Role of the Leafcutter Bee in Dissemination of *Verticillium albo-atrum* in Alfalfa

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ABSTRACT

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An investigation of the role of the alfalfa leafcutter bee (*Megachile rotundata*) as a dispersal agent for *Verticillium albo-atrum* was carried out in a commercial field of alfalfa known to have Verticillium wilt. Results showed that the bees use leaf pieces from both healthy and diseased plants to construct brood cells. Isolations from 69 female bees foraging in the field showed that 30% of the bees were contaminated with *V. albo-atrum*. Conidia were present in cuticle depressions of the abdomen, around the mouth, and on various other parts of the body. Alfalfa pollen grains covered with an amorphous mucilaginous substance were often adherent in these locations. On some bees, the conidia had germinated and developed into hyphal mats intermingled with pollen grains. Many of the pollen grains were colonized by hyphae. *V. albo-atrum* was isolated from remnant stigmata and styles of seed pods harvested from plants near the hive. Incidence of infected pods was 1.7% from plants with symptoms and 2.1% from symptomless plants. The alfalfa leafcutter bee is potentially an important agent for dissemination of *V. albo-atrum* because it utilizes diseased leaf tissue for making brood cells, carries the pathogen on its body, and transports the pathogen to infection courts such as pollen grains and stigmata of alfalfa flowers.

Alfalfa (*Medicago sativa* L.) seed production is a rapidly expanding industry in Canada. The area of pedigreed alfalfa seed production increased from 2,270 ha in 1976 to 13,011 ha in 1982 (11). Of the total production area in 1982, 93% was located in the prairie provinces of Alberta, Saskatchewan, and Manitoba (11).

The rapid expansion of the alfalfa seed industry in Canada is attributable to the use of the alfalfa leafcutter bee, *Megachile rotundata* (Fabricius), as a pollinator. Up to 50,000 bees per hectare are used to pollinate irrigated alfalfa (3,12). Production of this domesticated bee, which is of Eurasian origin, has also become an important agricultural industry in western Canada.

Verticillium wilt of alfalfa, caused by *Verticillium albo-atrum* Reinke & Berthold, has recently become a serious problem for alfalfa producers in British Columbia, Alberta, and Ontario in Canada (1,13). Previous studies in a commercial field of alfalfa near Lethbridge, Alberta, indicated that nine species of alfalfa pests and six species of predatory insects served as agents for dispersing conidia of *V. albo-atrum* (4,7). The pea aphid (*Acyrthosiphon pisum* Harris) (7) and the alfalfa weevil (*Hypera postica* Gyllenhal) (4) were proven effective as vectors for spores that caused Verticillium wilt in alfalfa plants. Moreover, leaf-chewing insects such as grasshoppers and alfalfa weevils can spread the pathogen via their feces (6).

Results of a field study in 1982 revealed that alfalfa leaf pieces cut by leafcutter bees and transported to the hives for use in the construction of cells, into which pollen and nectar are placed and young are reared, were frequently contaminated with *V. albo-atrum* (9). A small percentage of the leaf pieces may have originated from infected leaves because surface sterilization in 70% ethanol for 90 sec was ineffective in preventing isolation of the pathogen from the tissue. Studies on the mechanisms of seed-borne *V. albo-atrum* in alfalfa indicate that alfalfa pollen on the stigma of alfalfa flowers (5) or on agar media (8) is susceptible to infection by the pathogen. This suggests that the alfalfa leafcutter bee might be an important agent for dissemination of *V. albo-atrum* because it trips alfalfa flowers to gather pollen and nectar and cuts pieces from alfalfa leaves to make bee cells.

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This paper reports the results of field investigations on the role of leafcutter bees in dissemination, inoculation, and preservation of the Verticillium-wilt pathogen of alfalfa.

MATERIALS AND METHODS

Experiments were carried out in 1983 in 0.8 ha of a commercial field used previously to study the relationships between insects and Verticillium wilt of alfalfa (4,7,9). The alfalfa cultivar Chimo was irrigated by a wheel-move system. A leafcutter bee hive was installed above the canopy near the edge of the field in mid-June prior to the beginning of the bloom stage. About 1,000 adult leafcutter bees were released into the field in early July.

Forty-two leaflets from which leaf pieces had been cut and having V-shaped lesions, a typical symptom of Verticillium wilt (Fig. 1), were collected from plants within a 10-m radius of the hive

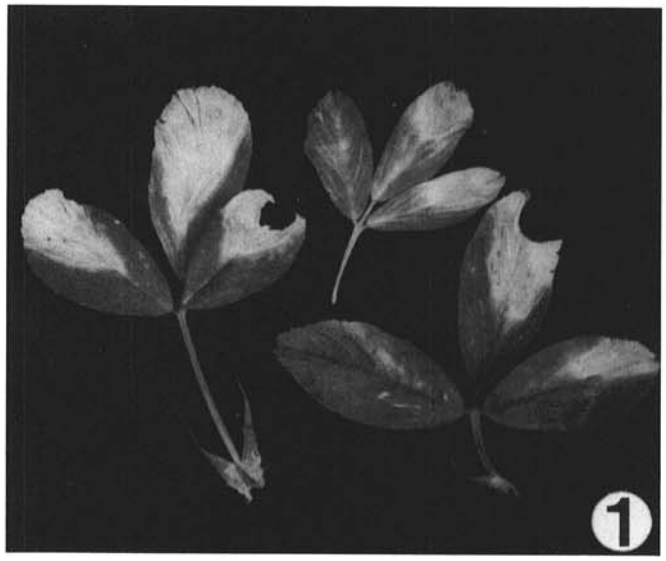
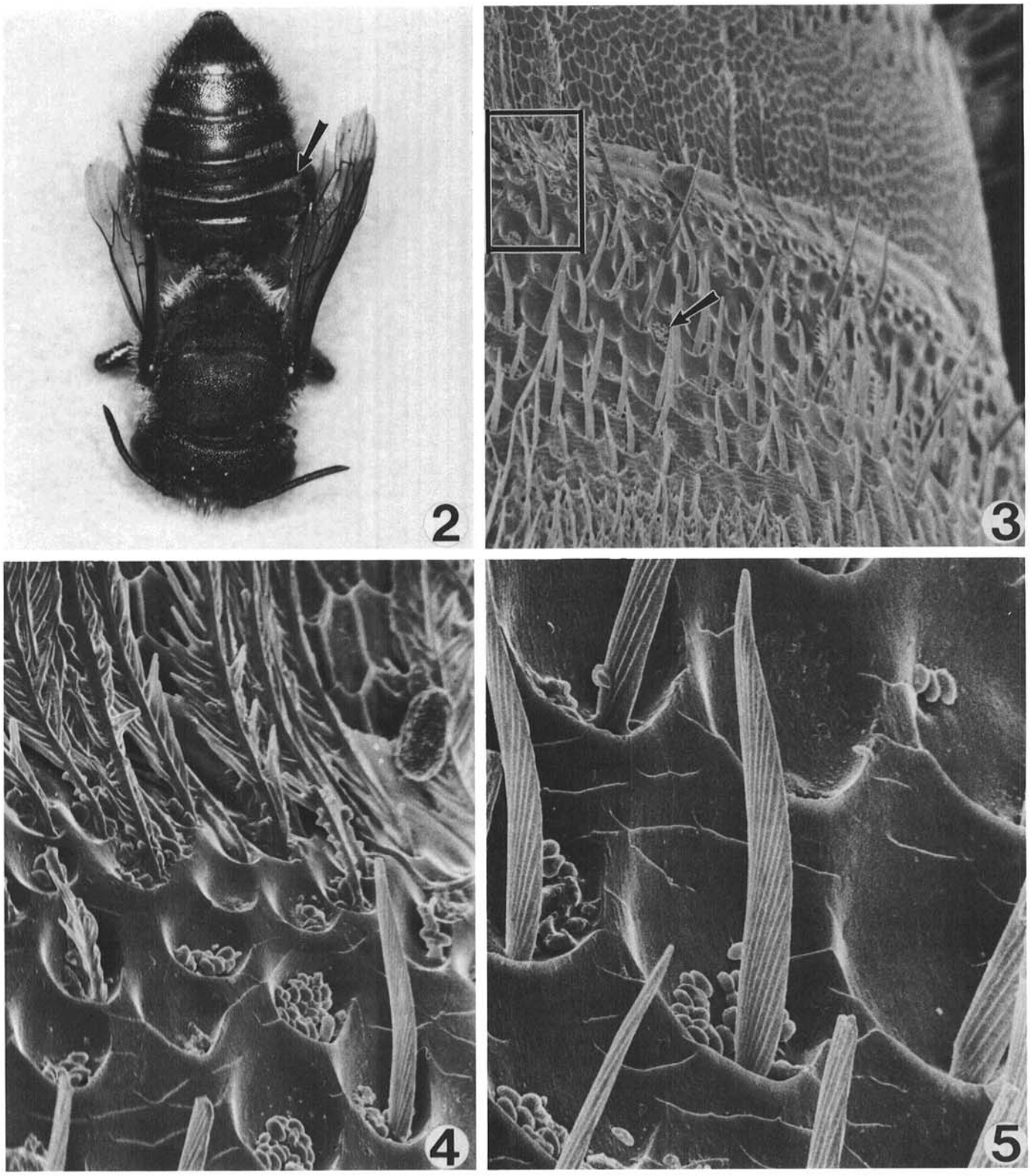


Fig. 1. Brown V-shaped lesions in alfalfa leaflets infected by *Verticillium albo-atrum*. The two semicircular excised areas are injuries caused by leafcutter bees. (×1.5).

to determine whether the leafcutter bee would cut diseased leaf pieces for making bee cells. Each leaflet was surface-sterilized in 70% ethanol for 90 sec, placed on a selective medium (2) in petri dishes, incubated at room temperature for 7 days, and examined for presence of *V. albo-atrum* under a stereomicroscope.

In mid-August, 81 female bees were collected individually in vials. Sixty-nine of the bees were killed, placed on the selective medium, incubated at room temperature for 10 days, and examined for *V. albo-atrum* by using a stereoscopic light microscope. The remaining 12 bees were processed for observation with scanning electron microscopy (SEM). These specimens were air-dried, mounted on aluminum stubs with colloidal silver paste and sputter-



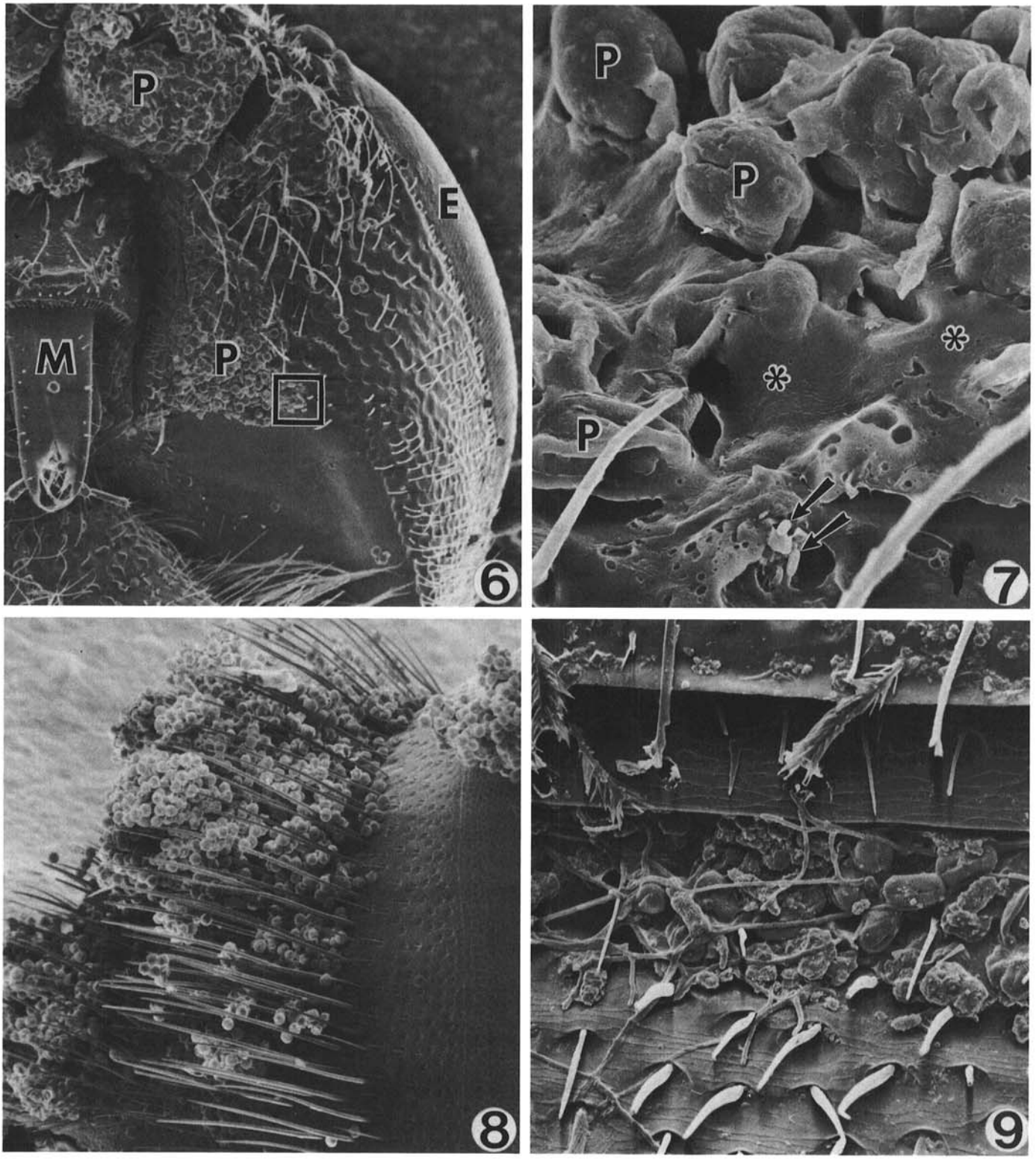
Figs. 2–5. Conidia of *Verticillium albo-atrum* on the dorsal side of a female leafcutter bee. **2,** Photomicrograph (×10) indicating the area of interest (arrow) enlarged in Fig. 3. **3–5,** Scanning electron micrographs showing alveoli of the cuticle filled with conidia (arrow) at low magnification (Fig. 3) and high magnifications (Figs. 4 and 5). Fig. 4 represents the inset area in Fig. 3. Note the spinelike and featherlike setae attached in the alveoli (Fig. 4). Magnifications: Fig. 3, ×260; Fig. 4, ×940; and Fig. 5, ×1,600.

coated with gold (15 nm thick) for examination and photography in a Hitachi S-500 scanning electron microscope.

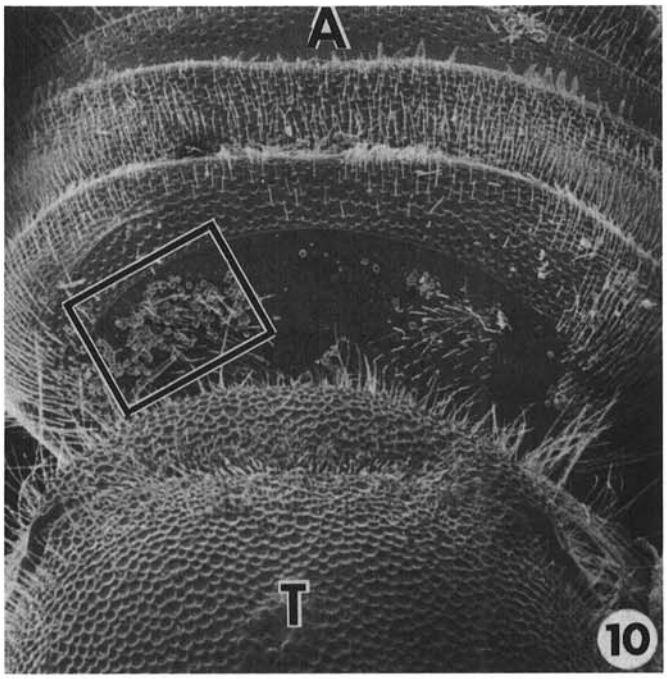
Alfalfa seed pods from plants with or without symptoms of Verticillium wilt were collected within a 12-m radius of the hive in mid-August. Isolation of *V. albo-atrum* from remnant stigma and style tissue on each pod was made by the same technique used for detecting the pathogen in leaflets.

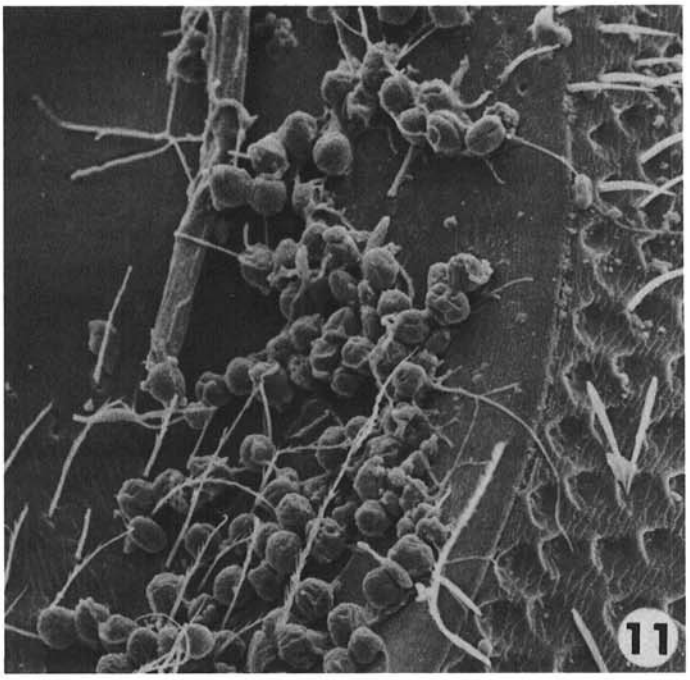
RESULTS

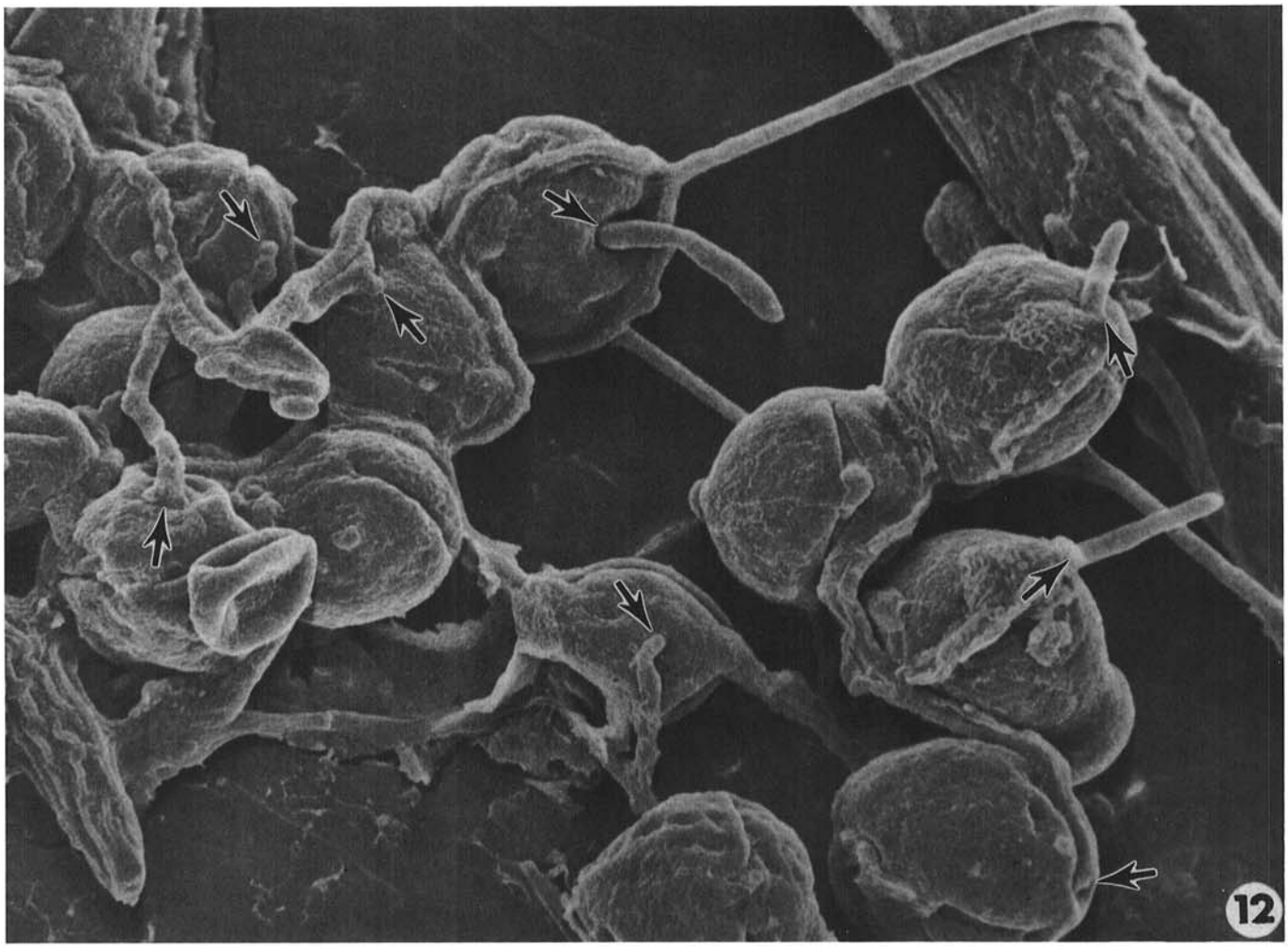
The results from surveying 10 random 1-m^2 locations in the field in early August showed that an average of 9% of the alfalfa plants were infected by *V. albo-atrum*. The diseased plants were often stunted and many of the leaflets showed typical V-shaped lesions. The leafcutter bees released in the field cut healthy as well as



Figs. 6–9. Scanning electron micrographs showing alfalfa pollen aggregates on **6 and 7,** the head; **8,** the abdominal sternites (especially the scopa); and **9,** the membrane between abdominal segments of a leafcutter bee. Fig. 7 is the enlargement of the inset area in Fig. 6. Note two conidia of *Verticillium albo-atrum* (arrows) on the mucilaginous matrix (star) which covers most of the pollen (P) (Fig. 7). Note also the development of a mycelial mat on the intersegmental membrane of the dorsal abdominal segments (Fig. 9). M = maxillae and E = eye. Magnifications: Fig. 6, ×65; Fig. 7, ×1,100; Fig. 8, ×80; and Fig. 9, ×440.







Figs. 10–12. Scanning electronic micrographs showing alfalfa pollen aggregates and hyphae of *Verticillium albo-atrum* on a leafcutter bee. **10,** Alfalfa pollen trapped on the propodeum, on the thorax (T), and on the first abdominal terga (A) of a bee; **11,** fungal hyphae intermingled with pollen; and **12,** pollen grains invaded by the hyphae. Figs. 11 and 12 are enlargements of the inset area in Fig. 10. Note the hyphae have either penetrated pollen grains (Fig. 12, arrows at lower left) or emerged from pollen grains (Fig. 12, arrows at upper right). Magnifications: Fig. 10, ×35; Fig. 11, ×220; and Fig. 12, ×1,250.

diseased leaves by removing a characteristic semi-circular portion of the leaflet (Fig. 1). Leaf-pieces were transported to the hive for the making of bee cells. Often the bee-injured area was in the brown, V-shaped lesion of an infected leaflet. The results of isolation showed that all the 42 field-collected leaflets with V-shaped lesions and signs of bee injury were infected by *V. albo-atrum*.

V. albo-atrum was detected on 21 of the 69 female leafcutter bees collected from the field. Typical verticillate conidiophores bearing spore droplets were found on various parts of the insect body, most frequently the mouth, abdomen, and legs. Other fungi such as *Rhizopus* spp. and *Penicillium* spp. also were observed on some of the field-collected insects.

The bodies of 12 female leafcutter bees that were examined under SEM were covered with setal sensilla (Fig. 2). The setae varied morphologically. For example, spinelike setae with fine spindle grooves (Figs. 3 to 5) were found on the more basal terga, but hairs were featherlike or plumose on the more distal terga (Fig. 4) of the abdominal segments. Each seta was attached in a shallow, oval-shaped cuticle depression known as a setal alveolus. The punctation of the abdominal terga varied from fine and close on the more basal terga to quite deep, distinct, and well separated on the more distal terga. Single-celled conidia of *V. albo-atrum*, 4-12 × 2.5-5 μm, were deposited in some of the alveoli of the tergum (Figs. 2 to 5). They were often arranged in a single layer behind the seta (Figs. 4 and 5). Conidia also were trapped on other parts of the body including the mouth (Figs. 6 and 7), legs, propodeum, first abdominal terga, and membranes between abdominal segments. Alfalfa pollen grains coated the bee, particularly near the mouth (Figs. 6 and 7), abdomen (especially the scopa [Fig. 8]), legs, intersegmental membranes (Fig. 9), and the propodeum and first abdominal terga (Fig. 10). Most of the pollen grains were aggregated and often covered with an amorphous mucilaginous substance (Fig. 7). Some of the pollen grains on the bee had germinated and produced pollen tubes about 4–5 μm in diameter. Conidia of *V. albo-atrum* on the bee also germinated frequently and produced hyphae which intermingled with the pollen grains on various sclerites of the bee body (Figs. 9 to 11). The hyphae infected pollen grains by direct penetration. Signs of the hyphae penetrating a pollen grain or emerging from a pollen grain, a phenomenon observed in the previous study in vitro (8), were common on the bee (Fig. 12).

V. albo-atrum was isolated from remnant stigma and stylar tissue of the seed pods harvested from plants within 12 m of the hive. However, the frequency of infected stylar tissue was low. Only 1.7% of the 1,427 pods collected from plants with symptoms and 2.1% of the 2,684 pods from the symptomless plants were infected by the pathogen.

DISCUSSION

This study reveals that alfalfa leafcutter bees are a potential vector of Verticillium wilt of alfalfa because they use infected leaf pieces for making the brood cells, carry conidia of *V. albo-atrum* on their bodies, and collect alfalfa pollen grains which provide an infection court for the pathogen.

Huang and Richards (9) observed a high frequency of contamination with *V. albo-atrum* of leaf pieces collected from the bee hives in a diseased field. They speculated that a small percentage (0.3-9%) of these leaf pieces may have originated from diseased leaves as they were unable to destroy the pathogen by surface sterilization. The hypothesis that the leafcutter bee will cut leaf pieces from diseased tissue is confirmed by the present study. Bee cells composed of such diseased leaf pieces may be of particular significance in long-distance dispersal of the pathogen via the trade of leafcutter bees within a country or between countries.

V. albo-atrum in the stylar tissue of the seed pods from both diseased and healthy plants probably resulted from inoculation of the stigma during flower-tripping by the pathogen-contaminated bees. This phenomenon is confirmed in a further greenhouse study with leafcutter bees artificially infested with spores of *V. albo-atrum* and allowed to forage on healthy alfalfa plants (H. C. Huang, K. W. Richards, and M. R. Hanna, *unpublished*). Huang et al (5) demonstrated that the pathogen in the style could spread further into the pod and the seed coat if the maturing pods were exposed to high humidity. Even though the frequency of infection was low in this field, seed pods with infected styles could still be a significant source of seed-borne *V. albo-atrum*.

The presence of numerous conidia of *V. albo-atrum* on the bees (Figs. 4 and 5) suggests that field conditions were conducive to both production and release of spores of the pathogen from diseased plants. One of the conducive factors may have been high humidity in the field because of irrigation. High humidity may accelerate the decomposition of diseased tissue and, in consequence, increase the production of conidia, thereby increasing the chance of the spores being trapped on insects, including leafcutter bees. In addition, irrigation may also create an ideal environment for germination of the conidia on insect bodies. Also, some parts of the bee's body, namely the propodeum and the first abdominal terga, tend to accumulate conidia and pollen as these areas are infrequently groomed when the pollen is manipulated by the bees (10). Because pollen grains of alfalfa are highly susceptible to *V. albo-atrum* (8), the growing hyphae of the pathogen can readily attack the pollen gathered by the bee (Fig. 12). The effectiveness of leafcutter bees contaminated with *V. albo-atrum* as pollinators may be reduced because some of the pollen grains they transport have been destroyed by the pathogen. If a high percentage of the bee population is contaminated by the pathogen and the conidia remain on the bee long enough to germinate and penetrate the pollen, then the alfalfa seed yield may be reduced as a result of poor pollination efficiency. Leafcutter bees can also transport the pathogen to the stigma during the flower-tripping process, either as conidia or as mycelia in infected pollen grains. This may result in the infection of stigma and style and aid in the introduction of the pathogen to alfalfa seeds (5).

Based on this and the previous study (9), it is concluded that the alfalfa leafcutter bee is potentially an important agent for dissemination of *V. albo-atrum* via bee cells transported in commerce and is a vector for transporting the pathogen to the infection courts such as pollen grains and stigma. To minimize the danger of spreading this important disease, alfalfa producers should avoid using fields infested with *V. albo-atrum* for alfalfa seed production.

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