Incidence of Infection of Asparagus Spears Marketed in Connecticut by *Fusarium* spp.

Wade H. Elmer, The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven 06504

ABSTRACT

Elmer, W. H. 2000. Incidence of infection of asparagus spears marketed in Connecticut by *Fusarium* spp. Plant Dis. 84:831-834.

Sixty-seven samples of asymptomatic asparagus spears (avg. 26.5 spears per sample) were obtained during 1995 and 1996 from 22 retail markets in New Haven County, CT, from three commercial growers in Connecticut, and from experimental field plots in Connecticut and North Carolina. Surface-disinfested basal and apical segments were placed on media selective for *Fusarium* spp. Eight species of *Fusarium* were identified among 418 isolates from 1,776 spears. Of these, 30 and 62% were *F. oxysporum* and *F. proliferatum*, respectively. The incidence of *Fusarium* colonization was greater from the basal segments (15.5%) than from apical segments (8.1%). Spears shipped from Mexico, Peru, and North Carolina had higher incidence of colonization by *F. proliferatum* than spears shipped from California, Washington, or the northeastern United States. In both years, spears purchased during June had the highest incidence of *F. proliferatum* compared with other months. Since *F. oxysporum* and *F. proliferatum* are pathogens and infect asparagus crowns and roots, it is probable that infection originated in the field. The less frequent species were found slightly more often on apical segments than on basal segments.

Postharvest disorders of asparagus (*Asparagus officinalis* L.) spears have been associated with infection by species of *Fusarium* (19,23,30). Three distinct species, *F. oxysporum* Schlechtend.:Fr. emend. Snyd. & Hans., *F. proliferatum* (T. Mat-sushima) Nirenberg, and *F. verticillioides* (Sacc.) Nirenberg (syn. *F. moniliforme* J. Sheld. emend. Snyd. & Hans.), have been associated with the crown and root rot disease (6,9,18,27), and in postharvest problems (11,12,19,23). Studies of spears harvested in California and Mexico demonstrated that the source of inoculum was infected crowns in the field (11,12,23). Another species, *F. redolens* Wollenweb. (Sacc.), causes Fusarium spear spot (4,5), but this disease is distinctly different from the postharvest problem cited above.

Fusarium species are isolated from asymptomatic crowns and roots as frequently as from diseased plants (10,15,18,27). This fact may hinder efforts to predict the incidence of *Fusarium* on the spear based on disease severity in the field. In addition, climate and age of the planting may also influence the composition of species on the spear. In warm climates, *F. proliferatum* is the dominant species on asparagus (10,23), but this species can be absent in cooler,

temperate climates (1). Nigh et al. (23) reported lower incidence of *F. proliferatum* infection of spears harvested from the more northerly fields in California than in Mexican fields. As asparagus plantings age, the incidence of *F. proliferatum* tends to increase, whereas *F. oxysporum* is more prevalent in younger plantings (9). Other factors that could govern the incidence of *Fusarium* on the spears are the time in storage and storage temperatures. Infected spears decline faster at warmer temperatures than noninfected spears. Nigh et al. (23) observed that *F. proliferatum* was capable of growing in the spear during storage temperatures of 26°C. Although Guerrero et al. (12) found no correlation between the area of production and incidence of *Fusarium* infection of spears, it is reasonable to assume that shipments from warmer regions take longer to arrive in northern markets and may have greater incidence of *F. proliferatum* than spears harvested from local areas.

There is also the concern that certain species of *Fusarium* could produce mycotoxins in the spear (21). Nigh et al. (24) reported that no fumonisin B1, B2, or B3

mycotoxins were found in Mexican and California spears that were colonized by *F. proliferatum*. Logrieco et al. (17), however, reported that the fumonisin B₁ and B₂ toxins were detectable in crown and stem tissue in Italy. Little is known about the relative incidence of *Fusarium* infection of asparagus spears grown in northern climates. The aim of this study was to determine the frequency of infection by *Fusarium* spp. of asparagus spears produced in local and foreign production centers and sold in Connecticut.

MATERIALS AND METHODS

Forty-five samples of salable asymptomatic asparagus spears were collected from retail markets in New Haven County, CT (Table 1). The origin of the spears was determined by querying store managers and distributors and from information obtained from the packaging material. Commercial fields near the Connecticut towns of Avon, East Canaan, and Madison were also sampled during 1995 and 1996. In 1996, 19 samples were collected over the cutting season from experimental fields in Hamden and Windsor, CT, and from Raleigh, NC. Each state or country from which spears were shipped was categorized as one of six regions. Samples from New Jersey and Connecticut were combined to represent one region.

Samples consisted of approximately 20 to 40 (mean = 26.5) spears and were collected weekly or biweekly when available (Table 1). Spears were washed in tap water, surface-disinfested in 10% household chlorine bleach (NaClO₂) for 1 min, and rinsed in distilled water. A transverse slice of the spear approximately 2 to 5 mm wide was cut approximately 5 to 7 cm from the apical and basal ends of each spear. In 1995, segments were placed on Komada's selective medium (14). Since Komada's medium is more selective for certain *Fusarium* species (14), peptone-PCNB agar medium (3) was used in 1996 to support growth of a wider range of *Fusarium*

Table 1. Origin and number of samples of asparagus spears

Origin	No. samples	No. spears	Dates samples taken
Connecticut and New Jersey	18	419	April to June, 1995 and 1996
Washington	4	93	Mar. to June, 1995 and 1996
California	17	407	Feb. to May, 1995 and 1996
North Carolina	7	199	Apr. to May 1996
Mexico	15	440	Mar., June to Aug., 1995 and 1996
Peru	6	218	Aug. to Dec., 1995 and 1996
Total	67	1,776	

Corresponding author: Wade H. Elmer
E-mail: Wade.Elmer@po.state.ct.us

Accepted for publication 12 April 2000.

Publication no. D-2000-0522-02R
© 2000 The American Phytopathological Society

species (3). A test comparing the two media found there were no significant differences in the number of colonies or species of *Fusarium* that grew from 30 spear segments placed on both media. Plates were incubated at room temperature for 5 to 8 days, whereupon every colony growing from a tissue segment was subcultured onto carnation leaf agar medium (CLA) (21). After 7 to 10 days, *Fusarium* colonies were identified and subcultured by transferring one conidium to CLA. CLA plates were incubated at 25°C day/20°C night under cool-white fluorescent lights with a 12-h photoperiod. After 10 to 14 days, all cultures were identified according to the taxonomic system outlined by Nelson et al. (22) and Burgess et al. (3). Nomenclature for species within the *Liseola* section followed that of Nirenberg and O'Donnell (25).

Representative isolates of *F. proliferatum* and *F. verticillioides* were further characterized into mating groups by pairing the isolates with known tester strains of the teleomorph, *Gibberella fujikuroi* (Sawada), in mating groups D and A (16). Methods were those described by Elmer (7) using the procedure of Klittich and Leslie (13). The "+" and "-" tester strains of each mating group were cultured on carrot agar medium for 10 to 14 days (13). These cultures were overseeded with conidial suspensions from 168 test isolates grown on CLA for 10 to 14 days. Plates were cultured as described above for 14 days. The presence of globose black

perithecia exuding a cirrhous of ascospores was evidence of sexual compatibility and was used as the basis for assigning an isolate to a mating group. The mating type (+ or -) of each test isolate was recorded.

The data from each sample were normalized as the number of *Fusarium* colonies or species per 100 spears. Because the number of samples from each region varied, and because the data did not fit a normal distribution, the analysis of variance was performed using the nonparametric Kruskal-Wallis test (29). The effects of tissue, region, and month of sampling on *Fusarium* spp. were statistically tested. Means were separated using the Kruskal-Wallis test at $P = 0.05$ (29).

RESULTS

Of 1,776 asparagus spears sampled, 418 (23%) were infected by a species of *Fusarium*. Sixty-two percent were identified as *F. proliferatum*, and 30% were *F. oxysporum* (Table 2). The remaining isolates were members of the species *F. verticillioides*, *F. subglutinans* (Wollenweb. & Reinking) P.E. Nelson, T.A. Toussoun, & Marasas, *F. solani* (Mart.) Appel & Wollenweb. emend. Syd. & Hans., *F. acuminatum* Ellis & Everh., *F. graminearum* Schwabe, *F. semitectum* Berk. & Ravenel, and *F. culmorum* (Wm.G. Sm.) Sacc. These species were recovered more frequently from the apical segments of spears than from basal segments. However, the total *Fusarium* incidence was significantly

higher on basal segments (15.5%) than on apical segments (8.1%) ($P = 0.03$).

When representative isolates of *F. verticillioides* and *F. proliferatum* were paired with known testers of the mating groups A and D of *G. fujikuroi*, respectively, 85% of the isolates were assigned to the D group (Table 3). Both "+" and "-" mating types were found among the *F. proliferatum* isolates, but the "+" was found twice as often as the "-" type. Three of the four *F. verticillioides* isolates were assigned to mating group A, and all were mating type "+".

The incidence of *Fusarium* on the basal segments of spears collected from different regions varied significantly ($P = 0.001$) (Table 4). More spears harvested in Peru, Mexico, and the experimental plots in North Carolina were colonized by *Fusarium* spp. than those from the other regions. Most of the variation in *Fusarium* incidence on the basal stem segments could be accounted for by two species, *F. oxysporum* and *F. proliferatum*. Spears shipped from Peru were more likely to be infected by *F. oxysporum* and *F. proliferatum* than were spears from the other regions (Table 4).

The incidence of *Fusarium* colonization of spears was greater in 1996 than in 1995 ($P = 0.001$). When isolation data were plotted against month, the peak period for *Fusarium* colonization of spears was in June for both years (Fig. 1). Other peaks occurred in September 1995 and August 1996. Spears from several sources were included in the June sample, but those responsible for the peaks observed during September 1995 and August 1996 were all from Peru.

Table 2. Species of *Fusarium* found colonizing apical and basal segments of asparagus spears

Species	No. of isolates		
	Apical ^z	Basal ^z	Total
<i>F. proliferatum</i>	78	189	267
<i>F. oxysporum</i>	43	76	119
<i>F. verticillioides</i>	1	3	4
<i>F. subglutinans</i>	4	2	6
<i>F. solani</i>	4	0	4
<i>F. acuminatum</i>	7	4	11
<i>F. graminearum</i>	4	0	4
<i>F. semitectum</i>	1	1	2
<i>F. culmorum</i>	1	0	1
Total	143	275	418

^z Segments were sampled 5 to 7 cm from the apical tip and from the base of the spear. Spears were between 20 and 22 cm long.

Table 3. Assignment of isolates of *Fusarium verticillioides* and *F. proliferatum* from asparagus spears into mating groups A and D, respectively, of *Gibberella fujikuroi*

	A	D
Isolates tested	4	162
Compatible with testers	3	137
+	3	92
-	0	45

Table 4. Colonization of apical and basal segments of asparagus spears from different regions by *Fusarium oxysporum* or *F. proliferatum*

Region	Apical (colonies per 100 segments) ^y			Basal (colonies per 100 segments) ^y		
	<i>F. oxysporum</i>	<i>F. proliferatum</i>	Total	<i>F. oxysporum</i>	<i>F. proliferatum</i>	Total
Connecticut, New Jersey	1.2	1.3	4.0	3.5 a ^z	5.1 a	9.0 a
Washington	2.3	0.0	2.3	4.8 ab	4.5 a	8.9 a
California	0.5	4.8	6.6	0.7 a	8.0 ab	9.1 a
North Carolina	0.6	14.1	16.1	0.0 a	19.0 c	19.4 ab
Mexico	0.6	2.7	4.7	5.7 ab	14.0 bc	22.3 b
Peru	12.4	5.0	17.4	12.8 b	16.9 bc	30.0 b
Mean	2.9	4.7	8.5	4.6	11.3	16.5
Kruskal Wallace test (P)	ns	ns	ns	0.001	0.002	0.001

^y Segments were sampled 5 to 7 cm from the apical tip and from the base of the spear. Spears were between 20 and 22 cm long. *Fusarium* spp. were recovered on Komada's medium in 1995, and on peptone PCNB medium in 1996.

^z Values followed by differing letters are significantly different by Kruskal-Wallis test ($P = 0.05$). ns = not significant.

DISCUSSION

The incidence of colonization of asparagus spears marketed in Connecticut by *Fusarium* spp. varied significantly by location on the spear, origin of the crop, and time the sample was taken. The majority of isolates were identified as *F. proliferatum* or *F. oxysporum*. These species of *Fusarium* are causal to the crown and root disease (9,10,15,26). The fact that these species probably originated in the crown, as suggested by Guerrero et al. (11,12), may explain why basal segments had more colonization than apical segments. The rapid growth of the spear may have allowed the apical segments to escape the advancing hyphae. Other species of *Fusarium* may have also originated in the crown, where they existed as nonpathogens. However, since the frequency of recovering these other species was slightly greater in the apical segments than in the basal segments, it is also probable that these species were introduced by aerial inoculum. Many of these species are common colonists of grasses and surface plant residues (2).

The colonization was also greater on spears shipped from Mexico and Peru than on those from the northern production sites. Although *F. oxysporum* is cosmopolitan, *F. proliferatum* tends to be more prevalent in soil in warmer regions than in cooler ones (26). In another survey that sampled asparagus crowns from different climates, *F. proliferatum* was observed more often on crowns from tropical regions than on those from temperate zones (10). These findings agree with the current study. Spears from warm areas had a higher incidence of *F. proliferatum* than spears from northerly sites.

The time the spears are in transit may also affect the amount of colonization. Nigh et al. (23) demonstrated that *Fusarium* spp. were capable of growth in the spear during transit. Although the time from harvest to market was not determined, it is also reasonable to assume that spears from warmer regions take longer to reach Connecticut than locally grown spears.

Although *F. verticillioides* (*F. moniliforme* mating group A) is a pathogen of

asparagus (6,8,18), its isolation frequency from spears was very low. In reports from Australia, the United States, and South Africa, *F. verticillioides* was either absent or present in low frequencies when compared with *F. proliferatum* (6,7,10,26). However, Montealegre and Palma (19) found that *F. verticillioides* was a major factor in postharvest decline of asparagus in Chile. In Argentina, Lori et al. (18) reported that *F. verticillioides* and *F. proliferatum* were both factors in asparagus decline.

Incidences of other species of *Fusarium* colonizing asparagus spears have not been reported. *F. solani* is frequently found colonizing root and crown tissue, but it has not been implicated as a pathogen. In the current study, *F. solani* was found infrequently on spears. Still, this species comprised between 11 and 20% of the isolates recovered from asparagus crowns in other surveys (10,15,26). Its low incidence on the spear is not understood. Its lack of pathogenicity on asparagus may restrict it from colonizing the spear.

A higher incidence of *Fusarium* spp. was observed from spears sampled in June. These spears were shipped from Connecticut, Washington, and Mexico. The higher summer incidence may be due to the warmer temperatures favoring infection and growth in the spears. Moreover, in Connecticut, New Jersey, and Washington, asparagus harvests end during the month of June. At this time, the carbohydrate reserves of the plant are at their lowest level, which may compromise host defenses in the plant and lead to greater invasion of the spear by *Fusarium* spp. (28).

Colonization of asparagus spears by *Fusarium* spp. may also be of concern due to the potential of these species to produce mycotoxins (21). For example, *F. proliferatum*, the more common colonist, can produce the toxins beauvericin, fumonisin B₁, B₂, B₃, and fusaproliferin (20). Nigh et al. (24) reported that the incidence of *F. proliferatum* can be high in the edible spear without any detectable level of the fumonisin mycotoxins. They suggested that the nutritional composition of the spear may be unfavorable for mycotoxin production or that their isolates of *F. proliferatum* may not produce fumonisins. Logrieco et al. (17), however, reported that the fumonisin B₁ and B₂ toxins were present in the *F. proliferatum*-infected crown tissue at 7.4 and 0.5 µg/g, respectively, and in the infested stems at 0.8 and 0.1 µg/g, respectively. Their isolates of *F. proliferatum* belonged to the D mating group of *G. fujikuroi* and produced fumonisins in quantities equal to those found on infested maize and rice. Given the high incidence of *Fusarium* spp. on spears observed in this study, additional testing may be warranted. It is possible that fumonisins and other toxins, such as beauvericin and fusaproliferin, may appear in asparagus spears.

ACKNOWLEDGMENTS

I thank E. O'Dowd for technical assistance and Doug Sanders, North Carolina State University, for providing asparagus spears from North Carolina.

LITERATURE CITED

1. Blok, W., and Bollen, G. J. 1995. Fungi on the roots and stem bases of asparagus in the Netherlands: Species and pathogenicity. *Eur. J. Plant Pathol.* 101:15-24.
2. Burgess, L. W. 1981. General ecology of *Fusaria*. Pages 225-235 in: *Fusarium: Diseases, Biology, and Taxonomy*. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. Pennsylvania State University, University Park.
3. Burgess, L. W., Summerell, B. A., Bullock, S., Gott, K. P., and Backhouse, D. 1994. Laboratory Manual for *Fusarium* Research. 3rd ed. *Fusarium* Research Laboratory, Department of Crop Sciences, University of Sydney, and Royal Botanic Gardens, Sydney, New South Wales.
4. Cheah, L. H. 1986. Spear spot of asparagus. Pages 228-229 in: *Proc. N.Z. Weed Pest Control Conf.*, 39th, New Zealand.
5. Dingley, J. M. 1965. New records of fungus diseases of plants in New Zealand, 1962-1964. *N.Z. J. Agric. Res.* 8:905-920.
6. Doan, M. C., and Carris, L. M. 1998. Characterization of *Fusarium* populations in asparagus fields in the Pacific Northwest. *Proc. Int. Asparagus Sympos.*, 9th. *Acta Hortic.* 479:219-225.
7. Elmer, W. H. 1995. A single mating population of *Gibberella fujikuroi* (*Fusarium proliferatum*) predominates in asparagus fields in Connecticut, Massachusetts, and Michigan. *Mycologia* 87:68-71.
8. Elmer, W. H., and Ferrandino, F. J. 1992. Pathogenicity of *Fusarium* species (Section *Liseola*) to asparagus. *Mycologia* 84:253-257.
9. Elmer, W. H., Johnson, D. A., and Mink, G. I. 1996. Epidemiology and management of the diseases causal to asparagus decline. *Plant Dis.* 80:117-125.
10. Elmer, W. H., Summerell, B. A., Burgess, L. W., Backhouse, D., and Abubaker, A. A. 1997. *Fusarium* species associated with asparagus crowns and soil in Australia and New Zealand. *Aust. Plant Pathol.* 28:255-261.
11. Guerrero, C., Nigh, E. L., and Stanghellini, M. E. 1999. *Fusarium oxysporum* and *F. proliferatum* infection of commercial asparagus spear originates from infected crown and the field. *Acta Hortic.* 479:227-230.
12. Guerrero, C., Nigh, E. L., Jr., Stanghellini, M. E., and Elmer, W. 1997. Incidence of *Fusarium* spp. in asparagus spears. *Plant Dis.* 81:957.
13. Klittich, C. J. R., and Leslie, J. L. 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). *Genetics* 118:417-423.
14. Komada, H. 1975. Development of selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Rev. Plant Prot. Res.* 8:114-124.
15. LaMondia, J. A., and Elmer, W. H. 1989. Pathogenicity and vegetative compatibility among isolates of *Fusarium oxysporum* and *F. moniliforme* colonizing asparagus. *Can. J. Bot.* 67:2420-2424.
16. Leslie, J. F. 1991. Mating populations in *Gibberella fujikuroi* (*Fusarium* Section *Liseola*). *Phytopathology* 81:1058-1060.
17. Logrieco, A., Doko, B., Moretti, A., Frisullo, S., and Visconti, A. 1998. Occurrence of fumonisin B1 and B2 in *Fusarium proliferatum* infected asparagus plants. *J. Agric. Food Chem.* 46:5201-5204.
18. Lori, G., Wolcan, S., and Mónaco, C. 1998. *Fusarium moniliforme* and *F. proliferatum*

Fig. 1. Incidence of *Fusarium* infection on asparagus spears sampled from commercial markets and fields and shipped to Connecticut during 1995 and 1996.

- isolated from crown and root rot of asparagus and their association with asparagus decline in Argentina. *Plant Dis.* 82:1405.
19. Montealegre, J. R., and Palma, M. J. 1994. Etiology of the main postharvest fungal rots in green asparagus in Chile. *Fitopatologia* 29:102-105.
 20. Moretti, A., Logrieco, A., Bottalico, A., Rittien, A., Fogliano, V., and Radazzo, G. 1996. Diversity in beauvericin and fusaproliferin production by different populations of *Gibberella fujikuroi* (*Fusarium* Section *Liseola*). *Sydowia* 48:45-56.
 21. Nelson, P. E., Desjardins, A. E., and Plattner, R. D. 1993. Fumonisin mycotoxins produced by *Fusarium* species. *Biology, Chemistry, and Significance. Annu. Rev. Phytopathol.* 32:233-252.
 22. Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium* Species: An Illustrated Manual for Identification. Pennsylvania State University, University Park.
 23. Nigh, E. L., Guerrero, C., and Stanghellini, M. E. 1999. Evaluation of asparagus spears infected with *Fusarium* species on shelf life and quality. *Acta Hort.* 479:271-274.
 24. Nigh, E. L., Guerrero, C., and Stanghellini, M. E. 1999. Evaluation of *Fusarium* spp. infected asparagus spears for fumonisin mycotoxins. *Acta Hort.* 479:247-252.
 25. Nirenberg, H. I., and O'Donnell, K. 1998. New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. *Mycologia* 90:434-438.
 26. Sangalang, A. E., Burgess, L. W., Backhouse, D., Dupp, J., and Wurst, M. 1995. Mycogeography of *Fusarium* species in soils from tropical, arid, and Mediterranean regions of Australia. *Mycol. Res.* 99:523-528.
 27. Schreuder, W., Lamprecht, S. C., Marasas, W. F. O., and Calitz, F. J. 1995. Pathogenicity of three *Fusarium* species associated with asparagus decline in South Africa. *Plant Dis.* 79:177-181.
 28. Shelton, D. R., and Lacy, M. L. 1980. Effect of harvest duration and yield depletion of storage carbohydrate in asparagus roots. *J. Am. Soc. Hort. Sci.* 105:332-335.
 29. Siegel, S., and Castellan, N. J. 1988. *Non-parametric Statistics for the Behavioral Sciences*. McGraw-Hill Book Co., New York.
 30. Snowden, A. L. 1992. *Postharvest diseases and disorders of fruit and vegetables*. Vol. 2. CRC Press, Ann Arbor, MI.