Host Range and Virulence of *Colletotrichum truncatum*, a Potential Mycoherbicide for Hemp Sesbania (*Sesbania exaltata*)

C. D. BOYETTE, Research Plant Pathologist, Southern Weed Science Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Stoneville, MS 38776

ABSTRACT

Boyette, C. D. 1991. Host range and virulence of *Colletotrichum truncatum*, a potential mycoherbicide for hemp sesbania (*Sesbania exaltata*). Plant Dis. 75:62-64.

A previously unreported anthracnose disease incited by *Colletotrichum truncatum* was discovered on hemp sesbania seedlings in Washington County, Mississippi, in 1987. Hemp sesbania inoculated with 2×10^7 spores per milliliter in the greenhouse were killed within 7 days. Soybean cvs. Bragg, Bedford, and Hill developed small, necrotic leaf spots, but no further symptoms occurred after 3 wk. Several cultivars of cotton, rice, and 23 other plant species representing eight plant families were immune to disease, as measured by visual assessment. Results of these tests indicate that this fungus has potential as a mycoherbicide to control hemp sesbania.

Additional keyword: bioherbicide

Hemp sesbania (*Sesbania exaltata* (Raf.) Cory) is a serious weed pest of soybean (*Glycine max* (L.) Merr.) and rice (*Oryza sativa* L.) in the southeastern United States (8–10), and infestations have also increased in cotton (*Gossypium hirsutum* L.) (1). As few as three hemp sesbania plants per meter of row for the full season will reduce seed cotton yields by 53% and boll weights by 9% (2).

Hemp sesbania can be controlled in soybeans and rice with postemergent applications of acifluorfen and imazaquin. However, there are no over-the-top postemergence herbicides currently registered for use in cotton, and control with postemergence directed sprays is erratic (7).

In June 1987, an anthracnose pathogen was isolated from leaf spots of hemp sesbania and was tentatively identified as *Colletotrichum truncatum* (Schwein.) Andrus & W. D. Moore. The disease was widespread among the hemp sesbania seedlings, mainly affecting the cotyledons. Symptoms were not found on older seedlings.

Experiments were conducted to determine the host range and virulence of this pathogen. This information is vital for the further testing of the bioherbicidal potential of *C. truncatum* for controlling hemp sesbania.

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Accepted for publication 25 June 1990.

MATERIALS AND METHODS

Collection, isolation, and culture. Diseased leaf samples were collected from the Southern Weed Science Laboratory weed nursery in Stoneville. Microscopic examinations revealed orange-colored masses of conidia being produced in acervuli in the lesions. A mass of spores was transferred into 10 ml of 10% (v/v) 0.525% sodium hypochlorite in sterile, distilled water. One milliliter of the spore suspension was pipetted into petri plates of potato-dextrose agar (PDA) amended with the antibiotics chloramphenicol (.75 mg/ml) and streptomycin sulfate (1.25 mg/L). The plates were incubated for 48 hr at 25 C. Advancing edges of fungal colonies were transferred to PDA and incubated for 5 days at 25 C under alternating 12-hr light/12-hr dark regimens, provided by fluorescent lights. The fungus was subcultured on PDA without antibiotics, preserved in screw-capped culture tubes of twice-autoclaved sandy loam soil, and stored at 4 C.

Inoculum was increased by flooding petri plates of PDA amended with L-proline (1.5 g/L [PDA-P]), with 1 ml of spore suspension from subculture plates. The plates were incubated for 3–5 days under the same temperature and lighting conditions as previously described. Conidia were washed from the plates with sterile, distilled water, rinsed twice by centrifugation (954 *g* for 10 min) and adjusted to 2×10^7 spores per milliliter by adding distilled water. Spore counts were determined with hemacytometers.

Test plant propagation. Hemp sesbania plants and host range test plants (Table 1) were grown from seed in a commercial potting mix contained in peat strips. Each strip contained 12 plants. The potting mix was supplemented with a slow-release fertilizer (14:14:14, NPK). The plants were placed on subirrigated benches in the greenhouse. Greenhouse temperatures ranged from 25 to 30 C with 40–90% relative humidity. The photoperiod was 12 hr with 1,650 $\mu E \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation as measured at midday.

Pathogenicity tests. Hemp sesbania plants from the cotyledonary stage through the seventh true-leaf stages of growth were inoculated by spraying until runoff with conidial preparations containing 2×10^7 conidia per milliliter and 0.02% Tween 80 surfactant. Control plants were sprayed with water and surfactant only.

Immediately after inoculation, the plants were placed in a darkened dew chamber for 12 hr at 25 C and transferred to subirrigated greenhouse benches. The plants were monitored for disease development for 1 wk.

Host-range tests. Test plants (Table 1) were grown under the same conditions as previously described for hemp sesbania. All plants were tested at the cotyledonary to third true-leaf stage of growth, with 36 plants used for each treatment. The plants were inoculated, incubated, and monitored for disease development for 3 wk.

Cross-inoculation tests. Cultures of *C. truncatum* and *C. dematium* isolates that have been reported as pathogens of other leguminous weeds or soybeans were obtained from the Northern Regional Research Laboratory (NRRL), Peoria, Illinois, and the American Type Culture Collection (ATCC), Rockville, Maryland, for pathogenicity comparisons (Table 2). Isolates NRRL 15933, pathogenic to Florida beggarweed (*Desmodium tortuosum* (Sw.) DC.); ATCC 22582, pathogenic to coffee senna (*Cassia occidentalis* L.); and ATCC 18013, pathogenic to soybeans, were cultured on petri plates of PDA. The spores were rinsed from the agar surfaces with distilled water, centrifuged for 10 min at 956 *g*, and adjusted to 2×10^7 spores per milliliter by adding distilled water, and the plants were sprayed with an atomizer (Table 2). The inoculated plants were given dew treatments as described previously and monitored for disease developed for 3 wk.

RESULTS

Collection, isolation, and culture. Leaf lesions, 2–4 mm in diameter with dark concentric rings, were observed on cotyledons of the collected plant material. Microscopic examinations revealed acervuli containing setae and truncate conidia measuring 18–28 × 3–5 μm. Based on characteristics observed in infected tissue and in artificial culture, the fungus was identified as *C. truncatum* (14), an identification subsequently confirmed by the Commonwealth Agricultural Bureau, International Mycological Institute, Kew, Surrey, England. The fungus has been deposited with the ARS Patent Culture Collection and has been assigned the number 18434.

Pathogenicity tests. CT isolate 18434 was highly virulent on hemp sesbania at all stages of growth that were tested. Cotyledonary plants were all killed within 3 days after inoculations while larger plants required 5–7 days to achieve this level of kill (Fig. 1).

Host-range tests. The reactions of the test plants to CT isolate 18434 are shown in Table 1. All evidence of infection was

Table 1. Response of various crop and weed species tested for susceptibility to *Colletotrichum truncatum*

Family Scientific name	Common name	Cultivar	Disease reaction[a]
Compositae			
Xanthium strumarium L.	Cocklebur		I
Convolvulaceae			
Ipomoea L.	Morning glory		I
Cucurbitaceae			
Cucurbita pepo L.	Pumpkin	Jack-O'-Lantern	I
Cucurbita pepo var. *melopepo* (L.) Alef.	Squash	Golden Summer Crookneck	I
Citrullus vulgaris Schrad.	Watermelon	Charleston Grey	I
Graminae			
Zea mays L.	Corn	Truckers Favorite	I
Sorghum halepense (L.) Pers.	Johnsongrass		I
Oryza sativa L.	Rice	LaBelle	I
		Starbonnet	I
Sorghum bicolor (L.) Moench	Grain sorghum	Texas C-124	I
Leguminosae			
Medicago sativa L.	Alfalfa	Delta	I
Cassia occidentalis L.	Coffee senna		I
Cassia obtusifolia L.	Sicklepod		I
Desmodium tortuosum (Sw.) DC.	Florida beggarweed		I
Crotalaria spectabilis Roth	Showy crotalaria		I
Sesbania exaltata (Raf.) Cory	Hemp sesbania		S-5
Sesbania drummondii (Rydb.) Cory	Rattlebox		I
Aeschynomene virginica (L.) B. S. P.	Northern jointvetch		I
Glycine max (L.) Merr.	Soybean	Bedford	S-2
		Bragg	S-2
		Dare	I
		Davis	I
		Forrest	I
		Hill	S-1
		Hood	I
		Centennial	I
		Tracy	I
Arachis hypogoea L.	Peanut	Tennessee Reds	I
Phaseolus vulgaris L.	Garden bean	Kentucky Wonder	I
		Romano Pole	I
		Ohio Pole	I
		Jackson Wonder	I
		Henderson Bush Lima	I
		Lady Cowpea	I
		White Crowder Pea	I
Malvaceae			
Gossypium hirsutum L.	Cotton	Stoneville 213	I
		Deltapine 61	I
Sida spinosa L.			
Abutilon theophrastii (Medik.)	Velvetleaf		I
Solanaceae			
Lycopersicon esculentum Mill.	Tomato	Beefsteak	I
		Marion	I
Datura stramonium L.	Jimsonweed		I

[a]Reaction: S = susceptible on a scale of 1–5, where 1 = small (2 mm), nonenlarging lesions and 5 = plant death; I = immune.

Table 2. Pathogenicity of *Colletotrichum truncatum* and *C. dematium* isolates in cross-inoculation experiments

Fungus	Isolate code	Disease reaction[a]			
		Hemp sesbania	Florida beggarweed	Coffee senna	Bragg soybean
C. truncatum	NRRL 18434	S-5	I	I	S-2
C. truncatum	NRRL 15933	I	S-5	I	S-2
C. dematium	ATCC 22852	I	I	S-5	S-3
C. truncatum	ATCC 18013	I	S-1	I	S-5

[a]Reaction: S = susceptible on a scale of 1–5, where 1 = small (1 mm), nonenlarging lesions and 5 = plant death; I = immune.

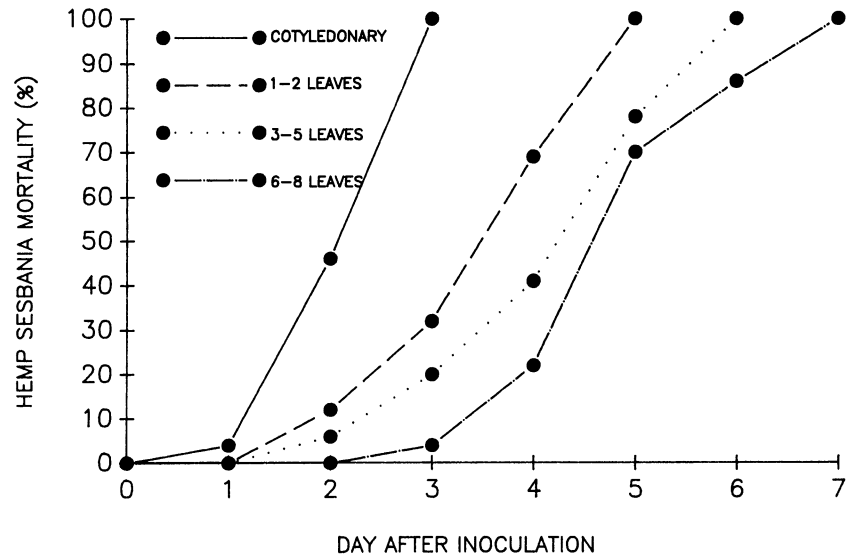

Fig. 1. Effect of *Colletotrichum truncatum* on hemp sesbania at various stages of growth. The plants were inoculated with *C. truncatum* spores at a rate of 2×10^7 spores per milliliter.

limited to plants in the Leguminosae. Some infection occurred on soybean cvs. Bragg, Bedford, and Hill, but it was restricted to small (<3 mm), nonenlarging lesions. The fungus was recovered from the lesions, but microscopic examinations revealed that it did not sporulate on the necrotic soybean tissue. A related species, *S. drummondii* (Rydb.) Cory, was not affected by the fungus.

Cross-inoculation tests. Of the isolates that were tested, only NRRL 18434 had visibly infected hemp sesbania after 3 wk. Neither Florida beggarweed nor coffee senna were visibly affected by this isolate. Bragg soybeans were slightly infected, but no mortality had occurred (Table 2). Florida beggarweed seedlings were infected and killed following inoculation with ATCC 22583. This isolate also caused slight infection on soybean. Infection and mortality of coffee senna and moderate infection of soybean occurred following inoculation of plants with ATCC 22852. ATCC 18013 was the most virulent on soybean, with severe infection and mortality occurring. This isolate also caused slight injury to Florida beggarweed, but it caused no visible infection to hemp sesbania or coffee senna (Table 2).

DISCUSSION

C. truncatum, the incitant of anthracnose on soybean, causes pre- and postemergence killing or blighting of soybean seedlings and anthracnose of older soybean plants (12,15). The isolates reported from those findings caused seedling death and veinal and petiole necrosis of larger plants on the soybean cultivars that were tested (5). The hemp sesbania isolate did not produce these symptoms on any of the soybean cultivars that were tested. Other researchers have isolated strains of *C. truncatum* pathogenic to soybean from various weed species that may serve as additional hosts for *C. truncatum* (6,8,11). However, this is the first report of the occurrence of *C. truncatum* on hemp sesbania.

The isolate of *C. truncatum* from hemp sesbania caused slight injury to some soybean cultivars, but it did not cause mortality nor did it sporulate on the soybean lesions. Although it is unlikely that this isolate of *C. truncatum* would pose a serious threat to soybeans or other nontarget species, more research is needed to verify these findings.

Other isolates of *C. truncatum* have been evaluated as biological herbicides for controlling the weeds coffee senna (5) and Florida beggarweed (3). In cross-inoculation tests, none of the isolates were pathogenic to hemp sesbania, nor was the hemp sesbania isolate pathogenic to either of these species.

From our results and from those of others, it appears that host specialization may occur within this species, although formae speciales of *C. truncatum* are not recognized by Sutton (13). Other fungal taxonomists, however, do recognize formae speciales based in part on host-range reactions (16).

These results indicate that *C. truncatum* has potential as a bioherbicide for controlling hemp sesbania. The fungus is highly pathogenic to the weed and either nonpathogenic or weakly pathogenic to the crop species that were tested. TeBeest (14) has discovered recently that *C. gloeosporioides* (Penz.) Penz. & Sacc. in Penz. f. sp. *aeschynomene*, the incitant of anthracnose on northern jointvetch (*Aeschynomene virginica* (L.) B. S. P.), has a much broader host range than originally reported by Daniel et al (4). Therefore, in order to further define the host range of *C. truncatum*, more research should be conducted with *C. truncatum* to include soybean cultivars and other leguminous crop species of more diverse genetic backgrounds (17). Additional studies will be conducted to determine the environmental and physical conditions necessary for this fungus to control hemp sesbania and to test the biocontrol efficacy of the pathogen under field conditions.

ACKNOWLEDGMENT

I thank Laurie B. Turfitt for providing valuable technical assistance.

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