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Colletotrichum gloeosporioides f. sp. *malvae* as a Bioherbicide for Round-Leaved Mallow (*Malva pusilla*): Conditions for Successful Control in the Field

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Abstract

Field trials were conducted to evaluate the efficacy of *Colletotrichum gloeosporioides* f. sp. *malvae* (C.g.m.) for control of *M. pusilla* under natural conditions. Moisture and temperature following application were the most important factors responsible for successful control. A 12 to 15 h dew period following application, precipitation greater than 6 mm within 48 h of application coupled with temperatures around 20°C and an overcast sky were required in the field for control. The combined stresses of competition and disease did not increase the control of *M. pusilla* over the pathogen alone. The pathogen developed equally well on *M. pusilla* in crop or alone. Under Saskatchewan conditions, crop canopy did not provide a better microclimate for disease development. Early application coupled with rapid disease development increased wheat and lentil yields significantly.

Introduction

In recent years, *Malva pusilla* Sm. (round-leaved mallow; Malvaceae) has been found in agricultural crops in the Canadian prairie provinces and is increasing in importance especially in less competitive crops such as lentil and flax (Makowski and Morrison 1989). The competitiveness of *M. pusilla* is dependent on that of the crop and the respective stages of development of the weed in relation to the crop. Significant yield reductions have been reported in less competitive crops such as lentil, but also in wheat when *M. pusilla* emerges before the crop (Makowski 1987). Thus, control measures for *M. pusilla* warrant development.

The fungus *Colletotrichum gloeosporioides* (Pen.) Sacc. f. sp. *malvae* (Coelomycetes) (*C.g.m.*), originally isolated from *M. pusilla* in 1982, has effectively controlled *M. pusilla* in greenhouse experiments and in field trials (Makowski 1987, Mortensen 1988). *C.g.m.* has been found in a number of locations in Saskatchewan and Manitoba (Mortensen 1988). However, under natural conditions, it has not developed into epidemic proportions until late in the growing season. The pathogen has potential as a biological herbicide or mycoherbicide if this low incidence of the disease in nature is due to low inoculum levels or to poor dissemination or overwintering rather than to low innate pathogenicity, host resistance, or constraining environmental requirements (Templeton *et al.* 1984).

Under controlled-environment conditions, *M. pusilla* was effectively controlled with *C.g.m.* at a concentration of 2 x 10^6 spores/ml, with a minimum of 20 h of dew or repetitive dew periods of 16 h, at temperatures below 30°C, and at all growth stages (Makowski 1987). These optimum temperatures for development of *C.g.m.* on *M. pusilla* were similar to those required for growth and germination of its seed. Knowledge of the environmental requirements for high levels of infection under field situations is also essential for determining its potential and its efficacy as a bioherbicide and will be required for registration purposes in Canada.

The use of pathogens as bioherbicides is one method of weed control and should be integrated into existing weed management systems (Quimby and Walker 1982). Plant pathogens can have a dramatic impact in reducing the competitive ability and survival of weeds. Other environmental stress such as intraspecific competition has been suggested as a major component in the reduction of competitiveness or death of weeds infected with plant pathogens (Charudattan and Walker 1982). Investigations into the field efficacy of pathogens as bioherbicides should also determine the interactions of stress due to infections and other stresses such as competition on yield of the weed and the crop.

The objectives of this study were to determine the efficacy of *C.g.m.* in controlling *M. pusilla* under field situations and the environmental conditions required for successful control. This was carried out by investigating: (1) the effect of date of application on crop yield and *M. pusilla* control; (2) the effect of crop canopy, which may provide a more suitable microclimate for disease development or prevent penetration to *M. pusilla*, on the efficacy of *C.g.m.*; and (3) the combined effect of crop competition and disease on control of *M. pusilla*.

Materials and Methods

General Procedure for Field Trials

Field experiments were conducted at the Agriculture Canada Indian Head Experimental Farm and Regina Research Station in Saskatchewan, in 1985 and 1986. Two crops were used in these trials; wheat as a good competitor and lentil as a poor competitor. At Indian Head, *M. pusilla* capsules were seeded with a cone seeder in the spring of 1985 just before seeding the crop, and in the fall of 1985 for the 1986 growing season at a rate of 6.3 g of seed capsules per 10 m row (approximately 5 cm between capsules). Plots could not be seeded in the fall of 1984 for the 1985 growing season due to a snowstorm in early October. At Regina, the plot area contained a natural infestation of *M. pusilla* which emerged at high densities every year. Wheat (cv. Katepwa) and lentil (cv. Laird) were seeded at recommended rates, 80 kg/ha respectively, across the *M. pusilla* rows. The plot area at Indian Head was sprayed in early June with diclofop-methyl (methyl-2-[4-(2,4-dichlorophenoxy)phenoxy] propionic acid) for control of grassy weeds at recommended rates and leaf stage, and also with carbaryl (1-naphthyl N-methylcarbamate) for grasshopper control in early June of each year at recommended rates and stage of development. The plot area at Regina was sprayed with deltamethrin for grasshopper control at recommended rates and stage of development in early June of each year. These treatments had no effect on *M. pusilla*.

Further details on the layout of field plots are presented below for each experiment. The number of crop and weed plants were recorded about 4 wk after seeding when tillering started in wheat. In addition to herbicide treatments, plots were hand weeded as required during the growing season.

Spores of *C.g.m.* were produced on solid Nz-amine agar (Veliky, I.A., pers. comm. 1987). Detailed methods of production are found in Makowski (1987). Spore suspensions were mixed with a drying agent (Kaolin: hydrated aluminium silicate) about 1:3 (v/v) into a thick slurry, then left to dry. Dried spores remained viable when refrigerated for over six months. When required, the spore material was resuspended in water and sprayed on field plots using a backpack sprayer with a four-nozzle (80° flat fan nozzles) boom supplied with a constant pressure of 275 kPa from a CO_2 tank. A spore concentration of 2×10^6 spores/ml in a water volume of 300 L/ha were used in all experiments. Plots were sprayed in the evening to ensure the longest possible dew period immediately following application. Since natural dew does not always form in Saskatchewan, plots were misted with water (250 ml/plot) at the time of application, and if conditions were hot and dry, plots were also misted on the morning following application. Disease development in field plots was monitored throughout the summer and rated at harvest in 1985 and 14 d after application and at harvest in 1986. A rating scale of 0 to 9 was used where 0 represents no symptoms and 9 over 95% plant kill (Mortensen 1988).

The effect of CO_2 from the tank on *C.g.m.* spores was tested by spraying the spore suspension with the backpack sprayer directly onto potato dextrose agar (PDA). Culture development was monitored and spore production determined after 10 d. Each batch of inoculum for spraying on field plots was also plated on PDA, and the culture development and spore production were recorded.

Temperature and relative humidity were monitored using a hygrothermograph and the period of dew using a leaf wetness recorder (Kahlsico International Corp.) placed directly in field plots for 48 h following application.

At harvest, crop and *M. pusilla* plants were counted, harvested, and total above ground shoot dry weights determined for each plot. *M. pusilla* and lentil were harvested separately by clipping plants at ground level. Wheat plants were pulled, the plants and tillers counted, then the roots were clipped off and discarded. All plants were oven dried for 24 h at 100°C and weighed. Crop plants were threshed to obtain seed weights and *M. pusilla* capsules were removed by hand from dried plants and weighed.

Experiment 1: Date of Application

At the Indian Head Experimental Farm, plots (1.5 m x 6 m, 6 crop rows) were established in a randomized complete block design with four treatments in 1985 and five in 1986 with four replicates/treatment. Alleys of 1 m between plots were kept weed-free by tilling throughout the summer. Treatments consisted of three spore applications in 1985 and four in 1986 at two week intervals plus a control (water only) with the first application July 5 in 1985 and June 11 in 1986. *M. pusilla* plants at all leaf stages were present in the field at time of application. Four m of the two center rows of crop were harvested in late August.

At Regina in 1986, 1 x 1-m plots were established in a randomized complete block design with four treatments and four replicates. Treatments consisted of three spore applications at two week intervals plus a control (water only) with the first application July 8. The plots were covered in plastic after application for 12 h, thus, ensuring at least 12 h of dew.

All counts and dry weights were converted to a m^2 basis, and analysed as randomized complete block design. Treatment means, when significantly different at the 5% level, were compared with a Fisher's LSD test.

Experiment 2: Effect of Competition and Crop Canopy on Disease Development

At Regina, treatments were established in a split plot design with four subplots/plot with three replicates in 1985, and three subplots per plot with six replicates in 1986. Subplots (1.5 m x 3 m, 6 crop rows) were seeded at different crop densities; 0, 0.5, 1, and 2 x normal seeding rate in 1985, and 0, 0.5, and 1 x normal seeding rate in 1986. Whole plots consisted of untreated and treated plots. *C.g.m.* was applied in late June of both years. *M. pusilla* plants at all leaf stages were present in the field at time of application. Two m of the four center rows of crop were harvested in late August.

All counts and dry weights were converted to a m^2 basis, and analysed as a split plot design. Treatment means, when significantly different at the 5% level, were compared with a Fisher's LSD test.

Results

Experiment 1: Date of Application

The CO_2 from the spray tank had no effect on *C.g.m.* Spore germination and production of the suspension sprayed on PDA plates with the CO_2 backpack sprayer was not significantly different from the spore suspension plated directly on PDA plates before spraying.

In 1985, few *M. pusilla* plants were present in field plots at Indian Head due to seeding of *M. pusilla* capsules in the spring. Growing conditions were hot and dry, and thus, little biomass was produced. In lentil, virtually no disease developed on *M. pusilla* and there were no crop yield differences between treated and control plots. In wheat, disease only developed after the last two *C.g.m.* applications, July 16 and 30, and the disease ratings of *M. pusilla* plants were significantly greater than with the first application. Temperatures (during dew and following application) were cooler and overcast conditions prevailed after these applications (Table 1). However, infections developed slowly and no crop yield differences were observed between treated and untreated wheat plots.

Table 1. Moisture and temperature conditions at time of application and for the next 48 h, at Indian Head in 1985 and 1986, and at Regina in 1986.

Site / Parameter	Dates			
Indian Head 1985	July 5	July 16	July 30	
Dew period: hrs/temp. (°C)				
night 1	2 / 15	6 / 9	4 / 10	
night 2	8 / 15	9 / 11	4 / 15	
Precipitation (mm)	1.0	0.2	0.0	
Sky	hot clear	overcast	partly cloudy	
Average day/night temp. (°C)	35 / 15	25 / 10	30 / 15	
Indian Head 1986	June 11	June 25	July 9	July 23
Dew period: hrs/temp. (°C)				
night 1	na / 11[1]	5 / 15	15 / 15	10 / 12
night 2	na / 8	na / 10	12 / 15	10 / 10
Precipitation (mm)	0.8	0.0	35.2	0.4
Average day/night temp. (°C)	26 / 10	28 / 15	25 / 15	30 / 10
Regina 1986	July 8	July 23	August 5	
Dew period: hrs/temp. (°C)				
night 1	12 / 12	12 / 14	12 / 5	
Precipitation (mm)	0.8	2.2	4.4	
Sky	overcast	overcast	partly cloudy	
Average day/night temp. (°C)	25 / 12	25 / 13	25 / 7	

[1] na = Not available due to equipment malfunction.

In 1986 at Indian Head, the treatment date did not affect yield (seed or total dry weight) of wheat or lentil (Figure 1A and 1C). However, in both wheat and lentil, the third *C.g.m.*

application July 9 significantly reduced *M. pusilla* total dry weight and capsule production. Disease incidence 14 d after application and at harvest was significantly greater than after the other treatments (Figure 1B and 1D). Moisture and temperature conditions were optimal for disease development with rain and overcast skies for a week following the July 9 application (Table 1). This third application of *C.g.m.* was the only treatment resulting in severe disease development 14 d after application. However, it developed too late in the season to affect crop yield. Competition from *M. pusilla* had already caused crop yield losses. At harvest, all plots were infected with *C.g.m.*, even the untreated control plots. Secondary infections occurred in the latter part of the growing season when favourable moisture conditions prevailed.

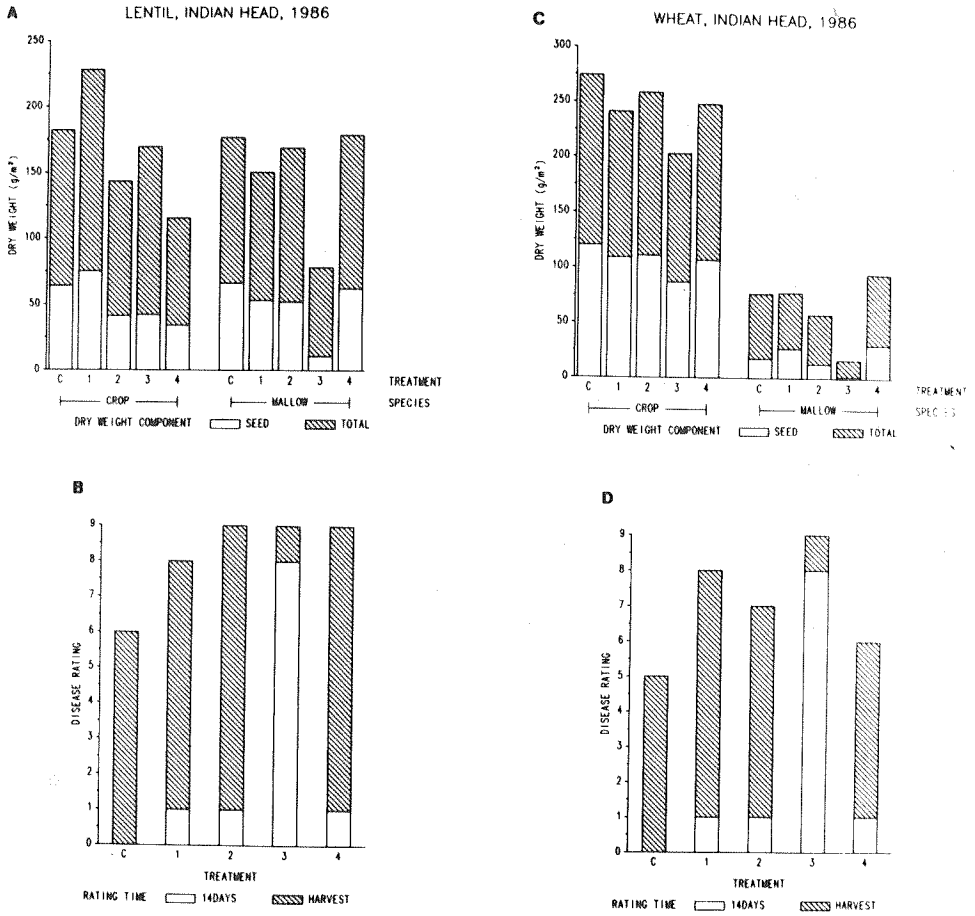

Figure 1. Effect of application date of *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *malvae* on crop and weed yield components in lentil (A) and wheat (C) at Indian Head in 1986 and disease rating on *Malva pusilla* Sm. in lentil (B) and wheat (D). Treatments represent a control (C) and applications on June 11 (1), June 25 (2), July 9 (3), and July 23 (4). Ratings are based on a scale from 0 to 9 where 0 = no symptoms, 9 = >95% of plant material killed.

In 1986 at Regina, the different treatments did not affect yield (seed or total dry weight) of wheat or lentil (Figure 2A and 2C). *M. pusilla* plants emerged before the crop, causing poor crop emergence and yield losses before *C.g.m.* applications. However, *C.g.m.* significantly reduced *M. pusilla* total dry weight and capsule production after the first application in lentil and the first and second application in wheat. The disease developed within 14 d of all three

applications due to a minimum 12 h of dew provided by the plastic covering. However, the most rapid development occurred after the first two applications (Figure 2B and 2D). Moisture and temperature were optimal for disease development at time of application (Table 1) as well as following application with 38 mm of rain during the week following the first application July 8, and 15 mm following the second application July 23. At harvest, all plots at Regina were infected with *C.g.m.*, even the untreated control plots. Spread of the disease again had occurred in the latter part of the growing season when favourable moisture conditions prevailed.

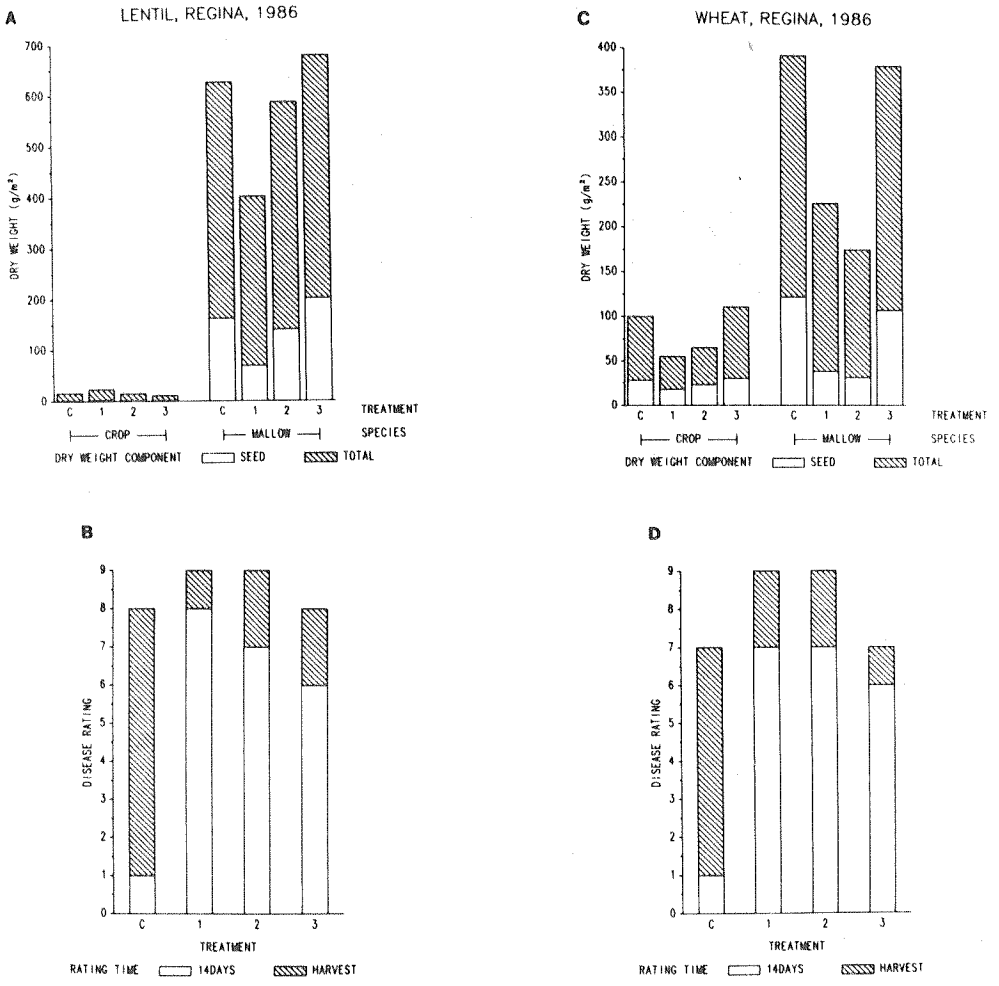

Figure 2. Effect of application date of *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *malvae* on crop and weed yield components in lentil (A) and wheat (C) at Regina in 1986 and disease rating on *Malva pusilla* Sm. in lentil (B) and wheat (D). Treatments represent a control (C) and applications on July 8 (1), July 23 (2), and August 5 (3). Ratings are based on a scale from 0 to 9 where 0 = no symptoms, 9 = >95% of plant material killed.

There were significant differences in wheat densities between plots at Indian Head in 1985 and at Regina in 1986. However, an analysis of covariance confirmed that these differences in density did not significantly affect yield components.

Experiment 2: Effect of Competition and Crop Canopy on Disease Development

In 1985, there were no significant differences in yield of seed or total dry weight of lentil or wheat between plots sprayed with *C.g.m.* and untreated control plots (Figure 3A and 3D). In sprayed plots, the disease developed slowly with a rating of only 2 (scale 0-9) in wheat plots and 5 in lentil plots 14 d following application (Figure 3C and 3F). Temperatures during the dew period after application were cool (5°C) with only limited rain (4 mm) occurring the week following application. However, at harvest, *M. pusilla* plants in sprayed plots were heavily infected with *C.g.m. M. pusilla* total dry weight was significantly reduced in sprayed plots in both lentil and wheat (except at the double density where differences were not significant) and capsule production was significantly reduced in lentil (Figure 3B and 3E).

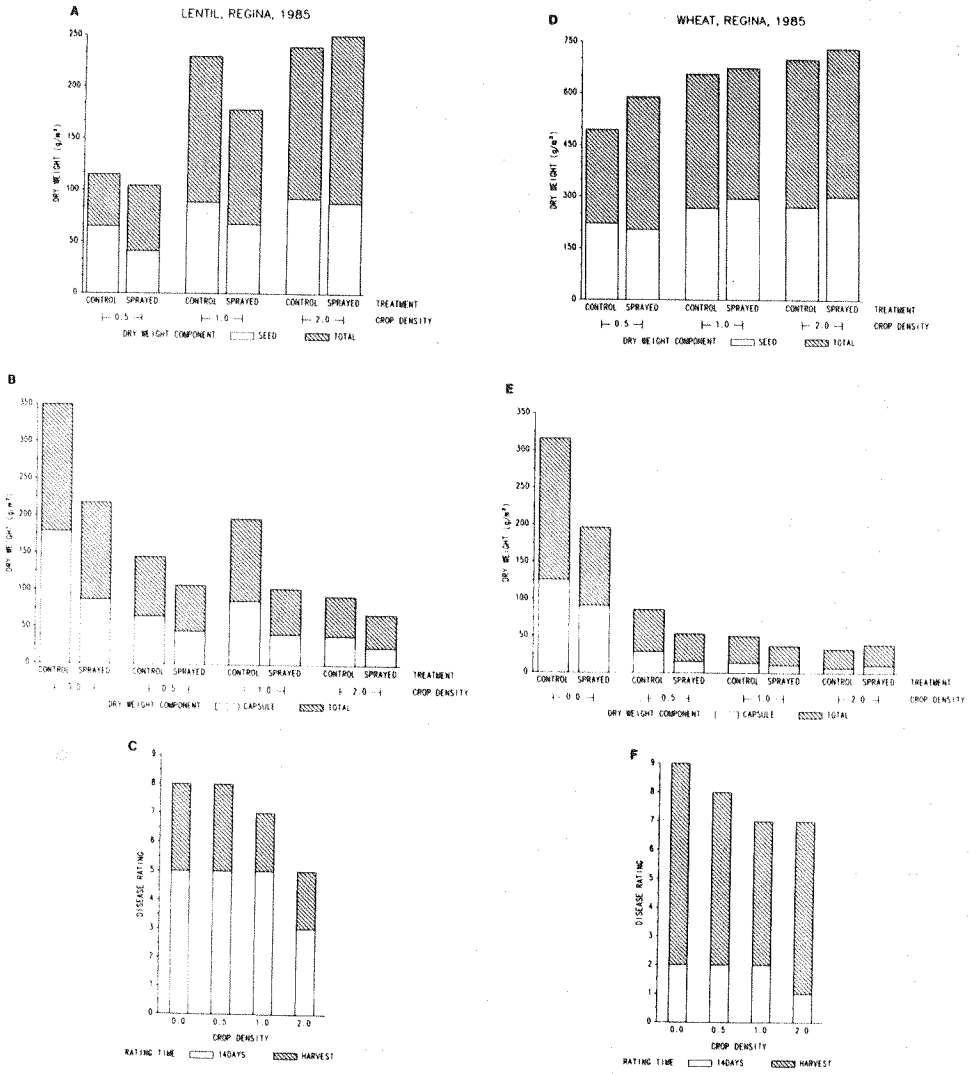

Figure 3. Effect of competition and crop canopy on disease development at Regina in 1985 presented as yield components of lentil (A), wheat (D), and *Malva pusilla* Sm. in lentil (B) and in wheat (E), and on disease rating of *M. pusilla* in lentil (C) and wheat (F) in sprayed plots. Crop densities are expressed as a factor normal seeding rate. Ratings are based on a scale from 0 to 9 where 0 = no symptom, 9 = >95% of plant material killed.

In 1985, there were significant differences in lentil and wheat yield between plots seeded at half rate and those seeded at normal rate, but not between plots seeded at normal rate and those seeded at twice the normal rate (Figure 3A and 3D). The various crop densities had no significant effect on *M. pusilla* yield components (Figure 3B and 3E). However, total dry weight and capsule production of *M. pusilla* were significantly greater in plots without any crop than those with crop. No infections were observed in any of the untreated plots. Disease development was not affected by the presence or the amount of crop canopy (Figure 3C and 3F).

In 1986, both lentil and wheat seed and total dry weights were significantly greater in plots that were sprayed with *C.g.m.* than in the control plots (Figure 4A and 4D). Applications of *C.g.m.* significantly reduced total dry weight and capsule production of *M. pusilla* in plots with wheat, lentil, and no crop (Figure 4B and 4E). Disease development was very rapid with a rating of 8, 14 d following application (Figure 4C and 4F). Control plots showed signs of infection 14 d after application and were severely infected with *C.g.m.* at harvest. Moisture and temperature conditions were optimal for disease development. Almost continuous moisture prevailed the week following application with 24 mm of rain being recorded.

In 1986, there were no significant differences in crop yield in either lentil or wheat between plots with different crop densities. There were no differences in total dry weight or capsule production of *M. pusilla* between any of the plots with or without crop. *M. pusilla* was responsible for decreased crop emergence in 1986 resulting in a poor crop stand. Total dry weight and capsule production of *M. pusilla* were not reduced due to competition in plots with crop as occurred in 1985. Similar to the 1985 growing season, disease development was not affected by the presence or amount of crop canopy.

Discussion and Conclusions

The most important factors responsible for successful control of *M. pusilla* with *C.g.m.* in the field were moisture and temperature conditions following application. The following conditions resulted in control of *M. pusilla*: 12 to 15 h of dew following application, precipitation greater than 6 mm within 48 h after application coupled with cool temperatures (about 20°C), and overcast skies. When hot (temperatures > 28°C), dry conditions prevailed after application, infections did not develop (Mortensen and Makowski 1989). The conditions resulting in successful control correspond to the optimal temperature and moisture requirements established under controlled environments (Makowski 1987): a minimum of 20 h dew or a 16 h dew on two consecutive nights, temperatures during dew and incubation around 20°C or a day/night temperature regime around 25/15°C.

Only a dense crop stand provided sufficient crop competition to reduce biomass and capsule production of *M. pusilla*. In the semi-arid Saskatchewan climate, the combined stresses of competition and disease did not increase control of *M. pusilla* over the disease alone. Crop canopy did not provide a better microclimate for disease development and did not impede penetration of *C.g.m.* to the target weed, *M. pusilla*. In a less arid climate such as in Manitoba where rainfall can be double that of Saskatchewan, the crop canopy may provide a better microclimate with longer dew periods and greater competition due to the denser stands from better soils and growing conditions. *M. pusilla* is also a much greater weed problem in Manitoba than in Saskatchewan (Makowski and Morrison 1989). Nevertheless, when conditions were favourable for infection, *C.g.m.* developed equally on *M. pusilla* in crop or alone. Thus, *M. pusilla* growing alone or in gardens and farmyards could be successfully controlled with *C.g.m.* with favourable moisture conditions.

M. pusilla continues to emerge during the entire growing season (Makowski 1987). With favourable moisture conditions, secondary infection of *C.g.m.* will control emerging plants throughout the growing season as also reported by Daniel *et al.* (1973) with *C. gloeosporioides* f. sp. *aeschynomene*, a bioherbicide for control of northern jointvetch, *Aeschynomeme virginica* (L.) B.S.P. (Fabaceae).

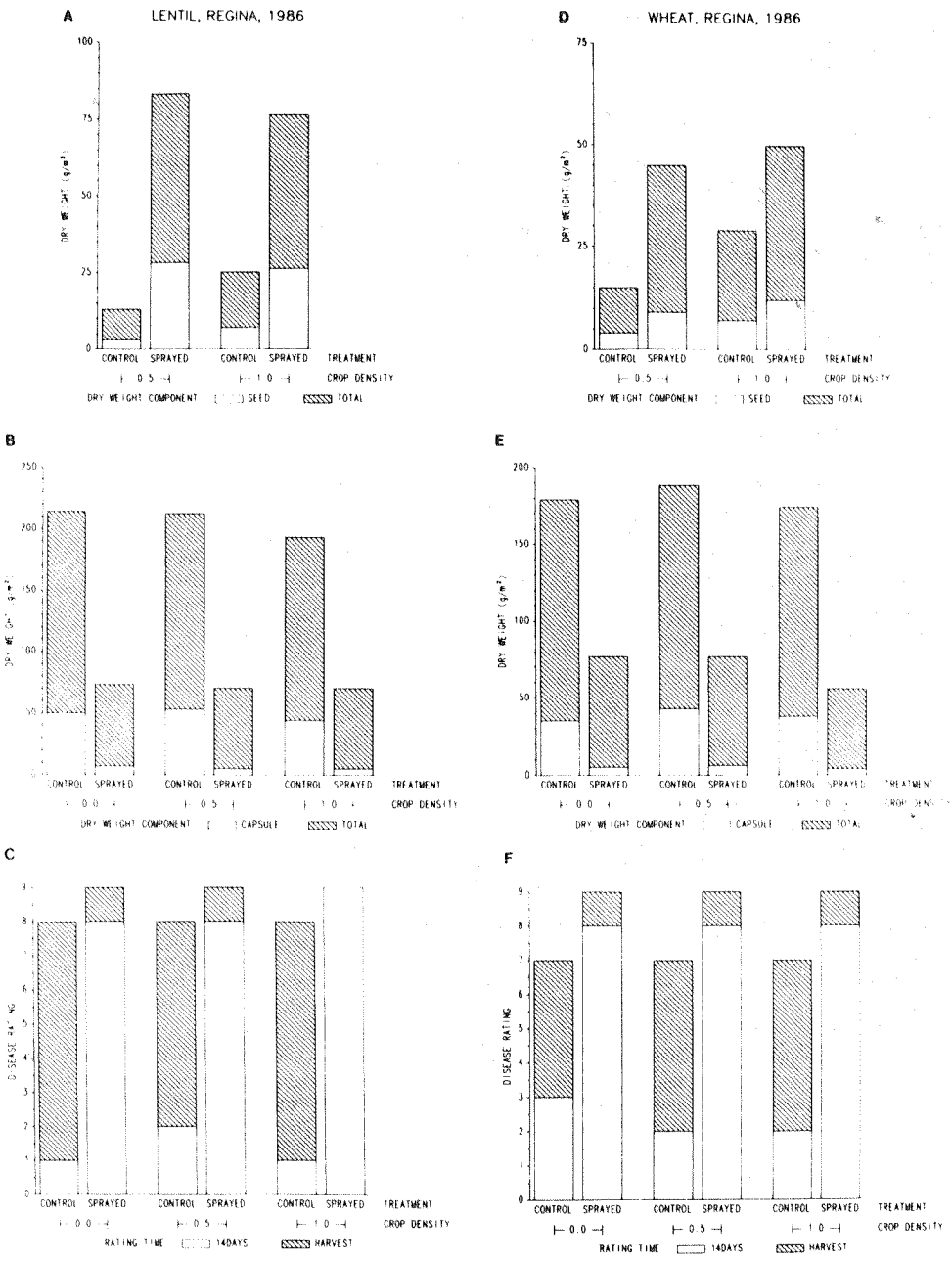

Figure 4. Effect of competition and crop canopy on disease development at Regina in 1986 presented as yield components of lentil (A), wheat (D), and *Malva pusilla* Sm. in lentil (B) and in wheat (E), and on disease rating of *M. pusilla* in lentil (C) and wheat (F). Crop densities are expressed as a factor of the normal seeding rate. Ratings are based on a scale from 0 to 9 where 0 = no symptoms, 9 = >95% of plant material killed.

M. pusilla, when well established, can cause significant yield reductions (Makowski 1987, Makowski and Morrison 1989). In 1985, little *M. pusilla* was present due to the spring seeding of the *M. pusilla* capsules. As reported by Makowski (1987), seed of *M. pusilla*

require at least one winter before their seed coat becomes permeable and they can germinate. Disease also developed slowly because of the hot and dry conditions, thus, no significant crop yield differences were observed. However, when *M. pusilla* was well established, the most important factors responsible for improved crop yields was early application of *C.g.m.* coupled with favourable weather conditions for rapid disease development and *M. pusilla* kill, as in Regina in 1986. From the present field trials, when disease development was rapid, applications in June resulted in increased crop yield. Applications in July and August were less beneficial as damage from *M. pusilla* competition had already occurred.

However, these late applications resulted in decreased *M. pusilla* biomass and capsule production at harvest and after harvest when healthy *M. pusilla* would continue to grow and produce seed even after the first few frosts. Thus, even late disease development reduces the amount of *M. pusilla* seed entering the seed bank decreasing future weed problems and would control plants of *M. pusilla* otherwise remaining healthy and green that interfere with machinery and harvesting operations.

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