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# Effect of seedbed steaming on *Cylindrocladium floridanum*, soil microbes and the development of white pine seedlings

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**PHYTOPROTECTION 79: 35-43**

The Egedal® bed steamer produced sufficient heat to kill microsclerotia of *Cylindrocladium floridanum* at 5 and 10 cm soil depths in one bareroot forest seedling nursery. At a second nursery the buried inoculum was killed only to a depth of 5 cm. Soil steaming did not affect the microsclerotia at 15 cm. The steaming reduced populations of fluorescent pseudomonads to undetectable levels to a depth of 20 cm and populations of *Trichoderma* species were significantly reduced in the upper 10 cm of the seedbed. Density of white pine seedlings sown in the steamed beds was significantly higher ($P = 0.05$), and height, root collar diameter, shoot weight and root weight were significantly greater ($P = 0.05$) 4 months after steaming than that of control seedlings sown in unsteamed beds.

**[Effet d'un traitement à la vapeur des plates-bandes sur le *Cylindrocladium floridanum*, les microbes du sol et le développement de semis de pin blanc]**

Dans une pépinière forestière à racines nues, l'appareil Egedal® de traitement à la valeur des plates-bandes, a produit suffisamment de chaleur pour détruire les microsclérotes du *Cylindrocladium floridanum* à 5 et 10 cm de profondeur. À une autre pépinière, l'inoculum dans le sol ne fut détruit qu'à une profondeur de 5 cm. Le traitement à la vapeur du sol n'a pas affecté les microsclérotes situés à 15 cm. Le traitement a réduit les populations des pseudomonas fluorescents à des niveaux non détectables jusqu'à une profondeur de 20 cm et les populations des espèces de *Trichoderma* furent réduites significativement dans les 10 cm supérieurs de la plate-bande. Quatre mois après le traitement, la densité des semis de pin blanc semés dans les plates-bandes traitées était significativement plus élevée ($P = 0,05$), et leur hauteur, leur diamètre au collet, le poids de leurs tiges et celui de leurs racines étaient significativement plus grands ($P = 0,05$) que ceux des semis témoins provenant des plates-bandes non traitées.

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## INTRODUCTION

*Cylindrocladium floridanum* Sob. & C.P. Seym. causes root disease in conifer and hardwood seedlings, and has resulted in substantial mortality in the bareroot nurseries of Ontario (Greifenhagen *et al.* 1991; Juzwik *et al.* 1988). The pathogen has also been isolated from asymptomatic seedlings (Juzwik *et al.* 1988). Sanitation practices and fumigation with dazomet (2H-1,3,5-thiadiazine-2-thione-tetrahydro-3,5- dimethyl) were used to reduce soil populations of this fungus in Ontario, however symptoms of root disease and dieback (caused by species of *Fusarium* and *Cylindrocarpon*) often became evident in the second growing season after fumigation (Honhart and Juzwik 1988). In the United States, control of soil pathogens in bareroot nurseries is primarily by soil fumigation with methyl bromide and/or chloropicrin (trichloronitromethane) (Enebak *et al.* 1990; Munnecke and Ferguson 1953; Newhall and Lear 1948). The decision by the United States Environmental Protection Agency to ban the use of methyl bromide has prompted research into potential alternative control measures (Smith and Fraedrich 1993).

Various non chemical control measures for *C. floridanum* are still in the experimental stages. Repeated soil cultivation during summer fallow (Testa and Juzwik 1989) was not effective in reducing soil populations of *C. floridanum* in Ontario. Soil solarization (Saunders, OMNR unpublished file report) significantly reduced fungal populations in the top 10 cm of soil in one southern Ontario nursery, although increased seedling transplant survival after treatment was not observed. Solarization may not be feasible in much of Ontario because of low solar radiation. Species of *Trichoderma* (Dumas *et al.* 1996 ) and *Phaeotheca dimorphospora* DesRochers & Ouellette (Yang *et al.* 1995) are being tested as potential biological control agents. Soil pasteurization using steam as a control for several diseases was used as early as the 1950's (Aldrich and Nelson 1969, Bartok 1994) and the imminent loss of chemical fumigation with methyl bromide has renewed interest in steam treatments for disease control (Awuah and Lorbeer 1991; Bartok 1994). Our objectives were to evaluate the feasibility of using steam as a method to reduce the inocula of *C. floridanum*, to determine its effect on the populations of other specific soil microbes and to determine its effect on the first year growth of white pine (*Pinus strobus* L.) seedlings.

## MATERIAL AND METHODS

The strain of *C. floridanum* used in this study was isolated from a diseased 1-0 white spruce (*Picea glauca* (Moench.) Voss) seedling from Kemptville, Ontario, on medium developed by Phipps *et al.* (1976). The isolate was transferred to 3% malt agar (Difco) and incubated at 25°C in the dark for 7 d, and then maintained in 10% glycerol in cryovials at -70°C.

To initiate microsclerotia formation, a cryovial of *C. floridanum* was thawed in a water bath at 30°C, transferred onto 3% malt agar and incubated in the dark for 1 wk at 25°C. Sand cultures were prepared as described by Hunter and Barnett (1976) using glucose as the carbon source, and potassium nitrate as the nitrogen source, in a ratio of 100:1 (w:w). Twenty five grams of this sand culture medium were added to 250-mL Erlenmeyer flasks, plugged with cotton plugs, and autoclaved at 121°C for 20 min. Following cooling, 10 mL of sterile distilled water and a 5 mm plug of *C. floridanum* taken from the edge of a 1-wk-old culture were added to each flask. The flasks were incubated at 25°C in the dark for 30 d.

The microsclerotia were aseptically harvested from the sand cultures using 1.5 *M* sucrose (Hunter and Barnett 1976) without the use of a flocculating agent. The microsclerotia were concentrated by centrifugation at 4500 RPM for 20 min in a swinging bucket centrifuge in 50-mL plastic aseptic conical tubes. The sucrose supernatant was discarded and any remaining sucrose was removed from the microsclerotia by re-suspend-

ing the pellet in 35 mL of sterile water followed by centrifugation as previously described. This procedure was repeated three times to ensure the complete removal of the sucrose. The pellets were re-suspended in a small volume of sterile water, and the concentration adjusted so that 50 µL contained 100 microsclerotia. Twenty mL of the suspension were added to 35 mL of sterile blasting sand (Nepheline Syenite, Unimin, Canada Ltd.) and stored at 4°C. The morning of the steam treatment, approximately 1 g of the sand-microsclerotia mix was added to small nylon bags and their tops folded over and stapled to prevent loss of inoculum. A piece of string with a tag was stapled to the inoculum bag to indicate the depth of burial (Fig.1).

During the latter part of May, tree beds naturally infected with *C. floridanum,* were randomly selected in each of two compartments at the G. Howard Ferguson Forest Station (Kemptville, Ontario) and one compartment at St. Williams nursery (St. Williams, Ontario). Soil texture at the Kemptville nursery was 82.4% sand, 8.5% silt, 9.1% clay, and at the St. Williams nursery soil texture was 74.6% sand, 14.0% silt and 11.4% clay. Two treated plots and two untreated plots (1.2 m x 15 m) were randomly located in each bed. Just prior to the treatment 10 locations within each plot were randomly determined and inocula bags were buried at 5, 10, and 15 cm in each sub-plot location. Ten inocula bags were buried for the same period of time in an untreated compartment to ensure that burial had no detrimental effects on the viability of the microsclerotia inocula. Steam was applied to the treatment plots with the Egedal® bed steamer (Fig. 2). This self-propelled machine travelled at a rate of 1 m per minute and the steam, produced by a high pressure generator, was forced under 1.2 m x 2 m stainless steel pads which covered the prepared beds. One compartment at the Kemptville nursery was seeded with stratified white pine seeds using a precision seeder at a rate of 389 seeds per m approximately 2 h after treatment. No seeding occurred at the St. Williams nursery.

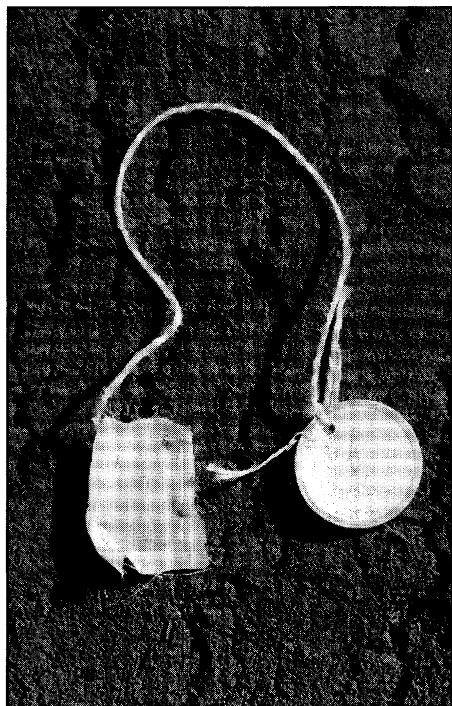

**Figure 1: Inoculum pouch containing the microsclerotia-sand mixture with the tag identifying the level to which it will be buried.**

Soil temperatures were monitored before, during and after the steam treatment using a Campbell Scientific 21X® micrologger. Thermocouples were buried at 5, 10, 15 and 20 cm at three randomly selected locations in the treatment plots at the Kemptville nursery, and two locations at St. Williams nursery. Soil temperatures were recorded every 10 s starting 30 min before the steam treatment and continuing for 2 h post-treatment.

The inocula bags were removed from each plot 1 h after the steamer had passed the end of the plot, and placed in a cooler at 4°C. To determine the viability of the microsclerotia, two samples were taken from each bag and sprinkled onto the surface of a 50 mm x 15 mm Petri dish containing Phipps *et al.* (1976) medium. The plates were

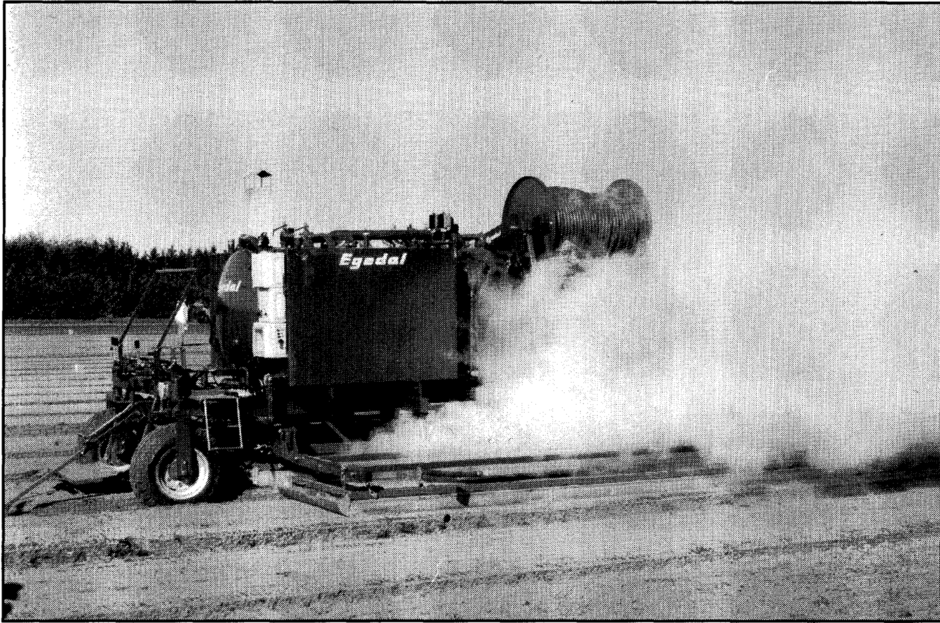


**Figure 2: The Egedal® steam generator treating three seedbeds at once.**

transported to the laboratory the following d and incubated at 25°C for 5 d. Viability was determined by assessing the germination rate of 50 randomly selected microsclerotia per plate using a stereomicroscope at 75 X magnification. Percent inhibition was calculated relative to the controls.

Populations of fluorescent pseudomonads and *Trichoderma* species were assessed just prior to the treatment, 1 h post-treatment and on a biweekly basis for 10 wk. Ten soil cores (2 cm x 20 cm) were collected on a diagonal line crossing each plot. Each core was divided into two sections, 0-10 cm and 11-20 cm. Populations of microbials were determined by shaking 10 g of soil in 25 mL of 0.1 *M* $MgSO_4$ on an orbital shaker for 10 min at 250 RPM. Serial dilution of the suspensions were done and the populations of fluorescent pseudomonads and *Trichoderma* were determined on Sands and Rovira (1970) and Papavizas and Lumsden (1982) media, respectively. The Petri dishes were incubated at 25°C in the dark. Populations of fluorescent pseudomonads were determined after 3 d and those of *Trichoderma* after 1 wk. Moisture contents of the soil samples were determined by drying 5 g of soil at 80°C until a constant weight was achieved. Populations were calculated as numbers of colony forming units (cfu) per g dry weight of soil.

Germination monitoring of white pine seeds began 4 wk after the steam treatments. Three randomly located 1-m long drill rows were permanently marked in each plot and the number of germinated seeds was counted twice a wk for 3 wk. Seedlings with symptoms of post-emergent damping-off were removed from the plots, placed in poly bags with damp paper towels and shipped to the laboratory. Damping-off samples were rinsed in sterile distilled water and plated on cornmeal agar and acidified potato dextrose agar. Cornmeal agar cultures were incubated at room temperature in the dark for 24-48 h before they were examined for pytheacious fungi. Potato dextrose cultures were incubated at room temperature at low light levels for 5-7 d prior to examination for growth of other damping-off fungi.

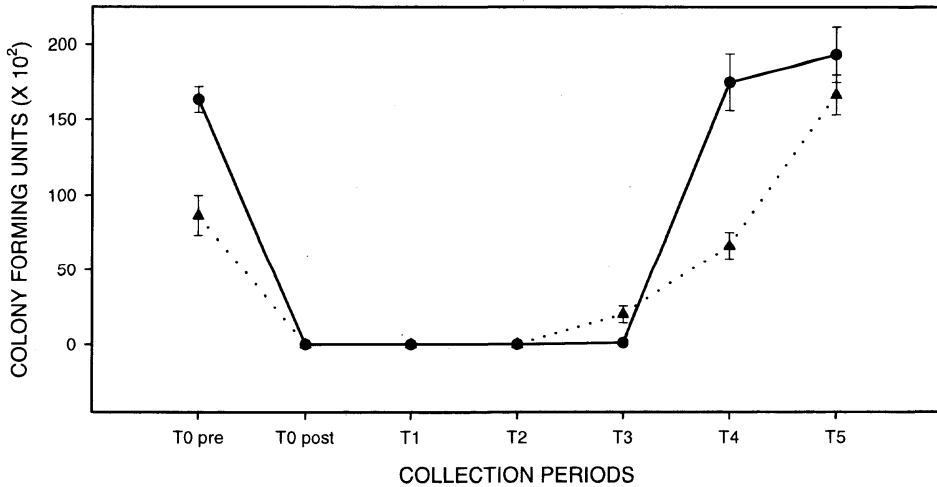


**Figure 3: Effect of steam treatment on the populations of fluorescent pseudomonads at 0-10(●) and 11-20(▲) cm depths.**

White pine seedling survival was assessed 4 mo after the treatment by counting the numbers of seedlings in two 1-m subplots in each plot. Growth was assessed by determining seedling height, root collar diam, shoot dry weight, and root dry weight measurements of 25 randomly selected seedlings from each plot.

Data were analyzed using SigmaStat statistical software for personal computers (Jandel Scientific Software, San Rafael, California).

## RESULTS

All microsclerotia buried at 5 and 10 cm in the Kemptville nursery were killed following the steam treatment whereas those buried at 15 cm were unaffected. In the St. Williams nursery mortality was obtained only at the 5 cm level and no effect was observed on microsclerotia buried at the 10 and 15 cm depths (Table 1). No differences in the germination rates of microsclertia buried at the different depths were found in the control plots.

Steam had a profound effect on the populations of the fluorescent pseudomonads at the two depths tested. Populations were reduced to 0 and began to recover 6 wk following the treatments (Fig. 3). *Trichoderma* species were undetectable at the 0 to 10 cm depths after the treatment. Populations started to increase after 8 wk and were similar

**Table 1. Effect of steam treatment on the germination rate of *Cylindrocladium floridanum* microsclerotia buried at 5, 10 and 15 cm in Kemptville and St. Williams nurseries**

| Depth | Germination rates (%) | |
|---|---|---|
| (cm) | Kemptville | St. Williams |
| 5 | 0 a[ψ] | 0 x |
| 10 | 0 a | 98.2 y |
| 15 | 84.8 b | 100.0 y |
| Control | 83.8 b | 99.4 y |

[ψ] Values in each column follwed by a different letter are significantly different according to the Student-Neuman-Keuls test ($P = 0.05$).

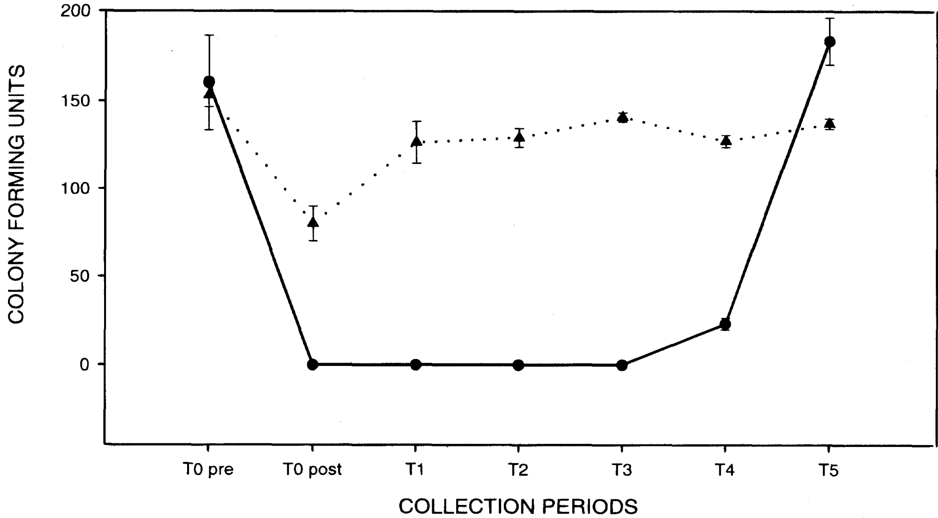


**Figure 4: Effect of steam treatment on the populations of *Trichoderma* species at 0-10(●) and 11-20(▲) cm depths.**

to the original populations after 10 wk. Effects on *Trichoderma* at the 10 to 20 cm level were marginal following the steam treatments and after 2 wk the populations increased and stayed relatively constant throughout the 10 wk of monitoring (Fig. 4).

Soil temperatures achieved by the steam treatment varied between plots and nurseries. Maximum soil temperatures recorded at 5 cm ranged from 49 to 86°C (Table 2). Maximum temperatures were retained for a very short time (< 1 min) and decreased substantially with increasing soil depth and also decreased gradually over the 2-h post-treatment period at the 5 and 10 cm depths. Temperatures at the 15 and 20 cm levels were still increasing slightly after 2 h.

Steam treatment neither enhanced nor reduced the germination rate of white pine when compared to the control plots 7 wk after seeding. No differences were observed in rate of damping-off (0.7 seedlings per m of bed in treated plots versus 1.4 seedlings in untreated plots).

A number of fungi were isolated from symptomatic seedlings from both treated and untreated plots on the acidified potato dextrose agar, including species of *Fusarium, Penicillium, Cylindrocar-*

**Table 2. Maximum soil temperatures obtained within a 2-h time period after the steam treatments at the Kemptville and St. Williams nurseries**

| | Temperature prior to treatment (°C) Soil depth | | | | Maximum temperature obtained (°C) Soil depth | | | |
|---|---|---|---|---|---|---|---|---|
| Nursery | 5 cm | 10 cm | 15 cm | 20 cm | 5 cm | 10 cm | 15 cm | 20 cm |
| Kemptville 1[a] | 8 | 7 | 7 | 7 | 68 | 37 | 25 | 16 |
| Kemptville 2 | 13 | 8 | 7 | 7 | 80 | 57 | 21 | 15 |
| Kemptville 3 | 12 | 8 | 7 | 7 | 86 | 36 | 23 | 17 |
| St. Williams 1 | 31 | 36 | 27 | 24 | 64 | 36 | 30 | 26 |
| St. Williams 2 | 31 | 29 | 26 | 24 | 49 | 33 | 29 | 26 |

[a] Soil probe

**Table 3. Effects of steam treatment, relative to the controls, on shoot height, root collar diameter, shoot weight and root weight of 4-month-old white pine seedlings**

| Height (cm) | | Root collar diameter (mm) | | Shoot weight (g) | | Root weight (g) | |
|---|---|---|---|---|---|---|---|
| Steamed | Control | Steamed | Control | Steamed | Control | Steamed | Control |
| 4.6 a[ψ] | 3.5 b | 1.5 a | 1.0 b | 4.0 a | 1.6 b | 2.1 a | 0.9 b |

[ψ] Values in each treatment followed by a different letter are significantly different, according to Tukey's test ($P = 0.05$).

*pon* and *Rhizopus*. Species of *Fusarium* were the most prevalent fungi isolated from seedlings in both the steamed and control plots. Fungal growth on cornmeal agar occurred only from seedlings from untreated plots These fungi were generally classed as pythiaceous fungi.

The survival of seedlings after 4 mo was significantly higher in steamed plots (262 seedlings per m) than in untreated plots (193 seedlings per m). Seedlings in treated plots were also significantly larger in height, root collar diam and root and shoot weight (Table 3).

## DISCUSSION

Treating preformed nursery seedbeds with steam can reduce the potential impacts of *C. floridanum,* but the effectiveness of the treatment is very sensitive to seedbed preparation technique and will vary with soil type. Soils in Ontario's nurseries are typically high in sand content and achieving sufficiently high temperatures to kill plant pathogens is more difficult in sandy and loamy soils than in clay soils (Runia 1983). The steamer's stainless steel pads create a seal at the edge of the seedbed, and a smooth flat bed is necessary to ensure that steam does not escape from beneath the pads. Irregularities in the bed will result in fluctuations in maximum temperature achieved at varying depths. The inability of the Egedal steam generator to produce enough heat in the soils at the St. Williams nursery, when compared to the Kemptville nursery, may have been caused by differences in soil and seedbed preparation techniques. Slower movement of the device or repetitive applications may be required before the soil temperatures would be high enough to have an impact on this pathogen.

Awuah and Lorbeer (1991) observed rapid recolonization of pasteurized soil by fungi. Under our conditions, microbial recolonization of the upper layer did not occur until 10 wk following the steam treatment. *Trichoderma* and fluorescent pseudomonads are inhibitory to *C. floridanum* (Dumas *et al.* 1996, Dumas unpublished data, respectively) and steaming could therefore prevent establishment of the normal biological control processes, but could also be used to prepare seedbeds for an application of *Trichoderma* isolates that are highly antagonistic to *C. floridanum.* This approach has been used in conjunction with soil solarization to control soil-borne diseases in potato fields (Elad *et al.* 1980).

Excessive heating can substantially increase the manganese content of soils (Jager *et al.* 1969), which can have a negative influence on crop development and quality (Sonneveld and Voogt 1973). However steam sterilization has also been shown to stimulate the growth of the subsequent crop due in part to the release of nutrients in the soil (Awuah and Lorbeer 1991; Runia 1983). The steam treatment had a positive effect on white pine seedling growth and survival. In all instances the white pine seedlings were larger and their density higher in the steamed plots compared to the controls, possibly due in part to this nutrient release effect as well as the reduction in fungal inoculum. The temperatures achieved with the Egedal steamer appeared sufficient to elimi-

nate *C. floridanum* from the upper layers of soil but were low enough (< 88°C) to limit the release of toxic amounts of manganese into the soil. Undoubtedly, once the roots of the seedlings reach a depth greater than 10 cm they will come into contact with viable inocula of *C. floridanum* but their ability to resist infection may be enhanced due to their increased vigor.

Further investigations are required to determine the effect of this treatment on the incidence of Cylindrocladium root disease in the second and third yr of seedling growth. Other susceptible seedling crops, especially black spruce (*Picea mariana* Lamb.) should also be tested on steamed beds. This type of site preparation method could be enhanced by integrating it with a biological control procedure.

## ACKNOWLEDGMENTS

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