Epiphytic growth and survival of *Tilletiopsis pallescens*, a potential biological control agent of *Sphaerotheca fuliginea*, on cucumber leaves

E.J. Urquhart and Z.K. Punja

Abstract: The influence of low (70%) and high (90%) relative humidity on epiphytic growth, development, and survival of *Tilletiopsis pallescens*, a ballistospore-forming yeast-like fungus, on cucumber leaves was investigated. In addition, survival of the fungus in the presence or absence of powdery mildew (*Sphaerotheca fuliginea*) colonies was determined. Growth and development were visualized by scanning electron microscopy of the leaf surface, and survival was quantified as colony-forming units recovered on a semiselective medium. Development of *T. pallescens* from blastospores that were applied to healthy leaves at 70% relative humidity was limited to small colonies that grew adjacent to leaf veins 7 days after application. At 90% relative humidity, extensive hyphal networks had developed within 3 days of blastospore germination, and ballistospores were produced within 7 days. Growth and sporulation of *T. pallescens* were most extensive at the base and on the surface of leaf trichomes. In the presence of *S. fuliginea*, *T. pallescens* mycelium developed adjacent to hyphae and conidiophores of the pathogen within 3 days at both 70 and 90% relative humidity. However, at 90% relative humidity, growth of *T. pallescens* was more extensive and ballistospores were produced within 5 days, and there was visible collapse of mildew hyphae. There was no evidence of penetration of the leaf or mildew hyphae by *T. pallescens*. Survival of *T. pallescens* was significantly (*P* = 0.05) increased at 1 and 5 days postapplication at 70% relative humidity when blastospores were amended with 1% (v/v) canola oil — lecithin. Survival at 90% relative humidity was also significantly increased with canola oil — lecithin and by the presence of *S. fuliginea*. The addition of liquid paraffin — lecithin or liquid paraffin — Tween had no effect on survival when compared to the control. The results from this study indicate that growth and survival of *T. pallescens* are enhanced by high relative humidity and by the presence of powdery mildew, and canola oil — lecithin amendment improved survival on the leaf surface at reduced ambient humidity.

Key words: antagonism, biological control, powdery mildew, yeast.

Résumé : Les auteurs ont déterminé l'influence d'une humidité relative faible (70%) ou élevée (90%) sur la croissance épiphyte, le développement, et la survie du *Tilletiopsis pallescens*, un champignon levuriforme formant des ballistospores, sur les feuilles du concombre. Ils ont également étudié la survie de ce champignon en présence ou en absence de colonies du mildiou poudreux (*Sphaerotheca fuliginea*). Ils ont observé la croissance et le développement sur les surfaces foliaires avec la microscopie électronique par balayage, alors que la survie a été quantifiée en terme d'unités formant des colonies sur un milieu semi-sélectif. Le développement du *T. pallescens*, à partir de blastospores appliquées aux feuilles saines en présence de 70% d'humidité relative, se limite à de petites colonies qui poussent accolées aux nervures, 7 j après l'application. Avec 90% d'humidité relative, on observe le développement de réseaux extensifs d'hyphes, moins de 3 j après la germination des blastospores, et les ballistospores apparaissent en moins de 7 j. La croissance et la sporulation du *T. pallescens* sont les plus fréquentes à la base et sur la surface des trichomes. En présence du *S. fuliginea*, le mycélium du *T. pallescens* se développe accolé aux hyphes et aux conidiophores du champignon pathogène, en moins de 3 j, en présence de 70% aussi bien que de 90% d'humidité relative. Cependant, à 90% d'humidité relative, la croissance du *T. pallescens* est plus étendue, les ballistospores apparaissent en moins de 5 j, et on observe un affaissement perceptible des hyphes du mildiou. Il n'y a pas de preuve de pénétration de la feuille ou des hyphes du mildiou par le *T. pallescens*. La survie du *T. pallescens* augmente de façon significative (*P* = 0.05) à 1 et 5 j après l'application, en présence de 70% d'humidité relative, lorsque les blastospores sont enrobées avec 1% (v/v) d'huile de canola/lecithine. La survie en présence de 90% d'humidité relative augmente également de façon significative avec le traitement huile de canola/lecithine et par la présence du *S. fuliginea*. L'addition de paraffine liquide/lecithine ou de paraffine liquide/Tween reste sans effet sur la survie lorsque comparée au témoing. Les résultats de cette étude indiquent que la croissance et la survie du *T. pallescens* sont stimulées par une humidité relative élevée et par la présence de mildiou poudreux, alors qu'un apport d'huile de canola/lecithine en améliore la survie sur la surface foliaire, en présence d'une humidité ambiante réduite.

*Mots clés* : antagonisme, lutte biologique, mildiou poudreux, levure.

Received August 14, 1996.

E.J. Urquhart and Z.K. Punja. Centre for Pest Management, Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

1 Author to whom all correspondence should be addressed. e-mail: punja@sfu.ca
Introduction

Species of the genus *Tilletiopsis* Derx (Class Basidiomycetes, Order Tilletiales) are frequently found in nature as spores. The spores germinate to produce regularly branched ally by forming blastospores, ballistospores, or chlamydo-spores. Yeast cells can be formed by powdery mildew and rust fungi (Boekhout 1991; Nyland 1950). Members of the genus *Tilletiopsis* reproduce asexually by forming blastospores, ballistospores, or chlamydo-spores. The spores germinate to produce regularly branched monokaryotic hyphae, which often are partly lysed with retraction septa. Yeast cells can be formed by *T. fulvescens*, *T. minor*, and *T. washingtonensis*, and no clamp connections are present (Boekhout 1991).

In nature, species of *Tilletiopsis* were reported to be isolated at a higher frequency from powdery mildew infected leaves compared with healthy leaves during the dry summer months (Urquhart et al. 1994). The interactions between the mildew pathogen and the yeast on the leaf surface have not been previously studied. Common phylloplane yeasts, such as *Sporobolomyces*, can successfully colonize leaf surfaces, and more colonies were formed near fungal infections or physically damaged areas (Last 1970). The survival of these yeasts is dependent on nutrient availability on the leaf surface, which may originate as leakage from the leaf (Pugh and Buckley 1971) or from other sources, such as aphid honey-dew secretions (Fokkema et al. 1983).

The abiotic environment, in particular moisture availability and ambient temperature, can also affect survival of yeasts such as *Sporobolomyces* on the leaf surface (Bashi and Fokkema 1977). In growth-chamber studies, recovery of *Tilletiopsis* following application of blastospores to cucumber leaves maintained at 95% relative humidity (RH) and 25°C was shown to decline within 7 days (Urquhart et al. 1994). The moisture available on the leaf surface also influenced the level of *Tilletiopsis* recovered, which was measured indirectly by a spore-fall method (Pennycook and Newhook 1981).

Several *Tilletiopsis* species have attracted interest because of their potential to act as biological control agents of powdery mildew on barley (Klecan et al. 1989; Knudsen and Skou 1993) and cucumber (Hijwegen 1986, 1992; Urquhart et al. 1994) caused by species of *Blumeria* and *Sphaerotheca*, respectively. For biological control agents to be effective against pathogen growth, survival in the phyllosphere or rhizosphere in sufficient numbers is an important requirement (Cook and Baker 1983). To enhance survival of phylloplane yeasts, formulation of inoculum with mineral or vegetable oils to reduce humidity stress or addition of nutrients to promote growth and survival could be attempted. The extent of survival of yeasts on the leaf surface can be determined by the leaf-print technique (Nyland 1950), by quantifying colony-forming units, or by visualizing the propagules in situ. There have been no studies to quantify the influence of ambient relative humidity on epiphytic growth and survival of *Tilletiopsis* species, or to determine the effects of various formulations of inoculum on promoting survival at reduced relative humidity. Furthermore, the significance of the association between *Tilletiopsis* and mildew occurrence in nature and its influence on growth and survival of the yeast have not been studied.

The objectives of this research were to determine (i) the influence of low (70%) and high (90%) relative humidity on growth and survival of *Tilletiopsis pallescens* on cucumber leaves, (ii) whether the presence or absence of powdery mildew influenced yeast growth and survival, and (iii) the effects of several amendments to yeast inoculum on survival. Both scanning electron microscopy and dilution plating were used to quantify survival. Preliminary results have been published (Urquhart and Punja 1996).

Materials and methods

Plant growth conditions

Cucumber plants (*Cucumis sativus* L. cv. Calypso) were grown from seeds in 9-cm^2^ pots containing sterilized medium (Sunshine Mix No. 1, Sungro Horticulture Canada Ltd., Delta, B.C.) in a controlled-environment chamber (Conviron, Winnipeg, Man.) at 25°C and 90% RH with a 16 h : 8 h light-dark photoperiod (provided by cool-white fluorescent lamps, intensity 100 μmol·m⁻²·s⁻¹) until five true leaves had developed, about 3 weeks after seeding. The plants were fertilized as needed with a commercial hydroponic solution for greenhouse cucumbers (British Columbia Ministry of Agriculture and Fisheries 1987). Powdery mildew (*Sphaerotheca fuliginea* (Schlechtend:Fr.) Pollacci) colonies were maintained on cucumber cv. Corona plants in a separate growth chamber under the same conditions as above. To inoculate cucumber plants, the procedure described by Reuveni et al. (1995) was used. Briefly, conidia were harvested from mildew-infected leaves by washing with distilled water containing 0.01% Tween 20 (polyoxyethylene (20) sorbitan monolaurate) (Sigma Chemical, St. Louis, Mo.). The spore concentration was adjusted to 15,000 spores·mL⁻¹ and lightly atomized onto cucumber leaves. The inoculated plants were maintained at 95% RH and 25°C until most of the leaf area was infected (about 2 weeks).

Inoculum production

An isolate of *T. pallescens* Gokhale (ATCC 96155) obtained from powdery mildew infected cucumber leaves in a greenhouse in Maple Ridge, British Columbia, was used. The culture was maintained on potato dextrose agar (PDA; Difco Laboratories, Detroit, Mich.) and placed at 4°C for long-term storage. For inoculum production, a broth medium composed of 10 g Bacto-peptone (Difco), 25 g glucose (BDH, Toronto), and 1 g microbiological yeast extract (Merck, BDH, Toronto) in 1 L distilled water was used (Urquhart et al. 1994). The broth (50 mL volume dispensed into a 125-mL flask) was inoculated with a 5 mm diameter plug of mycelium from a 3-week-old PDA culture and incubated on a rotary shaker at 150 rpm for 72 h under ambient laboratory conditions. Subsequent cultures were initiated by aseptically transferring 0.1 mL of the broth culture to fresh medium. To recover a blastospore suspension, the culture was filtered through three layers of sterile cheesecloth to remove large masses of mycelium, and the spores were diluted to 2 × 10⁷/mL with sterile water.

Survival of *Tilletiopsis* on the leaf surface

The abaxial leaf surface of four expanded cucumber leaves on each of the three replicate plants was sprayed with a *T. pallescens* blastospore suspension using a hand-held atomizer to provide an even distribution of inoculum (about 0.3 mL/leaf). A group of plants was placed in controlled-environment chambers set at 70 or 90% RH. The treated plants were either healthy or were previously infected with powdery mildew as described. For dilution plating to quantify survival, sampling was done at 0, 1, and 5 days after application. A single leaf was removed from each of the three replicate plants grown at each humidity and with mildew—nonmildew treatment. The leaf was cut into sections (about 1 cm²) with sterilized scissors and aseptically transferred to a 250-mL bottle containing 100 mL of 0.1% Bacto-peptone solution (Difco). The suspension was
Fig. 1. Growth of *Tilletiopsis* on healthy cucumber leaf surfaces at two relative humidities. (A) Small *Tilletiopsis* colony (arrow) adjacent to leaf vein at day 3 at 70% RH. (B) *Tilletiopsis* hyphal mass at day 7 at 70% RH. (C) Blastospore germination and germ tube growth at 24 h at 90% RH. (D) Extensive networks of hyphae on the leaf surface at day 3 at 90% RH. Scale bars = 10 µm.

shaken at 250 rpm for 10 min, at which time serial dilutions (up to $10^{-3}$) were made and a 0.5-mL sample was plated onto a semi-selective medium (Urquhart et al. 1994). The semiselective medium was composed of 17 g cornmeal agar (Difco) in 1 L distilled water with 10 µg·mL$^{-1}$ dichloran (Botran 75 WP). The sterile medium was cooled to 50°C and sterile ampicillin (Sigma) was added at 100 mg·L$^{-1}$ (Urquhart et al. 1994). Two replicate dishes were used for each dilution at each sampling time for each treatment. The dishes were incubated at 20°C for 14 days, the number of *Tilletiopsis* colonies that developed was counted, and the replicate dish counts were averaged. The experiment was repeated three times with powdery mildew present in two of the three experiments. Data were converted to colony-forming units (cfu) recovered per leaf.

**Scanning electron microscopy**

Leaves with and without powdery mildew were treated with a *T. pallescens* spore suspension as above and sampled 1, 3, 5, and 7 days after application for scanning electron microscopy (SEM). Leaf sections (0.5 cm$^2$) were placed on a nylon screen in a water-saturated atmosphere for up to 2 h to enhance leaf turgor. The nylon screen was attached to Plexiglas tubing (5 cm high × 10 cm wide), and placed in a covered 1.5-L beaker lined with wet filter paper to provide the water-saturated atmosphere. The leaf sections were rapidly frozen in the vapour phase above liquid nitrogen without any chemical fixation and placed on an ice layer inside glass bottles and placed in a glass vacuum container prior to lyophilization. All glassware which was in contact with the frozen leaf segments was

© 1997 NRC Canada
previously cooled in liquid nitrogen to prevent thawing. The freeze-dried leaf segments were then mounted on aluminum stubs and coated with gold using vacuum evaporation prior to SEM examination.

**Survival of Tilletiopsis in formulations**

A suspension of blastospores \((2 \times 10^7 \text{ mL}^{-1})\) of *T. pallescens* from a 72-h-old culture was placed in 100-mL sterile glass bottles and either not amended or amended (at a concentration of 1.0% v/v) with one of three treatments: (i) liquid paraffin – lecithin containing 10 g fine-ground, food-grade soy bean lecithin (approx. 90% pure, Trophic Foods, Vancouver, B.C.) in 100 mL of liquid paraffin (light) {BDH}, prepared by heating with vigorous stirring on a magnetic stirrer at 140°C for 30 min; (ii) liquid paraffin – Tween 80, consisting of 2 mL Tween 80 (polyoxyethylene (20) sorbitan monooleate) surfactant in 18 mL of liquid paraffin, mixed by vortexing together for 2 min; and (iii) canola oil – lecithin, containing 10 g food-grade soy bean lecithin in 100 mL food-grade canola oil (Canbra Foods Ltd., Lethbridge, Alta.), prepared by heating with vigorous stirring on a magnetic stirrer at 140°C for 30 min. The amendments were prepared prior to mixing with the inoculum and were stored at 4 °C until required. Inoculum and amendments were combined, and the bottles were capped and vigorously mixed for 1 min and then incubated at 20°C for 24 h. The suspensions were subsequently serial diluted (up to \(10^{-5}\)) with 0.1% Bacto-peptone water, and recovery of inoculum was determined on semiselective medium. The experiment was repeated four times, each with two replicates.

**Effect of formulation on survival of Tilletiopsis on cucumber leaves**

A blastospore suspension \((1 \times 10^7 \text{ spores} \cdot \text{mL}^{-1})\) from a 72-h-old culture was either unamended or amended with liquid paraffin – lecithin, liquid paraffin – Tween 80, or canola oil – lecithin to provide a concentration of 1.0% v/v as described above. The inoculum was sprayed uniformly using a hand-held atomizer onto each of eight fully expanded leaves (total volume applied per leaf = 0.3 mL). The plants were returned to the growth chamber after the leaves had dried. The population level of *Tilletiopsis* was determined 12 h after application (day 1) and on day 5 after application by serial dilution onto the semiselective medium as described previously.

**Effect of canola oil and lecithin on sporulation**

Sterile culture broth was amended with either 1% canola oil (Canbra), 0.1% lecithin (Trophic), both oil and lecithin, or no amendments (control). Amendments were sterilized by autoclaving for 15 min and cooled prior to addition to broth. Three flasks were prepared for each treatment and were inoculated with 0.1 mL of a 72-h-old *T. pallescens* suspension culture and incubated on a rotary shaker at 150 rpm at 20°C for 72 h under fluorescent lamps. The spore concentration was determined by serial dilution and plating on semiselective medium as described previously.

**Data analysis**

The results of experiments on survival with and without amendment treatments on the leaf surface were blocked by replicates prior to analysis. Each combination of time of sampling and percent RH was analyzed separately. All other experimental results were grouped and tested for significant difference between treatments using the general linear model program with multiple comparison testing of treatment means performed using the Bonferroni least significant difference method present in the SAS statistical analysis program (SAS Institute Inc., Cary, N.C.).

**Results**

**Survival of Tilletiopsis on the leaf surface: scanning electron microscopy**

**Healthy leaves**

Growth and development of *T. pallescens* following application of blastospores to cucumber leaves were affected by relative humidity. At 70% RH, very small colonies were visible after 3 days, and these were generally found adjacent to major leaf veins and trichomes (Fig. 1A). The colonies were presumed to originate from germinated blastospores and developed closely associated hyphae by day 7 (Fig. 1B).
The colonies did not increase significantly in size after 7 days (not shown), and no sporulation or chlamydospore formation was observed at this time. At 90% RH, blastospore germination was observed within 24 h (Fig. 1C) and germ tube growth was extensive. At 3 days, hyphal growth resulted in the formation of a network of fine mycelium on the leaf surface (Fig. 1D). Growth was epiphytic and there was no evidence of leaf penetration by *Tilletiopsis*. Sporulation was observed on hyphae growing on leaf trichomes (Fig. 2A), and the characteristic crescent-shaped ballistospores of *Tilletiopsis* were produced by day 7 (Fig. 2B).

**Mildew-infected leaves**

Healthy hyphae and conidiophores of *Sphaerotheca fuliginea* were observed at 70% RH on day 1; the conidia appeared turgid and were formed in long chains. A small colony, presumed to be of *Tilletiopsis*, was also visible (Fig. 3A). By day 3, the mycelium of *Tilletiopsis* was extensive and an extracellular amorphous matrix was visible, which may have originated from the culture medium. The conidiophores of *Sphaerotheca fuliginea* remained intact even when growing in close contact with *Tilletiopsis* hyphae at days 1 and 3 (Figs. 3A and 3B). By day 5, however, *Sphaerotheca fuliginea* conidia and hyphae had collapsed when in direct contact with *Tilletiopsis* hyphae (Fig. 3C). Large amounts of the extracellular matrix were still visible at day 5. There was no ballistospore or chlamydospore formation by *Tilletiopsis* at 70% RH.

At 90% RH, hyphae of *Tilletiopsis* and the extracellular matrix were visible at day 1 (Fig. 4A). By day 5, the mycelium had grown over large sections of the leaf surface and on trichomes (Fig. 4B) and ballistospores were produced at the end of hyphae. The ballistospores were formed singly and eccentrically (Fig. 4C). Mildew hyphae and conidia were collapsed in areas adjacent to *Tilletiopsis* hyphae (Fig. 4C).

**Survival of Tilletiopsis in formulations**

*In vitro*

After 24 h of exposure, the canola oil – lecithin amendment significantly improved \( (P = 0.05) \) the number of *Tilletiopsis* colonies recovered (Fig. 5). Canola oil alone had no effect on spore survival when compared with the nonamended control \( (P = 0.05) \). Liquid paraffin – lecithin, liquid paraffin – Tween 80, and lecithin alone had a negative effect on blastospore survival \( (P = 0.05) \).

*Leaf surface*

At 70% RH, canola oil – lecithin was the most effective formulation tested on days 1 and day 5 (Fig. 6). On day 1, this amendment provided the highest survival \( (P = 0.05) \) among.
Fig. 4. Growth of *Tilletiopsis* on mildew-infected cucumber leaf surfaces at 90% RH. (A) Extracellular matrix surrounding *Tilletiopsis* hyphae and *Sphaerotheca fuliginea* hyphae on day 1. (B) Colonization of leaf trichomes by *Tilletiopsis* with ballistospore production on day 5. Scale bars = 10 µm. (C) Collapsed *Sphaerotheca fuliginea* hyphae and conidia with *Tilletiopsis* spore production (arrow) by day 5.

Fig. 5. Effect of amendments on survival of *Tilletiopsis* spores after 24 h exposure in vitro. Bars with the same letter are not significantly different at $P = 0.05$.

---

all the treatments. Liquid paraffin – Tween 80 and mildewed leaves were similar to the control treatment. Liquid paraffin – lecithin was the least effective at maintaining *Tilletiopsis* survival. On day 5, canola oil – lecithin was the only formulation which significantly ($P = 0.05$) enhanced survival compared with all the other treatments.

At 90% RH, canola oil – lecithin and mildewed leaves were significantly better ($P = 0.05$) than the other treatments, except for liquid paraffin – Tween 80, at maintaining survival on day 1. Liquid paraffin – lecithin and the control were equal in value. On day 5, canola oil – lecithin, liquid paraffin – Tween 80, and mildewed leaves were comparable to the control in their ability to maintain *Tilletiopsis* survival, whereas liquid paraffin – lecithin was significantly worse. Growth of *Tilletiopsis* colonies after recovery from the leaf surface was slow, requiring 2 weeks of incubation at room temperature for identifiable colonies to develop on the semi-selective medium (Fig. 7).
Fig. 6. Survival of *Tilletiopsis* on cucumber leaf surfaces at either 70 or 90% RH as influenced by amendments to blastospores prior to application to the leaves. All amendments were used at 1% v/v. Powdery mildew infected leaves are also one of the treatments. Bars with the same letter are not significantly different at $P = 0.05$.

![Graph showing survival of *Tilletiopsis* on cucumber leaf surfaces at different RH and treatments.](image)

Fig. 7. *Tilletiopsis pallescens* colonies growing on semiselective medium at 14 days after recovery from the inoculated leaf surface.

**Effect of canola oil and lecithin on sporulation**
The addition of either canola oil – lecithin, canola oil, or lecithin had no significant effect ($P = 0.05$) on sporulation of *T. pallescens* after 72 h (Fig. 8).

**Discussion**

Epiphytic growth and development of *T. pallescens* on healthy cucumber leaves was influenced by ambient relative humidity. At 70% RH, colonies were observed only adjacent to major leaf veins and trichomes, and there was no visible expansion from these areas. The colonies were comprised of small, dense hyphal masses, and no ballistospores or chlamydospores developed over the 7-day examination period. A similar spatial pattern of colony development was observed with another common phylloplane yeast, *Sporobolomyces roseus*, in which colonies were formed principally above the leaf veins (Pugh and Buckley 1971). These regions may be more conducive to growth by providing a microenvironment with higher moisture availability and nutrients.

At 90% RH, blastospore germination of *T. pallescens* was followed by rapid germ tube growth, and large sections of...
the leaf surface were colonized within 3 days, especially on trichomes. Leaf trichomes can provide both structural and storage functions, including secretory and storage sites for secondary metabolites, which act as defensive compounds (Beckman et al. 1972). They can also provide a habitat for spore release (Knudsen and Skou 1993). The extent of storage functions— including secretory and storage sites for (Hajlaoui and Belanger 1991). In other research, growth of the greatest at 26 °C and 90% RH but was reduced at 70% RH (Hajlaoui and Belanger 1991). In other research, growth of Tilletiopsis on the trichomes could be the result of more nutrients or growth stimulants being available at these sites.

Previous studies on the effect of percent RH on survival of Tilletiopsis albescens have indicated that over 70% was needed to maintain a stable population as indicated by blastospore release (Knudsen and Skou 1993). The extent of colonization of Sphaerotheca pannosa var. rosea by Tilletiopsis washingtonensis on leaf disks was reported to be greatest at 26°C and 90% RH but was reduced at 70% RH (Hajlaoui and Belanger 1991). In other research, growth of T. albescens was found to be better when inoculated onto barley leaves parasitized with Blumeria graminis f.sp. hordei (Knudsen and Skou 1993), suggesting that the presence of powdery mildew in some manner enhanced Tilletiopsis development.

In this study, growth of T. pallescens was more extensive at both 70% RH and 90% RH when Sphaerotheca fuliginea was present compared to healthy leaves maintained under similar conditions. At 70% RH, hyphae and conidiophores of Sphaerotheca fuliginea in direct contact with Tilletiopsis hyphae showed signs of collapse after 5 days but development of Tilletiopsis was limited to hyphal growth. At 90% RH, hyphae of Tilletiopsis with extracellular matrix was evident 1 day after application, and adjacent mildew hyphae showed some collapse. Within 5 days, numerous fine Tilletiopsis hyphae were observed growing around Sphaerotheca fuliginea and mildew conidiophores and hyphae appeared collapsed.

There was no evidence of penetration of mildew hyphae by Tilletiopsis at either 70% RH or 90% RH. Previous studies reported changes in the cell organelles of B. graminis near the interface with T. pallescens using transmission electron microscopy (Klecan et al. 1989), while T. albescens was reported to cause perforations in S. fuliginea hyphae (Knudsen and Skou 1990). Stephanoascus flocculosus (Pseudozygma flocculosa (Boekhout 1995)), another yeast-like fungus with biocontrol potential, caused retraction of cytoplasm in S. pannosa var. rosea cells and hyphal plasmolysis and breakdown occurred, leaving only the cell wall after 24 h at 100% RH (Hajlaoui et al. 1992). Antibiotic production was the proposed mode of action for P. flocculosus (Hajlaoui et al. 1994), and two novel antibiotics have been isolated which showed activity against several fungi and bacteria (Choudhury and Traquair 1994). The effect of Pseudozyma on mildew resembles that of Tilletiopsis, although the production of antibiotics in the latter has not been reported. Instead, cell wall degrading enzymes, such as β-1,3-glucanase, are produced by Tilletiopsis, which may be involved in causing the collapse of mildew hyphae (Urquhart et al. 1994). An extracellular matrix that was present adjacent to Tilletiopsis hyphae was likely derived from the culture medium or was the result of polysaccharide materials produced during growth.

In previous studies, the protectant effects of formulation of T. minor and Ampelomyces quisqualis inoculum was demonstrated indirectly through improved biological control activity. A mineral oil-based adjuvant enhanced antagonism of T. minor to S. fuliginea on cucumber (Hijwegen 1992), while liquid paraffin combined with various Tween surfactants increased parasitization of Blumeria polyphaga and Sphaerotheca fuliginea by A. quisqualis (Philipp et al. 1990). An unrefined corn oil to water (1:1) emulsion also enhanced germination and subsequent infection by the mycobiherbicide Colletotrichum truncatum of Sesbania exaltata (Egley and Boyette 1995). Cottonseed oil provided superior infectivity of Metarhizium flavoviride on desert locusts (Schistocerca gregaria) when compared to water with 0.05% Tween 80 at 35% RH (Bateman et al. 1993). In our study, liquid paraffin—Tween 80 improved survival of Tilletiopsis inoculum at days 1 and 5 at 90% RH but not at 70% RH. Addition of 0.1 or 0.5% commercial liquid paraffin emulsion (Hora Olea 11E) or 0.1% coffee cream (Nutricia) was shown to significantly increase biocontrol activity of T. minor against Sphaerotheca fuliginea (at 60–80% RH) compared with T. minor alone (Hijwegen 1992).

Published information on the effects of amendments, either positive or negative, on survival of biological control agents is very limited. Anionic surfactants were suggested to be least toxic (Ward 1984), but if the carbon chain contained more than 14–16 carbons, there was phytotoxicity to plant cell cultures as the molecules became more hydrophilic (Ernst et al. 1982). A short-term exposure (1 day) to canola oil—lecithin significantly improved the recovery of Tilleti-
oposis compared with the unamended control or canola oil alone. This formulation was also the most effective when tested on whole plants at both 70 and 90% RH. Parasitization of *Sphaerotheca fuliginea* by *A. quisqualis* at 80% RH was equivalent to that at 95% RH when spores were applied in a water emulsion containing 1% liquid paraffin (Philipp and Hellstern 1986). However, when percent RH varied between 70 and 85% in greenhouse trials, a combination of liquid paraffin with the emulsifier MRJ 59 was the most effective, but was significantly less effective compared with that at 95% RH only (Philipp et al. 1990).

The liquid paraffin – lecithin amendment reduced *Tilletiopsis* survival at 70% RH on day 1. The addition of 0.1% lecithin to broth cultures had no effect on *Tilletiopsis* blastospore production over 3 days. Phospholipids are components of all cell membranes (Ansell et al. 1973), and commercial lecithin preparations are generally recognized as safe in foodstuffs and are unregulated in Canada and the United States (Maga and Tu 1995), suggesting that they could be readily used in commercial formulations.

In our study, development of *T. pallescens* at 70% RH was shown to be limited to growth of mycelium without any spore production, both on healthy leaves and on those with *Sphaerotheca fuliginea*, whereas high humidity resulted in rapid mycelium development and spore production on cucumber leaf surfaces. However, previous trials had shown that *T. pallescens* populations were reduced over time, even at 95% RH (Urquhart et al. 1994). The amount of growth achieved should positively correlate with the level of biocontrol, since there was more rapid collapse of *Sphaerotheca fuliginea* at 90% RH than at 70% RH. Therefore, providing amendments to inoculum which would moderate the effect of low humidity could provide a means of improving biological control activity against *Sphaerotheca fuliginea*. We found canola oil combined with soy lecithin was superior to light paraffin oil in promoting survival of *T. pallescens* at reduced relative humidity. These results indicate that such amendments can provide relief from moderate humidity stress and enhance growth and development of this biocontrol agent on the phylloplane.

Acknowledgements

This study was supported by a grant from the Natural Sciences and Engineering Research Council of Canada. We thank Vic Bourne for advice on scanning electron microscopy.

References


