New Forests **15:** 181–191, 1998.
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Interactions among *Streptomyces griseoviridis*, *Fusarium* root disease, and Douglas-fir seedlings

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Accepted 1 October 1997

Key words: germinants, root disease, seedling growth, seedling infection, virulence

Application. Our laboratory and greenhouse experiments failed to provide any evidence of protection afforded by *Streptomyces griseoviridis* to Douglas-fir seeds and seedlings against *Fusarium*-caused damping-off and root disease.

Abstract. In a laboratory experiment, Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) seedlings had similar disease ratings when treated with known *Fusarium* isolates or concurrently with *Fusarium* and *Streptomyces griseoviridis*. When tested under greenhouse conditions and against known *Fusarium* isolates, more seeds germinated and survived as seedlings in control medium than survived in *S. griseoviridis*-inoculated medium or when *S. griseoviridis* and *Fusarium* were added together. A series of applications of *S. griseoviridis* as a soil drench to a crop of Douglas-fir seedlings did not affect seedling morphology. However, against resident levels of *Fusarium*, *S. griseoviridis* reduced *Fusarium* infection by 16%, but increased infection by *F. oxysporum* and *F. proliferatum*, two potentially pathogenic fungi, by 40%.

Introduction

In container nurseries in Idaho, Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) seedlings can be seriously damaged or killed by *Fusarium*-caused disease (James et al. 1991). On older seedlings, controlling disease with medium-incorporated fungicides or fungicidal drenches is usually unsuccessful (James et al. 1987, Dumroese et al. 1990a). Recently, horticulturists have focused more attention on biological control of pathogens, although not explicitly for conifer seedlings (James et al. 1993). *Streptomyces* spp. or extracts from them have shown control of several root-inhabiting pathogens (Kundu and Nandi 1984, Rothrock and Gottlieb 1984), including *Fusarium* spp. (Lahdenperä et al. 1991, Elson et al. 1994). One commercially-available product is Mycostop® biofungicide containing *Streptomyces griseoviridis* L. Anderson (K61). This organism was iso-

lated from light colored, Finnish sphagnum peat used for horticultural crops (Tahvonen 1982). Our objective was to determine if either a seed coating or medium top-dressing of Mycostop was effective in reducing *Fusarium*-caused damping-off and root disease on container-grown Douglas-fir seedlings, and if *S. griseoviridis* affected seedling growth.

Methods

We used Mycostop biofungicide, a formulation containing dried mycelium and spores of the actinomycete *S. griseoviridis*, at rates recommended by the manufacturer (Kemira Agro Oy, Helsinki, Finland). The 1:1 peat:vermiculite medium was supplied by Grace/Sierra, Portland, Oregon. The greenhouse damping-off and seedling experiments were conducted at the University of Idaho Research Nursery, Moscow, Idaho.

Laboratory pathogenicity experiment

We tested *S. griseoviridis* against selected isolates of *Fusarium oxysporum* Schlecht. (9043B, 9065I), *F. proliferatum* (Matsushima) Nirenberg (9112F, 9201E), and *F. sporotrichioides* Sherb. (9114A) using five treatments: Mycostop treated, Mycostop treated and *Fusarium* inoculum, *Fusarium* inoculum alone, inoculum control, and control. *Fusarium oxysporum* and *F. proliferatum* isolates were collected from container-grown Douglas-fir seedlings while *F. sporotrichioides* was isolated from commercially-prepared peat:vermiculite growing medium. Isolates were used because of their different levels of virulence, as determined by laboratory assays (James 1996).

Glass vials (23 ml) were filled to two-thirds capacity with non-sterile, 1:1 peat:vermiculite. Mycostop treatment consisted of mixing 0.2 g granular Mycostop with 10 g seed before germination, and an additional 4 ml of Mycostop suspension (0.05 g / 50 ml water) when germinants were placed into vials. Four ml of plain water were added as the Mycostop control. *Fusarium* inoculum was prepared using techniques of Miles and Wilcoxson (1984) as modified by James et al. (1989), an effective method in previous pathogenicity tests on conifer seedlings (James et al. 1989, Dumroese et al. 1996). Perlite was the inoculum matrix and moistened cornmeal and 1% potato dextrose agar (PDA) provided nutrients for fungal growth. Perlite/cornmeal/PDA mixtures were inoculated with spore suspensions of selected *Fusarium* isolates and incubated in the dark at ≈24 °C at least 24 days. After incubation, inoculum was air dried and ground to a powder with mortar and pestle. Inoculum powder was added to vials and mixed thoroughly at a rate of 0.05 g (2.2% w/w). For the *Fusarium* control, non-inoculated perlite powder was added to

vials at the same rate as *Fusarium* inoculum. Control germinants were grown only with non-sterilized peat:vermiculite.

Douglas-fir seeds (seedlot Bovill 3) were surfaced sterilized 10 min in a solution of 2 parts bleach (5.25% sodium hypochlorite) with 3 parts tap water (Wenny and Dumroese 1987), rinsed 48 h in running tap water and chilled 28 days at 3 °C. After chilling, seeds were rinsed 24 h in running tap water prior to sowing. Half the seeds were mixed with Mycostop granular powder (0.2 g Mycostop / 10 g seed). Treated and control seeds were placed on moistened filter paper (Whatman No. 3, Whatman International Ltd., Maidstone, England) within petri dishes and incubated in the dark at ≈24 °C. Nearly 75% of germination occured in the first 14 days (70% cummulative germination). Germinants, selected when their primary roots were at least 5 mm long, were carefully placed with their primary root pointing downward, one per vial. Each treatment consisted of 24 vials with germinants, incubated under cool fluorescent, diurnal light (12 h photoperiod) at ≈24 °C. Germinants within inoculation vials were examined daily for disease symptoms (damping-off and hypocotyl rot). Two ml of sterile water were added to germinants surviving 7 days. Tests concluded at 14 days, and all surviving germinants were removed and examined for disease symptoms. Reisolations were made from all inoculated germinants onto Komada's (1975) medium to determine if infection by inoculated isolates occurred.

A numerical rating system was used to compare levels of *Fusarium* virulence and protection afforded by *S. griseoviridis* on inoculated germinants (James 1996). Briefly, the rating system awarded points based on duration of germinant survival within inoculated vials, occurrence and type of disease, reisolation of inoculated fungal isolate, and primary root growth within the vial. Germinants received one point for each day of survival from day 3–14 (12 points maximum), another 1, 2 or 4 points depending on type of disease apparent (maximum points if non-diseased), an additional 1, 2 or 4 points based on fungal reisolation (maximum points if no organism reisolated), and 3 points more if the root grew to the bottom of the vial. The range of possible points was 3–23, with higher point values reflecting less aggression by the tested isolate against the germinant. To convert points to a score in which higher numbers represent greater virulence, we devised a reciprocal rating. Ratings ranged from 0 to 100; 0 indicating germinants were uninfected within 14 days and 100 indicating all germinants killed within 3 days by the inoculated isolate.

Greenhouse damping-off experiment

Twelve clean metal pans (38 × 23 × 5 cm), filled with 1:1 peat:vermiculite medium, were autoclaved 1 h at 121 °C. Unground *Fusarium* inoculum,

prepared as described above and using the same isolates from the laboratory pathogenicity experiment, was added to medium on a dry-weight basis at 1:50 (James et al. 1989). Control pans were inoculated with perlite/cornmeal/PDA mixtures lacking *Fusarium*. Growing medium with inoculum was thoroughly moistened and allowed to drain to field capacity. Bovill 3 seeds were pre-treated as described above and surfaced dried. For the treatment, half of the seeds were coated with 5 g Mycostop per kg of seed (10^8 cfu / 1 g Mycostop). Non-coated seeds were controls. For each isolate/pan combination, 100 treated or control seeds were sown the first week of April and covered with 1 cm coarse silica grit and placed on greenhouse benches and maintained under operational growing conditions. Average minimum and maximum greenhouse temperatures were 17° and 26 °C. Damped-off seedlings were removed daily and emergence determined 28 days after planting. Trays were watered as necessary and no fungicides were applied. To determine if infection by inoculated isolates occurred, reisolations were made from dead seedlings, and after 30 days from remaining live seedlings, onto a selective medium for *Fusarium* (Komada 1975).

Seedling experiment

We used 36 non-sterilized trays, each containing 200 individual Ray Leach Pine Cell® containers (66 ml) in which Douglas-fir seedlings were previously grown. Background inoculum levels of *Fusarium* on container surfaces were assayed. Two pieces, each about 0.5 cm^2, were removed from the bottom drainage hole of 10 randomly selected cells per tray, placed on Komada's (1975) medium, and incubated as described below. All containers were filled with 1:1 peat:vermiculite. Three Douglas-fir seedlots (Bovill 3, MO21 and KO27) were pre-treated as described above. For these seedlots, germination rate was similar; about 75% of cummulative germination occurred within 14 days. Three seeds per cell were sown in April and covered with 1 cm coarse silica grit. Filled trays were randomly assigned to either the Mycostop treatment or control and placed on greenhouse benches in a completely randomized design. Each of the six trays per seedlot served as a replicate. As soon as the peat:vermiculite medium was thoroughly moistened, the control trays (18 total) were removed from benches and the remaining trays received Mycostop at a rate of 0.1 g / liter (each cell received 14 ml of suspension) applied with a traveling irrigation system. Control trays were returned and seedlings were grown using a standard growing regime (Wenny and Dumroese 1992) under operational conditions. Three more Mycostop applications were made with the traveling irrigation system at the same rate and at monthly intervals (May, June, July). No other fungicide applications were made. Seedling emergence was determined 28 days after sowing. From April

through July, average minimum and maximum greenhouse temperatures were 17 ° and 26 °C.

Eight months after sowing (November), 10 non-diseased seedlings from each replicate/treatment/seedlot combination were measured for root collar diameter (RCD), height from groundline to the tip of the terminal bud, and root volume using Burdett's (1979) water displacement technique. Ten root tips (each 1 cm long) from each non-diseased seedling were randomly removed. Roots were washed, surface sterilized in a 1:10 bleach:water solution for 1 min, rinsed in sterile water, and aseptically placed on Komada's (1975) medium. These were incubated 7 days under cool fluorescent light at ≈24 °C. Samples were assayed for infection; percentage root colonization of infected seedlings was calculated by counting root pieces colonized. Oven-dry shoot and root weights were determined after drying 48 h at 60 °C.

Statistics

ANOVA (PROC GLM) was used to analyze seedling morphological characteristics and seedling infection levels (SAS Institute Inc. 1989). Greenhouse emergence data, *Fusarium* colonization data, and laboratory pathogenicity ratings were analyzed with a one-way analysis of variance (Snedecor and Cochran 1989). Data from the damping-off study were pooled (all control vs. all treated) prior to one-way analysis of variance. Residuals were plotted and their distribution was normal, independent, and homogenous, making data transformation unnecessary. When appropriate, means were compared with Tukey's HSD at the 0.05 level of significance.

Results and discussion

Inoculum for *Fusarium* infection in the seedling experiment was provided by natural levels on seed and non-sterile containers and by natural introduction of airborne spores. About 80% of the cells in each tray were colonized with one of seven *Fusarium* spp., including *F. acuminatum* Ell. & Ev. (found in 35% of colonized cells), *F. proliferatum* (30%), *F. sambucinum* Fuckel (21%), *F. culmorum* (W.G. Smith) Sacc. (9%), *F. sporotrichioides* (6%), *F. chlamydosporum* Wollenw. & Reinking (3%), and *F. oxysporum* Schlect. (1%). *Fusarium* inoculum was almost certainly not uniformly distributed throughout all treatments at the beginning of the experiment. However, our experience indicates that a large percentage of seedlings become infected with *Fusarium* from natural inoculum, although not necessarily resulting in disease, by the end of the growth cycle (James et al. 1987, 1988b). Therefore, we believe valid

Table 1. Laboratory pathogenicity ratings (mean ± S.E.) by individual *Fusarium* isolates either alone (control) or added concurrently with *S. griseoviridis*

Treatment	*Fusarium* isolate					
		F. oxysporum		*F. proliferatum*		*F. sporotrichioides*
	None	9043B	9065I	9112F	9201E	9114A
Control	20 ± 5	30 ± 4	55 ± 6	77 ± 2	84 ± 1	86 ± 1
S. griseoviridis	29 ± 6	27 ± 2	44 ± 6	73 ± 3	83 ± 1	89 ± 1
p value	0.3	0.5	0.2	0.3	0.5	0.02

24 observations/isolate/treatment.

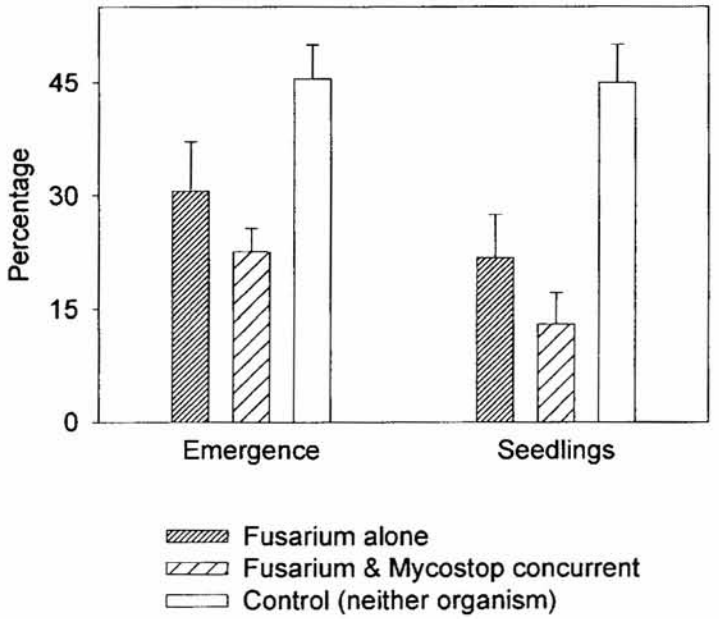

Figure 1. Greenhouse damping-off experiment. The percentage of germinating seeds (emergence) and the resulting percentage of seedlings after 28 days (±S.E.) after growing medium was either inoculated with *Fusarium*, *Fusarium* and *S. griseoviridis*, or neither (control). Values shown are averages for the 5 *Fusarium* isolates.

comparisons can be made among treatments based on prevalence of natural inoculum.

Laboratory virulence ratings between the control and *S. griseoviridis*-inoculated were similar (Table 1). In this respect, and compared with other potential biological control organisms, *S. griseoviridis* was similar to *Trichoderma harzianum* (Mousseaux et al. 1998) but unlike *Gliocladium virens*

(Dumroese et al. 1996). Early work with *S. griseoviridis* showed the organism to be highly effective in controlling damping-off by *Alternaria brassicicola* on artificially and naturally infected seeds of cruciferous plants (Tahvonen and Avikainen 1987). Further, Tahvonen and Avikainen (1987) speculated *S. griseoviridis* applied as a seed dressing could be effective against *Fusarium* spp. However, in the laboratory experiment, significant differences in disease ratings were lacking between germinants inoculated with *S. griseoviridis* and *Fusarium* concurrently, and germinants exposed only to *Fusarium*. An exception was *S. griseoviridis*-treated germinants exposed to *F. sporotrichioides* (isolate 9114A) that showed a significantly higher disease rating when concurrently treated with *S. griseoviridis* and *Fusarium*. These results are similar to Merriman et al. (1974) who found root disease was not effectively controlled by a *Streptomyces* spp. Further, in the damping-off experiment, *S. griseoviridis* failed to improve germination when tested against known *Fusarium* isolates; emergence was similar between treatments ($p = 0.1$), but after 28 days, more live germinants were in the control when compared to *S. griseoviridis*-inoculated or *S. griseoviridis*/*Fusarium* inoculated ($p = 0.02$) (Figure 1). However, for all seedlots in the seedling experiment where *S. griseoviridis* was challenged with resident levels of *Fusarium* spp., emergence was unaffected by treatment ($p = 0.2$; data not shown).

In the seedling experiment, *S. griseoviridis* had little effect on seedling morphological characteristics, although slight reductions in height ($p = 0.08$) were observed (Table 2). Ocamb et al. (1996) observed that height of bareroot *Pinus strobus* L. seedlings treated with Mycostop was similar to the control. When tested against resident *Fusarium* spp. in containers, *S. griseoviridis* reduced the number of seedlings infected by *Fusarium* spp. 16%, but root colonization levels were similar between treatments (Table 2). Although the number of seedlings infected by all *Fusarium* spp. was reduced, the number of seedlings infected by *F. proliferatum* and *F. oxysporum* (species found during the container assay) was significantly increased about 40% in the *S. griseoviridis* treatment, and nearly 85% of the increase was by *F. proliferatum*. Increased occurrence of *F. proliferatum* and *F. oxysporum* on non-diseased seedlings in the *S. griseoviridis* treatment was similar to our findings with *G. virens* (Dumroese et al. 1996). *Fusarium proliferatum* is an important cause of root disease in older container-grown seedlings (James et al. 1995). In recent experiments, isolates of *F. proliferatum* were consistently high in virulence with little variation among tested isolates, whereas *F. oxysporum* showed a range of virulence from low to high (James et al. 1997). In our experiments, *S. griseoviridis* failed to reduce colonization of roots by the most probable pathogenic *Fusarium* species, *F. proliferatum*.

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