Ingestion-Egestion and Aerial Transmission of *Pythium aphanidermatum* by Shore Flies (Ephydrinae: *Scatella stagnalis*)

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ABSTRACT

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Aerial transmission of *Pythium aphanidermatum* by shore flies (*Scatella stagnalis*) is documented for the first time. Shore flies, which were thought to feed only on blue-green algae and diatoms, also fed on cucumber roots colonized by the fungus. Ninety-seven percent of the first and second instar larvae, 20% of pupae/third instar larvae, and 10% of adult flies carried mature oospores in their gut. Oospores excreted by larvae and adults were capable of germinating. *P. aphanidermatum* was transmitted to healthy cucumber plants by naturally infested larvae and adult flies. Adult flies infested with *P. aphanidermatum* may account for pathogen introduction and spread within commercial greenhouse facilities.

Additional keywords: hydroponics, vector.

One advantage to growing plants hydroponically is the presumed avoidance of root diseases; however, hydroponic cultural systems are not devoid of disease problems. The most common root diseases that occur in hydroponic culture are caused by various species of *Pythium* (1,7,12,13). Determination and elimination of the source(s) responsible for the introduction of these root pathogens into commercial greenhouses is requisite for the development of effective disease control.

During the course of investigations on nonchemical methods for the control of Pythium root rot of cucumbers (*Cucumis sativus* L. 'Toska 70') cultivated in a recirculating hydroponic system, we occasionally encountered infection of plants by *Pythium aphanidermatum* (Edson) Fitzp. in presumed pathogen-free areas of the system. It also was observed that the facility was infested with shore flies (Ephydrinae: *Scatella stagnalis* Fallen). Eggs, larvae, pupae, and adult flies of this common greenhouse pest, which reportedly feeds primarily on algae (4), were noted in great abundance around the base of cucumber plants. In preliminary studies, *P. aphanidermatum* was isolated from one out of 13 adult flies collected in the greenhouse facility. Documentation of the ingestion-egestion and transmission of *P. aphanidermatum* by larvae and adult shore flies is presented herein. A preliminary report has been published (6).

MATERIALS AND METHODS

Collection of insects and transmission studies, unless otherwise specified, were conducted in a greenhouse containing an aboveground, recirculating, hydroponic system in which cucumber plants, approximately 3 wk old, were being grown. Many plants were infected with *P. aphanidermatum,* and roots of infected plants contained numerous oospores of the fungus (Fig. 1). The recirculating hydroponic system consisted of three interconnected cultural tanks, each containing 850 L of a complete, aerated, nutrient solution. The nutrient solution consisted of Hydrosol (a Peter's fertilizer product, W. R. Grace and Co., Foelsville, PA), magnesium sulfate, potassium nitrate, and calcium nitrate. The fertilizer was added to each hydroponic tank at the rate of 972 g of Hydrosol, 200 g of magnesium sulfate, 544 g of potassium nitrate, and 875 g of calcium nitrate. Plants, 20 per tank and spaced 30 cm apart, were anchored in a Styrofoam flotation board, measuring 2.4 × 1.2 × 0.03 m (1).

Pathogen infestation of adult flies, pupae, and larvae. To determine the extent of insect infestation by *P. aphanidermatum*, adult flies were collected daily by aspiration from the greenhouse over a 6-day period. Additionally, larvae and pupae, which were located around the base of infected plants, were collected daily over a 14-day period. Adult flies, larvae, and pupae were transported to the laboratory, aseptically squashed, and plated on water agar containing streptomycin sulfate at 200 μg/ml (Sigma, St. Louis, MO). After 48 hr of incubation at 37 C, isolated fungi were identified. The frequency of isolation of *P. aphanidermatum* from each life stage of the insect was recorded. Additionally, all squashed insect specimens were examined microscopically for direct evidence of the location and nature of fungal structures on or in the various life stages of the insect.

Germinability of oospores recovered from larvae, pupae, and adult flies. To test their germinability, oospores released from squashed larvae, pupae, and adult flies were syringed onto a selective agar medium (2). After 48 hr of incubation at 37 C, the number of colonies of *P. aphanidermatum* were recorded, and the origin (nature of the propagule) of each colony was microscopically determined.

Germinability of oospores in insect frass. Live larvae and adult flies were collected from around the base of plants in the greenhouse and placed in petri dishes containing selective medium. Within 1 hr, numerous discrete frass deposits from larvae and adults were observed in all dishes. The plates then were placed in an incubator at 37 C. After 48 hr of incubation, the origin of colonies of *P. aphanidermatum* developing on the medium were determined microscopically.

Larval transmission of *P. aphanidermatum*. Ten larvae, collected from around the base of cucumber plants in the infested greenhouse, were placed around the base of a healthy 2-wk-old cucumber plant housed in a small, hydroponic chamber. The chamber then was sealed with Parafilm with air holes. Control plants were placed in identical chambers with no larvae added. After 4 days at room temperature in the laboratory, the chambers were transferred to an incubator at 27 C. After an additional 3 days, the entire root system was divided into 4-cm segments. Segments were blotted dry on sterile paper towels and plated on water agar. After 48 hr at 37 C, isolated fungi were identified. Isolation of *P. aphanidermatum* from roots was considered as proof of insect transmission. The experiment was repeated twice with four replicates.

Adult transmission of *P. aphanidermatum*. Transmission

studies with adult flies were conducted in the laboratory and in the fly-infested greenhouse. First, 15 adult flies, collected in the greenhouse by aspiration, were immediately placed in each of five separate hydroponic chambers. Each chamber contained a healthy 2-wk-old cucumber plant. Two additional chambers containing plants without flies served as controls. The chambers were sealed with Parafilm with air holes and assessed for transmission as outlined above. Second, seven 2-wk-old healthy cucumber seedlings, reared individually in portable hydroponic chambers, were transported to the fly-infested greenhouse. These chambers were placed randomly throughout the facility. Two of the chambers, however, were covered with insect-proof cages. After 6 days, 10 root segments (each 4 cm long) from each plant were individually assessed for infection by *P. aphanidermatum* as described above.

RESULTS

Pathogen infestation of adult flies, pupae, and larvae. *P. aphanidermatum* was isolated from larvae, pupae, and adult flies. However, the level of infestation varied depending on the specific life stage of the insect (Table 1). Oospores, but no hyphae, of *P. aphanidermatum* were observed microscopically in the gut of squashed specimens of larvae (Fig. 2A and B), pupae, and adult flies. Additionally, movement of ingested oospores, as well as algal cells, within the gut of live larvae was observed microscopically during peristaltic contractions of the alimentary canal. The number of oospores varied from 25 to 680 per larva. Oospores in the gut of infested adult flies ranged in number from 1 to 25 per individual.

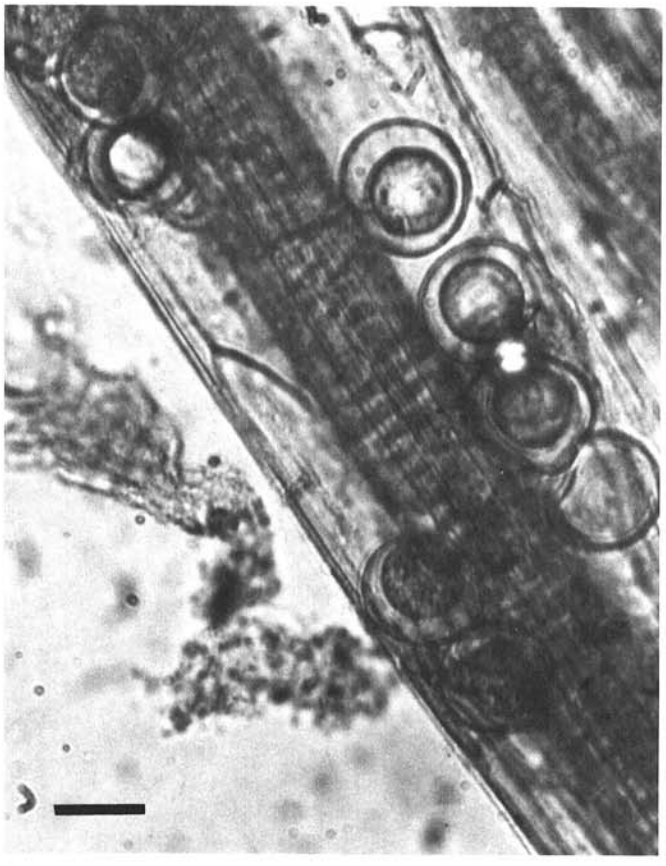

Fig 1. Oospores of *Pythium aphanidermatum* in cucumber roots. Bar = 20 μm.

TABLE 1. Percent of adult flies, pupae, and larvae infested with *Pythium aphanidermatum*[a]

	No. sampled	No. infested	Percent infested
Adult flies	263	27	10
pupae/3rd instar	315	65	20
larvae (1st and 2nd instar)	30	29	97

[a] Infestation was documented by isolation on a selective medium and by microscopic observation.

Germinability of oospores recovered from larvae, pupae, and adult flies. Oospores in the gut of larvae, pupae, and adult flies appeared to be normal, mature oospores. Oospores that were washed from squashed adult flies, larvae, or pupae germinated readily on selective medium, and developing colonies were traced to germinating oospores (Fig. 2C). In addition, oospores in squashed larvae were counted and then washed onto selective medium. In three separate washings, 38 of 115, 9 of 36, and 10 of 30 oospores from infested larvae germinated and developed into colonies.

Germinability of oospores in frass. Excreted oospores were observed microscopically in frass deposited by larvae and adult flies. Colonies of *P. aphanidermatum* were obtained on selective medium from both the larval and the adult frass (Fig. 2D), and the origins of the colonies were traced to germinated oospores in frass droplets.

Larval and adult transmission of *P. aphanidermatum*. *P. aphanidermatum* was isolated from the roots of one of five plants in the first adult fly experiment, but not from roots of the control plant. Transmission of the fungus also occurred when larvae were placed around the base of all healthy seedlings.

In the second experiment, adult flies were observed around the base of the uncaged plants within 5 min after the individual hydroponic chambers were placed in the fly-infested greenhouse. After 1 day, frass clearly was visible around the base of the plants and on the stem of the cucumber seedlings, and, within 6 days, the fungus was isolated from roots (some of which were necrotic) of all uncaged plants in the fly-infested greenhouse. *P. aphanidermatum* was not isolated from the roots of plants that had been enclosed in insect-proof cages. Within 6 days after the cages were removed from control plants, they also were colonized by *P. aphanidermatum*.

DISCUSSION

Commercial peat-based propagation mixes (3,9) and naturally infested sand (1) are primary inoculum sources of various species of *Pythium* and other root-infecting fungi in greenhouses. We document, for the first time, acquisition and aerial transmission of *P. aphanidermatum* by shore flies from infected to healthy plants in a greenhouse. Oospores of *P. aphanidermatum* were observed microscopically in the gut of larvae, pupae, and adult shore flies, and viable oospores were excreted in frass produced by larvae as well as adult flies. Larvae, which fed on infected roots laden with oospores (the only source of oospores in our investigation), acquired the fungus. Because adult flies did not have access to infected roots, the oospores observed in the gut of adult flies likely reflect residual oospore populations retained after pupation. These residual oospore populations, however, were sufficient to infect all plants frequented by naturally infested adult flies.

The presence of fungal propagules in the gut of insects has been reported (10). Thus, any insect stage that feeds on infested plant material could ingest propagules of any fungus present and could be potential vectors of that organism. For example, we observed sporangia of *Plasmopara lactucae-radicis*, a newly described root pathogen (11), in the gut of shore fly larvae recovered from roots of infected lettuce plants grown under hydroponic conditions. Additionally, externally infested, adult fungus gnats (*Bradysia impatiens*) experimentally disseminated *Verticillium albo-atrum* to soil-grown plants in a greenhouse (8), and this insect also is suspected to be a potential vector of *Pythium* in greenhouses (3). Their potential to function as vectors for *Pythium* was strengthened by the recent discovery that laboratory-reared larvae of fungus gnats, fed in the laboratory on pure cultures of *P. aphanidermatum* and other species of *Pythium*, ingest and excrete germinable oospores (5).

Increased germinability of oospores of *P. aphanidermatum* after

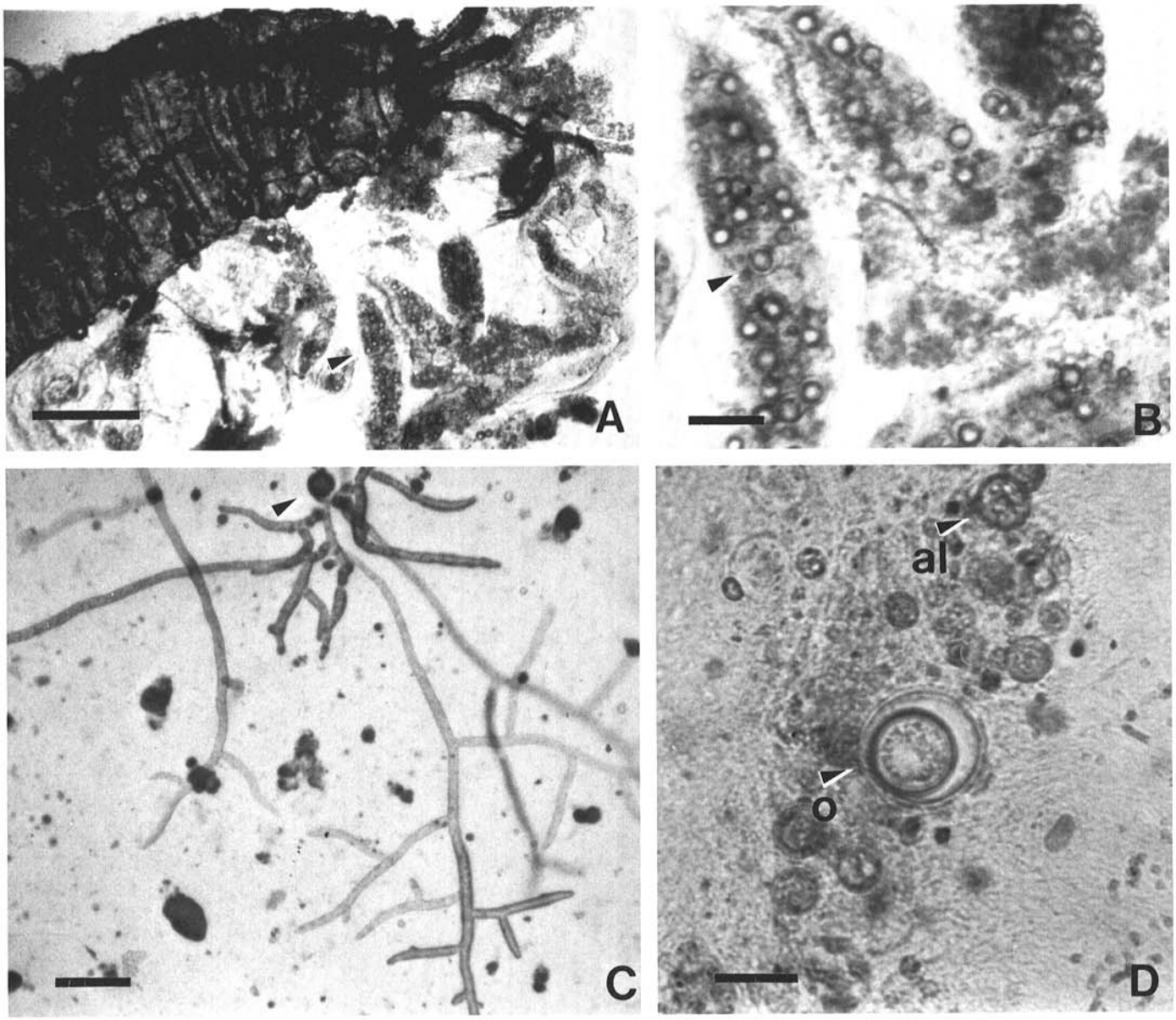

Fig 2. Oospores of *Pythium aphanidermatum*. **A and B,** oospores in the gut of a shore fly larva (2A, bar = 400 μm; 2B, bar = 60 μm). **C,** Colony originating from a larva-ingested oospore (arrow); bar = 60 μm. **D,** Oospore in adult fly frass (bar = 20 μm); al = algal cell. o = oospore.

passage through the alimentary canal of snails has been reported (14). Whether oospore germinability was altered by passage through the gut of shore flies is not known; however, approximately 30% of the oospores recovered from the gut of shore fly larvae were capable of germination.

Shore flies are common pests in many commercial greenhouses and, in light of their newly documented role as a vector of *Pythium*, strategies for control of infestations by this insect should be implemented. Infested adult flies may account for introduction and spread of *Pythium* within commercial greenhouse facilities.

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