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Comparative effects of temperature and humidity on the activity of three potential antagonists of rose powdery mildew

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Abstract

Three reported antagonists of cucumber powdery mildew, *Stephanoascus flocculosus*, *Stephanoascus rugulosus*, and *Tilletiopsis washingtonensis*, were tested and compared under different environmental conditions for their potential for controlling rose powdery mildew, caused by *Sphaerotheca pannosa* var. *rosae*. Under controlled conditions in vitro, all three fungi induced a rapid collapse of conidia, conidiophores and hyphae of *S. pannosa* var. *rosae* on detached leaflets of miniature roses within 48 h following their application, as observed under a SEM. Both temperature and relative humidity (r.h.) affected the activity of the antagonists differently. The colonization of powdery mildew was maximal at 26 °C, especially for *St. rugulosus* and *T. washingtonensis*. Maximal colonization was achieved at the highest r.h. tested (90%) for all three antagonists but only *St. flocculosus* maintained a colonization of 80% or better under lower r.h. These observations stress the importance of considering environmental conditions when assessing the activity of antagonistic microorganisms.

Additional keywords: Biological control, hyperparasites, *Sphaerotheca pannosa* var. *rosae*, *Stephanoascus* spp., *Tilletiopsis washingtonensis*.

Introduction

The current efforts to eliminate pesticides from agricultural crops have led to new developments in the biological control of diseases (Mukerji and Garg, 1988). Although in most cases biocontrol of plant diseases is still at the experimental stage, antagonistic relationships between fungi and plant pathogens have been observed in several instances (Blakeman and Fokkema, 1982; Fokkema, 1983; Sundheim and Tronsmo, 1988).

For the powdery mildew fungi, the existence of hyperparasites and antagonists has been clearly established (Hijwegen and Buchenauer, 1984). For example, species of *Tilletiopsis* Derx have been reported to control *Sphaerotheca fuliginea* (Schlecht.: Fr.) Poll. (cucumber powdery mildew) (Hoch and Provvidenti, 1979; Hijwegen, 1986, 1988), *Erysiphe graminis* DC var. *hordei* Ém. March. (barley powdery mildew) (Klecan et al., 1990) and powdery mildews of other plant species (Hijwegen and Buchenauer,

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1984). More recently, two newly identified fungi, *Stephanoascus flocculosus* Traquair, Shaw & Jarvis (anamorph: *Sporothrix flocculosa* Traquair, Shaw & Jarvis) and *Stephanoascus rugulosus* Traquair, Shaw & Jarvis (anamorph: *Sp. rugulosa* Traquair, Shaw & Jarvis) (Traquair et al., 1988) were found to colonize and inactivate *S. fuliginea* on cucumber (*Cucumis sativus* L.) leaves (Jarvis et al., 1989). It was further shown that the activity of these antagonists was greatly influenced by environmental conditions, especially relative humidity (r.h.) (Jarvis et al., 1989). This observation stresses the importance of understanding the autecology of potential antagonists in order to achieve successful biological control.

Powdery mildew of roses (*Rosa hybrida*), caused by *Sphaerotheca pannosa* (Wallr.: Fr.) Lév. var. *rosae* Wor., is the single most important disease of greenhouse roses (Paulus and Nelson, 1988). In spite of the recent developments in the biocontrol of powdery mildews, none of the aforementioned antagonists has been tested against *S. pannosa* var. *rosae*. In light of these observations, the objectives of this study were twofold: 1) to determine and compare the potential of *St. flocculosus*, *St. rugulosus* and *T. washingtonensis* Nyland for controlling powdery mildew of roses and 2) to determine the effects of temperature and humidity on the activity of these putative antagonists.

Materials and methods

Antagonistic activity of Stephanoascus flocculosus, St. rugulosus and Tilletiopsis washingtonensis. The strain of *T. washingtonensis* was obtained from the University of Alberta Microfungus Collection (UAMH 1738) and *St. flocculosus* and *St. rugulosus* were kindly provided by Drs W.R. Jarvis and J.A. Traquair, Agriculture Canada, Research Station, Harrow, Ontario, Canada. Conidial suspensions of all three fungi were collected from 5-day-old cultures on 2% malt extract agar by washing the colonies with sterile distilled water and filtering the suspensions through cheesecloth. Conidial concentrations were determined with a haemocytometer and adjusted to 1×10^6 conidia ml^{-1}.

Miniature roses cv. Ruiredro were purchased from a local grower and grown in a greenhouse until natural development of the disease. Leaflets were selected when approximately half the area was covered by mycelium of powdery mildew. Four individual leaflets were placed on a moist filter paper in each of eight 9-cm Petri dishes. For each antagonist, the leaflets in two Petri dishes were sprayed lightly with the conidial suspension. The last two dishes, the controls, were sprayed with distilled water. The dishes were left open for ca. 30 min to evaporate the inoculum droplets then closed and sealed with Parafilm (American Can Co. Inc.). The dishes were incubated at 25 °C in mixed fluorescent and incandescent light at 30 $\mu E\ m^{-2}\ s^{-1}$ for a 12-h photoperiod. Following the method of Jarvis et al. (1989), the leaflets were observed under a stereomicroscope at 24-h intervals over a period of 96 h.

For scanning electron microscopy (SEM), five leaflets from each treatment were collected at 48 h and vapour-fixed overnight with osmium tetroxide in a sealed moist chamber at 26 °C. Following two washes with distilled water, the specimens were dehydrated in a graded ethanol series. They were then mounted on aluminium studs and sputter-coated with gold to a thickness of about 20 nm, and examined with a Cambridge Stereoscan 5-150 microscope at 20 KV.

Effect of temperature. Mildewed plants were transferred to growth chambers equipped with humidity and temperature controls (Conviron model 3028, Controlled Environments Ltd., Winnipeg, Canada) maintained at 18, 22, 26, 30, or 34 °C for a week under 90% r.h. and a 12-h photoperiod under a light intensity of 300 μE m^{-2} s^{-1}. Four plants were placed in each of the five chambers and received one of the following treatments: application of a conidial suspension of *St. flocculosus*, or *St. rugulosus*, or *T. washingtonensis* or distilled water. The chambers were compartmentalized to prevent cross-contamination. Each day, five leaves from each plant were sampled randomly and colonization was scored according to an arbitrary scale described by Jarvis et al. (1989) where 0 = no colonization and 5 = 81-100% colonization. This experiment was repeated three times.

Effect of humidity. To determine the effect of r.h., mildewed plants were placed in growth chambers similar to the ones described above maintained at r.h. of 70, 80 or 90% at a temperature of 26 ± 1 °C and a 12-h photoperiod for one week. The r.h. was checked with a portable hygrometer. The experimental design and growth conditions were as described above.

Results

Antagonistic activity of Stephanoascus flocculosus, St. rugulosus and Tilletiopsis washingtonensis. Under in vitro conditions, all three putative antagonists developed profusely on conidia and mycelium of *S. pannosa* var. *rosae* as observed under the stereomicroscope. After 24 h, most chains of conidia had collapsed on all leaflets treated with the antagonists whereas they were abundant on leaflets treated with water alone. After 96 h, inoculated powdery mildew colonies were completely covered by the antagonists, taking a flat and cottony appearance.

Under SEM, after 48 h, the mycelium and conidia of the pathogen had collapsed and were completely covered by dense mycelium and abundant conidia of all three antagonists (Figs. 1B-D). The mycelium of *S. pannosa* var. *rosae* appeared to have lost all turgor but no hyphal penetration by any antagonist was observed. The powdery mildew colonies treated with water alone showed no sign of collapse (Fig. 1A).

Effect of temperature. The best colonization occurred at 26 °C with *St. flocculosus* achieving complete overgrowth in approximately 48 h and *St. rugulosus* and *T. washingtonensis* in 5 days (Fig. 2). Complete colonization was also achieved at 30 °C in 5 days by *St. flocculosus* while a colonization of 80% was noted after 3 and 7 days at temperatures of 22 and 34 °C respectively (Fig. 2A). For *St. rugulosus* and *T. washingtonensis* temperatures below 22 °C and above 30 °C achieved less than 50% colonization of *S. pannosa* var. *rosae* (Figs. 2B-C). At any given temperature, *St. flocculosus* achieved a higher rate of colonization than the other two antagonists.

Effect of humidity. Only a r.h. of 90% achieved complete colonization of *S. pannosa* var. *rosae* by all three antagonists (Fig. 3). Again *St. flocculosus* exhibited a faster activity than the other two antagonists tested. The superior activity of *St. flocculosus* was also noticeable at r.h. 80% and 70% where it reached complete overgrowth within 7 days at r.h. 80% and 80% colonization at r.h. 70% (Fig. 3A). By contrast, *St. rugu-*

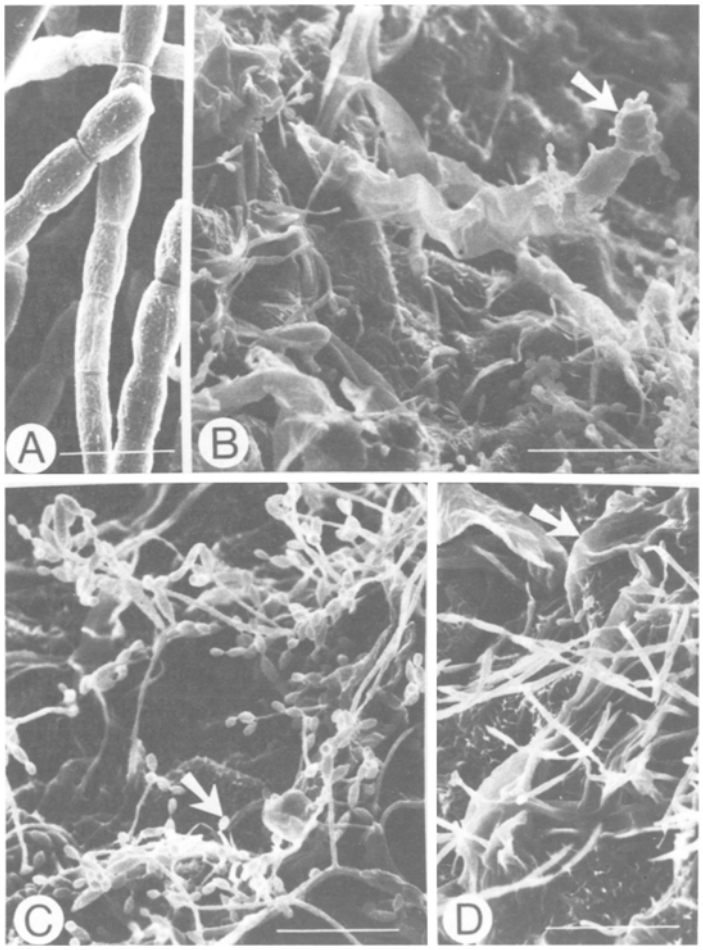

Fig. 1. Colonization of *Sphaerotheca pannosa* var. *rosae* 48 h after application of (A) water, (B) *Stephanoascus flocculosus*; note collapsed host terminal conidia (arrow) and mycelium, (C) *St. rugulosus*; note warted conidia (arrow) and collapsed host conidia, and (D) *Tilletiopsis washingtonensis*; note collapsed host mycelium (arrow). Scale bar represents 20 μm.

losus and *T. washingtonensis* never attained maximum colonization below r.h. 90% with the latter being the least active especially at r.h. 70% (Figs. 3B-C).

Discussion

Our results clearly indicate that *St. flocculosus*, *St. rugulosus* and *T. washingtonensis* can impede the development of *S. pannosa* var. *rosae*. The antagonistic effects of the *Stephanoascus* species had been reported previously only on one other species of powdery mildew, *Sphaerotheca fuliginea* (4). *Tilletiopsis* spp. had shown antagonistic activities against a larger number of powdery mildew species (Hijwegen and Buchenauer, 1984), but evidently had not been tested against *S. pannosa* var. *rosae*. It thus

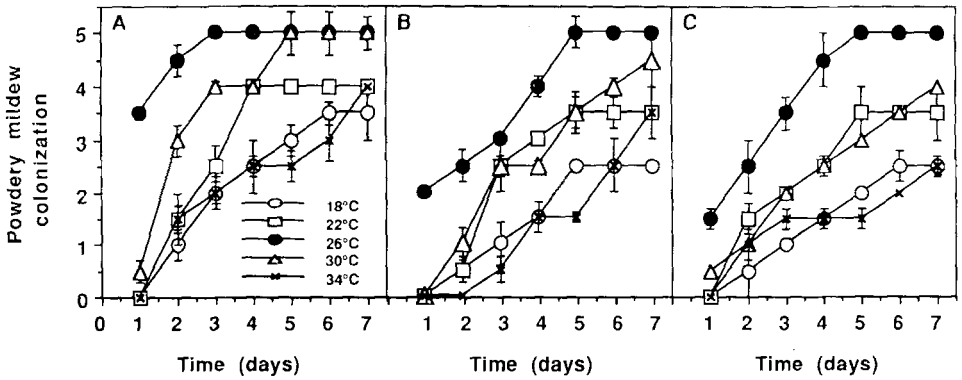

Fig. 2. Effect of temperature on colonization of *Sphaerotheca pannosa* var. *rosae* by (A) *Stephanoascus flocculosus*, (B) *St. rugulosus*, and (C) *Tilletiopsis washingtonensis*. Vertical bars represent standard error of the mean. Relative humidity was maintained at 90%.

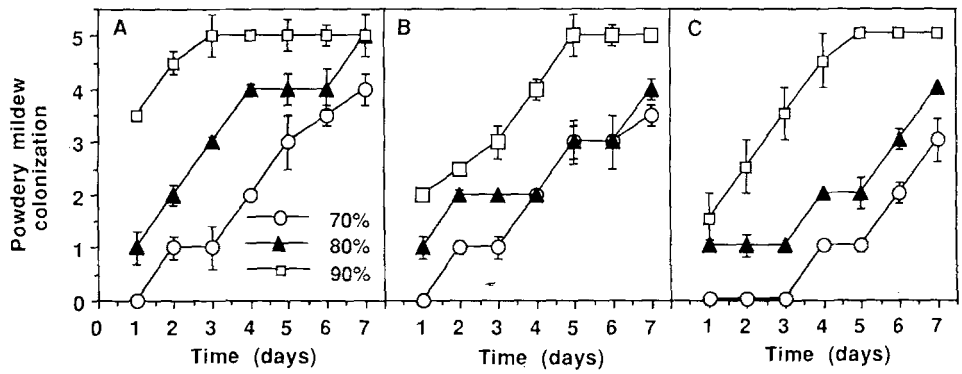

Fig. 3. Effect of relative humidity on colonization of *Sphaerotheca pannosa* var. *rosae* by (A) *Stephanoascus flocculosus*, (B) *St. rugulosus*, and (C) *Tilletiopsis washingtonensis*. Vertical bars represent standard error of the mean. Temperature was maintained at 26 °C.

appears that all three antagonists tested here are probably active against a large number of powdery mildew fungi. Both *Stephanoascus* species also showed antagonistic activities against *Oidium begoniae* Link and *E. graminis* DC. f.sp. *tritici* Em. Marchal (unpublished).

Based on SEM observations, the mode of action of all three antagonists is primarily directed to powdery mildew conidia and conidiophores, limiting the spread of the disease. No evidence of direct penetration of *S. pannosa* var. *rosae* was observed thus confirming observations by Jarvis et al. for *Stephanoascus* spp. (1988) and Hijwegen (1986) and Klecan et al. (1990) working with *T. minor* Nyland and *T. pallescens* Gokhale respectively. The rapid collapse of the host fungus suggests a plasmolysis of the cell or an alteration of the plasmalemma which would implicate the production of enzymes or specific antibiotics by the antagonists. This mode of action is in accordance with observations by Hoch an Provvidenti (1979) and Jarvis et al. (1989).

Although all three fungi tested were shown to be antagonistic to rose powdery mildew under in vitro conditions, tests on mature plants revealed that the acitivity of

the antagonists was considerably decreased under low ambient humidity conditions and temperatures outside a range of 22 to 30 °C. These observations stress the importance of considering environmental conditions when assessing the activity of antagonistic microorganisms. For instance, we were able, through modifying the environment, to estimate quantitatively the relative activity of the tested organisms. Our results matched very well the ones obtained by Jarvis et al. (1989) who tested *St. flocculosus* and *St. rugulosus* on cucumber powdery mildew. They too found that *St. flocculosus* was a faster colonizer than *St. rugulosus* and was less affected by environmental changes. We were further able to demonstrate that *Tilletiopsis washingtonensis*, despite its reported antagonism against several powdery mildew species, lost its activity appreaciably in low environmental humidity and proved to be less active than *St. flocculosus*.

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