# Association of the Asparagus Miner with Stem Rot Caused in Asparagus by *Fusarium* species

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## ABSTRACT

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The relationship between the asparagus stem rot pathogens *Fusarium moniliforme* and *F. oxysporum* f. sp. *asparagi* and the asparagus miner (*Ophiomyia simplex*) was investigated. Feeding by larvae of *O. simplex* resulted in extensive stem mining of asparagus, leading to increased stem rot, primarily caused by *F. moniliforme*. Inoculum of *F. moniliforme* increased dramatically when the fungus sporulated on dead and dying epidermal and cortical tissues damaged by larval feeding. Fusaria were associated with all life stages of *O. simplex*. Successful management of Fusarium stem rot of asparagus must also include management of *O. simplex*.

*Additional key words*: *Fusarium solani*, *F. tricinctum*, insect dissemination.

Production of asparagus (*Asparagus officinalis* L.) in Massachusetts has declined by more than 50% in the last 15 yr due to crown and stem rot caused by *Fusarium moniliforme* Sheldon and *F. oxysporum* f. sp. *asparagi* (Cohen) (7). *F. moniliforme* is the most common species involved in stem rot because its inoculum is both airborne and waterborne (7,8,11).

We have observed for several years that stem mining by larvae of the asparagus miner *Ophiomyia simplex* Loew is frequently associated with extensive Fusarium stem rot. The adult is a small, dark fly, 2–3 mm long. Females oviposit in stem bases, either near or just above the soil surface. Larvae of *O. simplex* (white maggots up to 5 mm long) feed exclusively in asparagus stems, creating raised multiple mines and destroying cortical tissue as they tunnel up and down beneath the epidermis. New mines are pale green, whereas older mines are brown and cracked due to death of epidermal tissue. Margins of older mines are reddish brown and are similar in appearance to stem lesions caused by *F. moniliforme*. Two generations of *O. simplex* are possible, with larvae from the second generation overwintering as pupae in stem tissue (5).

*O. simplex* was discounted as a pest in Washington by Eichmann (4), who concluded that cortical destruction caused by larval mining did not affect the basic physiological functions of the plant. Cohen and Heald (1) suggested that *O. simplex* predisposed stems to infection by soilborne *Fusarium* spp. in Washington. Van Bakel and Bethe (14) found more *F.o.* f. sp. *asparagi* on mined than on unmined stems in Germany. The objectives of our study were to determine if pathogenic isolates of *F. moniliforme* and *F.o.* f. sp. *asparagi* were associated with stem mines and insect life stages of *O. simplex*, and whether adults of *O. simplex* could transmit *Fusarium* from stem mines to aseptically grown seedlings in the laboratory.

## MATERIALS AND METHODS

**Fusaria associated with mines and life stages of *O. simplex*.** Four asparagus fields were sampled in September, and 375 mature stems were randomly chosen for laboratory evaluations. All stems were evaluated for total number of mines and puparia, and average numbers per stem were determined. Puparia were crushed to determine if adult flies had emerged. In the course of this evaluation, fungal sporulation often was noted in epidermal and cortical tissues, especially in older, lower, stem-mine areas. Fifty randomly chosen sporulating tissue pieces from stem-mine areas were placed on plates of potato-carrot agar (13) acidified with 50% lactic acid to pH 4.0 (PCA). After 14 days at 24 C, resultant colonies either were identified directly, or single-spored and identified later after growth on carnation leaf agar (CLA) (6,12). PCA and CLA were used routinely for isolation and identification of fusaria. Mined areas with intact epidermis were selected at the midpoints of 200 randomly chosen stems. The epidermis was removed, and individual sections of underlying cortical tissue were excised, soaked in 10% chlorine bleach solution for 5 min, and placed on PCA plates. An additional 200 nonmined stem lesions, and 200 symptomless stem areas (controls), were processed similarly. Larval frass samples from 50 stem-mined areas, one sample per stem, were removed and placed on PCA plates.

Larvae and puparia of *O. simplex* were individually collected from stem mines. Two hundred live larvae were individually washed five times in sterile distilled water and individually placed on PCA plates. To determine internally associated fungi in pupae, 200 puparia were treated with 95% ethanol for 5 min, in 25% chlorine bleach solution for 15 min, plated on PCA, and incubated for 4 days at 24 C. Puparia free of visible microbial growth then were transferred to new PCA plates, where they were crushed with sterilized forceps to allow pupae to come in contact with the medium. Six hundred additional puparia were divided into six lots of 100 each. One hundred were individually washed five times in sterile distilled water, 100 were soaked in 10% chlorine bleach for 5 min, and 100 were soaked in ethanol and then chlorine bleach solution as previously described. All were then placed on PCA plates. The remaining three groups of 100 puparia were either not treated, soaked in 10% chlorine bleach for 5 min, or soaked in ethanol, then treated with chlorine bleach as before. These puparia were stored for 4 mo at 5 C to simulate a winter period. The puparia were placed on PCA plates, and the ethanol-bleach-treated puparia were transferred to new PCA plates and crushed as previously described. During a single 1-wk period, 200 adult flies were individually captured by aspiration from asparagus ferns in the field. The adults were cooled in a freezer to slow their motion, placed on PCA plates, and crushed with sterilized forceps.

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**Dissemination of *F. moniliforme* by adults of *O. simplex*.** Adults of *O. simplex* were reared in the laboratory from ethanol-bleach-treated, field-collected puparia, or collected individually in the field by aspiration. Seeds of asparagus cultivar Rutgers Beacon were treated with 25,000 ppm benomyl in acetone (to eradicate seedborne fusaria), rinsed in sterile water, soaked in 10% chlorine bleach for 10 min, germinated on water agar, and transferred to agar slants of Hoagland's solution in 20 × 150-mm tubes (2). Two-week-old seedlings were used. A large single experiment was then devised to determine dissemination of *F. moniliforme* by adults of *O. simplex*. Thirty seedlings were not inoculated (controls), and 30 were inoculated with a pathogenic isolate of *F. moniliforme* from a stem mine by placing mycelial disks from PCA cultures adjacent to seedling stem bases. Thirty adults of *O. simplex* were cooled and then placed in a petri dish containing a sporulating PCA culture of *F. moniliforme* for 5 min. The culture was then cooled and individual flies were removed with sterilized forceps and transferred individually to 30 uninoculated asparagus seedlings in tubes. Thirty field-captured adults also were added individually to 30 uninoculated asparagus seedlings in tubes. Adult flies were allowed to forage on asparagus seedlings for 24 hr and then they were removed. All seedlings were allowed to continue to grow in tubes for 3 wk after fly removal. Seedlings then were removed from tubes and given a visual disease rating of 0–5, in which 0 = no symptoms, 1 = crown discoloration, 2 = crown discoloration + one to two root lesions, 3 = crown discoloration + more than three stem or root lesions, 4 = crown discoloration + extensive root and stem rot and wilting, and 5 = dead plants. All crowns were excised, soaked in 5% bleach solution for 5 min, and placed on PCA plates.

**Pathogenicity of fusaria associated with *O. simplex*.** Sixteen single-spore isolates of *F. moniliforme* and *F.o.* f. sp. *asparagi* isolated originally from stem mines, larvae, puparia, and adults of *O. simplex* were used in a single pathogenicity test with asparagus seedlings, using methods described in the previous experiment. Two isolates of each *Fusarium* were used from each source, with 10 seedlings inoculated per isolate for a total of 20 per source.

## RESULTS

**Fusaria associated with mines and life stages of *O. simplex*.** Large populations of asparagus miners were found in the four sampled commercial fields. We found 706 mines in 375 stems which averaged 1.9 per stem. Associated with the mines were 1,068 pupae, or 2.9 per stem, with 584 (54%) emerged. As many as 15 pupae were found on individual stems.

Stem mines were found below the soil line near the crowns and well above ground. Individual mines extended as high as 1 m on stems, although most mines and pupae occurred in the lower 10 cm of the stem. Examination of the mines showed that overlying epidermal tissue had died and turned brown. The epidermis often was cracked and detached from entire lower stem areas where mines had girdled the stems. Examination of stem sections through mine areas showed that the mines extended 1–2 mm into the cortex, with no apparent vascular involvement. Mines observed early in the season initially appeared pale green, and later showed red-brown discoloration (Fig. 1). Mycelium, sporodochia, and larval frass were observed in mines, but not in surrounding healthy tissue. Isolations from epidermal fragments, larval frass, and cortical tissues from mined areas yielded *F. moniliforme*, *F.o. asparagi*, *F. tricinctum*, and *F. solani* (Table 1). *F. moniliforme* occurred most frequently.

Results of isolations of fusaria from life stages of *O. simplex* are summarized in Table 2. *F. moniliforme* and *F.o. asparagi* were found associated with washed larvae and with washed and chlorine

TABLE 1. Frequency of isolation of fusaria from field-collected asparagus stems with mines (caused by *Ophiomyia simplex*), stem lesions, and symptom-free tissue

| Tissue sources | Colonies of *Fusarium* isolated[a] (%) | | | |
|---|---|---|---|---|
| | *F. moniliforme* | *F.o. asparagi* | *F. tricinctum* | *F. solani* |
| Stem mines[a] | 76 | 10 | 6 | 1 |
| Stem lesions[a] | 16 | 9 | 0.5 | 0.5 |
| Symptom-free stems[a] | 0 | 0 | 0 | 0 |
| Larval frass[b] | 72 | 16 | 0.6 | 0.8 |

[a]Two hundred individually collected sections of cortical tissue per source, treated in 10% chlorine bleach for 5 min, and placed on acidified potato-carrot agar plates.

[b]Larval frass from 50 stem-mine areas, one sample per stem, placed on acidified potato-carrot agar plates.

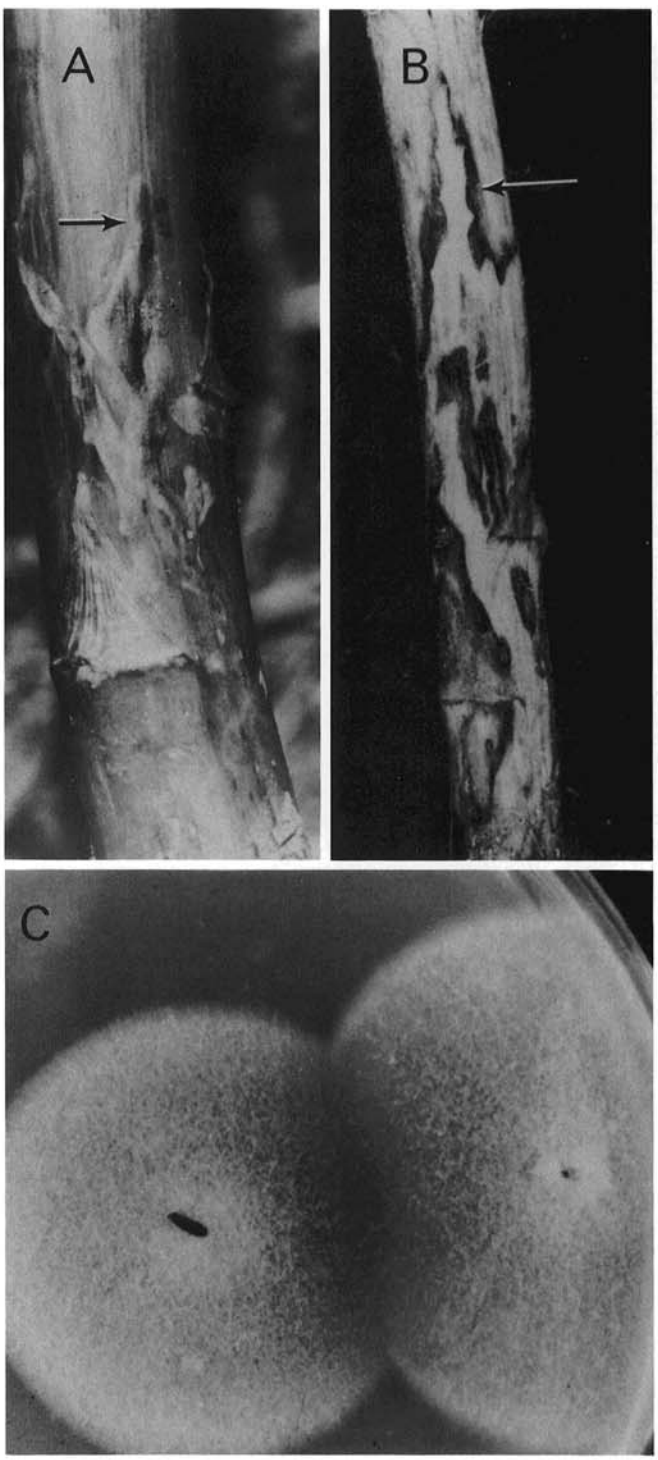


**Fig. 1.** Association of *Ophiomyia simplex* with asparagus. **A,** Early stages of mine development and **B,** later stages, showing epidermal destruction and red-brown margins. **C,** Colonies of *Fusarium moniliforme* that developed from a pupa (left) and an adult fly of *O. simplex* (right).

bleach-treated puparia (Fig. 1), but not with puparia treated with 95% ethanol and 25% chlorine bleach. When ethanol and bleach-treated puparia were crushed on PCA plates, however, both fusaria were isolated when crushed pupae came in contact with the medium. Storing puparia at 5 C for 4 mo did not affect isolation of both fusaria, regardless of treatment. *F. moniliforme* was isolated from 6% of field-captured adult flies. Three percent carried *F.o. asparagi* (Table 2).

**Dissemination of *F. moniliforme* by adults of *O. simplex*.** Foraging by laboratory-reared adults artificially contaminated with *F. moniliforme* resulted in infection of 53% of the asparagus seedlings for a mean disease rating of 2.2 (Table 3). Exposure to field-captured adults resulted in infections in 16% of seedling crowns for a mean disease rating of 1.1. All seedlings inoculated with agar disks containing *F. moniliforme* were infected, for a mean disease rating of 4.3. Controls were not infected.

**Pathogenicity of fusaria associated with *O. simplex*.** Isolates of *F. moniliforme* from mine tissue, puparia, washed puparia, and adults of *O. simplex* resulted in mean seedling disease ratings of 3.5, 4.0, 4.0, and 5.0, respectively, while isolates of *F.o. asparagi*, from corresponding sources, resulted in mean seedling disease ratings of 3.5, 3.5, 2.5, and 3.0. Control seedlings were not infected. Isolates of *F.o. asparagi* were less pathogenic than those of *F. moniliforme*.

## DISCUSSION

*F. moniliforme* was the most frequently isolated species from stem mines caused by *O. simplex*. Feeding by larvae of *O. simplex* tended to favor increased infections of asparagus stems by *F. moniliforme*, and (to a lesser extent) *F. oxysporum*. External sporulation by *F. moniliforme* was noted in stem mines as early as mid-June, whereas sporulating lesions on nonmined stem areas were not observed until late in the growing season (7). This is consistent with the idea that *F. moniliforme* is the principal pathogen of asparagus stems (7,11).

Graham (9) found that *F. moniliforme* from asparagus stems in Ontario was able to utilize cellulose as a substrate. *F. moniliforme* may initially utilize cellulose of mined asparagus tissue and larval frass to colonize mines. Mining predisposes the stem to invasion, allowing *F. moniliforme* to grow in mines, sporulate, and increase inoculum early in the growing season. This inoculum can become airborne or waterborne and infect nearby stems and plants (7,8). Low incidence of *F. moniliforme* and *F.o. asparagi* from upper stem lesions indicated that mines are a favored growth substrate. Upper stem lesions can also be caused by *Phoma asparagi* Sacc. and *Botrytis cinerea* Pers., especially in September, or may be invaded by saprophytic fungi. This would also decrease isolation of *F. moniliforme* and *F.o. asparagi* from stem lesions. The red-brown discoloration of stem mine margins, typical of Fusarium infections, extended aboveground with the mines. Higher incidence of *F.o. asparagi* on live larvae from mines is probably due to larval contact with the fungus during belowground feeding at mine initiation. Contaminated larvae may carry fungal propagules during mining, resulting in a more rapid colonization of mines, or mycelium may simply grow along in tissue behind the advancing larvae. Higher incidence of *F. moniliforme* from puparia may be due to their aboveground location. Puparial contamination occurred either during larval feeding, or was due to *F. moniliforme* colonizing mined tissue and puparial surfaces.

Fungal propagules were closely associated with external surfaces of puparia, and were only eliminated by 10% bleach solution used in combination with ethanol. The surfaces of puparia may provide crevices where propagules lodge and escape sterilization, as was shown for spores of fusaria on asparagus seed surfaces by Inglis (10). Association of the fungi with the pupae in the puparia shows more prolonged association, as does survival through cold storage. Puparia and pupae in senescent stems are likely overwintering sites for both fusaria and potential inoculum sources for the next growing season. Adult flies emerging from contaminated puparia can become contaminated and possibly disseminate spores of *Fusarium* to other stems. Adults are active flyers and readily contact aerial plant parts (5). Adult contamination also may occur in the field through contact with sporulating stem infections.

The importance of the association between *Fusarium* and *O. simplex* is unknown. Our results indicated that colonization of mines and life stages by fusaria is widespread in western Massachusetts and our laboratory experiment indicated that adult asparagus miner flies can transmit *F. moniliforme* to asparagus seedlings. The association is favored by the high incidence of *Fusarium* and *O. simplex* in asparagus in western Massachusetts (7). *O. simplex* is not the major means of dissemination for *Fusarium*, particularly for soilborne *F.o. asparagi*, but its feeding activities definitely favor stem rot caused by *F. moniliforme*. We feel the high incidence of Fusarium stem rot in declining asparagus beds is due to the extensive occurrence of associations of *Fusarium* and *O. simplex* in commercial fields in western Massachusetts.

Unlike Eichmann (4), we conclude that *O. simplex* is a significant pest of asparagus in Massachusetts, and that it plays a significant role in increasing stem rot caused by *F. moniliforme* and, to a lesser extent, by *F.o. asparagi*. Cohen and Heald (1) suggested that similar results occurred in Washington, and van Bakel and Bethe (14) found more stem rot caused by *F.o. asparagi* in mined than nonmined stems in Germany.

The use of insecticides, or removal of senescent ferns in the fall, should decrease larval damage by *O. simplex* the following year. Such management of *O. simplex* has been studied in an integrated pest management program where the use of diazinon reduced the stem mining by 75% and stem rot by 40% (3). These results further substantiate the idea that the feeding activities of *O. simplex* result in increased stem rot in asparagus and increased sporulation by

TABLE 2. Isolation of fusaria from life stages of *Ophiomyia simplex*

| Life stages | Number used | Colonies of *Fusarium* isolated (%) | |
|---|---|---|---|
| | | *F. moniliforme* | *F.o. asparagi* |
| Plated after treatment | | | |
| Larvae[a] | 200 | 8 | 17 |
| Pupae in puparia[b] | 200 | 6.5 | 5.5 |
| Puparia[a] | 100 | 24 | 22 |
| Puparia[c] | 100 | 33 | 13 |
| Puparia[d] | 100 | 0 | 0 |
| Stored 4 mo at 5 C, then placed on PCA plates | | | |
| Puparia[c] | 100 | 22 | 19 |
| Puparia[a] | 100 | 8 | 14 |
| Pupae in puparia[b] | 100 | 8 | 4 |
| Adults from field[e] | 200 | 6 | 3 |

[a] Five sterile water washes.
[b] Soaked in 95% ethanol for 5 min then in 25% chlorine bleach solution for 15 min, moved to a plate of acidified potato-carrot agar, and crushed with sterile forceps.
[c] Soaked in 10% chlorine bleach for 5 min.
[d] Soaked in 95% ethanol for 5 min, then in 25% chlorine bleach for 15 min.
[e] No treatment.

TABLE 3. Dissemination of *Fusarium moniliforme* by adults of *Ophiomyia simplex* to 2-wk-old, individual, aseptically grown cultivar Rutgers Beacon asparagus seedlings

| Treatment[a] | Mean seedling disease rating[b] | Seedling crowns (%) invaded by *F. moniliforme* |
|---|---|---|
| Field-captured adults[c] | 1.1 | 16.6 |
| Laboratory-reared adults exposed to culture of *F. moniliforme* | 2.3 | 53 |
| Seedlings inoculated with *F. moniliforme* | 4.3 | 100 |
| Seedlings not inoculated or exposed to *O. simplex* (controls) | 0 | 0 |

[a] Thirty individual seedlings per treatment.
[b] Based on a 0–5 scale, in which 0 = no symptoms and 5 = dead seedling.
[c] Flies allowed to forage among seedlings for 24 hr, then removed.

*Fusarium* species, particularly *F. moniliforme*. Successful management of Fusarium stem rot of asparagus must also include management of *O. simplex*.

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