Etiology

Pathogenicity of Sclerotia- and Nonsclerotia-forming Isolates of Colletotrichum truncatum on Soybean Plants and Roots

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ABSTRACT

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Three isolates (Ct-1, Ct-3, Ct-4) of Colletotrichum truncatum from soybean (Glycine max) soil or seeds produced microsclerotia in culture and in soybean tissues. The three sclerotia-forming and three nonsclerotiaforming isolates (Ct-2, Ct-AU, Ct-HL) were pathogenic to soybean roots in an aeroponics growth chamber. Sclerotia-forming isolates of C. truncatum and the pathogenicity of C. truncatum on soybean roots were not reported previously. Soybean cvs. A. K. (Kansas), Boone, Corsoy 79, and Williams 82, which differed in resistance to foliar anthracnose caused by C. truncatum, were all susceptible to root infection by all isolates;

Williams 82 was most susceptible, followed, in descending order, by Corsoy 79, A. K. (Kansas), and Boone. Lesions on roots of plants growing in an aeroponics growth chamber were significantly longer with Corsoy 79 and Williams 82 inoculated with isolates Ct-1, Ct-AU, and Ct-HL than on roots inoculated with isolates Ct-2, Ct-3, or Ct-4. In field studies, yields were significantly suppressed below noninoculated plants for all cultivars except Boone when inoculated separately with isolates Ct-1, Ct-2, and Ct-3. Estimated yield loss due to anthracnose was 17% for A. K. (Kansas), 23% for Corsoy 79, and 30% for Williams 82.

Anthracnose of soybeans (Glycine max (L.) Merr.), caused by Colletotrichum truncatum (Schwein.) Andrus & W. D. Moore, has been reported as a disease of the above-ground plant parts wherever soybeans are grown. The disease is economically important in the humid tropics and subtropics and occasionally in the temperate zones after prolonged warm, rainy periods (15,18). The above-ground parts of soybean plants may be infected by C. truncatum at any growth stage, but symptoms generally appear either during early seedling or late reproductive stages and at senescence. The infection remains latent during vegetative stages (3,4,17). The disease can reduce seedling stands and seed quality, and yields can be suppressed by 16-26% in the United States (1). All studies on pathogenicity and resistance of cultivars to C. truncatum have been based on foliar infection (11-13). Root infection by C. truncatum has not been reported. Designation of C. truncatum as the causal agent of soybean anthracnose includes a key in which no reference is made to sclerotia-forming isolates of C. truncatum (20). We isolated three sclerotia-forming isolates of C. truncatum. We report on the pathogenicity of these three sclerotia-forming and three nonsclerotia-forming isolates on soybean seedling roots, the variation in infection and disease development of the three sclerotia-forming isolates on soybean cultivars under growth chamber and field conditions, and the effect of these isolates on plant growth and yield in the field. A portion of this work was reported (9).

MATERIALS AND METHODS

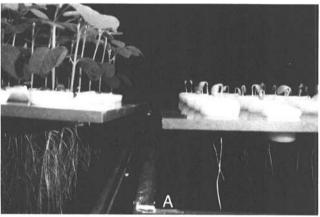
Isolates used. The six isolates of C. truncatum were: three sclerotia-forming isolates with their American Type Culture Collection acquisition number (Ct-1 [ATCC 76263], from a soildilution plate of field soil from a soybean field on the Cruse Farm, Urbana; Ct-3 [ATCC 76264] and Ct-4 [ATCC 76265], from infected soybean seeds grown on the Agronomy/Plant Pathology South Farm, Urbana) and three nonsclerotia-forming isolates (Ct-2, isolated from a soybean seed grown on the South Farm; and Ct-AU and Ct-HL, received from P. A. Backman, Auburn University, Auburn, AL). Single conidial cultures of isolates Ct-1, -2, -3, and -4 were obtained from dilution plates on water agar (WA) incubated for 8-10 h at 25 C, then transferred to acidified (pH 4.5) Difco (Detroit, MI) potato-dextrose agar (PDA). Identification was verified by comparison with isolate ATCC-18013 of C. truncatum. Stock cultures were maintained on PDA in the dark at 5 C.

Inoculum preparation. All isolates were grown on NaCl yeastextract agar (SYA). To induce conidia formation, isolates were transferred onto SYA in 9-cm-diameter petri dishes and incubated under 12 h alternating dark and cool-white fluorescent light (800 μmol·m⁻²·sec⁻¹) at 26 C. Inoculum was prepared with conidia

harvested from 8- to 10-day-old SYA plates. Cultures were flooded with 5-10 ml of sterile, deionized distilled water and brushed with a sterile rubber policeman. The conidial suspension was filtered through a double layer of cheesecloth and diluted with water to a final concentration of 2.5×10^6 conidia per milliliter.

In a second method, which required less time than the first, five to eight pieces of soybean leaf, petiole, or stem pieces were autoclaved for 15 min at 121 C and placed on WA in 9-cm-diameter petri dishes. Several plugs of an isolate were placed 1.0 mm from the tissue samples. All plates were incubated from 3 to 6 days under continuous cool-white fluorescent light at 26 C. Each isolate produced abundant acervuli on the plant tissues. A conidial suspension was prepared by aseptically removing the tissue samples and vigorously shaking them in sterile, deionized distilled water.

Inoculation of roots. Roots were inoculated by immersing them in a conidial suspension without or with methylcellulose (1.5%) for 10 s or by insertion of the root zone of differentiation into a 2-cm-diameter sponge soaked with SYA with or without fungus inoculum. The sponge pieces were split radially so that seedlings could be inserted, autoclaved, then dipped into autoclaved SYA before solidification and placed on SYA culture plates. Mycelial plugs from 7-day-old cultures were placed on the sponge pieces and incubated for 7-10 days at 25 C.



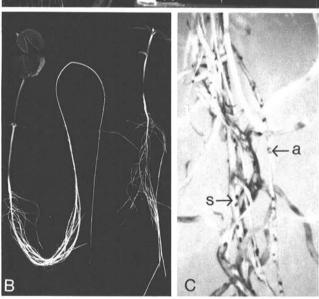


Fig. 1. A, emerging soybean seedlings (right) and seedlings in the V1-V2 growth stages (left) in trays of an aeroponics system (R. W. Wagner, 1990, Ph.D. thesis, University of Illinois, Urbana, 146 pp.); B, soybean seedlings (growth stage V1) from the aeroponics chamber noninoculated (left) or inoculated with Colletotrichum truncatum isolate Ct-1, showing anthracnose symptoms (right); and C, roots of a soybean seedling (growth state V3) from the aeroponics chamber showing anthracnose symptoms and the presence of acervuli (a) and sclerotia (s) of C. truncatum isolate Ct-1.

Pathogenicity studies. Pathogenicity studies on soybean seedling roots were conducted in an aeroponics chamber, which consisted of a root growth chamber that contained liquid-atomizing nozzles (21) (Fig. 1A). The chamber was fitted with a lid with plant ports in which individual plants were secured by polyurethane-based foam plugs (Dispo, American Scientific, McGaw Park, IL), so that seedling roots extended into the chamber. Roots were misted for 1 s every 5 min with a half-strength, sterile nutrient solution (6). The entire system was placed in a growth chamber with alternating 12 h light and dark at 25 C. The temperature within the root-misting chamber and in the seedling canopy was monitored with a copper-constantine thermocouple control module (model CR7, Campbell Scientific Instruments, Logan, UT). Photon flux density per unit area for the growth chamber was 255 μmol·m⁻²·s⁻¹. This value was based on the mean of 10 measurements throughout the chamber.

The pathogenicity of sclerotia- and nonsclerotia-forming isolates was assessed on soybean cvs. Corsoy 79 (maturity group III) and Williams 82 (maturity group IV), which are both susceptible to foliar infection by C. truncatum; Boone (maturity group IV), which is resistant to foliar infection; and A. K. (Kansas) (maturity group IV), which is moderately resistant to foliar infection. All seeds were surface-disinfested for 3-4 min in 0.05% NaOCl and planted in vermiculite in plastic trays in a growth chamber at 28 ± 1 C. Three-day-old seedlings were removed, washed in deionized distilled water, and individually secured in a foam sponge plug so that the cotyledons were in contact with the upper surface of the plug. The plug was inserted into a plant port so that roots extended into the chamber, and the roots were misted as described previously. To study lesion development, misting intervals were changed to 1 s every 30 min.

Roots were inoculated 2 days after placement in the aeroponics chamber. Roots were immersed into a conidial suspension, or a piece of foam sponge infiltrated with SYA with or without (control) fungus was attached. Roots dipped in water also served as a control. Two experiments were conducted, the first using inoculum of each of the six isolates alone and the second using isolates Ct-1 and Ct-2 alone or in combination. The inoculum concentration was the same whether one or a mixture of two isolates was used. Each treatment was replicated eight times and arranged in a completely randomized design in the chamber. Taproot and shoot length were recorded before roots were harvested for assay. Each of the two experiments was done three times.

Root assay. Six days after inoculation, a 5-cm piece was cut from the differentiation zone of each root, washed in deionized distilled water, and surface-disinfested with 0.05% NaOCl and rinsed twice in sterilized, deionized distilled water. These were placed individually on moist double-layered Whatman No. 2 filter paper in sterile 9-cm-diameter petri dishes and incubated at 25 $\pm\,2$ C. The number of visible sclerotia per 5-cm root was recorded 5 days later.

The same procedure was used to evaluate soybean cultivars for relative resistance to root infection. For these studies, lesion length on taproots was measured 6 days after inoculation.

Field studies. All field studies were conducted on the Agronomy/Plant Pathology South Farm, Urbana, during the 1988-1989 growing seasons. Soybean cvs. A. K. (Kansas), Boone, Corsoy 79, and Williams 82 were seeded 6 June 1988 or 30 May 1989 at a rate of 20 seeds per meter in five rows per cultivar with each row 4 m long and 0.76 apart. Stands were thinned to 10 plants per meter. To reduce interplot interference and fungal inoculum drift, plots were separated on all sides by a 1.5-m band of cv. Williams 82. The experimental design was split-plot in a randomized complete block with four replications. Cultivars were assigned to main plots, and isolates of C. truncatum were assigned to subplots. Plants at growth stage R1 were inoculated once in each year with conidial suspensions $(3-5 \times 10^6)$ conidia per milliliter) of Ct-1, -2, or -3 either on 27 July 1988 or 1 August 1989. Inoculum was prepared in deionized distilled water and applied with an atomizer-sprayer to the three center rows of each cultivar/subplot. Plants were sprayed until runoff. Control plants were sprayed with distilled water. Plants were inoculated just

before sunset, when the air was calm, and humidity was increasing. Plants were harvested by hand, each plot was threshed separately, and yield data were recorded as grams per plot. In 1988, yields were suppressed because of drought, and only 1,000-seed weights were recorded.

Typical symptoms of anthracnose appeared during growth stages R1-R2 on lower leaves, petioles, and stems as irregular brown lesions. Symptoms progressed acropetally as plants aged. Laminar vein necrosis, petiole cankers, and premature defoliation were recorded on Corsoy 79 and Williams 82 in the late R4 and early R5 growth stages. Plants can be asymptomatic (17), so disease severity was rated on leaf petioles in the laboratory. Leaf petioles were sampled at the R7 growth stage from bottom, middle, and upper portions of 10 randomly selected plants from each row of the three central rows in each plot. Petioles were cut into 5-cm pieces, washed under running tap water for 8 h, and treated with paraguat to aid in the detection of latent colonization by the fungus (3). Disease severity was rated on a scale of 0-5, in which 1 = trace to 5%, 2 = 6-25%, 3 = 26-65%, 4 = 66-90%, and 5 = 91-100% of the area of individual pieces was covered with acervuli.

Data from all experiments were analyzed using ANOVA, and treatment means were compared by LSD using SAS GLM procedures (16). Disease and yield data were subjected to regression analysis (19).

RESULTS

Root infection. Roots of inoculated Corsoy 79 seedlings misted for 1 s every 5 min did not develop symptoms after 6-7 days in the aeroponics chamber. However, roots of inoculated seedlings misted for 1 s every 30 min developed lesions within 4-6 days. Lesions first developed on seedling roots in the differentiation zone. A light yellow discoloration appeared before lesion appearance. This area developed into an irregularly shaped brown lesion, which later became water-soaked, dark-brown to black, and extended above and below the point of injection (Fig. 1B). Acervuli and sclerotia developed on secondary roots misted for 1 s every 30 min (Fig. 1C), but neither acervuli nor sclerotia formed on roots of any inoculated or noninoculated plant under the misting regime for 1 s every 5 min.

Acervulus and sclerotium formation. Acervuli developed on the root pieces of all inoculated seedlings of Corsoy 79 within 5-days after placement on moist filter paper. Acervulus initials developed subepidermally, then erupted through the epidermis

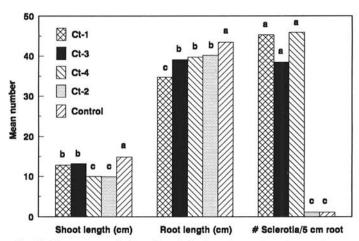


Fig. 2. Mean root and shoot length (cm), and number of sclerotia per 5 cm of taproots of cv. Corsoy 79 soybean seedlings either noninoculated (control) or at 7 days after root inoculation with Colletotrichum truncatum isolates Ct-1, -2, -3, or -4. No sclerotia developed either on taproots inoculated with Ct-2 or the control. Bars labeled with the same letter do not differ significantly (P = 0.05) according to least significant difference (LSD) values. LSD values were valid only for comparing the isolates within a specific variable.

with maturity and produced characteristic setae and conidia. No acervuli developed on roots of noninoculated seedlings.

On root pieces from seedlings inoculated with isolates Ct-1, -3, and -4, sclerotia initials and sclerotia developed subepidermally, frequently in proximity to acervuli. However, production of sclerotia was independent of acervulus production. No sclerotia were produced on roots of seedlings inoculated with Ct-2 nor on roots of the noninoculated control.

Inoculation methods. Methylcellulose had no effect on root and shoot length nor on the number of microsclerotia per 5-cm root length. Therefore, conidial suspensions without methylcellulose were used for all succeeding experiments, even though the sponge inoculation method established root infections more effectively than the conidial suspension with or without methylcellulose.

Comparison of isolates. Roots and shoots of all inoculated seedlings were significantly shorter than the control (Fig. 2). Roots were shorter on seedlings of Corsoy 79 inoculated with isolate Ct-1 than on those inoculated with Ct-2, -3, or -4. However, seedling shoots inoculated with isolates Ct-2 and Ct-4 were significantly shorter than those inoculated with Ct-1 and Ct-3.

Sclerotia developed on root pieces from seedlings inoculated with isolates Ct-1, -3, and -4 but not on those inoculated with Ct-2 nor the control (Fig. 2). The number of sclerotia produced by Ct-1, -3, and -4 did not differ significantly.

Root pieces from seedlings inoculated with Ct-2 developed a mean of 25.1 acervuli per 5-cm length. Because we were originally interested in sclerotia formation, the number of acervuli produced on root pieces of seedlings inoculated with Ct-1, -3, and -4 was not recorded. No acervuli were produced on root pieces from control seedlings.

Lesion development. Lesions developed on taproots of the four soybean cultivars inoculated with each of the four isolates of C. truncatum (Fig. 3). Brown, sunken, and water-soaked lesions developed 4-5 days after inoculation. Lesion length was longer on roots of all cultivars inoculated with isolates Ct-1, -AU, or -HL than on roots inoculated with the other three isolates. Seedlings of Williams 82 inoculated with Ct-1 had significantly longer lesions than those inoculated with any of the other five isolates. Mean lesion length on taproots of the four cultivars was significantly longer when inoculated with a combination of Ct-1 and Ct-3 than either isolate alone with the same inoculum concentration (Table 1).

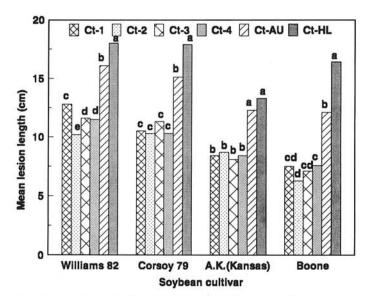


Fig. 3. Mean lesion length (cm) on the taproot of seedlings of four soybean cultivars 6 days after inoculation with one of six isolates (Ct-1, -2, -3, -4, -AU, -HL) of Colletotrichum truncatum. Bars labeled with the same letter do not differ significantly (P = 0.05) according to least significance difference (LSD) values. LSD values are valid only for comparing the isolates within a specific cultivar.

The mean lesion length on secondary roots 2, 4, and 6 days after inoculation with isolate Ct-1 varied among cultivars (Table 2). Lesion length was longer 2 and 4 days after inoculation on roots of Corsoy 79 and Williams 82 than on Boone and A. K. (Kansas); and those on roots of A. K. (Kansas) were longer than those on Boone. A similar relationship was recorded at 6 days after inoculation, except the difference between lesion length on roots of A. K. (Kansas) and Boone was not different. Seedlings of Corsoy 79 and Williams 82 inoculated with isolates Ct-AU or Ct-HL had longer taproot lesions than those on Boone and A. K. (Kansas).

Taproot and shoot length. The mean taproot and shoot lengths of inoculated seedlings of all cultivars were shorter than the respective controls for all isolates (Table 3). Mean taproot lengths tended to be shorter, and mean shoot lengths tended to be longer on all cultivars inoculated with isolates Ct-AU or Ct-HL.

Field studies. Disease symptoms appeared in the R1-R2 growth stages as brown, irregularly shaped lesions on lower leaves, petioles, and some lower internodes. The disease progressed acropetally with plant age. Laminar vein necrosis, petiole cankers, and some premature defoliation occurred in the late R4 and early

TABLE 1. Mean lesion length (cm) on taproots of seedlings of four soybean cultivars 6 days after inoculation with two sclerotia-forming isolates of *Colletotrichum truncatum* alone or in combination

Treatment	Cultivar ^a						
(isolates)	A.K. (Kansas)	Boone	Corsoy 79	Williams 82			
Ct-1 + Ct-3	11.8	8.6	15.9	15.3			
Ct-1	8.4	7.3	10.8	12.8			
Ct-3	8.1	7.1	11.3	11.6			
LSD ($P = 0.05$)	0.9	0.9	1.1	1.1			

^a Combined means of eight plants from two experiments.

TABLE 2. Mean lesion length (cm) on secondary roots of seedlings of four soybean cultivars at 2, 4, and 6 days after inoculation with *Colletotrichum truncatum* isolates Ct-1, Ct-Au, and Ct-HL

	Days after inoculation ^a						
Cultivar	Ct-1	Ct-1	Ct-1	Ct-Au	Ct-HL		
Williams 82	2.9	6.3	8.1	16.1	18.0		
Corsoy 79	3.0	6.4	7.6	15.1	17.9		
A.K. (Kansas)	2.1	4.6	6.1	12.3	13.3		
Boone	1.5	4.1	5.3	12.1	16.4		
LSD $(P = 0.05)^{b}$	0.4	0.4	0.8	0.9	1.0		

Mean lesion lengths on the longest secondary rot based on nine replicates.
 LSD values based on univariate analysis of variance for specific days after inoculation.

R5 growth stages. At this time, the fungus was recovered from pods, and pod infection increased as the disease severity increased.

The combined data for the four cultivars inoculated with isolates Ct-1, -2, or -3 in 1989 showed that inoculated plants had a higher disease rating and a reduced yield compared to noninoculated plants (Table 4). In 1988, isolates had a similar effect on 1,000-seed weights over cultivars. The highest disease rating occurred on plants inoculated with Ct-3, followed by those inoculated with Ct-1 and Ct-2. A trace amount of disease was recorded from control plants. Plants inoculated with Ct-1 had the lowest yield followed by those inoculated with Ct-3 and Ct-2 compared to the control.

The mean disease severity rating varied among isolates and between cultivars in 1989 (Fig. 4). Plants of all four cultivars inoculated with isolate Ct-3 generally had a higher disease rating than those inoculated with Ct-2. The disease rating for A. K. (Kansas) and Boone inoculated with Ct-3 was higher than that for plants inoculated with either Ct-1 or Ct-2. The disease rating for Corsoy 79 inoculated with either Ct-1 or Ct-3 was higher than plants inoculated with Ct-2. The disease rating was similar for Corsoy 79 inoculated with either Ct-1 or Ct-3 and for Williams 82 plants inoculated with either Ct-1 or Ct-2.

Yields were lower than the noninoculated control for A. K. (Kansas), Corsoy 79, and Williams 82 inoculated with each of three isolates of *C. truncatum* (Fig. 5). Yields averaged over all isolates were inversely and significantly related to disease severity for A. K. (Kansas), Corsoy 79, and Williams 82, but not Boone (Fig. 6). The estimated loss due to anthracnose for A. K. (Kansas), Corsoy 79, and Williams 82 was 17, 23, and 30%, respectively. Losses were estimated from the regression analysis based on the mean disease severity for each cultivar.

DISCUSSION

When fungal diseases of above-ground soybean plant parts were described or studied, little attention was given to the possibility that the pathogen involved may also infect and cause disease on the roots. Soybean anthracnose caused by *C. truncatum* has been considered a disease of only the above-ground part of soybean plants. However, six isolates of *C. truncatum* infected and caused disease on roots of four soybean cultivars. Thus, soybean anthracnose is a disease of the entire soybean plant. Root infection, like that of above-ground plant parts, may be initially latent and therefore overlooked. Asymptomatic infection of *C. truncatum* of above-ground soybean plant parts has been reviewed (17).

Three of the isolates pathogenic to soybean roots and seedlings (Ct-1, -2, -3) formed sclerotia in culture and on plants. These isolates and the three nonsclerotia-forming isolates were pathogenic to soybean foliage and roots. Apparently, one other pathogenic species of *Colletotrichum* forms sclerotia, *C. coccodes*

TABLE 3. Combined mean taproot length (TRL) and shoot length (SL) (cm) of seedlings of four soybean cultivars 6 days after inoculation with six isolates of Colletrotrichum truncatum

	Isolate ^a											
	Ct	-1	Ct	-2	Ct	-3	Ct	-4	Ct-A	\U	Ct-	HL
Treatment	TRL	SL	TRL	SL	TRL	SL	TRL	SL	TRL	SL	TRL	SL
Williams 82							1.41000			(2000)	17(0-0100)	
Inoculated	40	12	39	10	38	13	38	10	37	14	33	13
Control	46	15	44	13	47	17	44	13	45	18	44	17
Corsoy 79									43	10	44	17
Inoculated	35	12	41	10	38	12	40	10	35	13	32	13
Control	43	15	45	13	44	14	46	13	41	17	42	17
A.K. (Kansas)							. 40	13	71	17	42	17
Inoculated	36	10	40	10	37	10	39	10	35	12	31	12
Control	43	13	43	13	43	12	43	13	41	15	42	15
Boone							15	13	7.1	13	42	13
Inoculated	37	14	37	11	38	13	40	11	36	16	32	13
Control	44	18	43	14	42	17	43	12	41	20	43	18
LSD ($P = 0.05$)	3	1	2	1	3	2	2	1	3	20	3	10

^a Taproot and shoot length are combined linear measurements of eight plants from two experiments.

(Wallr.) S. J. Hughes on tomato (7). Sclerotia production by an isolate of *C. truncatum* easily could have been overlooked because not all isolates produce sclerotia, sclerotia resemble old acervuli, and formation of sclerotia occurs primarily in mature and senescent tissues (8,10). Sclerotia production allows the fungus to overseason in soil and crop debris in a more resistant form than mycelia. Thus, the epidemiology of anthracnose may be more complicated than previously understood. Crop rotation and incorporation of crop debris into soil may not be as effective disease control procedures as previously thought.

Differences were noted in the pathogenicity of the six isolates of *C. truncatum*. In general, Ct-AU and Ct-HL from Alabama tended to be more virulent than the four isolates from Illinois. In addition, each isolate caused disease levels significantly above noninoculated controls in 2-yr field studies. Regression lines based on data confirmed that Corsoy 79 and Williams 82 were more susceptible to anthracnose than A. K. (Kansas) and Boone. The quadratic regression for A. K. (Kansas) showed that anthracnose caused yield losses when the disease severity rating increased to 2.8. Similar results occurred in field studies in Alabama (1). Variation in pathogenicity was also reported among isolates of *C. truncatum* causing foliar anthracnose of soybeans (5,14).

Lesions that developed on secondary roots within 2 days after inoculation on Corsoy 79 and Williams 82 inoculated with isolate Ct-1 were longer than on A. K. (Kansas) and Boone, but not significantly so; however, lesion length was significantly different on A. K. (Kansas) and Boone at 2 and 4 days but not 6 days

TABLE 4. Combined means disease severity rating and yields at harvest from four soybean cultivars noninoculated (control) or inoculated separately with one of three sclerotia-forming isolates of *Colletotrichum truncatum* in the field, 1989

Treatment	Disease severity rating ^a	Yield (grams per plot) ^b
Ct-1	3.5	1,016
Ct-2	3.1	1,080
Ct-3	4.1	1,026
Control	1.4	1,239
LSD $(P = 0.05)$	0.2	53

^a Disease severity based on a scale of 0-5 for which 1 = trace to 5%, 2 = 6-25%, 3 = 26-65%, 4 = 66-90%, and 5 = 91-100% of the area covered with acervuli.

^b Means from 16 plots (four cvs. [A. K. (Kansas), Boone, Corsoy 79, Williams 82] replicated four times).

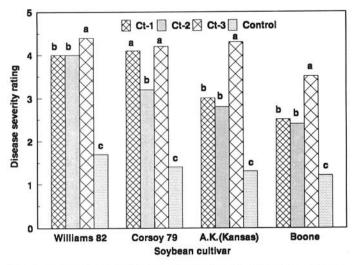


Fig. 4. Mean disease severity on petioles from four soybean cultivars noninoculated (control) or inoculated separately in the field with one of three isolates (Ct-1, -2, -3) of Colletorichum truncatum in 1989. Ratings based on a scale of 0-5, in which 1 = trace to 5%, and 5 = 91-100% of the area covered with acervuli. Means based on four replications of each treatment.

after inoculation. This suggested that the rate of lesion development was different with time.

Soybean seedlings inoculated with a combination of two isolates developed longer lesions on the four cultivars tested compared to inoculation with a single isolate. This additive effect suggested that disease development under field conditions would be more severe when more than one isolate of the fungus might be established. The quantity of inoculum of *C. coccodes* on tomato roots influenced disease development (8). Furthermore, a single sclerotium of *C. coccodes* on tomato root resulted in delayed disease development, whereas the use of many sclerotia resulted in rapid disease development.

In addition to variation in pathogenicity among isolates, resistance to root infection varied among the four cultivars tested.

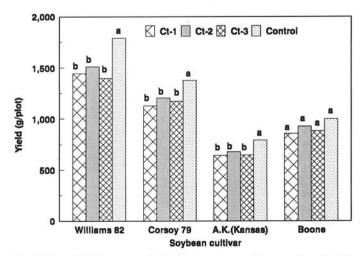


Fig. 5. Mean yield (grams per plot) of four soybean cultivars noninoculated (control) or inoculated separately with one of three isolates (Ct-1, -2, -3) of Colletotrichum truncatum in 1989. Means based on four replications of each treatment.

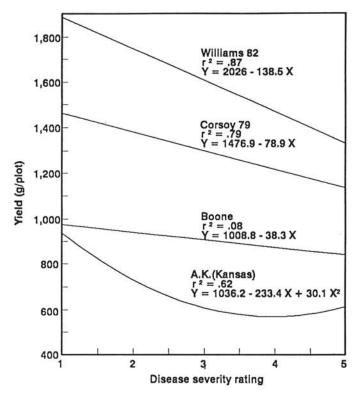


Fig. 6. Relationship of anthracnose (*Colletotrichum truncatum*) severity to seed yield of four soybean cultivars. Selected models were tested for the lack-of-fit and were significantly greater than zero, except for Boone, which was resistant to foliar anthracnose.

For example, Corsoy 79 and Williams 82, both susceptible to foliar anthracnose, were susceptible to root infection by isolates Ct-2, -3, or -4, but Corsoy 79 was less susceptible than Williams 82 to root infection by Ct-1, -AU, or -HL. Similarly, roots of A. K. (Kansas), moderately resistant to foliar anthracnose, were more resistant than those of Boone, which is resistant to foliar anthracnose, inoculated with isolates Ct-2, -3, or -HL.

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