Pathogenicity of Three *Fusarium* Species Associated with Asparagus Decline in South Africa

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ABSTRACT

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Isolations were made from crown, root, and stem lesions of symptomatic U.C. 157 F2 asparagus plants and from soil debris sampled in a declining asparagus field. *Fusarium oxysporum, F. proliferatum,* and to a lesser extent *F. solani* were the three dominant fungi isolated. Isolates of each species were evaluated for pathogenicity by inoculating U.C. 157 F2 plants in a greenhouse assay and an in vitro agar-test-tube assay. All three *Fusarium* spp. were pathogenic to asparagus, although they differed significantly in their disease-causing ability. In an in vitro assay, *F. proliferatum* isolates had a mean disease rating class of 4 compared with a class 3 for *F. oxysporum* and 2 for *F. solani,* on a scale of 1–5. Significant differences between *F. proliferatum* isolates were also detected. The high frequency of isolation and virulence of both *F. oxysporum* and *F. proliferatum* indicate that both species are important pathogens associated with asparagus decline in South Africa.

Additional keywords: *Asparagus officinalis,* Fusarium crown and root rot

In South Africa, asparagus (*Asparagus officinalis* L.) has been commercially grown since the early 1970s. By 1990, there were about 3,000 ha under cultivation, but due to the increasing demand of both the local and export markets for fresh and canned asparagus, cultivation areas have increased during the last 3 yr up to about 4,500 ha (H. Viljoen, *personal communication*). Plantations are generally started using 1-yr-old crowns, or to a lesser extent, 4-mo-old seedlings produced in nurseries. No direct seeding is done.

A rapid decline of new plantings with no history of asparagus cultivation, produced from 4-mo-old seedlings, was observed in the 1990 growth season near Ficksburg in the Orange Free State. Symptoms noted were extensive rotting of feeder and storage roots, vascular discoloration in the crown and base of infected stems, fern chlorosis, wilt, and death. Reddish-brown lesions were also present on the exterior of stems and roots. Similar symptoms were present on seedlings produced in a nursery located near Pietermaritzburg in Natal, from which the seedling transplants used at Ficksburg had originated.

The objectives of this study were to determine the fungi associated with the disease symptoms as described above, and to determine the pathogenicity of three *Fusarium* species that were isolated predominantly from the diseased plants and soil debris. Preliminary reports have been published (16,17).

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MATERIALS AND METHODS

Fungal isolation and identification. Twenty-seven symptomatic U.C. 157 F2 plants, transplanted in December 1989, were removed from a field near Ficksburg in February (S1), November (S2) and December (S3) 1990. In December 1990, 30 seedlings with disease symptoms were also sampled at a nursery in Natal (S4) from which the Ficksburg transplants originated. Plants were washed under a fine spray of tap water, then surface disinfested with 0.5% NaOCl for 1 min, rinsed in distilled water, and again surface disinfested with 70% ethanol for 1 min and left to dry in a flow bench. Sections for isolation were made from crown, root, and stem lesions, and pieces of tissue (±2–4 mm) were placed on a *Fusarium*-selective medium (19), Komada's medium (KM) (10), potato-dextrose agar with 0.02% novostreptomycin (PDA+), and water agar with 0.02% novostreptomycin.

Soil samples, between plants within rows, were also taken in November and December of 1990 to determine the number of *Fusarium* spp. present in soil debris as described by Marasas et al (13). Ten pieces of soil debris (±2 mm, mainly root fragments) were plated on each of 10 petri dishes of *Fusarium*-selective medium. Two hundred U.C. 157 F2 seeds, of the same seed lot used to produce seedlings for the 1989 plantings in Ficksburg, were plated on KM to determine the presence of *Fusarium* spp. Petri dishes with plant tissue and soil debris were incubated at 20–25 C on a laboratory bench for 5–10 days. Petri dishes with seed were incubated on the laboratory bench for 21 days. All colonies of fungi that grew from the tissue and seeds were transferred to divided petri dishes containing PDA+ in one half of the dish and carnation-leaf agar (8) with 0.02% novostreptomycin in the other, for identification. Plates were incubated at 20 C with a 12-hr photoperiod under 40 W cool-white fluorescent light and 40 W black light (near ultraviolet) for 14–21 days and then examined for morphological characteristics. Single spores of all *Fusarium* isolates were subcultured and identified according to Nelson et al (15). Single-conidial isolates were maintained on PDA+ slants at 10 C, and representative isolates of *Fusarium* spp. were lyophilized.

Pathogenicity tests. Ten representative single-conidial isolates each of *F. oxysporum* Schlecht. emend. Snyd. & Hans. and *F. proliferatum* (Matsushima) Nirenberg and six of *F. solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans. that were obtained from the above samplings, were assayed for pathogenicity by inoculating U.C. 157 F2 asparagus seedlings. Greenhouse-grown asparagus plants commonly become cross-infected by windblown and insect-vectored *Fusarium* spp. (2). Therefore, pathogenicity tests were also conducted on axenically cultured seedlings in an agar-test-tube assay as described by Stephens and Elmer (18). For both the greenhouse and agar-test-tube assays, seed of U.C. 157 F2 (supplied by Langeberg Foods Ltd., South Africa) was treated (18) and incubated for germination on 0.6% water agar (17).

Greenhouse assay. One germinated seed was transplanted into a plastic bag (140 × 70 mm) containing a pasteurized commercial potting mixture (18:12:1, m/m, peat/vermiculite/polystyrene, pH 5.8, Hygrotech Seed [Pty]Ltd., South Africa) and placed in a greenhouse with an average day/night temperature of 27/16 C. Each plant received approximately 100 ml of a 0.2% soluble fertilizer (Chemicult hydroponic nutrient, macro elements 6.5% N, 2.7% P, 13% K, 7% Ca, 2.2% Mg, 7.5% S, micro elements 0.15% Fe, 0.024% B, 0.005% Zn, 0.002% Cu, 0.001% Mo, Chemicult Products [Pty]Ltd., South Africa) once a week.

At 14 wk, healthy plants were transplanted into 170 × 150 mm plastic pots (one plant/pot) containing 1.4 kg of pasteurized soil mix (1:1:1, v/v, sand/perlite/topsoil) supplemented with 35 g of sand-bran inoculum (12) and incubated as before. Ten pots per *Fusarium* isolate were used, with an equal number of control plants inoculated with sterile sand-bran mixture. The treatments were completely randomized with 10 replications. An experimental unit consisted of one plant/pot. Thirteen weeks later, plants were removed, washed and rated on a scale of 1–5, modified from that of Stephens and Elmer (18), in which 1 = no root lesions or vascular discoloration in crown and roots, 2 = fewer than five root lesions with no vascular discoloration in crown and roots, 3 = five to 10 root lesions with slight vascular discoloration in crown and roots, 4 = more than 10 root lesions with vascular discoloration in crown and roots, 5 = heavily rotted crown or death of plant. Two plants of each treatment were used for reisolation on KM, and the rest were excised just above the hypocotyl and the root and shoot dry mass were individually determined as with the test tube assay seedlings.

Agar-test-tube assay. Fourteen days after incubation, a single sterile germinated seed was transferred to each test tube (200 × 28 mm) containing 20 ml of solidified sterile Hoagland's No. 2 (1). The tubes were sealed with sterile nonabsorbent cottonwool and incubated for 10 days at 25 C day and 18 C night temperature under cool-white fluorescent light with a 14-hr photoperiod. Ten uniform seedlings were selected per treatment for inoculation. Mycelial plugs (3 mm in diameter) from a 5-day-old colony cultured on PDA+ of each of the *Fusarium* isolates were transferred to 500-ml flasks containing 100 ml of potato-dextrose-broth (10 g/L). Flasks were placed on a shaker (150 rpm) maintained at 25 C. After 5 days, contents of the flasks were filtered through four layers of sterile cheesecloth. The filtrate was diluted with sterile distilled water and adjusted to an inoculum concentration of 1×10^6 conidia per milliliter by means of a hemacytometer. Each seedling in each treatment received 0.5 ml of inoculum suspension and 0.5 ml of sterile diluted potato-dextrose broth was applied to the control. The inoculated seedlings were incubated as before. The treatments were completely randomized with 10 tubes in each treatment. After 30 days, the root systems were examined for disease development and rated on a 1–5 scale (18), in which 1 = no disease, 2 = lesions present on >0–25% of the root system, 3 = lesions present on >25–50% of the root system, 4 = lesions present on >50–75% of the root system, 5 = lesions present on >75–100% of the root system. After the seedlings had been rated, they were removed from the tubes and two of the 10 seedlings of each treatment were used for reisolation on KM, while the rest were excised just above the hypocotyl and the root and shoot dry mass were individually determined. The above procedures were conducted twice and each experiment was treated as an experimental block.

Statistical procedures. The root and shoot dry masses in the greenhouse assay, were analyzed by a completely randomized design. The program PC-PLUM (14) was used to analyze the disease ratings. For the agar-test-tube assay, standard analysis of variance for a randomized block design was performed on the root and shoot dry masses. The disease ratings (ordinal data) were analyzed using a general linear model technique. The program PC-PLUM was also used to analyze the data. Meaningful comparisons were tested, as shown in the analyses of deviance and variances table (Table 1). For both assays, the disease rating figures (Figs. 1A and 2A) are represented as means on the underlying scale (14); the treatment means of root (Figs. 1B and 2B) and shoot (Fig. 2B, C) dry masses were calculated and are also represented.

RESULTS

Fungal isolation and identification. A total of 778 fungal isolates were obtained from diseased asparagus plants from the field and nursery, and were associated with specific plant parts (Tables 2 and 3). The mean percentage fungal recovery from diseased asparagus plants showed that *F. oxysporum* (35.6%), *F. proliferatum* (32.7%), and *F. solani* (8.6%) were the three dominant fungi present (Table 2). *F. oxysporum* and *F. proliferatum* were the two most prevalent *Fusarium* spp. isolated from the crowns, roots, and stems of asparagus plants (Table 3). An *Alternaria* sp. occurred at relatively high frequency (12.5%) in plants collected in December 1990 (S3) and was isolated mainly from the stems (Table 3). Plants from the nursery (S4) also had a high incidence of a *Trichoderma* sp. (24.2%), which was isolated mainly from the below-ground parts. A very low incidence of a *Phoma* sp. was recorded from the root and stems of plants collected in February (S1) and December 1990 (S3).

Isolations from soil debris also indicated that *F. oxysporum* (36.4%), *F. proliferatum* (18.2%), and *F. solani* (10.9%) were the predominant fungi. No *Fusarium* spp. were isolated from the U.C. 157 F2 seeds plated on KM.

Pathogenicity tests in greenhouse

Table 1. Analysis of deviance and analysis of variance results of disease ratings, root dry mass, and shoot dry mass of asparagus seedlings inoculated with three *Fusarium* spp. in an agar-test-tube assay

		Disease rating		Root dry mass		Shoot dry mass	
Source	DF	MD	P^a	MS	P^a	MS	P^a
Blocks	1	3.48	0.041	107.53	<0.001	906.10	<0.001
Treatments	26	8.01	<0.001	2.22	0.135	16.93	0.077
Control vs species	1	56.83	<0.001	20.95	<0.001	16.80	0.197
Control vs FP[b]	1	96.69	<0.001	18.62	0.001	42.68	0.045
Control vs FO[c]	1	40.73	<0.001	25.64	<0.001	18.36	0.178
Control vs FS[d]	1	20.96	<0.001	12.50	0.007	0.75	0.810
Between species	3						
FP vs FO	1	65.49	<0.001	3.08	0.154	27.72	0.101
FP vs FS	1	108.05	<0.001	1.88	0.262	220.32	<0.001
FO vs FS	1	11.47	<0.001	8.35	0.023	105.75	0.003
Within species	23						
Isolates within FP	9	1.81	0.039	1.46	0.447	13.20	0.249
Isolates within FO	9	0.85	0.374	1.51	0.616	8.73	0.531
Isolates within FS	5	0.73	0.454	0.89	0.680	0.78	0.995
Error	26	0.750		1.429		9.583	
Total (Corrected)	53						

[a] Probability (a probability less than 0.05 is considered as significant).
[b] *F. proliferatum.*
[c] *F. oxysporum.*
[d] *F. solani.*

assay. Although *F. oxysporum, F. proliferatum,* and *F. solani* were recovered from correspondingly inoculated plants 13 wk after incubation, *F. proliferatum* was isolated from crowns, roots, and stems of all plants including the controls. The root system of most plants had reddish lesions and in some cases a few roots were completely rotted. The root systems of seven and four plants, inoculated with *F. proliferatum* isolate nos. 3 and 5, respectively, had completely collapsed and died (Fig. 1). Due to the aerial contamination between treatments with *F. proliferatum* isolates, this experiment was not repeated and statistical comparisons were not done, but despite the contamination, it was evident that the *F. proliferatum*–inoculated plants had numerically higher disease ratings than the control, *F. oxysporum*– and *F. solani*–treated plants (Fig. 1A). The *F. proliferatum* isolates caused a mean disease rating of 3, while the control, *F. oxysporum,* and *F. solani* isolates caused a mean disease rating of 2. The root and shoot dry masses of plants inoculated with *F. proliferatum* isolate nos. 3 and 5 were reduced compared with the control plants (Fig. 1B and C).

Pathogenicity tests in agar-test-tube assay. Koch's postulates were confirmed with all fungal isolates tested, and no fungi were isolated from the control seedlings. Reddish brown lesions were observed on primary and secondary roots 12–15 days after inoculation, but ratings were made after 30 days to allow sufficient time for disease to develop in all treatments. All isolates of *F. oxysporum, F. proliferatum,* and *F. solani* caused root lesions while the uninoculated control seedlings had no root lesions. Only a few inoculated seedlings showed chlorosis, and fern tissue of most of the seedlings was green after the incubation period. The disease ratings and root and shoot dry masses are shown in Fig. 2A–C; statistical data are presented in Table 1. The agar-test-tube assay detected significant differences ($P < 0.001$) between the disease ratings of the controls and the inoculated seedlings (Fig. 2A and Table 1), indicating the pathogenicity of all three *Fusarium* spp. to asparagus. There were significant differences ($P < 0.001$) among the *Fusarium* spp. in their disease-causing ability, and significant differences ($P = 0.039$) between isolates of *F. proliferatum* were also detected. Seedlings inoculated with isolates of *F. proliferatum* had significantly ($P < 0.001$) higher disease ratings than those inoculated with isolates of *F. oxysporum* and *F. solani.* Isolates of *F. oxysporum* were significantly ($P < 0.001$) more virulent than *F. solani* isolates. *F. proliferatum* isolates had a mean disease rating of 4 compared with 3 for *F. oxysporum* and 2 for *F. solani* isolates (Fig. 2A). A significant reduction in root dry

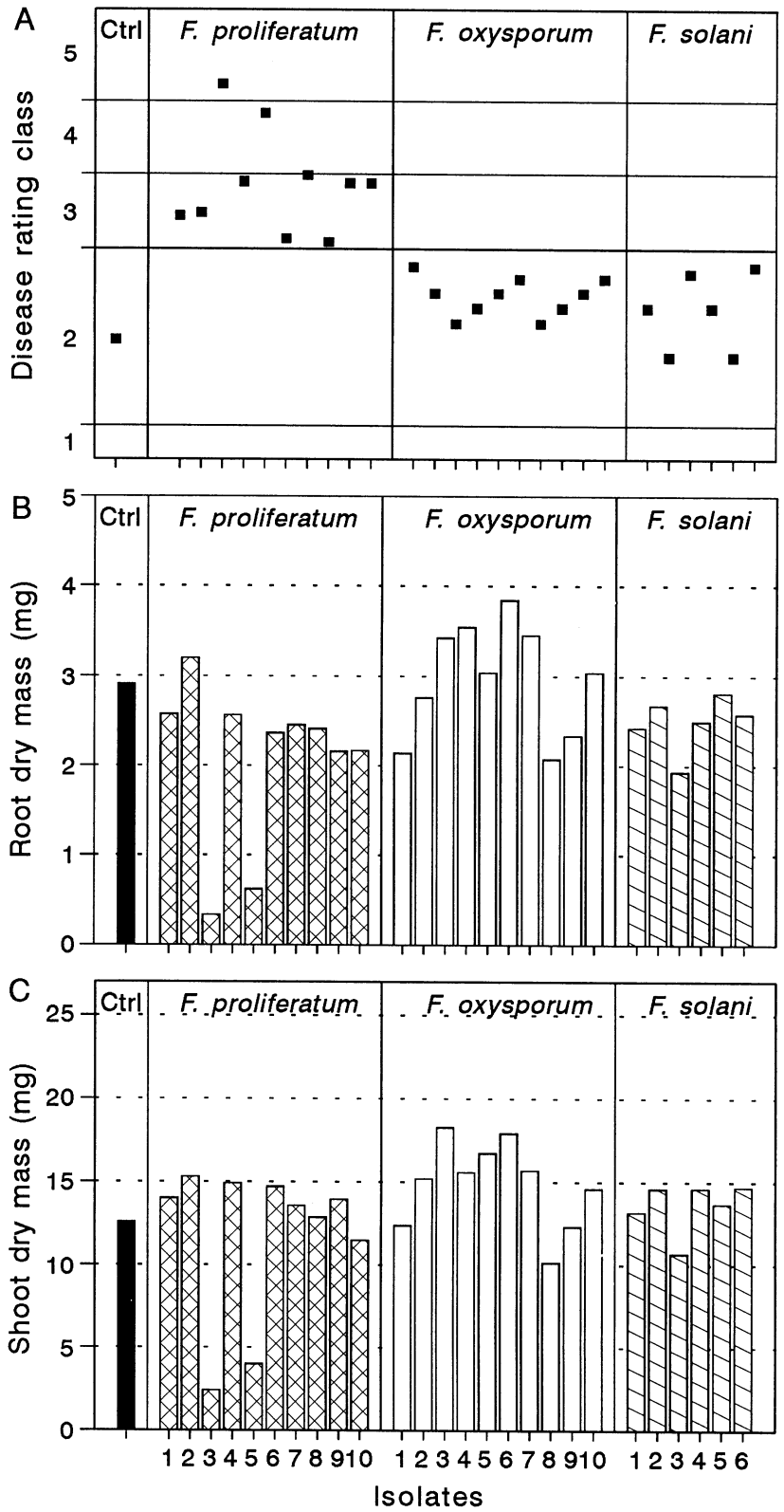

Fig. 1. (**A**) disease ratings, (**B**) root dry mass, and (**C**) shoot dry mass of *Asparagus officinalis* U.C. 157 F2 plants inoculated with isolates of *Fusarium oxysporum, F. proliferatum,* and *F. solani* in a greenhouse assay. Disease ratings (ordinal data) represent treatment means of 10 samples on underlying scale. Treatment means of root and shoot dry mass calculated from eight samples. Disease rating scale: 1 = no root lesions or vascular discoloration in crown and roots; 2 = fewer than five root lesions with no vascular discoloration in crown and roots; 3 = five to 10 root lesions with slight vascular discoloration in crown and roots; 4 = more than 10 root lesions with vascular discoloration in crown and roots; 5 = heavily rotted crown or death of plant.

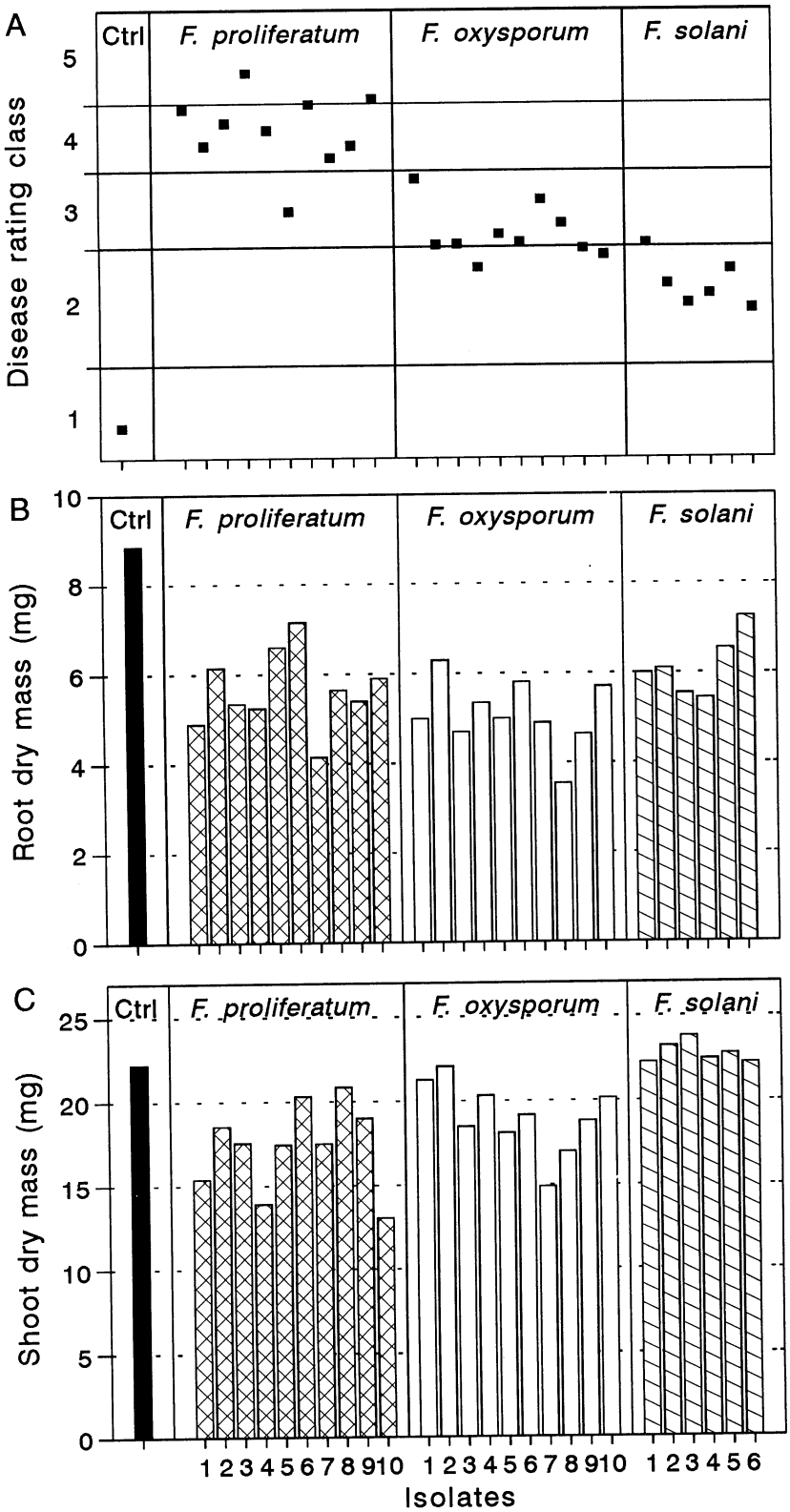

Fig. 2. (**A**) disease ratings, (**B**) root dry mass, and (**C**) shoot dry mass of *Asparagus officinalis* U.C. 157 F2 seedlings inoculated with isolates of *Fusarium oxysporum, F. proliferatum*, and *F. solani* in an agar-test-tube assay. Disease ratings (ordinal data) represent treatment means of 10 samples with two replicates on underlying scale. Treatment means of the root and shoot dry mass calculated from eight samples and two replicates. Disease rating scale: 1 = no disease, 2 = lesions present on >0–25% of root system, 3 = lesions present on >25–50% of root system, 4 = lesions present on >50–75% of root system, 5 = lesions present on >75–100% of root system.

mass was caused by isolates of each *Fusarium* sp. when compared with control seedlings, but only isolates of *F. proliferatum* caused a significant reduction in shoot dry mass ($P = 0.045$) when compared with control seedlings. The root dry mass of seedlings inoculated with *F. proliferatum* did not differ significantly from that of seedlings inoculated with *F. oxysporum* and *F. solani*. However, root dry mass of *F. oxysporum*–inoculated seedlings was significantly ($P = 0.023$) lower than that of seedlings inoculated with *F. solani*. Shoot dry mass of seedlings inoculated with *F. proliferatum* and *F. oxysporum* did not differ significantly, but shoot dry mass of seedlings inoculated with *F. proliferatum* and *F. oxysporum* was significantly lower than that of seedlings inoculated with *F. solani*.

DISCUSSION

The major cause of asparagus decline is Fusarium crown and root rot caused by *F. moniliforme* Sheldon, *F. oxysporum* Schlecht. emend. Snyd. & Hans. f. sp. *asparagi* Cohen & Heald (FOA), *F. proliferatum,* and to a lesser extent *F. solani* (2,4,5,9,11,18). Although *F. moniliforme* and *F. proliferatum* both produce microconidia in chains, the two species can be separated on the basis of the presence of polyphialides in *F. proliferatum* since *F. moniliforme* produces monophialides only (15). Isolates from asparagus previously referred to as *F. moniliforme* (11) have recently been reclassified as *F. proliferatum* (5). Therefore it appears that the pathogen of asparagus previously referred to in the literature as *F. moniliforme* is probably *F. proliferatum.*

The most prevalent fungi isolated from all symptomatic U.C. 157 F2 plants and soil debris samples in the present study were *F. oxysporum* and *F. proliferatum.* Of the two species, *F. proliferatum* was more frequently isolated from plant samples collected from a field near Ficksburg in February (S1) and December 1990 (S3), while *F. oxysporum* was more frequently isolated from plant samples collected from the field in November 1990 (S2) and from seedlings collected from a nursery at Pietermaritzburg in December 1990 (S4). LaMondia and Elmer (11) showed that *F. moniliforme,* later redesignated as *F. proliferatum* (5), was more frequently isolated from basal stem segments, crown tissue, and roots than was *F. oxysporum.* Elmer (5) also indicated that *F. proliferatum* was the species most frequently isolated from the base of chlorotic asparagus ferns from fields of varying ages in Connecticut, Massachusetts, and Michigan. Johnston et al (9), on the other hand, showed that *F. oxysporum* was the most prevalent *Fusarium* sp. isolated from 1- and 2-yr-old plantings, while *F. moniliforme* was the dominant species in a 12-

Table 2. Incidence of fungi isolated from diseased asparagus plants obtained from a field near Ficksburg and a nursery in Pietermaritzburg, South Africa

Fungus	Incidence of fungi (%)[a]				
	S1[b]	S2[c]	S3[d]	S4[e]	Mean
Alternaria sp.	0.0	0.0	12.5	3.8	4.1
F. oxysporum	30.5	42.9	21.0	48.1	35.6
F. proliferatum	41.8	33.3	47.9	7.7	32.7
F. solani	8.3	23.0	0.4	2.7	8.6
Other *Fusarium* spp.[f]	12.4	0.0	8.8	8.2	7.4
Phoma sp.	7.0	0.0	2.2	0.0	2.3
Trichoderma sp.	0.0	0.0	0.0	24.2	6.0
Other fungi[g]	0.0	0.8	7.2	5.3	3.3

[a] Percentage of incidence out of 778 fungal isolates obtained from crowns, roots, and stems of diseased asparagus plants.
[b] Plant samples collected from a field near Ficksburg in February 1990.
[c] Plant samples collected from a field near Ficksburg in November 1990.
[d] Plant samples collected from a field near Ficksburg in December 1990.
[e] Asparagus seedlings collected from a nursery at Pietermaritzburg in December 1990.
[f] *F. acuminatum*, *F. avenaceum*, *F. chlamydosporum*, *F. equiseti*, and *F. scirpi*.
[g] Species of *Curvularia*, *Epicoccum*, *Gliocladium*, *Neocosmospora*, *Penicillium*, *Phomopsis*, *Rhizopus*, and other unidentified fungi.

Table 3. Incidence of fungi isolated from affected crown, root, and stem parts of asparagus plants obtained from a field near Ficksburg and a nursery in Pietermaritzburg, South Africa

Fungus	Incidence of fungi (%) in affected parts[a]		
	Crown	Root	Stem
Alternaria sp.	0.7	0.0	17.6
F. oxysporum	38.4	36.4	34.8
F. proliferatum	30.6	34.2	17.7
F. solani	11.1	5.2	1.3
Other *Fusarium* spp.[b]	9.9	2.7	14.6
Phoma sp.	0.0	3.9	2.3
Trichoderma sp.	6.3	15.1	3.6
Other fungi[c]	3.0	2.5	8.1

[a] Percentage of incidence out of 239, 301, and 238 fungal isolates from crown, root, and stem, respectively.
[b] *F. acuminatum*, *F. avenaceum*, *F. chlamydosporum*, *F. equiseti*, and *F. scirpi*.
[c] Species of *Curvularia*, *Epicoccum*, *Gliocladium*, *Neocosmospora*, *Penicillium*, *Phomopsis*, *Rhizopus*, and other unidentified fungi.

yr-old planting.

The method for conducting pathogenicity tests with asparagus seedlings in an in vitro agar-test-tube assay, developed by Davis (3) and modified by Stephens and Elmer (18), is generally used by researchers (6,7,11) because results are similar to greenhouse tests (6,18), and it avoids inconsistencies in results originating from cross-contamination in greenhouse tests (2,18) as our greenhouse assay reconfirmed.

Our results agree with those of others (5,11) that *F. proliferatum* is more virulent than *F. oxysporum* to asparagus seedlings. Elmer (4) reported that the average reduction in weight of asparagus plants (cv. Mary Washington) grown in soil infested with *F. proliferatum* was greater than with *F. moniliforme*. Elmer and Stephens (7) considered *F. oxysporum* strains to be nonpathogenic on asparagus if plants had a mean disease rating of less than 2.0 in the in vitro assay. Using this criterion, all 10 South African *F. oxysporum* isolates tested should be designated FOA. Our results also agree with previous reports (2,11) that *F. solani* does not appear to be an important pathogen of asparagus due to its low frequency of isolation and virulence.

Although the *Fusarium* spp. significantly differed among each other in disease-causing ability, significant differences between the South African isolates of *F. proliferatum* were also detected. Elmer (5) did not find significant differences in virulence between 26 *F. proliferatum* isolates from different origins and vegetative compatibility groups. However, it has been shown that individual isolates of *F. oxysporum* and *F. moniliforme* can differ in their virulence to asparagus seedlings in pathogenicity tests (2,18). Stephens and Elmer (18) found that among FOA isolates, FOA5 (mean disease rating 3.7) incited significantly more root lesions than F-10 (mean disease rating 1.5). However, using the criterion of Elmer and Stephens (7), it appears that isolate F-10 is not an FOA strain.

The high frequency of isolation and virulence of *F. oxysporum* and *F. proliferatum* in our study led us to conclude that both these *Fusarium* spp. are important pathogens associated with asparagus decline in South Africa, and that pathogens associated with asparagus decline in South Africa are similar to those in other asparagus-producing countries of the world, except for the absence of *F. moniliforme* (9).

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