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Virulence of *Fusarium oxysporum* on Douglas fir germinants: comparison of isolates from nursery soil and roots of healthy and diseased seedlings

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Abstract

Using *in vitro* techniques, we determined pathogenicity and virulence of 179 isolates of *Fusarium oxysporum* on young Douglas-fir seedlings. Our isolates were from soil or from healthy or diseased conifer seedling roots at two bareroot nurseries in the Inland Northwest of the United States. Isolates from diseased seedling roots were more virulent than those from healthy seedling roots; all isolates from diseased seedling roots were virulent. Virulence of isolates was different between nurseries; soil-derived isolates were more virulent at one nursery, while the other nursery had more virulent root-derived isolates. At one nursery, soil-derived isolates were more virulent than those found on conifer roots. Nearly half of the soil isolates were highly or moderately virulent on Douglas-fir germinants. We concluded that both virulent and non-pathogenic *F. oxysporum* isolates are common in forest nursery soil. Further, conifer seedling root infection is common but most isolates from healthy seedlings are either non-pathogenic or exhibit low virulence.

Keywords: *Fusarium oxysporum*, root diseases, conifer seedlings, pathogenicity, bareroot forest nurseries

1 Introduction

Fusarium oxysporum Schlecht. is one of the most important soil-borne pathogens of forest nurseries in western North America. The fungus causes several types of diseases including pre- and post-emergence damping-off, cotyledon blight, and root disease (Bloomberg 1971). Although common in both container and bareroot nurseries, *F. oxysporum* most often causes greater impact in bareroot nurseries (Bloomberg 1976, James et al. 1991). This fungus is commonly isolated from nursery soil as well as roots of apparently healthy and

diseased seedlings (Chi et al. 1964, Smith 1967, Vaartaja 1967, Vaartaja and Bumbieris 1967). In agricultural soils, a relatively large proportion of the *F. oxysporum* population is usually comprised of non-pathogenic isolates, although most of these readily colonize plant organic matter and cortical cells of crop plant roots (Elias et al. 1991, Fiely et al. 1995, Gordon and Martyn 1997, Gordon and Okamoto 1992, Gordon et al. 1989, Katan et al 1994, Kistler 1997, Park 1959). Pathogenic and non-pathogenic isolates of *F. oxysporum* are morphologically indistinguishable (Bloomberg 1966, Bosland and Williams 1987, Correll et al. 1986a, Elmer and Stephens 1989, Katan et al. 1994). Pathogenic isolates of *F. oxysporum* are classified into formae speciales (f.sp.) on the basis of plant host range (Correll et al. 1986b, DiPietro et al. 1994, Gerlagh and Blok 1988, Gordon and Martyn 1997, Kistler 1997, Kuninaga and Tokosawa 1989). Pathogenic isolates on seedlings of all conifer seedlings are designated within f.sp. *pini* (Lock 1973, McCain 1978, Wright et al. 1963). However, there is apparently little evidence of host specialization within *F. oxysporum* associated with forest nurseries (Bloomberg 1981).

Evaluation of soil populations of *Fusarium* spp. in general and *F. oxysporum* in particular are often necessary to determine efficacy of different soil treatments in disease control experiments. Recently, much work has centered on developing alternatives to chemical pre-plant soil fumigation for production of high quality forest seedlings (James et al. 1993, Stone et al. 1997). Soil dilution techniques are implemented to evaluate how different treatments influence populations of *F. oxysporum*. Unfortunately, current techniques only give estimates of total population densities rather than elucidating pathogenic populations (Gordon et al. 1989). However, high overall populations of *F. oxysporum* in soil often correlate well with high disease levels on susceptible plants (Timmer 1982). New molecular techniques have sometimes been successful in separating pathogens from non-pathogens (Edel et al. 1995, Gordon and Martyn 1997, Kelly et al. 1994). However, such procedures are not always effective (Gordon and Martyn 1997) and require highly-trained personnel and expensive laboratory equipment.

Traditionally, laboratory or greenhouse pathogenicity tests have been used to separate pathogenic and non-pathogenic *Fusarium* isolates (Baayen et al. 1988, Manicom et al. 1990). Such tests are also used to evaluate relative susceptibility of certain plant cultivars to specific pathogenic races (Assigbetse et al. 1994, Baayen et al. 1988, Gordon and Martyn 1997, Gordon and Okamoto 1992a, Kistler et al. 1987). As a result, investigators are able to better characterize *Fusarium* populations and make more accurate predictions of disease potential.

Our objective was to screen a relatively large sample of *F. oxysporum* isolates obtained from soil and conifer seedling roots to determine proportion of pathogenic strains and elucidate relative virulence of isolates on an important conifer species. We hoped to obtain greater understanding of the ecological interactions among *F. oxysporum* strains commonly encountered in forest nurseries.

2 Materials and methods

A total of 179 *F. oxysporum* isolates were evaluated for pathogenicity on Douglas-fir germinants in laboratory tests. Isolates were from two bareroot forest nurseries in the Inland Pacific Northwest of the United States. Isolates were collected from nursery soil and the roots of either healthy (non-symptomatic) or diseased conifer seedlings during a 5 year period (Table 1). Selected isolates were incubated on a selective agar medium for *Fusarium* (Komada 1975) and single-spored and transferred to carnation leaf agar (Fisher et al. 1982). Isolates were stored for extended periods on colonized carnation leaves within sterile water (Fisher et al. 1982).

Table 1. Number of *Fusarium oxysporum* isolates tested for pathogenicity on Douglas-fir germinants.

Isolate Source	Nursery 1	Nursery 2	Total
Soil	90	39	129
Healthy Seedlings	8	25	33
Diseased Seedlings	13	4	17
Totals	111	68	179

We conducted pathogenicity tests using a technique for rapid laboratory assessment of virulence (James 1996). The basic approach of our pathogenicity tests was to expose Douglas-fir germinants to fungal isolates and record production of disease symptoms. Cornmeal-perlite inoculum was prepared for each tested isolate 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. Yellow cornmeal (150 g) was moistened with 300 ml warm 1% potato dextrose agar (PDA), to which 75 g of perlite was added. The mixture was placed into 25 ml glass vials to about 2/3 capacity which were then autoclaved for 60 min at 121°C. After cooling, vials were inoculated with about 10 ml of a spore suspension of each test fungus. The spore suspension was produced by adding sterile, distilled water to 14-day-old cultures grown on PDA. Vial caps were left loose to allow aeration and incubated in the dark for at least 21 days, after which the fungus had thoroughly colonized the cornmeal-perlite mixture. After incubation, inoculum was removed from vials and dried 5-7 days in open petri plates within a cabinet. Inoculum did

not become contaminated with other organisms during drying because the food base was completely colonized by the test fungus (Miles and Wilcoxin 1984). Once dry, inoculum was stored in plastic vials and refrigerated until needed. This type of inoculum remained viable in previous tests for at least two years (James 1996).

We used 24 vials (25 ml capacity) to test each fungal isolate. Each vial was filled to about 2/3 capacity with dried 1:1 (v/v) coir pith medium (Grace/Sierra Horticultural Products, Milpitas, CA). This medium has periodically been used by some growers to produce many different container-grown plants including conifer seedlings. Vials with media were autoclaved at 121°C for 60 min and cooled before being used in tests.

We used Douglas-fir seedlots with high germination capacity and energy. Seeds were initially soaked in an aqueous solution of sodium hypochlorite (2 parts bleach with 3 parts water) for 10 min (Wenny and Dumroese 1987), rinsed 48 h in running tap water, and stratified 21 days at 2-3°C. After stratification, seeds were placed in sterile petri plates on filter paper moistened with sterile water. Seeds were incubated under 12-h 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.

Cornmeal-perlite inoculum for each test isolate was ground to a fine powder with mortar and pestle and 0.05 g of the powder was added to each vial containing dried media. This resulted in an approximately 1:50 w/w mixture of inoculum to media. Inoculum was distributed throughout the media by shaking. One recently-germinated seed (germinant) was carefully placed into each vial with its primary root placed downward into the medium. Sterile water (4 ml) was added to each vial with caps replaced loosely to allow aeration. Adding water activated the inoculum (Miles and Wilcoxin 1984). Controls consisted of 24 vials with non-inoculated perlite added instead of inoculum.

Vials containing germinants were incubated at about 20-24°C on a lab bench with 8-12 h daily fluorescent light. Each isolate was evaluated for its ability to cause germinant disease within 14 days. After 3 days, germinants were first checked for disease and checked daily thereafter. Standard post-emergence damping-off was the most common disease encountered. A wet-rot type of disease sometimes occurred where the root was decayed but the above-ground portion of the germinant was not affected. Diseased seedlings were removed, their roots washed thoroughly and placed directly on Komada's medium for re-isolation of inoculated isolates. After 14 days, surviving germinants were examined to determine if their roots grew to the bottom of the vial. Roots of surviving seedlings were also analysed for infection by inoculated isolates as described above.

A numerical rating system for isolate comparisons was used which awards points based on duration of germinant survival, occurrence and type of disease, re-isolation of the inoculated test isolate, and primary root growth within vials (James 1996). The range of possible points was 3-23, with higher point values reflecting less virulence by the tested isolate. To convert points to a score in which higher numbers represented greater virulence, a reciprocal rating system was used (James 1996). In this system, the maximum score was 100 and the minimum score was zero.

Average virulence ratings of isolates on germinants were used to compare isolates. Based on previous experience (James and Perez 1999, James et al. 1995, 1997), highly virulent isolates exhibited scores of 80-100, moderately virulent isolates from 60-80, isolates with low virulence from 40-60, and isolates with average scores below 40 were considered non-pathogenic. Average virulence scores were compared using one-way analysis of variance; comparisons were made between nurseries and between isolate sources (soil vs. root tissue, healthy vs. diseased seedlings). Significant means (P=0.05) were separate using Tukey's HSD test.

3 Results

Virulence on Douglas-fir germinants varied widely among the *F. oxysporum* isolates tested. Some soil isolates were quite virulent, whereas many others were non-pathogenic. At one nursery, overall average virulence of soil isolates was similar to virulence of seedling root isolates (Table 2). However, at the other

Table 2. Comparisons of average virulence scores of *Fusarium oxysporum* isolates obtained from nursery soil and conifer seedling roots at two nurseries in the inland Pacific Northwest[1].

Isolate Source	Number Tested	Nursery 1	Nursery 2	Both Nurseries
Soil	129	57.6 A	63.6 A	59.5 A
Root Tissue[2]	50	58.4 A	51.8 B	54.8 B

[1] Within each column, means followed by the same capital letter are not significantly different (P=0.05) using Tukey's HSD.

[2] Included healthy and diseased seedlings.

nursery, soil isolates were significantly more virulent than isolates obtained from seedling roots. At both nurseries, *F. oxysporum* isolates obtained from diseased seedlings were more virulent than those from non-diseased seedling roots (Table 3).

Table 3. Comparisons of average virulence scores of *Fusarium oxysporum* isolates obtained from roots of healthy and diseased conifer seedlings at two nurseries in the inland Pacific Northwest[1].

Isolate Source	Number Tested	Nursery 1	Nursery 2	Both Nurseries
Healthy Roots	33	45.6 B	51.7 B	50.2 B
Diseased Roots	17	66.5 A	56.2 A	64.1 A

[1] Within each column, means followed by the same capital letter are not significantly different (P=0.05) using Tukey's HSD.

About 45% of the tested soil isolates were either highly or moderately virulent on germinants (Table 4). Almost 18% of these isolates were non-pathogenic in our tests. A higher percentage of isolates from roots of healthy seedlings were non-pathogenic when compared to soil isolates. None of the isolates from roots of diseased seedlings were non-pathogenic (Table 4).

Table 4. Virulence of tested *Fusarium oxysporum* isolates from two inland Pacific Northwest nurseries.

		Percent of isolates within virulence category			
Isolate Source	No. tested	High	Medium	Low	Non-Pathogen
Soil	129	19.4	28.7	34.9	17.0
Healthy Roots	33	6.1	27.3	36.4	30.2
Diseased Root	17	23.5	23.5	53.0	0
All Isolates	179	17.3	27.9	36.9	17.9

4 Discussion

Our results indicated that populations of *F. oxysporum* in forest nurseries vary widely in their ability to incite disease on young Douglas-fir germinants. Isolates classified as virulent were capable of eliciting typical post-emergence damping-off. It is possible that these isolates may not be capable of causing root disease on older seedlings. Most *Fusarium*-associated disease losses occur during the first growing season of bareroot production (Bloomberg 1971, 1976, 1981, James et al. 1991, Lock 1973). Shortly after emergence, seedlings are particularly susceptible to attack and mortality from damping-off fungi, including *F. oxysporum*. Once their stems become lignified and less succulent,

seedlings are not as susceptible and losses decrease (Bloomberg 1971, 1973, 1985, Brownell and Schneider 1983, Hartley and/ Merrill 1918, Spaulding 1914). Therefore, it is important to reduce populations of potentially-pathogenic *Fusarium* to levels where disease is less impacting. Also, seedlings should not be stressed, thus reducing their susceptibility. Restricting fertilization, especially nitrogen, is important when seedlings are particularly susceptible to damping-off (Salisbury 1954, Sinclair et al. 1975, Stoddard 1947, Walker and Foster 1946, Wensley and McKeen 1964). Also, seedlings should not be water-stressed; reduction of excessively high temperatures by shading may reduce seedling susceptibility to damping-off (Lock 1973, Park 1963, Reyes 1970, Salisbury 1952, Shea and Rediske 1961).

Reducing soil populations of potentially pathogenic *F. oxysporum* may be difficult, especially without soil fumigation. Because soil fumigation is expensive and methyl bromide will not be available for use in the near future (Evans and Greczy 1995, Shaheen 1996), nurseries are seeking alternatives to chemical fumigation for production of conifer seedlings (James et al. 1993, Stone et al. 1997). Possible alternatives include fallowing with periodic soil cultivation, amending with organic materials (particularly composts), alternative cover-green manure crop management, implementing biological control, and growing more seedlings in containers (Hansen et al. 1990, Stone et al. 1997). Some alternatives may be more successful than others at particular nurseries. Much additional work is needed to develop site-specific fumigation alternatives at particular nurseries.

Populations of *F. oxysporum* have been characterized using molecular analyses of nucleic acids (Appel and Gordon 1995, DiPietro et al. 1994, Edel et al. 1995, Kelley et al. 1994, Kistler et al. 1991), vegetative compatibility groups (Bentley et al. 1998, Correll et al. 1986b, Elias et al. 1991, Elmer et al. 1994, Gordon and Okamoto 1992a, 1992b, Jacobson and Gordon 1990a), isozymes (Ho et al. 1985), serology (Iannelli et al. 1962, Rataj-Guranowska and Wolko 1991), and production of volatile odors (Moore et al. 1991). *F. oxysporum* reproduces only asexually so populations consist of distinct clonal lineages (Kistler 1997, Kistler et al. 1991). In some cases, genetic characterization has shown direct relationships with pathogenicity (Bosland and Williams 1987, Coddington et al. 1987, Correll et al. 1986a, Gordon and Okamoto 1992a, Katan et al. 1989, Larkin et al. 1990). However, in other cases, genetic diversity was not related to pathogenic differences (Ho et al. 1985, Jacobson and Gordon 1990b, Salgado and Schwartz 1993, Venter et al. 1992). An important research goal is to identify the proportion of *F. oxysporum* isolates within a population that are pathogens on particular hosts without reverting to labor and time-intensive pathogenicity testing. If genetic markers directly related to pathogenicity can be identified, then large numbers of isolates can be quickly screened for these markers, giving investigators important information regarding pathogenic potential of populations. However, thus far such genetic markers have been difficult to obtain (Jacobson and Gordon 1988). Therefore, currently the proportion of the

population comprising pathogens can only be accurately determined using standard pathogenicity tests.

Isolates of *F. oxysporum* that commonly cause diseases of particular hosts are designated as "formae speciales" (f.sp.) (Correll et al. 1986a, Gerlagh and Block 1988, Gordon and Martyn 1997, Kistler 1997). Some conifer species appear more susceptible to *F. oxysporum*-associated diseases than others (James et al. 1991). Therefore, important questions include: (1) are all these species infected with the same f.sp.? (2) are there separate f.sp. that cause diseases of different conifer species? (3) do specific races of pathogenic *F. oxysporum* cause disease on different conifer species as they do on some agricultural crops (Gordon and Martyn 1997, Kistler 1997). Different strains of *F. oxysporum* have been identified based on their relative virulence on specific tree crops (Bloomberg and Lock 1972, Brownell and Schneider 1983, Matuo and Chiba 1966). However, they have not generally been assigned acceptable sub-specific taxons.

Our work only identified isolates that were pathogenic on Douglas-fir, it is possible that some isolates within the sampled population were more virulent on other conifer species. Perhaps some of the isolates we classified as "non-pathogenic" on Douglas-fir were pathogenic on other conifer species. This becomes important when putative non-pathogenic *F. oxysporum* isolates are selected for potential biological control (Elmer and Stephens 1989, Fuchs et al. 1997, Hervas et al. 1995, Larkin et al. 1996, Nagao et al. 1990). Non-pathogenic isolates of *F. oxysporum* may be excellent biological control agents against pathogenic isolates, particularly because they are well adapted to niches that might be occupied by pathogens (Alabouvette et al. 1993, Elias et al. 1991, Guillino et al. 1995, Mandeel and Baker 1991) and successfully compete with pathogens for nutrients (Appel and Gordon 1994, Damicone and Manning 1982, Edel et al. 1995, Lemanceau et al. 1993). However, if isolates selected for biological control are only non-pathogenic on one or a few conifer species, their applicability will be greatly limited. Pathogenic characters of *F. oxsporum* are controlled by a small number of gene sets (Kuninaga and Yokosawa 1989). Pathogenic isolates have been shown to be derived from populations of non-pathogens in the field (Gordon and Martyn 1997, Gordon and Okamoto 1992c). However, genetic exchange between pathogens and non-pathogens has not been detected *in vitro* (Guillino et al. 1995).

In vitro techniques we used to assess virulence of *F. oxysporum* isolates were ideal for infection and disease initiation by test fungi (Duchesne et al. 1989, Farquhar and Peterson 1990, James 1996). Under such conditions, differences in susceptibility was best related to length of seedling survival (Bloomberg and Lock 1972, James 1996). Under more normal field or greenhouse conditions, it is possible some of the tested isolates could behave differently. We suspect that at least a proportion of the isolates we called "virulent" would probably not cause disease under normal nursery conditions where competing micoroorganisms might ameliorate disease development. Although we know that some portion of the soil population is probably pathogenic, we still cannot easily quantitatively determine pathogens. Further work involving molecular genetic

analyses coupled with pathogenicity testing under normal nursery conditions, may help us accurately predict disease severity from soil population densities.

References

Alabouvette, C., Lemanceau P. & Steinberg C. 1993. Recent advances in biological control of *Fusarium* wilts. Pesticide Science 37: 365-373.

Appel, D. J. & Gordon, T. R. 1994. Local and regional variation in populations of *Fusarium oxysporum* from agricultural field soils. Phytopathology 84: 786-791.

— & Gordon, T. R. 1995. Intraspecific variation within populations of *Fusarium oxysporum* based on RFLP analysis of the intergenic spacer region of the DNA. Experimental Mycology 19: 120-128.

Assigbetse, K. R., Fernandez, D., Dubois, M. P. & Geiger, J.-P. 1994. Differentiation of *Fusarium oxysporum* f.sp. *vasinfectum* races on cotton by random amplified polymorphic DNA (RAPD) analysis. Phytopathology 84: 622-626.

Baayen, R. P., Elergsma, D.M., Demmink, J. F. & Sparnaaij, L. D. 1988. Differences in pathogenesis observed among susceptible interactions of carnation with four races of *Fusarium oxyporum* f.sp. *dianthi*. Netherlands Journal of Plant Pathology 94: 81-94.

Bentley, S., Pegg, K. G., Moore, N. Y., Davis, R. D. & Buddenhagen, I. W. 1998. Genetic variation among vegetative compatibility groups of *Fusarium oxysporum* f.sp. *cubense* analyzed by DNA fingerprinting. Phytopathology 88: 1282-1293.

Bloomberg, W. J. 1966. The occurrence of endophytic fungi in Douglas-fir seedlings and seeds. Canadian Journal of Botany 44: 413-420.

— , 1971. Diseases of Douglas-fir seedlings caused by *Fusarium oxysporum*. Phytopathology 61: 467-470.

— , 1973. Fusarium root rot of Douglas-fir seedlings. Phytopathology 63: 337-341.

— , 1976. Distribution and pathogenicity of *Fusarium oxysporum* in a forest nursery soil. Phytopathology 66: 1090-1092.

—, 1981. Diseases caused by *Fusarium* in forest nurseries. In: Nelson, P. E., Toussoun T. A. & Cook R. J. (eds.). *Fusarium*: Diseases, Biology, and Taxonomy. The Pennsylvania State University Press, University Park. pp. 178-187.

— , 1985. The epidemiology of forest nursery diseases. Annual Review of Phytopathology 23: 83-96.

— & Lock, W. 1972. Strain differences in *Fusarium oxysporum* causing diseases of Douglas-fir seedlings. Phytopathology 62: 481-485.

Bosland, P. W. & Williams, P. H. 1987. An evaluation of *Fusarium oxysporum* from crucifers based on pathogenicity, isozyme polymorphisms, vegetative compatibility, and geographic origin. Canadian Journal of Botany 65: 2067-2073.

Brownell, K.H. and R.W. Schneider. 1983. *Fusarium* hypocotyl rot of sugar pine in California forest nurseries. Plant Disease 67:105-107.

Chi, C. C., Childers, W. R. & Hanson, E. W. 1964. Penetration and subsequent development of three *Fusarium* species in alfalfa and red clover. . Phytopathology 54: 434-437.

Coddington, A., Matthews, P. M., Cullis, C. & Smith, K.H. 1987. Restriction digest patterns of total DNA from different races of *Fusarium oxysporum* f.sp. *pisi* - an improved method for race classification. Journal of Phytopathology 118: 9-20.

Correll, J.C., Puhalla, J. E. & Schneider, R. W. 1986a. Identification of *Fusarium oxysporum* f.sp. *apii* on the basis of colony size, virulence, and vegetative compatibility. Phytopathology 76: 396-400.

— , Puhalla, J. E. & Schneider, R. W. 1986b. Vegetative compatibility groups among nonpathogenic root-colonizing strains of *Fusarium oxysporum*. Canadian Journal of Botany 64: 2358-2361.

Damicone, J. P. & Manning, W. J. 1982. Avirulent strains of *Fusarium oxysporum* protect asparagus seedlings from crown rot. Canadian Journal of Plant Pathology 4: 143-146.

DiPietro, A., Anaya, N & Roncero, M. I. G. 1994. Occurrence of a retrotransposon-like sequence among different formae speciales and races of *Fusarium oxysporum*. Mycological Research 98: 993-996.

Duchesne, L. C., Campbell, S. E., Koehler, H. & Peterson, R. L. 1989. Pine species influence suppression of *Fusarium* root rot by the ectomycorrhizal fungus *Paxillus involutus*. Symbiosis 7: 139-148.

Edel, V., Steinberg, C., Avelange, I., Laguerre, G. & Alabouvette, C. 1995. Comparison of three molecular methods for the characterization of *Fusarium oxysporum* strains. Phytopathology 85: 579-585.

Elias, K. S., Schneider, R. W. & Lear, M. M. 1991. Analysis of vegetative compatibility groups in nonpathogenic populations of *Fusarium oxysporum* isolated from symptomless tomato roots. Canadian Journal of Botany 69: 2089-2094.

Elmer, W. H. & Stephens, C. T. 1989. Classification of *Fusarium oxysporum* f.sp. *asparagi* into vegetative compatiblity groups. Phytopathology 79: 88-93.

— , Wick, R. L. & Haviland, P. 1994. Vegetative compatibility among *Fusarium oxysporum* f.sp. *basilicum* isolates recovered from basil seed and infected plants. Plant Disease 78: 789-791.

Evans, G. R. & Greczy, L. M. 1995. Methyl bromide: the cure-all of the horticulture industry will be banned by 2001. When this happens, what, if anything, will take its place. American Nurseryman 182(7): 95-105.

Farquhar, M. L. & Peterson, R. L. 1990. Early effects of the ectomycorrhizal fungus *Paxillus involutus* on the root rot organism *Fusarium* associated with *Pinus resinosa*. Canadian Journal of Botany 68: 1589-1596.

Fiely, M. B., Correll, J. C. & Morelock, T. E. 1995. Vegetative compatiblilty, pathogenicity, and virulence diversity of *Fusarium oxysporum* recovered from spinach. Plant Disease 79: 990-993.

Fisher, N. L., Burgess, L. W., Toussoun T. A. & Nelson, P. E. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology 72: 151-153.

Fuchs, J.-G., Moenne-Loccoz, Y. & Defago, G. 1997. Nonpathogenic *Fusarium oxysporum* strain Fo47 induces resistance to Fusarium wilt in tomato. Plant Disease 81: 492-496.

Gerlagh, W. & Blok, W. J. 1988. *Fusarium oxysporum* f.sp. *cucurbitacearum* n.f. embracing all formae speciales of *Fusarium oxysporum* attacking cucurbitaceous crops. Netherlands Journal of Plant Pathology 94: 17-31.

Gordon, T. R. & Martyn, R. D. 1997. The evolutionary biology of *Fusarium oxysporum*. Annual Review of Phytopathology 35: 111-128.

Gordon, T. R. & Okamoto, D. 1992a. Population structure and the relationship between pathogenic and nonpathogenic strains of *Fusarium oxysporum*. Phytopathology 82: 73-77.

Gordon, T. R. & Okamoto, D. 1992b. Variation in mitochondrial DNA among vegetatively compatible isolates of *Fusarium oxysporum*. Experimental Mycology 16: 245-250.

Gordon, T. R. & Okamoto, D. 1992c. Variation within and between populations of *Fusarium oxysporum* based on vegetative compatibility and mitochondrial DNA. Canadian Journal of Botany 70: 1211-1217.

— , Okamoto, D. & Jacobson, D. J. 1989. Colonization of muskmelon and nonsusceptible crops by *Fusarium oxysporum* f.sp. *melonis* and other species of *Fusarium*. Phytopathology 79: 1095-1100.

Guillino, M. L., Q. Migheli, Q. & Mezzalama, M. 1995. Risk analysis in the release of biological control agents: antagonistic *Fusarium oxysporum* as a case study. Plant Disease 79: 1193-1199.

Hansen, E. M., Myrold D. D., & Hamm, P. H. 1990. Effects of soil fumigation and cover crops on potential pathogens, microbial activity, nitrogen availability, and seedling quality in conifer nurseries. Phytopathology 80: 698-704.

Hartley, C. & Merrill, T. C. 1918. Seedling diseases of conifers. Journal of Agricultural Research 15: 521-558.

Hervas, A., Trapero-Casas, J. L. & Jimenez-Diaz, R. M. 1995. Induced resistance against Fusarium wilt of chickpea by nonpathogenic races of *Fusarium oxysporum* f.sp. *ciceris* and nonpathogenic isolates of *F. oxysporum*. Plant Disease 79: 1110-1116.

Ho, Y. W., Varghese, G. & Taylor, G. S. 1985. Protein and esterase patterns of pathogenic *Fusarium oxysporum* f.sp. *elaeides* and *F. oxysporum* var. *redolens* from Africa and non-pathogenic *F. oxysporum* from Malaysia. Phytopthologishe Zietschrift 11: 301-311.

Iannelli, D., Capparelli, R., Critinizo, G., Marziano, F., Scala, F. & Noviello, C. 1962. Serological differentiation among formae speciales and physiological races of *Fusarium oxysporum*. Mycologia 74: 313-319.

Jacobson, D. J. & Gordon, T. R. 1988. Vegetative compatibility and self-incompatibility with *Fusarium oxysporum* f.sp. *melonis*. Phytopathology 78: 668-672.

— & Gordon, T. R. 1990a. Further investigations of vegetative compatibility within *Fusarium oxysporum* f.sp. *melonis*. Canadian Journal of Botany 68: 1245-1248.

— & Gordon, T. R. 1990b. Variability of mitochondrial DNA as an indicator of relationships between populations of *Fusarium oxysporum* f.sp. *melonis*. Mycological Research 94: 734-744.

James, R. L. 1996. Technique for quantifying virulence of *Fusarium* and *Cylindrocarpon* spp. on conifer germinants. USDA Forest Service, Northern Region, Forest Health Protection. Nursery Disease Notes No. 132. 8p.

— , Dumroese, R. K. & Wenny, D. L. 1991. *Fusarium* diseases of conifer seedlings. In: Sutherland, J. R. & Glover, S. G. (eds.). Proceedings of the first meeting of IUFRO Working Party S2.07.09 (Diseasees and Insects in Forest Nurseries). Forestry Canada, Pacific and Yukon Region. Information Report BC-X-331. pp. 181-190.

— , Dumroese, R. K. & Wenny, D. L. 1995. *Fusarium proliferatum* is a common, aggressive pathogen of container-grown conifer seedlings. Phytopathology 85: 1129.

— , Dumroese, R. K. & Wenny, D. L. 1997. Pathogenicity of *Fusarium proliferatum* in container-grown Douglas-fir seedlings. In: James, R. L. (ed.). Proceedings of the third meeting of IUFRO Working Party S7.03-04 (Diseases and Insects in Forest Nurseries). USDA Forest Service, Northern Region, Forest Health Protection. Report 97-4. pp. 26-33.

— , Hildebrand, D. M., Frankel, S. J., Cram M. M. & O'Brien, J. G. 1993. Alternative technologies for management of soil-borne diseases in bareroot forest nurseries in the United States. *In*: Sutherland, J.R. and R. Perrin (eds.). Diseases and Insects in Forest Nurseries. Proceedings of the second meeting of IUFRO Working Party S7.03.04. Institut National de la Recherche Agronomique. Les Colloques No. 68. pp. 237-246.

— & Perez, R. 1999. Pathogenic characteristics of *Fusarium sporotrichioides* isolated from inland Pacific Northwest forest nurseries. USDA Forest Service, Northern Region, Forest Health Protection. Report 99p.

Katan, T., Berliner, R. & Katan, J. 1994. Vegetative compatibility in populations of *Fusarium oxysporum* from wild carnation. Mycological Research 98: 1415-1418.

— , Hadar, E. & Katan, J. 1989. Vegetative compatibility of *Fusarium oxysporum* f.sp. *dianthi* from carnation in Israel. Plant Pathology 38:376-381.

Kelly, A., Alcala-Jimenez, A. R., Bainbridge, B. W., Heale, J. B., Perez-Artes, E. & Jimenez-Diaz, R. M. 1994. Use of genetic fingerprinting and random amplified polymorphic DNA to characterize pathotypes of *Fusarium oxysporum* f.sp. *ciceris* infecting chickpea. Phytopathology 84: 1293-1298.

Kistler, H. C. 1997. Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum*. Phytopathology 87: 474-479.

— , Bosland, P. W., Benny, U., Leong, S. & Williams, P. H. 1987. Relatedness of strains of *Fusarium oxysporum* from crucifers measured by examination of mitochondrial and ribosomal DNA. Phytopathology 77: 1289-1293.

— , Momol, E. A. & Benny, U. 1991. Repetitive genomic sequences for determining relatedness among strains of *Fusarium oxysporum*. Phytopathology 81: 331-336.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review Plant Protection Research (Japan) 8: 114-125.

Kuninaga, S. & Yokosawa, R. 1989. Genetic relatedness within and between formae speciales of *Fusarium oxysporum* as measured by DNA-DNA reassociation kinetics. Annals of the Phytopathological Society of Japan 55: 216-223.

Larkin, R. P., Hopkins, D. L. & Martin, F. N. 1990. Vegetative compatibility within *Fusarium oxysporum* f.sp. *niveum* and its relationship to virulence, aggressiveness, and race. Canadian Journal of Microbiology 36:352-358.

Larkin, R. P., Hopkins, D. L. & Martin, F. N. 1996. Suppression of Fusarium wilt of watermelon by nonpathogenic *Fusarium oxysporum* and other microorganisms recovered from a disease-suppressive soil. Phytopathology 86: 812-819.

Lemanceau, P., Bakker, P. A. H. M., Dekogel, W. J., Alabouvette, C. & Schippers, C. 1993. Antagonistic effect of nonpathogenic *Fusarium oxysporum* Fo47 and pseudobactin 358 upon pathogenic *Fusarium oxysporum* f.sp. *dianthi.* Applied and Environmental Microbiology 59: 74-82.

Lock, W. 1973. Fusarium root rot of Douglas-fir nursery seedlings. Canadian Forestry Service, Forest Pest Leaflet. No. 61. 7p.

Mandeel, Q. & Baker, R. 1991. Mechanism involved in biological control of Fusarium wilt of cucumber with strains of nonpathogenic *Fusarium oxysporum*. Phytopathology 81: 462-469.

Manicom, B. Q., Bar-Joseph, M., Kotze, J. M. & Becker, M. M. 1990. A restriction fragment length polymorphism probe relating vegetative compatibility groups and pathogenicity in *Fusarium oxysporum* f.sp. *dianthi*. Phytopathology 80: 336-339.

Matuo, T. & Chiba, O. 1966. Species and formae speciales of *Fusarium* causing damping-off and root rot of coniferous seedlings in Japan. Annals of the Phytopathological Society of Japan 32: 14-22.

McCain, A. H. 1978. Nursery disease problems - containerized nurseries. *In*: Gustafson, R.W. (ed.). Proceedings 1978 Nurseryman's Conference & Seed Processing Workshop. Western Forest Nursery Council & Intermountain Nurseryman's Association. pp. B-138 - B-142.

Miles, M. R. & Wilcoxin, R. D. 1984. Production of fungal inoculum using a substrate of perlite, cornmeal, and potato dextrose agar. Plant Disease 68: 310.

Moore, N. Y., Hargreaves, P. A., Pegg, K. G. & Irwin, J. A. G. 1991. Characterisation of strains of *Fusarium oxysporum* f.sp. *cubense* by production of volatiles. Australian Journal of Botany 39: 161-166.

Nagao, H., Couteaudier Y. & Alabouvette, C. 1990. Colonization of sterilized soil and flax roots by strains of *Fusarium oxysporum* and *Fusarium solani*. Symbiosis 9: 343-354.

Park, D. 1959. Some aspects of the biology of *Fusarium oxysporum* in soil. Annals of Botany 23: 35-49.

— , 1963. The presence of *Fusarium oxysporum* in soil. Transactions of the British Mycological Society 46: 444-448.

Rataj-Guranowska, M. & Wolko, B. 1991. Comparison of *Fusarium oxysporum* and *Fusarium oxysporum* var. *redolens* by analyzing the isozyme and serological patterns. Journal of Phytopathology 132: 287-293.

Reyes, A. A. 1970. The effect of soil temperature on *Fusarium* yellows of cabbage. Proceedings of the Canadian Phytopathological Society 37:28.

Salgado, M. O. & Schwartz, H. F. 1993. Physiological specialization and effects of inoculum concentration of *Fusarium oxysporum* f.sp. *phaseoli* on common beans. Plant Disease 77: 492-496.

Salisbury, P. J. 1952. The effect of temperature and hydrogen-ion concentration on growth of certain cultures of *Fusarium oxysporum* isolated from Douglas-fir seedlings. Canadian Department of Agriculture, Division of Forest Biology Report. 7 p.

— , 1954. A review of damping-off of Douglas-fir seedlings in British Columbia. Forestry Chronicle 30: 407-410.

Shaheen, L. 1996. Potential loss of methyl bromide to prompt changes in Clean Air Act. Pest Control 64(5): 68,74.

Shea, K. R. & Rediske, J. H. 1961. Pathological aspects of germination and survival of Douglas-fir in controlled environments. Weyerhauser Company Forest Research Note 41. 8 p.

Sinclair, W.A., Cowles, D. P. & Hee, S. M. 1975. Fusarium root rot of Douglas-fir seedlings: suppression by soil fumigation, fertility management, and inoculation with spores of the fungal symbiont *Laccaria laccata*. Forest Science 21: 390-398.

Smith, R. S. 1967. Decline of *Fusarium oxysporum* in roots of *Pinus lambertiana* seedlings transplanted into forest soils. Phytopathology 57: 1265.

Spaulding, P. 1914. The damping-off of coniferous seedlings. Phytopathology 4: 73-88.

Stoddard, D. L. 1947. Nitrogen, potassium, and calcium in relation to Fusarium wilt of muskmelon. Phytopathology 37: 875-884.

Stone, J. K., Hildebrand, D., James, R. L. & Frankel, S. J. 1997. Alternatives to chemical fumigation in bareroot forest nurseries: effects on pathogen levels and seedling density, mortality, and quality. *In*: James, R.L. (ed.). Proceedings of the third meeting of IUFRO Working Party S7.03-04. USDA Forest Service, Northern Region, Forest Health Protection. Report 97-4. pp. 59-69.

Timmer, L. W. 1982. Host range and host colonization, temperature effects, and dispersal of *Fusarium oxysporum* f.sp. *citri*. Phytopathology 72: 698-702.

Vaartaja, O. 1967. Damping-off pathogens in South Australian nurseries. Phytopathology 57: 765-768.

Vaartaja, O. & Bumbieris, M. 1967. Organisms association with roots rots of conifers in South Australian nurseries. Plant Disease Reporter 51: 473-476.

Venter, S. L., Theron, D. J., Steyn, P. J., Ferreira D. I. & Eicker, A. 1992. Relationship between vegetative compatibility and pathogenicty of isolates of *Fusarium oxysporum* f.sp. *tuberosi* from potato. Phytopathology 82: 858-862.

Walker, J. E. & Foster, R. E. 1946. Plant nutrition in relation to disease development. III. Fusarium wilt of tomato. American Journal of Botany 33: 259-264.

Wenny, D. L. & Dumroese, R. K. 1987. Germination of conifer seeds surface sterilized with bleach. Tree Planters' Notes 38(3): 18-21.

Wensley, R. N. & McKeen, C. D. 1964. Some relationship between plant nutrition, fungal populations, and incidence of Fusarium wilt of muskmelon. Canadian Journal of Microbiology 11: 581-594.

Wright, E., Harvey, G. M. & Bigelow, C. A. 1963. Tests to control Fusarium root rot of ponderosa pine in the Pacific Northwest. Tree Planters' Notes 59: 15-20.