NURSERY DISEASE NOTES **USDA FOREST SERVICE**

NORTHERN REGION
FOREST HEALTH PROTECTION

No. 158 April 2004

PATHOGENIC CHARACTERISTICS OF *FUSARIUM CULMORUM* AND *FUSARIUM ANTHOPHILUM* ISOLATED FROM INLAND PACIFIC NORTHWEST FOREST NURSERIES

R.L. James

ABSTRACT

Five isolates each of *Fusarium culmorum* and *F. anthophilum* obtained from forest nurseries in the Inland Pacific Northwest were tested for pathogenicity on young Douglas-fir germinants under controlled laboratory conditions. Although sample sizes were small, all tested isolates were pathogenic and all but one highly virulent in lab tests. Both *Fusarium* species have not been previously recognized as important forest nursery pathogens, but apparently have potential to cause diseases on conifer seedlings. Further tests screening more isolates from diverse nurseries and follow-up greenhouse tests are warranted.

INTRODUCTION

Fungal species in the genus *Fusarium* are routinely associated with diseased host plants in forest nurseries. These fungi are often isolated from diseased, seedlings, especially those with root disease, as well as seedlings without disease symptoms (James et al. 1991). They are also isolated from nursery soil and containers used to grow seedlings in greenhouses. Some species have been isolated more frequently than others, and not all species are considered pathogens of nursery seedlings.

Many *Fusarium* species are associated with important diseases of agricultural crops. These are often the same species isolated from seedlings in forest

nurseries and some may have entered nurseries from surrounding agricultural areas (Bruehl 1989). Because forest nurseries are essentially agricultural operations, it is not surprising to find agricultural pests causing problems in these nurseries.

Isolating microorganisms from diseased hosts is the first step in proving that such organisms are causing disease. However, to confirm this, Koch's Postulates (Agrios 1969) must be completed. These procedures were initially developed to confirm etiology of microorganisms on animals. They were adapted for plant diseases and require that isolated organisms cause the original disease symptoms when inoculated onto healthy plants. Reisolation of inoculated isolates confirms pathogenicity and thus completes Koch's Postulates. Because *Fusarium* spp. are routinely isolated from nursery seedlings, their ability to cause disease has often been taken for granted. However, there is wide diversity within this genus and even within individual species in their ability to cause disease (James et al. 1988, 1991). Previous tests have shown that some isolates are inherently pathogenic whereas others are either strictly saprophytic or only weakly virulent under conditions of host stress (James 2000; James and Perez 1999, 2000; James et al. 1986, 1988, 1997, 2000). Therefore, care must be taken in categorizing all *Fusarium* as "pathogens" in forest nurseries. In most cases, many individual isolates in the nursery population are not pathogens.

Techniques have been developed to quantify virulence of *Fusarium* isolates under controlled laboratory conditions (James 1996). These have previously been applied to five *Fusarium* spp.: *F. oxysporum* Schlecht., *F. proliferatum* (Matsushima) Nirenberg, *F. solani* (Mart.) Apple & Wollenw., *F. acuminatum* Ell. & Ev., and *F. sporotrichioides* Sherb. (James 2000; James and Perez 1999, 2000; James et al. 1997, 2000). Based on these tests, the most virulent isolates were within *F. proliferatum*, followed by *F. oxysporum* and *F. solani*. Tested isolates of *F. acuminatum* and *F. sporotrichioides* were mostly nonpathogenic.

Several other *Fusarium* species isolated from forest nursery seedlings have not been tested for pathogenicity. This report describes tests on two of these species: *F. culmorum* (W.G. Smith) Sacc. and *F. anthophilum* (A. Braun) Wollenw. Compared to several other *Fusarium* species, *F. culmorum* and *F. anthophilum* are isolated infrequently in forest nurseries.

MATERIALS AND METHODS

The technique outlined by James (1996) was used in 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 and Gilligan 1984; James et al. 1988, 1989b), cornmeal-perlite inoculum was used for all tests; this inoculum was prepared using the procedures 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 75 g of perlite were added. The mixture was placed into 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 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-hr. 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. Exactly 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. Controls consisted of exposing 24 seedlings to non-inoculated perlite within vials; they were evaluated like seedlings inoculated with fungal isolates.

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 the 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 for *Fusarium* (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 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: non-pathogenic = below 40; low virulence = 41-60; moderate virulence = 61-80; high virulence = above 80.

Five different *F. culmorum* isolates were evaluated (table 1). They were from diseased or healthy-appearing container-grown Douglas-fir seedlings, forest nursery soil, or a styroblock container used to grow seedlings in greenhouses at a north Idaho nursery. All five tested *F. anthophilum* isolates were from diseased Douglas-fir germinants being grown in a container nursery.

Table 1. Characteristics of *Fusarium culmorum* isolates evaluated for virulence on young Douglas-fir germinants.

Isolate	Host	Host Condition	Nursery Type
9202P	Styroblock Container	Not Applicable	Container
9217B	Douglas-fir	Diseased	Container
9217I	Douglas-fir	Diseased	Container
9245D	Soil	Not Applicable	Bareroot
9257H	Douglas-fir	Healthy	Container

RESULTS

All tested *F. culmorum* isolates were pathogenic to young Douglas-fir germinants; 4 of the 5 tested isolates exhibited high levels of virulence (table 2). Likewise, all tested *F. anthophilum* isolates were pathogenic and all were highly virulent on young Douglas-fir germinants (table 3).

Examination of radicles of recently-inoculated Douglas-fir germinants indicated that both *F. culmorum* (figure 1) and *F. anthophilum* (figure 2) readily colonized young, succulent tissues. After colonization, tissue decay resulted and germinants quickly died.

Table 2. Virulence of selected *Fusarium culmorum* isolates on young Douglas-fir germinants.

Isolate[1]	Percent Diseased[2]	Percent Damped-off	Percent Wet Rot[3]	Average Survival[4]	Average Virulence Score[5]
9202P	95.8	87.5	8.3	4.7	87.7 [High]
9217B	86.9	60.9	26.1	11.3	47.4 [Low]
9217I	100.0	58.3	41.7	6.5	79.8 [High]
9245D	87.5	79.2	8.3	6.0	81.7 [High]
9257H	100.0	79.2	20.8	4.0	92.7 [High]
Control	20.8	4.2	16.7	13.9	11.9[Non]

[1] See table 1 for isolate characteristics.
[2] After 14 days of exposure to inoculum.
[3] Seed radicle was decayed although seedling remained upright.
[4] Days of survival while being exposed to inoculum.
[5] Ranges from 0 – 100; 80-100: high; 60-80: moderate; 40-60: low; below 40: non-pathogenic.

Table 3. Virulence of selected *Fusarium anthophilum* isolates on young Douglas-fir germinants.

Isolate[1]	Percent Diseased[2]	Percent Damped-off	Percent Wet Rot[3]	Average Survival[4]	Average Virulence Score[5]
9244A	100.0	58.3	41.7	6.5	80.2 [High]
9244B	100.0	37.5	62.5	6.3	80.4 [High]
9244C	100.0	66.7	33.3	6.3	82.1 [High]
9244F	100.0	87.5	12.5	5.2	88.5[High]
9244G	100.0	70.8	29.2	5.6	85.6 [High]
Control	20.8	4.2	16.7	13.9	11.9[Non]

[1] All isolates from diseased Douglas-fir seedlings in a container nursery.
[2] After 14 days of exposure to inoculum.
[3] Seed radicle was decayed although seedling remained upright.
[4] Days of survival (out of a maximum of 14) while being exposed to inoculum.
[5] Ranges from 0 – 100; 80-100: high; 60-80: moderate; 40-60: low; below 40: non-pathogenic.

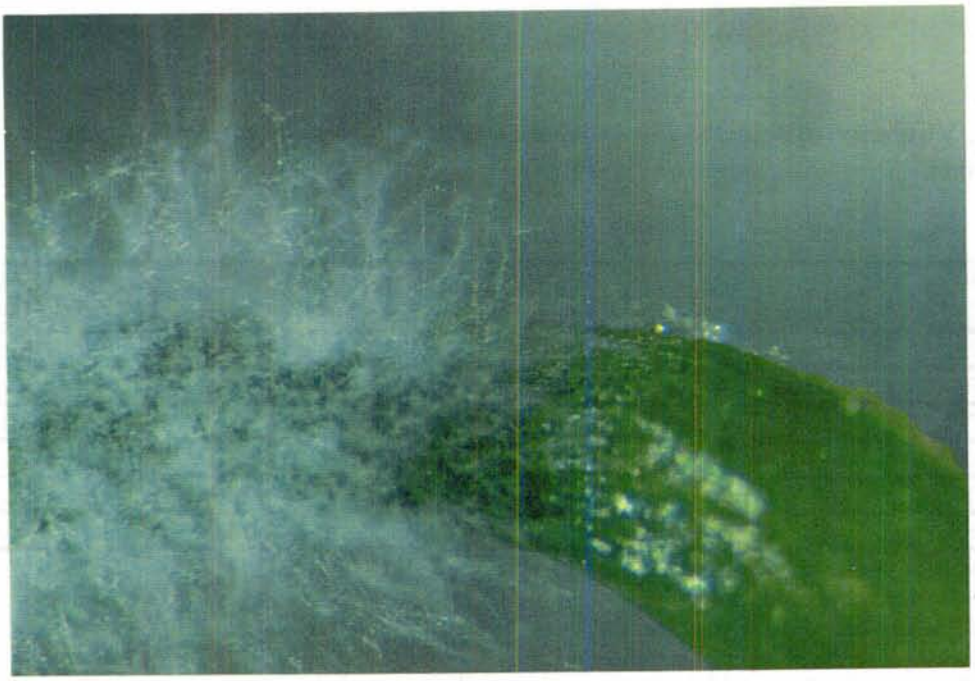

Figure 1. *Fusarium culmorum* infecting the radicle of a young Douglas-fir germinant in pathogenicity tests under controlled, laboratory conditions.

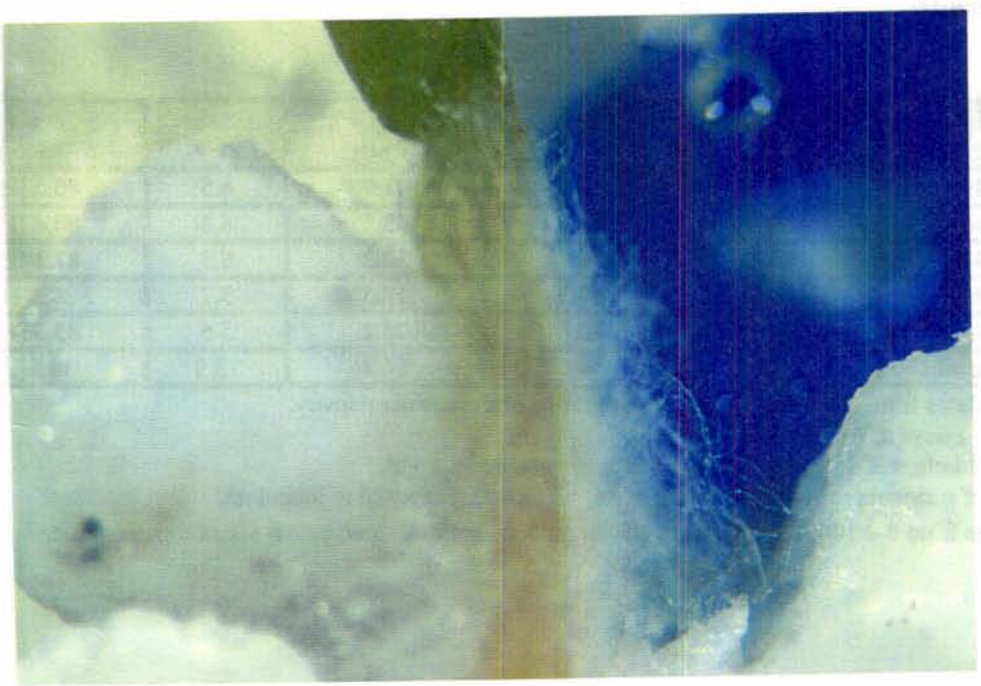

Figure 2. *Fusarium anthophilum* infecting the radicle of a young Douglas-fir germinant in pathogenicity tests under controlled, laboratory conditions.

DISCUSSION

Several different *Fusarium* spp. have routinely been isolated within forest nurseries in the Inland Pacific Northwest (James et al. 1989a; 1991). Some are commonly encountered whereas others are rare. *Fusarium culmorum* and *F. anthophilum* are in the latter category and have been isolated from root diseased and healthy-appearing seedlings, nursery soil, and styroblock containers used to grow seedlings in greenhouses. Because of their infrequent occurrence, it is unknown what, if any, role they might play in disease etiology on nursery seedlings. Techniques have been developed to quickly determine potential of specific *Fusarium* isolates to cause disease on young Douglas-fir germinants (James 1996). If isolates are capable of causing post-emergence damping-off under controlled, laboratory conditions, they probably have potential as pathogens under normal nursery conditions (James et al. 1986, 1997, 2000). At the very least, they warrant further evaluation such as in greenhouse inoculation trials (James et al. 1989b, 2000).

The 10 isolates evaluated in this study (five each of *F. culmorum* and *F. anthophilum*) were highly virulent, with one exception, in laboratory inoculation tests. This is in contrast with previous tests of some other *Fusarium* species, such as *F. acuminatum* and *F. sporotrichioides*, which indicated that many isolates were either non-pathogenic or exhibited low virulence (James 2000; James and Perez 1999). Tests evaluating more commonly-encountered species, such as *F. oxysporum*, *F. proliferatum*, and *F. solani* indicated that highly-virulent isolates of these species may be more common (James and Perez 2000; James et al. 1997, 2000). The small number of *F. culmorum* and *F. anthophilum* isolates tested precludes definitive conclusions regarding the overall potential of these species as pathogens in forest nurseries.

Fusarium culmorum is best known as an important agricultural pathogen, particularly on cereals, other grasses, and alfalfa (Bonish and Di Menna 1993; Burgess et al. 1975; Carter et al. 2000; Couch and Bedford 1966; Liu et al. 1997; Miedaner et al. 1996). It is an important component of the Fusarium head blight syndrome on wheat, barley, and other cereals (Chelkowski et al. 2000; Golinski et al. 2002; Miedaner and Lreinbrecht 2001; Siranidou et al. 2002; Stack and McMullen 1985; Tan and Niessen 2003). In addition, it has been commonly associated with cereal seedling damping-off and root diseases (Elliott et al. 1969; Gonzalez and Treyathan 2000; Gordon and Sprague 1941; Graham et al. 1979; Hancock 1983, 1985; Oswald 1949). A major reason for importance of *F. culmorum* on these crops is its ability to produce powerful trichothecene toxins which aid in pathogenicity (Liu et al. 1997; Mesterhazy 2002; Miedaner et al. 1997) and are of concern because of their extreme toxicity at low concentrations to animals and humans (Desjardins et al. 1993; Gang et al. 1998; Hestbjerg et al. 2002; Kang and Buchenauer 2000, 2002). There are only a few records of *F. culmorum* in forest nurseries (Asiegbu et

al. 1999; Hoefnagels and Linderman 1999; James et al. 1989a, 1990; Warcup 1951); most of these do not consider this fungus to be an important cause of seedling diseases (James et al. 1989a, 1990). However, based on the results of this investigation, potential importance of *F. culmorum* in forest nurseries may require reas-sessment.

Because of its high prevalence in pastures, grasses, and wheat fields, it is possible that *F. culmorum* invaded forest nurseries from nearby agricultural lands (Bruehl 1989). The fungus is very well adapted to survival in soil (Burgess 1981; Burgess and Summerell 1992; Larsen et al. 1998) and may occur at relatively high populations, especially near the soil surface. *Fusarium culmorum* produces both macroconidia and chlamydospores (James et al. 1989a; Nelson et al. 1983; Smither-Kopperl et al. 1998); macroconidia are disseminated fairly long distances, both vertically and horizontally, by rain splash (Horberg 2002; Jenkinson and Parry 1994b; Miedaner and Lreinbrecht 2001). Therefore, if the fungus becomes well established in soil, inoculum is readily available for infection of seedling crops.

In contrast to *F. culmorum*, little is known about *F. anthophilum*. It was once reported on Douglas-fir and ponderosa pine seeds (Lori and Salerno 2002), but little is known about its potential as a forest nursery or agricultural pest. This may partly be due to problems of taxonomy. *Fusarium anthophilum* was placed within the *Fusarium* section *Liseola* by Nelson et al. (1983) based on morphological and microscopic characteristics. The species lacks chlamydospores and produces microconidia on monophialides only within false heads (no microconidial chains). The major characteristic separating this taxon from other species within *Liseola* is production of oval to napiform microconidia. This characterization may not necessarily have taxonomic validity, i.e., spore morphology may be related to natural variability in culture. Because of this, *F. anthophilum* has been incorporated into other taxa in subsequent treatments of this section (O' Donnell and Cigelnik 1997; O'Donnell et al. 1998). Therefore, isolates tested in this study may not necessarily be taxonomically different from other more common members of *Liseola*, such as *F. proliferatum, F. verticillioides* (Sacc.) Nirenberg (= *F. moniliforme* Sheldon), or *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas. Previous pathogenicity tests of *F. proliferatum* isolates found that most isolates were highly virulent on Douglas-fir germinants (James et al. 1995, 1997), similar to what was found in this study for isolates designated as *F. anthophilum*. Therefore, it is possible that most *Fusarium* isolates within section *Liseola* have relatively high potential to cause important diseases on seedlings in forest nurseries. Additional work is needed to confirm this hypothesis.

Results of this study provided the basis for further investigations into the relative importance of *F. culmorum* and *F. anthophilum* as pathogens of forest nursery seedlings. These fungi may be more common in nurseries than previously thought. Recently, polymerase chain reaction (PCR) amplifications have been successful in quantifying *F. culmorum* within symptomatic and non-symptomatic host

plants (Simpson et al. 2001). Since *F. culmorum* is morphologically and genetically very stable (Booth 1975; Miedaner and Schilling. 1996), probes developed on other cropping systems should be useful in detecting the species within forest nursery seedlings. If *F. culmorum* is relatively common in forest nurseries, it will be of increased concern because of its aggressiveness as a pathogen on agricultural hosts (Jenkinson and Parry 1994a; Miedaner and Schilling 1996; Miedaner et al. 2001; Siranidou et al. 2002; Teperi et al. 1998) and its apparent potential for causing forest seedling diseases.

LITERATURE CITED

Agrios, G.N. 1969. Plant Pathology. Academic Press, New York. 629p.

Asiegbu, F.O., M. Kacprzak, G. Daniel, M. Johansson, J. Stenlid and M. Manka. 1999. Biochemical interactions of conifer seedling roots with *Fusarium* spp. Canadian Journal of Microbiology 45:923-935.

Bonish, P.M. and M.E. Di Menna. 1993. A comparison of two methods for isolating *Fusarium* spp. from pasture herbage. Mycological Research 97:91-94.

Booth, C. 1975. The present status of *Fusarium* taxonomy. Annual Review of Phytopathology 13:83-93..

Bruehl, G.W. 1989. Integrated control of soilborne plant pathogens: an overview. Canadian Journal of Plant Pathology 11:153-157.

Burgess, L.W. 1981. General ecology of the Fusaria. *In:* Nelson, P.E., T.A. Toussoun and R.J. Cook (eds.). *Fusarium:* Diseases, Biology & Taxonomy. The Pennsylvania State University Press, University Park. pp. 225-235.

Burgess, L.W. and B.A. Summerell. 1992. Mycogeography of *Fusarium*: survey of *Fusarium* species in subtropical and semi-arid grassland soils from Queensland, Australia. Mycological Research 96:780-784.

Burgess, L.W., A.H. Wearing and T.A. Toussoun. 1975. Surveys of Fusaria associated with crown rot of wheat in eastern Australia. Australian Journal of Agricultural Research 26:791-799.

Carter, J.P., H.N. Rezanoor, A.E. Desjardins and P. Nicholson. 2000. Variation in *Fusarium graminearum* isolates from Nepal associated with their host of origin. Plant Pathology 49:452-460.

Chelkowski, J., H. Wisniewska, T. Adamski, P. Golinski, Z. Kaczmarek, M. Kostecki, J. Perkowski and M. Surma. 2000. Effects of *Fusarium culmorum* head blight on mycotoxin accumulation and yield traits in barely double haploids. Journal of Phytopathology 148:541-545.

Couch, H.B. and E.R. Bedford. 1966. Fusarium blight of turf grasses. Phytopathology 56:781-786.

Desjardins, A.E., T.M. Hohn and S.P. McCormick. 1993. Trichothecene biosynthesis in *Fusarium* species: chemistry, genetics and significance. Microbiological Reviews 57:595-604.

Elliott, E.S., R.E. Baldwin and R.B. Carroll. 1969. Root rots of alfalfa and red clover. West Virginia Agricultural Experiment Station Bulletin 585T. 32p.

Gang, G., T. Miedaner, U. Schuhmacher, M. Schollenberger and H.H. Geiger. 1998. Deoxynivalenol and nivalenol production by *Fusarium culmorum* isolates differing in aggressiveness toward winter rye. Phytopathology 88:879-884.

Golinski, P., Z. Kaczmarek, I. Kiecana, H. Wisniewska, P. Kaptur, M. Kostecki and J. Chelkowski. 2002. Fusarium head blight of common Polish winter wheat cultivars – comparison of effects of *Fusarium avenaceum* and *Fusarium culmorum* on yield components. Journal of Phytopathology 150:135-141.

Gonzalez, M.S. and L.E. Trevathan. 2000. Identity and pathogenicity of fungi associated

with root and crown rot of soft red winter wheat grown on the upper Coastal Plain land resource area of Mississippi. Journal of Phytopathology 148:77-85.

Gordon, W.L. and R. Sprague. 1941. Species of *Fusarium* associated with root rots of the Gramineae in the northern Great Plains. Plant Disease Reporter 25:168-180.

Graham, J.H., D.L. Stuteville, F.I. Frosheiser and D.C. Erwin. 1979. Compendium of alfalfa diseases. The American Phytopathological Society, St. Paul, MN. 65p.

Hancock, J.G. 1983. Seedling diseases of alfalfa in California. Plant Disease 67:1203-1208

Hancock, J.G. 1985. Fungal infection of feeder rootlets of alfalfa. Phytopathology 75:1112-1120.

Hestbjerg, H., G. Felding and S. Elmholt. 2002. *Fusarium culmorum* infection of barley seedlings: correlation between aggressiveness and deoxynivalenol content. Journal of Phytopathology 150:308-312.

Hoefnagels, M.H. and R.G. Linderman. 1999. Biological suppression of seedborne *Fusarium* spp. during cold stratification of Douglas-fir seeds. Plant Disease 83:845-852.

Horberg, H.M. 2002. Patterns of splash dispersed conidia of *Fusarium poae* and *Fusarium culmorum*. European Journal of Plant Pathology 108:73-80.

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

James, R.L. 2000. Pathogenic characteristics of *Fusarium acuminatum* isolated from inland Pacific Northwest nurseries. USDA Forest Service, Northern Region, Forest Health Protection. Report 00-16. 8p.

James, R.L., R.K. Dumroese, C.J. Gilligan and D.L. Wenny. 1989b. Pathogenicity of *Fusarium* isolates from Douglas-fir seed and container-grown seedlings. Idaho Forest, Wildlife and Range Experiment Station Bulletin. No. 52. 10p.

James, R.L., R.K. Dumroese and D. L. Wenny. 1988. *Fusarium* diseases of containerized conifer seedlings in northern Rocky Mountain nurseries: infection, symptom production and pathogenicity of associated fusaria. Phytopathology 78(12):1533.

James, R.L., R.K. Dumroese and D.L. Wenny. 1989a. Occurrence, characteristics, and descriptions of *Fusarium* isolates from Douglas-fir seed and seedlings. USDA Forest Service, Northern Region, Forest Pest Management. Report 90-4. 23p.

James, R.L., R.K. Dumroese and D L. Wenny. 1991. *Fusarium* diseases of conifer seedlings. *In*: Sutherland, J. R. and S. G. Glover (eds.). Proceedings of the first meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Forestry Canada. Pacific and Yukon Region. Information Report BC-X-331. pp. 181-190.

James, R.L., R.K. Dumroese and D.L. Wenny. 1995. *Fusarium proliferatum* is a common, aggressive pathogen of container-grown conifer seedlings. Phytopathology 85(10): 1129.

James, R.L., R.K. Dumroese and D.L. Wenny. 1997. Pathogenicity of *Fusarium proliferatum* in container-grown Douglas-fir seedlings. *In:* James, R. L. (editor). 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.

James, R.L. and C.J. Gilligan. 1984. Studies of *Fusarium* associated with containerized conifer seedling diseases: pathogenicity tests of isolates from the Alpine Nursery, Kalispell, Montana. USDA Forest Service, Northern Region, Forest Pest Management. Report 84-14. 29p.

James, R.L., S. Metzger and C.J. Gilligan. 1990. Effects of soil fumigation on conifer seedling production at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 90-11. 18p.

James, R L., E.P. Militante, J.Y. Woo and C.J. Gilligan. 1986. Pathogenicity of *Fusarium* from forest seedling nurseries on Douglas-fir and ponderosa pine seedlings. USDA Forest Service, Northern Region, Forest Pest Management. Report 86-8. 12p.

James, R.L., R. Perez, R.K. Dumroese and D.L. Wenny. 2000. Virulence of *Fusarium oxysporum* on Douglas-fir germinants: comparison of isolates from nursery soil and roots of healthy and diseased seedlings. *In*: Lilja, A. and J:R. Sutherland (eds.). Proceedings of the 4th Meeing of IUFRO Working Party 7.03.04 – Diseases and Insects in Forest Nurseries. Finnish Forest Research Institute, Research Papers 781. pp. 49-64.

James, R.L. and R. Perez. 1999. Pathogenic characteristics of *Fusarium sporotrichioides* isolated from inland Pacific Northwest forest nurseries. USDA Forest Service, Northern Region, Forest Health Protection. Report 99-8. 11p.

James, R.L. and R. Perez. 2000. Pathogenic characteristics of *Fusarium solani* isolated from inland Pacific Northwest forest nurseries. USDA Forest Service, Northern Region, Forest Health Protection. Report 00-15. 12p.

Jenkinson, P. and D.W. Parry. 1994a Isolation of *Fusarium* species from common broad-leaved weeds and their pathogenicity to winter wheat. Mycological Research 98:776-780.

Jenkinson, P. and D.W. Parry. 1994b. Splash dispersal of conidia of *Fusarium culmorum* and *Fusarium avenaceum*. Mycological Research 98:506-510.

Kang, Z. and H. Buchenauer. 2000. Ultrastructural and cytochemical studies on cellulose, xylan and pectin degradation in wheat spikes infected by *Fusarium culmorum*. Journal of Phytopathology 148:263-275.

Kang, Z.and H. Buchenauer. 2002. Studies on the infection process of *Fusarium culmorum* in wheat spikes: degradation of host cell wall components and localization of trichothecene toxins in infected tissue. European Journal of Plant Pathology 108:653-660.

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

Larsen, J., P.A. Olsson and I. Jakobsen. 1998. The use of fatty acid signatures to study mycelial interactions between the arbuscular mycorrhizal fungus *Glomus intraradices* and the saprotrophic fungus *Fusarium culmorum* in root-free soil. Mycological Research 102:1491-1496.

Liu, W., W. Langseth, H. Skinnes, O.N. Elen and L. Sundheim. 1997. Comparison of visual head blight ratings, seed infection levels, and deoxynivalenol production for assessment of resistance in cereals inoculated with *Fusarium culmorum*. European Journal of Plant Pathology 103:589-595.

Lori, G. and M.I. Salerno. 2002. Fusaria population associated with Douglas-fir and ponderosa pine seeds in Argentina. Seed Science & Technology 30(3): 559-566.

Mesterhazy, A. 2002. Role of deoxynivalenol in aggressiveness of *Fusarium graminearum* and *F. culmorum* and in resistance to Fusarium head blight. European Journal of Plant Pathology 108:675-684.

Miedaner, T., G. Gang and H.H. Geiger. 1996. Quantitative-genetic basis of aggressiveness of 42 isolates of *Fusarium culmorum* for winter rye head blight. Plant Disease 80:500-504.

Miedaner, T., G. Gang, A.G. Schilling and H.H. Geiger. 1997. Aggressiveness and mycotoxin production of populations of *Fusarium culmorum* and *Fusarium graminearum* in winter rye. Fifth European *Fusarium* Seminar, Szeged, Hungary. pp. 471-475.

Miedaner, T. and C. Lreinbrecht. 2001. Trichothecene content of rye and wheat genotypes inoculated with a deoxynivalenol- and a nivalenol-producing isolate of *Fusarium culmorum*. Journal of Phytopathology 149:245-251.

Miedaner, T. and A.G. Schilling. 1996. Genetic variation of aggressiveness in individual field populations of *Fusarium graminearum* and *Fusarium culmorum* tested on young plants of winter rye. European Journal of Plant Pathology 102:823-830.

Miedaner, T., A.G. Schilling and H.H. Geiger. 2001. Molecular genetic diversity and variation for aggressiveness in populations of *Fusarium graminearum* and *Fusarium culmorum* sampled from wheat field in different countries. Journal of Phytopathology 149:641-648.

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

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium* species: an illustrated manual for identification. The Pennsylvania State University Press, University Park. 193p.

O'Donnell, K. and E. Cigelnik. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Molecular Phylogenetics and Evolution 7:103-116 [

O'Donnell, K., E. Cigelnik and H.I. Nirenberg. 1998. Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. Mycologia 90:465-493.

Oswald, J.W. 1949. Cultural variation, taxonomy and pathogenicity of *Fusarium* species associated with cereal root rots. Phytopathology 39:359-376.

Simpson, D.R., G.E. Weston, J.A. Turner, P. Jennings and P. Nicholson. 2001. Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. European Journal of Plant Pathology 107:421-431.

Siranidou, E., Z. Kang and H. Buchenauer. 2002. Studies on symptom development, phenolic compounds and morphological defense responses in wheat cultivars differing in resistance to Fusarium head blight. Journal of Phytopathology 150:200-208.

Smither-Kopperl, M.L., R. Charudattan and R.D. Berger. 1998. Dispersal of spores of *Fusarium culmorum* in aquatic systems. Phytopathology 88:382-388.

Stack, R.W. and M.P. McMullen. 1985. Head blighting potential of *Fusarium* species associated with spring wheat heads. Canadian Journal of Plant Pathology 7:79-82.

Tan, M.-K. and L.M. Niessen. 2003. Analysis of rDNA ITS sequences to determine genetic relationships among, and provide a basis for simplified diagnosis of, *Fusarium* species causing crown rot and head blight of cereals. Mycological Research 107:811-821.

Teperi, E., M. Keskinen, E. Ketoja and R. Tahvonen. 1998. Screening for fungal antagonistis of seed-borne *Fusarium culmorum* on wheat using *in vivo* tests. European Journal of Plant Pathology 104:243-251.

Warcup, J.H. 1951. Effect of partial sterilization by steam or formalin on the fungus flora of an old forest nursery soil. Transactions of the British Mycological Society 34:519-532.

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

R.L. James is Plant Pathologist, USDA Forest Service, Northern Region, Forest Health Protection. Address: USDA Forest Service, 3815 Schreiber Way, Coeur d'Alene, ID 83814; email rjames@fs.fed.us.