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Interactions of Virulent and Avirulent *Fusarium* Species on Clonal Asparagus Plantlets and Mechanisms Involved in Protection of Asparagus with Avirulent *Fusarium* Species Against Stem and Crown Rots

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아스파라거스에서 병원성 및 비병원성 *Fusarium*균의 상호작용과 비병원성 *Fusarium*을 이용한 아스파라거스 줄기 및 뿌리썩음병 방제 기작 연구

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ABSTRACT : Protection of asparagus plantlets against *Fusarium*-induced diseases was greatest when pathogenic isolates were inoculated five or seven days after inoculation with avirulent *Fusarium* species. Avirulent *F. oxysporum* (AVFO) was a more effective protectant against *F. moniliforme* than against *F. oxysporum*. In contrast, *F. solani* was more effective against infection by *F. oxysporum* than it was against *F. moniliforme*. All the tested *Fusarium* species infected asparagus plantlets through primary and lateral root tips, natural wounds, and between the walls of the epidermal cells. Some penetration was possible through appressorium-like structures and some penetration was direct. It was assumed that the meristematic region could act as a major infection site for both virulent and avirulent *Fusarium* isolates. Virulent *Fusarium* species grew faster and more abundantly inside and outside epidermal areas of the plantlet than avirulent *Fusarium* species. *F. solani* grew slowest among the tested *Fusarium* species. Within a short period, virulent species caused cortical rots. Over extended periods, they eventually invaded tracheary elements, and caused extensive damage. AVFO accumulated heavily on and around the epidermal areas even if it invaded a part of cortical cells inside the epidermal regions. *F. solani* caused proliferation of lateral roots and increased the surface area of primary and secondary roots. In conclusion, it was found AVFO and *F. solani* could be used as biological control agents against the infection of virulent *Fusarium* species on asparagus plantlets.

***Key words* : avirulent *Fusarium* species, protection tests, cortical rots, biological control.**

It was found that *Fusarium moniliforme* J. Sheld and *F. oxysporum* Schlechtend: Fr. were responsible for asparagus (*Asparagus officinalis* L.) decline in Massachusetts (3). Both pathogens caused asparagus seedling death, and root and stem lesions on mature plants. *F. oxysporum* parasitizes storage and feeder roots, cortical tissues of stem bases, and vascular crown tissues. It exists in soil as saprophyte in the form of chlamydospores or in association with volunteer plants or weed hosts. *F. moniliforme* attacks aboveground plant parts and survives on seeds, volunteer plants, and in association with asparagus miner flies (9, 10, 15).

In Connecticut, LaMondia and Elmer (22) isolated *F. moniliforme*, *F. oxysporum* and *F. solani* (Mart.) Sacc. from symptomatic and asymptomatic feeder roots, storage roots, crown and basal stem segments of asparagus. They found that *F. moniliforme* was more virulent than *F. oxysporum* in asparagus seedlings; *F. solani* was nonpathogenic.

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Few attempts for cross-protection of asparagus against *Fusarium*-induced diseases have been successful. Graham (16) demonstrated that mixed inoculations of asparagus with both virulent *F. oxysporum* and *F. moniliforme* resulted in greater reductions in seedling blight than when either isolate was used alone. Damicone and Manning (10), and Manning (25) used an avirulent isolate of *F. oxysporum* (1B) from bean hypocotyl to protect asparagus seedlings against virulent *F. oxysporum*. Tu *et al.* (39) used a nonpathogenic isolate of *F. oxysporum* to reduce disease severity in pot tests. Lee and Manning (24) achieved reduction of root and crown rot of tissue-cultured asparagus plantlets *in vitro* by prior inoculation with 1B.

There have been studies of penetration, ingress, and subsequent colonization and systemic distribution of *F. oxysporum* within the roots of susceptible host plants including asparagus (36, 37). Cortical decay caused by *Fusarium* spp. is confined principally to the cortex of their hosts. Fungal growth in the tissue may be intercellular, intracellular or a combination of both, and is frequently facilitated by enzymatic degradation of the middle lamella and cell walls. The pathogens may be confined to the cortex by the endodermis or may penetrate the vascular system during the late stages of infection (30). The host responds to infection in many cases by the production of hypertrophied, hyperplastic cells resembling peridermal cells. This response occasionally limits the spread of the pathogen. Graham (16) reported delineation of the lesion area by a periderm-like layer of cortical cells. These cells appeared to play a role in restricting *F. oxysporum* var. *redolens* to the cortex of asparagus. Similar results for *Fusarium* spp. which caused cortical rot were reported (1, 7, 31). No host response leads to the complete maceration and collapse of the cortical cells, vascular system, and eventual death of the plant or plantlets (30).

There are no effective strategies available against asparagus diseases caused by *Fusarium* spp. No resistant cultivars are available, and chemical control has met with limited success (21, 35). Therefore, a study was undertaken: 1) to reexamine etiology of *Fusarium* diseases on asparagus; 2) to study infections of virulent and avirulent *Fusarium* species or isolates and determine the possible use of avirulent *Fusarium* species to protect against infection on asparagus plantlets by virulent *Fusarium* species; and 3) to study the infection process of virulent and avirulent *Fusarium* species within a short period and over extended periods.

MATERIALS AND METHODS

Isolation of *Fusarium* spp. and pathogenicity tests on asparagus plantlets. Numerous isolates of *Fusarium* species were obtained from asparagus plants grown in the field in Amherst and Sunderland, MA. Identification of all isolates to species was based on the taxonomic key by Nelson *et al.* (27). Avirulent *F. oxysporum* (AVFO) was previously obtained from bean hypocotyl surfaces (25). All selected isolates were preserved in silica gel (40) for further experiments. Asparagus plantlets (female clone, NJ362M) were obtained through meristem-tip culture as described previously (13, 19, 26, 41, 42), and placed on filter paper slants in test tubes (25 mm) containing Hoagland solution. Pathogenicity tests on plantlets were performed *in vitro* according to the methods described previously (23, 24), and virulent *Fusarium* species were selected. Statistical analysis of the results were performed with SAS program (ver 6.1) (33).

Interactions of virulent and avirulent *Fusarium* species on asparagus plantlets. One thousand cfus of conidial spores of virulent *F. oxysporum*, or *F. moniliforme* were inoculated on asparagus plantlets 1, 3, 5, or 7 days after the initial inoculation of 1,000 cfus of AVFO or *F. solani*. Inoculum was prepared by growing *Fusarium* species on potato carrot agar (PCA) medium and washing with distilled sterile water. Asparagus plantlets (female clone, NJ362M) were obtained through meristem-tip culture as described previously (13, 19, 26, 41, 42), and placed on filter paper slants in test tubes (25 mm) containing Hoagland solution. Evaluation and sample selection for microscopic observation were conducted four weeks after challenge inoculations. Root rot ratings were based on 0~5 scale where 0 = no disease, and 5 = death of plantlets. Root discoloration ratings were based on 0~5 scale where 0 = no color change, and 5 = severe discoloration. Statistical analyses of the results were performed with t-test on SAS program (ver 6.1).

Infection process of virulent and avirulent *Fusarium* species on asparagus. Asparagus plantlets were transplanted into a modified sterile glass-board unit (20) or into a sterile 15-cm-diameter plastic petri-dish moist-chamber apparatus. Glass-board units were washed and autoclaved before each use, and 15-cm-diameter plastic petri dishes were sterilized with 70% ethanol before use. Inoculum was prepared by growing *Fusarium* species on potato carrot agar (PCA) medium and washing with

distilled sterile water. Several designated parts of roots and stems in each plantlet were inoculated with 1,000 cfus of each *Fusarium* isolate a day after the plantlets were conducted 1, 3, 5, and 7 days after inoculation.

Stems and roots of plantlets were also inoculated with 1,000 cfus of avirulent and virulent *Fusarium* spp. a day after the plantlets were transplanted. Evaluation and sample collection for microscopic observation were conducted over time within four weeks. Root and stem pieces were embedded in paraffin, sectioned (10 μm), stained with aniline blue W. S. without removal of paraffin, and observed under light microscopy.

RESULTS

Isolation of *Fusarium* spp. and pathogenicity tests on asparagus plantlets. Seventeen isolates of *F. oxysporum*, three isolates of *F. moniliforme*, one isolate of *F. solani*, and thirteen isolates of unknown *Fusarium* species from asparagus were selected for pathogenicity tests.

In pathogenicity tests (Table 1), plantlets inoculated with AVFO did not appear different from those of the control treatments. All *F. moniliforme* isolates caused severe to very severe root rot symptoms and death of plantlets. Some *F. oxysporum* isolates caused severe rot symptoms on plantlets, and some caused death. The *F. solani* isolate did not induce root rots. Isolates of *F. solani*, *F. moniliforme*, and *F. oxysporum* caused moderate to very severe root discoloration, and varied depending on *Fusarium* species tested.

Interactions of virulent and avirulent *Fusarium* species on asparagus plantlets. All treatments showed significant differences in root rot ratings in all different interval inoculations except in control and *F. solani* combination treatments in one- and three-day-interval inoculations (Table 2). Combination treatments of control and AVFO, *F. solani* and *F. oxysporum*, and *F. solani* and *F. solani* did not show visible differences in root rots compared to the control treatment. The *F. solani* isolate provided significantly better protection against *F. oxysporum* than against *F. moniliforme* in all inoculations of different interval days. *F. solani* also reduced the mild virulence of single or double inoculations of an AVFO isolate in all inoculations of different interval days. The AVFO isolate did not provide much protection against *F. oxysporum* in all interval days except in seven-day-interval inoculation. The *F. solani* isolate provided much better protection against *F. oxysporum* than AVFO provided significant protection against virulent *F. oxysporum* at five- and seven-day-interval inoculations. The AVFO isolate provided much better protection against *F. moniliforme* in three-, five- and seven-day-interval inoculations than *F. solani* isolate did. *Fusarium solani* and *F. moniliforme* combination inoculations at all different interval days did not show any visible difference in root rot symptoms from those caused by *F. solani* and *F. moniliforme* combination inoculations. *F. moniliforme* and *F. solani* combination inoculations caused more severe root rot symptoms than those caused by *F. oxysporum* and *F. solani* combination inoculations in all different interval days. AVFO and *F. solani* combination inoculations caused less severe root rot symptoms than those caused by *F. moniliforme* and *F. solani* combination inoculations did. AVFO, *F. oxysporum*, and *F. moniliforme* combination inoculations did not show much visible difference compared to the control treatment at seven-day-interval inoculation, and its combination inoculation reduced root rot symptoms at five- and seven-day-interval inoculations compared to other

Table 1. *In vitro* pathogenicity test of *Fusarium* isolates on asparagus plantlets[a]

Fusarium isolate[b]	Root rot rating means[c]	Root discoloration rating means[d]
AVFO-1	0.8c	2.5bcd
AVFO-2	0.5c	2.3cd
FM24	5.0a	5.0a
FM28	5.0a	5.0a
FM37	5.0a	5.0a
FO19	2.5b	2.3cd
FO45	4.0a	2.0d
FO48	5.0a	3.0bc
FO49	5.0a	3.0bc
FS	0.0c	3.3b
Control	0.0c	0.0e
LSD (p=0.05)	1.01	0.08

[a] Results of each replication were the mean of four observations.

[b] AVFO=avirulent *F. oxysporum*, FM=*F. moniliforme*, FO=virulent *F. oxysporum*, and FS=*F. solani*.

[c] Root rot ratings were based on 0~5 scale where 0=no disease, 5=death of plantlets. Means with the same letter are not significantly different at p=0.05 by the least significance difference (LSD) test.

[d] Root discoloration ratings were based on 0~5 scale where 0=no color change and 5=severe discoloration. Means with the same latter are not significantly different at p=0.05 by the least significance (LSD) test.

Table 2. *In vitro* protection test with asparagus plantlets[a]

Isolate combination[e]	Root rot rating means[b]				Root discoloration means[c]			
	Day(s) of interval[d]				Day(s) of interval[d]			
	1	3	5	7	1	3	5	7
CA	0.5de[f]	1.8ef	2.5cd	0.9fgh	4.0ab	4.0abcd	4.3ad	4.5abc
AA	1.8bc	3.5bcd	3.5bc	0.6fgh	4.0ab	3.8abcd	4.8ab	3.2cdef
SA	1.3cd	0.5gh	0.8efg	0.8fgh	3.5bcd	3.8abcd	4.0ab	4.3abcd
LSD[g]	1.66	0.96	0.96	0.43	1.00	0.77	1.66	1.50
CO	4.5a	4.5ab	3.5bc	3.3de	3.0bcd	3.5bcd	3.5bc	2.8efg
AO	4.3a	4.0abc	2.5cd	1.5f	3.3bcd	4.5ab	4.8ab	4.3ab
SO	0.8de	2.8de	1.3ef	1.3fg	3.3bcd	3.0cde	3.8abc	4.8ab
OO	5.0a	5.0a	5.0a	4.8ab	2.8cde	3.3bcd	3.5bc	3.5bcde
LSD[g]	1.03	1.85	1.56	1.14	1.07	1.31	1.22	1.07
CM	5.0a	5.0a	5.0a	4.8ab	4.8a	4.5ab	4.5ab	3.8abcde
AM	4.8a	3.0dc	3.3bc	2.5e	4.0ab	3.5bcd	4.8ab	5.0a
SM	5.0a	4.8ab	4.5ab	4.3abc	1.3g	2.8de	2.8c	2.5efgh
MM	5.0a	4.8ab	5.0a	5.0a	3.8bc	4.3abc	4.3ab	3.5bcde
LSD[g]	0.40	0.80	1.00	0.70	0.67	1.51	1.31	1.51
CS	0.3e	0.0h	0.3fg	0.8de	3.3bcd	4.0bcd	3.8abc	2.0fgh
AS	2.0bc	3.5bcd	4.3ab	3.8cd	4.0ab	5.0a	4.8ab	4.3abcd
SS	0.8de	1.3fg	1.8de	0.5gh	2.8cde	4.3abc	3.8abc	2.5efgh
MS	5.0a	5.0a	5.0a	5.0a	1.3g	1.0fg	1.0d	1.3h
OS	2.5b	2.5de	4.5ab	4.0bcd	2.0efg	3.3bcd	3.5bc	1.8gh
LSD[g]	1.28	1.21	1.18	1.27	0.98	0.94	0.92	1.31
COM	5.0a	5.0a	4.5ab	4.8ab	3.3bcd	4.0abcd	4.3ab	3.8abcde
AOM	5.0a	4.5ab	4.0ab	2.8e	2.5def	3.8abcd	4.5ab	4.5abc
SOM	5.0a	4.5ab	3.8abc	4.8ab	1.8efg	2.0ef	3.8abc	3.0edfg
OM[2]	5.0a	5.0a	5.0a	5.0a	3.0bcd	4.5ab	5.0a	3.3cdef
LSD[g]	0.00	0.75	0.93	0.67	1.33	0.93	1.22	1.79
Con.	0.0e	0.0h	0.0g	0.0h	0.0h	0.0g	0.0d	0.0i
LSD[h]	0.9	1.1	1.1	0.8	0.9	1.0	1.0	1.2

[a] Results of each treatment were the mean of four replications. Results were obtained four weeks after the challenge inoculation.

[b] Disease ratings were based on 0~5 scale where 0=no disease, and 5=death of plantlets.

[c] Root discoloration ratings were based on 0~5 scale where 0=no color change, and 5=severe root discoloration.

[d] Day(s) of interval indicate(s) the day intervals between protection and challenge inoculations.

[e] Isolate combination inoculations as follows: CA=Con/Av, AA=Av/Av, SA=Fs/Av, CO=Con/Fo, AO=Av/Fo, SO=Fs/Fo, OO=Fo/Fo, CM=Con/Fm, Am=Av/Fm, Sm=Fs/Fm, MM=Fm/Fm, CS=Con/Fs, AS=Av/Fs, SS=Fs/Fs, MS=Fm/Fs, OS=Fo/FS, COM=Con/Fo+Fm, AOM=Av/Fo+Fm, SOM=Fs/Fo+Fm, and OM[2]=Fo+Fm/Fo+Fm, where Con=control, Av=avirulent *F. oxysporum* (AVFO), Fs=*F. solani*, Fo=virulent *F. oxysporum*, and Fm=*F. moniliforme*.

[f] Means with the same letter are not significantly different within the same interval day at p=0.05.

[g] Comparison of different treatment within each group within the same interval day (LSD at p=0.05).

[h] Comparison of different treatments within the same interval day (LSD at p=0.05).

combination inoculations at all different interval days.

All treatments showed significant differences in root discoloration in all different interval day inoculations except in three-day-interval inoculation of *F. moniliforme* and *F. solani* combination. Almost all different combinations of dual or triple *Fusarium* species caused visibly and statistically significant differences compared to the control treatment. Combinations of *F. moniliforme* and *F. solani* did not cause severe root discolorations at all different interval day inoculations. In one-day-in-

terval inoculation test, *F. solani* and *F. moniliforme* combination, *F. oxysporum* and *F. solani* combination, and *F. solani*, *F. oxysporum* and *F. moniliforme* combination treatments showed less significantly different root discoloration ratings compared to the control treatments. In three-day-interval inoculation tests, only *F. moniliforme* and *F. solani* combination, and *F. solani*, *F. oxysporum* and *F. moniliforme* combination showed less severe root discolorations. In five-day-interval inoculation test, only *F. moniliforme* and *F. solani* combination inoculation showed less severe root discolorations. In seven-day-interval inoculation, combinations of control and *F. solani*, *F. moniliforme* and *F. solani*, and *F. oxysporum* and *F. solani* caused less severe root discolorations compared to other combination treatments within the same interval day.

In a combination inoculation of AVFO and *F. moniliforme* where AVFO was used as a protection agent, there was no extensive heavy damage within the vicinity of epidermal areas, but there was heavy damage near tracheary elements and endodermal areas (Fig. 1A). In a combination inoculation of AVFO and *F. oxysporum*, there was heavy damage not only in vessel elements and parenchyma cells but also in epidermal areas (Fig. 1B). *F. solani* and *F. oxysporum* combination inoculation caused no extensive damage in parenchyma cells even if there was minor damage in epidermal regions and to vessel elements (Fig. 1C). *F. solani* and *F. moniliforme* in combination caused overall heavy damage in tracheary elements and tissues inside the ep-

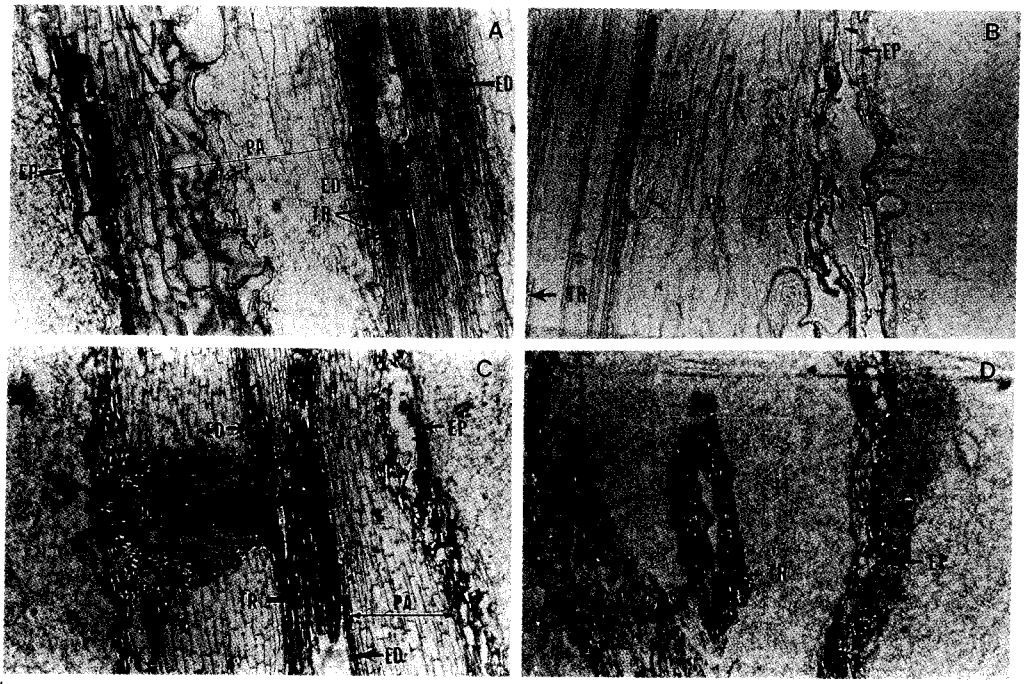

Fig. 1. Interactions of virulent and avirulent fusarial species on asparagus plantlet protection against *Fusarium* species with avirulent *Fusarium* species. A. Asparagus plantlet root tissues protected with avirulent *F. oxysporum* (AVFO) against *F. moniliforme* infection (×100). Note almost intact epidermal region, partially damaged parenchyma, and intact endodermal areas. B. Asparagus plantlet root tissues protected with avirulent *F. oxysporum* (AVFO) against virulent *F. oxysporum* (×100). Note heavy damages in tracheary elements, parenchyma cells, and epidermal areas. C. Asparagus plantlet root tissues protected with *F. solani* against *F. oxysporum* (×40). Note intact parenchyma cell regions, and minor damages in epidermal areas and vessel elements. D. Asparagus plantlet root tissues protected with *F. solani* against *F. moniliforme* (×30). Note overall heavy damage in tracheary element, and tissues inside the epidermis. Also, tissues inside the epidermal areas were totally dissolved with few cells left. Root pieces were embedded in paraffin, sectioned (10 μm), stained with aniline blue W. S. without removal of paraffin, and observed under light microscopy. ED : endodermis, EP : epidermis, IT : internal tissues destroyed, LR : lateral root, N : nuclei, PA : parenchyma, TR : tracheary element.

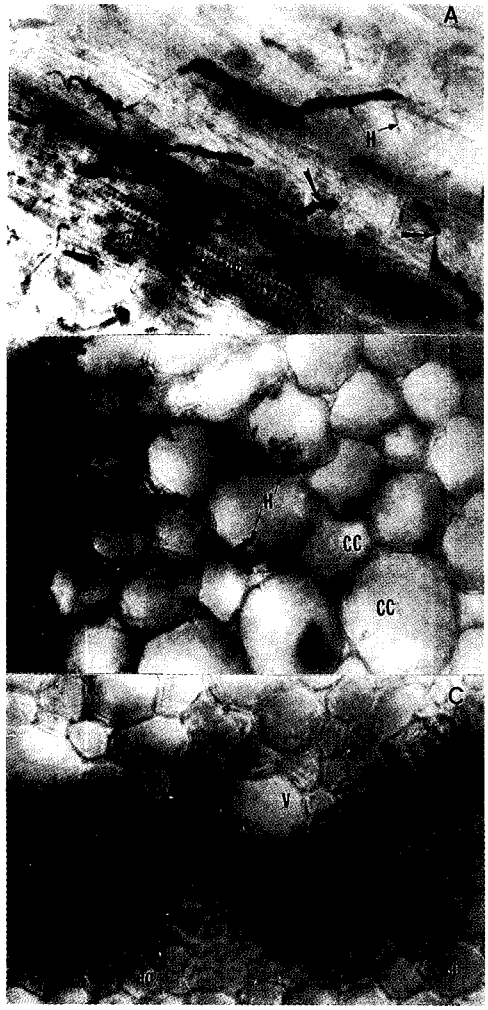

Fig. 2. Infection process of virulent and avirulent fusarial species within a short period. A. Longitudinal section of asparagus plantlet root infected with *F. oxysporum* 24 hours after inoculation (×370). Note appressorium-like structures (<---). Root pieces were cut by hand, mounted on slides, stained with aniline blue W. S., and observed under light microscopy. B. Avirulent *F. oxysporum* (AVFO) infection on cortical cells of asparagus plantlet stem seven days after inoculation (× 980). Note hyphal masses within cortical cells. Stem pieces were cut by hand, mounted on slides, stained with aniline blue W. S., and observed under light microscopy. C. *Fusarium moniliforme* infection on stem of asparagus plantlet two weeks after inoculation (×560). Note hyphal growth near or within vessel elements. Stem pieces were cut in cross section by hand, mounted on slides, stained with aniline blue W. S., and observed under light microscopy. CC : cortical cells, H : hyphae, V : vessel elements, XY : xylem.

idermal areas. This combination totally dissolved tissues inside the epidermal areas (Fig. 1D).

Infection process of virulent and avirulent *Fusarium* species on asparagus. All *Fusarium* species infected asparagus plantlets through primary and lateral root tips, natural wounds, and between walls of the epidermal tissues directly. Some penetration was possible through appressorium-like structure (Fig. 2A). Virulent *Fusarium* species penetrated tissues very quickly and infected the tissues faster than avirulent *Fusarium* species did. *F. moniliforme* grew abundantly within and outside the plantlet tissues, and *F. oxysporum* grew more abundantly within the cortical and epidermal tissues than outside of the epidermal and cortical tissues. AVFO showed similar growth pattern as virulent *F. oxysporum* did within a short period of growth stage (Figs. 2B and 2C). *Fusarium solani* did not show any growth within twenty-four hours after inoculation, and started to grow two days after inoculation. Most of infections in this early stage were intercellular and confined to cortical regions. This result supports previous reports by Christou and Snyder (6), Hancock (18), and Sparnicht and Roncadori (38). Some species grew outward from epidermal and cortical tissue regions. At later stages of infection, some species started to cause more intracellular infections as reported previously.

At the later stages of infection, virulent *Fusarium* species such as *F. oxysporum* and *F. moniliforme* caused heavy damage in epidermal, cortical, and xylem tissues. Highly virulent *F. moniliforme* infection dissolved the whole root tissues completely except the epidermal areas (Fig. 3A). AVFO isolate did not cause any major damage on plantlet tissues (Fig. 3B) except minor damage in root tip areas. Hyphae of AVFO isolate accumulated heavily in epidermal regions as evidenced by heavy staining with aniline blue W. S. (Fig. 3C). *F. solani* infection did not cause any damage on plantlet tissues except in limited areas of the epidermal region. *F. solani* promoted lateral root formation, and increased surface area of primary and lateral roots (Fig. 3D)

Histopathological studies showed that AVFO provided protection in asparagus plantlets by accumulation of hyphae on or around the epidermal area (Fig. 4), thus forming barriers against virulent *Fusarium* infections.

DISCUSSION

Results of pathogenicity tests indicate the difference

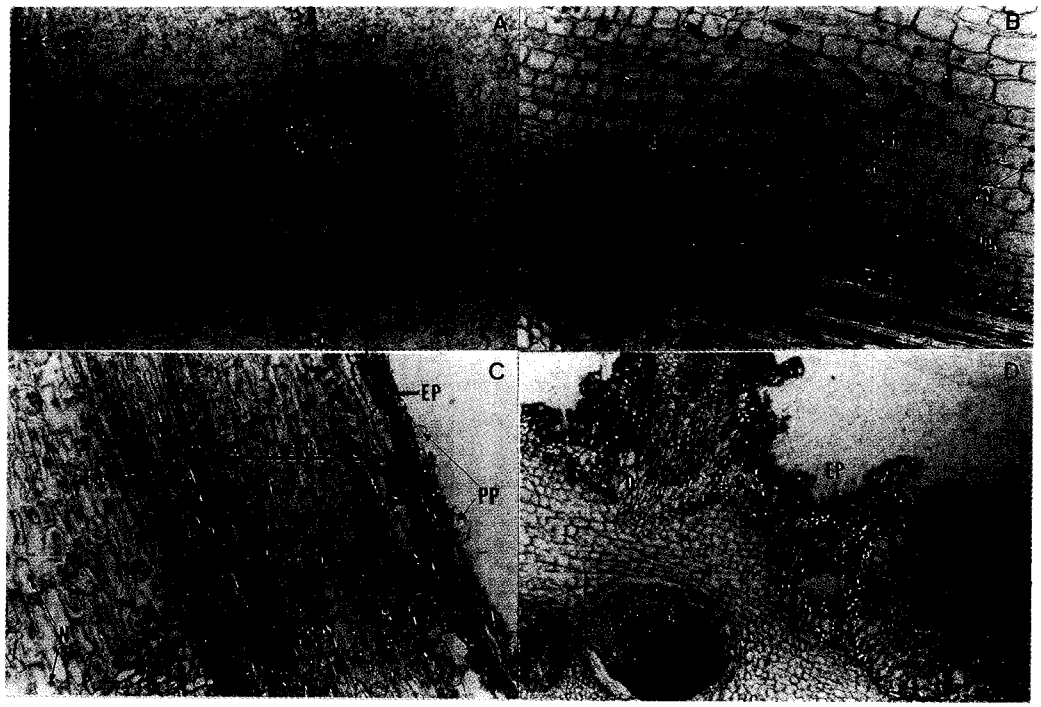

Fig. 3. Infection process of virulent and avirulent fusarial species on asparagus plantlets over extended period. A. *Fusarium moniliforme* infected stem tissues of asparagus plantlet four weeks after inoculation (×40). Note total destruction of tissues inside the epidermal areas. B. *Fusarium solani* infected root tissues of asparagus plantlet four weeks after inoculation (×100). Note intact parenchyma cells, vascular system, and endodermis. Lateral roots are growing vigorously as indicated by increased cells near lateral root growing regions. C. Avirulent *F. oxysporum* (AVFO) infected root tissues of asparagus plantlet four weeks after inoculation (×90). Note heavily stained areas within and around the epidermal area, intact tracheary element, and intact parenchyma cells. D. *Fusarium solani* infected root tissues of asparagus plantlet four weeks after inoculation (×40). Note proliferation of lateral roots, and minor damages on epidermal region. Also, note the increased surface area of plantlet root. Root pieces were embedded in paraffin, sectioned (10 μm), stained with aniline blue W. S. without removal of paraffin, and observed under light microscopy. CR : crystals, D : damaged area, ED : endodermis, EP : epidermis, IT : internal tissues destroyed, LR : lateral root, N : nuclei, PA : parenchyma, PP : paraffin particles, TR : tracheary element, VA : vascular system.

of infection capabilities among three different *Fusarium* species affecting asparagus in the field, which support previous reports by Damicone and Manning (11), Gibertson (15), and Manning (25) in Massachusetts, and LaMondia and Elmer (22) in Connecticut. Nigh (28) showed that pathogenicity on asparagus varies among the different *Fusarium* isolates.

Based on the results it is highly possible to use avirulent *Fusarium* species such as *F. oxysporum* and *F. solani* for the protection of asparagus plantlet against virulent *Fusarium* species such as *F. oxysporum*, and *F. moniliforme*. In this study, avirulent isolates successfully controlled virulent isolates of *Fusarium* species.

Fusarium species infect asparagus plantlets through primary and lateral root tips, natural wounds, and between the walls of the epidermal cells directly. Some penetration was appressorium-like and direct and the penetration was taking place between the walls of the epidermal cells, not directly through the outer walls unless there was a damaged tissue area. The same observations were reported previously by Graham (16). The meristematic region of the root was regarded as a major infection site because of heavy mycelial aggregation in that region as reported in previous studies (16, 36, 37). However, there were differences among virulent and avirulent *Fusarium* species in their speed of infection on asparagus plantlet root and stem tissues.

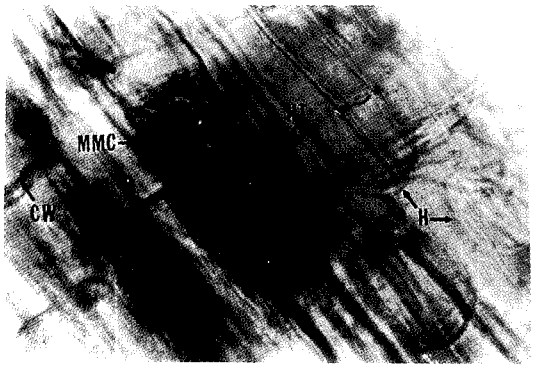

Fig. 4. Avirulent *F. oxysporum* (AVFO) infection on epidermal area of asparagus plantlet root seven days after inoculation (×100). Note mycelial coils (MMC) within a cell after penetration. Root pieces were cut longitudinally by hand, mounted on slides, stained with aniline blue W. S., and observed by light microscopy. CW : cell walls, H : hyphae, HT : hyphal tip, MMC : mycelial mats coiled.

F. moniliforme infected root tissues slightly faster than *F. oxysporum* did, not only within a twenty-four-hour periods but also during the whole infection processes. Hyphae of *F. moniliforme* grew more abundantly inside and outside of the epidermal and cortical tissues of asparagus plantlets than *F. oxysporum* did during the whole infection processes. Where *F. moniliforme* was infecting root tip areas, the hyphae were concentrated in the area of the root cap and along the epidermal area immediately basipetal to the root cap forming a heavy thallus network around the apex. The same phenomenon was observed in inoculations of *F. oxysporum* in root tissues. Where *F. moniliforme* was infecting stem tissues, heavy hyphal growth on the surface of asparagus plantlet stems was also observed. Hyphae of *F. oxysporum* grew more abundantly inside the tissues than outside of the tissues. Hyphae of *F. oxysporum* grew mainly intracellularly in root tissues. Hyphal growth and infection processes of AVFO were similar to those of virulent isolates of *F. oxysporum* except that AVFO accumulated more on the epidermal area than virulent *F. oxysporum* isolates did over a longer period. No fungal infection was observed within twenty-four hours of inoculation of the *F. solani* isolate, penetration was observed about 2 days after inoculation, and hyphae of *F. solani* grew very slowly and mostly remained in the epidermal and cortical areas in early stages of infection. This delayed penetration of *F. solani* is in contrast to the report by Buxton and Perry (4) who reported that *F. solani* was an aggressive colonizer of the root cortex of pea and reduced the pea wilt disease by *F. oxysporum*. However, results we have obtained support previous observations by Chi *et al.* (5).

During and after the initial infection by virulent *Fusarium* species, there were clear signs of symptom development in primary and lateral root tips, and epidermal tissues of roots and stems. In areas where symptoms were weakly expressed, there was a presence of small brownish elliptical regions of infection. Dark brown lesions were visible especially in root tip areas and epidermal tissues of stems near crown regions. These lesions seemed to represent a primary stage in the development of the symptoms. Some isolates of virulent *Fusarium* species also caused lesions near the points of origin of the lateral roots. Examination of roots and stems revealed masses of fungal hyphae in the cortical tissue. At this early stage of disease development, hyphae were developed externally from epidermal and cortical cells of asparagus plantlets in some occasions, and this result supports previous observations by Bennett (2), Dahl (8), and Pugh *et al.* (32).

As the disease progressed, hyphae penetrated into both intracellular and intercellular spaces. Some of the *F. moniliforme* and *F. oxysporum* hyphae were coiled just inside or outside of the cells before penetration into nearby cells. Some isolates of *F. moniliforme* and *F. oxysporum* formed coils within the plantlet cells just after penetration. Hyphae were located in the peripheral areas between the cells, and the hyphae extended for long distances in longitudinal sections of stems. Occasionally, lateral hyphae were found entering still intact cortical cells by means of a constriction and padlike swellings or appressorium-like structures.

Infected and stunted cells stained deeply with aniline blue W. S. and lactophenol (34), presumably because of the greater permeability of their walls. After the hyphae penetrated, they were generally intercellular. However, at later stages, they penetrated intercellularly and intracellularly, in lateral and vertical directions. The hyphae were variable in their manner of growth within the tissues of the asparagus root and stem and on the surface of root and stem, and formed numerous small swellings as they progressed through the cortex. These swellings can be regarded as appressoria because their formation apparently precedes the passage

of an extremely small intercellular space, or the penetration of a cell wall. The colonization of the host asparagus plantlet extends to the parenchyma and vessel elements.

AVFO did not cause any damage in xylem and parenchyma cell regions, and there were heavy accumulations of mycelial mats within and around the epidermis compared to the control treatment. This epidermal area was heavily stained with aniline blue W. S. In a *F. solani* infection, there was no extensive damage on cells except in limited areas of the epidermal region. *F. solani* infection caused minor damages on the epidermal regions even if it promoted lateral root formation. Also, *F. solani* increased the surface area of plantlet primary and lateral roots. Therefore, it was clear that *F. solani* reduced asparagus plantlet root rot and wilt diseases by promoting lateral root formation, and increasing the root surface areas. This promoted lateral root formation and increased surface area of primary and lateral roots might have been caused by undefined toxins produced by *F. solani*, or by mechanisms similar to those observed in mycorrhizal fungi.

In contrast to AVFO and *F. solani* infections, however, virulent *F. oxysporum* and *F. moniliforme* caused extensive damages on plantlet tissues. Both *F. oxysporum* and *F. moniliforme* caused heavy damages on vessel elements and epidermal regions. Highly virulent *F. moniliforme* infection caused quite extensive damage on plantlet tissues, and dissolved the tissues inside the epidermal areas completely. These observations support the facts that *F. oxysporum* and *F. moniliforme* cause cortical rots in the early stage and vascular system destruction over longer periods (17).

AVFO provided slightly better protection against *F. moniliforme* than it did against virulent *F. oxysporum*. In a combination of AVFO and *F. moniliforme* inoculation where AVFO was used as a protection agent, there was no extensive damage within the vicinity of epidermal areas, and there was heavy damage near tracheary elements and endodermal areas. However, this combination inoculation caused less damage compared to AVFO and *F. oxysporum* combination inoculation where AVFO was used as a protection agent. In this AVFO and *F. oxysporum* inoculation, not only vessel elements and parenchyma cells but also epidermal areas were damaged heavily. Therefore, AVFO formed mechanical barriers on the epidermal areas, and thus provided protection against virulent *Fusarium* infections. There are many reports of use of avirulent or weakly virulent isolates of *F. oxysporum* formae speciales for the control of diseases for each of different host plants, and they explained the mechanisms involved as competition for nutrients or space, or antagonisms among virulent and avirulent (or weakly virulent) isolates (12, 14, 29).

F. solani provided much better protection against *F. oxysporum* than it did against *F. moniliforme*. In a combination of *F. solani* and *F. oxysporum* inoculation, there was no extensive damage in parenchyma cell regions even if there were minor damages on epidermal areas and vessel elements. Compared to this combination inoculation, there was overall heavy damage of plantlet cells in a combination inoculation of *F. solani* and *F. moniliforme*. In this *F. solani* and *F. moniliforme* combination inoculation, there was damage in tracheary elements and tissues inside the epidermal areas. In this combination inoculation, tissues inside the epidermal areas were totally dissolved with few cells left, suggesting that *F. solani* reduced asparagus plantlet root rot and wilt diseases by promoting lateral root formation and increasing the root surface areas.

Histopathological studies showed that AVFO provided protection in asparagus plantlets by the accumulation of hyphae on or around the epidermal area (Fig. 4), thus forming barriers against virulent *Fusarium* infections. Similar results were reported previously (1, 7, 16, 31). However, these previous reports dealt mainly with periderm-like layer of cortical cells formed in reaction to fungal infections, instead of accumulation of hyphae on epidermal areas. *Fusarium solani* provided protection of asparagus plantlets by increasing lateral roots and increasing surface area of primary and secondary roots of asparagus plantlets.

These results are the first report of AVFO, isolated from bean hypocotyl, and *F. solani*, isolated from asparagus plants grown in the field, as protection agents on tissue-cultured asparagus plantlets against virulent *F. oxysporum* and *F. moniliforme* infections. Also, these results are the first report that elucidates the mechanical mechanisms of protection of AVFO and *F. solani* against virulent *F. oxysporum* and *F. moniliforme* infections on asparagus plantlets.

요 약

병원성 *Fusarium*에 의한 아스파라거스 감염은 비병원성 *Fusarium*을 5일과 7일 전에 접종하였을 때 방제효과가 있었다. 비병원성 *F. oxysporum*은 *F. monil-*

*iforme*에 대하여 방제 효과가 있었고, *F. solani*는 *F. oxysporum*에 대하여 방제효과가 있음이 밝혀졌다. 실험에 사용된 *Fusarium* 균들은 모두 주근과 측근의 말단 부위, 상처부위, 그리고 표피의 세포벽 사이를 통하여 감염하였다. 경우에 따라 감염하는 동안 appressorium과 유사한 구조를 형성하기도 하였고, 직접 감염하는 경우도 있었다. 병원성 그리고 비병원성 *Fusarium* 균 모두 공통적으로 생장점 부위를 통하여 감염하였다. 병원성이 강한 *Fusarium* 균의 경우 비병원성 균들보다 감염의 속도가 빨랐고 더욱 생장이 왕성하였다. *F. solani*는 생장속도나 기주 조직 침입속도가 매우 느렸다. 기주 감염의 결과 처음에는 cortical rot을 유발시켰고 나중에는 tracheary elements를 감염하고 결국은 조직의 괴사를 유발하는 것이 관찰되었다. 비병원성 *F. oxysporum*은 표피조직에 두터운 균사층을 형성하였고, 이는 병원성 *Fusarium* 균에 대한 방제효과를 나타내는 원인을 제공한 것으로 여겨진다. *F. solani*는 측근의 생성을 촉진시켜 표면적을 증대시킨 것으로 여겨진다. 결론적으로 AVFO와 *F. solani*를 이용하여 아스파라거스에 발생하는 병원성 *Fusarium* 균의 침입을 저지할 수 있는 생물적 방제가 가능함이 밝혀졌다.

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