

## Reduced Growth of *Erysiphe graminis* f. sp. *hordei* Induced by *Tilletiopsis pallescens*

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Advice provided by G. Adams, Department of Botany and Plant Pathology, Michigan State University, is greatly appreciated.

We thank M. Hermanns for sectioning samples for transmission electron microscopy.

Supported in part by grants from the U.S. Department of Energy (DE-AC02-76-ER01338) and the National Institute of Health (GM 40084). The financial support of the "Dr. Christian Dräger Stiftung" is gratefully acknowledged.

Michigan Agriculture Experiment Station Journal Article Number 13042.

Accepted for publication 20 September 1989 (submitted for electronic processing).

### ABSTRACT

Klecan, A. L., Hippe, S., and Somerville, S. C. 1990. Reduced growth of *Erysiphe graminis* f. sp. *hordei* induced by *Tilletiopsis pallescens*. *Phytopathology* 80: 325-331.

The leaf epiphyte, *Tilletiopsis*, which possesses hyaline ballistoconidia, was found contaminating barley seedlings infected with the obligate parasite *Erysiphe graminis* f. sp. *hordei*. The contaminant was identified as an isolate of the rare *Tilletiopsis pallescens* based on morphological, physiological, and biochemical characteristics. An antagonistic relationship between *E. g. hordei* and *T. pallescens* was demonstrated

on the surface of barley leaf segments. On a gross level, *T. pallescens* caused severe reduction of mycelial expansion and spore production by *E. g. hordei*, whereas *T. minor* was antagonistic to a lesser extent. Low-temperature scanning and conventional transmission electron microscopy showed that hyphae of *E. g. hordei* were collapsed and degenerated in the presence of *T. pallescens*.

*Additional keywords:* biocontrol, isozyme, mycoparasite, powdery mildew.

Yeastlike, haploid *Tilletiopsis* spp. are common epiphytes identified primarily by possession of hyaline ballistoconidia formed on short sterigmata (1,6,20,29). During the warmer months, ballistoconidia can be obtained in large numbers from air samples taken above cereal crops (13,22). In addition, *Tilletiopsis* spp. have been isolated from a wide range of habitats, including sewage, a fruiting body of *Sirobasidium* sp., living or dead aerial plant parts, and, especially, leaves infected with powdery mildew, rust, or smut (3,6,14,19-21,29). Identification of *Tilletiopsis* Derx to the species level is difficult when based on ballistoconidia size, colony morphology, or physiological criteria. Isozyme electrophoresis patterns, though, are sufficient to uniquely describe each species of *Tilletiopsis* (33).

Another inhabitant of barley aerial surfaces is *Erysiphe graminis* DC. f. sp. *hordei* Ém. Marchal, an obligate fungal pathogen causing powdery mildew of barley. During the maintenance of laboratory stock cultures of *E. g. hordei*, an isolate of *Tilletiopsis*

was recovered that appeared to interfere with the growth of *E. g. hordei*. Cultures of *E. g. hordei* on uniformly inoculated leaves exhibited atypical patches of clearing. With a stereo microscope, the white, "fuzzy" mycelial growth of *Tilletiopsis* was observed covering the hyphae of *E. g. hordei* surrounding the cleared patches. This observation suggested that *Tilletiopsis* might be a mycoparasite and exploitable as a biocontrol organism. In this report, the isolate of *Tilletiopsis* is identified to species, and an antagonistic relationship between this fungus and *E. g. hordei* on barley leaves is described.

### MATERIALS AND METHODS

**Plant material.** Near isogenic barley (*Hordeum vulgare* L.) lines CI-2330, CI-16137, CI-16139, and CI-16141 (17), each carrying a different powdery mildew resistance allele, were provided by J. G. Moseman, U.S. Department of Agriculture-Agricultural Research Service, Bethesda, MD. To establish aseptic seedlings, barley seeds were dehulled, surface disinfested with 5% sodium hypochlorite for 28 min, and rinsed with sterile water. These seeds were germinated in the dark at room temperature on potato-

dextrose agar (PDA) (Difco Laboratories, Detroit, MI) plates adjusted to pH 6.5 with NaOH. After 2 days, healthy seedlings were transferred aseptically to test tubes (25 × 200 mm) containing 7.5 ml of nutrient medium for *Arabidopsis* (26) solidified with 0.25% Gel Gro (ICN Biomedical, Inc., Cleveland, OH). These seedlings were grown at approximately 22–26 C under cool white fluorescent lamps (100 μE m<sup>-2</sup> s<sup>-1</sup>) with a 16-hr day cycle.

**Fungal material.** Races 59.3 (= 061) and 59.2 (= 12A1) of *E. g. hordei* (16) were received from J. G. Moseman. Race CR3 was obtained from R. Wise, Michigan State University, East Lansing, and race NCI was recovered locally. Aseptic cultures were maintained by transferring conidia weekly to barley leaf segments on 1.2% agar, 1 mM Ca(NO<sub>3</sub>)<sub>2</sub>, and 1 mM benzimidazole (CBA) in petri dishes (4). Races of *E. g. hordei* were grown on selective barley lines: race 59.3 on line CI-16141, race 59.2 on line CI-16139, race NCI on line CI-16137, and race CR3 on line CI-2330. Cultures of *E. g. hordei* were grown in a growth cabinet set at 20 C with a 15-hr day and a light intensity of 20 μE m<sup>-2</sup> s<sup>-1</sup> (15).

Four species of *Tilletiopsis* were obtained from the American Type Culture Collection (ATCC), Rockville, MD: *T. fulvescens* Gokhale (ATCC 24344), isolated from *Forsythia* sp.; *T. minor* Nyland (ATCC 10764), obtained from leaves; *T. pallescens* Gokhale (ATCC 24345), isolated from a fruiting body of *Sirobasidium* sp.; and *T. washingtonensis* Nyland (ATCC 36489), isolated from a leaf surface. The Michigan isolate of *Tilletiopsis* was isolated in our laboratory from cultures of *E. g. hordei* maintained on barley seedlings from field-grown seed lots. Cultures of *Tilletiopsis* were maintained at room temperature on cornmeal agar (CMA). For the isozyme analysis, mycelial mats grown on the surface of the liquid culture medium described by Yamazaki and Komagata (34) were used. For leaf segment antagonism experiments, all species of *Tilletiopsis* were grown on Czapek's agar modified to contain 0.3% (w/v) sucrose (30). Environmental conditions were the same as those used to maintain the cultures of *E. g. hordei*. The Michigan isolate of *T. pallescens* has been deposited in the American Type Culture Collection, and a voucher specimen has been deposited in the Michigan State University Fungal Herbarium. *Penicillium* sp., *Alternaria* sp., and *Cladosporium* sp. were isolated from barley seeds.

**Physiological properties of *Tilletiopsis* spp.** The methods of Van der Walt and Yarrow (32) were used for nutrient assimilation and temperature growth tests. Use of glucose, α-methyl-D-glucoside, galactose, phenylalanine, and sorbose, along with growth in vitamin-free medium, were tested in standing liquid cultures at room temperature. The ability to hydrolyze arbutin was observed in agar slant tubes. Inoculum (25 μl) consisted of hyphal fragments from 1-mo-old mycelial mats of *Tilletiopsis* sheared by agitation. In addition, growth at 30 C on malt extract agar plates (Difco Laboratories) was assessed. To initiate this experiment, a malt extract agar culture block was dragged over a malt extract agar plate. The experimental design was a completely random design with three replicates. The experiment was performed twice.

**Microscopic examination of anastomosis.** As a modification of the method of Parmeter et al (23), single-layer sections of autoclaved dialysis tubing were placed on microscope slides coated with clarified CMA. Loops of hyphal fragments and ballistocidia from *T. pallescens* or the Michigan isolate were transferred to the piece of dialysis tubing. The loops were streaked over the surface in two parallel lines 0.5 cm apart, and the slides were placed in a sterile moist chamber. During the following week, the line interface was observed for hyphal fusions at ×400 magnification with a compound microscope equipped with Nomarski optics (E. Leitz, Inc., Rockleigh, NJ).

**Preparation of cell-free extract of *Tilletiopsis* spp.** Eight-day-old mycelial mats of all cultures, except *T. washingtonensis*, were harvested by vacuum filtration. Mycelia of *T. washingtonensis* were collected by centrifugation at 8,000 g for 10 min. After weighing, mycelia were frozen with liquid nitrogen and ground to a fine powder with a mortar and pestle. The powder was extracted on ice with 50 mM Tris-HCl, pH 7.5, containing 10%

glycerol and 0.1% 2-mercaptoethanol at a rate of approximately 1.5 ml of buffer per gram fresh weight. The extract was centrifuged 4 min at 13,600 g, and the supernatant was concentrated fourfold by placing it in dialysis tubing (*M<sub>r</sub>* cutoff of 6,000–8,000) surrounded by solid polyethylene glycol (*M<sub>r</sub>* 10,000) at 4 C. The concentrated fraction was stored at –80 C. Protein content of the fungal extracts was determined by the Bradford method (2) with reagents purchased from Bio-Rad Laboratories, Richmond, CA; bovine serum albumin served as a standard.

**Cellulose-acetate gel electrophoresis.** The cellulose-acetate gels (Titan III), sample applicator, and the electrophoresis tray were purchased from Helena Laboratories, Beaumont, TX. Previously described methods were used for the isozyme electrophoresis and analysis (24). The running buffer, 20 mM sodium phosphate, pH 7.0, gave the best resolution of glucose-6-phosphate dehydrogenase (EC 1.1.1.49), glutamate-oxaloacetate transaminase (EC 2.6.1.1), and malate dehydrogenase (EC 1.1.1.37). The enzymes, acid phosphatase (EC 3.1.3.2), and isocitrate dehydrogenase (EC 1.1.1.42) were separated with 10 mM citrate-phosphate, pH 6.4, as the running buffer. For 6-phosphogluconate dehydrogenase (EC 1.1.1.44) and alkaline phosphatase (EC 3.1.3.1), 15 mM Tris-HCl, pH 7.8, containing 10 mM MgCl<sub>2</sub> and 5 mM ethylenediaminetetraacetic acid, was used as the running buffer. A fixed volume of 0.25 μl, containing a minimum of 93 ng of protein, was applied to the cellulose-acetate gels. The electrophoretograms were developed at 250 V for 20 min at 4 C. The recipes for enzyme activity staining are described in Richardson et al (24). For the enzyme glutamate-oxaloacetate transaminase, the method of Vallejos (31) was followed. Each experiment was repeated three times.

**Antagonism experiments.** A 1-cm<sup>2</sup> block from PDA cultures of the Michigan isolate and one of either *Alternaria* sp., *Penicillium* sp., or *Cladosporium* sp. were co-plated on PDA or CMA on opposite sides of 9-cm-diameter petri dishes. Colony interactions at room temperature were observed for 1 mo for evidence of reduced growth.

Leaf segments, 3 cm long, were taken 3–6 cm from the tip of the first leaf of 10-day-old, axenically grown barley seedlings. Segments were inoculated with hyphal fragments and ballistocidia of individual *Tilletiopsis* spp. by cutting a 1-cm<sup>2</sup> block from a 2-mo-old culture and dragging the block across the adaxial surface of the leaf three to four times. Uninoculated control segments were treated similarly with a block of modified Czapek's agar. After this treatment, the leaf segments were inoculated with conidia of *E. g. hordei* by means of a settling tower modified to fit a petri dish (5,18). Inoculated leaf segments were arranged in a pinwheel formation on CBA plates, with one treatment each of the control (no fungal inoculation), *Tilletiopsis* alone, *E. g. hordei* alone, and both *Tilletiopsis* and *E. g. hordei*. The plates were placed in a growth cabinet with conditions for growth of *E. g. hordei* as described previously. At 7 days postinoculation, leaf segments were examined by eye and with a stereo microscope (×31 magnification). The specific pairings of *E. g. hordei* and *Tilletiopsis* are given in each figure. A randomized complete block

TABLE 1. Physiological properties of species of *Tilletiopsis*

Test <sup>a</sup>	Isolate <sup>b</sup>				
	FUL	PAL	MICH	MINOR	WASH
Glucose	+ <sup>c</sup>	+	+	+	+
Galactose	+	+	+	–	–
α-methyl-D-glucoside	–	+	+	–	–
Phenylalanine	+	+	+	–	W
Sorbose	–	+	+	–	–
Vitamin free	+	+	+	–	+
Arbutin	+	+	+	+	+
30 C	–	–	W	W	W

<sup>a</sup> Growth was monitored 1 mo after fungi were transferred to medium.

<sup>b</sup> FUL = *T. fulvescens*; PAL = *T. pallescens*; MICH = Michigan isolate; MINOR = *T. minor*; WASH = *T. washingtonensis*.

<sup>c</sup> Growth evaluations are +, growth; W, weak growth; –, no growth.

design with four replicates was used for these experiments. Each experiment was repeated at least twice.

**Low-temperature scanning electron microscopy.** Leaf segments were treated as described for the antagonism experiments. At 7 days postinoculation, leaf segments, inoculated with race 59.3 of *E. g. hordei* alone or and with race 59.3 of *E. g. hordei* paired with either *T. pallescens* or the Michigan isolate, were cut into pieces 2.5- to 5-mm in length and mounted with Tissue-Tek O.C.T. compound (Miles Laboratories, Inc., Elkhart, IN) on a stub. The samples were frozen in a nitrogen slush in a SP-2000 cryosystem (Emscope, Asford, Kent, UK). After etching at -65 C and sputter coating with gold, samples were viewed at 15 kV with a JSM35Z scanning electron microscope (JEOL, Tokyo, Japan) equipped with a cryogenic stage cooled to -150 or -95 C. At the higher temperature, the samples appeared more conductive and less subject to charging. Therefore, most samples were viewed at -95 C. This experiment was repeated three times.

**Conventional transmission electron microscopy.** Barley leaf segments were inoculated either with race 59.3 of *E. g. hordei* alone or with both race 59.3 of *E. g. hordei* and the type isolate of *T. pallescens*, as described previously for the antagonism experiments. After 7 days of incubation, 0.5-mm<sup>2</sup> leaf segments were cut and fixed at 4 C overnight in a solution of 2% glutaraldehyde and 1.5% paraformaldehyde in 0.1 M sodium phosphate at pH 7.2. After extensive rinsing in 0.1 M sodium phosphate, pH 7.2, samples were incubated for 3 hr in 1.5% OsO<sub>4</sub> in the same buffer at room temperature. Samples were dehydrated in a graded series of ethanol concentrations, followed by 30 min of incubation in acetone. These tissue samples were infiltrated with Spurr's resin (27), embedded in Beem capsules (size 00, Ted Pella, Redding, CA), and thin sectioned to give silver sections. Finally, sections from three blocks of each treatment were mounted on one-hole grids coated with pioloform (Polaron, Cambridge, MA) and carbon, stained with 8% aqueous uranyl acetate and alkaline lead citrate for 10 min each, and examined with an EM10 Electron Microscope (Zeiss, Oberkochen, Federal Republic of Germany) operating at 60 kV.

## RESULTS

**Identification of the isolate of *Tilletiopsis*.** On CMA, the cream-colored colonies of the Michigan isolate possessed septate hyphae with no clamp connections. Hyphae were surprisingly brittle, possibly because periodic sections of the hyphae were devoid of protoplasm. Solitary, curved ballistoconidia, 13-16.5 × 2.5 μm, were borne on short sterigmata at the apex of aerial hyphae. Terminal and intercalary chlamydospores were observed in 1-month old PDA cultures (1). These features are characteristic of *T.*

*pallescens* (1,6). The Michigan isolate deviated slightly in colony morphology from the type isolate of *T. pallescens* in having more expansive colonies, shorter aerial hyphae, and whiter colony centers.

To categorize the Michigan isolate to species, a series of physiological characteristics were assayed for comparison with four known species of *Tilletiopsis* (Table 1); results corresponded well with previous reports (1,34). It is notable that the Michigan isolate and the type isolate of *T. pallescens* had identical physiological profiles, which were distinct from those of the remaining three *Tilletiopsis* spp. The use of sorbose and α-methyl-D-glucoside were diagnostic characteristics of *T. pallescens* and the Michigan isolate. The only physiological difference between the two isolates was that *T. pallescens* did not grow at 30 C, whereas the Michigan isolate grew slightly at this temperature. No anastomosis was observed between *T. pallescens* and the Michigan isolate. In control experiments in which the Michigan isolate was paired with itself or *T. pallescens* was paired with itself, no anastomosis was detected.

Isozyme electrophoresis patterns were used to determine unequivocally the relationship of the Michigan isolate to the other species of *Tilletiopsis*. Initial attempts to separate isozymes by native polyacrylamide gel electrophoresis (33) produced only smeared multiple bands, which could not be analyzed. Electrophoresis on cellulose-acetate plates, however, proved to be a quick method with superior separation and resolution. For example, the two bands representing the cytosolic and mitochondrial malate dehydrogenase isozymes could be separated readily on cellulose-acetate plates (Table 2).

Electrophoretic polymorphisms suitable for distinguishing among the species of *Tilletiopsis* were exhibited by five enzymes (Table 2). Not included in the table were two enzymes, acid phosphatase and isocitrate dehydrogenase, which had patterns characteristic of this genus. Profiles for five enzymes from *T. pallescens* and the Michigan isolate were essentially identical and distinct from those of the remaining three species. Unlike the other species, *T. fulvescens* was characterized by multiple malate dehydrogenase and glutamate-oxaloacetate transaminase bands. Curiously, no alkaline phosphatase band was observed in the extract from *T. washingtonensis* when conditions that resolved this enzyme in extracts from the other species were used.

**Antagonism experiments.** Ballistospore ejection from an agar block of cultures of *Tilletiopsis* onto barley leaf segments did not provide inoculum coverage adequate to produce a measurable antagonistic effect on the growth of *E. g. hordei*. However, dragging an agar block of a culture of *Tilletiopsis* across the leaf surface just before inoculating with *E. g. hordei* proved suitable for these experiments. By itself, the Michigan isolate caused no obvious effect, except for altering light reflectance from

TABLE 2. Relative electrophoretic mobilities of five enzymes from cell-free extracts of species of *Tilletiopsis*<sup>a</sup>

Species	Relative mobility of enzyme <sup>b,c</sup>				
	ALP	GOT	G6PD	PGD	MDH
<i>T. fulvescens</i>	0.65	0.43 0.72 1.19	1.36	0.70	0.49 0.80 0.96 1.12
<i>T. pallescens</i>	1.01	1.00	1.02	1.00	0.34 1.00
Michigan isolate	1.00	1.00	1.00	1.00	0.34 1.00
<i>T. minor</i>	0.56	1.17	1.11	0.64	0.53 0.64
<i>T. washingtonensis</i>	None	0.35	0.98	1.24	0.67 1.04

<sup>a</sup> The values given represent the means calculated from three experiments.

<sup>b</sup> Data expressed as migration from the origin divided by the distance traveled by the isozyme band of the Michigan isolate.

<sup>c</sup> ALP = alkaline phosphatase; GOT = glutamate-oxaloacetate transaminase; G6PD = glucose-6-phosphate dehydrogenase; PGD = 6-phosphogluconate dehydrogenase; MDH = malate dehydrogenase.

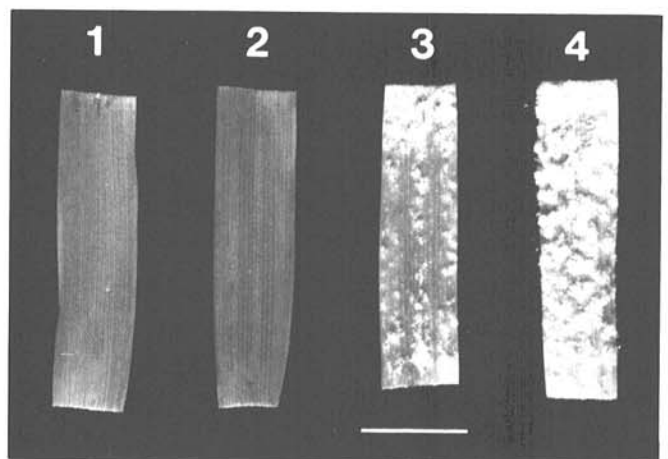


Fig. 1. Antagonism between race 59.3 of *Erysiphe graminis* f. sp. *hordei* and a Michigan isolate of *Tilletiopsis* 7 days after co-inoculation. Barley leaf segments were inoculated with: 1, neither fungus; 2, Michigan isolate; 3, Michigan isolate and *E. g. hordei*; and 4, *E. g. hordei*. Bar = 1 cm.

the leaf segment surface (Fig. 1). By staining leaf segments with lactophenol blue (25), a hyphal net was observed at  $\times 250$  magnification (not shown). With co-inoculation, growth of *E. g. hordei* was severely retarded in the presence of the Michigan isolate (Fig. 1). The number of colonies of *E. g. hordei* was reduced, and those colonies that did develop appeared to produce fewer conidia and had matted, dull-brown mycelia. Visible growth of *E. g. hordei* on co-inoculated leaf segments occurred at the leaf edges and midrib, which probably received a lesser amount of inoculum of the Michigan isolate due to curling of the leaf segment (Fig. 1).

Because hyphae of *Erysiphe* are superficial on leaf surfaces, this pathogen lends itself to observation by scanning electron microscopy. The use of cryogenic methods for sample preparation and microscopy was judged successful because the structure of the wax plates on the barley leaf surface was preserved (Fig. 2). Additionally, the hyphae of *E. g. hordei* were smooth and rounded in appearance without the shrinkage often associated with conventional preparation methods (Fig. 2A). At high magnifications, the collapse of hyphae of *E. g. hordei* growing in association with the Michigan isolate was readily apparent (Fig. 2B). The smaller diameter hyphae of *Tilletiopsis* were found closely intertwined with the much larger hyphae of *E. g. hordei*. No appressorial structures of *Tilletiopsis* were observed; no preferential growth of the Michigan isolate towards *E. g. hordei* was apparent. Interestingly, the wax plates of the barley surface often were absent in areas adjacent to hyphae of the Michigan isolate (Fig. 2C).

The ultrastructural features of the fungiculous relationship between *E. g. hordei* and *T. pallescens* were examined by trans-

mission electron microscopy. The hyphae of *Tilletiopsis* were septate and highly electron dense, with periodic degenerated sections (Fig. 3D and E), confirming observations made by light microscopy. *Erysiphe* growing in association with *Tilletiopsis* appeared moribund. At early stages of degeneration, hyphae of *Erysiphe* were less electron dense than healthy hyphae (compare Fig. 3A and E). Figure 3E depicts vacuolation of the hyphal cytoplasm observed in *Erysiphe* antagonized by *Tilletiopsis*. At advanced stages of degeneration, extensive vacuolation was accompanied by an aggregation of damaged cytoplasmic components and lysis (Fig. 3E). Superficially, this vacuolation resembled the senescence-associated vacuolation of older hyphae of *E. g. hordei* (Fig. 3B) (7). Though not in direct contact with hyphae of *Tilletiopsis*, haustoria of *E. g. hordei* exhibited signs of necrosis similar to those observed in hyphae. Haustoria from healthy *E. g. hordei* had a homogeneous cytoplasm, whereas haustoria of *Erysiphe* antagonized by *T. pallescens* were characterized by vacuolation and cytoplasmic disintegration (compare Fig. 3C and F). With serial sectioning, no evidence for appressoria of *Tilletiopsis* or penetration of the hyphae of *E. g. hordei* by *Tilletiopsis* was found, supporting observations made with scanning electron microscopy and by Hoch and Provvidenti (8). In addition, no degradation of hyphal walls of *E. g. hordei* at sites of contact between the two fungi was detected (Fig. 3E).

Because the physiological characteristics and isozyme patterns suggested that the Michigan isolate was a member of species *T. pallescens*, it was important to test whether the type isolate of *T. pallescens* was similarly antagonistic to *E. g. hordei*. In experiments similar to the one presented in Figure 1, only *T.*

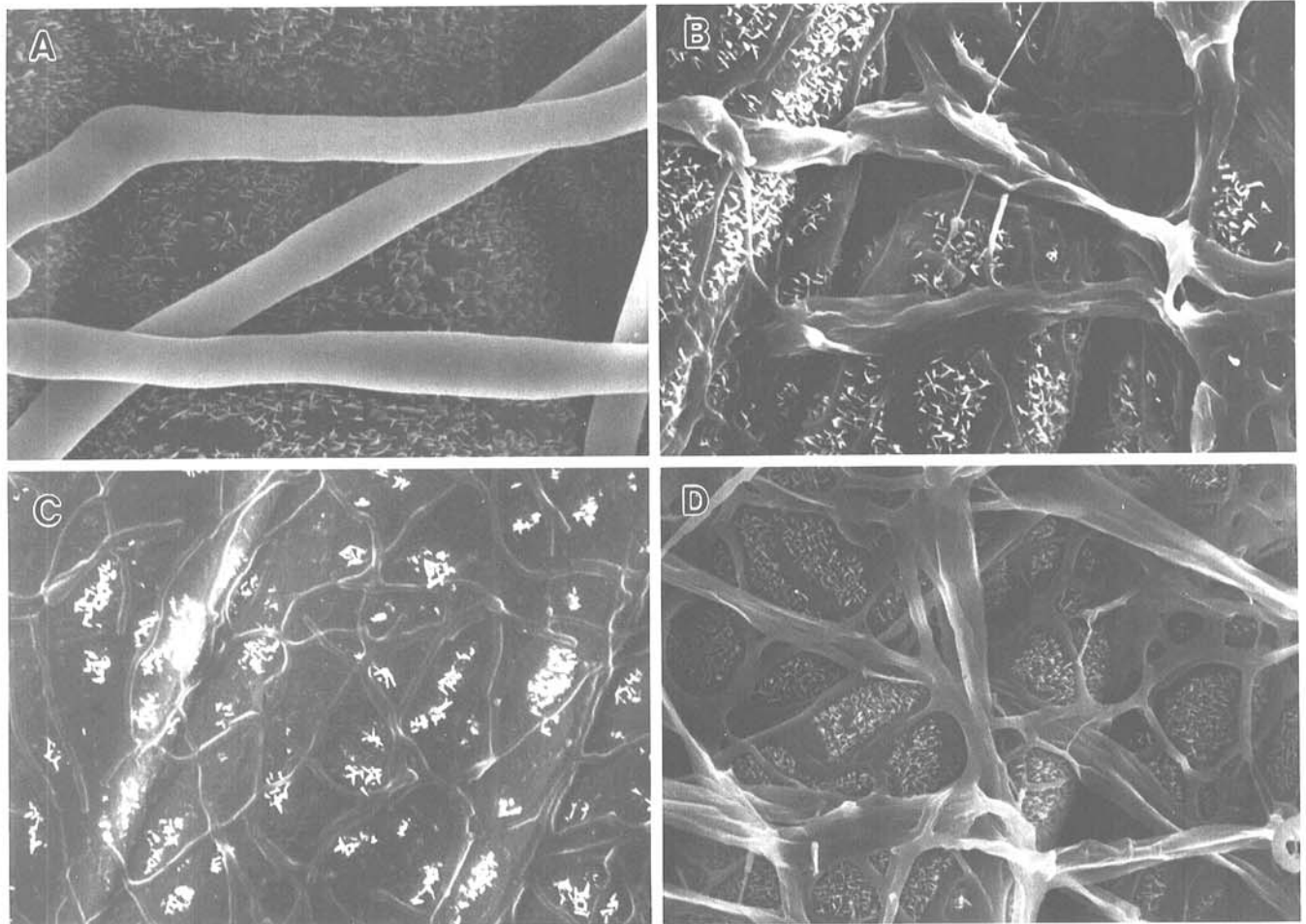


Fig. 2. Scanning electron micrographs demonstrating the antagonistic interaction between race 59.3 of *Erysiphe graminis* f. sp. *hordei* and *Tilletiopsis* on barley leaf segments ( $\times 800$ ). A, Healthy hyphae of *E. g. hordei* with the barley cuticular wax plates in the background. B, Collapsed hyphae of *E. g. hordei* associated with the Michigan isolate hyphae. A typical example of extreme destruction of *E. g. hordei*. C, Netlike saprophytic growth of the Michigan isolate with no apparent hyphal anastomosis. Some areas adjacent to hyphae lack cuticular wax plates. D, Collapse of hyphae of *E. g. hordei* caused by the type specimen of *T. pallescens*.

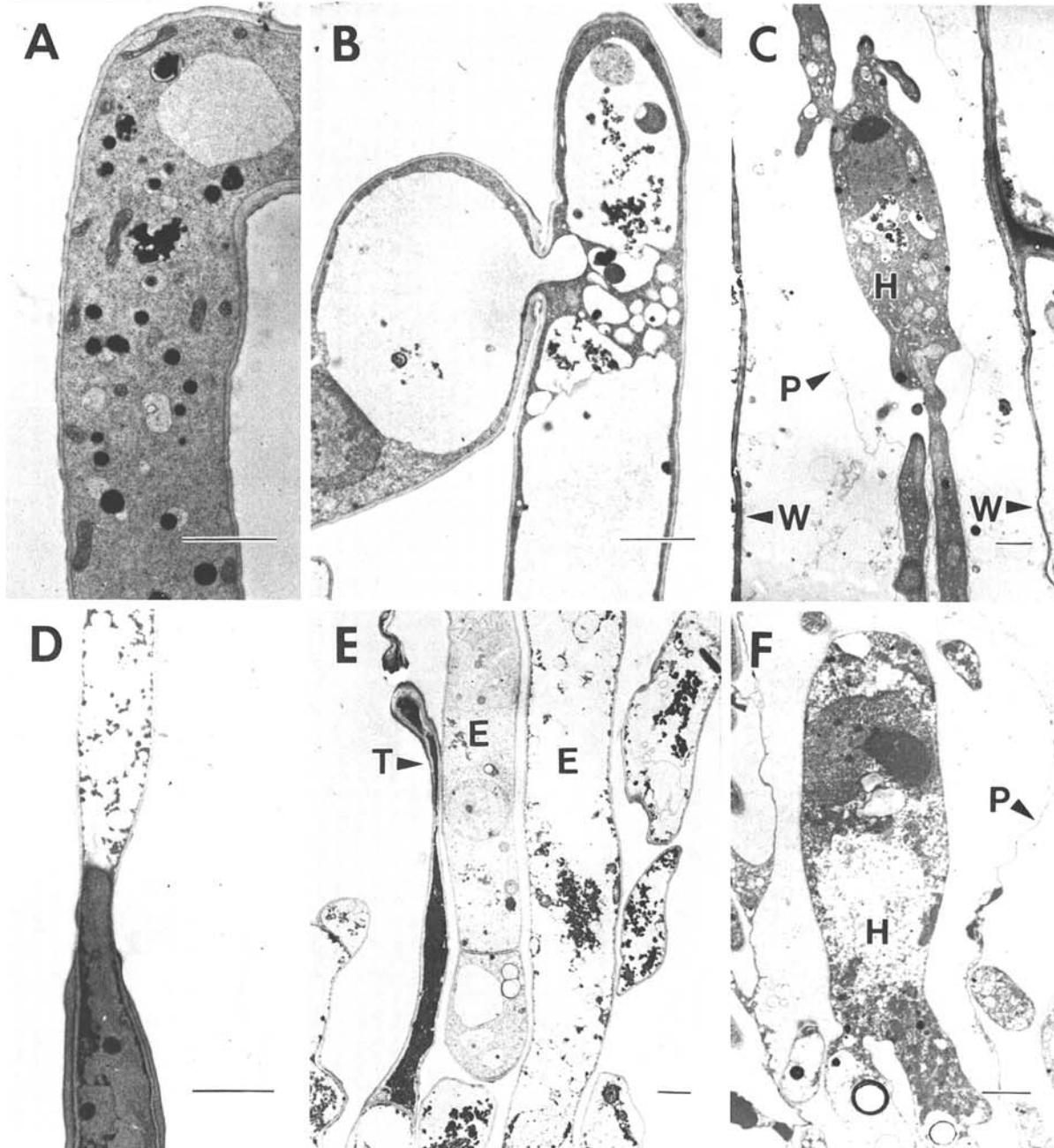
*pallescens* and the Michigan isolate severely inhibited the growth of race 59.3 of *E. g. hordei*. In contrast, *T. minor* caused a slight inhibition in growth of *E. g. hordei*, whereas *T. fulvescens* and *T. washingtonensis* had no antagonistic effect on the growth of *Erysiphe* (data not shown). In addition, races 59.3, CR3, NCI, and 59.2 of *E. g. hordei* appeared equally susceptible to growth inhibition by the Michigan isolate (data not shown).

In *in vitro* experiments to test whether the Michigan isolate might be inhibitory to other fungi, the hyphae of the Michigan isolate grew slowly from the agar inoculum block and colonized the agar chiefly by spore ejection. The three test fungi, *Penicillium* sp., *Alternaria* sp., and *Cladosporium* sp., quickly overgrew the

Michigan isolate. No evidence for antibiotic release, such as a cleared zone or limited growth around the Michigan isolate, was observed.

## DISCUSSION

In this paper, evidence for an antagonistic relationship between *E. g. hordei* and *T. pallescens* was demonstrated. A Michigan isolate of *T. pallescens* was found contaminating laboratory cultures of *E. g. hordei*. Previously, no *T. pallescens* had been recovered other than the type specimen found on a fruiting body of the jelly fungus, *Sirobasidium* sp., in Japan (6).



**Fig. 3.** Transmission electron micrographs detailing the interaction between race 59.3 of *Erysiphe graminis* f. sp. *hordei* and the type specimen of *Tilletiopsis pallescens*. **A**, Healthy hypha of *E. g. hordei*. **B**, Hyphae of *E. g. hordei* displaying senescence-related vacuolation. A rare occurrence of anastomosis is presented. **C**, Healthy haustorium of *E. g. hordei* with a central body and several fingerlike lobes. A host-derived extrahaustorial membrane surrounds the haustorium, isolating it from the host cytoplasm. **D**, A septate hypha of *Tilletiopsis* showing a region of electron-dense cytoplasm and a moribund, electron-translucent section of cytoplasm. **E**, A smaller diameter, electron-dense hypha of *Tilletiopsis* in close association with larger hyphae of *E. g. hordei*. The hypha of *E. g. hordei* adjacent to that of *Tilletiopsis* exhibits limited vacuolation associated with an early stage of antagonism. The highly vacuolate and necrotic condition of the distant hypha of *E. g. hordei* suggests a late stage of antagonism. **F**, Haustorium from a colony of *E. g. hordei* antagonized by *T. pallescens*. The necrotic, electron-translucent areas are typical of haustoria in an advanced stage of degeneration. E = hypha of *E. g. hordei*; H = haustorial central body; P = extrahaustorial membrane; T = hypha of *T. pallescens*; W = barley epidermal cell wall. Bar = 0.5  $\mu$ m.

Morphological, physiological, and biochemical characters were used to classify the Michigan isolate as *T. pallescens*. Consistent with previous taxonomic studies of the genus *Tilletiopsis*, sorbose utilization proved to be a distinguishing physiological characteristic of *T. pallescens* and also the Michigan isolate (1,6,33). The use of isozyme profiles furnished convincing evidence of the identity of the Michigan isolate. Such patterns reportedly vary less than 10% among isolates of the same species (24,33). Based on the relative mobilities of six isozyme bands, *T. pallescens* and the Michigan isolate exhibited essentially no differences and, thus, can be considered members of the same species.

The co-identity of the two species could not be confirmed by the hyphal anastomosis test. However, in earlier experiments by Tubaki (29), no anastomosis between other species of *Tilletiopsis* was observed, suggesting that hyphal fusion does not occur readily. When all the criteria are considered, the Michigan isolate is most similar to the type culture of *T. pallescens* and, therefore, is an isolate of *T. pallescens*.

However, the Michigan isolate and the type isolate from Japan were not identical. Slight differences in colony morphology and in the ability to grow at 30 C were recorded. Such differences were expected, given the geographic separation of the two isolates and the apparent lack of sexual propagation by species of *Tilletiopsis*.

Although *Tilletiopsis* spp., especially *T. minor*, are recovered routinely in spore air-trap and spore-fall experiments, no such reports have been made for *T. pallescens* (13,14,19-22,29). It is possible that *T. pallescens* occurs outside the range tested in earlier reports or that it occurs at relatively low densities. Because the classification of organisms isolated in these earlier experiments was based solely on morphological features, *T. pallescens* may have been recovered but misidentified. Field studies are required to identify the source of the Michigan isolate.

*T. pallescens* and, to a lesser extent, *T. minor* were clearly antagonistic to *E. g. hordei*. The basis for this antagonistic interaction is unknown; however, it does not depend on a nonspecific toxin (29). No evidence of appressoria or of direct penetration of the hyphae of *E. g. hordei* was observed, a result similar to the observations of Hoch and Providenti (8) for the interaction between *T. minor* and *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci. These structures, however, are not required to cause cell permeability changes. For instance, in hyphal interference interactions, close proximity to hyphae of *Coprinus heptemerus* M. Lange et A. H. Smith resulted in loss of hydrostatic pressure, vacuolation, and death of hyphae of *Ascobolus crenulatus* Karsten (9-11). Because comparable ultrastructural changes in *E. g. hordei* were observed by transmission electron microscopy, *T. pallescens* may act in a similar manner to inhibit the growth of *E. g. hordei*.

Although *Tilletiopsis* is a common leaf epiphyte, there have been only three previous reports that members of this genus are antagonistic to other fungi. Last (13) demonstrated that *T. minor* limited the growth of the saprophytic yeast *Sporobolomyces* on field-grown barley. Typically occupying the entire leaf surface, *Sporobolomyces* apparently was restricted to the leaf margins. As observed by Pady (21), *T. minor* appeared to inhibit basidiospore formation of the rust *Puccinia malvacearum* Bertero ex Mont. in Gay. Hoch and Providenti (8) reported that *T. minor* depressed the growth of *S. fuliginea* (cucumber powdery mildew), *Podosphaera leucotricha* (Ellis & Everh.) E. S. Salmon (apple powdery mildew), and *Uncinula necator* (Schwein.) Burrill (grape powdery mildew) in detached leaf culture. Interestingly, *T. minor* caused a slight inhibition of growth of *E. g. hordei* when co-inoculated onto barley leaf segments.

Although *Erysiphe cichoracearum* DC. was shown to be susceptible to the hyperparasites *Ampelomyces quisqualis* Cesati and *Cladosporium cladosporioides* (Fresen.) G. A. De Vries, there have been no previous reports that *E. g. hordei* is similarly susceptible to a mycoparasite (28). To date, only *A. quisqualis* has been advanced as a biocontrol organism for use against *S. fuliginea* on greenhouse cucumbers (12). The ability to evaluate *T. pallescens* as a potential biocontrol agent for *E. g. hordei* is dependent on a complete understanding of the antagonistic

interaction between the two fungi and of the population interactions on the leaf surface.

**Note added in proof:** We have recently been made aware of the report by Dr. Skou that *T. albescens* Gokhale is a mycoparasite of *E. g. hordei* and an effective biocontrol agent for powdery mildew on barley (Skou, J.P. 1986. Nordisk Jordbrugforskning 68:331-332). Also, Dr. Hijwegen has demonstrated that *T. minor* is an effective biocontrol agent for powdery mildew of cucumber (Hijwegen, J. 1986. Neth. J. Plant Pathol. 92:93-95).

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