Effect of Inoculum Concentration, Temperature, Dew Period, and Plant Growth Stage on Disease of Round-Leaved Mallow and Velvetleaf by *Colletotrichum gloeosporioides* f. sp. *malvae*

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ABSTRACT

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In greenhouse and controlled-environment tests, disease development on round-leaved mallow (*Malva pusilla*) and velvetleaf (*Abutilon theophrasti*) of *Colletotrichum gloeosporioides* f. sp. *malvae* was affected by inoculum concentration and plant growth stage at time of inoculation, postinoculation air temperature, and dew-period duration and temperature. The highest levels of control were obtained with an inoculum concentration of 2×10^6 spores per milliliter for *M. pusilla* and 4×10^6 spores per milliliter for *A. theophrasti*. All growth stages were susceptible, although younger seedlings were less susceptible than older plants. Postinoculation air temperatures of 30 C for *M. pusilla* and >15 C for *A. theophrasti* reduced disease development significantly. A minimum dew period of 20 h at 20–25 C was required to achieve satisfactory control of *M. pusilla*, whereas a dew period of 48 h was required to achieve a comparable level of control of *A. theophrasti* with the same inoculum concentration. Shorter repetitive dew periods of 16 h, which resemble field conditions more than a long, single dew period, also adequately controlled *M. pusilla*. However, infection on *A. theophrasti* was not affected by any of the repetitive dew periods. *C. g. malvae* meets the epidemiological characteristics of a good bioherbicide for control of *M. pusilla*, whereas its potential for control of *A. theophrasti* is questionable and remains to be determined.

Additional keywords: biological control of weeds, mycoherbicide.

Velvetleaf (*Abutilon theophrasti* Medic.) is one of the most serious weed problems of corn and soybean fields in eastern Canada and the United States (9,10,25). It also reduces cotton yields significantly (5). Recently, round-leaved mallow (*Malva pusilla* Smith) also has become a major weed problem, mainly in less-competitive field crops in the Canadian prairies. *M. pusilla* can cause yield reductions of up to 85% in flax and lentil and up to 15% in wheat (11,16,17).

The pathogen *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. f. sp. *malvae* was registered for round-leaved mallow control in 1992 as the first bioherbicide in Canada, under the tradename BioMal (19). In the original host-specificity tests, *M. pusilla* was fully susceptible with lesions girdling all stems and branches, causing more than 90% plant wilt and kill, and *A. theophrasti* was less susceptible with lesions girdling medium stem branches and petioles, causing 50–75% plant wilt and kill (23). For a pathogen to be successful as a bioherbicide, a high level of weed mortality is desirable (4,6), and, thus, it is essential to have a clear understanding of the conditions under which such levels can be achieved.

The objectives of this study were to investigate the optimum conditions for infection, disease development, and satisfactory control of *M. pusilla* and *A. theophrasti* with *C. g. malvae* under the following controlled parameters: 1) inoculum concentration, 2) plant growth stage, 3) dew-period duration, 4) repetitive dew-period treatments, which represent conditions in the field, and 5) temperature during dew period and after inoculation.

MATERIALS AND METHODS

Plant production. *M. pusilla* seed was collected during the fall prior to conducting the experiments from a natural infestation at the Agriculture Canada Regina Research Station, SK. Plants were air-dried on greenhouse benches; the capsules were collected and stored in paper bags at room temperature. As seed was required, capsules were crushed, and seeds were sorted for homogeneity of size and color. *A. theophrasti* seed was obtained from infested field plots at McDonald College Campus, McGill University, Ste. Anne de Bellevue, PQ, Canada.

A. theophrasti seeds and dark *M. pusilla* seeds, scarified by nicking the seed coat with a scalpel, were placed on filter paper (Whatman No. 3, Maidstone, England) in 9-cm glass petri dishes with 5 ml of distilled water and germinated in a dark incubator at 20 C. After 48 h, the germinated seeds were planted in 20-cm pots in a 3:1:1 (v/v) soil/vermiculite/peat moss mixture and were placed in a greenhouse at 18–25 C with a 14-h day that was extended with fluorescent and incandescent light at an intensity of 280 μmol m^{-2} s^{-1} at plant canopy. Plants were watered daily or as required and weekly with a nutrient solution (N-P-K, 30-10-10). When specific, controlled conditions were required, seeded pots were placed in a growth chamber with a 16-h photoperiod at an intensity of 280 μmol m^{-2} s^{-1} from fluorescent tubes and incandescent bulbs. Temperatures varied with the experiment and are specified.

Inoculum production. *C. g. malvae* was originally isolated from diseased *M. pusilla* collected at the Agriculture Canada Regina Research Station. Diseased stem sections were surface-sterilized for 1 min in 0.6% sodium hypochlorite (10% Javex), rinsed in sterile distilled water, and plated on potato dextrose agar (PDA; Difco Laboratories, St. Louis, MO) containing streptomycin sulfate and penicillin at 10 mg/L. Spores of *C. g. malvae* were produced on PDA plates incubated for 24 h at 25/20 C (day/night) with a 12-h photoperiod provided by fluorescent light at an intensity of 28 μmol m^{-2} s^{-1}, then incubated for 1 wk in a dark incubator at 20 C. Spores were transferred to sterile water with a bacterial loop. The spore concentration was determined with a hemacytometer. The spore suspension or inoculum was sprayed on plants with an air brush (type H-5; Paasche Airbrush Ltd., Chicago, IL) at a constant air pressure of 200 kPa until

runoff. After inoculation, plants were placed in a dark, dew chamber (Percival model E-54U-DL, Boone, IA) that provided constant dew without runoff for the required time, then plants were returned to the greenhouse or growth chamber.

Unless otherwise specified, a spore concentration of 2×10^6 spores per milliliter was sprayed until runoff on plants at the one- to two-leaf stage and a 24-h dew period at 20 C were used. Plants were monitored for disease, and the number of plants killed was recorded every 7–10 days until 28–30 days after inoculation.

Spore viability. To ensure that the spores used were viable, germination in each batch of inoculum was measured by plating 1 ml of spore suspension on PDA, incubating for 24 h at 25–20 C, and counting the number of germinated and nongerminated spores from five 0.5- $\times$ 0.5-mm grids from the underside of the PDA plate with a light microscope at 120$\times$ magnification. For the inoculum-concentration experiment, each concentration also was plated.

Growth stage and inoculum concentration. Plants were sprayed at each of five leaf stages with six concentrations of inoculum. *M. pusilla* plants were inoculated at the cotyledon- to one-, two- to three-, four- to five-, six- to seven-, and eight- to 10-leaf stages, and *A. theophrasti* plants were inoculated at the cotyledon, one- to two-, three- to four-, five- to six-, and seven- to 10-leaf stage. *M. pusilla* plants were inoculated at each leaf stage with spore suspensions containing 0.25, 0.5, 1, 2, 4, or 8×10^6 spores per milliliter. *A. theophrasti* plants were inoculated at each leaf stage with spore suspensions containing 0.5, 1, 2, 4, 8, or 16×10^6 spores per milliliter. After inoculation, plants were placed in a dark dew chamber at 20 C for 24 h and then were returned to the greenhouse (conditions are described above). Disease development was monitored for 30 days after inoculation; ratings were done every 10 days.
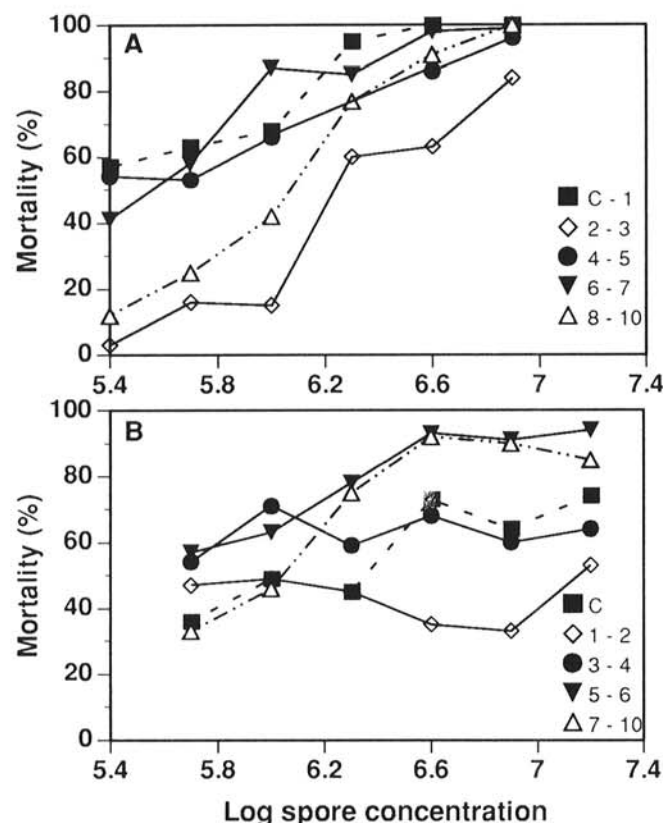

Fig. 1. Effect of *Colletotrichum gloeosporioides* f. sp. *malvae* inoculum concentrations and plant growth stages on control of **A,** *Malva pusilla* and **B,** *Abutilon theophrasti*, expressed as the mean percentage of plants killed 20 days after inoculation. Plants were sprayed with suspensions at six concentrations (average of two trials, each replicated three times with 10 plants per replicate) of *C. g. malvae* spores until runoff at each of five growth stages (C = cotyledon, 1–10 = leaf stage).

Dew-period duration and temperature during dew period. After inoculation, seedlings were incubated in dark dew chambers at temperatures of 10, 15, 20, or 25 C for durations of 4, 8, 12, 16, 20, 24, or 48 h and then were placed on greenhouse benches (conditions as above). Disease development was monitored for 28 days after inoculation; ratings were done every 7 days.

Repetitive dew periods. After inoculation, seedlings were subjected to various dew-period treatments: 1) one period of dew on the first night (night 1) after inoculation; 2) two sequential periods of dew, one on each of the first and second nights (nights 1 and 2) after inoculation; 3) three sequential periods of dew, one on each of the first, second, and third nights (nights 1, 2, and 3) after inoculation; 4) three periods of dew, one on each of the first, third, and fourth nights (nights 1, 3, and 4) after inoculation; 5) one period of dew on the second night (night 2) after inoculation; and 6) two periods of dew, one on each of the second and third nights (nights 2 and 3) after inoculation. Each treatment was subjected to dew periods lasting 4, 8, 12, or 16 h at 20 C then was placed on greenhouse benches (conditions as above). Disease development was monitored for 28 days after inoculation; ratings were done every 7 days.

Air temperature during incubation. After inoculation and a 24-h dew period at 20 C, seedlings were placed in growth chambers with either a constant air temperature of 10, 15, 20, 25, or 30 C or with a day/night temperature regime of 20/15, 25/10, 25/15, or 25/20 C. Disease development was monitored for 28 days after inoculation; ratings were done every 7 days.

Data analyses. For each plant species, all experiments consisted of two trials, and all treatments in each experiment were replicated three times with 10 plants per replicate. All data were analyzed with the Statistical Analysis System (SAS Institute, Cary, NC). Analysis of variance (ANOVA) was performed for each experiment. Percentage data were arcsine transformed, and spore concentrations were log transformed prior to analysis. Regression analyses on the means, orthogonal polynomial contrasts (linear, quadratic, and cubic), and protected Fisher's LSD were performed when appropriate.

RESULTS

Spore viability. Spore germination was used to verify the viability of spores in each batch of inoculum and was greater than 75% in the inoculum used in all experiments, except in the inoculum-concentration experiment. In the inoculum-concentration experiment on *A. theophrasti*, the highest mean percentage of germination (94%) occurred at the lowest concentration of inoculum at 0.5×10^6 spores per milliliter. Germination decreased from 88, 78, and 68, to 55% as concentrations increased from 2, 4, and 8, to 16×10^6 spores per milliliter, respectively. When the experiment was performed with *M. pusilla*, a different batch of spores was used; however, a similar trend was observed, especially at the higher concentrations. For *M. pusilla*, germination decreased from 79 and 69 to 57% as concentrations increased from 2 and 4 to 8×10^6 spores per milliliter.

Growth stage and inoculum concentration. Mortality of both species increased with increasing inoculum concentrations up to 2×10^6 spores per milliliter for *M. pusilla* and 4×10^6 spores per milliliter for *A. theophrasti* before leveling off (Fig. 1). All growth stages of both species were susceptible to *C. g. malvae*. All growth stages of *M. pusilla* responded to inoculum density (Fig. 1A). However, for *A. theophrasti*, only the cotyledon and the higher leaf stages (greater than five leaves) responded to inoculum density (Fig. 1B; Table 1).

For *M. pusilla*, over 80% mortality was obtained with inoculum concentrations greater than 2×10^6 spores per milliliter at growth stages of cotyledon to one leaf and more than six leaves (Fig. 1A). *A. theophrasti* displayed similar mortality when concentrations greater than 4×10^6 spores per milliliter were used (Fig. 1B). The two- to three-leaf stage for *M. pusilla* and the one- to two-leaf stage for *A. theophrasti* were less susceptible to *C. g. malvae* than were the other leaf stages. Plant growth stage had a greater effect on *A. theophrasti* mortality than on *M. pusilla*

mortality; plants with more than five leaves on *A. theophrasti* were the most susceptible (Fig. 1B). Over 90% of the *A. theophrasti* plants with more than five leaves at the cotyledon and three- to four-leaf stages were killed with an inoculum concentration of 4×10^6 spores per milliliter. Whereas, over 85% of the *M. pusilla* plants at all leaf stages except the two- to three-leaf stages were killed with 4×10^6 spores per milliliter. The mortality of *M. pusilla* at the four- to five-leaf stage with an

inoculum concentration of 2×10^6 spores per milliliter was significantly lower than with concentrations of 0.25, 0.5, and 1×10^6 spores per milliliter and was not included in the regression analysis. Orthogonal polynomials (linear, quadratic, and cubic) were fit to the data, of which only the linear contrasts were significant. The linear regression equations adequately described the effects of spore concentration on mortality of plants at different growth stages (Table 1).

At 30 days after inoculation (data not shown), mortality of *M. pusilla* and *A. theophrasti* was greater than 80% when inoculated with 2×10^6 and 4×10^6 spores per milliliter, respectively. However, only 60% mortality was achieved for the two- to three-leaf stage of *M. pusilla* and the one- to two-leaf stage of *A. theophrasti*. The initial infection on *A. theophrasti* at the one- to two-leaf stage with 4×10^6 spores per milliliter was lower than for the other growth stages (15% mortality after 10 days), whereas the initial infection on *M. pusilla* at the two- to three-leaf stage with 2×10^6 spores per milliliter was the same as that for the eight- to 10-leaf stage, giving 50% mortality after 10 days. For *M. pusilla*, the rate of disease development over 30 days was slower for this leaf stage, but all plants of each species were infected.

Dew-period duration and temperature during dew period. For *M. pusilla*, greater initial infections 1 wk after inoculation resulted when dew periods were greater than 20 h at temperatures of 20 and 25 C (Fig. 2A). After 48 h of dew, over 80% mortality was observed at temperatures of 15, 20, and 25 C 1 wk after inoculation. At least 20 h of dew was required at 20 or 25 C to achieve satisfactory control (over 80% kill) of *M. pusilla* by 3 wk after inoculation, whereas, at 15 C, a dew period of 48 h was required to achieve the same result. At a temperature of 10 C during the dew period, few signs of infection were observed 1 wk after inoculation, and only 50% kill occurred 4 wk after inoculation.

TABLE 1. Estimated regression parameters and associated statistics for the regression on the means of arcsine transformed percent plant mortality of *Malva pusilla* and *Abutilon theophrasti* at various growth stages as a function of the log of spore concentration 20 days after inoculation

Growth stage	Error (df)	F (P value)	r^2	Estimate (SE)[a] b_0	b_1
M. pusilla					
Cotyledon to 1[b]	4	0.0027	0.92	−1.80 (0.02)	0.50 (0.003)
2–3	4	0.0020	0.93	−4.45 (0.72)	0.83 (0.12)
4–5	4	0.0050	0.95	−1.28 (0.33)	0.39 (0.5)
6–7	4	0.0044	0.89	−2.35 (0.61)	0.58 (0.10)
8–10	4	0.0001	0.99	−4.55 (0.30)	0.89 (0.05)
A. theophrasti					
Cotyledon	4	0.0239	0.76	−1.25 (0.59)	0.33 (0.09)
1–2	4	NS[c]	0.004	0.79 (0.54)	−0.01 (0.08)
3–4	4	NS	0.15	0.59 (0.41)	0.05 (0.06)
5–6	4	0.0103	0.84	−1.11 (0.50)	0.36 (0.08)
7–8	4	0.0194	0.78	−2.33 (0.89)	0.53 (0.14)

[a]SE = standard error of the estimate.
[b]Leaf stages.
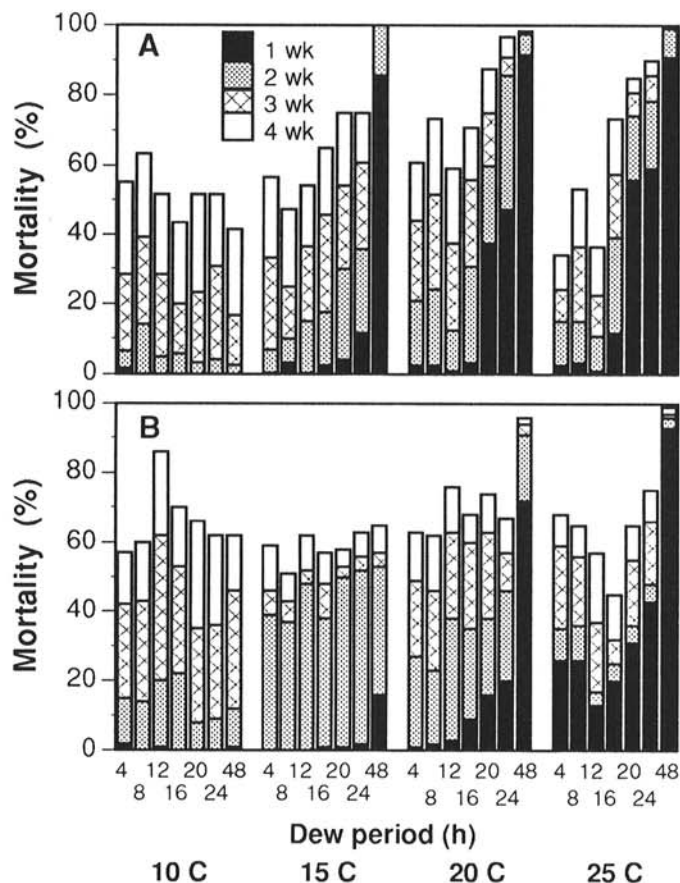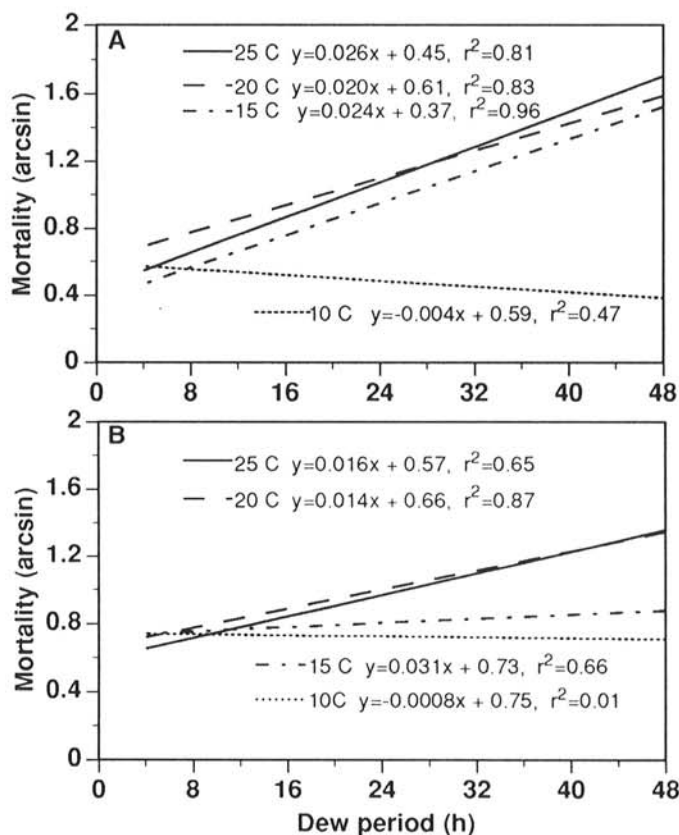[c]NS = not significant at $P > 0.05$.

Fig. 2. Effect of dew-period duration and temperature during dew period on disease development on **A,** *Malva pusilla* and **B,** *Abutilon theophrasti* by *Colletotrichum gloeosporioides* f. sp. *malvae*, expressed as the mean percentage of plants killed 1, 2, 3, and 4 wk after inoculation. A spore suspension of *C. g. malvae* was sprayed at 2×10^6 spores per milliliter until runoff on seedlings in the one- to two-leaf stage and given seven dew-period treatments at each of four temperatures.

Fig. 3. Response of plant mortality (arcsine transformed) of **A,** *Malva pusilla* and **B,** *Abutilon theophrasti* 3 wk after inoculation to increasing dew periods at each of four temperatures and to inoculation of *Colletotrichum gloeosporioides* f. sp. *malvae* calculated by fitting a linear regression to the means (two trials, each replicated three times with 10 plants per replicate).

For *A. theophrasti*, greater initial infections 1 wk after inoculation resulted from 48 h of dew at 15, 20, and 25 C (Fig. 2B). Satisfactory control (over 80% kill) of *A. theophrasti* was achieved only after 48 h of dew at 25 C 1 wk after inoculation and at 20 and 25 C 2 wk after inoculation. At a dew-period temperature of 10 C, few signs of infection were observed 1 wk after inoculation, as with *M. pusilla*, and approximately 60% plant kill was achieved 4 wk after inoculation at 10 and 15 C.

Regression analysis adequately described the effects of dew-period duration on mortality of plants subjected to different dew temperatures (Fig. 3). At 10 C, increasing dew-period duration had no effect on mortality for either species 3 wk after inoculation. For *M. pusilla*, there were no significant differences in mortality from dew temperatures of 15, 20, or 25 C (Fig. 3A, simple linear regression comparison [35]). However, the amount of initial infection and the disease progression over 4 wk was lower for 15 C (Fig. 2A). For *A. theophrasti*, there was no significant difference in mortality in response to dew duration for 10 and 15 C nor for 20 and 25 C; however, the two higher temperatures were significantly different than the two lower temperatures (Fig. 3B, simple linear regression comparison [35]). The amount of initial infection and the disease progression over 4 wk increased with increasing dew duration and temperature during dew period (Fig. 2B).

Disease development on both weeds was greater when the dew-period temperature was 20 or 25 C than for the lower temperatures (Figs. 2 and 3). *M. pusilla* did not require as long a dew period (20 h) to achieve satisfactory control with *C. g. malvae* as did *A. theophrasti* seedlings (48 h).
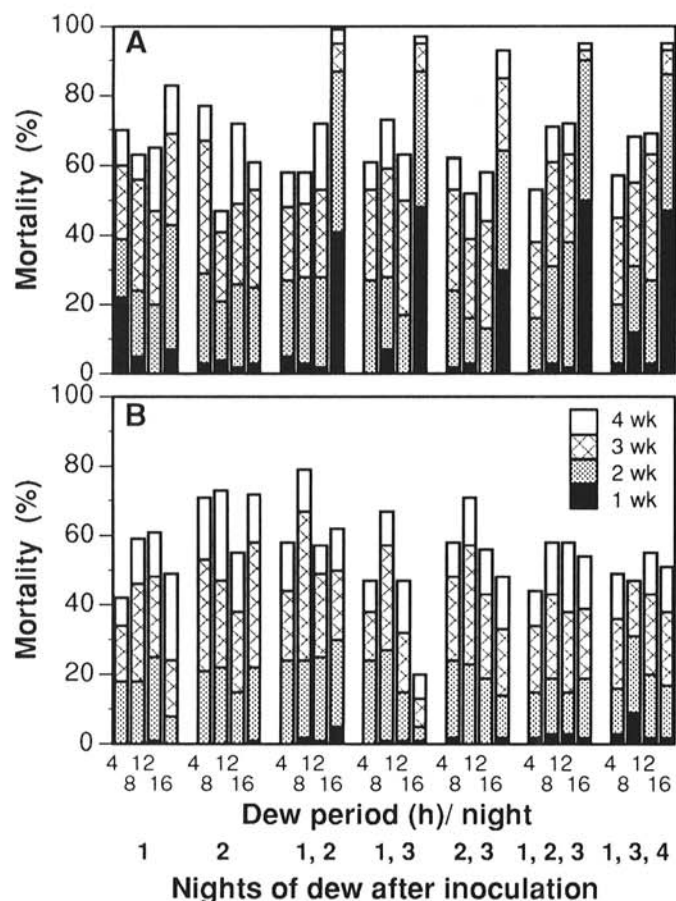
Repetitive dew periods. The effect of repetitive dew periods on infection by *C. g. malvae* was investigated to determine if the dew period-duration requirement could be shortened if repetitive or sequential dew periods were given instead of one longer dew period, i.e., treatments that would more readily represent conditions in the field. Mortality of *M. pusilla* increased with increasing dew-period duration for treatments with more than one night of dew (Fig. 4A). Orthogonal polynomials (linear and quadratic) were fit to the effect of dew-period duration on mortality (arcsine transformed) at 4 wk, rating for each night of dew treatment (significance of linear and quadratic trend for each night of dew treatment: night 1, $P = 0.18$, $P = 0.17$; night 2, $P = 0.61$, $P = 0.36$; nights 1 and 2, $P < 0.01$, $P < 0.01$; nights 1 and 3, $P = 0.01$, $P = 0.18$; nights 2 and 3, $P = 0.03$, $P = 0.02$; nights 1, 2, and 3, $P < 0.01$, $P = 0.71$; nights 1, 3, and 4, $P < 0.01$, $P = 0.11$). The analysis was not significant for the single nights of dew treatments, linear contrasts were significant for all other treatments, and quadratic contrasts were significant for treatments with dew treatments on nights 1 and 2 and 2 and 3. Control of *M. pusilla* was not increased to satisfactory levels (over 80% kill) with any of the repetitive dew treatments of 4, 8, or 12 h. There were significant differences between treatments with dew-period duration of 16 h. With 16 h of dew, significantly more kill was achieved with a single dew period immediately after inoculation (night 1) than if it was delayed until night 2. With 16 h of dew, two or three nights of dew significantly increased plant kill as early as 1 wk after inoculation over treatments with a single dew period. With two or more nights of 16 h of dew, disease development was more rapid when the initial dew period immediately followed inoculation. Treatments with three nights of dew did not significantly increase plant kill over treatments with two nights of dew periods.

There was no effect of number of dew periods or dew-period duration on mortality of *A. theophrasti* (Fig. 4B). None of the treatments controlled *A. theophrasti* effectively. All treatments had less than 80% plant kill.

Air temperature during incubation. For *M. pusilla* in constant-temperature regimes, initial infection and disease progression was greater at 20 and 25 C (Fig. 5); however, 2 wk after inoculation, plant mortality was over 90% at 10, 15, 20, or 25 C. At a constant temperature of 30 C, infections were significantly reduced for the duration of the experiment. Orthogonal polynomials (linear, quadratic, and cubic) were fit to the data at the 4-wk rating. There was no lack of fit for any of the contrasts ($P < 0.0001$).

For *M. pusilla* at alternating temperatures of 25/15 and 25/20 C (Fig. 5), initial infection and disease progression was similar
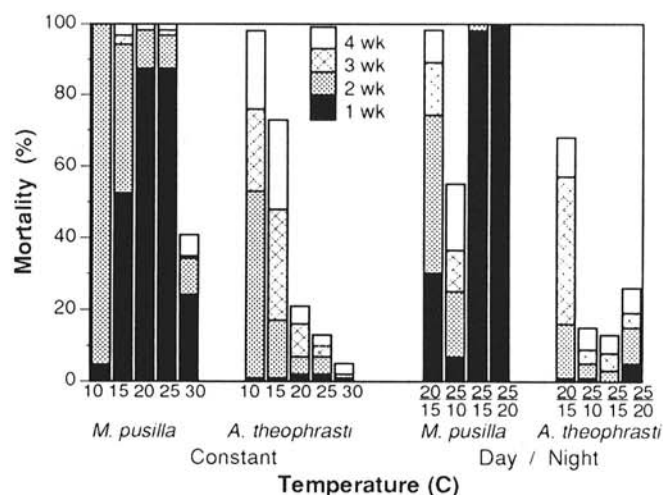

Fig. 4. Effect of single or repetitive nights of dew on control of **A**, *Malva pusilla* and **B**, *Abutilon theophrasti* by *Colletotrichum gloeosporioides* f. sp. *malvae*, expressed as the mean percentage of plants killed 1, 2, 3, and 4 wk after inoculation. A spore suspension of *C. g. malvae* was sprayed at 2×10^6 spores per milliliter until runoff on seedlings in the one- to two-leaf stage and given seven nights of dew treatments at each of four dew periods.

Fig. 5. Effect of constant and alternating (day/night) postinoculation air temperatures on control of *Malva pusilla* and *Abutilon theophrasti* with *Colletotrichum gloeosporioides* f. sp. *malvae*, expressed as the mean percentage of plants killed 1, 2, 3, and 4 wk after inoculation. A spore suspension of *C. g. malvae* was sprayed at 2×10^6 spores per milliliter until runoff on seedlings in the one- to two-leaf stage.

to that at constant temperatures of 20 and 25 C. The percent plant kill at the 25/10 C regime 4 wk after inoculation was significantly lower than at the other three alternating temperature regimes after which all plants were dead.

For *A. theophrasti*, the response to various incubation temperatures was distinctly different than for *M. pusilla* (Fig. 5). Under constant-temperature regimes, 1 wk after inoculation there were few signs of infection and no differences between the various temperatures. The percent plant kill was significantly greater at 10 C and decreased with increasing temperatures 2 wk after inoculation and during the rest of the experiment. Orthogonal polynomials (linear, quadratic, and cubic) were fit to the data, of which only the linear contrast was significant. The regression equation adequately described the effect of incubation temperature on mortality (arcsine transformed means of percent mortality, 3 wk after inoculation): $y = 1.54 - 0.05x$, $r^2 = 0.92$.

For *A. theophrasti*, initial infection and disease development was significantly reduced at alternating temperatures compared with constant 10 C (Fig. 5). The percent plant kill was significantly greater at the 20/15 C regime than at the three other alternating temperature regimes 3 wk after inoculation and during the rest of the experiment.

DISCUSSION

The optimum conditions for achieving satisfactory control (80% mortality) (4,6) of *M. pusilla* and *A. theophrasti* are as follows. *M. pusilla* was controlled by *C. g. malvae* at a concentration of 2×10^6 spores per milliliter, whereas *A. theophrasti* required a higher concentration of at least 4×10^6 spores per milliliter. In all subsequent experiments, an inoculum concentration of 2×10^6 spores per milliliter was used to allow for comparisons between both plant species. *M. pusilla* and *A. theophrasti* plants at all leaf stages were susceptible to *C. g. malvae*. A minimum of 20 h of dew was required for satisfactory control of *M. pusilla* or repetitive dew periods of 16 h, whereas 48 h of dew was required for *A. theophrasti*. Satisfactory control was achieved during dew at 20 or 25 C. Postinoculation temperatures below 30 C were preferable for *M. pusilla*, whereas 10 C provided the best control of *A. theophrasti*. It appeared that *A. theophrasti* outgrew the disease at temperatures greater than 10 C.

The volume and concentration of inoculum at 2×10^6 spores per milliliter that was required to spray seedlings is equivalent to 60×10^6 spores per square meter, a more applicable measurement for comparison with field-trial results (21). Under field conditions, 60×10^6 spores per square meter also was adequate to achieve satisfactory control of *M. pusilla* (24). At the higher inoculum concentrations, disease was not reduced further because germination was lower. This autoinhibition of germination at high concentrations also was reported by Heiny and Templeton (14) for *Phoma proboscis* on field bindweed (*Convolvulus arvensis*). It is possible the plant surface may compensate for this growth inhibition through interaction with the chemical characteristics of the plant cuticle or dilution due to spatial redistribution on the plant surface (1).

Even though all growth stages were susceptible, the younger two- to three-leaf stage of *M. pusilla* and the one- two-leaf stage of *A. theophrasti* were less susceptible than were the other growth stages, contrary to reports of other potential bioherbicides in which younger seedlings showed the greatest susceptibility (3,6, 28). However, penetration by *C. gloeosporioides* was inhibited in mitotically active cells of papaya fruit (26) and in young unexpanded leaves of *Hakea sericea* (22). The decreased susceptibility of the younger leaf stages may be similarly due to the actively growing tissue that partially outgrew the disease.

C. gloeosporioides develops over a wide range of temperatures, but moisture is the most important requirement for germination and infection (22,30,32). The optimum dew requirement for control of *M. pusilla* was longer than the dew-period durations required for other mycoherbicides or potential mycoherbicides such as *C. gloeosporioides* f. sp. *aeschynomene* (28), *C. coccodes* (2), *Fusarium lateritium* (3), or *Alternaria cassiae* (31). Shorter

repetitive dew periods (i.e., 16 h rather than 20 h), which resembled field conditions more than a long single dew period, gave adequate control of *M. pusilla*. Sequential dew periods also have been reported to shorten the optimal dew period required for high efficacy of *A. cassiae* for control of sicklepod, *Cassia obtusifolia* (31). The 16–20 h dew period required for satisfactory control of *M. pusilla* corresponds to the timing of appressoria formation and the beginning of penetration into cells (16). Under field conditions, a long period of dew, shorter repetitive dew periods, cool overcast conditions, or rain immediately after inoculation are required to obtain satisfactory control. Therefore, the timing of field applications for effective control with *C. g. malvae* is crucial. When hot, dry conditions prevail after field application of *C. g. malvae*, infections are low, and disease development is slow, giving poor control of round-leaved mallow. However, when applications are properly timed, optimum conditions can be achieved in the field even in the semiarid climate of the Canadian prairies and give satisfactory control of *M. pusilla* (18,23). Adequate moisture conditions for effective control with *C. g. malvae* could be provided by irrigation in orchards and crops. Complete kill of *M. pusilla* plants was achieved after direct application of *C. g. malvae* to the irrigation system in field plots at the Agriculture Canada, Research Station, Swift Current, SK, Canada (K. Mortensen, *personal communication*).

The dew period required for *A. theophrasti* control was much longer than for *M. pusilla* given the same inoculum concentration, and *A. theophrasti* was not affected by any of the repetitive dew periods tested. A longer dew period also was required for control of *A. theophrasti* compared to prickly sida (*Sida spinosa*) with *F. lateritium* (3). Fungal colonization of leaves and stems of *A. theophrasti* may be impeded because of the presence of large numbers of trichomes covering their entire surfaces (1), requiring additional dew time for prepenetration interactions and penetration and increased inoculum.

Temperatures during the dew period that provided satisfactory control of *M. pusilla* were similar to those during incubation but were different for *A. theophrasti*. *C. g. malvae* infected and developed rapidly on *M. pusilla* over a wide range of constant and alternating temperatures. This is contrary to reports on the development of *C. g. aeschynomene* on northern jointvetch in which higher optimal temperatures were reported and alternating temperature regimes decreased infection significantly over constant temperatures (28). The optimum temperatures for development of *C. g. malvae* on *M. pusilla* were similar to those required for germination (16) and those found under field conditions in the natural habitat in Saskatchewan and Manitoba where *M. pusilla* grows (17). However, for *A. theophrasti*, temperatures for infection of *C. g. malvae* had a very narrow range and were cooler than temperatures required for germination and growth of this weed, which is a more southerly species and thrives at higher temperatures. This explains the failure of preliminary field trials of *C. g. malvae* applied to *A. theophrasti*-infested fields in southern Ontario (23). Under greenhouse conditions in this study, *A. theophrasti* seedlings appeared to outgrow the disease at temperatures greater than 15 C. Although lesions developed at these temperatures, they remained at the surface of the stem epidermis within the first few layers of cells, and lesions did not enlarge nor girdle the stem. Some cell proliferation around the lesions also was observed. These symptoms were similar to those reported for species resistant to *C. gloeosporioides* (20,26).

From the results presented, *C. g. malvae* meets the epidemiological characteristics of a potential bioherbicide, including high virulence, broad range of tolerance of environmental conditions (at least in the range in which the weed is a problem), capacity to damage its host plant and achieve high levels of disease, and rapid control (6–8,27,29,33). However, the potential of *C. g. malvae* as a bioherbicide for *A. theophrasti* remains questionable. Greater spore concentrations and/or longer dew periods may be necessary for control of *A. theophrasti*. Different formulations of *C. g. malvae* or application in conjunction with a herbicide (12,13), as also reported with *C. coccodes* (34), require further investigation. The *C. g. malvae* isolate used may be adapted to

the cooler conditions of the prairies, so this problem may be overcome by isolation of *C. gloeosporioides* directly from *A. theophrasti* in its natural habitat where it has been reported (15).

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