Increased Susceptibility to Fusarium Crown and Root Rot in Virus-Infected Asparagus

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ABSTRACT


Asparagus (Asparagus officinalis L.) seedlings infected with asparagus virus II (AV-II) became significantly more diseased when inoculated with Fusarium oxysporum f. sp. asparagi than did virus-free seedlings. Asparagus seedlings doubly infected with both AV-I and AV-II became more diseased when inoculated with F. o. asparagi than seedlings infected with either virus alone. Increased levels of Fusarium crown and root rot in asparagus infected with either or both viruses were correlated with an increase in root exudation and an increase in susceptibility of root tissues to infection by the pathogen. Root exudates from AV-II-infected asparagus plants grown in liquid culture contained more electrolytes (3%), carbohydrates (1.7%), and amino acids (8%) than those from virus-free plants. Germination and subsequent germ tube growth from conidia of F. o. asparagi was stimulated more by root exudates from virus-infected plants than from virus-free plants. Histopathology studies indicated that virus-infected asparagus seedlings were less able than virus-free plants to wall-off and lignify the infection courts of F. o. asparagi.

Asparagus "decline" occurs worldwide and has been described as the reduction in the profitable life of an asparagus (Asparagus officinalis L.) plant (19). Both Fusarium oxysporum f. sp. asparagi Schlecht. and Fusarium moniliforme Sheldon are believed to contribute to reduced plant populations, low vigor of surviving plants, and reduced yields (22). The results of a number of studies indicate that F. o. asparagi is the predominant pathogen associated with asparagus decline (19,22). However, the association of F. moniliforme in asparagus decline is more important than it appears from accounts in the literature (18,22). Several cultural, environmental, or biological stresses acting alone or in combination may predispose the asparagus plant to infection by F. o. asparagi and F. moniliforme, including herbicide application (23), tillage (31), insect feeding (6,16), alelopathic compounds (21), defoliation caused by rust (M. L. Lacy, unpublished), overharvesting of spears (32), and virus infections (9,39).

Several viruses have been reported to infect asparagus in Europe, North America, and Asia. In Europe, asparagus virus I (AV-I, a member of the potyvirus group) and asparagus virus II (AV-II, a member of the ilarvirus group) are very common and easily detectable in older commercial fields by virus-indexing procedures (39). Both AV-I and AV-II are present in all asparagus-growing areas of the United States. In Michigan and Washington, AV-II has been detected widely within most commercial asparagus plantings, and AV-I has also been determined to be present, but not widespread (9,20,25,37). Both asparagus viruses are widespread in all asparagus-growing areas of California (11), but in New Jersey AV-I is far more commonly detected than AV-II (26). On the other hand, in Japan AV-II is predominant and AV-I is less widespread (14,15).

Virus infection has been demonstrated to negatively affect the vigor and productivity of asparagus. Asparagus plantings in Germany infected with AV-I, AV-II, or cucumber mosaic virus (a cucumovirus) showed a 29-44% reduction in yield and a 15-20% decrease in number of spears and height of ferns (39). In Washington, asparagus plants infected with either AV-I or AV-II exhibited a mild reduction in vigor and productivity (40). Plants infected with both viruses showed severe decline and mortality in the second year in the field. These viruses may constitute important biological stress factors leading to an increased incidence and severity of Fusarium crown and root rot in asparagus.

A number of studies indicated that fungal infections of plant roots are increased when plants are also infected with one or more viruses (1,2,3,7,12,32,34,38). Two possible mechanisms have been proposed for the increased fungal root rot induced by virus infection: that the inherent susceptibility of root tissue is increased by virus infection, and that increased leakage of nutrients from roots of virus-infected plants increases the inoculum potential of the fungi in the rhizosphere (2).

The objective of this investigation was to evaluate the effect of infection by AV-I and AV-II on disease severity caused by the Fusarium crown and root-rotting organisms in asparagus and to investigate changes in root exudate composition and the ability of the root to lignify the area surrounding infection courts.

MATERIALS AND METHODS

Preparation of seed. All asparagus seed was first surface-disinfested of Fusarium with a solution of 5 g/ml of benomyl in acetone on an orbital shaker overnight (5). Seeds were washed free of benomyl with 100–200 ml of acetone and air dried. Surface-disinfested asparagus seeds were pregerminated on moistened sterile filter paper in petri dishes in the dark. When radicles emerged, seedlings were transferred to flats containing standard commercial potting mix and maintained in a greenhouse at 21–24 C and a 16-hr photoperiod.

Preparation of seedlings. Virus-free and AV-II-infected asparagus seedlings were prepared by using a seedlot of cultivar Mary Washington 500 (M 500) that had been chosen because it was determined to be approximately 50% infected with AV-II (provided by G. I. Mink). Six wk after germination, seedlings were indexed for AV-II by using the indirect enzyme-linked immunosorbent assay method (36).

Asparagus seedlings (M 500) either infected with AV-I or doubly infected with both AV-I and AV-II were obtained from healthy and AV-II-infected plants by aphid transmission of AV-I (9). Thirty to 50 green peach aphids (Myzus persicae Sulz.) were allowed to feed for 2 wk on asparagus plants known to be infected only with AV-I, then transferred to healthy or AV-II-infected 6-wk-old asparagus plants. An equal number of nonviraliferous green peach aphids were allowed to feed on a second group of
healthy or AV-II-infected asparagus seedlings as a control. After 2 wk all stems were cut at soil level and plants were transferred to aphid-free cages. Plants were indexed for the presence of AV-I and AV-II after the emergence of the second new shoot. Shoot tips were triturated in a small volume of 0.01 M sodium phosphate buffer, pH 7.0, containing 0.01% 2-mercaptoethanol and rub inoculated onto the surface of Chenopodium quinoa (Willd.) leaves previously dusted with carborundum (320 mesh). Plants were maintained in a greenhouse at 21–24°C and a 16-hr photoperiod for 14 days and observed for the development of symptoms (25). AV-II typically produces chlorotic ringspots on inoculated leaves in 5–10 days, and AV-I produces necrotic lesions on inoculated leaves in 10–14 days. Single necrotic lesions developing in 10–14 days were examined with serologically specific electron microscopy (SSEM) for AV-I particles (8). (Antiserum to AV-I was provided by G. I. Mink.)

Preparation of tissue culture clones. Healthy and AV-II-infected asparagus lines were prepared by culturing shoot tips and apical meristems from field-grown asparagus plants of cultivar Viking K83 known to be infected only with AV-II (4,27,41). Tissue culture clones infected with AV-I or doubly infected with AV-I and AV-II could not be produced for use in this study. Tissue explants derived from AV-I or AV-I- and AV-II-infected plants were slow to develop roots and often died in culture (10). Explants of tissue were obtained from shoots about 20 cm long and 2 cm in diameter. All subsequent procedures were carried out in a laminar flow hood with sterile technique. The surface of each shoot was washed with water and then sterilized with 0.5% sodium hypochlorite solution (v/v) for 10 min. The scales on the tip of each shoot were removed and meristems and shoot tips were excised under a binocular dissecting microscope with a microscalpel.

Two types of tissue explants were used. Apical domes less than 0.1 mm in height and free of leaf primordia were used for the production of virus-free plants, and shoot tips greater than 2 mm in height with several leaf primordia for the production of AV-II-infected plants. Isolated meristems and shoot tips were placed in Pyrex tubes (10 × 2.5 cm) on 10 ml of modified Murashige and Skoog’s medium (MMS) (30) with 0.1 ppm of naphthalenacetic acid (NAA) and 0.1 ppm of kinetin (6-furfurylimidione). The medium was adjusted to pH 5.7 with 1 N NaOH or 1 N HCl and autoclaved at 121°C and 15 psi for 20 min. Tubes containing meristems and shoot tips were placed in a growth chamber and maintained at 25°C with a 16-hr photoperiod.

After 2 mo the plantlets were transferred to 125-ml flasks containing 50 ml of MMS medium with 0.1 ppm of NAA and 0.3 ppm of kinetin. One plantlet was placed into each flask, and after 2–3 mo plantlets were indexed for AV-II as previously described.

Source and maintenance of fungi. Isolates of F. o. asparagi and F. moniliforme were originally isolated from diseased asparagus by M. L. Lacy, Michigan State University, and were maintained in sterile soil at 4°C until use.

Preparation of inoculum. Soil containing F. o. asparagi and F. moniliforme was sprinkled onto potato-dextrose agar (PDA; Difco Laboratories, Detroit) plates and grown 14–21 days at 24°C. Glass-distilled water (100 ml) was added to 200 g of millet seed in a flask and autoclaved for 1 hr at 121 Cand 15 psi on two consecutive days (17). After each autoclaving, millet seed was first allowed to cool, and the flask was shaken vigorously to break up large clumps of millet seed. Sterile millet seed was inoculated with the appropriate Fusarium isolate from PDA, and the flask was incubated at 21–24°C for 10–14 days. Flasks were shaken daily to ensure an even distribution of the fungus within the inoculum.

Inoculation of plants. Two-mo-old asparagus seedlings or tissue-cultured asparagus clones of comparable size were inoculated with 8 g of colonized millet seed per 1,000 g of pasteurized sandy-loam soil. This amount of inoculum was determined to cause a moderate level of disease. A comparable amount of sterilized but uncolonized millet seed was mixed with soil as a control. Plants were maintained in a greenhouse at 21–24°C with a 16-hr photoperiod.

Evaluation of disease. Disease severity of roots was evaluated 2 mo after inoculation on a scale of 1–5: 1 = healthy plant, no visible root lesions, no rotted roots or crown discoloration; 2 = few root lesions (1–5) and/or rotted roots, no crown discoloration, no reduction in number of shoots or roots; 3 = moderate number of root lesions (6–10) and/or rotted roots, no or slight crown discoloration, shoots reduced, feeder roots reduced; 4 = many root lesions (>10) and/or rotted roots, crown discoloration, shoots reduced, feeder roots sparse; 5 = many root lesions (>10) and/or rotted roots, crown discoloration, shoots greatly reduced, feeder roots greatly reduced, absent, or dead.

Effect of virus infections on root rot severity. Two-mo-old healthy and AV-II-infected asparagus seedlings or asparagus clones were inoculated with F. o. asparagi- or F. moniliforme- colonized millet seed in the greenhouse. The interaction between infection with AV-I and/or AV-II and subsequent infection by F. o. asparagi was evaluated by using the procedures described above. Inoculated plants were maintained in the greenhouse at 21–24°C and 16-hr photoperiod and were fertilized at 1 mo with 100 ml of Peter’s 20-20-20 fertilizer (W. R. Grace and Company, Fogelsville, PA). Plants were evaluated for root rot severity 2 mo after inoculation, and both F. o. asparagi or F. moniliforme were routinely isolated from inoculated plants. The experiment was repeated twice.

Effect of root exudates on root rot severity. The effect of root exudates from healthy and virus-infected asparagus plants on root rot severity was investigated in the greenhouse. Five 2-mo-old healthy or AV-II-infected asparagus seedlings were grown in pasteurized sandy-loam soil in 25.4-cm plastic pots and watered twice daily with 100 ml of glass-distilled water for 2 mo. Leachates from these pots were allowed to drain onto the soil in pots containing virus-free asparagus plants inoculated with either F. o. asparagi or F. moniliforme-colonized millet seed. After 2 mo, plants that were watered with leachates were evaluated for disease severity. The experiment was repeated twice.

Collection of root exudates. Healthy and AV-II-infected clones were grown in axenic, liquid culture supported by nylon screens in staining dishes (10 × 8 × 6 cm). Four plantlets were grown in each dish with four replicates per treatment, then placed in autoclaved polypropylene bags (Bel-Art Products, Pequannock, NJ) and maintained in a growth chamber with 16 hr of light (240–310 µE/m²/sec) at 25 ± 2°C. Plants were grown in full-strength Hoagland’s solution (4) for 6 days, washed with sterile glass-distilled water, and then transferred to 100 ml of double-distilled water for 24 hr for exudate collection. Plants were returned to fresh, full-strength Hoagland’s solution, and this pattern of exudate collection was continued for 4 wk.

All solutions containing root exudate samples were passed through 0.20 µm filters (Nalgene Sterilization Unit, Type S, Nalgene Company, Rochester, NY) and stored at 4°C. Solutions were concentrated to 1/10 volume in a flash evaporator at 50°C and passed through 0.45 µm Millipore filters and stored in sterile tubes at 4°C or frozen.

Chemical analysis of root exudates. The conductivity of culture solutions containing root exudates was determined with a standard conductivity bridge, and the results were expressed in µhos. Total carbohydrate of root exudates were determined by using Dreywood’s anthrone reagent (29). The anthrone reagent was prepared by dissolving 0.4 g of anthrone in 200 ml of 9.3 M H₂SO₄. One ml of root exudate sample was mixed with 2 ml of anthrone reagent and then placed into a boiling water bath for 3 min. Samples were quickly cooled, and the optical density at 620 nm was measured. A standard curve was prepared from 1-ml samples containing 10, 20, 40, 80, and 160 µg of glucose. Glucose content of root exudates was determined with an enzymatic colorimetric test (no. 115, Sigma Chemical Co., St. Louis). Optical densities were read at 450 nm and compared with a standard curve prepared by using 1-ml samples containing 10, 20, 40, 80, and 160 µg of glucose.

Total amino acids were determined by using the ninhydrin method (28). A 2-ml sample of root exudate was mixed with 1 ml of a ninhydrin reagent solution and heated in a boiling water bath for 15 min. Samples were quickly cooled to below 30°C, vigorously shaken, and optical density measured at 570 nm. A standard curve was prepared from 2-ml samples containing 4, 8, 16, and 32 µg of
glycine. Protein was determined with Folin Ciocalteu’s Phenol Reagent (24). A 2.2-ml aliquot of Biuret Reagent was added to 0.2 ml of diluted test solutions and to 0.2 ml of the sodium chloride solution, mixed thoroughly, and allowed to stand at room temperature for 10 min. Then 0.1 ml of Folin Ciocalteu’s Phenol Reagent was added, mixed thoroughly, and allowed to stand at room temperature for 30 min. Optical density at 725 nm was determined. A standard curve was prepared from 0.2-ml samples containing 5, 10, 20, 40, and 80 μg of bovine serum albumin.

**Biological activity of root exudates.** Microconidia of *F. o. asparagi* and *F. moniliforme* were removed from the agar surface and filtered through two layers of sterile cheesecloth to remove most mycelial fragments. Microconidia were washed by centrifuging at 6,000 g for 15 min and resuspending the pellet in 50 ml of sterile glass-distilled water. This process was repeated three times, and the final suspension was adjusted to the appropriate microconidia concentration. Concentrated root exudates from virus-infected and healthy asparagus were diluted 10-fold by adding 0.1 ml of sterile root exudate to a solution containing 0.1 g of agar (Difco Laboratories, Detroit) in 9.9 ml of sterile glass-distilled water at 55°C in a water bath. Washed microconidia were spread uniformly onto the surface of the water agar plates and incubated at 24°C. Germination of microconidia and subsequent germ tube growth were measured hourly by using a dissecting microscope and ocular micrometer. A minimum of 50 microconidia were monitored on duplicate plates for each experiment, and the experiment was repeated twice.

**Effect of virus infection on lignin formation within asparagus roots in response to infection by *Fusarium* spp.** The effect of virus infection on the ability of asparagus seedling roots to lignify and wall-off infections of *F. o. asparagi* and *F. moniliforme* was evaluated in the greenhouse. Healthy and AV-II-infected 2-mo-old asparagus seedlings were inoculated with *F. o. asparagi* and *F. moniliforme* as previously described. Two mo after inoculation, plants were evaluated for disease severity and root tissue with distinct lesions was excised. Root lesions were separated into two categories by size: 1–5 mm in length or 6–10 mm in length. Root tissue with lesions was hand sectioned by using a razor blade and stained with 1% phloroglucinol in 50% HCl (13) and rated for relative degree of lignification in the region surrounding the infection court. At least 100 sections were evaluated on a scale of 1–5.

**RESULTS**

**Effect of virus infection on root rot severity.** Tissue culture clones infected with AV-II became significantly (*P = 0.05*) more diseased than virus-free plants when inoculated with *F. o. asparagi* (Table 1). The difference between disease severities of AV-II-infected and virus-free asparagus plants was smaller and not statistically significant when inoculated with *F. moniliforme* (Table 1). In a second experiment, asparagus seedlings infected with AV-I or AV-II were significantly (*P = 0.05*) more diseased than virus-free seedlings when inoculated with *F. o. asparagi* (Table 2). Asparagus seedlings doubly infected with AV-I and AV-II became significantly (*P = 0.05*) more diseased when inoculated with *F. o. asparagi* than seedlings infected with either virus alone.

**Effect of root leachates on root rot severity.** Root rot severity in virus-free asparagus plants watered with leachates from AV-II-infected asparagus was significantly (*P = 0.05*) greater than that of plants watered with leachates from virus-free asparagus (Fig. 1). Differences in root rot severity were no greater for plants inoculated with *F. o. asparagi* than with *F. moniliforme*.

**Changes in root exudates of virus-infected asparagus clones.** Asparagus clones infected with AV-II and grown in liquid culture released three times more electrolytes in a 24-hr period than did virus-free clones (Fig. 2). AV-II-infected asparagus clones grown in liquid culture released more glucose (3X), total carbohydrates (1.7X), and amino acids (8X) than did virus-free asparagus clones (Fig. 3). There were no differences in the amount of protein released by the roots of virus-infected and healthy asparagus plants. Each healthy asparagus plant released approximately 27 μg of carbohydrate (2.5 μg of this determined to be glucose) and less than 1 μg of amino acids per day. Asparagus plants infected with AV-II each released about 50 μg of carbohydrate (10 μg of this determined to be glucose) and 8 μg of amino acids over the same period. Similar results were obtained in a second experiment (data not shown).

**TABLE 2.** The effect of infection with asparagus virus I or asparagus virus II alone or in combination on disease severity in seedlings of *Asparagus officinalis* inoculated with *Fusarium oxysporum* f. sp. *asparagi*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease index*</th>
<th>Control†</th>
<th>*F. o. asparagi†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus-free</td>
<td>1.1 a</td>
<td>2.2 a</td>
<td></td>
</tr>
<tr>
<td>Asparagus virus I</td>
<td>1.2 a</td>
<td>3.2 b</td>
<td></td>
</tr>
<tr>
<td>Asparagus virus II</td>
<td>1.1 a</td>
<td>3.2 b</td>
<td></td>
</tr>
<tr>
<td>Asparagus virus I and II</td>
<td>1.3 a</td>
<td>4.1 c</td>
<td></td>
</tr>
</tbody>
</table>

* = Disease index of 1–5; 1 = healthy plant; 2 = few root lesions and/or rotted roots; 3 = moderate number root and/or rotted roots; 4 = many lesions and/or rotted roots; 5 = many root lesions and/or rotted roots or plant dead.

†Values are means for 10 plants.

Within columns, numbers followed by a common letter are not significantly different according to Duncan’s multiple-range test (*P = 0.05*).

**TABLE 1.** Disease severity in clones of *Asparagus officinalis* 'KB3' with and without asparagus virus II when inoculated with *Fusarium oxysporum* f. sp. *asparagi* or *F. moniliforme*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus-free plant</td>
<td>1.3 ± 0.4</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>Asparagus virus II</td>
<td>1.2 ± 0.3</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>Virus-free plants</td>
<td>2.3 ± 0.3</td>
<td>3.0 ± 0.4 ns</td>
</tr>
<tr>
<td>Asparagus virus II</td>
<td>2.0 ± 0.3</td>
<td>2.8 ± 0.4 ns</td>
</tr>
<tr>
<td>Control</td>
<td>1.0 ± 0.1</td>
<td>1.4 ± 0.4 ns</td>
</tr>
</tbody>
</table>

* = Disease index of 1–5; 1 = healthy plant; 2 = few root lesions and/or rotted roots; 3 = moderate number root lesions and/or rotted roots; 4 = many lesions and/or rotted roots; 5 = many root lesions and/or rotted roots or plant dead.

Values are means ± the standard deviation.

ns = not significantly different from its respective control according to LSD test (*P = 0.05*).
Biological activity of root exudates. A significantly greater number of microconidia of *F. o. asparagi* and *F. moniliforme* germinated 6-12 hr on water agar supplemented with root exudates from AV-II-infected asparagus than on that supplemented with root exudates from healthy plants (Fig. 4). Germ tubes from microconidia of *F. o. asparagi* were significantly (P = 0.05) longer after 12 hr in the presence of root exudates from AV-II-infected asparagus than microconidia grown on water agar supplemented with root exudates of virus-free plants or water agar alone (Table 3).

Effect of virus infection on lignin formation within asparagus roots in response to infection by *Fusarium* spp. Virus infection of asparagus seedlings significantly (P = 0.05) reduced the amount of lignin produced in the areas of the root surrounding the infection courts of *F. o. asparagi* and *F. moniliforme*. Virus-free asparagus plants had a mean lignification rating of 3.5-4.0, whereas virus-infected plants had a mean rating of only 1.3-1.8 (Table 4). There were no apparent differences in the amount of lignin produced in response to infection by either *F. o. asparagi* or *F. moniliforme*.

**DISCUSSION**

Field observations in Germany, Washington, and New Jersey indicate that virus infection has a negative effect on the vigor and productivity of asparagus (39,40). Our studies indicate that asparagus seedlings infected with AV-I or AV-II become significantly more diseased when inoculated with *F. o. asparagi* than do virus-free seedlings. Asparagus seedlings infected with both AV-I and AV-II become the most diseased when infected with *F. o. asparagi*. Evidence is presented to suggest that virus-induced increase in root rot severity may be due in part to an alteration in the quantity of certain root exudate components. A direct effect of root exudates on disease severity was demonstrated by watering

![Graph](Fig. 2). Increased exudation of electrolytes by asparagus clones infected with asparagus virus II and grown in liquid culture relative to the exudation of virus-free clones. Values are the mean release by four plantlets over three separate 24-hr periods. Columns headed by different letters are significantly different according to Duncan’s multiple-range test (P = 0.05).

![Graph](Fig. 3). Exudation of glucose, total carbohydrates, amino acids, and protein from roots of asparagus clones infected with asparagus virus II and grown in liquid culture, relative to exudation of virus-free clones. Values are the mean release by four plantlets over three separate 24-hr periods.

![Graph](Fig. 4). The effect of root exudates from virus-free and asparagus virus II-infected asparagus on the germination of microconidia of *Fusarium oxysporum* f. sp. *asparagi* and *Fusarium moniliforme*. Values are the mean disease rating plus or minus the standard error of the mean.

**TABLE 3.** The effect of root exudates from virus-free and AV-II-infected asparagus clones on the growth of *Fusarium oxysporum* f. sp. *asparagi* and *F. moniliforme* germ tubes

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>F. o. asparagi</em></th>
<th><em>F. moniliforme</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Root exudates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virus-free</td>
<td>0.94 a</td>
<td>3.50 a</td>
</tr>
<tr>
<td>AV-II-infected</td>
<td>2.50 b</td>
<td>3.90 a</td>
</tr>
<tr>
<td>Water agar alone</td>
<td>1.05 a</td>
<td>3.50 a</td>
</tr>
<tr>
<td>Potato-dextrose agar alone</td>
<td>2.20 b</td>
<td>3.80 a</td>
</tr>
</tbody>
</table>

*Values are the mean germ tube lengths at 12 hr for 50 spores per treatment.*

*Exudates from plants grown in liquid culture were diluted 10-fold in water agar.*

*Within columns, numbers followed by a common letter are not significantly different according to Duncan’s multiple-range test (P = 0.05).*
TABLE 4. Effect of infection with asparagus virus II on lignification within asparagus seedling roots in response to infection by Fusarium oxysporum f. sp. asparagi or F. moniliforme

<table>
<thead>
<tr>
<th>Virus content</th>
<th>Root lesions</th>
<th>Root lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1–5 mm in length</td>
<td>6–10 mm in length</td>
</tr>
<tr>
<td><strong>F. o. asparagi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>3.7 ± 0.8</td>
<td>4.1 ± 0.7</td>
</tr>
<tr>
<td>AV-II</td>
<td>1.8 ± 0.7*</td>
<td>1.3 ± 0.7*</td>
</tr>
<tr>
<td><strong>F. moniliforme</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>3.6 ± 0.5</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>AV-II</td>
<td>1.3 ± 0.4*</td>
<td>1.5 ± 0.6*</td>
</tr>
</tbody>
</table>

*Hand-sections of root lesions were first stained with 1% phloroglucinol in 50% HCl. Lignification was rated on a scale of 1–5: 1 = no lignification; 2 = light lignification; 3 = moderate lignification; 4 = heavy lignification; and 5 = very heavy lignification. Values are means of 100 sections ± the standard deviation.

*Asterisks = means for asparagus virus-II-infected plants differ from corresponding virus-free plants according to the LSD test (P = 0.05).

However, the authors report that virus-free asparagus seedlings inoculated with *F. o. asparagi* or *F. moniliforme* with root exudates from virus-infected plants. Carbohydrates and amino acids were exuded in larger amounts from the roots of asparagus plants infected with AV-II than from healthy plants and may provide an increased nutrient base for rhizosphere organisms.

Soilborne pathogens such as *F. o. asparagi* and *F. moniliforme* attack their host in a sequence of steps, including germination of spores, mycelial growth, penetration of host tissue, and pathogenesis. Each step is expected to be influenced by the nutrient environment. Nitrogen and carbon are clearly the most important nutrients in the soil and can greatly affect the pathogenic development of fungi (33). Toussoun et al. (35) showed that the germination of conidia of *Fusarium solani* f.sp. *phaseoli* was favored by glucose. Glucose also stimulated saprophytic mycelial growth but delayed the penetration of the bean hypocotyl and pathogenesis. On the other hand, colonization of bean hypocotyl and pathogenesis was favored by nitrogen. Similarly, Beute and Lockwood (3) observed that disease severity in *F. solani*-infected peas was increased by the addition of amino acids and that glucose had no effect.

Healthy asparagus roots have the ability to ward off colonization by *Fusarium* before infection of the stele. Histological studies by Smith and Peterson (33) have revealed that wall appositions are produced by healthy asparagus roots in response to penetration by *F. o. asparagi*. These apposition materials were produced in the region immediately basipetal of the root meristems and were composed of polysaccharides, acid mucopolysaccaride, calloso, and phenolic substances. The deposition of these materials may serve to block the penetration of *F. o. asparagi* hyphae. The reduced ability of virus-infected asparagus plants to wall-off and lignify infection courts of pathogenic *Fusarium* spp. may contribute to the increase in disease severity observed in these plants.

The mechanism by which virus infection leads to an increase in root rot appears to be twofold. First, virus infection leads to an increased permeability of cell membranes of the root and an increased leakage of nutrients, including carbohydrates and amino acids. This may result in an increase in the inoculum level of the pathogens in the rhizosphere. Second, the roots of virus-infected asparagus may have an increased susceptibility to infection by *F. o. asparagi* and *F. moniliforme* because of a reduced ability to synthesize lignin barriers. We believe virus infection to be one of several stress factors that predispose asparagus to infection by the *Fusarium* crown and root-rotting organism. Further data is needed to assess the relative importance of virus infection to other stresses.

LITERATURE CITED


