SOYBEAN SEED DECAY: STUDIES ON DISEASE CYCLES, EFFECTS OF CULTURAL PRACTICES ON DISEASE SEVERITY AND DIFFERENTIATION OF THE PATHOGENS PHOMOPSIS SP., DIAPORTHE PHASEOLORUM VAR. SCJAE AND D. PHASEOLORUM VAR. CAULIVORA.

DISSERTATION

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

by

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****

The Ohio State University

1975

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TO MOM AND DAD, YOUR LOVE AND PRAYERS HAVE
HELPED ME REACH MY GOAL. GOD BLESS YOU ALWAYS.
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God bless my wife, Marilyn, for love, patience and understanding throughout this investigation.
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INTRODUCTION

Soybean (Glycine max (L.) Merr.) seed quality is affected by species of Phomopsis and by Diaporthe phaseolorum in Ohio and other major soybean growing areas (2, 11, 27, 28, 30, 34, 36, 48, 49). Seed infection is more prevalent in wet than in dry years (30, 56) but the pathogens are associated yearly with above-ground parts of immature soybeans and may sporulate on mature plants (2, 11, 21, 30, 31, 54).

Since Diaporthe was first associated with soybean seed (30,) most studies have centered on the relationship between Diaporthe phaseolorum (Cke. & Ell.) var. sojae (Lehman) and mature seed or seedlings and senescent plants (6, 11, 12, 14, 22, 26, 34, 36, 37, 48, 49, 55, 56). However, the author recently isolated a species of Phomopsis and D. phaseolorum var. caulivora (Ahow and Caldwell) in addition to D. phaseolorum var. sojae from poor quality seed in Ohio (28). All three fungi were associated with immature as well as mature plants and seed. This study presents additional evidence for the relationship of Phomopsis and Diaporthe with immature plants and seed. The work centers on the disease cycle and effects of culture practices commonly used in Ohio soybean production on the incidence of Phomopsis and Diaporthe. Also, the differentiation of the species of Phomopsis and Diaporthe pathogenic on soybean seed is examined.
PART I

SOYBEAN SEED DECAY: PATHOGENS INVOLVED
AND DISEASE CYCLE
INTRODUCTION

*Diaporthe phaseolorum* (Cke. & Ell.) var. *sojeae* (Lehman) (Dps) causes pod and stem blight of soybean (*Glycine max* (L) Merr.) and is involved in lowering seed quality in several major soybean areas (2, 11, 27, 28, 30, 34, 36, 48, 49). Reports on seed infection focus on the relationship between Dps and mature plants and seed (2, 8, 21, 22, 25, 30, 31, 34, 36, 48, 49). In these studies isolates forming only the *Phomopsis* stage were commonly recovered but were considered heterothallic and named Dps. Recently, the widespread occurrence of *Phomopsis* sp. and *Diaporthe* on immature soybeans was reported (28) along with evidence for separating seed isolates into *Phomopsis* sp., Dps and *D. phaseolorum* var. *cauliypora* (Dpc)(Athow and Caldwell). The latter study (28) suggested a need for additional investigations on the association of *Phomopsis* sp., Dps and Dpc with soybeans.

This study further examines *Phomopsis* and *Diaporthe* isolates from soybean and their association with plants and seed. The report includes: 1) additional evidence of widespread occurrence and frequency of *Phomopsis* and *Diaporthe* in immature plants and seed, 11) frequency of the fungi in mature seed and the relationship of seed infection to plant symptoms and seed quality, 111) the source of inoculum and spread of infective propagules during plant growth and iv) spread of the pathogens within immature plants.
MATERIALS AND METHODS

Field plot design

Soybean varieties Amsoy 71, Wayne and Calland were planted on May 17, 1973 and May 10, 1974 at The Ohio State University Farm Science Review area. Seed were relatively Phomopsis-free. Each variety was replicated four times in each of 2 blocks in a randomized block design with an individual replication consisting of 3 rows 9.3 m long and 75 cm apart. The block had been cropped to soybeans for two years prior to 1973. In one experiment Wayne soybeans were planted in a block previously cropped to corn. The same block was also used in the 1974 tests.

Studies on immature plants and seed

Plant parts sampled were treated as follows before plating or incubation in moist chambers. Vegetative material and mature seeds were thoroughly rinsed in tap water, soaked for 1 minute in 70% ethanol, agitated in 1.3% sodium hypochlorite (NaClO) for 2 minutes to eliminate surface organisms, then air dried on sterile paper towels. One cm cross sections of cotyledons, hypocotyls, stems, pods and petioles, and entire flowers, ovules and seeds were plated on Difco potato dextrose agar, acidified to pH 4.5 with 85% lactic acid (PDA-L). Ovules and immature seeds were removed from NaClO-soaked pods and dipped in 1.3% NaClO for 5 sec before plating. Incubation period and identification of Phomopsis or Diaporthe on PDA-L
were made as described previously (28). Growth stages indicated are based on a key developed by Fehr et al. (15).

During 1973 and 1974 seedlings were sampled daily beginning 48 hours after planting and continuing until emergence (10 days). In the first experiment, PDA-L was used for plating. However, high populations of Pythium spp. masked the presence of Phomopsis and Diaporthe. Subsequently, PDA-L was amended with 50 mg/l ethazole (5-ethoxy-3-trichloromethyl 1, 2, 4-thiadiazole) 30% WP) added prior to pouring plates to control Pythium.

Five plants per replication in 3 varieties growing in two cropping rotations were sampled weekly in 1973 and at the following stages in 1974: i) the first trifoliate (V1 according to Fehr et al. (15), ii) full bloom, iii) lower pod green bean (R5) and iv) harvest maturity i.e., 95% of pods were brown (R8). A single 1 cm hypocotyl and cotyledon sections were plated. Stem and pod sections and whole flowers and seeds were sampled from top, middle and lower plant areas. During 1974 petioles were sampled weekly from top and bottom of plants. Also, 100 pycnidia from fallen petioles were plated on PDA-L.

One axil with an attached pod was removed from the top third of each of 5 Calland plants from each of 4 replications. After surface disinfection, the axil and pod were cut in half perpendicular to the pod sutures. The cut seed were removed and plated. Incidence of Phomopsis and Diaporthe growing from the peduncle, apeduncle, proximal, or distal sections of the pod was recorded.
In addition to isolation on PDA-L Phomopsis and Diaporthe were detected by a moist chamber incubation method (MCI). Cotyledons, pods, petioles, and 10 cm sections of hypocotyls and stems were surface disinfected and placed in plastic bags or petri dishes with wet paper toweling or filter paper, respectively. Bags or plates were incubated in stacks at 24-28 C. Positive identification of Phomopsis or Diaporthe was based on formation of pycnidia and presence of alpha spores. Classification of each pycnidium as Phomopsis sp. or Dps was not possible but frequent plating of randomly selected pycnidia on PDA-L gave a good indication of the predominant species present. In 1973 sampling began at stage V1 and in 1974 at emergence and continued through maturity in both years.

Studies on mature plants and seed

Frequency of Phomopsis sp., Dps and Dpc in mature seed, possible correlation between incidence of each fungus and plant symptoms, and effects on seed quality were examined in 1974. Plants and pods were hand-harvested and-shelled at stage R8, dry down (13-14% moisture, ca. 7-10 days after maturity), one month and two months after maturity. Each variety was tested at the four harvests as follows: 1) Seeds were soaked for 1 minute in 1.3% NaCl0, air dried and plated on FDA-L. Percentage Phomopsis sp., Dps and Dpc were recorded from 100 seed per replication. Also, frequency of the fungi in seed from proximal, middle and distal pod locations was obtained from 10 pods per replication; 2) Twenty-five stems and 100 pods per replication were divided into 4 categories:
1) Blotching only, ii) pycnidia only, iii) blotching and pycnidia and iv) no symptoms; 3) Percentage visible mold from 100 seed per replication was recorded; 4) Five plants per replication were placed in the four symptom groups described in (2) above, pods were selected from 10 nodes from top to the bottom of plants and seeds sampled from proximal, middle and distal pod sections; 5) Weight of one hundred seed was recorded per replication and 6) A standard germination test was performed on 100 seed per replication by the Ohio Seed Improvement Association. In addition, yield was determined from the 3 varieties at maturity.

**Inoculum studies: sources of inoculum**

During 1974 soybean stubble from the previous crop was examined weekly for viable pycnidia and perithecia beginning May 10 and continuing through maturity. As cotyledons and petioles dropped from the plants, the time required for pycnidia to form in the field and in moist chambers was recorded. At maturity a survey was made to determine percentage of fallen petioles bearing *Phomopsis*-type pycnidia.

Beginning in February and continuing into July, 1975, soybean stubble in the field was sampled weekly to determine when *Phomopsis* pycnidia and *Diaporthe* perithecia matured and how long pycnidiospores and ascospores were produced.

**Inoculum studies: spread in the field**

Soybean stubble bearing pycnidia and blotched areas was placed on the soil surface at the time of planting Wayne seed in soil previously cropped to corn. The stubble was spread over a 1 m square area at the center in the middle row of four
3 row plantings. At stages V1, V2, V4, R2, R4, R5, and R6 three plants were sampled from each replication from each of the following sites: i) debris site, ii) directly across in each adjacent row, iii) 1 to 2 m along the row in each direction, iv) 4 m along the row in each direction, v) in adjacent rows opposite site 3, and vi) site 20 m away from nearest debris plot to serve as control.

Inoculum spread was studied under controlled conditions on Corsoy soybeans in growth chambers. Seed were planted in wood boxes of steamed Wooster soil mix (WSM) containing Wooster silt loam, muck and Canadian peat (5:5:2 V/V). At stage V1 pycnidia-bearing petioles were spread at the base of plants. Two boxes containing 4 twelve plant rows were treated with: i) overhead sprinkling for 2 minutes, 3 times daily until maturity, ii) as (i) for 2 weeks, then surface watered as needed through maturity, iii) as (i) with debris removed after 2 weeks, iv) continuous surface watering as needed, v) as (iv) but also four, 3 minute simulated rainstorms (plants sprinkled from height of 12 m) every two weeks from stage V3 through maturity and vi) no debris, surface watering as needed. Plants were sampled every two weeks through maturity. The test was performed twice.

Distribution of Phomopsis-type spores on stems and pods in the field was examined during 1974. Three plants in 4 replications of Amsoy 71 were sampled at 14 dates from stages V1 through R8. A single cotyledon, lower, middle or top stem section and flower or pod was placed in 10 ml water plus Tween 20 in 250 ml flasks at the sample site.
Environmental conditions for the previous 72 hours were recorded. Flasks were shaken for 15 minutes at 150 rpm on a rotary shaker. The suspension was filtered by a vacuum pump through 5.0 micron Nuclepore filter (Nuclepore Membrane filters clear, plain 47 mm) and the filtrate passed through 0.22 micron Millipore filter (Millipore Filter Corp. white, plain 25 mm). The Millipore filter was stained with lactophenol cotton blue and observed under a light microscope for Phomopsis-type spores.

Spread of the pathogens in soybean plants

Several experimental attempts were made to determine the spread of *Phomopsis* and *Diaporthe* in soybeans at various growth stages. The toothpick tip method of Crall (7) was employed on stems and pods in the field and cotyledons, hypocotyls and stems in growth chambers. A modification of this method was used on seed (28). Field inoculations with 9, 6 and 4 isolates of *Phomopsis* sp., Dps and Dpe, respectively, were performed on variety Wayne in soil previously cropped to corn. Twenty-five pods from stages R3, R5 and R7 and ten stems 78-85 days old were inoculated. Inoculated parts and stem sections 10 cm above and below the inoculation point were plated on PDA-L 1 week after inoculation and at maturity. In the growth chamber, tests were performed on 2 sets of 5 plants in 4 replications of emerged, two-week old and three-week old Calland soybeans growing in WSM in clay pots. The plants were inoculated with a single *Phomopsis* sp. isolate in the hypocotyl and one cotyledon on separate plants.
One set was incubated at 50% relative humidity (RH) the other at 100% RH. Reisolation of *Phomopsis* sp. from inoculated parts and stem sections 10 cm above and below the inoculation point were attempted one and two weeks after inoculation.

Spore suspensions for field and growth chamber inoculation were prepared from 14-21 day-old *Phomopsis* sp. cultures and contained ca. 5,000 spores/ml water. Suspensions were applied with an atomizer. In the field, twenty-five Calland flowers or pods were sprayed with a single isolate of *Phomopsis* sp. or *Dps* in 4 replications at stages R1, R2, R3, R4, R5, R7 and R8. Pods were plated on PDA-L 2 weeks after inoculation. Growth chamber studies were conducted on Corsoy and Beeson soybeans growing in WSM in boxes. Plants were grown at 22-24 C day time and 16-18 C night time temperatures at 50-60% RH. A single isolate of *Phomopsis* sp. and *Dps* was employed in all tests. Spores were atomized onto cotyledons, hypocotyls and stems of 4 plants for each part in 3 replications (total 12 plants per part). Also, 5 petioles, axils, flowers, and pods on each of 5 plants in 3 replications (total 15 plants per part) were inoculated. In tests on both varieties inoculated parts were sampled one week after inoculation and at maturity. An exception was plants of Beeson inoculated at the base of main axis every two weeks; these plants were sampled only at maturity. Hypocotyls were sprayed at stage V4 and cotyledons at stages V1 and V4. Green petioles, leaves and axils were inoculated at stage V4 and yellow petioles and axils at stage V7.
Flowers were atomized at stage R2. At 75 days after planting in Corsoy and 71 days in Beeson, pods less than 2.54 cm, 2.54 cm to 3.81 cm long and full size were inoculated. Full size pods in both varieties were sprayed at stage R7. As plants approached maturity, particular note was made of premature ripening for all the inoculation times. Plants were harvested at 130 days in Corsoy and 123 days in Beeson and record made of pycnidia and blotching on stems and pods. Movement of Phomopsis sp. and Dps through moribund tissue was determined by plating out plant sections proximal and distal to inoculation points.

**Root infection**

Infection of roots and movement of Phomopsis and Dps from roots to the upper parts of plants was studied by planting Beeson soybeans in steamed WSM in clay pots infected with the fungi cultured on sterile pods. Randomly selected plants were removed bi-weekly and the roots and stems plated on PDA-L to determine presence of Phomopsis. The experiment was performed once. In a second experiment, Phomopsis and Diaporthe root infection of young seedlings was examined on variety Wayne growing in WSM in wood flats. Four 0.76 m rows in each flat were inoculated with ca. 5,000 spores/ml of a single isolate. Three isolates each of Phomopsis sp., Dps and Dpc were used. Percentage emergence was recorded and seedlings were selected one, two and three weeks after planting and plated on PDA-L. The experiment was repeated twice.

An analysis of variance was performed on all data and an LDS computed when F values were significant at .01 or .05 level.
RESULTS

Widespread distribution of Phomopsis and Diaporthe on immature plants.

Our preliminary investigations showed isolation of Phomopsis and Diaporthe from immature soybean plants beginning 4 weeks after planting (28). In the present work, Phomopsis sp., Dps or Dpc were not recovered from preemergent soybeans. In 1973, Phomopsis sp. was isolated from 12 day-old seedlings but was not recovered in 1974 until 26 days after planting. Diaporthe phaseolorum var. sojae was isolated in both years by stage V2 (30-33 days after planting) but Dpc was not isolated from immature plants until yellow leaf stage (R7). Phomopsis sp. was isolated significantly more often than Dps from plants at all growth stages (Table 1).

Our data reaffirm previous results on the widespread occurrence of Phomopsis on immature soybeans (28). Also, as reported previously (28), the frequency of Phomopsis was higher in stems than in green pods, and higher in green pods than in immature seed (Table 2). In addition, the frequency was higher in lower portions of the plant than the upper portions (Table 1). As plants approached maturity, differences in percentage Phomopsis isolated from lower and upper parts of the plant were less pronounced.
Table 1. Season-long presence of *Phomopsis* sp. and *Diaporthe phaseolorum* var. *sojae* (Dps) in developing soybean stems.

<table>
<thead>
<tr>
<th>Growth Stage&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Phomopsis sp.</th>
<th>Dps</th>
<th>LSD&lt;sup&gt;c&lt;/sup&gt;</th>
<th>LSD&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td>(.01)</td>
<td>Lower</td>
</tr>
<tr>
<td>Emergence</td>
<td>1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>V1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>V2</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>V3</td>
<td>44</td>
<td>24</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>V5</td>
<td>60</td>
<td>35</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>R1</td>
<td>76</td>
<td>24</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>R2</td>
<td>78</td>
<td>10</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>R2R3</td>
<td>100</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>R3</td>
<td>70</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>R3R4</td>
<td>84</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>R4</td>
<td>92</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>R4R5</td>
<td>78</td>
<td>4</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>R5</td>
<td>100</td>
<td>30</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>R6</td>
<td>86</td>
<td>36</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>R7</td>
<td>64</td>
<td>32</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>R8</td>
<td>54</td>
<td>30</td>
<td>25</td>
<td>28</td>
</tr>
</tbody>
</table>

<sup>a</sup>Based on Fehr et al. (15).

<sup>b</sup>Mean from 5 plants in 4 replications of varieties Amsoy 71, Wayne and Calland.

<sup>c</sup>Separate analysis was performed on data for Emergence through stage V5 and on data for stages R1 through R8, respectively.
Table 2. Percentage *Phomopsis* sp. isolated from pods and seed of Amsoy 71 soybeans over a three year period at 4 stages of pod and seed development.

Percentage plants with *Phomopsis* sp. at reproductive stages

<table>
<thead>
<tr>
<th>Year</th>
<th>Plant part</th>
<th>R3&lt;sup&gt;b&lt;/sup&gt;</th>
<th>R5</th>
<th>R7</th>
<th>R8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1972</td>
<td>Lower pod</td>
<td>20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Lower seed</td>
<td>0</td>
<td>10</td>
<td>20</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Upper pod</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Upper seed</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>LSD (.01)</td>
<td>NS</td>
<td>8</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>1973</td>
<td>Lower pod</td>
<td>15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35</td>
<td>30</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Lower seed</td>
<td>5</td>
<td>3</td>
<td>10</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Upper pod</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Upper seed</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>LSD (.01)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>1974</td>
<td>Lower pod</td>
<td>10</td>
<td>5</td>
<td>69</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Lower seed</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Upper pod</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Upper seed</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>LSD (.01)</td>
<td>NS</td>
<td>NS</td>
<td>11</td>
<td>17</td>
</tr>
</tbody>
</table>

<sup>a</sup>Reproductive stage based on system of Fehr et al. (15).

<sup>b</sup>Isolations from 10 plants.

<sup>c</sup>Isolations from 4 replications of 5 plants each in 1973 and 1974.
Isolation of *Phomopsis* from flowers was relatively infrequent. The fungus was first isolated from pods at stage R2 (flowers at the top plant nodes and pods ca. 0.5 cm long and some flowers at the remaining nodes). *Phomopsis* incidence from young pods was low but the fungus was isolated with greater frequency from all pods as plants matured irrespective of the stage of pod development. There was no significant difference in *Phomopsis* frequency in proximal or distal and placental and aplacental sections of pods and seeds. *Phomopsis* was isolated as frequently from floral bracts and remnants as from walls of pods at all stages of development.

*Phomopsis* sp. and *Dpa* was not isolated from green petioles but the fungi were recovered with increasing frequency as petioles yellowed and dropped from plants. Also, *Dpa* was isolated infrequently from senescent petioles.

The occurrence of *Phomopsis*-type pycnidia on detached cotyledons, stems, pods and petioles in moist chambers is further evidence for the widespread distribution of *Phomopsis* on immature soybeans. Pycnidia were induced to form on cotyledons, hypocotyls and stems at stage V1, in pods at stage R3, and in petioles at stage R7. Pycnidia frequency increased as plants aged and was recorded on greater than 90% lower stems and pods by maturity (Table 3). Pycnidia formed within 11-21 days and were scattered or in linear rows and usually dispersed over the entire plant part. In general, plant sections approaching maturity developed pycnidia sooner
Table 3. Frequency of Phomopsis-type pycnidia formed on detached soybean plant parts placed in moist chambers (MCI method).

<table>
<thead>
<tr>
<th>Plant part incubated</th>
<th>V1</th>
<th>V5</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
<th>R8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotyledon</td>
<td>64</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypocotyl</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower Stem</td>
<td>28</td>
<td>33</td>
<td>38</td>
<td>21</td>
<td>23</td>
<td>58</td>
<td>55</td>
<td>53</td>
<td>97</td>
</tr>
<tr>
<td>Mid Stem</td>
<td>6</td>
<td>32</td>
<td>8</td>
<td>8</td>
<td>2</td>
<td>31</td>
<td>35</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Upper Stem</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>30</td>
<td>25</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Lower pod</td>
<td>6</td>
<td>8</td>
<td>29</td>
<td>62</td>
<td>86</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid pod</td>
<td>0</td>
<td>8</td>
<td>17</td>
<td>40</td>
<td>73</td>
<td>83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper pod</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>37</td>
<td>44</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD (.05)</td>
<td>16</td>
<td>8</td>
<td>10</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>

aGrowth Stages based on Fehr et al. (15).

bMean percentage of varieties Amsoy 71, Wayne and Calland replicated 4 times each.
than actively growing parts. Randomly selected pycnidia or pycnidial ooze plated on PDA-L proved to be pycnidia of *Phomopsis* sp. in more than 90% of the cases. No *Diaporthe* perithecia were found even on parts incubated over one month.

**Phomopsis sp., Dps and Dpc in seed**

*Phomopsis* and/or *Diaporthe* were previously found associated with seed in green bean stage on the lower pods (28). However, the frequency of *Phomopsis* sp., *Dps* and *Dpc* in immature seed was not shown. In 1973 and 1974, 85% of the total *Phomopsis*-type isolates were *Phomopsis* sp., 14% were *Dps* and 1% were *Dpc*. Frequency of *Phomopsis* sp. increased with time but yearly values differed at various stages of development (Table 2). Also, between stages B7 and B8 frequency of *Phomopsis* and *Diaporthe* increased significantly. *Phomopsis* sp. was isolated more frequently than *Dps* or *Dpc* from seed at maturity (Table 4) and at various times after maturity (Table 5). Eighty-two percent of all isolates from mature seed were *Phomopsis* sp., 10% *Dps* and 8% *Dpc* in 1973 and 68%, 20%, and 12% were *Phomopsis* sp., *Dps* and *Dpc*, respectively, in 1974.

Seed infected with *Phomopsis* sp. or *Dpc* were completely covered with mycelia and did not germinate on PDA-L. Seed harvested up to one month after maturity that produced *Dps* colonies usually germinated on PDA-L but *Dps*-infected seed harvested later did not germinate. Also, various combinations of *Phomopsis* sp., *Dps* and *Dpc* were frequently isolated from
Table 4. Frequency of *Phomopsis* sp., *Diaporthe phaseolorum* var. *sojae* (Dps) and *D. phaseolorum* var. *caulivora* (Dpc) in Amsoy 71 seed at maturity in 1972, 1973 and 1974.

<table>
<thead>
<tr>
<th>Year</th>
<th>Percentage seed infected</th>
<th>Percentage infected with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Phomopsis</td>
</tr>
<tr>
<td>1972</td>
<td>52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58</td>
</tr>
<tr>
<td>1973</td>
<td>52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86</td>
</tr>
<tr>
<td>1974</td>
<td>44&lt;sup&gt;c&lt;/sup&gt;</td>
<td>80</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean of 100 seed at Columbus plots.

<sup>b</sup>Mean of 4 replications of 100 seed each at Columbus plots.

<sup>c</sup>Mean of 4 replications of 100 seed each at Columbus, South Charleston, Hoytville and Wooster, Ohio plots.
Table 5. Frequency of *Phomopsis* sp., *Diaporthe phaseolorum* var. *sojae* (Dps) and *D. phaseolorum* var. *caulivora* (Dpc) in Wayne seed harvested at 4 times in 1973 from plants growing at Columbus, Ohio.

<table>
<thead>
<tr>
<th>Harvesta</th>
<th>Percentage seed infected</th>
<th>Phomopsis sp.</th>
<th>Dps</th>
<th>Dpc</th>
</tr>
</thead>
<tbody>
<tr>
<td>R8</td>
<td>34b</td>
<td>82</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Dry down</td>
<td>44</td>
<td>82</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>One month</td>
<td>72</td>
<td>88</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Two months</td>
<td>97</td>
<td>77</td>
<td>18</td>
<td>5</td>
</tr>
</tbody>
</table>

---

Harvest times

R8 - Growth stage based on Fehr et al. (15): harvest maturity, pods 95% brown.
One month - One month after R8.
Dry down - Seed with 13-14% moisture at 7 days after R8.
Two months - Two months after R8.

bMean of 4 replications of 100 seed each.
single seed.

*Phomopsis* sp. and *Diaporthe* were isolated as frequently from seed in the pod locule nearest the main stem axis (proximal) as from seed in the middle or distal locules. *Phomopsis* sp. was isolated significantly more often from seeds in pods on the lower two-thirds of plants than from pods on the upper one-third at dry down (Table 6). However, *Phomopsis* sp. was isolated with similar frequency from all pod nodes one month after maturity or later. In general, *Dps* and *Dnc* were isolated more frequently from seed harvested from the top than from the bottom of plants at both delayed harvests (Table 6). The 3 fungi were isolated from seed sampled from pods showing blotching and/or pycnidia, and from pods without symptoms at the 4 harvest dates. Frequency of *Phomopsis* and *Diaporthe* at maturity and dry down was slightly higher in seed from pods with symptoms than those without symptoms. However, in delayed harvested seed there was no differences in isolation frequency from pods with or without symptoms. Despite a significant increase in *Phomopsis* and *Diaporthe* frequency in seed and a significant decrease in seed germination with harvest delay, few pods showed PSE symptoms until two months after maturity (Table 7).

Pods covered with *Phomopsis*-type pycnidia always contained visibly moldy seed. Seed in pods from early harvests with blotches usually were visibly mold-free, but *Phomopsis* or *Diaporthe* was isolated from them. Percentage of plants bearing *Phomopsis*-type pycnidia, blotching, or both, increased
Table 6. Incidence of *Phomopsis* sp., *Diaporthe phaseolorum* var. *sojae* (*Dps*) and *D. phaseolorum* var. *caulivora* (*Dpc*) in Calland seed from pods located from the bottom to the top of plants.

<table>
<thead>
<tr>
<th>Pod Position&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Phomopsis</th>
<th>Dps</th>
<th>Dpc</th>
<th>Phomopsis</th>
<th>Dps</th>
<th>Dpc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4</td>
<td>0</td>
<td>66</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>66</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>0</td>
<td>0</td>
<td>37</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>0</td>
<td>0</td>
<td>63</td>
<td>5</td>
<td>0</td>
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<tr>
<td>5</td>
<td>26</td>
<td>0</td>
<td>0</td>
<td>43</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>2</td>
<td>2</td>
<td>40</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>2</td>
<td>0</td>
<td>32</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>17</td>
<td>0</td>
<td>4</td>
<td>15</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>31</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

LSD (.01) = 24 NS NS 22 11 (.05) = 7

<sup>a</sup>1 through 10: 1 = lowest pod node, 10 = highest pod node.

<sup>b</sup>Approximately 7 days after maturity, seed 13-14% moisture.

<sup>c</sup>Mean of 10 pods in each of 4 replications.
Table 7. Percentage pods with symptoms, incidence of *Phomopsis* and *Diaporthe* in seed and seed germination in variety Wayne.

<table>
<thead>
<tr>
<th>Harvest stage</th>
<th>Pycnidia</th>
<th>Blotching</th>
<th>Pycnidia and Blotching</th>
<th>No Symptoms</th>
<th>Percentage Seed Infected</th>
<th>Percentage Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>R8</td>
<td>1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5</td>
<td>0</td>
<td>94</td>
<td>34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>66&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dry down</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>94</td>
<td>44</td>
<td>71</td>
</tr>
<tr>
<td>One month</td>
<td>2</td>
<td>9</td>
<td>1</td>
<td>88</td>
<td>72</td>
<td>55</td>
</tr>
<tr>
<td>Two months</td>
<td>0</td>
<td>62</td>
<td>29</td>
<td>9</td>
<td>97</td>
<td>10</td>
</tr>
<tr>
<td>LSD (.01) = NS</td>
<td>14</td>
<td>4</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>10</td>
</tr>
</tbody>
</table>

<sup>a</sup>Harvest stage

R8 - harvest maturity, 95% pods brown (based on Fehr et al. (15)).
Dry down - seed at 13-14% moisture at ca. 10 days after maturity.
One month - one month after maturity.
Two months - two months after maturity.

<sup>b</sup>Symptoms on 100 pods in each of four replications.

<sup>c</sup>Isolations from 100 seed in each of four replications plated on PDA-L.

<sup>d</sup>Standard germination test by OSIA on 100 seeds in each of four replications.
significantly with harvest delay (Table 7) but only a slight increase in visibly moldy seed was observed. Visibly moldy seed were generally located in distal pod locules at early harvest and throughout the pod by one month after maturity. Moldy seed plated on PDA-L were colonized by *Phomopsis* sp. in most cases and occasionally by *Dpc*. All 3 fungi were isolated from late harvest moldy seed.

Yield and 100 seed weight were comparable in seed lots with high or low frequencies of *Phomopsis* and *Diaporthe*. Soybean stubble and debris as sources of inoculum

Soybean stubble sampled weekly from March through November in 1974 and February through July in 1975 contained immature pycnidia until late May in both years. By the first week in June more than 85% of pycnidia examined contained alpha spores. *Phomopsis* sp. was isolated from 98% of 200 randomly sampled pycnidia. Perithecia could be induced to form in moist chambers on 80% of samples examined in the Spring of 1974 and 1975. Perithecia were not found in the field in 1974 but during the first and second weeks of June in 1975 perithecia were observed in the field in 13% of the samples and 10% contained mature ascospores.

Soybeans planted in early May, 1975 dropped lower leaves during the second week of July. Seven to 10 days after the petioles fell, mature alpha spores were found in 90% of 200 pycnidia-laden petioles examined. Pycnidia formed on fallen petioles within 10 days in the field and on yellow petioles removed from plants and placed in moist chambers.
In a survey of debris at plant maturity in 1974, greater than 90% of abscised petioles bore pycnidia. Perithecia were not observed on petiole debris during sampling in 1974 or 1975.

Plants killed prematurely are rapidly colonized by *Phomopsis* and *Diaporthe*. For example, pycnidia were produced on hail damaged soybeans in 1974. Within 3 weeks after the hailstorm 82% of dead stem remnants contained pycnidia.

*Phomopsis* sp. was found more frequently than *Dps* on soybean debris and stubble. Ninety-two percent of 1020 pycnidia picked off stems, petioles and pods during a one year period and plated on PDA-L produced *Phomopsis* sp. colonies and the remaining 8% formed *Dps*. *Diaporthe phaseolorum* var. *sojae* was isolated from 97% of 200 perithecia induced on stem sections in moist chambers but was found only infrequently on overwintered stems in the field.

*Phomopsis* and *Diaporthe* were found exclusively on soybean debris and were not isolated from corn stubble or on weeds.

**Spread of inoculum**

Spread of *Phomopsis* and *Diaporthe* inoculum from soybean stubble was studied in the field. *Phomopsis* sp. was isolated at stage V1 from plants emerging through soybean stubble. The fungus was isolated from 20% of plants directly across from and 2m in any direction from the stubble by the time cotyledons dropped from plants. *Phomopsis* sp. was not recovered from control plants until stage R7 in areas where
debris was not spread. Also, *Phomopsis* sp. was not isolated from plants beyond 2m from the debris until the same stage.

Attempts were made to simulate spread of *Phomopsis* in the field using several watering variables in the growth chamber. *Phomopsis* sp. was isolated from cotyledons, hypocotyls and lower stems of plants growing in WSM laden with petioles bearing *Phomopsis*-type pycnidia and watered with a fine mist for at least two weeks. The fungus was recovered from the lower main axis only throughout plant development. Frequency of *Phomopsis* sp. in mature plants under continuous overhead sprinkling, in those receiving drip watering after two weeks overhead watering, and in plants receiving simulated heavy rain was 48, 42 and 40%, respectively. No *Phomopsis* was recovered from plants watered continuously without splashing or from plants without debris at the base. The fungus was not isolated from the top one-third of plants in any treatment until maturity and was never recovered from flowers, pods or seeds.

*Phomopsis*-type spores were found on plants in the field under wet and dry conditions. The number of plants bearing spores was highest when at least 2 cm rain was recorded 72 hours prior to sampling. More than 80% of alpha spore identifications were made from the lower one-third of plants. However, in several cases spores were found only from the mid-and top sections washed. Spores were obtained on at least one occasion from stems, flowers, pods and petioles. In most cases, *Phomopsis* sp. was found in the same plant section from which alpha spores were washed, indicating that other spores
had infected the stem earlier. Spores from unidentified species of *Santoria* and *Fusarium* were frequently observed at all sample dates.

**Phomopsis and Diaporthe spread in immature plants**

*Phomopsis* sp. and *Dps* usually caused local infection when introduced into immature stems and pods in the field on mycelial-covered toothpick tips. Limited movement of the fungi from the inoculation site occurred within growing tissues but the pathogens spread rapidly in senescent tissues. *Phomopsis* sp. was recovered more frequently one week after inoculation than *Dps* and was more aggressive in spreading throughout maturing pods and subsequently infecting seed. *Diaporthe phaseolorum* var. *caulivora* caused stem canker and killed an average of 70% of 78-85 day old Wayne soybeans. One isolate each of *Phomopsis* sp. and *Dps* killed 10% of the plants. *Phomopsis* sp. infected an average of 36% green pods, *Dps* 70% and *Dpc* 58%.

In the growth chamber, *Phomopsis* sp. was isolated at the inoculation point one week after introduction into one-two- or three-week old cotyledons and hypocotyls using the toothpick method. The fungus did not spread from cotyledons into the main stem axis at 50% or 100% RH. Hypocotyl infection by *Phomopsis* sp. was local in one-and two-week old plants at 50% RH but the fungus was isolated from the cotyledonary node in all plants placed in 100% RH and in three-week old plants in 50% RH.

In field studies using spore suspensions as inoculum, *Phomopsis* was recovered two weeks after inoculation from 6-10%
flowers and 5-20% pods inoculated at stages R3, 4 and 7. The fungus was isolated from 10% of mature pods developing from inoculated flowers and 20% each of mature pods developing from pods inoculated at stages R3, 4 and 7. A few pods with pycnidia were noted at maturity from all inoculations.

Phomopsis sp. was recovered in less than 20% of Beeson or Corsoy pods inoculated at stages R3, 4 and 7 in the growth chamber. Frequency of Phomopsis and Dps was higher at maturity than at one week after inoculation in all parts inoculated. Also, the fungus was found in parts adjacent to the inoculation site in many cases (Table 8). Phomopsis sp. was isolated from less than 5% of seeds of inoculated flowers, stage R5 pods (all sizes) or yellow pods. Diaporthe phaseolorum var. sojae was isolated from 5% of seed from full size pods at stage R5. In most cases, both varieties showed some premature ripening and blotched and linear row pycnidia-bearing plants were found in at least 10% of plants from all inoculations (Table 8). Pods showed pycnidia or blotching in only a few cases.

Root infection

Phomopsis sp. and Dps were first isolated from plants growing in soil infested with Phomopsis-infected pods when leaves were yellow and pods turning brown. Phomopsis sp. was isolated from 50% of roots and hypocotyls sampled and Dps from 8% of each. A reddish lesion on stems at the soil line was noted on 25% of Phomopsis-infected plants. Phomopsis sp.
Table 8. Frequency of *Phomopsis* sp. and *Diaporthe phaseolorum* var. *sojae* (Dps) at maturity in inoculated Beeson pods and petioles, and in adjacent stems and pods, and percent inoculated plants with pod and stem blight symptoms (PSE).

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Part</th>
<th>Growth Stage</th>
<th>Percentage plant parts with fungi</th>
<th>Percentage PSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phomopsis sp.</td>
<td>Petiole</td>
<td>R7</td>
<td>50&lt;sup&gt;d&lt;/sup&gt; 8 8 16&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pod</td>
<td>R3</td>
<td>- 43 17 43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pod</td>
<td>R5</td>
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<tr>
<td></td>
<td>Pod</td>
<td>R7</td>
<td>- 8 42 8</td>
<td></td>
</tr>
<tr>
<td>Dps</td>
<td>Petiole</td>
<td>R7</td>
<td>0 8 8 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pod</td>
<td>R3</td>
<td>- 8 0 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pod</td>
<td>R5</td>
<td>- 8 25 8</td>
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<tr>
<td></td>
<td>Pod</td>
<td>R7</td>
<td>- 17 0 17</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Based on Fehr et al. (15).

<sup>b</sup>Stem section adjacent to pod or petiole inoculated.

<sup>c</sup>Pod adjacent to petiole inoculated or pod inoculated.

<sup>d</sup>Mean percent of 10 parts in each of 3 replications.

<sup>e</sup>Mean percent of 10 plants in 3 replications showing linear row and scattered pycnidia or blotching, or both.
was recovered from each lesioned plant. By 106 days after planting, Phomopsis-infected plants had mature seed. Beeson usually matures in the field in 120 days. Also, 54% of plants with brown stems had Phomopsis-type pycnidia. Control and Dps-infected plants had green stems, brown pods, and immature seed. When all plants were mature (124 days after planting), Phomopsis-type pycnidia on brown stems were found in 79% and 9% of Phomopsis- and Dps-infected plants, respectively. Phomopsis sp. was recovered from 16% of roots and hypocotyls of plants with no pycnidia, and 33% and 75% roots and hypocotyls, respectively, of pycnidia-bearing plants. The fungus was not recovered from stems, pods or seeds. Dps was reisolated from roots of only one plant.

Phomopsis sp., Dps or Dpc could not be isolated from Wayne seedlings up to 3 weeks old growing in soil inoculated with spore suspensions of the fungi. Percent emergence in all inoculated treatments was comparable to controls.
DISCUSSION

Phomopsis and Diaporthe species have been recognized as soybean seed pathogens for over 50 years. Despite this fact Phomopsis and Diaporthe seed decay has not been recognized as a distinct disease but considered part of the pod and stem blight disease complex (11, 13, 21, 22, 23, 30, 36, 37, 43, 49). The current study shows that Phomopsis sp. and Diaporthe phaseolorum var. sojae (Dps) cause pod and stem blight and, in certain isolates, stem canker, while D. phaeolorum var. caulivora (Dpc) causes only stem canker. However, Phomopsis sp. and both varieties of D. phaseolorum cause seed decay. Soybean seed infected by Phomopsis or Diaporthe are found in plants with or without visible symptoms of pod and stem blight or stem canker. Therefore, the author recognizes soybean seed decay as a distinct disease caused by Phomopsis sp., Dps and Dpc. The studies herein have elucidated various parts of the disease cycle which will now be discussed.

Phomopsis and Diaporthe recovered from mature soybean seed are frequently isolated from immature plants and seed in Ohio. In the two years that isolations were made from soybeans in this study Phomopsis sp. was obtained more frequently than Dps or Dpc as indicated earlier (28). Also, there may be a higher incidence of Phomopsis in immature plants than isolation frequency from plant sections on PDA-L indicate.
Detection of *Phomopsis* was higher using the MCI method than isolating on PDA-L. The limiting factor was probably the size of the tissue sample (stem sections 10 cm for MCI method and 0.5 cm for plating on PDA-L).

Although *Phomopsis* was readily isolated from immature plants, the fungus produced no visible disease symptoms. However, premature killing of plants with stem and root inoculations were noted in growth chamber studies. Further research is needed to determine the role of *Phomopsis* in causing premature plant death in the field. *Phomopsis*-infected plants, prematurely or naturally ripened, produce distinct symptoms at maturity. These symptoms characterize pod and stem blight of soybean and are typified by linear rows of pycnidia on stems, petioles and pods (2). However, pod and stem blight disease ratings have included severity of blotching on plants (13), intensity of pycnidia (37) and percentage of plants showing linear row and scattered pycnidia (28). In this study plants inoculated with spore suspensions and mycelia-infested toothpick tips of *Phomopsis* sp. and *Dps* or growing in soil containing *Phomopsis*-infested pods were blotched and had pycnidia scattered and in linear rows on stems at maturity. This evidence suggests that future pod and stem blight symptom ratings should include intensity of blotching and pycnidia development.

The type of association between *Phomopsis* and immature plants was not directly established in this study. Seedborne *Phomopsis* can be carried over into the postemergence phase of development in seedlings (50).
However, studies of Phomopsis movement in soybean plants growing from infected seed have not been pursued. All seed used in this study were relatively Phomopsis-free. Therefore, most immature plant colonization probably occurred from inoculum deposited on the plants. The surface disinfection method used should have eliminated all surface organisms. No Phomopsis was isolated when surface disinfested stems were imprinted on PDA-L. The conclusion was that the fungus was established in the cortex beneath the epidermis. In inoculation studies using pycnidiospores, Phomopsis was re-isolated after one week from inoculation sites following surface disinfestation which indicates that the fungus had become established deeply enough to survive the alcohol-NaCl0 treatment. The fungus did not spread when introduced into immature plants with mycelia-infested toothpick tips or spore inoculations. Phomopsis could have persisted either as an internal resident or as a latent pathogen. Following the principles of Verhoeff (47) who describes latency as a dormant or quiescent parasitic relationship which after a time can change into an active one, Phomopsis is probably a latent pathogen. In greenhouse inoculations, premature ripening and production of pod and stem blight symptoms was evidence that the fungus does have an active stage in senescent plants. Phomopsis probably becomes established in pods as latent infection under most field conditions. However, the fungus has been reported as an aggressive pathogen on very young to full grown and mature pods when the pods were placed in moist chambers and sprayed with spore suspensions (30).
Pods of all sizes inoculated with spores in the study were not incubated in high moisture and did not produce pycnidia in 9 to 12 days after inoculation as reported by Lehman (30). Phomopsis and Diaporthe were reisolated, however, from many of the inoculated pods and PSB symptoms were evident in some cases at maturity. Kilpatrick (25) suggested systemic penetration of pods by Dps because he isolated the fungus more often from seed nearest the peduncle than at the tip of the pod. However, in this study, the fungus was isolated with equal frequency from all pod sections.

Several reports speculate on the movement of Phomopsis from pod to seed. Lehman (30) reported mycelia from pods invades the seed cavity at the same time that pycnidia form on pods. Athrow and Caldwell (3) concluded that most seed infection was associated with pod wall deterioration. Indirect evidence was obtained in this study that direct penetration from the pod wall into the testa occurs while seed are immature. In mature seed, mycelia were found abundant in the hourglass cell layer, less so in the parenchyma cell layer and least in the palisade cell layer of Dps-infected seed coats (23). Also, mycelia were found occasionally in the cotyledonary and embryonic tissues. The location of Phomopsis and Diaporthe was not determined in this research but the data show that the pathogens were isolated with equal frequency from seed in the proximal, middle and distal pod locules and from seed sections attached to the pod wall as often as sections distal to the attachment point.
No *Phomopsis* symptoms were noted on immature seed indicating that latent infection is highly probable. The time of fungus transition from latency to active growth is not clear. However, the most likely transition period is during ripening. Lehman (30) suggested that the mycelia attacks the developing seed at the same time pycnidia develop on stems and pods but gave no evidence for seed colonization prior to symptom expression on plants. In some fruit diseases changes in the middle lamella during ripening lead to breaking of pathogen dormancy because the pathogen enzymes can now disrupt the middle lamella and penetrate the unprotected cell wall (47). The situation may be similar in soybean seed but at the present time we can only assume that transition is connected with physiological changes in maturing host tissue.

Excessive rain and high temperature and relative humidity are environmental conditions which may enhance movement of *Phomopsis* from pod to seed. In field tests seed from pods at the top of the plant sprayed with pycnidiospores were infected with *Phomopsis* at maturity. The fungus was not isolated from seed in control pods which were inoculated with water. Rainfall was 0.6 cm above normal during the 7 days following inoculations while temperatures averaged 26°C and hH 80%. In growth chamber tests similar pod inoculations did not result in seed infection at maturity. The growth chamber experiment was watered by dripping water on the surface and field experiment by natural rainfall. Lehman (30) observed that infection and dissemination of *D. sojae* is strongly
dependent on high humidity, the soybean pod and stem blight disease and subsequent seed infection being markedly more abundant during rainy than dry summers. Wilcox et al. (56) suggested high temperature and humidity in September and early October probably favor the development of Dps and other fungi in seed. Further studies should be performed on the impact of excessive moisture and high humidity and temperature on movement of Phomopsis from pod to seed.

The association of Phomopsis sp. with immature soybean plants indicates that the fungus is one of the "pioneer colonists" (5) of soybeans in Ohio. This possession of potential residue prior to the death of tissue is probably a great advantage for survival in soybean debris. Complete colonization of soybean stems prior to maturity may aid in excluding other saprophytic fungi. Stems infested with Phomopsis are a potential source of late-season inoculum, an overwintering site for the fungus, and the primary source of inoculum the next Spring. Field samples collected during 1974 and 1975 show the major sources of Phomopsis and Diaporthe inoculum in the field are overwintered soybean stubble and fallen parts from the current crop (debris). Phomopsis sp. is disseminated in the form of alpha spores and Dps predominately as ascospores. Viable pycnidia were found on 90% of stubble and debris from June through mid-September. Perithecia were not found in 1974 and only in 10-20% of the samples during 1975. The season-long concentration and availability of Phomopsis sp. inoculum and the paucity of Dps inoculum may be the primary reason for higher incidence of
*Phomopsis* than *Dps* under Ohio conditions. However, *Dps* incidence does increase slightly in seed at, and after maturity. The source of this late season inoculum is not known. Perithecia were not found on debris from the current crop. Although old stubble was not examined, new perithecia were probably formed or existing perithecia produced ascospores. The concepts governing pycnidiospore and ascospore concentration and availability may help explain the higher incidence of *Phomopsis* sp. than *Dps* and the more frequent isolation of both fungi from plants near maturity than at other stages of growth. Growth chamber and field inoculations with *Phomopsis* and *Diaporthe* demonstrated that the fungi can colonize plant parts at many growth stages. There are no reports showing whether all pycnidiospores or ascospores mature simultaneously within a pycnidium and perithecium, respectively, or whether continued sporulation occurs throughout the season. Ascii within a perithecium do not mature simultaneously within many pyrenomycetes (24). In *Calonectria crotalariae* asci continue to mature and be discharged for ca. two weeks (38). In *Gibberella zea* the number of ascospores discharged is regulated not only at the rate the perithecia dry but also by the availability of mature ascospores, which in turn is affected by the conditions that regulate ascospore maturation (46). High concentration and availability of *Phomopsis* sp. and *Dps* inoculum could result in a high incidence of young plant infection and potential epiphytotic seed infection under optimum seed decay conditions.
The major emphasis in this study has been on the association of *Phomopsis* and *Diaporthe* with above-ground plant parts of soybean. However, preliminary experiments show the seed decay pathogens may infect plants through the roots late in the season. Reasons for root susceptibility to *Phomopsis* and *Diaporthe* as plants mature are not clear. Bacteria causing decline in root resistance to rhizosphere microflora normally not pathogenic on roots is one possibility. Microbial associations have been found to predispose root to root rot in beans (33). Also, an important cause of poor seed quality in soybeans is related to root deterioration initiated by high bacterial populations (10). Further studies on root infection by *Phomopsis* and *Diaporthe* and a possible interaction between seed decay pathogens and bacteria in seed and roots should be investigated.
PART II

SOYBEAN SEED DECAY: EFFECT OF CULTURAL
PRACTICES ON DISEASE SEVERITY
INTRODUCTION

Soybean seed decay caused by *Phomopsis* sp., *Diaporthe phaseolorum* (Cke. & Ell.) var. *sojae* (Lehman) (Dps) and *D. phaseolorum* var. *ceulivora* (Dpc) occurs in all soybean growing areas of Ohio. A wide variety of cultural practices are used throughout the state. Some of these practices may have a profound effect on seed quality by affecting the incidence of seed decay. Intensive cropping of soil to soybeans is now, and will in the future, result in problems that affect soybean yield and quality. Intensive cultivation of a crop results in uniformity rather than diversity in an ecosystem which in turn favors serious disease problems. Crop rotation is a cultural practice often used to reduce disease. Crop monoculture can result in disease enhancement (17) or may favor buildup of secondary pathogens (50). At the other extreme, reduction in take-all disease of wheat and increase in yield has been shown with extended monoculture (40). Also, crop rotation does not eliminate certain pathogens in the absence of host tissue. In a California cotton field planted for several years to cereals, the population of the pathogen *Fusarium oxysorum* f. sp. *vasinfectum* increased more rapidly in the absence of cotton than when previously planted to cotton (41). Because of the wide range of effects crop rotation might have on soybean seed decay, the effect of crop rotation on the incidence of
Phomopsis and Diaporthe in soybean plants seed and the subsequent effect on seed quality was studied.

Planting date (19, 26, 35, 42, 56), delayed harvest (28, 56) and varietal differences (12, 19, 42, 56) in relation to soybean seed quality have been studied. The interrelationship of the three cultural practices may help to predict the onset of the seed decay disease. In a study by Wilcox et al. (56) greater deterioration in seed quality with delayed harvest was more apparent in early maturing than in late maturing varieties. Early varieties are exposed to more days of mean daily temperatures higher than 10C, more days of precipitation higher than 0.25 cm. and more days with relative humidity higher than 60%, than are late varieties. In this study effects of planting date, delayed harvest and differences in variety maturity are evaluated in conjunction with crop rotation.

A preliminary study was conducted on pathogen survival in fall versus spring plowed fields. There are no reports on the over-winter survival of Phomopsis and Diaporthe in stubble remaining on, or plowed under the soil surface. Plowing under infested debris may be beneficial or detrimental to pathogen survival through adverse conditions (31). There are no reports on how well Phomopsis and Diaporthe compete with soil-borne inhabitants. Therefore, the time of plowing may aid in controlling the inoculum density within a field.
MATERIALS AND METHODS

Plot designs

The effect of crop rotation and post-maturity irrigation on incidence of Phomopsis and Diaporthe was studied at Columbus in 1973 in two blocks previously cropped to corn (CR) and two blocks previously cropped to soybeans (SR) for two years. Phomopsis-free Amsoy 71, Wayne and Calland seed treated with Captan (N-[(trichloromethyl) thio]-4-cyclohexene-1, 2-dicarboxyimide) were planted on May 17 at The Ohio State University Farm Science Review Area. Each variety was planted in 3 row plots 9.3 m long with rows spaced 75 cm apart. The plots were replicated four times in each block in a randomized block design. Plants from one block in each rotation were sampled during immature plant studies. The other block of each rotation was irrigated between September 24 and November 5 by sprinkling with 63 mm water daily except on days of rainfall greater than 25 mm. The non-irrigated blocks received only natural rainfall. Both irrigated and non-irrigated blocks were sampled after September 24.

Soybeans from SR and CR rotations were sampled for Phomopsis and Diaporthe from four locations in Ohio in 1974. At Columbus, representing central Ohio, Amsoy, Wayne and Calland were planted on May 17 in plots similar to those used in 1973. At South Charleston, representing southwestern Ohio
Amsoy 71, Wayne and Calland were planted on May 7 in SR soil and were part of The Ohio State University Soybean Variety Field Trials. The CR studies were performed on one variety at each of 3 farms located within 10 miles of South Charleston. At each farm 4 replications consisting of 3 rows 9.3 m long and 75 cm apart were staked in each field. Planting dates for the 3 varieties were: Amsoy 71, May 14; Wayne, May 11 and Calland, May 29. Amsoy 71, Beeson and Williams were planted in SR and CR at Hoytville (northwestern, Ohio) and Wooster (northern, Ohio) on May 20 and May 13 respectively, and were part of the University Variety Field Trials.

The effect of planting date on incidence of Phomopsis and Diaporthe in Amsoy 71, Beeson and Williams in CR and SR soil was studied at Columbus. Planting dates were May 2, May 16, June 3 and June 14. Each variety was planted in three 20 m rows at each date. Four 9.3 m sections were marked for sampling in each 3-row planting.

Sampling and isolation

The corn rotation versus soybean rotation effect on incidence of Phomopsis and Diaporthe in soybean plants and seed was examined weekly in 1973 beginning at emergence and continuing through maturity. Five plants per replication were sampled weekly from non-irrigated CR and SR blocks. Surface disinfestation, schedule for planting plant parts, media employed, incubation and identification of isolates were as described in Materials and Methods, Part I.
The effects of high moisture on pod and stem blight (FSB) symptoms, *Phomopsis* and *Diaporthe* incidence in mature seed and seed germination were studied in both irrigated and non-irrigated CR and SR plots in 1973. The following data were recorded at maturity, dry down (13-14% moisture, ca. 7-10 days after maturity), one month and two months after maturity from each replication: i) Percentage of 25 plants per replication with FSB symptoms (scattered or rows of pycnidia with or without biotching); ii) incidence of *Phomopsis* sp., *Dps* and *Dpc* in randomly harvested seed; iii) incidence of the 3 fungi in seed from plants with and without FSB symptoms; iv) percentage visibly moldy seed; v) seed germination (Official Ohio Seed Improvement Association Test); and vi) the location (proximal, middle, distal) of seed infection in each of 25 pods. Data were obtained from 100 seed per replication in tests (ii) through (v).

Plants from the state-wide survey and date of planting studies in CR and SR in 1974 were sampled at the 1st trifoliolate, V1 according to Fehr et al. (15); full bloom stage, R2; lower pod green bean stage, R5, and maturity stage, R8. Immature stems, pods and seeds were plated, incubated and isolates identified using the same methods listed in Part I. Sampling and plating at maturity differed from immature plant isolations. Four plants per replication from each variety growing in both CR and SR soil were harvested at stage R8. Ten seed and four 0.5 cm stem and pod sections from the bottom, middle and top of plants were plated on PDA-L.
Pod and stem blight symptom percentages of 4 plants per replication were made in the date of planting study.

Pycnidia formation on immature plants

The MCI method described in Part I was used to induce pycnidia on stems and pods sampled from CR and SR plots in 1973 and 1974. Five plants per replication were sampled weekly from stage V1 through stage R8. A 10 cm section from the lower, middle and top of each plant was placed in a moist chamber and percentage of pycnidia was recorded after 14 days. When pods developed, one from the lower, middle and top of each plant was incubated separately.

Effects of plowing on pathogen survival

A preliminary investigation was made on the effects of Spring and Fall plowing on the survival of Phomopsis sp. Dpe and Dpc in soybean stubble from the previous crop. Soybean stubble was sampled on March 18 and April 15, 1975 from one field each at The Ohio State University Farm Science Review area having the following crop rotation -- plowing time combinations: 1) monoculture soybeans, Fall plowed; 2) monoculture soybeans, Spring plowed; 3) soybeans following corn, Fall plowed; 4) soybeans following corn, Spring plowed and 5) soybeans following corn, not plowed. The fields were subdivided into three sections and 10 pieces of stubble were removed from each. The stubble was surface disinfested, a 0.5 cm section was plated on PDA-L and a 2.0 cm section was placed in a moist chamber to induce perithecial formation.
RESULTS

Effects of crop rotation on immature plants and seed

Phomopsis sp. was isolated from all above-ground plant parts of soybeans growing in soil cropped two years to soybeans (SR) or cropped two years to corn (CR) at Columbus in 1973 and 1974. The fungus was initially recovered from hypocotyls and cotyledons at emergence in 1973 and by stage V1 in 1974 in SR plants but not until stage V2 in CR plants both years (Table 9). Diaporthe phaseolorum var. sojae was isolated at stage V2 in both CR and SR plants but Dpo was not isolated from plants in either rotation until stage R7 and was recovered from ca. 5% of plants and seed from then until maturity.

Phomopsis sp. was isolated from more than 75% of SR plants by stage R2 but was found infrequently in CR plants throughout the growing season (Table 9 and Fig. 1). Dps was isolated infrequently from SR plants but increased gradually in CR as plants approached maturity.

Phomopsis sp. and Dps frequency in stems, pods and seeds was typical of the recovery patterns reported previously (28). In CR and SR plants the fungi were found more often in stems than pods, and more frequently in pods than seed throughout plant development. By maturity differences in frequency of Phomopsis sp and Dps in stems and pods were less pronounced but isolations from seed were significantly lower than from
Table 9. The incidence of Phomopsis sp. and Diaporthe phaseolorum var. acacia (Dps) in immature soybean plants growing in soil previously cropped to soybeans (Sa) and corn (Cr).

<table>
<thead>
<tr>
<th>Growth Stage</th>
<th>Phomopsis sp.</th>
<th>Dps</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SR</td>
<td>CR</td>
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<tr>
<td>Emergence</td>
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<tr>
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<tr>
<td>R8</td>
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</tr>
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</table>

*aBased on Fehr et al. (15).

*bMean of 4 replications of varieties Amsoy 71, Wayne and Calland.

cData for emergence through stage V3 analyzed together, for V5 analyzed separately and for R1 through R8 analyzed together.
Figure 1. Incidence of *Phomopsis* sp. in Wayne soybeans planted in soil previously cropped to soybeans (S₅) and corn (Ch) and sampled at four growth stages (based on Fehr et al. (15)) in 1973 and 1974.
stems or pods.

The formation of Phomopsis-type pycnidia or detached stems and incubated in the moist chamber (MCI method) was presented in Part I as a second line of evidence for Phomopsis presence in immature plants. Significantly more pycnidia formed on stems and pods from SR plants than from CR plants sampled at all stages of plant growth (Table 10). Also, pycnidia formed on a significantly higher number of green stem sections than on green pods. Pycnidia formed with equivalent frequency on detached stems and pods sampled from plants approaching, or at maturity.

No varietal differences were noted in incidence of Phomopsis and Diaporthe in immature plants. At maturity Phomopsis and Diaporthe in seed fluctuated among the three varieties (Table 11). In 1973 there was no differences in seed infection among varieties from either SR or CR soil but in 1974 Phomopsis sp. was isolated significantly more often from Amsoy 71 than Wayne or Calland in both rotations. Amsoy 71 is a Maturity Group II variety having a maturity date of ca. 120 days. Wayne and Calland are Maturity Group III varieties with maturity dates of ca. 134 and 136 days, respectively. The recovery of Dps or Dpc from seed of the 3 varieties was comparable in both 1973 (Table 12) and 1974.

Effect of irrigation on mature seed

Irrigation in September and October had no effect on incidence of Phomopsis and Diaporthe in seed. Total rainfall was 5.1 mm below normal. Overhead irrigation applied was 6.3 mm/day (total 27.50 cm).
Table 10. Percentage lower pods forming pycnidia in moist chambers (MCI method) after being harvested from plants growing in soil previously cropped to soybeans (SR) and corn (CR) at Columbus in 1973 and 1974.

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>1973</th>
<th>1974</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SR</td>
<td>CR</td>
</tr>
<tr>
<td>R4</td>
<td>50(^b)</td>
<td>6</td>
</tr>
<tr>
<td>R5</td>
<td>65</td>
<td>28</td>
</tr>
<tr>
<td>R6</td>
<td>88</td>
<td>35</td>
</tr>
<tr>
<td>R7</td>
<td>76</td>
<td>40</td>
</tr>
<tr>
<td>R8</td>
<td>93</td>
<td>86</td>
</tr>
<tr>
<td>LSD (.01)</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Growth stages bases on Fehr et al. (15).

\(^b\)Mean of 5 pods in each of four replications.
Table 11. Incidence of *Phomopsis* sp. in seed harvested at maturity from varieties Amsoy 71, Wayne and Calland growing in soil previously cropped to soybeans (SR) and corn (CR) during 1973 and 1974.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Amsoy 71</td>
<td>44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15</td>
<td>66</td>
<td>25</td>
</tr>
<tr>
<td>Wayne</td>
<td>28</td>
<td>12</td>
<td>50</td>
<td>8</td>
</tr>
<tr>
<td>Calland</td>
<td>40</td>
<td>19</td>
<td>35</td>
<td>3</td>
</tr>
<tr>
<td>LSD (.01) =</td>
<td>11</td>
<td></td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean of 100 seed in each of four replications.
Table 12. Isolation of *Phomopsis* sp. (Phom), *Diaporthe phaseolorum* var. *sojae* (Dps) and *D. phaseolorum* var. *caulivora* (Dpc) from seed at 4 harvest dates from 3 varieties in two crop rotations.

| Fungus isolated | Harvest time | Amsoy 71 | | | Wayne | | | Calland | | |
|----|--------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Phom | Maturity | 44<sup>d</sup> | 15 | 28 | 12 | 40 | 19 |  |  |  |  |
| | Dry down | 43 | 12 | 36 | 35 | 30 | 15 |  |  |  |  |
| | One month | 62 | 25 | 65 | 28 | 60 | 48 |  |  |  |  |
| | Two months | 62 | 37 | 75 | 43 | 70 | 45 |  |  |  |  |
| | LSD (.05)=11 |  |  |  |  |  |  |  |  |  |  |
| Dps | Maturity | 3 | 2 | 3 | 4 | 3 | 10 |  |  |  |  |
| | Dry down | 2 | 7 | 4 | 3 | 2 | 5 |  |  |  |  |
| | One month | 3 | 10 | 4 | 13 | 8 | 11 |  |  |  |  |
| | Two months | 8 | 10 | 17 | 30 | 16 | 29 |  |  |  |  |
| | LSD (.05)=4 |  |  |  |  |  |  |  |  |  |  |
| Dpc | Maturity | 5 | 3 | 3 | 3 | 2 | 3 |  |  |  |  |
| | Dry down | 2 | 2 | 4 | 6 | 3 | 2 |  |  |  |  |
| | One month | 4 | 4 | 4 | 5 | 8 | 14 |  |  |  |  |
| | Two months | 10 | 14 | 5 | 10 | 8 | 12 |  |  |  |  |
| | LSD (.05)=NS |  |  |  |  |  |  |  |  |  |  |

<sup>a</sup>Harvest time

Maturity = Stage R8 based on Fehr et al. (15).

Dry down = 13-14% seed moisture at ca. 7-10 days after maturity.

One month = One month after maturity.

Two months = Two months after maturity.

<sup>b</sup>Soil previously cropped to soybeans.

<sup>c</sup>Soil cropped to corn two years prior to soybeans.

<sup>d</sup>Mean of 100 seed in each of four replications.
Phomopsis and Diaporthe incidence in seed, PSB symptoms on plants or percent seed germination did not significantly differ between irrigated and non-irrigated plots irrespective of variety or crop rotation. Only the data from the non-irrigated plots was used to evaluate the effect of delayed harvest on the incidence of Phomopsis and Diaporthe in this paper.

Effects of crop rotation on plants and seed at and after maturity

The data evaluating the effect of harvesting seed at maturity and at three intervals after maturity on soybean seed decay included: 1) percentage plants with PSB symptoms, 2) percentage visibly moldy seed, 3) incidence of Phomopsis sp., Dps and Dpc in seed, 4) PSB symptoms versus incidence of Phomopsis and Diaporthe in seed and 5) seed germination.

Pod and stem blight symptoms were observed on 10–47% SR and CH plants at maturity, dry down and one month after maturity harvest. Two months after maturity PSB symptoms occurred in 60% or more of the plants in both rotations. For example, PSB symptoms in Amsoy 71 at maturity, dry down, one month and two months after maturity were recorded on 13, 22, 47 and 95% of plants, respectively, in SR soil and 11, 19, 35 and 94%, respectively, in CR plots.

Amsoy 71 had significantly higher incidence of visible mold on seed than the two late maturing varieties through one month after maturity. Two months after maturity the 3 varieties tested had comparable percents visible mold on seed.
Higher percent of visibly moldy seed was recorded from SR than from CR plants but the maximum percentage found in either rotation was 15%.

Although percentage PSB symptoms on plants were similar in SR and CR soils, total Phomopsis and Diaporthe were recovered more frequently from SR seed than from CR seed (Table 12). Phomopsis sp. was significantly more frequent in SR than in CR seed but levels of Dps were similar from the two rotations until one month after maturity. Diaporthe phaseolorum var. caulivora was isolated from seed with equal frequency in both rotations. A significant increase in total Phomopsis and Diaporthe in seed was noted between dry down and one month after maturity but percentage of plants with PSB symptoms did not significantly increase. However, between one and two months after maturity total Phomopsis and Diaporthe in seed and plants with PSB symptoms dramatically increased. Data presented in Part I showed incidence of Phomopsis and Diaporthe higher in seed from plants with PSB symptoms than plants without symptoms at maturity and dry down but equal incidence of infection from symptom and symptomless plants grown in SR soil at the two delayed harvests. A similar relationship was also found in CR plants in 1973.

Crop rotation had a significant effect on seed germination. Germination varied from 11% in Wayne harvested two months after maturity in SR to over 80% in Wayne harvested at maturity from CR. The mean germination of all 3 varieties over 4 harvest dates was 53% for seed from SR and 86% for
seed from CH. Harvest time had a dramatic effect on seed germination. Mean germination of Wayne seed harvested two months after maturity from both crop rotations was 14%, whereas mean germination at maturity was 74%. The variety-harvest date interaction (Table 13) and the variety-rotation interaction were both significant (Table 14).

**Effects of sampling from different regions**

Additional evidence for association of *Phomopsis* and *Diaporthe* with developing and mature soybeans was obtained from sampling soybeans from SR and CR soil from Columbus, South Charleston, Hoytville and Wooster. Both fungi were isolated from cotyledons, hypocotyls, stems, pods and seed and were recovered more frequently with plant age. However, *Phomopsis* was isolated significantly more often than *Dps* or *Dpc*. The data for *Phomopsis* sp. is presented in Table 15. At all locations *Phomopsis* sp. was isolated more frequently from plants in SR than from plants from CR soil from emergence through the green bean stage. At maturity *Phomopsis* sp. was isolated from plants in SR more frequently than plants in CR soil at 3 of 4 locations. At the fourth location, Hoytville, the incidence of both fungi was extremely low. Varietal difference of *Phomopsis* or *Diaporthe* in seed at maturity was noted at the various locations. (Table 16). In 3 out of 4 locations *Phomopsis* sp. was dominant over *Dps* in mature seed but no differences were found between seed in CR soil (Tables 17 and 18). Less than 7% of seed were infected with *Dpc* at all locations.
Table 13. Percentage germination of Amsoy 71, Wayne and Calland seed harvested from plants grown in soil previously cropped to soybeans (SR) and to corn (CR).

<table>
<thead>
<tr>
<th>Variety</th>
<th>SR</th>
<th>CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amsoy 71</td>
<td>51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74</td>
</tr>
<tr>
<td>Wayne</td>
<td>53</td>
<td>65</td>
</tr>
<tr>
<td>Calland</td>
<td>55</td>
<td>65</td>
</tr>
<tr>
<td>LSD (.01) =</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Harvest time</th>
<th>Amsoy 71</th>
<th>Wayne</th>
<th>Calland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maturity</td>
<td>68(^b)</td>
<td>74</td>
<td>77</td>
</tr>
<tr>
<td>Dry down</td>
<td>78</td>
<td>66</td>
<td>82</td>
</tr>
<tr>
<td>One month</td>
<td>70</td>
<td>83</td>
<td>67</td>
</tr>
<tr>
<td>Two months</td>
<td>34</td>
<td>14</td>
<td>19</td>
</tr>
</tbody>
</table>

LSD (.01) = 10

\(^a\)Harvest time

- Maturity = Stage R3 based on Fehr et al. (15).
- Dry down = 13-14% seed moisture at ca. 7-10 days after maturity.
- One month = One month after maturity.
- Two months = Two months after maturity.

\(^b\)Mean of four replications of 100 seed each.
Table 15. *Phomopsis* sp. incidence in plants of Amsoy 71 at 4 stages of growth at 4 locations in Ohio.

<table>
<thead>
<tr>
<th>Growth Stage</th>
<th>Columbus</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SR(^a)</td>
<td>CH(^b)</td>
<td>SR</td>
<td>CH</td>
<td>SR</td>
<td>CR</td>
<td>SR</td>
<td>CR</td>
<td>SR</td>
<td>CR</td>
<td>SR</td>
<td>CR</td>
<td></td>
</tr>
<tr>
<td>1st Trifoliate</td>
<td>46(^c)</td>
<td>6</td>
<td>37</td>
<td>0</td>
<td>16</td>
<td>0</td>
<td>41</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full Bloom</td>
<td>23</td>
<td>8</td>
<td>12</td>
<td>15</td>
<td>35</td>
<td>0</td>
<td>31</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green Bean</td>
<td>39</td>
<td>4</td>
<td>55</td>
<td>32</td>
<td>73</td>
<td>19</td>
<td>46</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maturity</td>
<td>98</td>
<td>32</td>
<td>81</td>
<td>78</td>
<td>100</td>
<td>62</td>
<td>85</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD (.01) =</td>
<td>20</td>
<td></td>
<td>21</td>
<td></td>
<td>(.05)</td>
<td>21</td>
<td></td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Soil previously cropped to soybeans.

\(^b\)Soil cropped to corn two years prior to soybeans.

\(^c\)Mean of 4 plants in each of four replications.
Table 16. Phomopsis and Diaporthe incidence in seed at maturity at 4 locations in Ohio.

**Percentage seed with Phomopsis or Diaporthe**

<table>
<thead>
<tr>
<th>Variety</th>
<th>Columbus</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SR(^a)</td>
<td>CR(^b)</td>
<td>SR</td>
<td>CR</td>
<td>SR</td>
<td>CR</td>
<td>SR</td>
<td>CR</td>
<td>SR</td>
<td>CR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amsoy 71</td>
<td>66(^c)</td>
<td>25</td>
<td>26</td>
<td>2</td>
<td>72</td>
<td>18</td>
<td>14</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wayne</td>
<td>50</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calland</td>
<td>35</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beeson</td>
<td>-</td>
<td>-</td>
<td>32</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>31</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Williams</td>
<td>-</td>
<td>-</td>
<td>18</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD (.01)=</td>
<td>NS</td>
<td></td>
<td>7</td>
<td></td>
<td>15</td>
<td></td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Soil previously cropped to soybeans.

\(^b\) Soil cropped to corn two years prior to soybeans.

\(^c\) Mean of 100 seed in each of four replications.
Table 17. Incidence of *Phomopsis* sp. and *Diaporthe phaseolorum* var. *sojae* (Dps) in mature seed harvested from plants grown in soil previously cropped to soybeans (SR) and corn (Ch) at Columbus and South Charleston, Ohio.

Percentage seed with fungi

<table>
<thead>
<tr>
<th>Site b</th>
<th>Rotation</th>
<th>Pathogen</th>
<th>Amsoy 71</th>
<th>Wayne</th>
<th>Calland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Col</td>
<td>SR</td>
<td>Phomopsis</td>
<td>66 c</td>
<td>43</td>
<td>34</td>
</tr>
<tr>
<td>Col</td>
<td>SR</td>
<td>Dps</td>
<td>0</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Col</td>
<td>Ch</td>
<td>Phomopsis</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Col</td>
<td>CR</td>
<td>Dps</td>
<td>22</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>SC</td>
<td>SR</td>
<td>Phomopsis</td>
<td>52</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>SC</td>
<td>SR</td>
<td>Dps</td>
<td>11</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>SC</td>
<td>CH</td>
<td>Phomopsis</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>SC</td>
<td>CR</td>
<td>Dps</td>
<td>3</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

*aMean percentage isolated from 100 seed in each of four replications.*

*bCol = Columbus  
SC = South Charleston*
Table 18. Incidence of *Phomopsis* sp. and *Diaporthe* phaseolorum var. *sojae* (*Dps*) in mature seed harvested from plants grown in soil previously cropped to soybeans (SH) and corn (CR) at Hoytville and Wooster, Ohio.

<table>
<thead>
<tr>
<th>Site</th>
<th>Rotation</th>
<th>Pathogen</th>
<th>Amsoy 71</th>
<th>Beeson</th>
<th>Williams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoyt</td>
<td>SH</td>
<td><em>Phomopsis</em></td>
<td>6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Hoyt</td>
<td>SR</td>
<td><em>Dps</em></td>
<td>8</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Hoyt</td>
<td>CR</td>
<td><em>Phomopsis</em></td>
<td>8</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Hoyt</td>
<td>CR</td>
<td><em>Dps</em></td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Woos</td>
<td>SR</td>
<td><em>Phomopsis</em></td>
<td>17</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>Woos</td>
<td>SR</td>
<td><em>Dps</em></td>
<td>8</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Woos</td>
<td>CR</td>
<td><em>Phomopsis</em></td>
<td>2</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Woos</td>
<td>CR</td>
<td><em>Dps</em></td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Nean percentage isolated from 100 seed in each of four replications.

<sup>b</sup>Hoyt = Hoytville
Woos = Wooster
Distinct varietal differences in seed infection were noticed at each location. Less *Phomopsis* sp. and *Dps* were isolated from late maturing varieties Wayne, Calland and Williams than from Amsoy 71 at Columbus and South Charleston. Incidence of the fungi in seed at Hoytville and Wooster were too low to detect varietal differences.

**Effects of planting date**

The effect of different planting dates on the incidence of *Phomopsis* and *Diaporthe* in plants and seed was studied in 1974 at Columbus. Fungi isolated from immature plants and seed were typical of previous isolation trends (28). Also, *Phomopsis* sp. was isolated more frequently than *Dps* in SR and *Dps* more often than *Phomopsis* sp. in plants in CR from each planting date. Total *Phomopsis* and *Diaporthe* frequency was always higher in SR than CR plants and seed. No significant difference in *Phomopsis* and *Diaporthe* incidence was noted among the four planting dates in plants sampled at stages V1, R2 and R5. At stage R8 percentage plants with PSB symptoms were higher in SR than in CR plants in the 3 varieties (Table 19). Higher frequency PSB was found in Amsoy 71 plants than in Beeson or Williams irrespective of planting date. Amsoy 71 and Beeson had significantly lower severity of PSB symptoms on soybeans planted on May 16 or June 3 than on those planted on May 2. Also, severity of PSB in Williams increased significantly between the May 2 and June 14 planting dates. Significantly higher frequency of *Phomopsis* sp. than *Dps* was found in plants of all varieties
Table 19. Percentage stems and pods with pod and stem blight symptoms (PSB) at maturity in three varieties planted at four dates in two crop rotations.

<table>
<thead>
<tr>
<th>Planting Date</th>
<th>SR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CR&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amsoy 71 Beeson Williams</td>
<td>Amsoy 71 Beeson Williams</td>
</tr>
<tr>
<td>May 2</td>
<td>75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>55</td>
</tr>
<tr>
<td>May 16</td>
<td>25</td>
<td>22</td>
</tr>
<tr>
<td>June 3</td>
<td>28</td>
<td>13</td>
</tr>
<tr>
<td>June 14</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>LSD (.01)=16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Soil previously cropped to soybeans.

<sup>b</sup> Soil cropped to corn at least two years prior to soybeans.

<sup>c</sup> Mean of 10 plants in each of four replications.
and in seed of Amsoy 71 and Beeson from SR soil (Table 20). However, the two pathogens were isolated with equal frequency from CR plants and seed (Table 21). Diaporthe phaseolorum var. caulivora was isolated from ca. 5% of mature plants and seed in all samples. Incidence of Phomopsis sp. decreased significantly between May 2 and May 16 in SR and CR plants of Amsoy 71, in CR seed of Beeson and in SR plants of Williams. In general, there was no significant difference in incidence of Phomopsis sp. in soybeans planted after May 16 in SR or CR soils. Also, there was little difference in frequency of Dps in plants and seed at maturity across planting dates. The incidence of Phomopsis and Diaporthe from stems and pods of Williams planted in May or June was generally equal to Amsoy 71 or Beeson but incidence in seed of Williams was significantly lower than the two early varieties.

Effects of plowing on pathogen survival

A preliminary examination was made on the occurrence of Phomopsis and Diaporthe in soybean fields Spring and Fall plowed. Phomopsis sp. was isolated most often from the plot not plowed (Table 22). There was no difference in Phomopsis sp. frequency in SR soil Fall or Spring plowed but Diaporthe (perithecia of Dps or Dpo) frequency was higher in Fall than in Spring plowed soil. In CR soil Phomopsis sp. was isolated more often in Spring plowed than in Fall plowed soil but again slightly higher incidence of Dps was noted in Fall than in Spring plowed plots (Table 22).
Table 20. Isolation of *Phomopsis* sp. from mature stems and pods, and seed of three varieties planted at four dates in two crop rotations.

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Variety</th>
<th>SR&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th>CR</th>
<th></th>
<th></th>
<th>LSD (&lt;.01)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Stems and pods</td>
<td>Amsoy 71</td>
<td>73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>45</td>
<td>58</td>
<td>50</td>
<td>43</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Beeson</td>
<td>68</td>
<td>55</td>
<td>48</td>
<td>53</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Williams</td>
<td>55</td>
<td>28</td>
<td>35</td>
<td>48</td>
<td>23</td>
<td>28</td>
</tr>
<tr>
<td>Seed</td>
<td>Amsoy 71</td>
<td>70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>26</td>
<td>22</td>
<td>10</td>
<td>43</td>
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<tr>
<td></td>
<td>Beeson</td>
<td>28</td>
<td>24</td>
<td>3</td>
<td>5</td>
<td>21</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Williams</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>SR = soil previously cropped to soybeans.  
<sup>c</sup>CR = soil previously cropped to corn.  
<sup>b</sup>1 = May 2  2 = May 16  
<sup>c</sup>3 = June 3  4 = June 14  
<sup>d</sup>Mean of 4 plants in each of four replications.  
<sup>d</sup>Mean of 100 seed in each of four replications.
Table 21. Isolation of *Diaporthe phaseolorum* var. *sojae* (Dps) from mature stems and pods, and seed of three varieties planted at four dates in two crop rotations.

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Variety</th>
<th>SR&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th></th>
<th></th>
<th>CR</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>LSD&lt;sup&gt;b&lt;/sup&gt; (&lt;,01)</th>
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<tbody>
<tr>
<td></td>
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<td>4</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stems and</td>
<td>Amsoy</td>
<td>71</td>
<td>12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23</td>
<td>8</td>
<td>7</td>
<td>25</td>
<td>47</td>
<td>28</td>
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<td>21</td>
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<td>14</td>
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<td>22</td>
<td>22</td>
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<td>5</td>
<td>6</td>
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<td></td>
</tr>
<tr>
<td></td>
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<td>0</td>
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<td>1</td>
<td></td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
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</table>

<sup>a</sup>SR = soil previously cropped to soybeans.  
CR = soil previously cropped to corn.

<sup>b</sup>1 = May 2  2 = May 16  
3 = June 3  4 = June 14

<sup>c</sup>Mean of 4 plants in each of four replications.

<sup>d</sup>Mean of 100 seed in each of four replications.
<table>
<thead>
<tr>
<th>Rotation</th>
<th>Phom</th>
<th>Diap</th>
<th>Peri</th>
<th>Phom</th>
<th>Diap</th>
<th>Peri</th>
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<td>SR-Fall</td>
<td>60f</td>
<td>10f</td>
<td>70g</td>
<td>70f</td>
<td>10f</td>
<td>70g</td>
</tr>
<tr>
<td>SR-Spring</td>
<td>60</td>
<td>10</td>
<td>40</td>
<td>70</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>CH-Fall</td>
<td>20</td>
<td>20</td>
<td>70</td>
<td>20</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>CH-Spring</td>
<td>60</td>
<td>10</td>
<td>50</td>
<td>60</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>CH-No Flow</td>
<td>80</td>
<td>10</td>
<td>10</td>
<td>90</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

March 18

April 15

aSH = soil previously cropped to soybeans.

bFall -- plowed in October.
Spring -- plowed in March.
No Flow -- not plowed as of April 15.

cPhomopsis sp.

dD. phaseolorum var. sojae (Dps) or var. caulivora (Dpc).

ePerithecia of Dps or Dpc found in moist chambers.

fMean of 10 pieces stubble per three replications in each block plated on PDA-L.

gMean of 10 pieces stubble per three replications in each block placed in moist chambers.
DISCUSSION

Certain consistent associations between *Phomopsis* and *Diaporthe* and soybeans were noted in these studies that were not affected by the cropping history of the soil:
1) the fungi could be isolated from at least a few plants beginning at emergence and continuing through maturity;
2) *Phomopsis* sp. was located more frequently in lower and middle plant sections than in upper sections and *Dps* more frequently near the top than at the bottom of plants;
3) as shown previously (28), *Phomopsis* and *Diaporthe* were isolated more frequently from stems than pods, and more often from pods than from seed with differences decreasing with plant age; 4) *Dpc* was not isolated until stage H7 and was recovered from 5% or less of stems, pods and seeds and 5) Maturity Group II varieties had higher incidence of seed infection at maturity than Maturity Group III varieties.

There were some consistent differences between incidence of *Phomopsis* and *Diaporthe* related to crop rotation: 1) total *Phomopsis* and *Diaporthe* was higher from SR than from CR plants and seed; 2) *Phomopsis* sp. was recovered more frequently than *Dps* from SH and *Dps* usually isolated more often than *Phomopsis* sp. from CR plants and seed; 3) *Phomopsis* and *Diaporthe* were "pioneer" colonists (5) of stems, pods and seeds from SH but not from CR and 4) PSB symptoms were similar on plants growing in either rotation but incidence
was always greater in SH than in CH plants. Several factors contribute to the pattern of development of \textit{Phomopsis} and \textit{Diaporthe} in soybean. Inoculum availability, environmental conditions at critical infection times, a nutritional factor in the host and the number of days after planting when pod and seed development occurs in different varieties are considered the most critical factors relating to this study.

The higher frequency of \textit{Phomopsis} sp. than \textit{Dps} in SH plants and the reverse situation in CH plants appears to be the result of available inoculum within the rotations. In Part I, stubble observation data showed the domination of \textit{Phomopsis} sp. on the dead tissue from the previous growing season. Subsequently, \textit{Phomopsis} sp. was isolated more often from immature plants growing in SH plots. However, growth chamber studies show that both \textit{Phomopsis} sp. and \textit{Dps} can be reisolated from plants inoculated throughout plant development. This indicates that the primary reason for the high recovery rate of \textit{Phomopsis} sp. from soybeans is due to inoculum availability. The preliminary study on Spring versus Fall plowing provides further evidence for the importance of inoculum availability. \textit{Phomopsis} sp. survives equally well on stubble plowed under in the Fall or left to overwinter on the soil surface but \textit{Dps} and \textit{Dpc} survival is enhanced if stubble is turned under the soil surface in the Fall. \textit{Phomopsis} and \textit{Diaporthe} on soybean stubble is probably the primary source of inoculum in SH soil but the source of the fungi in CH soil remains unknown. Luttrell (31) isolated \textit{Phomopsis} and \textit{Diaporthe} from several weed hosts in soybean.
fields. Also, the fungi could be colonizing the corn stubble. However, they were not isolated from pieces of corn tissue plated on PDA-L. Long-term survival of Dps and Dpc as free ascospores or Phomopsis sp. in stromatic tissue remaining from some previous host tissue are distinct possibilities. Further work is needed to correlate Phomopsis and Diaporthe survival and sources of inoculum with soybean seed decay. Also, control measures might be aimed at reducing the potential inoculum by reducing the over-wintering stubble.

When inoculum is available, environmental and nutritional factors and variety maturity dates interplay to promote or deter pathogen infection and spread within the host. Phomopsis and Diaporthe infected pods receiving limited moisture in growth chambers but did not colonize seed. Incidence of diseased seed in the field is usually higher during years having excessive precipitation than in years receiving average rainfall. However, seeds from plants kept continually wet after maturity in the irrigation study were as highly infected as seed from plants receiving natural rainfall, which was below normal in 1973. This indicates that moisture on plants after maturity probably must interact with optimum relative humidity and temperature to enhance severe seed decay. In addition, environmental conditions for green pod infection are probably different than environmental conditions for seed infection from infected pods after maturity. Optimum environment probably coincides with some nutrient factor(s) in the host and pathogen before pod or seed infection occurs.
Crittenden (8) showed a significant decrease in D. sojae-infected seed when potassium (K) levels in upper pods were increased. A marked difference in late-season K depletion between pods on the upper parts of the plant and pods on the lower parts of the plant was found previously (20). A possible relationship between low K and high D. sojae was supported when Crittenden found higher levels of D. sojae in top pods than in lower pods. Data in this study show a significant increase in Dps and Phomopsis in upper stems and pods between stages R7 and R8. Evidence presented in Part I showed that mature pods and seeds located from the bottom to the top of the plant are equally susceptible to Phomopsis and Diaporthe. However, Phomopsis sp. was isolated more frequently from seed in middle and lower sections of the plant than from the top of plants. The reverse situation was true for Dps. The author is uncertain whether the D. sojae identified by Crittenden (8) includes Phomopsis sp. and Dps but the lower level of D. sojae-infected seed obtained when potassium amendments are supplied to plants suggests this concept merits further consideration.

Phomopsis and Diaporthe seed infection is influenced by environment and nutrition but seed decay severity appears to be dependent on date of maturity in varieties grown in Ohio. Planting date influences the number of days to flowering and to maturity (32) and may in turn affect seed quality. Maturity Group II varieties Amsoy 71 and Beeson generally had higher incidence of seed infection than Maturity Group III varieties Wayne, Calland and Williams. The subsequent
result was lower quality seed from Amsoy 71 and Beeson. However, frequency of *Phomopsis* and *Diaporthe* and seed germination was also affected by harvest date, rotation and planting date. Seed from plants from CR soil germinated higher than SR seed and had less severe seed infection and lower PSB symptoms on plants. Planting studies have shown soybean seed quality higher from late than from early dates of planting (19, 42). In this study, seed from Amsoy 71 and Beeson planted from mid-May to mid-June were less infected than seed from early May planting. Williams seed from CR plants was low in *Phomopsis* and *Diaporthe* irrespective of planting date. Although CR seed were generally higher quality than SR seed at maturity, delay in harvest resulted in poor germination and high *Phomopsis* and *Diaporthe* in seed from both rotations. Also, the high germination of seed from Maturity Group III recorded at maturity and dry down was reduced at one month and significantly reduced at two months after maturity. With extreme delay in harvest after maturity, seed of later varieties (Calland, Wayne and Williams) deteriorated more rapidly than seed of earlier varieties (Amsoy 71 and Beeson).

The evidence presented in this study and in a previous report (28) indicate varieties commonly used in Ohio lack resistance to soybean seed decay. However, a variety may escape severe seed decay if optimum seed infection conditions are circumvented. These optimum conditions include the previously described environmental and nutritional factors.
contributing to a compatible host-pathogen relationship and poor culture practices which enhance disease progress. Therefore, low frequency of seed decay is possible in Ohio if farmers consider modification of intensive cropping of soybeans and delay planting early varieties from early until mid or late May.
PART III

SOYBEAN SEED DECAY: DIFFERENTIATION OF PHOMOPSIS AND DIAPORTHE SPECIES INVOLVED BY COLONY MORPHOLOGY, SPOHOCARP PRODUCTION, PATHOGENICITY AND SYMPTOMS INDUCED
INTRODUCTION

Separation of Diaporthe phaseolorum on soybean into D. phaseolorum (Cke. & Ell.) Sacc. var. sojae (Dps) and D. phaseolorum var. caulivora (Ahow and Caldwell) (Dpc) is well documented (2, 21, 43, 53). Despite sufficient morphological and pathological evidence to warrant separation of the stem canker pathogen as Dpc (2, 46) and the pod and stem blight organism as Dps (2, 21, 30), the validity of the separation has been questioned (45, 54). In addition, stem canker is found infrequently among currently used varieties, and pod and stem blight, though widespread, is not considered to be economically important (2, 31, 53). The major disease problem caused by Dps and Dpc is lowering soybean seed quality (3, 21, 28, 31, 34, 36, 43, 48, 49). Diaporthe phaseolorum var. sojae has been associated with soybean seed more frequently than Dpc (8, 28, 36, 43). However, the identification of all Diaporthe and Phomopsis isolates from soybean as Dps has been questioned (28). Isolates forming only the imperfect stage are found more frequently than isolates forming the perfect stage (8, 18, 21, 28, 31, 34). A number of workers have suggested that isolates which do not form perithecia are heterothallic strains of Diaporthe (21, 30, 31, 52, 53). However, there is no experimental evidence which demonstrates successful mating of monoconidial isolates of these "heterothallic" strains.
Isolates of *Phomopsis* from soybeans which form only pycnidia were shown to be different in morphology and pathogenicity than isolates producing both pycnidia and perithecia (28). In this study further evidence is presented indicating that *Phomopsis* sp. commonly found in soybean seed differs from both *D. phaseolorum* var. *sojae* and *D. phaseolorum* var. *caulivora* previously described.
MATERIALS AND METHODS

Isolation and Identification

The possibility that more than one Phomopsis or Diaporthe species is associated with soybean seed decay was recognized when morphologically different colonies were isolated from soybean seed (28). Subsequently, these different types of Phomopsis and Diaporthe were isolated from immature plants using the techniques in Part I. Identification of plant and seed isolates as Phomopsis sp., Dps and Dpc was based on cultural characteristics of single spore isolates obtained from plants and seed plated on PDA-L. The characters studied included colony type, sporocarp morphology, type of spores formed, stability of characters during subculturing, and the effect of light on sporulation. Additional isolates for study were obtained from whole sporocarps, pycnidial ocze and single spores from pycnidia and perithecia, all of which were produced on naturally infected plants in the field.

Single spore isolates were obtained as follows: i) mycelia growing out from host tissue on PDA-L were transferred to a fresh PDA-L plate, ii) the agar was inverted so that the transferred mycelia were touching the bottom of the dish, iii) plates were incubated at 24-26 C, iv) mycelia tips were removed as they grew through to the top of the inverted agar and placed on fresh PDA-L, v) beginning 4 days after
transfer, plates were placed in continuous light from a 15-watt cool-white fluorescent lamp, hereafter referred to as continuous fluorescent light, and vi) transferred single spores to fresh PDA-L after sporocarps formed. Spores were obtained from three sources: i) alpha spores from isolates forming pycnidia, ii) alpha spores from isolates producing both pycnidia and perithecia in the same stroma and iii) ascospores from isolates producing perithecia only. The plates were incubated as described in (iii) and (v) above. In these studies, resulting colonies were identified as *Phomopsis* sp. when only pycnidia with alpha spores (and occasionally beta spores) formed, as *Dps* when pycnidia with alpha and beta spores and perithecia formed, and as *Dpc* when perithecia and pycnidia with stylospores or no pycnidia were formed. Colony, sporocarp and spore morphology were characterized on PDA-L for 100 alpha spore isolates of *Phomopsis* sp., 50 alpha spore isolates of *Dps* and 50 ascospore isolates of *Dpc*. Also, 3 single ascospores were obtained from 6 perithecia on each of 4 over-wintered stems (total 72 isolates) and characterized as described above.

The stability of cultural characters was observed on 10 isolates of each fungus during 7 successive transfers on PDA-L. The test was repeated twice.

The effect of light on sporulation was evaluated on 3 isolates of each fungus. Mycelial transfers were incubated at 24-26 C in i) continuous fluorescent light, ii) normal daylight for four days, then continuous light thereafter, and iii) continuous dark. The experiment was repeated twice.
Frequency of *Phomopsis* sp. *Dps* and *Dpc* in naturally infected plants was determined from isolations of 1,020 pycnidia and 200 perithecia plated on PDA-L.

**Production of symptoms in vitro**

Symptoms produced by 20 single spore isolates each of *Phomopsis* sp. *Dps* and *Dpc* on stem sections and whole pods detached from plants were recorded. Ten cm stem sections and full sized green pods were harvested from 87-day-old Beeson soybeans growing in wooden boxes of steamed Wooster soil mix (WSM) containing Wooster silt loam, muck and Canadian peat (5:5:2 V/V). The stem sections and pods were rinsed in tap water, soaked for one minute each in 70% ethanol and 1.3% NaClO, rinsed in sterile water and air dried. They were then placed on water agar amended with 1 ml propylene oxide (Baker grade) per plate (WAP). The 20 isolates of each fungus included 5 single spore isolates from stems, pods or seed of plants collected from one of four locations in Ohio -- Columbus, South Charleston, Hoytville and Wooster. This group of isolates were used in further studies and were designated Statewide isolates. A one ml spore suspension containing ca. 5000 spores/ml was pipetted onto each of 5 stems and 3 pods in four replications per isolate. Plates were incubated at 24-26 C. Four days after inoculation plates were placed in continuous fluorescent light. Symptoms produced by the fungi were observed over a 30-day period.

**Pathogenicity**

Three types of inoculations were made to determine pathogenicity: 1) mycelial-infested toothpick tips were
introduced into wounded immature stems and pods and into
green and mature seed, 11) spore suspensions were atomized
on mature seed and 11) seed were placed on PDA-L cultures
then planted in non-sterile soil.

Wound inoculations

The toothpick tip method of Crall (7) was employed
on immature stems and pods in the field and on stems in
growth chambers. The isolates used in field tests were as
follows: 9 isolates of *Phomopsis* sp. (3 each from stems,
pods and seeds), 6 isolates of *Dpe* (2 each from stems, pods
and seeds) and 4 isolates of *Dpc* (3 from seed and 1 from a
stem). This group of isolates was originally from soybeans
in research plots at The Ohio State University farms and
designated as Columbus isolates in subsequent studies. Host
plants for the field studies were 75-80 day-old Amsoy 71
soybeans growing in soil previously cropped to corn at The
Ohio State University farms. Each isolate was introduced
into 5 full size green pods on each of 5 plants and into
stems of 10 additional plants. The experiment was repli-
cated four times in each of two tests.

In growth chamber inoculations, 5 different single spore
isolates of each fungus were inoculated into 70-75 day-old
Beeson stems growing in WSM. The growth chamber was main-
tained at 22 C daytime and 16 C nighttime, 12 hr. day, 12 hr.
night regime and 50-60% relative humidity. The test was
performed three times. Sterile toothpick tips inoculated
into stems or pods served as the control in all toothpick
inoculation tests. Symptoms in field and growth chamber
experiments were recorded four weeks after inoculation and again at maturity.

A modification of Crall's toothpick tip method (28) was employed on green and mature seed. Full size green seed were inoculated with the Columbus isolates. Results were recorded as percentage seed decayed seven days after inoculation. The test was performed twice. For mature seed tests the Statewide and Columbus isolates were used. Seed of variety Calland were inoculated with the Columbus isolates and seed of variety Wayne with the Statewide isolates. The experiment was repeated three times, with three replications per experiment. Seed inoculated with sterile toothpick tips served as controls. The method of recording the results of this experiment differed from those used in an earlier study (28). Results eight days after inoculation were recorded as follows: 1) non-germinated - seed completely covered with mycelia or radicle emerged but less than 1 1/2 times the length of the seed, 11) germination arrested - radicle greater than 1 1/2 times the length of the seed but browned and beginning to decay, and 111) germinated - radicle longer than 1 1/2 times the length of the seed and not discolored. Isolates were classified as virulent if greater than 50% of inoculated seed did not germinate as described in (1) above, as moderately virulent if 30-50% of seed did not germinate and as avirulent if less than 30% of seed did not germinate. Uninoculated seed germinated 70% or higher.
Inoculations with spores

Pathogenicity of Phomopsis sp., Dps and Dpc on mature seed was further tested using spore suspensions of Phomopsis and Diaporthe Statewide isolates. Three replications of ten seed each of variety Wayne were soaked for one hour in sterile distilled water and immersed for 30 sec. in a spore suspension containing ca. 5,000 spores/ml. The seed were placed on moist filter paper in sterile glass petri plates (10 seed/plate) and incubated at 24-26 C. Observations were completed 10 days after inoculation. Seed immersed in sterile water and placed on a clean PDA-L served as controls. Results were recorded in the way described for the toothpick tip method. The test was performed twice.

Seed placed on actively growing cultures

An experiment was designed to determine the effect of Phomopsis sp., Dps and Dpc inoculated seed on emergence. Wayne soybean seed were surface disinfested in 1.3% NaClO for one minute and rinsed three times in sterile distilled water. Thirty seed were placed at the perimeter of an actively growing 4 day-old culture of Phomopsis sp., Dps or Dpc. Three isolates of each fungus selected from the Statewide isolates were used. Control seed were placed on a clean PDA-L plate. Seed were removed from plates at 1, 24, 48, 72 and 96 hours after introduction and planted in a non-sterile greenhouse mix (GSM) containing soil, peat and perlite (1:1:1 V/V). Thirty seed per isolate were planted in 3 six-inch pots (10 per pot). Plants were watered every other day. The growth chamber was kept at 22 C daytime,
16°C at night in a 14 hour day, 10 hour night light regime and 60-70% relative humidity. Emergence was recorded eight days after planting. The test was repeated three times.

**Mating Phomopsis sp. isolates**

Three experiments were devised to induce perithecia formation by mating Phomopsis sp. isolates. Eight single spore isolates were used for each test. All mating combinations were incubated at 24-26°C receiving normal daylight for four days and continuous fluorescent light thereafter. The tests were terminated 35 days after inoculation.

The first method consisted of placing a 1 cm plug of each of two isolates at opposite ends of a petri plate with FDA-L and observing for perithecia where the colonies grew together. The test was performed three times.

In the second experiment 1 ml spore suspensions (ca. 5,000 spores/ml) of two isolates were mixed and pureed over 5 sterile Wayne seed in a screw cap vial. All possible combinations of the 8 single spore isolates were tested in this way. The test was repeated twice, five replications per test.

In the third method, 87 day-old Beeson stems on WAP media were inoculated with all possible combinations of the 8 isolates. A 0.1 ml spore suspension (ca. 5,000 spores/ml) of one isolate was pipetted onto one end of a 10 cm section and a 0.1 ml aliquot of another isolate at the other end of the same section. Five stem sections per plate on each of three plates were inoculated for each combination. The inoculated stem pieces were checked closely for perithecia. The test was performed twice.
RESULTS

Several thousand colonies of *Phomopsis* and *Diaporthe* were observed growing out from soybean plant parts and seed over a two year period. Single spore isolates obtained from 100 colonies tentatively identified as *Phomopsis* sp., 50 as *D. phaseolorum* var. *sojae* (Dps) and 50 as *D. phaseolorum* var. *caulivora* (Dpc) were studied and are probably representative of the total isolated. The two varieties of *D. phaseolorum* and the *Phomopsis* species were distinguished on basis of colony morphology on PDA-L, symptoms produced on naturally infected and inoculated plants, pathogenicity and mating studies.

Colony morphology

The cultural characteristics of *Phomopsis* sp., *Dps* and *Dpc* on PDA-L were distinctly different. Single alpha spore isolates of *Phomopsis* sp. produced erumpent, beaked and multichambered pycnidia which exuded spores in a gelatinous matrix (Fig. 2). The pycnidia were borne in dense, carbonaceous stromata surrounded by dingy yellow and white mycelia. Perithecia were not formed. Single spore isolates of *Dps* produced colonies of loose-growing mycelia at first white, turning orange-yellow, with aerial hyphae becoming loosely united into tufts (Fig. 3). Usually, single pycnidia formed within echinulate stromata with single beaks slightly
Figure 2. Pycnidia of *Phomopsis* sp. Note gelatinous matrix at the tip of ostioles (arrow).
Figure 3. Colony appearance of *Diaporthe phaseolorum* var. *sojae* on PDA-L.
protruding (Fig. 4). Multi-beaked pycnidia were occasionally found but these formed only after cultures were stored for more than one month. Spherical or turbinate perithecia developed at the base or intermingled with pycnidia, or grew independently within mycelial tufts (Fig. 5). Single ascospores of Dps produced colonies similar to Dps but mycelia remained white and pycnidia formed infrequently and contained only beta spores. Perithecia were borne singly or in clusters below pycnidia or within mycelial tufts (Fig. 6). Sporocarp and spore sizes of Phomopsis sp., Dps and Dpc overlapped (Table 23). Size of pycnidia, perithecia, beta spores, asci and ascospores (Fig. 7) of Dps and Dpc were similar. Also, size of alpha spores of Phomopsis sp. and Dps were similar (Fig. 8).

Phomopsis sp. continued to produce alpha spores after 7 successive transfers on PDA-L and infrequently beta spores. However, Dps pycnidia produced increasing numbers of beta spores with successive subcultures, having more than 90% stylospores after 4 transfers. Six of 10 Dps isolates and 9 of 10 Dpc isolates always formed perithecia. The 4 remaining Dps and the 1 Dpc isolates failed to produce perithecia after subculturings. Mycelial morphology remained stable in all three fungi throughout the transfers.

The effect of light on in vitro sporulation and colony morphology of Phomopsis and Diaporthe was studied. Cultures placed in continuous dark grew rapidly and produced tufts of white, fluffy mycelia without pigmentation. One isolate of Phomopsis sp. formed several scattered pycnidia with few
Figure 4. Pycnidia (arrow) of *Diaporthe phaseolorum* var. *sojae* in culture.
Figure 5. Perithecia (arrow) of Diaporthe phaseolorum var. sojae in culture.
Figure 6. Perithecia of *Diaporthe phaseolorum* var. *caulivora* showing caespitose group of sporocarps.
Table 23. Comparison of morphology in culture of *Phomopsis* sp., *Dianorthe phaseolorum* var. *sojae* (Dps) and *D. phaseolorum* var. *caulivora* (Dpc).

<table>
<thead>
<tr>
<th>Structure</th>
<th>Phomopsis sp.</th>
<th>Dps</th>
<th>Dpc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Microns&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Microns&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Microns&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pycnidia</td>
<td>50-290 x 55-300</td>
<td>50-320 x 60-320</td>
<td>90-290 x 80-310</td>
</tr>
<tr>
<td>mean</td>
<td>220 x 190</td>
<td>260 x 200</td>
<td>200 x 170</td>
</tr>
<tr>
<td>Alpha spores</td>
<td>4.5-11.0 x 2.0-3.0</td>
<td>4.8-11.0 x 2.0-2.8</td>
<td>...</td>
</tr>
<tr>
<td>mean</td>
<td>6.9 x 2.3</td>
<td>6.8 x 2.3</td>
<td>...</td>
</tr>
<tr>
<td>Beta spores</td>
<td>...</td>
<td>12-27 x 0.8-1.8</td>
<td>16-30 x 1.3-2.0</td>
</tr>
<tr>
<td>mean</td>
<td>...</td>
<td>16 x 1.1</td>
<td>19 x 1.4</td>
</tr>
<tr>
<td>Perithecia</td>
<td>...</td>
<td>155-290 x 180-306</td>
<td>204-334 x 295-357</td>
</tr>
<tr>
<td>mean</td>
<td>...</td>
<td>210 x 240</td>
<td>240 x 310</td>
</tr>
<tr>
<td>Ascii</td>
<td>...</td>
<td>26-55 x 5.1-7.5</td>
<td>26-50 x 5.0-8.1</td>
</tr>
<tr>
<td>mean</td>
<td>...</td>
<td>38 x 5.6</td>
<td>36 x 5.3</td>
</tr>
<tr>
<td>Ascospores</td>
<td>...</td>
<td>9-13 x 2.6-4.3</td>
<td>7.5-14.5 x 2.8-3.3</td>
</tr>
<tr>
<td>mean</td>
<td>...</td>
<td>11 x 3.2</td>
<td>12.2 x 3</td>
</tr>
</tbody>
</table>

<sup>a</sup>Based on 100 measurements of each structure.
Figure 7. Ascii and ascospores of *Diaporthe phaseolorum* var. *sojae*.
Figure 8. Alpha and beta spores of *Phomopsis* sp. or *Diaporthe phaseolorum* var. *sojae*. 
alpha spores and a single isolate of Dps formed a few sterile pycnidia. Isolates of Dpo did not form sporocarps. Colony morphology of isolates in continuous light throughout the experiment was similar in appearance to the single spore isolates described earlier. Phomopsis sp. produced pycnidia with alpha spores. Dps produced pycnidia with alpha and beta spores and perithecia with ascospores, and Dpo produced perithecia with ascospores. Isolates of the 3 fungi incubated in light and dark for four days followed by continuous light for the duration of the experiment were similar to those incubated in continuous light in appearance and sporulation characteristics.

Phomopsis and Diaporthe was further characterized using isolates from sporocarps formed in the field. Phomopsis sp. was isolated from 92% of 1,020 pycnidia from mature soybean tissue and Dps from the remaining 8%. Phomopsis sp. was recovered from the gelatinous matrix or entire sporocarp of all erumpent, beaked and sometimes multichambered pycnidia sampled. The small percentage of Dps was obtained from subglobose pycnidia with short, rounded to conical beaks. Occasionally, sickle-shaped stylospores were found in Dps pycnidia but not observed in Phomopsis sp. pycnidia.

Perithecia were not observed on immature or mature soybean plants but formed on mature stem sections incubated in moist chambers 30 days or more. Two hundred cultures were obtained from perithecia formed in these moist chambers. Ninety-seven percent of these cultures were identified as Dps and the remaining 3% as Dpo. Perithecia were observed
on soybean stubble in the field in the Spring (Fig. 9). Of 72 single ascospores obtained from 6 of these perithecia on each of 4 over-wintered stubble pieces, three were identified as Dps while the remaining 69 isolates produced colonies of Dpc.

**Sporulation and development on detached stems and pods**

Phomopsis sp. and Dps did not sporulate on green plants inoculated in the field. However, the fungi did sporulate readily on mature plants. Therefore, an experiment was designed to monitor the sporulation of various Phomopsis sp., Dps and Dpc isolates on senescent or dying tissue. Twenty isolates of each fungus from the Statewide group were inoculated onto detached stems and pods placed on WAP media. Phomopsis sp. produced pycnidia on significantly more stems and pods than Dps (Table 24) but Dps produced blotching more frequently on stems than Phomopsis sp. Also, pycnidia were noticed as early as four days after inoculation in several Phomopsis sp. isolates but not until seven days after in Dps isolates. At seven days after inoculation 58% and 80% of Phomopsis sp.-infected stems and pods and 13% and 25% of Dps-infected stems and pods, respectively, had pycnidia. Nineteen isolates each of Phomopsis sp. and Dps produced pycnidia by 30 days after inoculation. However, the incidence of pycnidia formation varied widely among isolates of the two fungi. Frequency of pycnidia formation by Dps isolates ranged from 0 to 100% and by Phomopsis isolates from 50 to 100%. Pycnidia of Phomopsis sp. and Dps were morphologically similar until 30 days after
Figure 9. Perithecia of *Diaporthe phaseolorum* var. *sojae* on a dead soybean stem.
Table 24. Percentage of detached stems with pycnidia, perithecia and blotching and of detached pods with pycnidia 7 and 30 days after inoculations with *Phomopsis* sp. and *Diaporthe phaseolorum* var. *sojae* (Dps).

<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Plant part</th>
<th>Symptoms</th>
<th>Percentages</th>
<th>Phomopsis</th>
<th>Dps</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>stems</td>
<td>pycnidia</td>
<td>58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>stems</td>
<td>perithecia</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>stems</td>
<td>blotching</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pods</td>
<td>pycnidia</td>
<td>80</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>stems</td>
<td>pycnidia</td>
<td>90</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>stems</td>
<td>perithecia</td>
<td>0</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>stems</td>
<td>blotching</td>
<td>19</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pods</td>
<td>pycnidia</td>
<td>92</td>
<td>73</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean of two experiments testing 20 isolates of each fungus.
inoculation. At that time most isolates of *Phomopsis* sp. had produced some beaked, multichambered pycnidia while *Dps* pycnidia remained subglobose. Isolates of *Dps* produced blotching on 2% of pods and *Phomopsis* sp. failed to blotch pods. Nine percent of *Dps* infected stems had perithecia by 30 days after inoculation. Stems and pods inoculated with *Dpc* isolates were free from pycnidia, perithecia and blotching.

**Pathogenicity**

**Wound Inoculations**

Immature stems and pods of plants in the field and of plants in the growth chamber were inoculated with *Phomopsis* sp., *Dps* and *Dpc*. The stem canker pathogen, *Dpc*, was more highly pathogenic on stems of field plants than *Phomopsis* sp. or *Dps* (Table 25). Isolates of *Phomopsis* sp. decayed significantly more green pods of these field plants than *Dps* or *Dpc*. Green and mature seed were as frequently decayed by *Phomopsis* sp. as by *Dpc* and significantly less decayed by *Dps*. In the growth chamber, isolates of *Dpc, Phomopsis* sp. and *Dps* killed 30, 8 and 2%, respectively, of stem-inoculated plants. Also, 90% of surviving plants inoculated with *Dpc* formed cankers whereas 36% and 30% of surviving *Dps* and *Phomopsis* sp.-inoculated plants formed cankers, respectively. Pod and stem blight symptoms or premature ripening was found on 44, 31 and 39% of *Dps*-Dpc- and *Phomopsis* sp.-inoculated stems, respectively. *Phomopsis* sp. was reisolated from 67% of inoculated plants at maturity and
Table 25. Percentage plants killed and pods and seed decayed by isolates of *Phomopsis* sp., *Diaporthe phaseolorum* var. *sojae* (Dps) and *D. phaseolorum* var. *caulivora* (Dpc).

<table>
<thead>
<tr>
<th>Plant part inoculated</th>
<th>Phomopsis&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Dps&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Dpc&lt;sup&gt;c&lt;/sup&gt;</th>
<th>LSD (.01)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stems</td>
<td>0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>80</td>
<td>-</td>
</tr>
<tr>
<td>Pods</td>
<td>74&lt;sup&gt;e&lt;/sup&gt;</td>
<td>25</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>Green seed</td>
<td>90&lt;sup&gt;f&lt;/sup&gt;</td>
<td>49</td>
<td>80</td>
<td>11</td>
</tr>
<tr>
<td>Mature seed</td>
<td>64</td>
<td>20</td>
<td>54</td>
<td>33</td>
</tr>
</tbody>
</table>

<sup>a</sup>Nine isolates tested.

<sup>b</sup>Six isolates tested.

<sup>c</sup>Four isolates tested.

<sup>d</sup>Mean number plants killed one month after inoculation.

<sup>e</sup>Mean number pods decayed one month after inoculation.

<sup>f</sup>Mean number seed decayed seven days after inoculation.
Dps and Dpc from 33% each.

Mature seed inoculated with toothpick tips infested with the Phomopsis and Diaporthe Statewide isolates showed a wide range of virulence among the isolates. However, Phomopsis sp. isolates were usually more pathogenic on seed than Dps or Dpc. Sixteen of 20 Phomopsis sp. isolates were virulent on seed whereas 9 and 7 of 20 Dps and Dpc isolates, respectively, were virulent (Table 26). Also, a qualitative difference in non-germinated seed was noted between seed inoculated with Phomopsis sp. or Dpc and seed inoculated with Dps. The former isolates usually completely colonized seed preventing the radicle from emerging. With Dps, the radicle emerged from seed not visibly colonized but was killed before it attained the length of the seed. In seed with germination arrested, Phomopsis sp.-infected radicles browned completely while Dps-and Dpc-infected radicles browned at the tip only. There was no difference in virulence among Phomopsis sp. isolates from the four Statewide locations. However, Dps and Dpc isolates from Hoytville were more pathogenic than the remaining isolates. Hoytville Dps isolates prevented germination in 58% of seeds while Wooster, Columbus and South Charleston isolates prevented 40, 34, and 50%, respectively. Hoytville Dpc isolates prevented germination in 73% of seeds while Wooster, Columbus and South Charleston prevented 40, 34 and 36% respectively.
Table 26. Number virulent, moderately virulent and avirulent isolates of *Phomopsis* sp., *Diaporthe phaseolorum* var. *solaee* (Dps) and *D. phaseolorum* var. *caulivora* (Dpc) on soybean seed inoculated with mycelia-infested toothpick tips.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Virulent&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Moderately virulent&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Avirulent&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phomopsis</em></td>
<td>16&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Dps</td>
<td>9</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Dpc</td>
<td>7</td>
<td>9</td>
<td>4</td>
</tr>
</tbody>
</table>

<sup>a</sup>More than 50% of inoculated seed decayed and non-germinated.

<sup>b</sup>Thirty to 50% of inoculated seed decayed and non-germinated.

<sup>c</sup>Less than 30% of inoculated seed decayed and non-germinated. Uninoculated seed germinated 70% or higher.

<sup>d</sup>Number out of 20 isolates.
Inoculations with spores

Mature Wayne seed were inoculated with the Statewide isolates. Phomopsis sp. was more pathogenic than Dps or Dpc in most cases. Phomopsis sp.-and Dpc-inoculated seed decayed more rapidly than seed inoculated with Dps but the seed were not covered with mycelia. The radicle in seeds with germination arrested browned completely when inoculated with Phomopsis sp., Dps or Dpc. Differences among isolates of each fungus from the four locations in Ohio were minimal.

Effect of incubation time on emergence when seed were inoculated with Phomopsis and Diaporthe

Wayne soybean seed were incubated on rapidly growing cultures of Phomopsis sp., Dps and Dpc for 1, 24, 48, 72 and 96 hours before planting in non-sterile WSM soil. The three isolates of each fungus used were the most pathogenic isolates on mature seed from the Statewide group. There was no effect on emergence from seed incubated for 1 and 24 hours. At 48 hours Phomopsis sp. had significantly reduced emergence while only slight reductions were noted with Dps and Dpc (Fig. 10). Isolates of all three fungi reduced emergence with increased time of incubation but Phomopsis sp. was the most virulent.

Mating Studies with Phomopsis sp. Isolates

Three experimental attempts were made to mate Phomopsis sp. isolates. Perithecia were not produced in any of these experiments. Pycnidia were produced but they were characteristic of the original isolates.
Figure 10. Seedling emergence from Wayne soybean seed planted in non-sterile soil after incubation for 1, 24, 48, 72 and 96 hours on actively growing cultures of Phomopsis sp., Diaporthe phaseolorum var. sojae (Dps) and D. phaseolorum var. caulivora (Dpc).
DISCUSSION

This study presents evidence that three different fungi are causal organisms of soybean seed decay. A species of Phomopsis, Diaporthe phaseolorum var. sojae and D. phaseolorum var. caulivora have been associated with soybean seed during isolations over a three year period. Also, the Phomopsis sp. and Dps have been associated with immature plants from emergence through maturity while the soybean stem canker pathogen, Dpc, was isolated infrequently from senescent and mature plants. These three pathogens are distinguished from differences in sporocarp type and morphology, frequency of alpha and beta spores formed, appearance in culture and differences in pathogenicity. The two varieties Dps and Dpc have been previously described (2) and the data reported herein further support the validity for separation of these two varieties. The species of Phomopsis described in this research is distinct from both Dps and Dpc in morphology and pathogenicity.

Different forms of Phomopsis and Diaporthe associated with soybean plants and seed have been variously described. Luttrell (31) reported isolating perithecial and non-perithecial strains of Dps. Morphological variants of D. phaseolorum were found in Delaware (36, 43) and hetero- and homothallic types of Diaporthe were reported from single
ascospore isolates (21, 53). *Phomopsis* sp., as characterized here, appears similar to Lehman's strain 14 (30), the non-perithecial strains of Luttrell (31), types 2, 3 and 5 described by Strelecki (43) and isolates of Hildebrand (21) forming multichambered pycnidia with distorted beaks. Isolates of *Dps* correspond to descriptions presented in several reports (2, 31, 51, 52, 53, 54) and most notably strain 17 of Lehman (30). Strain 17 produced perithecia regularly in culture with alpha spores less abundant and stylospores more numerous than in imperfect strains. The *Phomopsis* described in this report is distinct from *Dps* since perithecia have not been found in culture or in soybean tissues. Perithecia from soybean stems or cultures always gave rise to *Diplarthra* colonies. Colonies forming *Dps* pycnidia and perithecia were isolated from 8% of 1,020 pycnidia removed from stems, pods, and petioles. The remaining 92% grew into cultures of *Phomopsis* sp. The *Phomopsis* isolated from soybean is possibly a heterothallic form of *Dps*. However, from previous investigations (2, 21, 30, 54) and in this study, *Diplarthra* could not be obtained by mating single alpha spore isolates of *Phomopsis* sp.. Thus it appears that *Dps* is homothallic and that the beaked pycnidial, stromatic type *Phomopsis* has not been demonstrated to be heterothallic. The only contradictory evidence is by Welch and Gillman (53) who obtained perithecia from mating single ascospores of *Dps*. Their single ascospore cultures produced only pycnidia.
However, it is not clear from their report if the pycnidia were of the Dps-type or the Phomopsis-type described in this paper. Until it is clearly demonstrated that the beaked, erumpent pycnidia and stromatic type of Phomopsis is heterothallic and capable of producing perithecia when mated, this fungus should be considered a distinct entity.

Phomopsis sp. and Dps are distinct in several other cultural characteristics. Phomopsis sp. produces erumpent, beaked, multichambered pycnidia and Dps subglobose pycnidia with slightly protruding beaks, although multichambered pycnidia have been observed on old cultures. The pycnidia of Phomopsis sp. are borne in carbonaceous, pulvinate stromata in contrast to the echinulate stromata bearing Dps pycnidia. A gelatinous matrix is commonly exuded from Phomopsis sp. pycnidia but rarely from Dps pycnidia. Stylospores were seen in Phomopsis sp. pycnidia on only two occasions but Dps pycnidia contained them often, especially after several subculturings. Phomopsis sp. alpha spore production persists through several subcultures in most isolates but Dps forms fewer such spores with increasing transfer of isolates on PDA-L. Mycelia pigmentation was variable in some instances, but in general Phomopsis sp. was a dingy yellow or yellow-green and Dps an orange or orange-yellow color. The differences in culture appearance that the author believes separates Phomopsis sp. from Dps agree remarkably well with the characteristics Lehman found different between imperfect strains 7, 14, and 19 and the
ascospore strain 17 (30).

Despite the above differences, Phomopsis sp. and Dps were similar on plants and in culture in several characteristics. The dimensions of young pycnidia of the two species are indistinguishable on stems, pods, cotyledons and petioles. Pycnidia of both fungi were boat-shaped on stems and spherical on pods in naturally and artificially infected samples. These observations agree with results reported by Lehman (30). Also, alpha spore sizes overlapped and were similar in culture and in nature. Light was unnecessary for sporocarp formation but the number formed was greater in the presence than in the absence of light.

Separation of fungal species based on cultural characteristics has definite shortcomings. Media composition, incubation temperature and quality and quantity of light during incubation may have profound effects on vegetative and reproductive structures in culture (4, 29, 44). Admittedly, a number of media types and a range of light and temperature conditions should be tested on Phomopsis and Diaporthe isolates before the separation is totally valid. In spite of these deficiencies, the consistent differences observed in this study indicate taxonomic distinction should be considered for the Phomopsis and Diaporthe described herein.

In addition to morphological distinctions, Phomopsis sp. and Dps differed in pathogenicity on soybeans. Phomopsis sp. is more pathogenic and virulent than Dps on green
pods and green and mature seed. *Phomopsis* sp. isolates produced pycnidia in 4 days on green pods incubated on WAP media whereas Dps-infected pods did not form pycnidia until 7 to 10 days after inoculation. Lehman noted pycnidia forming on pods in greenhouse-grown plants inoculated with imperfect strain 7 by five days after inoculation and not until 7 days with perfect strain 17 (30). In both the present study and in Lehman's (30) work, pycnidia were produced sooner and on a higher number of *Phomopsis*-inoculated pods than on Dps-inoculated pods. Therefore, *Phomopsis* sp. is generally more virulent than Dps on green pods. *Phomopsis* sp. completely colonizes seed and prevents the radicle from emerging whether the fungus is introduced naturally or by toothpick tip inoculation. Seed infected naturally or artificially by Dps are usually devoid of mycelia and the radicle generally emerges even in seed inoculated with a virulent isolate. There are variations in pathogenicity among isolates of both *Phomopsis* sp. and Dps but degrees of pathogenicity among plant pathogens are common in nature (9, 39). Despite a wide virulence range, 16 of 20 *Phomopsis* sp. isolates decayed more than 50% of seed inoculated in contrast to only 9 of 20 highly virulent isolates of Dps. Although *Phomopsis* sp. and Dps differed in pathogenicity on seed, they induced similar reactions on soybean plants. This evidence supports the findings of Lehman (30) in which his ascospore strain 17 was as pathogenic on pods as the imperfect strain 14. In the present study, a single
isolate of both fungi caused stem canker and killed plants in growth chamber studies. Also, the two pathogens caused pod and stem blight symptoms in the field and in the growth chamber.

Positive separation of the soybean seed decay pathogens as *Phomopsis* sp., *Diaporthe phaseolorum* var. *sojae* and *D. phaseolorum* var. *caulivora* is warranted by evidence presented in this study. However, the criteria used may not be upheld by certain taxonomic standards. In ascomycetes having a pleomorphic life cycle, the first valid name or epithet applied to the perfect state takes precedence over a name or names applied to the imperfect state (1). Names of imperfect states may, however, still be used on appropriate occasions. The author feels the recognition of *Phomopsis* sp. and *Dps* as distinct pathogens is justified based on natural and cultural morphology and on qualitative and quantitative differences in pathogenicity.
SUMMARY AND CONCLUSIONS

Soybean seed decay is caused by a species of *Phomopsis*, *Dipotthe phaseolorum* var. *sojae* (Dps) and *D. phaseolorum* var. *caulivora* (Dpc). Evidence presented in this paper suggests the involvement of 3 pathogens based on differences in sporocarp and colony morphology, sexual reproduction, symptom production, and pathogenicity. In addition to further data supporting the separation of Dps and Dpc the author proposes recognizing the *Phomopsis* species causing soybean seed decay as an entity distinct from Dps or Dpc. Although *Phomopsis* and Dps are similar in several morphologic characters, differences in pathogenicity and sexual reproduction, or the lack of it, justify the recognition of two distinct fungi. Until further taxonomic studies are made, the fungus lacking perithecia and highly pathogenic on seed should be named *Phomopsis* sp.

In addition to their role as seed decay pathogens, *Phomopsis* sp., Dps and Dpc are associated with immature and mature soybean plants. *Phomopsis* sp. and Dps are consistently recovered from plants beginning at emergence and continuing long after maturity. A single isolate, each of *Phomopsis* sp. and Dps, were actively pathogenic, i.e. causing stem canker, in the growth chamber but in most cases the fungi are associated with immature plants as residents.
or quiescent, latent pathogens. On mature plants *Phomopsis* sp. and *Dps* are highly saprophytic. *Diaporthe phaseolorum* var. *caullivora* was not isolated from immature plants until near maturity and was recovered infrequently from most samples. However, it was isolated from all plants with stem canker disease in the field.

The frequency and condition of association of *Phomopsis* sp., *Dps* and *Dpc* in immature and mature soybeans is regulated by several cultural practices and epidemiological factors. Higher frequency of *Phomopsis* sp. than *Dps* or *Dpc* in plants and seed is determined by availability of inoculum. Soybeans can be infected by *Dps* or *Dpc* but sporocarp production in the field is significantly greater in *Phomopsis* sp. than in either *Diaporthe* species. The high level of *Phomopsis* sp. inoculum is found in pycnidia on soybean stubble. The practice of crop rotation appears to reduce the inoculum level of *Phomopsis* sp. but the same practice results in increased incidence of *Dps*. Subsequently, *Dps* is found more often in plants growing in soil previously cropped to corn than is *Phomopsis* sp. Conversely, *Phomopsis* sp. is isolated significantly more often than *Dps* from plants growing in soil monocultured to soybeans. Plowing soybean debris in the fall results in lower frequency of *Phomopsis* sp. on stubble than if debris is not plowed under until spring while the reverse is true for *Dps* incidence. Further work is needed on survival of the pathogens.
Phomopsis and Diaporthe show a number of constant epidemiological features in their association with soybeans. The fungi are recovered from plants as early as 12 days after planting and increase in frequency as plants and seed matured. In addition, harvest delay results in nearly 100% seed infection by the pathogens. Although Phomopsis and Diaporthe are widespread on soybeans, Phomopsis sp. is isolated most often from middle and lower areas of the plant while the Diaporthe species are recovered most often from near the top of plants. The 3 pathogens are usually splashed onto plants, cause local infection, remain quiescent in green tissue and spread rapidly throughout the plant as senescence occurs.

Date of planting and variety influence the severity of plant and seed infection by Phomopsis and Diaporthe. In early season varieties, the fungi were recovered more often from soybeans planted in early May than from those planted in mid May or in June. Maturity Group III varieties were usually less infected during development and at maturity than Group II varieties irrespective of planting date, crop rotation, or location of planting.

The pathogens causing soybean seed decay are omnipresent in Ohio soybean fields. Since resistance has not been found in commonly used varieties and chemical control is still being evaluated, several cultural practices are suggested for disease control: 1) modify intensive cropping of soybeans, 11) plow under soybean debris in the fall,
iii) use varieties from Maturity Group III or later when possible and iv) harvest seed soon after maturity.
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