Incidence of *Colletotrichum dematium* on Prickly Sida, Spotted Spurge, and Smooth Pigweed and Pathogenicity to Soybean

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ABSTRACT

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Field collections of prickly sida (*Sida spinosa*), spotted spurge (*Euphorbia maculata*), and smooth pigweed (*Amaranthus hybridus*) from various soybean (*Glycine max*) fields in Mississippi were assayed for *Colletotrichum*. Isolates from weeds were identified as either *C. dematium* var. *truncata* or *C. dematium*. Mean incidence of *C. d.* var. *truncata* on prickly sida, spotted spurge, and smooth pigweed was 19, 6, and 10%, respectively. Mean incidence of *C. dematium* on prickly sida and spotted spurge was 2%, and on smooth pigweed was 6%. In greenhouse pathogenicity tests, isolates of *C. d.* var. *truncata* from prickly sida and spotted spurge caused damping-off, seedling blight, and reduced root volume of soybean seedlings. The same isolates colonized the two weeds and, although they caused no symptoms, they produced conidia on dead weed tissue. Dispersal of conidia from the dead weed tissue to emerging soybean seedlings and the subsequent infection of the seedlings was demonstrated. Isolates of *C. d.* var. *truncata* from smooth pigweed and *C. dematium* from prickly sida, spotted spurge, and smooth pigweed were not pathogenic on soybean.

Additional keywords: cotton, soybean anthracnose, tomato

Anthracnose of soybean (*Glycine max* (L.) Merr.) is found in all soybean-producing areas of the United States and is especially damaging in humid regions (1,4,15). Several species of *Colletotrichum* or *Glomerella* are capable of causing the disease but *C. dematium* (Pers. ex Fr.) Grove var. *truncata* (Schw.) Arx (19) (= *C. truncatum* (Schw.) Andrus & Moore sensu Sutton [17]) is the prevalent anthracnose fungus on soybean (12,14,15). *C. d.* var. *truncata* infects seeds and causes preemergence and postemergence killing or blighting of seedlings and anthracnose of older soybean plants (1,14,15,18).

C. d. var. *truncata* overwinters in soybean seeds and debris (1,15). It has been isolated from several weeds (3,7,9,14) which presumably may also serve as sources of inoculum. Following infection of soybean plants, the fungus remains quiescent in tissues. Signs and symptoms of latent infection usually become evident only in senescent or mature plants (1,4,15,18). Desiccant herbicides, such as paraquat, have been shown to hasten the production of signs and symptoms in plants infected with *C. d.* var. *truncata* (4).

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Soybean fields in Mississippi were surveyed to determine the incidence of fungi on selected weed species, emphasizing the identification of fungi pathogenic to soybean. Results of isolations from three economically important weeds and pathogenicity tests with a selected species, *C. dematium*, are reported.

MATERIALS AND METHODS

Isolation of fungi. Weeds were collected from 24 soybean fields in Mississippi in September and October of 1984 and 1985. An "X" sampling pattern with each arm covering approximately 50 m was used to randomly sample 10 plants each of prickly sida (*Sida spinosa* L.), spotted spurge (*Euphorbia maculata* L.), and smooth pigweed (*Amaranthus hybridus* L.) per field. After storage at 5 C for no longer than 36 hr, 10 0.5-cm-long tissue sections were excised from leaves and stems of each plant. Tissue pieces first were surface-sterilized in 95% ethanol for 10 sec and then in 1% sodium hypochlorite (NaOCl) for 1 min, and were aseptically plated on Difco potato-dextrose agar (PDA) in 9-cm petri dishes at the rate of 10 pieces per plate. Cultures were incubated at 24 C for 1 wk, during which time fungi growing from tissue were identified or subcultured for later identification.

Identification of *C. dematium* and *C. d.* var. *truncata* was based on morphological descriptions reported by von Arx (19). Sutton (17) is of the opinion that the nomenclature of this fungus should be *C. truncatum*. We, however, have adopted von Arx's concept of this fungus (19).

Increase of inoculum. To obtain conidia for inoculation of soybean seeds and weed seedlings, mycelial plugs from colonies on PDA were transferred to clarified V-8 juice agar. Cultures were exposed to a 12-hr photoperiod of fluorescent light (1,634 lx) for 10 days at 22 C. Sporulating colonies were flooded with sterile water and conidia were dislodged with a glass rod. Conidial suspensions were filtered through two layers of cheesecloth and adjusted to 5×10^5 conidia/ml using a hemacytometer.

Pathogenicity of *C. d.* var. *truncata* and *C. dematium* to soybean. Two isolates each of *C. d.* var. *truncata* and *C. dematium* from each weed species were tested (Table 1). Isolates of *C. d.* var.

Table 1. Sources and isolate codes of *Colletotrichum dematium* var. *truncata* and *C. dematium* used for inoculation of soybeans

Isolate	Isolate code	Host
C. d. var. *truncata*	SB CDT-1	Soybean
C. d. var. *truncata*	PW CDT-1	Smooth pigweed
C. d. var. *truncata*	PW CDT-2	Smooth pigweed
C. d. var. *truncata*	PS CDT-1	Prickly sida
C. d. var. *truncata*	PS CDT-2	Prickly sida
C. d. var. *truncata*	SS CDT-1	Spotted spurge
C. d. var. *truncata*	SS CDT-2	Spotted spurge
C. dematium	SB CD-1	Soybean
C. dematium	PW CD-1	Smooth pigweed
C. dematium	PW CD-2	Smooth pigweed
C. dematium	PS CD-1	Prickly sida
C. dematium	PS CD-2	Prickly sida
C. dematium	SS CD-1	Spotted spurge
C. dematium	SS CD-2	Spotted spurge

truncata and *C. dematium* from soybean were included for comparison. Isolates of *C. dematium* were included in these initial tests because another falcate-spored *Colletotrichum* provisionally identified as *C. dematium* was reported as occurring on soybean (3).

Bragg soybean seeds were placed in petri dishes and inoculated with 2 ml of the appropriate conidial suspension, then they were shaken vigorously to obtain a uniform distribution of inoculum.

In the greenhouse, 20 inoculated or noninoculated (control) seeds were planted in sand contained in 15-cm-diameter plastic pots, and were replicated four times. Pots were randomized and plants incubated in a mist chamber at 29 ± 3 C for 4 days, then transferred to a greenhouse bench. Seedling emergence, symptom development, and root volume were determined 2 wk after inoculation. The disease index was based on a 0–5 scale where 0 = no symptoms, 1 = cotyledon necrosis, 2 = stem necrosis, 3 = root necrosis, 4 = postemergence damping-off, and 5 = preemergence damping-off. Root volume was determined by submerging roots in water and measuring water displacement. Attempts were made to reisolate *C. d.* var. *truncata* and *C. dematium* from seedlings by culturing tissue on PDA as described previously. This experiment was conducted twice.

Comparative pathogenicity of original isolates and reisolates of *C. d.* var. *truncata* to soybean. Each isolate of *C. d.* var. *truncata* was reisolated from plants in the above experiment and included with the original isolates in seed and pod inoculation tests. The procedure described above was used for inoculation of Bragg seeds in the greenhouse. Seedling emergence, symptom development, and root volume were recorded 2 wk after inoculation. Attempts were made to reisolate the fungus from seedlings by culturing tissue on PDA as described previously.

A detached-pod technique (9) was used in the laboratory to determine the pathogenicity of *C. d.* var. *truncata* isolates to pods. Greenhouse-grown Bragg soybean pods containing full-size green beans (R6 growth stage) (6) were harvested, surface-sterilized as described previously, and rinsed three times in sterile water. Six pods in each of three replications were placed in moisture chambers containing two layers of Whatman No. 2 filter paper. Pods were inoculated by application of 0.1 ml of an inoculum (5×10^5 conidia/ml) to the pod surface above each developing seed. Pods similarly treated with sterile water served as controls. Pods were randomized and incubated at 22 C under a 12-hr photoperiod. Relative humidity was maintained at 100% by periodic addition of water to the filter paper. After 10 days, pods were observed for symptoms, dissected, and a 5-mm-diameter disk was excised from the peduncle end of each. Pod disks and seeds were surfaced-sterilized and plated on PDA as described previously. Colonies of *C. d.* var. *truncata* growing from pod disks and seeds, and seed germination were determined after 7 days of incubation at 22 C.

Pathogenicity of *C. d.* var. *truncata* to weeds. To determine pathogenicity to original weed hosts (prickly sida, spotted spurge, and smooth pigweed), five seedlings of each species were grown in each of four replications in sterile soil in 10-cm-diameter plastic pots. Conidial suspensions of the *C. d.* var. *truncata* isolates were atomized to runoff on seedlings in the V1 growth stage (6). Seedlings sprayed with sterile water served as controls. Pots were randomized and plants were incubated in a mist chamber at 29 ± 3 C for 4 days, then placed on a greehouse bench and observed for development of signs and symptoms. After 2 wk, plants were sprayed with paraquat (1,1-dimethyl-4-4-bipyridinium dichloride) at the rate of 2.34 kg/ha. Two days later, the dead weeds resulting from the paraquat treatment were returned to the mist chamber for 4 days and again observed for development of signs and symptoms. Production of acervuli, setae, and conidia was determined by using a binocular dissecting microscope and by viewing slide mounts of conidia using a compound light microscope. Immediately before the paraquat treatment, a subsample of living seedlings was bioassayed for *C. d.* var. *truncata* by plating tissue on PDA as described previously.

Infection of soybeans exposed to *C. d.* var. *truncata* inoculum from weeds. To demonstrate infection of soybean by inoculum dispersed from paraquat-treated, dead weed tissue, Bragg soybean seeds were planted in sterile soil in 10-cm-diameter plastic pots (10 seeds per pot) and were placed in a mist chamber. Two grams of shredded, dead weed tissue from the previous test, which contained acervuli and conidia of *C. d.* var. *truncata*, were placed in pots on the soil surface either at planting (treatment 1) or when seedlings emerged 5 days later (treatment 2). Paraquat-treated, non-inoculated weed tissue from the previous test served as controls. Treatments were replicated three times and arranged in a completely random design. In treatment 1, the effect of percolating water on dispersal of conidia and the subsequent infection of germinating seeds and developing seedlings was determined. To determine the effect of splashing rain on dispersal of conidia and the subsequent infection of emerged seedlings (treatment 2), rain was simulated for 2 min using a water-breaker hose attachment. Plants from treatments 1 and 2 were incubated in the mist chamber at 29 ± 3 C for 10 days, then seedlings were observed for signs and symptoms of anthracnose. Attempts were made to reisolate the fungus by culturing seedling tissue on PDA as described previously. Data were subjected to analysis of variance, and means were separated using Duncan's multiple range test.

RESULTS

Incidence of *C. d.* var. *truncata* and *C. dematium* on weeds. *C. d.* var. *truncata* was isolated from a mean of 19% (0–35%

Table 2. Pathogenicity to soybean seedlings of *Colletotrichum dematium* var. *truncata* and *C. dematium* weed isolates inoculated on soybean seeds

		Test 1		Test 2	
Fungus	Isolate code[x]	Disease severity index[y]	Root volume (cc^3)	Disease severity index	Root volume (cc^3)
C. d. var. *truncata*	PS CDT-2	4.4 a[z]	0.5 d	4.4 ab	3.4 d
C. d. var. *truncata*	SS CDT-1	4.2 a	5.2 c	4.4 ab	3.1 d
C. d. var. *truncata*	SB CDT-1	4.2 a	4.4 c	4.2 b	2.8 d
C. d. var. *truncata*	PS CDT-1	3.9 b	2.3 cd	4.4 ab	3.4 d
C. d. var. *truncata*	PW CDT-2	0.3 c	13.6 ab	0.1 c	17.5 abc
C. d. var. *truncata*	SS CDT-2	0.2 c	15.2 ab	4.2 b	4.2 d
C. d. var. *truncata*	PW CDT-1	0.1 c	12.2 b	0.2 c	20.2 abc
C. dematium	PS CD-1	0.1 c	14.8 ab	0.2 c	18.4 abc
C. dematium	PS CD-2	0.1 c	15.6 ab	0.1 c	22.0 ab
C. dematium	SB CD-1	0.1 c	14.7 ab	0.2 c	22.6 ab
C. dematium	PW CD-2	0.1 c	13.0 ab	0.1 c	17.8 abc
C. dematium	PW CD-1	0.1 c	14.2 ab	0.1 c	19.2 abc
C. dematium	SS CD-1	0.1 c	14.5 ab	0.1 c	21.7 ab
C. dematium	SS CD-2	0.1 c	14.5 ab	0.1 c	18.6 abc
Control		0.1 c	19.6 a	0.1 c	18.5 abc

[x] PS, SS, SB, and PW designate isolates from prickly sida, spotted spurge, soybean, and smooth pigweed, respectively. CDT and CD designate *C. d.* var. *truncata* and *C. dematium*, respectively.
[y] 0 = No symptoms, 1 = cotyledon necrosis, 2 = stem necrosis, 3 = root necrosis, 4 = postemergence damping-off, and 5 = preemergence damping-off.
[z] Within a column, figures followed by the same letter are not significantly different ($P = 0.05$), according to Duncan's multiple range test.

range), 6% (0–10% range), and 10% (0–18% range), and *C. dematium* from a mean of 2% (0–6% range), 2% (0–9% range), and 4% (0–8% range), of the prickly sida, spotted spurge, and smooth pigweed plants sampled, respectively.

Pathogenicity of *C. d.* var. *truncata* and *C. dematium* to soybean. Isolates of *C. d.* var. *truncata* from prickly sida and spotted spurge were pathogenic to soybean seedlings (Table 2). Isolate SS CDT-2 did not cause symptoms or reduce root volume in the first test, but did so in the second test. The major symptoms caused by isolates of *C. d.* var. *truncata* from the two weeds were preemergence damping-off, reduced root systems, and dark, sunken lesions on hypocotyls and cotyledons.

Isolates of *C. d.* var. *truncata* from smooth pigweed were not pathogenic (Table 2). Isolate PW CDT-1 reduced the root volume of seedlings in the first test but not in the second. None of the isolates of *C. dematium* were pathogenic.

Because of variation in pathogenicity of isolates SS CDT-2 and PW CDT-1 in the first two seed inoculation tests, a third test was conducted with *C. d.* var. *truncata* (Table 3). In this test, each isolate of the fungus from prickly sida and spotted spurge was pathogenic and reduced the root volume of seedlings. Isolates of *C. d.* var. *truncata* from smooth pigweed were not pathogenic.

Comparative pathogenicity of original isolates and reisolates of *C. d.* var. *truncata* to soybean. Of the prickly sida and spotted spurge isolates tested, all original isolates and the reisolates of *C. d.* var. *truncata* from previously inoculated soybean seedlings were pathogenic to seedlings (Table 4). Isolate SS CDT-2 was the least virulent of the pathogenic isolates and less virulent than SS CDT-2R. None of the original isolates and reisolates from smooth pigweed were pathogenic. None of the *C. d.* var. *truncata* isolates from weeds were pathogenic to soybean pods and seeds.

Pathogenicity of *C. d.* var. *truncata* to weeds. None of the isolates tested caused symptoms on prickly sida, spotted spurge, or smooth pigweed seedlings. However, each was reisolated from its respective weed host. In addition, each isolate produced fertile acervuli on stems after seedlings were killed with paraquat and incubated in a mist chamber.

Infection of soybeans exposed to *C. d.* var. *truncata* inoculum from weeds. The percentage of soybean seedlings infected by inoculum dispersed from dead seedlings of prickly sida and spotted spurge was 16.8 and 9% (treatment 1, percolating water) and 20.5 and 16.0% (treatment 2, simulated rainfall), respectively. Each mean was significantly different ($P = 0.05$) from the control, which had no symptomatic plants. Symptomatic seedlings had dark, sunken lesions on hypocotyls and cotyledons. Isolates of *C. d.* var. *truncata* from prickly sida and spotted spurge were reisolated from these lesions. Most of the lesions on hypocotyls appeared to occur at the point of contact between infested weed tissue and the developing soybean seedlings. None of the soybean seedlings exposed to infested smooth pigweed tissue became infected.

Table 3. Pathogenicity to soybean seedlings of *Colletotrichum dematium* var. *truncata* weed isolates inoculated on seeds

Isolate code[x]	Disease index[y]	Root volume (cc^3)
SS CDT-1	4.3 a[z]	2.0 c
SB CDT-1	4.1 a	2.0 c
PS CDT-2	4.0 ab	2.8 c
PS CDT-1	4.0 ab	3.4 c
SS CDT-2	3.6 b	8.0 b
PW CDT-1	0.2 c	22.1 a
PW CDT-2	0.1 c	20.8 a
Control	0.1 c	19.8 a

[x] PS, SS, SB, and PW designate isolates from prickly sida, spotted spurge, soybean, and smooth pigweed, respectively. CDT and CD designate *C. d.* var. *truncata* and *C. dematium*, respectively.
[y] 0 = No symptoms, 1 = cotyledon necrosis, 2 = stem necrosis, 3 = root necrosis, 4 = postemergence damping-off, and 5 = preemergence damping-off.
[z] Within a column, figures followed by the same letter are not significantly different ($P = 0.05$), according to Duncan's multiple range test.

Table 4. Pathogenicity to soybean seedlings of *Colletotrichum dematium* var. *truncata* weed isolates and reisolates inoculated on seeds

Isolate code[x]	Disease index[y]	Root volume (cc^3)
SS CDT-1R	4.6 a[z]	0.3 c
SB CDT-2R	4.3 a	1.9 c
PS CDT-2R	4.3 a	1.8 c
SS CDT-1	4.2 a	1.0 c
PS CDT-1R	4.1 a	2.0 c
SB CDT-1	4.1 a	1.2 c
PS CDT-2	3.8 ab	2.1 c
PS CDT-1	3.6 ab	3.3 c
SS CDT-2	3.1 b	11.7 b
PW CDT-1R	0.4 c	23.9 a
PW CDT-2R	0.2 c	21.0 a
PW CDT-2	0.2 c	22.0 a
PW CDT-1	0.2 c	24.0 a
Control	0.1 c	23.1 a

[x] PS, SS, SB, and PW designate isolates from prickly sida, spotted spurge, soybean, and smooth pigweed, respectively. CDT and CD designate *C. d.* var. *truncata* and *C. dematium*, respectively. R = Reisolated from inoculated soybean seedlings in previous tests.
[y] 0 = No symptoms, 1 = cotyledon necrosis, 2 = stem necrosis, 3 = root necrosis, 4 = postemergence damping-off, and 5 = preemergence damping-off.
[z] Within a column, figures followed by the same letter are not significantly different ($P = 0.05$), according to Duncan's multiple range test.

DISCUSSION

C. d. var. *truncata* and *C. dematium* were found to occur on prickly sida, spotted spurge, and smooth pigweed. This is the first report of their occurrence on these weeds.

Isolates of *C. d.* var. *truncata* from prickly sida and spotted spurge were highly pathogenic to soybean seedlings when inoculated on seeds. They caused symptoms indistinguishable from those caused by *C. d.* var. *truncata* from soybean. Apparently this fungus is able to colonize the weeds and still retain its pathogenicity to soybean. Others (5,8,10,11) working with different fungi and hosts reported similar results.

Based on a previous study (8), the pathogenicity of *C. d.* var. *truncata* from weeds was expected to increase after passage through soybean. However, only one reisolate (Table 4) increased in virulence upon inoculation into soybean a second time.

Isolates of *C. d.* var. *truncata* from smooth pigweed were not pathogenic to soybean seedlings. It is possible that such isolates are simply less aggressive on soybean. Perhaps a longer incubation period than was used in our tests would have allowed the isolates to express their pathogenicity. Differences in virulence among isolates of *C. d.* var. *truncata* were observed in this and another study (14). It is also possible that the isolates from pigweed are a subspecies of *C. dematium* with a host range exclusive of soybean. Formae speciales have been described for *Colletotrichum* (19).

The isolates of *C. dematium* tested were not pathogenic to soybean. These are identical in colony and morphological characteristics to isolates of *C. dematium*, shown by Batson and Roy (3) to be pathogenic to tomato fruit. *C. dematium* (2,3,16) and *C. capsici* (Sydow) Butler & Bisby (13), considered synonymous by von Arx (19), are reported to be pathogenic to tomato fruit, and the latter species also to cotton and numerous other hosts (13). Therefore, it is probable that the *C. dematium* isolates from the weeds are capable of infecting tomato, and, if the synonymy (19) is indeed correct, perhaps other economic hosts as well. The possibility of prickly sida, spotted spurge, and smooth pigweed as sources of inoculum for infection of tomato and cotton should be investigated.

The inability of *C. d.* var. *truncata* isolates to cause symptoms on inoculated prickly sida and spotted spurge seedlings and their reisolation from living seedlings inoculated with the fungus indicate latent colonization of the weeds by the fungus. Nevertheless, sporulation of the isolates on dead tissues of the weed seedlings and dispersal of the inoculum to soybean seedlings was demonstrated. The killing of these weeds by preemergence and postemergence herbicides or by dessicant-type herbicides used as harvest aids, such

as paraquat (4), occurs commonly in soybean production. Under conditions of high humidity or moisture the death of prickly sida and spotted spurge plants due to such herbicides or to natural senescence could stimulate sporulation and thereby provide inoculum for infection of soybeans.

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