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BIOLOGICAL CONTROL OF *FUSARIUM OXYSPORUM* AND *FUSARIUM PROLIFERATUM* ON YOUNG DOUGLAS-FIR SEEDLINGS BY A NONPATHOGENIC STRAIN OF *FUSARIUM OXYSPORUM*

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ABSTRACT

Tests were conducted to evaluate detrimental effects of *Fusarium oxysporum* strain Fo47, which has been developed as a potential biological control for *Fusarium* root diseases, on young Douglas-fir seedlings. Further tests evaluated the ability strain Fo47 to reduce *Fusarium* disease severity on young seedlings under laboratory conditions. Strain Fo47 elicited disease symptoms on about 50% of the inoculated seedlings at a 2% (w/w) inoculum level; at twice this rate, almost three-fourths of the seedlings displayed disease symptoms. Strain Fo47 significantly reduced disease severity caused by pathogenic *F. oxysporum* and *F. proliferatum* on young, Douglas-fir seedlings in five of six tests. These tests indicated that strain Fo47 warrants further greenhouse and/or field testing to evaluate its efficacy in controlling *Fusarium* diseases on conifer seedlings.

INTRODUCTION

Fusarium spp. cause important diseases of conifer seedlings in forest tree nurseries (Bloomberg 1971, 1981; Brownell and Schneider 1983, 1985; Enebak et al. 1990; James 1985; James et al. 1991; Lock 1973; Salisbury 1954). Most of these diseases are caused by soilborne isolates of *Fusarium* that infect and colonize host root cortical cells (Bloomberg 1966; Chakravarty et al. 1991; Duda and Sierota 1987; Farquhar and Peterson 1990; James and Perez 1999; Matuo and Chiba 1966; Strobel and Sinclair 1991). In many cases, root are colonized by *Fusarium* isolates that are either not capable of eliciting disease symptoms (nonpathogenic) or can only cause disease when hosts are severely stressed (Amir and Alabouvette 1993; Axelrood et al. 1995; Ben-Yephet and Shtienberg 1994; Brownell and Schneider 1985; Jorqe-Silva et al. 1989). However, some isolates of *Fusarium* can be aggressive pathogens and quickly kill host tissues (Armstrong and Armstrong 1975; Gordon and Martyn 1997; Gordon and Okamoto 1992; James et al. 1997, 2000; Vaartaja and Bumbieris 1967; Wenner and Merrill 1984).

When assays for soilborne populations of *Fusarium* are conducted, reported levels of these organisms include both pathogenic and nonpathogenic populations. Unfortunately, both types of isolates appear morphologically similar and cannot easily be differentiated (Appel and Gordon 1994; James et al. 1991; Kistler 1997). In some cases, molecular analyses have been successful in separating pathogenic from nonpathogenic isolates (Appel and Gordon 1995; Baayen et al. 2000; Edel et al. 1995; Gordon and Okamoto 1992; Ho et al. 1985; Kistler et al. 1991; Paavanen-Huhtala et al. 1999), but these techniques are expensive, require sophisticated equipment, and are not always effective.

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One of the most important pathogenic species of *Fusarium* is *F. oxysporum* Schlecht. This common soilborne species has a very wide host range; individual pathogenic strains of this species are recognized as particular formae speciales based on their ability to elicit disease on specific host plants (Armstrong and Armstrong 1975; Gordon and Martyn 1997; Kistler 1997; Kuninaga and Yokosawa 1989; Matuo and Chiba 1966). Races are delineated on the basis of pathogenicity to specific cultivars of certain hosts (Gordon and Martyn 1997; Jacobson and Gordon 1990; Kaiser et al. 1994). Previous reports (Amir and Alabouvette 1993; Elad and Baker 1985; Larkin et al. 1993, 1996) have indicated that in some cases where populations of pathogenic *F. oxysporum* are high and susceptible plant species are present, disease does not occur. Soil with these characteristics is identified as disease "suppressive"(Amir and Albouvette 1993; Duchesne et al. 1989a; Larkin et al. 1996; Sinclair et al. 1975). Investigations of these soils indicate that the suppressiveness is due primarily to high levels of biotic agents (Alabouvette et al. 1984; Amir and Alabouvette 1993; Hansen et al. 1990; Hocking and Cook 1972). Recently, workers have found that some disease suppressive soils have very high populations of *F. oxysporum* that seem to interfere with the ability of pathogenic isolates of this and other *Fusarium* species to initiate diseases on susceptible hosts (Alabouvette et al. 1993; Appel and Gordon 1994; Correll et al. 1986; Gordon and Okamoto 1992; Hillocks 1986). These nonpathogenic strains of *F. oxysporum* seem able to effectively compete with pathogenic strains because they occupy the same niches (readily infect root cortical cells), have the same food requirements, and can proliferate saprophytically on a wide range of soil organic matter (Benhamou and Garand 2001; Damicone and Manning 1982; Duijff et al. 1999; Elias et al. 1991; Hervas et al. 1995, 1997; Larkin and Fravel 1998, 1999). One particular nonpathogenic isolate of *F. oxysporum* (designated Fo47) was isolated from suppressive soils in France and is currently available as a potential biological control agent against pathogenic *Fusarium* isolates, including *F. oxysporum* (Duijff et al. 1999; Fuchs et al. 1997; Lemanceau et al. 1992, 1993). Because of the importance of *Fusarium*-associated diseases in western forest nurseries, investigations were initiated to determine how strain Fo47 of *F. oxysporum* might perform in controlling *Fusarium* diseases. This work was especially timely because of the anticipated loss of methyl bromide as a pre-plant soil fumigant by January 2005 (Stone et al. 1997). Methyl bromide in combination with chloropicrin, has effectively controlled soilborne pathogens, including *Fusarium* spp., in bareroot nurseries for many years (Hansen et al. 1990; James. 1989). However, most alternatives are often not nearly as satisfactory in keeping pathogen populations low and diseases under control (Hansen et al. 1990; Stone et al. 1997). Likewise, chemical fungicides are usually not effective in controlling *Fusarium* diseases, particularly once disease symptoms appear (James et al. 1991; Williams 1989). Therefore, treatments with effective biological control agents may enhance current strategies for disease control without relying on chemical pesticides.

MATERIALS AND METHODS

Techniques outlined by James (1996) were adapted for this investigation. The basic approach was to expose young Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) seedlings to test fungal isolates and record production of disease symptoms. Because of past success (James 1996; James et al. 1997, 2000), cornmeal-perlite inoculum was used for all tests. Fungal inoculum was prepared using the techniques of Miles and Wilcoxin (1984). Perlite, an inert, inorganic, siliceous rock of volcanic origin commonly used in potting mixtures, was the matrix for fungal growth. 150 g of yellow cornmeal was moistened with 300 mL warm 1% potato dextrose agar (PDA), to which 75g of perlite were added. The mixture was placed into glass vials to about two-thirds capacity which were then autoclaved for 60 minutesF12- hour at 121°C. After cooling, vials were inoculated with about 10 mL spore suspension of the test fungus (produced by

adding sterile, distilled water to 14 day-old cultures grown on PDA). Vial caps were left loose to allow aeration. Vials were incubated in the dark for at least 21 days, after which the fungus had thoroughly colonized the perlite/cornmeal mixture. After incubation, inoculum was removed from vials and dried in open petri plates within a cabinet. Inoculum dried within 5-7 days and did not become contaminated with other organisms because the food base was completely colonized by the inoculated fungus. Once dry, inoculum was stored in sterile, plastic vials and refrigerated until needed.

Each test involved exposing 24 seedlings to specific fungal isolates within 23 mL vials. Each vial was filled to about 2/3 capacity (2.5 g) with dried coconut-vermiculite (coir) media (Grace/Sierra Horticultural Products, Milpitas, CA) and autoclaved at 121°C for 60 min. Vial lids were replaced loosely before sterilization. One high germination Douglas-fir seedlot was used for all tests (designated Flat Creek 78-10, Moscow Mountain – courtesy of the University of Idaho Research Nursery). Seeds were soaked in a 2-part bleach and 3-part water solution for 10 min. (Wenny and Dumroese 1987), rinsed 48 hrs. in running tap water, and stratified 21 days at 2-3°C. After stratification, seeds were placed on filter paper moistened with sterile water in petri plates. Seeds were incubated under 12-hour diurnal fluorescent light cycles at about 24°C and monitored daily for germination. Seeds were considered germinated when their primary root was at least 3 mm long.

Fungal inoculum (colonized perlite/cornmeal) was ground to fine powder with mortar and pestle. Fungal isolates selected for testing included the nonpathogenic *F. oxysporum* isolate Fo47 (courtesy of C. Alabouvette, Laboratoire de Recherches sur la Flore pathogene dans le sol, INRA, Dijon, France), four isolates of *F. oxysporum* and two of *F. proliferatum* (Matsushima) Nirenberg which were shown to be aggressive pathogens in previous tests (James et al. 1997, 2000). The four pathogenic *F. oxysporum* isolates were designated 9051C (isolated from diseased container-grown lodgepole pine seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho), 9306M (isolated from diseased container-grown western white pine seedlings – Potlatch Corporation Nursery, Lewiston, Idaho), 9243G (isolated from diseased container-grown Douglas-fir seedlings – Colville Reservation Nursery, Nespelem, Washington) and 9224G (isolated from diseased container-grown Douglas-fir seedlings – Bitterroot Native Growers Nursery, Hamilton, Montana). The two *F. proliferatum* isolates were designated 9202T (isolated from contaminated styroblock containers – Potlatch Corporation Nursery, Lewiston, Idaho) and 9306C (isolated from diseased container-grown western white pine seedlings – Potlatch Corporation Nursery, Lewiston, Idaho).

The first tests involved exposing young Douglas-fir seedlings to four different concentrations of *F. oxysporum* isolate Fo47 to evaluate possible pathogenicity or phytotoxicity to this isolate alone. The four inoculum concentrations tested were 0.4% (0.01 g), 1.2% (0.03 g), 2% (0.05 g) and 4% (0.1 g). The appropriate amount of dried inoculum was added to each vial containing dried, sterilized coconut-vermiculite media. Inoculum was distributed throughout the media by shaking. One recently-germinated Douglas-fir seed was placed in each vial with its radicle placed downward into the media. Four mL sterile water was added to each vial and caps were replaced loosely to allow aeration. Adding water activated the inoculum (Miles and Wilcoxin 1984). Each inoculum concentration was replicated once (24 seedlings per replication) except the 2% concentration which was replicated three times.

Vials containing inoculated seedlings were incubated at about 24°C within an incubator that provided fluorescent light for 12 hours daily. Tests ran a maximum of 14 days. Three days after inoculation, seedlings were first checked for disease symptoms. During this inspection, seedling roots were reoriented downward into the medium if necessary. Seedlings were then checked for disease symptoms daily until the end of the test. Standard post-emergence

damping-off was the most common disease symptom. In some cases, root decay occurred below ground line without visible mycelial production. After 14 days, surviving seedlings (without noticeable disease symptoms) were examined to determine if their roots had grown to the bottom of the inoculation vial; their roots were also examined for decay and/or necrotic lesions. Roots from all inoculated seedlings were washed, surface sterilized in 10% bleach (0.525% aqueous sodium hypochlorite) and incubated on a selective agar medium (Komada 1975) to determine if they were infected by the inoculated isolate.

A numerical test score was assigned to each inoculated seedling based on duration of seedling survival (without disease symptoms) within inoculated vials, occurrence and type of disease, reisolation of inoculated fungal isolate, and primary root growth within the vial (James 1996). The maximum score possible (all seedlings killed within 3 days by the test isolate) was 100; the minimum (all seedlings were not infected within 14 days) was zero. The average rating for all seedlings tested for a particular isolate was used to compare isolates. Virulence ratings were assigned based on average test scores: nonpathogenic = below 40; low virulence = 41-60; moderate virulence = 61-80; high virulence = above 80. Average percent disease, days of survival (without disease symptoms) and test scores for the Fo47 tests were compared with an analysis of variance. Significant differences (P=0.05) were located using Tukey's HSD test.

The second test involved initially exposing seedlings to *F. oxysporum* isolate Fo47 and then challenging seedlings with pathogenic isolates of either *F. oxysporum* or *F. proliferatum*. Inoculum concentrations for both Fo47 and pathogenic isolates were 2% (0.05 g). Exposure of seedlings to the pathogenic isolates alone was also done for comparisons. Tests were conducted as outline above with Fo47 inoculum introduced 5 days prior to the pathogenic isolates. Tests ran for 14 days after inoculation with the pathogenic isolates. Paired T tests were used to statistically compare inoculations with the pathogenic isolates only to those with strain Fo47 followed by the pathogenic isolates. Significant differences in average test scores were set at P=0.05.

RESULTS

The biological control strain of *F. oxysporum* (Fo47) initiated low levels of disease symptoms and exhibited either low virulence or was deemed nonpathogenic to young Douglas-fir seedlings (table 1). Detrimental effects on tested seedlings seemed related to level of Fo47 inoculum exposed to seedlings. For example, when the amount of inoculum was doubled from 2% to 4%, much more disease was encountered and higher average test scores, which measured general aggressiveness of isolates, were measured. However, even very low inoculum levels (0.4-1.2%) elicited some level of disease symptoms in about half the seedlings tested, although many of the symptoms did not appear until late in the 14-day test.

All putative pathogenic isolates of *F. oxysporum* and *F. proliferatum* were highly virulent against young Douglas-fir seedlings in the second test (table 2). All caused disease symptoms on all exposed seedlings, producing very high average test scores. In five of six cases, the *F. oxysporum* isolate Fo47 significantly reduced disease, as indicated by lowered average test scores caused by the pathogenic *F. oxysporum* and *F. proliferatum* isolates (table 2). Average seedling survival of young seedlings (without producing disease symptoms) was increased in four of the six tests. Therefore, isolate Fo47 exerted some level of biological control, when seedlings were first exposed to this isolate and then challenged with highly virulent *Fusarium* isolates.

Table 1. Effects of *Fusarium oxysporum* isolate Fo47 on Douglas-fir seedlings under controlled laboratory conditions.

Inoculum Rate – Replication[1]	Percent Disease[2]	Average Survival[3]	Average Test Score[4]	Virulence Rating[5]
0.4%-1	49.2 A	12.5 A	44.6 AB	Low
1.2%-1	50.0 A	12.2A	38.3 AB	Non-Path.
2%-1	54.2 A	10.9 AB	46.3 AB	Low
2%-2	56.5 A	12.5 A	48.9 AB	Low
2%-3	54.2 A	9.3 B	36.0 A	Non-Path.
4%-1	73.9 B		58.0 B	Low

[1]Inoculum rate (w/w/ added to coir-vermimulite growing media per vial: 0.4% = 0.01 g; 1.2% = 0.03g; 2% = 0.05 g; 4% = 0.10 g.

[2]Based on percent of inoculated seedlings (24 per replication) displaying disease symptoms by the end of the test (14 days after inoculation). Means followed by the same capital letter are not significantly different (P-0.05) using Tukey's HSD Test.

[3]Average number of days seedlings remained alive following inoculation; tests lasted a maximum of 14 days. Means followed by the same capital letter are not significantly different (P-0.05) using Tukey's HSD Test.

[4]Test scores based on seedling survival, disease production, reisolation of inoculated isolates, and extension of seedling radicle within inoculation vials; the maximum score was 100, the minimum was 0. Means followed by the same capital letter are not significantly different (P-0.05) using Tukey's HSD Test.

[5]Based on the average test score: 0-40 = nonpathogenic; 41-60 = low virulence; 61-80 = moderate virulence; 81-100 = high virulence.

Table 2. Effects of pre-inoculation of Douglas-fir seedlings with *Fusarium oxysporum* isolate Fo47 on disease caused by virulent isolates of *F. oxysporum* and *F. proliferatum*.

Fusarium Isolate[1]	Percent Disease[2]	Average Survival[3]	Average Test Score[4]	Virulence Rating[5]
FOXY-9051C	100.0	4.4	92.8	High
9051C-Fo47	91.3	6.8*	76.5*	Moderate
FOXY-9306M	100.0	5.0	89.1	High
9306M- Fo47	90.0	5.7	81.0	High
FOXY-9243G	100.0	4.7	91.0	High
9243G- Fo47	95.0	7.7*	79.2*	Moderate
FOXY-9224G	100.0	4.6	878.5	High
9224G- Fo47	100.0	6.3*	79.2*	Moderate
FPRO-9202T	100.0	3.1	99.4	High
9202T- Fo47	100.0	4.2*	90.4*	High
FPRO-9306c	100.0	3.2	99.2	High
9306c- Fo47	100.0	3.6	94.1*	High

[1]FOXY – *F. oxysporum*; FPRO – *F. proliferatum* Fo47 = biological control *F. oxysporum* isolate. Seedlings were inoculated with isolate Fo47 5 days prior to challenge by the pathogenic isolates.

[2]Based on percent of inoculated seedlings (24 per test) displaying disease symptoms by the end of the test (14 days after inoculation).

[3]Average number of days seedlings remained alive following inoculation; tests lasted a maximum of 14 days. Means followed by an asterisk are significant different (P-0.05) than those jut above that represent inoculation with only pathogenic strains using a paired T Test.

[4]Test scores passed on seedling survival, disease production, re-isolation of inoculated isolates, and extension of germinant radicle within inoculation vials; the maximum score was 100, the minimum was 0. Means followed by an asterisk are significant different (P-0.05) than those jut above that represent inoculation with only pathogenic strains using a paired T Test.

[5]Based on the average test score: 0-40 = nonpathogenic; 41-60 = low virulence; 61-80 = moderate virulence; 80-100 = high virulence.

DISCUSSION

Fusarium oxysporum is actually a complex of fungi that include important pathogens on a very wide range of plant hosts (Gordon and Martyn 1997; Kistler 1997; Migheli et al. 1993). It also comprises many strains that are usually nonpathogenic, although they may readily inhabit the rhizosphere and colonize plant roots (Benhamou et al. 2001; Correll et al. 1986; Laflamme et al. 1999; Mandeel and Baker 1991; Ogawa and Komada 1984;

Olivain and Alabouvette 1997; Schneider 1984). In general, the level of microbiological activity is very high in soils that are suppressive to *F. oxysporum*-caused diseases (Larkin et al. 1993, 1996; Sneh et al. 1984). Much of this biomass may include non-pathogenic or competitive strains of *F. oxysporum* as well as other fungi and many different types of bacteria (Duijff et al. 1999; Hervas et al. 1997; Hocking and Cook 1972; Larkin and Fravel 1998; Lemanceu and Alabouvette 1991). One major characteristic of suppressive soils is that they tend to be biologically stable (Alabouvette et al. 1984; Amir and Alabouvette 1993), i.e., their microbiological populations will remain high and stable unless they are disturbed by nonbiological effects such as treatment with pesticides or fumigants (Hansen et al. 1990; James 1989).

The putative biological control strain of *F. oxysporum* (Fo47) evaluated in this test was obtained from a *Fusarium*-suppressive soil in France several years ago (Alabouvette et al. 1993; Lemanceu and Alabouvette 1991). Many tests have been conducted on a wide range of agricultural crops showing the efficacy of this strain to control diseases elicited by pathogenic isolates of *F. oxysporum* within greenhouses or fields (Duijff et al. 1999; Fuchs et al. 1997; Hervas et al. 1995, 1997; Larkin and Fravel 1999; Larkin et al. 1996; Lemanceau et al. 1992, 1993; Postma and Rattink 1992; Salerno et al. 2000). Strain Fo47 is an rhizosphere-competent, aggressive colonizer of the surface of roots as well as epidermal and cortical tissues (Benhamou and Garand 2001; Lemanceau and Alabouvette 1991; Olivain and Alabouvette 1997; Steinberg et al. 1999b). Generally the fungus does not colonize the vascular tissues of infected plants (Benhamou and Garand 2001; Olivain and Alabouvette 1997). Strain Fo47 seems to colonize root tissues quickly and provides a protective barrier against pathogenic strains (Olivain and Alabouvette 1997; Steinberg et al. 1999a), similar to the Hartig net produced within roots by ectomyorrhizal fungal symbionts (Chakravarty et al. 1991; Duchesne et al. 1989b). Several investigations (Chakravarty et al. 1990; Duchesne et al. 1989a; Farquhar and Peterson 1990, 1991; Sinclair et al. 1975; Stack and Sinclair 1975; Strobel and Sinclair 1991a; Sylvia 1983) have shown that certain mycorrhizal symbionts protect young conifer seedling roots from disease caused by *F. oxysporum*. The protective effects are both physical and chemical (production of antibiotics) (Chakravarty et al. 1991; Farquhar and Peterson 1990, 1991; Strobel and Sinclair 1991b; Sylvia and Sinclair 1983). Protection of roots by strain Fo47 may be similar.

Strain Fo47 elicited disease symptoms on some young Douglas-fir seedlings evaluated in this test under controlled laboratory conditions. This "phytotoxicity" was at higher than expected levels for a putative non-pathogenic isolate. It appeared that this strain was able to adversely affect some seedlings when other microorganisms were either absent or present at very low levels (care was taken to avoid introduction of other microorganisms on both the growing medium used and the seed introduced into inoculation chambers). Apparently without the ameliorating effects of other microorganisms, high levels of strain Fo47 exerted some toxicity to vulnerable young seedlings. Similar phytotoxicity was found in previous tests of other potential biological control agents (*Gliocladium virens* and *Trichoderma harzianum*) under similar test conditions (Dumroese et al. 1996; Mousseaux et al. 1998).

Strain Fo47 was able to significantly reduce disease severity in most tests when pathogenic

isolates of *F. oxysporum* or *F. proliferatum* were inoculated onto seedlings after they had been exposed to the biocontrol agent for a few days. However, level of disease reduction was not extensive. One reason why strain Fo47 was unable to reduce disease severity more was that the tested pathogenic isolates were highly virulent. Under more normal field or greenhouse conditions, where seedlings would likely be exposed to a wide variety of potentially-pathogenic isolates, levels of disease protection may be greater. Under the laboratory conditions reported here, highly virulent isolates were able to quickly invade and induce disease on vulnerable young seedlings and were only marginally restricted by strain Fo47. Greater disease control may have been possible if strain Fo47 had been allowed to colonize seedling tissues more thoroughly than was possible in the 5 days allowed in this test.

The *Fusarium oxysporum* complex of fungi presents extensive challenges for reducing their impact on important plant crops. They cannot be easily distinguished morphologically, but new molecular techniques provide potential for rapid detection of pathogenic strains within infected plant tissues (Chiocchetti et al. 1999; Edel et al. 2000; Ho et al. 1985; Kelly et al. 1988; Manulis et al. 1994; Paavenen-Huhtala et al. 1999) and populations within particular fields can be characterized as to their potential to cause disease problems (Appel and Gordon 1994; Baayen et al. 2000; Gordon and Okamoto 1992; Jacobson and Gordon 1990). This complex of fungi exist as specific clones that reproduce asexually (Armstrong and Armstrong 1975; Gordon and Martyn 1997; Molnar et al. 1990; Puhalla 1985) and can be spread easily throughout a nursery. Fortunately, the general lack of sexual recombination should help preclude introduction of new, increasingly-virulent strains in a specific nursery (Gordon and Martyn 1997; Kistler 1997; Molnar et al. 1990). However, existing virulent strains may increase at the expense of less virulent or non-pathogenic strains depending on cropping practices (James and Perez 1999; James et al. 1991). If soils are repeatedly fumigated, any "biological balance" that may be achieved by soil microorganisms will be destroyed (Hansen et al. 1990; James 1989) Under these conditions, populations of non-pathogenic organisms that might naturally increase over time would be expected to decrease. If pathogens rapidly recolonize fumigated soil, their numbers will be much higher than if the soil had not been fumigated (Hansen et al. 1990; James 1989; Vaartaja and Bumbieris 1967).

Ideally, it would be very useful to characterize the *Fusarium* population within nursery soil prior to any prescribed treatment. If this population contained fairly high levels of non-pathogens, then soil treatments may be postponed or canceled. However, if the population was comprised of a relatively large proportion of pathogenic strains, soil treatments may be prescribed. Currently, only molecular techniques are able to properly characterize soil microorganisms populations (Gordon and Martyn 1997; Gordon and Okamoto 1992; Kistler 1997; Kistler et al. 1991) and such techniques have not been routinely applied to forest tree nurseries. Adding non-pathogenic and pathogen-antagonistic organisms to soils would help increase disease suppressiveness (Fuchs et al. 1997; Guillino et al. 1995; Larkin et al. 1993, 1996; Nagao et al. 1990; Yamaguchi et al. 1992) and thus ameliorate some of the potential problems of not fumigating. If a disease suppressive soil could be established and maintained, there would be no need for further soil treatments and diseases could be controlled more "naturally" than with introduced chemical pesticides.

In most cases, biological control of plant pathogens under agricultural conditions has not been as effective as chemical pesticides (Alabouvette et al. 1993; James et al. 1993; Larkin and Fravel 1998; Lemanceau and Alabouvette 1991). This may be due to the fact that most pesticides are non-organism specific and are very lethal to most soil microorganisms (Duda and Sierota 1987; Enebak et al. 1990; Williams 1989). On the other hand, biological agents are generally either competitive with or

antagonistic toward specific pathogens and are much less lethal (De Cal et al. 2000; Duda and Sierota 1987; Hock and Fuller 1977; James 2000; Louter and Edgington 1990; Park et al. 1988; van Peer et al. 1991). Therefore, when using biological agents, one goal is to achieve a more balanced biological community in which pathogens, although present, are not able to aggressively kill host plants because of the ameliorating effects of other organisms (Alabouvette et al. 1993; James et al. 1993). Under such conditions, the proportion of populations comprised of pathogens naturally decreases because pathogens have to compete with and generally reproduce less than non-pathogens (Lemanceau and Alabouvette 1991). This is the ideal situation for forest nurseries.

This work indicated that biological control of highly virulent *F. oxysporum* and *F. proliferatum* was possible using strain Fo47 on Douglas-fir seedlings. Further greenhouse and/or field tests of strain Fo47 are warranted. Such tests on container-grown seedlings might involve mixing Fo47 inoculum with container growing media. Young emerging seedlings could then be colonized by this organism shortly after seed germination. Later challenge with pathogenic *Fusarium* isolates could be used to assess biocontrol efficacy within greenhouses. In the field, strain Fo47 could be applied directly over seed following sowing. Pathogen inoculum could either be introduced artificially or naturally-occurring levels of soil pathogens could be used to assess biocontrol efficacy. A major problem with using strain Fo47 is to convince growers that introducing "*F. oxysporum*" into their nurseries will not result in more severe disease. Specialists have indicated that there is very little likelihood of genetic mutations which might result in converting nonpathogenic *F. oxysporum* isolates into virulent ones (Gordon and Martyn 1997; Gordon and Okamoto 1992; Hadar et al. 1989; Kistler 1997; Migheli et al. 1993; Molnar et al. 1990). Also there is a very remote chance of lateral gene transfer from pathogenic isolates to nonpathogens (Guillino et al. 1995). Therefore, widespread application of strain Fo47 within nurseries should be safe.

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