Preliminary Assessment of *Colletotrichum capsici* as a Potential Mycoherbicide for Control of Pitted Morningglory

D. K. CARTWRIGHT, Former Graduate Assistant, and G. E. TEMPLETON, Distinguished Professor, Department of Plant Pathology, University of Arkansas, Fayetteville 72701

ABSTRACT

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An isolate of the indigenous fungus *Colletotrichum capsici*, pathogenic on pitted morningglory (*Ipomoea lacunosa*), was isolated in the summer of 1989 and evaluated in laboratory and growth chamber tests as a potential mycoherbicide. The fungus grew well at 20, 25, and 30 C on potato-dextrose agar containing streptomycin. Conidia germinated at 15, 20, 25, 30, and 35 C, with optimal germination at 25 and 30 C. Disease symptoms developed in 2–3 days on pitted morningglory seedlings at the cotyledonary stage when seedlings were inoculated with 1×10^6 conidia per milliliter and incubated in a dew chamber for 24 hr at 30 C. All seedlings were killed within 5–7 days after inoculation. Two sequential 12-hr dew periods resulted in 86% mortality. The only other crop or weed species tested that was susceptible was sharppod morningglory (*I. trichocarpa* var. *trichocarpa*), which was as susceptible as pitted morningglory to the pathogen. Results from controlled environmental studies support field testing and further development of this isolate of *C. capsici* as a potential commercial mycoherbicide.

Pitted morningglory (*Ipomoea lacunosa* L.) is a serious and widespread weed in soybean (*Glycine max* (L.) Merr.), cotton (*Gossypium hirsutum* L.), and peanut (*Arachis hypogaea* L.) fields throughout the southeastern United States (1,6,10). It causes yield loss, increased lodging, seed quality degradation, and difficulty with harvest (6). Control is difficult because of the weed's tolerance to many commonly used soil and foliar-applied herbicides, prolific growth habit, and season-long emergence (5,8,9). Alternative controls are needed to replace or supplement existing methods. The practicality of mycoherbicides for specific weed control has been established, and the method warrants consideration for control of this serious weed (3,4,11,12).

In the summer of 1989, an anthracnose disease of pitted morningglory caused by *Colletotrichum capsici* (Syd.) E.J. Butler & Bisby was discovered in Benton County, Arkansas. Pitted morningglory seedlings sprayed with conidial suspensions of the fungus developed severe symptoms under controlled conditions. The potential of this fungus as a mycoherbicide for pitted morningglory was evaluated in laboratory and growth chamber studies. Research was conducted on the effect of temperature on germination and growth of the fungus in vitro; the effect of dew period, dew temperature, and inoculum concentration on disease development; and host range of the pathogen.

Present address of first author: Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

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MATERIALS AND METHODS

Isolation from tissue. The fungus was isolated from diseased pitted morningglory seedlings by rinsing sections of diseased tissue in 0.5% NaOCl for 1 min and placing sections on potato-dextrose agar (2% agar) supplemented with either 300 mg/ml of streptomycin sulfate or 12.5 mg/ml of chlortetracycline (PDA+). Pure cultures were maintained on PDA+.

Stock cultures. Several isolates were screened for pathogenicity on pitted morningglory seedlings, and the most virulent (isolate 4C) was used in further tests. Conidia were collected from 8- to 11-day-old colonies grown on PDA+, placed in cryopreservative (1:1 solution of 40% glycerol and 10% skim milk) contained in 1.5-ml plastic cryovials, and stored at −80 C. Aliquots from frozen stock cultures were utilized for all inoculum increases.

Effect of temperature on conidia germination and radial growth rate. Germination of conidia of *C. capsici* was measured by spreading 0.1 ml of a suspension (1×10^6 conidia per milliliter) onto PDA+ plates, wrapping the plates in foil, and incubating the plates at 15, 20, 25, 30, or 35 C. Germinated conidia (100 per plate) were counted after 8 hr with a compound light microscope at 10×; three replicate plates per temperature were utilized.

For radial growth, a 5-mm plug taken from the margin of an actively growing colony of *C. capsici* was placed mycelia side down in the center of a PDA+ plate, and the plate was wrapped in foil. Three replicate plates were incubated at 15, 20,

25, 30, or 35 C. Colony diameter was measured after 4, 7, and 10 days. Both experiments were repeated once.

Inoculum production. Conidia from the frozen stock cultures were streaked onto PDA+ plates and incubated on a laboratory bench under two 20W cool-white fluorescent lights (12-hr photoperiod) suspended 27 cm above the cultures at room temperature. After 8–11 days, conidia were gently scraped from the colony surface with a microscope coverslip while being rinsed with distilled water. The suspension was filtered through cheesecloth, quantified with a hemacytometer, and adjusted to the desired conidial concentration.

Plant production. Seeds from mature pitted morningglory plants were collected from naturally occurring field populations in the fall of 1989 at the Main Agricultural Experiment Station, University of Arkansas, Fayetteville, and stored at 12 C. Seedlings were obtained by planting seeds in commercial potting soil and vermiculite (3:1, v/v) in 7.6-cm-diameter plastic pots. All host range test plants except sweetpotato (*I. batatas* (L.) Lam.) were grown from seed; sweetpotato cuttings were taken from mature plants and allowed to root in water before being potted. Plants were grown in a greenhouse under natural light until inoculation. Plants were watered daily and fertilized weekly (Peter's Fertilizer Products, Fogelsville, PA). Pitted morningglory seedlings were thinned to four plants per pot immediately before inoculation.

Effect of dew period and dew temperature. An aerosol atomizer was used to spray 3- to 5-day-old seedlings of pitted morningglory until runoff with $5–7 \times 10^6$ conidia per milliliter. Control plants were sprayed with distilled water. Seedlings were placed either in a controlled environmental growth chamber (Conviron, Model E-7, Pembina, ND) maintained at 28/24 C (day/night) with a daily 14-hr photoperiod or in a dew chamber (Percival Manufacturing Co., Model I-35D, Boone, IA) in the dark at 100% RH for 6, 12, 18, or 24 hr at temperatures of 15, 20, 25, 30, or 35 C before transfer to a growth chamber. Noninoculated controls were maintained in the dew chamber for 24 hr at each temperature level. Seedlings in the growth chamber were monitored daily for disease development and mortality. Experiments were conducted at least twice, with three replications per treatment.

After 10 days, plants were removed from pots, washed gently to remove soil from the roots, and air-dried for 1 hr. Plants were separated by pot (replication), cut up, and weighed in aluminum weighing pans. After treated and control plants were dried for 24 hr at 85–90 C in a mechanical convection oven (Precision Scientific Group, Model 26, Chicago, IL), the dry weights were recorded as a percentage of the highest dry weight value within each experiment.

Effect of sequential dew periods on disease. Pitted morningglory seedlings were inoculated and incubated in the dark for 12 hr in a 30 C dew chamber, placed in a growth chamber for 12 hr of light, and then subjected to another 12 hr of dew at 30 C. Inoculated seedlings held for single 12- or 24-hr dew periods were included as controls. Plants were transferred to a growth chamber, and mortality was recorded after 10 days. Experiments were conducted twice, with three replications per treatment.

Effect of inoculum concentration on disease. Pitted morningglory seedlings were sprayed with conidial suspensions of 5.5×10^3 to 5.5×10^7 conidia per milliliter and held in a dew chamber for 24 hr at 30 C. Control plants were sprayed with distilled water. Plants were removed to a growth chamber, and mortality was recorded after 10 days. Experiments were conducted twice, with three replications per treatment.

Host range. Crop plants inoculated with *C. capsici* were lettuce (*Lactuca sativa* L.) cvs. Black-Seeded Simpson and Butterhead; garden beet (*Beta vulgaris* L.) cvs. Ruby Queen and Detroit Dark Red; sweetpotato cvs. Centennial, Georgia Jet, Redmar, and Travis; cultivated morningglory (*I. tricolor* Cav.) cvs. Scarlet O'Hara and Heavenly Blue; soybean cv. Forrest; cotton cv. Stoneville 506; rice (*Oryza sativa* L.) cvs. Labelle and Skybonnet; wheat (*Triticum aestivum* L.) cv. Ben Hur; sweet corn (*Zea mays* L.) cvs. Honey Butter and Jubilee; and tomato (*Lycopersicon esculentum* Mill.) cvs. Better Boy and Tomatillo.

Weeds inoculated were palmer amaranth (*Amaranthus palmeri* S. Wats.), Queen Anne's lace (*Daucus carota* L.), purple morningglory (*I. hederacea* (L.) Jacq.), entireleaf morningglory (*I. hederacea* var. *integriuscula* Gray), tall morningglory (*I. purpurea* (L.) Roth), bigroot morningglory (*I. pandurata* (L.) G. Meyer), palmleaf morningglory (*I.*

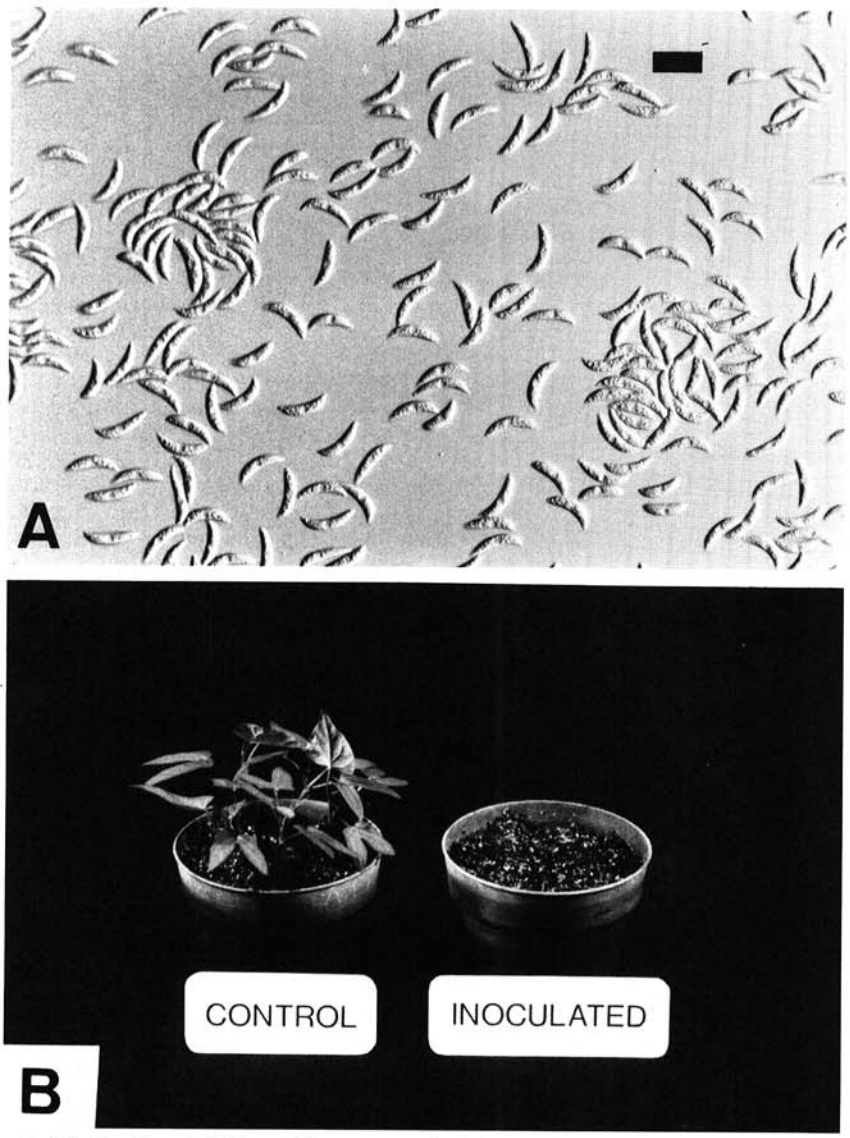

Fig. 1. (A) Conidia of *Colletotrichum capsici*. Scale bar = 20 μm. **(B)** Pitted morningglory seedlings (left) noninoculated and (right) 7 days after inoculation with *C. capsici*.

wrightii Gray), red morningglory (*I. coccinea* L.), cypressvine morningglory (*I. quamoclit* L.), smallflower morningglory (*Jacquemontia tamnifolia* (L.) Griseb.), field bindweed (*Convolvulus arvensis* L.), dodder (*Cuscuta* spp.), lawnleaf (*Dichondra repens* J.R. Forster & G. Forster), northern jointvetch (*Aeschynomene virginica* (L.) B.S.P.), prickly sida (*Sida spinosa* L.), and jimsonweed (*Datura stramonium* L.).

Seedlings ranging from the cotyledonary to the fourth true-leaf stage were sprayed with suspensions of 5×10^6 conidia per milliliter and incubated in a dew chamber for 24 hr at 30 C. One noninoculated pot of each species was included as a control. Pitted morningglory seedlings were included in all experiments as susceptible controls. Plants were considered either resistant (no visible reaction) or susceptible (necrotic areas, flecking, stunting) by visual observation after 10 days of incubation in the growth chamber at 30 C.

Statistical procedures. All treatments were randomized, with a minimum of three pots (replications) per treatment. Experiments were conducted at least twice. Three pots of control plants were included in all tests except in the host-range tests. Data were subjected to ANOVA or GLM analysis and presented as the means of replicated experiments. Duncan's multiple range test was used to separate means at the $P = 0.05$ level, and regression analysis was used to determine growth rates of the fungus at different temperatures.

RESULTS

From microscopic examination of diseased tissue and cultural characteristics, the fungus was identified as *C. capsici*. Identification was confirmed by B. C. Sutton at the International Mycological Institute in Kew, England. In nature, the fungus produces small, irregular necrotic spots with distinct margins on cotyledons and mature leaves of pitted morningglory seedlings and occasional stem lesions. The fungus was readily isolated from diseased tissue and sporulated abundantly on PDA+. In culture, the fungus formed dark, smooth, flat colonies with regular margins and produced pink to orange conidial masses with numerous setae. Conidia produced on PDA+ were hyaline, falcate, and 16.8–26.4 (21.2) μm long × 4.0–5.6 (4.7) μm wide (Fig. 1A).

Conidia germinated well on PDA+ at 25 (82.5%) and 30 C (84%) (Table 1). Growth rate was fastest at 30, 25, and 20 C (6.8, 6.7, and 5.8 mm per day, respectively) and limited at 15 (1.1 mm per day) and 35 C (0.4 mm per day).

The fungus infected pitted morningglory seedlings at all dew temperatures tested when incubated for 24 hr, but optimum dew temperatures for disease progress, mortality, and dry weight reduction were 25 and 30 C. First symptoms were slight cupping or twisting of cotyledons 48–60 hr after inoculation. These symptoms were followed by necrosis of the entire cotyledon, which progressed rapidly down the petioles and girdled the stem within 3–4 days. Plant death occurred 5–7 days after inoculation (Fig. 1B). At dew temperatures of 30, 25, and 35 C, mortality was 92, 89, and 12.5%, respectively, after 24 hr of dew and 72, 33, and 4%, respectively, after 18 hr of dew. A dew period of 12 hr or less resulted in no significant mortality at any temperature, and no mortality occurred at 15 or 20 C, regardless of dew period (Fig. 2). Greatest reduction in dry weight occurred at 25 and 30 C after 24 hr of dew, at which time weights were 15.1 and 16.4%, respectively, of the maximum dry weight value. Results were similar with an 18-hr dew period, with reductions to 35.4 and 19.6% at 25 and 30 C, respectively. Dry weight was not reduced at any temperature when seedlings were given a dew period of 12 hr or less.

Two sequential 12-hr dew periods resulted in 85.7% mortality at 30 C, compared with 4.2 and 100% mortality of seedlings held for a single 12-hr and a single 24-hr dew period, respectively (Table 2).

Complete kill was achieved with conidial concentrations of 5.5×10^5 to 5.5×10^7 conidia per milliliter when seedlings were given a 24-hr dew period at 30 C (Table 3).

The fungus had no effect on the other plants tested except sharppod morningglory, which was as susceptible as pitted morningglory. Severe disease developed

Table 1. Germination of *Colletotrichum capsici* conidia after 8 hr of incubation at different temperatures

Temperature (C)	Percent germination
15	13.4 a[z]
20	60.2 b
25	82.5 c
30	84.0 c
35	55.8 b

[z] Mean values of two experiments, with three replications per treatment. Means followed by different letters are significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 2. Effect of sequential dew periods on mortality of pitted morningglory seedlings inoculated with *Colletotrichum capsici*

Dew period (hr)	Percent mortality
12	4.2 a[z]
12 + 12	85.7 b
24	100.0 c

[z] Mean values of two experiments, with three replications per treatment. Means followed by different letters are significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 3. Effect of inoculum concentration on mortality of pitted morningglory seedlings held for 24 hr of dew at 30 C and incubated in a growth chamber for 10 days

Conidia/ml	Percent mortality
5.5×10^3	4.2 a[z]
5.5×10^4	54.2 b
5.5×10^5	100.0 c
5.5×10^6	100.0 c
5.5×10^7	100.0 c

[z] Mean values of two experiments, with three replications per treatment. Means followed by different letters are significantly different at $P = 0.05$ according to Duncan's multiple range test.

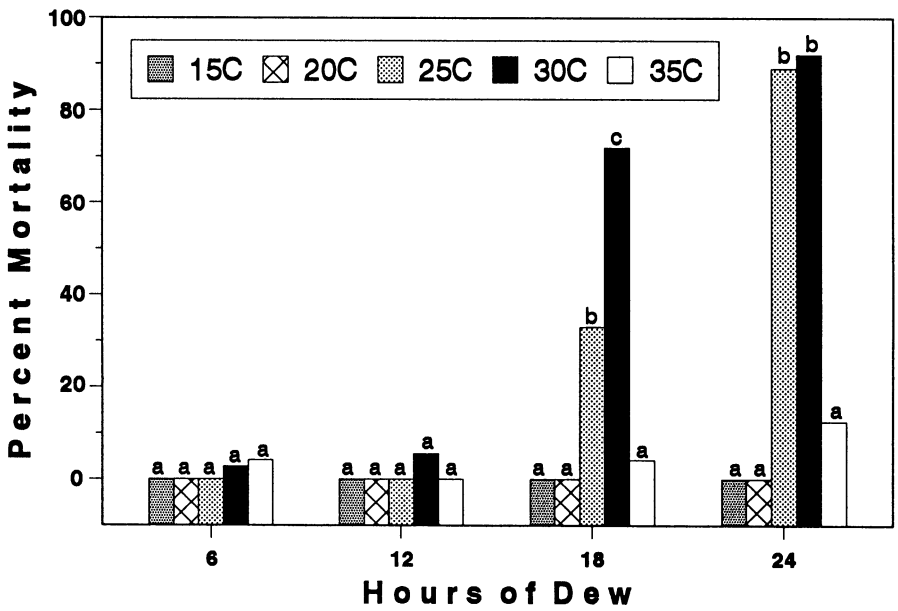

Fig. 2. Mortality of pitted morningglory seedlings after 10 days of incubation at different temperatures and periods of dew.

on sharppod morningglory within 2–3 days, and seedlings were killed within 4–6 days.

DISCUSSION

This isolate of *C. capsici* produces abundant conidia in culture, is highly specific and virulent, and is pathogenic over a reasonably wide temperature range—characteristics that overcome several important barriers to mycoherbicide development (11). Thus, this fungus appears to have potential to control pitted morningglory in the seedling stage.

Consistent infection was achieved over a wide range of temperatures and inoculum concentrations. Under the proper environmental conditions, the fungus was virulent enough to kill pitted morningglory seedlings. However, mortality occurred only at 25, 30, and 35 C, indicating a requirement for warm temperatures to achieve adequate control. This correlated with its behavior in nature, since the disease was found most abundantly in mid-July and flourished in the field in late summer. The optimum temperature for germination, growth, and disease progress (30 C) was similar to that of the Collego pathogen, *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *aeschynomene* (4). This temperature range would be typical of much of the Mississippi Delta and southeastern United States, where pitted morningglory is a serious problem (6).

The fungus proved to be relatively host-specific, but its inability to control other weedy morningglory species is a disadvantage. Different morningglory species may be present in a single field during the growing season, and most chemical herbicides control more than one species (2). Genetic manipulation might be used to broaden the virulence of this isolate on other morningglory species (13). Also, the possibility exists that naturally occurring isolates with equivalent potential can be found on other morningglory species and combined for control of multiple species. The fungus was pathogenic on sharppod morningglory, and evaluation of it as a mycoherbicide for this weed is warranted.

The principal constraint to development of this fungus is its requirement of long periods of free moisture for maximum infection. At least 18 hr of continuous dew was required for sufficient control at optimum temperatures. Dew periods of this length would be unusual in many field crops. The use of the fungus in rice fields or other irrigated crops could overcome this barrier. Timing of applications to ensure maximum periods of moisture could also overcome this constraint. This has proved to be an important strategy for improving efficacy in the field for such pathogens as *C. g.* f. sp. *malvae* to control round-leaved mallow (7). Recent improvements in spore formulations or carriers such as invert emulsions (12), as well as the use of surfactants, might also reduce the dew period requirement. Two 12-hr sequential dew periods were comparable to 24 hr of constant dew in terms of seedling control; thus, the use of consecutive dew periods of shorter lengths should be evaluated.

The fungus grew well and sporulated abundantly on solid media, but sporulation in submerged culture should be evaluated before field trials are initiated. On the basis of experience with similar fungi, conidia production in submerged culture should be possible. However, other *Colletotrichum* species, such as *C. malvarum* (A. Braun & Casp.) Southworth, are restricted in ability to sporulate in submerged culture (D. O. TeBeest, *personal communication*). Storage viability of inoculum should also be investigated before field trials and further development.

The economic importance of pitted morningglory and other morningglory species justifies further research with *C. capsici* as a potential mycoherbicide.

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