Seedling Blight of Sicklepod Caused by *Alternaria cassiae*

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ABSTRACT

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Alternaria cassiae isolated from diseased sicklepod (*Cassia obtusifolia*) was pathogenic to sicklepod, coffee senna (*Cassia occidentalis*), and showy crotalaria (*Crotalaria spectabilis*). Soybean (*Glycine max*), peanut (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), corn (*Zea mays*), and 26 other crop and weed species in nine families were resistant to the pathogen. Host range and virulence studies indicated this fungus to have potential for use as a biological herbicide.

Alternaria cassiae Jurair and Khan (1) was isolated from a diseased sicklepod (*Cassia obtusifolia* L.) seedling that was greenhouse grown from a seed collection that was harvested in the fall of 1977 near Richton (Perry County), MS. In 1981, the pathogen was isolated from diseased sicklepod seedlings that were collected at several locations in Claiborne County, MS. Many of these diseased seedlings exhibited severe stem and leaf lesions, and infected plants were frequently killed or severely stunted.

These studies were conducted to evaluate the host range, virulence, and inoculum production of the sicklepod isolate of *A. cassiae*. This information is necessary to determine the potential of the pathogen as a biocontrol agent for sicklepod, which is a problem weed species in 11 states in the southeastern United States (3).

MATERIALS AND METHODS

The single culture of *A. cassiae* used in this study was isolated on potato-dextrose agar, then subcultured and maintained on V-8 juice agar (2). The pathogen was preserved in screw-capped culture tubes of twice-autoclaved sandy loam soil and stored at room temperature (23–26 C) and at 4 C. The fungus sporulated profusely on V-8 juice agar in plastic petri dishes incubated for 2 or more days at 25 C with 12-hr diurnal light supplied by two 40-W cool-white fluorescent bulbs suspended 45 cm above the cultures.

Conidia from petri dish cultures were used to inoculate 10 L of liquid growth medium (V-8 juice, 200 ml/L; calcium carbonate, 3 g/L; and sucrose, 30 g/L in distilled water) in 14-L vessels of a New Brunswick model 214 fermentor. A silicone-based antifoaming agent (Dow Corning Antifoam C) was added as needed to prevent excessive foaming

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Table 1. Reaction of various plant species to *Alternaria cassiae* isolated from sicklepod[a]

Family Species	Disease reaction[b]
Caryophyllaceae	
Carnation (*Dianthus chinensis* L.) 'Dwarf Baby Mixed'	R^+
Compositae	
Chrysanthemum (*Chrysanthemum morifolium* (Ramat. Hemsl.) 'Korean'	R^+
Cocklebur (*Xanthium pensylvanicum* Wallr.)	R^+
Dandelion (*Taraxacum officinale* Weber)	R^+
Sunflower (*Helianthus annuus* L.) 'Sungold'	R^+
Zinnia (*Zinnia elegans* Jacq.) 'B's Best'	R^+
Convolvulaceae	
Morning glory (*Ipomoea* spp.)	R^+
Cucurbitaceae	
Cantaloupe (*Cucumis melo* L.) 'Harley's Best Jumbo'	R
Pumpkin (*Cucurbita pepo* L.) 'Jack-O'Lantern'	R^+
Squash (*Cucurbita pepo* var. *melopepo* (L.) Alef.) 'Golden Summer Crookneck'	R^+
Watermelon (*Citrullus vulgaris* Schrad.) 'Charleston Grey'	R
Gramineae	
Corn (*Zea mays* L.) 'Truckers Favorite'	R^+
Oats (*Avena sativa* L.)	R
Sorghum (*Sorghum bicolor* (L.) Moench) 'Texas C 424'	R^+
Wheat (*Triticum aestivum* L.) 'Coker 68-15'	R
Leguminosae	
Alfalfa (*Medicago sativa* L.) 'Delta'	R^+
Bean, Lima (*Phaseolus limensis* Macf.) 'Burpee's Fordhook'	R^+
Bean, Pole (*Phaseolus vulgaris* L.) 'Romano'	R^+
Coffee senna (*Cassia occidentalis* L.)	S
Cowpea (*Vigna sinensis* (Torner) Savi) 'Early Ramshorn'	R^+
Hemp sesbania (*Sesbania exaltata* (Raf.) Cory)	R^+
Peanut (*Arachis hypogaea* L.) 'Tennessee Reds'	R^+
Showy crotalaria (*Crotalaria spectabilis* Roth)	S
Sicklepod (*Cassia obtusifolia* L.)	S
Soybean (*Glycine max* (L.) Merr.)	
'Bragg'	R
'Tracey'	R^+
'Forrest'	R^+
Liliaceae	
Onion (*Allium cepa* L.) 'Yellow Globe'	R^+
Malvaceae	
Cotton (*Gossypium hirsutum* L.) 'Stoneville 213'	R^+
Okra (*Abelmoschus esculentus* (L.) Moench) 'Clemson Spineless'	R^+
Prickly sida (*Sida spinosa* L.)	R^+
Spurred anoda (*Anoda cristata* (L.) Schlecht.)	R^+
Velvetleaf (*Abutilon theophrasti* Medic.)	R^+
Solanaceae	
Pepper (*Capsicum frutescens* L.) 'Large Cherry'	R
Tomato (*Lycopersicon esculentum* Mill.)	
'Heinz 1439'	R
'Rutger'	R

[a] Twelve plants of each variety were sprayed with inoculum containing 1×10^5 conidia per milliliter and surfactant. Controls were sprayed with surfactant and water only. Plants were evaluated daily for 14 days.

[b] R = resistant and S = susceptible to the pathogen; + = phytotoxic injury by the pathogen limited to flecking or small, nondamaging burning of the leaves.

during fermentation. Conidia used in the greenhouse and field studies were produced from mycelia that were grown at 26 C in the submerged liquid culture with vigorous agitation and aeration. The mycelia were harvested 48–72 hr after inoculation, comminuted for 30 sec in a Waring Blendor, then poured to a depth of 2–3 mm into 12 to 18 pans (41 × 27 × 5.5 cm) and exposed to direct sunlight for 20–30 min. After exposure to sunlight, the pans of mycelia were placed in unlighted chambers at 23–25 C for 48–72 hr to permit the development of conidiophores and conidia; then the conidia and mycelia were air-dried at 35 C. The conidia were harvested from the surfaces of the mycelia with a cyclone collector (4), dried over calcium chloride for 48 hr at 25 C, and stored in glass vials at 4 C. The procedure for production of *A. cassiae* conidia was adapted from methods previously described (5). Conidial concentrations were determined with a hemacytometer.

Greenhouse studies. The plant species included in the greenhouse studies are listed in Table 1. Plants were grown in a commercial potting mix in peat strips that contained 12 plants each and were fertilized weekly with a water-soluble fertilizer (14-14-14). Temperatures ranged from 28 to 32 C with 40–60% relative humidity. The day length was approximately 12 hr with photosynthetically active radiation of 1,650 $\mu E/m^2$ per second, as measured at noon with a Lambda PAR meter (Lambda Instruments Corporation, Lincoln, NE).

Plants in the cotyledon to first-leaf stage of growth were sprayed to runoff with conidial inoculum of *A. cassiae* (1×10^5 conidia per milliliter) applied with an atomizer. The conidia were suspended in 0.02% (v/v) surfactant, nonoxynol (9 to 10 POE) [*a*-(*p*-nonyl-phenyl)-*w*-hydroxypoly(oxyethylene)] in distilled water. Control plants were sprayed with water and 0.02% surfactant only. All plants were placed in dew chambers for 8–10 hr at 20 C. The plants were then moved to greenhouse benches, and disease development was evaluated daily for 14 days. All tests were repeated on at least two dates, and 12 plants were used for each treatment in each test.

Field studies. The control of sicklepod by *A. cassiae* was studied in small field plots. Randomized field plots 2.8 m square were replicated three times and planted with 100 *Glycine max* (L.) Merr. 'Forrest' seeds and about 800 sicklepod seeds. The soybean seeds were planted in two rows per plot, and the sicklepod seeds were broadcast. The plots were watered to field capacity by sprinkler irrigation to promote seed germination. Soybean and sicklepod plants in the cotyledon to first-leaf stage of growth were sprayed to runoff with a conidial suspension consisting of 0.1% surfactant and 2.5×10^5 conidia per milliliter in distilled water that was applied with a garden-type, small-volume sprayer. Control plots were sprayed with distilled water and 0.1% surfactant only.

At 7–15 and 31–39 hr after inoculation, an overhead sprinkler sprayed water on the test area for 3 min/hr from 2200 to 0600 hr. Thus, at least 8 hr of free moisture was present on the plants to stimulate conidial germination and subsequent infection of the host plants. Eight days after treatment, plants within a randomly selected, 1-m^2 area in each plot were evaluated for symptoms of disease. The *t* test was used to indicate differences between means.

RESULTS AND DISCUSSION

Culture of the pathogen. *A. cassiae* sporulated profusely; 1.5 g, consisting primarily of conidia, was commonly harvested from each pan of comminuted mycelia. These dried conidial preparations contained approximately 9×10^7 conidia per gram. Germination of the conidia was >90% on water agar when incubated at 25 C for 18 hr. The average dimensions of 100 conidia from 10-day-old cultures grown on V-8 juice agar were 25×81 μm for the spore body, with an overall length of 145 μm (Fig. 1). A description of the species *A. cassiae* has been published previously (1).

Greenhouse studies. The fungus was highly virulent to sicklepod seedlings

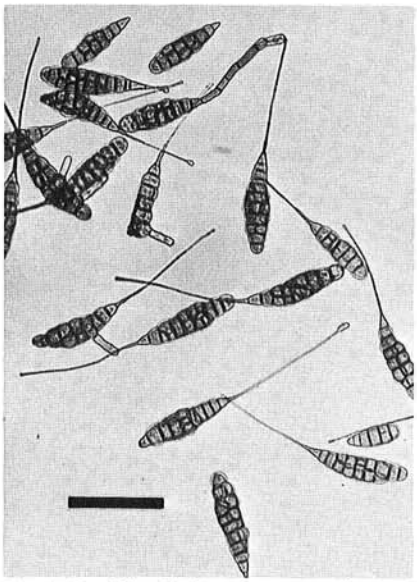

Fig. 1. Conidia of *Alternaria cassiae* isolated from sicklepod; from a culture grown on V-8 juice agar for 4 days at 25 C and 12 hr of light per day. (Bar represents 75 μm).

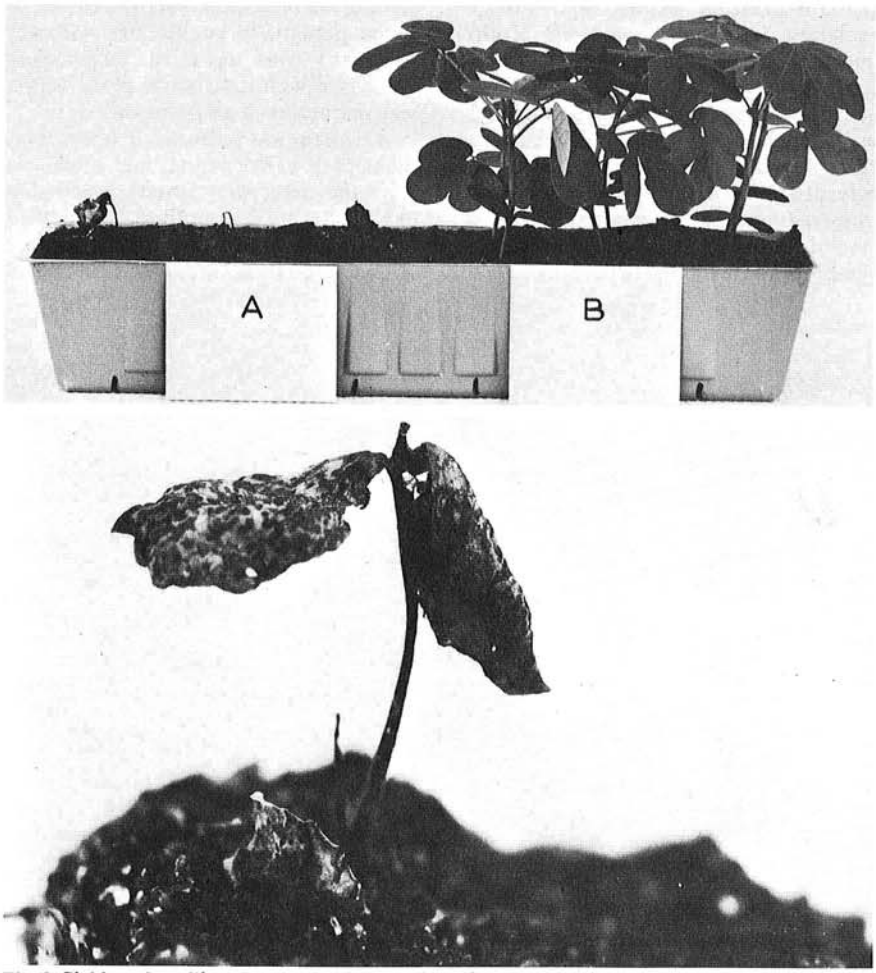

Fig. 2. Sicklepod seedlings 7 days after inoculation with *Alternaria cassiae*. **(Top)** Plants on the left **(A)** were sprayed with a mixture containing 1×10^5 conidia per milliliter and 0.02% surfactant in water. Plants on the right **(B)** were sprayed with surfactant and water only. **(Bottom)** Close-up of a diseased sicklepod seedling.

Table 2. Effect of a foliar application of *Alternaria cassiae* conidia to sicklepod in the field[a]

Treatment	Percentage of plants		
	Diseased	Severely stunted	Dead
Inoculated[b]	100[c]	48[c]	32[c]
Control[d]	<1	0	0

[a] Plants from a 1-m^2 area within each plot were evaluated. Each value represents the average of three replicates, 8 days after treatment. Each plot contained an average of 65 sicklepod plants per square meter.

[b] Plants were sprayed to runoff with a suspension of 2.5×10^5 conidia per milliliter, 0.1% surfactant, and water.

[c] The two values within columns are significantly different as determined by the *t* test ($P = 0.01$).

[d] Plants were sprayed to runoff with surfactant and water only.

(Fig. 2). Most seedlings in the cotyledon to first-leaf stage of growth were killed in 2–7 days after inoculation. The pathogen produced dark brown to black lesions 1–5 mm in diameter on the leaves and stems within 2 days. The lesions enlarged with time on any remaining plants and produced severe stem cankers and defoliation within 7 days. Coffee senna (*Cassia occidentalis* L.) and showy crotalaria (*Crotalaria spectabilis* Roth) appeared to be as susceptible as sicklepod to the pathogen.

Thirty other representative crop and weed species in nine families were resistant to the pathogen; however, phytotoxic damage was occasionally observed on the inoculated leaves of several plant species (Table 1). Phytotoxic symptoms were limited to flecking or to a marginal or interveinal 'burn' of inoculated leaves. These symptoms appeared within 48–72 hr after inoculation and did not increase in number or severity with time. Succulent tissues were most susceptible to phytotoxic damage; however, injury was seldom severe enough to induce defoliation.

Greenhouse-grown soybeans occasionally exhibited phytotoxic symptoms, whereas these symptoms were greatly reduced or absent on field-grown soybeans. The phytotoxicity is attributed to the high concentrations of conidia contained in the inoculation mixtures. Phytotoxic injury was not observed in every test, and this injury was never observed on the control plants. In other greenhouse studies, reduction of conidial concentrations from 1×10^5 to 2.5×10^4 conidia per milliliter decreased phytotoxic injury, even though sicklepod seedlings exhibited severe disease symptoms. The cause for the phytotoxicity observed on some of the nontarget species needs further investigation.

Field studies. The field plots contained an average of 65 sicklepod seedlings per square meter. All sicklepod plants in the treated plots exhibited disease symptoms (Table 2). The number of sicklepod plants in the treated plots was reduced 32% and the number of sicklepod plants considered to be potentially competitive with the soybeans was reduced 80% when compared with the control plots. Injury was not observed on soybeans.

A. cassiae was restricted in host range. Sicklepod, coffee senna, and crotalaria were the only plant species tested that exhibited disease symptoms. The limited host range and highly virulent nature of *A. cassiae* on these three species indicated that this pathogen has potential for use as a biological control agent for sicklepod. These studies also indicated that the methodology developed to produce field inoculum for other biocontrol agents (5) can be adapted to produce inoculum for further studies with *A. cassiae*. Additional studies are needed to determine the potential of this fungus for the control of coffee senna and showy crotalaria.

This is the first report of sicklepod, coffee senna, and showy crotalaria as hosts for *A. cassiae*, and this is the first report of *A. cassiae* in the western hemisphere. Cultures of the pathogen have been deposited in the Agricultural Research Culture Collection, U.S. Department of Agriculture, Peoria, IL (Accession NRRL-12553).

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