Effect of Soil Temperature on Infection of Soybean Roots by Sclerotia-Forming Isolates of *Colletotrichum truncatum*

MAHMOOD KHAN and J. B. SINCLAIR, Department of Plant Pathology, University of Illinois at Urbana-Champaign, 1102 S. Goodwin Avenue, Urbana 61801-4709

ABSTRACT

Khan, M., and Sinclair, J. B. 1991. Effect of soil temperature on infection of soybean roots by sclerotia-forming isolates of *Colletotrichum truncatum*. Plant Dis. 75:1282-1285.

Soybean (*Glycine max*) cvs. A. K. (Kansas), Boone, and Williams 82 were grown in sand infested with sclerotia from two sclerotia-forming isolates of *Colletotrichum truncatum* in soil temperature tanks at 20, 25, 30, or 35 C and at greenhouse ambient temperature (21–28 C). Root and hypocotyl infection were recorded on all cultivars at all temperatures. Lesion size and number generally increased with an increase in soil temperature up to 30 C and then declined. Williams 82 had the highest disease rating, Boone the lowest, and A. K. (Kansas) was intermediate over all temperatures.

Anthracnose of soybeans (*Glycine max* (L.) Merr.), caused by *Colletotrichum truncatum* (Schwein.) Andrus & W. D. Moore, is a disease of aboveground plant parts and is found wherever soybeans are grown. It is of economic importance in the humid tropics and subtropics and occasionally in the temperate zones after prolonged warm, rainy periods (14). The aboveground parts of soybean plants may be infected by *C. truncatum* at any growth stage, but symptoms generally appear either during growth stages V1–V3 (early seedling) or R7–R8 (late reproductive) and senescence and are latent during vegetative stages (3,4,13). The disease can reduce seedling stands and seed quality and yields by 16–26% in the United States (1). Pathogenicity and cultivar resistance studies with *C. truncatum* have relied on foliar infection (6–8).

Manuscript 1588 of the Department of Plant Pathology, University of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

This study was part of Project 68-0244 of the Agricultural Experiment Station, College of Agriculture, University of Illinois at Urbana-Champaign. It was supported in part by a grant from the Archer Daniels Midland Foundation, Decatur, IL.; the Illinois Crop Improvement Association, Champaign; and the Illinois Soybean Program Operating Board, Bloomington.

Accepted for publication 24 July 1991 (submitted for electronic processing).

Three sclerotia-forming and three nonsclerotia-forming isolates of *C. truncatum* from soybeans, soybean seeds, or soil in which soybeans were grown were reported to be pathogenic to roots of soybean cvs. A. K. (Kansas), Boone, Corsoy 79, and Williams 82, each differentially resistant to foliar anthracnose (9,10).

We report on the effect of four controlled soil temperatures and ambient greenhouse temperature on root infection by two of the sclerotia-forming isolates of *C. truncatum* and the response of three soybean cultivars differentially resistant to foliage anthracnose. A portion of this work was reported as an abstract (9).

MATERIALS AND METHODS

Inoculum preparation. Two sclerotia-forming isolates of *C. truncatum*, Ct-1 (ATCC 76263) and Ct-3 (ATCC 76264), recovered from soybean field soil or a soybean seed, respectively, were used. To produce sclerotia, cultures were grown on Difco potato-dextrose agar (PDA) plastic culture plates incubated for 3 wk in the dark at 28 C. To collect sclerotia, cultures were homogenized in a Waring blender, washed through sieves (no. 60

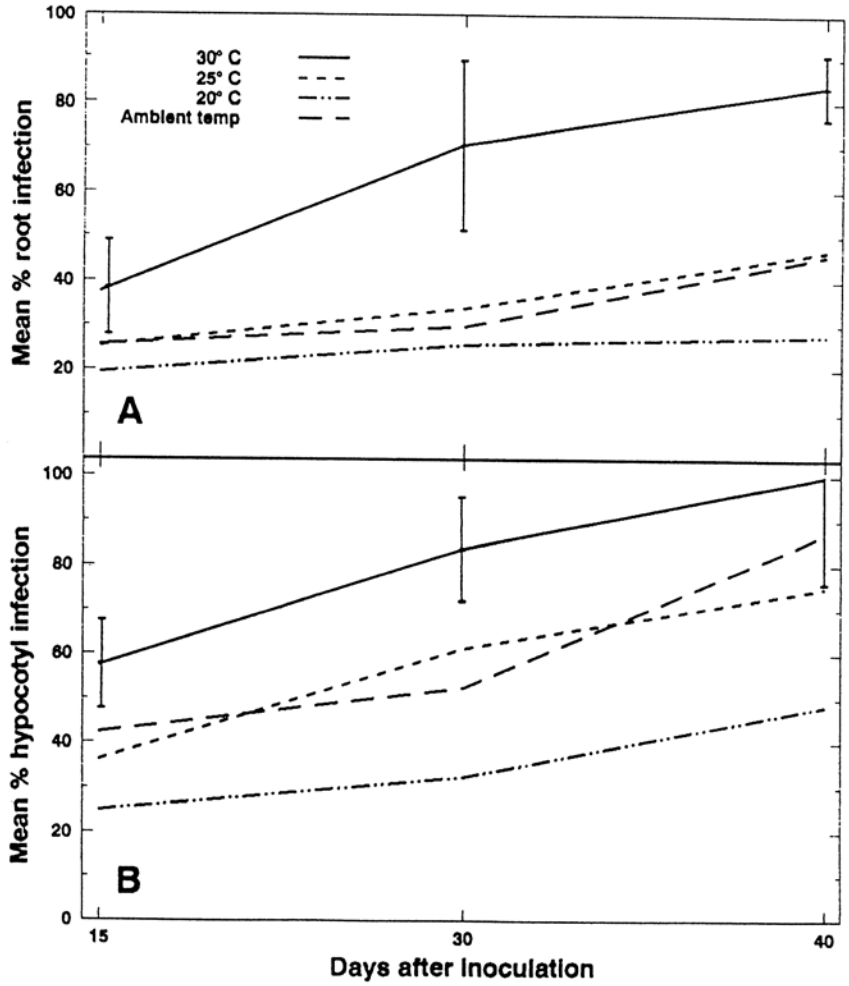

Fig. 1. Combined mean percent **(A)** root and **(B)** hypocotyl infection of Williams 82 soybean seedlings at 15, 30, or 40 days after planting (inoculation) in sand infested with sclerotia of *Colletotrichum truncatum* isolates Ct-1 and Ct-3 at three controlled soil temperatures and ambient temperatures (21–28 C). Each point represents a mean of four replications. Bars represent the least significant difference (LSD) for each point ($P = 0.05$).

and 170) under tap water, and the sclerotia were resuspended in sterile deionized, distilled water. The suspension was centrifuged for 15 min at 630 *g* to remove excess agar. The pellet was then washed with sterile deionized, distilled water. The sclerotia were air-dried for 24 hr at room temperature (27 ± 1 C) and stored at 5 C until needed.

Inoculation. Sclerotia of isolates Ct-1 and Ct-3 were mixed with steamed sand (pH 6.7) at 80 sclerotia per gram of sand. Before adding sclerotia, the sand was steamed for 8 hr and then allowed to stand for 1 wk. Sand (about 600 g), with or without sclerotia (control), was placed in 1-L plastic greenhouse pots. Each pot was placed in a larger plastic pot that contained zinc weights to prevent the pots from floating in the water of the soil-temperature tanks. A thin layer of uninfested soil was spread on top of infested soil to separate seeds from direct contact with the inoculum. Ten seeds of soybean cv. Williams 82 were placed in each pot and covered with uninfested, steamed sand. All pots were placed on a greenhouse bench until seedling emergence, thinned to six plants per pot, and then transferred to the soil-temperature tanks. Twenty-four pots under ambient temperature (21–28 C) were left on the bench.

Soil temperature studies. Soil temperature tanks contained water with temperatures maintained by an auxiliary heating element regulated by a thermostatically controlled mercury switch and continuous water circulation. Three tanks were used, one each at 20, 25, or 30 C. Pots with either uninfested or infested sand were randomized in each

Fig. 2. Representative cv. Williams 82 soybean seedlings grown at two soil temperatures. Uninoculated seedling at 25 C (left), two seedlings each grown in sand infested with sclerotia of *Colletotrichum truncatum*, isolates Ct-1 and Ct-3, at 25 C (center) or 30 C (right).

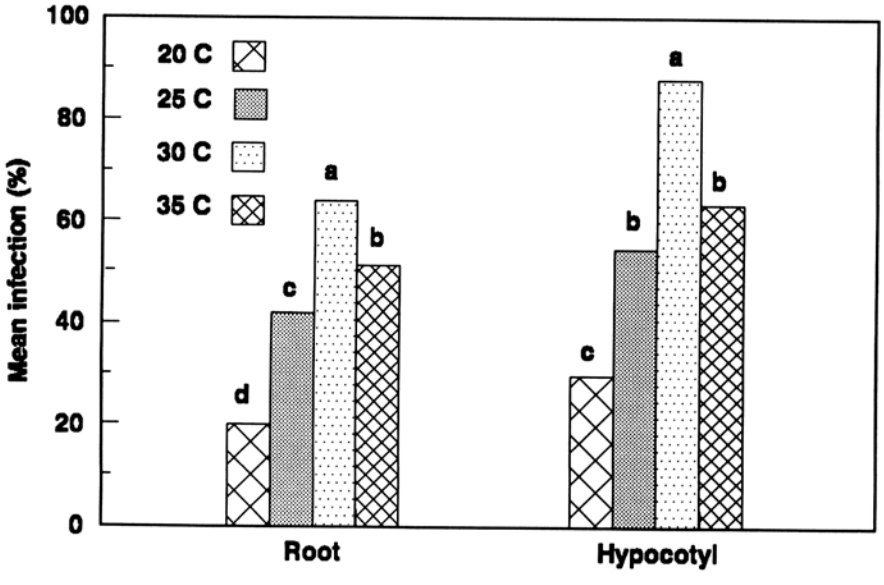

Fig. 3. Combined mean infection of seedling root and hypocotyl tissues over three soybean cultivars at four soil temperatures (20, 25, 30, or 35 C) at 30 days after planting in sand infested with sclerotia-forming isolates of *Colletotrichum truncatum*, Ct-1 and Ct-3. Bars labeled with the same letter do not differ significantly ($P = 0.05$) according to least significant differences (LSD) values.

tank. A total of 24 pots, 12 pots per treatment, were placed in each tank. At 15, 30, and 40 days after placing pots in the tanks, four pots each were removed for evaluation of root and hypocotyl infection and measurement of plant growth parameters. The experiment was terminated after 40 days and considered as one replication. Four such replicates were conducted, and the overall experiment was sequentially performed twice under natural light in the spring.

Cultivar resistance studies. Three soybean cultivars—Williams 82 (maturity group [MG] III), susceptible to foliar infection by *C. truncatum*; A. K. (Kansas) (MG IV), moderately resistant to foliar infection; and Boone (MG IV), resistant to foliar infection—were planted in greenhouse pots as described previously. To determine the effect of soil temperature on cultivar susceptibility to infection of seedling roots and hypocotyls, 10 seeds of each cultivar were planted in each 1-L plastic pot containing steamed sand with or without sclerotia as described previously. Four soil temperature tanks, one each set at 20, 25, 30, or 35 C were used as described previously. The experiment was terminated after 30 days.

Root and hypocotyl assay. Root and hypocotyl pieces were assayed on NaCl yeast-extract agar (5 g of NaCl, 3 g of yeast extract, and 20 g of agar per liter of deionized water) (11) amended with tetracycline (100 mg/L) (SYA) in 9-cm-diameter culture plates. Pots were taken from the tanks at specified intervals, and plant roots were removed and washed under running tap water. Half of all plants from each treatment were used to evaluate root and hypocotyl infection and half were used to record dry weights. Three pieces, each 1 cm long, were cut from each root beginning at 0.5 cm below the soil line, surface-disinfested with 0.05% NaOCl for 1 min, and then rinsed twice with sterile deionized, distilled water. Root pieces were placed on SYA in 9-cm-diameter culture plates and incubated for 3–4 days at room temperature (27 ± 1 C). The number of infected roots was recorded and the percent root infection was calculated.

Because symptoms did not develop on hypocotyl tissues at any temperature throughout this study, symptoms were induced by treating 1-cm-long pieces of hypocotyl cut from the crown of each seedling under running tap water for 4–6 hr, surface-sterilizing as described previously, and treating with paraquat (11.64% paraquat dichloride) (3,4). The pieces were then placed on SYA culture plates, and the percent infection was recorded after 4–6 days at room temperature (23 ± 2 C). Roots and shoots were dried in oven for 48 hr at 70 C and the dry weight was recorded in grams per plant for each treatment.

To determine differences in disease severity among cultivars, roots were rated using a scale of 0–5, where 0 = no symptoms, 1 = trace to 5, 2 = 6–25, 3 = 26–65, 4 = 66–90, and 5 = 91–100% of area covered with acervuli of *C. truncatum*.

Experimental design and analysis. A randomized complete block design with four replicates was used in the root infection studies. The resistant experiment used a split plot with temperatures as the main plot and cultivar as the subplot. All data were analyzed by the SAS ANOVA procedure (SAS Institute, Inc., Cary, NC) (12). Means were separated using least significance difference (LSD) ($P = 0.05$) (15).

Table 1. Mean shoot length and root and shoot dry weight of Williams 82 soybean seedlings grown in sand uninfested or infested with sclerotia of *Colletotrichum truncatum* isolates Ct-1 and Ct-3, at soil temperatures of 20, 25, 30 C, or ambient temperatures at 40 days after inoculation

Treatment	Shoot length[x] (cm)	Root weight[y] (g/plant)	Shoot weight[y] (g/plant)
30 C infested	35.6 b[z]	0.7 a–c	1.6 bc
Uninfested	40.2 a	0.8 a	1.7 ab
25 C infested	35.4 bc	0.5 d	1.5 bc
Uninfested	41.2 a	0.6 cd	1.8 ab
20 C infested	31.2 cd	0.6 cd	1.4 c
Uninfested	34.6 b–d	0.7 a–c	1.6 bc
Ambient (21-28 C)			
Infested	25.0 e	0.6 cd	1.4 c
Uninfested	30.4 d	0.7 a–c	1.9 a
LSD ($P = 0.05$)	4.3	0.1	0.3

[x]Combined means of 32 plants from two experiments.
[y]Means of 16 plants recorded 48 hr after drying at 70 C in an oven.
[z]Means in the same column followed by the same letter do not differ significantly ($P = 0.05$) from each other according to least significant difference (LSD) values.

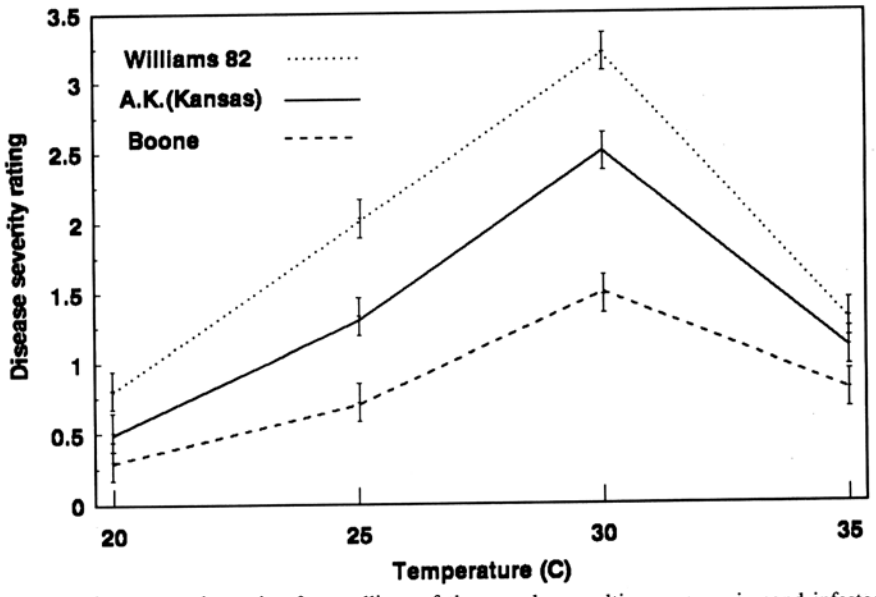

Fig. 4. Disease severity rating for seedlings of three soybean cultivars grown in sand infested with sclerotia-forming isolates of *Colletotrichum truncatum*, Ct-1 and Ct-3, at four soil temperatures at 30 days after inoculation. Mean disease severity ratings based on a scale of 0–5, where 0 = no symptoms, 1 = trace to 5, 2 = 6–25, 3 = 26–65, 4 = 66–90, and 5 = 91–100% of area covered with acervuli of *C. truncatum*. Bars represent 95% confidence levels.

RESULTS

Symptoms developed on excised roots and were induced on hypocotyls of seedlings of Williams 82 soybeans at all temperatures tested. The optimum for root and hypocotyl infection was 30 C, the minimum was 20 C, and 25 C and the ambient temperature were intermediate at 15, 30, or 40 days after planting (inoculation) (Fig. 1). The most severe symptoms developed on seedlings grown in infested sand at 30 C (Fig. 2). Overall, a significantly higher level of root and hypocotyl infection occurred at 30 than at 20 C, and 25 C and the ambient temperature were intermediate. The combined means for infection of seedling roots and hypocotyls over all cultivars was highest at 30 C, followed by 35, 25, and 20 C, in descending order (Fig. 3). Shoot lengths and root and shoot weights of seedlings of Williams 82 were less for all plants, regardless of temperature in infested sand compared with those in uninfested sand (Table 1). Means for shoot lengths of plants from infested sand were lower than those from unin-

fested sand at 25 C, 30 C, and at ambient temperatures and for shoot weight only at ambient temperatures. The differences between means for root weights were not significant.

Comparison of cultivars. The disease severity ratings at all temperatures were consistently highest for Williams 82, lowest for Boone, and intermediate for A. K. (Kansas), with the greatest differences occurring at 30 C and the least at 20 and 35 C (Fig. 4). Ratings did not differ significantly between A. K. (Kansas) and Boone at 20 C, nor between A. K. (Kansas) and Williams 82 at 35 C.

Root and shoot dry weights. The combined means for root and shoot dry weights and shoot lengths over the four temperatures showed the greatest reduction in both root and hypocotyl dry weights at 30 C, the least at 20 C, and intermediate reductions for those at 25 and 35 C (Table 2). The differences between inoculated and uninoculated plants was greatest for Williams 82, least for Boone, and intermediate for A. K. (Kansas).

Table 2. Combined means over four temperatures for shoot length and root and shoot dry weight of three soybean cultivars grown in sand uninfested or infested with sclerotia of *Colletotrichum truncatum* isolates Ct-1 and Ct-3

Treatment	Shoot length[y] (cm)	Root weight[y] (g/plant)	Shoot weight[y] (g/plant)
Boone			
Infested	22.8 b[z]	0.18	0.53 c
Uninfested	24.4 a	0.19 b	0.56 b
A.K. (Kansas)			
Infested	16.7 e	0.13 d	0.39 e
Uninfested	18.6 d	0.15 c	0.43 d
Williams 82			
Infested	20.7 c	0.19 b	0.55 bc
Uninfested	23.5 ab	0.23 a	0.60 a
LSD ($P = 0.05$)	1.4	0.02	0.03

[y] Combined data for four temperatures (20, 25, 30, and 35 C) 1 mo after inoculation. Mean of four replicates with 12 samples per replicate.

[z] Means in the same column followed by the same letter do not differ significantly ($P = 0.05$) from each other according to least significant difference (LSD) values.

DISCUSSION

C. truncatum has been considered a pathogen of only the aboveground parts of soybean plants. Infected seeds, crop, and weed debris serve as the sources of primary inoculum (5,13). We have shown that three sclerotia-producing and three nonsclerotia-producing isolates of *C. truncatum* are pathogenic to soybean roots and hypocotyls (9). Data from the present report confirm the pathogenicity of two of the three sclerotia-forming isolates and demonstrate that the sclerotia from the two isolates may serve as a source of primary inoculum for root and hypocotyl infection of three soybean cultivars differentially resistant to foliar anthracnose. We have also shown that *C. truncatum* may cause latent infection in soybean roots. Infection of soybean seedling roots and hypocotyls may have been overlooked because of the latent infection by this fungus.

Increased soil temperature resulted in an increase in disease development on soybean seedling roots and hypocotyls with an optimum at 30 C. The optimum temperature for growth of *C. truncatum* in culture is 28–30 C (1,2,14), which is similar to that for disease development on soybean seedlings. Soybean anthracnose is most serious in the tropic and subtropic growing areas of the world. This may be attributable, in part, to the high temperature optima required for both fungal growth and disease development in the field.

The relationship of root and hypocotyl infection to that of foliar infection by *C. truncatum* was similar for all temperatures, with Williams 82 being most susceptible, Boone least, and A. K. (Kansas) intermediate. The difference among cultivars was greatest at 30 C, least at 20 and 35 C, and intermediate at 25 C, which is the same relationship found for the optimum growth of *C. truncatum* in culture (34). Thus, we recommend that any evaluation of soybean cultivars for resistance to *C. truncatum* should be done at 30 C or as close to this temperature as possible.

ACKNOWLEDGMENT

We thank R. McClary for technical assistance and advice.

LITERATURE CITED

1. Abney, T. S., and Richards, T. L. 1982. Fungal colonization of soybean stems. (Abstr.) Phytopathology 72:995.
2. Bryant, R. S., and Walter, H. J. 1980. Mycelial growth and conidial production of *Colletotrichum dematium* var. *truncata* in culture. Phytopathology 70:565-566.
3. Cerkauskas, R. F., Dhingra, O. D., and Sinclair, J. B. 1983. Effect of three desiccant-type herbicides on fruiting structures of *Colletotrichum truncatum* and *Phomopsis* spp. on soybean stems. Plant Dis. 67:620-622.
4. Cerkauskas, R. F., and Sinclair, J. B. 1980. Use of paraquat to aid detection of fungi in soybean tissues. Phytopathology 70:1036-1038.
5. Hepperly, P. R. 1985. Soybean anthracnose. Pages 547-554 in: Proc. World Soybean Res. Conf., 3rd. R. Shibles, ed. Westview Press, Boulder, CO.
6. Hepperly, P. R., Mignucci, J. S., Sinclair, J. B., and Mendoza, J. B. 1982. Soybean anthracnose and its seed assay in Puerto Rico. Seed Sci. Technol. 11:371-380.
7. Kackley, K. E., Grybauskas, A. P., and Dernoeden, P. H. 1990. Growth of *Magnaporthe poae* and *Gaeumannomyces incrustans* as affected by temperature-osmotic potential interaction. Phytopathology 80:646-650.
8. Kackley, K. E., Grybauskas, A. P., Hill, R. L., and Dernoeden, P. H. 1990. Influence of temperature-soil water status interactions on the development of summer patch in *Poa* spp. Phytopathology 80:650-655.
9. Khan, M., and Sinclair, J. B. 1990. Effect of soil temperature on infection of soybean roots by sclerotia-forming isolates of *Colletotrichum truncatum*. (Abstr.) Phytopathology 80:1040.
10. Khan, M., Wagner, R. E., and Sinclair, J. B. 1990. Infection of soybean roots with sclerotia-forming isolates of *Colletotrichum truncatum*. (Abstr.) Phytopathology 80:1044.
11. Manandhar, J. B., Hartman, G. L., and Sinclair, J. B. 1986. *Colletotrichum destructivum*, the anamorph of *Glomerella glycines*. Phytopathology 76:282-285.
12. SAS Institute, Inc. 1985. SAS User's Guide: Statistics. Version 5 Ed. SAS Institute Inc. Cary, NC. 956 pp.
13. Sinclair, J. B. 1991. Latent infection of soybean plants and seeds by fungi. Plant Dis. 75:220-224.
14. Sinclair, J. B., and Backman, P. A. 1989. Compendium of Soybean Diseases. 3rd ed. American Phytopathological Society, St. Paul, MN. 106 pp.
15. Steele, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics. 2nd ed. McGraw-Hill Book Co., New York. 633 pp.
16. Wong, C. F. J., Nik, W. Z., and Lim, T. K. 1983. Studies of *Colletotrichum dematium* f. sp. *truncata* on soybeans. Pertanika 6:28-33.