Fusarium spp. and Pinus strobus seedlings: root disease pathogens and taxa associated with seed

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Abstract. Eastern white pine (Pinus strobus L.) seeds were sown in soil infested with Fusarium proliferatum, root necrosis developed on seedling roots, and F. proliferatum was reisolated from symptomatic roots; thus, demonstrating that F. proliferatum is pathogenic to eastern white pine seedlings. Soils infested with F. acuminatum or F. sporotrichioides resulted in few diseased seedlings. Seedlings with root rot generally showed reductions in seedling height. All Fusarium species tested were recovered from rhizosphere soil samples. Three seedlots of Pinus strobus were examined for Fusarium infestation. Fusarium species were recovered from most seeds in two seedlots. Fusarium proliferatum and F. sporotrichioides were the predominant species isolated. Additional species not previously reported from P. strobus included: F. acuminatum, F. chlamydosporum, F. equisetii, F. graminearum, F. moniliforme, F. poae, F. polychaetidicum, F. heterosporum, F. saubinetum, and F. semitectum.

Introduction

Eastern white pine (Pinus strobus L.) seedlings are susceptible to a root rot incited by several Fusarium species (Riffle and Strong 1960). A primary causal agent, F. oxysporum Schlechtend.: Fr., has been isolated from root lesions of P. strobus seedlings in Wisconsin, USA (Enebak 1988), Michigan, USA (Riffle and Strong 1960), and Ontario, Canada (Honhart and Juzwik 1988) nurseries. Two other Fusarium species have been reported in association with affected eastern white pine seedlings; Fusarium moniliforme J. Sheld. and F. solani (Mart.) Sacc. (Riffle 1959; Riffle and Strong 1960). Evaluations of fields in Wisconsin state forest nurseries and an Ontario provincial nursery revealed 10–25% seedling mortality due to root rot (Juzwik and Rugg 1996; Renlund 1980; Prey et al. 1985; Ocamb et al. 1991). Affected trees may survive, be lifted from the beds, and then culled due to unacceptable levels of root rot (Prey et al. 1985).

Symptomatic roots from problematic white pine nursery fields were examined for Fusarium species and the predominant species isolated included F. proliferatum, F. oxysporum, and F. oxysporum var. redolens (Ocamb and Juzwik 1995). In this study, we examined the pathogenicity of Fusarium species isolated from proble-
matic forest nurseries, evaluating the ability of representative isolates of *F. acuminatum* Ell. and Ev. *sensu* Gordon, *F. oxysporum*, *F. oxysporum* var. *redolens* (W. L. Gordon), *F. proliferatum* (T. Matsushima) Nirenberg, *F. solani*, and *F. sporotrichioides* Sherb. to cause root rot, colonize rhizosphere soil, and affect seedling growth in greenhouse studies. We also evaluated *Fusarium* spp. associated with *P. strobus* seeds.

**Materials and methods**

*Experimental design and data analyses*

The greenhouse pathogenicity tests were conducted three different times (= three separate runs or replicate experiments). A completely randomized design was used, with each run containing one pot of 30 seedlings per *Fusarium* sp. isolate. Data were analyzed in a two-way factorial structure (replication by isolate). The per pot means of shoot height, shoot dry weight, root dry weight, and root rot rating were calculated for each replicate. Analyses of variance were conducted to determine treatment differences (SAS Institute 1990). For each of these variables, the difference from the replicate mean was calculated for each *Fusarium* sp. isolate of each run. These differences were used in calculating correlation coefficients. Isolates were tested for significant effects (*P* = 0.05) on number of colony-forming units (CFU) using a general linear model for an analysis of variance of colony-forming units present in rhizosphere soil samples; means were compared with Tukey’s W statistic (SAS Institute 1990).

An analysis of variance was performed on disease incidence of each isolate using a general linear model and means were compared using Tukey’s W (SAS Institute 1990).

*Inoculum production*

Isolates of *Fusarium* species obtained from fields at the Wilson State Forest Nursery, Boscobel, Wisconsin, included one each of *F. sporotrichioides* (Fsp) and *F. acuminatum* (Fac); three of *F. oxysporum* (Fox1, Fox2, Fox3), *F. proliferatum* (Fp1, Fp2, Fp3), and *F. solani* (Fsol1, Fsol2, Fsol3); and four of *F. oxysporum* var. *redolens* (For1, For2, For3, For4). Isolates were obtained from symptomatic roots or soil, purified by the single-spore method, and stored on silica gel at 5 °C (Windels and Burnes 1988).

Fungal isolates were grown from silica gel crystals on carnation leaf agar (CLA) under fluorescent lamps (three General Electric or Sylvania 40W tubes) supplemented with black light (one Sylvania 40W tube, BLB series) in a 12-hr photoperiod at 25 °C (Nelson et al. 1983). Agar plugs (5-mm diameter) were transferred from 10- to 14-day-old CLA cultures to sterile cornmeal-sand medium (97 g sand, 3 g cornmeal, 40 ml distilled water) for inoculum production. Inoculated
cultures were incubated 4–6 weeks at 25 °C under the light regime previously described and dried in a horizontal laminar flow hood.

Seed stratification

White pine seeds of lot H-158 (courtesy of T. Marty, Wisconsin Department of Natural Resources) were used. Seeds were surface-disinfested by agitation in 0.5% NaOCl solution on an orbital shaker at 120 rpm for 25 minutes and rinsed four times in sterile, distilled water. Seeds were wrapped in moistened, sterile cheesecloth, enclosed in foil, and stored at 4–5 °C for 8 weeks.

Soil infestation and seedling culture

Loamy sand soil with an organic content of 1–2% and average bulk density of 1.20 g/cm³ was collected from a white pine field in the Wisconsin nursery. The soil was placed in autoclavable plastic shoeboxes, covered, and autoclaved at 10 psi for 45 min on each of two consecutive days, and then allowed to rest for 2 days. *Fusarium* inoculum was added to rested soil and thoroughly mixed to give a final concentration of about 20000 CFU/g oven-dried soil. One clay pot (19.5 cm diameter by 9.5 cm deep) per *Fusarium* isolate was filled first with 400 cm³ pasteurized field soil, then 400 cm³ of infested soil, and finally 800 cm³ of pasteurized field soil, making three distinct layers. The control treatment consisted of a pot filled with 1600 cm³ pasteurized field soil (non-infested control). Stratified white pine seeds (30 seeds/pot) were randomly selected and sown 0.5 cm below the soil surface, after which a 2-cm layer of sterile vermiculite was added. Pots were placed on a greenhouse bench in a room maintained at 24 °C days/18 °C nights on a 12-hour photoperiod. Plants were watered daily using local tap water and were fertilized on a weekly basis with 0.61 g/L of a 20-19-18 Peter’s fertilizer beginning 6 weeks after sowing. Each pot received about 100 ml of fertilizer solution.

Seedling and rhizosphere soil sampling

After 16 weeks, seedlings were carefully removed from pots and excess soil was gently shaken from roots; the remaining soil was considered rhizosphere soil. Ten seedlings from each pot were randomly selected, each placed in individual 2-cm glass tubes (40-ml volume) containing 15 ml of 0.01% water agar, and the remaining 20 plants in each pot were discarded. Each seedling sampled was agitated in the tube for 15 s at a setting of 5 on a Fisher Scientific Vortex Genie 2 (Pittsburgh, PA, USA) to remove the rhizosphere soil. The seedlings were then removed and washed under running tap water for 2 min. Shoot height was recorded for each seedling, and root systems were rated on a 1–5 rating system: 1 = apparently healthy; 2 = over 50% length of one lateral root exhibited necrosis; 3 = lower 1/3 of primary root exhibited necrosis or greater than 50% of two or more lateral roots exhibited necrosis; 4 = lower 2/3 of primary root exhibited necrosis (with or
without lateral root injury); and 5 = upper third of primary root exhibited necrosis or entire root system was necrotic. Shoot and root dry weight of each seedling was determined after drying at 105 °C for 48 hrs.

If roots exhibited necrosis then small segments of tissues were excised from the edge of necrotic areas, disinfested in 0.5% NaOCl for 1 min. and embedded into solidified Nash medium (Nash and Snyder 1962) supplemented with aureomycin (Kommedahl et al. 1979). The colony-forming units present in the rhizosphere soil was determined through a dilution series pipetted onto solidified Nash medium. Dilution plates were incubated at 24 °C with indirect lighting for up to 21 days. Putative Fusarium species were transferred from the Nash medium to potato dextrose agar (PDA) (Dhingra and Sinclair 1985) and CLA and the cultures incubated under the light and temperature regimes previously described. Each isolate was examined microscopically and identified to species according to the system of Nelson, Toussoun, and Marasas (1983). The subgroup, *F. oxysporum* var. *redolens* (Booth 1971), was delineated from other *F. oxysporum* isolates (Matuo and Chiba 1966; Ocamb and Juzwik 1995), though its status as a separate taxon is not universally accepted (Messiaen and Cassini 1981).

**Seedlot evaluations**

During 1992 and 1993, non-stratified eastern white pine seeds were obtained from two state forest nurseries in Wisconsin: Griffith State Nursery, Wisconsin Rapids, and F. G. Wilson State Nursery, Boscobel. Seedlots are seeds collected from a wide area (county-wide) of cone production. A seed lot represents a wide genetic base from a number of different trees. The WI DNR collects seeds and seedlots are distributed to public nurseries. Each nursery stores their seeds. Fall seeding is practiced; seeds undergo stratification in the field. For this study, two seedlots were sampled from each nursery, one seedlot was common to both nurseries. Samples from seedlots A and B were collected from one nursery while samples from seedlots B and C were collected from the other. Seed samples were obtained from the seeder, just prior to sowing. Dry seeds were placed in sterile, plastic centrifuge tubes (50-ml volume), brought to the laboratory, and stored at −10 °C until assayed.

From each site during both years of sampling, 200 seeds/lot were placed on supplemented Nash medium then incubated at 24 °C for 18 days. Ten seeds were embedded in each culture plate. Putative *Fusarium* colonies were transferred to PDA and CLA, and then incubated under the light and temperature regimes previously described. Isolates were identified to species through microscopic examinations according to the taxonomic system described by Nelson et al. (1983). The numbers of seeds from which *Fusarium* species were isolated, as well as the incidence of each *Fusarium* species, were recorded.

**Results**

Root rot incidence, severity, and recovery of *Fusarium* spp. *Fusarium oxysporum*. 
F. proliferatum, and F. solani soil infestations were associated with greater disease incidence compared to non-infested soil (Table 1). The numbers of seedlings exhibiting root rot (RR > 1) were greater (P = 0.05) for all Fusarium spp. isolates assayed, except for F. acuminatum, compared to the non-infested treatment (control). Figure 1 shows the frequency of each root rot rating class for each Fusarium isolate. Only a small proportion of seedlings grown in soil infested with F. acuminatum or F. sporotrichioides exhibited more than minimal root rot (RR > 2).

Fusarium isolates that caused root rot were not consistently associated with reductions in root and shoot dry weights or shoot height. In general, root dry weight, shoot dry weight, and shoot height were negatively correlated with root rot ratings; only shoot height was significantly (P = 0.05) correlated in this group of isolates (Table 2). A scatter plot of root dry weights and shoot dry weights illustrates the possibility of seedling growth enhancement associated with some isolates of pathogenic Fusarium species (Figure 2A). The data points representing the F. sporotrichioides, F. acuminatum, and the non-infested treatments were in the same proximity on scatter plots of root rot rating and seedling height (Figure 2B).

Fusarium species were recovered from all rhizosphere soil samples (Table 3). Higher numbers (P = 0.05) of CFUs were recovered from F. proliferatum treatments, Fp-2 and Fp-3, than any other treatment.

Seedlot evaluations

The percentage of seeds from which Fusarium species were isolated in seedlot B

Table 1. Disease incidence in greenhouse studies with Pinus strobus seedlings grown in nursery soil artificially-infested with Fusarium species

| Soil Treatment      | Percent seedlings with root rot
<table>
<thead>
<tr>
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<th></th>
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</thead>
<tbody>
<tr>
<td>None</td>
<td>17c</td>
</tr>
<tr>
<td>F. acuminatum</td>
<td>43bc</td>
</tr>
<tr>
<td>F. oxysporum var. redolens-1</td>
<td>97a</td>
</tr>
<tr>
<td>F. oxysporum var. redolens-2</td>
<td>93a</td>
</tr>
<tr>
<td>F. oxysporum var. redolens-3</td>
<td>83ab</td>
</tr>
<tr>
<td>F. oxysporum var. redolens-4</td>
<td>83ab</td>
</tr>
<tr>
<td>F. oxysporum-1</td>
<td>97a</td>
</tr>
<tr>
<td>F. oxysporum-2</td>
<td>100a</td>
</tr>
<tr>
<td>F. oxysporum-3</td>
<td>97a</td>
</tr>
<tr>
<td>F. proliferatum-1</td>
<td>83ab</td>
</tr>
<tr>
<td>F. proliferatum-2</td>
<td>95a</td>
</tr>
<tr>
<td>F. proliferatum-3</td>
<td>80ab</td>
</tr>
<tr>
<td>F. solani-1</td>
<td>97a</td>
</tr>
<tr>
<td>F. solani-2</td>
<td>97a</td>
</tr>
<tr>
<td>F. solani-3</td>
<td>97a</td>
</tr>
<tr>
<td>F. sporotrichioides</td>
<td>57abc</td>
</tr>
</tbody>
</table>

1 Numbers represent means based on ten seedlings per soil treatment per trial (30 seedlings total per soil treatment). 2 Mean disease incidence = (no. seedlings with RR >1/ total no. seedlings evaluated) x 100. 3 Reading down, values followed by the same letters are not significantly different (P = 0.05) according to Tukey’s W statistic.
Figure 1. Mean number of *Pinus strobus* seedlings in four disease severity classes (RR1-RR4) after growing in pasteurized nursery soil artificially-infested with *Fusarium acuminatum* (Fac), *F. oxysporum* var. *reidents* (For1, For2, For3, For4), *F. oxysporum* (Fox1 Fox2, Fox3), *F. proliferatum* (Fp1, Fp2, Fp3), *F. solani* (Fsol1, Fsol2, Fsol3), *F. sporotrichioides* (Fsp), or no *Fusarium* species (None). Disease severity classes are: RR1 = apparently healthy; RR2 = over 50% length of one lateral root exhibiting rot; RR3 = lower 1/3 of tap root is symptomatic or greater than 50% of two or more lateral roots is necrotic; RR4 = lower 2/3 of tap root is rotted (with or without lateral root injury); and RR5 = upper third of tap root is rotted or entire root system is affected. Bars represent means based on ten seedlings per soil isolate per three experimental runs (30 seedlings per soil isolate). Bars labeled with the same letters are not significantly different ($P = 0.05$) according to Tukey’s W statistic.
Table 2. Pearson correlation matrix for variables measured in greenhouse pathogenicity experiments with *Fusarium* species and *Pinus strobus* seedlings.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Root rot rating r</th>
<th>Root rot rating P</th>
<th>Root dry weight r</th>
<th>Root dry weight P</th>
<th>Shoot height r</th>
<th>Shoot height P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot dry weight</td>
<td>-0.423</td>
<td>0.103</td>
<td>0.797</td>
<td>0.001</td>
<td>0.571</td>
<td>0.021</td>
</tr>
<tr>
<td>Shoot height</td>
<td>-0.606</td>
<td>0.013</td>
<td>0.480</td>
<td>0.060</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root dry weight</td>
<td>-0.188</td>
<td>0.486</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Correlation coefficient. *P* values based on a sample size of n=30.

Figure 2. Scatter plot of mean isolate deviation from overall run mean for root dry weight-shoot dry weight (A) and root rot severity rating – seedling height (B). Soil infestation isolates included *F. acuminatum* (Fac), *F. oxysporum* var. *redolens* (For1, For2, For3, For4), *F. oxysporum* (Fox1 Fox2, Fox3), *F. proliferatum* (Fp1, Fp2, Fp3), *F. solani* (Fsol1, Fsol2, Fsol3), *F. sporotrichoides* (Fsp), and no *Fusarium* species (N). Numbers represent means based on ten *Pinus strobus* seedlings per soil treatment per run (30 seedlings per soil treatment).
samples from Griffith and Wilson nurseries during 1992 was 91 and 73%, respectively, and 70 and 88% for 1993 samples, respectively. In seedlot C from Griffith nursery, 43% of the seeds yielded Fusarium isolations for both years sampled. Fusarium species were isolated from 69 and 90% of seeds from seedlot A collected in Wilson nursery during 1992 and 1993, respectively.

Fusarium spp. isolations from seedlot B, sampled each year from both nurseries, varied by both year and nursery site. In general, F. proliferatum and F. sporotrichioides were the predominant species isolated from seedlot C (Table 4). Fusarium polyphialidicum, newly-associated with seeds in this report and never before reported in the U.S. or Canada, was relatively abundant in isolations made from seedlot C in both years sampled. During 1993, F. oxysporum within seedlot A and F. chlamydosporum within seedlot C were found at relatively greater levels compared to other Fusarium species, except for F. proliferatum. We found the following Fusarium species never previously reported from seeds of any Pinus species grown in the U.S.: F. chlamydosporum, F. equiseti, F. graminearum, F. poae, F. polyphialidicum, and F. heterosporum. Mixtures of Fusarium species were found. During 1992, 19, 3, 15, and 15%; in 1993, 12, 1, 8, and 18% of the total Fusarium isolations were mixtures from seedlots B (Griffith), C, A, and B (Wilson), respectively. A mixture is comprised of one seed with multiple Fusarium species. Nearly all of the mixtures obtained consisted of F. proliferatum with F. sporotrichioides or F. oxysporum.
Table 4. Isolation frequencies (% of Fusarium species from three seedlots of Pinus strobus immediately prior to sowing in two nurseries \textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Fusarium taxa</th>
<th>1992 Seed Collection</th>
<th>1993 Seed Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wilson\textsuperscript{a}</td>
<td>Griffith\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Seed lot A</td>
<td>Seed lot B</td>
</tr>
<tr>
<td>\textit{F. acuminatum}</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>\textit{F. avenaceum}</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>\textit{F. chlamydosporum}</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>\textit{F. equiseti}</td>
<td>&lt; 1</td>
<td>2</td>
</tr>
<tr>
<td>\textit{F. graminearum}</td>
<td>1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>\textit{F. moniliforme}</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>\textit{F. oxysporum}</td>
<td>2</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>\textit{F. porae}</td>
<td>&lt; 1</td>
<td>-</td>
</tr>
<tr>
<td>\textit{F. polyphialdiicum}</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>\textit{F. proliferatum}</td>
<td>55</td>
<td>62</td>
</tr>
<tr>
<td>\textit{F. heterosporum}</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>\textit{F. sambuciforme}</td>
<td>-</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>\textit{F. semitectum}</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>\textit{F. solani}</td>
<td>&lt; 1</td>
<td>-</td>
</tr>
<tr>
<td>\textit{F. sporotrichioides}</td>
<td>39</td>
<td>30</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Griffith = Griffith State Nursery, Wisconsin Rapids and Wilson = F. G. Wilson State Nursery, Boscobel, Wisconsin, USA. \textsuperscript{2} Two hundred seed were sampled from each seedlot at each site on each date.

Discussion

Our work expands the list of known \textit{Fusarium} taxa confirmed as causal agents of \textit{P. strobus} root rot (Enebak 1988; Honhart and Juzwik 1988). In particular, our studies confirm that isolates of \textit{F. proliferatum} can cause root rot on eastern white pine seedlings and further support Riffle and Strong’s (Riffle and Strong 1960) implication of \textit{F. moniliforme}, \textit{F. oxysporum} and \textit{F. solani} in root rot of eastern white pine. Since their report, \textit{F. proliferatum} has been taxonomically separated from \textit{F. moniliforme} (Nelson et al. 1983), and it is likely that the isolates Riffle and Strong (1960) tested were actually \textit{F. proliferatum}. A previous report on Fusarium root rot of \textit{P. strobus} did not confirm Koch’s postulates for \textit{Fusarium proliferatum} as a cause of \textit{P. strobus} root rot (Wenner and Merrill 1984). \textit{Fusarium acuminatum} and \textit{F. sporotrichioides} were associated with less severe root rot symptoms relative to other \textit{Fusarium} species we studied. These two \textit{Fusarium} species have been isolated at low frequencies from the roots of symptomatic \textit{P. strobus} seedlings in the field (Ocamb and Juzwik 1995).

We also recovered \textit{Fusarium} species from rhizosphere soil samples. The relatively high numbers of \textit{F. proliferatum} propagules associated with rhizosphere soil suggest that this species may be producing greater amounts of hyphae and/or conidia compared to the other \textit{Fusarium} species studied. Low numbers of \textit{Fusarium} colony-forming units were detected in rhizosphere soil sampled from the untreated
control seedlings. Seed-borne Fusarium not removed by the surface-disinfection treatment may be the source of these isolates.

Root rot severity was not strongly correlated with seedling growth parameters in this study. Genetic variation may account for the lack of significant differences in seedling growth. Seeds used in the pathogenicity studies were not genetically uniform, representing a countywide collection of seeds resulting from open pollination. Testing of Fusarium strains on clonal P. strobus stock may yield a better measure of the effects of these Fusarium species on seedling growth.

This is the first report of F. acuminatum, F. avenaceum, F. chlamydosporum, F. equiseti, F. graminearum, F. moniliforme, F. poae, F. polyphialidicum, F. heterosporum, F. sambucinum, or F. semitectum being isolated from any P. strobus plant part. Our findings expand the range of Fusarium species reported on seeds of P. strobus; only F. sporotrichoides was previously reported on seeds of P. strobus (Mittal and Wang 1986, 1986).

To find additional seed-associated Fusarium species is not unexpected, as other Fusarium species have been isolated from seeds of other conifers grown in the USA and Canada. Our findings further suggest that pathogenic Fusarium species may be associated with seed of P. strobus and could contribute to nursery disease problems. In previous reports, Pinus species other than P. strobus have yielded F. acuminatum, F. avenaceum, F. moniliforme, F. oxysporum, F. proliferatum, F. roseum, F. sambucinum, F. semitectum, F. solani, F. subglutinans, and F. tricinctum in seed isolations (James and Genz 1982; Anderson 1986; James 1986; Fraedrich and Miller 1995; Lilja et al. 1995). Fusarium acuminatum, F. avenaceum, F. culmorum, F. lateritium, F. moniliforme, F. oxysporum, F. poae, F. proliferatum, F. roseum, F. sambucinum, F. solani, and F. tricinctum were reported from seeds of Pseudotsuga species (Bloomberg and Lock 1972; Graham and Linderman 1983; James 1984; Anderson 1985, 1986; James 1986; Lilke and Browning 1991; Axelrood et al. 1995; Hoefnagels and Linderman 1999). Fusarium oxysporum was found on seeds of Abies species (Lilke and Browning 1991) while F. oxysporum, F. solani, F. tricinctum were isolated from seeds of Picea species (James 1985, 1985, 1986; Mittal and Wang 1986, 1986; Lilke and Browning 1991). Our findings expand the species range associated with conifer seed in the USA and Canada. Also, this is the first report of F. polyphialidicum associated with seeds. Fusarium polyphialidicum was previously isolated from debris in African soil (Marasas et al. 1986).

The presence of Fusarium species on pine seed is probably established after seed formation or cone opening (Mittal and Wang 1986, 1986), perhaps during seed extraction, transport, storage, or sowing. The variation in Fusarium isolated within one seedlot (B) sampled from two nurseries suggests that seed-borne Fusarium levels associated with this seedlot were affected during seed handling, storage, and/or sowing. Furthermore, some Fusarium species found in seedlot B were detected from only one nursery. Fusarium populations are affected by handling and/or sowing, since this seedlot was stored at only one site, it suggests that Fusarium may be introduced during storage or handling. Reduced fungal viability on seed probably does not account for the variation between years or sites (Mittal and Wang 1989).
We have shown that many *P. strobus* seeds may be potentially contaminated with *Fusarium* species and pathogenic strains may be introduced into fumigated nursery beds in this way. We have shown one of the predominant species obtained, *F. proliferatum*, to be pathogenic on roots of eastern white pine seedlings. Another species that also dominated our isolations, *F. sporotrichioides*, reportedly causes pre- and post-emergence death of white pine seedlings (Mittal and Wang 1986, 1986; Enebak 1988). Examinations of the pathogenicity of these seed-borne isolates are needed to confirm the potential problem.

Some *Fusarium* species associated with root rot of eastern white pine were rarely isolated from seeds in our study and primary inocula of these pathogens are probably introduced from other sources, such as surrounding soil unaffected by fumigation. *Fusarium* propagules may also survive in plant debris through the fumigation process (Juzwik et al. 1995a). Thus, pathogen infestation in nurseries is probably not solely through seed-borne introduction but seed-borne sources could augment the pathogenic *Fusarium* population in a nursery.

Future work should examine *Fusarium* root rot development under different environmental conditions, including various temperature, soil matric potentials, or soil types, since root rot incidence or severity may change as environment or host condition varies (Juzwik et al. 1995b). Also, the role of *Fusarium* species isolated from seeds in both root rot and damping-off of eastern white pine needs to be studied, since not all forms found associated with plant parts are pathogenic (Axelrood et al. 1995). Finally, control measures that are devised for *Fusarium* root rot must take into account the taxonomic range of the potential pathogens plus the inoculum sources for these *Fusarium* diseases. Eastern white pine seeds are generally not treated with fungicides nor chemically-disinfested prior to sowing. If these seed-borne *Fusarium* isolates are pathogenic, development of seed treatments may be warranted.

**Acknowledgements**

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