Dispersal of *Verticillium albo-atrum* by the Fungus Gnat (*Bradysia impatiens*)

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ABSTRACT

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Adult flies of *Bradysia impatiens* collected from a greenhouse or growth chamber that contained alfalfa plants affected with Verticillium wilt were contaminated 79% with propagules of the alfalfa strain of *Verticillium albo-atrum*. Individual flies washed in 1 ml of sterile distilled water yielded 0–> 100 colony-forming-units of *V. albo-atrum* on ethanol-streptomycin agar. When *Bradysia* flies either naturally or artificially infested with *V. albo-atrum* were caged on clipped, 10- to 12-wk-old plants of Iroquois alfalfa for 5–7 days, the incidence of *Verticillium*-infected plants ranged from 31 to 41%. Plants caged with *Trichoderma*-infested flies or with uninfested flies did not become infected.

Verticillium wilt of alfalfa was detected for the first time in New York State in 1981 (R. L. Millar, *unpublished*). Investigations on the disease were begun immediately and involved experiments done in greenhouses and controlled-environment chambers. On many occasions, uninoculated alfalfa plants growing in Cornell Peat-lite Mix B (1) became infected with *Verticillium albo-atrum* Reinke & Berthold and developed symptoms of Verticillium wilt. A possible explanation was suggested by observing the activities of fungus gnats (*Bradysia impatiens* Johannsen) and shore flies (*Scatella* sp.), which can be pests in greenhouses and growth chambers (2,3,9,10,13). Adult flies were observed to emerge from the potting medium, walk over necrotic stems or leaflets that had abscised from diseased plants, then fly to nearby healthy plants. Examination of the leaflets and stems often revealed sporulation of *V. albo-atrum* on necrotic tissues. On the basis of these observations, we investigated the possibility that *Bradysia* flies were serving as a vector of *V. albo-atrum*.

MATERIALS AND METHODS

Cultures of *V. albo-atrum* were maintained on prune-lactose-yeast extract agar (12) at 21 C. An isolate of *Trichoderma* sp., which was used as a control in some experiments, was maintained on potato-dextrose agar (PDA).

Adult flies of *B. impatiens* and *Scatella* sp. (used only in a preliminary experiment) were collected in the greenhouse or growth chamber by aspiration into large test tubes. The flies were used immediately or stored up to 8 hr at 5 C. Flies were anesthetized with carbon dioxide and transferred between containers by grasping a wing or leg with a fine-pointed forceps. *Bradysia* was reared in the laboratory at 21–25 C on cultures of *V. albo-atrum* or *Trichoderma* in large test tubes. Cultures consisted of prune-lactose-yeast extract agar or PDA covered with an autoclaved mixture of grass clippings and brewer's yeast (6). Because progeny of a single female are either all male or all female, populations were mixed to maximize mating and egg laying.

Plants of alfalfa cultivar Iroquois, which is susceptible to Verticillium wilt, were grown from seed and transplanted singly into Cornell Peat-Lite Mix B (1) in 7.5-cm-diameter clay pots. Plants were grown in a greenhouse free of *Verticillium* or in a chamber programmed to 20/18 C day/night, 70–80% relative humidity, and 16-hr/day photoperiod of about 18 klux fluorescent and incandescent light. Air in the chamber was monitored with an Anderson spore sampler to detect airborne *Verticillium* conidia; none was detected. When the plants were 10–12 wk old, the stems were clipped at 5–8 cm above the crown and each plant was enclosed in an insect cage. The cages were cylinders (10 cm long by 7.5 cm in diameter) made from 4-mil acetate fitted at one end by two layers of cheesecloth held by a rubber band. They were secured over the plant by pressing the open end of the cylinder into the Peat-lite mix. Diazinon (1.2 g/L, 50WP) was applied as a soil drench at about 20 ml per pot to obtain insect-free plants and for particular treatments to eliminate all stages of the flies at appropriate intervals after infesting the plants. Each pot was placed in a container that was used to subirrigate the plants and thereby minimize the possibility of splash dispersal of inoculum. Plants infected with *V. albo-atrum* were identified by excising the stems, disinfesting them in 0.5% NaOCl for 2 min, and plating a 2-cm section from the base on ethanol-streptomycin agar (ESA) (11). The plates were kept at 21–25 C and observed for growth of *V. albo-atrum*.

RESULTS

Natural infestation of flies. In a preliminary experiment, 20 each of fungus gnats and shore flies were collected from a growth chamber that contained alfalfa plants infected with *V. albo-atrum*. Individual flies were added to test tubes containing 1 ml of sterile distilled water, the tubes were agitated for 30 sec on a Vortex-Genie (Scientific Industries, Inc., Springfield, MA), and the liquid was poured over the surface of ESA in a petri plate. The plates were kept 7–10 days at 21–25 C and examined for colonies of *V. albo-atrum*. Both fungus gnats (79%) and shore flies (50%) were contaminated with the pathogen. Because shore flies feed on algae (9) whereas fungus gnats feed on fungi, plant parts, and residues and have been reported to cause damage on shallow-rooted crops and seedlings (7,8), only fungus gnats were investigated in additional experiments.

In a second experiment, two lots of 100 *Bradysia* flies were collected from a chamber that contained plants affected with Verticillium wilt. Individual flies were washed and the washings added to plates of ESA. Recovery of *V. albo-atrum* was 33 and 51%. The number of colonies obtained per fly for each lot, respectively, was: 0 colonies, 67 and 49 flies; 1–5 colonies, 19 and 23 flies; 6–10 colonies, 4 and 8 flies; 11–20 colonies, 3 and 6 flies; 21–50 colonies, 2 and 8 flies; 51–100 colonies, 4 and 5 flies; and more than 100 colonies, 1 and 1 flies.

To determine if *Bradysia* could serve as a vector, adult flies were collected from growth chambers containing alfalfa plants infected with *V. albo-atrum*. Based on a sample of 40 flies, 75% were contaminated with the pathogen. Groups of 12–15 flies were shaken from a test tube onto the surface of a petri dish lid and transferred to a healthy alfalfa plant that had been clipped and then caged. Flies were collected and transferred to individual plants over a 10-day period. One hundred plants received flies presumed to be infested with *V. albo-atrum*; 10 plants that received no flies and 10 plants that

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received flies from a greenhouse free of plants affected with Verticillium wilt served as controls. A sample of the flies used for the control plants was checked for contamination; none was detected. After 5–7 days, the plants were treated with diazinon and the cages were removed; 4 wk later, each plant was assayed for infection. For the treatment with flies presumed to be infested with the pathogen, 31% were infected; none of the controls was infected.

Artificial infestation of flies. Adult flies were collected from plants grown in a greenhouse or growth chamber and added to fungal cultures of *V. albo-atrum* or *Trichoderma* sp. After the number had increased over several generations, adult flies were anesthetized and transferred to 2-wk-old cultures of *V. albo-atrum* or *Trichoderma* with profuse sporulation. After 16–24 hr, the flies were anesthetized and 10–12 flies were transferred individually to healthy alfalfa plants that had been clipped 2, 8, 24, or 48 hr before receiving the flies. Based on a sample of 10 flies, 100% were contaminated with *V. albo-atrum* and colony-forming units averaged 7,700 per fly. Eighty plants received *Verticillium*-contaminated flies; 10 plants that received no flies but otherwise were treated similarly served as controls. Plants were caged and treated with diazinon after 5–7 days. When the plants had grown for 5 wk, stems from each plant were assayed for *V. albo-atrum*.

The numbers of plants infected with *V. albo-atrum* were 32 of 80, 0 of 10, and 0 of 10 for plants that received *V. albo-atrum*-infested, *Trichoderma*-infested, or no flies, respectively. For a second trial of the experiment, the results were 31 of 100, 0 of 10, and 0 of 10, respectively. The length of time between cutting the plants and infesting them with flies did not affect the number of infected plants (7 of 29, 7 of 20, 9 of 24, and 8 of 27 infected plants for plants cut 2, 8, 24, or 48 hr, respectively, before receiving the flies).

DISCUSSION

The fungus gnat (*Bradysia* sp.) has been reported to cause damage of economic importance to plants grown both in the greenhouse and the field (2,3,10,13). Moreover, Leath and Newton (9) determined that larval feeding, when severe, could cause death of alfalfa seedlings, and when less severe, could predispose seedlings to invasion and death from *Fusarium oxysporum* Schlect. emend. Snyd. & Hans. f. sp. *medicaginis*. However, that *Bradysia* might also be important as a dispersal agent and vector for fungal pathogens apparently has not been reported previously.

Harper and Huang (4) and Huang et al (5) have determined that several other insects may be important for dispersal of the alfalfa strain of *V. albo-atrum* and that some that are sucking and biting and chewing insects could serve as vectors; presumably by injuring the leaves, they facilitate ingress into the vascular elements. *Bradysia* larvae, which feed on fungi, fibrous roots, and organic matter, usually inhabit the top 2 cm of soil (6), but under favorable conditions, they may also feed on stems and leaves (9). They, therefore, may serve both to disperse *V. albo-atrum* and facilitate *V. albo-atrum* colonization of the potting medium and to provide ample access to vascular tissue, thereby facilitating systemic invasion of the plants. Ingress by *V. albo-atrum* could have occurred via feeding injuries to roots, stems, or leaves or via the cut surfaces of stems clipped before infesting the plants. However, we have found (*unpublished*) that the receptiveness of freshly cut stems to invasion by *V. albo-atrum* decreases sharply within minutes after cutting.

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