Soybean Germ Plasm Evaluation for Resistance to *Colletotrichum truncatum*

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ABSTRACT

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The number of conidia per square centimeter of potato-dextrose agar (PDA) disk produced by *Colletotrichum truncatum*, cause of soybean (*Glycine max*) anthracnose, and its pathogenicity on soybean seedlings varied among seven isolates. Isolate Ct-1 from soybeans produced more conidia than other isolates and was highly pathogenic on inoculated soybean seedlings and seeds. Foliar anthracnose in susceptible plants inoculated with a conidial suspension of isolate Ct-1 progressed curvilinearly with increased time in a mist chamber. Seedlings inoculated at the V1 growth stage were killed within 72 hr. Plants inoculated at the V2, V6, and R4 growth stages were defoliated within 72 hr. Necrosis in stem tips occurred most often at V2, intermediately at V6, and least at R4 growth stages. Disease development and survival of *C. truncatum* in soybean cultivars remained high after 0–98 hr under dark conditions but decreased with increasing periods under light conditions. None of the 414 germ plasm accessions of maturity groups 000–X inoculated at the V1 growth stage and evaluated for foliar anthracnose was immune. Several lines in maturity groups 00–IV, PI 96.860 (maturity group VI), and Tarheel Black (maturity group VII) were resistant.

Anthracose of soybeans (*Glycine max* (L.) Merr.) caused by *Colletotrichum truncatum* (Schw.) Andrus & W. D. Moore is an economically important disease in the humid tropics and subtropics (21). It is an important foliar pathogen of soybeans in the southern United States (1) and causes an estimated 0.1–5% annual yield loss (16). Grain yield losses from 16 to 26% have been reported in susceptible cultivars (1).

The pathogen is found within the seed coats of soybeans (20). It causes preemergence and postemergence damping-off and seedling blight (5–8,10,19). Symptomatology was described by Ling (8) for seedling blight and by Hepperly et al (5) for foliar anthracnose. Conidia of *C. truncatum* form appressoria on leaves (14,15,17).

Isolates of *C. truncatum* vary in their pathogenicity on soybeans (7,18). Soybean cultivars vary in their susceptibility to anthracnose (2,5,6,10); however, no commercial soybean cultivars are immune to anthracnose. Bowers (2) evaluated soybean germ plasm in the field in Texas. Other workers (6,10) have evaluated a few cultivars and lines by field inoculation. There is no report on the evaluation of soybean germ plasm for resistance to anthracnose under controlled environmental conditions.

We report on the pathogenicity on soybean seedlings and variation in production of conidia in culture of six isolates of *C. truncatum*, the development of a uniform inoculation technique to evaluate soybean germ plasm for anthracnose susceptibility, the effects of length of darkness on disease severity, and variation of disease severity at various growth stages of soybean cultivars. A portion of this work was reported earlier (11).

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MATERIALS AND METHODS

Inoculum maintenance and preparation. Seven isolates of *C. truncatum* from soybeans were used and designated Ct-1, Ct-2, Ct-3, Ct-4, Ct-5, Ct-6, and Ct-7. Identification of the seven isolates was verified by comparison with the ATCC 18013 culture of *C. truncatum*. Stock cultures of all isolates were maintained on Difco potato-dextrose agar (PDA) at 5 C in the dark to restrict growth. Isolates were subcultured by transferring hyphal tips onto 9-cm-diameter PDA culture plates and incubated under 12 hr of alternating dark and cool-white fluorescent light (800 $\mu E/m^2/sec$) at 26 C to induce sporulation. Inoculum was prepared by harvesting conidia from 8- to 10-day-old PDA plates by first flooding each plate with 5–10 ml of sterile distilled water, then brushing the surface of the fungal colonies with a sterile camel's-hair brush. An inoculum concentration of $3–5 \times 10^6$ conidia per milliliter determined with a hemacytometer was adequate to cause severe disease and was used for inoculation experiments.

Pathogenicity of seven isolates. Conidial suspensions of the seven isolates of *C. truncatum* were prepared by shaking 1-cm-diameter disks cut from 8-day-old sporulating PDA culture plates of *C. truncatum* in 10 ml of sterilized distilled deionized water. Pathogenicity of the seven isolates was assessed on cultivars Corsoy 79 and Williams 79 by measuring percent stand reduction from the control and by measuring disease severity on foliage of inoculated seedlings.

Seeds (300/cultivar) were soaked for 4 min in the spore suspension and planted in trays (100/tray and three replicates per cultivar) and incubated on a greenhouse bench at 26 ± 2 C. Seeds soaked in water for 4 min served as controls. Stand counts were recorded as the number of plants with healthy trifoliolates 15 days after planting. Percent stand reduction was calculated by the formula: percent stand reduction = [(stand of control − stand of inoculated plants)/stand of control] × 100.

Five seeds each of Corsoy 79 and Williams 79 were planted in 12-cm-diameter pots containing steam-sterilized (steamed for 4 hr on two consecutive days) Drummer silt loam and sand mixed in equal volumes (v/v). Seedlings were thinned to three plants per pot after emergence at the cotyledonary stage. An atomizer (DeVilbiss no. 15) was used to spray seedlings at the V1 growth stage (4) to incipient runoff with a conidial suspension of the seven isolates. Seedlings sprayed with sterile deionized distilled water served as controls. Plants were incubated for 24 hr in a mist chamber with 15-min mist cycles per hour and 12-hr alternating dark and cool-white fluorescent light (800 $\mu E/m^2/sec$) at 25 ± 2 C. Pots were placed on a greenhouse bench, and individual plants were rated 1–3 days later on a scale of 0–4, where 0 = asymptomatic; 1 = veinal necrosis on the leaves; 2 = leaf veinal and petiole necrosis; 3 = necrosis on stems, petioles, and leaves; and 4 = plants dead.

Disease severity. Five seeds each of Corsoy 79 and Williams 79 were planted in 12-cm-diameter pots containing steam-sterilized (steamed 4 hr on two consecutive days) Drummer silt loam and sand mixed in equal volumes (v/v). Seedlings were thinned to three seedlings per pot, not sprayed or sprayed with a conidial suspension of isolate Ct-1, and incubated in a mist chamber as described previously. Five pots of each cultivar

were removed from the mist chamber after 6 hr and at 6-hr intervals up to 72 hr. Pots were placed on a greenhouse bench and rated 1–3 days later as described previously. The experiment was done three times. Data were analyzed as a split plot with incubation time as main plot and experiments as subplots.

Soybean growth stage. Five seeds each of Corsoy 79 or Williams 79 were planted in 30-cm-diameter pots containing steam-sterilized soil each week during June and July. Seedlings were grown outside the greenhouse and thinned to three plants per pot at the cotyledonary stage. Pots containing plants in the V1, V2, V6, and R4 growth stages were not inoculated (control) or inoculated with a conidial suspension of isolate Ct-1. Three pots were used for each treatment. Plants were incubated in the mist chamber for 10 hr with 15-min mist cycles per hour, and disease severity was rated as described previously. This study was done twice. Data were analyzed as a split plot with inoculation at various growth stages as the main plots and experiments as subplots.

Shading studies. To determine the effects of light vs. darkness on disease severity, Corsoy 79 and Williams 79 plants were grown in 12-cm-diameter pots. Seedlings were not inoculated (control) or inoculated at the V1 growth stage with isolate Ct-1 and incubated in the mist chamber for 10 hr with 15-min mist cycles per hour. After removal from the mist chamber, the pots were divided into two equal lots. One lot was placed in the dark at 26 ± 2 C, and the other lot was placed on a greenhouse bench at 28 ± 2 C. After 0, 24, 44, 66, and 98 hr of exposure, five pots of each cultivar were returned to the mist chamber and incubated for an additional 72 hr with 12-hr photoperiods. Plants were rated for disease severity 1–3 days after misting. The experiment was conducted twice. Data were analyzed as a split-split plot with darkness and light as the main plot, time of exposure the subplot, and experiments as sub-subplots.

Germ plasm evaluation for foliar anthracnose. We evaluated 414 soybean germ plasm accessions or lines representing maturity groups (MG) 000–IV (supplied by R. L. Bernard, USDA/ARS, University of Illinois, Urbana) and V–X (supplied by E. E. Hartwig, USDA/ARS, Mississippi Agricultural Forestry Experiment Station, Stoneville, MS). Eight seeds of each line were planted in continuous rows in trays 48 × 32 cm containing 5-cm depth of steam-sterilized soil. Seedlings were thinned at the cotyledonary stage to five per row. A completely randomized design with three replicates was used. Seven accessions including Corsoy 79 and Williams 79, which were used for comparison, were grown in each tray. Plants were inoculated at the V1 growth stage with isolate Ct-1 and incubated in the mist chamber for 72 hr at 28 ± 2 C; disease severity was rated as described previously. Uninoculated plants served as controls. Plants retaining green foliage despite veinal and petiole necrosis were termed resistant. Data from cultivars of similar maturity groups were analyzed together.

RESULTS

Isolates Ct-1, Ct-2, and Ct-3 produced significantly more conidia per square centimeter of agar disk than did the other isolates tested (Table 1). Isolates Ct-1, Ct-3, Ct-4, and Ct-5 induced the most severe foliar symptoms (Table 1). Isolate Ct-1 caused the greatest reduction in stand in the seed-inoculation test (Table 1).

Corsoy 79 and Williams 79, at the V1 growth stage, did not differ significantly from one another in disease severity for any duration of incubation period. Both cultivars were considered susceptible to *C. truncatum*. No symptoms developed on inoculated plants incubated for 6 hr, but veinal necrosis was evident in plants incubated for 12 hr. Lesions appeared on petioles and stems of inoculated plants after 18 and 42 hr of incubation, respectively. Death of inoculated plants occurred after 66 hr or longer. The combined data from Corsoy 79 and Williams 79 showed a curvilinear disease increase with increased misting time (Fig. 1). No disease developed on uninoculated

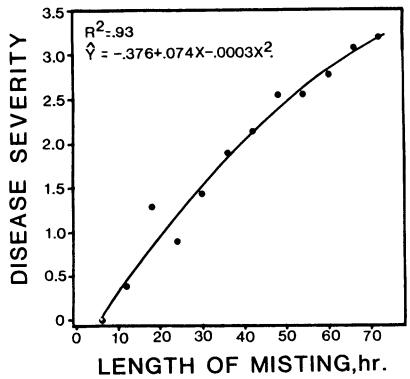

Fig. 1. The relation of anthracnose (*Colletotrichum truncatum*) severity (0 = asymptomatic; 1 = leaf veinal necrosis; 2 = lesions on leaves and petioles; 3 = lesions on leaves, petioles, and stems; and 4 = plants dead) on soybean plants at the V1 growth stage to hours of incubation in a mist chamber at 25 ± 2 C. Combined means of three experiments and two cultivars, Corsoy 79 and Williams 79. Uninoculated controls did not develop symptoms.

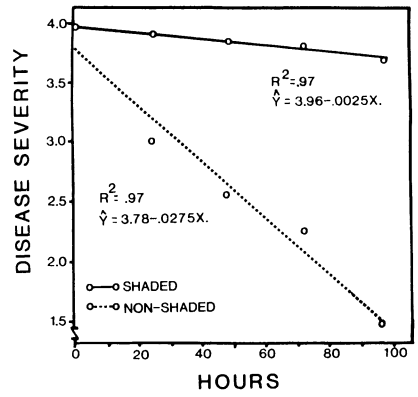

Fig. 2. Effects of hours of exposure (after initial inoculation and incubation for 10 hr in a mist chamber at 25 ± 2 C) under darkness (shaded) and light (unshaded, greenhouse) on severity of anthracnose (*Colletotrichum truncatum*) on soybean (*Glycine max*) seedlings (0 = asymptomatic; 1 = leaf veinal necrosis; 2 = lesions on leaves and petioles; 3 = lesions on leaves, petioles, and stems; and 4 = plants dead). Combined means of two experiments and two cultivars, Corsoy 79 and Williams 79, after an additional 10-hr incubation in the mist chamber. Uninoculated controls did not develop symptoms.

Table 1. Variation among isolates of *Colletotrichum truncatum* in the number of conidia produced per square centimeter of potato-dextrose agar disk, in soybean foliar anthracnose severity, and mean stand reduction of inoculated soybean seeds

Isolate	Conidia produced[w] (× 10^6)	Disease severity[x]	Stand reduction[y] (%)
Ct-1	2.8 a[z]	3.8 a	90.3 a
Ct-2	2.8 a	2.8 b	64.1 b
Ct-3	2.9 a	3.7 a	25.8 c
Ct-4	–[z]	3.9 a	26.0 c
Ct-5	0.5 c	3.6 a	–
Ct-6	–	3.2 ab	6.9 d
Ct-7	1.9 b	–	5.8 de
Control	–	0.3 c	0 e

[w] Number of conidia produced per square centimeter of a potato-dextrose agar disk taken between the center and margin of the colony.

[x] Based on a severity scale of 0–4, where 0 = asymptomatic; 1 = leaf veinal necrosis; 2 = lesions on leaves and petioles; 3 = lesions on leaves, petioles, and stem; and 4 = plant dead. Combined data from three experiments.

[y] Combined analysis for two cultivars, Corsoy 79 and Williams 79, recorded 15 days after planting compared with an autoclaved deionized distilled water percent stand reduction = [(stand of control − stand of inoculated plants)/stand of control] × 100.

[z] Means followed by the same letter are not significantly different (FLSD, $P = 0.05$). – = Data not available.

controls.

Inoculated Corsoy 79 and Williams 79 plants at growth stages V1, V2, V6, and R4 developed veinal necrosis, petiole lesions, and defoliation after 72 hr. All plants at the V1 growth stage died. At the R4 growth stage, plants developed necrosis on stem tips and pods on the lower nodes with some defoliation. At the V2 and V6 growth stages, disease severity was intermediate between those at V1 and R4 growth stages. No significant differences were recorded in disease severity between growth stages V2 and V6.

After the initial inoculation followed by reincubation in the mist chamber for various times, severity of foliar anthracnose did not significantly increase with increasing hours of darkness, whereas it decreased significantly with increasing hours of light in the greenhouse. Disease severity at any given period of exposure was significantly higher for plants kept in the dark than for those in the greenhouse (Fig. 2). Inoculated plants exposed to darkness for 98 hr and reincubated died, whereas only leaf and petiole necrosis developed in plants under greenhouse conditions.

All cultivars, including Corsoy 79 and Williams 79, were susceptible in the greenhouse mist chamber trials, with 217 cultivars ranging from 3.6 to 4 in anthracnose severity, 79 ranging from 3.1 to 3.5, 68 ranging from 2 to 3.4, 42 ranging from 2.1 to 2.5, and eight with a rating of less than 2 (12,13). None of the 414 germ plasm accessions was immune. Lines within maturity groups 00, 0, I, II, III, and IV had a wide range of disease ratings (Table 2). Cultivar susceptibility varied significantly within each maturity group. Maturity groups 000, V, VI, VII, VIII, IX, and X had narrower ranges of susceptibility and a higher mean disease score. PI 95.860 (MG VI) and Tarheel Black (MG VII) were the only accessions with a low disease rating. These lines retained their foliage.

DISCUSSION

In agreement with other studies (7,15), our isolates of *C. truncatum* varied in the number of conidia produced per square centimeter of agar disk and in pathogenicity. Inoculation vs. noninoculation of soybean plants at the V1 growth stage in a mist chamber appeared ideal for the evaluation of foliar disease resistance. A film of free moisture on the plant surface is necessary for *C. truncatum* to infect host plants (5,7,9,23). Frequent misting may enhance penetration and infection of inoculated plants by promoting certain biochemical events, such as accelerated dilution of inhibitors present on the plant surface, decreasing host membrane permeability, or releasing soluble nutrients for *Colletotrichum* spp. (3,24).

Inoculation at the V1 growth stage resulted in death of plants, whereas inoculation at the V2, V6, and R4 growth stages caused lesions in stem tips. This may be due to the susceptibility of young tissues of stem tips. Therefore, we assume that greater crop losses caused by anthracnose may occur in fields of younger, more tender soybean plants than in mature ones, depending on availability of inoculum and environmental conditions.

Plants inoculated with *C. truncatum* at the V1 growth stage and incubated for 10 hr in the mist chamber developed symptoms after the 98-hr treatment whether in the dark or light after reincubation in the mist chamber. The initial misting may have promoted conidial germination and appressorium formation, penetration, and infection (14,22). Increased disease severity and survival of the pathogen under dark conditions, but not in light, may be due to a combination of factors, such as weakening of plants because of reduced photosynthesis, shorter appressorial dormancy because of reduced desiccation, and secretion of less inhibitory substances by the host (17). Appressorium formation on plant surfaces was promoted by free moisture and cool temperature (22). However, attachment of appressoria to host cuticle and penetration into host cells was stimulated by unknown compounds including leachates from either or both the host and the pathogen (3).

The low disease severity rating in some plants was due to retention of leaves even though leaf veinal necrosis and necrotic lesions developed on petioles and stems. Production of acervuli and conidia were restricted on such plants (*unpublished*), which may be an expression of resistance. Most germ plasm lines of maturity groups V–X, including lines reported resistant by Bowers (2), were highly susceptible with the exceptions of PI 95.860 (VI) and Tarheel Black (VII).

Table 2. Anthracnose severity ratings at the V1 growth stage of soybean germ plasm lines from 13 maturity groups 72 hr after inoculation and incubation in a mist chamber

Maturity group	No. of lines	Disease severity[a]	
		Range	Mean
000	3	2.9–3.8	3.4*[b]
00	6	2.4–3.8	3.3*
0	8	1.5–4.0	2.6**
I	21	1.7–4.0	3.0**
II	44	1.4–4.0	3.3**
III	75	2.0–4.0	3.4**
IV	61	1.5–4.0	3.4**
V	9	3.7–4.0	3.9
VI	24	2.7–4.0	3.7**
VII	23	2.9–4.0	3.8**
VIII	39	3.7–4.0	3.9
IX	91	3.3–4.0	3.9**
X	9	3.6–4.0	3.9

[a] Disease severity based on a scale of 0–4, where 0 = asymptomatic; 1 = leaf veinal necrosis; 2 = lesions on leaves and petioles; 3 = lesions on leaves, petioles, and stem; and 4 = plants dead.
[b] * = FLSD, $P = 0.05$, and ** = FLSD, $P = 0.01$. Data analysis for germ plasm entries within maturity group.

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