Effect of *Microsphaeropsis* sp. on the Production of Perithecia and Ascospores of *Gibberella zeae*

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ABSTRACT

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The potential of *Microsphaeropsis* sp. (isolate P130A) as an antagonist of *Gibberella zeae* was tested under in vitro and field conditions. Firstly, an in vitro method of ascospore production was developed on wheat and corn residues. The plant type (corn or wheat), residue type (straw/stalk or grain), and incubation conditions (closed or open) had a significant effect on ascospore production. Perithecia were more abundant on wheat and corn grain incubated under open conditions. On these two substrates, the application of *Microsphaeropsis* sp. significantly reduced ascospore production. On wheat, the antagonist had a significant effect when applied 2 weeks before (-2), at the same time (0), and 4 weeks after (+4) inoculation with *G. zeae*, with 1.73, 0.31, 1.11, and 1.36 log ascospores per cm² for the control, -2, 0, and +4 weeks treatments, respectively. On corn, *Microsphaeropsis* sp. had a significant effect when applied 2 weeks before, at the same time, 4 weeks after, and 6 weeks after inoculation with *G. zeae* with 3.02, 0.23, 1.29, 2.35, and 2.22 log ascospores per cm² for the control, -2, 0, +4, and +6 weeks treatments, respectively. When applied to crop residues in the field as postharvest or preplanting applications, *Microsphaeropsis* sp. had no effect on the pattern of perithecia maturation, but significantly reduced the number of perithecia produced on two sampling dates, May 1998 and July 1999. There is a potential to biologically reduce the initial inoculum of *G. zeae*; however, more work is needed to optimize the efficacy of the biocontrol agent.

Gibberella zeae (Schwein.) Petch (anamorph: *Fusarium graminearum* Schwabe) is known to be the causal agent of several cereal diseases, including Fusarium head blight (or scab) of wheat (FHB) and *Gibberella* ear rot of corn. The economic implications of these diseases are a worldwide concern, especially in Canada and the United States where several epidemics have occurred over the last decade. In 1996, in Ontario (Canada), 30% of the winter wheat crop was lost due to the disease. With all economic factors taken into account, losses for the 1996 crop were estimated to be over \$100 million (Cdn\$) for Ontario alone (29). In 1993, North Dakota, South Dakota, and Minnesota experienced an epidemic that caused losses of over \$1 billion (US\$) (21). *Gibberella* ear rot of corn has caused chronic problems over the last several decades. In 1990,

an epidemic in New York State left 40% of the corn crop unharvested, equaling a loss of \$12 million (US\$) (4).

Currently, there are no efficient methods for the control of Fusarium head blight (FHB) or *Gibberella* ear rot. Some fungicides have been registered for the control of FHB, but even if these chemicals are effective in limiting infection, reductions in disease severity are not always accompanied by a reduction of deoxynivalenol levels (12,19,22,30). It has been demonstrated that cultural control methods, such as crop rotation and residue burial, can markedly reduce the level of initial inoculum (7,16,32). However, reduced tillage offers many agronomic advantages, such as reduced energy costs and soil conservation; consequently, producers are unlikely to change their practices.

Biological control of FHB has been investigated over the past 10 years, with two major approaches being reported in the literature. The potential of *Bacillus subtilis*, *Bacillus* sp., *Sporobolomyces roseus*, and *Streptomyces* sp. sprayed on wheat, at or close to anthesis, was studied (5,11,20,25). Different researchers (14,15,23; N. I. Khan, D. A. Schisler, M. J. Boehm, P. J. Slininger, and R. J. Bothast, unpublished) investigated the potential of choline-utilizing bacteria as antagonists. To our knowledge, there was only one study on the possibility of applying the antagonist on crop residues with the aim of reducing primary inoculum during the saprophytic

stage (9). Biological control through the interruption of the saprophytic stage of *G. zeae* has several major advantages. Fusarium head blight and *Gibberella* ear rot are both monocyclic diseases, and the main source of inoculum comes from infected residues that are left on the soil surface during harvest. The pathogen can overwinter on these residues for a long period of time, even under adverse conditions. Consequently, the use of a biocontrol agent adapted to winter conditions and capable of significantly decreasing the level of initial inoculum on crop residues would be a useful tool in an integrated pest management system.

This approach has been successfully employed for the apple scab pathogen *Venturia inaequalis* using a fall treatment of *Microsphaeropsis* sp. (isolate P130A) on diseased apple leaves. *V. inaequalis* produced pseudothecia in the fall that overwinter and mature in the spring, as opposed to *G. zeae*, which produces ephemeral perithecia. FHB and *Gibberella* ear rot are similar to apple scab because the major inoculum source comes from melanized structures, such as pseudothecia or perithecia, produced on crop residues. In addition, the antagonist *Microsphaeropsis* sp. is a saprophyte, well adapted to winter conditions (3,28). It is believed that the similarities between the two disease systems make *Microsphaeropsis* sp. (isolate P130A) a good candidate for use as a biocontrol agent to decrease the inoculum level of *G. zeae* on crop residues.

There is, to our knowledge, no reliable in vitro method published for the evaluation of biocontrol agents against *G. zeae* during its saprophytic stage. Therefore, a method for producing perithecia and ascospores of *G. zeae* on residues of wheat and corn under in vitro conditions is needed as the first step in evaluating biocontrol agents. Currently, few methods have been described for the in vitro production of *G. zeae* perithecia. However, none of these methods is appropriate for the evaluation of biocontrol agents. The most widely known method is based on the use of carnation leaves placed on water agar and was developed by Tschanz et al. (34). This method does not involve wheat or corn as substrate and fails to share similarities with field conditions. Khong and Sutton (17) developed another method using wheat and corn pieces, but it was not reproducible under the conditions of our experiment. Finally, Jin and Zhang (13) reported a third

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method using oat grains as substrate. The method, although reliable, does not use wheat and corn residues, the substrates to be tested.

The objectives of this study were: (i) to find appropriate substrates and incubation conditions for the *in vitro* production of perithecia and ascospores of *G. zeae* on wheat and corn residues and; (ii) to evaluate the potential of *Microsphaeropsis* sp. (isolate P130A) to reduce ascospore production by *G. zeae* under *in vitro* and field conditions.

MATERIALS AND METHODS

Perithecial production. Conidia of *Fusarium graminearum* (anamorph of *G. zeae*) (strain DAOM 178148) were produced in tomato and salt broth (100 g of tomatoes and 16 g of NaCl per liter of distilled water). The broth (1.5 liters) was inoculated with the entire contents of two petri dishes of *F. graminearum* grown on potato dextrose agar for 2 weeks. Sterile air, introduced into the medium by a tubing system equipped with air filters, was used to oxygenate the broth. The flasks were incubated under black light and cool-white fluorescent light (F40 BLB:F40 CW, 2:2) (General Electric). After 7 to 10 days, the broth turned pink, a sign of a high macroconidia concentration. The broth was then aseptically filtered through cheesecloth, leaving only the conidial suspension. The conidial concentration was assessed using a Fuch's Rosenthal hemacytometer.

Different types of residues were evaluated for their ability to support *in vitro* perithecia production of *G. zeae*. These types of residues were: wheat straw, corn-stalk, wheat grain, and corn kernel. The wheat residues originated from the cultivar Roblin, while the corn was produced from Pioneer hybrid 3893. Six pieces of autoclaved residues (3 cm long) were placed in petri dishes and inoculated with 200 μ l of a conidial suspension of *F. graminearum* DAOM 178148 at a concentration of 5×10^4 spores per milliliter. The petri dishes containing the residues were sealed with Parafilm and incubated under sterile conditions on a bench top ($22^\circ\text{C} \pm 1^\circ\text{C}$) for 3 weeks to allow mycelial growth.

After colonization, residues were transferred to new sterile petri dishes containing sterile vermiculite (about 3 g per dish) and moistened with sterile distilled water. They were then incubated under near fluorescent UV light (F40 BLB:F40 CW, 2:2) (General Electric) for 3 weeks, or until the production of mature perithecia and ascospores on the residues. The vermiculite was periodically remoistened (13). During this step, depending on treatment, the petri dish was closed (but not sealed with Parafilm) or open (lid removed).

Ascospore trapping was done by re-wetting the vermiculite in all the petri dishes, placing a 25×75 mm microscope slide greased with silicon 10 mm above the

bottom of the petri dish, and finally putting the petri dish lid on top. The residues and vermiculite were allowed to dry out and then were re-wetted 4 days later. This period of humidity followed by 4 days of drought was considered an ejection cycle. The slides were left in place for 2 to 3 cycles of optimal ejection conditions, until there was abundant spore ejection on the slides or until the perithecia were empty (determined by destructively sampling extra controls). Estimation of the number of ascospores produced in each treatment was done by counting 8 transects of 50 mm on the microscope slide (30% of the slide surface). When, in certain fields, the number of ascospores was too high to be counted, the number was estimated using a scale function of uncountable surface area, ascospore size, and density of the aggregation.

The experiment was performed as a $2 \times 2 \times 2$ factorial design with blocks. It consisted of 3 factors (plant type, residue type and incubation conditions), each one with two levels, and 15 blocks per trial. Levels of plant type were wheat and corn. Levels of residue type were grain and stalks, and the levels of incubation conditions were open and closed. The whole experiment was conducted twice. Data were analyzed using the Friedman two-way analysis of variance by ranks and the multiple comparison procedure for use with the Friedman test (6).

Evaluation of the antagonist. The pathogen was produced and established on crop residues following the methodology described in the previous experiment; however, only one combination of factors was used for each substrate. This combination of factors was selected based on the analysis of the results from the perithecia production experiment based on reproducibility (variation among blocks) and on spore production. For both corn and wheat, *G. zeae* was established under open conditions, on cornstalks and on wheat straw. Frozen spores (-80°C) of *Microsphaeropsis* sp. (isolate P130A) (provided by Bios Philom, Saskatoon, Canada) were used for inoculation. The antagonist was inoculated on residues at different time intervals relative to pathogen application at a concentration of 5.5×10^5 spores per milliliter. Antagonist application times were 2 weeks before *G. zeae* (-2), at the same time as *G. zeae* (0), and at 2, 4, and 6 weeks after pathogen application (+2, +4, +6).

The petri dishes were incubated under the same conditions as in the *in vitro* perithecia production experiment. Ascospore trapping was performed using the methodology described in the previous experiment. To evaluate ascospore concentrations, the total ascospore number of 8 complete transects (50 mm) was counted on each slide. If ascospore concentration was too high for reliable counting, the area and/or microscope magnification was

modified. For each treatment, the total area counted was recorded and used as covariable in the statistical analysis. The covariable for a slide on which 8 complete transects (30%) were counted was equal to 1. The experimental design was a randomized complete design with 6 residues per sampling unit, 5 replicates per treatment, and 7 treatments per trial. The treatments were not treated (control), inoculated with *G. zeae* alone (*G. zeae*), inoculated with *G. zeae* and *Microsphaeropsis* sp. 2 weeks before *G. zeae* (-2), at the same time as *G. zeae* (0), and at 2, 4, and 6 weeks after pathogen application (+2, +4, +6).

The whole experiment was conducted twice. The effect of the covariable on the dependent variable was tested with an analysis of variance, and data were analyzed with an analysis of covariance. LSD tests were used to detect significant differences among treatment means.

Field trials. To produce infected debris, in the spring of 1997 and 1998, wheat (*Triticum aestivum*) cv. Roblin was sown in 2×2 m plots. Before anthesis at growth stage 45 in Zadoks's scale (35), corn kernels (Pioneer hybrid 3893) infected with *F. graminearum* DAOM 178148 were dispersed in the plots at a rate of 4 liters of grain per plot. The heads were also sprayed until runoff with a conidial suspension of *F. graminearum* DAOM 178148 adjusted to 50,000 spores per milliliter. In the first trial, they were sprayed at anthesis (growth stage 69 in Zadoks's scale) and a week later. In the second trial, the heads were sprayed only once, at anthesis. About 2 weeks after anthesis, disease severity was assessed by counting the number of infected spikelets and the total number of spikelets on 160 heads in each plot. This assessment was done to check the uniformity of disease among the different locations before treating the plots with the antagonist in the fall. Hand harvesting took place on 5 September 1997 for the first trial and on 10 September 1998 for the second trial.

The residues from the plots were treated with the antagonist according to the following four treatments: control (no application), fall application, spring application, and fall+spring applications. The antagonist was applied on the residues at a rate of 10^{12} spores per hectare. The fall application was made soon after harvest (15 September 1997 for the first trial and 17 September 1998 for the second trial). The spring application was made after snow had completely melted (24 April 1998 for the first trial and 13 May 1999 for the second trial). The spore suspension was applied using a gasoline powered Solo backpack sprayer (model 422, Solo Sindelfingen, Newport News, VA) and a hand-held gun mounted with a single nozzle with a 80° flat spray tip operated at 620 kPa with a flow rate of approx. 2.2 liters/min. To evaluate the effect of the antagonist on debris-borne inoculum, 20 g of

residue was put in 15 × 15 cm mesh sachets fixed on the soil surface using metal sticks. A sufficient number of sachets for each plot was made to enable a once monthly recovery of plant material during December and from March to July.

During the next season, the pathogen incidence (presence of the pathogen on the residues) was assessed monthly from April to July based on 25 residue pieces randomly chosen in a sachet from each plot. The crop residues were surface-sterilized for 6 min in 0.05% NaOCl and rinsed for 15 min in sterile distilled water. They were dried in a laminar flow hood and plated on the semi-selective medium (8) for *F. graminearum*. On this medium, *F. graminearum* DAOM 178148 produced a characteristic yellow pigment, differentiating it from other *Fusarium* strains and species (8). Using the residues from the sachets, the number of perithecia produced was estimated. Data for two types of residues were collected from the sachets: straw and spikelets. Perithecia counts were separated into immature (beige to partially blue in color) or mature (purple-black color). For the straw, estimation was done by counting 10 dissecting scope fields at ×30 (7 mm in diameter) on 10 randomly chosen pieces of straw. For spikelets, 10 heads were randomly picked, and one spikelet was randomly chosen on each of these heads. Perithecia were counted on the entire surface (two sides) of the spikelet. The perithecia maturity was evaluated by squashing five randomly chosen perithecia on 10 debris pieces. These 50 perithecia were evaluated based on a maturity scale (Table 1). The experiment was conducted as a randomized complete block design consisting of four treatments per block and three blocks per trial. The whole experiment was conducted twice (in 1997-98 and 1998-99). The data for disease severity, pathogen incidence, and number of perithecia were analyzed with ANOVA and LSD tests. The perithecia maturation data were analyzed with the chi-square test for goodness-of-fit.

RESULTS

Perithecial production. Results from this experiment demonstrated that all the factors tested had a significant effect ($P < 0.05$) on ascospore production (Table 2). Ascospore production was significantly higher on wheat in comparison to corn residues ($P < 0.05$) (Table 2). Ascospores were significantly less abundant on stalk than on grain residues ($P < 0.05$) (Table 2). The effect of incubation conditions was also significant ($P < 0.05$), with more ascospores being produced under open conditions (Table 2). Corn kernel under open conditions and wheat grain under open conditions gave a significantly ($P < 0.05$) higher production than the other treatment combinations (Table 3). There was a significant interaction between the

plant type and the incubation conditions ($P < 0.05$).

Evaluation of the antagonist. On both substrates, the application of the biocontrol agent *Microsphaeropsis* sp. (isolate P130A) had a significant effect on ascospore production (Fig. 1). On wheat, the effect was significant ($P < 0.05$) for the treatments (−2) weeks, (0) week, and (+4) weeks with, respectively, 0.31, 1.11, and 1.36 log ascospores per cm² of substrate produced in comparison to the positive control (1.73 ascospores per cm² of substrate). When the antagonist was applied 2 weeks before the pathogen, the number of ascospores was significantly lower ($P < 0.05$) than for the positive control (pathogen alone) but was not significantly different ($P < 0.05$) from the negative control (antagonist alone). When the antagonist was applied at the same time as the pathogen, the number of ascospores produced was significantly different ($P < 0.05$) from the number of ascospores produced when the biological control agent was applied later. On wheat, the number of ascospores produced decreased as the time between the pathogen and the antagonist increased. However, (+2) and (+6) weeks treatments were not significantly different ($P < 0.05$) from the positive control (pathogen alone) (Fig. 1A).

On corn, a significant effect ($P < 0.05$) of the antagonist was observed for the treatments (−2), (0), (+4), and (+6) weeks

with, respectively, 0.23, 1.29, 2.35, and 2.22 log ascospores per cm² of substrate in comparison to the positive control (3.02 ascospores per cm² of substrate). At (−2) weeks, the number of ascospores was significantly lower ($P < 0.05$) than the positive control (pathogen alone) but was not significantly different ($P < 0.05$) from the negative control (antagonist alone). At (0) week, a significant effect was observed, with an ascospore production significantly lower ($P < 0.05$) than the production with later applications of the antagonist (Fig. 1B).

Field trials. In the first trial, disease severity in each plot varied from 51.5 to 80.7%, while in the second trial it varied between 56.7 and 87.6% in the plots prior to the application of the antagonist. In both trials, a significant difference ($P < 0.01$) was observed among the plots. In the plots used to produce the infected residues, 64 and 55% of the spikelets showed FHB symptoms in the first and second trial, respectively. Both years, the antagonist had no significant effect ($P < 0.05$) on the pathogen incidence on crop residues. Percentage of pathogen recovery was high and varied from 89 to 100% in 1997-98 and from 86 to 100% in 1998-99 (data not shown).

Microsphaeropsis sp. had a significant effect, in both May and June 1998, on immature and mature perithecia formed on spikelets (Fig. 2C and D). In May, the fall

Table 1. Modified Paulitz's scale for *Gibberella zeae* perithecial maturity evaluation

Maturity stage	Description
1.0	Beige
1.5	Partially light blue
2.0	All light blue
2.5	Dark blue
3.0	Purple-black in color without asci or ascospores
4.0	Purple-black in color with asci but without mature ascospores
5.0	Purple-black in color with asci and mature ascospores
6.0	Purple-black in color without asci, empty or ascospores of irregular appearance (shrunken or swollen)

Table 2. Effect of plant type, debris type, and incubation conditions on ascospore production by *Gibberella zeae* on crop residues under in vitro conditions

Plant	Ascospore (×10 ⁵) per cm ² substrate ^z	Debris	Ascospore (×10 ⁵) per cm ² substrate ^z	Incubation	Ascospore (×10 ⁵) per cm ² substrate ^z
Corn	5.77 a	Stalk	2.49 a	Open	15.81 b
Wheat	13.89 b	Grain	17.18 b	Closed	3.84 a

^z Values within the same column with the same letter are not significantly different according to the Friedman test at the 0.05 level of confidence.

Table 3. Effect of treatment combinations on the number of ascospores of *Gibberella zeae* produced under in vitro conditions on wheat and on corn

Treatments	Ascospore (×10 ⁵) per cm ² of substrate ^z	Treatments	Ascospore (×10 ⁵) per cm ² of substrate ^z
Grain-closed	0.48 a	Kernel-closed	3.22 a
Grain-open	3.71 b	Kernel-open	9.70 b
Straw-closed	0.07 a	Stalk-closed	0.07 a
Straw-open	1.45 a	Stalk-open	0.89 a

^z Values within the same column with the same letter are not significantly different according to the Friedman test at the 0.05 level of confidence.

and fall+spring applications significantly reduced ($P < 0.05$) both immature and mature perithecia numbers. The spring applications had a significant impact ($P < 0.05$) on mature perithecia only. In 1999, the antagonist had a significant effect on perithecia production ($P < 0.05$) in April and July (Fig. 2). In April, on spikelets, significantly higher numbers ($P < 0.05$) of immature and mature perithecia were observed in the fall treatment in comparison with other treatments, including the control (Fig. 2C and D). In July, a significant effect ($P < 0.05$) was observed on all residue types and at all maturity levels. The fall application significantly reduced ($P < 0.05$) the number of perithecia under all conditions. On straw, the fall+spring appli-

cations had a significant effect ($P < 0.05$) on the number of immature and mature perithecia (Fig. 2A and B). On spikelets, the spring application had a significant effect ($P < 0.05$) on both mature and immature perithecia (Fig. 2C and D). In 1998 and 1999, the application of *Microsphaeropsis* sp. had no significant effect ($P < 0.05$) on frequency distributions of maturity stages of *G. zeae* perithecia at any of the sampling dates (Figs. 3 and 4).

DISCUSSION

In this study, an in vitro method for the production of perithecia and ascospores of *G. zeae* on wheat and corn residues was developed. Ascospores were produced on both types of residues and under all the

incubation conditions tested, although variations in productivity were observed. Differences in productivity can be explained by a number of reasons, including the C:N ratio of the residues and variations in relative humidity and moisture inside the residue. In the closed environment, the residues took twice as long to dry in comparison to the open environment. Thus, one possible explanation is that alternating high relative humidity and drought periods could trigger perithecia and ascospore production. Preliminary studies confirmed that cycles of moisture and drought were necessary for consistent and sufficient ascospore ejection (24).

The method of perithecia and ascospore production developed offers several advantages in comparison to the other methods available. First, it more closely simulates field conditions than methods using agar media (34). Consequently, it would be a better predictor of interactions that could occur under field conditions. Secondly, the method has been successfully experimented with using several types of residues and incubation conditions, giving it flexibility for different in vitro tests. Finally, although the production of perithecia and ascospores with this method is slower than for other techniques (13,18,34), we believe that its consistency and flexibility make it an interesting new tool for the evaluation of biocontrol agents against *G. zeae*. The results of the first experiment made possible the selection of two combinations of factors for the experiment used to evaluate the potential antagonist *Microsphaeropsis* sp. These factors were cornstalks under open conditions and wheat straw under open conditions. These substrates were selected for their sufficient and consistent ascospore production. On these substrates, ascospore production was high but still countable.

The reduction in ascospore production observed after treatment with *Microsphaeropsis* is in accordance with the results of other tests conducted with *Microsphaeropsis* sp. (isolate P130A) with melanized fungal structures. Philion et al. (28) reported that on leaf disks, *Microsphaeropsis* sp. (isolate P130A) inhibited more than 98% of the ascospore production by *V. inaequalis*, the causal agent of apple scab. Our results are similar to those reported for the antagonist *Limonomyces roseipellis* against the tan spot pathogen *Pyrenophora tritici-repentis* (26,27), where early applications of the antagonist provided a better control. The same author reported that an application of the antagonist made more than 4 weeks after harvest was not effective. Similarly, Fernandez (9) reported that early applications of *Trichoderma harzianum* allowed the antagonist to occupy the residue before the decomposition process took place and before the establishment of saprophytes.

Fig. 1. Effect of *Microsphaeropsis* sp. (isolate P130A) on in vitro ascospore production by *Gibberella zeae* as a function of timing of application, A, on wheat, and B, on corn residues. The timings of application were not treated (control), inoculated with *G. zeae* alone (*G. zeae*), inoculated with *G. zeae* and *Microsphaeropsis* sp. 2 weeks before *G. zeae* (-2), at the same time as *G. zeae* (0), and at 2, 4, and 6 weeks after pathogen application (+2, +4, +6). Values of the bars with the same letter are not significantly different according to the LSD test at the 0.05 level of confidence.

Antagonistic mechanisms other than competition may be involved. In 1998, Benyagoub et al. suggested that *Microsphaeropsis* sp. (isolate P130A) could interfere with the maturation of perithecia, since the antagonist was thought to be

attracted to melanized fungal structures such as pseudothecia or perithecia (1). The antagonist may have also used competition or antibiosis, these capacities having been demonstrated by *Microsphaeropsis* sp. toward other fungi (1). For further biocon-

trol field studies, this in vitro experiment suggests that the antagonist would have to be applied as soon as possible after harvest for maximizing ascospore reduction.

In this study, the antagonist *Microsphaeropsis* sp. was tested under severe

Fig. 2. Effect of *Microsphaeropsis* sp. (isolate P130A) on the number of perithecia produced by *Gibberella zeae* on different wheat residues in 1998 (left) and 1999 (right). A, Immature perithecia on straw, B, mature perithecia on straw, C, immature perithecia on spikelet, and D, mature perithecia on spikelet. Values of the bars with the same letter are not significantly different according to the LSD test at the 0.05 level of confidence.

epidemic conditions. When Fernandez (9) tested an isolate of *Trichoderma harzianum* under field conditions, inoculum level was lower than in our experiment. In this experiment, 36% of crop residues were infected by *F. graminearum*. Thus, inoculum level in our experiment was considerably higher, which may explain in part the absence of effect of *Microsphaeropsis* sp. on pathogen incidence, although it did have an effect on the number of residue-borne perithecia for at least some of the sampling dates. Since this antagonist was able to reduce the number of perithecia produced under field conditions, we would probably have observed an effect on disease incidence. For some sampling dates, the antagonist successfully reduced the number of both immature and mature perithecia. For these dates, the fall application was the most consistent treatment, while the effect of the spring application was variable. On a given sampling date, when a significant

reduction in the number of perithecia was observed, the fall application of the antagonist was one of the treatments involved in the reduction, in all cases. These results are in accordance with the in vitro experiment, where the efficacy of the antagonist was higher when it was applied before the pathogen. Because of the variation in the effect of the spring application and of the reliability of the fall application, the antagonist probably required a certain amount of time to establish on the substrate.

Because data were collected over time, it has been possible to observe that the biocontrol agent was efficient at only one date each year (May in 1998 and July in 1999). Looking at the weather conditions of previous months, we noted that the biocontrol agent was efficient after a period of drought and when the majority of the perithecia had reached maturity. April 1998 and June 1999 were very dry

months, with 36.7 mm and 28 mm of rain, respectively. In addition, the months where the antagonist was effective were months when the proportion of mature perithecia in the population was the highest during that year. This observation supports previous work from Benyagoub et al. (1) suggesting that the antagonistic effect exerted by *Microsphaeropsis* sp. against other fungi was due to an attraction to melanized structures. Consequently, there may be a relation between the efficacy of the antagonist, the weather conditions, and the maturity level of the perithecia.

Although our work is one of the first done on the biological control of FHB through the management of crop residues, this approach had been previously used on wheat against *Pyrenophora tritici-repentis*, the causal agent of tan spot (26,27). These field biocontrol studies demonstrated that it was possible to reduce the initial inoculum

Fig. 3. Frequency distributions of the maturity stages (Table 1) of *Gibberella zeae* perithecia at different dates (April, May, June, and July) and treatments with the antagonist *Microsphaeropsis* sp. (isolate P130A) in 1998. The residues from the plots were treated with the antagonist according to the following four treatments: control (no application), fall application, spring application, and fall+spring applications.

of a residue-borne plant pathogen using a biocontrol agent applied to crop residues. This work also determined that the success of this approach was strongly influenced by several factors related to the epidemiology of the disease, the environmental conditions after application, the formulation of the antagonist, and the timing of application. Bockus and Shroyer (2) also suggested that this method of attacking the pathogen during its saprophytic stage would probably be more appropriate and efficient for residue-borne monocyclic diseases.

FHB is known to be a monocyclic disease for which residue-borne ascospore inoculum is most probably the only significant inoculum source under eastern Canadian climatic conditions (10,24,33). Consequently, more than for polycyclic diseases, an effective management of crop debris should have a greater impact on disease and should diminish infection

pressure during seasons where weather conditions favor epidemics. However, a study by Stack (31) demonstrated that large inoculum differences were required to obtain measurable changes in FHB and a tenfold decrease of inoculum was necessary for a two-fold reduction in disease severity.

Although *Microsphaeropsis* sp. demonstrated the capacity to decrease perithecia production by *G. zeae*, the size of inoculum reductions required for an appropriate control of FHB is too high to consider the use of this biocontrol agent alone. However, 5% urea, propionic acid, and acetic acid demonstrated an ability to reduce perithecia number on wheat and maize debris and to favor colonization of the residues by saprophytes (17). In addition, tests conducted with *Microsphaeropsis* sp. indicated that the antagonist was not affected by urea at concentrations up to 5% (O. Carisse, unpublished data). Conse-

quently, further studies on the biocontrol of FHB with this antagonist should consider the combination of the antagonist and one of the chemicals tested by Khonga and Sutton (17). Such combinations could give rise to a more important control of *G. zeae* initial inoculum.

For improving the efficacy of biological control of FHB residue-borne inoculum, other aspects need to be further studied. Spray technologies need to be improved to increase coverage of residues both horizontally and vertically. A better knowledge of the ecological relations between the pathogen and the biological control agent will also permit an optimization of application timing. It will allow the determination of substances that could be added into the formulation to favor antagonist colonization, which would be detrimental to the pathogen and would allow a better retention of the biocontrol agent on the debris.

Fig. 4. Frequency distributions of the maturity stages (Table 1) of *Gibberella zeae* perithecia at different dates (April, May, June, and July) and treatments with the antagonist *Microsphaeropsis* sp. (isolate P130A) in 1999. The residues from the plots were treated with the antagonist according to the following four treatments: control (no application), fall application, spring application, and fall+spring applications.

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