

## ***Colletotrichum coccodes*, a Potential Bioherbicide for Control of Velvetleaf (*Abutilon theophrasti*)**

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### **ABSTRACT**

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A velvetleaf (*Abutilon theophrasti*) isolate of *Colletotrichum coccodes* was evaluated as a potential bioherbicide for velvetleaf. On velvetleaf, disease caused by this isolate developed over a wide range of dew period durations and temperatures, but the most rapid and destructive disease development occurred following a 24-hr dew period at 24 C. With cooler temperatures and/or shorter dew durations, the pathogen caused premature defoliation of inoculated leaves. An inoculum density of  $10^7$  spores/ml applied to runoff or an application rate of  $2.3 \times 10^8$  spores/m<sup>2</sup> was necessary to produce the most destructive levels of disease. Velvetleaf plants at all growth stages were susceptible and were reduced in vigor following inoculation. Plants inoculated at the cotyledon stage were killed.

Additional keywords: biological weed control

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A pathogen identified as *Colletotrichum coccodes* (Wallr.) Hughes was isolated from foliar lesions on velvetleaf

(*Abutilon theophrasti* Medik.) collected near Burlington, VT in 1981 (4). This isolate has potential as a bioherbicide for velvetleaf (3,8,9), and it meets many of the important criteria for successful biocontrol agents (11,13). Although this species is known to have a wide host

range (7), our laboratory and field studies (*unpublished*) have shown that the host range of the velvetleaf isolate of *C. coccodes* is restricted and this fungus does not damage other plants.

The objective of this study was to determine the optimum conditions for infection of velvetleaf by this fungus. The potential of the velvetleaf isolate of *C. coccodes* as a bioherbicide was evaluated under growth chamber, greenhouse, and field conditions.

### **MATERIALS AND METHODS**

**Stock culture of *C. coccodes* and inoculum production.** A stock culture of *C. coccodes* (DAOM 183088, deposited in the Biosystematics Research Institute, Ottawa) was established and maintained on several potato-dextrose agar (PDA) slants in small glass vials under oil at 3 C. Mycelium from the stock culture was periodically transferred to PDA plates. Plates were incubated for 7–21 days at

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24 C and agar plugs were removed and transferred to liquid media.

Inoculum was produced in modified beef peptone (MBP) medium (10 g of dextrose, 4 g of beef extract, 11 g of bacto-peptone, 1,000 ml of H<sub>2</sub>O) or V-8 medium (14). One hundred-ml aliquots were dispensed to 250-ml Erlenmeyer flasks, autoclaved, and cooled before seeding with agar plugs. Cultures were grown for 7 days on a rotary shaker (250 rpm), harvested by filtering through cheesecloth, and centrifuging (6,500 g) the resulting spore suspension. The spore pellet was resuspended in distilled water and diluted to the desired concentration in distilled water or a gelatin solution (0.75% w/v gelatin powder in water).

**Inoculation of velvetleaf seedlings.** Velvetleaf seed dormancy was broken by placing seeds on moist filter paper for 48 hr at 5 C and then incubating for 24 hr at 30 C. Germinated seeds were sown, four per pot, in potting medium (Pro-Mix BX, Premier Brands, Inc., New York, NY) in 10-cm plastic pots. Seedlings were grown in growth chambers (14-hr photoperiod, 300  $\mu$ Em<sup>-2</sup>s<sup>-1</sup>, 24/18 C day/night temperature) and thinned to one or three per pot before use. For some experiments, plants were inoculated by spraying the spore suspension to runoff with a hand-held atomizer. For other experiments, potted plants in trays were moved beneath two tandem DeVilbiss atomizers (Model 163, DeVilbiss, Inc., Somerset, PA) with nozzles 10 cm apart and 20 cm above the plant canopy. Atomizers were powered with compressed air at 83 kPa, and the system was calibrated to apply 115 L of H<sub>2</sub>O/ha and 2.3  $\times$  10<sup>8</sup> spores/m<sup>2</sup> (concentration of spore suspension = 2  $\times$  10<sup>7</sup> spores/ml). Controls were sprayed with distilled water or a sterile gelatin solution, as appropriate. The age of plants, fungus culture medium (MBP or V-8), inoculation method (sprayed to runoff or sprayed with a known rate), and spore suspension diluent (water or gelatin) are indicated for each experiment. Except as indicated, inoculated seedlings were given an 18-hr dew period in a dark dew chamber (Percival Model E-54UDL, Boone, IA) at 24 C. Leaf disease severity was rated in three ways: the Horsfall-Barratt scale (6), an arbitrary rating scale (0 = no disease symptoms, 1 = light infection, 2 = moderate infection, 3 = severe infection, 4 = complete desiccation and abscission of the leaf), or by comparing the necrotic area of the leaves to known percentage standards. These standards consisted of a series of leaf-shaped grids representing various proportions of a leaf covered with necrotic tissue (8).

**Effect of dew period temperature and duration on disease.** Velvetleaf seedlings at the 2- to 3-leaf stage (14–21 days after planting) were inoculated by spraying to runoff with 10<sup>7</sup> spores/ml in gelatin

solution. Inoculum was produced in MBP medium. Dew periods were provided by placing the plants in dark growth chambers for 24 hr at 18, 24, or 30 C and covering the plants with plastic bags for 12, 16, 20, or 24 hr. All plants then were returned to the initial growing conditions for 2 wk. There were four pots per treatment with one plant per pot. The percentage of total inoculated leaf area that became necrotic for each plant was determined once daily by comparison with percentage standards. Abscission of diseased leaves also was recorded for each plant.

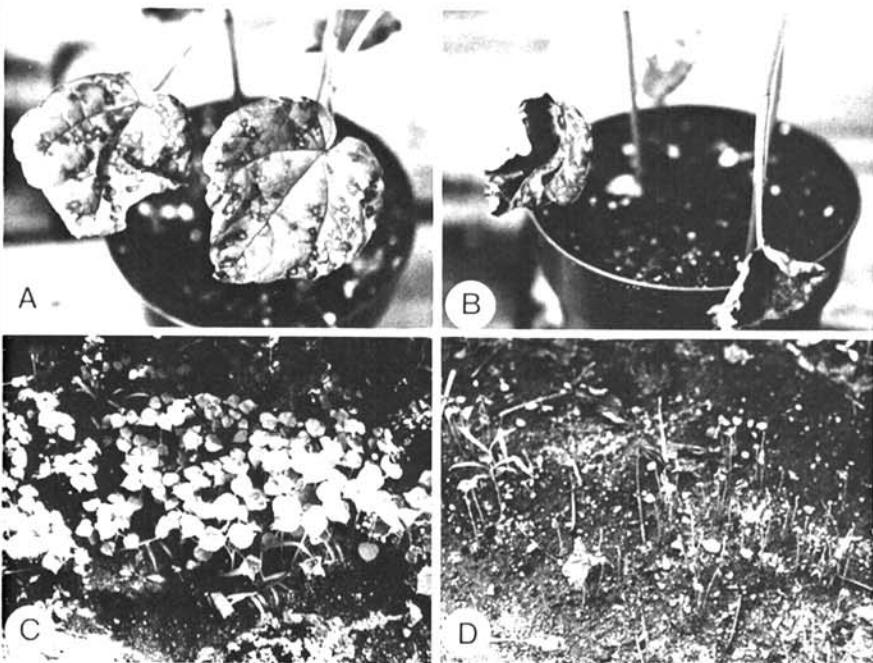
In a second experiment, velvetleaf seedlings at the 3-leaf stage (18 days after planting) were inoculated with 2.3  $\times$  10<sup>8</sup> spores/m<sup>2</sup> in 115 L of H<sub>2</sub>O/ha. Inoculum produced in V-8 medium. Plants were placed in the dew chamber for 0, 3, 6, 9, 12, 15, 18, 21, or 24 hr at 24 C. Plants were then incubated in the growth chamber and rated for disease development after 7 and 10 days using the Horsfall-Barratt scale. Inoculated leaves that had been shed were rated "11" since corresponding leaves on uninoculated plants were rarely shed at the time of rating. There were four pots per treatment with three plants per pot. Mean ratings for the first two leaves on each of the three plants (total of six leaves) in each pot were determined and the data were analyzed before converting to percentages.

**Effect of incubation temperature regime on disease development.** Velvetleaf seedlings at the 2- to 3-leaf stage

(14–21 days after planting) were inoculated by spraying to runoff with 10<sup>7</sup> spores/ml with gelatin. Inoculum was produced in MBP medium. Plants were covered with plastic bags to provide a dew period and were placed in a dark growth chamber for 24 hr at 25 C. Plastic bags then were removed and plants were incubated for 2 wk in growth chambers with the following day/night temperature regimes: 15/10 C, 25/20 C, and 35/30 C. There were five pots per treatment with one plant per pot. The mean number of days required for the second leaf from the apex of each plant (tagged at the time of inoculation) to reach severity ratings of 3 and 4 were determined for each treatment.

**Effect of inoculum density on disease development.** Velvetleaf plants at the 1- to 2-leaf stage (8–14 days after planting) were inoculated by spraying to runoff with four different spore suspensions (10<sup>4</sup>, 10<sup>5</sup>, 10<sup>6</sup>, and 10<sup>7</sup> spores/ml) with gelatin. Inoculum was produced in MBP medium. There were four pots per treatment with one plant per pot. Following inoculation, plants were covered with plastic bags and placed in a dark growth chamber for 24 hr at 25 C. Plastic bags then were removed and plants were returned to initial growing conditions. The percentage of total inoculated leaf area covered with necrosis for each plant was determined by comparison with percentage standards at 4 and 6 days after inoculation.

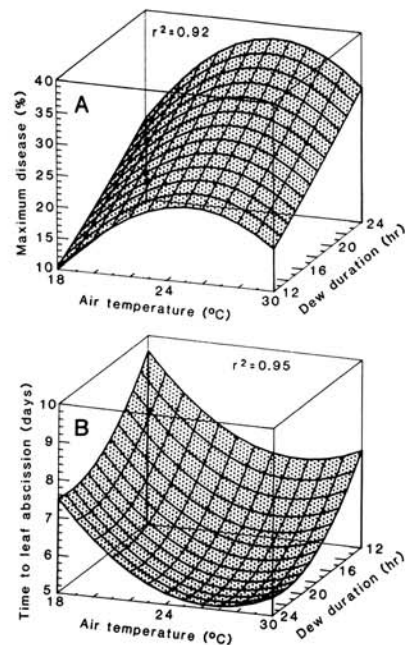
**Effect of a supplementary dew period on conidia production by *C. coccodes*.** Velvetleaf seedlings at the 2- to 3-leaf



**Fig. 1.** Leaf blight symptoms on velvetleaf inoculated with the velvetleaf isolate of *Colletotrichum coccodes*. (A) Lesions on leaves 7 days after inoculation. (B) Advanced symptoms showing necrosis and desiccation of leaves prior to abscission 10 days after inoculation. (C) Field trial control plot. (D) Field trial inoculated plot (10<sup>9</sup> spores/m<sup>2</sup> in 300 L/ha of 0.1% gelatin in water). Photos of field plots were taken 3 wk after inoculation.

stage (15 days after planting) were inoculated with  $2.3 \times 10^8$  spores/m<sup>2</sup> in 115 L of H<sub>2</sub>O/ha. Inoculum was produced in V-8 medium. Plants were provided with an 18-hr dew period in the dew chamber at 24 C and then incubated for 6 days in the growth chamber. Plants then were returned to the dew chamber and heavily infected leaves were removed periodically for 42 hr, rinsed in 15-ml distilled H<sub>2</sub>O, and the numbers of conidia present in the wash water were determined with the aid of a hemacytometer. Only leaves of similar size and with similar amounts of necrotic tissue were selected. At least four leaves were taken at each sample time.

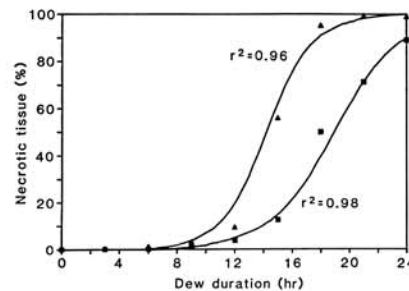
**Effect of velvetleaf growth stage and incubation in a mist frame on weed control.** Velvetleaf seedlings at the cotyledon, 1-leaf, 2-leaf, and 3-leaf stages (3, 8, 12, and 18 days after planting, respectively) were inoculated with  $2.3 \times 10^8$  spores/m<sup>2</sup> in 115 L H<sub>2</sub>O/ha. Inoculum was produced in V-8 medium.



**Fig. 2.** Effect of dew period duration and air temperature on (A) maximum disease levels caused by *Colletotrichum coccodes* on velvetleaf, and (B) number of days from inoculation to leaf abscission (dew period duration axis reversed to allow optimum viewing of the surface). Pooled results of two experiments. Plants were inoculated at the 2- to 3-leaf stage by spraying with a spore suspension of *C. coccodes* at  $10^7$  spores/ml. Dew periods were provided by placing the plants in growth chambers and covering the plants with plastic bags. Arc sine transformed regression equations: maximum disease level =  $-92.12 + (8.71 \cdot t) - (0.17 \cdot t^2) + (0.83 \cdot d)$ , and days to leaf abscission =  $38.44 - (1.77 \cdot t) + (0.03 \cdot t^2) - (0.91 \cdot d) + (0.02 \cdot d^2)$  where  $t$  = dew period air temperature in °C and  $d$  = dew period duration in hr. For graphing purposes, data were converted back to percentages following statistical analysis.

Plants were placed in the dew chamber for 18 hr and then incubated either in the growth chamber or in a greenhouse mist frame where moisture was maintained on the leaves for a 12-hr period each night (1900–0700 hr). The minimum greenhouse temperature was 16 C and lighting was supplemented with 400 W high pressure sodium lamps (14-hr photoperiod, 0600–1800 hr, approximately  $50 \mu\text{Em}^{-2}\text{s}^{-1}$  supplemental light). Plants were rated for weed control and harvested after 2 wk. There were four pots per treatment with three plants per pot. Height was measured from the cotyledonary node to the growing point and dead plants were given a height of 0 mm. Height data were recorded as mean height for each of the four pots. Mortality data were recorded as percentages for each of the four pots. Biomass of aboveground living tissue was determined by cutting the live plants at the cotyledonary node, drying in paper bags for 7 days at 60 C, and weighing. Biomass data were recorded as total yield for each of the four pots.

**Field trials.** Germinated velvetleaf seeds were planted (200 seeds/m<sup>2</sup>) in 1.0-m<sup>2</sup> plots at three different dates to provide plants at different growth stages (2- to 3-leaf, 6- to 8-leaf, and 12-leaf) at the time of application. Plots were spray-inoculated with *C. coccodes* (produced in V-8 medium) at three rates (0,  $10^7$ , and  $10^9$  spores/m<sup>2</sup>) and in three spray volumes (10, 100, and 200 L/ha of 0.1% gelatin in water). There were four replicate plots per treatment. Disease was rated (0–4 scale) 21 days after inoculation and velvetleaf plants were harvested to



**Fig. 3.** Effect of dew period duration on disease development caused by *Colletotrichum coccodes* on velvetleaf. Pooled results of two experiments. Plants were inoculated at the 3-leaf stage ( $2.3 \times 10^8$  spores/m<sup>2</sup>), then given a dew period in a dew chamber at 24 C. Disease was rated on the second and third inoculated leaves using the Horsfall-Barratt scale and data were analyzed before converting to percentages. ■ = Disease ratings taken at 7 days after inoculation. ▲ = disease ratings taken at 10 days after inoculation. Regression equations: disease (scale rating) at 7 days =  $0.26 - (0.32 \cdot t) + (0.05 \cdot t^2) - (0.0008 \cdot t^3)$ , and disease (scale rating) at 10 days =  $0.59 - (0.62 \cdot t) + (0.10 \cdot t^2) - (0.002 \cdot t^3)$  where  $t$  = dew period duration in hr. For graphing purposes, data were converted to percentages following statistical analysis.

determine aboveground biomass on a per plant basis. Mortality was determined at this time by counting all velvetleaf plants in each plot, dividing the number of dead plants by the total, and multiplying by 100.

**Data analysis.** Data were analyzed with an analysis of variance (ANOVA) and mean separation test suitable to the experimental design. All growth chamber and greenhouse experiments were repeated at least once. Results were pooled when the analysis indicated no significant difference due to experiment. Where appropriate, treatment sums of squares were broken into single degree of freedom sums of squares, and polynomial regression equations were generated using treatment means and only those terms that were significant at the 5% level of probability. Percentage data were arc sine transformed and field trial biomass data were  $\log_{10}$  transformed before analysis.

## RESULTS

**Symptomatology.** On velvetleaf, this isolate of *C. coccodes* caused gray-brown leaf lesions and black, sunken, elongate stem lesions. Leaf lesions were observed within 5 days of inoculation and appeared as small water-soaked spots that enlarged and became necrotic (Fig. 1A,B). Areas surrounding lesions became desiccated, and diseased leaves were shed prematurely within 5–14 days after inoculation. Stem lesions were infrequent and failed to enlarge sufficiently to girdle the stem.

**Effect of dew period temperature and duration on disease development.** The most severe damage to velvetleaf caused by this isolate of *C. coccodes* occurred following dew periods of 18–24 hr at an air temperature of 24 or 30 C (Figs. 2A,B and 3). These results agree with the optimum temperatures for mycelial growth and spore germination by the velvetleaf isolate of *C. coccodes* of 27 and 24 C, respectively (*unpublished*). The factorial ANOVA indicated significant effects on disease level due to air temperature and dew period duration, but no significant interaction.

In experiments in which dew periods were maintained with plastic bags, maximum levels of necrosis reached only 35–40% (Fig. 2A), but this was sufficient to cause abscission of inoculated leaves within 5 days of inoculation (Fig. 2B). Cooler or warmer air temperatures and shorter dew periods resulted in less disease (Fig. 2A) and longer times for inoculated and diseased leaves to be shed from the plants (up to 10 days following inoculation) (Fig. 2B). Similar leaves on uninoculated control plants remained green and healthy and had not been shed 2 wk after treatment applications.

In experiments in which dew periods were provided in the dew chamber, low levels of disease occurred with dew

periods of up to 12 hr (Fig. 3). For dew periods from 15–24 hr rated 7 days after inoculation, there was an increase in the amount of necrotic tissue as length of dew period increased. This confirmed the results obtained with plastic bags. Ten days following inoculation, however, necrotic leaves had been shed and there was little difference in disease ratings between the 18- and 24-hr dew periods. An 18-hr dew period in the dew chamber at 24 C was selected as the optimal treatment for further experiments.

**Effect of incubation temperature regime on disease development.** Pooled results from two experiments indicated that disease development was more rapid at warmer incubation temperatures (25/20 C and 35/30 C day/night temperature) than at 15/10 C. Inoculated leaves were rated as “severely diseased” within 4–5 days and had been shed from the plants within 5–6 days when plants were incubated under the two warmer temperature regimes. Under the cooler regime, 7 and 10–11 days were required to reach a severe disease rating and to shed diseased leaves, respectively. Both values for the cooler regime were significantly greater ( $\alpha = 0.05$ ) than corresponding values for the warmer regimes, according to the Waller-Duncan  $k$ -ratio  $t$  test. There were no significant differences between the two warmer regimes.

**Effect of inoculum density on disease development.** Increasing the inoculum density from  $10^4$  to  $10^7$  spores/ml resulted in an increase in necrotic tissue (Fig. 4). Only leaves inoculated with  $10^6$  or  $10^7$  spores/ml were sufficiently diseased to be prematurely shed from the plants during the course of this experiment. Higher inoculum densities (e.g.,  $2.3 \times 10^9$  spores/ $m^2$  [ $2 \times 10^8$  spores/ml]) did not improve the efficacy of *C. coccodes* (unpublished), and therefore, the optimum inoculum concentration of  $10^7$  spores/ml was chosen for subsequent experiments.

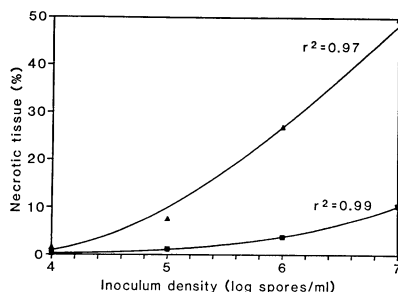
**Production of conidia on diseased leaves.** *Colletotrichum coccodes* sporulated but produced few conidia on diseased leaves without additional moist periods. Numerous conidia, however, were produced following exposure of diseased leaves to moist conditions in the dew chamber. With increasing duration of a supplementary dew period, conidia production increased with a maximum following 42 hr of moisture (Fig. 5). After 42 hr, velvetleaf leaves were decomposing and the numbers of conidia recovered decreased with longer dew durations.

**Effect of velvetleaf growth stage and incubation in the mist frame on weed control.** Incubation of inoculated plants in the mist frame increased the efficacy of *C. coccodes* (Table 1). Velvetleaf biomass and height were significantly reduced for inoculated compared with uninoculated plants at all growth stages in both the

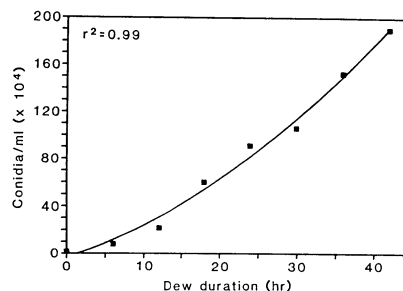
growth chamber and mist frame. The effect on biomass and height was significantly greater in the mist frame than in the growth chamber. Although inoculated velvetleaf plants at all growth stages were stunted and reduced in vigor, mortality occurred only when plants were inoculated at the cotyledon stage, and there was a significant increase in mortality when plants were incubated in the mist frame compared with those incubated in the growth chamber. Plants not killed within 2 wk continued to grow.

Inoculated leaves that were 30% necrotic or more were shed from plants within 2 wk while corresponding leaves on control plants had not been shed.

**Field trials.** Leaf lesions were observed on all inoculated velvetleaf plants within 7–10 days after application. When applied at  $10^9$  spores/ $m^2$  to plants at the 2- to 3-leaf stage, 46% of the plants were killed and biomass per plant was significantly reduced compared with the control (Table 2, Fig. 1C,D). Mortality of older plants (6- to 12-leaf stage) was



**Fig. 4.** Effect of *Colletotrichum coccodes* inoculum density on disease development on velvetleaf. Pooled results of two experiments. Plants were inoculated at the 1- to 2-leaf stage by spraying to runoff with a spore suspension of *C. coccodes*. Dew periods were provided for 24 hr at 25 C by placing the plants in a growth chamber and then covering them with plastic bags. ■ = Data collected 4 days after inoculation, ▲ = data collected 6 days after inoculation. Arc sine transformed regression equations: disease at 4 days =  $13.64 - (7.14 \cdot i) + (1.12 \cdot i^2)$ , and disease at 6 days =  $-45.94 + (12.87 \cdot i)$  where  $i = \log$  spores/ml. For graphing purposes, data were converted back to percentages following statistical analysis.



**Fig. 5.** Effect of supplementary dew period duration on conidia production by *Colletotrichum coccodes* on diseased velvetleaf leaves. Pooled results of two experiments. Plants were inoculated at the 4- to 5-leaf stage ( $2.3 \times 10^8$  spores/ $m^2$ ), incubated 7 days at 24/18 C day/night temperature, then given a supplementary dew period in a dew chamber at 24 C for the indicated length of time. Conidia produced on diseased leaves were recovered in 15 ml distilled  $H_2O$  and counted. Regression equation: conidia/ml  $\times 10,000 = -2.95 + (2.09 \cdot t) + (0.06 \cdot t^2)$  where  $t =$  supplementary dew period duration in hr.

**Table 1.** Effect of incubation in the mist frame or growth chamber on suppression of velvetleaf by *Colletotrichum coccodes*<sup>w</sup>

Location	Age when inoculated (days) <sup>x</sup>	Mortality (%)		Biomass (g dry wt/pot) <sup>z</sup>		Height (mm) <sup>z</sup>	
		Control	Inoc. <sup>y</sup>	Control	Inoc.	Control	Inoc.
Growth chamber	3	0	33	0.16	0.01	28	3
	8	0	0	0.69	0.35	82	55
	11	0	0	1.26	0.83	113	103
Mist frame	18	0	0	2.38	1.83	203	199
	3	0	100	0.07	0	12	0
	8	0	0	0.31	0.04	57	15
	11	0	0	0.67	0.14	108	41
	18	0	0	1.91	0.96	196	158

<sup>w</sup> Plants were inoculated with *C. coccodes* at the indicated ages, given a dew period for 18 hr at 24 C and then incubated in the indicated location for 2 wk. Values are means of the four pots for each treatment.

<sup>x</sup> Age and stage when inoculated: 3 at cotyledon stage, 8 at 1-leaf stage, 11 at 2-leaf stage, 18 at 3-leaf stage.

<sup>y</sup> Inoc. = Inoculated treatment.

<sup>z</sup> Factorial analysis of variance indicated a significant difference ( $P = 0.05$ ) between controls and inoculated plants, between the growth chamber and mist frame, and between the different ages at the time of inoculation. Regression equations: biomass (g, growth chamber control) =  $-0.38 + (0.15 \cdot t)$ , biomass (g, growth chamber inoculated) =  $0.32 - (0.19 \cdot t) + (0.03 \cdot t^2) - (0.001 \cdot t^3)$ , biomass (g, mist frame control) =  $0.07 - (0.03 \cdot t) + (0.01 \cdot t^2)$ , biomass (g, mist frame inoculated) =  $0.16 - (0.07 \cdot t) + (0.01 \cdot t^2)$ , height (mm, growth chamber control) =  $-10.19 + (11.67 \cdot t)$ , height (mm, growth chamber inoculated) =  $-42.54 + (13.25 \cdot t)$ , height (mm, mist frame control) =  $-31.58 + (12.50 \cdot t)$ , height (mm, mist frame inoculated) =  $9.87 - (5.54 \cdot t) + (0.76 \cdot t^2)$ , where  $t =$  time after planting in days.  $r^2 = 0.99$  for each equation.

**Table 2.** Effect of *Colletotrichum coccodes* on velvetleaf in the field<sup>1</sup>

Application rate (spores/m <sup>2</sup> )	Mean disease rating (0-4)	Biomass (g/plant) <sup>2</sup>	Mortality (%) <sup>2</sup>
0	2.2	0.050 a	3 a
10 <sup>7</sup>	2.3	0.040 a	3 a
10 <sup>9</sup>	3.3	0.025 b	46 b

<sup>1</sup>Data are for velvetleaf plants inoculated at the 2- to 3-leaf stage.

<sup>2</sup>Values followed by the same letter are not significantly different ( $\alpha = 0.05$ ), according to the method of contrasts. Values were log (biomass) or arc sin (mortality) transformed before analysis.

not observed. Spray volume had no significant effect on mortality, biomass, or disease rating. Some mortality and measurable levels of disease occurred even in uninoculated controls, probably due to spread of inoculum from adjacent inoculated plots.

## DISCUSSION

Velvetleaf is a major weed in corn (*Zea mays* L.) and soybeans (*Glycine max* (L.) Merr.) in the United States corn belt and southern Ontario, and is increasing in Quebec (1,2,5,12). Velvetleaf is difficult to control by chemical, mechanical, or cultural methods. Bioherbicides are an alternative that may control this weed.

The velvetleaf isolate of *C. coccodes* causes a severe foliage blight of velvetleaf resulting in premature leaf abscission. It grows well on a variety of solid and liquid media, and large-scale commercial production using agricultural by-products as nutrient sources should be practical. The inoculum density required for optimum disease development was high but spore germination is variable (*unpublished*), and if consistently high germination could be obtained inoculum density might be reduced. The pathogen grows and sporulates, and spores germinate over a wide range of temperatures. Although optimum dew periods in growth chamber and greenhouse experiments are long (18-24 hr), the disease did develop under cool temperatures and with short dew durations. Also, the disease occurs in the field with dew periods presumably shorter than 24 hr. High levels of disease may be obtained with short dew periods through the use of adjuvants in the spore suspension (16) or a follow-up application of an invert emulsion (10).

Incubation of plants in a greenhouse mist frame provides conditions more favorable for pathogens and allows a more accurate assessment of the potential of candidate bioherbicide organisms. Growth chamber incubation does not allow secondary disease cycles to occur and these may be important for some bioherbicide organisms. *Colletotrichum coccodes* produces numerous conidia on diseased leaves during exposure to moist conditions, and mist frame incubation should allow these conidia to be produced, redistributed, germinate, and infect. Initial experiments with bioherbicide organisms designed to determine optimum conditions for disease development should be incubated under the more controlled conditions of the growth chamber. Experiments designed to test the efficacy of a bioherbicide organism for actual weed control, however, should be carried out under conditions that periodically supply additional moist periods and more accurately reflect field conditions.

In addition to weed control efficacy, host specificity of a candidate bioherbicide pathogen is a major concern (15). The velvetleaf isolate of *C. coccodes* has a restricted host range compared with published data on other *C. coccodes* isolates, and it does not damage other plants. Details of the laboratory and field host range of this isolate of *C. coccodes* are being published elsewhere.

When conditions are optimum, *C. coccodes* is highly effective in killing and/or reducing the biomass of velvetleaf as demonstrated in laboratory experiments. All velvetleaf tissue was susceptible and inoculated leaves were readily killed and shed from the plants prematurely. Plants were killed, however, only when inoculated at a young growth stage. When applied to velvetleaf at later growth stages the pathogen caused a severe blight on inoculated leaves. Velvetleaf is a vigorous weed, however, and continues to grow after shedding diseased leaves. These inoculated plants were stunted and their development was delayed. Additional field testing is needed to determine if this reduction in competitiveness is sufficient to adequately control velvetleaf in corn and/or soybeans. In the field experiments reported here, the velvetleaf isolate of *C. coccodes* effectively suppressed velvetleaf. These results with the velvetleaf isolate of *C. coccodes* suggest that it is a potential bioherbicide that should be tested further.

## ACKNOWLEDGMENTS

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