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Seedling mortality and development of root rot in white pine seedlings in two bare-root nurseries

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Seedling mortality and development of root rot in white pine (*Pinus strobus*) were followed across locations and over time within three operational nursery fields with loamy sand soils at a provincial nursery in southwestern Ontario, Canada, and a state nursery in southern Wisconsin, USA. One Ontario field was fumigated with dazomet; the other was not fumigated. The Wisconsin field was fumigated with methyl bromide-chloropicrin. Mortality and disease severity data were collected; the latter were based on visual assessment of seedling roots. White pine root rot (10 to > 30% incidence) was observed in the fields regardless of soil fumigation treatment. Systematically placed plots in the outer two seedling beds in the Wisconsin field had higher mortality levels than those in the other beds during the second growing season, and they had higher disease severity in July and October of the same year. Plot locations in the Ontario fields were stratified according to topographic features. Cumulative seedling mortality level during the second growing season in the Ontario fumigated field was highest in the low areas ($P < 0.001$) and equal in the mid-slope and high areas; cumulative mortality in the nonfumigated field did not differ among location types. Higher disease severity was observed in the low areas of the nonfumigated field in July and October of the same growing season ($P = 0.03$) compared to pooled mid-slope and high areas. Seedling mortality was higher than expected in mid-spring and less than expected in October of the second growing season in the outermost bed of the Wisconsin field and for all topographic areas in the Ontario fields.

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On a surveillé la mortalité des plants de semis et le développement de nécrose racinaire chez le pin blanc (*Pinus strobus*) à des emplacements divers et dans le temps dans trois champs en exploitation sur loam sableux d'une pépinière provinciale au sud-ouest de l'Ontario (Canada) et d'une pépinière d'état du sud du Wisconsin (USA). Un champ ontarien a été fumigé au dazomet et l'autre pas. Le champ du Wisconsin a été fumigé au bromure de méthyle-chloropicrine. On a recueilli les données de mortalité et d'intensité de maladie; celle-ci était basée sur un examen visuel des racines de jeunes plants. La nécrose racinaire du pin blanc s'est manifestée dans les champs (10 à > 30% de mortalité), qu'ils aient été fumigés ou non. Les parcelles ayant été localisées dans les deux planches de semis à l'extrémité du champ du Wisconsin ont subi des mortalités plus élevées que celles dans les autres planches pendant la deuxième saison de croissance, et avaient une intensité de maladie plus élevée en juillet et octobre de la même année. La position des parcelles dans les champs ontariens a été déterminée selon les caractéristiques topographiques. L'accroissement de la mortalité des jeunes plants durant la deuxième saison de croissance dans le champ fumigé de l'Ontario était plus élevée dans les terrains bas ($P < 0{,}001$) que dans les terrains intermédiaires et les terrains hauts; la mortalité cumulative dans le champ non-fumigé n'a pas varié selon l'emplacement des parcelles. Dans les terrains bas du champ non fumigé, on a observé une intensité de maladie plus élevée en juillet et octobre de la même saison de croissance ($P = 0{,}03$) que dans l'ensemble des terrains hauts et intermédiaires. La mortalité des jeunes plants a été plus élevée que prévue à la mi-printemps et plus basse que prévue en octobre de la deuxième saison de croissance dans la planche la plus extrême du champ du Wisconsin et dans toutes les zones topographiques des champs de l'Ontario.

Root rot has been observed in eastern white pine seedlings (*Pinus strobus* L.) of various ages and transplants in bare-root nurseries in the north central states of the USA and in Ontario, Canada, for many years. Consistent losses in white pine seedbeds in the USDA Forest Service Chittenden Nursery, Wellston, MI, occurred over a number of years in the 1940s and 1950s (Riffle & Strong 1960). Root rot of white pine has been occurring in Wisconsin state forest tree nurseries since 1976 (Prey et al. 1985). Extent and significance of losses have varied annually among the three Wisconsin state nurseries and have occurred in fields fumigated with either dazomet or methyl bromide - chloropicrin (MC-33) prior to sowing or transplanting. In 1986 64% (480 000) and in 1987 68% (571 000) of white pine seedlings were culled at Wilson State Forest Nursery due to root rot (Prey et al. 1986). White pine root rot was also observed in 1994 in a field that had been fumigated with metam sodium at General Andrews Nursery, Willow River, MN (Gust, pers. comm.); however, the disease has not historically been a problem at that nursery. White pine root rot was commonly observed by the first author in the Midhurst and St. Williams provincial nurseries in Ontario from 1984 through 1991.

Foliar symptoms of the problem are as Riffle and Strong (1960) described, " ... chlorosis and stunting of foliage, and a stunting of the tree itself." Chlorotic needles later dry and turn reddish brown. The shoot symptoms, however, are not highly correlated with root symptoms. Lesions may be found on primary roots of 2 and 3-year-old seedlings with either chlorotic or healthy-appearing foliage (Palmer & Nicholls 1979). Necrosis in the secondary roots is common and may allow for disease spread to the primary root at the point of lateral root attachment. Necrotic lesions may also be found at any point on primary roots without evidence of lateral root necrosis. The lower portion of the primary root may also be necrotic, with subsequent upward colonization by the pathogen.

At least three different genera of fungi have been consistently associated with white pine root rot in Michigan, Ontario, and Wisconsin nurseries. *Fusarium* species were the predominant potential pathogens recovered from diseased seedlings in a Michigan (Riffle & Strong 1960) and an Ontario and Wisconsin nursery study (Ocamb & Juzwik 1995). *Cylindrocladium* spp. were considered a major cause of root rot at Griffith Nursery, Wisconsin Rapids, WI, in 1980 (Palmer & Nicholls 1980) and at Wilson Nursery in 1981 (Palmer & Nicholls 1981). *Cylindrocarpon* sp. and *Fusarium* spp. were also commonly recovered by the first author from affected white pine seedlings in fields at Midhurst and St. Williams nurseries in Ontario between 1985 and 1991.

Information on the patterns or trends in root rot development by location and over time within a nursery field is limited. In both Ontario and Wisconsin nurseries, foliar symptoms first appear in the fall of the first growing season in small, localized centers in white pine fields. The disease intensifies and develops in larger areas during the second and any subsequent growing seasons in the nursery. A survey of a 0.2 ha field of 2-year-old white pine at Wilson Nursery in 1983 found overall mortality of 19%. However, levels differed by location within the field, with 33% mortality occurring in the two outer beds compared to 4% in the two inner ones (Cummings 1983). In a subsequent survey of 3-year-old white pine seedlings in 1985 at the same nursery, 100 trees from each of 34 plots in 15 bed-rows of two fields were examined. Disease incidence varied from 8 to 75%, but there was no observable pattern of disease occurrence in the fields (Prey et al. 1985).

The objectives of this research were to document how root disease develops and mortality occurs by location and over time within three operational nursery fields that had received different fumigation treatments prior to crop establishment. A report on *Fusarium* species associated with diseased seedlings in these same fields has recently been published (Ocamb & Juzwik 1995). Pathogenicity of these *Fusarium* species on white pine has also been determined (Ocamb, pers. comm.). For these reasons, the disease assessed in this study is considered to be *Fusarium*-associated root rot.

Materials and methods

Study sites. Operational fields in two nurseries were used for the investigation: C50 at Wilson State Forest Nursery, Boscobel, WI, USA, and two sections of A14 at St. Williams Provincial Nursery, St. Williams, Ontario, Canada. The nurseries are similar in latitude, species grown, and soil type. The soils in all fields are loamy sands with an organic content of 1–2% and average bulk densities of 1.2 g/cm^2. The maximum water holding capacity was 20% in the Wisconsin field and 18% in the Ontario field; average soil pH was 5.6 in the Wisconsin field and 5.4 in the Ontario field. Both sites have a history of root rot in white pine seedling crops.

The Wisconsin field (168 m × 8 m) was fumigated with methyl bromide - chloropicrin (MC-33 at 392 kg/ha) in August 1989. White pine seed was sown in four beds (168 m × 1.1 m) in early October 1989, and the field maintained through two growing seasons according to standard nursery practices. One section (130 m × 42 m) of the Ontario study area was fumigated with dazomet (400 kg prod/ha) in August 1989. The other section was not fumigated. These sections are hereafter referred to as the fumigated and nonfumigated Ontario fields. Ten beds (130 m × 1.1 m) in both sections were sown with stratified white pine seed of the same seedlot in April 1990 and the fields maintained through each growing season according to standard nursery practice.

Field sampling and seedling assessment. Plots were established in May 1990, the beginning of the first growing season in the Wisconsin field, where little topographic variance was found. Twenty-four 1.1 m^2 plots were systematically located down the length of the field. In each of the Ontario fields, twelve 1.1 m^2 plots were established in April 1991, the beginning of the second growing season. Plots were placed according to a stratified random sample design with an equal number of plots located in low, mid-slope, and high areas of each section.

Data and seedling samples were collected during the first and second growing season from the Wisconsin field, and during the second for the Ontario fields. Specific dates differed by nursery: 31 July 1990, 18 October 1990, 14 May, 21 July, and 8 October 1991 in the Wisconsin field; 30 April, 2 July, and 2 October 1991 in the Ontario fields. Number of living (healthy or symptomatic) and dead seedlings were recorded for each plot on each sampling date in each field. The shoots of all dead seedlings were cut at the ground line and removed on each assessment date. Ten randomly selected, living seedlings with intact root systems were then removed from each plot on each sampling date. The seedlings were stored in polyethylene bags at 5°C until processed.

In the laboratory, root systems were washed to remove soil and examined for location and extent of necrosis. Individual plants were rated for root rot severity on a 1–5 scale where 1 = no evidence of necrotic tissue; 2 = one lateral with 50% of the root necrotic; 3 = two or more laterals with 50% of the roots necrotic, or lower third of primary root necrotic; 4 = necrotic lesion or extensive necrosis in middle third of primary root; and 5 = necrotic lesion or extensive necrosis in upper third of primary root, or entire root system necrotic.

Data analyses. To investigate how root disease and mortality developed by location within a field, data from each sampling date were analyzed separately with standard loglinear models (Agresti 1990), using the likelihood ratio test statistic (G^2). The analyses were performed using StatXact Turbo version 2.11 (Mehta & Patel 1992) and Systat for Windows version 5.01 (Wilkinson 1992). StatXact computes exact, small-sample P-values, rather than relying on the asymptotic χ^2 distribution. When significant effects were found in the tables, subtables were created to determine which beds (Wisconsin field) or topographic areas (Ontario fields) were causing the differences.

Possible location differences across fields reflecting the effect of fumigation were also considered for the two Ontario fields. The mortality data were analyzed as above using loglinear models. Disease severity was analyzed through testing for homogeneity of odds ratios and whether the common odds ratios were equal to 1 (Agresti 1990). The odds ratio testing was done using StatXact Turbo because of the low cell counts involved at higher levels of disease severity.

After location trends were determined, data were analyzed for trends over time. The G^2 goodness-of-fit statistic was used to assess a null hypothesis of a constant underlying rate for the development of each variable, e.g. rate of change in seedling mortality occurrence, rate of change in disease severity rating. Because time endpoints were necessary for this analysis, we arbitrarily set the beginning of the growing season as April 15, and the end of the growing season as September 15 (other endpoints were also tested to determine the sensitivity of results to the dates chosen).

Results

Wisconsin nursery. The average densities (no. per m^2) of seedlings in the six plots of each bed on the first observation date were: 358, bed 1; 416, bed 2; 349, bed 3; and 279, bed 4. Average densities (per m^2) in the same plots of each bed on the last observation date were: 287, bed 1; 349, bed 2; 305, bed 3; and 204, bed 4. The lower number of seedlings in plots of bed 4 is largely attributed to washout of seeds and soil between sowing and end of the germination period.

Seedling mortality expressed as percentage of dead seedlings per total seedlings (living + dead) was < 6% for the plots within the four beds on all dates in

Table 1. Results of likelihood ratio test of goodness-of-fit analyses for variability of mortality during the second growing season in three white pine nursery fields

Field	Days to observation date* 1	2	3	Bed or area†	Seedling status	Date 1 counted	Date 1 expected	Date 2 counted	Date 2 expected	Date 3 counted	Date 3 expected	df	G^2	P value
Wisconsin fumigated	29	68	56	1	dead	70	48.8	101	105.2	63	79.7	2	27.11	0.0001
					live	1943	1964.2	1782	1777.8	1659	1642.3			
				2	dead	22	43.7	97	97.7	97	75.1	2	12.47	0.002
					live	2309	2287.3	2152	2151.3	1995	2016.9			
				3	dead	2	7.3	8	16.6	27	13.2	2	22.21	0.0001
					live	1958	1952.7	1890	1881.4	1803	1816.8			
				4	dead	15	16.8	36	36.9	31	28.3	2	0.48	0.79
					live	1379	1377.2	1283	1282.1	1192	1194.7			
Ontario fumigated	15	63	75	low	dead	43	8.5	32	33.6	5	37.9	2	117.4	0.0001
					live	1490	1524.5	1418	1416.4	1373	1340.1			
				ms+high	dead	39	6.9	17	27.4	10	31.7	2	96.83	0.0001
					live	3073	3105.2	2956	2945.5	2886	2864.2			
Ontario non-fumigated	15	63	75	low	dead	17	7.7	35	29.5	17	31.7	2	18.02	0.0002
					live	819	828.3	744	749.5	687	672.3			
				ms+high	dead	32	11	55	42.7	13	46.2	2	64.06	0.0001
					live	1598	1619	1463	1475.3	1370	1336.8			

(Number of seedlings on observation date: 1, 2, 3 — counted and expected.)

*Based on arbitrary April 15–Sept. 15 growing season endpoints; days to observation date 1 is number from April 15 to the first observation date; days to second observation date is number from first observation date to second one, etc. Observation dates for Wisconsin field: 1 = 14 May; 2 = 21 July; and 3 = 8 October. Observation dates for Ontario fields: 1 = 30 April; 2 = 2 July; and 3 = 15 October.

†Topographic areas in Ontario fields were: low, ms = mid-slope, and high.

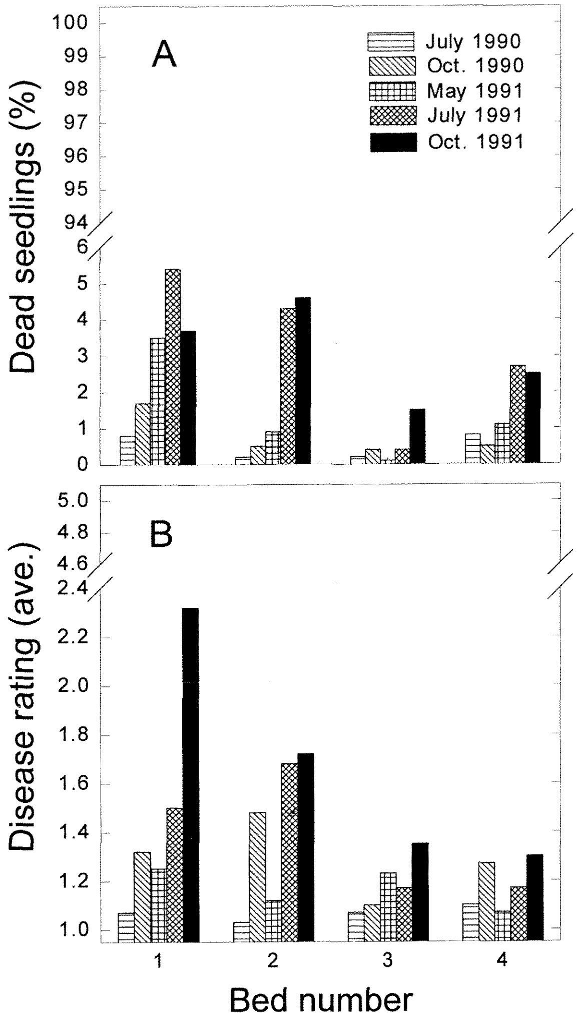

Figure 1. **A**) Mortality, and **B**) root disease severity in eastern white pine seedlings growing in a methyl bromide - chloropicrin-fumigated field at Wilson State Forest Nursery, Boscobel, WI, 1990–91.

the Wisconsin field (Fig. 1A). However, significant differences were found in mortality among the beds on the five different observation dates. Mortality was highest in October 1990 and May 1991 in bed 1 ($P = 0.0001$); thereafter, mortality was highest in and did not differ between beds 1 and 2. Mortality was lowest in bed 3 on all observation dates. However, cumulative mortality over the second growing season was different for each bed ($P \leq 0.01$), showing a rank order (highest to lowest) of bed 1 (12.4%), bed 2 (9.8%), bed 4 (6.4%), and bed 3 (2.0%).

The rate of seedling mortality occurrence during the second growing season was not constant for plots in beds 1, 2, and 3; however, a constant rate was found for plots in bed 4 (Table 1). These results were not sensitive to different growing season endpoints tested.

No differences in disease severity ratings were observed among rows for the first three observation dates (Fig. 1B). Bed differences for this variable occurred in July and October 1991 ($P \leq 0.003$). July severity ratings were similar for plots in beds 1 and 2, and plots in beds 3 and 4; plots in beds 1 and 2 had greater severity than plots in beds 3 and 4 ($P < 0.045$). A gradient in disease severity across the field was observed in October 1991, when similar severity was found for plots in beds 1 and 2 and beds 2, 3, 4. Ratings for plots in bed 1 were quite different from those in beds 3 and 4 ($P \leq 0.002$).

Highest average rating for all rows occurred at the end of the second growing season (Fig. 1B). The rate of change in disease severity over the second growing season was analyzed using combined data for beds 1 and 2, and for beds 3 and 4. Non-constant rates were apparent only for beds 3 and 4 ($P = 0.011$). These results were not sensitive to different growing season endpoints tested.

Ontario nursery, fumigated field. Seedling densities in the fumigated field were approximately twice those found in the Ontario nonfumigated field. Field preparation prior to sowing (with the exception of fumigation), seeding rate, seedlot, and seedling maintenance practices were the same for these adjacent fields; therefore, the difference in density was attributed to fumigant use. The average densities (per m^2) of seedlings in the four plots of each topographic area on the first observation date were: 383, low; 434, mid-slope; and 344, high. Average densities (per m^2) in the same plots of each area on the October 1991 sampling date were: 344, low; 406, mid-slope; and 318, high.

Seedling mortality, expressed as percentage of dead seedlings per total seedlings (living + dead), was ≤ 2.8% for all plots within the three areas on all dates (Fig. 2A). On the first two observation dates, levels were highest in the low areas ($P \leq 0.002$), while levels in mid-slope and high areas did not differ. No differences in mortality were found among areas in October 1991. Cumulative mortality for 1991 was highest ($P < 0.001$) in the low areas (5.5%), and equal in the mid-slope and high areas (2.2%). Mortality rates were not constant over the second growing season for plots in the low or combined mid-slope/high areas (Table 1). For all areas, the underlying mortality rate was higher than expected in April, and lower than expected in October. Results were not sensitive to different growing season endpoints tested.

Disease ratings (Fig. 2B) across areas showed no differences in April or October 1991. Interestingly, although the mid-slope and high areas did not differ in July, they did have higher severity than the low areas ($P = 0.02$). Changes in the rate of development

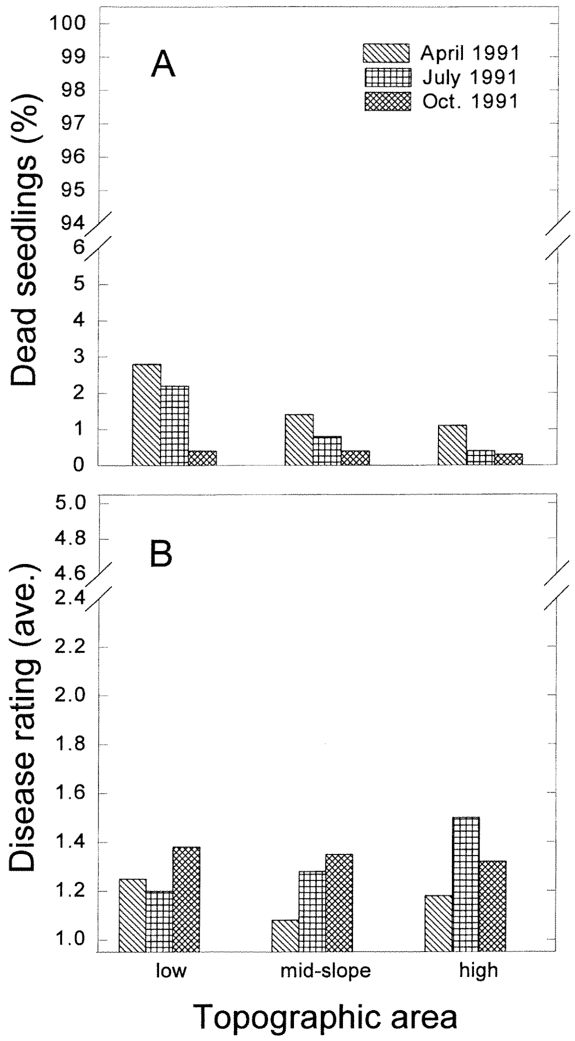

Figure 2. A) Mortality, and **B**) root disease severity in eastern white pine seedlings growing in a dazomet-fumigated field at St. Williams Nursery, St. Williams, Ontario, 1991.

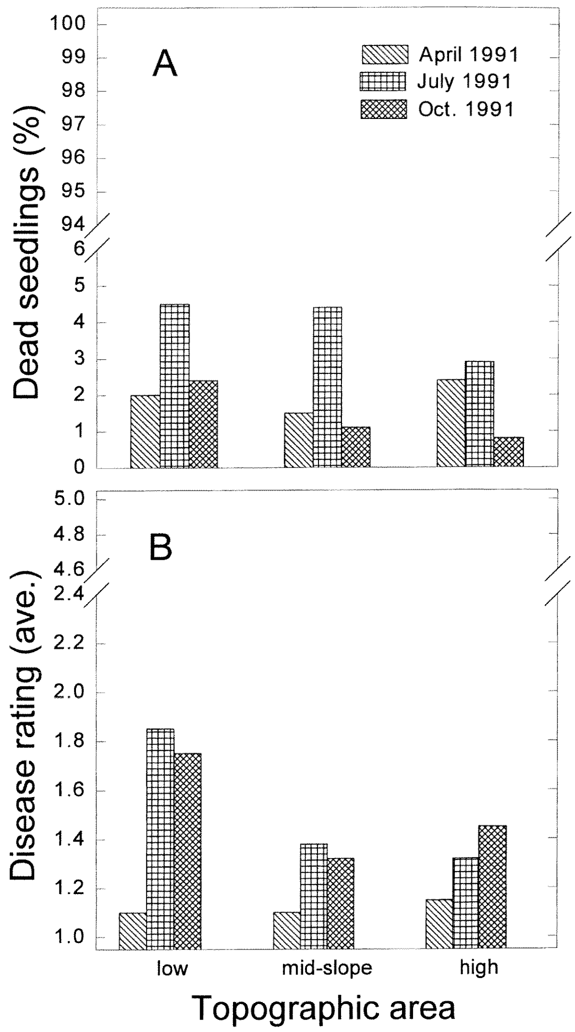

Figure 3. A) Mortality, **B**) and root disease severity in eastern white pine seedlings growing in a nonfumigated field at St. Williams Nursery, St. Williams, Ontario, 1991.

of disease severity over the season were assessed for the low areas, and the combined mid-slope and high areas. No changes were found in the low areas. However, there were seasonal changes in the rates for plots in mid-slope/high areas ($P = 0.0002$) due to higher than expected severity in July and lower than expected severity in October. Results for the low areas were sensitive to choice of arbitrary season start date; results for mid-slope and high areas were not sensitive to different growing season endpoints tested.

Ontario nursery, nonfumigated field. The average densities (per m^2) of seedlings in the four plots of each topographic area on the first observation date were: 209, low; 196, mid-slope; and 211, high. Average densities (per m^2) in the same plots of each area on the last observation date were: 176, low; 165, mid-slope; and 180, high.

Seedling mortality expressed as percentage of dead seedlings per total seedlings (living + dead) was < 5% for all plots within the three areas on all dates (Fig. 3A). In April 1991 mortality ranged from 1.5 to 2.4% and no differences were found among locations. Levels were higher in July (2.9 to 4.8%), but again no differences were observed. Seedling mortality in October was higher in the low areas than the high areas ($P = 0.021$), but not different from the mid-slope areas ($P = 0.065$); no difference was found between mortality in mid-slope and high areas ($P = 0.79$). Cumulative mortality over the 1991 season (7.6 %) did not differ among location types. In general, the highest mortality levels in the field were observed in July. Mortality rate was not constant for plots in the low areas over the second growing season, nor for rates determined for combined mid-slope and high areas (Table 1). Specifically, mortality was higher than expected in April and lower than expected in October.

Disease ratings were highest for the low area in July and October 1991, and different from mid-slope and high areas ($P = 0.03$) when data for the two dates were pooled (Fig. 3B). Rating data were similar between mid-slope and high areas on the three sampling dates during 1991. The largest change in disease severity in each area occurred between April and July 1991. Overall, there did not appear to be changes in severity over time, but for the mid-slope/high area this conclusion is dependent on selection of the beginning date for the arbitrary growing season endpoints.

Ontario nursery, field comparisons. Mortality levels in the dazomet fumigated and the nonfumigated field were not different in April 1991 in the low areas, but there was some evidence that the fumigated field had less mortality in the mid-slope/high areas ($P = 0.061$). In July 1991, the fumigated field had less mortality in the low area ($P = 0.003$), and much less mortality in the mid-slope/high areas ($P < 0.0001$) than the nonfumigated field. In October 1991, the fumigated field again had less mortality than the nonfumigated field ($P < 0.001$). However, this difference in mortality did not differ by topographic area.

Disease severity ratings in 1991 were not different between fields for the mid-slope/high areas in April, July, or October ($P > 0.27$). For the low areas, there was no difference in April. In July and October, however, there was lower severity in the fumigated field ($P < 0.035$).

Discussion

White pine root rot was observed in three bareroot nursery fields regardless of whether field soil had been fumigated prior to sowing of the conifer crop. Based on percentage of number of seedlings with root rot ratings ≥ 2 per total number of seedlings, high disease incidence levels (> 30% ave.) occurred in both methyl bromide-chloropicrin and nonfumigated fields during the second growing season; moderate levels (10–20% ave.) were found in a dazomet treated field. Soil fumigation with dazomet had a significant, positive effect based on comparison of the two Ontario nursery fields. In general, higher mortality was found in the nonfumigated field compared to the fumigated one. Also, higher root disease severity generally occurred in low areas of the nonfumigated field compared to the dazomet treated one. Comparisons between the Ontario and Wisconsin fumigated fields were not made due to differences in cultural practices and site.

The fact that white pine root rot occurred at significant levels in operationally fumigated fields may be attributed to recolonization of treated fields by the pathogen(s), insufficient fumigation of soil, and/or elimination of beneficial and antagonistic microorganisms by the fumigants. Recolonization by *Fusarium* spp. may occur via infested wind-blown soil from headlands or adjacent fields (Vaartaja 1964), via white pine seed infested with *Fusarium* spp. (Ocamb & Juzwik 1993), or other factors (Bloomberg 1985). Insufficient injection depth during methyl bromide fumigation may partially account for the periodic high levels of white pine root rot that have been observed in fumigated fields in two Wisconsin nurseries (Juzwik et al. 1995). Incorporation of dazomet granules by rotary tillers in forest nurseries may also result in insufficient fumigation depth for root disease control (Kelpas & Campbell 1994, Juzwik et al. 1994).

Trends in occurrence of mortality and disease severity were observed by location within each nursery field. Seedling mortality was generally highest in the outer beds in the Wisconsin field; highest disease severity also occurred in these same beds. These results agree with those of Cummings (1983) and with observations of the first author. Low areas of beds in the nonfumigated Ontario fields were generally associated with higher disease severity. In both fields we suspect that the longer length of time that seedlings are exposed to high soil moisture levels in the identified areas is contributing to disease development and seedling death. Preliminary analysis of data on soil water content and soil matric potential indices in these two fields indicates a correlation with higher disease levels (Juzwik 1993, Juzwik et al. 1994).

Finally, root disease that occurs during the first growing season does not become visually evident (brown or dry, dead shoot) until the spring of the second season. This conclusion is based on observations of higher than expected mortality in mid-spring and lower than expected mortality in October in the outermost bed of the Wisconsin field and for all topographic areas in the Ontario fields. Based on visual evaluation of seedling roots, no consistent trends were observed in disease severity over time during the second growing season for the three fields.

In summary, white pine root rot was observed at moderate to high levels in three bareroot nursery fields regardless of fumigation treatment, and within the fields trends were found in mortality and root disease development across locations.

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