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Attraction and oviposition responses of the fungus gnat *Bradysia impatiens* to microbes and microbe-inoculated seedlings in laboratory bioassays

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Abstract

Laboratory tests were conducted to examine preferences of *Bradysia impatiens* Johannsen (Diptera: Sciaridae) larvae and adults for various microbes associated with greenhouse crops. Fungus gnat larvae and adults exhibited a preference for cultures of *Pythium* spp. over the medium used to grow the pathogens. Larvae also exhibited a preference for geranium seedlings infected with pathogenic *Pythium* spp. [*P. aphanidermatum* (Edson) Fitz., *P. ultimum* Trow, and *P. irregulare* Buis. (Oomycota: Peronosporales)] over non-inoculated plants. Adult fungus gnats exhibited a strong ovipositional preference for the aforementioned *Pythium* spp. as well as a variety of other microorganisms, including the pathogenic fungus *Thielaviopsis basicola* (Berk. & Br.) (Ascomycota: Microascales), the geranium-infecting bacterium *Xanthomonas campestris* pv. *pelargonii* (Brown) Dye (Proteobacteria: Xanthomonadales), the non-pathogenic species *Pythium torulosum* Coker & P. Patt. and *Pythium graminicola* Subramaniam, the pathogen-suppressive fungus *Trichoderma harzianum* Rifai (Ascomycota: Hypocreales), and the insect pathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales). Our study is the first to demonstrate that fungus gnats are attracted to and/or stimulated to oviposit by a wide array of living microorganisms both in pure culture and in association with plant seedlings. These findings have important implications with respect to the potential role of fungus gnats in plant pathogen transmission.

Introduction

Fungivorous insects are abundant and include a variety of Collembola and larval and adult Coleoptera and Diptera that vary in the specificity of their fungal feeding. Among the Diptera, the dark-winged fungus gnats (Sciaridae) are diverse and speciose, and fungi, including various Ascomycetes, Basidiomycetes, and Myxomycetes, are thought to make up an essential component of their diets (Kennedy, 1974; Harris et al., 1996). Sciarids generally live in moist shady habitats; the larvae of most species live in fungi, but some inhabit soils, and a few are agricultural pests in field crops, greenhouses, or mushroom cellars (Steffan, 1966; Harris et al., 1996; Triplehorn & Johnson, 2005). Kennedy (1976) described sciarid adults as generally aphagous, although they have been reported to feed on liquids, including nectar (Mercier, 1911), molasses (Hungerford, 1916), and 'organic ooze' (Steffan, 1966). In greenhouses, fungus gnat larvae feed primarily on decaying roots and stems of many plant species (Johannsen, 1912; Hungerford, 1916; Kennedy, 1974; Harris et al., 1996). However, larvae of some pest species also sometimes consume healthy root, stem, and leaf tissues of vascular plants in greenhouses (Wilkinson & Daugherty, 1970).

In general, fungus gnat survival is reduced when fungal abundance is low (Harris et al., 1996). Kennedy (1974) demonstrated that *Bradysia impatiens* (Johannsen) larvae developed more rapidly and exhibited greater survivorship when reared on diets containing the fungus *Alternaria tenuis* Nees or brewer's yeast (*Saccharomyces cerevisiae* Meyen ex E.C. Hansen) than on non-fungal diets of lima bean agar, potato dextrose agar, or agar-agar. Anas &

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Reeleder (1988) also found that *Bradysia coprophila* (Comstock) larvae developed into fertile adults when raised on plants inoculated with *Botrytis porri* Buckw., *Rhizoctonia solani* Kühn, or *Sclerotinia minor* Jagger, but failed to survive on non-infected plants. Olson et al. (2002) revealed that a significantly greater number of *Bradysia* sp. adults emerged from soilless media composed of coconut coir or sphagnum peat moss when a food source of 1 g of yeast was added.

Very little is known about the capacity of sciarid fungus gnats to subsist on microorganisms other than true fungi. In recent years, observations have pointed to a possible correlation between *Bradysia* fungus gnat infestations and root rot diseases caused by many common oomycetes, especially *Pythium* spp. (Gardiner et al., 1990; Jarvis et al., 1993). *Bradysia* and *Pythium* spp. seem to be constantly associated with greenhouses (JP Sanderson & ML Daughtrey, pers. obs.), and such observations have fostered the general perception that these insects are significant *Pythium* vectors (Gardiner et al., 1990; Jarvis et al., 1993). However, potential interactions among fungus gnats, *Pythium* spp., and their host plants have not been thoroughly investigated. It may be that the close association between fungus gnats and diseased plants is based primarily on a strong attraction of the fungus gnats to microbial activity in diseased plant tissues. Fungus gnats thus may become associated with plants infected by microbes that they do not play a significant role in vectoring. The overall goal of the current study was to improve our understanding of the role of fungus gnats in the epidemiology of plant diseases, particularly in greenhouses where these insects are ubiquitious pests. The specific objective was to determine if attraction and oviposition responses of fungus gnats to potential host plants and other substrates are enhanced by the presence of active microbes, including various pathogenic and non-pathogenic *Pythium* spp.

Materials and methods

Insect rearing

A laboratory colony of *B. impatiens* was established with adults collected from a greenhouse on the Cornell University campus (Ithaca, NY, USA). Identity was determined from the species description by Johannsen (1912) and confirmed by comparing colony-reared insects to the type specimens of *B. impatiens* in the Cornell University Insect Collection. Adults and larvae were routinely reared in plastic containers (9.8 cm diameter × 6.2 cm depth; Pioneer Plastics, Dixon, KY, USA) with lids bearing holes (5.5 cm diameter) covered with nylon mesh (95 μm) for ventilation. Each container was provided 40 g of growing medium (Premier Pro-Mix® BX; Premier Horticulture, Quakertown, PA, USA) mixed with 10 g (dry weight) of ground pinto beans [*Phaseolus vulgaris* L. (Fabaceae)] (Goya® Foods, Secaucus, NJ, USA). The mix was then saturated with tap water, and 50 fungus gnat adults (predominately gravid females, which are physically larger than the males) were introduced for egg production. Colony containers were maintained at 27 ± 1 °C and L14:D10 photoperiod. In the following description of methods, these specific temperature/light conditions will be referred to as the 'standard conditions.' New containers were established daily to provide a constant supply of all fungus gnat life stages. To obtain an even-aged cohort of third-instar fungus gnats for use in bioassays, approximately 200 adult fungus gnats were collected from colony containers and released into a 45 × 45 × 45.5 cm cage in the laboratory at ambient temperature and allowed to oviposit on 90 mm-diameter Petri dishes (Becton Dickinson Labware, Franklin Lakes, NJ, USA) that contained water-saturated cotton covered with a piece of black filter paper spread with a thin layer (approximately 1.6 g) of ground pinto beans. After 24 h, Petri dishes were removed from cages and the fungus gnat cultures maintained until the larvae completed development to the third instar. Additional water and pinto beans were added to the dishes as needed. Third instars were readily identified on the surface of the filter paper (based on the relative sizes of head capsules) and transferred to bioassay containers using a fine brush.

Plant propagation

Geranium seeds [*Pelargonium* × *hortorum* L.H. Bailey 'Orbit White' (Geraniaceae); Goldsmith Seeds®, Gilroy, CA, USA] were surface sterilized in a 0.5%-sodium hypochlorite solution and germinated individually on top of a filter paper disk (42.5 mm diameter) saturated with 400 μl sterile distilled water (SDH_2O) in 47 mm diameter friction-lid Petri dishes (Fisher Scientific, Pittsburgh, PA, USA). The seeds were incubated in darkness for 3 days at room temperature. Seedlings were maintained under standard conditions and watered daily with 200 μl SDH_2O until used in bioassays. The seedlings were oriented, for the most part, horizontally in the Petri dishes (lying on the filter paper).

Seeds used in plug cell bioassays were planted in tap water-saturated Redi-earth Plug and Seedling Mix (Sun Gro Horticulture Canada, Vancouver, BC, Canada) within individual plug cells (ca. 14 ml volume) that were cut from 128-well plastic plug trays (Dillen Products/Myers Industries, Middlefield, OH, USA) and incubated in darkness at 23 °C until germination (4 days). Seedlings were maintained under standard conditions until used in bioassays.

Microbe maintenance

Various microbes, including oomycetes, fungi, and bacteria, that fungus gnats typically encounter in the greenhouse were chosen for use in bioassays to examine the attraction and oviposition responses of the insect. *Pythium aphanidermatum* (Edson) Fitzp. (Oomycota: Peronosporales) strain Pa58 (isolated from *Gypsophila paniculata* L.) and *Pythium ultimum* Trow strain P4 (isolated from *Phaseolus vulgaris* L.) were obtained from a culture collection maintained at Cornell University, Ithaca, NY, USA (EBN lab), and *Pythium irregulare* Buis. strain Par 1 (isolated from *Pelargonium* sp.) was obtained from a collection maintained at the Long Island Horticultural Research and Extension Center (LIHREC), Riverhead, NY, USA (MLD lab). Cultures were grown in 60 mm-diameter Petri dishes on V8 juice agar [100 ml V8 juice (Campbell Soup Company, Camden, NJ, USA), 400 ml H_20, 1.5 g $CaCO_3$, and 10 g Bacto^{TM} Agar] and maintained in the dark at 27 ± 1 °C. For some experiments with *P. aphanidermatum*, cultures were grown in 60 mm-diameter Petri dishes in potato dextrose (PD) broth, comprising 6 g PD powder (MP Biomedicals, Solon, OH, USA) in 250 ml SDH_2O, and maintained in the dark at 27 ± 1 °C. One-week-old *Pythium* cultures were used in all experiments and for inoculating plants for periodic re-isolation of the pathogens. Every 2 weeks, the pathogens were re-isolated from 11-day-old geranium seedlings that had been inoculated 3 days earlier by placing a 6 mm-diameter *Pythium*-colonized agar disk on the root tip. Each infected seedling was placed in a 90 mm-diameter Petri dish and covered with 2% (wt/vol) molten water agar supplemented with the antibiotics rifampicin, penicillin G, and kanamycin (1.0, 1.0, and 2.5 ml l^{-1} SDH_2O, respectively, and all at 0.01 g ml^{-1}) (WARP). *Pythium* hyphae that grew rapidly through the agar were excised from the surface of the medium and transferred to a fresh WARP plate. *Pythium torulosum* Coker & P. Patt. (isolated from *Impatiens* sp.) and *P. graminicola* Subramaniam (isolated from soil) (LIHREC strains Pt-1 and L-59, respectively, both of which are not pathogenic on geranium), were grown on V8 juice agar.

Thielaviopsis basicola (Berk. & Br.) (Ascomycota: Microascales), obtained from LIHREC and isolated from *Calibrachoa*, was grown in the dark on PD agar (2% agar) at 24 ± 1 °C. One-week-old cultures of *T. basicola* were used in all experiments. Every 2 weeks, the pathogen was reisolated from 18-day-old geranium seedlings that had been inoculated 10 days earlier as described above for *Pythium*-infected plants. The bacterium *Xanthomonas campestris* (Brown) Dye (Proteobacteria: Xanthomonadales) strain 98–024 (obtained from DW Kalb of Cornell University, Ithaca, NY, USA and isolated from *Pelargonium* sp.) was grown in the dark at 24 ± 1 °C. Two-day-old cultures of *X. campestris* were used in all experiments. The biocontrol agents *Trichoderma harzianum* Rifai (Ascomycota: Hypocreales) strain T-22 and *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) strain GHA [isolated from the commercial products Rootshield® and BotaniGard® (BioWorks, Victor, NY, USA), respectively] were grown in the dark either in potato dextrose broth or on potato dextrose agar at 24 ± 1 °C.

Infection of seedlings

Eight-day-old seedlings in the previously described friction-lid Petri dishes were inoculated by placing a 6 mm-diameter agar disk colonized by either *P. aphanidermatum*, *P. ultimum*, or *P. irregulare* directly onto the root tips. The seedlings were then maintained under standard conditions and watered daily (200 µl SDH_2O) until they were 11 days old and exhibited signs of infection, including wilting and water-soaking that made the tissue appear darker green. Assays conducted in Petri dishes allowed for the direct observation of microbial growth on the shoots and roots of the seedlings. Using the same protocol, additional seedlings were inoculated with *T. basicola* or *X. campestris* and incubated at 24 ± 1 °C under the standard light regime. Seedlings infected with *T. basicola* were maintained until they were 18 days old and exhibited black and water-soaked roots; *X. campestris*-infected seedlings were maintained until they were 12 days old and exhibited wilting and yellowing. Seedlings were also inoculated with the non-plant-pathogenic microbes *P. graminicola*, *P. torulosum*, *T. harzianum*, or *B. bassiana* as described above and maintained under standard conditions until they were 11 days old. At this time, epiphytic hyphal growth of the microbes was evident on the seedling roots.

Seven-day-old seedlings in plug cells were inoculated by placing a 6 mm-diameter agar disk of either *P. aphanidermatum* or *T. harzianum* in contact with the crown of the plant. Seedlings were watered (with 2 ml tap water) after inoculation and maintained under standard conditions until the plants were 10 days old. Inoculation disks were removed from seedlings prior to the initiation of experiments.

Larval choice: *Pythium*-colonized vs. non-colonized agar disks

Assays were conducted to determine the attraction of larval fungus gnats to a *Pythium*-colonized vs. non-colonized agar substrate. Tests were initially conducted using pure cultures to provide fungus gnats with a sufficient amount of *Pythium* biomass when making choices among substrates. All replicate assays were conducted on the same laboratory bench under room

temperature conditions (25 ± 1 °C). Each replicate was conducted on the wet surface of a 90 mm-diameter disk of filter paper (saturated with 1.5 ml SDH_2O) lining a Petri-dish lid with inside diameter of 91 mm (Becton Dickinson Labware). Four 12 mm-diameter V8 agar disks – one colonized with *P. aphanidermatum*, one with *P. ultimum*, one with *P. irregulare,* and one non-colonized – were randomly assigned a position around the perimeter of the filter paper, equidistant from one another and from the center of the dish (centers of the disks were ca. 5.2 cm apart and 3.7 cm from the center of the dish). In each dish, the locations to which the four disks were randomly assigned corresponded to the extreme back, right, front, and left from the fixed viewpoint of the observer (0/360º, 90º, 180º, and 270º). Approximately 20 min. after placement of the disks, one third-instar fungus gnat was deposited (oriented indiscriminately) in the center of the arena using a fine paint brush, and the arena was covered with the dish bottom (87 mm inside diameter). Movements of the larva were then visually monitored for the entire assay. The first agar disk a larva contacted was recorded, and the assay was terminated. Preliminary assays revealed that 30 min was a sufficient amount of time for most larvae (>75%) to contact an agar disk. Larvae that did not contact a disk within 30 min were classified as non-responders. A total of 71 replicate assays were conducted on three different days (23–24 assays per day) using different batches of fungus gnats.

Larval choice: *Pythium*-infected vs. non-infected geranium seedlings

Larval fungus gnat preference for *Pythium*-infected vs. non-infected geranium seedling roots was assessed using the same arena and general protocol as described above for the agar disk assays. Pieces of infected root tip (2 cm in length) were cut from 11-day-old geranium seedlings infected with either *P. aphanidermatum*, *P. ultimum*, or *P. irregulare* as previously described. One root tip from each of the three *Pythium*-infected seedlings and one from an 11-day-old non-infected seedling were randomly assigned to the four previously described positions on the wet filter paper (root pieces were oriented approximately parallel to the wall of the dish). One third-instar fungus gnat larva was placed in the center of the arena, and its movements were visually monitored for the entire assay. The first root tip contacted by a larva was noted, and the assay was terminated. Preliminary assays revealed a markedly slower response than in the above-described assays with *Pythium*-colonized agar disks; 90 min was required for >75% of the insects to contact a root tip. Larvae that did not contact a root tip within 90 min were considered non-responders. Eighty-two replicate assays were conducted on four different days (ca. 10–24 assays per day).

Adult choice: *Pythium*-colonized vs. non-colonized agar disks

Assays were conducted to determine fungus gnat adult preference for disks of *P. aphanidermatum*. Assays were conducted in 150 × 25 mm Petri dishes (Becton Dickinson Labware) (arena without filter paper). One V8-agar disk colonized with *P. aphanidermatum* and one non-colonized V8-agar disk (each 12 mm diameter.) were placed opposite one another, equidistant from the center of the Petri dish. The centers of the disks were ca. 6 cm from the center of the dish and 12 cm apart. In each assay, the fungus-colonized disk was first randomly assigned to one of the four positions described for the larval-choice assays, and then the uncolonized agar disk was placed in the opposite location. Two-day-old adult female fungus gnats were collected individually into small (20 ml) glass vials and chilled at 4 °C for 10 min. The vial was then inverted over the center of the arena and tapped sharply to dislodge the test subject. The protocol then followed that of the larval choice assays, except that the dishes were monitored for a longer period, as the released adults tended to remain motionless for some time before responding. Two hours were required for >75% of the insects to contact an agar disk. Seventy-six replicate assays were conducted on four different days (17–20 assays per day).

Adult choice: oviposition on *Pythium*-infected, heat-killed, or non-infected plants

Thirty replicate assays were conducted to determine fungus gnat ovipositional preference for *Pythium*-infected, non-infected, or heat-killed geranium seedlings using the 90 mm-diameter Petri dishes with wet filter paper as described for the larval choice tests. This experiment was conducted to determine whether fungus gnats are attracted to dead plants in general or only those that have been infected by a pathogen. Treatments included 11-day-old seedlings that were either non-infected, freshly killed via a 30-s microwave treatment (microwave oven set on high; Model R-120DK; Sharp Electronics, Mahwah, NJ, USA), or infected with *Pythium* (*P. aphanidermatum*, *P. ultimum*, or *P. irregulare*). One *Pythium*-infected, one heat-killed, and one non-infected seedling were placed randomly in parallel non-overlapping positions in the Petri dish, and one mated pair of 2-day-old fungus gnats (one male and one female) was added in the middle at the base of the dish. The dishes were sealed with Parafilm,® placed randomly on trays, and maintained under standard incubation conditions for 36 h. Eggs deposited on the seedlings and filter paper were then enumerated using a dissecting microscope. Each *Pythium* treatment

(individual *Pythium* species) was replicated 10 times. Ten replicate control assays also compared preference for *P. aphanidermatum*-infected seedlings vs. seedlings sham-inoculated with V8 agar. *Pythium* spp. were re-isolated from infected seedlings at the end of the experiments.

Oviposition assays in all additional experiments to be described below were based on the above-described protocol, unless otherwise indicated. In those assays testing only two substrates in addition to the filter paper surface, the substrates were randomly assigned to opposite positions in the arena as described for the experiments investigating adult fungus gnat choice of *Pythium*-colonized agar disks. Assays in all oviposition experiments were conducted on two or three different days, with an average of ca. five assays conducted each day.

Adult choice: oviposition on dead *Pythium* vs. live *Pythium* or healthy geranium seedlings

A series of assays was conducted to determine fungus gnat ovipositional preference for live vs. dead (dried or frozen) *P. aphanidermatum* and live or dead *P. aphanidermatum* vs. non-infected 7-day-old geranium seedlings. These treatment combinations were examined to determine if killed pathogens would stimulate oviposition. Mats of *P. aphanidermatum* mycelium were removed from the surface of PD broth, washed three times with SDH_2O, and cut into 1 × 1 cm pieces. Subsets of these pieces were then treated (killed) either by drying for 2 h in an open Petri dish in a sterile laminar flow hood or by freezing for 3 h at −18 °C; non-viability of the dried/frozen pieces was confirmed by incubation on V8 agar. Two of the above-described substrates were randomly assigned to opposite positions in each assay arena. The SDH_2O used for filter paper saturation contained the antibiotic gentamicin sulfate at a concentration equivalent to 0.1 mg gentamicin base per ml to prevent bacterial growth over the course of the assays. Substrate combinations and numbers of assays (in parentheses) included: live vs. dried *Pythium* (18), live vs. frozen *Pythium* (13), live *Pythium* vs. non-infected plant (15), dried *Pythium* vs. non-infected plant (12), and frozen *Pythium* vs. non-infected plant (13). An additional 10 assays testing live vs. frozen *Pythium* were conducted without addition of gentamicin.

Adult choice: oviposition under no-choice conditions

A series of assays was conducted to compare rates of oviposition on treated seedlings in no-choice assays. In these assays, a single non-infected, microwave-killed, or *Pythium*-infected 11-day-old geranium seedling was placed in the center of the Petri dish, and a pair of fungus gnats (one male, one female) was added. Assays included non-infected seedlings (11), microwave-killed seedlings (11), and seedlings infected with *P. ultimum* (10), *P. irregulare* (10), or *P. aphanidermatum* (11). These assays demonstrated fungus gnat preference for oviposition on specific substrates.

Adult choice: oviposition on fungus- or bacterium-infected vs. non-infected plants

Twenty-two replicate assays were conducted with *T. basicola*-infected geranium seedlings to determine fungus gnat ovipositional preference for fungus-infected vs. non-infected 18-day-old geranium seedlings, and 20 replicate assays were conducted to determine preference for *X. campestris*-infected vs. non-infected 12-day-old geranium seedlings.

Adult choice: oviposition on non-pathogenic *Pythium* spp. vs. non-infected plants

Assays were conducted with *P. torulosum*-inoculated geranium seedlings (11 assays) and *P. graminicola*-inoculated geranium seedlings (10 assays) to compare fungus gnat ovipositional preference for geranium seedlings inoculated with *Pythium* spp. non-pathogenic on geranium vs. non-inoculated plants. Controls (11 assays) compared preference for seedlings sham-inoculated with V8 agar vs. non-inoculated seedlings. Either one 11-day-old *P. torulosum*-inoculated, *P. graminicola*-inoculated, or V8 agar-inoculated geranium seedling and one 11-day-old non-inoculated geranium seedling were randomly assigned to opposite positions in the assay arena. Both *P. torulosum* and *P. graminicola* were re-isolated from stems and roots of inoculated seedlings at the end of the assays.

Adult choice: oviposition on beneficial microbes vs. non-infected plants

Oviposition assays were conducted to assess the attractiveness of two commercially available beneficial microbes, the mycoparasite *T. harzianum*, and the insect pathogen *B. bassiana*. Fungus gnat ovipositional preference was determined for live *T. harzianum* or *B. bassiana* vs. non-infected 7-day-old geranium seedlings and *T. harzianum* or *B. bassiana*-inoculated vs. non-infected 11-day-old geranium seedlings. Mats of *T. harzianum* or *B. bassiana* mycelium were removed from the surface of PD broth, washed three times with SDH_2O, and cut into 1 × 1 cm pieces. Two of the above-described substrates were randomly assigned to opposite positions in each assay arena. Substrate combinations and numbers of assays (in parentheses) included: live *T. harzianum* vs. non-infected plant (19), *T. harzianum*-inoculated plant vs. non-inoculated plant (12), live *B. bassiana* vs. non-infected plant (16), and *B. bassiana*-inoculated plant vs. non-inoculated plant (12).

An additional 16 assays testing PD agar-inoculated plants vs. non-inoculated plants were conducted to determine if fungus gnats exhibit a preference for the inoculation medium. Both *T. harzianum* and *B. bassiana* were re-isolated from stems and roots of inoculated seedlings at the end of the assays.

Adult choice: oviposition on plants grown in plug cells

Assays were conducted with *P. aphanidermatum*-infected geranium seedlings (14 assays) and *T. harzianum*-inoculated geranium seedlings (10 assays) to compare fungus gnat ovipositional preference for plug cell-grown seedlings inoculated with microorganisms compared to plug cell-grown sham-inoculated seedlings. This more complex assay was used to confirm that the Petri dish assay results were reflective of reality in a peat-based growing system. Assays were conducted in plastic containers (9.8 cm diameter × 6.2 cm depth; Pioneer Plastics) with lids. Either one 10-day-old *P. aphanidermatum*-inoculated and one V8 agar-inoculated geranium seedling or one 10-day-old *T. harzianum*-inoculated and one potato dextrose agar-inoculated geranium seedling were randomly assigned to opposite positions in each assay arena. One mated pair of 2-day-old fungus gnats (one male and one female) was added. Each plug cell was watered once with 1 ml tap water, and the containers were maintained under standard incubation conditions for 36 h. Both *P. aphanidermatum* and *T. harzianum* were re-isolated from inoculated seedlings and from the plug mix at the end of the experiment.

Statistical analysis

All statistical tests were conducted using the JMP® software version 7.0 (Littell et al., 2002). It was generally not possible to conduct large numbers of assays at one time (especially with the multiple species of pathogens and other microbes), and rigorous comparisons among the various assays is therefore not possible. Our analyses are thus focused on the responses to the different substrates tested within assays. Proportions of larval or adult fungus gnats responding (attracted) to the various substrates within assays were initially tested for differences across test days by heterogeneity χ^2 analysis, and differences among proportions of the test population attracted to the various substrates were then examined via conventional goodness-of-fit χ^2 analysis testing frequencies observed vs. frequencies expected from a show of no preference (equal numbers of gnats attracted to each substrate) (Sokal & Rohlf, 1995; Zar, 1999). Alpha in all tests was set at 0.05.

Egg counts from the adult oviposition experiments were analyzed as repeated measures, with each female confined (confronted simultaneously) with multiple substrates. We opted to employ MANOVA for the analysis, as it does not require selection of a specific correlation structure; MANOVA assumes that each of the correlations between the repeated observations is unique (Littell et al., 2002). This flexibility comes at the cost of considerable power compared to the alternative mixed model approach (which is based on selection of an appropriate, simpler covariance model); however, we found the MANOVA to have adequate power to detect differences between responses to infected/contaminated vs. non-infected/non-contaminated plants.

All percentages were arcsine transformed prior to MANOVA. In experiments with more than two treatments, means comparisons were made via multiple pairwise tests (χ^2 goodness-of-fit or repeated measures MANOVA) with sequential Bonferroni adjustments to maintain the experimentwise alpha level at 0.05 (Sokal & Rohlf, 1995). No more than four means were directly compared in any test.

Results

Larval choice: *Pythium*-colonized vs. non-colonized agar disks

The heterogeneity χ^2 test indicated no differences in responses across days ($\chi^2 = 3.20$, d.f. = 2, $P>0.05$), and data from all replicates were therefore pooled. The *Pythium*-colonized substrates (all *Pythium* species

Table 1 Number of fungus gnats attracted to *Pythium*-colonized vs. non-colonized V8 agar disks

Assay – substrates tested	No. fungus gnats[1]	% total responses[2]
Larval fungus gnats		
Pythium aphanidermatum-colonized agar	25	42.4a
Pythium irregulare-colonized agar	20	33.9a
Pythium ultimum-colonized agar	12	20.3a
Non-colonized agar	2	3.4b
Total responding	59	100
Adult fungus gnats		
Pythium aphanidermatum-colonized agar	37	63.8a
Non-colonized agar	21	36.2b
Total responding	58	100

[1]Number of fungus gnats that selected each substrate (excluding 12 larvae that were non-responsive (did not make contact with a test substrate) within 30 min and 18 adults that were non-responsive within 2 h).

[2]Larval assays: percentages followed by same letter are not significantly different (sequential Bonferroni test: experimentwise $\alpha = 0.05$); adult assays: percentages followed by a different letter are significantly different (χ^2 goodness-of-fit test: $\alpha = 0.05$).

combined) attracted nearly 97% of all responding fungus gnats, with the V8 agar alone attracting <4% (Table 1). No differences in attractiveness were observed among the substrates colonized with the three *Pythium* spp. ($\chi^2 = 4.53$, d.f. = 2, P = 0.10). Upon contact with the chosen substrates, larvae typically began feeding. Seventeen percent of the larvae (12/71) did not choose a disk within 30 min (Table 1).

Larval choice: *Pythium*-infected vs. non-infected geranium seedlings

As observed in the tests with agar disks, response was homogeneous across days, and data from all replicates were pooled ($\chi^2 = 3.53$, d.f. = 3, P>0.05). Ninety-four percent of the responding larvae were attracted to the *Pythium*-infected geranium seedlings vs. only 6% to the non-inoculated seedlings (Table 2). Seedlings infected with the three *Pythium* spp. were equally attractive ($\chi^2 = 1.41$, d.f. = 2, P = 0.49). Upon contact with the chosen substrates, larvae typically began feeding. The seedlings always exhibited signs of infection, including wilting and water-soaking that made the tissue appear darker green. Twenty-one percent of larvae (17/82) did not choose a seedling within 90 min.

Adult choice: *Pythium*-colonized vs. non-colonized agar disks

Results did not vary significantly across days ($\chi^2 = 0.24$, d.f. = 3, P>0.05), and data from all replicates were pooled. Among 58 responding adult fungus gnats, a significantly greater number (64%) were attracted to the *P. aphanidermatum*-colonized disks vs. the disks of V8 agar alone ($\chi^2 = 4.41$, d.f. = 1, P = 0.036). Upon contact with the chosen substrates, adults typically remained sedentary for a brief period of time (up to 1 min) and then continued to fly around the bioassay arena. Among all flies tested, 24% (18/76) did not choose either disk within 2 h (Table 1).

Table 2 Number of fungus gnat larvae attracted to *Pythium*-infected vs. non-infected geranium roots

Treatment	No. larvae[1]	% total responses[2]
Pythium aphanidermatum-infected root	23	35.4a
Pythium irregulare-infected root	16	24.6a
Pythium ultimum-infected root	22	33.8a
Non-infected root	4	6.2b
Total responding	65	100

[1]Excluding 17 larvae that were non-responsive (did not make contact with a geranium root) within 90 min.
[2]Percentages followed by same letter are not significantly different (sequential Bonferroni test: experimentwise $\alpha = 0.05$).

Adult choice: oviposition on *Pythium*-infected, heat-killed, or non-infected plants

There was a highly significant effect of oviposition substrate on the number of eggs laid in each assay (P-values <0.0001) (Table 3), and the overall oviposition responses were similar in the assays with the three different *Pythium* species. Approximately 86, 89, and 92% of all eggs were deposited on the *P. aphanidermatum*-, *P. irregulare*-, and *P. ultimum*-infected seedlings, respectively, whereas low, equivalent numbers of eggs were laid on the non-infected live and killed seedlings. In the control assay, <3% of all eggs were deposited on the sham-inoculated seedlings.

Adult choice: oviposition on dead *Pythium* vs. live *Pythium* or healthy geranium seedlings

In each assay there was a highly significant effect of oviposition substrate on the number of eggs laid (all P-values ≤ 0.0003) (Table 4). Across all assays with desiccation- or freeze-killed *Pythium* mycelium (with and without gentamicin) vs. live *Pythium* mycelium, only 2–5% of all eggs were deposited on the dead mycelium. In assays of killed *Pythium* vs. healthy geranium seedlings, significantly more eggs (75–79%) were deposited on the seedlings than on the killed *Pythium* (3–7%) or filter paper (18%). In assays of live *Pythium* mycelium vs. healthy geranium seedlings, significantly more eggs (62%) were deposited on the live *Pythium* than on the seedlings (26%) or filter paper (12%).

Adult choice: oviposition under no-choice conditions

Results of assays in which fungus gnats were presented with just a single substrate (either a *Pythium*-infected, a non-infected, or a microwave-killed seedling) are presented in Table 5. Seedlings infected with *P. aphanidermatum, P. irregulare*, and *P. ultimum* received 86, 86, and 81% of all eggs, respectively, with the remaining eggs in each assay being deposited on the wet filter paper. When confronted with the choice between non-infected (healthy) seedlings and filter paper, the fungus gnats deposited statistically equivalent numbers on each substrate (44 vs. 57%). In contrast, microwave-killed seedlings attracted <7% of egg deposition.

Adult choice: oviposition on fungus- or bacterium-infected vs. non-infected plants

There was a highly significant effect of oviposition substrate on the number of eggs laid in each assay (P-values ≤ 0.0002) (Table 6), and the overall oviposition responses were similar in the assays with fungal and bacterial plant pathogens. Approximately 80 and 62% of all eggs were deposited on the *T. basicola*- and *X. campestris*-infected seedlings, respectively, whereas lower numbers of eggs

Table 3 Oviposition by fungus gnats on *Pythium*-infected vs. non-infected (healthy) geranium seedlings

Assay – substrates tested	No. replicate assays	Mean (± SE) no. eggs	% eggs[2]	MANOVA F-test
Pythium aphanidermatum-infected seedling	10	46.3 ± 10.1	85.9 ± 6.5a	$F_{3,7}$ = 51.1, P<0.0001
Non-infected seedling		0.9 ± 0.41	2.1 ± 0.8b	
Non-infected, microwave-killed seedling		1.9 ± 1.32	3.7 ± 2.6b	
Filter paper		2.7 ± 1.87	8.3 ± 6.2b	
Pythium irregulare-infected seedling	10	62.2 ± 6.37	88.7 ± 4.7a	$F_{3,7}$ = 42.0, P<0.0001
Non-infected seedling		5.4 ± 3.14	6.1 ± 3.5b	
Non-infected, microwave-killed seedling		1.6 ± 0.79	3.4 ± 2.1b	
Filter paper		1.3 ± 0.67	1.8 ± 0.8b	
Pythium ultimum-infected seedling	10	58.2 ± 9.8	91.8 ± 3.5a	$F_{3,7}$ = 43.2, P<0.0001
Non-infected seedling		1.2 ± 0.68	2.7 ± 1.9b	
Non-infected, microwave-killed seedling		0.9 ± 0.48	2.5 ± 1.6b	
Filter paper		1.8 ± 1.29	2.9 ± 2.3b	
Pythium aphanidermatum-infected seedling	10	60.4 ± 10.1	88.5 ± 5.9a	$F_{3,8}$ = 76.2, P<0.0001
Sham-inoculated seedling[1]		1.6 ± 0.95	2.5 ± 1.4b	
Filter paper		4.7 ± 2.39	9.0 ± 5.0b	

[1]Healthy geranium seedling sham-inoculated with V8 agar.
[2]Mean percentage of eggs (± SE) laid on each substrate (one female fungus gnat per replicate assay). Means within each assay followed by the same letter are not significantly different (sequential Bonferroni test: experimentwise α = 0.05).

Table 4 Effects of *Pythium aphanidermatum* viability (live vs. dead) on attractiveness to ovipositing fungus gnats

Assay – substrates tested[1]	No. replicate assays	Mean (± SE) no. eggs	% eggs[2]	MANOVA F-test
Live *Pythium*	18	50.8 ± 8.68	82.3 ± 5.0a	$F_{2,16}$ = 49.7
Desiccation-killed *Pythium*		1.1 ± 0.51	4.6 ± 2.8b	P<0.0001
Filter paper		6.4 ± 2.16	13.1 ± 3.3c	
Live *Pythium*	13	55.2 ± 7.65	85.4 ± 4.9a	$F_{2,11}$ = 79.5
Freeze-killed *Pythium*		1.1 ± 0.47	2.6 ± 1.2b	P<0.0001
Filter paper		5.4 ± 1.94	12.0 ± 4.7b	
Live *Pythium*	10	30.6 ± 4.39	69.7 ± 5.4a	$F_{2,8}$ = 34.5
Freeze-killed *Pythium*		2.8 ± 1.31	5.1 ± 2.2b	P = 0.0001
Filter paper (without antibiotic)		10.8 ± 1.9	25.2 ± 4.7c	
Live *Pythium*	15	23.7 ± 3.26	61.6 ± 7.3a	$F_{2,13}$ = 16.3
Geranium seedling		12.6 ± 3.94	26.3 ± 6.8b	P = 0.0003
Filter paper		5.2 ± 1.28	12.0 ± 2.5b	
Desiccation-killed *Pythium*	12	1.8 ± 0.95	6.9 ± 4.8a	$F_{2,10}$ = 21.0
Geranium seedling		31.0 ± 5.11	75.4 ± 6.0b	P = 0.0003
Filter paper		6.8 ± 1.77	17.7 ± 3.9c	
Freeze-killed *Pythium*	13	0.9 ± 0.42	3.1 ± 1.5a	$F_{2,11}$ = 82.5
Geranium seedling		37.2 ± 8.18	78.6 ± 4.7b	P<0.0001
Filter paper		6.2 ± 1.67	18.3 ± 4.4c	

[1]Substrates included 1 × 1 cm mats of live or dead *Pythium aphanidermatum* mycelium, healthy (non-infected) 7-day-old geranium seedlings, and water-saturated filter paper; the filter paper substrate (upon which all other substrates were placed) was saturated with water containing the antibiotic gentamycin in all but the indicated series of assays.
[2]Mean percentage of eggs (± SE) laid on each substrate (one female fungus gnat per replicate assay). Means within each assay followed by the same letter are not significantly different (sequential Bonferroni test: experimentwise α = 0.05).

were laid on the non-infected seedlings (11–26%) and filter paper (9–12%). Seedlings infected with *T. basicola* exhibited black and water-soaked roots; *X. campestris*-infected seedlings exhibited wilting and yellowing.

Adult choice: oviposition on non-pathogenic *Pythium* spp. or non-infected plants

There was a highly significant effect of oviposition substrate on the number of eggs laid in each assay

Table 5 Oviposition by fungus gnats on filter paper vs. *Pythium*-infected geranium seedlings, non-infected seedlings, or microwave-killed seedlings

Assay – substrates tested	No. replicate assays	Mean (± SE) no. eggs[1]	% eggs[1]	MANOVA F-test
Pythium aphanidermatum-infected seedling	11	44.9 ± 6.68	85.5 ± 5.7a	$F_{1,10} = 20.1$
Filter paper		7.1 ± 3.41	14.5 ± 5.7b	$P = 0.0003$
Pythium irregulare-infected seedling	10	50.5 ± 7.95	86.4 ± 4.7a	$F_{1,9} = 39.6$
Filter paper		5.4 ± 1.18	13.6 ± 4.7b	$P = 0.0001$
Pythium ultimum-infected seedling	10	58.5 ± 8.03	80.7 ± 6.6a	$F_{1,9} = 17.0$
Filter paper		11.9 ± 4.16	19.3 ± 6.6b	$P = 0.003$
Non-infected (healthy) seedling	11	25.7 ± 8.19	43.5 ± 12.6a	$F_{1,10} = 0.32$
Filter paper		35.7 ± 9.6	56.5 ± 12.6a	$P = 0.58$
Microwave-killed seedling	11	2.2 ± 0.96	6.8 ± 3.1a	$F_{1,10} = 84.5$
Filter paper		57.1 ± 9.18	93.2 ± 3.1b	$P<0.0001$

[1]Mean percentage of eggs (± SE) laid on each substrate (one female fungus gnat per replicate assay). Means within each assay followed by the same letter are not significantly different (MANOVA F-test: $\alpha = 0.05$).

Table 6 Oviposition by fungus gnats on *Thielaviopsis basicola*- or *Xanthomonas campestris*-infected vs. non-infected (healthy) geranium seedlings

Assay – substrates tested	No. replicate assays	Mean (± SE) no. eggs	% eggs[1]	MANOVA F-test
Thielaviopsis basicola-infected seedling	22	40.1 ± 4.4	80.1 ± 4.4a	$F_{2,20} = 38.1$
Non-infected seedling		4.8 ± 1.2	11.0 ± 3.0b	$P<0.0001$
Filter paper		3.9 ± 1.2	8.8 ± 2.8b	
Xanthomonas campestris-infected seedling	20	35.0 ± 5.9	62.2 ± 7.7a	$F_{2,18} = 14.2$
Non-infected seedling		13.0 ± 4.0	25.7 ± 7.2b	$P = 0.0002$
Filter paper		6.3 ± 1.6	12.2 ± 2.9b	

[1]Mean percentage of eggs (± SE) laid on each substrate (one female fungus gnat per replicate assay). Means within each assay followed by the same letter are not significantly different (sequential Bonferroni test: experimentwise $\alpha = 0.05$).

Table 7 Oviposition by fungus gnats on *Pythium torulosum*-, *P. graminicola*-, or V8 agar-inoculated vs. non-inoculated geranium seedlings

Assay – substrates tested	No. replicate assays	Mean (± SE) no. eggs	% eggs[1]	MANOVA F-test
Pythium torulosum-inoculated seedling	11	32.5 ± 5.0	62.7 ± 7.4a	$F_{2,9} = 9.1$
Non-inoculated seedling		10.8 ± 3.7	21.4 ± 7.6b	$P = 0.007$
Filter paper		8.7 ± 2.9	16.0 ± 4.0b	
Pythium graminicola-inoculated seedling	10	75.0 ± 9.2	85.8 ± 5.6a	$F_{2,8} = 54.5$
Non-inoculated seedling		9.7 ± 4.8	10.0 ± 4.7b	$P<0.0001$
Filter paper		3.5 ± 1.2	4.2 ± 1.4b	
V8 agar-inoculated seedling	11	39.3 ± 12.1	46.3 ± 13.8a	$F_{2,9} = 48.5$
Non-inoculated seedling		28.2 ± 7.5	51.3 ± 13.4a	$P<0.0001$
Filter paper		1.9 ± 1.2	2.4 ± 1.5b	

[1]Mean percentage of eggs (± SE) laid on each substrate (one female fungus gnat per replicate assay). Means within each assay followed by the same letter are not significantly different (sequential Bonferroni test: experimentwise $\alpha = 0.05$).

(P-values ≤ 0.007) (Table 7), and the overall oviposition responses were similar in the assays with the two different *Pythium* species. Approximately 63 and 86% of all eggs were deposited on the *P. torulosum*- and *P. graminicola*-inoculated seedlings, respectively, whereas lower numbers of eggs were laid on the non-inoculated seedlings (10–21%) and filter paper (4–16%). Epiphytic hyphal growth of the microbes was evident on the seedling roots used in

these assays. When confronted with the choice between sham-inoculated vs. non-inoculated seedlings, the fungus gnats deposited statistically equivalent numbers on each substrate (46 vs. 51%).

Adult choice: oviposition on beneficial microbes or non-infected plants

In each assay there was a highly significant effect of oviposition substrate on the number of eggs laid (all P-values ≤ 0.004) (Table 8). Across all assays with live *T. harzianum* or *B. bassiana* mycelia, significantly more eggs were laid on the mycelia (60 and 77%, respectively) than on the non-inoculated seedlings (29 and 18%) or filter paper (11 and 5%). In assays with *T. harzianum* - or *B. bassiana* - inoculated vs. non-inoculated seedlings, significantly more eggs were laid on fungus-inoculated seedlings (85 and 80%, respectively) than on non-inoculated seedlings (8 and 14%) or filter paper (7 and 6%). Epiphytic hyphal growth of the microbes was evident on the seedling roots used in these assays. When confronted with the choice between sham-inoculated and non-inoculated seedlings, the fungus gnats deposited statistically equivalent numbers on each substrate (35 vs. 54%, respectively).

Adult choice: oviposition on plants grown in plug cells

There was a significant effect of oviposition substrate on the number of eggs laid in each assay (P-values ≤ 0.03) (Table 9), and the overall oviposition responses were similar in the assays with *P. aphanidermatum* and *T. harzianum*. Approximately 72 and 79% of all eggs were deposited on the *P. aphanidermatum*-infected and *T. harzianum*-inoculated seedlings, respectively, whereas lower numbers of eggs were laid on the sham-inoculated seedlings (28 and 21%, respectively).

Discussion

Our results demonstrated that attractiveness of geranium seedlings to larval and adult female *B. impatiens* was greatly enhanced by presence of plant pathogenic oomycetes (*Pythium* spp.). Seedlings infected/infested with various microbes, including oomycetes, fungi, and bacteria, were also strong oviposition stimulants, as were pure-culture mycelial preparations of *Pythium* and two beneficial fungi (*T. harzianum* and *B. bassiana*). Inactivation of *Pythium* mycelia via freezing or desiccation rendered the mycelia unattractive as oviposition substrates even in comparison to healthy seedlings. This is the first study to demonstrate fungus gnat attraction to a broad range of pathogenic and non-pathogenic microorganisms. We did not conduct studies to identify the fundamental agent of attraction; however, the above-described findings with mycelia suggest that one or more chemical by-products of microbial metabolism and growth may be key. The more rapid response of the larvae to the *Pythium*-colonized agar disks than to the *Pythium*-infected seedlings (response within 30 vs. 90 min) is noteworthy in this regard. The colonized agar disks likely presented a larger

Table 8 Oviposition by fungus gnats on non-inoculated geranium seedlings vs. *Trichoderma harzianum*- or *Beauveria bassiana*-inoculated seedlings or culture mats

Assay – substrates tested[1]	No. replicate assays	Mean no. eggs	% eggs[2]	MANOVA F-test
Live *Trichoderma*	19	36.2 ± 5.3	60.0 ± 6.9a	$F_{2,17} = 18.0$, P<0.0001
Non-inoculated seedling		19.1 ± 4.0	28.8 ± 4.9b	
Filter paper		6.5 ± 1.8	11.2 ± 3.2c	
Trichoderma-inoculated seedling	12	42.5 ± 4.7	85.1 ± 4.2a	$F_{2,10} = 36.5$, P<0.0001
Non-inoculated seedling		4.7 ± 2.1	7.9 ± 3.7b	
Filter paper		3.8 ± 1.8	6.9 ± 2.7b	
Live *Beauveria*	16	35.6 ± 4.2	77.4 ± 6.0a	$F_{2,14} = 62.0$, P<0.0001
Non-inoculated seedling		9.4 ± 3.1	17.8 ± 5.2b	
Filter paper		2.3 ± 0.7	4.7 ± 1.5c	
Beauveria-inoculated seedling	12	36.4 ± 6.0	80.0 ± 6.2a	$F_{2,10} = 34.4$, P<0.0001
Non-inoculated seedling		6.2 ± 2.3	14.0 ± 5.6b	
Filter paper		2.0 ± 0.6	6.1 ± 1.9b	
Potato dextrose agar (PDA)-inoculated seedling	16	14.6 ± 4.5	34.6 ± 9.0ab	$F_{2,14} = 8.59$, P = 0.0037
Non-inoculated seedling		25.9 ± 6.5	53.5 ± 10.0a	
Filter paper		4.2 ± 1.5	11.9 ± 4.3b	

[1]Substrates included 1 × 1 cm mats of live *Trichoderma harzianum* or *Beauveria bassiana* mycelium, *T. harzianum*-, *B. bassiana*-, or PDA-inoculated geranium seedlings, healthy (non-infected) geranium seedlings, and water-saturated filter paper.

[2]Mean percentage of eggs (± SE) deposited on each substrate in each assay (one female fungus gnat per replicate assay). Means within each assay followed by the same letter are not significantly different (sequential Bonferroni test: experimentwise $\alpha = 0.05$).

Table 9 Oviposition by fungus gnats on *Pythium aphanidermatum*- or *Trichoderma harzianum*-inoculated vs. non-inoculated (healthy) geranium seedlings grown in plug cells

Assay – substrates tested	No. replicate assays	No. eggs[1]	% eggs[1]	MANOVA F-test
Pythium aphanidermatum-infected seedling	14	16.6 ± 4.2	71.9 ± 9.3a	$F_{1,13} = 6.0, P = 0.03$
V8 agar-inoculated seedling		7.2 ± 2.6	28.1 ± 9.3b	
Trichoderma harzianum-inoculated seedling	10	18.1 ± 3.6	79.3 ± 9.1a	$F_{1,9} = 11.1, P = 0.009$
PDA-inoculated seedling		5.8 ± 2.8	20.7 ± 9.1b	

[1]Mean percentage of eggs (± SE) laid on each substrate in each assay (one female fungus gnat per replicate assay). Means within each assay followed by the same letter are not significantly different (MANOVA F-test: $\alpha = 0.05$).

and more concentrated biomass of the microbe than the infected seedlings.

Volatiles generated as a result of microbial activity have been implicated as oviposition stimulants for another agriculturally important dipteran, the onion maggot fly, *Hylemya antiqua* (Meigen) (Ellis et al., 1979). In contrast to our finding, however, investigators found that these flies were not stimulated to oviposit by the test microbe (*Pseudomonas cepacia*) presented in pure culture; rather, oviposition stimulation was the result of an interaction between the microbe and the onion plant. It was posited that the bacteria metabolize precursors in onion plants, converting them to volatile sulfide attractants/oviposition stimulants (Hough et al., 1981, 1982).

Relatively few studies have investigated factors mediating substrate selection by sciarid fungus gnats. With respect to our results, a reasonable hypothesis would be that *B. impatiens* is attracted to or stimulated to oviposit by some factor broadly associated with microbial activity. This may explain the observations of Meers & Cloyd (2005), who found that female fungus gnats did not exhibit an oviposition preference among a choice of three different un-sterilized growing media and that greater numbers of eggs were laid on the growing media than on filter paper. Meers & Cloyd (2005) ultimately suggested that fungus gnats may rely on factors such as 'volatiles emitted from growing media' in selecting an oviposition substrate. One obvious candidate for future investigations is the metabolic by-product CO_2. This compound is an important attractant of various insects, including other nematocerous diptera (e.g., foraging mosquitoes) (see Guerenstein & Hildebrand, 2008). In addition, studies of *Lycoriella ingenua* (Dufour), a common fungus gnat pest of mushroom cultures, revealed that oviposition on fungus-colonized litter increased when the litter was derived from plants grown in CO_2-enriched vs. normal environments (Frouz et al., 2002). Other studies, however, have indicated that *L. ingenua* does not find all microbes equally attractive. Frouz & Nováková (2001) reported that oviposition attractiveness of various fungi to this insect was highly variable, even among species of the same genus (in some cases, one species of a genus was highly attractive while another was completely unattractive). Additional studies will be required to elucidate the precise mechanism (s) by which microbial activity elicits the observed responses from *Bradysia* fungus gnats.

We must recognize that oviposition site selection is a complex process, likely involving an interaction of physical, biological, and chemical factors. For some dipterans, oviposition attractants that result in oriented movement toward a source are the same (Jang & Light, 1991; Gouinguené et al., 2005) as oviposition stimulants that elicit oviposition, whereas in other instances the chemicals may differ (Bentley & Day, 1989; Navarro-Silva et al., 2009). In this study, we did not isolate any compounds associated with infected/inoculated plants so it is not possible to discern whether the ovipositional attractant(s) and stimulant (s) are the same. Also, it is well known that visual cues play a pivotal role in oviposition for many insects (Kelber, 2001), and future studies should examine whether or not this sensory modality is important in fungus gnat oviposition site selection.

Mechanisms underlying fungus gnat ovipositional preferences for beneficial microbes such as *Trichoderma* and *Beauveria* are also important to consider in greenhouse management. Microorganisms such as these are often applied to growing media to control soil-borne pathogens and insects (Harman, 2000; Ugine et al., 2005), and they may impact various aspects of the fungus gnat life cycle. However, demonstrated ovipositional preferences of sciarids for *Trichoderma* are quite variable in the literature. For example, Frouz & Nováková (2001) found that some *Trichoderma* species were highly attractive to *L. ingenua* whereas others were completely unattractive. We observed attraction to *T. harzianum* pure cultures and *T. harzianum*-inoculated seedlings growing in Petri dishes and in a commercial growing medium, but Cloyd et al. (2007) did not see attraction to *T. harzianum*-inoculated growing media. Potential for currently marketed beneficial microbes like *Trichoderma* and *Beauveria* to attract fungus gnats has implications for insect pest and disease management programs, and studies conducted under actual crop production conditions are needed.

Our study is the first to demonstrate that *B. impatiens* larvae and adults respond to a wide variety of living microorganisms. This suggests that the commonly observed associations between fungus gnats and diseased plants are primarily the result of this attraction and will occur even in the absence of pathogen transmission by fungus gnats. Indeed, although fungus gnats are commonly associated with *Pythium* root rot disease outbreaks, we have found that these pests are not significant *Pythium* vectors, as the infectious propagules of these pathogens are produced primarily below ground (Braun et al., 2010) so are less likely to be acquired and transported on the bodies of adults. On the other hand, fungus gnat attraction to diseased/decaying plants obviously enhances contact between these insects and the readily acquired (and subsequently vectored) spores of many other pathogens produced in abundance on aerial plant parts (Kalb & Millar, 1986; Gillespie & Menzies, 1993; Harris, 1995; El-Hamalawi & Stanghellini, 2005; El-Hamalawi, 2008; Elmer, 2008; Braun et al., 2010). Although the microbial community to which fungus gnats are exposed likely changes with plant species, potting medium, and fertilization and irrigation practices, management strategies for these pests might include efforts to reduce unnecessary sources of microbial activity in the vicinity of potted plants (e.g., basic sanitation measures to remove plant debris) or to employ substrates supporting high microbial activity as trap baits. Such tactics will need to be balanced with providing crop plants with conditions that promote their defense and fitness. Overall, additional studies should be conducted to understand the relative attractiveness of various microbes for sciarid fungus gnats and the factors mediating substrate selection. Nonetheless, the findings of this study have enhanced our understanding of basic fungus gnat biology and the ways these insects interact with other organisms.

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