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PATHOGENIC CHARACTERISTICS OF *FUSARIUM ACUMINATUM* ISOLATED FROM INLAND PACIFIC NORTHWEST NURSERIES

R.L. James
Plant Pathologist

ABSTRACT

Thirty-three isolates of *Fusarium acuminatum* obtained from inland Pacific Northwest forest nurseries were tested for their pathogenicity on young Douglas-fir germinants under controlled laboratory conditions. Tested isolates were from forest nursery soil, roots of healthy-appearing and diseased conifer seedlings, styrofoam and hard plastic containers, conifer seeds, and adult fungus gnats. The vast majority of isolates were non-pathogenic under test conditions. A few isolates from roots of healthy-appearing or diseased seedlings displayed low or moderate virulence. Although isolates of *F. acuminatum* are routinely encountered in forest nurseries, they are probably not important pathogens under most conditions and should usually not be considered a threat to seedling production.

INTRODUCTION

Fusarium spp. are commonly associated with diseased seedlings in forest nurseries in the inland Pacific Northwest. These fungi are frequently isolated from roots and stems of diseased seedlings, roots of healthy-appearing seedlings, forest nursery soil, conifer seeds, and containers used to grow seedlings in greenhouses (James et al. 1991b). *Fusarium* spp. occupy several niches and play differing roles in eliciting seedling diseases in forest nurseries (James et al. 1991b).

One of the most frequently isolated *Fusarium* spp. in forest nurseries is *F. acuminatum* Ell. & Ev. (teleomorph: *Gibberella acuminata* Wollenw.). This species is taxonomically delimited by its characteristic macroconidial morphology, ability to form chlamydospores, and characteristic growth pattern (including pigment formation) in culture (Nelson et al. 1983). *F. acuminatum* most closely resembles and can often be confused with *F. avenaceum* in culture

(James et al. 1989). However, the most distinctive differentiating characteristic separating the two taxa is production of chlamydospores, i.e., isolates of *F. avenaceum* do not form typical chlamydospores. Unfortunately, some isolates of *F. acuminatum* form chlamydospores infrequently or very slowly (Burgess et al. 1993; Nelson et al. 1983). Although *F. acuminatum* is morphologically most similar to *F. avenaceum*, it is most closely related (based on DNA homology) to three other *Fusarium* spp.: *F. culmorum*, *F. sambucinum*, and *F. oxysporum* (Szecsi and Dobrovolsky 1985). Taxonomy of *F. avenaceum* is further confused by the fact that most mycologists differentiate this species into two distinct subspecies (*acuminatum* and *armeniacum*)(Altomare et al 1997; Nagy and Hornok 1994). Subspecies *armeniacum* is distinguished from subspecies 07by having longer macroconidia, more rapid formation of chlamydospores, formation of apricot-colored sporodochia in culture, and more rapid growth on potato dextrose agar (PDA)(Burgess et al. 1993). Based on these differentiating factors, the great majority of isolates tested in this investigation were probably from the subspecies *armeniacum*.

Isolates of *F. acuminatum* are predominantly found in cold and temperate-cold environments (Burgess 1981; Sangalang et al. 1995). It is a common soil inhabitant, most often colonizing either grassland or desert soils, but very infrequently in forest soils (Christensen 1981; McMullen and Stack 1983; Wacha and Tiffany 1979). *Fusarium acuminatum* frequently occurs in agricultural settings where it is often isolated from roots and stems of different crops. Examples include alfalfa (Elliott et al. 1969; Graham et al. 1979; Hancock 1983, 1985) and other forage legumes (Stutz et al. 1985), wheat (Fernandez et al. 1985; Hill and Blunt 1984; Sturz and Bernier 1991), maize (Francis and Burgess 1975), bananas (Lukezic and Kaiser 1966) and soybeans

(Killebrew et al. 1993). The fungus has also been frequently isolated from seeds of wheat (Duthie et al. 1986; Gordon 1952), barley (Salas et al. 1999), and other cereals (Duthie et al. 1986; Gordon 1952). In general, *F. acuminatum* has been considered mostly a saprophyte and secondary colonizer of agricultural plants (Altomare et al. 1997). However, it may be moderately pathogenic on maize (Francis and Burgess 1975), cause damping-off of alfalfa seedlings (Hancock 1983), and pathogenic on banana crowns (Lukezic and Kaiser 1966).

Fusarium acuminatum is frequently encountered in forest nurseries. It has been isolated from many different conifer species including Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) (James 1989b, 1990a), western white pine (*Pinus monticola*) (James 1988, 1991b), western larch (*Larix occidentalis*)(James 1989a), ponderosa pine (*Pinus ponderosa*)(James 1989c, 1990c; James and Cooley 1987), lodgepole pine (*Pinus contorta*)(James 1987b; James and Cooley 1987), limber pine (*Pinus flexilis*)(James 1990d), Colorado blue spruce (*Picea pungens*)(Gordon 1959), Engelmann spruce (*Picea engelmanni*)(James et al. 1990a) red pine (*Pinus resinosa*)(Gordon 1959), Scots pine (*Pinus sylvestris*)(Gordon 1959; James 1987c), and true fir (*Abies* spp.)(James 1987a, 1990d, 1990f). It has also been isolated from other nursery species including Russian-olive (*Elaeagnus angustifolia*) (Hildebrand 1986) and *Caragana* spp. (James 1991a). *Fusarium acuminatum* is often isolated from roots of both diseased (James 1993; James et al. 1990b, 1991a) and healthy-appearing (Dumroese et al. 1990, 1996; James and Gilligan 1988c) seedlings grown as either bareroot or container stock. It is also frequently isolated, along with many other *Fusarium* spp., from forest nursery soil (James and Beall 1999, 2000), sometimes at relatively high populations. The fungus is a frequent colonizer of conifer seeds (James 1986; James and Genz 1982; James et al. 1995b) and may routinely be isolated from both styrofoam and hard plastic containers (James and Gilligan 1998a, 1998b; James and Woollen 1989).

Previous tests indicated that some isolates of *F. acuminatum* may be capable of eliciting diseases on forest nursery seedlings (Gordon 1959; James and Gilligan 1984; James et al. 1986; Rathbun-Gravatt 1925). However, other reports (Hartley et al. 1918; Hildebrand 1986; Rathbun-Gravatt 1931; Vaartaja and Cram 1956) indicated that this species was not an active pathogen in forest nurseries under most conditions. Because of these disparities, tests were conducted to elucidate the ability of a wide range of

isolates from different forest nursery sources to elicit disease under controlled laboratory conditions. Results of these tests will help provide information regarding the potential risk of *F. acuminatum* in forest nurseries.

MATERIALS AND METHODS

A total of 33 isolates of *F.acuminatum* were tested for their ability to elicit typical post-emergence damping-off on young Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) germinants using a standard inoculation technique (James 1996). Tested isolates were obtained from forest nursery soil, roots of healthy-appearing and diseased conifer seedlings, conifer seeds, bodies of adult fungus gnats, and both styrofoam and hard plastic containers. Isolates were usually obtained using a selective agar medium for *Fusarium* and closely related fungi (Komada 1975). Isolates were typically transferred from the selective medium, single-spored, and grown on carnation leaf agar (Fisher et al. 1982) and potato dextrose agar (PDA) to facilitate their identification using the taxonomy of Nelson et al. (1983). *Fusarium* isolates were stored for long periods either as spore suspensions in sterile soil or on carnation leaves within sterile water. Under these conditions, isolates remained viable for at least 10 years.

The basic approach of tests was to expose young Douglas-fir germinants to isolates of *F. acuminatum* and record production of disease symptoms. Cornmeal-perlite 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 percent PDA, to which 75 g of perlite were added. The mixture was placed into glass vials (23 ml capacity) to about two-thirds capacity which were then autoclaved for 60 minutes at 121°C. After cooling, vials were inoculated with about 10 ml spore suspension of the test isolate (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 fungal isolates 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 was not contaminated with other organisms because the food base was completely colonized by the inoculated test isolate. Once dry, inoculum was refrigerated in plastic vials until needed.

Twenty-four vials (23 ml capacity each) were used to test each fungal isolate. Each vial was filled to about 2/3 capacity (about 2.5 g) with dried coconut-vermiculite media (Grace/Sierra Horticultural Products, Milpitas, CA) and autoclaved at 121°C for 60 minutes. Lids were placed loosely on vials before sterilization.

One Douglas-fir seedlot with high germination capacity (designated Flat Creek 78-10, Moscow Mountain – courtesy of the University of Idaho Research Nursery) was used throughout pathogenicity tests. Seeds were soaked in a 2-part bleach and 3-part water solution for 10 minutes. (Wenny and Dumroese 1987), rinsed 48 hours 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 radicles were at least 3 mm long.

For each tested isolate, perlite/cornmeal inoculum 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 approximate 1:50 w/w mixture of inoculum to media. Inoculum was distributed throughout the media by shaking. One recently germinated seed (germinant) was placed in each vial with its radicle placed downward into the media. Four ml of sterile water were added to each vial with caps replaced loosely to allow aeration. Adding water activated inoculum (Miles and Wilcoxin 1984). At least 20 of the 24 vials must have had germinants with normally extending radicles for a valid test (occasionally, a germinant's radicle would abort or decay because of infection by seed-borne microorganisms). One set of 24 control vials were included; each vial contained non-inoculated perlite instead of fungal inoculum.

Vials containing germinants were incubated at about 24°C on a lab bench, providing fluorescent light for 8-12 hours daily. Each test ran for 14 days. After 3 days, germinants were first checked for disease. Germinants were then checked for disease daily throughout the 14-day test period. Diseases were categorized as either standard post-emergence damping-off with fungal growth appearing just above the groundline or root decay in which fungal growth at or above the groundline was lacking, but the radicle was decayed while growing within the media. When germinates appeared diseased, they were carefully removed with forceps, rinsed thoroughly in tap water, and placed on Komada's medium for reisolation of inoculated isolates. After 14 days, surviving germinants were examined to determine if their roots were diseased (decayed or with necrotic lesions) and had grown to the bottom of the vial. Roots were then washed and plated on Komada's medium for reisolation of inoculated isolates.

A numerical rating system for isolate comparisons was used. This system awarded points based on duration of germinant survival within inoculated vials, occurrence and type of disease, reisolation of inoculated fungal isolates, and root growth within the vial. The range of possible points was 3-23, with higher point values reflecting less aggression by the tested isolates. To convert points to a score in which higher numbers represented greater virulence, a reciprocal rating was devised. In this system, the maximum score (germinants killed within 3 days by the test isolate) was 100 and the minimum score (indicating germinants were not diseased or infected by the test isolate within 14 days) was zero. Based on previous tests (James 1996; James and Perez 1999b; James et al. 1995a, 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.

The average rating and average number of days' survival (without noticeable disease) were used to compare isolates. Averages were compared using one-way analysis of variance; comparisons were made among isolate sources (soil vs. healthy roots vs. diseased roots). Significant means (P=0.05) were separated using Tukey's HSD test.

RESULTS

Two primary criteria were used to characterize pathogenic ability of tested *F.acuminatum* isolates. Probably the most revealing was average virulence scores, summarized in table 1. The other criterion was average number of days' survival of germinants exposed to tested fungal isolates, summarized in table 2. As indicated above, isolates with average virulence scores of less than 40 were considered non-pathogenic. Therefore, on average, *F. acuminatum* isolates from all nursery sources were classied as non-pathogenic in these tests (table 1). However, some individual isolates from roots of healthy-appearing seedlings (table 3 – Appendix) and diseased seedlings (table 4 – Appendix) displayed low or moderate levels of virulence. On the other hand, none of the isolates from nursery soil, containers, seeds, or fungus gnats were pathogenic under these tests (table 5 – Appendix). There were no statistically significant differences in average virulence scores or average germinant survival among tested isolates from forest nursery soil or roots

of healthy or diseased seedlings (tables 1 and 2). Many germinants survived the entire period of the pathogenicity test (14 days); the most virulent isolates caused reduced germinant survial (tables 6, 7, and 8 – Appendix). Although most germinants were not diseased or killed as a result of exposure to tested *F. acuminatum* isolates, they generally became infected and inoculated isolates were routinely reisolated from colonized plants

DISCUSSION

These pathogenicity tests confirmed previous work (Hartley et al. 1918; James and Gilligan 1984; James et al. 1986; Vaartaja and Cram 1956) indicating that the vast majority of *F. acuminatum* isolates obtained from seedling stock or growing environments in forest nurseries are probably not pathogenic to important commercial seedling crops. This fungus therefore does not pose an important risk to seedling production, even though it is frequently isolated. There are probably some individual isolates that may be pathogenic, but these are in the minority. Although seedling roots may often become colonized with isolates of *F. acuminatum*, this fungus is restricted to either epidermal or cortical tissues and does not penetrate vascular tissues, nor usually induce disease-associated tissue necrosis (Hill and Blunt 1994; Hill et al., 1987. It may in fact occupy host tissues to such an extent as

Table 1. Comparisons of average virulence scores of *Fusarium acuminatum* isolates obtaind from roots of healthy and diseased conifer seedlings, seeds, fungus gnats, containers, and soil at nurseries in the inland Pacific Northwest.

Isolate Source	No. Isolates Tested	Virulence Rating	
		Average[1]	Range
Nursery Soil	6	29.9 A	23.9-34.0
Healthy Roots	11	33.8 A	17.3-70.8
Diseased Roots	12	34.8 A	17.7-58.5
Seeds	2	32.5 A	26.5-38.5
Fungus Gnats	1	31.3 A	31.3
Containers	1	32.7 A	32.7
All Isolates	33	33.2	
Control	-	19.8	19.8

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

Table 2. Comparisons of average number of days' survival of young Douglas-fir germinants inoculated with *Fusarium acuminatum* isolates obtained from roots of healthy and diseased conifer seedlings, seeds, fungus gnats, containers, and soil at nurseries in the Pacific Northwest.

Isolate Source	Number of Isolates Tested	Average Survival[1]
Nursery Soil	6	12.9 A
Healthy Roots	11	12.9 A
Diseased Roots	12	13.5 A
Seeds	2	13.0 A
Fungus Gnats	1	12.9 A
Containers	1	13.9 A
All Isolates	33	13.0
Control	-	14.0

[1] Maximum survival of 14 days (length of pathogenicity tests); within this column, means followed by the same capital letter are not significantly different (P=0.05) using Tukey's HSD.

to prevent colonization by more aggressive *Fusarium* pathogens such as *F. oxysporum* on bareroot and *F. proliferatum* on container-grown seedlings (James and Perez 1999a; James et al. 1991b). In such cases, *F. acuminatum* may act as a biological control of more pathogenic *Fusarium* strains, although this has not been shown experimentally.

Results of these and some other tests indicated that not all *Fusarium* isolates in forest nursery environments are "bad" from the standpoint of disease potential (James et al. 1991b). It is important that the *Fusarium* populations be differentiated and characterized for different nursery situations; if the majority of isolates comprise species, such as *F. acuminatum*, that are generally non-pathogenic, little disease potential exists. Growers should not necessarily be concerned just because "*Fusarium*" is present at high populations in their nursery. Disease amelioration efforts should be based on proper characterization of the *Fusarium* population rather than only on presence of these fungi.

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