Disease Development on *Lisianthus* Following Aerial Transmission of *Fusarium avenaceum* by Adult Shore Flies, Fungus Gnats, and Moth Flies

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ABSTRACT

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Fusarium crown and stem rot of lisianthus (*Eustoma grandiflorum*), caused by *Fusarium avenaceum*, is a destructive disease in California. The pathogen produces large masses of orange-colored macroconidia on stem lesions that extend up to 35 cm in length from the soil surface. Populations of macroconidia (97% viability) range from 1.1×10^8 to 1.9×10^8 per cm of infected stem tissue. An aboveground life stage for a soilborne pathogen could serve as a source for acquisition and aerial dissemination by adult shore flies, fungus gnats, and moth flies. Our results provide evidence that these three insects are attracted to and readily acquire (either externally and/or internally) macroconidia of *F. avenaceum* produced on naturally infected lisianthus stems and then disseminate acquired macroconidia to healthy plants, which subsequently died, or to an abiotic substrate (Komada's medium, KM). The high percentage of transmission, as evidenced by both the number of KM plates colonized by the pathogen (up to 68.5% within 18 h) and the number of plants infected (75% within 4 days), reflects the efficiency of these insects as vectors.

Additional keywords: insect vectors

Fusarium crown and stem rot is a destructive disease of lisianthus (*Eustoma grandiflorum* Raf. (Shinn.)) with mortality rates frequently reaching 70% in California and Florida (9,10). The causal agent of this disease, *Fusarium avenaceum* (Fr.:Fr.) Sacc., infects the roots, crown, and lower stem tissues of lisianthus. Wilting and stem lesions occur as disease progresses, and masses of orange-colored spores, consisting primarily of macroconidia, are produced on mature stem lesions that can extend up to 35 cm in length from the soil surface (Fig. 1).

Little is known concerning the epidemiology of this disease. However, the existence of an aboveground life stage for this soilborne pathogen could serve as a source of inoculum for airspora or for acquisition and aerial dissemination by common greenhouse insects. Both airspora (3,8,12) and greenhouse insects (i.e., shore flies [*Scatella* spp.] and fungus gnats [*Bradysia* spp.]) have been implicated (1,5,6,7,15) in the spread of soilborne fungal pathogens, many of which reproduce aboveground (3,8,11,12,15,16). Aerial dissemination

would change the epidemiology of diseases caused by these soilborne pathogens from a typical monocyclic to a polycyclic pattern, which, in turn, would necessitate development of different and more appropriate disease management strategies.

The potential role of insects in the epidemiology of *F. avenaceum* on lisianthus, which is widespread in southern California, was therefore investigated. Specifically, our objectives were (i) to document the acquisition of the pathogen from naturally infected plant tissue by adults of three common greenhouse insect pests, i.e., shore flies (*Scatella* spp.), fungus gnats (*Bradysia* spp.), and (ii) to evaluate pathogen dissemination and transmission to healthy plants by these adult insects following pathogen acquisition. A preliminary report has been published (2).

MATERIALS AND METHODS

Fungal and insect culture maintenance and plant establishment. A single-spore culture of *F. avenaceum*, obtained from a naturally infected lisianthus plant (cv. Echo Pink) from a commercial nursery in San Diego, was maintained on potato dextrose agar (PDA). A subculture of this isolate was used for soil infestation.

A colony of shore flies was established and maintained on algal-infested rock wool blocks in a growth chamber maintained at 30°C with a 12-h photoperiod. Algae originated from adult shore flies

used to establish the colony. The rock wool blocks were fertilized weekly with Miracle-Gro (3 g of product per liter of water; Scotts Miracle-Gro Products, Inc., Marysville, OH). Colonies of fungus gnats and moth flies were established and reared on a pinto bean substrate (4) in separate growth chambers maintained at 30°C with a 12-h photoperiod.

One-month-old, disease-free lisianthus seedlings (cv. Echo Pink) were obtained from a commercial nursery. Seedlings were transplanted into 6-cm-diameter plastic pots containing a commercial potting mix and grown in the greenhouse for 14 days before use in transmission studies. Potted plants were irrigated daily and fertilized weekly with Miracle-Gro.

Inoculum for insect acquisition and transmission studies. Naturally infected lisianthus stems, bearing macroconidia of *F. avenaceum*, were used in all insect acquisition and transmission studies. Infected stems were collected from a commercial nursery and stored (maximum of 2 days) in a moist chamber until used. The populations of macroconidia on infected stems were estimated as follows: infected stems were cut into 1-cm-long segments. Each segment was then placed in 5.0 ml of ster-

Fig. 1. Sporulation of *Fusarium avenaceum* on the stem of an infected lisianthus plant.

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ile distilled water in a centrifuge tube and agitated for 5 min on a vortex mixer. Macroconidia in the suspension were then serially diluted, and populations were estimated with the aid of a hemacytometer. Two stem segments from each of five different plants were assayed. Additionally,

the viability of macroconidia was determined by plating aliquots (0.1 ml) of the highest dilution onto water agar medium (2.0%) in 9-cm-diameter petri dishes. Seeded dishes were then incubated in the dark at 24°C, and percent spore germination (minimum of 200 spores counted per

plate) was determined microscopically (×400) after 18 h.

Acquisition and transmission of *F. avenaceum* by shore flies, fungus gnats, and moth flies. Two different experimental methods were used to assess the efficacy of shore flies, fungus gnats, and moth

Fig. 2. Experimental system used to assess the acquisition and dissemination of *Fusarium avenaceum* by adult insects from naturally infected host tissue to an abiotic substrate. **A**, An insect screen cage containing Komada's medium (KM) in petri plates located at varying distances from lisianthus stem segments bearing macroconidia of *F. avenaceum* (arrow). **B**, Actual and **C**, diagrammatic distribution of KM plates colonized (shaded spots) by the pathogen following acquisition and dissemination by 10 adult shore flies placed in the cage for 18 h.

Fig. 3. Experimental system used to assess the acquisition and transmission of *Fusarium avenaceum* by adult insects from naturally infected host tissue to a biotic substrate. **A**, An insect screen cage containing healthy lisianthus seedlings in pots located at varying distances from infected lisianthus stem segments bearing macroconidia of *F. avenaceum* (arrow). **B**, Distribution of diseased plants following acquisition and transmission by 20 adult shore flies placed in the cage for 4 days. **C**, Control plants caged with 20 insects without infected stems or infected stems without insects.

flies in transmitting *F. avenaceum* following acquisition.

Ten adults of each insect were collected by aspiration from their respective colonies and released into separate insect screen cages (50 cm L, 40 cm W, and 50 cm H) located on a greenhouse bench. Each cage contained a metal tray (45 cm L, 35 cm W, and 2 cm H) upon which 54 plastic petri plates (each 35 mm diameter) were arranged without lids. Plates contained Komada's medium (KM), a *Fusarium*-selective medium (13). The plates were arranged in six columns and nine rows (Fig. 2). An open petri dish with two 5-cm-long, naturally infected lisianthus stem segments bearing *F. avenaceum* macroconidia was placed at one end of the metal tray. These infected stem segments served as the source of inoculum for acquisition by the adult insects. Two controls, consisting of KM plates in cages with infected stems but no insects and KM plates in cages with insects but no infected stems, were also included in the study. After 18 h, the KM plates were covered with lids, removed from the screen cage, and incubated at 24°C in the dark in the laboratory. After 8 days, the number and location of each colonized plate relative to the location of the source of inoculum (i.e., the infected stem segments) was recorded. Preliminary studies showed that there was no increase in either the number of colonies per plate or the number of plates colonized beyond the 8-day incubation period. All colonies on KM plates, which ranged in numbers from 1 to 29, exhibited the morphology of *F. avenaceum*, and the identity of five colonies per treatment was confirmed by transfer to PDA and microscopic examination. The experiment was repeated once.

Twenty adults of each insect were placed in separate screen cages containing two 5-cm-long, naturally infected lisianthus stem segments bearing macroconidia of *F. avenaceum* and 20 healthy lisianthus seedlings in 6-cm-diameter pots (Fig. 3). Controls consisted of healthy plants caged with insects without infected stems and healthy plants caged with infected stems without insects. After 4 days in their respective cages, plants were removed from the cages and incubated on a greenhouse bench (ambient temperatures ranged from 18 to 28°C, with a 14-h photoperiod). An additional control consisted of 20 healthy lisianthus plants that were inoculated, via a soil drench, with a spore suspension (2 ml per pot, 1×10^5 macroconidia per ml) obtained from a 20-day-old culture of *F. avenaceum*. Potted plants were irrigated daily and fertilized weekly with Miracle-Gro. Disease symptoms, which consisted of wilting and necrosis of stem tissue followed by plant death, were recorded daily in the various treatments over a 70-day postinoculation period. Isolations from crowns and stems of three symptomatic

and asymptomatic plants per treatment were conducted to verify the presence and identity of the pathogen. The experiment was repeated once.

Origin of *F. avenaceum* inoculum transmitted by adult insects. Subsequent to removal of the KM plates from cages used in the above experiments, 10 insects of each species were captured and placed individually in petri dishes containing KM and incubated for 18 h at 24°C in the dark in the laboratory. The insects were then removed from each petri dish, and external body parts were examined microscopically ($\times 100$) for macroconidia. Colonies of *F. avenaceum* were microscopically ($\times 10$) visible after 24 h of incubation, and colony origin was determined microscopically at that time, as well as daily over the next 7 days.

Statistical analysis. Data were analyzed using the general linear models (GLM) procedure of SAS. Treatment means were compared using Fisher's protected least significant difference test ($\alpha = 0.05$).

RESULTS

Inoculum for insect acquisition and transmission studies. The populations of macroconidia of *F. avenaceum* on naturally infected lisianthus stems used in our investigation ranged from 1.1×10^8 to 1.9×10^8 per cm of infected stem tissue. The viability of macroconidia recovered from those naturally infected stems was $96.7 \pm 0.2\%$.

Acquisition and transmission of *Fusarium* by shore flies, fungus gnats, and moth flies. Within 18 h after placement of

adult insects in cages with *F. avenaceum*, the percentage of KM plates colonized by *F. avenaceum* was 68.5, 45.5, and 39.5% for shore flies, fungus gnats, and moth flies, respectively (Fig. 4). The spatial distribution of colonized plates relative to the location of the inoculum source for shore flies is presented in Figure 2B and C. Similar distribution patterns were recorded for fungus gnats and moth flies (Fig. 5). Only the moth fly failed to transmit the pathogen to petri plates located at distances greater than 26 cm from the inoculum source. None of the KM plates were colonized by *F. avenaceum* in any of the control treatments, which included KM plates in cages with infected stems but no insects and KM plates in cages with insects but no infected stems.

After 4 days exposure to adult insects in cages with a source of inoculum (i.e., infected stem tissue bearing *F. avenaceum* macroconidia), a high percentage of the lisianthus plants were subsequently shown to be infected by the pathogen. Specifically, *Fusarium* crown and stem symptoms developed on 75, 55.5, and 45% of the plants caged with shore flies, fungus gnats, and moth flies, respectively (Fig. 6). Disease symptoms were first observed 40 days after insect inoculation, and disease incidence increased progressively to a maximum at 66 days after inoculation (Fig. 3B). The pathogen was isolated consistently from symptomatic crown and stem tissues. None of the plants in the various control treatments (i.e., healthy plants caged with insects without infected stems

Fig. 4. Comparison of the efficiency of transmission of *Fusarium avenaceum* by adult shore flies, fungus gnats, and moth flies after acquisition of the pathogen from an inoculum source (an infected lisianthus stem bearing macroconidia) to Komada's medium (KM) in petri plates located at varying distances from the inoculum source. Data are means of two experiments, 54 plates per experiment per treatment. Vertical bars represent standard error of the mean. Columns with the same letter are not significantly different based on the *F* test of the analysis of variance.

Fig. 5. Comparison of the spatial distribution of *Fusarium avenaceum* by adult shore flies, fungus gnats, and moth flies after acquisition of the pathogen from an inoculum source (an infected lisianthus stem bearing macroconidia) and dissemination to Komada's medium (KM) in petri plates located at varying distances from the inoculum source. Data are means of two experiments, 54 plates per experiment per treatment. Vertical bars represent standard error of the mean. Within each insect group, columns with the same letter are not significantly different based on the *F* test of the analysis of variance. For moth flies, means were separated using Fisher's LSD ($\alpha = 0.05$).

Fig. 6. Progression of stem rot disease symptoms following acquisition and transmission of *Fusarium avenaceum* macroconidia on infected lisianthus stems to healthy lisianthus seedlings by adult shore flies, fungus gnats, and moth flies. Controls consisted of healthy plants inoculated with macroconidia applied to soil, healthy plants exposed to insects without infected stems, and infected stems without insects. Data are means of two experiments, 20 plants per experiment per treatment. Vertical bars represent standard error of the mean.

and healthy plants caged with infected stems without insects) developed disease symptoms (Fig. 3C), and the pathogen could not be isolated from asymptomatic crown and stem tissues.

The development of disease symptoms following the soil drench inoculation

method was faster than the insect inoculation method. Specifically, disease symptoms were first observed 25 days after soil drench inoculation and reached an asymptote of 95% by day 40 after inoculation (Fig. 6).

Origin of *F. avenaceum* inoculum transmitted by adult insects. After 24 h

incubation, microscopic examination ($\times 10$) of KM plates that contained shore flies revealed that all *F. avenaceum* colonies originated from frass deposits. Subsequent colonies that grew over the next 7 days originated primarily from solitary or small clusters of macroconidia not associated with frass deposits. This was in contrast to the origin of colonies of the pathogen on KM plates containing either fungus gnats or moth flies. All colonies of *F. avenaceum*, which developed only after 3 or more days of incubation, originated from solitary or small clusters of macroconidia. None originated from frass deposits of either fungus gnats or moth flies.

Microscopic examination ($\times 40$) of frass deposits from shore flies on KM revealed the presence of abundant macroconidia of *F. avenaceum* (Fig. 7). Estimates of the number of macroconidia per frass deposit were $7.7 \times 10^4 \pm 1.5 \times 10^5$ macroconidia whose viability was $95.9 \pm 0.6\%$. Additionally, microscopic examination ($\times 100$) of adults removed from the KM plates after 18 h incubation revealed the presence of numerous macroconidia of *Fusarium* externally on various body parts of all three adult insect species (data not presented).

DISCUSSION

Our objectives were to document the acquisition of *F. avenaceum* from naturally infected plant tissue by adults of three common greenhouse insects and to evaluate pathogen dissemination and transmission to healthy plants by these insects subsequent to pathogen acquisition. Our data, obtained using an abiotic and a biotic experimental approach, support and extend our concerns that these insects function as vectors of soilborne plant pathogens. Specifically, results provide conclusive evidence that shore flies, fungus gnats, and moth flies readily acquire (externally and/or internally) macroconidia of *F. avenaceum* produced on naturally infected stems of the host plant (i.e., lisianthus) and then disseminate acquired macroconidia to healthy plants, which subsequently die, or to an abiotic substrate (i.e., KM). The high percentage of transmission, as evidenced by both the number of KM plates colonized by the pathogen (up to 68.5% within 18 h) and the number of plants infected (75% within 4 days), reflects the efficiency of these insects as vectors. Shore flies appeared to be more efficient in transmission compared with either fungus gnats or moth flies, but the differences were not statistically significant. The latter may be due to the fact that shore flies acquire the pathogen both externally and internally, whereas adult fungus gnats and moth flies are only contaminated externally with spores of the pathogen. Data to support these conclusions are based upon the origin of *F. avenaceum* colonies on KM. In the case of shore flies, colonies originated

Fig. 7. Macroconidia of *Fusarium avenaceum* in frass excreted by an adult shore fly. (Bar = 0.1 mm)

from both frass deposits (i.e., via excretion of ingested macroconidia or “internal contamination”) and solitary or clustered macroconidia (i.e., via shedding of externally borne macroconidia or “external contamination”), whereas all colonies associated with fungus gnats and moth flies originated from solitary or clustered macroconidia (i.e., external contamination). Irrespective of the method of acquisition, all three insects, in the relatively short time span of 4 days, were able to transmit acquired macroconidia to a minimum of 45% of the plants. Given the omnipresence of one or more of these insects in many commercial greenhouses, assignment of a prioritized level of significance to any one insect species would not be appropriate. Under a commercial situation, a high level of transmission will be governed by the population density of the primary insect pest(s) in the facility, the percentage of infected plants, and the inoculum density of the pathogen available for acquisition by those insects. Lack of inoculum does not appear to be a limiting factor. The population of viable macroconidia ranged from 1.1 to 1.9×10^8 per cm of naturally infected stem tissue or 6.7×10^9 per infected plant. The populations of macroco-

nidia on naturally infected lisianthus stems are consistent with those reported for other crops infected by stem-colonizing *Fusarium* spp. (3,8,11,12).

Recognition of the high probability that insects, as well as airspora, are significant factors in the epidemiology of soilborne pathogens that reproduce aboveground will necessitate the development of different and more appropriate disease management strategies than those normally employed for this group of pathogens. Roguing of infected plants prior to inoculum production is an obvious strategy that will reduce or eliminate the source of inoculum for airspora or insect acquisition. Insect control is another obvious management strategy. Various cultural, chemical, and biological methods of control are available for some but not all of these insect pests, particularly shore flies. We recently reported on the use and efficacy of *Beauveria bassiana* (an entomopathogen) on colonized millet seed as a biopesticide for the control of shore flies (14).

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