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Note

Comparison of Susceptibility of Asparagus (*Asparagus officinalis* L.) Plantlets and Seedlings to Different *Fusarium* Species

Youn Su Lee*
Institute of Agricultural Science, College of Agriculture,
Kangwon National University, Chuncheon 200-701, Korea

아스파라거스(*Asparagus officinalis* L.) 유묘와 기내배양 식물체의 *Fusarium* species에 대한 감수성 비교

이 윤 수*
강원대학교 농과대학 농업과학연구소

ABSTRACT : Comparison of susceptibility of asparagus (*Asparagus officinalis* L.) seedlings and plantlets to different fusarial species was made to determine whether *in vitro* propagated asparagus plantlets can be used as a substitute for seedlings in histopathological study on the infection processes of *Fusarium* species to asparagus. *Fusarium oxysporum* was isolated most frequently (50% of the total) from lesions of root and crown rot of asparagus cultivated in the field followed by *F. moniliforme* (8.8% of the total) and *F. solani* (2.9% of the total). Plantlets and seedlings of all asparagus were susceptible to *F. moniliforme* and *F. oxysporum* isolates, but those were not susceptible to both avirulent *F. oxysporum* (AVFO) and *F. solani* in pathogenicity tests. Overall, there were no differences between seedlings and plantlets in the susceptibility to virulent fusarial infections. *In vitro* propagated asparagus plantlets, therefore, could be used as a substitute for seedlings in histopathological study on the infection processes of *Fusarium* species to asparagus.

***Key words* : *Asparagus officinalis*, *Fusarium* species, susceptibility, seedling, plantlet.**

A histopathological study program to determine the mechanisms involved in biocontol of virulent species of *Fusarium* with avirulent fusarial species on asparagus plants was planned. As a initial step for the research, the susceptibility of asparagus plantlets and seedlings (2, 8, 9, 13), and the difference of their susceptibility to virulent *Fusarium* species were to be determined because the research work was intended to be performed with clean, soil-free asparagus plantlet roots. Also, it is generally known that plantlets are genetically more stable than seedlings, and the research work was determined to be performed with *in vitro* propagated plantlets to obtain uniform results. Therefore, the purposes of the research were to perform pathogenicity tests to determine the susceptibility of asparagus seedlings and plantlets to different *Fusarium* species isolated from field cultivated asparagus roots and crowns, and to determine whether asparagus plantlets, instead of seedlings, can be used as plant materials for *in vitro* studies to investigate the mechanisms of interactions between virulent and avirulent *Fusarium* species during infection on asparagus.

Asparagus shoots (clone 'New Jersy', female, NJ 362M) were obtained from asparagus field in Amherst, MA, and washed with 10% Clorox bleach (v/v) for 3 minutes and rinsed three times with sterile distilled water before meristems were harvested with forcepts under sterile conditions. Obtained meristems were *in vitro* propagated using previously reported methods (5, 11, 15, 18, 20, 21) for 2~3 months before pathogenicity test. Asparagus plants showing typical root and crown rot symptoms were obtained from several locations in Amherst and Sunderland, MA. Isolation and identification of *Fusarium* species were made based on previously reported methods (1, 6, 7, 12, 16, 19). Selected isolates were preserved in silica (19) for further experiments. Surface-steriliza-

*Corresponding author.

tion of seeds (clone 'Mary Washington') was done following methods described by Damicone *et al.* (3). 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 transplanted on Hoagland solution (10) slants (25 mm test tubes) and were established on the growth bench for 3 days at room temperature. 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* (Isolate 19, 45, 48, and 49), three isolates of *F. moniliforme* (Isolate 24, 28, and 37), an avirulent *F. oxysporum* (AVFO) isolate obtained from bean hypocotyl surfaces (14), and an isolate of *F. solani* were used as inocula. Seedlings were evaluated for disease incidence four weeks after inoculation.

Table 1. Isolation frequency of *Fusarium* species from necrotic lesions of root and crown rot of asparagus (*Asparagus officinalis* L.) cultivated in the field

Fusarium species	Isolation frequency (%)
F. oxysporum	50.0%
F. moniliforme	8.8%
F. solani	2.9%
Unidentified *Fusarium* spp.	38.3%

In vitro propagated asparagus plantlets were placed on filter paper slants in test tubes (25 mm) containing Hoagland solution. Two agar discs without inoculum (control) or two agar discs containing a *Fusarium* isolate, were placed in contact with roots, just below the crown. The same isolates as those used for seedling pathogenicity test were used for plantlet pathogenicity test. Plantlets were evaluated for disease incidence 4 weeks after inoculation.

Almost all the meristems (98% of the total) placed on the nutrient rooting media produced roots within four weeks. Subsequent *in vitro* propagation was successful enough to have ample quantity of plantlet materials. Seventeen isolates of *F. oxysporum*, three isolates of *F. moniliforme*, one isolate of *F. solani*, and thirteen isolates of unidentified *Fusarium* sp. were obtained. An avirulent *F. oxysporum* (AVFO) isolate was obtained previously from bean hypocotyl surfaces (14). *Fusarium oxysporum* was isolated most frequently (50%), followed by *F. moniliforme* (8.8%), and *F. solani* (2.9%) (Table 1). 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 inoculum (control), and from seedlings inoculated with avirulent *F. oxysporum* (AVFO) or *F. solani* (Table 2). *Fusarium moniliforme* and *F. oxysporum* did not show significant differen-

Table 2. Pathogenicity of *Fusarium* species to asparagus (*Asparagus officinalis* L.) seedlings and plantlets[a]

Isolates	Replication	Disease ratings[b]									
		Seedlings					Plantlets				
		1	2	3	4	Mean[c]	1	2	3	4	Mean[c]
F. moniliforme	24	3	3	3	4	3.3 b	5	5	5	5	5.0 a
F. moniliforme	28	3	4	4	5	4.0 a	5	5	5	5	5.0 a
F. moniliforme	37	4	4	4	4	4.0 a	5	5	5	5	5.0 a
F. oxysporum	19	4	3	3	4	3.5 a	2	2	3	3	2.5 b
F. oxysporum	45	4	3	4	3	3.5 a	1	5	5	5	4.0 a
F. oxysporum	48	4	3	3	3	3.3 b	5	5	5	5	5.0 a
F. oxysporum	49	3	4	3	4	3.5 a	5	5	5	5	5.0 a
Avirulent *F. oxysporum*		1	1	1	1	1.0 c	1	0	0	1	0.5 c
F. solani		1	2	1	1	1.3 c	0	0	0	0	0.0 c
Control		0	0	0	0	0.0 d	0	0	0	0	0.0 c
LSD (p=0.05)						0.67					1.01

[a] Seedlings and plantlets were inoculated with *Fusarium* species in test tubes with Hoagland solution.
[b] Disease ratings were based on 0~5 scales; 0=no diseases, and 5=death of seedlings and plantlets.
[c] Means with the same letter are not significantly different at p=0.05 (t-test)

ces in disease incidence between the two. However, *F. moniliforme* caused slightly more severe stunting of seedlings than *F. oxysporum* did. *Fusarium solani* isolates caused no severe disease symptoms, and appeared to mildly stimulate seedling growth by showing increased seedling vigor. All plantlets inoculated with *F. moniliforme* isolates were killed (Table 2). Isolates of virulent *F. oxysporum* killed some plantlets, but caused moderate disease in others. In the test, 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) isolate and control plantlets. Also, there were no significant differences between plantlets inoculated with *F. solani* and control plantlets. In conclusion, it was found that isolates of *F. moniliforme* and *F. oxysporum* caused highly severe disease symptoms on plantlets, whereas those of avirulent *F. oxysporum* (AVFO) and *F. solani* did not cause much damage or no damage at all on plantlets.

Differences in isolation rate of *Fusarium* species might indicate the differences of saprobic capabilities among three different *Fusarium* species affecting asparagus in the field. This result support previous reports by Gilbertson (8), Manning (14), and Damicone and Manning (4) in Western Massachusetts, and LaMondia and Elmer (13) in Connecticut area. In pathogenicity test on seedlings and plantlets (Table 2), avirulent *F. oxysporum* (AVFO) did not show any difference from seedlings and plantlets with control treatments, respectively. *Fusarium moniliforme* isolates caused very severe root rot symptoms on both seedlings and plantlets. *Fusarium moniliforme* isolates caused severe rot symptoms or death of seedlings, and all *F. moniliforme* isolates caused death of plantlets. Overall, *F. moniliforme* isolates showed higher virulence on plantlets than on seedlings. Isolates of *F. oxysporum* showed minor differences in pathogenicity on seedlings or plantlets with different treatments. Some *F. oxysporum* isolates caused severe rot symptoms on both seedlings and plantlets, and some caused death of plantlets. However, *F. oxysporum* isolates showed higher virulence on plantlets than on seedlings. *Fusarium solani* isolates showed weak virulence on seedlings, and did not show any sign of virulence on plantlets. Nigh (17) 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 (clone 'Mary Washington') and plantlet (clone '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 disease symptoms on both seedlings and plantlets, whereas those of avirulent *F. oxysporum* (AVFO) and *F. solani* did not cause damage on both seedlings and plantlets. There were no differences between seedlings and plantlets in susceptibility to virulent fusarial infections. It was also found that both avirulent *F. oxysporum* (AVFO) and *F. solani* showed no differences in infectivity on both seedlings and plantlets. Overall, it was found that *in vitro* propagated asparagus plantlets, instead of seedlings, can be used for studies to investigate the infection processes of *Fusarium* species and the mechanisms of interactions between virulent and avirulent *Fusarium* species infection on asparagus.

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요 약

Fusarium species가 아스파라거스를 침입할 경우 병원성과 비병원성 *Fusarium*이 어떻게 상호작용 하는가를 구명하는 연구에 기내에서 계대배양된 아스파라거스를 포장에서 재배된 아스파라거스와 동일하게 *Fusarium* species의 기주감염 과정연구에 공시재료로 사용 할 수 있는지를 알고자 두 재료식물의 병에대한 감수성을 비교하였다. 경작지에서 생장된 아스파라거스의 이병조직으로부터 *F. oxysporum*이 가장많이 분리되었고 (전체의 50%), *F. moniliforme*와 *F. solani*가 각각 8.8%와 2.9% 분리되었다. 분리된 병균의 병원성 검정을 통해 종자를 발아시켜 재배한 유묘와 기내에서 계대배양을 통해 얻은 아스파라거스 모두 *Fusarium oxysporum*과 *F. moniliforme*의 감염에 대해 높은 감수성을 나타내었고, 비병원성 *F. oxysporum* (AVFO)과 *F. solani*의 감염에 대해서는

감수성을 나타내지 않았다. 따라서 병원성과 비병원성 *Fusarium* species가 아스파라거스를 감염시킬 경우 기내 배양한 아스파라거스는 포장에서 재배된 아스파라거스와 마찬가지로 상호작용연구에 공시재료로 사용될 수 있을 것으로 생각된다.

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