Effect of repeated cultivation during summer fallow on *Cylindrocladium floridanurn* in two Ontario forest nurseries

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The effect of repeated cultivation on microsclerotial densities of Cylindrocladiurn floridanurn Sob. & C.P. Seym. in field soil was determined during three trials at two Ontario provincial nurseries. Repeated treatment during summer months of 1986 and 1987 did not significantly alter the germinable propagule levels in the field soil at Midhurst Nursery and Kemptville (= Howard J. Ferguson) Nursery. Although cylindrocladium root rot incidence and mortality of black spruce transplanted into one trial area were higher and the root collar diameters and seedling heights were lower in cultivated plots than untreated plots, the differences were not significant. Disease incidence and mortality of transplants in cultivated plots were, however, significantly higher than in spruce in fumigated plots ($p > 0.05$), while root collar diameters were significantly lower ($p > 0.05$). Repeated cultivation is not recommended as a means of reducing inoculum levels of *C.* floridanurn in Ontario nursery soils. Its use may also negatively affect survival and growth of spruce transplants.

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On a determine l'effet du travail répété du sol sur la densité de microsclerotes de Cylindrocladiurn floridanurn Sob. et C.P. Seym. dans le sol, au cours de trois essais realises a deux pepinieres provinciales de l'Ontario. L'application repetee de traitements au cours des mois d'été de 1986 et 1987 n'a pas permis de modifier significativement le taux de germination des propagules presents dans le sol aux pepinieres de Midhurst et de Kenptville (= Howard J. Ferguson). L'incidence du pourridie cause par Cylindrocladiurn et le taux de mortalite des epinettes noires repiquees dans une parcelle d'essai etaient plus eleves dans les parcelles où l'on avait travaille le sol que dans les parcelles non traitees. En revanche, le diamètre des collets et la hauteur des plantures y étaient inférieures. Ces differences n'etaient toutefois pas significatives. Par contre, l'incidence de la maladie et le taux de mortalite des epinettes repiquees etaient significativement plus eleves dans les parcelles où l'on avait travaille le sol que dans les parcelles fumigees ($P > 0,05$), tandis que le diametre des collets y était significativement plus faible ($P > 0,05$). On ne recommande pas de répéter le travail du sol en vue de reduire la quantite d'inoculum de C. floridanum present dans les sols des pepinieres de l'Ontario. Cet usage peut aussi nuire a la survie et a la coissance des epinettes repiquees.

Introduction

Cylindrocladiurn floridanurn Sob. & C.P. Seym. causes an important root rot of conifers in forest nurseries (Bugbee and Anderson, 1963; Cox, 1954; Thies and Patton, 1970). All conifer species grown in Ontario provincial forest nurseries are susceptible with the possible exception of eastern white cedar (*Thuja occidentalis* L.).

The distribution of and losses due to the disease appear to have increased since it was first detected in a provincial nursery in 1974 (Juzwik *et al.*, 1987). Approximately 430,000 spruce seedlings were identified as *culls* due to the root rot in an assessment of six compartments in five nurseries (Juzwik *et al.*, 1988).

Chemical fumigation has been the main means of controlling cylindrocladium root rot in conifer nurseries (Berbee, 1973). The use of flax, corn, and sorghum-sudangrass as organic amendments has also been found to reduce populations of C. *floridanurn* in soil in Minnesota and Wisconsin studies (Thies, 1969; Berbee, 1973; Hadi, 1974; Menge and French, 1976). The effect of other cultural practices on fungus populations in the soil and on disease incidence in seedlings is not clear.

Tilling of fallow fields is practiced in nurseries for physical weed control (Owston and Abrahamson, 1984). Fallowing and cultivation have been used to reduce soil populations of fungi such as *Fusarium*, and *Phytophthora* in British Columbia bareroot nurseries (J. Sutherland, pers. comm.). In a preliminary trial conducted in 1982 at Kemptville Nursery in Ontario, a reduction in recovery of *C. floridanum* from soil was associated with repeated cultivation of a fallow field during July and August (OMNR, unpub. file report). An alfalfa bioassay was used for assessing fungus presence.

Repeated plowing and discing of nursery soils during summer months would expose propagules of the fungus to higher temperatures at the soil surface and to drying. These environmental factors may result in a reduction in infective propagule levels of C. *floridanurn* in the soil (Thies and Patton, 1970; Zarnstorff, 1983). Thies and Patton (1970) reported a decreased recovery of C. *scoparium* microsclerotia when soil samples were subjected to air-drying. Similar treatment of field soil resulted in no recovery of *C. crotalariae* microsclerotia, but after re-wetting of the soil to near field capacity for 1-4 weeks, partial recovery of microsclerotia occurred (Griffin, *et al.*, 1978). The reduced recovery of microsclerotia may have been caused by a temporary decrease in propagule germinability, not a true loss in viability.

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Two bareroot nurseries began using repeated cultivation during summer fallow on a limited basis in an attempt to reduce the level of *C. floridanurn* in field soil. Although published reports and results of the 1982 preliminary trial suggested a value for the practice, further investigation was warranted because detrimental effects of the treatment (*e.g.* dispersal of inoculum, breakdown of soil structure) could outweigh any benefits. The effect of repeated cultivation on levels of *C. floridanurn* in field soil in three trials at two Ontario nurseries are reported here. Results on treatment effect on subsequent root rot incidence, mortality, and growth of spruce transplants in one trial area are also included.

Materials and methods

Nursery compartments

Field trials were conducted in compartment A33 (CA33) at Midhurst Nursery, near Barrie, in 1986 and 1987, and in compartment 30 (C30) at Kemptville (= Howard J. Ferguson) Nursery, near Ottawa, in 1987. The previous crop in CA33 was 1.5 + 1.5 black spruce (*Picea mariana* [Mill.] B.S.P.) which was lifted in April 1986. Approximately 55% of the stock had been culled due to cylindrocladium root rot (Juzwik *et al.*, 1987). Little was known about the distribution of the fungus in the compartment soil so the compartment was graded in May 1986 in an attempt to minimize the clustering of *C. floridanurn* propagules prior to trial establishment. The grading of the field involved wind-rowing the top 12-14 cm of soil and re-distributing the wind-rows until the field was level again. This was completed in both a N-S direction and an E-W direction. The soil in CA33 is a sandy loam with a pH of 6.2, maximum water holding capacity of 23%, and an average bulk density of 1.3 g/cm^3.

Previous soil sampling work had revealed a wide and fairly even distribution of *C. floridanurn* in C30 (Juzwik, unpub. data) and grading was not considered necessary. The previous crop, 3 + 0 white spruce (*Picea glauca* (Moench) Voss), had been lifted in April 1987. The estimated cull in the spruce due to *C. floridanurn* at time of lifting was 13.2% (Juzwik *et al.*, 1988). The soil in C30 is a sandy loam with a pH of 5.5, maximum water capacity of 20%, and an average bulk density of 1.00 g/cm^3.

Experimental design

The 1986 cultivation trial was conducted in the north end of A33. A randomized complete block (RCB) design with nine replications of each treatment was used. The trial was repeated in the south end of CA33 in 1987 using the same design. All treatment plots were 12 m x 1 m with a 1 m wide buffer surrounding each plot which also allowed for access to the plots. The RCB trial at Kemptville Nursery was established in the middle of the compartment where data on the fungus distribution had previously been obtained. Ten replications of the cultivation and the control treatments were included in one block. The plots were 10 m x 4.5 m and oriented in a N-S manner. Buffer areas at the end of each plot allowed for turning of equipment during treatment.

Treatment

The treatments at Midhurst Nursery involved tilling the plots with a rotary cultivator equipped with L-shaped tines. The cultivator has a swath width of **40** cm, therefore, three passes were required to treat the 1 m wide plots. The soil in each plot was cultivated to a 20 cm tine depth once per week for 8 consecutive weeks. Treatment periods were July 8 - September 1 in 1986 and July 10 - August 28 in 1987. Control plots were left undisturbed. Weed control in the trial area in 1986 was achieved through early August by one application of a tank-mix of chlorthal-dimethal (5 kg a.i./ha) and prometryne (0.5 kg a.i./ha). Plots were hand-weeded as required in the latter part of the season. In 1987, both trial areas were treated with glyphosate (5.0 - 5.6 L prod./ha) on April 24 and June 23. Additional glyphosate was applied July 22 in the south area and September 15 in the north.

At Kemptville Nursery, a tractor-mounted cultivator ('Do-It-All', Laning Farm Equipment Distrib.) equipped with harrows, rotating blades and plow teeth was used to turn the soil to a 20 cm depth. The cultivator width (4.5 m) required only one pass per treatment plot. The soil in the plots was tilled once per week between June 9 and July 21, and on August 21. Effort was made to schedule the treatment during the hottest, driest period of each week. The control plots were left undisturbed. Because weed control was required for the control plots and buffer areas, the entire trial area received an application of glyphosate (1.24 kg a.i./ha) plus 2,4-D (0.8 kg a.i./ha) in late June 1987.

Seedling transplanting

In a preliminary effort to evaluate effect of repeated cultivation on infection, disease development, and plant growth and survival, black spruce seedlings were transplanted in the 1986 treatment plots in CA33. Seedlings were also transplanted into two other sets of nine replicated plots for comparison. The soil in one set had been undisturbed except for rototilling on June 17, 1986, to incorporate 0.25 m^3 of sphagnum peat per plot. The remaining plots had been fumigated with dazomet 98% (Basamid, P.C.P. no. 15032) at a rate of 400 kg prod./ha in July 1986. A polyethylene tarp was used as a seal during fumigation. The mean density of *C. floridanurn* in the fumigated plots prior to transplanting was 0.08 prop./g dry soil.

The 1.5 + 0 spruce used for transplanting were from a small portion of one row in compartment DE7 at Swastika Nursery. The trees were obtained during an operational lifting of stock from the compartment on July 6, 1987. The majority of the seedlings lifted were destined for transplant fields at the nursery. The seedlings were considered to have no or a very low level of cylindrocladium root rot because the disease had not been detected during 1986 and 1987 seedling surveys and the fungus was not recovered from soil during a fall 1986 soil survey.

The seedlings were immediately transported to Midhurst Nursery after lifting and were transplanted in the three sets of plots on July 7. Four 12 m long drill lines (16 seedlings/m) were established in each plot. The seedlings were considered to be in fair condition based on usual nursery parameters of shoot height and root development. Although isolations were not conducted, no evidence of *C. floridanurn* infection (*i.e.* root lesions) was observed. Normal nursery practices, such as fertilization and irrigation, were conducted in the plots with the transplants.

Sample collection

Pre-, mid-, and post-treatment soil samples were collected from the cultivated and the control plots in both areas in CA33. Samples were taken with a soil sampling tube (2.0 cm dia) to a vertical depth of 20 cm, yielding about 50 cm^3 of soil per core. Five soil cores were collected at 2 m intervals along the longitudinal centreline of each plot. The five cores for each plot were then bulked in a polyethylene bag, sealed, labelled, and stored at room temperature until further processed. Pre-, mid-, and post-treatment samples were collected on June 9, July 21, and August 21 in C30. Four soil cores were systematically taken from each plot in a manner similar to that described previously. Storage was the same as for samples from CA33.

Transplant seedlings were observed on August 18,1987, and sampled on July 19, 1988, for root rot caused by *C. floridanurn.* Disease incidence was determined by randomly locating three 0.25 m^2 subplots in each plot, collecting all living seedlings in the subplots, and isolating for the fungus from the trees. Morphological characteristics and mortality were also recorded for the seedlings on June 9 and July 18, 1988, respectively. Height and root collar diameter were measured on 10 randomly selected seedlings in each plot. Seedling mortality was determined for a 33% area sample of the 12 m^2 plots.

Sample processing

A modified wet-sieving technique was used to determine the number of *C. floridanurn* propagules in the soil (Juzwik *et al.*, 1988). The propagules (primarily microsclerotia) were first separated on the basis of size and density, and then allowed to germinate on a selective medium. Following 10 days of incubation at 20°C, the *C. floridanurn* colonies were counted. The number of fungus propagules per gram of dry soil (prop./g) were then determined.

Fungus isolations were made from the roots and lower stems of each seedling with lesions. Plant tissue segments (approx. 1 cm long) were immersed in 10% NaOCl for 3 min, rinsed in sterile distilled water, and small pieces of tissue excised and placed on a *Cylindrocladiurn* selective medium in petri dishes (Phipps *et al.*, 1976). The dishes were examined for *C. floridanurn* after 10 days of incubation at room temperature.

Data analyses

The fungus population values were individually transformed using the sum of the square root of each subsample value plus 1.0 x 10^{-8}. Analyses of covariance (ANCOVA) were performed and treatment comparisons were made using standard t-tests (Steel and Torrie, 1980). If adjustment of variate values was not warranted, analysis of variance (ANOVA) was performed. ANOVA and ranking of means by Least Significant Difference method (Ray, 1982) were used to analyze the seedling transplant data. The disease incidence data were first transformed by an arcsine square root method.

Results

Analysis of population values from the Midhurst trials did not reveal significant differences between the mid- and post-treatment populations in the 1986 cultivated plots compared to the untreated one (Table 1). Less variability in populations was observed with the 1987 trial data, but no significant differences were found in *C. floridanurn* levels in the mid- and post-treatment samples between the cultivation and untreated plots. No significant differences in soil moisture content existed between cultivation and untreated plots for the pre-, mid-, and post-treatment soil samples for the 1986 and 1987 trials at Midhurst. In the Kemptville trial, there were no significant differences in the transformed numbers of propagules recovered from cultivated plots versus untreated plots at the mid- and post-treatment sampling dates. The actual mean number of recovered propagules in the cultivation and untreated plots increased from 3.97 to 5.08 prop./g soil and 3.21 to 4.21 prop./g soil, respectively, during the time of the treatment period. The cultivation treatments significantly reduced soil moisture content in comparison to that of the untreated plots at both the mid- (13.8 vs. 15.2%) and post-treatment (11.5 vs. 13.0%) sample dates ($p = 0.001$).

Table 1. Number of *Cylindrocladium floridanurn* propagules recovered from plots before, during and after cultivation treatment in compartment A33, Midhurst Nursery.

Trial area	Sampling time[a]	Treatment	
		Cultivation	Control
North	Pre-treatment	5.69	3.85
	Mid-treatment	6.40	3.79
	Post-treatment	4.85	3.65
	April 1987	6.51	4.62
	April 1988	5.63	4.85
South	Pre-treatment	4.98	4.19
	Mid-treatment	4.30	2.79
	Post-treatment	4.10	4.07
	April 1988	4.00	4.20

NOTE: Values expressed as mean numbers of propagules per gram of dry soil. Each value is the mean number of nine replications.

[a]Cultivation treatment conducted between July 8 and September 1, 1986, in north area and July 10 and August 28, 1987, in south area.

Weather data recorded during the cultivation periods were obtained from the two nurseries. Maximum daily ambient air temperature ranged from 9.8 to 26.0°C in 1986 and 11.6 to 27.0 in 1987 at Midhurst and from 12.4 to 26.7 at Kemptville Nursery in 1987. Rainfall events were more frequent during the trial periods at Midhurst (23 in 1986 and 16 in 1987) than at Kemptville (9 events). Similarly, cumulative rainfall over the trial period was higher for the Midhurst trials (194 mm in 1986 and 220 mm in 1987) than the Kemptville one (134 mm).

Table 2. Disease incidence (DI) and mortality of black spruce transplants in repeated cultivation, control, and fumigation plots in north end of compartment A33, Midhurst Nursery.

Treatment	DI (%)	Mortality (%)
Cultivation	11.9 y[a]	51.3 y
Control	6.7 y	40.1 yz
Fumigation	1.4 z	29.5 z

NOTE: Values expressed as mean of nine replications,

[a]Means with the same letter within a column are not significantly different (LSD, alpha= 0.05).

Table 3. Growth parameters of transplants in cultivation, control and fumigation plots in north end of compartment A33, Midhurst Nursery.

Treatment	Growth parameter	
	RCD[a] (mm)	Height (cm)
Cultivation	2.25 y[b]	14.62 y
Control	2.36 y	14.90 y
Fumigation	2.59 z	16.19 y

NOTE: Values are means of nine replications.

[a]RCD = root collar diameter measured with stem caliper.

[b]Means with the same letter in a column are not significantly different (LSD, alpha = 0.05).

Root rot incidence due to *C. floridanurn* and transplant seedling mortality in the north CA33 plots were highest in the tilled plots (Table 2). The differences in disease incidence and mortality were not significant between the tilled and untreated plots, but were between the tilled and fumigated ones ($p < 0.05$). The smallest root collar diameters and seedlings heights were found in the transplants in the tilled plots (Table 3). The root collar diameters of the trees in the tilled and non-treated plots were significantly less compared to trees in the fumigated plots ($p < 0.05$). Seedling heights of transplants in tilled plots were also less than in the fumigated ones, but differences were not significant ($p = 0.06$).

Discussion

Repeated tilling of fallow nursery soil during summer did not significantly alter *C. floridanurn* populations in three trials conducted at two Ontario nurseries. The effect of fallowing on the fungus in the soil has been previously reported. Although some reduction in propagules does occur, *C. floridanurn* can survive for 9 years in the field in fallow nursery soil and may be capable of surviving as microsclerotia for 15 years or more (Thies and Patton, 1970; French and Menge, 1978).

Maximum ambient air temperature recorded by the nurseries during the trial periods was 28°C. The optimum range for microsclerotial production by five *Cylindrocladiurn* spp., including *C. floridanurn,* is 24-28°C (Hunter and Barnett, 1976). Stevens *et al.* (1986) reported 27°C as the optimum temperature for *Cylindrocladium scoparium*. Therefore, the maximum daily temperatures during the three trials would not have been deleterious to microsclerotia survival, and in fact, were probably optimal for their production.

Cumulative rainfall was significantly less during the Kemptville trial than during the Midhurst ones. This may have contributed to the significant reduction in soil moisture content found in the cultivated plots compared to the controls. However, lower microsclerotial densities were not associated with this reduction. Drought during June 1975 was associated with a reduction in microsclerotial populations of *C. crotalariae* in the upper 13 cm of field soil (Taylor *et al.*, 1981).

Although differences were not significant, disease incidence and mortality were higher while root collar diameters and seedling heights were smaller for spruce transplants in cultivated plots than those in untreated plots. However, disease incidence and mortality of spruce in cultivated plots were significantly higher than that observed in fumigated plots, while root collar diameters were significantly smaller. Based on these results, it appears that repeated cultivation may be detrimental to subsequent seedling survival and growth. Excessive tillage in nursery soils generally results in detrimental changes, such as soil compaction (Warkentin, 1984). The aggregates created by rototilling are too small for optimum seedling growth to be achieved.

A significant part of the spruce mortality observed in all treatment plots may have been due to the condition of the seedlings at time of lifting or related to the lapse of time between lifting and transplanting (24 to 30 hr). The level of tree mortality observed in fumigated plots supports either hypothesis. The average survival rate of black spruce transplants following operational transplanting at Swastika Nursery is 80 to 85% (E. Reitenan, pers. comm.).

In summary, repeated cultivation of nursery soil during summer fallow did not significantly alter populations of *C. floridanurn* and was correlated with poorer seedling survival and growth. The practice is not recommended as a means of reducing inoculum levels in Ontario nursery soils.

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