Potential of *Colletotrichum malvarum* for Biological Control of Prickly Sida

T. L. KIRKPATRICK, Graduate Assistant, Department of Plant Pathology, North Carolina State University, Raleigh 27650; G. E. TEMPLETON, Professor, and D. O. TeBEEST, Assistant Professor, Department of Plant Pathology, University of Arkansas, Fayetteville 72701; and R. J. SMITH, JR., Research Agronomist, Agricultural Research Service, U.S. Department of Agriculture, Stuttgart, AR 72160

ABSTRACT

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Colletotrichum malvarum was evaluated as a bioherbicide for prickly sida control in agronomic crops. Of 38 plant species tested, only hollyhock (*Althaea rosea*) and prickly sida (*Sida spinosa*) were susceptible. In growth chamber experiments, disease symptoms on prickly sida seedlings were severe after single and multiple dew periods of 16 hr. In small field plot studies, inoculations with an aqueous suspension of 4×10^6 conidia per milliliter applied at 378 L/ha killed 84–95% of the prickly sida plants after 3 wk.

Additional key words: endemic disease, epidemiology, microbial herbicide

Although the concept of weed control with plant pathogens is not new (11), the bioherbicide or microbial herbicide tactic (6) has increased the scope and efficacy of using plant pathogens for weed control. The microbial herbicide tactic involves the application of massive levels of host-specific pathogens to target weed populations. Success has been achieved with this technique in controlling winged waterprimrose in rice (1) and northern jointvetch in rice and soybeans (6) with two indigenous special forms of *Colletotrichum gloeosporioides* (Penz.) Sacc. that normally occur at endemic levels.

Success of the bioherbicide tactic has increased interest in using other weed pathogens as bioherbicides. *C. malvarum* (A. Braun & Casp.) Southworth is a possible bioherbicide for prickly sida (*Sida spinosa* L.) (7), a persistent and troublesome weed in cotton (3) that reduces yields severely (2,4). Apparently natural epidemics of this fungus have occurred in weed populations. Buchanan and Burns (2) reported that an epidemic of *Colletotrichum* sp. occurred on prickly sida used in competition experiments with cotton; the disease was so severe that it had to be controlled with fungicide applications. The epidemic was probably caused by *C. malvarum* because it is the only species of *Colletotrichum* reported on prickly sida.

The objective of this study was to evaluate *C. malvarum* as a bioherbicide for control of prickly sida.

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MATERIALS AND METHODS

Inoculum. An isolate of *C. malvarum* from Chicot County, AR, was used for all experiments. Inoculum for all tests was conidia harvested from 7- to 10-day-old cultures grown in petri dishes at 24 C on a modified Emersons medium (8), with torula yeast substituted for yeast extract and cereal solids (MOR-REX, Corn Products Corporation, Argo, IL) substituted for soluble starch. Conidia were washed from the agar with distilled water and strained through two layers of cheesecloth. Conidial concentration was adjusted to 4×10^6/ml using a counter (Model Z-BI, Coulter Electronics, Inc., Hialeah, FL).

Host range. Ten seedlings of each of 38 plant species, representing 13 families, were inoculated by being sprayed with a conidial suspension (atomizer) until leaves and stems were thoroughly wet. Immediately after inoculation, plants were placed in a dew chamber (Model I-35D, Percival Mfg. Co., Boone, IA) at 28 C and 100% relative humidity for 16 hr and then transferred to a greenhouse bench. All plants were examined for lesions 3 wk after inoculation.

Disease development. The effects of dew temperature and dew periods on disease development were studied in a growth chamber. Dew temperatures ranged from 16 to 36 C (Table 1), and one to three dew periods were applied before and after inoculation. Seedlings were grown to a height of 10 cm in a growth chamber (Model M-31, Environmental Growth Chambers, Chagrin Falls, OH) maintained at a diurnal regime of 15 hr of daylight at 24 C and 9 hr of darkness at 15 C. Plants were exposed to dew periods and then inoculated, or they were inoculated and then subjected to dew periods. Dew periods consisted of 16 hr in all studies. After exposure to dew, plants were returned to a growth chamber for 8 hr at 28 C. In multiple dew-exposure treatments, plants were cycled between the dew and growth chambers.

Seedlings were rated for disease severity 2 wk after inoculation according to a four-point scale: 1 = no disease; 2 = slight to moderate, with one to several stem lesions and minor reduction in plant vigor; 3 = severe, with multiple lesions or lesions encircling the stem, flaccid stem, wilted leaves, and severe reduction in plant vigor; and 4 = plant dead. Ratings for 10 seedlings were averaged and each experiment was repeated. Mean values were compared by Duncan's multiple range test (5).

Field studies. A field of Rex 713 cotton, naturally infested with prickly sida at an average density of 14 plants per square meter, was used to test the efficacy of *C. malvarum* as a bioherbicide. Plots were four rows of cotton, 9.14 m long × 3.88 m wide, and treatments were arranged in a randomized complete block

Table 1. *Colletotrichum malvarum* infection of prickly sida seedlings after 16-hr dew periods at various temperatures

Dew temperature (C)	Disease severity[a]
16	1.0 a
20	2.1 b
24	3.6 d
28	2.9 c
32	1.2 a
36	1.0 a

[a] Rating at 2 wk after inoculation: 1 = no disease, 2 = slight to moderate, 3 = severe, 4 = plant dead. Each value is the mean rating of 10 seedlings. Means followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

with four replicates that were separated by a 3-m alley. Weeds were inoculated with a suspension of 4×10^6 conidia per milliliter of water applied at 378 L/ha with a backpack plot sprayer (constant air-pressure) when sida seedlings were 4 or 20 cm tall.

Treatments were one or two weekly applications while sida seedlings were emerging and growing to an average height of 4 cm, one application to 20-cm-tall seedlings, and an uninoculated control. Temperature and relative humidity were recorded with a hygrothermograph (Belfort Instrument Co., Baltimore, MD) at the top of the crop canopy for 1 wk after each inoculation. Disease severity was rated on each plant in two areas, each one a square meter in the center middle of each plot, 2 wk after inoculation; the percentage of control was rated 3 wk after inoculation. Cotton plants increased in height from 40 cm at the first inoculation to 100 cm at the time of control ratings. Mean values were compared by Duncan's multiple range test.

RESULTS

Host range. Of the 38 plant species tested, only *Althaea rosea* (L.) Cav. (hollyhock) and prickly sida, both in the Malvaceae, became infected. Disease severity was moderate (2.5) on the former and severe (3.0) on the latter. The following plants were immune: Chenopodiaceae: *Chenopodium album* L. (lambsquarter); Compositae: *Eclipta alba* L. (eclipta) and *Helianthus annuus* L. (Russian sunflower); Convolvulaceae: *Ipomoea* sp. (morningglory); Cucurbitaceae: *Citrullus vulgaris* Schrad. (Dixie watermelon), *Cucumis melo* L. (Spartan Rock muskmelon), *Cucumis sativus* L. (Pic-of-the Pickle cucumber), and *Cucurbita pepo* L. (Jack O'Lantern pumpkin); Euphorbiaceae: *Euphorbia maculata* L. (spotted spurge); Gramineae: *Avena fatua* L. (wild oat), *A. sativa* L. (Nora oat), *Digitaria sanguinalis* (L.) Scop. (large crabgrass), *Eleusine indica* (L.) Gaertn. (goosegrass), *Panicum texanum* Buckl. (Texas panicum), *Setaria faberi* Herrm. (giant foxtail), *Sorghum halepense* (L.) Pers. (johnsongrass), *Triticum aestivum* L. (Potomac wheat), and *Zea mays* L. (Golden Cross corn); Leguminosae: *Aeschynomene virginica* (L.) B.S.P. (northern jointvetch), *Cassia obtusifolia* L. (sicklepod), *Glycine max* (L.) Merr. (Lee 74 soybean), *Phaseolus limensis* Macf. (Early Thoroughgreen lima bean), *Phaseolus vulgaris* L. (White Half Runner garden bean), and *Sesbania exaltata* (Raf.) Cory (hemp sesbania); Liliaceae: *Allium cepa* L. (Italian Red onion); Malvaceae: *Abutilon theophrasti* Medico (velvetleaf), *Anoda cristata* (L.) Schlecht. (spurred anoda), *Gossypium hirsutum* L. (Rex 713 cotton), *Hibiscus esculentus* L. (Green Dwarf okra), and *H. trionum* L. (Venice mallow); Phytolaccaceae: *Phytolacca americana* L. (common pokeweed); Polygonaceae: *Polygonum pensylvanicum* L. (Pennsylvania smartweed); Sapindaceae: *Cardiospermum halicacabum* L. (balloonvine); Solanaceae: *Datura stramonium* L. (jimsonweed), *Lycopersicon esculentum* L. (Earliana tomato), and *Physalis angulata* L. (cutleaf groundcherry).

Disease development. The temperature during the dew period influenced disease severity. Disease was more severe with a 16-hr dew period at 24 or 28 C constant temperature than at higher or lower temperatures (Table 1). Seedlings that received dew at 24 C had an average disease severity rating of 3.6, compared with a rating of 2.9 when exposed at 28 C. Seedlings that received dew at temperatures of 20 or 32 C were slightly diseased, but no lesions were found on seedlings that received dew treatments at 16 or 36 C.

Exposure to two or three dew periods before inoculation had no effect on disease severity (Table 2). However, two or three exposures after inoculation increased disease severity when compared with seedlings that received no dew, received all dew periods before inoculation, or received only one dew period immediately after inoculation.

Field studies. Severe disease developed on prickly sida in field plots within 2 wk after inoculation (Table 3). Inoculations made when prickly sida plants were 4 or 20 cm tall were equally effective. Disease severity in plants inoculated twice at 1-wk intervals was not significantly different from that after one inoculation. Plant mortality ranged from 84 to 95% 3 wk after inoculation; highest mortality occurred in plots that received one inoculation when prickly sida plants were 20 cm tall.

Table 2. Influence of dew periods before or after inoculation on *Colletotrichum malvarum* infection of prickly sida seedlings

Dew period[a]		Disease
No.	Applied	severity[b]
0	...	1.5 a
1	Before	1.7 ab
2	inoculation	1.4 a
3		1.9 ab
1	After	2.0 b
2	inoculation	3.7 c
3		3.8 c

[a] Each dew period consisted of 16 hr at 100% relative humidity and 28 C, with 8 hr at 28 C in a growth chamber between successive dew periods.
[b] Rating at 2 wk after inoculation: 1 = no disease, 2 = slight to moderate, 3 = severe, 4 = plant dead. Each value is the mean rating of 10 seedlings. Means followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Table 3. Disease severity and mortality in prickly sida plants in cotton plots inoculated with *Colletotrichum malvarum*

	Prickly sida[b]		
Inoculations[a] (no.)	Plant height[c] (cm)	Disease severity[d]	Plants killed[e] (%)
0		1.0 a	0 a
1	4	3.3 b	84 b
2	4	3.5 b	91 b
1	20	3.8 b	95 b

[a] Inoculated over the top with a water suspension of 4×10^6 conidia per milliliter applied at 378 L/ha.
[b] Means within a column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[c] Average height at the time of inoculation.
[d] Average rating at 2 wk after inoculation: 1 = no disease, 2 = slight to moderate, 3 = severe, 4 = plant dead.
[e] At 3 wk after inoculation.

DISCUSSION

C. malvarum is considered a "substrate form" of *C. gloeosporioides* by von Arx (10), who retained its classical name on the basis of distinctive morphology; distinctive growth in pure culture; ascigerous state unknown but presumed to be *Glomerella;* and host range limited to a certain species, genus, or family. The isolate of *C. malvarum* used in this study was less host-specific than the *C. gloeosporioides* strains previously demonstrated to be effective bioherbicides (1,6). Pathogenicity of *C. malvarum* to hollyhock, an ornamental grown as an annual or perennial, may cause some conflict of interest between gardeners and cotton or soybean growers (9). Regional studies on the epidemiology of the disease caused by *C. malvarum* are needed to assess properly its potential as a bioherbicide.

Disease was most severe when plants were exposed to a 16-hr dew period at 24 C. However, disease severity was comparable on plants exposed to multiple dew periods after inoculation at 28 C. The influence of repeated dew cycles on disease development may be important in controlling prickly sida in the field. The probability of dew formation in the field each night is high. Formation of dew at regular intervals may permit infection at temperatures that are above or below the optimum for disease development.

The level of control of prickly sida in the field with *C. malvarum* was encouraging. Environmental conditions were favorable for epiphytotic development during these tests. Temperature and relative humidity were 30 C and 86% at the time of inoculation of 4-cm-tall plants and 26 C and 94% at the time of inoculation of 20-cm-tall plants. Temperatures ranged from 20 to 36 C for 1 wk following inoculations, and relative humidities ranged from 50 to 95%.

Practical use of *C. malvarum* as a bioherbicide for weed management in

cotton or soybeans appears feasible; however, additional research and development are needed before it can be used commercially.

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