

Survival of *Verticillium albo-atrum* from Alfalfa in Feces of Leaf-Chewing Insects

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ABSTRACT

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Alfalfa leaves infected with *Verticillium albo-atrum* were fed to leaf-chewing insects—grasshoppers (*Melanoplus sanguinipes* and *M. bivittatus*), alfalfa weevil (*Hypera postica*), and woolly bear (*Apantesis blakei*)—to determine survival of the pathogen after passage through their digestive tracts. *V. albo-atrum* survived in the digestive tracts of all tested species, usually first appearing in the feces 1 day after feeding. The percentage of feces contaminated by *V. albo-atrum* varied among individuals within species and was related to the duration of feeding on diseased tissues. The average percentage of contaminated feces in adult *M. sanguinipes* was less than 14% after 1 day of feeding the insects with diseased tissue but increased to 49% after 3 days of feeding. *V. albo-atrum*

was eliminated from the digestive tracts of grasshoppers by changing the diet to uninfected alfalfa leaves. The time required for complete removal of the pathogen appeared to be related to the level of contamination in digestive tracts, averaging 1.6 days for individuals with less than 25% of pathogen-contaminated feces and 6.1 days for those with more than 75% of contaminated feces in their digestive tracts. When grasshopper feces contaminated with *V. albo-atrum* were buried near roots of alfalfa seedlings, 20.8 and 13% of plants became infected and developed wilt symptoms after 6 wk in experiments 1 and 2, respectively. The role of leaf-chewing insects in the dissemination of *V. albo-atrum* in alfalfa and other crops is discussed.

Verticillium wilt of alfalfa (*Medicago sativa* L.), which is caused by *V. albo-atrum* Reinke & Berthold, is a destructive disease in Europe (13,18) and North America (2,9,19). Infection of alfalfa by *V. albo-atrum* results in drastic reductions in yield (8,16), life of stands (3,6,13), and quality (20). Although the disease is still relatively new on this continent, it has already become a serious problem for alfalfa production in British Columbia, Alberta, and Ontario, Canada (2,19) and in many northern states of the United States (9,17).

Conidia of *V. albo-atrum* have been found in diseased alfalfa fields, both on infected stems (11,13) and in the air (7,15). Production of conidia is apparently dependent upon the stage of the disease development, as sporulation is much heavier on stems that have been infected for some time than on newly infected ones (11). Recent investigations in an irrigated alfalfa hay field naturally infested with *V. albo-atrum* revealed that numerous species of insects including pests, predators (10,11), and pollinators (12) are important dispersal agents for spores of the pathogen. Pests such as pea aphids (11) and alfalfa weevils (10) were proven to be effective vectors capable of transmitting viable, disease-inducing spores from diseased stems to healthy plants.

Previous studies indicated that the fungus *V. albo-atrum* can be isolated readily from freshly infected alfalfa stems (6,17), petioles, and leaflets (6). However, production of spores from newly infected leaflets with typical V-shaped lesions was not observed in the field or in the greenhouse. Leaf-chewing insects such as grasshoppers and alfalfa weevils are important pests of alfalfa and cause extensive damage to leaves. There are no reports on the dissemination of *V. albo-atrum* or other species of *Verticillium* via feces of insects, although this phenomenon has been documented with other fungal pathogens such as *Sclerotium rolfsii* Sacc. in feces of cockroaches (1) and *Phytophthora palmivora* (Butl.) Butler in feces of snails (14).

This study was undertaken to investigate the role of the leaf-chewing insects in the dissemination of *V. albo-atrum* from infected alfalfa leaves.

MATERIALS AND METHODS

Alfalfa plants, cultivar Vernal, were grown in Cornell Mix (4) in pots for 8 wk in a greenhouse. They were inoculated with a Canadian strain of *V. albo-atrum* (LAWS-82-055) from alfalfa by soaking the roots in a suspension containing 3.8×10^6 spores per milliliter. Most of the plants developed wilt symptoms within 6 wk. Plants with at least one leaflet with wedged lesions were used for insect feeding tests in the laboratory.

Insects, including the migratory grasshopper (*Melanoplus sanguinipes* Fabricius), the two-striped grasshopper (*Melanoplus bivittatus* Say), the alfalfa weevil (*Hypera postica* Gyllenhal), and the woolly bear larva (*Apantesis blakei* Grote), either collected from a field near Lethbridge or reared in the laboratory, were fed with healthy alfalfa leaves for 1 day and then with diseased leaves for 3 days in petri dishes. Feces were collected daily and plated directly on a selective medium (5). The plates were incubated at room temperature for 3–7 days and the feces were examined for *V. albo-atrum* by using a stereomicroscope. Feces from insects fed with healthy alfalfa leaves were used as a control. During the feeding period, the diseased leaves in petri dishes were examined microscopically for production of conidia.

In another experiment, the grasshopper, *M. sanguinipes*, was used to study how long *V. albo-atrum* survives in the digestive tract. This was done by feeding 43 insects with diseased leaves for 3 or 6 days and then with healthy leaves for 8 days. Feces were collected daily and assessed for contamination with *V. albo-atrum* by using the technique described above. Feces collected prior to the feeding schedule were also examined for presence of the pathogen.

To determine whether the feces contaminated with *V. albo-atrum* could serve as primary inoculum for the disease, grasshopper feces collected during a 10-day period of feeding on diseased leaves were pooled and stored in a paper bag. Feces from grasshoppers feeding on healthy leaves were used as a control. Four-week-old alfalfa seedlings, cultivar Grimm, grown in Cornell Mix in individual cells of the Roottrainer Books (Spencer-Lemaire Industries Ltd., Edmonton, Alta), one seedling per cell, were used for inoculation. Inoculation was done in the greenhouse by burying 10 fecal pellets at a depth of 1–2 cm near the tap root of each seedling. Treatments were: feces contaminated with *V. albo-atrum*, uncontaminated feces, and the control (no feces). There were three replicates of each treatment and 32 seedlings in each replicate. Wilt symptoms appeared on some plants about 4 wk after inoculation, but isolation of the pathogen from each plant was made at 6 wk

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TABLE 1. Survival of *Verticillium albo-atrum* after passage through the digestive tracts of leaf-chewing insects

Insect ^a	Stage	No. tested	Fecal pellets with <i>V. albo-atrum</i> (%)			
			Pre-feeding	Day 1 ^b	Day 2	Day 3
				Range (mean)	Range (mean)	Range (mean)
<i>Melanoplus sanguinipes</i>	Adult	48	0	0-50 (13.5)	0-100 (34.1)	0-100 (49.0)
	Nymph	13	0	0-45 (20.6)	26-92 (48.6)	54-100 (64.8)
<i>M. bivittatus</i>	Nymph	6	0	46-60 (53.0)	53-78 (65.5)	89-100 (94.5)
<i>Hypera postica</i>	Adult	48	0	0-38 (12.8)	0-17 (4.6)	0-35 (7.8)
	Larva	40	0	0-96 (39.1)	0-84 (32.8)	1-82 (46.8) ^c
<i>Apantesis blakei</i>	Larva	6	0	0-11 (5.5)	6-83 (45.0)	0-100 (61.0)

^aNo *V. albo-atrum* was detected in fecal pellets from the control insects fed with healthy alfalfa leaves.

^bFeces collected 1 day after feeding the insects with diseased alfalfa leaves.

^cBased on the results from 25 larvae due to pupation of 15 larvae.

after inoculation. For isolation, stem segments from each plant were surface sterilized in 70% ethanol for 90 sec, plated on selective media in petri dishes, incubated at room temperature for 1 wk, and then examined under a stereomicroscope for the presence of *V. albo-atrum*. The experiment was repeated in the same greenhouse with feces collected from another grasshopper feeding trial. The same technique as above was applied, but the level of inoculum was 12 fecal pellets per seedling. A subsample of 100 fecal pellets from each of the experiments was examined by using the plating technique described previously and the frequency of feces contaminated with *V. albo-atrum* from the feeding with diseased tissue was 71 and 25% for experiments 1 and 2, respectively.

RESULTS

All four species of insects ate both the green and chlorotic tissues of diseased alfalfa leaves. Grasshoppers ate more tissue than did alfalfa weevils or woolly bears. Some grasshoppers ate half of a diseased leaflet within 3 min. The number of fecal pellets produced each day varied with individuals, ranging from 11 to 58 and 6 to 17 for grasshopper adults and nymphs, respectively; from 8 to 21 and 3 to 31 for alfalfa weevil adults and larvae; and from 3 to 17 for woolly bear larvae.

V. albo-atrum was detected in feces of all test species of insects fed with diseased alfalfa leaves (Table 1) but not in feces from the insects fed with healthy tissue. The pathogen appeared in the fecal pellets within 1 day after feeding, but the frequency generally remained low. The frequency of feces contaminated with *V. albo-atrum* increased in most of the samples collected on the second and third day of feeding (Table 1). In some individuals, all the feces collected after the third day of feeding with diseased tissue were contaminated with *V. albo-atrum*.

Viable *V. albo-atrum* in insect feces were identified easily by the characteristic verticillate conidiophores bearing spore droplets which formed after 3-7 days of incubation on the selective medium. The distribution of conidiophores on the feces was also a good criterion for comparing the difference between the time of feeding with diseased tissue and the extent of contamination of feces by the pathogen. *V. albo-atrum* was generally localized in the feces collected 1 day after feeding, but it heavily colonized those samples collected 2 or 3 days later. No sporulation on infected leaflets was observed during the 3-day feeding period.

The persistence of *V. albo-atrum* in the digestive tract of grasshoppers was affected by the type of material consumed. Of the 43 adults of *M. sanguinipes* fed with diseased alfalfa leaves, the frequency of *V. albo-atrum* in feces collected at the third or sixth day varied from less than 25% in 14 individuals to more than 75% in nine individuals (Table 2). When the diseased alfalfa leaves were replaced with healthy leaves, the level of feces contaminated with *V. albo-atrum* decreased in most of the tested individuals within 1 day after changing the diet. However, the time required to completely eliminate the pathogen from the digestive tract appeared to be associated with the original level of contamination in each individual. For instance, an average of 1.6 days of feeding on

TABLE 2. Persistence of *Verticillium albo-atrum* in the digestive tracts of adult *Melanoplus sanguinipes*

Feces with <i>V. albo-atrum</i> ^a (%)	No. of insects ^b	Disappearance of pathogen from digestive tracts (days) ^c	
		Range	Mean
<25	14	1-5	(1.6)
26-50	10	1-4	(1.9)
51-75	10	1-9	(3.5)
>76	9	3-9	(6.1)

^aFeces collected one day before changing the diet from diseased to healthy alfalfa.

^bCombined results from two experiments with 19 and 24 insects for experiments 1 and 2, respectively.

^cDays after changing the diet from diseased to healthy alfalfa.

healthy alfalfa was required to eliminate the *V. albo-atrum* from the digestive tract if the original contamination rate was below 25%; but an average of 6.1 days was required if the contamination rate was over 75% (Table 2).

When the grasshopper feces contaminated with *V. albo-atrum* were buried near alfalfa roots, wilt symptoms developed on leaflets about 4 wk after inoculation. Isolations from the stems 6 wk after inoculation showed that the frequency of diseased plants was 20.8 and 13% for experiments 1 and 2, respectively. No *Verticillium* wilt was found in plants inoculated with feces from insects fed healthy tissue or in the uninoculated control.

DISCUSSION

Previous reports indicate that alfalfa pests and predatory insects (10,11) are effective agents for spreading spores of *V. albo-atrum* in diseased alfalfa fields. This study demonstrates that where field conditions are not conducive to spore production, the pathogen can be transmitted via the feces of leaf-chewing insects, particularly grasshoppers. Therefore, leaf-chewing insects are significant to the epidemiology of *Verticillium* wilt in alfalfa and perhaps also to many vascular wilt diseases in other crops because they may carry the pathogen both externally on their bodies (10) and internally in feces in their intestinal tracts.

Laboratory studies show that *V. albo-atrum* is detected in feces within one day of feeding the insects with diseased leaves. Whether the viable propagules of the pathogen in the feces are resting mycelia or other forms, such as thin-walled hyphae and conidia, remains unknown. Nevertheless, the contaminated feces can serve as potential sources of inoculum for new infections in alfalfa plants. Because grasshoppers are highly mobile, dissemination of *V. albo-atrum* by this insect would be effective both within and between fields.

Despite the same source of infected leaflets used for feeding, there was a difference in frequency of feces contaminated with *V. albo-atrum* among individual insects (Tables 1, 2). Such a difference may have resulted from different feeding behaviors as well as the amount of tissue consumed by the insect. An alfalfa

leaflet may be infected with *V. albo-atrum* showing a V-shaped lesion at the leaf tip. However, the distribution of the pathogen is restricted to the midrib and the chlorotic lesion (H. C. Huang, unpublished). Unless the insect is feeding on chlorotic tissue, midribs, and veins of diseased leaflets, the chance of *V. albo-atrum* being carried in digestive tracts is very unlikely.

Grasshoppers attack many important crops. The possibility for this insect to serve as a vector of *V. albo-atrum* and perhaps *V. dahliae* on other crops warrants further investigation.

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