Viability of Oomycete Propagules Following Ingestion and Excretion by Fungus Gnats, Shore Flies, and Snails

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ABSTRACT

Hyder, N., Coffey, M. D., and Stanghellini, M. E. 2009. Viability of oomycete propagules following ingestion and excretion by fungus gnats, shore flies, and snails. *Plant Dis.* 93:720-726.

Sporangia of *Phytophthora capsici* and *P. nicotianae*, as well as hyphal swellings of *Pythium splendens*, *P. sylvaticum*, and *P. ultimum*, were ingested by adult shore flies but none were viable after passing through the digestive tract. Oospores of *Pythium aphanidermatum* retained their viability following ingestion by adult shore flies. Larval stages of fungus gnats and shore flies ingested sporangia of *Phytophthora capsici*, *P. nicotianae*, and *P. ramorum*, but they were not viable upon excretion. In contrast, hyphal swellings of *Pythium splendens*, *P. sylvaticum*, and *P. ultimum*, chlamydospores of *Phytophthora ramorum*, and oospores of *Pythium aphanidermatum*, retained their viability after passage through the digestive tract of these larvae. Snails were capable of ingesting and excreting viable sporangia and chlamydospores of *P. ramorum*, which upon excretion infected detached leaves. Although the impact of larvae and snails in the rapid dissemination of pathogen propagules is unknown, this work does highlight the possibility that some often-ignored animal–fungus interactions should be considered in long-range dispersal of pathogen propagules via food webs.

Adult shore flies (*Scatella stagnalis* (Fallen)) and fungus gnats (*Bradysia imptiens* (Johannsen)) have been reported to function as aerial vectors of several plant pathogenic fungi, i.e., *Fusarium*, *Verticillium*, and *Thielaviopsis* (3,5,13,21). Although these fungi are soilborne, root-infecting pathogens, they also sporulate on aboveground portions of their hosts. The aboveground propagules of these fungi (hyphal swellings, microsclerotia, and/or chlamydospores) serve as a source of inoculum for insect acquisition, via internal and external contamination, and subsequent aerial dissemination. Aerial dissemination by insects can provide an additional means of spread for some soilborne pathogens and can greatly impact the management of such diseases under field and greenhouse production systems. However, with the exception of a single report on the aerial transmission of *Pythium aphanidermatum* by adult shore flies (8), there is no information regarding the role of these insects as vectors of other oomycetes, a major group of extremely destructive root-infecting pathogens. These pathogens produce diverse reproductive structures (i.e., oospores, hyphal swellings, sporangia, and chlamydospores) ranging in size from 20

to 60 μ m on colonized roots. Their belowground location, however, could preclude acquisition by adult life stages of both shore flies and fungus gnats, which live strictly aboveground. However, some species of oomycetes also produce aboveground reproductive structures that could be vectored by these insects by ingestion and defecation or casual contact. For example, sporangia and chlamydospores of *Phytophthora ramorum* are produced on leaves of colonized hosts (2), and sporangia/oospores of *P. capsici* and *P. nicotianae* can be produced on aerial parts of colonized hosts (1,6,11). Additionally, larvae of shore flies and fungus gnats feed belowground and could acquire propagules of *Pythium* species in infected plant roots.

In addition to insects, snails also have been implicated as vectors of oomycetes (16,23). The role of snails in transmitting *Phytophthora citricola* among avocado trees has been documented by El-Hamalawi and Menge (4). Here, feces of snails collected from avocado groves contained viable oospores and hyphal fragments of *P. citricola*, and in greenhouse

experiments, snails exposed to infected avocado plants or infected avocado cuttings were able to transmit the disease to healthy avocado plants via feces or direct contact.

Phytophthora ramorum, the causal agent of Ramorum blight, thrives in moist conditions that also favor high snail populations, making it potentially possible for snails to ingest aboveground propagules of *P. ramorum* and spread the pathogen via infected feces.

The specific objectives of our research were to (i) assess the potential of fungus gnats and shore flies to ingest and excrete propagules of *Phytophthora capsici*, *P. nicotianae*, *P. ramorum*, *Pythium aphanidermatum*, *P. splendens*, *P. sylvaticum*, and *P. ultimum* and the ability of these excreted propagules to initiate infection, and (ii) evaluate ingestion, excretion, and transmission of *Phytophthora ramorum* by brown garden snails (European brown snail), *Helix (Cytomyphalus) aspersa* Müller.

MATERIALS AND METHODS

Oomycete pathogens. Three species of *Phytophthora* and four species of *Pythium* were selected for this study (Table 1). These oomycetes were specifically chosen because they are recognized as major pathogens of diverse greenhouse crops. Additionally, the various propagules produced by these oomycetes provided us the opportunity to assess the survivability of these propagules in the digestive tract of the insects investigated. Stock cultures of the oomycetes were maintained on 10% V8 juice agar unless otherwise specified.

Pathogen cultures. Two methods were employed to produce large quantities of oomycete propagules: cultivation in liquid culture and cultivation in/on infected host tissue. For cultivation of *Phytophthora nicotianae*, *Pythium aphanidermatum*, *P. splendens*, *P. sylvaticum*, and *P. ultimum*,

Table 1. Characteristics of Oomycetes selected for investigation

Oomycete	Propagule type	Average size (μ)	Source
<i>Phytophthora capsici</i>	Sporangia	60 $\times$ 36	Pepper
<i>P. nicotianae</i>	Sporangia	40 $\times$ 29	Citrus
<i>P. ramorum</i>	Sporangia	55 $\times$ 25	Recycled water
<i>P. ramorum</i>	Chlamydospores	46–60	Camellias
<i>Pythium aphanidermatum</i>	Oospores	20	Cucumbers
<i>P. splendens</i>	Hyphal swellings	43	Easter lily
<i>P. sylvaticum</i>	Hyphal swellings	32	Lettuce
<i>P. ultimum</i>	Hyphal swellings	25	Lettuce

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Accepted for publication 20 March 2009.

doi:10.1094/PDIS-93-7-0720
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7-mm-diameter plugs of 48-h-old V8 agar (10% V8 juice, 2% agar, 0.1% CaCO₃) cultures of each pathogen were placed separately in 90-mm-diameter petri plates containing 15 ml of sterile distilled water (SDW) and two autoclaved rolled oats (0.7 × 0.4 cm) (Quaker Oats Company, Chicago, IL). Cultures were incubated at 25°C for 14 days, during which time the various propagules (sporangia, oospores, or hyphal swellings [Table 1]) were produced on the mycelial mats.

For cultivation of *Phytophthora ramorum*, isolates P10467 (which readily produced chlamydospores in culture) and P10102 (which readily produced sporangia in culture) (The World Phytophthora Genetic Resource Collection [WPC]), 7-mm-diameter plugs of 7-day-old cultures maintained on PARPH selective media (cornmeal agar amended with pimarin, ampicillin, rifampicin, PCNB, and hymexazol [14]) were transferred to 15 ml of 5% V8 broth in petri plates and incubated at 25°C for 30 days, during which time abundant chlamydospores and/or sporangia were produced on the mycelial mats.

For cultivation of *P. capsici*, eggplant fruits (*Solanum melongena* L.) were inoculated with 8-mm-diameter plugs of 48-h-old V8 agar cultures and placed in a moist chamber. After 8 days at 25°C, numerous sporangia were produced on the surface of infected fruit.

Maintenance and collection of insects. A colony of shore flies (*S. stagnalis*) naturally occurring in the greenhouse was maintained on algal-infested rockwool blocks (7 × 14 × 90 mm) in a greenhouse at 25 to 30°C with a 12-h photoperiod at 100% relative humidity. Algae originating from adult shore flies were used to establish the colony. The rockwool blocks were fertilized weekly with Miracle-Gro (3 g product per liter of water, Scotts Miracle-Gro Products, Inc., Marysville, OH). Adult shore flies were collected by aspiration, and larvae and pupae were picked up with a dissecting needle under a dissecting microscope, both from insect-infested rockwool blocks.

Fungus gnats (*B. impatiens*) naturally occurring in the greenhouse were maintained on disease-free pepper plants (*Capsicum annuum* L. cv. Joe E. Parker) growing in UC mix (a 1:1 mixture of #30 silica sand and peat moss) in 11 × 11 cm plastic pots, at 25 to 30°C with a 12-h photoperiod at 100% relative humidity. Larvae were periodically examined microscopically to ensure that they were free of external and internal contamination (examination of frass deposits) by fungal pathogens. Larvae and pupae were collected from the top 2 cm of the potting mix around the base of plants by rinsing the mix through a 32-mesh sieve. The larvae and pupae retained on the sieve were backwashed into petri plates with 15 ml of water and collected with a dissecting nee-

dle under a dissecting microscope. Adult fungus gnats do not feed on fungi (9) and thus were not evaluated for ingestion and egestion of pathogen propagules.

In all experiments, insects fed on rolled oats were included as controls. These insects excreted frass but were found not to be internally infested by the pathogens. The numbers of fungus gnat and shore fly larvae and shore fly adults varied among experiments due to their availability and/or mortality at and during the time the experiment was performed. Additionally, for the same reasons mentioned above, not all life stages of both insects were used for all the studies.

Pathogen ingestion and excretion by adult shore flies. All test species except *P. ramorum* were evaluated for their ability to survive ingestion and egestion by adult shore flies. Currently, *P. ramorum* is a quarantined pest (24), and therefore to avoid the risk of contaminated shore flies escaping and spreading the pathogen outside of the containment area, *P. ramorum* was not included in this set of studies. Mycelial mats of *Phytophthora nicotianae*, *Pythium aphanidermatum*, *P. splendens*, *P. sylvaticum*, and *P. ultimum* bearing numerous propagules (Table 1), produced as described above, were removed from petri dishes, rinsed in SDW, placed on a paper towel to remove excess moisture, and then placed in separate petri plates (90 mm diameter). Additionally, four pieces of the colonized eggplant fruit (2 × 2 cm) bearing sporulating colonies of *P. capsici* were also placed in a separate petri plate (90 mm diameter). Fifteen to 132 adult shore flies from colonies maintained in the greenhouse were temporarily incapacitated by exposing them to 0°C for 5 min and then transferred to each petri plate to feed on the above cultures. The petri plates were incubated at room temperature (25°C) for 24 h, during which time hundreds of frass deposits were observed on the undersides of the lids of the petri plates. Approximately 10 to 33 frass deposits from each lid were randomly examined microscopically (×200) for the presence of propagules. Then, sections of solidified ½-strength PDA, measuring 3 × 3 × 0.2 cm, were overlaid on the frass deposits to facilitate transfer of the deposits to the agar. After 24 h, the agar sections were removed and placed in separate petri plates and incubated at 25°C. Propagule germination was recorded after 48 h. These experiments were conducted three times for each pathogen.

Pathogen ingestion and excretion by larvae of fungus gnats and shore flies. Mycelial mats bearing propagules of *Phytophthora capsici*, *P. nicotianae*, *P. ramorum* [isolates P10102 and P10467], *Pythium aphanidermatum*, *P. splendens*, *P. sylvaticum*, and *P. ultimum* were obtained by the procedures described above (Table 1). Fungus gnat and shore fly larvae were allowed to feed on these propagules for 24

h. The larvae were collected, rinsed twice in SDW, and examined microscopically (×200) for the presence of propagules in their intestinal tracts. Larvae which were internally infested with propagules were placed individually on petri plates containing water agar and incubated at 25°C. Within 2 h, one to 13 discrete frass deposits were observed on the surface of the agar in each petri plate. Frass deposits were examined microscopically (×200) to confirm the presence, condition, and number of propagules in each deposit; each frass speck was then incubated at 25°C for 24 h to assess propagule viability, i.e., germination. Frass deposits containing propagules of *P. ramorum* were overlaid with PARPH medium and incubated at 25°C for 24 to 72 h to assess propagule viability. The experiment with *P. aphanidermatum* was conducted twice, and all the other experiments were conducted 3 times per pathogen, and 10 to 20 insects were assayed per experiment.

Pathogen acquisition and excretion by garden snails. Brown garden snails were collected from a natural population at a residential landscape location in southern California and fed on moistened rolled oats for 18 h to clean out their digestive tracts. Six snails were then rinsed in SDW and placed individually in 90-mm-diameter petri plates containing mycelial mats of *P. ramorum* (isolate P10467) bearing abundant sporangia and chlamydospores for 24 h. Frass ribbons excreted by these snails were collected, gently rinsed with SDW, and microscopically examined (×40) for the presence and condition of excreted propagules of *P. ramorum*. Frass ribbons containing propagules were added to a 2-ml microcentrifuge tube containing 1 ml of SDW and vortexed for 60 s to dislodge the propagules from the ribbon. Three 0.1-ml aliquots of the suspension were then dispensed on the surface of a 90-mm-diameter petri plate containing PARPH medium and incubated at 25°C to observe germination of excreted propagules. Controls consisted of six snails whose frass was harvested after they were fed on rolled oats. The experiment was conducted three times.

Transmission studies. *Transmission of Pythium by larvae of fungus gnats.* All transmission studies were conducted using a modified method developed by Misaghi (20). Approximately one-fourth of a 4-mm-thick water agar slab was removed from one side of a 90-mm-diameter petri plate and discarded. Five surface-sterilized pepper seeds (*Capsicum annuum* L. cv. Joe E. Parker), susceptible to all species of *Pythium* used in this study, were inserted equidistantly into the cut edge of the agar retained in the petri plate. The petri plates were stacked on edge in a plastic crisper (29.8 × 12.4 × 6.2 cm) (Tri-State Plastics, Inc., Latonia, KY) with the seeds facing up and incubated at 28°C in the dark until

germination occurred. Fungus gnat larvae were allowed to feed on sporulating cultures of *P. splendens*, *P. sylvaticum*, and *P. ultimum*, produced as described above, for 24 h. The larvae were then rinsed with SDW and microscopically examined for the presence of hyphal swellings in their digestive tracts. Two internally infested larvae were then placed in each petri plate containing 9-day-old pepper seedlings. Petri plates with pepper seedlings without fungus gnat larvae and petri plates with pepper seedlings with fungus gnat larvae not fed with *Pythium* spp. served as negative controls. Pepper seedlings inoculated by placing 7-mm-diameter plugs from the

edge of actively growing cultures of each *Pythium* species on the agar substrate in which the seedlings were growing served as positive controls. All plates were incubated at 28°C in a growth chamber (12 h photoperiod), and seedling mortality data were collected over the next 2 weeks. Experiments with each pathogen were conducted twice, and three petri plates (5 seedlings/petri plate) were used in each experiment.

Transmission of *Phytophthora ramorum* by larvae of fungus gnats. Detached rhododendron leaves (*Rhododendron* spp. L. cv. Cunningham's Blush) were surface-sterilized (0.5% NaOCl for 2 min and rinsed with SDW) and wounded by cutting

off the margins at the apical and the basal ends. Each leaf was placed in a 90-mm-diameter petri plate containing 10 ml of SDW. One moist sterile rolled oat seed was placed on the leaf as a food source for the fungus gnat larvae. Two fungus gnat larvae, internally infested with chlamydospores of *P. ramorum* (isolate P10467) (fed on *P. ramorum* for 24 h), were placed on each leaf. The petri plates were incubated at 25°C for 10 days, after which time the leaves were examined microscopically (×100 and ×200) for necrosis of tissue and colonization (presence of hyphae and chlamydospores either on leaf margins or embedded in cleared leaf tissue). In order to observe the mycelium, chlamydospores, and sporangia of *P. ramorum* inside the leaf tissue, the leaves were cleared in 1:1 acetic acid and ethanol and stained with lactic acid and aniline blue using a modified technique developed by McLean and Byth (19). Rhododendron leaves placed in SDW without fungus gnat larvae and leaves in SDW with non-*P. ramorum*-infested fungus gnat larvae served as controls. The experiment was conducted three times with 12 replicates (2 fungus gnat larvae/rhododendron leaf/petri plate) each.

Transmission of *P. ramorum* by garden snails. Snails, collected as previously described, were fed overnight on moistened rolled oats to clean out their digestive tracts, rinsed in SDW, and then allowed to feed on mycelial mats bearing sporangia and chlamydospores of either *P. ramorum* isolate P10467 or P10102. After a 24-h acquisition period, snails were rinsed with SDW and placed individually in a petri plate containing one surface-sterilized wounded rhododendron leaf and 10 ml of SDW. Additionally, one frass ribbon excreted by each snail was collected, rinsed in SDW, and microscopically examined (×200) for the presence of *P. ramorum* propagules. Frass ribbons containing propagules of *P. ramorum* were transferred to a petri plate containing a surface-sterilized rhododendron leaf and 10 ml of SDW. Plates were incubated at 25°C for 7 to 10 days, and the leaves were periodically examined for necrosis of tissue and colonization (presence of hyphae and chlamydospores either on leaf margins or embedded in leaf tissue). Experiments with isolate P10467 were conducted 5 times with 8 replicates (1 snail/rhododendron leaf/petri plate) each, and experiments with isolate P10102 was conducted twice with 7 replicates (1 snail/rhododendron leaf/petri plate) each. Snails not fed on *P. ramorum* and snail frass from noninfested snails placed on rhododendrons leaves served as controls.

RESULTS

Pathogen ingestion and excretion by shore fly adults. Sporangia and/or hyphal swellings of *Phytophthora capsici*, *P. nicotianae*, *Pythium splendens*, *P. sylvaticum*, and *P. ultimum* were ingested by adult

Fig. 1. Plasmolyzed/empty sporangia of *Phytophthora capsici* in the frass deposit of an adult shore fly. Bar = 60 µm.

Fig. 2. Frass excreted by adult shore fly showing germinated oospores (arrows) of *Pythium aphidematum*. Bar = 25 µm.

shore flies, but none of those propagules retained their viability after passage through the digestive tract. All frass deposits excreted by these adults (approximately 9 frass deposits/adult/24-h period) were microscopically examined, and no viable-looking propagules were detected nor did any propagules germinate. Excreted propagules were observed in various stages of lysis/plasmolysis (dissolved papillae, disrupted plasma membrane, digested cytoplasm) in frass deposits (Fig. 1).

In contrast, oospores of *P. aphanidermatum* ingested by adult shore flies were viable upon excretion (Fig. 2). In three experiments, an average of 65 to 78% (mean 73) of the frass deposits examined contained viable oospores. The number of oospores in each infested deposit ranged from 10 to 70 (mean 32). Thirty-six percent of the excreted frass deposits germinated upon incubation at 35°C. Morphological and physiological characteristics were used to identify and confirm the identity of the fungus emerging from the frass deposits as *P. aphanidermatum* (6,25).

In all assays, no test pathogens were ever detected on or in insects that were not allowed to feed on the pathogens.

Pathogen ingestion and excretion by larvae of fungus gnats and shore flies. Fungus gnat and shore fly larvae were capable of ingesting sporangia of *Phytophthora capsici*, *P. nicotianae*, and *P. ramorum* (isolates P10467 and P10102), but excreted sporangia were either empty or in various stages of plasmolysis (Fig. 3A to C). In contrast, chlamydospores, hyphal swellings, and oospores of these oomycetes retained their viability after passing through the digestive tract of both fungus gnat and shore fly larvae and were able to germinate upon incubation (Fig. 4A to E, Table 2).

Sixty-two to 71% (mean = 67) of the shore fly larvae assayed (45 in three experiments per pathogen) for each of the three pathogens, *P. splendens*, *P. sylvaticum*, and *P. ultimum*, excreted at least one frass deposit that contained viable hyphal swellings. The number of hyphal swellings per frass deposit (1 to 5 frass deposits/insect) ranged from 6 to 60 (Table 2). Developing colonies were traced back to hyphal swellings in each frass deposit. Between 51 and 89% (mean = 74) of the fungus gnat larvae tested (45 in three experiments per pathogen) excreted at least one frass deposit containing viable hyphal swellings of each of the three *Pythium* species.

A mean of 6% of the shore fly larvae that fed on *P. ramorum* (isolate P10102) (34 in three experiments) excreted viable chlamydospores, and the number of chlamydospores per frass deposit (1 to 3 deposits/larvae) ranged from 2 to 5 chlamydospores. Developing colonies were traced back to chlamydospores in each frass deposit.

A mean of 40% of the fungus gnat larvae (60 in 3 experiments) that fed on *P. ramorum* (isolate P10467) excreted viable

chlamydospores, and the number of chlamydospores per frass deposit (2 to 13 deposits/larvae) ranged from 1 to 25. Developing colonies were traced back to chlamydospores in the frass deposits. No attempt was made to estimate the percent germination of the excreted propagules.

Additionally, a mean of 77% of the fungus gnat larvae (30 in three experiments) that fed on *Pythium aphanidermatum* excreted at least one frass deposit containing viable oospores. The number of oospores per frass deposit (2 to 8) ranged from 10 to 55. Developing colo-

Fig. 3. A and B, Plasmolyzed/empty sporangia of *Phytophthora capsici* in the digestive tracts of shore fly larvae. Bar = 60 μ m. C, Plasmolyzed/empty sporangia of *Phytophthora ramorum* in the frass deposit of a fungus gnat larvae. Bar = 55 μ m.

nies were traced back to hyphal swellings in each frass deposit.

Pathogen acquisition and excretion by garden snails. Garden snails ingested and excreted chlamydospores as well as sporangia of *P. ramorum*. In three experiments (6 snails/experiment), a mean of 67% of the snails ingested and excreted viable sporan-

gia and chlamydospores of *P. ramorum* (isolate P10467). An average of 200 chlamydospores obtained from each frass sample germinated with multiple germ tubes (2 to 7 per propagule within 24 h of excretion). An average of 200 sporangia from each frass sample germinated directly with a single germ tube (Fig. 5). Frass excreted by

snails not fed on *P. ramorum* was devoid of propagules of *P. ramorum*.

Transmission studies. *Transmission of Pythium by larvae of fungus gnats.* Fungus gnat larvae fed on either *P. splendens*, *P. sylvaticum*, or *P. ultimum* were able to transmit the pathogens to healthy pepper seedlings grown in petri plates (Fig. 6).

Fig. 4. **A,** Hyphal swellings of *Pythium splendens* in the digestive tract of a fungus gnat larva. **B,** Hyphal swellings of *P. splendens* in the frass deposit of a fungus gnat larva. **C,** Chlamydospores of *Phytophthora ramorum* in the frass deposit of a fungus gnat larva. **D,** Frass excreted by a fungus gnat larva showing germinated hyphal swellings of *P. splendens*. **E,** Germinated chlamydospores of *P. ramorum* in frass of a fungus gnat larva. Bars in A, B, and D = 43 μ m. Bars in C and E = 55 μ m.

The mean percentages of seedlings killed by *P. splendens*, *P. sylvaticum*, and *P. ultimum* (in both experiments per pathogen) within 2 weeks of introduction of pathogen-infested larvae to the seedlings were 28.6, 35.7, and 66.7%, respectively. Symptoms included necrosis of stem tissue and root rot. The respective pathogens were reisolated from diseased seedlings. All control plants remained healthy.

Transmission of *Phytophthora ramorum* by larvae of fungus gnats. Chlamydospores of *P. ramorum* (isolate P10467) excreted by fungus gnat larvae were able to infect and colonize detached wounded rhododendron leaves. Abundant chlamydospores, as well as a few sporangia, were observed along the wounded leaf margins and/or within cleared leaf tissue. In three experiments (12 fungus gnat larvae/experiment), a mean of 44% of the rhododendron leaves were symptomatically infected with *P. ramorum* within 10 days after the introduction of the pathogen-infested larvae.

Transmission of *P. ramorum* by garden snails. A mean of 23% of snails (40 in five experiments) internally infested with sporangia and chlamydospores of *P. ramorum* (isolate P10467) infected healthy rhododendron leaves. A mean of 31% of frass samples (one from each of 36 snails in five experiments) collected from these snails contained viable propagules that infected healthy rhododendron leaves. Additionally, a mean of 14% of snails (14 in two experiments) internally infested with *P. ramorum* (isolate P10102) and a mean of 43% frass samples (one from each of 14 snails in two experiments) collected from these snails contained viable sporangia which infected healthy rhododendron leaves. Snails not fed on *P. ramorum* and frass excreted by these snails did not contain propagules of *P. ramorum* and therefore were not able to initiate infections in rhododendron leaves.

DISCUSSION

Exclusion of plant pathogens from greenhouse production systems is dependent upon understanding all avenues of pathogen introduction and spread. Once

mechanisms of introduction and dispersal are identified, specific management strategies can be developed to exclude pathogens from production areas. We demonstrated that common greenhouse pests and snails are capable of vectoring several oomycetes. Our findings complement and extend earlier work that implicates common greenhouse insects as vectors of several plant pathogenic fungi (3,5,13,21). With the exception of oospores of *P. aphanidermatum*, adult shore flies are not capable of ingesting and excreting viable hyphal swellings, chlamydospores, or sporangia of three species of *Pythium* and two species of *Phytophthora* (Table 3). Thus, they are unlikely to function as aerial vectors of the oomycetes we investigated. However, larval stages of both fungus gnats and shore flies ingested and excreted viable hyphal swellings of *P. splendens*, *P. sylvaticum*, and *P. ultimum* as well as chlamydospores of *P. ramorum*, implicating them as potential vectors of these oomycetes (Table 3). Sporangia of *P. aphanidermatum*, *P. ramorum*, *P. capsici*, and *P. nicotianae*, although ingested by larvae, were not viable upon excretion. The papillae of all three species of *Phytophthora*, as well as the sporangial cytoplasm, were digested during passage through the intestinal tract of the insect. The papillae of sporangia are composed of a gel-like substance (18) that appeared to have been dissolved upon passing through the intestinal tract of the larvae.

Although larvae of these greenhouse insect pests may not contribute to the rapid transmission of these oomycetes, they may play a role in the introduction of the pathogen into a production facility via larval-infested cuttings and horticultural substrates. Once introduced, these oomycetes could be dispersed by the recirculating irrigation water or through high-density "community flats" via root-to-root contact and infect the entire crop within the system. In a commercial nursery where *P. ramorum* was found in 2003, most infections originated in community flats where nursery seedlings were raised (B. Reddy, Monrovia Growers, personal communication).

Our studies indicate that land snails, which are common nursery, greenhouse, and garden pests, are capable of ingesting and excreting viable propagules of *Phytophthora ramorum*. *P. ramorum* is pathogenic to numerous ornamental plants, and environmental conditions favorable to this pathogen, i.e., cool, moist, and shady conditions, are also conducive to snails. Thus, snails have the opportunity to acquire and the ability to disperse the pathogen, albeit slowly, within a facility and serve as transient vectors for long-distance movement during the marketing of plants.

The ability of vertebrates and invertebrates to disperse ectomycorrhizal fungi has been documented (17). Fungivores such as mites, springtails, millipedes, beetles, and fly larvae were found to acquire spores of *Tomentella subulilacina* as a result of feeding on mature sporocarps of this mycorrhizal fungus. Vertebrate and invertebrate predators of these fungivores can indirectly acquire these pathogen propagules. Several fungivores and their predators contain fungal spores in their intestinal tracts and/or their excreta (17). For example, viable spores of aquatic phycomycetes were found in the excreta of birds that had fed on worms that in turn had ingested various soil fungi (22). Birds have also

Fig. 5. Germinated sporangia of *Phytophthora ramorum* in snail frass. Bar = 55 μ m.

Table 2. Propagules of *Phytophthora* and *Pythium* species and their survival through the intestinal tract of larvae of fungus gnats and shore flies^a

Oomycete	Propagule type	Fungus gnat larvae		Shore fly larvae	
		% that excreted propagules	% that excreted viable propagules	% that excreted propagules	% that excreted viable propagules
<i>Phytophthora capsici</i>	Sporangia	100	0	100	0
<i>P. nicotianae</i>	Sporangia	100	0	100	0
<i>P. ramorum</i> (10467)	Sporangia	-	-	nt ^b	nt
	Chlamydospores	48 $\pm$ 5.8	40 $\pm$ 5.8	nt	nt
<i>P. ramorum</i> (10102)	Sporangia	73 $\pm$ 5.8	0	44 $\pm$ 5.4	0
	Chlamydospores	-	-	21 $\pm$ 7.7	6 $\pm$ 5.4
<i>Pythium aphanidermatum</i>	Oospores	83 $\pm$ 5.8	77 $\pm$ 5.8	nt	nt
<i>P. splendens</i>	Hyphal swellings	89 $\pm$ 10.2	89 $\pm$ 10.2	78 $\pm$ 3.9	71 $\pm$ 10.2
<i>P. sylvaticum</i>	Hyphal swellings	82 $\pm$ 7.7	82 $\pm$ 7.7	71 $\pm$ 13.9	62 $\pm$ 10.2
<i>P. ultimum</i>	Hyphal swellings	69 $\pm$ 7.7	51 $\pm$ 10.2	71 $\pm$ 20.4	67 $\pm$ 17.7

^a Each value is a mean of three experiments, each with 10 to 20 replications $\pm$ SD.

^b nt = Not tested.

Fig. 6. Growth chamber transmission studies demonstrating that hyphal swellings of *Pythium splendens*, *P. sylvaticum*, and *P. ultimum* excreted by fungus gnat larvae were able to infect pepper seedlings and cause disease. Controls show healthy seedlings.

Table 3. Summary of ingestion and excretion of viable pathogen propagules by various life stages of shore flies and fungus gnats

Pathogen (propagule)	Fungus gnats ^a		Shore flies ^a	
	Adult	Larvae	Adult	Larvae
<i>Phytophthora capsici</i> (sporangia)	nt	No	No	No
<i>Phytophthora nicotianae</i> (sporangia)	nt	No	No	No
<i>Phytophthora ramorum</i> (sporangia)	nt	No	nt	No
<i>Phytophthora ramorum</i> (chlamydospores)	nt	Yes	nt	Yes
<i>Pythium aphanidermatum</i> (oospores)	nt	Yes	Yes	nt
<i>Pythium splendens</i> (hyphal swellings)	nt	Yes	No	Yes
<i>Pythium sylvaticum</i> (hyphal swellings)	nt	Yes	No	Yes
<i>Pythium ultimum</i> (hyphal swellings)	nt	Yes	No	Yes

^a No = propagules not ingested or excreted by the insect. Yes = propagules ingested and excreted by the insect in a viable form. nt = not tested.

been implicated in disseminating *Cylindrocladium parasiticum* by ingesting infected peanuts and depositing its microsclerotia in droppings (12). Ants have been shown to move *Phytophthora palmivora* vertically through infected cocoa trees (7), and chlamydospores of *P. cinnamomi* have been shown to be vectored by birds and termites (15). *Colletotrichum lagenarium* has also been shown to survive the gut of white garden snails (10). Thus, pathogen infested insects and snails could potentially facilitate dispersal of propagules in food webs. If birds were to prey on snails and/or larvae that are internally infested with sporangia or chlamydospores of *P. ramorum*, they can potentially disperse the propagules over longer distances during flight albeit at a very low probability. Birds have been suspected of vectoring *P. ramorum* (2).

Control of fungus gnats, shore flies, and snails is an ongoing problem in greenhouse systems. Historically, fungus gnats and shore flies have been considered a nuisance, i.e., deposition of their feces on aerial parts of plants may subsequently diminish the aesthetic and/or market value of the crop. The possibility that they may also serve as vectors of some major patho-

gens gives growers another reason to exclude them from production areas. Snails could allow *P. ramorum* to enter the food chain and be transmitted over relatively long distances by birds or snail-eating mammals. Quarantine measures to limit spread of pathogens should take into consideration the possibility that passive vectors can serve as another avenue of pathogen introduction or spread.

ACKNOWLEDGMENTS

We wish to thank I. J. Misaghi and F. P. Wong for editorial assistance and encouragement.

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