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非病原性 *Fusarium*菌을 利用한 아스파라거스의 病原性 *Fusarium*菌의 生物的 防除

이윤수

Biological Management of Virulent *Fusarium* Species on Asparagus with Avirulent *Fusarium* Species *In Vitro*

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Abstract

Fusarium oxysporum was isolated most frequently, followed by *F. moniliforme*, and *F. solani* from infected asparagus plants grown in the field. In pathogenicity tests both with seedlings and plantlets, *F. moniliforme* showed higher virulence than *Fusarium oxysporum* did in general. *Fusarium moniliforme* showed more consistent virulence on both seedlings and plantlets than *F. oxysporum* did. *Fusarium oxysporum* showed higher virulence on plantlets than on seedlings. *Fusarium solani* showed very weak or no sign of virulence on seedlings and plantlets, respectively, in both tests. In protection tests with plantlets, most protection of asparagus against virulent fusarial infections occurred when challenge isolates were inoculated five or seven days after inoculation of protective fusarial species. Avirulent *F. oxysporum* was a more effective protective agent against infection of *F. moniliforme* than it was against *F. oxysporum*. *Fusarium solani* was more effective against infection of *F. oxysporum* than it was against *F. moniliforme*.

Introduction

Fusarium species associated with diseased asparagus were first reported in Massachusetts by Stone and Chapman[1], who isolated a *Fusarium* species from young, wilted asparagus shoots. Boyd[2,3] reported fusaria associated with diseased asparagus in Massachusetts, while

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Armstrong[4] described a very similar fusarial disease in other parts of U.S.A. Since Cohen and Heald[5] first described the causal agent of the wilt and root rot disease of asparagus to be *Fusarium oxysporum* (Schlect) var. *asparagi*, there were many studies on the root and crown rot diseases of asparagus by virulent fusarial species in California[6,7,8], Canada[9], Connecticut[10], Massachusetts[11,12,13,14,15,16], Michigan[17,18], New Jersey[19,20], and Washington[21,22]. Cohen and Heald[5] reported that the fungus was able to initiate a symptom complexes in the host, and colonize roots, crowns, and stems. They hypothesized that the pathogen survived as a saprophyte on senescing asparagus tissue during the winter and spring, and attacked living tissue in the summer.

It was found that *F. moniliforme* and *F. oxysporum* to be responsible for asparagus decline in Western Massachusetts[11]. *Fusarium oxysporum* was the major causal agent in the study. Both pathogens caused asparagus seedling death, and root and stem lesions on mature plants. *Fusarium oxysporum* parasitize storage and feeder roots, cortical tissue of stem bases, and vascular crown tissues; and exist in soil as saprophyte, as chlamydospores or in association with volunteer plants or weed hosts, while *F. moniliforme* attacks aboveground plant parts and survives on seeds, volunteer plants, and in association with asparagus miner flies[12,13,14].

LaMondia and Elmer[10] isolated *Fusarium moniliforme, F. oxysporum* and *F. solani* from symptomatic and asymptomatic feeder roots, storage roots, crown and basal stem segments. In their study, they found that *F. moniliforme* was more virulent than *F. oxysporum* on asparagus seedlings and *F. solani* was considered nonpathogenic, and they concluded that virulence on asparagus was a common trait with few exceptions among genetically distinct populations of *F. moniliforme* and *F. oxysporum* colonizing asparagus. In Western Massachusetts, Lee and Manning[15] found that *F. oxysporum* was isolated more frequently than *F. moniliforme* from diseased plants grown in the field, and *F. moniliforme* and *F. oxysporum*, isolated from diseased asparagus plants grown in the field, were the major causal agents of root and stem rot of asparagus seedlings and plantlets *in vitro*.

Malta[23] described the use of saprophytic or mildly pathogenic species, form species, or races of the pathogen for biological management of root rot diseases to protect the host against virulent forms. This so called "immunization"[23], "induced resistance"[23], or "cross protection"[9,24] has been the focus of many studies involving *Fusarium* wilt diseases. This method uses a pre-inoculation with the mild saprophytic parasite, certain periods of incubation, and challenge inoculations. The mild saprophyte colonizes the cortex and/or vascular system without causing severe symptoms and is able to ward off colonization by the pathogens.

There were attempts at cross protection and few success has been reported in the field and/or greenhouse. Graham[9] used mixed inoculations of asparagus with both *F. oxysporum* and *F. moniliforme* which resulted in reduced seedling blight than when either isolate was used alone. Damicone and Manning[25], and Manning[26] developed methods to obtain aviru-

lent isolates of *F. oxysporum* (Isolate 1B) from bean hypocotyl surfaces and to determine their efficacy in protecting asparagus seedlings in gnotobiotic culture and in naturally-infested soil in the greenhouse. In their study, use of isolate 1B resulted in significantly larger plants with reduced disease symptoms compared with the control. Treatment with isolate 1B conferred protection comparable to that achieved with a 0.1% benomyl fungicide treatment. Tu *et al.*[27] used a saprophytic isolate of *Fusarium oxysporum* nonpathogenic to asparagus to reduce the severity of the disease in pot tests. Lee and Manning[16] achieved reduction of root and crown rot of tissue-cultured asparagus plantlets *in vitro* by prior inoculation with an avirulent isolate of *F. oxysporum* (AVFO) obtained from bean hypocotyl surfaces[26].

There are no effective strategies available against *Fusarium* disease on asparagus. There are no effective resistant varieties available, and chemical control has met with limited success[18,28]. Also, there were few reports of successful biological management of *Fusarium* disease on asparagus. Therefore, based on reports on stem, crown and root rot of asparagus in Western Massachusetts[14] and in Connecticut[10], and on biological management of *Fusarium* disease on asparagus with antagonistic soil microorganisms[12] or avirulent *Fusarium oxysporum*[26], a study was undertaken 1) to isolate *Fusarium* species from field grown asparagus plants, 2) to perform pathogenicity tests and compare susceptibility of seedlings and plantlets to different *Fusarium* species obtained, 3) to determine the possible use of avirulent *Fusarium* species to protect against infection on asparagus plantlets by virulent *Fusarium* species, and 4) to determine the time between induction and challenge inoculation that gives the highest levels of protection, if there is any, through *in vitro* tests with plantlets.

Materials and Methods

Isolation of fusarial species from infected asparagus plants. Asparagus plants showing typical crown and root rot symptoms were obtained from several locations in Amherst, MA and Sunderland, MA. For chlamydospore formation, edges of crown and root rot lesions were cut out and surface sterilized with 10% Clorox bleach (v/v) for 3 minutes and rinsed three times with sterile distilled water before plating on 2% water agar medium. Komada's medium[29] was chosen for selective isolation of *Fusarium oxysporum*, and KCl medium[30] was used for conidial chain formation by *Fusarium moniliforme*. Carnation leaf agar (carnation leaves on 2% water agar)[31,32], for conidia and conidiophore formations, was used for accurate identification. Also, different colors of fungal growth on PDA medium[33] among different species of *Fusarium* were used as criteria for identification. Isolation of *Fusarium* species from original plates, and subsequent transfers were made by using single spore isolation technique[33] to eliminate most of the problems associated with variability and difficulty of identification. Final identification of each species was based on the taxonomic key by Nelson *et al*[33]. Selected isolates were preserved in silica [34] for further experiments.

Pathogenicity tests with seedlings. Seeds of asparagus clone Mary Washington were surface-sterilized with 25% benomyl in acetone (100%) for overnight followed by washing with sterile distilled water three times to remove benomyl residues[35]. Washed seeds were treated with 10% Clorox bleach (v/v) for a few minutes and washed with sterile distilled water three times. Cleaned seeds were soaked for 24 hours in sterile distilled water to facilitate seed germination. Soaked seeds were then blotted on sterile filter paper before plating on 0.6% water agar for 2 weeks for germination and initial growth. Germinated seedlings were aseptically trasplanted on Hoagland solution[36] slants (25mm test tubes) and were established on the growth bench for 3 days at room temperatures. Two agar discs (control) or two agar discs bearing a *Fusarium* isolate, were placed in contact with roots, just below the crown. Four isolates of *Fusarium oxysporum* (Isolates 19, 45, 48, and 49), three isolates of *F. moniliforme* (Isolates 24, 28, and 37), two avirulent *F. oxysporum* (AVFO) isolates (Isolates AVFO-1 and AVFO-2) obtained previously from bean hypocotyl surfaces[26], and one isolate of *F. solani* were used as inocula. Seedlings were evaluated for disease incidence four weeks after the inoculation. The experiment was repeated twice.

Pathogenicity tests with plantlets. Asparagus plantlets (Female clone, NJ362M) were obtained through meristem tip culture[37,38], rooted on rooting medium, increased on multiplication medium[39,40,41], and placed on filter paper slants in test tubes (25mm) containing Hoagland solution. For the inoculations and evaluations, the same isolates and methods as described for seedling pathogenicity tests were used.

Interactions of virulent and avirulent *Fusarium* species on asparagus plantlets *in vitro*. Asparagus plantlets obtained through meristem tip cultrue were cultivated on multiplication medium for 2−3 months, and placed on filter paper slants in test tubes containing Hoagland solution[36]. Two control agar discs, or two agar discs bearing a *Fusarium* isolate, were placed in contact with roots, just below the crown. Platlets were inoculated with an avirulent isolate of *F. oxysporum* (AVFO) or *F. solani* isolate, and then challenged with an isolate of *F. moniliforme* or a virulent *F. oxysporum* isolate at 1-, 3-, 5-, and 7-day intervals after inoculation of an avirulent *F. oxysporum* (AVFO) or *F. solani*.

Results and Discussion

Isolation of fusarial species. Seventeen isolates of *F. oxysporum* (50% of the total), three isolates of *F. moniliforme* (9% of the total), one isolate of *F. solani* (3% of the total), and thirteen isolates of unknowns (38% of the total) were obtained from a total of thirty-four selected subculture plates (Table 1). These results indicate the difference of saprobic capabilities among three different *Fusarium* species affecting asparagus in the field. They support previously reported results by Damicone and Manning[13], Gilbertson[14], and Manning[26] in Western Massachusetts, and LaMondia and Elmer[10] in Connecticut area. An avirulent *F. oxysporum* (AVFO) isolate was obtained from

Table 1. *Fusarium* species isolated from root and stem pieces of asparagus plants grown in the field.

ID.#	Fungal species	ID.#	Fungal species
AS17	*F. oxysporum* 17	AS34	Unknown *Fusarium* sp.
AS18	*F. solani*	AS35	*F. oxysporum* 35
AS19	*F. oxysporum* 19	AS36	*F. oxysporum* 36
AS20	Unknown *Fusarium* sp.	AS37	*F. moniliforme* 37
AS21	*F. oxysporum* 21	AS38	*F. oxysporum* 38
AS22	*F. oxysporum* 22	AS39	Unknown *Fusarium* sp.
AS23	*F. oxysporum* 23	AS40	Unknown *Fusarium* sp.
AS24	*F. moniliforme* 24	AS41	Unknown *Fusarium* sp.
AS25	*F. oxysporum* 25	AS42	Unknown *Fusarium* sp.
AS26	*F. oxysporum* 26	AS43	Unknown *Fusarium* sp.
AS27	*F. oxysporum* 27	AS44	Unknown *Fusarium* sp.
AS28	*F. moniliforme* 28	AS45	*F. oxysporum* 45
AS29	Unknown *Fusarium* sp.	AS46	Unknmown *Fusarium* sp.
AS30	Unknown *Fusarium* sp.	AS47	*F. oxysporum* 47
AS31	*F. oxysporum* 31	AS48	*F. oxysporum* 48
AS32	Unknown *Fusarium* sp.	AS49	*F. oxysporum* 49
AS33	Unknown *Fusarium* sp.	AS50	*F. oxysporum* 50

bean hypocotyl surfaces[26].

Pathogenicity tests with seedlings. Seedlings inoculated with all isolates of *F. moniliforme* and *F. oxysporum* showed severe stunting of growth, and showed significant differences from seedlings treated with agar plugs without fusarial species (control), and from seedlings inoculated with avirulent *F. oxysporum* (AVFO) or *F. solani* (Table 2). *Fusarium moniliforme* and *F. oxysporum* did not show significant differences in disease incidence between the two. *Fusarium moniliforme* killed all tested seedlings, and *Fusarium oxysporum* caused severe stunting or death of seedlings. Even though there were statistically significant differences from controls, avirulent *F. oxysporum* (AVFO) isolates and *F. solani* isolates caused mild disease symptoms occasionally with increased root discoloration. *Fusarium solani* isolates caused severe root discoloration, no severe disease symptoms, and appeared to mildly stimulate seedling growth by showing increased seedling vigor.

Pathogenicity tests with plantlets. All plantlets inoculated with *F. moniliforme* isolates were killed (Table 2). Isolates of virulent *F. oxysporum* killed some plantlets, but caused only moderate disease in others for tests with plantlets. There were significant differences between plantlets inoculated with all isolates of *F. moniliforme* or *F. oxysporum* isolates 48 and 49, and control plantlets, and there were no significant differences between plantlets inoculated with avirulent *F. oxysporum* (AVFO) iso-

Table 2. Results of *in vitro* pathogenicity test with asparagus seedlings[u].

\Rep[v]	Root rot rating means[w]				
Iso[x]\	1	2	3	4	Ave[y]
AVFO-1	1	2	1	1	1.3b
AVFO-2	1	1	2	1	1.3b
FM 24	5	5	5	5	5.0a
FM 28	5	5	5	5	5.0a
FM 37	5	5	5	5	5.0a
FO 19	4	5	5	4	4.5a
FO 45	5	5	4	3	4.3a
FO 48	5	5	4	3	4.3a
FO 49	5	5	5	5	5.0a
FS	1	2	1	1	1.3b
CTR[z]	0	0	0	0	0c
LSD(P=0.05)					0.74

[u] Results of each replication were the mean of four observations.
[v] Rep=replications.
[w] Disease ratings were based on 0-5 scale where 0=no disease, and 5=death of seedlings.
[x] Iso=isolates as follows; AVFO=avirulent *F. oxysporum*, FM=*F. moniliforme*, FO=virulent *F. oxysporum*, and FS=*F. solani*.
[y] Ave=average. Means with the same letter are not significantly different at P=0.05, as etermined by a t-test.
[z] CTR=control.

late and control plantlets. Also, there were no significant differences between plantlets inoculated with *F. solani* and control plantlets. Isolates of *F. solani*, *F. moniliforme*, and *F. oxysporum* caused moderate to very severe root discolorations. All isolates of *Fusarium* species caused significant differences in root discoloration compared to those of control plantlets. However, there were no visible root discoloration differences between plantlets inoculated with avirulent *F. oxysporum* (AVFO) and those inoculated with pure agar plugs (control).

Susceptibility of asparagus seedlings and plantlets to different *Fusarium* species infection. Avirulent *F. oxysporum* (AVFO) isolates showed negligible virulence on seedlings even if there was a statistical difference when compared to control treatment (Table 2). However, in pathogenicity test on plants (Table 3), avirulent *F. oxysporum* (AVFO) did not show any difference from plantlets with control treatments. *Fusarium moniliforme* isolates caused serve to very severe root rot symptoms on both tests with seedlings and plantlets. *Fusarium moniliforme* isolates caused severe rot symptoms or death of seedlings (Table 2), and all *F. moniliforme* isolates caused

Table 3. Results of *in vitro* pathogenicity test with asparagus plantlets[t].

\Rep[u]	Root Rot Rating Means[v]					Root Discoloration Rating Means[w]				
Iso[x]\	1	2	3	4	Ave[y]	1	2	3	4	Ave[y]
AVFO-1	0	1	1	1	0.8c	2	3	3	2	2.5bcd
AVFO-2	0	1	1	0	0.5c	2	2	2	3	2.3cd
FM24	5	5	5	5	5.0a	5	5	5	5	5.0a
FM28	5	5	5	5	5.0a	5	5	5	5	5.0a
FM37	5	5	5	5	5.0a	5	5	5	5	5.0a
FO19	1	5	2	5	3.3b	1	3	2	3	2.3cd
FO45	1	5	5	5	4.0ab	1	2	2	3	2.0d
FO48	5	5	5	5	5.0a	2	3	3	4	3.0bc
FO49	5	5	5	5	5.0a	2	3	3	4	3.0bc
FS	0	0	0	0	0c	3	3	4	3	3.3b
CTR[z]	0	0	0	0	0c	0	0	0	0	0e
LSD(P=0.05)					1.35					0.80

[t] Results of each replication were the mean of four observations.
[u] Rep=replications.
[v] Root rot ratings were based on 0−5 scale where 0=no disease, and 5=death of plantlets.
[w] Root discoloration ratings were based on 0−5 scale where 0=no color change and 5=severe discoloration.
[x] Iso=isolates as follows; AVFO=avirulent *F. oxysporum*, FM=*F. moniliforme*, FO=virulent *F. oxysporum*, and FS=*F. solani*.
[y] Ave=average. Means with the same letter are not significantly different at P=0.05, as determined by a t-test.
[z] CTR=control.

death of plantlets (Table 3). Overall, *F. moniliforme* isolates showed higher virulence on plantlets than on seedlings. Isolates of *F. oxysporum* showed slightly different pathogenicity on seedlings or plantlets with different treatments (Tables 2 and 3). Some *F. oxysporum* isolates caused severe rot symptoms on both seedlings and plantlets, and some caused death of seedlings and plantlets. However, *F. oxysporum* isolates showed higher virulence on plantlets than on seedlings. *Fusarium solani* isolates showed weak virulence on seedlings (Table 2), and did not show any sign of virulence on plantlets (Table 3). Nigh[42] showed that pathogenic variability on asparagus plant exists between the different fusarial isolates from diverse geographic areas. Also, different clones of asparagus used for seedling (Mary Washington) and plantlet (New Jersey) pathogenicity tests might caused minor differences in susceptibility of seedlings and plantlets to infections of different fusarial species.

In conclusion, however, it was found that isolates of *F. moniliforme* and *F. oxysporum* caused severe to highly severe disease symptoms on both seedlings and plantlets, whereas

Table 4. Results of *in vitro* protection test with asparagus plantlets[s].

Is＼Cm[v]	Root Rot Rating Means[t]				Root Discoloration Means[u]			
	Day(s) of Interval				Day(s) of Interval			
	1	3	5	7	1	3	5	7
CA	0.5de[w]	1.8ef	2.5cd	0.9fgh	4.0ab	4.0abcd	4.3ab	4.5abc
AA	1.8bc	3.5bcd	3.3bc	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[x]	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[x]	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[x]	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	2.5d	2.5de	4.5ab	4.0bcd	2.0efg	3.3bcd	3.5bc	1.8gh
LSD[x]	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[z]	5.0a	5.0a	5.0a	5.0a	3.0bcd	4.5ab	5.0a	3.3cdef
LSD[x]	0.00	0.75	0.93	0.67	1.33	0.93	1.22	1.79
CTR[y]	0.0e	0.0h	0.0g	0.0h	0.0h	0.0g	0.0d	0.0i
LSD[z]	0.9	1.1	1.1	0.8	0.9	1.0	1.0	1.2

[s] Results of each treatment were the mean of four replications.

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

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

[v] 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, OM[z]=Fo+Fm/Fo+Fm, and CON=control where Con=control, AV=avirulent *F. oxysporum* (AVFO), Fs=*F. solani*, Fo=virulent *F. oxysporum*, and Fm=*F. moniliforme*.

[w] Means with the same letter are not significantly different within the same interval day at P=0.05, as determined by a t-test.

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

[y] CTR=control.

[z] Comparison of different treatment within the same interval day (LSD at P=0.05).

those of avirulent *F. oxysporum* (AVFO) and *F. solani* did not cause much damage or no damage at all on both seedlings and plantlets. Overall, there were no differences between seedlings and plantlets in susceptibility by virulent fusarial infections. It was also found that there was no difference in infectivity of avirulent *F. oxysporum* (AVFO) and *F. solani* on both seedlings and plantlets.

Interactions of virulent and avirulent *Fusarium* species on asparagus plantlet infection. Root rots. All different treatments showed significant differences in root rot ratings in all different interval day inoculations except in conrol and *F. solani* combination treatments in one and three day interval inoculations (Table 4). Combination treatments of control and avirulent *F. oxysporum* (AVFO), *F. solani* and *F. oxysporum*, and *F. solani* and *F. solani* did not show visible differences compared to control treatment. In these treatments, it has been shown that *F. solani* isolate provided better protection against *F. oxysporum* than against *F. moniliforme* in all inoculations of different interval days. *Fusarium solani* also reduced the mild virulence of single or double inoculations of an avirulent *F. oxysporum* (AVFO) isolate in all inoculations of different interval days. Avirulent *F. oxysporum* (AVFO) isolate did not provide much protection against *F. oxysporum* in all interval days except in seven days interval inoculation. *Fusarium solani* isolate provided much better protection against *F. oxysporum* than avirulent *F. oxysporum* (AVFO) did. However, avirulent *F. oxysporum* (AVFO) provided significant protection against virulent *F. oxysporum* at five and seven days interval inoculations. Avirulent *F. oxysporum* (AVFO) isolate provided much better protection against *F. moniliforme* in three, five and seven days 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. *Fusarium 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. Avirulent *F. oxysporum* (AVFO) and *F. solani* combination inoculations caused less severe root rot symptoms than those caused by *F. moniliforme* and *F. solani* combination inoculations. Avirulent *F. oxysporum* (AVFO), *F. oxysporum*, and *F. moniliforme* combination inoculations did not show much visible difference compared to control treatment at seven days interval inoculation, and its combination inoculation reduced root rot symptoms at five and seven days interval inoculations compared to other combination inoculations at all different interval days.

Root discoloration. All different treatments showed significant differences in all different interval day inoculations except in three days 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 control treatment. Combination of *F. moniliforme* and *F. solani* did not cause severe root discolorations at all different interval day inoculations. In one day interval inoculation test, *F. solani* and *F. moniliforme*

combination, *F. oxysporum* and *F. solani* combination, and *F. solani*, *F. oxysporum* and *F. moniliforme* combination showed less significantly different root discoloration ratings compared to control treatments. In three days 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.

Based on these results it is highly possible to use avirulent fusarial species such as avirulent *F. oxysporum* (AVFO) and *F. solani* for the protection of asparagus plantlet against virulent fusarial species such as *F. oxysporum* and *F. moniliforme*. Based on previous studies [43,44] it is desirable to study possible mechanical exclusion or localized resistance by non-pathogen. Buxton and Perry[45] used *F. solani* to reduce pea wilt disease caused by *F. oxysporum*. In their study, they concluded that *F. solani* was a more aggressive colonizer of the root cortex which resulted in a hypersensitive reaction in the host tissue that prevented entrance by the wilt fungus *F. oxysporum*. However, in this study with asparagus plantlets, *F. solani* was not an aggressive colonizer of any tissue. Therefore, it is highly possible that some other mechanisms might be operative in protection against virulent fusarial species infections. This requires further investigation on the role of *F. solani* in protection of asparagus plantlet against virulent fusarial species infections.

Also, further study is necessary on the role of avirulent *F. oxysporum* (AVFO) in protection of asparagus plantlet against virulent fusarial species infections. Furthermore, studies to maximize the most effective combination of avirulent *F. oxysporum* (AVFO) and *F. solani* isolates to obtain maximum protection against individual *F. oxysporum* or *F. moniliforme* infection, or their combination infections are necessary. These further studies are especially essential for the management of fusarial infection on asparagus seedlings or plantlets in field or greenhouse conditions.

요 약

재배지에서 경작된 아스파라거스의 이병조직으로 부터 *Fusarium oxysporum*이 가장 많이 분리되었고 *F. moniliforme*와 *F. solani*가 그다음 순으로 분리되었다. 유묘와 조직배양을 통해 얻은 개체를 이용한 병원성 검정결과, *F. oxysporum*과 *F. moniliforme*는 병원성이 강한 것으로 밝혀졌고, *F. solani*와 잠두의 배축으로 부터 분리한 비병원성 *F. oxysporum* (AVFO)은 병원성이 약하거나 없는 것으로 밝혀졌다. 또한, *F. moniliforme*가 *F. oxysporum* 보다 병원성이 더 강한 것으로 밝혀졌고, *F. moniliforme*가 *F. oxysporum* 보다 더욱 일관된 병원성을 지니고 있음이 밝혀졌다. 비병원성 *Fusarium*균을 이용한 생물적 방제 실험 결과, 병원성 균주를 접종하기 5－7일 전에 비병원성 균주들을 접종하여 방제효과를 거둘 수 있었다. 비병원성 *F. oxysporum* (AVFO)은 *F. oxysporum*에 대해서 보다 *F. moniliforme*에 대해 더욱 효과적인 방제효과를 나타냈고, *F. so-*

*lani*는 *F. moniliforme*에 대해서 보다 *F. oxysporum*의 방제에 더욱 효과적이었다.

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