Pseudocercospora nigricans, a Pathogen of Sicklepod (*Cassia obtusifolia*) with Biocontrol Potential

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ABSTRACT

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A fungus, *Pseudocercospora nigricans*, isolated from foliar lesions on diseased sicklepod (*Cassia obtusifolia*) was determined to be the causal agent of the observed disease. Symptoms consisted of chlorosis, necrotic mottling of leaves, and accelerated leaf drop, which contributed to overall reduction of plant size. In host range studies conducted in the greenhouse, the fungus was pathogenic only to sicklepod but not to 23 crop and weed species in 10 plant families. Results of both greenhouse host range studies and pathogenicity studies in the field indicated this fungus may be useful as a classical biological control agent.

Additional key words: weed control

A fungus identified as *Pseudocercospora nigricans* (Cooke) Deighton (syn. *Cercospora nigricans* Cooke) (3) was isolated from foliar lesions on mature sicklepod plants collected in the fall of 1982 in Columbia County near Lake City, FL. The fungus did not appear lethal to its host but caused extensive destruction of photosynthetic area resulting from mottling and chlorosis of the leaves.

The objectives of this study were to assess the host range and effectiveness of this fungus in reducing plant growth when applied to sicklepod plants in the field, using mycelial inoculum produced in the laboratory. Such information is essential in determining the potential and possible role this pathogen has in the overall control of sicklepod infestations in the southeastern United States (5).

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MATERIALS AND METHODS

Pathogen isolation and culture. The isolate tested in this study, PN82-1, was one of several obtained by selective removal of conidia from sporulating foliar lesions. The conidia were removed using a heat-drawn glass microneedle under a dissecting microscope and transferred to Difco potato-dextrose agar containing 0.5% yeast extract (PDAY). The isolate was subcultured and stored on PDAY plates at room temperature and at 5 C in PDAY slant culture tubes.

Greenhouse studies. Host range studies involving sicklepod and 23 crop and weed species in 10 plant families were conducted on various dates under greenhouse conditions (Table 1). Inoculum was grown in potato-dextrose broth plus 0.5% yeast extract (PDBY) in Roux bottles, each containing 100 ml of the medium. Three- to 4-wk-old mycelial mats were removed from the culture

bottles, and 15 g (wet weight) mycelium was comminuted for 15 sec in 50 ml of the original culture broth filtrate plus 150 ml of deionized water in a Waring Blendor. Tween 20 (polyoxyethylene sorbitan monolaurate) was added, 0.1 ml per 200 ml blended suspension, as a surfactant. After straining through a stainless steel screen (mesh size: six wires per centimeter), the suspension was transferred to a portable hand-operated piston-type sprayer for inoculation of the plants. Plants were maintained in 15-cm clay pots on greenhouse benches at 25–30 C and were inoculated at ages ranging from 1 to 2 wk postgermination. Plants were inoculated by spraying the leaf and stem surfaces to runoff with the inoculum suspension. Controls were sprayed with water and surfactant only. Plants were then placed in a dew chamber, incubated for 12 hr in the dark at 25 C and 100% relative humidity, and removed to the greenhouse bench. Plants were observed for symptoms for the following 3-wk period.

Field studies. The effects of inoculation and subsequent infection of sicklepod plants with *P. nigricans* at two stages of growth were studied in nine 1-m^2 field miniplots with three replicates per treatment and a randomized complete block design. Each plot was sown with 2,000 scarified sicklepod seeds. Seeds were acid-scarified for 15 min in 15 N H_2SO_4, rinsed 3 min in running tap water, and blotted dry. Seeds were broadcast within the boundaries of each plot and raked under the freshly disked soil. Plots were then watered to field capacity by sprinkler irrigation. Seven days after planting, when most plants were at the cotyledon to first-leaf stage, the first inoculation was made (early-stage inoculation). The second inoculation (late-stage inoculation) was carried out on another three plots 50 days after planting, when plants were at two- to six-leaf stages. The inoculum in both treatments was based on 4.4 g wet weight of mycelium per square meter and delivered to the plots in a volume of tap water corresponding to 935 L/ha (4.4 g of mycelium delivered in 94 ml/plot). Triton X-100 (octyl phenoxy polyethoxyethanol) was included as a surfactant at a concentration of 0.05%. Control plots received 94 ml of tap water containing 0.05% Triton X-100 at each inoculation date. The treatments were applied with a calibrated CO_2-powered regulated pressure sprayer at 138 kPa. The inoculum was applied to the plots within 2 hr of sunset to avoid drying and to allow for a natural dew period shortly thereafter. Plants were observed weekly for disease symptoms after the first inoculation. Date of appearance of infection was recorded from the onset of typical symptoms. All sicklepod plants within all plots were harvested by hand 105 days after planting. Immediately before harvesting, five measurements of maximum sicklepod canopy height at four corners and the center of each plot were recorded and averaged. The total plant number and number of fruits produced in each plot were determined after the plants were harvested. Plants were then oven-dried to constant weight, and dry weight biomass for each plot was recorded. Field data were subjected to analysis of variance, and the means were separated into significant ranges by Duncan's multiple range test at $P = 0.05$ using the Statistical Analysis System general linear models procedure (4). All count data were subjected to the square root transformation.

RESULTS

Symptomatology. Symptoms were observed about 2 wk after inoculation. Lesions initially appeared as diffuse, light brown blotches, which eventually darkened to more definite, irregular grayish brown to black necrotic spots 3–10 mm in diameter (Fig. 1) containing numerous conidia. Chlorosis of leaf area adjacent to the necrotic areas was frequently observed on infected leaves followed by premature abscission.

Greenhouse studies. Of the 24 plant species in 10 plant families tested, all were nonsusceptible to the pathogen, with the exception of sicklepod (Table 1). Sicklepod plants were always included in each trial, and any trial in which sicklepod plants failed to show typical symptoms of infection was considered invalid. Symptoms similar to those originally observed in the field developed on all inoculated sicklepod plants. Uninoculated sicklepod controls and all other plant varieties, whether inoculated or control, failed to show symptoms at any time. These results indicate that *P. nigricans* has a restricted host range. However, more extensive host range studies must be conducted to ensure safety in the use of the pathogen as a biocontrol agent and its release into areas other than its present range.

Field studies. The onset of visible foliar symptoms was observed in all inoculated plots about 2 wk after respective applications of the fungus. Early application of inoculum gave consistently greater reductions than the later application in the categories of plant height, number of fruits produced, and biomass (Table 2). However, only the

Table 1. Reactions of plants tested for susceptibility to *Pseudocercospora nigricans* in greenhouse inoculation tests[a]

Family Scientific name, cultivar, common name	No. infected/ no. inoculated
Alliacae	
Allium cepa L. 'Texas Grano' (onion)	0/4
Chenopodiacae	
Spinacia oleracea L. 'Bloomsdale Long Standing' (spinach)	0/20
Compositae	
Helianthus annuus L. 'Giant Grey Stripe' (sunflower)	0/10
Cruciferae	
Brassica oleracea L. 'New Jersey Wakefield' (cabbage)	0/27
Raphanus sativus L. 'Champion' (radish)	0/32
Cucurbitacae	
Cucurbita pepo L. 'Early Summer Crookneck' (squash)	0/10
Cucumis sativus L. 'Poinsett' (cucumber)	0/18
Gramineae	
Paspalum notatum Fluegge 'Argentine Bahia' (bahiagrass)	0/26
Triticum aestivum L. 'Nebeoka' (wheat)	0/21
Zea mays L. 'Golden Bantam' (corn)	0/13
Leguminosae	
Arachis hypogea L. 'Florunner' (peanut)	0/7
Cassia obtusifolia L. (sicklepod)	34/34
C. occidentalis L. (coffee senna)	0/5
Glycine max (L.) Merr. 'Bragg' (soybean)	0/12
	0/4
Phaseolus limensis Macf. 'Fordhook' (lima bean)	0/7
P. vulgaris L. 'Blue Lake' (pole bean)	0/7
Pisum sativum L. 'Acre Cream' (pea)	0/12
Sesbania exaltata (Raf.) Cory (hemp sesbania)	0/5
Vigna unguiculata L. 'Knuckle Purple Hull' (cowpea)	0/8
Malvaceae	
Gossypium hirsutum L. 'Deltapine' (cotton)	0/3
Solanaceae	
Capsicum annuum L. 'Early Cal. Wonder' (pepper)	0/38
Lycopersicon esculentum Mill. 'Burpee's Big Boy' (tomato)	0/9
Umbelliferae	
Apium graveolens L. 'Pascal' (celery)	0/8

[a]Plants were sprayed with inoculum containing 15 g (wet weight) of mycelium in 200 ml of a 1:3 solution of culture broth:deionized water and 0.05% surfactant. Controls were sprayed with surfactant and water only. Plants were incubated in a dew chamber (100% relative humidity) at 25 C for 12 hr, then removed to a greenhouse bench. They were evaluated 3 wk after inoculation.

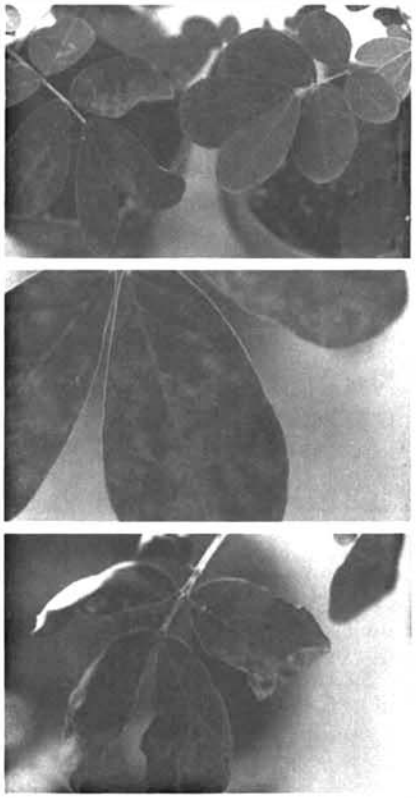

Fig. 1. Symptoms on sicklepod caused by *Pseudocercospora nigricans*. **(Top)** Comparison of (right) control and (left) inoculated leaves at onset of symptoms. Close-up of symptoms at **(middle)** onset and **(bottom)** advanced stage.

difference in canopy height between inoculated and control treatments proved significant (Table 2). Compared with control plots, early application of inoculum gave a 36% reduction in canopy height and late application gave a 15% reduction for the same parameter. Of the number of sicklepod plants remaining in each plot at the end of the experiment, the least were in the control plots even though the control plots contained the greatest biomass.

Table 2. Effects on sicklepod of early and late field applications of *Pseudocercospora nigricans*[y]

Treatment	Av. no. plants remaining per plot	Av. no. fruits produced per plot	Av. canopy height (cm)	Av. biomass per plot (g)
Control	273.0 a[z]	350.7 a	70.3 a	328.7 a
Late	347.7 a	310.3 a	60.0 b	270.7 a
Early	289.7 a	258.0 a	45.3 b	194.0 a

[y] Data are averages of three replicates. Each plot of the late and early treatments was sprayed with a suspension of 4.4 g of mycelium in 94 ml of tap water and 0.05% surfactant. Control plots were sprayed with surfactant and water only. Plots were rated 105 days after planting.

[z] Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

DISCUSSION

A foliar disease of sicklepod caused by *P. nigricans* was observed in the field in the autumn months of 1982. It was observed again in subsequent years and was most evident from early September until the onset of killing frosts in November. Even though the natural occurrence of the disease was observed in autumn, the disease could be induced artificially in the field through inoculation of the plants as early as June and July. The most notable effects of the disease were destruction of photosynthetic leaf area, localized chlorosis, and accelerated leaf abscission, which ultimately resulted in stunted plant growth.

In culture on PDAY, the fungus grew slowly at an optimum temperature of 25 C. The dense, dark gray mycelium sporulated very sparsely on PDAY and not at all in liquid culture (PDBY). To our knowledge, this is the first culturing and pathogenicity testing of this organism (2).

On the basis of field study of the parameters measured, the only significant effect of *P. nigricans* was on plant stunting. In view of the slow onset of symptoms and foliar damage, which is not lethal to the sicklepod plant as a whole, the efficacy of this pathogen in reducing sicklepod infestations seems to limit its use to employment as a classical biocontrol agent (1). This is in contrast, for instance, to the herbicidal activity of *Alternaria cassiae*, which shows more dramatic and lethal results over a very short period when properly applied as a mycoherbicidal preparation to sicklepod seedlings during very early growth stages (6). However, in areas with nonseasonal, relatively permanent stands of sicklepod, such as in tropical pasture areas of the islands of the South Pacific (2), it seems possible that *P. nigricans* would give significant reductions in sicklepod canopy cover through continuous defoliation if a high level of disease could be established.

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