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THE ROLE OF SOYBEAN DEBRIS IN SURVIVAL AND INOCULUM PRODUCTION OF *DIAPORTHE PHASEOLORUM* VAR *CAULIVORA*, THE CAUSE OF STEM CANKER ON SOYBEAN

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ABSTRACT

Diaporthe phaseolorum (Cke. and Ell.) Sacc. var. *caulivora* Athow and Caldwell, the cause of soybean stem canker, overwintered and sporulated on soybean stem residue whether it was buried, on the surface, or elevated above the soil surface. Sporocarps were observed as early as February at all positions. Conidia emanating from overwintered plant residues on the surface were collected continuously on spore traps from late June to late August with peak numbers in August. No ascospores were observed. Soybean seedlings exposed to *Diaporthe*-infested residues on the soil surface were inoculated as early as June. Soybean plants growing in soil containing buried soybean debris infested with the pathogen were inoculated in root or stem areas below the soil surface.

INTRODUCTION

Stem canker, caused by *Diaporthe phaseolorum* (Cke. and Ell.) Sacc. var. *caulivora* Athow and Caldwell (Dpc), is one of several diseases of soybeans (*Glycines max* L.) which may occur from midseason to maturity. One symptom is a dark brown, sunken canker which extends several centimeters along the stem as plants approach senescence. Stems are eventually girdled causing premature death.

Until the middle 1970's and early 1980's, stem canker had been primarily limited to northern agricultural regions where it overwintered on soybean debris remaining in the field following harvest (Frosheiser, 1957; Hildebrand, 1954; Kmetz, et al., 1979). Recently, the disease has been observed in a number of southeastern states (Boerma, et al., 1977; Keeling, 1982; Krausz and Fortnum, 1983) including Tennessee (Hilty, 1981). The pathogen in the southeast is distinct from that in the north (Hobbs and Phillips, 1985; Morgan-Jones and Backman, 1984) and several isolates from Tennessee have been identified as the "southern type."

The prevalence of the pathogen in Tennessee is associated with the increased practice of conservation tillage which allows soybean debris and stubble of harvested plants to remain exposed. This implies that high inoculum levels developing on debris are important in disease development. Rain splashed or wind-blown spores can infect plants. Burying crop residue, as in conventional tillage has reduced the incidence and severity of stem canker (Rothrock, et al., 1985; Tyler, et al., 1983).

However, in a greenhouse test it was suspected that soybeans became infected by the pathogen buried in the root area. Inoculation of soybeans with buried inoculum of a midwestern isolate of the pathogen was attempted by Frosheiser, 1957, and Dunleavy, 1958, with varying results.

This study was conducted to examine the role of soybean debris in the disease cycle of the pathogen causing stem canker in Tennessee.

MATERIALS AND METHODS

Survival on Debris

Plant debris from a field of stem canker-diseased soybeans (cv. Forrest) was harvested in Madison County, Tennessee, in November of 1982 and stored outside in loose bales on the soil surface in Knoxville. In January of 1983, diseased stems with a gray-black blotch symptom were cut into 15-20 cm lengths and wrapped into bundles of five in fiberglass screen. Bundles were placed in each of three positions: on the soil surface; buried at approximately 15 cm; elevated approximately 15 cm above the soil surface. Rainfall, air temperature and soil temperature at 15 cm depth were recorded daily (Table 1). Approximately once a month from February to July a bundle of stems was retrieved from each position. Stems were rinsed in running tap water and examined for perithecia and pycnidia development with a dissecting microscope. Sporocarps were removed and examined for spore development with a light microscope.

Spore Dispersal

Spore dispersal from soybean debris and time of plant inoculation was examined in the growing season of 1984. Overwintered stem canker-diseased soybean debris, including stems, petioles and leaves, from Madison County, was distributed about 2.54 cm deep over 15 square feet of soil surface in Knoxville in June. The test plot was 8.0 km from the nearest soybean field.

Table 1. Climatological data for January to June, 1983, Knoxville.

Date	Temperature (C) Lowest	Highest	Avg. Max.	Avg. Min.	Avg.	Avg. Soil[a]	Precip. mm.
January	-6.1	16.1	9.2	-0.2	4.4	0.8	15.2
February	-5.6	23.3	12.7	2.2	7.4	3.2	94.0
March	-1.1	31.1	18.4	5.9	12.1	7.1	40.6
April	-1.1	31.1	21.8	7.7	14.7	10.0	165.1
May	7.8	32.2	28.4	13.5	20.4	16.8	162.6
June	12.2	37.7	32.7	18.2	25.4	24.7	73.7

[a]Temperature at 15 cm depth, 9:00am

Table 2. Average number of conidia trapped per day, weekly precipitation, isolation of pathogen from seedlings, symptoms on senescent plants and weekly average air temperature at trapping site, Knoxville, 1984.

Date	Spores Trapped Per day	PPT (MM)	Isolation From Seedlings	Symptoms on Senescent Plants	Temp. Avg(C)
7/4	4.7	66.0	+[b]	+[b]	25.0
7/11	20.6	61.0	-[c]	+	27.5
7/18	13.4	81.3	+	+	25.4
7/25	14.4[a]	0.0	+	-[c]	25.9
8/1	15.0[a]	33.0	-	-	25.5
8/8	90.9	5.1	+	-	27.0
8/15	4.0	10.2	-	-	28.0
8/22	5.6[a]	18.0	+	+	26.4
8/29	4.1[a]	5.1	+	-	25.5
9/4	0.0	33.0	+	-	25.5

[a]one day of rotorod malfunction
[b] +=positive isolation or symptoms
[c] -=negative isolation or symptoms

Every seven days from 27 June to 5 September 1984, five 10 cm pots, each with two 9 day-old greenhouse-grown soybean seedlings (cv. Forrest) were placed randomly on the debris. After a seven day exposure the plants were returned to the greenhouse. One seedling from each pot was cut at the soil line and all petioles, the second and fourth node and hypocotyl were rinsed in gently flowing tap water and plated on acidified potato dextrose agar (Tuite, 1969). The remaining seedlings were transplanted to 20 cm containers and allowed to grow to maturity in the greenhouse.

A rotorod sampler (Ted Brown Associates, Los Altos, CA, 94002) was used to collect airborne spores above the infested debris near the seedlings. The retracting-head collector, while in the closed position, was located 20 cm above the debris at the level of the seedling terminus. The sampler was timed to operate 15 min each hour. Silicone-coated collecting rods were changed every 2-3 days, affixed to a stage adapter and examined for spores using a light microscope. Rainfall was recorded at the spore collecting site using a standard gauge (Table 2).

Buried Inoculum

In a greenhouse study, soybeans were grown in soil containing soybean debris artificially infested with Dpc. Soybean debris, including leaves, petioles, stems and empty pods, was autoclaved in 946 ml glass jars with 400 ml half-strength potato dextrose broth (Tuite, 1969). The debris was inoculated with pieces of PDA on which the pathogen was growing. The pathogen used was isolated from a diseased plant (cv Essex) in west Tennessee and maintained on PDA. After nine days in stationary culture at 25 C the infested debris was drained, air-dried 72 hr, and broken in a blender until the largest pieces were 2-3 cm in length. The infested debris was mixed with a soil medium consisting of 1/2 silt-loam and 1/2 sharp sand at the rate of 10 g in 454 g of medium.

Plastic pots (10 cm diameter) were filled with infested soil to 2.5 cm below the rim and watered. Seeds of cultivars Tracy and Essex, resistant and susceptible to stem canker, respectively, were planted in 10 pots, 10 seeds per pot, and gently pressed into the surface. Seeds and inoculum were covered to a depth of 2.0 cm with sterile sharp sand, assuring that inoculum was not exposed. Controls consisted of seeds planted in five pots of autoclaved infested soil. Plants were gently watered as needed and a 20-20-20 aqueous fertilizer was added weekly.

Ten to 14 days after planting, percent emergence was recorded and seedlings were thinned to two per pot and transplanted to 20 cm terracotta pots for maturation.

Samples of seed lots were plated on acidified PDA to detect the seed-borne phase of Dpc.

RESULTS AND DISCUSSION

Survival on Debris

It is well-established that the incidence of stem canker is associated with inoculum emanating from soybean debris. The pathogen survived on soybean stems at the three positions from February to July 1983. Pycnidia were present on all stems at each position as early as 3 February but conidia (alpha spores) were observed only in crushed pycnidia from buried stems on that date. Perithecial necks 1.0 mm in length were first observed on 18 February only on buried stems, and discernible ascospores were first detected 15 March on buried stems. Perithecia without discernible ascospores were first observed on stems on the surface at mid-March. Perithecia were first detected on elevated stems in July but ascospores were not developed. Perithecia were more prevalent than pycnidia on stems at all positions from April to late July. Buried stems were not unduly decomposed and fungus survival was not diminished.

Spore Dispersal

It is believed that airborne spores initiate most infections by Dpc. Wind-blown or splashed conidia were abundant in the test and were collected as soon as the collecting rods were in operation in June 1984. Conidia were observed in relatively low concentrations through 1 August (Table 2). A sudden peak in spore numbers occurred during the sampling period ending 8 August, followed by an immediate decline. No conidia were collected during the period of 29 August to 4 September, and ascospores were never observed. The dearth of ascospores has been reported by others and their importance as inoculum questioned (Kmetz et al., 1979), although their infectivity has been demonstrated in artificial conditions (Frosheiser, 1957; Smith et al, 1986).

The pathogen was isolated from one or more seedling parts exposed to surface debris throughout the test and as early as the period ending 4 July. It is unlikely that inoculum from a source external to the test plot occurred, since stem canker has not developed in east Tennessee. Pathogen cultures developing from seedling parts probably originated from spores adhering to the plant surface, since tissue was gently washed and no surface sterilants were used, or latent infections occurred. The delay in canker development until plants reached reproductive stages supports findings of others (Kmets et al., 1979).

Buried Inoculum

Prominent cankers developed on stems on Essex growing in soil containing inoculum as infested soybean debris in the greenhouse (Table 3). Lesions, which emanated from below the covering sand, were evident after plants reached reproductive stages (Fehr and Caveness, 1977). Some plants developed severe wilting of youngest leaves and eventually the entire plant. Symptoms did not develop on uninoculated controls or on Tracy. Typical Perithecia developed on all diseased stem segments when excised and incubated 14-16 days in a moist chamber but the pathogen was isolated from only a few discolored roots.

There were no differences in emergence between inoculated and uninoculated plants and no symptoms of infection were evident before lesions appeared at the base of stems. The seedborne phase of the pathogen was not detected. Thus, since the only source of inoculum was buried in the soil medium, it is believed that plants became infected through the below-ground parts.

Gerdemann detected partially decomposed soybean stems above and below ground in Illinois fields devoid of soybean crops for three years, and buried soybeans can be identified in Tennessee soils in the spring when fall plowing has been practiced (Tyler, personal communication).

These conventional tillage operations have been practiced on an average of 77% of Tennessee soybean acreage from 1984 to 1988 (TN Crop Rept. Ser. 1988) and an increasingly common practice in Tennessee is spring plowing of overwintered soybean debris and stubble just prior to planting. Conceivably, the pathogen existing on diseased stems tilled into the seedbed at planting could serve as an inoculum source in addition to aerial spores. The quantity of infested debris and its proximity to seeds and developing seedlings in the field could affect inoculations of below-ground plant tissue. Although the condition and fate of mycelium of Dpc on plant residue tilled into the soil is unknown, in the long term, such inoculum may serve as a source of infection.

Table 3. Stem canker development on Essex soybeans growing in soil containing buried soybean debris infested with *Diaporthe phaselorum* var. *Caulivora.*

Canker detected (growth stage)[a]	Percent plants with canker[b]
R1	8.0
R2	16.0
R3	8.0
R4	33.3
R5	16.0
R7	8.0

[a]R = reproductive stages according to Fehr and Cavinnes, 1977.
[b]Cankers did not occur on control plants without inoculum.

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