3	3.6	7.3
	100	24.0	28.9	16.1	23.2	4.0	10.2
	150	22.0	27.6	17.8	24.4	5.9	13.4
	200	16.8	23.7	14.9	22.1	7.1	15.0
24	0	34.6	35.9	16.9	23.1	4.0	8.7
	50	29.1	32.3	14.8	22.2	2.9	7.2
	100	21.6	27.2	15.0	22.4	4.8	10.5
	150	16.6	23.8	11.0	18.9	4.6	9.5
	200	11.9	19.9	9.4	17.3	7.5	15.3
40	0	27.8	31.4	15.3	22.0	2.8	7.9
	50	19.0	25.4	10.8	17.3	3.1	8.9
	100	13.5	21.3	8.0	16.1	3.4	9.3
	150	8.3	16.3	6.9	13.4	4.5	10.2
	200	10.1	18.2	7.9	15.2	9.3	16.9
FLSD (0.05)		4.3	3.2	3.6	3.1	NS <sup>d</sup>	NS

<sup>a</sup> Based on initial *P. longicolla* levels in the seed lot. High = 21-30%; Medium = 11-20%; low = 0-10%.

<sup>b</sup> Mean of 4 replications of 4 cultivars each.

<sup>c</sup>  $\sin^{-1} (p_i)^{0.5}$ ; where  $p_i$  = proportion of seeds from which *P. longicolla* was recovered.

<sup>d</sup> Not significant at  $p = 0.05$

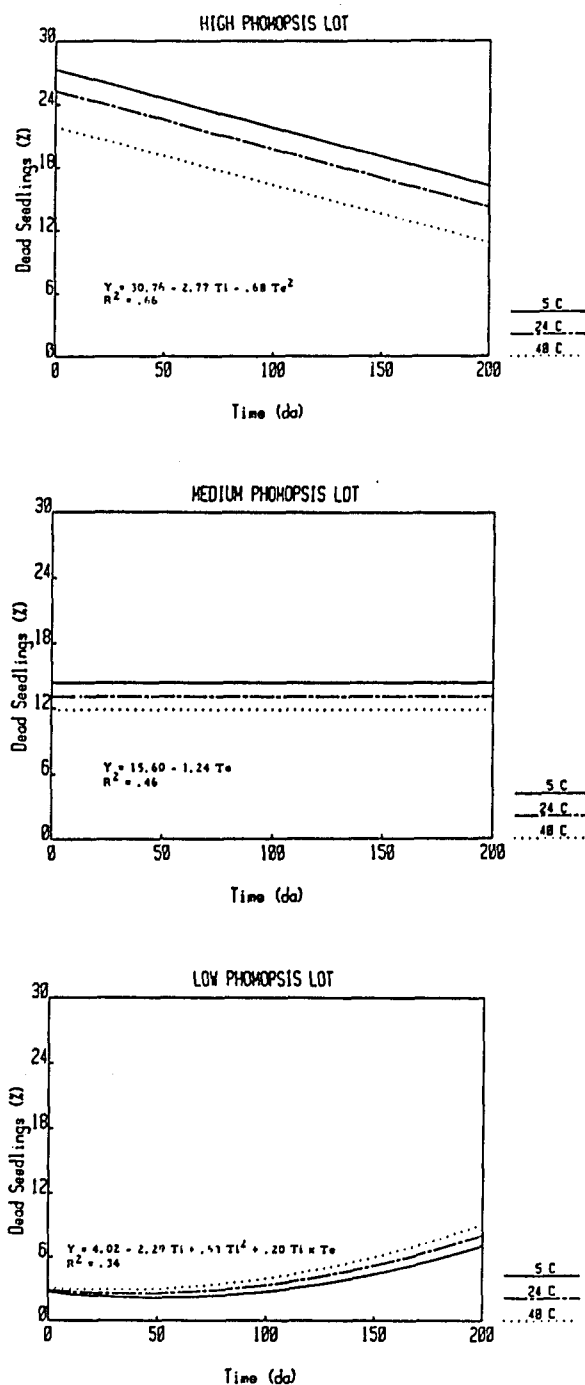


Fig. 16. Stepwise regression of percent dead seedlings produced with time at three temperatures (5, 24, 40C) for 12 seed lots with different initial levels of *Phomopsis longicolla* infection. Upper: High (21%-30%) *Phomopsis* lot. Middle: Medium (11-20%) *Phomopsis* lot. Lower: Low (0-10%) *Phomopsis* lot.

affect the relationship predicted by regression (Fig. 16).

The low Phomopsis lot produced similar percentages of dead seedlings regardless of time or temperature (Table 7). Regression, however, predicted a slight increase at all storage temperatures as storage time increased (Fig. 16).

The percentages of fungi other than Phomopsis present in the lots were always low (<10%) and followed trends similar to those already discussed for that pathogen. Therefore, these data will not be presented.

Other germination components were examined in the modified test, but were found to be of lesser significance than those already discussed. These components were: percent 4-day germination (= % normal seedlings after 4 days); percent total seedlings (= % normal + % abnormal seedlings); and percent hard seed. The 4-day germination trends were essentially similar to those found for percent normal seedlings. Trends found for total seedlings were intermediate between those for normal seedlings and those for abnormal seedlings, and opposite of those for dead seedlings. Hard seed were virtually absent from these seed lots.

Vigor components studied with the modified test were not found to have strong relationships to percent Phomopsis infection. In the seedling classification test, which categorizes normal seedlings as strong and weak based on seedling morphology and size, both categories were found to

have trends similar to those already discussed for normal seedlings, but the coefficients of determination were lower. Speed of germination ( $= [\% \text{ 4-day germination}/4] + [\% \text{ normal seedlings}/7]$ ) also followed the trends found for normal seedlings, but again the coefficients of determination were low. Average dry weight of normal seedlings, used in this study to determine the seedling growth rate, was essentially the same throughout this test, although there was a significant increase of about 6 mg in the seeds stored at 5 C from time zero to 50 days. Phomopsis levels correlated very poorly with this vigor component ( $r = -0.10$  overall, significant at  $p = 0.01$ ).

Moisture content of the seed was independent of the level of Phomopsis infection; therefore, results for the three lots were combined during statistical analysis (Table 8). Moisture content of seed stored at 5 C increased, and that of seed stored at 40 C decreased, by the end of the study. Moisture content of the seed stored at 24 C remained unchanged.

#### DISCUSSION

Numerous studies have indicated a decline or inactivation of seedborne pathogens during storage (136). The present work has shown that recovery of P. longicolla from soybean seed decreased as storage time increased and that a concomitant rise in germination percentage also

Table 8. The effect of storage time and temperature on moisture content of soybean seeds.

Storage Temperature(c)	Storage Time(DA)	Moisture Content (%) <sup>a</sup>
5	0	6.7
	200	7.2
24	0	6.7
	200	6.6
40	0	6.7
	200	3.8
FLSD (0.05)		0.2

<sup>a</sup> Mean of 4 replications of 12 cultivars each.

occurred, as has been noted for SSD pathogens in general (68, 198, 199, 236). The mechanism for this phenomenon has not been investigated, but the effect was augmented in this study by storing the seed at 40 C. However, the pathogen was never entirely eliminated at that temperature, even in seeds where Phomopsis was initially low. Furthermore, continued treatment was detrimental to seed quality. It is interesting to note that germination improved even when Phomopsis levels were below 25%, usually considered the level at which damage by SSD pathogens occurs (137).

Soybeans are usually considered to be of low quality if germination is below 80%. Although germination of lots with appreciable levels of Phomopsis (>10%) improved when seed were stored at 24 or 40 C, it was never greater than 80% at any time. The improvement in germination was not as dramatic as that noted by some (68, 236), whereas others have indicated similar results (198, 199). The improvement in germination in the current work was also not as marked as that obtained with fungicide seed treatments, where 20% improvement in germination has often been reported (30, 58, 235, 236). No tests were performed to determine if fungicides would improve germination of seed after heat treatment.

The greatest improvement of germination in lots with >10% Phomopsis occurred when seed were stored at 24 or 40 C for 100-150 days. Van Toai (232) found that germination of

soybean seeds stored under ambient conditions improved after three months. These results imply that soybean seed testing, often made soon after harvest (112, 113), should be delayed at least 100 days after harvest, and may partially explain the variable results encountered in the literature. Also, germination in the present study was best when low Phomopsis lots were stored at 5 C. Based on this work, the best method for handling Phomopsis-infected seed would be to eliminate Phomopsis with a heat treatment and then store Phomopsis-free seed at 5 C. More research will be necessary to optimize such a procedure.



### CHAPTER III

## Potential for the Use of Resident Seedborne Fungi of Soybean to Control Phomopsis longicolla and Improve Seed Quality

### INTRODUCTION

Soybean (Glycine max [L.] Merr.) seed can be protected from decay due to Diaporthe and Phomopsis species by using a physical barrier (11), or using chemical (58, 59, 206) or biological (166) seed or plant treatments. The physical barrier method used by Athow and Laviolette (11) may have usefulness in research experiments, but is too expensive and laborious for commercial application. Foliar fungicide treatments can result in the development of fungicide-resistant pathogens (14). Resistance to benomyl (methyl - 1 - [butylcarbamoyl] - 2 - benzimidazolecarbamate), a fungicide commonly used to control Diaporthe and Phomopsis diseases on soybean, has been reported for various fungi (27, 125). The fungicidal activity of benomyl also decreases as time after application increases (62). Also, fungicide-treated seed cannot be used for any purpose other than planting. For these reasons, fungal and bacterial antagonists have been investigated for their biological

control capabilities as either spray or seed treatments for soybean (45, 110, 166, 195, 196, 251).

Several studies have been conducted to evaluate the effects of seed treatment with microorganisms on soybean. Kommedahl and co-workers (110, 195, 196) reported variable results using fungal and bacterial seed treatments. Stand and yield of the soybeans treated were 65-182% and 50-124%, respectively, of those of the untreated control (110). Seeds treated with a fungicide in the same test gave 134% of the stand and 75-125% of the yield when compared to the untreated soybeans. Datnoff et al (45) examined the effectiveness of five fungal seed treatments and found that all five treatments decreased soybean emergence in comparison to nontreated seed. One treatment also significantly decreased yield. Yeh and Sinclair (251) examined the effect of Chaetomium cupreum Ames on several seedborne pathogens, including Phomopsis sp. sensu Kmetz et al (106, 197) (= P. longicolla Hobbs). They found that the former organism was very antagonistic to the pathogens, apparently producing a diffusible toxin which inhibited colony growth of the pathogens in dual cultures. Unfortunately, culture filtrates of the antagonist also inhibited germination of soybean seed.

The use of Cercospora kikuchii (Mats. & Tom.) Gardner by Roy and Abney (166) was the first demonstration that infection by Diaporthe and Phomopsis species pathogenic on

soybean could be controlled effectively with spray applications of an antagonistic fungus. However, C. kikuchii has been shown to reduce germination and increase the number of abnormal seedlings produced by soybean seeds (77, 247, 252).

Various other studies have demonstrated the effectiveness of biologic agents for the control of both aerial and soilborne pathogens (see [14] and [37] for examples). Among the fungal antagonists used in these studies were species of Alternaria, Aspergillus, Chaetomium, Cladosporium, Epicoccum, and Trichoderma. These anamorph genera are also represented in the seed and phylloplane mycota of soybean (52, 102, 103, 104, 123, 216), and generally are not considered to be pathogenic to seed (123).

The present study was undertaken to determine if isolates of the above genera, obtained from soybean seed, were antagonistic in vitro to P. longicolla (hereafter referred to as Phomopsis), the primary soybean seed decay pathogen found in Ohio. Experiments were also made to determine the effectiveness of these fungi as seed treatments to mollify the negative effects of Phomopsis on seed quality or as foliar sprays to prevent seed infection by Phomopsis.

## MATERIALS AND METHODS

### Culture Information.

The various isolates used in these experiments were identified as Alternaria alternata (Fr.) Keissler, Aspergillus niger Van Tieghem, Chaetomium globosum Kunze, Cladosporium herbarum (Pers.) Link, Epicoccum nigrum Link, Penicillium verrucosum Dierckx var. cyclopium (Westling) Samson, Stock, & Hadlok, and Trichoderma harzianum Rafai. Hereafter, these will be referred to only by the genus names. All isolates were obtained from soybean seeds in 1978, except for one isolate of Chaetomium obtained from D.C. McGee (Iowa) that year. Stock cultures were maintained on Difco potato dextrose agar (PDA) at 5 C. Isolates used in seed treatment or foliar spray experiments were grown on PDA plates for two weeks under intermittent fluorescent light (about 12 hr daily) at 22-25 C on a laboratory bench.

### Antagonism In Vitro.

Inhibition of Phomopsis by test antagonists was evaluated in dual culture on PDA plates using a modification of the method of Royce and Ries (1978). Mycelial plugs, 5 mm in diameter, were obtained from the periphery of 4-day old PDA cultures of test antagonists in the genera Alternaria, Chaetomium, Cladosporium, Epicoccum, Penicillium, and Trichoderma. One plug was then placed at the periphery of each of four replicate PDA plates and these plates incubated in the dark at 25 C for 2 days. At that

time, 5 mm plugs were taken from the periphery of 4-day old PDA cultures of Phomopsis and placed 5 cm from the initial test antagonist plug. The cultures were then incubated a further 7 days in the dark. At the end of this incubation period, the zone of inhibition between the test antagonist and the Phomopsis colonies was measured, and the percent inhibition of pathogen growth (PIPG) determined. PIPG was calculated as  $[(r_1 + 1) - (r_s)] / (r_1 + 1) \times 100$ , where  $r_1$  and  $r_s$  are the longest and shortest distance, respectively, from the Phomopsis plug to the edge of the Phomopsis colony. The test was performed three times.

#### Seed Treatments.

a) Laboratory tests for germination and vigor. A modification of the standard rolled towel test (9) was used to assess the effects of test antagonists in the genera Alternaria, Chaetomium, Epicoccum, and Trichoderma on germination and vigor of a soybean seed lot (cultivar Wells) that had about 30% Phomopsis. Test antagonists were incubated as already described. At the end of the incubation period, one half of the cultures of each test antagonist were exposed to propylene oxide (0.5 ml per plate) in a sealed container for 24 hr to kill the spores. Spore suspensions of each isolate were then prepared from both living and dead cultures by flooding the PDA plates with sterile distilled water and gently scraping the surface with a rubber policeman. The number of spores was estimated

with the aid of a haemocytometer, and the suspensions made so that the final concentrations were  $1.5 \times 10^5$  spores per ml in 2% sodium carboxymethylcellulose (W/V) (cellulose gum, type 7MF, Hercules Inc., Wilmington, DE 19899) (hereafter, CMC) each. This concentration was chosen because of limitations in spore numbers of one test antagonist (Alternaria). For each seed treatment, 1 ml of suspension was applied per 50 gm of seed. Besides the dead spores, other controls included 2% CMC alone and untreated seed. Fifty seed were treated for each of four replications. The germination and vigor components measured using this test included percent germination after 4 days, and the following components, all determined after 7 days: percentages of strong normal and weak normal seedlings, total normal seedling, abnormal seedlings, dead seedlings, and ungerminated seeds. The average normal seedling length to the nearest mm was also determined at 7 days. Average normal seedling dry weight in mg was determined after drying the seedlings for 24 hrs at 40 C. The experiment was repeated once.

b) Emergence in sand. Emergence of antagonist-treated seed was examined in a greenhouse study using a randomized complete block design. Test antagonists in the genera Chaetomium, Epicoccum, and Trichoderma were incubated as before, and spore suspensions of each organism, containing  $5.0 \times 10^4$ ,  $9.0 \times 10^4$ , and  $2.0 \times 10^4$  spores per ml, prepared

in sterile distilled water. Seed of the 'Wells' seed lot previously described were planted in sterile sand (hammermilled, washed sandstone) in plastic pots (20 cm in diameter; 1 pot per treatment, 25 seed per.pot). Uniform depth and spacing of seeds in the pots were maintained with a dibble board. For fungal seed treatments, 1 ml of the appropriate suspension was pipeted onto each seed at planting. Captan 80% WP (N-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide) (hereafter, Captan 80) and Vitavax 200 flowable (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide plus tetramethylthiuram disulfide) (hereafter, Vitavax 200) were additional seed treatments. Both fungicides were applied to the seed prior to planting at a rate of 1 gm of formulation to 200 gm of seed. Control seeds were treated with 1 ml of sterile distilled water at planting. The treatments were replicated four times.

Emergence was determined 10 days after planting. Seedlings that emerged were classified as either strong or weak. Strong seedlings were those with two healthy cotyledons; weak seedlings had only one cotyledon, or had necrotic lesions on at least one cotyledon.

c)Field emergence and yield. Eight Epicoccum isolates were incubated as previously described and used in a field trial in 1981. Seed treatments consisted of living and dead spore suspensions prepared in 2% CMC as before, except that spore concentrations were  $2.0 \times 10^5$ . Additional living and

dead spore suspensions of  $1.0 \times 10^5$  and  $3.0 \times 10^6$  were prepared for one and two isolates, respectively. Captan 80 and Vitavax 200, applied as previously noted, served as fungicide standards. Seed treated with 2% CMC alone and untreated seed were controls. Two hundred 'Wells' seeds that had about 25% Phomopsis were used per treatment, and the treatments replicated five times in a randomized complete block design. Seedling emergence was determined 20 days after planting. The plants were harvested in the fall and the seed weighed. Yields were calculated on a dry weight basis.

#### Prophylactic Foliar Spray Treatments.

Spore suspensions prepared in sterile distilled water were applied to soybean plants at various growth stages in 1979, 1980, and 1981 in order to determine if Phomopsis seed infection could be prevented by colonization of the seed by nonpathogens. In each year, test antagonists were grown as already described and spore suspensions prepared by comminuting cultures of the appropriate organism in 500 ml of sterile distilled water for 30 sec in a Waring blender at low speed, and then filtering each suspension through a sterile nylon mesh screen (125  $\mu$ m mesh opening) (Tetko Inc., Elmsford, NY 10523) to remove mycelial fragments and agar. The final suspensions consisted of  $1.0 \times 10^6$  in 1979 and 1980, and  $1.0 \times 10^8$  spores per ml in 1981. Spray treatments were applied to run-off using a Hudson hand-



sprayer in 1979 and a low pressure (20 psi) carbon dioxide sprayer in 1980 and 1981. All treatments were applied late in the afternoon. A randomized complete block design was used each year. Individual experiment treatments for each year are discussed below.

Following harvest each year, 100 seeds per treatment were surface-sterilized in 1.05% sodium hypochlorite for 1 min and plated on PDA acidified to pH 4.5 with 85% lactic acid after autoclaving. Following a 2 wk incubation period on a laboratory bench under intermittent fluorescent light (about 12 hr daily) at 22-25 C, the incidences of Phomopsis and other seedborne mycota were determined. In 1980 and 1981, germination was estimated from the seed plated on PDA using the method of Nicholson et al (137).

a) 1979. Factors examined in 1979 included: 1) Time of application (R3, R4, R5, R6, and R7; based on the stage of development descriptions for soybean by Fehr et al [67]); 2) Spray treatment (Alternaria, Chaetomium, Phomopsis, Alternaria + Phomopsis, Chaetomium + Phomopsis, Alternaria + Chaetomium + Phomopsis, and a sterile water control); and 3) Harvest date (at maturity and maturity + 1 mo). Treated rows were bordered on each side by untreated guard rows. Treatments were replicated six times.

b) 1980. Factors examined in 1980 included: 1) Time of application (R4, R7, and R4 + R7); and 2) Spray treatment (Alternaria, Aspergillus, Chaetomium [2 isolates],

Cladosporium, Epicoccum [2 isolates], Phomopsis, Trichoderma, and a sterile water control). Adjacent treatments were protected from different sprays with a plastic tarp during spray applications. The treatments were replicated five times. In addition to testing for seedborne mycota, 100 seeds from each treatment were sealed in polypropylene bags and stored at 5 C until the next spring. They were then planted using the same experimental design and percent emergence determined after 20 days.

c) 1981. Spray treatments in 1981 were applied at R4 and again two weeks later. Fungal treatments consisted of living and dead spore suspensions of Chaetomium and Epicoccum, prepared as described earlier. Benomyl, applied each time at a rate of 113.4 gm per acre, was included as a fungicide standard. Sterile distilled water and untreated controls were also included in the test. Adjacent treatments were again protected from each other by a plastic tarp during spray application. The treatments were replicated twice within each of eight blocks.

## RESULTS

### Antagonism In Vitro.

Zones of inhibition formed between Phomopsis colonies and colonies of either Chaetomium or Epicoccum, whereas isolates of Alternaria, Cladosporium, Penicillium, and Trichoderma were not antagonistic to Phomopsis by this test

(Table 9). Isolates of Epicoccum produced significantly larger zones of inhibition than those obtained from any other test antagonist.

Trichoderma completely inhibited colony growth of Phomopsis in dual cultures (Table 10). Alternaria, Chaetomium, and Epicoccum inhibited slightly more or less than 50% of Phomopsis colony growth.

#### Seed Treatments.

a) Laboratory tests for germination and vigor. Germination at four days, strong normal seedlings, abnormal seedlings, and ungerminated seeds, average normal seedling length and dry weight all were affected by seed treatments with test antagonists (Table 11). The percentages of total normal, weak, and dead seedlings were not affected by any of the seed treatments in the two trials.

Viable Trichoderma significantly increased germination at four days, but also increased abnormal seedling and decreased average normal seedling length and dry weight compared to the controls (Table 11). In contrast, viable Epicoccum significantly decreased the percentage of abnormal seedlings, and increased the percentage of strong normal seedlings and the average length and dry weight of the normal seedlings. Only the viable Alternaria, Epicoccum, and 2% CMC treatments did not significantly increase the percentage of ungerminated seeds over that of untreated seeds.

Table 9. Zones of inhibition between test antagonists and *Phomopsis longicolla* in dual cultures.

Test antagonist	Isolate	Zone of inhibition (mm)			
		Trial <sup>a</sup>			Overall <sup>b</sup> Mean
		1	2	3	
Alternaria	A10	0.0	0.0	0.5	0.2
Alternaria	A32	0.0	1.0	1.5	0.8
Chaetomium	C25	0.0	0.0	0.0	0.0
Chaetomium	C103	1.9	2.8	2.4	2.3
Chaetomium	C104	2.3	0.5	0.0	0.9
Cladosporium	C99	0.0	0.0	0.0	0.0
Epicoccum	E11	9.6	9.5	10.4	9.8
Epicoccum	E18	9.0	7.8	9.9	8.9
Epicoccum	E20	8.5	8.1	9.5	8.7
Penicillium	P76	0.0	0.0	-- <sup>c</sup>	0.0
Trichoderma	T2	0.0	0.0	0.0	0.0
FLSD (0.05)		1.1	1.5	1.9	0.9

<sup>a</sup> Mean of 4 observations.

<sup>b</sup> Mean of 12 observations, except for isolate P76 = mean of 8 observations.

<sup>c</sup> Contamination precluded measurement.

Table 10. Percent inhibition of colony growth of Phomopsis longicolla by test antagonists in dual cultures.

Test antagonist	Isolate	Inhibition of colony growth (%) <sup>a</sup>			Overall <sup>c</sup> Mean
		Trial <sup>b</sup>			
		1	2	3	
Alternaria	A10	55.6	54.3	46.3	52.1
Alternaria	A32	51.8	49.0	44.6	48.5
Chaetomium	C25	60.0	57.4	55.5	57.6
Chaetomium	C103	40.3	41.2	37.4	39.6
Chaetomium	C104	58.0	56.4	55.4	56.6
Cladosporium	C99	43.7	45.7	42.0	43.8
Epicoccum	E11	52.4	55.3	45.6	51.1
Epicoccum	E18	56.0	52.6	48.7	52.5
Epicoccum	E20	54.0	48.0	41.9	48.0
Penicillium	P76	44.0	41.8	-- <sup>d</sup>	42.8
Trichoderma	T2	100.0	100.0	100.0	100.0
FLSD (0.05)		2.8	4.6	9.3	4.2

- a Calculated as  $[(r_1 + 1) - r_s] / (r_1 + 1) \times 100$ , where  $r_1$  and  $r_s$  are the longest and shortest radii, resp., from its inoculation point to the edge of the Phomopsis colony.
- b Mean of 4 observations.
- c Mean of 12 observations, except isolate P76 = mean of 8 observations.
- d Contamination precluded calculation.

Table 11. Effects of seed treatments on germination and vigor components of a soybean seed lot with 30% natural *Phomopsis longicolla* infection.

Component measured	Treatment (isolate)										FLSD (0.05)
	(A32) Alternaria		(C104) Chaetomium		(E11) Epicoccum		(T2) Trichoderma		2% CMC <sup>a</sup>	None	
	viability										
	+ <sup>b</sup>	-	+	-	+	-	+	-			
% 4-day germination	50.0 <sup>c</sup>	47.0	45.5	54.0	52.5	48.5	38.5	56.5	44.0	46.0	10.4
% strong normal seedlings	36.0	36.5	35.5	40.5	49.5	43.0	15.5	38.0	36.5	38.0	9.5
% abnormal seedlings	11.5	8.0	10.5	12.0	6.0	7.5	22.5	12.5	13.0	14.0	6.6
% underdetermined seeds	6.0	7.0	11.5	8.5	3.5	9.0	6.5	7.0	5.5	1.0	5.3
Average normal seedling length (mm)	280.0	278.3	292.0	294.0	316.3	269.0	213.3	291.5	262.5	283.0	26.2
Average normal seedling dry weight (mg)	35.8	35.8	37.0	36.3	41.5	38.8	30.3	36.0	37.8	39.0	2.7

<sup>a</sup> Sodium carboxymethylcellulose.

<sup>b</sup> + = living spores; - = spores killed by exposure to propylene oxide.

<sup>c</sup> Mean of 8 replications of 50 seed each.

b) Emergence in sand. Treatment of seeds with Vitavax 200 resulted in significantly higher emergence and strong seedling percentages than those found for the control seeds (Table 12). Treatment of seeds with Captan 80 was as good as treatment with Vitavax 200, but not better than any other treatment. The percentage of weak seedlings found was unaffected by seed treatment. Fungal spore seed treatments, irrespective of rate, did not influence the level of any component measured in this test.

c) Field emergence and yield. Results for field emergence were similar to those found in the greenhouse for emergence in sand (Table 13). More of the seed treated with Vitavax 200 emerged than did those of any other treatment. Treatment of seed with Captan 80 resulted in an emergence higher than that of 2% CMC-treated seed, but not better than untreated seed. Treatment of seeds with Epicoccum spores did not result in statistically different emergence compared to the 2% CMC-treated seed or untreated seed. One dead Epicoccum spore treatment significantly reduced yield.

#### Prophylactic Foliar Spray Treatments.

a) 1979. Application of fungal spores to plants in the field did not significantly change the incidences of the different seedborne fungi isolated in 1979. Both the time of application and harvest date, however, significantly influenced seedborne fungal incidences. Most of the fungal populations examined followed trends similar to those found

Table 12. Effect of seed treatments on emergence in sand of a soybean seed lot with 30% natural *Phomopsis longicolla* infection.

Treatment	Isolate	Rate <sup>b</sup>	Total Seedling Emergence		Strong Seedlings <sup>a</sup>		Weak Seedlings <sup>a</sup>	
			% <sup>c</sup>	trans. <sup>cd</sup>	%	trans.	%	trans.
Chaetomium	C25	1	63.0	52.9	48.0	43.9	15.0	22.5
		2	58.0	49.6	43.0	40.9	15.0	22.5
		3	53.0	46.8	44.0	41.5	9.0	17.1
	C104	1	55.0	47.9	44.0	41.5	11.0	18.9
		2	62.0	52.0	49.0	44.4	13.0	20.9
		3	63.0	52.6	53.0	46.8	10.0	16.0
Epicoccum	E11	1	62.0	52.0	52.0	46.2	10.0	17.9
		2	52.0	46.3	45.0	42.1	7.0	14.9
		3	56.0	48.6	51.0	45.6	5.0	11.1
	E18	1	63.0	52.7	56.0	48.5	7.0	15.2
		2	57.0	49.1	48.0	43.8	9.0	17.0
		3	54.0	47.3	49.0	44.4	5.0	11.1
	E20	1	57.0	49.1	52.0	46.2	5.0	12.7
		2	55.0	48.0	47.0	43.3	8.0	16.2
		3	61.0	51.4	55.0	47.3	7.0	14.9
Trichoderma	T2	1	56.0	48.5	45.0	42.1	11.0	19.3
		2	54.0	47.4	44.0	41.5	10.0	18.2
		3	64.0	53.2	55.0	47.9	9.0	14.7
Captan 80		4	72.0	58.5	64.0	53.3	8.0	14.2
Vitavax 200		4	82.0	65.1	75.0	60.2	7.0	12.9
Sterile water		—	55.0	47.9	45.0	42.1	10.0	17.7
FLSD (0.05)			14.3	8.5	13.9	8.2	NS <sup>e</sup>	NS

- a Strong = seedling with two healthy cotyledons; weak = seedling with one cotyledon or necrotic lesions on at least one cotyledon.
- b 1 =  $2.0 \times 10^5$ ; 2 =  $9.0 \times 10^4$ ; and 3 =  $1.0 \times 10^4$  spores/ml; 4 = 1 g formulation/200 g seed.
- c Mean of 4 replications of 25 seed each.
- d  $\sin^{-1}(p_i)^{0.5}$ , where  $p_i$  is the proportion of emergent, strong emergent, or weak emergent seeds.
- e Not significant at  $p = 0.05$ .



Table 13. Effect of seed treatments on field emergence and yield of a soybean seed lot with 25% natural *Phomopsis longicolla* infection.

Treatment	Isolate	Viability <sup>d</sup>	Rate <sup>c</sup>	Emergence <sup>a</sup>		Yield <sup>e</sup> (gm dry weight)
				%	trans. <sup>d</sup>	
Epicoccum	E1	+	2	71.4	57.9	376.5
		-	2	69.6	56.8	300.4
	E2	+	2	69.8	56.7	320.2
		-	2	69.4	56.5	257.5
	E4	+	2	69.6	56.7	258.7
		-	2	65.8	54.4	279.7
	E5	+	2	63.6	53.0	299.0
		-	2	63.6	53.0	247.4
	E6	+	2	64.6	53.6	297.7
		-	2	64.0	53.2	298.7
	E7	+	2	64.2	53.4	336.4
		-	2	67.6	55.4	326.3
	E10	+	2	65.6	58.0	354.0
		-	2	59.4	57.0	325.9
	E11	+	3	71.8	54.2	262.8
		-	3	70.2	50.6	234.3
		+	1	63.6	58.9	278.2
		-	1	70.4	58.4	268.0
		+	2	70.8	57.7	312.9
		-	2	63.4	52.9	321.3
		+	3	73.0	53.0	298.1
		-	3	63.4	57.2	325.5
Captan 80			4	73.8	59.7	334.1
Vitavax 200			4	83.8	66.6	332.3
2 % CMC ef			—	63.2	52.8	259.3
None			—	68.2	55.8	346.4
FLSD (.05)				9.9	6.2	NS <sup>g</sup>

a Mean of 5 replications of 200 seed each.

b + = living spores; - = spores killed by exposure to propylene oxide.

c 1 =  $1.0 \times 10^5$ ; 2 =  $2.0 \times 10^5$ ; and 3 =  $3.0 \times 10^6$  spores/ml; 4 = 1 g formulation/200 g seed.

d  $\sin^{-1} (p_i)^{0.5}$ , where  $p_i$  is the proportion of germination seeds.

e Mean of 5 replications.

f Sodium carboxymethylcellulose.

g Not significant at  $p = 0.05$ .

for Phomopsis, which are discussed below, but the incidences were not as high.

Phomopsis incidence in seeds was significantly higher when applications were made at R3 than when they were made at R4 (60.0 and 46.8%, respectively;  $LSD_{0.05} = 3.2$ ), or at R5, R6, and R7 (41.9, 43.4, and 42.2%, respectively). Phomopsis incidence was also higher when harvest was delayed for one month than when it was made promptly (60.1 and 25.1%, respectively;  $LSD_{0.05} = 1.5$ ). The interaction of application time and harvest date on Phomopsis incidence in seed is summarized in Table 14. Generally, Phomopsis incidence was higher when applications were made earlier. Delaying harvest resulted in increases in Phomopsis to levels that were statistically similar at all but the earliest application time (R3), which had the highest incidence of Phomopsis found.

b)1980. Neither spray treatments nor application time significantly influenced incidences of fungi in seed in 1980. However, germination was significantly higher when seed were from sprayed plants than from the sprayed control (Table 15). There were no statistical differences in germination between seeds from different spray treatments. When seed from this experiment were saved and planted the next spring, no statistical differences in field emergence were detected (range 36.2-55.4%, average 45.7%).

Table 14. Interaction of application time and harvest date on the incidence of *Phomopsis longicolla* in seed in 1979.

Harvest Date	Application Time <sup>b</sup>	Incidence of <i>Phomopsis</i> <sup>a</sup>	
		%	Transformed <sup>c</sup>
Maturity	R3	27.0	30.8
	R4	18.9	25.3
	R5	16.4	23.3
	R6	16.5	23.2
	R7	16.4	23.3
1-mo delay	R3	92.7	75.3
	R4	74.6	60.2
	R5	67.4	55.4
	R6	70.4	57.2
	R7	68.0	55.9
FLSD (0.05)		3.4	2.4

a Mean of 42 replications of 100 seed each.

b Based on stage of development descriptions by Fehr et al (64).

c  $\sin^{-1} (p_i)^{0.5}$ , where  $p_i$  is the proportion of infected seeds.

Table 15. Effect of spray treatments on germination of soybean seed in 1980.

Test antagonist	Isolate	Germination <sup>a,b</sup>	
		%	Transformed <sup>c</sup>
Alternaria	A10	45.9	42.6
Alternaria	A32	44.5	43.4
Chaetomium	C25	51.4	45.8
Chaetomium	C103	51.3	45.8
Chaetomium	C104	51.9	46.2
Cladosporium	C99	46.4	42.5
Epicoccum	E11	53.0	46.8
Epicoccum	E18	43.4	41.0
Epicoccum	E20	44.2	41.7
Penicillium	P76	46.6	42.5
Trichoderma	T2	31.4	32.9
FLSD (G.05)		12.3	7.7

a Mean of 15 replications of 100 seeds each.

b Based on the method of Nicholson et al (137).

c  $\sin^{-1} (p_i)^{0.5}$ , where  $p_i$  is the proportion of germinating seeds.

c)1981. Spray treatments applied in 1981 significantly influenced the incidences of Alternaria, Chaetomium, and Phomopsis found in seed that year (Table 16). Alternaria was significantly higher, and Phomopsis lower, in seeds from plants that had been sprayed with benomyl than in those from untreated plants. Chaetomium incidence was higher when seeds were from plants sprayed with either viable Chaetomium or sterile distilled water, than when they were from untreated plants. Germination of the seeds from the variously treated plants was statistically similar to that of seed from untreated plants after data were transformed.

#### DISCUSSION

"Internally seedborne pathogens...are unlikely to be controlled biologically other than through host resistance" (14). Such appears to be the case for Phomopsis. Although most of the test antagonists were inhibitory to Phomopsis in dual cultures, none of them improved germination of Phomopsis-infected seed, either in the laboratory or in the field. Epicoccum improved seed vigor compared to that of untreated seed in the modified rolled towel test, but was ineffective for improving either emergence or yield in other tests. Trichoderma, frequently used as a seed treatment to control soilborne diseases (14, 37, 110), was likewise ineffective against Phomopsis, and was detrimental to both seed germination and vigor as measured in the modified

Table 16. Effect of spray treatments on incidence of *Alternaria alternata*, *Chaetomium globosum*, and *Phomopsis longicolla* in seeds and on germination in 1981.

Treatment	Isolate	Viability <sup>c</sup>	Incidence of <i>Alternaria</i> <sup>a</sup>		Incidence of <i>Chaetomium</i>		Incidence of <i>Phomopsis</i>		Germination <sup>b</sup>	
			%	Trans. <sup>d</sup>	%	Trans.	%	Trans.	%	Trans.
Chaetomium	C25	+	2.6	25.2	2.9	28.4	4.2	34.7	92.8	88.4
		-	2.6	24.3	1.6	14.0	3.7	29.1	93.2	88.7
Epicoccum		+	1.8	18.3	1.8	16.1	4.6	32.4	92.6	89.1
		-	3.0	25.6	2.5	19.8	3.5	33.0	94.0	89.0
Benomyl			4.1	35.5	2.6	24.3	1.6	16.4	97.2	89.1
Sterile water			2.8	28.0	3.3	33.0	4.4	33.5	92.1	88.5
None			2.3	24.2	1.9	15.9	3.4	28.4	94.4	89.0
FLSD (0.05)			1.1	7.4	NS <sup>e</sup>	11.0	1.5	10.1	2.9	NS

<sup>a</sup> Mean of 16 replications of 100 seed each.

<sup>b</sup> Based on the method of Nicholson et al (137).

<sup>c</sup> + = living spores; - = spores killed by exposure to propylene oxide.

<sup>d</sup>  $\sin^{-1} (p_i)^{0.5}$ , where  $p_i$  is the proportion of seeds infected (for *Alternaria*, *Chaetomium*, and *Phomopsis*) or germinated.

<sup>e</sup> Not significant at  $p = 0.05$ .

rolled towel test.

Part of the present study was an attempt to increase resident mycota in pods or seeds in order to reduce Phomopsis seed infection. Resident mycota that were selected, however, usually were not increased in seeds by spray application of viable spores, and Phomopsis was not reduced. This may be due in part to the effect of weather after inoculation on infection by test antagonists. Early sprays made in 1979 were followed by 48 hours of dryness with a temperature range of 12-31 C (mean 21.6 C), which could have resulted in death of spores due to desiccation. Also, applications in other years may have been too late. Baker and Cook (14) indicate that one possible opportunity to control seedborne diseases is at the time of seed infection by airborne inocula. Since Phomopsis invades seed from infected pods and pods on lower plant parts can be infected as soon as they form (108), prophylactic sprays would have to begin as early as possible and continue through the season because pod development is not synchronous. Thirdly, these resident fungi may not be adapted to latent infection, as is Phomopsis (106, 107). Chaetomium incidence in seed did increase one out of three years, but no other test antagonist ever increased. In order for this approach to work, other antagonists better adapted to latent colonization of pods and seeds would have to be found. Fungi of this type, such as Colletotrichum

dematium (Pers.) Grove var. truncata (Schw.) Arx (218), are, unfortunately, usually pathogenic (233).

Some results reported here indicate a potential for the use of Epicoccum to improve the vigor of soybean seeds and thereby possibly minimize the detrimental effects of Phomopsis infection on seed quality. As far as can be determined, this is the first report of using Epicoccum as a seed treatment. Epicoccum has previously been successfully used as a biological treatment on tree pruning cuts to reduce damage due to canker pathogens (167). It should be noted, however, that the results of this research indicate that a combination of chemical sprays during the growing season and prompt harvest at maturity would give the best reduction of Phomopsis in seed. Fungicide seed treatments were also effective in improving stands from Phomopsis-infected seed. Therefore, the overall results from this study indicate that the general conclusion made by Baker and Cook (14), quoted above, is applicable to Phomopsis infection of soybean seed.



## GENERAL SUMMARY AND CONCLUSIONS

A new species of Phomopsis isolated from soybean was described. The species, P. longicolla, differs from P. sojae, the anamorph of Diaporthe phaseolorum var. sojae, in several morphological characteristics in culture. Conidiomata of the new species are produced in massive, effuse stromata and are markedly beaked. Phialidic alpha-conidia are produced on conidiophores that are frequently branched. Alpha-conidium length and width measurements for the two species overlapped, but the length-to-width ratios were always distinct.

As storage time increased, recovery of P. longicolla (hereafter, Phomopsis) from seeds decreased, indicating the pathogen died during storage. A concomitant rise in the germination percentage of the same seed lots suggested that Phomopsis contributed to decreases in germination in the laboratory test used. The simultaneous decline in Phomopsis and rise in germination was augmented by storing seed at 40 C, but the pathogen was never completely eliminated and continued treatment was detrimental to seed germination and vigor. Germination also improved, and Phomopsis decreased, in lots stored at 24 C, but improvement at either

temperature was never as good as that reported for fungicide-treated seed. Phomopsis and germination were fairly stable when seed were stored at 5 C, indicating that the best method for handling seed highly-infected by Phomopsis would be a short high temperature treatment to kill the pathogen, followed by low temperature storage of the same seed to maintain seed quality.

Studies designed to examine the effectiveness of resident seedborne mycota for control of Phomopsis in soybean showed that isolates in several different genera were antagonistic to Phomopsis in vitro. Epicoccum nigrum as a seed treatment improved vigor of Phomopsis-infected seed in the laboratory, but not in the field. Trichoderma harzianum was pathogenic to seed in laboratory tests.

Attempts were made to increase resident mycota in pods or seeds by spray applications with spores, but did not result in reduction of seedborne Phomopsis. It was concluded that a combination of chemical sprays during the growing season and prompt harvest at maturity would result in the least seedborne Phomopsis. Fungicide treatments of infected seed were also effective in improving stands. Biological treatments for the control of seedborne Phomopsis will only be feasible if organisms better adapted to either pod or seed colonization than Phomopsis can be found.

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APPENDIX A

Reports of Diaporthe and Phomopsis Species  
on Legume Hosts, Including Anamorphs, Teleomorphs,  
Homonyms, Synonyms, and Unnamed States.



Appendix A. Reports of *Diaporthe* and *Phomopsis* Species on Legume Hosts,  
Including Anamorphs, Teleomorphs, Homonyms,  
Synonyms, and Unnamed States.

<u>Diaporthe species<sup>a</sup></u>	<u>Legume Host(s)</u>	<u>Perithecia/Ostioles/Asci/ Ascospores (microns)<sup>b</sup></u>
acaciae Tilak 1966 (anamorph unknown)  ref. 219	Acacia arabica	330-420x225-350/—/64-88x6.4-8/ 14.4-17.6x4-5
aggerum Sacc. & Speg. in Sacc. 1878c (anamorph unknown; synonym of D. arctii)  ref. 172, 178, 221, 242	Lotus corniculatus	333 diam./—/50-60x7/14-16x3-4
amorphae Ellis & Everh. 1894 (anamorph unknown)  ref. 56, 109, 181, 242	Amorpha fruticosa Maackia amurensis var. buergeri	(320-560x240-320) (333-500) (300-500) diam./900-1100 long/(50-55x6-8) (55-80x7-9) (58-64.8-70x7.5-8.4-10)/ (8-10x3-4) (12-16[17]x3-5) (11-12.4-15x3.5-3.9-4.5)

<sup>a</sup> ? = possibly; ref. = reference[s].

<sup>b</sup> — = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<i>Diaporthe</i> species <sup>a</sup>	Legume Host(s)	<del>Perithecia/Ostioles/Asci/</del> <del>Ascospores (microns)</del> <sup>b</sup> Perithecia/Ostioles/Asci/ Ascospores (microns) <sup>b</sup>
arctii (Lasch)Nits. 1870 (teleomorph of <i>Phomopsis arctii</i> ; basionym for <i>D. aggerum</i> , <i>D. baptisiae</i> , <i>D. desmodiana</i> , <i>D. desmodii</i> , <i>D. meliloti</i> , <i>D. pratensis</i> , ? <i>D. psoraleae</i> - <i>bituminosae</i> , <i>D. tulasnei</i> , and <i>D. winteri</i> )  ref. 242	<i>Dorycnium pentaphyllum</i> (as <i>D. suffruticosum</i> )	(320-400x160-200) (280-480x160-320)/ —/(47-54x6-7) ([40] 47-60x7-10)/ (11-12x2.5-3.5) ([11] 12-15[17]x2.5-4)
baptisiae Rehm 1908 (anamorph unknown; synonym of <i>D. arctii</i> )  ref. 164, 193, 242	<i>Baptisia tinctoria</i>	(150 diam.) (240-320x160-240)/ (500 long) (160-200x120)/40-45x6-9/ (10-12x3.5) (10.5-12x2.5-3)
caraganae Jacz. 1895 (teleomorph of <i>Phomopsis caraganae</i> and <i>P. serebrianikowii</i> )  ref. 99, 181, 242	<i>Caragana arborescens</i>	320-800x320-640/180-240 diam./(80x12) (65-75x9-11)/(20x5-6) (14-19x3.5-5.5)

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Diaporthe species<sup>a</sup></u>	<u>Legume Host(s)</u>	<u>Perithecia/Ostioles/Asci/ Ascospores (microns)<sup>b</sup></u>
chrysoides (Tul.) Sacc. 1882 (anamorph unnamed; doubtful species; ?synonym of <i>D. inaequalis</i> )  ref. 178, 228, 242	Cytisus laburnum	—/—/100-130x7/10x6.5
coronillae Sacc. 1876 (teleomorph of <i>Phomopsis coronillae</i> ?= <i>P. oblonga</i> ; synonym of <i>D. eres</i> )  ref. 169, 178, 221, 242	Coronillia emerus	(500 diam.) (600-640x400-480)/—/ 70x10/(14x5-6) (11.5-14x3-4)
crotalariae Weber 1933 (teleomorph of <i>Phomopsis crotalariae</i> )  ref. 240	Crotalaria spectabilis	220-380 diam./1000-2000x250-500/ 34.01x6.85/9.12-10.95-12.31x2.28-2.5-3
desmodiana (Cooke & Ellis) Sacc. 1882 (anamorph unknown; synonym of <i>D. desmodii</i> = <i>D. arctii</i> )  ref. 40, 178, 242	Desmodium sp.	230-440x160-200/—/23-40x5.5-7/ (18 long) (8-12x2.5-3)

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<i>Diaporthe</i> species <sup>a</sup>	Legume Host(s)	Perithecia/Ostioles/Asci/ Ascospores (microns) <sup>b</sup>
desmodii (Peck) Sacc. 1882 (anamorph unknown; synonym of D. desmodiana = D. arctii)	Desmodium sp.	230-440x160-200/—/(23-40x5.5-7) (35x5)/ (@ 9-10 long) (8-12x2.5-3) (8-10x2.5)
ref. 53, 141, 178, 242		
digitfera Mouten 1889 var. digitfera (anamorph unknown; ?synonym of D. inaequalis)	Cytisus scoparius (as Sarothamnus scoparius)	to 500x500/—/110x15/ (25-32x8.5) (25-35x8.5)
ref. 130, 180, 242		
digitfera var. lignicola Sacc. 1891 (anamorph unknown)	Cytisus scoparius (as Sarothamnus scoparius)	—/—/—/—
ref. 180		
dolosa Sacc. & Roum. 1883 (anamorph unknown; synonym of D. oncostoma)	Robinia pseudacacia	500 diam./—/60-70x10-12/10-12x4
ref. 180, 189, 221, 242		
dorycnii Fabre 1878 non Sacc. 1882 (anamorph unknown)	Dorycnium pentaphyllum (as D. suffruticosum)	—/—/60-70x14-16/15-27x4-6
ref. 63, 178, 242		

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Diaporthe species</u> <sup>a</sup>	<u>Legume Host(s)</u>	<u>Perithecia/Ostioles/Asci/ Ascospores (microns)</u> <sup>b</sup>
dorycnii (Mont.) Sacc. 1882 non Fabre 1878 (anamorph unknown; ?true Diaporthe)  ref. 129, 178, 242	Dorycnium pentaphyllum (as D. suffruticosum)	—/—/80-100x10/(13-14x5) (13.5x5)
enteroleuca (Curr.) Sacc. 1882 (anamorph unknown; synonym of D. oncostoma)  ref. 38, 44, 178, 242	Robinia pseudacacia Robinia sp.	—/—/—/15-17 long
eres Nits. 1870 (teleomorph of Phomopsis oblonga; basionym for D. coronillae, D. genisticola, D. ligulata, ?D. mendax, D. nucleata, D. occidentalis, D. seposita, and ?D. tropicalis)  ref. 109, 127, 132, 242	Amorpha fruticosa Laburnum anagyroides	(600-640x400-480) (240-800x160-500) (250-800) (200-550) (350-500) diam./ (180-820 long) (80-90 thick)/ (38-44.5-59x5-7-9) (40-50x5-7)/ (13x3-4) (8.5-11.5-15x2.5-3.1-4) (11-14x2.5-4) (11.5-14x3-4) (10-12x2.5-3) (9.5-15x2.5-4) (11-13x2.5-3.5)
eumorpha (Durieu & Mont.) Maire 1917a (teleomorph of Phomopsis stromatigena; basionym for D. lirellaeformis)  ref. 21, 120, 242	Lupinus sp.	200-320x120-240/—/40-47x6-8/9-15x2.5-4

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<u>Diaporthe species<sup>a</sup></u>	<u>Legume Host(s)</u>	<u>Perithecia/Ostioles/Asci/ Ascospores (microns)<sup>b</sup></u>
fasciculata Nits. 1870 var. fasciculata (teleomorph of <i>Phomopsis pseudacaciae</i> ; synonym of <i>D. oncostoma</i> )	Robinia pseudacacia	500 diam./—/(53-56[60]x8-9) (53-60x8-9) (53-66x8-9) (53-56[66]x8-9)/ 12-14x3-4
ref. 139, 178, 200, 221, 242, 249		
genistae Rehm 1913 (teleomorph of <i>Phomopsis genistae- tinctoriae</i> )	Genista pilosa	(150x150) (320-480x240-320)/—/ (to 50x5) (40-47x4-7)/(10-12x2) (10-14x1.5-2)
ref. 165, 223, 242		
genistae Ade 1923 (later homonym)	Genista tinctoria	300-400 diam./350x100-160/ 33-42x4.5-6.5/10-13x2-2.5
ref. 2, 148, 242		
genisticola Rehm 1892 (anamorph unnamed; ?synonym of <i>D. eres</i> )	Genista tinctoria	—/—/—/13x3-4
ref. 242		

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<u>Diaporthe species<sup>a</sup></u>	<u>Legume Host(s)</u>	<u>Perithecia/Ostioles/Asci/ Ascospores (microns)<sup>b</sup></u>
gorgonoidea Cooke & Harkn. 1884 (anamorph unknown; synonym of D. medusaea)  ref. 42, 53, 180, 242	Acacia sp.	—/—/—/(15-17x3) (10-13x2.5-3.5)
hemicypta (Durieu & Mont.) Sacc. 1891 (anamorph unknown)  ref. 21, 180, 242	Anagyris foetidae	333 diam./500x125/50x12-13/10x2.5
inaequalis (Curr.) Nits. 1870 (teleomorph of <i>Phomopsis inaequalis</i> ; basionym for ?D. chrysoides, ?D. digitifera, D. neglecta, and <i>Melanconis cytisi</i> Naumov 1914)  ref. 44, 117, 134, 139, 178, 200, 221, 242, 243, 249	Amorpha fruticosa Cytisus capitatus C. hirsutus C. ratisbonnensis C. scoparius (as <i>Sarothamnus scoparius</i> ) Cytisus sp. Genista germanica G. tinctoria Genista sp. Sarothamnus vulgaris Sarothamnus sp. Ulex europaeus Ulex sp.	(450-720x240-500) (350-630 diam.)/ 750-120x200/(120-180x8-14) (96x13.8) (70-100x8-11) (70-110x9-15)/(14-18x7-10) (15-20[24]x[7]8-10[12]) (15-20x8-12) ([12]13-17[18]x5.5-9) (15-24x8-12) (13.8-17.4x7-8.7) (15 long or a little over)

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Diaporthe species</u> <sup>a</sup>	<u>Legume Host(s)</u>	<u>Perithecia/Ostioles/Asci/ Ascospores (microns)</u> <sup>b</sup>
indigoferae E. Mull. & Ahmad 1958 (anamorph unknown)  ref. 131	Indigofera gerardiana	350-450 diam./@ 100 wide/40-50x6-7/ 9-11x2-2.5
interrupta (Mont.) Sacc. 1882 (anamorph unknown; synonym of D. sarothamni)  ref. 128, 177, 242	Cytisus scoparius	—/—/—/—
ligulata Nits. 1870 (teleomorph of Phomopsis ligulata ?= P. oblonga; synonym of D. eres)  ref. 139, 173, 178, 242, 249	Ulex europaeus	—/—/(60x9-10) (60x8)/([11]12[13]x4) (12-13x4) (11-14x2.5-4) (10-11x3-3.5)
lirellaeformis Pat. 1897 (teleomorph of Phomopsis stromatigena; (as Phaca baetica) synonym of D. eumorpha)  ref. 140, 191, 242	Astragalus lusitanicus	200-240x120-160/—/(80x6-8) (40-47x7-8)/ 11-15x3-4
lupinii Harkn. 1884 (anamorph unknown)  ref. 53, 78, 160, 180, 242	Lupinus arboreus	(400-550x200-380) (333 diam.)/ 320x160-200/(55-60x9) (50-55x10) (50-60x9)/(15x4) (12-16x4-4.5) (12-16[18]x5-6.5)

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic. 6



Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<u>Diaporthe species<sup>a</sup></u>	<u>Legume Host(s)</u>	<u>Perithecia/Ostioles/Asci/ Ascospores (microns)<sup>b</sup></u>
medusaea Nits. 1870 (teleomorph of <i>Phomopsis rudis</i> ; basionym for <i>D. gorgonoidea</i> , ? <i>D. rhynchophora</i> , and <i>D. rudis</i> )  ref. 139, 178, 200, 242, 249	<i>Cytisus laburnum</i>	200-500 diam./—/(46-52x7) (40-47x6-9)/ (10-13x3) (10-15x2.5-3.5)
meliloti (Sacc.) Trav. 1906 (teleomorph of <i>Phomopsis meliloti</i> ?= <i>P. arctii</i> ; synonym of <i>D. fasciculata</i> var. <i>meliloti</i> [Sacc.] Sacc. in Sacc. & Trott. 1913 = <i>D. arctii</i> )  ref. 193, 221, 242	<i>Melilotus officinalis</i>	333 diam./—/50-54x8-10/(12-15x3.5-4) (9-10.5x2.5)
melonis Beraha & O'Brien 1979 (teleomorph of <i>Phomopsis cucurbitae</i> )  ref. 16	<i>Glottidium</i> sp. (sterile)	100-125 diam./325-1200x50-75/ 24.6-30.8-36.3x3.6-4.8-5.7/ 7.2-9.6-11x2.2-3.1-4.7

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Diaporthe species</u> <sup>a</sup>	<u>Legume Host(s)</u>	<u>Perithecia/Ostioles/Asci/ Ascospores (microns)</u> <sup>b</sup>
mendax Sacc. 1875 (teleomorph of <i>Phomopsis mendax</i> ?= <i>P. oblonga</i> ; ?synonym of <i>D. eres</i> )  ref. 168, 178, 221, 242	<i>Albizia julibrissin</i> (as <i>Albizzia julibrissin</i> )	333-500 diam./—/60x8/12-15x5
microcarpa Rehm in Voss 1891 (anamorph unknown)  ref. 180, 234, 242	<i>Cytisus nigricans</i>	@ 300 diam./—/50x8/15x4, appendaged
micromegala Ellis & Everh. 1893b nomen sed non planta (anamorph unknown; excluded species = <i>Gnomonia</i> sp.[ref. 242])  ref. 55, 181, 242	<i>Desmodium</i> sp.	(250-333) (200-300)diam. (320-480x200-320)/1000 long/(50x20) (67x20-22)/(25-28x5-6) (38-40x6-7)
neglecta (Duby) Berl. & Vogl. 1886 (anamorph unknown; synonym of <i>D. inaequalis</i> )  ref. 18, 39, 180, 242	<i>Genista tinctoria</i>	—/—/—/—

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<u>Diaporthe species<sup>a</sup></u>	<u>Legume Host(s)</u>	<u>Perithecia/Ostioles/Asci/ Ascospores (microns)<sup>b</sup></u>
nucleata (Curr.) Sacc. 1882 (anamorph unknown; synonym of <i>D. eres</i> )  ref. 44, 178, 242	<i>Ulex</i> sp.	—/—/—/(17-20 long) (11-14x2.5-4)
occidentalis Sacc. & Speg. in Sacc. 1878c Sacc. 1878c (teleomorph of <i>Phomopsis occidentalis</i> ?= <i>P. oblonga</i> ; synonym of <i>D. eres</i> )  ref. 172, 178, 221, 242	<i>Gleditsia triacanthos</i>	500 diam. or greater/—/45-55x7-8/ 12-14x3-4
oncostoma (Duby) Fuckel 1870 (teleomorph of <i>Phomopsis oncostoma</i> = <i>P. pseudacaciae</i> ; basionym for <i>D. dolosa</i> , <i>D. enteroleuca</i> , <i>D. fasciculata</i> , and <i>D. personata</i> )  ref. 38, 53, 67, 132, 133, 178, 200, 221, 241, 242, 249	<i>Robinia macrophylla</i> <i>R. microphylla</i> <i>R. viscosa</i>	(350-600x350-550) (450-600x400-450) (500-1000) (to 700) (350-600) diam./—/ (65-72x8-9) (65x9) (45-50x8-9) (60-67x7-8) (64x8) (@ 75x10) (60-70x6-9) (60-80x6-9) (50-56.4-71x7.5-8.5-9.5)/(16x4) (12-16x3-3.5) (14-16x3.5) (18x4.5-5) (13-17[25]x3-4[5]) (13-17x3-4) (13-16x3-4) (16-20[22]x4-5.5[7.5]) (14-18x2-2.5) (14-16x3-3.5) (14-18x3.5-4.5)
parvula Tschern. 1929 (anamorph unknown)  ref. 226	<i>Caragana arborescens</i>	400 diam./500 long/35-45x7-9/9-14x3

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<u>Diaporthe species<sup>a</sup></u>	<u>Legume Host(s)</u>	<u>Perithecia/Ostioles/Asci/ Ascospores (microns)<sup>b</sup></u>
personata (Cooke & Ellis) Sacc. 1882 (anamorph unknown; synonym of D. oncostoma)  ref. 41, 178, 242	Robinia pseudacacia	—/—/—/25-28x6
phaseolorum (Cooke & Ellis) Sacc. 1882 var. phaseolorum (teleomorph of <i>Phomopsis phaseoli</i> [Desm.] Sacc. {homonym <i>P. phaseoli</i> [Desm.] Grove})  ref. 40, 53, 79, 178, 242	Melilotus alba (sterile) Phaseolus lunatus Phaseolus sp. Vigna radiata (as Phaseolus aureus)	(160-350x110-200) (158-251.9-355.5) (158-215.6-237) diam./ (120-400x50-80) (250-500 long)/ (28-46x5.5-8) (30-35x6-7) (28-37.4-46.2x5.2-6.73-8) (28-33.6-44x4.8-7-8)/ (16 long) (10-12x3) (8-12x2-3.5) (6.4-9.5-12x2.3-2.93-4) (8-10-12x2.4-3.3-4)
phaseolorum var. batatatis (Hart. & Field) Wehm. 1933 (teleomorph of <i>Phomopsis batatae</i> ; isolates did not form anamorph and are now believed to have been D. phaseolorum var. caulivora)  ref. 10, 80, 242, 245	Glycine max	275-390x178-325/280-546 long/ 27.2-40.8x6.8-8.5/8.5-10.2x3.4-5.1

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<i>Diaporthe</i> species <sup>a</sup>	Legume Host(s)	<i>Perithecia</i> / <i>Ostioles</i> / <i>Asci</i> / <i>Ascospores</i> (microns) <sup>b</sup>
phaseolorum var. caulivora Athow & Caldw. 1954 (anamorph unnamed)  ref. 10, 66, 105	Glycine max Medicago sativa Melilotus alba Phaseolus vulgaris Pisum sativum Trifolium incarnatum T. pratense T. repens	(282-412x165-340) (295-310-357x204-240-334)/ 240-518x58-192/(29.8-40.2x4.5-7) (24-36-50x5-5.3-8.1)/(8.6-11.8x3-3.9) (7.5-12.2-14.5x2.8-3-3.3)
phaseolorum var. sojae (Lehm.) Wehm. 1933 (teleomorph of <i>Phomopsis sojae</i> )  ref. 7, 10, 36, 105, 116, 119, 202, 242, 245, 246	Arachis hypogaea Glycine max (as Soja max) Glycine ussuriensis (as Glycine soja) Lespedeza spp. Lotus corniculatus Lupinus hirsutus Melilotus alba (sterile) Phaseolus helvolus (as <i>Strophostyles helvola</i> ) P. limensis P. lunatus P. vulgaris Vigna radiata (as <i>Phaseolus aureus</i> ) V. unguiculata (as V. sinensis)	(172-270-330x151-210-265) (145-348x116-318) (192-335x156-260) (190-254-340x163-218-272) (185-346x148-282) (180-240-306x155-210-290)/(1500x40-60) (1300-1600x35-56) (350-1500x60-142) (347-521 long)/(28-41x6.5-10) (21.1-36.9-46.4x7.2-8.3-11.5) (37.2-44.9-50.2x7.2-8.3-12.2) (28-38.4-44.8x7.8-8.6-10.6) (37-52x7.4-12.9) (38-51.2x5-10.3) (26-38-55x5.1-5.6-7.5)/(11.5x3.5) (8.6-11.3-12.8x2.8-3.5-5) (9.6-11.4-12.4x2.4-3.5-4.2) (9-11.1-13.5x3-3.7-4.8) (10.4-18.5x3.7-5.5) (9.2-13.5x3.3-5.6) (9-11-13x2.6-3.2-4.3)

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Diaporthe species</u> <sup>a</sup>	<u>Legume Host(s)</u>	<u>Perithecia/Ostioles/Asci/ Ascospores (microns)</u> <sup>b</sup>
pratensis Sacc. & Speg. in Sacc. 1878c (anamorph unknown; synonym of D. arctii)  ref. 172, 221, 242	Medicago lupulina M. sativa	333-500 diam./—/(35-40x6.5-7.5) (40-50x6.5-7.5)/14-15x3-4
psoraleae-bituminosae Petr. 1922 (teleomorph of Phomopsis psoraleae ?= P. arctii; ?synonym of D. arctii)  ref. 142, 242	Psoralea bituminosa	150-300 diam./—/45-50x9-11/10-13x3-4
recondita Sacc. 1916 (anamorph unknown; later homonym of D. recondita [Schw.]Ellis & Everh. 1892; ?= D. pardolata [Mont.]Fuckel 1870)  ref. 188, 222, 242	Gliricidia maculata	120-130 diam./—/30-35x4.5-5/—
rhynchophora Fabre 1878 (anamorph unknown; ?synonym of D. medusaea)  ref. 63, 178, 242	Coronilla minima	250 diam./1000 or longer/45-50x8/16x4

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<i>Diaporthe</i> species <sup>a</sup>	Legume Host(s)	<i>Perithecia</i> / <i>Ostioles</i> / <i>Asci</i> / <i>Ascospores</i> (microns) <sup>b</sup>
<i>rudis</i> (Fr.) Nits. 1870 (teleomorph of <i>Phomopsis rudis</i> ; synonym of <i>D. medusaea</i> )  ref. 65, 139, 178, 200, 221, 227, 242	<i>Cytisus laburnum</i> <i>C. nigricans</i> <i>C. sessifolius</i> (as <i>C. sessiflorus</i> )	—/—/(62-70x8-9) (62-90x8-9)/(14-16x4) (13x3-4) (10-13.5x2.5-4)
<i>sarothamni</i> (Auersw.) Nits. 1870 (teleomorph of <i>Phomopsis sarothamni</i> = <i>P. spartii</i> ; basionym for <i>D. interrupta</i> )  ref. 133, 139, 178, 200, 242, 249	<i>Cytisus scoparius</i> (as <i>Sarothamnus scoparius</i> or <i>Spartium scoparium</i> )	(320-720x240-350) (300-400 diam.)/ 150-400x100/(60-69x7-8) (60-70x7-8) (45-58x7-14) (60-70x8-10)/(14-15x3-4) (15-18x3-4) (13-16[17]x3-4[4.5]) (14-15x3-5)
<i>seposita</i> Sacc. 1875 (teleomorph of <i>Phomopsis seposita</i> ?= <i>P. oblonga</i> ; synonym of <i>D. eres</i> )  ref. 178, 221, 242	<i>Wisteria sinensis</i> (as <i>Wistaria chinensis</i> or <i>W. sinensis</i> )	250-333 diam./—/70x7-8/16-18x5-6
<i>sheariana</i> Petr. 1952 (anamorph unknown)  ref. 150	<i>Acacia koa</i>	400-700 diam./—/60-75x10-13/ 17-20-25x5-7; an appendage 5-8x1.5 on each end

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -# = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<u>Diaporthe species<sup>a</sup></u>	<u>Legume Host(s)</u>	<u>Perithecia/Ostioles/Asci/ Ascospores (microns)<sup>b</sup></u>
sophorae Sacc. 1878c (teleomorph of <i>Phomopsis sophorae</i> )  ref. 172, 178, 221, 242	<i>Sophora japonica</i>	333 diam./—/50-60x8-10/12-15x5-6
sparsa Niessl 1883 (teleomorph of <i>Phomopsis sparsa</i> )  ref. 138, 180, 222, 242	<i>Hardenbergia monophylla</i> (as <i>Glycine violacea</i> )	—/—/52-60x8-10/10-12x3-4
tropicalis Speg. 1880a (anamorph unknown; ?synonym of D. eres)  ref. 178, 207, 242	<i>Bauhinia grandiflora</i> (as <i>B. aculeata</i> )	250-333 diam./—/45x8-10/12-14x4-5
tulasnei Nits. 1870 (teleomorph of <i>Phomopsis tulasnei</i> ?= <i>P. arctii</i> ; synonym of <i>D. arctii</i> )  ref. 183, 139, 178, 221, 242, 249	<i>Medicago</i> sp. <i>Melilotus</i> sp.	500-600 diam./@ 50 thick/(46-53x6-7) (45-55x7-10)/(10-14x3) (10-16x3-3.5) (10-14x3-3.5).

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.



Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<u>Diaporthe species<sup>a</sup></u>	<u>Legume Host(s)</u>	<u>Perithecia/Ostioles/Asci/ Ascospores (microns)<sup>b</sup></u>
tulasnei forma galegae Wint. 1884 (teleomorph of Phoma galegae)  ref. 180, 248	Galega officinalis	—/—/—/—
vaccinii Shear 1931 (teleomorph of Phomopsis vaccinii)  ref. 204, 242	Melilotus sp. (sterile)	300-500x200-400 to 1500-2000 diam./ to 500 long/37-51x6.8-11.7/ 8.8-11.8x2.4-3.4
winteri Kunze 1878 (teleomorph of Phomopsis winteri ?= P. arctii; synonym of D. arctii)  ref. 178, 242, 249	Ononis spinosa (also as O. repens) Ononis sp.	370-490 wide/—/(47-52x8-9) (42-46x7-9)/ (12-13x3-3.25) (10x3.5) (9-13x2.5-3.5)
woodii Punith. 1974b (teleomorph of Phomopsis leptostromiformis and P. rossiana)  ref. 160	Lupinus albus L. angustifolius L. arboreus L. digitatus L. luteus	to 500 wide/to 2000 long/35-45x5-6/ 8-10x2.5-3.5

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Phomopsis species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/Conidiophores (microns)</u> <sup>b</sup>
acaciae Chen 1967 (teleomorph unknown)  ref. 31	Acacia confusa	166.6-397.5x107.1-197.5/6.2-7.9x2.1-3.1/ 19.4-28.6x1.9-2.9/3.8-11.9x1-1.9
acaciicola (P. Henn.)Died. 1911 (teleomorph unknown)  ref. 3, 19, 49, 50, 83	Acacia albicans A. dealbata A. longifolia A. lunata var. brevifolia A. retinodes A. spectabilis Acacia spp.	(@ 200) (to 500) diam./ (7-9x3-3.5) (6-10x3.5-4)/—/22-28x1.5
amherstiae Ponn. 1971 (teleomorph unknown)  ref. 156	Amherstia nobilis	243-292x194-240/7.5-10.25x1.75-2/—/ 18.5-30[56]x1.8
anthyllidicola (Hennings)Diedicke 1911 (teleomorph unknown)  ref. 3, 49, 50, 83, 192	Anthyllis barba-jovis	@ 400 diam./ (7-9x4) (7-9x3-4)/—/ 15-20x1-2

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<i>Phomopsis</i> species <sup>a</sup>	Legume host(s)	Pycnidia/alpha-conidia/Beta-conidia/ Conidiophores (microns) <sup>b</sup>
arctii (Sacc.) Trav. 1906 (anamorph of <i>Diaporthe arctii</i> ; ?basionym for <i>P. meliloti</i> , <i>P. psoraleae</i> , and <i>P. tulasnei</i> )  ref. 142		—/(7x3-3.5) (7-8x3.5) (8-10x2-2.5)/ (25x1.5) (18-25x1)/—
batatae (Ellis & Hals.) Hart. & Field 1912 (anamorph of <i>Diaporthe phaseolorum</i> var. <i>batatatis</i> )  ref. 10, 76, 80, 111, 242	<i>Canavalia ensiformis</i> <i>Glycine max</i> <i>Lathyrus latifolius</i> <i>L. sylvestris</i> <i>Lens culinaris</i> <i>Lotus corniculatus</i> <i>Phaseolus coccineus</i> <i>P. lunatus</i> <i>P. vulgaris</i> <i>Pisum sativum</i> <i>Trigonella foenum-graecum</i> <i>Vicia faba</i> <i>Vigna angularis</i> <i>V. radiata</i> var. <i>radiata</i> <i>V. unguiculata</i> subsp. <i>unguiculata</i>	65-154x58-128/5.7-10.2x2.8-4.2/ 15-33x1.1-1.9/—; "C"-conidia: 6.4-14 long; (measurements from agar cultures)

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Phomopsis species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/Conidiophores (microns)</u> <sup>b</sup>
bauhinia Bausa Alc. 1952 (teleomorph unknown)  ref. 15, 157	Bauhinia purpureae Bauhinia sp.	(120-432 diam.x40-150 high) (75-185x75-125)/ (6-{usw. 7.5-8.5}-9.5x1.8-2-2.2) (5.5-9.5{5.5-11.5 in culture}x2-3)/ 15-20.5x1.5-2/10-14x1.8-2.4
brachyseomatis (P. Henn.)Died. 1911 (teleomorph unknown)  ref. 3, 49, 50, 83, 192	Brachysema undulatum Brachysema sp.	200-225 diam./7-9x2-2.5/—/about as long as alpha-conidia x 1-2
buteae Sahni 1968 (teleomorph unknown)  ref. 194	Butea monosperma	73.5-133.6-177 diam./ 3.3-5.7-9.9x2.1-3-4/ 7.9-12.9-15.5x1-1.3-1.6/—
cajani H. & P. Syd. in H. Syd., P. Syd., & Butler 1916 (teleomorph unknown)  ref. 213, 224	Cajanus cajan (as C. indicus)	150-225 diam./6-8x1.5-2/—/12-15x1

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; #- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Phomopsis species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/Conidiophores (microns)</u> <sup>b</sup>
cajani (Rang.) Cif. 1962 (teleomorph unknown; later homonym)  ref. 35, 161	Cajanus cajan (as C. indicus)	(160-240) (150-300) diam./ (5-8x1.5-2) (4-9x1.5-2)/—/(9-60 long) (30-50x1.5-2)
calophacae (P. Henn.) Died. 1911 (teleomorph unknown)  ref. 3, 49, 50, 84, 192	Calophaca wolgarica	180-220 diam./7-10x2.5-3.5/—/as long as alpha-conidia or shorter
cancrici (Punith.) Punith. 1974a (teleomorph unknown)  ref. 158, 159	Tipuana tipu	to 500 diam./6-9x2-3/—/10-20x2-4
caraganae Bond. 1922 (?anamorph of Diaporthe caraganae; ?synonym of P. serebrianikowii)  ref. 20, 162	Caragana arborescens Caragana sp.	@ 1000 diam./ (13-18x3-3.5) (7.5-13.5x3-3.5)/14-20x1.5/ (about as long as alpha-conidia) (30x2.5-3.5)
cassiae Camara 1951 (teleomorph unknown)  ref. 29, 118	Cassia sp.	(215-335 diam.) (200-280x200-270)/ (8-9.3x3) (8-10x2.5-3)/20-21x1.3/ (18-22.5x1.7-2.2) (13-19x1-2)

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<u>Phomopsis species<sup>a</sup></u>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/ Conidiophores (microns)<sup>b</sup></u>
cassiae (Sacc.)Rat. 1968 (teleomorph unknown; later homonym)  ref. 163, 173, 179	Cassia corymbosa (as C. carymbosa) C. occidentalis	(110 diam.) (300-420x190-255)/ (6-8x1.75-2) (7.5-8.7x3)/9-30x1-1.5/ 20x2
cladrastidis Petr. 1934 (teleomorph unknown)  ref. 149, 179	Maackia amurensis (as Cladrastis amurensis)	750-1500 diam. x 500 high/7-13x2-3/—/ 8-15[20]x1.5
coluteae (Sacc. & Roum.)Died. 1911 var. coluteae (teleomorph unknown)  ref. 3, 49, 70, 74, 127, 177, 179	Colutea arborescens Colutea sp.	(333) (@ 350) diam./ (7x3) (7-9x2.5-3) (7-8x2-2.75[3.5])/—/about as long as alpha-conidia
coluteae var. longipes Amorim & Camara 1954 (teleomorph unknown)  ref. 6	Colutea sp.	204-333x154-179/6-12x3-4/—/16-23x2.5

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<u>Phomopsis species<sup>a</sup></u>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/ Conidiophores (microns)<sup>b</sup></u>
coronillae (Westend.) Bubak 1906 (anamorph of <i>Diaporthe coronillae</i> = <i>D. eres</i> ; ?synonym of <i>P. oblonga</i> )  ref. 3, 22, 23, 49, 70, 74, 170, 179, 193, 221, 242	<i>Coronillia emerus</i>	—/(7-8x3) (7-8x2-3) (9-11x2-2.5)/ (20-26x1.5) (15-16x1)/(20x1.5) (10-20 long)
crotalariae Weber 1933 (anamorph of <i>Diaporthe crotalariae</i> )  ref. 240	<i>Crotalaria spectabilis</i>	200-450 diam./ 5.9-7.73-10x1.9-2.88!-2.8/ 16.6-30.94x1.8-2.3/15.4-26.6 long
cucurbitae McKeen 1957 (anamorph of <i>Diaporthe melonis</i> )  ref. 16, 124	<i>Glycine max</i> (sterile) <i>Glottidium sp.</i> (sterile)	(140-400) (100-500) diam./ (6.8-14.5x2.8-4.2) (6.3-8.3-10.3x2.16-2.6-3)/ (14-25x1.15-1.35) (18.6-24.7-27.7x1-1.3-2)/—

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Phomopsis species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/Conidiophores (microns)</u> <sup>b</sup>
cunninghamii H. Syd. 1924 (teleomorph unknown)  ref. 211	Carmichaelia grandifolia Notospartium carmichaeliae	250-400 diam./6-8x2-2.5/—/ 8-12[15]x1-1.5
cuspariae Gutner 1933 (teleomorph unknown)  ref. 75, 225	Bauhinia odoratissima (as Cusparia odoratissima)	225 diam.x100 high/6-10x1.5-3/—/—
cyamopsidis Petr. & H. Syd. 1923 (teleomorph unknown)  ref. 154	Cyamopsis tetragonolobus (as C. psoraleoides)	100-200 diam./4-7x1.75-2.5/—/ 10-15x1.5-2
cytisi Gonz. Frag. 1914 (teleomorph unknown)  ref. 69, 224	Cytisus purgans	—/6-7x2/—/—
cytisi (P. Henn.)Died. ex Rat. 1967 (teleomorph unknown; ?later homonym [authorities incorrect?])  ref. 162	Cytisus caucasicus	300-375x225-285/—/12-25.5x1.5/12 long

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; #- = average measurement; ! = sic.



<u>Phomopsis species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/ Conidiophores (microns)</u> <sup>b</sup>
dalbergiae Sacc. 1915 (teleomorph unknown)  ref. 187	Dalbergia sissoo	178-194 diam./7-8x7/—/12x2
dalbergiae Sahni 1968 (teleomorph unknown; later homonym)  ref. 194	Dalbergia sissoo	96.9-136-220.4 diam./ 4-5.7-6.7x1.3-2.3-3.3/ 6-11.1-13.2x1.2-1.5-2.5/—
dorycnii Petr. 1921 (teleomorph unknown)  ref. 146	Dorycnium pentaphyllum (as D. suffruticosum)	200-300 diam./7-11x3-4/—/12-18x1-2
epicarpa Sacc. 1909 (teleomorph unknown)  ref. 184, 193	Robinia pseudacacia	250-300 diam./8-9x2.5/—/15-17x1.5
erythrinae (Berk.) Trav. in Trav. & Spes. 1910 (teleomorph unknown)  ref. 17, 24, 222	Erythrina crista-galli	—/(8.5x1.4) (7-8 long) (7-9x2.5-3)/ 20-30x1.5/(twice as long as alpha-conidia x just as thin) (8-15x3)

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Phomopsis species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/Conidiophores (microns)</u> <sup>b</sup>
erythrinae (Petch) Petr. 1957 (teleomorph unknown; later homonym)  ref. 142, 152	Erythrina lithosperma Erythrina sp.	90-180x60-70/(6-8x2) (6-9x2-2.5)/—/ (@ 10 long) (10-16x2)
genistae-tinctoriae Petr. 1916 (anamorph of Diaporthe genistae)  ref. 144	Genista tinctoria	400-600x350-500/4-7x2-3/—/—
gliricidia H. & P. Syd. 1913 (teleomorph unknown)  ref. 157, 212	Gliricidia sepium G. maculata	(120-180 diam.) (121.5-194.5x73-267.5)/ (6.5-8.5[7.5-9.5 in culture]x1.5-2.5) (10-16x3)/17-22x1/8-12 long
glycines Petr. in Petr. & Syd. 1936 (teleomorph unknown)  ref. 155	Glycine max (as G. hispidae)	100-350 diam./5-7.5x1.5-2.5/—/5-12x1.5

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported from legume hosts CONT'D

<i>Phomopsis</i> species <sup>a</sup>	Legume host(s)	Pycnidia/alpha-conidia/Beta-conidia/ Conidiophores (microns) <sup>b</sup>
<i>gulabii</i> Lal & Arya 1981 (teleomorph unknown)  ref. 114	<i>Dolichos lablab</i> (artificial inoculation)	to 2000 wide/6.6-7.7-8.9x2.2-3.07-3.3/ 15.5-14.8!-22.2x1.11/17.7-22.2x3.3; "C"-conidia: 11-13x1.6-3.3
<i>gymnocladi</i> Byz. in Byz. et al. 1968 (teleomorph unknown)  ref. 28	<i>Gymnocladus dioica</i>	137-440x75-275/5-10x2-3/12.5-25x1-2/ 22-27x1-1.5
<i>inaequalis</i> (Speg.) Trav. 1906 (anamorph of <i>Diaporthe inaequalis</i> ; ?basionym for the unnamed anamorph of <i>D. chrysoides</i> )  ref. 19, 50, 74, 139, 178, 179, 200, 221, 242	<i>Amorpha fruticosa</i> <i>Cytisus capitatus</i> <i>C. scoparius</i> (as <i>Sarothamnus scoparius</i> ) <i>Cytisus</i> sp. <i>Genista germanica</i> <i>G. tinctoria</i> <i>Genista</i> sp. <i>Sarothamnus vulgaris</i> <i>Sarothamnus</i> sp. <i>Ulex europaeus</i> <i>Ulex gallii</i> <i>Ulex</i> sp.	—/(7-10x2-3) (5.7-10x2.8)/21-27x2/ (15-20 long) (21-27x2)

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Phomopsis species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/Conidiophores (microns)</u> <sup>b</sup>
lathyrina (Sacc.)Grove 1919b (teleomorph unknown)  ref. 3, 72, 74, 162, 176, 179	Astragalus sp. Lathyrus latifolius L. silvestris	(250) (to 400) diam. (900x600)/(9-10x2.5) (8-10x2-2.5) (7.5-12[15]x2-3)/12-24x1.5/ (16-20x1-1.5) (18-27 long)
leptostromiformis (Kuhn)Bubak ex Lind 1913 (anamorph of Diaporthe woodii; basionym for P. rossiana)  ref. 74, 97, 98, 101, 117, 118, 160, 239	Lupinus albus L. arboreus L. angustifolius L. angustifolius var. uniwhite L. digitatus L. luteus L. mutabilis (sterile) L. polyphyllus Lupinus sp.	(100-200 diam.) (to 2000 wide) (180-200x100-150)/(7-8.5x2) (8-9x2-2.5) (8-9x2-2.5) (7-9.5x2-2.5) (8-10x1.5-2) (6-8-12x1.5-2!-2)/present/(13-15.5 long) (as long as alpha-conidia or 15-20x1.5) (8-14x1.4-2.5)
ligulata Grove 1935 (anamorph of Diaporthe ligulata = D. eres; ?synonym of P. oblonga)  ref. 48, 74	Ulex europaeus U. minor	380-500x170-330/(6-8x1.7-2.5) (7.5-10x2-2.5)/10-21x1/(10-15x1) (13.5-17.5x1.25)

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Phomopsis species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/Conidiophores (microns)</u> <sup>b</sup>
loti Upadh. 1966 (teleomorph unknown)	Lotus angust	40-306-650x25-232-500/4-8.5-17x1-4-6/
	L. angustissimus	8-19-28x1-2-3/7-14-25x2-2.5-3
	L. arabicus	
ref. 230, 231	L. carmeli	
	L. corniculatus	
	L. corniculatus var. arvensis	
	L. corniculatus var. ciliatus	
	L. corniculatus var. glaber	
	L. corniculatus var. hirsutus	
	L. edulis	
	L. hispidus	
	L. lamprocarpus	
	L. maroccanus	
	L. mearnsii	
	L. ornithopodioides	
	L. pedunculatus	
	L. peregrinus	
	L. prushianus	
	L. pusillus	

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<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Phomopsis species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/ Conidiophores (microns)</u> <sup>b</sup>
loti (continued)	L. tenuis L. tetragonolobus L. weillerii Vicia villosa Vigna unguiculata subsp. unguiculata (as V. sinensis)	
machaeriicola Petr. 1953 (teleomorph unknown)	Machaerium sp.	150-250 diam./4.5-7x2-3/—/5-10x1.5
ref. 151		

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Phomopsis species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/</u> <u>Conidiophores (microns)</u> <sup>b</sup>
mediterranea Sacc. 1913 (teleomorph unknown)  ref. 185, 187	Medicago arborea	@ 300 diam./8x2.5/—/12-15x2
meliloti Grove 1930 (anamorph of Diaporthe meliloti = D. arctii; ?synonym of P. arctii)  ref. 73, 74, 242	Melilotus officinalis Melilotus sp.	@ 500 long/7x3/—/16-20 long
mendax (Sacc.)Trav. 1906 (anamorph of Diaporthe mendax = D. eres; ?synonym of P. oblonga)  ref. 3, 162, 173, 179, 187, 221, 242	Albizia julibrissin (as Albizzia julibrissin)	396-420x240-276/(10x2) (6.8-10x2.5-3)/ —/(25x1) (10-20x1.5)
millettiae Swar., Chauh. & Trip. 1966 (teleomorph unknown)  ref. 210	Millettia ovalifolia	95.1-151.05-190.2x95.1-127.3-190.2/ 4.8-6.7x2.4-2.9/—/—

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<u>Phomopsis species<sup>a</sup></u>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/ Conidiophores (microns)<sup>b</sup></u>
myriosticta Sacc. 1914 (teleomorph unknown)  ref. 162, 186, 203, 224	Caesalpinia gilliesii C. pulcherrima Caesalpinia sp.	(80-120 diam.) (285-420x285-375)/ (9-10x2.5) (7.5-11.5x[2.5]3)/—/ (9-12x2) (19 long)
oblonga (Desm.) Hohn. 1906 (anamorph of <i>Diaporthe eres</i> ; type described from <i>Ulmus americanus</i> and <i>U. campestris</i> ; ?basionym for <i>P. coronillae</i> , <i>P. ligulata</i> , <i>P. mendax</i> , <i>P. occidentalis</i> , <i>P. seposita</i> , and the unnamed anamorph of <i>Diaporthe genistinicola</i> )  ref. 90, 109, 127, 132, 242	<i>Amorpha fruticosa</i> <i>Laburnum anagyroides</i>	250-350 diam./ (6-9x2-2.5) (14-15x5) (7-8x3) (4-5x2) (9-11x2-2.5) (6-7x2-2.5) (5-8.2-11x1.5-2.6-4) (5.5-7.3-8.5x2-2.4-3)/(33x1) (15-18x1) (20x1.5) (20-26x1.5) (15-16x1) (23-27.7-33x0.5-1.1-1.5) (17-24.6-30x0.5-0.9-1)/(5-10) (3-8) (10-20) long
occidentalis (Sacc.) Sacc. in Sacc. & D. Sacc. 1906 var. <i>occidentalis</i> (anamorph of <i>Diaporthe occidentalis</i> = <i>D. eres</i> ; ?synonym of <i>P. oblonga</i> )  ref. 48, 49, 174, 179, 221, 242	<i>Gleditsia triacanthos</i> <i>Gleditsia</i> sp. (as <i>Gleditschia</i> sp.)	370-430x260-300/(10x2) (8-10x2-2.5)/—/ 25-32.5x2

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.



Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<i>Phomopsis</i> species <sup>a</sup>	Legume host(s)	Pycnidia/alpha-conidia/Beta-conidia/ Conidiophores (microns) <sup>b</sup>
occidentalis var. irregularis (Trav.) Sacc. in Sacc. & D. Sacc. 1906 (anamorph of <i>Diaporthe occidentalis</i> = <i>D. eres</i> ; ?synonym of <i>P. oblonga</i> )  ref. 190, 220	<i>Gleditsia triacanthos</i>	(250-333) (250-300) diam./6-8x2.5-3.5/ 18-22x2/—
oncostoma (Thum.) Hohn. 1906 (anamorph of <i>Diaporthe oncostoma</i> ; synonym of <i>P. pseudacaciae</i> )  ref. 38, 49, 50, 67, 71, 74, 90, 170, 179, 200, 221, 241, 242	<i>Robinia macrophylla</i> <i>R. microphylla</i> <i>R. pseudacacia</i> <i>R. viscosa</i> <i>Robinia</i> sp.	(to 300) (to 500) diam./ (10x2-3) (10x2) (8-10x2-3) (8-10x2-2.5) (10x2-2.5)/ (18-30x1) (13-22x1-1.5) (18-20x1)/ (twice as long as alpha-conidia) (20-25x2)
ononidicola (Holl.) Moez 1930 (teleomorph unknown)  ref. 93, 126, 225	<i>Ononis spinosa</i>	(240-300x210-230) (187-375 diam.)/ (10-12x3.5-4) (11-12.5x2.5-3.5)/ 15-30x1-1.5/—
pehenningsii Sharma 1982 (teleomorph unknown)  ref. 203	<i>Arachis hypogaea</i>	140-180 diam./5-6.5-7x2-2.5-3/—/—

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<i>Phomopsis</i> species <sup>a</sup>	Legume host(s)	Pycnidia/alpha-conidia/Beta-conidia/ Conidiophores (microns) <sup>b</sup>
petiolorum (Desm.)Grove 1917 (anamorph of <i>Diaporthe fasciculata</i> = <i>D. oncostoma</i> ; synonym of <i>P. pseudacaciae</i> )  ref. 3, 47, 174, 179, 242	Cytisus sp. Gleditsia sp. Sophora sp. Robinia pseudacacia Robinia sp.	—/(7-8x3) (8x3) (7-8x2-2.5)/—/ (20-23x1) (12-15x2.5-3)
phaseoli (Desm.)Sacc. 1915 (anamorph of <i>Diaporthe phaseolorum</i> var. <i>phaseolorum</i> ; basionym for <i>P. phaseoli</i> [Desm.]Grove and <i>P. subcircinata</i> )  ref. 3, 46, 111, 117, 179, 187, 237, 238	Canavalia ensiformis Glycine max Lathyrus latifolius L. sylvestris Lens culinaris Lotus corniculatus Macroptilium atropurpureum Macrotyloma axillare Phaseolus coccineus P. lunatus P. vulgaris Phaseolus sp. Pisum sativum Trigonella foenum-graecum Vicia faba Vigna angularis V. caracalla (as <i>Phaseolus caracalla</i> ) V. radiata var. radiata V. unguiculata subsp. unguiculata	136-267-639x122-230-354/(10-12 long) (5.3-8.6-12x2.5-3.1-4.5)/ 14-21.4-30.8x1.4/—

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<u>Phomopsis species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/ Conidiophores (microns)</u> <sup>b</sup>
phaseoli (Desm.)Grove 1917 (later synonym) ref. 70, 74, 242	Phaseolus sp.	300-500 long/(7-9x2.5-3) (5.1-8.5[10]x1.7-4)/(11-31[54]x1.3-2.4) (to 15x2-2.5)/—
phaseoli Petch 1922 (teleomorph unknown; later homonym) ref. 143	Glycine max (as Phaseolus max)	250 diam./3-6x1.5-2/14-16 long/—
phyllophila Petr. 1919 (teleomorph unknown) ref. 145, 224	Trifolium repens	200-300 diam./—/16-30x0.5-1/—
pisicola Petr. & Cif. 1930 (teleomorph unknown) ref. 153	Pisum sativum	120-200 diam./5-7.5x1.5-2.5/—/ 5-8[10]x1-1.5
podalyriae (P. Henn.)Desm. 1911 (teleomorph unknown) ref. 3, 49, 50, 83, 192	Podalyria sp.	to 500 diam. x 300-400 high/(9-11x2-3) (8-13x2-3)/—/15x1.5

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Phomopsis species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/Conidiophores (microns)</u> <sup>b</sup>
pseudacaciae (Sacc.)Hohn. 1906 (anamorph of <u>Diaporthe fasciculata</u> = <u>D. oncostoma</u> ; basionym for <u>P. oncostoma</u> and <u>P. petiolorum</u> )  ref. 3, 49, 50, 74, 90, 117, 139, 178, 179, 200, 221, 242	<u>Robinia pseudacacia</u> <u>Robinia sp.</u>	(@ 1000) (to greater than 1000)wide/ (8-10x2.5-3) (10-16x2.5-3)/ (20-22[24]xl) (15-20[longer]xl-1.5)/ (20xl) (20-22xl) (20 long) (20-24xl) (slightly longer than alpha-conidia)
psoraleae Bubak 1906 (anamorph of <u>Diaporthe psoraleae-bituminosae</u> ?= <u>D. arctii</u> ; ?synonym <u>P. arctii</u> )  ref. 22, 23, 147, 193, 242	<u>Psoralea bituminosa</u>	180 long/5.5-9x2-3/20-25xl-1.5/ to 12 long
pterocarpi Hughes 1953 (teleomorph unknown)  ref. 94	<u>Pterocarpus erinaceus</u>	200 diam.x100 high/6-9x2.5-3/—/10x2-3
rhynchosiae Nov. in Bond.-Mont. et al. 1936 (teleomorph unknown)  ref. 19	<u>Rhynchosia sp.</u>	250-300 diam./5.7-8.5x2-3/—/17-43x2

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Phomopsis species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/Conidiophores (microns)</u> <sup>b</sup>
rossiana (Sacc.) Sacc. in Sacc. & D. Sacc. 1906 (anamorph of <u>Diaporthe woodii</u> ; synonym of <u>P. leptostromiformis</u> )  ref. 122, 160	Lupinus albus L. digitatus Lupinus sp.	300-500 diam./8x2.5/—/11-16x1.5-2
rudis (Sacc.) Hohn. 1906 (anamorph of <u>Diaporthe rudis</u> = <u>D. medusaea</u> )  ref. 3, 49, 50, 70, 74, 90, 91, 117, 139, 170, 178, 200, 221, 227, 242, 249	Cytisus laburnum C. nigricans C. sessifolius (as C. sessiflorus) Cytisus sp. Laburnum alpinum (as Cytisus alpinus) L. vulgare Laburnum sp.	(to 600 diam.) (to 500 wide) / (6-7x2) (6.5x2) (6-8x2-2.5) (7-9x2) (7-9x2-2.5) / (21-30x1.5) (21-30x0.5) / (20-30x1.3) (21-30x1.3) (20-24x1-1.5) (21-30x1.5) (20 long) (20-30x1-1.5)
sarothamni (Sacc.) Hohn. 1906 (anamorph of <u>Diaporthe sarothamni</u> ; synonym of <u>P. spartii</u> )  ref. 3, 49, 70, 73, 74, 90, 139, 163, 178, 179, 200, 242	Cytisus scoparius (as <u>Sarothamnus scoparius</u> or <u>Spartium scoparium</u> ) Sarothamnus sp. Spartium junceum	(300-600 diam.) (420-450x105) / (8-10[12]x2) (8-12x2) (8-12x2-2.5) ([4.5]6-9x2-3) / (18x1) (15-27x1.5-2) (30-33x1) / (30x1) (15x1) (15-20x1-1.5)

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<i>Phomopsis</i> species <sup>a</sup>	Legume host(s)	Pycnidia/alpha-conidia/Beta-conidia/ Conidiophores (microns) <sup>b</sup>
seposita (Sacc.) Trav. 1906 (anamorph of <i>Diaporthe seposita</i> = <i>D. eres</i> ; ?synonym of <i>P. oblonga</i> )  ref. 3, 5, 169, 173, 178, 179 221, 242	<i>Hardenbergia monophylla</i> (as <i>Glycine violacea</i> ) <i>Wisteria sinensis</i> (as <i>Wistaria chinensis</i> or <i>W. sinensis</i> )	—/(6x3) (7-10x3) (10x3) (5-7x2.5-3)/ (22x1)/(20-24x1-2) (10-24x1-2)
serebrianikowii (Bubak) Hohn. 1917b (anamorph of <i>Diaporthe caraganae</i> ; ?basionym for <i>P. caraganae</i> )  ref. 25, 92	<i>Caragana arborescens</i>	—/—/15-23x2/20-40[longer]x1.5-2
sojae Lehm. 1922 (anamorph of <i>Diaporthe phaseolorum</i> var. <i>sojae</i> )  ref. 7, 10, 12, 96, 105, 111, 115, 116, 119, 202, 242, 246	<i>Alysicarpus vaginalis</i> <i>Arachis hypogaea</i> <i>Canavalia ensiformis</i> <i>Glycine max</i> (also as <i>Soja max</i> ) <i>Lathyrus latifolius</i> <i>L. sylvestris</i> <i>Lens culinaris</i> <i>Lespedeza striata</i> <i>Lespedeza</i> spp. <i>Lotus corniculatus</i> <i>Lupinus hirsutus</i> <i>Melilotus alba</i>	(165-278.7-472x59-136.8-213) (82-375x82-225) (95-240-598x82-185-408) (112-542x98-385) (50-260-320x60-200-320)/ (6.1-8.1-10.6x1.9-2.9-3.5) (7.5x3) (6.27-7.15x2.18-2.31) (4.9-9.8x1.8-3.2) (4.5-7.3-9.8x1.1-2.7-3.9) (4.8-6.8-11x2-2.3-2.8)/(16.6x1.6) (7.5-16.2-21.8x0.9-1.5-1.8) (9-14.3-21x0.8-1.3-1.8) (14.1-35.1x1.2-1.7) (12-16-27x0.8-1.1-1.8)/1.5-3 times alpha-conidia length

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; #- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Phomopsis species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/ Conidiophores (microns)</u> <sup>b</sup>
sojae (continued)	Phaseolus acutifolius P. coccineus P. helvolus (as Strophostyles helvola) P. limensis P. lunatus P. vulgaris Pisum sativum Sesbania exaltata Trifolium pratense Trigonella foenum-graecum Vicia faba Vigna angularis V. radiata var. radiata V. unguiculata subsp. unguiculata (as V. sinensis)	

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<i>Phomopsis</i> species <sup>a</sup>	Legume host(s)	<i>Pycnidia</i> / <i>alpha-conidia</i> / <i>Beta-conidia</i> / <i>Conidiophores</i> (microns) <sup>b</sup>
sophorae (Sacc.) Trav. 1906 (anamorph of <i>Diaporthe</i> sophorae)  ref. 3, 48, 49, 50, 70, 74, 117, 118, 169, 179, 187, 221, 242	Sophora japonica S. japonica var. pendulae Sophora sp.	(250) (180-480) (to 500) (750) (200-400) diam./ (8-10x3.5-4) (6-10x3-4) (8-11x2-2.5) (8-9x2.5-3) (7.5-10x2.25-3)/ 25x0.5/(25x0.5) (15-20x2) (13-15.5x1-1.5) (25x1) (usw. 20 long)
sparsa Trav. & Spes. 1910 (anamorph of <i>Diaporthe</i> sparsa)  ref. 138, 180, 222	Hardenbergia monophylla (as <i>Glycine</i> violacea)	—/12-15x4-5/—/—
spartii (Sacc.) Bubak 1906 (?anamorph of <i>D. sarothamni</i> ; basionym for <i>P. sarothamni</i> )  ref. 3, 19, 22, 23, 49, 50, 171, 179, 242	Cytisus scoparius (as <i>Spartium</i> scopar or <i>S. scoparium</i> ) <i>Spartium junceum</i> <i>Spartium</i> sp.	(333x200) (420) diam. (to 750 diam. x 350 high)/(10-11x2-2.5) (7-8.5x2.8-3)/—/(20-22x1.5-2) (20-22x1-1.5) (15-20 long)
stromatigena Maire 1917b (anamorph of <i>Diaporthe</i> lirellaeformis = <i>D. eumorpha</i> )  ref. 121	Astragalus lusitanicus (as <i>Erophaca baetica</i> )	800-1100x600-800/6-8x1.5-2/—/ 17-25x1-1.5

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.



Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

<i>Phomopsis</i> species <sup>a</sup>	Legume host(s)	Pycnidia/alpha-conidia/Beta-conidia/ Conidiophores (microns) <sup>b</sup>
subcircinata (Ellis & Everh.)Hart. 1917 (anamorph of <i>Diaporthe phaseolorum</i> var. <i>phaseolorum</i> ; synonym of <i>P. phaseoli</i> [Desm.]Sacc.)  ref. 54, 79, 82, 242	<i>Melilotus alba</i> (sterile) <i>Phaseolus lunatus</i> <i>Phaseolus</i> sp.	(158-245.86-475) (197.5-219-260) (70-90) diam./ (5.6-7.82-10x2.4-3.11-4) (6-7.5-8.6x2.4-3.23-4.1) (5-6x2-2.5)/ (20.6-32.44-54.4x1.38-2-2.4) (11.7-22.83-31x1.4-1.73-2)/ (1.5-3 times alpha-conidia length) (longer than alpha-conidia)
swainsoniae (P. Henn.)Died. 1911 (teleomorph unknown)  ref. 3, 49, 50, 83, 192	<i>Swainsona fernandi</i> (as <i>Swainsonia fernandi</i> ) <i>Swainsona</i> sp. (as <i>Swainsonia</i> sp.)	@ 120-150 diam./7-10x3.5-4/—/ 12-15 long
tabernaemontanae Ponn. & Nag Raj 1974 (teleomorph unknown)  ref. 157	<i>Gliricidia</i> sp. (sterile)	—/7-9.5x2.5-4/24-28x1.5-2.5/—
templetoniae (P. Henn.)Died. 1911 (teleomorph unknown)  ref. 3, 49, 50, 83, 192	<i>Templetonia glauca</i> <i>Templetonia</i> sp.	—/6-8x3-4/—/as long as alpha-conidia
tephrosiae Chowdh. 1967 (teleomorph unknown)  ref. 32	<i>Tephrosia purpurea</i>	to 135(108 ave.)diam./ 2.6-4.2-5.3x1.3-2-2.7/—/—

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

<u>Phomopsis species<sup>a</sup></u>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/ Conidiophores (microns)<sup>b</sup></u>
teramni Hasijs 1963 (teleomorph unknown)  ref. 81	Teramnus labialis	60-155 diam./6.6-11.6x5-9.2/—/small
thermopsidis Buchw. in Moll. 1958 (teleomorph unknown)  ref. 127	Thermopsis fabacea	140-150x75-100/6-7x2/—/—
tipuanae (Tassi) Lucas & Camara 1952 (teleomorph unknown)  ref. 118, 192, 215	Tipuana tipu (as T. speciosae)	(333-500 diam.) (180-300x80-100)/ (6-7x2-2.5) (6.5-8x2.5-3)/—/ 13-15.5 long
tulasnei (Sacc.) Sacc. in Sacc. & D. Sacc. 1906 (anamorph of Diaporthe tulasnei = D. arctii; ?synonym of P. arctii)  ref. 49, 74, 182, 221, 242	Medicago sp. Melilotus sp.	200 diam./ (7-8x2.5-3) (7-8x2-2.5) (10-11x2-2.5) (7-8x2)/—/15-18x1.5
vaccinii Shear 1931 (anamorph of Diaporthe vaccinii)  ref. 204, 242	Melilotus sp. (sterile)	300-500 to 1000-2000 diam./6-11x2-5/ 14-20x0.35/15-25 long or longer

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

<u>Phomopsis species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/</u> <u>Conidiophores (microns)</u> <sup>b</sup>
viciae Bubak in Bubak & Kabat 1915 (teleomorph unknown)  ref. 26, 224	Vicia sepium	150-220 diam./7-9.5x3-4/—/8-15x4
winteri Petr. 1919 (anamorph of Diaporthe winteri = D. arctii; ?synonym of P. arctii)  ref. 145	Ononis sp.? (host not named; only called "substrate")	@ 250-400 diam./10-14x2-3/20-28x1/ 15-20x1-1.5

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

Unnamed Anamorphs of Diaporthe Species

<u>Diaporthe species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/alpha-conidia/Beta-conidia/Conidiophores (microns)</u> <sup>b</sup>
chrysoides (Tul.) Sacc. 1882 (anamorph ?= <u>Phomopsis inaequalis</u> )  ref. 178, 228, 242	Cytisus laburnum	—/(@ 3.5 long) (3-4 long)/—/30 long
genisticola Rehm 1892 (anamorph ?= <u>Phomopsis oblonga</u> )  ref. 242	Genista tinctoria	—/6-7x2-2.5/—/—
phaseolorum (Cooke & Ellis) Sacc. 1882 var. caulivora Athow & Cald. 1954 (originally described without an anamorph)  ref. 66, 87, 105	Glycine max Medicago sativa Melilotus alba Phaseolus vulgaris Pisum sativum Trifolium incarnatum T. pratense T. repens	(198-528 diam.) (90-200-290x80-170-310)/ (6x2.8) (5.2-7.5x2.2-2.8) (2.4-8.3x1.5-3.9)/16-19-30x1.3-1.4-2/ —; "C"-conidia: 16.3x2.1

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. Diaporthe and Phomopsis species reported on legume hosts, continued.

Anamorphs of <u>Diaporthe</u> Species in Genera Other Than <u>Phomopsis</u>		
<u>Anamorph species</u> <sup>a</sup>	<u>Legume host(s)</u>	<u>Pycnidia/Conidia/Conidiophores</u> <u>(microns)</u> <sup>b</sup>
<u>Phoma galegae</u> Thum. 1880 (anamorph of <u>Diaporthe tulasnei</u> forma <u>galegae</u> )  ref. 3, 179, 217, 248	<u>Galega officinalis</u>	—/6-7x3-3.5/—

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

Appendix A. *Diaporthe* and *Phomopsis* species reported on legume hosts, continued.

Unnamed *Phomopsis* Species Not Associated with a Teleomorph

<u>Researcher<sup>a</sup></u>	<u>Legume Host(s)</u>	<u>Pycnidia/Alpha-conidia/Beta-conidia/ Conidiophores (microns)<sup>b</sup></u>
Kmetz 1975 ref. 105	Glycine max	50-220-290x55-190-300/ 4.5-6.9-11x2-2.3-3/—/—
Bondartzeva-Monteverde et al. 1936 ref. 19	Myroxylon toluiferum	140 diam./6-8.5x2-2.5/—/15-20 long

<sup>a</sup>? = possibly; ref. = reference[s].

<sup>b</sup>— = absent, not observed, or not reported; [#] = rare measurement; -#- = average measurement; ! = sic.

## APPENDIX B

Stepwise Multiple Regression Equations for Storage Time  
and Temperature Study.

Appendix B. Stepwise Multiple Regression Equations for Storage Time and Temperature Study.

Abbreviations: P=% Phomopsis longicolla; DPC=% Diaporthe phaseolorum var. caulivora; DPS=% D. phaseolorum var. sojae;  
 TSD=Total % Seed Decay fungi;  
 TMM=Total % Miscellaneous Mycota;  
 TSM=Total % Seedborne Mycota;  
 Temp=Storage Temperature;  
 Time=Length of Storage;  
 \* = interaction of independent variables.

Average Values of Microorganism Groups Over All Times and Temperatures Used to Simplify Regression Equations.

Microorganism Group	High	Medium	Low	Combined
P	21.3	11.3	2.2	11.6
DPC	1.3	1.1	0.4	0.9
DPS	0.7	0.7	0.3	0.5
TSD	23.2	12.8	2.9	13.0
TMM	12.1	10.9	10.0	11.0
TSM	35.4	23.8	12.9	24.0

<sup>a</sup> See Chapter II.



## 1) 4-DAY GERMINATION

$$\begin{aligned} \text{All Lots: GERM4} = & 89.11 - 2.48 P + .02 P^2 + .16 P \cdot \text{Time} \\ & + .20 P \cdot \text{Temp} - 1.27 \text{Time} \cdot \text{Temp} - .91 \text{Temp}^2 \\ & - .12 \text{Time} \cdot \text{TMM} + .01 \text{TMM} \cdot \text{TSM} - .46 \text{DPC} \cdot \text{DPS} \end{aligned}$$

$$(R = .74; R^2 = .53)$$

$$\text{High P Lot: GERM4} = 51.61 - .35 \text{TSD} + .01 \text{TMM}^2 + .05 \text{TSD} \cdot \text{Time}$$

$$(R = .36; R^2 = .12)$$

$$\text{Medium P Lot: GERM4} = 70.35 - 2.61 \text{Temp} - .02 P^2$$

$$(R = .31; R^2 = .09)$$

$$\text{Low P Lot: GERM4} = 84.31 - .40 P \cdot \text{Temp} - 1.65 \text{Time} \cdot \text{Temp}$$

$$(R = .61; R^2 = .37)$$

## 2) STRONG SEEDLINGS, SEVEN DAYS

$$\begin{aligned} \text{All Lots: STRONG} = & 58.68 + 10.16 \text{Time} - 1.69 \text{Time}^2 - 1.66 P \\ & - .03 P^2 - 24.00 \text{Temp} + 4.37 \text{Temp}^2 \\ & + .15 \text{TSD} \cdot \text{Temp} - .01 \text{TSD} \cdot \text{TSM} \end{aligned}$$

$$(R = .65; R^2 = .41)$$

$$\begin{aligned} \text{High P Lot: STRONG} = & 6.93 + 14.53 \text{Time} - 1.53 \text{Time}^2 \\ & + .003 P^2 + .24 \text{DPC}^2 - .09 P \cdot \text{DPC} \\ & - .89 \text{Time} \cdot \text{Temp} - .10 \text{TSD} \cdot \text{Time} \end{aligned}$$

$$(R = .58; R^2 = .32)$$

$$\begin{aligned} \text{Medium P Lot: STRONG} = & 36.62 + .70 \text{Temp}^2 + 1.53 \text{DPC} \cdot \text{DPS} \\ & - .26 \text{Temp} \cdot \text{TSM} \end{aligned}$$

$$(R = .45; R^2 = .19)$$

$$\begin{aligned} \text{Low P Lot: STRONG} = & 61.78 - 18.01 \text{Temp} + 3.30 \text{Temp}^2 \\ & + 1.16 \text{DPC}^2 \end{aligned}$$

$$(R = .40; R^2 = .15)$$

## 3) WEAK SEEDLINGS, SEVEN DAYS

$$\text{All Lots: WEAK} = 50.97 - 6.45 \text{ Time} + .55 \text{ Time}^2 - .02 \text{ P}^2 \\ + .09 \text{ TSD} * \text{Time} + .11 \text{ Temp} * \text{TSM}$$

$$(R = .46; R^2 = .20)$$

$$\text{High P Lot: WEAK} = 39.23 - .01 \text{ P}^2 + .07 \text{ P} * \text{DPC} + .10 \text{ Temp} * \text{TSM}$$

$$(R = .54; R^2 = .28)$$

$$\text{Medium P Lot: WEAK} = 27.69 + 25.27 \text{ Temp} - 6.81 \text{ Temp}^2 - 2.31 \text{ Time} \\ - .02 \text{ TSD}^2 + .21 \text{ Temp} * \text{TMM} + .22 \text{ TSD} * \text{Temp}$$

$$(R = .58; R^2 = .32)$$

$$\text{Low P Lot: WEAK} = 52.27 - 4.34 \text{ Time} - 2.28 \text{ DPC}^2 + .21 \text{ DPC} * \text{TSM} \\ - .11 \text{ P} * \text{TMM} + .91 \text{ P} * \text{Temp}$$

$$(R = .58; R^2 = .32)$$

## 4) NORMAL SEEDLINGS, SEVEN DAYS

$$\text{All Lots: NORMAL} = 88.82 - 1.98 \text{ P} + 4.50 \text{ Time} - .96 \text{ Time}^2 \\ - 1.34 \text{ Time} * \text{Temp} + .19 \text{ P} * \text{Temp} + .17 \text{ P} * \text{Time} \\ + .01 \text{ P} * \text{TSM} - .35 \text{ DPC} * \text{DPS}$$

$$(R = .74; R^2 = .55)$$

$$\text{High P Lot: NORMAL} = 65.54 - .01 \text{ P}^2 - .07 \text{ DPS} * \text{TMM} \\ + .10 \text{ Time} * \text{TMM} + .04 \text{ Temp} * \text{TSM}$$

$$(R = .53; R^2 = .28)$$

$$\text{Medium P Lot: NORMAL} = 81.04 - .04 \text{ P}^2 .42 \text{ Time}^2 \\ + .09 \text{ TSD} * \text{Time}$$

$$(R = .47; R^2 = .21)$$

$$\text{Low P Lot: NORMAL} = 81.53 + 10.69 \text{ Temp} - 2.01 \text{ Temp}^2 \\ - 2.01 \text{ Time} * \text{Temp}$$

$$(R = .72; R^2 = .51)$$

## 5) ABNORMAL SEEDLINGS, SEVEN DAYS

$$\text{All Lots: ABNORMAL} = 8.01 + .001 \text{ TSM}^2 - .01 \text{ Time} * \text{TSM} \\ - .05 \text{ Temp} * \text{TSM} + 1.22 \text{ Time} * \text{Temp}$$

$$(R = .63; R^2 = .40)$$

$$\text{High P Lot: ABNORMAL} = 8.56 + 1.16 \text{ Time} * \text{Temp} - .11 \text{ Temp} * \text{TMM}$$

$$(R = .67; R^2 = .44)$$

$$\text{Medium P Lot: ABNORMAL} = 5.99 + .01 \text{ TMM}^2 + .12 \text{ P} * \text{DPS} \\ - 1.39 \text{ DPS} * \text{Temp} + 1.11 \text{ Time} * \text{Temp}$$

$$(R = .58; R^2 = .32)$$

$$\text{Low P Lot: ABNORMAL} = 7.15 - .30 \text{ TSD} * \text{Temp} + 1.36 \text{ Time} * \text{Temp}$$

$$(R = .70; R^2 = .48)$$

## 6) DEAD SEEDLINGS, SEVEN DAYS

$$\begin{aligned} \text{All Lots: DEAD} = & -1.92 + 1.92 P - .17 \text{DPC}^2 + .30 \text{Time}^2 \\ & - .17 P*\text{Temp} + .04 \text{Temp}*\text{TSM} + .36 \text{DPC}*\text{Temp} \\ & - .17 P*\text{Time} - .01 P*\text{TSM} + .06 \text{DPS}*\text{TMM} \end{aligned}$$

$$(R = .86; R^2 = .74)$$

$$\begin{aligned} \text{High P Lot: DEAD} = & 11.05 + 1.29 P - .68 \text{Temp}^2 - .13 P*\text{Time} \\ & + .06 \text{DPS}*\text{TMM} - .01 \text{TSD}*\text{TSM} \end{aligned}$$

$$(R = .82; R^2 = .66)$$

$$\text{Medium P Lot: DEAD} = 4.87 + .95 P - .11 P*\text{Temp}$$

$$(R = .68; R^2 = .46)$$

$$\begin{aligned} \text{Low P Lot: DEAD} = & 2.84 + .10 \text{TMM} - 2.29 \text{Time} + .53 \text{Time}^2 \\ & + .20 \text{Time}*\text{Temp} + .06 P*\text{TSD} \end{aligned}$$

$$(R = .60; R^2 = .34)$$

## 7) TOTAL SEEDLINGS, SEVEN DAYS

$$\begin{aligned} \text{All Lots: TSEED} = & 101.83 - 1.91 P + .01 P^2 + .20 \text{DPC}^2 \\ & - .30 \text{Time}^2 + .17 P*\text{Time} + .13 P*\text{Temp} \\ & + .01 P*\text{TMM} - .05 \text{Temp}*\text{TMM} - .37 \text{DPC}*\text{Temp} \\ & - .05 \text{DPS}*\text{TMM} \end{aligned}$$

$$(R = .86; R^2 = .74)$$

$$\begin{aligned} \text{High P Lot: TSEED} = & 88.97 - 1.30 P + .68 \text{Temp}^2 + .14 P*\text{Time} \\ & - .06 \text{DPS}*\text{TMM} + .01 \text{TSD}*\text{TSM} \end{aligned}$$

$$(R = .82; R^2 = .66)$$

$$\text{Medium P Lot: TSEED} = 95.18 - .95 P + .11 P*\text{Temp}$$

$$(R = .68; R^2 = .46)$$

$$\begin{aligned} \text{Low P Lot: TSEED} = & 97.11 - .10 \text{TMM} + 2.27 \text{Time} - .53 \text{Time}^2 \\ & - .19 \text{Time}*\text{Temp} - .06 P*\text{TSD} \end{aligned}$$

$$(R = .59; R^2 = .34)$$

## 8) SPEED OF GERMINATION (= GERM4/4 + NORMAL/7)

$$\begin{aligned} \text{All Lots: SPEED} = & 34.20 - .85 P + .01 P^2 + 1.18 \text{ Time} \\ & - .28 \text{ Time}^2 - .24 \text{ Temp}^2 - .46 \text{ Time*Temp} \\ & + .05 P*\text{Time} + .07 P*\text{Temp} - .13 \text{ DPC*DPS} \end{aligned}$$

$$(R = .77; R^2 = .59)$$

$$\begin{aligned} \text{High P Lot: SPEED} = & 22.63 - .21 P + .01 \text{ TMM}^2 + .03 P*\text{Time} \\ & + .02 \text{ TSD*Temp} \end{aligned}$$

$$(R = .48; R^2 = .22)$$

$$\text{Medium P Lot: SPEED} = 28.54 - .01 P^2 - .18 \text{ Time*Temp}$$

$$(R = .36; R^2 = .12)$$

$$\text{Low P Lot: SPEED} = 34.37 - .21 P - .68 \text{ Time*Temp}$$

$$(R = .69; R^2 = .47)$$

## 9) AVERAGE DRY WEIGHT OF SEEDLINGS, SEVEN DAYS

$$\begin{aligned} \text{All Lots: AVGWT} = & 51.61 + .91 \text{ Temp} + .02 \text{ DPS}^2 + .01 \text{ DPC*TSM} \\ & - .30 \text{ Time*Temp} + .04 \text{ Time*TMM} - .01 \text{ TMM*TSM} \end{aligned}$$

$$(R = .34; R^2 = .11)$$

$$\begin{aligned} \text{High P Lot: AVGWT} = & 53.82 + .02 P*\text{DPC} - .01 P*\text{TMM} \\ & - .17 \text{ Time*Temp} \end{aligned}$$

$$(R = .46; R^2 = .21)$$

$$\begin{aligned} \text{Medium P Lot: AVGWT} = & 52.32 - .01 \text{ TMM}^2 + .05 P*\text{Temp} - .04 \text{ DPC*TMM} \\ & - .22 \text{ Time*Temp} + .03 \text{ Time*TMM} \end{aligned}$$

$$(R = .42; R^2 = .16)$$

$$\text{Low P Lot: AVGWT} = 51.57 + .05 \text{ TSD}^2 - .02 P*\text{TMM}$$

$$(R = .26; R^2 = .06)$$

10) PHOMOPSIS ISOLATION PERCENTAGE

$$\text{All Lots: } \text{PHO} = 14.91 + 3.31 \text{ Time} - .65 \text{ Time}^2 - 1.02 \text{ Time*Temp}$$

$$(R = .43; R^2 = .18)$$

$$\text{High P Lot: } \text{PHO} = 28.16 + 6.13 \text{ Time} - 1.34 \text{ Time}^2 - 1.76 \text{ Time*Temp}$$

$$(R = .78; R^2 = .60)$$

$$\text{Medium P Lot: } \text{PHO} = 17.72 - 1.07 \text{ Time*Temp}$$

$$(R = .64; R^2 = .41)$$

$$\text{Low P Lot: } \text{PHO} = 3.32 - .19 \text{ Time*Temp}$$

$$(R = .31; R^2 = .09)$$

11) DIAPORTHE PHASEOLORUM VAR. CAULIVORA ISOLATION PERCENTAGE

$$\text{All Lots: } \text{DPC} = 1.26 + .10 \text{ Time} - .10 \text{ Time*Temp}$$

$$(R = .25; R^2 = .06)$$

$$\text{High P Lot: } \text{DPC} = 1.92 - .10 \text{ Time*Temp}$$

$$(R = .25; R^2 = .06)$$

$$\text{Medium P Lot: } \text{DPC} = 2.06 - .31 \text{ Temp} - .07 \text{ Time*Temp}$$

$$(R = .39; R^2 = .15)$$

$$\text{Low P Lot: } \text{DPC} = .59 - .03 \text{ Time*Temp}$$

$$(R = .15; R^2 = .02)$$

12) DIAPORTHE PHASEOLORUM VAR. SOJAE ISOLATION PERCENTAGE

$$\text{All Lots: } \text{DPS} = .63 - .04 \text{ Time}^2 - .10 \text{ Time*Temp}$$

$$(R = .24; R^2 = .05)$$

$$\text{High P Lot: } \text{DPS} = 1.63 - .44 \text{ Temp}$$

$$(R = .23; R^2 = .05)$$

12) DIAPORTE PHASEOLORUM VAR. SOJAE ISOLATION PERCENTAGE (cont.)

Medium P Lot:  $DPS = .20 + .37 \text{ Time} - .13 \text{ Time*Temp}$

$$(R = .34; R^2 = .11)$$

Low P Lot: No regression steps (y-intercept = .267)

## 13) TOTAL SEED DECAY FUNGI (= P + DPC + DPS) ISOLATION PERCENTAGE

All Lots:  $TSD = 20.59 - 1.27 \text{ Time*Temp}$

$$(R = .42; R^2 = .18)$$

High P Lot:  $TSD = 30.79 + 6.25 \text{ Time} - 1.36 \text{ Time}^2 - 1.90 \text{ Time*Temp}$

$$(R = .80; R^2 = .64)$$

Medium P Lot:  $TSD = 20.01 - 1.20 \text{ Time*Temp}$

$$(R = .65; R^2 = .42)$$

Low P Lot:  $TSD = 4.19 - .22 \text{ Time*Temp}$

$$(R = .33; R^2 = .11)$$

## 14) TOTAL MISCELLANEOUS MYCOTA ISOLATION PERCENTAGE

All Lots:  $TMM = 15.45 - .75 \text{ Time*Temp}$

$$(R = .42; R^2 = .18)$$

High P Lot:  $TMM = 16.47 - .72 \text{ Time*Temp}$

$$(R = .44; R^2 = .19)$$

Medium P Lot:  $TMM = 15.83 - .82 \text{ Time*Temp}$

$$(R = .47; R^2 = .22)$$

Low P Lot:  $TMM = 14.34 - .72 \text{ Time*Temp}$

$$(R = .37; R^2 = .14)$$

## 15) TOTAL SEEDBORNE MYCOTA ISOLATION PERCENTAGE

All Lots:  $TSM = 36.16 - 2.02 \text{ Time*Temp}$

$(R = .53; R^2 = .28)$

High P Lot:  $TSM = 56.65 - .73 \text{ Time}^2 - .92 \text{ Temp}^2$   
 $- 1.49 \text{ Time*Temp}$

$(R = .80; R^2 = .63)$

Medium P Lot:  $TSM = 35.91 - 2.03 \text{ Time*Temp}$

$(R = .78; R^2 = .61)$

Low P Lot:  $TSM = 18.52 - .93 \text{ Time*Temp}$

$(R = .42; R^2 = .17)$