Biological Control of Rose Powdery Mildew with the Antagonist Yeast *Tilletiopsis pallescens*

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Abstract. The efficacy of *Tilletiopsis pallescens* Gokhale, a naturally occurring ballistospore-forming yeast isolated from mildew-infected leaves, was evaluated as a biological control agent against rose powdery mildew [*Sphaerotheca pannosa* (Wallr.:Fr.) Lévl. var. *rosae* Woronichin]. Two trials were conducted on potted rose (*Rosa* sp.) plants (1-year-old cv. Cardinal Pink) under commercial greenhouse-growing conditions during the summer (June to September) when mildew was most severe. Mildew-infected plants were subjected to one of four treatments: a *T. pallescens* spore suspension applied three times (3–4 d apart), distilled water (applied three times), one application of *T. pallescens* spore suspension, or one application of culture filtrate without spores. Two weeks after treatment began, mildew development was evaluated by enumerating conidial density on sampled leaflets. Sporulation was significantly reduced (by 97%–98%) on plants treated with three applications of *T. pallescens* spore suspension, compared to a 47%–57% reduction on plants treated with three applications of distilled water. There was no significant difference in conidial density between plants treated with one application of *T. pallescens* spore suspension and plants treated with one application of its culture filtrate, with a 78%–94% reduction in conidia, which was significantly higher than for the water treatment. The mode(s) of action of *T. pallescens* appears to be eradicator and associated with enzymes or metabolites produced in the culture filtrate. The results from this study demonstrate the potential for biological control of rose powdery mildew under commercial growing conditions in British Columbia.

Rose powdery mildew, caused by *Sphaerotheca pannosa*, is a serious disease problem in the commercial production of greenhouse roses grown for cut flowers in the Fraser Valley of British Columbia. Disease control measures include the application of fungicides such as dodemorph-acetate (Meltatox) or dinocap (Karathane), use of dormant lime sulfur (Lime Sulphur) spray, or the use of less susceptible varieties (Price, 1970). In addition, inorganic salts, such as sodium bicarbonate (Horst et al., 1992), potassium silicate (Menzie et al., 1992), and phosphate and

potassium salts (Reuveni et al., 1995), have been tested against various powdery mildews with promising results. Increasing public concern over the use of chemical pesticides has made the development of biological control agents for powdery mildew highly desirable, since they could provide an alternative to or reduce dependency on currently used control measures. Moreover, the emergence of fungicide-insensitive variants of *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci, the fungus responsible for powdery mildew on cucumber (*Cucumis sativus* L.), has been reported (McGrath, 1991; Schepers, 1983).

There is accumulating evidence that several biological control agents can be effective antagonists of powdery mildews. *Ampelomyces quisqualis* Ces. (Sundheim, 1982), *Acremonium alternatum* Linc.:Fr. (Malathrakakis, 1985), *Tilletiopsis* spp. (Hijwegen, 1986; Klecan et al., 1990), and *Sporothrix flocculosa* Traquair, Shaw and Jarvis (Bélanger et al., 1994; Hajlaoui and Bélanger, 1993) have been reported to attack mycelia and reproductive structures of mildew fungi. *Tilletiopsis pallescens*, a yeast-like fungus, which occurs naturally on mildew-infected leaves of various plant species, was shown to provide excellent control of

powdery mildew on cucumber plants under commercial greenhouse-growing conditions (Urquhart et al., 1994). This antagonistic ballistospore-forming yeast reduced mildew sporulation and hyphal growth of *S. fuliginea*.

The objective of this study was to evaluate the potential of *T. pallescens* as a biological control agent for rose powdery mildew under greenhouse-growing conditions.

Materials and Methods

Culture conditions. The isolate of *T. pallescens* (ATCC 96155) was provided by E.J. Urquhart, Centre for Pest Management, Dept. of Biological Sciences, Simon Fraser Univ., and was grown in liquid T II medium, consisting of 10 g peptone (Bacto), 25 g glucose, and 1 g yeast extract (Difco) (per liter of water) (Urquhart et al., 1994). A 50-mL aliquot was dispensed into a 125-mL flask, sterilized by autoclaving, and inoculated with a 4-mm-diameter plug from a 3-week-old malt extract agar colony. The flask was placed on a rotary shaker (150 rpm) at 20 °C under a 12-h photoperiod provided by fluorescent lights. After 5 days of growth, a 2-mL aliquot of the culture was inoculated into 2-L flasks containing 1 L of T II broth and incubated under the same environmental conditions as above. After 72 h, the culture was filtered through three layers of cheesecloth to exclude mycelial masses. Spore density was adjusted to $\approx 10^7$ cells/mL by dilution with the culture medium, based on spore counts made with the aid of a haemocytometer. The 72-h culture filtrate (without spores) was obtained by filtering the culture through No. 540 Whatman filter paper (using a Buchner funnel) under vacuum.

Growth of plants. One-year-old rose plants (cv. Cardinal Pink) were grown in 2-gallon nursery pots containing three parts composted bark and one part peat moss (v/v) (Augustine Bark, Pitt Meadows, B.C.) for use in all experiments. Plants were maintained in two ebb-and-flood beds in a greenhouse equipped with fogging systems and a computer-controlled environment monitoring system at the British Columbia Ministry of Agriculture, Fisheries and Food station in Abbotsford, B.C. The plants were watered and fertilized every other day with a complete in-house hydroponic solution through flooding. They were pruned 4 weeks earlier to promote new shoot development and inoculated with mildew conidia obtained from naturally infected rose plants by gently tapping or rubbing infected leaflets onto healthy 3- to 4-day-old leaflets. The relative humidity (RH) in the greenhouse was maintained above 90% for 7 days to allow mildew conidia to germinate and then was reduced to 50% to encourage mycelial development and sporulation. Within 2 weeks after inoculation, powdery mildew began to establish on young leaves and stems, pedicels, and sepals of unopened flower buds. For the experiments described below, daily ambient max/min temperatures and RH were monitored using a thermistor (Fenwall Electronics, Conn.) and a RH sensor (General Eastern, Mass.), respectively, placed at canopy level.

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Treatments. Plants were subjected to one of four treatments: 1) three applications (3–4 days apart) of *T. pallescens* spore suspension (10^7 cells/mL); 2) three applications (3–4 days apart) of distilled water; 3) one application of *T. pallescens* spore suspension (10^7 cells/mL); or 4) one application of 72-h culture filtrate. Treatments were applied to run-off on both sides of the leaflets, stems, pedicels, and sepals of unopened flower buds, generally in late afternoon, using a hand-held sprayer. About 100 mL was applied to each potted plant. Each treatment was replicated four times, with two potted plants in each replicate, to allow for a sufficient number of infected leaflets to be sampled; pots were arranged in a completely randomized design. All treated leaflets were tagged and arbitrarily assigned one of three sampling times (0, 1, or 2 weeks after the first application), to assess mildew development.

Disease assessment. The average mildew conidial density was estimated by sampling three arbitrarily assigned, infected leaflets from two randomly selected replications in each treatment. The method developed by Urquhart et al. (1994) was used to quantify conidial density and to determine the effect of *T. pallescens* application on powdery mildew development. Clear cellophane tape (20 mm wide) was gently pressed onto the leaflet surface, removed, and subsequently transferred to a glass microscope slide. At each sampling time, six subsamples were obtained from each replication, one from each leaflet. The conidial density was determined by counting 10 fields per slide at equal distances along two parallel lengths of the slide. Conidial counts per microscope field were averaged and converted to conidial density per cm^2 of leaf surface. Statistical analyses to determine treatment effects were conducted using the general linear modeling program (GLM) in the SAS statistical software package (version 6.08, SAS Institute, Cary, N.C.). The average mildew conidial density per cm^2 of leaf surface in each treatment was compared using a nonparametric analysis of variance (Kruskal–Wallis test) followed by Student–Newman–Keul's test. In all analyses, levels of significance of $P < 0.05$ were used.

Results and Discussion

Plants treated with a spore suspension of *T. pallescens* had a significant reduction ($P < 0.05$) in the development of powdery mildew when compared to plants treated with distilled water. In the first trial (27 June–11 July), initial mildew conidial density was reduced by 97% (from 29.7×10^3 to 0.8×10^3 cells/ cm^2 of leaf surface) on plants treated thrice with the spore suspension, when sampled 2 weeks after the first application (27 June). In comparison, there was a 57.3% reduction on plants treated with distilled water (Fig. 1). During this trial, the daily average temperature and RH were 24.5°C and 60%, respectively (Fig. 2). In the second trial (12 Sept.–26 Sept.), the initial mildew conidial density was reduced by 99% (from 18.8×10^3 to 0.24×10^3 cells/ cm^2 of leaf surface) on plants treated thrice with the spore

Fig. 1. Biological control activity of *Tilletiopsis pallescens* (*T. p.*) on rose powdery mildew under greenhouse-growing conditions (Trial 1). Mean separation at $P \leq 0.05$ for mildew conidial density at the same sampling date as verified by Student–Newman–Keul's test.

Fig. 2. Ambient temperature (A) and relative humidity (B) during the experiment on biological control activity of *Tilletiopsis pallescens* on rose powdery mildew (Trial 1).

suspension when sampled 2 weeks after the first application. On plants treated with distilled water, mildew sporulation was reduced by 47% (Fig. 3). During this trial, the average

daily temperature and RH were 21°C and 72%, respectively (Fig. 4).

Distilled water was chosen as one of the treatments because it has been shown that

repeated exposure to water has deleterious effects on mildew spore germination (Perera and Wheeler, 1975) and development (Yarwood, 1939). This was evident in both trials as mildew spore density was reduced after water treatments. However, water did not provide satisfactory control of mildew, and conidiophores re-established within 72 h after each application (Fig. 5A). In contrast, the spore suspension of *T. pallescens* provided complete control of mildew, and hyphae and conidiophores were observed to collapse and disintegrate within 24 h after treatment (Fig. 5B). When the leaflets were examined under a dissecting microscope, mildew structures were absent on the surface 72 h after treatment (Fig. 5C), and only necrotic lesions at primary infection sites were visible on the leaflets.

The culture filtrate of *T. pallescens* was also tested to compare its activity with that of the spore suspension. In the first trial (Fig. 1), plants treated with one application of spore suspension had conidial density reduced at 1 and 2 weeks after application from 29.7×10^3 to 8.8×10^3 and 6.5×10^3 cells/cm² of leaf surface, a reduction of 70% and 78%, respectively. In contrast, the mildew conidial density on plants treated with one application of culture filtrate was reduced by 59% and 85%, respectively, on the two sampling dates. Similar results were obtained in the second trial with both treatments (Fig. 3). The spore suspension of *T. pallescens* or culture filtrate reduced the initial mildew conidia density (16.8×10^3 cells/cm²) 1 week after application by 87% and 80% respectively, and on the second week by 98% and 94%, respectively. There were no significant differences ($P \leq 0.05$) in conidial density between plants treated with one application of *T. pallescens* spore suspension and plants treated with one application of the culture filtrate, except on 4 July in the first trial. Both treatments resulted in collapse and disintegration of the mildew hyphae and conidiophores within 24 h after application. Similar results were reported by Hijwegen (1986) and Knudsen and Skou (1993) when evaluating *T. minor* Nyland and *T. albescentis*, respectively, against cucumber powdery mildew. Also, Bélanger et al. (1994) have shown that the application of *S. flocculosa*, another antagonistic yeast, provided control of rose powdery mildew in Quebec that was similar to that of currently used fungicides when tested under commercial conditions.

The biocontrol activity of these yeasts may be due to enzymes (Hijwegen, 1992; Urquhart et al., 1994), metabolites (Hajlaoui and Bélanger, 1993), or a combination of both. The presence of β -1,3 glucanase activity in the culture filtrate of *T. pallescens* may suggest this to be one of the potential modes of action of this biological control agent against powdery mildew (Urquhart et al., 1994). Antibiotic production, reported in *S. flocculosa* (Hajlaoui et al., 1992; Hajlaoui and Bélanger, 1993), is a second possible mode of action. The effect of culture filtrate alone (without spores) on mildew sporulation was shown to be better than water in this study, suggesting that compounds secreted into the medium had

Fig. 3. Biological control activity of *Tilletiopsis pallescens* (*T. p.*) on rose powdery mildew under greenhouse-growing conditions (Trial 2). Mean separation at $P \leq 0.05$ for mildew conidial density at the same sampling date as verified by Student–Newman–Keul's test.

Fig. 4. (A) Ambient temperature and (B) relative humidity during the experiment on biological control activity of *Tilletiopsis pallescens* on rose powdery mildew (Trial 2).

antifungal activity. Choudhury et al. (1994) have isolated two new secondary metabolites with antimycotic and antibacterial activity from liquid cultures of *S. flocculosa* and *S. rugulosa* Traquair, Shaw and Jarvis, although these

compounds were not tested on powdery mildew.

When the trials were completed, most plants had some newly developed leaves with mildew infection, except for those treated with

Fig. 5. Comparison of mildew-infected rose leaves treated with water (A) or with a *Tilletiopsis* spore suspension (B and C). Photographs were taken after 24 h (B) or 72 h (A, C) under a dissecting microscope at magnifications of $\times 25$ (A, B) and $\times 40$ (C). In (C), the necrotic (black) spots represent infection sites where the mildew fungus had penetrated the leaflet.

three applications of *T. pallescens* spore suspension. Re-infection likely had occurred as a result of spore dispersal from control plants. No phytotoxic symptoms were evident on plants treated with *T. pallescens* spores or the culture filtrate. However, both treatments left a sticky residue on leaflets, which was especially apparent on plants treated with three

applications of *T. pallescens* spore suspension. The residue may have resulted from unutilized glucose and peptone in the culture medium, or from polysaccharide material and other compounds produced by the yeast during growth. Some saprophytic fungi were growing on older leaflets in the second trial 1 week after the third application was made. This trial

was conducted under conditions of high RH (Fig. 4) that could have enhanced saprophytic fungal growth. Similar fungi were absent on treated plants in the first trial, which was conducted under conditions of moderate RH (Fig. 2). In many commercial greenhouses, RH is not easily controlled, and saprophytic fungi could become a problem after treatment under high RH. Application of culture filtrate, which is diluted, could circumvent the problem, provided the metabolites produced by *T. pallescens* in the culture medium are in sufficient concentration.

Previous reports have shown the need to maintain RH at 70% or higher to achieve good growth of *T. albescens* on the phylloplane and achieve good control of *S. fuliginea* (Knudsen and Skou, 1993). The high humidity ($\leq 70\%$) requirement for optimal growth of the yeast biocontrol agents was also reported by Hajlaoui and Bélanger (1991) and Hijwegen (1992). Therefore, appropriate formulations may need to be developed to deliver the biocontrol agent and have it survive on the leaf surface in the greenhouse environment. A formulation consisting of canola oil (1%) and lecithin (0.1%) has been shown to enhance survival of *T. pallescens* on cucumber leaves (Urquhart and Punja, 1996) and was not phytotoxic.

This study has demonstrated that *T. pallescens* spore suspension or culture filtrate provided excellent control of *S. pannosa* var. *rosae*, and demonstrated the potential for biological control of rose powdery mildew in the commercial greenhouse environment. Additional research is needed to identify the active metabolite(s) involved and to develop an appropriate formulation that will enhance the survival of *T. pallescens* on mildew-infected foliage.

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