In Vitro Control of *Fusarium* Diseases of *Asparagus officinalis* L. with a *Streptomyces* or Its Polyene Antibiotic, Faeriefungin†

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Faeriefungin, a novel antibiotic from *Streptomyces griseus* var. autotrophicus (ATCC 53668), effectively controlled root rot and stem wilt of *Asparagus officinalis* L. caused by *Fusarium oxysporum* (Schlecht.) emend. synd. & Hans. f. sp *asparagi* (FOA) Cohen and Heald in the laboratory. Faeriefungin did not adversely affect UC157 asparagus seed germination or plant growth. This antibiotic at 25 ppm decreased the severity of disease in sterile asparagus plants grown on water agar and increased fibrous root weight of plants grown in the absence of *F. oxysporum*. The producer organism, grown on several media, was found to be antagonistic to *F. oxysporum*. Root dip treatments of UC157 seedlings in *S. griseus* cell suspension resulted in a greater fleshly root dry weight, total root dry weight, and total plant dry weight for plants challenged with FOA compared to controls.

INTRODUCTION

A number of stresses increase the susceptibility of asparagus to infection by *Fusarium oxysporum* f. sp. *asparagi* (FOA) and *F. moniliformae* (FM), the casual agents of *Fusarium* wilt and root rot and *Fusarium* stem and crown rot, respectively. Living asparagus plants and decaying root tissue have been shown to release allelochemicals and autotoxins (Hartung and Stephens, 1983; Hartung and Putnam, 1985; Laufer and Garrison, 1986; Yang, 1982, 1985; Young, 1986). Furthermore, these phytotoxic compounds are not toxic to the fungal pathogens (Hartung, 1983). Pierce and Colby (1987) determined that an autotoxin present in asparagus root filtrate predisposed the young radicles and hypocotyls to increased infection by FOA while simultaneously stimulating the pathogen. Viral infection also enhances the fungal disease in asparagus plants (Evans, 1985). Tolerance of asparagus to *Fusarium* spp. may vary with soil moisture (Wilcox, 1985). Vesicular arbuscular mycorrhizae (VAM) increased survival and growth of asparagus plants in seedbed trials (Powell and Bagyaraj, 1983).

No effective method exists for the control of *Fusarium* stem and crown rot and *Fusarium* wilt and root rot diseases in open pollinated cultivars of asparagus (Takatori and Souther, 1978). Lewis and Shoemaker (1964) demonstrated that Mary Washington, Viking, and Eden cultivars of asparagus are highly susceptible to root rot caused by FOA. Tolerant or resistant plants have not emerged from a program of identification of healthy asparagus plants from declining fields with subsequent asexual propagation using tissue culture techniques (Stephens, 1985). Chemical treatment is not effective in controlling crown rot of asparagus (Zandstra and Price, 1984). Benomyl [methyl 1-(butylcarbamoyl)-1*H*-benzimidazol-2-ylcarbamate] and Iprodione [3-(3,5-dichlorophenyl)-*N*-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboxamide], when applied as a crown dip, produced no measurable differences from controls (Stephens et al., 1986). However, Cassini et al. (1985) successfully employed an antagonistic microbe to control *Fusarium* infection of field-grown asparagus.

Streptomyces griseus var. autotrophicus (ATCC 53668), an actinomycete isolated from the soil obtained from a fairy ring, is reported to produce a strong antifungal antibiotic, faeriefungin (Nair et al., 1989). Faeriefungin is a polyene macrolide antibiotic with broad-spectrum biological activity. In vitro antifungal assay on *Fusarium* species with faeriefungin gave minimum inhibitory concentration at 3.2 μg/mL (Nair et al., 1989). In this paper, we report the use of faeriefungin (Figure 1) and its producer organism, *S. griseus*, to inhibit *Fusarium* diseases of asparagus.

MATERIALS AND METHODS

Microorganisms. FOA cultures were obtained from diseased asparagus plants in Michigan by M. L. Lacy of Michigan State University (isolate F10). Cultures of FM were also obtained from the above source. These isolates were stored according to a method described by Nelson et al. (1983) and propagated on potato dextrose agar (PDA) by Difco. The conidia of these fungi were harvested according to a method described by Stephens and Elmer (1988). *S. griseus* was grown in A-9 medium (Nair et al., 1989) (peptone 10 g/L, molasses 20 g/L, and dextrose 10 g/L) in 2-L baffled flasks on a rotary shaker (150 rpm) at 26 °C for 7 days. Soil extracts for growing *S. griseus* were prepared as follows: Loamy sand soil (500 g) was stirred with distilled water (1 L) at 90 °C (1 h) and filtered (Whatman, No. 1) under vacuum. The soil was rinsed with an additional 700 mL of water. The combined extracts were filtered again (Whatman, No. 5) and finally filter sterilized (2 μm). Agar (14 g) was dissolved in 140 mL of water, autoclaved, and combined with the above extract. The final adjusted volume of the medium was 2 L.

Plant Materials. The seeds of UC157 asparagus, a sensitive indicator species to *Fusarium*, were sterilized according to the method described by Stephens et al. (1986) and were transferred asceptically to sterile 100 × 15 mm Petri plates containing water agar (7 g of agar/L of water) followed by germination in the dark at 26 °C for 7 days. Growth chamber experiments with asparagus plants were at 16-h photoperiod (27 °C day temperature and 16 °C night temperature, light intensity 118.8 microEinsteins). Nutrient solution used for growing plants was Hoagland's No. 2 at $^1/_2$ strength modified to contain 7 g of agar/L (Hoagland and Arnon, 1938).

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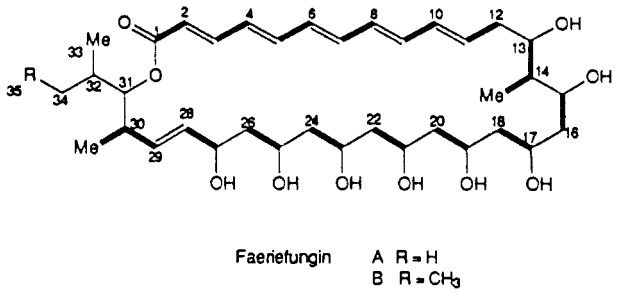

Figure 1. Faeriefungin, a 1:1 mixture of isomers A and B differing only in their side chain.

Determination of Minimum Inhibitory Concentration (MIC) of Faeriefungin. A solution of faeriefungin in DMSO (100 μL) was incorporated into 1.9 mL of liquid yeast maltose glucose (YMG) agar in 16 × 125 mm sterile, capped glass culture tubes. The tubes were slanted and allowed to solidify. The final concentrations of faeriefungin in the tubes were 0, 1, 2, 3, 4, 5, 6, 12.5, 25, and 50 ppm. A conidial suspension of FOA (50 μL) was pipetted onto the agar surface and incubated at 26 °C for 96 h.

Phytotoxicity Assay of Faeriefungin on Asparagus. Surface-sterilized seeds (10) were placed on 60 × 15 mm glass Petri plates lined with Whatman filter paper. Solutions of 0.5% DMSO–H_2O (1 mL) containing 0, 5, 25, and 50 ppm faeriefungin were pipetted into each plate and incubated in the dark at 26 °C. At the end of 12 days, shoot and root lengths were recorded. The experiment was arranged in a randomized complete block design.

Asceptically grown 7-day-old asparagus seedlings were transferred, five per box, to sterile magenta boxes (No. 10) containing nutrient solution and faeriefungin at concentrations of 0, 10, 50, and 100 ppm. Shoot and root dry weights were recorded at 2-, 3-, and 4-week intervals.

In Vitro Assay of Faeriefungin against FOA with Asparagus Plants. Faeriefungin was diluted with liquid agar to achieve concentrations of 0, 12.5, 25, and 50 ppm. Aliquots (50 mL) were then poured into sterile magenta boxes and allowed to solidify. Seven-day-old asparagus seedlings were planted, three per box, and placed in a growth chamber for 7 days. The seedlings were challenged with 1 mL of FOA suspension containing 2 × 10^6 conidia and allowed to grow for another 14 days, after which time the lengths and dry weights of the fleshy root, fibrous root, and shoots were recorded. The experiment was a 2 × 4 factorial arranged in a randomized complete block design.

Antagonism of *S. griseus* to FOA under Different Nutrient Conditions. Each of the following media was pipetted into sterile polystyrene 60 mm × 15 mm Petri plates: PDA (Difco), soil extract, soil extract plus peptone (5 g/L), soil extract plus dextrose (4 g/L), and soil extract plus peptone plus dextrose. The media were allowed to solidify and were subsequently inoculated with 0.1 mL of a suspension of *S. griseus* (approximately 6 × 10^4 CFU/mL). The inoculum was spread evenly on the entire surface. The plates were then incubated at 26 °C for 8 days in the dark. On the eighth day, a sterile spatula was used to remove the agar from each plate, taking care not to contaminate the bottom of the agar with the antagonist, and the inverted disk was placed in a serile 100 mm × 15 mm polystyrene plate. A PDA plug was cut from the edge of a 6-day-old FOA culture with a sterile 0.75-cm cork borer and was placed in the center of the newly exposed agar surface. The plates were then placed in an incubator at 26 °C for 5 days. The distance from the edge of the plug to the edge of the hyphal proliferation was then measured. The experiment was a 2 × 5 factorial arranged in a randomized complete block design with five blocks.

Disease Control Experiments with *S. griseus.* *In Vitro Agar Assay.* Three methods were employed to introduce the antagonist into the biological system: surface inoculation, incorporation of the antagonist into the agar, and root dip of the asparagus seedlings in a suspension of the antagonist. Cultures of *S. griseus* (7 days old) were centrifuged, and the cells were used for inoculation. The incorporation experiments were conducted in magenta boxes containing 50 mL of amended nutrient agar (dextrose 1 g/L, agar 7 g/L) and 2 × 10^6 CFU/mL of *S. griseus*, while the control was devoid of the antagonist. The root-dip treatments were achieved by dipping plants (7 days old) in normal saline solution or normal saline suspensions of *S. griseus* (8 × 10^5 CFU/mL). Surface inoculation treatments were conducted by pipetting 0.5-mL suspensions of *S. griseus* (4 × 10^5 CFU/mL) on nutrient agar solidified in magenta boxes. Treated and control plants were kept in a growth chamber for 7 days, after which time half of the plants were challenged with 1 mL of a conidial suspension of FOA (2 × 10^6 CFU/mL) and returned to the growth chamber for another 2 weeks. Dry weights of the shoots and roots were recorded. The experiment was a 2 × 4 factorial arranged in a randomized complete block design.

In Vitro Soil Assay. The ability of *S. griseus* to control disease in sterile soil was evaluated by challenging the asparagus plants with FOA after a root-dip treatment or a soil-drench treatment. The seedlings (7 days old) were transplanted to steam-sterilized sandy loam soil in sterile 8.9-cm metric pots and kept in a growth chamber at 26 °C for 8 days. One set of plants was then drenched with a distilled water suspension of *S. griseus* (37 mL, 9 × 10^5 CFU) followed by an additional 20-mL drench 2 days later. Two lots of soil were then prepared: a sterile batch containing 5 g of sterile millet/pot and a batch containing pathogen colonized millet and sterile soil. Plants that received drench treatment were then transplanted into the soils described above. Root dip treatment plants were dipped in a distilled water suspension of *S. griseus* (8 × 10^5 CFU/mL) for 2 s before they were transplanted. Control plants were also transplanted into the sterile and pathogen-inoculated soil. Treated and control plants were kept in a growth chamber for 14 days followed by visual evaluation for disease control and determination of shoot and root dry weights.

Greenhouse Assay. Cells from a YMG liquid culture (400 mL, 8 days) of *S. griseus* were collected by centrifugation and resuspended in normal saline (100 mL). An aliquot of this stock solution was diluted for root-dip experiment (1 × 10^6 CFU/mL). A 72-cell 10 × 20 flat was used for this experiment. Each cell was lined with two layers of cheesecloth and received 63 g of sterile soil, 0.12 g of yeast extract, 0.12 g of dextrose, and 2.5 g of millet (sterile or colonized with FOA). The cells intended for drench treatment received 10 mL of the antagonist stock solution. Seven-day-old plants were either planted directly into the appropriate cells or dipped in the cell suspension for 2 s prior to planting. The flats were kept in the greenhouse for 4 weeks under fluorescent lights. Fleshy root length and dry weight of fleshy roots, shoots, and fibrous roots were measured.

RESULTS AND DISCUSSION

Faeriefungin (Figure 1) was found to have a minimum inhibitory concentration of 12.5 ppm against *F. oxysporum* f.sp. *asparagi* and 5 ppm against *Aspergillus flavus* (Figure 2). In contrast, asparagus seedlings showed good tolerance to the chemical. In fact, the shoot length (77 mm) for germinating seedlings exposed to 50 ppm of faeriefungin was significantly greater than the mean shoot length (41 mm) of the controls (Table I), while root lengths and percent of germination were unaffected. Treatment of asparagus plants with 0, 10, and 100 ppm of faeriefungin resulted in no detectable difference in plant shoot weight and total plant weight when measured after 2, 3, and 4 weeks of growth (Figure 3). Extraction of the agar, in which the plants were grown with methanol at the end of the experiment, demonstrated the absence of faeriefungin.

Plants treated with 25 ppm of faeriefungin and challenged with FOA produced more than double the dry weight of fibrous roots, when compared to inoculated controls (Table II). Also, the fibrous root weights of these plants did not differ from that of the noninoculated plants.

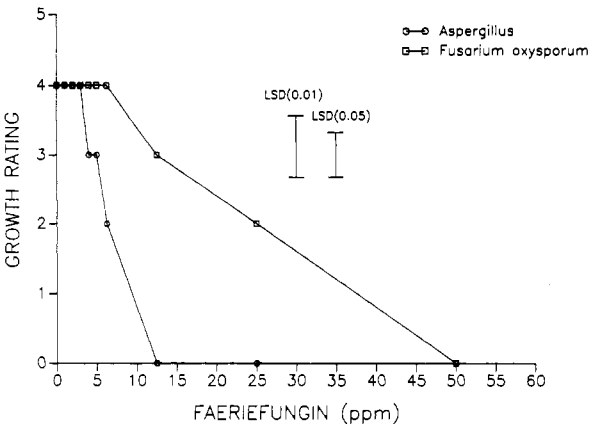

Figure 2. Response of two fungi, *A. flavus* and *F. oxysporum*, to several concentrations of faerifungin.

Table I. Effect of Faeriefungin on Germination and Seedling Growth of UC157 Asparagus Seeds[a]

treatment, ppm	shoot length, cm	root length, cm	germination, %
0	4.1 a	21.4 a	94 a
5	4.9 a	19.9 a	92 a
25	6.2 ab	15.7 a	86 a
50	7.7 b	18.3 a	94 a

[a] Average of 50 seeds. Means accompanied by the same letter are not significantly different at $p = 0.05$ by Duncan's multiple range test.

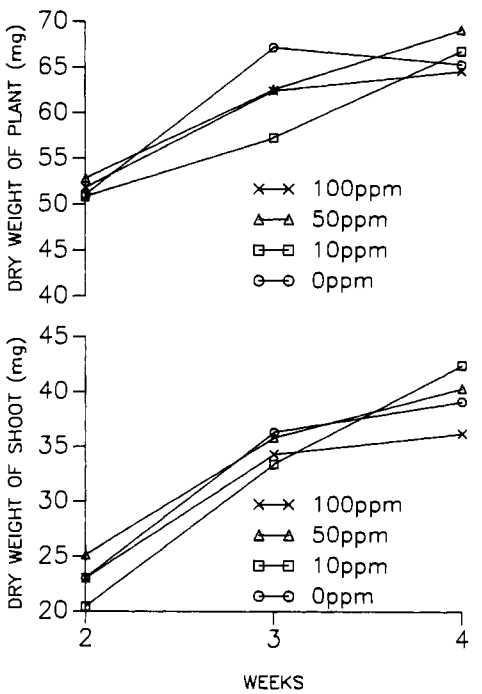

Figure 3. Effect of faeriefungin on UC157 asparagus seedlings.

The increase in the dry weight of the fibrous roots with the 25 ppm treatment was attributred to an increase in the length of the fibrous roots rather than an increase in the number. This suggests that faeriefungin interferes with infection of the root tip and allows increased growth. Additionally, the fibrous root dry weight (25 ppm) for faeriefungin-treated controls was greater than that for untreated controls. This is another indication that the compound is acting directly on the fibrous roots (Table II). The total root dry weight of FOA-inoculated plants treated with 25 ppm of faeriefungin did not differ from that of noninoculated plants with the 25 ppm treatment. The inoculated plants receiving 25 ppm of faeriefungin also showed increased dry weights of the entire root and whole plant when compared to the inoculated controls. While the lengths of the fleshy roots for all inoculated treatments were reduced, the dry weights of the fleshy roots receiving faeriefungin at 0, 12.5, and 25 ppm were not significantly different from those of the noninoculated controls. This could imply that fleshy root dry weight fails to accurately describe the extent of disease. More likely, however, is that the parameter represents a morphological change induced by infection that does not necessarily confer a competitive disadvantage. The ability of the fleshy roots to store nutrients may not be limited as evidenced by their dry weight status. Also, the increased surface area of the fibrous roots, due to their increase in length, quite likely confers a nutrient uptake advantage for those plants with a greater fibrous root dry weight. This may also explain why the fleshy root dry weight of the FOA-inoculated plants receiving 50 ppm of faeriefungin treatment was significantly less than that of the noninoculated controls.

Table II. Control of Root Rot and Stem Wilt with Faeriefungin in an in Vitro Agar Assay[a,b]

faeriefungin, ppm	fleshy root length, mm	fleshy root dry wt, mg	fibrous root dry wt, mg	total root dry wt, mg	total plant dry wt, mg
-FOA					
0.0	135.0	5.0	1.2	6.2	15.4
12.5	115.1	3.9	1.9	5.8	18.0
25.0	125.9	4.3	1.9	6.2	18.3
50.0	93.0	3.3	1.5	4.7	13.1
+FOA					
0.0	63.2	3.9	0.6	4.6	12.6
12.5	51.0	4.3	1.0	5.3	14.8
25.0	68.7	4.9	1.4	6.2	17.0
50.0	38.6	3.0	0.8	3.8	10.7
LSD (0.05)	31.4	1.2	0.5	1.2	3.5
LSD (0.01)	42.7	1.6	0.7	1.6	4.7

[a] Average for 12 seedlings. [b] The interaction of FOA vs faeriefungin was not significant at $p = 0.05$.

Table III. Control of Root Rot and Stem Wilt with *S. griseus* in Vitro Agar Assay[a]

treatment	fleshy root length, mm	fleshy root dry wt, mg	fibrous root dry wt, mg	total root dry wt, mg	total plant dry wt, mg
-FOA					
control	134.5 a	4.9 a	2.51 a	7.5 a	25.9 a
root dip	67.1 b	4.8 ab	0.91 ab	5.7 b	16.5 b
incorporate	15.6 d	1.8 c	0.0 d	1.8 c	6.6 cd
surface	21.5 d	3.1 bc	0.0 cd	3.1 c	7.8 c
+FOA					
control	47.9 c	5.7 a	0.76 ab	6.5 b	18.7 b
root dip	55.9 bc	4.2 ab	0.65 bc	4.9 b	15.2 b
incorporate	14.5 d	1.5 c	0.0 d	1.5 c	5.3 cd
surface	17.8 d	1.7 c	0.02 d	1.7 c	2.5 d

[a] Average for eight seedlings. Means accompanied by the same letter are not significantly different at $p = 0.05$ according to Duncan's multiple range test.

The antagonist completely inhibited the growth of FOA on all media assayed but did not show any protection to plants against disease on the basis of the variables measured (Table III). All of the *S. griseus* treatments reduced asparagus seedling growth in the absence of FOA. The root-dip treatments challenged with the pathogen did not differ from the treatments with the pathogen alone. Incorporation and inoculation on the surface of the agar with *S. griseus* resulted in plants that were less healthy by all parameters measured, whether or not they were challenged with the pathogen. This might perhaps be

Table IV. Control of Root Rot and Stem Wilt of Asparagus Grown in Greenhouse in Sterile Soil with *S. griseus* Antagonist Treatments[a]

treatment	fleshy root length, mm	fleshy root dry wt, mg	fibrous root dry wt, mg	total root dry wt, mg	total plant dry wt, mg
-FOA					
control	54.2 a	18.4 a	16.1 a	34.5 a	67.6 a
root dip	51.6 ab	11.4 abc	19.7 abc	19.7 b	40.8 b
incorporate	50.6 ab	11.6 abc	2.0 bc	13.7 bc	25.8 bc
+FOA					
control	16.8 b	4.1 c	1.0 c	5.1 c	10.1 c
root dip	36.6 ab	14.1 ab	5.5 b	19.6 bc	31.3 b
incorporate	29.2 ab	8.4 bc	5.1 bc	13.5 bc	28.8 bc

[a] Average of five seedlings. Means accompanied by the same letter are not significantly different at $p = 0.0$ according to Duncan's multiple range test.

explained by an accumulation of toxic metabolites in the closed magenta box system supplied with a large antagonist inoculum, since the root-dip treatments previously did not demonstrate toxicity.

Data for dip treatments of growth chamber grown plants were not processed due to severe plant injury or death. No differences were seen in plants receiving soil drench treatments of antagonist followed by introduction of the pathogen compared to plants challenged only with the pathogen. The death of the plants after dipping may have been due to inadequate rinsing of cells to remove broth. In contrast, soil drenching may not have exposed the roots to high concentrations of toxins because they may have been diluted in the relatively large volume of soil or they may have been adsorbed to the soil particles.

In the greenhouse experiments, inoculation with FOA reduced growth for all variables measured (Table IV). *S. griseus* incorporation into sterile soil provided no reduction in disease as evidenced by all variables measured. Dry weights of fibrous root, total root, and total plant weight of the incorporated treatment challenged with the pathogen were all significantly lower than the pathogen-free control plants. Pathogen-free root-dip treatments provided an increase in dry weight of total root, fibrous root, and total plant dry weight compared to inoculated controls. Moreover, inoculated root-dip treatments were higher than inoculated controls in terms of fleshy root dry weight, total root dry weight, and total plant dry weight. These data suggested that disease control, using root dip as the method of inoculation, might be achieved by using *S. griseus*. Despite the favorable influence of the inoculated root-dip treatments over inoculated controls, the pathogen-free dip treatments still performed more poorly than pathogen-free control plants in terms of total root dry weight, fibrous root dry weight, and total plant dry weight. This reduction may be the result of the toxic effects of the antagonist. The increased efficacy of the root-dip treatment in comparison to that in the agar system may be due to a reduced accumulation of toxic microbial metabolites in the open soil system.

Faeriefungin showed no phytotoxic effects against *A. officinalis* in the concentration ranges assayed. In fact, faeriefungin showed some stimulatory effect on the growth of asparagus seedlings. Additionally, the chemical provided disease protection as evidenced by an increase in several growth parameters used to assess FOA infection. *S. griseus* was a strong antagonist against FOA regardless of nutrient conditions. However, *S. griseus* showed toxicity to asparagus plants and no disease control was achieved with *S. griseus* in the growth chamber whether the plants were grown in agar or in sterile soil. Some degree of control was achieved, however, in the greenhouse experiments. These experiments indicate that the antibiotic might be manipulated more effectively than the organism for control of FOA. Additional tests, particularly field trials, are necessary to ascertain the efficacy of these approaches.

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