Top Dieback of Soybean Caused by *Diaporthe phaseolorum* var. *caulivora*

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**ABSTRACT**

Top dieback of soybean caused by *Diaporthe phaseolorum* var. *caulivora (Dpc)* developed late in the season and was distinct from soybean stem canker, which is also incited by *Dpc*. Symptoms of top dieback were premature death of the upper five or six internodes. In field studies, isolates of *Dpc* induced dieback in tip-inoculated plants and stem canker in plants inoculated in lower internodes. *Dpc* was isolated three times more frequently from seeds than from pods from which seeds were taken.

Additional key words: *D. phaseolorum* var. *sojae*, *Phomopsis* sp., pod and stem blight

In the last several decades, species of *Diaporthe* and *Phomopsis* have been associated with seedling blights, pod and stem blight, stem canker, and seed decay of soybean (*Glycine max* (L.) Merrill) (1,2,7,9,15). Pod and stem blight and seed decay at harvest have been the most prevalent symptoms observed in Ohio in recent years (10-16).

Late in 1977, we observed an unusual dieback of maturing soybean plants in some fields in Ohio. The upper five or six internodes of these plants were distinctly darker brown than the lower internodes of the same plants. Pod and stem blight symptoms were evident in lower internodes but not in the upper internodes where dieback was apparent. Seeds collected at upper nodes were often visibly moldy (Fig. 1), but fewer seeds from lower nodes were moldy.

Initial isolates from pods and seeds from dieback sections of affected plants were mostly the stem canker pathogen, *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* Athrow & Caldwell (*Dpc*) (Fig. 1). *D. phaseolorum* var. *sojae* (Lehman) Wehmeyer and *Phomopsis* sp. (13) were also present.

Plants with similar dieback symptoms were observed in Ohio again in 1979 and described from Michigan in 1980 (Schmitttenner and Hobbs, unpublished). *D. phaseolorum* var. *caulivora* was frequently isolated from seeds collected from these plants in both years.

The objective of this study was to investigate the etiology of the top dieback and its relationship to the *Diaporthe* and *Phomopsis* spp. known to be soybean pathogens. A preliminary report has been published (11).

**MATERIALS AND METHODS**
In 1977, 30 soybean cultivars growing in central Ohio were sampled to determine the relative frequencies of *Dpc* and *Phomopsis* sp. plus *D. phaseolorum* var. *sojae* (Ps) associated with different symptoms. Six cultivars had only pod and stem blight symptoms, and 24 had both dieback and pod and stem blight. Fifty or more mature pods were collected from the five uppermost nodes of all cultivars; a second sample was obtained from the five lowermost nodes of cultivars with dieback symptoms. In

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**Fig. 1.** Pods and moldy seed from upper internodes of soybean plants with dieback and isolates of *Diaporthe phaseolorum* var. *caulivora* from seed growing on potato-dextrose agar.
addition, similar “upper” and “lower” samples were collected from plants with dieback symptoms from six fields in five counties in Ohio.

Pods were rinsed thoroughly in tap water containing Tween 20. Seeds were removed from the pods, and disks of pod tissue were cut with a paper punch. Disks were surface-sterilized for 1 min in 70% ethanol, followed by 2 min in 1.05% sodium hypochlorite, then drained on sterile filter paper, and plated on potato-dextrose agar acidified to pH 4.5 after autoclaving with 85% lactic acid (PDA-L). Seeds were surface-sterilized for 1 min in the sodium hypochlorite solution, drained, and placed on PDA-L with the disks (three disks plus three seeds per plate). One hundred disks and 100 seeds per sample were plated unless otherwise noted.

Plates were incubated at room temperature (23–26 C) 4 days in the dark, then 11 days under continuous fluorescent light. Isolates of Dpc and Ps were identified by colony morphology as described by Kmetz (12) and isolation percentages determined.

Pathogenicity of Dpc isolates. Five Dpc isolates from seed were maintained at room temperature on PDA-L. Mycelial agar blocks (0.5 cm) were transferred from stock cultures to fresh PDA-L plates, incubated 4 days in darkness and then under fluorescent light supplied 12 hr per day. After 5 wk, a culture of each isolate, containing mycelia and mature perithecia with ascospores, was blended in 50 ml of sterile distilled water for 20 sec in a Sorvall Omnimixer at setting 6 and used as inoculum in pathogenicity tests.

Single row plots of soybean cultivars Calland and Williams were planted in the spring of 1979 at the Ohio Agricultural Research and Development Center in north central Ohio. The cultivars were separated and bordered by single rows of other cultivars. Seventy-eight days after planting, 10 blocks of 12 plants each were marked off in each cultivar. Each block was further divided into six pairs of plants, and each pair was randomly assigned one of the five Dpc treatments or a sterile water control. One plant of each pair was inoculated at the stem tip and the other at a lower internode. Inoculation positions were alternated in each block.

For stem tip inoculations with Dpc, plant tops were severely where the stem diameter was about 5 mm; a sterile steel probe was inserted there and rotated slightly to create a channel. Stem tips were then injected with 0.4 ml of inoculum from a hypodermic syringe as the needle was slowly withdrawn from the channel. For lower internode inoculations, a channel was made below the third or fourth node and Dpc inoculum injected as described. Control plants were injected with sterile distilled water. After injection, wounds were covered with petroleum to prevent desiccation.

Injected plants were hand-harvested 113 days after planting and numbers of plants with discolorations or cankers recorded. Plant segments were assayed for Dpc as follows. Stem tip and lower internode segments were scrubbed in a solution of tap water and Tween 20 to remove surface dirt and petroleum, air-dried, and surface-sterilized as described for pod disks. Ten transverse sections about 0.5 cm long were removed from each segment and plated on PDA-L. Plates were incubated as noted before, but light was supplied only 12 hr per day. Fungal isolates were identified as described.

RESULTS

Dpc was isolated twice as often from cultivars with dieback as from cultivars with pod and stem blight only (Table 1). Conversely, Ps occurred three times more frequently in cultivars that had only pod and stem blight. Dpc occurred nearly four times more frequently in pod disks and seeds from dieback sections than in lower node sections of the same plants. Ps was isolated three times more frequently from lower nodes than from upper nodes. Dpc was isolated nearly three times more frequently from seeds taken from pods of upper nodes of all cultivars than from the pods themselves; Ps levels were not significantly different.

Pathogenicity data from the Calland and Williams cultivars were combined (Table 2) because the percentages of plants with symptoms (67.5 and 65.8%, respectively) were similar, as were the percentages of plants from which Dpc was recovered (55.0 and 63.3%, respectively). Data for the five Dpc isolates tested were also combined because no differences were found among them for percentages of plants with symptoms.

Although percentage recovery of Dpc varied among some isolates, in all cases recovery was significantly higher from plants inoculated with a Dpc isolate than from the controls.

Of the plants inoculated at the stem tip with Dpc, 81% had discolored internodes by the time the study ended, whereas 5% of the comparable controls had similar symptoms (Table 2). Dpc was recovered from 85% of inoculated plants and 5% of the controls. Of the plants inoculated with Dpc at a lower internode, 74% developed cankers at the treated internode, and Dpc was recovered from 56% of the inoculated plants. Cankerlike symptoms developed in 20% of the control plants for this group, but Dpc was not recovered from any of these plants.

DISCUSSION

This is the first detailed confirmation that Dpc causes a late season dieback of soybean. Dunleavy (6) inoculated stem tips of greenhouse-grown soybeans with Dpc after flowering to determine cultivar resistance to stem canker but did not report Dpc infection of stem tips occurring naturally in the field. Dpc previously has been associated only with soybean seedling blights (7,9) and with midseason stem canker (1,2,4,7,9,17).

Top dieback occurs later in the growing season than stem canker, which can first be found 62 days after planting (1). Crall (4) and Hildebrand (9) found perithecia of Dpc on infected plants during the growing season and postulated that they might provide inoculum for later infections, a view supported by Dunleavy (7). However, other workers (2,14,17) have reported that Dpc perithecia are found only on overwintered soybean stems. Kmetz et al (15) did not find Dpc perithecia on detached vegetative soybean parts that were incubated 1 mo in a moist chamber. Another study (10) indicated that overwintered debris may be the most important source of Dpc inoculum. Additional studies are needed to determine when perithecia mature and ascospores are liberated.

Soybean plants may be resistant to Dpc infection during rapid vegetative development but become susceptible as physiologic changes associated with flowering occur. Athow and Caldwell (2)

| Table 1. Isolation of Diaporthe phaseolorum var. caulivora (Dpc) and Phomopsis sp. plus D. phaseolorum var. sojae (Ps) from soybean pods and seeds |
|------------------|------------------|------------------|
| Category         | Percent isolation |
| Symptom          | Dpc              | Ps               |
| Dieback          | 24.7            | 14.8             |
| Pod and stem blight | 12.2          | 46.2             |
| LSD (0.05)       | 10.3            | 7.1              |
| Pod position     |                  |                  |
| Upper            | 24.7            | 14.8             |
| Lower            | 6.3             | 53.2             |
| LSD (0.01)       | 8.4             | 8.9              |
| Plant part       |                  |                  |
| Seeds            | 32.5            | 22.3             |
| Pods             | 12.7            | 17.8             |
| LSD (0.01)       | 10.4            | NS*              |

*Based on isolations from pods and seeds from the five uppermost nodes. Data are least-squares means of 60 replications of 100 units each of either pod disks or seeds (one replication was of 50 seeds) for cultivars with dieback and 12 replications of 100 units each for cultivars with only pod and stem blight.

*Based on isolations from pods and seeds from the five uppermost or lowermost nodes of soybean cultivars with dieback. Data are means of 30 replications of 200 units each (100 seeds plus 100 pod disks), except that one upper and one lower replication had 150 units each (50 seeds plus 100 pod disks).

*Based on isolations from pods and seeds from the five uppermost nodes of various soybean cultivars. Data are means of 36 replications of 100 units each, except that one seed replication had only 50 units.

*Not significant at P = 0.05.
Table 2. Pathogenicity of Diaporthe phaseolorum var. caulivora (Dpc) to soybean plants inoculated at stem tips or lower internodes

<table>
<thead>
<tr>
<th>Inoculation position</th>
<th>Treatment</th>
<th>Plants with symptoms (%)</th>
<th>Recovery of Dpc (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem tip</td>
<td>Dpc</td>
<td>81.0</td>
<td>85.0</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>LSD (0.01)</td>
<td>13.8</td>
<td>12.6</td>
</tr>
<tr>
<td>Lower internode</td>
<td>Dpc</td>
<td>74.0</td>
<td>56.0</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>20.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>LSD (0.01)</td>
<td>16.1</td>
<td>16.9</td>
</tr>
</tbody>
</table>

*Discoloration or canker of inoculated internode.
*Data for Dpc treatment are means of 10 plants for each of five Dpc isolates for each of two soybean cultivars (Calland and Williams). Data for the water treatment are means of 10 plants for each of the two cultivars.
*Based on isolations on potato-dextrose agar, pH 4.5, acidified after autoclaving with lactic acid.
*Based on total number of plants inoculated with Dpc.

Inoculated stems of 28- to 86-day-old greenhouse-grown soybean plants with Dpc and found that stem canker development was restricted in most of the younger plants but that the older plants were more susceptible. Inoculation of 62- to 91-day-old soybean plants indicated that susceptibility to stem canker decreases as the plants mature (3.9). Upper internodes would be the most susceptible since they mature later than lower parts of the plant.

In previous studies on Dpc, D. phaseolorum var. sojae, and Phomopsis sp. in immature and mature soybean plants, Phomopsis sp. was more prevalent on lower sections of the plants (8,13,15), and the two D. phaseolorum varieties were isolated with similar frequency from all sections sampled (15). In the present work, Dpc was isolated more frequently from the upper sections and Ps from the lower sections. Possible differences in the mechanisms of dissemination of ascospores of Dpc and conidia of Ps could account for these results. However, no comparative studies of this aspect have been made. It seems more likely that Ps spores are disseminated early, with infection remaining latent until late in the growing season (14), whereas Dpc spores are disseminated late in the season and immediately penetrate and colonize susceptible plant tissues. Kmetz et al (15) did not isolate Dpc from vegetative tissues of soybean until near maturity, although Phomopsis sp. was initially isolated from 12-day-old seedlings and D. phaseolorum var. sojae from 30- to 33-day-old plants. Dpc was first isolated from immature soybean seeds as pods began to develop at the upper nodes.

The full impact of Dpc infection on soybean production and seed quality is not known, although stem canker is recognized as an economically important disease (1.2,9,17). The planting of Dpc-infected seed has not been shown to reduce soybean yields or to influence the incidence of stem canker (9). However, previous studies have reported relatively low (5–12%) levels of Dpc-infected seed (9,15), whereas in this study, Dpc was isolated from 22.3% of the seeds from cultivars with dieback. Dpc seed infection may be a more important factor in soybean production than has been previously realized. Further research on the economic and epidemiological aspects of infection of soybeans by Dpc is needed.

LITERATURE CITED