

Mycoparasitic Relationships: Cytology of the *Sphaerotheca fuliginea*-*Tilletiopsis* sp. Interaction

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ABSTRACT

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Sphaerotheca fuliginea (the cause of powdery mildew of cucumber) was controlled by an isolate of *Tilletiopsis* sp. on cucumber leaves in detached leaf culture. *S. fuliginea* was eradicated from infected cucumber leaves sprayed with a spore suspension of *Tilletiopsis* sp. The antagonist

also protected cucumber leaves from establishment of powdery mildew. Light and electron microscopy of the *Tilletiopsis* sp.-*S. fuliginea* interaction revealed primarily a leaf surface phenomenon and not one involving direct cell penetration.

Additional key words: biological control, *Cucumis sativus*.

Leaf surfaces abound with microorganisms, which are variously antagonistic to other microorganisms, including plant pathogens. Often these antagonisms are overlooked because they occur on such a small scale and are difficult to detect. Antagonism between *Sphaerotheca fuliginea* (Schl.) Pollacci, (the causal agent of powdery mildew of cucurbits) and *Tilletiopsis* a common phylloplane fungus (1,7-10) recently was observed in our laboratory. Preliminary observations indicated that *Tilletiopsis* sp., a ballistosporic yeast of the Sporobolomycetaceae, might control powdery mildew of cucumber.

We report here the ability of *Tilletiopsis* sp. to control powdery mildew in detached cucumber leaf culture, and the cytological relationship between the two fungi.

MATERIALS AND METHODS

S. fuliginea was maintained on detached leaves of *Cucumis sativus* L. 'Marketer'. The leaves were sustained in covered plastic dishes (15 cm diameter \times 3 cm deep) on blotter paper saturated with half-strength Hoagland's solution. The ends of the excised petioles were covered with absorbent cotton soaked with the diluted Hoagland's solution. All leaf cultures were grown under continuous fluorescent lighting at \sim 21 C.

Tilletiopsis sp. isolated from powdery-mildewed cucumber leaves was maintained on potato-dextrose agar (PDA) at 22 C. It has been deposited with the American Type Culture Collection as ATCC 36535.

Inoculation of *Sphaerotheca fuliginea*. Spore suspensions of *Tilletiopsis* sp. were prepared by adding sterile distilled water and scraping the surface of 2 wk old culture. The suspension was filtered through three layers of cheesecloth and the volume adjusted with sterile distilled water so that \sim 200 spores/cm² would be deposited by spraying the leaf with an atomizer.

One leaf of a triplicate set of detached cucumber leaves (three-quarters expanded) from greenhouse-grown plants free of powdery mildew was sprayed with sterile water and two were sprayed with *Tilletiopsis* sp. spore suspension at timed intervals up to 21 days. One leaf treated with sterile water and another with *Tilletiopsis* sp. spores were inoculated with *S. fuliginea*. The second leaf sprayed with *Tilletiopsis* sp. was not treated further. The treatments were replicated five times on two occasions.

Leaves previously inoculated with *S. fuliginea* that exhibited a uniform distribution of colonies of the powdery mildew fungus (with conidial chains) were half-leaf treated with sterile water and with a suspension of *Tilletiopsis* sp. spores.

The effect of the treatments on powdery mildew development or on eradication were observed with a dissecting microscope. These treatments or similar ones were repeated several times.

Microscopy. For scanning electron microscopy (SEM), 5-mm² leaf segments were excised from the leaves and prepared as previously described (5). For transmission electron microscopy (TEM), 1 \times 5-mm transverse leaf segments were excised and fixed in 4% glutaraldehyde buffered with 0.1 M PO₄ (K⁺), pH 6.8, for 10 min. Then the material was embedded in agar (4) to prevent further disturbance of the leaf surface microflora by subsequent treatments. The specimens were fixed in glutaraldehyde for an additional 60 min, postfixed in aqueous 1% OsO₄, dehydrated via an acetone series, and embedded in Spurr's medium (11). Thin sections were stained with uranium acetate and lead citrate.

RESULTS

Hyphae that developed from spores of *Tilletiopsis* sp. atomized onto detached-leaf cultures infected with *S. fuliginea* eliminated all phylloplane hyphae and conidial inoculum of the phytopathogen. The development of *Tilletiopsis* sp. on *S. fuliginea* was first observable with the dissecting microscope 48 hr after inoculation. By 5 days, *S. fuliginea* was eradicated (Fig. 1). When *Tilletiopsis* spores were sprayed onto colonies of the powdery mildew fungus at lower rates, ie, 10 spores/cm², the time required for eradication was increased by several days. The increased time was apparently required for a buildup of *Tilletiopsis* sp. inoculum.

Leaves not inoculated with *S. fuliginea* but sprayed with conidia of *Tilletiopsis* sp. showed no growth of *Tilletiopsis* sp. for periods as long as 21 days. Leaves with powdery mildew not inoculated with *Tilletiopsis* sp. continued to support *S. fuliginea* growth for 2-3 wk, then became chlorotic, senesced, and were rapidly overcome by various saprophytic microorganisms.

Treatment of cucumber leaves with *Tilletiopsis* sp. conidia up to 8 days before inoculation with *S. fuliginea* completely prevented powdery mildew development. Beyond 8 days and up to 21 days, only a few isolated colonies of the powdery mildew fungus developed, but they seldom spread to noncolonized leaf areas.

When examined microscopically, hyphae of *Tilletiopsis* sp. were entwined around *S. fuliginea* hyphae, conidiophores, and conidia

(Fig. 2,5,7,8). *Tilletiopsis* sp. hyphae are 1–1.8 μm in diameter, approximately one-tenth that of *S. fuliginea* hyphae. The spores (hyaline by light microscopy) are curved, approximately $10 \times 1.6 \mu\text{m}$, and attached to sterigmata (Fig. 8). Based on spore size, the organism is most similar to *T. minor* (8). Frequently, leaf trichomes were entwined by *Tilletiopsis* sp. hyphae (Fig. 6); however, this growth probably was supported by adjacent moribund *S. fuliginea* hyphae and conidia and not by leaf nutrients.

The close spatial association of *Tilletiopsis* sp. and *S. fuliginea* was examined further by transmission electron microscopy (Fig. 9,10). Cells of *S. fuliginea* in contact with *Tilletiopsis* usually were necrotic, although occasional *S. fuliginea* cells appeared to be unaffected (not shown). This latter situation possibly represented early contact between the two microorganisms. Penetration of *S. fuliginea* structures by hyphae of *Tilletiopsis* sp. (Fig. 10) was rarely observed and probably represented postnecrotic invasion.

DISCUSSION

The eradication and prevention of *S. fuliginea* development on cucumber leaves suggest that *Tilletiopsis* sp. might be a useful biological control agent against *S. fuliginea*. However, our results are based on detached leaf culture in an enclosed environment with nearly 100% relative humidity. The relative humidity and

temperature optima for *Tilletiopsis* sp. growth and antagonism of *S. fuliginea* must be determined before a possible biological control program can be contemplated. Some information regarding ballistospore production in *T. minor* and *T. washingtonis* is available (9). Optimum temperature for *T. minor* is 16–21 C, but *T. minor* also produces spores well at 27 C. *T. washingtonis* has a considerably lower temperature optimum. Biological control and mycoparasitism of *S. fuliginea* and other powdery mildew fungi with *Cicinnobolus* and *Cicinnobella* spp. have been reported (2,3,6,13–15). Biological control practices have not been generally adopted, however, in part because effective fungicides have been readily available and complete biological control of a mildew pathogen has not been achieved. In addition, specific temperatures often are required for growth and parasitism by *Cicinnobolus* spp. antagonists.

Unlike the relations between *Cicinnobolus* spp. and their hosts, those of the *Tilletiopsis* sp.-*S. fuliginea* interaction apparently do not involve penetration of the host fungus. Some other factor, presumably a substance secreted by *Tilletiopsis* sp., is involved. A *Sporobolomyces* sp. relative (Sporobolomycetaceae) of *Tilletiopsis* produces fungistatic antibiotics in culture (12). *Tilletiopsis* sp. probably subsists on nutrients subsequently released from necrosed *S. fuliginea* hyphae and conidia. The relationship between *Tilletiopsis* sp. and *S. fuliginea* is not unique, since the antagonist

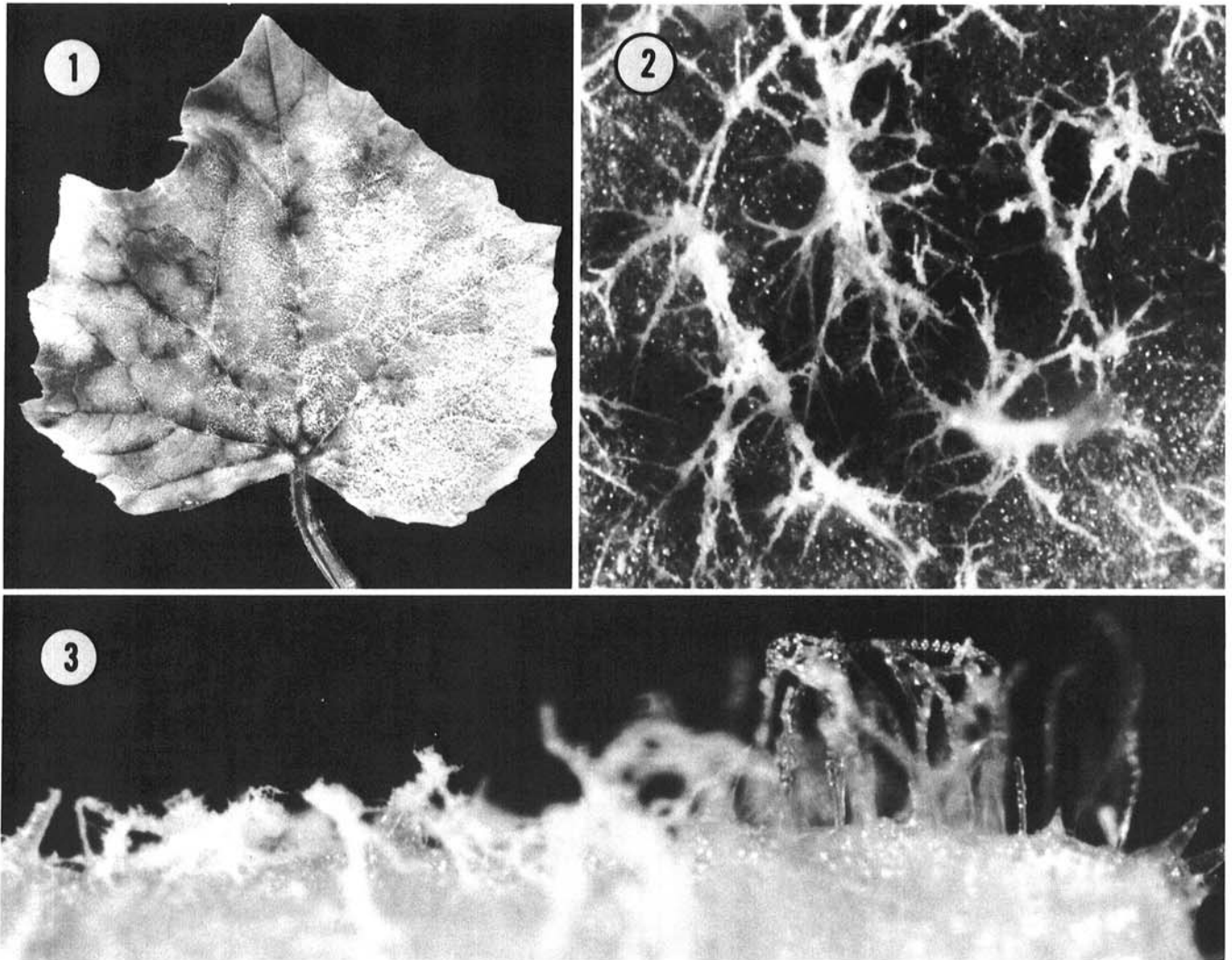


Fig. 1–3. Antagonism of *Sphaerotheca fuliginea* (cause of powdery mildew) by *Tilletiopsis* sp. on cucumber leaves. 1) The left half was sprayed 5 days previously with a *Tilletiopsis* sp. spore suspension and the right half was sprayed with water. *Tilletiopsis* sp. has eradicated *S. fuliginea* from the left half and begun to invade the right half. 2, 3) *Tilletiopsis*-colonized *S. fuliginea*. The *S. fuliginea* conidia and conidiophores in Fig. 3 (right side) were not yet colonized. Both approximately $\times 60$.

also will effectively control powdery mildew of apple (caused by *Podosphaera leucotricha* (Ell. & Ev.) Salm.) and grape (caused by *Uncinula necator* (Schw.) Burr.) leaves in detached leaf culture (Hoch, unpublished).

Both *Tilletiopsis* spp. and *Sporobolomyces* spp. have been reported to be abundant on diseased leaves of various plants (7,9). *Sporobolomyces* spp. but not *Tilletiopsis* spp. have been reported to occur in large numbers on rusted leaves. However, Last (7)

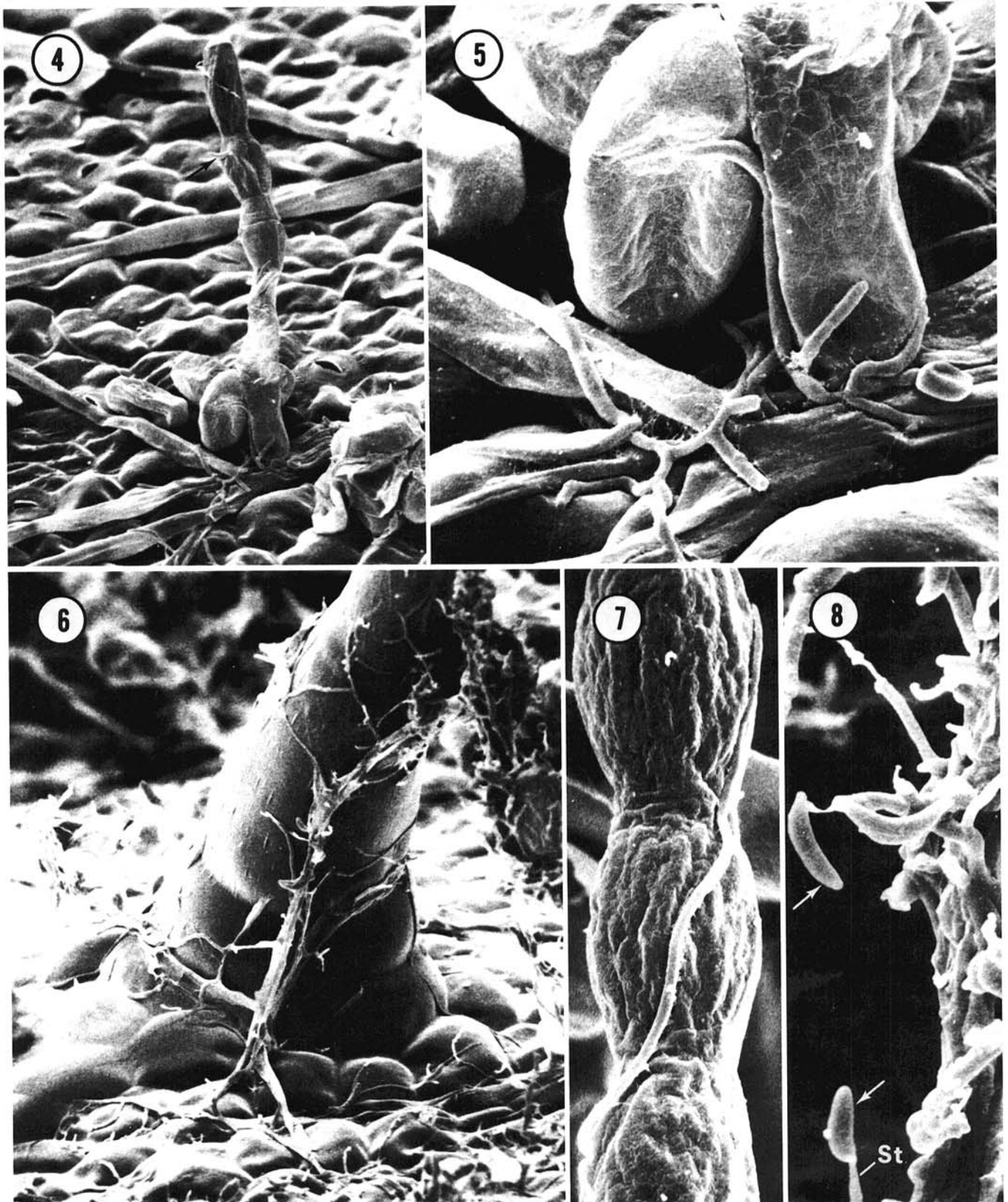


Fig. 4-8. Antagonism of *Sphaerotheca fuliginea* (cause of powdery mildew) by *Tilletiopsis* sp. on cucumber leaves. 4,5) Scanning electron micrograph of *S. fuliginea*-infected cucumber leaf surface. Base of *S. fuliginea* conidiophore and adjacent hyphae (Fig. 4 and detailed in Fig. 5) invaded by *Tilletiopsis* sp. hyphae. Arrow = conidium of *Tilletiopsis* sp. on immature *S. fuliginea* conidium, $\times 690$ and $\times 2,760$, respectively. 6) Cucumber leaf surface and trichome covered with *Tilletiopsis* sp. hyphae, $\times 670$. 7) Immature chain of *S. fuliginea* conidia on which a hypha of *Tilletiopsis* sp. was growing, $\times 2,600$. 8) *Tilletiopsis* sp.-colonized *S. fuliginea* conidiophore. Spores (arrows) of *Tilletiopsis* are slightly curved and attached to sterigma, $\times 2,500$.

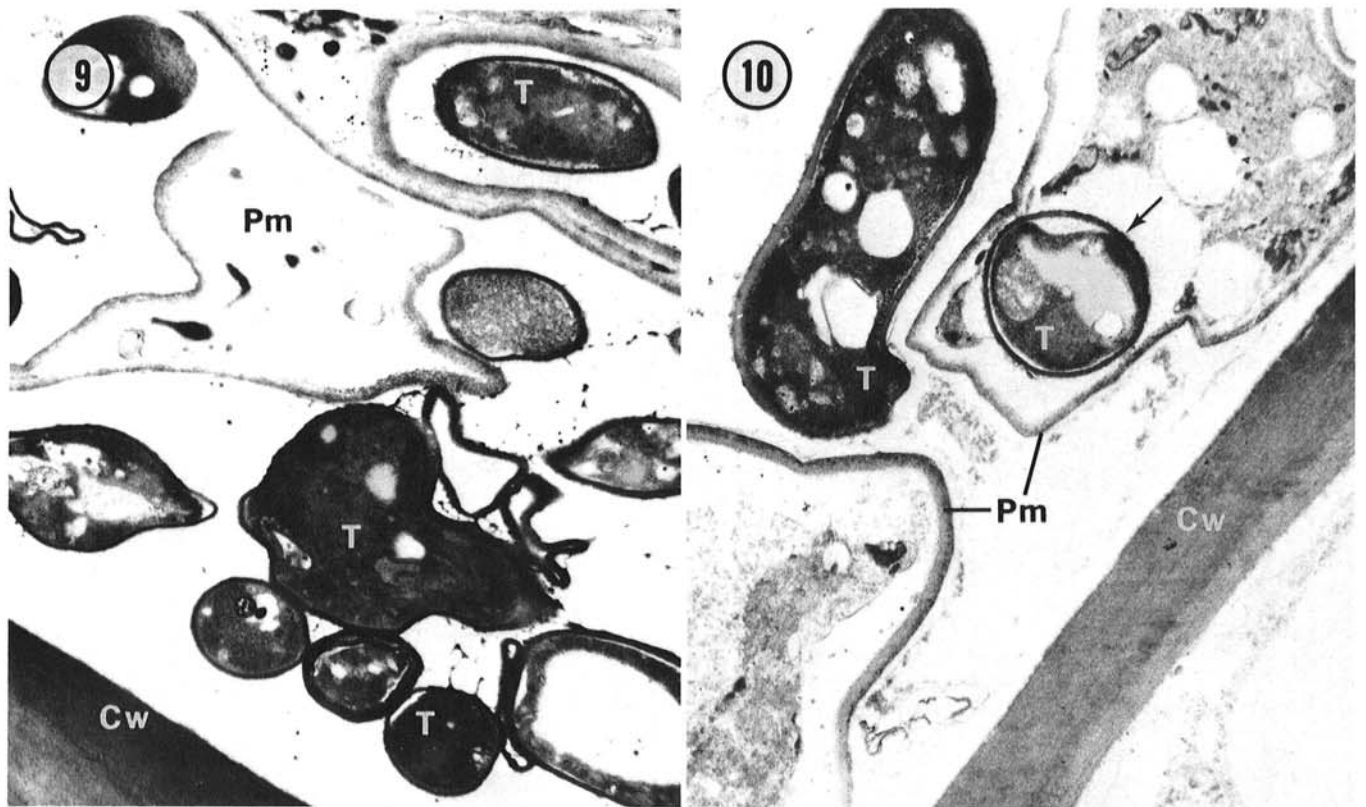


Fig. 9, 10. Transmission electron micrograph of the *Tilletiopsis-Sphaerotheca fuliginea* relationship. All cells of the powdery mildew fungus are necrosed or moribund. *Tilletiopsis* sp. hyphae were rarely observed in *S. fuliginea* cells (arrow, Fig. 10). Cw = epidermal cell wall of cucumber; Pm = *S. fuliginea* hyphae; T = *Tilletiopsis* sp. hyphae. Both $\times 16,700$.

reported that *Tilletiopsis* spp., as well as *Sporobolomyces* spp., occur in greater numbers on leaves with mildew than on leaves free of the disease. He also reported that these phylloplane fungi occurred in greater numbers on apple and pear leaves infected with *Venturia* spp. Biological control of these important plant pathogens should also be investigated with *Tilletiopsis* sp.

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