*New Forests* **24:** 67–79, 2002.


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

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Received 5 July 2001; accepted in revised form 19 November 2001

**Key words:** *F. moniliforme, F. oxysporum, F. polyphialidicum, F. proliferatum, F. sporotrichioides, Fusarium equiseti*

**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. equiseti, F. graminearum, F. moniliforme, F. poae, F. polyphialidicum, F. heterosporum, F. sambucinum*, 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 $g/cm^2$ 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 20 000 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 $cm^3$ pasteurized field soil, then 400 $cm^3$ of infested soil, and finally 800 $cm^3$ of pasteurized field soil, making three distinct layers. The control treatment consisted of a pot filled with 1600 $cm^3$ 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-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[x]

| Soil Treatment | Percent seedlings with root rot[y] |
|---|---|
| None | 17c [z] |
| F. acuminatum | 43bc |
| F. oxysporum var. redolens-1 | 97a |
| F. oxysporum var. redolens-2 | 93a |
| F. oxysporum var. redolens-3 | 83ab |
| F. oxysporum var. redolens-4 | 83ab |
| F. oxysporum-1 | 97a |
| F. oxysporum-2 | 100a |
| F. oxysporum-3 | 97a |
| *F. proliferatum*-1 | 83ab |
| *F. proliferatum*-2 | 95a |
| *F. proliferatum*-3 | 80ab |
| *F. solani*-1 | 97a |
| *F. solani*-2 | 97a |
| *F. solani*-3 | 97a |
| *F. sporotrichioides* | 57abc |

[x] Numbers represent means based on ten seedlings per soil treatment per trial (30 seedlings total per soil treatment). [y] Mean disease incidence = (no. seedlings with RR >1/ total no. seedlings evaluated) x 100. [z] Reading down, values followed by the same letters are not significantly different ($P = 0.05$) according to Tukey's W statistic.

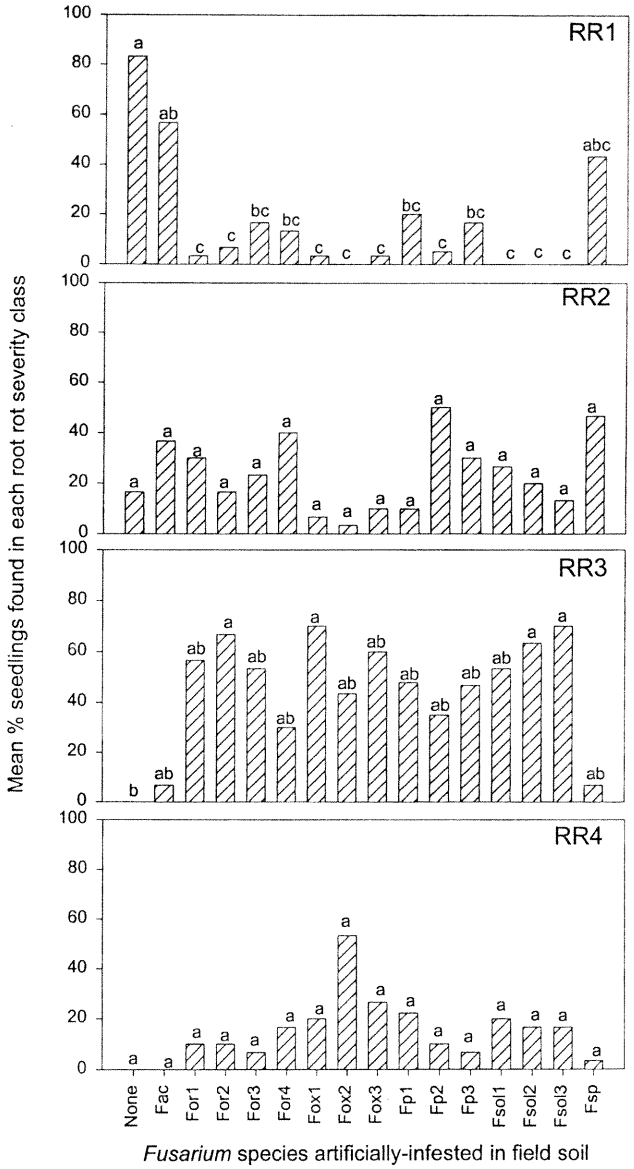


*Figure 1.* Mean number of *Pinus strobus* seedlings in four disease severity classes (RR1-RR4) after growing in pasteurized nursery soil artificially-infested with *Fusarium acuminatum* (Fac), *F. oxysporum* var. *redolens* (For1, For2, For3, For4), *F. oxysporum* (Fox1 Fox2, Fox3), *F. proliferatum* (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.

| Variable | Root rot rating r[a] | Root rot rating *P*[b] | Root dry weight r | Root dry weight *P* | Shoot height r | Shoot height *P* |
|---|---|---|---|---|---|---|
| Shoot dry weight | −0.423 | 0.103 | 0.797 | 0.001 | 0.571 | 0.021 |
| Shoot height | −0.606 | 0.013 | 0.480 | 0.060 | | |
| Root dry weight | −0.188 | 0.486 | | | | |

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

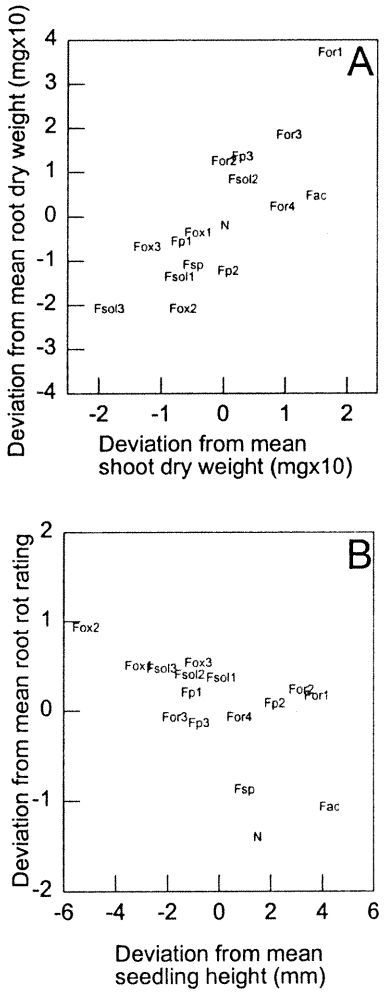


*Figure 2.* Scatter plot of mean isolate deviation from overall run mean for root dry weight-shoot dry weight (A) and root rot severity rating – seedling height (B). Soil infestation isolates included *F. acuminatum* (Fac), *F. oxysporum* var. *redolens* (For1, For2, For3, For4), *F. oxysporum* (Fox1 Fox2, Fox3), *F. proliferatum* (Fp1, Fp2, Fp3), *F. solani* (Fsol1, Fsol2, Fsol3), *F. sporotrichioides* (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).

*Table 3.* Mean number of colony-forming units (CFU) of *Fusarium* species per g of dried rhizosphere soil collected from *Pinus strobus* seedlings grown in various *Fusarium* species – infested soil

| Soil treatment | CFU x $10^{3y}$ |
|---|---|
| F. acuminatum | 550 cd $^{z}$ |
| F. oxysporum var. redolens-1 | 35 d |
| F. oxysporum var. redolens-2 | 290 d |
| F. oxysporum var. redolens-3 | 220 d |
| F. oxysporum var. redolens-4 | 520 cd |
| F. oxysporum-1 | 190 d |
| F. oxysporum-2 | 190 d |
| F. oxysporum-3 | 190 d |
| *F. proliferatum*-1 | 970 c |
| *F. proliferatum*-2 | 1600 b |
| *F. proliferatum*-3 | 2300 a |
| *F. solani*-1 | 210 d |
| *F. solani*-2 | 290 d |
| *F. solani*-3 | 470 cd |
| *F. sporotrichioides* | 220 d |
| non-infested | 6 d |

$^{y}$ Numbers represent means based on ten seedlings per soil treatment per run (30 seedlings per soil treatment). $^{z}$ Reading down, values followed by the same letters are not significantly different ($P = 0.05$) according to Tukey's W statistic.

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 [a,b]

| *Fusarium* taxa | 1992 Seed Collection | | | | 1993 Seed Collection | | | |
|---|---|---|---|---|---|---|---|---|
| | Wilson[a] | | Griffith[a] | | Wilson | | Griffith | |
| | Seed lot A[b] | Seed lot B | Seed lot B | Seed lot C | Seed lot A | Seed lot B | Seed lot B | Seed lot C |
| *F. acuminatum* | - | 4 | 1 | 1 | < 1 | - | - | 2 |
| *F. avenaceum* | - | - | 3 | - | - | - | < 1 | - |
| *F. chlamydosporum* | - | - | - | - | - | 7 | 12 | 16 |
| *F. equiseti* | < 1 | 2 | 1 | - | < 1 | - | < 1 | - |
| *F. graminearum* | 1 | < 1 | 1 | 2 | < 1 | 2 | < 1 | 1 |
| *F. moniliforme* | - | - | < 1 | - | < 1 | 10 | 4 | - |
| *F. oxysporum* | 2 | < 1 | < 1 | 7 | 10 | 3 | 1 | 4 |
| *F. poae* | < 1 | - | 1 | - | 1 | 3 | 3 | 2 |
| *F. polyphialidicum* | - | - | - | 14 | < 1 | 2 | 6 | 24 |
| *F. proliferatum* | 55 | 62 | 59 | 39 | 75 | 54 | 49 | 41 |
| *F. heterosporum* | < 1 | < 1 | - | - | - | - | - | - |
| *F. sambucinum* | - | < 1 | 1 | - | - | - | - | - |
| *F. semitectum* | < 1 | < 1 | - | - | < 1 | - | - | 1 |
| *F. solani* | < 1 | - | - | - | < 1 | < 1 | < 1 | 1 |
| *F. sporotrichioides* | 39 | 30 | 31 | 37 | 10 | 19 | 22 | 7 |

[a] Griffith = Griffith State Nursery, Wisconsin Rapids and Wilson = F. G. Wilson State Nursery, Boscobel, Wisconsin, USA. [b] Two hundred seed were sampled from each seedlot at each site on each date.

## Discussion

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

We also recovered *Fusarium* species from rhizosphere soil samples. The relatively high numbers of *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 *Fusarium* species studied. Low numbers of *Fusarium* colony-forming units were detected in rhizosphere soil sampled from the untreated

control seedlings. Seed-borne *Fusarium* not removed by the surface-disinfestation 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. sporotrichioides* 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; Littke and Browning 1991; Axelrood et al. 1995; Hoefnagels and Linderman 1999). *Fusarium oxysporum* was found on seeds of *Abies* species (Littke 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; Littke and Browning 1991). Our findings expand the species range associated with conifer seed in the USA and Canada. A lso, 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

We thank Trenten Marty of the Wisconsin Department of Natural Resources for providing seed; and Paul E. Nelson and Jean Juba, Pennsylvania State University, for confirming the identity of *F. polyphialidicum*. Thanks also to J. Patrick Martinez, Joseph O'Brien, and Jeff Stone for their valuable suggestions for this manuscript.

## References

Anderson R.L. 1985. Checklist of micro-organisms associated with true seeds in the world. USDA For. Ser., 34 pp.

Anderson R.L. 1986. New method for assessing contamination of slash and loblolly pine seeds by *Fusarium moniliforme* var.*subglutinans*. Plant Disease 70: 452–453.

Axelrood P., Neumann M., Trotter D., Radley R., Shrimpton G. and Dennis J. 1995. Seedborne *Fusarium*

on Douglas-fir: pathogenicity and seed stratification method to decrease *Fusarium*contamination. New Forests 9: 35–51.

Bloomberg W.J. and Lock W. 1972. Strain differences in *Fusarium oxysporum* causing diseases of Douglas-fir seedlings. Phytopathology 62: 481–485.

Booth C. 1971. The Genus *Fusarium*. Commonwealth Mycol. Instit., Kew, England 237.

Dhingra O.D. and Sinclair J.B. 1985. Basic Plant Pathology Methods. CRC Press, Boca Raton, Florida, 355 pp.

Enebak S.A. 1988. Control of Soilborne Pathogens in Forest Tree Nurseries, M.S., University of Minnesota, St. Paul 55 pp.

Fraedrich S.W. and Miller T. 1995. Mycoflora associated with slash-pine seeds from cones collected at seed orchards and cone-processing facilities in the south-eastern USA. Eur. J. For. Path. 25: 73–82.

Graham J.H. and Linderman R.G. 1983. Pathogenic seed-borne *Fusarium* from Douglas-fir. Plant Disease 67: 323–325.

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

Honhart C. and Juzwik J. 1988. The impact of Fusarium, Cylindrocarpon and Cylindrocladium root rot on white pine seedlings in compartment A59, Midhurst Nursery, 1987. In: Ont. Min. Nat. Res, Pest Control Section, Sault Ste. Marie, Ontario, Canada, Pest Control Sec. Rep. PC-32. .

James R.L. 1984. Fungi colonizing Douglas-fir seed at the Champion Timberlands Nursery, Plains, Montana Northern Region Report 84–13. 3.

James R.L. 1985. Diseases of conifer seedlings caused by seed-borne*Fusarium* species. In: Shearer R.C. (ed.), Conifer tree seed in the inland mountain west symposium, Missoula, MT, August 5–6, 1985. USDA Forest Service General Tech. Report INT-203., pp. 267–271.

James R.L. 1985. Pathogenic *Fusarium* on spruce seed from the Towner Nursery, North Dakota. Northern Region Report 85-23. 9 pp.

James R.L. 1986. Occurrence of *Fusarium* on conifer tree seed from northern rocky mountain nurseries Western Forest Nursery Meeting, Turnwater, WA, Aug. 12-15, 1986. USDA Forest Service Report Rm-137., pp. 109–114.

James R.L. and Genz D. 1982. Evaluation of fungal populations on ponderosa pine seed. USDA Forest Service.

Juzwik J. and Rugg D. 1996. Seedling mortality and development of root rot in white pine seedlings in two bare-root nurseries. Can. J. Plant Pathol. 18: 335–341.

Juzwik J., Ocamb C.M. and Cease K.R. 1995a. Effect of cover-cropping and fumigation on *Fusarium*-species populations in bare-root nursery soils. Phytopathology 85: 1198.

Juzwik J., Rugg D.J., Menes P.J. and Cease K.R. 1995b. Soil water management and Fusarium root rot in eastern white pine seedlings Proc. of 1995 Int. Res. Conf. on Methyl Bromide Alt. and Emissions Reduction, Nov. 6-8, 1995. Elsevier, San Diego, CA, USA available online:www.mbao.org/mbrpro95.html., pp. 76−1−76−2.

Kommedahl T., Windels C.E. and Strucker R.E. 1979. Occurrence of *Fusarium* species in roots and stalks of symptomless corn plants during the growing season. Phytopathology 69: 961–966.

Lilja A., Hallaksela A.M. and Heinonen R. 1995. Fungi colonizing Scots-pine cone scales and seeds and their pathogenicity. Eur. J. For. Path. 25: 38–46.

Littke W.R. and Browning J.E. 1991. Assaying for seed-borne fungi of Douglas-fir and true fir species. In: Sutherland J.R. and Glover S.G. (eds), Proc. of the First Meeting of IUFRO Working Party S2.07-09. Forestry Canada, Victoria, British Columbia, Canada, pp. 253–258.

Marasas W., Nelson P., Toussoun T. and Van Wyk P. 1986. *Fusarium polyphialidicum*, a new species from South Africa. Mycologia 78: 678–682.

Matuo T. and Chiba O. 1966. Species and formae speciales of *Fusaria* causing damping-off and root-rot of coniferous seedlings in Japan. Ann. Phytopath. Soc. Japan 32: 14–22.

Messiaen C.M. and Cassini R. 1981. Taxonomy of *Fusarium*. In: Nelson T.A.T.P.E. and Cook R.J. (eds), *Fusarium*: Diseases, Biology, and Taxonomy. The Pennsylvania University Press, University Park, Pennsylvania.

Mittal R.K. and Wang B.S.P. 1986. Fungi associated with seeds of eastern white pine and white spruce during cone processing and seed extraction. Can. J. For. Res. 17: 1026–1034.

Mittal R.K. and Wang B.S.P. 1986. Pathogenicity of seed-borne fungi in *Pinus strobus*. Phytopathology 76: 1113.

Mittal R.K. and Wang B.S.P. 1989. Fungi on eastern white pine seeds with high moisture content can survive ultralow temperatures. Tree Planters' Notes 40: 34–36.

Nash S.M. and Snyder W.C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot Fusarium in field soils. Phytopathology 52: 567–572.

Nelson P.E., Toussoun T.A. and Marasas W.F.O. 1983. *Fusarium* species: An Illustrated Guide for Identification. Penn. State University Press, University Park, PA, USA, 193pp.

Ocamb C.M. and Juzwik J. 1995. *Fusarium* species associated with rhizosphere soil and diseased roots of eastern white pine seedlings and associated nursery soil. Can. J. Plant Pathol. 17: 325–330.

Ocamb C.M., Juzwik J. and Stolhammer K.S. 1991. *Fusarium* species associated with root lesions on eastern white pine seedlings in a Wisconsin nursery. Phytopathology 81: 1241.

Prey A.J., Hall D.J. and Cummings J.E., Department of Natural Resources, Div. of Resource Management 1985. Forest Pest Conditions in Wisconsin, Annual Report. Bureau of Forestry, Madison, WI, 44 pp.

Renlund D.W., Department of Natural Resources, Div. of Resource Management 1980. Forest Pest Conditions in Wisconsin, Annual Report. Bureau of Forestry, Madison, WI, 37 pp.

Riffle J.W. 1959. Studies on White Pine Seedling Root Rot, M.S, University of Michigan, E. Lansing, MI 78 pp.

Riffle J.W. and Strong F.C. 1960. Studies in white pine seedling root rot. Quarterly Bull. MI Agric. Expt. Sta 42: 845–853.

SAS Institute 1990. SAS User's Guide: Statistics. SAS Institute, Inc., Cary, NC, 1686 pp.

Wenner N. and Merrill W. 1984. New conifer hosts for Fusarium root rot in Pennsylvania. Plant Disease 68: 536.

Windels C. and Burnes P. 1988. Five-year preservation of *Fusarium* species on silica gel and soil. Phytopathology 78: 107–109.