Commercial-Scale Control of Rose Powdery Mildew with a Fungal Antagonist

R. R. BÉLANGER, Assistant Professor, and C. LABBÉ, Research Assistant, Département de phytologie, Faculté des Sciences de l'Agriculture et de l'Alimentation, Université Laval, Québec, Canada G1K 7P4; and W. R. JARVIS, Senior Research Scientist, Harrow Research Station, Agriculture Canada, Harrow, Ontario N0R 1G0

ABSTRACT

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The yeastlike fungus *Sporothrix flocculosa*, reported to be antagonistic against several members of the Erysiphales, was used under strict commercial conditions to control rose powdery mildew caused by *Sphaerotheca pannosa* var. *rosae*. The antagonist was applied in two separate trials on nearly 1,000 plants of rose cultivars Preference and Samantha, and results were compared with those obtained from plants treated with registered fungicides. In the first trial, treatment with the antagonist exerted control comparable to that of the fungicide, but its efficacy varied with the level of relative humidity prevailing in the greenhouse. In the second trial, *S. flocculosa* was again as effective as fungicides at controlling powdery mildew, and amendment of the antagonist suspension with a surfactant to reduce dependency on humidity yielded even better results than *S. flocculosa* alone or fungicides. The quality of harvested roses was similar following either chemical or biological treatment in cv. Samantha and was higher following biocontrol treatment in cv. Preference.

There is accumulating evidence that some biocontrol agents can be as effective as chemicals in repressing fungal pathogens. However, reports of success beyond experimental conditions in the laboratory remain scarce (12). This phenomenon can be attributed to several factors, including loss of efficacy under large-scale conditions or technical and legal difficulties related to commercial-scale testing (8).

Powdery mildew remains one of the most conspicuous diseases on roses and cucurbits and is still managed by chemical measures even though several natural antagonists have shown excellent potential for replacing fungicides (9,12). Indeed, *Ampelomyces quisqualis* Ces. (11), *Tilletiopsis* spp. (4,7), and *Stephanoascus* spp. (6) all can attack mycelial and reproductive structures of powdery mildew fungi. However, large-scale experiments with these antagonists either have yielded unsuccessful results or have not been attempted because of the high relative humidity (RH) conditions required to achieve maximum control, especially in the case of *A. quisqualis* and *Tilletiopsis* spp., which lose their efficacy rapidly below 90% RH (10). In a recent study, Hajlaoui and Bélanger (2) showed that the yeastlike fungus *Sporothrix flocculosa* Traquair, Shaw, & Jarvis was less demanding in terms of environmental conditions than *Tilletiopsis washingtonensis* Nyland or *S. rugulosa* Traquair, Shaw, & Jarvis and was also a much faster colonizer of rose powdery mildew caused by *Sphaerotheca pannosa* (Wallr.:Fr.) Lév. var. *rosae* Woronichin. Furthermore, its activity was observed on several members of the Erysiphales, including *S. fuliginea* (Schlechtend.:Fr.) Pollacci (6) and *Erysiphe graminis* DC. f. sp. *tritici* Ém. Marchal (3).

In light of these results and the need to develop prophylactic measures that are less dependent on fungicides, a research permit was sought to conduct experiments with *S. flocculosa* under commercial conditions. In this study, we present results of the first commercial trials to control rose powdery mildew with a natural antagonist.

MATERIALS AND METHODS

Inoculum. An isolate of *Stephanoascus flocculosus* Traquair, Shaw, & Jarvis (anamorph: *Sporothrix flocculosa*) (ATCC 64874) (13) was used for all experiments. The fungal cultures, initiated from 2-mo-old slant cultures incubated at 4 C, were first transferred to yeast-malt agar in 9-cm petri dishes for 2 wk in the dark at 24 C. Subsequently, three 15-mm plugs taken from the margins of actively growing colonies were inoculated into 150-ml conical flasks containing 75 ml of yeast-malt-peptone broth. Five-day-old cultures averaging 5×10^8 cfu/ml were used as sources of inocula. The fungal propagules were resuspended in 12 L of tap water and adjusted to 1×10^6 cfu/ml. Before use, a few drops (0.02%) of the surfactant Aqua-Aid (Ken Crowe Inc., Montreal) were added to the suspension.

Commercial-scale experiments. The experiments were conducted at Rose Drummond, Drummondville, Québec, the largest greenhouse rose producer in the province, with a compound covering 3 ha. The greenhouse facilities were equipped with the latest in modern technology, including fogging systems and computer control of environmental parameters. These studies were undertaken under a restrictive research permit (No. 13-RP-91) issued by Agriculture Canada under the Pest Control Acts, which prohibited the commercial sale of the treated roses. The experimental area was completely surrounded by a plastic curtain and covered approximately 100 m^2 enclosing nearly 1,000 rose plants under continuous production. Access to the experimental area was restricted to authorized personnel. Our management of the experimental area was limited to applications of the antagonist and assessment of powdery mildew incidence. Rose Drummond personnel were in charge of harvest, quality grading, insect control, temperature and humidity control, and general plant management. After harvest and grading, the treated flowers were destroyed, so no market studies were possible. The experimental design was defined to reflect as closely as possible commercial conditions encountered by producers daily.

Trial 1. Trial 1 was established 12 May 1991 (Julian day [JD] 132) and lasted 8 wk. The treated area contained cv. Preference, which was selected on the basis of its high level of susceptibility to *S. p. rosae*. The fungal antagonist *S. flocculosa* was applied with a 12-L electric sprayer (Flot Jet 3500, Machineries Simard, Drummondville). Leaves were sprayed to runoff, and 48 L of *S. flocculosa* suspension was required for each application to cover the experimental area. This volume was equal to the one used to cover a similar area with the fungicide dodemorph acetate (Meltatox), 2.5 g/L, 40% a.i. (5,000 L/ha). The antagonist was applied weekly unless otherwise specified. The incidence of powdery mildew was assessed on an arbitrary scale of 0–3, where 0 = no signs of powdery mildew, 1 = few visible signs on lower leaves of the flower stem, 2 = visible signs concentrated on lower leaves, and 3 = visible signs on most leaves. A grade exceeding 1.5 was considered as nonmarketable by Rose Drummond. At each harvest, all roses collected from the treated area were rated for the incidence of powdery mildew. They were subsequently graded for quality with an automatic commercial grader. Our results were compared with those obtained in areas adjacent to the

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experimental plot where cv. Preference was treated for powdery mildew with Meltatox or occasionally with microfine sulfur (Micro-Niasul) by Rose Drummond personnel. The fungicide was applied on an as-needed basis. In those commercial areas, all roses were evaluated for powdery mildew incidence and graded in the same manner as described above. At the request of Rose Drummond, there was no untreated control, to avoid excessive disease pressure and to prevent spread of the pathogen to other areas. Temperature and RH were recorded daily to assess the influence of those variables on the efficacy of our treatments. At the end of the trial, powdery mildew incidence was plotted on a weekly basis, and the quality and the total number of roses were computed and compared to harvest from an equal area of cv. Preference treated with fungicides.

Trial 2. The second trial was established 31 October 1991 (JD 304) and also lasted 8 wk. The experimental protocol was similar to the one described in trial 1 with the exceptions that both cvs. Preference and Samantha were treated, the latter representing 70% of the market in Canada and reported to be more resistant to *S. p. rosae*. Further, for each cultivar, one-half of the area was treated with an antagonist suspension amended with 1% paraffin to alleviate humidity requirements by the antagonist, as reported by Philipp et al (10). Powdery mildew incidence, flower quality, and RH were recorded as previously described and compared to results obtained with fungicide treatments on both cultivars.

RESULTS

Commercial-scale experiments. *Trial 1.* At our request, fungicide applications were stopped in the experimental area on JD 125, or 1 wk before the start of the experiments. This period coincided with a severe outbreak of powdery mildew that necessitated two applications of Meltatox in the commercial area and resulted in an unusually high incidence of powdery mildew in the experimental area before the first application of *S. flocculosa* (Fig. 1). Then, on JD 127, the fogging system broke down and the RH level fell near 60%, creating conditions extremely favorable for spread of powdery mildew and unfavorable for development of *S. flocculosa*. These conditions created a situation where the incidence of powdery mildew reached a near maximum level (2.5) 6 days into the experiment (Fig. 1). However, once the misting problems were solved and following the second application of the antagonist, the activity of *S. flocculosa* on *S. p. rosae* became readily apparent. In fact, within 2 wk, the incidence of powdery mildew in the experimental area was brought down from a maximum of 2.5 to nearly 0 on JD 151, exceeding by far the control level obtained with Meltatox (Fig. 1). As a matter of fact, the incidence of powdery mildew remained rather constant under chemical treatment, ranging between 1 and 1.5, for the duration of the experiment. Interestingly, in the week from JD 144 to JD 151, the *S. flocculosa* treatment brought the disease incidence to its lowest level, whereas Rose Drummond personnel had to use three Meltatox applications to maintain powdery mildew below an acceptable level (Fig. 1). On JD 156, the fogging system broke down again, which quickly resulted in an increase of disease incidence in the experimental area. The fogging system worked sporadically for the remainder of the experiment, but the *S. flocculosa* treatment was nonetheless as efficient as, or more efficient than, the fungicide treatment during that period. On the last day of the trial, powdery mildew incidence was down to 0.5 in the treated area compared to 1.5 in the commercial area. In all, *S. flocculosa* was applied seven times during the trial, while 10 Meltatox applications were necessary in the commercial area. A total of 3,534 roses were harvested in the experimental area for the duration of the trial, compared to a mean of 3,498 for an area of the same size treated with fungicides.

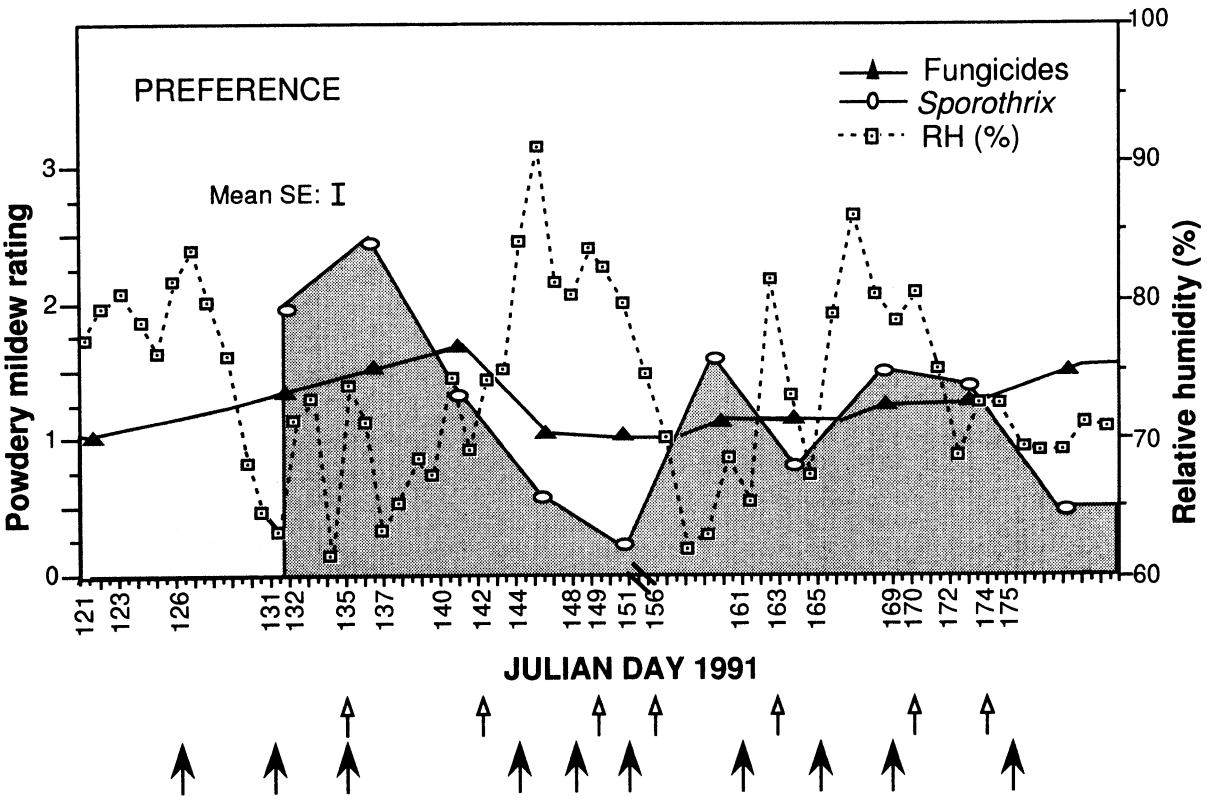

Fig. 1. Evolution of powdery mildew incidence over time in a commercial greenhouse on harvested cv. Preference roses under treatments with the antagonist *Sporothrix flocculosa* or with the fungicide Meltatox. White arrows indicate an application of *S. flocculosa* and black arrows indicate an application of the fungicide. Relative humidity is based on the daily average. Powdery mildew rating was assessed on an arbitrary scale of 0–3, where 0 = no signs of powdery mildew, 1 = few visible signs on lower leaves of the flower stem, 2 = visible signs concentrated on lower leaves, and 3 = visible signs on most leaves.

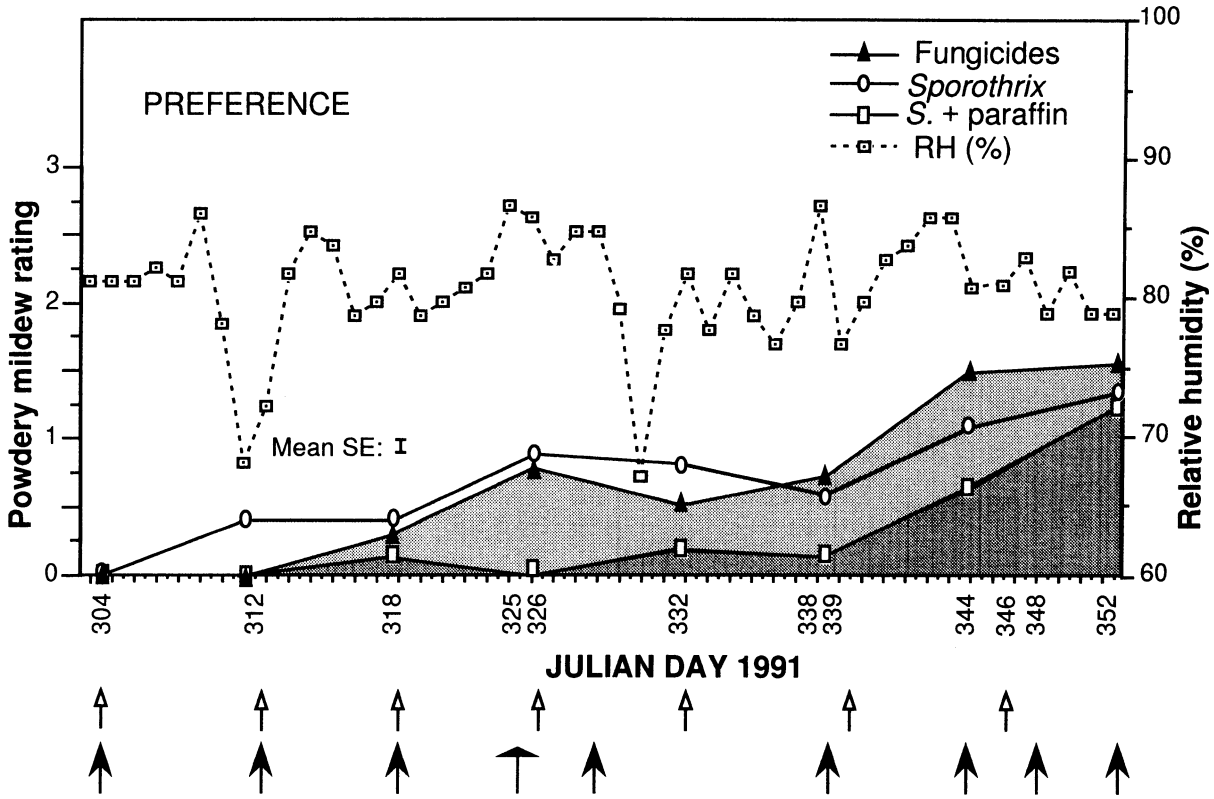

Fig. 2. Evolution of powdery mildew incidence over time in a commercial greenhouse on harvested cv. Preference roses under treatments with fungicides or with the antagonist *Sporothrix flocculosa* amended or not with 1% paraffin (*S.* + paraffin). White arrows indicate an application of *S. flocculosa*, narrow black arrows indicate an application of Meltatox, and wide black arrows indicate an application of sulfur. Relative humidity is based on the daily average. Powdery mildew rating was assessed on an arbitrary scale of 0–3, where 0 = no signs of powdery mildew, 1 = few visible signs on lower leaves of the flower stem, 2 = visible signs concentrated on lower leaves, and 3 = visible signs on most leaves.

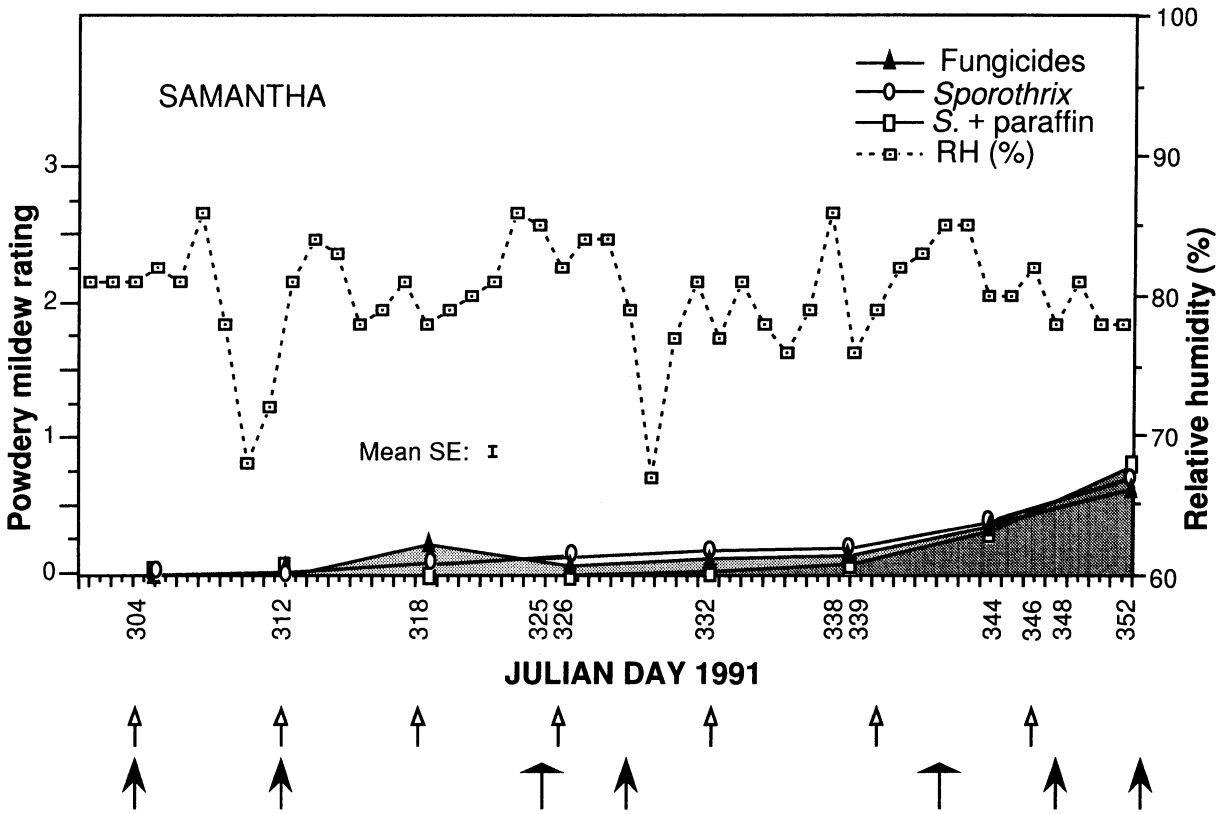

Fig. 3. Evolution of powdery mildew incidence over time in a commercial greenhouse on harvested cv. Samantha roses under treatments with fungicides or with the antagonist *Sporothrix flocculosa* amended or not with 1% paraffin (*S.* + paraffin). White arrows indicate an application of *S. flocculosa*, narrow black arrows indicate an application of Meltatox, and wide black arrows indicate an application of sulfur. Relative humidity is based on the daily average. Powdery mildew rating was assessed on an arbitrary scale of 0–3, where 0 = no signs of powdery mildew, 1 = few visible signs on lower leaves of the flower stem, 2 = visible signs concentrated on lower leaves, and 3 = visible signs on most leaves.

Trial 2. In the second trial, the first application of *S. flocculosa* was synchronized with treatments in the commercial area, which prevented an initial outbreak of powdery mildew in the experimental area. The overall incidence of powdery mildew was lower inside the greenhouse compound, and the fogging system was in operation for the duration of the trial. These conditions yielded a much better control of the disease in both the commmercial and the experimental areas (Figs. 2 and 3).

With cv. Preference, the disease level was kept below 1 for the better part of the trial regardless of the treatment (Fig. 2). *S. flocculosa* alone was as effective as the fungicide throughout the experiment, yielding even slightly better results in the last 2 wk of the trial. Interestingly, this latter period coincided with a general increase of powdery mildew throughout the compound. But the biocontrol treatments kept the disease level below that of the Meltatox treatment despite the fact that three fungicide applications were necessary during that period compared to one with *S. flocculosa.*

Amendment of the antagonist suspension with 1% paraffin improved the level of control, keeping the disease rating near 0 for the first 6 wk of the trial and significantly below the fungicide treatment throughout the experimental period (Fig. 2). However, small blisters (edema) on the underside of some leaves of cv. Preference were diagnosed after 4 wk and the amendment was interrupted by concern over affecting the quality of harvested flowers. These symptoms were not observed on cv. Samantha. A total of 1,623 (822 with *S. flocculosa* and 801 with *S. flocculosa* + paraffin) cv. Preference roses were harvested in the experimental area compared to 1,502 in the commercial area. As in the first trial, cv. Preference received seven applications of *S. flocculosa* in the treated area. During the same period, one sulfur and eight Meltatox treatments were used in the commercial area.

The incidence of powdery mildew remained near 0 throughout the trial on cv. Samantha, which is reported to be more resistant than cv. Preference (Fig. 3). All treatments, biological and chemical, were equally effective at controlling the disease. Incidence of powdery mildew was slightly increased during the last 2 wk, as observed throughout the compound, but the level never exceeded 0.7 on the arbitrary scale. A total of 1,175 roses were harvested in the experimental area compared to 1,051 in the commercial area. For this cultivar, both areas, treated and commercial, were given the same number of treatments (Fig. 3).

Assessment of flower quality was indicative of the nonphytotoxic nature of *S. flocculosa* (Fig. 4). In fact, the biological treatment improved the overall quality of cv. Preference, yielding more than 10% of grade Select and grade 1 flowers (Fig. 4A). By comparison, no flowers produced under chemical treatments exceeded grade 2, with the bulk of the production being of grade 3X and 3. This quality improvement observed with the biological treatment was explained by the sensitivity of that particular cultivar to Meltatox. The results were remarkedly similar for cv. Samantha, whether the flowers were treated with *S. flocculosa* (amended or not with paraffin) or with the fungicide (Fig. 4B).

DISCUSSION

Results presented in this study summarize the first large-scale experiment of powdery mildew biocontrol to be conducted under strict commercial conditions and for which a research permit had been issued. The fungal antagonist *S. flocculosa*, which had been reported

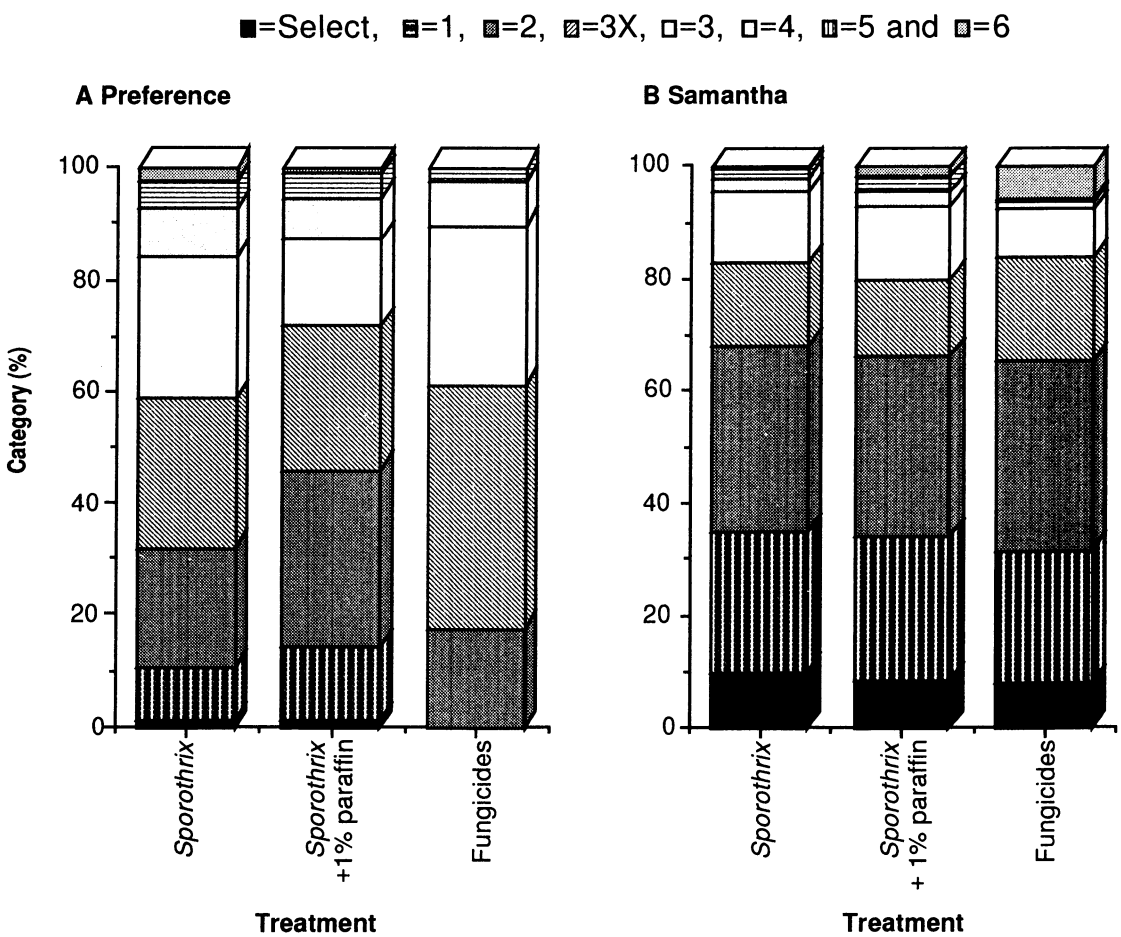

Fig. 4. Comparative assessment of flower quality for **(A)** cv. Preference roses and **(B)** cv. Samantha roses, harvested in a commercial greenhouse under treatments for powdery mildew with fungicides or with the antagonist *Sporothrix flocculosa* amended or not with 1% paraffin. Quality grading is based on stem length on a scale of decreasing value from Select to 6 as defined by the Canadian Flower Association.

as an effective biocontrol agent of several members of the Erysiphales, proved to be as effective as registered fungicides in controlling *S. p. rosae* on commercial greenhouse roses.

Because of their dependency on specific environmental conditions for development, biocontrol agents are usually effective within a limited range of temperature and RH (1). In the case of powdery mildew antagonists, the most limiting factor consistently has been their requirement for high RH. In this context, the greenhouse environment provides conditions conducive for the development of microbial antagonists and should be the first target of commercial exploitation of biological control (5). This was well exemplified in our experiments where, under controlled RH conditions, *S. flocculosa* was able to outperform fungicides in the control of rose powdery mildew. However, it is important to note that when RH conditions fell below 60% in the first trial, the incidence of powdery mildew increased rapidly, highlighting the importance of this parameter in the efficacy of the organism. Under those adverse conditions, *S. flocculosa* was nonetheless able to maintain the disease incidence at a level comparable to that of the fungicide, reinforcing its greater spectrum of activity compared to other known powdery mildew antagonists, as reported by Hajlaoui and Bélanger (2).

Amendment of the antagonist suspension with 1% paraffin in the second trial was effective at lowering the incidence of powdery mildew, especially with cv. Preference. This technique was proposed by Philipp et al (10) to alleviate high humidity requirements of the hyperparasite *A. quisqualis* for control of cucumber powdery mildew in the field. While these authors made no reference to the phytotoxicity of the treatment, Jarvis (*unpublished*) observed phytotoxic symptoms on greenhouse cucumber with the same concentration. In our experiment, we were able to treat cv. Samantha for the duration of the experiment without observing any adverse effects; minor symptoms of edema were noted on cv. Preference after the fourth application. This approach warrants further study, as it could broaden the range of application of *S. flocculosa* by lowering its dependency on humidity conditions. Additionally, the use of oil and antitranspirants has been reported to lower the incidence of powdery mildew and other diseases (14).

It is noteworthy that the *S. flocculosa* treatment has helped improve the overall quality of harvested roses in the case of cv. Preference. According to Rose Drummond personnel, this cultivar, in addition to its susceptibility to powdery mildew, is very sensitive to Meltatox. Considering the nonphytotoxic nature of *S. flocculosa* when applied to roses, it appears that a biological treatment, for a similar efficacy, would have another added advantage over chemical products.

In conclusion, this study, conducted under the rigorous conditions of commercial production, has shown that the use of a natural antagonist could achieve as good a control of rose powdery mildew as currently used fungicides. On the basis of our results, it appears that the greenhouse environment is compatible with the development of *S. flocculosa* and that this biocontrol treatment, if proved economical, could adequately replace the use of fungicides for the control of *S. p. rosae*.

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