MANAGEMENT OF CONIFER SEEDLING ROOT ROT IN PROVINCIAL BAREROOT NURSERIES

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The views, conclusions and recommendations are those of the author(s) and should not be construed as policy nor endorsement by the Ontario Ministry of Natural Resources nor Forestry Canada.

SUMMARY

The management of conifer seedling root rot in Ontario bareroot nurseries was investigated by implementing several developmental field trials. Trials were designed to monitor the impact of solarization, fumigation, and chemical treatment on populations of soil borne fungi. Mortality associated with infected seedlings was determined for the initial six months following outplanting.

Soil solarization has been used for several decades in various parts of the world as a method of soil disinfestation. The technique was investigated for efficacy in control of Cylindrocladium floridanum Sobers & Seymour at the forest nursery in St. Williams Ontario. Solarization with 2 ml plastic resulted in a significant reduction in pathogen population in the 0-10 cm soil depth. In the 10-20 cm soil depth, solarization did not effect the fungi. Since the root zone of the seedlings lie within the top 10 cm, the technique has potential as a method of soil disinfestation.

Pruning and wrenching is a technique used to produce superior seedlings conditioned for overwinter storage and ultimately outplanting. The practice involves cutting the roots and simultaneously stressing the tree. Under certain environmental conditions, and provided the soil is infected, pruning and wrenching could lead to greater disease incidence. In this study, a correlation between pruning and wrenching and increased disease

incidence was not found.

Black spruce seedlings were lifted from compartments known to have Cylindrocladium root rot at two Ontario forest nurseries. After six months, significantly higher levels of mortality caused by Cylindrocladium floridanum Sob. & C.P. Seym. ($p<0.05$) occurred in seedlings with symptomatic shoots or with main root lesions and non-symptomatic shoots, compared to seedlings with only lateral root lesions and non-symptomatic shoots or non-symptomatic roots and shoots.

The most common means of controlling nursery diseases in seedbeds is by application of broad spectrum biocides. Basamid granular (active ingredient: dazomet) was investigated for its ability to control Fusarium oxysporum and Cylindrocarpon destructans. Results suggested that although an initial population reduction resulted from high application rates, reinvasion of the soil is a hazard. The technique may be enhanced by combining with tarping, but such a modification is prohibitively expensive.

A complex involving the stubby root nematode Paratrichodorus pachydermis and the fungi Cylindrocarpon destructans and Fusarium oxysporum is suspected to be the cause of losses in white pine compartments at the St. Williams nursery. A study was carried out to investigate the effectiveness of selected chemicals in controlling nematode and fungal populations. Plots treated with benomyl produced the best 3+0 crop when compared to seedlings in

plots treated with nematicides Telone IIB and Experimental chemical SN55N. This finding will be reassessed in light of nematode population data when it becomes available.

Information gained from these trials will be of immediate value to nurserymen and will contribute to the development of strategies for managing root rots in bareroot nurseries.

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INTRODUCTION

Solarization is a technique being investigated as a method of soil disinfestation. Also called solar heating of soil, soil pasteurization, polyethylene mulching, solar tarping, solarization is a technique in which moist soil is covered with a clear polyethylene tarp for several weeks during the hottest time of the year. The term solarization refers to the thermal, chemical, and biological changes in soil caused by solar radiation when it is tarped with polyethylene (Stapleton and Devay, 1986). This 'greenhouse effect' is enhanced by moistening the field before and during solarization (Katan, 1980).

The modes of action of soil solarization extend well beyond thermal death of the pathogens (Stapleton and DeVay, 1986). Solarization causes changes in soil biota and substrate, providing a favourable environment for colonization by saprophytic micro-organisms which may subsequently inactivate surviving phytopathogenic fungi that were damaged or weakened by solarization (Katan, 1976; Stapleton and DeVay, 1986).

Interest in solarization as a method of soil disinfestation has gained considerable momentum over the past decade, and has been investigated in at least 24 countries (Katan, 1987). The success of solarization is directly related to the local climate, although various other factors (soil type, pathogen, soil microflora) are also intricately involved. To date, solarization has proven itself to be an effective pest management technique, especially in the warmer regions of the world. Control of

pathogens such as Verticillium spp., and Rhizoctonia solani has been achieved in Israel (Katan, 1980), and California (Pullman et. al., 1981b). Solarization has also been investigated in temperate regions of the world. White and Buczacki (1979), in Wellsbourne, UK (approximately 52°N latitude) investigated the effect of solarization on the clubroot pathogen Plasmodiophora brassicae Wor. They recorded a reduction in clubroot incidence on cabbage seedlings grown in soil samples recovered from beneath the tarps. Yet, existing conditions (wind, rainfall, wildlife) render the technique impractical for the UK. Hopefully, new research into solarization will increase its applicability to regions that consider it unfeasible because of economic and climatic limitations.

In the past few years, solarization has been investigated in conifer nurseries in the United States. The results have varied with the region, and the particular pathogen investigated. At the Colorado State Forest Service Nursery, Hildebrand (1985) found that heating with 2 ml clear polyethylene for 55 days beginning in early July resulted in significant reductions in Pythium spp., and Fusarium spp. In Central Point, Oregon,(Cooley, 1985) solarization significantly reduced populations of Fusarium spp., but the reduction did not bring about a corresponding decrease in incidence of Fusarium root rot. In a trial in Wisconsin, Zarnstorff (1983) found that solarization did not reduce populations of Fusarium oxysporum, Rhizoctonia solani, or Cylindrocladium floridanum Sobers &

Seymour.

The objectives of this study were to determine the increases in soil temperature obtained with various combinations of tarping and irrigation and to determine the impact of solarization on populations of the fungus Cylindrocladium floridanum Sobers & Seymour at the provincial government tree nursery at St. Williams (42° 40' N latitude) situated in southwestern Ontario. Treatments included one and two layers of 2 ml plastic, with, and without an irrigation system installed under the tarps.

MATERIALS AND METHODS

The trial design used was a complete randomized block with four treatments. Each treatment was replicated seven times and each replicate consisted of a 3.3 X 3.3 meters separated from each other by a one meter buffer area. The four treatments were:

1) 1 layer of plastic and soaker hose irrigation: 1p+i
2) 1 layer of plastic, no irrigation system: 1p
3) 2 layers of plastic and soaker hose irrigation: 2p+i
4) soaker hose irrigation system only: control

The trial area was tilled with a tractor mounted, three point hitch rototiller to a depth of 20 cm on June 6, 1988, and soil samples were taken immediately following plot establishment. All 28 plots were sampled, and each sample consisted of ten soil cores taken at random with a 2 cm diameter Oakfield sampling tube. Soil samples were divided into a top layer (0-10 cm), and a lower layer (10-20 cm). The depths were divided to determine the vertical distribution of the fungi as well as the treatment effect in the two vertical zones. The soil samples were kept in polyethylene bags and stored at room temperature until processing within four weeks.

Following soil sampling, the area was irrigated to soil capacity, and a drip irrigation system consisting of soaker hoses was laid out in the designated treatment plots. Tarps (2 ml plastic) were placed on the plots on June 7, and anchored down at the edges with soil, and remained in place for 83 days. Soil samples were taken once the plastic was removed.

Single point source thermometers were placed at 7.5 and 17.5 cm depths in two plots of each treatment. Soil tension was recorded with fixed tensiometers (Soilmoisture Equipment Corp., Santa Barbara, Ca.), the ceramic tip was placed at a depth of 15 cm. The tensiometers were placed in two plots of each treatment.

Soil samples were processed using a modified wet sieving technique adapted from Thies and Patton (1970) which separates fungal propagules on the basis of size and density. Soil samples (approx. 200-g) were thoroughly mixed. Three subsamples were taken from the sample, one 10-g sample was used for determination of soil moisture content, two separate 3-g subsamples were used in the isolation procedures. Each 3-g subsample was put into an Oster Blender (Sunbeam Corp. Ltd.) mini jar containing 250 ml of distilled water. The mixture was blended for one minute. The suspension was allowed to settle for 15 seconds, the supernatant was decanted onto nested 100 (150 um) and 200 (75 um) mesh screens (C.E. Tyler, St. Catherines, Ontario). Distilled water was added to the residue and stirred into suspension on a Mag-Mix (Precision Scientific Co.). The suspension was allowed to settle for 15 seconds, and then decanted onto the screens. The decanting procedure was repeated five times. The material collected on the 200 mesh screen was washed thoroughly and the remaining residue was immersed in a 10% NaOCl solution for 15 sec. The residue was then washed with water, and collected into a 250 ml beaker and termed the 'inoculum concentrate'. 50 ml of dilute water agar (0.6%) was added to the inoculum concentrate.

The total suspension was added to a bottle containing 90 ml of a Cylindrocladium selective medium (Phipps et. al., 1976) kept at 45° C in a water bath. The contents of each bottle was mixed and poured into 20-25 petri-plates. The plates were incubated at room temperature, colonies were examined ten days later. Colony counts from each subsample were totalled and corrected for moisture content and reported as propagules/gram of dry soil. Soil moisture was calculated for every soil sample using the following formula:

(Sw - Sd)/Sd X 100 = % moisture content (%mc)

where Sw = weight of moist soil

Sd = weight of oven dried soil

propagule density was calculated as:

propagules in 3 grams/ 3 X (1-mc)

The two subsample variables reported as propagules/gram of dry soil were individually transformed with a square root transformation: $\sqrt{p/g + 1 \text{ X } 10^{-8}}$

The sum of the two transformed variables represented one sample. Since pre-trial populations in the treatment plots were not significantly different from each other (alpha = 0.05), analysis of variance (ANOVA) was used for data analysis. Populations before and after treatment were compared for both the top and the lower level data. Means were separated out at the 0.05 level using Duncan's multiple range test.

RESULTS

a) Populations of *Cylindrocladium floridanum*:

Solarization resulted in a significant ($P < 0.05$) decrease in population levels of *C. floridanum* in the top 10 cm of soil. Populations of *C. floridanum* pre- and post- treatment are given in Table 1. In the top 10 cm, the effect of solarization did not seem to be enhanced by the addition of a second layer of plastic, or the addition of continuous irrigation (Figure 1).

At the 10-20 cm level, population levels of *C. floridanum* were unaffected by solarization (Table 2). In this level, the effect was not significant for any of the three tarped treatments compared to the control (Figure 2).

Table 1. Recovery of Cylindrocladium floridanum microsclerotia from the 0-10 cm soil depth in treatment plots, compartment E7, St. Williams Nursery, 1988

Treatments[c]	Microsclerotia recovered[ab] Pre-treatment	Post-treatment
1p+i	1.75a	0.18b
2p+i	2.35a	0.26b
1p	2.03a	0.25b
i(control)	2.16a	2.36a

[a] values are transformed means of propagules per g of dry soil.

[b] means in each vertical column followed by the same letter do not differ significantly according to Duncan's multiple range test ($P < .05$)

[c] 1p+i= 1 layer of 2 ml plastic + irrigation, 2p+i= 2 layers of 2 ml plastic + irrigation, 1p= 1 layer of 2 ml plastic + no irrigation, i= irrigated control.

Table 2. Recovery of Cylindrocladium floridanum microsclerotia from 10-20 cm soil depth in treatment plots, compartment E7, St Williams Nursery, 1988.

Treatments[c]	Microsclerotia recovered[ab] Pre-Treatment	Post-Treatment
1p+i	1.91a	1.18a
2p+i	1.36a	0.98a
1p	1.25a	1.20a
i(control)	1.66a	2.04a

[a] values are transformed means of propagules per g of dry soil.

[b] means in each vertical column followed by the same letter do not differ significantly according to Duncan's multiple range test ($P < .05$).

[c] abbreviations as in Table 1.

b) Temperature:

Single point source thermometers were placed at 7.5 and 17.5 cm depths to record soil temperature. The maximum soil temperatures from June 7 to Aug 29 at depths of 7.5 and 17.5 cm were 46°C (1p +i) and 38°C (1p +i) respectively. The peaks, reached in early July, were maintained for three consecutive days. During the same period, peak temperatures in the control plots, at 7.5 and 17.5 cm were 40°C and 32°C, respectively.

At the 7.5 cm level, the temperature regimes of all three tarped treatments were strikingly similar, and all three differed from the control plot by approximately 8-10°C. Figure 3 illustrates the temperature profile of the control plots and the 1p+i plots. The remaining two treatments overlap the 1p+i treatment and have been left off the figure for the purpose of clarity.

At the 17.5 cm depth, the temperature regime of the 1p+i and 2p+i were strikingly similar. At this depth, the treatment 1p did not reach the temperatures recorded in the 1p+i and 2p+i treatments, but it did reach higher temperatures than those achieved in the control (i) plots. The temperature profiles of 1p+i and the control are illustrated in Figure 4. Maximum temperatures and their respective achievement dates are shown in Table 3.

Table 3. Maximum temperatures attained at two depths in solarized plots, compartment E7, St. Williams Nursery,1988.

Treatment[a]	7.5 cm. depth		17.5 cm. depth	
	temp °C	date	temp °C	date
1p+i	46	July 5-8	38	Aug 9
2p+i	45	July 6-8	37	July 8
1p-no i	45	July 4-8	35	July 8
i(control)	40	Aug 3, Aug 18	32	Aug 3

[a] abbreviations as in Table 1

c) Tarp Durability:

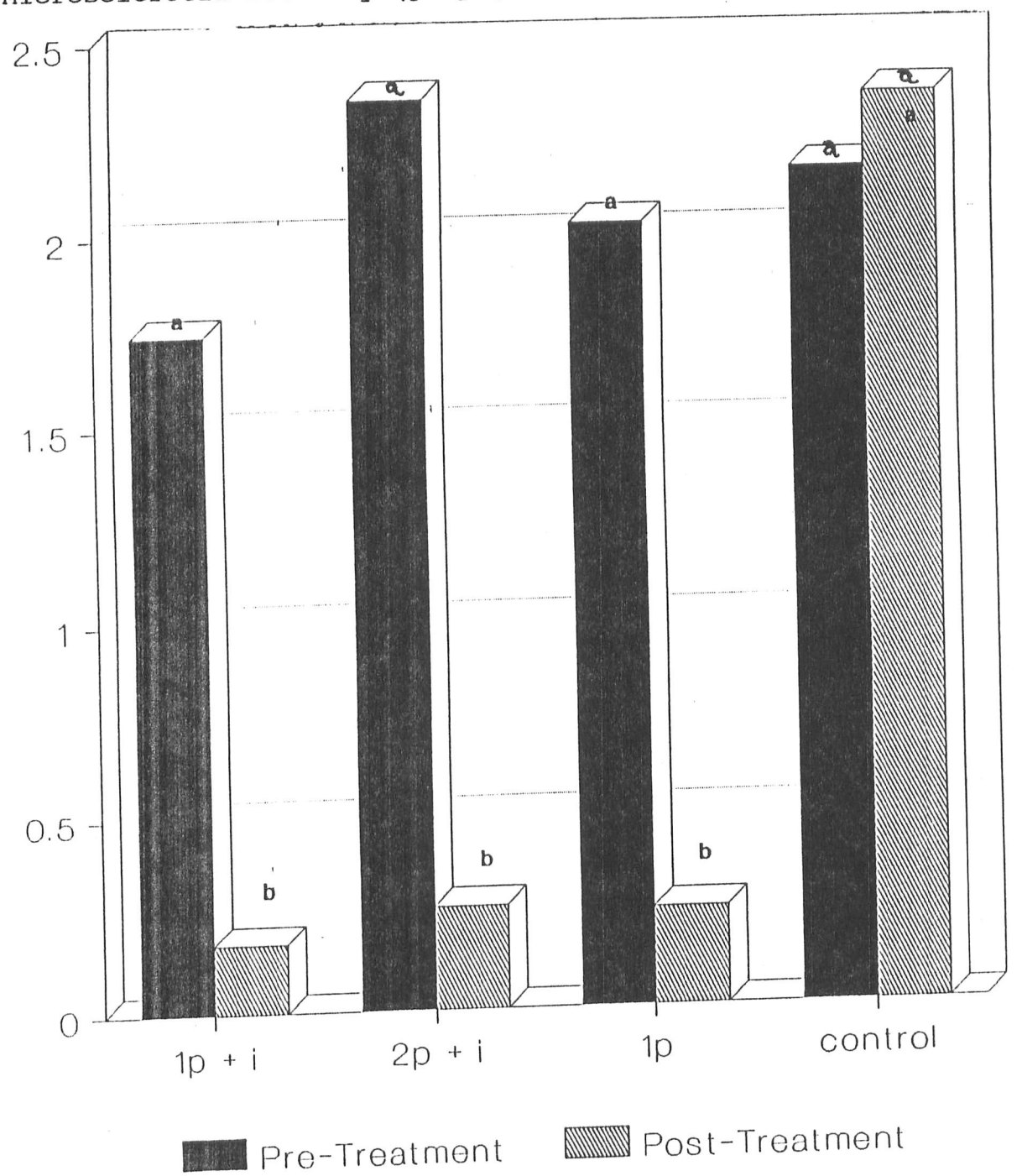
In the fields, polyethylene is exposed to direct sunlight, wind, rain, and condensation buildup beneath the bottom side. The 2 ml clear polyethylene used in this study remained intact for approximately 50 days, after which it became brittle, broke apart and had to be replaced.

d) Moisture:

Tension fluctuations in soil moisture for the 1p + i, 2p + i and 1p were similar. The control plots had erratic fluctuation due to the influence of natural rainfall as well as evaporation. Figure 5 illustrates the soil moisture tension patterns of 1p + i, and 1p plots. The two remaining treatments have been left out for the sake of clarity.

Until the tarps tore apart (day 50), moisture patterns were fairly consistent between tarped plots, with the extremes being 11.0 and 17.5 centibars. For comparison, a value of zero is field capacity, and is considered ideal for plant growth. A value of 100 is wilting point; 50 is the value at which irrigation is initially considered. After day 50, tension was somewhat erratic, yet the pattern was consistent for all tarped plots.

Figure 1. Effect of solarization (0-10 cm depth) on Cylindrocladium floridanum microsclerotia in compartment E7, St. Williams Nursery, 1988.

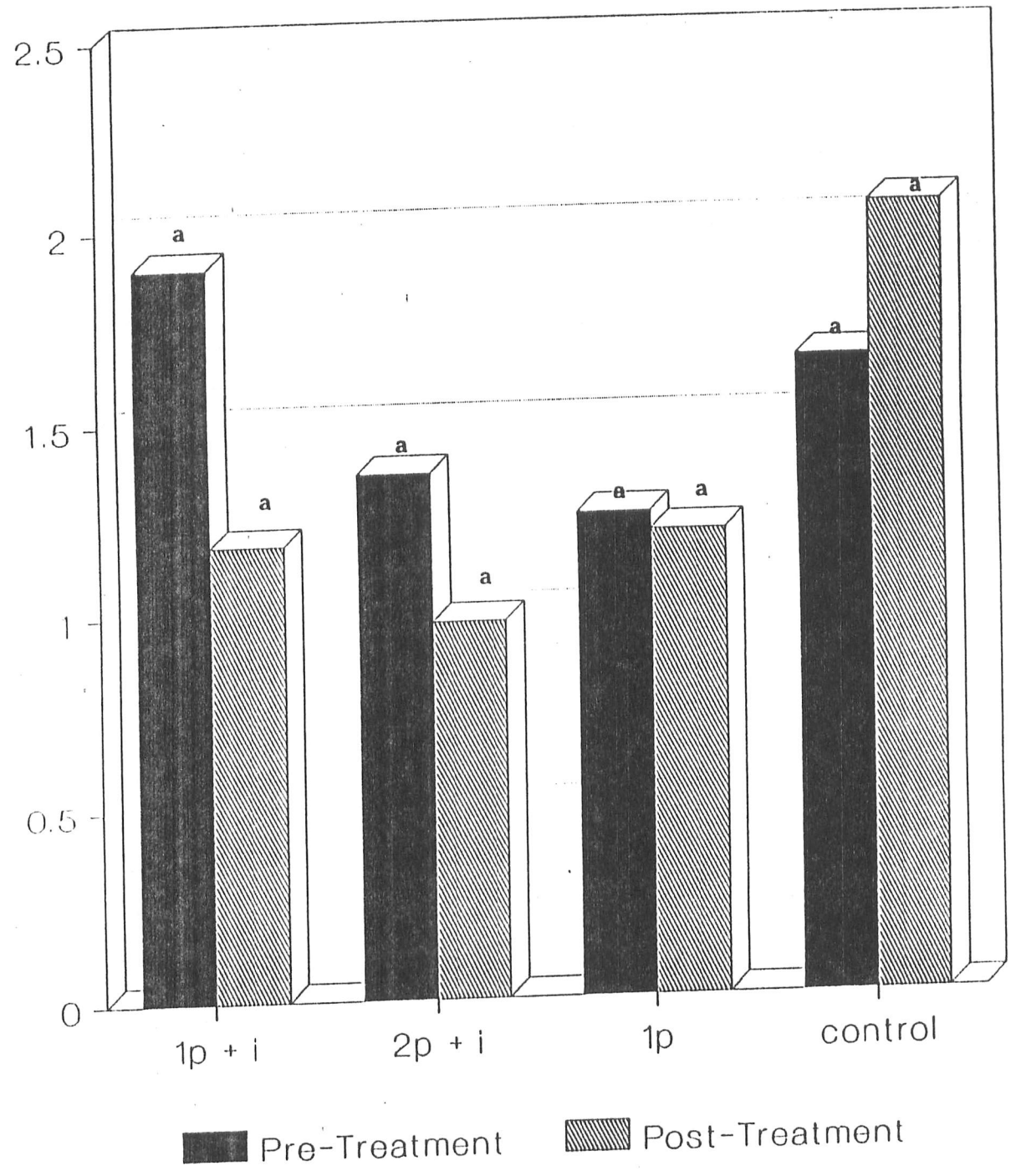


1 Values are transformed (square root) means of propagules per gram of dry soil.

The same letter above the bars indicates that the means (7 replications) of the the data are not significantly different (p=0.05) according to Duncan's multiple range test.

Figure 2. Effect of solarization (10-20 cm depth) on Cylindrocladium floridanum microsclerotia in compartment E7, St. Williams Nursery, 1988.

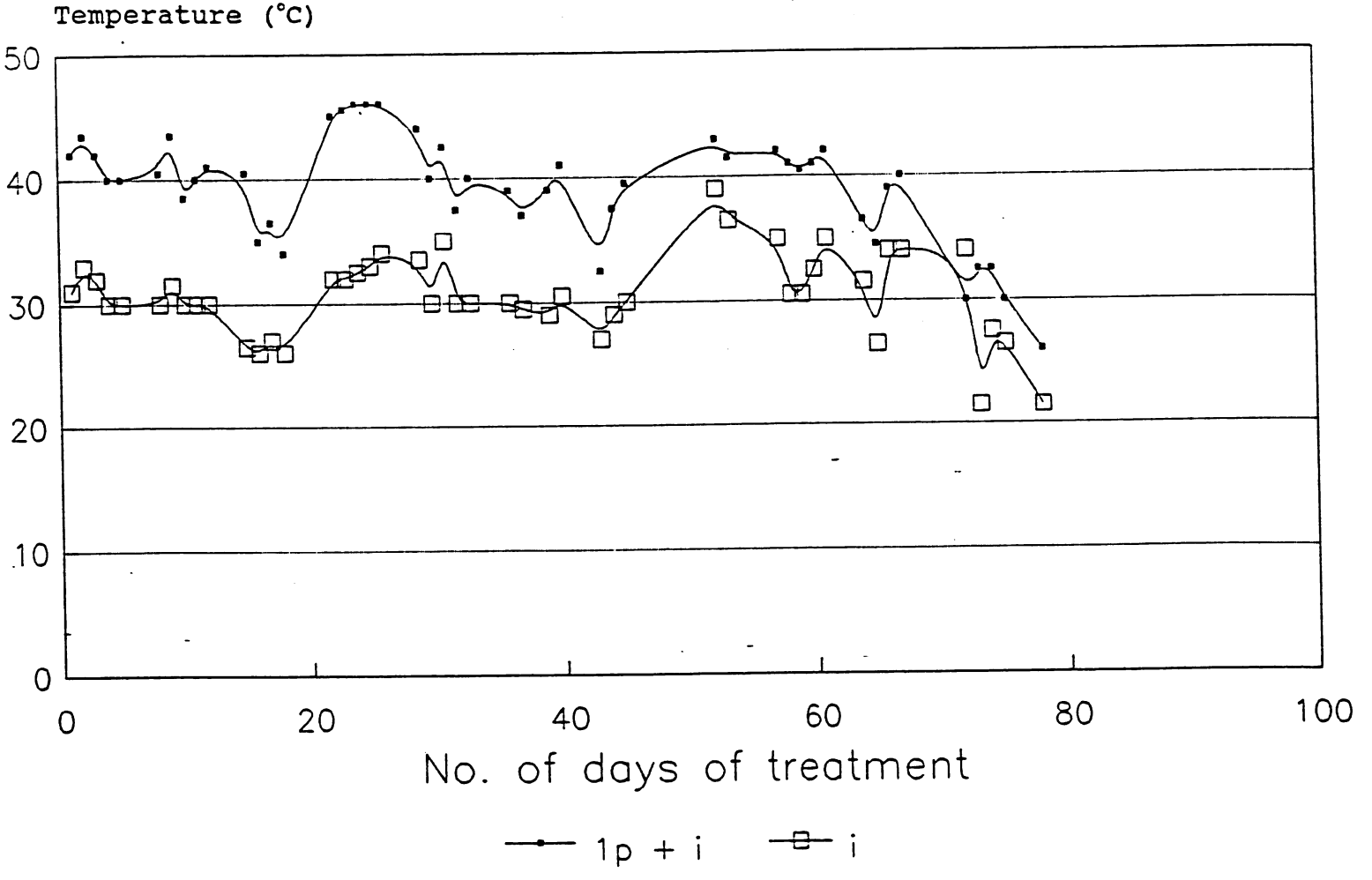
Microsclerotia recovery (propagules/g). [1]



[1] Values are transformed (square root) means of propagules per gram of dry soil.

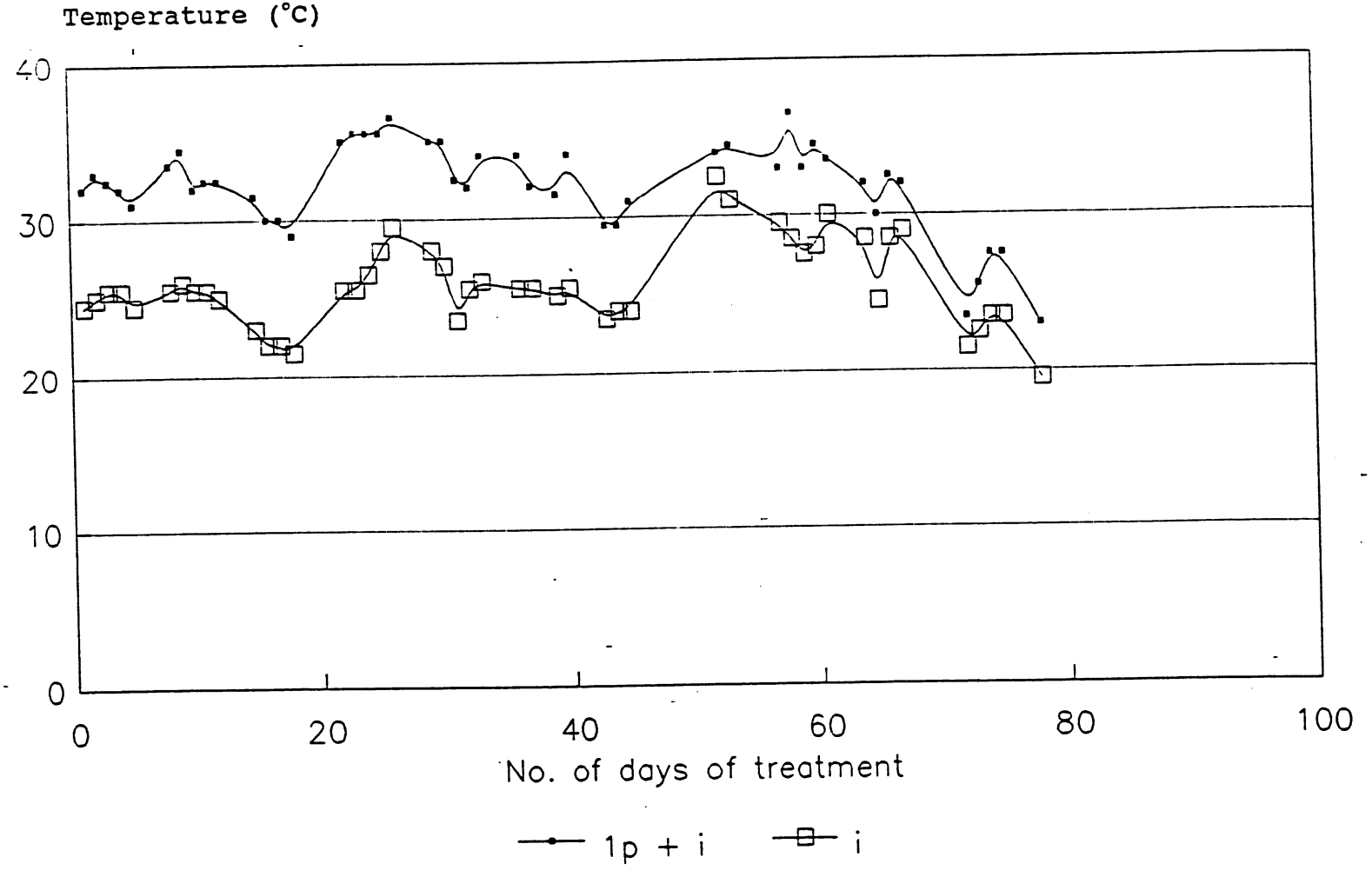
The same letter above the bars indicates that the means (7 replications) of the the data are not significantly different (p=0.05) according to Duncan's multiple range test.

Figure 3. Maximum daily temperatures recorded at the 7.5 cm depth in solarized plots, compartment E7, St. Williams Nursery, 1988.



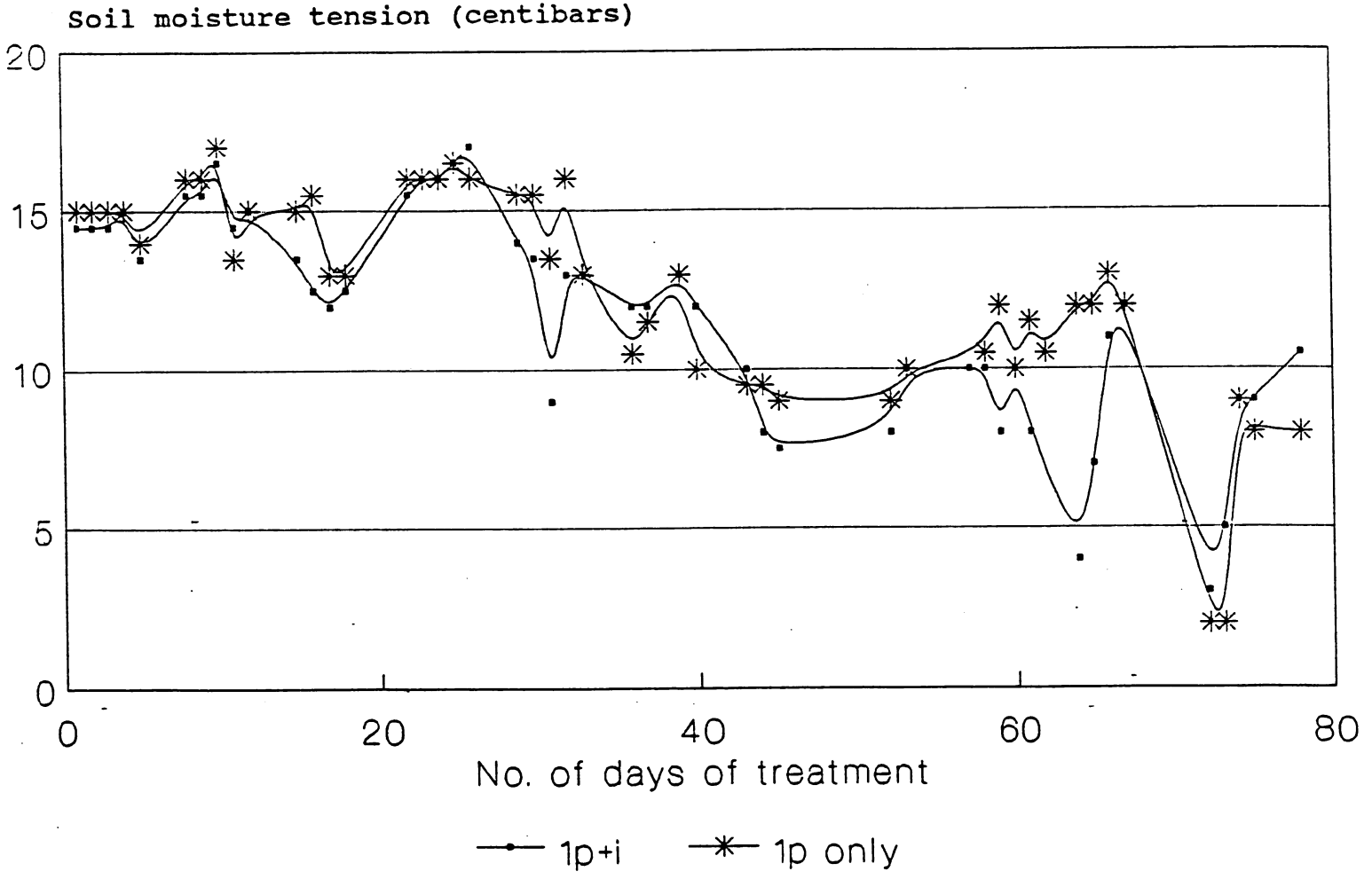
(p= layer of plastic; i = irrigation)

Figure 4. Maximum daily temperatures recorded at the 17.5 cm depth in solarized plots, compartment E7, St Williams Nursery, 1988.



(p=layer of plastic,i=irrigation)

Figure 5. Soil tension at 15 cm depth recorded solarized plots, compartment E7, St. Williams Nursery, 1988



(p = layer of plastic; i = irrigation)

DISCUSSION

a) Populations of Cylindrocladium floridanum:

Population levels of Cylindrocladium floridanum were significantly reduced in the top 10 cm of soil by the three tarped treatments. Between 10-20 cm depth, the solarization treatments did not significantly reduce C. floridanum populations.

Solarization did not control Cylindrocladium floridanum at a nursery in Boscobel, Wisconsin (Cummings-Zarnstorff, 1983). Temperatures at Boscobel were similar to those at St. Williams; the discrepancy in findings may be due to the low pre-trial population levels (< 0.5 propagules/gram) used in the Boscobel study.

Cooley (1983) found that populations of Fusarium spp. were significantly reduced between 0-30 cm. in an Oregon nursery . The reduction in population did not, however, result in better seedling survival. The short solarization period used (4 weeks) in the study and its late application (set up on July, 29) may have contributed to the limited success achieved. Cooley (1985) found similar results in a nursery in Central Point, Oregon. Solarization significantly reduced populations of Fusarium spp., yet the effect was limited to the top 15 cm, and the reduction did not result in a corresponding decrease in seedling mortality. The results suggested that solarization had potential, but the technique needed modification if it was to be used in a moderate climatic region.

In California, Pullman et. al. (1981b) found solarization to be highly effective in reducing soil populations of Verticillium dahliae, Pythium spp., Rhizoctonia solani, and Thielaviopsis basicola. The effect extended well beyond 15 cm into soils heated to maximum temperatures near 36-38°C. At temperatures of 37-50°C., populations can be greatly reduced, and sometimes eradicated within 2-5 weeks (Pullman, DeVay, and Garber, 1981a; Pullman et. al. 1981b; Porter and Merriman, 1983).

In the 10-20 cm depth, solarization did not significantly reduce populations of C. floridanum. Loss of viability as calculated by estimating the number of surviving propagules is the simplest method for assessing the effect of solarization. Yet, this method does not take into account the reduction in the inoculum potential of the affected propagules (ie: a decrease in germinability in the field). Weakened propagules which may not survive in the field, will give rise to a colony in culture, and thereby artificially inflate the propagule count. Weakening of propagules was not investigated in this paper, but suitable parameters are available such as speed of germination, and initial growth. The effect of sublethal heating on growth and germination has been investigated with various fungi (Munnecke et. al. 1976).

b) Temperature:

Temperatures achieved with tarping were comparable to those achieved in Oregon (Cooley,1983), and Colorado (Hildebrand, 1985), and lower than those achieved in California (Pullman et.

al. 1981) and Israel (Chen and Katan, 1980). Table 4 illustrates various temperatures associated with solarization treatments around the world.

When comparing either the top or the bottom layer, the temperature achievements and the fungal control are very similar for both the 1p+i and the 2p+i treatments. In this study, the second tarp of the 2p+i treatment was placed directly on top of the bottom tarp. It was later known that double layers of tarp, if separated by an air layer, can cause an increase in temperature greater than that attained with a single tarp (C. Stevens, pers. comm.; Ben-Yephet et. al.,1987). Ben-Yephet et. al. (1987) found that by using two layers of 1 ml (25 um) plastic separated by a 6 cm air layer, temperatures rose by 3.6°C (mean value) above the level attained with the single layer. This increase was achieved at both the 15 and 30 cm level. Although the increase seems minimal, it lead to a 97-98% reduction in Fusarium oxysporum f. spy. vasinfectum, as compared to a 57-60 % decrease attained with a single tarp. Although the optimum separation distance for the tarps is presently unknown, the mechanism is attributed to an decrease in moisture loss as well as an insulation factor (Ben-Yephet et. al., 1987).

Table 4. Record temperatures achieved throughout the world by soil solarization.

Location	Latitude (N)	Peak Temp°C 5-10 cm	Peak Temp°C 15-20cm	Reference
Israel(various)	32° 0'*	50	45	(Chen & Katan,1980)
Shafter CA.	35° 30'	60	50	(Pullman et.al.,1981)
Ft. Collins, CO.	40° 05'	41+	41+	(Hildebrand, 1985)
Central Point, OR.	42° 05'	44	41	(Cooley, 1985)
St. Williams, ON.	42° 40'	46	37	(Saunders&Juzwik,1988)
Boscobel, WN.	43° 07'	49	41	(Zarnstorff, 1970)
Bend, OR.	44° 04'	53	35	(Cooley, 1983)
Wellsbourne, War.	52° 0'	45	27.5	(White & Buczacki,79)

* approximate latitude of Israel.

c) Tarp Durability:

The 2 ml polyethylene used in this study had to be replaced after approximately 50 days in the field. The elements of sun, wind, and rain caused the plastic to become brittle. Correspondence is currently being carried out with plastic companies to gain information on plastics that are 1) more efficient in retaining and transferring heat; 2) more resistant to the physical elements. At present, plastic technology is virtually an untapped resource in investigations into solarization, it has the capacity to significantly increase the effectiveness of solarization (C. Stevens pers comm.).

d) Moisture:

All plots were irrigated to field capacity prior to tarping, but only treatments 1p+i and 2p+i were equipped with a drip irrigation system. Comparing the 'drip-irrigated' to the 'pre-tarp irrigation only', no significant difference is found. It seems that a drip irrigation systems are not required, the effect of solarization can be achieved provided the field is soaked to field capacity prior to tarping. This finding is supported by previous papers (Katan, 1981; Jacobsohn et. al., 1980).

e) Ecological implications of solarization:

The hydrothermal effect accompanying solarization is fundamental to soil disinfestation. Thermal death rate is dependant upon the nature of the pathogen, the temperature of the

length of time the pathogen is exposed to the elevated temperature. By definition, thermal death (complete kill) is a true loss of viability; care must be taken to differentiate a true loss of viability from a decrease in germinability. Griffin et. al. (1978) found that low temperatures may adversely affect the recovery of Cylindrocladium crotalariae microsclerotia in the laboratory. It is possible this low temperature phenomenon may be a low-temperature induced dormancy, or it may actually be a low temperature injury with loss of viability. The possibility of high-temperature induced dormancy which could be the pathogens' response to solarization has yet to be investigated. Preliminary investigations into temperature induced germinability/viability responses of C. floridanum are being carried out.

Besides causing thermal death, it is believed that solarization promotes biological control of soilborne pathogens (Katan, 1980). Biocontrol is achieved through reduction in inoculum density, and the subsequent inoculum supression.

Inoculum density may be reduced by microbes acting on weakened pathogens and parasitism stimulated by solarization. Based on studies involving Trichoderma harzianum, Katan (1981), and Stapleton and DeVay (1986) suggest that solarization causes changes in soil biota and substrate that provide a favourable environment for colonization by saprophytic micro-organisms which may subsequently inactivate surviving phytopathogenic fungi. The ability of Trichoderma spp. to rapidly colonize disinfected soil

has been reported (Mughogho, 1968; Warcup 1951). Inoculum suppression may be achieved by shifting the biologic equilibrium of the soil in favour of microorganisms that prevent the pathogen from reinvading (Katan, 1980).

Chemical properties of soil are also affected by solarization. Chen and Katan (1980) found that solarization increased concentrations of NO3-, NH4+, Ca2+, Mg2+, and Cl-. They found similar changes in a variety of soils, and propose that these chemical changes may be utilized to test the effectiveness of solarization. The chemical tests would be quicker and simpler than the biological tests currently used.

There are limitations to solarization. Some pathogens seem to remain relatively unaffected by considerable temperature achievements. Macrophomina phaseolina failed to be controlled in northern California (McCain et. al., 1982) and Arizona (Milhail and Alcorn, 1984). There is a remote possibility that repeated application will lead to the development of heat tolerant pathogens (Katan, 1981). Milhail and Alcorn (1984) found the incidence of Macrophomina phaseolina-associated plant mortality in tarped plots was always as least as high as in any control plot, insinuating that if certain conditions are met, solarization could eliminate a sufficient proportion of the soil microflora antagonistic to or competitive with M. phaseolina to result in a significant increase in disease incidence.

A third more credible limitation of solarization is its dependence on the prevailing climate. Reasonable success was

achieved in this trial, yet the season was considerably warmer than average, and future years may be less co-operative. The maximum temperature obtained in 1988 was 46°C, exceeding the 41°C (Honhart & Juzwik, 1987) peak achieved in the previous year.

f) Solarization in southwestern Ontario:

To date, solarization has had mixed success in forest nurseries. In St. Williams, control of the stubby root nematode Paratrichodorus pachydermis was achieved, as was control of C. floridanum in the upper 10 cm. Solarization is attractive in that it is economical, environmentally and ecologically acceptable, and seems to have potential in becoming a viable pest management tool.

Concurrently, it is apparent that solarization is limited in fungal control in that it reaches only the top profile of the soil, and fails to effect propagules existing in the deeper levels. Based on this limitation, there are two general directions to consider for solarization:

1) increase the effectiveness of solarization by combining it with fumigation. Although this method may work, it is self defeating in both economic and ecological considerations.

2) increase the efficacy of the technique of solarization. There are many possibilities for the enhancement of solarization, and they should be explored.

Proper usage of double layers of plastic will increase temperature achievements (C.L. Stevens, pers. comm.; Ben-Yephet et. al. 1987), this modification will be investigated at St.

Williams in 1989.

A major drawback of solarization is that upon plowing a solarized field, sclerotia from the lower soil depths that were not killed would be moved to the upper levels of the soil profile and cause infection (Adams, 1987). This 'drawback' could be used to the advantage of disease control by cultivating between treatments. St. Williams has a hot season of approximately 8 weeks. The field could be tarped mid-June to mid-July, cultivated, and tarped again. The cultivation should bring the propagules from the lower level to the upper level where they would be subjected to the higher temperatures. Disinfestation at the upper level may be accomplished in 4-6 weeks (Stapleton and DeVay, 1986; Katan, 1981). This modification of the technique will not be investigated in 1989, but it is an avenue that should be considered if a reduction in disease incidence is not realized with the 1988-89 solarization trial.

g) Further Investigations:

The temperatures attained in southwestern Ontario were comparable to those reached in the United States where solarization has been investigated. The potential seems to exist, but the technique must be altered to attain maximum effect.

At the nursery in St Williams, the efficacy of solarization will be investigated in 1989 by transplanting white spruce seedlings into the solarized and control plots established in

this study. Disease incidence and inoculum levels will be monitored throughout 1989. Growth rates will be compared by comparing dry root and shoot weights of seedlings from the solarized 1p +i and control plots. As well a second solarization trial will be set up to monitor the effect of solarization on population levels of Rhizoctonia solani, Cylindrocarpon destructans, and Fusarium oxysporum and the stubby root nematode Paratrichodorus pachydermis. The trial will utilize pre-trial irrigation, with no irrigation systems under the tarps. The treatments will consist of a control, one layer of 2 ml plastic, and two layers of 2 ml plastic separated by a gap of 5-10 cm. A datalogger will be used to monitor temperature changes at 5, 10, and 15 cm depths in one of each of the three treatment plots. Soil analysis will be done prior to and following solarization to determine the effect of tarping on the chemical properties of the soil. Soil moisture will be analyzed prior to and following solarization.

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Effect of pruning and wrenching treatments on infection of conifer seedling roots by *Cylindrocladium floridanum*

PROGRESS REPORT

J Saunders and J Juzwik

February, 1990

INTRODUCTION

Cylindrocladium floridanum, the causal agent of a conifer root rot, has been documented as a significant problem in Ontario's bareroot nurseries. In order to establish guidelines for managing root rots, cultural practices must be critically examined.

Pruning and wrenching of bareroot nursery stock is a technique that nurserymen have used for over half a century. According to Racey (1987), pruning and wrenching has five basic objectives:

- reduce top-root ratio
- increase root fibrosity
- produce seedlings with the attributes of transplants
- condition for outplanting
- condition for overwinter storage

Pruning and wrenching places large roots in contact with Cylindrocladium microsclerotia while simultaneously stressing the seedlings. Although seedlings were able to localize and fend off infections on small roots, infections of large roots cause death of the seedlings (Thies and Patton, 1970). Pruning and wrenching may be hazardous in heavily infested soil. The work reported here examined the effect of pruning and wrenching treatments on infection of conifer seedling roots by Cylindrocladium floridanum.

METHODS AND MATERIALS

The study was carried out at the provincial nurseries in Midhurst and Thessalon, Ontario.

Thessalon:

Compartment 3 at the Kirkwood Nursery (Thessalon Ontario) contained 3+0 red pine (Pinus resinosa Ait.). Two adjacent rows were used in the study. In each row, six 10 m plots were staked out. In each row, three plots were pruned and wrenched, the remaining three were used as controls. The seedlings were allowed to set bud prior to cutting. The pruner consisted of a sharp blade that cuts the seedling without shaking it. The seedlings were allowed to sit one week in moderate water stress prior to wrenching at 3". The wrenching blade was a thick 8" wide blade set on a 30° angle to cause a shaking action. The wrenching throws the bed, trees come down straight. Water stress was avoided, the seedlings were wrenched every three weeks.

Soil samples from all plots were collected in August 1988, each sample consisted of ten soil cores (15-20 cm) taken at random with a 2 cm diameter Oakfield sampling tube. The soil samples were kept in polyethylene bags and stored at room temperature until processing within four weeks.

Soil samples were processed using a modified wet sieving technique from Thies and Patton (1970) which separates fungal propagules on the basis of size and density. Soil samples (approx. 200-g) were thoroughly mixed. Three subsamples were taken from the sample, one 10-g subsample was used for determination of soil

moisture content, two separate 3-g subsamples were used in isolation procedures. Each 3-g subsample was put into an Oster Blender (Sunbeam Corp. Ltd.) mini jar containing 250 ml of distilled water. The mixture was blended for one minute. The suspension was allowed to settle for 15 seconds, the supernatant was decanted onto nested 100 (150 um) and 200 (75 um) mesh screens (C.E. Tyler, St. Catherines, Ontario). Distilled water was added to the residue and stirred into suspension on a Magmix(Precision Scientific Co.). The suspension was allowed to settle for 15 seconds, and then decanted onto the screens. The decanting procedure was repeated five times. The material collected on the 200 mesh screen was washed thoroughly and the remaining residue was immersed in a 10% NaOCl solution for 15 sec. The residue was then washed with water, and collected into a 250 ml beaker and termed the 'inoculum concentrate'. 50 ml. of dilute water agar (0.6%) was added to the inoculum concentrate. The total suspension was added to a bottle containing 90 ml. of a Cylindrocladium selective media (Phipps et. al. 1976) kept at 45 °C in a water bath. The contents of each bottle was mixed and poured into 20-25 petri-plates. The plates were incubated at room temperature, colonies were examined ten days later. Colony counts from each subsample were totalled and corrected for moisture content and reported as propagules/gram of dry soil. Soil moisture was calculated for every soil sample using the following formula:

(Sw-Sd)/Sd X 100 = % moisture content (%mc)

where: Sw = weight of moist soil

Sd = weight of oven dried soil

propagule density was calculated as:

propagules in 3 grams/ 3 X (1-mc)

The two subsample variables reported as propagules/gram were averaged, the value achieved represented one sample.

Although the media was selective for Cylindrocladium, the presence of Cylindrocarpon and Fusarium was also recorded.

Seedling counts were conducted in July 1988 (pre-treatment) and October 1988 (post-treatment). Two counts were conducted at one, three, and nine meters into the plot. Counting was done with a 10 cm X 1.07 m frame which was laid across the bed. The pretreatment counts assessed the density prior to pruning and wrenching, the post-treatment counts assessed the effect of pruning and wrenching. Final tree stand, expressed as a percentage of initial tree stand, was calculated by use of the following formula:

Final Tree Stand (%) = (Final Tree Count/Initial Tree Count) X 100

Midhurst:

A randomized block design was used to investigate the effect on pruning and wrenching on disease incidence in jack pine (Pinus banksiana Lamb., red pine (Pinus resinosa Ait., and white pine (Pinus strobus L.). Seedlings were planted in plots 10 m long. On May 20, 1987, the trial area was seeded using an eight drill seeder. On May 21, the area was mulched and sprayed with five lbs/acre 75W Dacthal. Mulch was applied according to operational practices. The field was irrigated after seeding, and then at intervals deemed to mimic operational practices.

In 1988, seedlings were pruned and wrenched as follows: June 17-pruned at 2.5-3", July 14-wrenched at 4-5",July 27-wrenched at 4-5", August 18-wrenched at 5-6", Sept 7-wrenched at 5-6".

Soil was collected in July 1987, 1988, 1989 and processed according to the method described earlier. 100 seedlings/plot were selected (stratified random sampling) from each plot in July, 1988 and 1989. Seedlings were sealed in a plastic bag and placed in cold storage (4 °C) until processing. Roots were separated from shoots, washed, and surface sterilized with 10% javex, and rinsed in sterile water. Symptomatic areas were sectioned from the root under sterile conditions and plated on selective media. After ten days, the plates were examined. Although the media used was selective for Cylindrocladium, the presence of Cylindrocarpon and Fusarium was also recorded. If Cylindrocladium, Cylindrocarpon, or Fusarium were recovered from any root section, the seedling was considered infected.

Seedling counts were conducted pretreatment (July,15,1988) and post-treatment (October, 1988). Seven counts/plot were taken using a 15 cm X 122.5 cm frame which was laid across the bed. The pretreatment counts assessed the density prior to pruning and wrenching, the post-treatment counts assessed the effect of pruning and wrenching. Final tree stand, expressed as a percentage of initial tree stand, was calculated by use of the following formula:

Final Tree Stand (%) = (Final Tree Count/Initial Tree Count) * 100

RESULTS

Thessalon:

Cylindrocladium floridanum was recovered from all 12 plots monitored in the study (Table 1). In both the pruned and the control plots, infection rate was low prior to pruning and wrenching (Table 2) and remained low following treatment (Table 3). The treatment had no effect on stand count (Table 4).

Midhurst:

Due to heaving that was not anticipated by the nurserymen, the seedbeds of red pine and white pine were lost by the spring of 1988. The jack pine did survive and the trial was carried out with the one remaining species.

Cylindrocladium floridanum was recovered from all eight plots used to assess the treatment effect on jack pine (Figure 1). In both the pruned and control plots, infection rate was low prior to pruning and wrenching (Table 5), and remained low after the treatment (Table 6 & 7). Pruning and wrenching had no effect on stand count (Table 8).

Table 1-Population (propagules/g dry soil) of Cylindrocladium floridanum in pruned and control plots, compartment 3, Thessalon Nursery, Aug 15, 1988.

plot	average	maximum	minimum
pruned	16.19	28.62	4.48
control	15.07	44.83	.48

Table 2- Incidence of root rot (%) prior to pruning and wrenching, compartment 3, Thessalon Nursery, 1988.

treatment	Cylindrocladium	Cylindrocarpon	Fusarium
pruned	1.5	6.5	1.5
control	2.0	6.5	2.2

Table 3-Incidence of root rot (%) following pruning and wrenching, compartment 3, Thessalon Nursery, 1988

treatment	Cylindrocladium	Cylindrocarpon	Fusarium
pruned	0	5.7	3.0
control	0.5	3.0	0.0

Table 4- Seedling density before and after pruning and wrenching treatment, compartment 3, Thessalon Nursery, 1988

treatment	July initial tree stand/ .1 m^2	October final tree stand/ .1 m^2	final tree stand %
pruned	41.7	44.7	107.2
nonpruned	41.0	41.3	100.7

Figure 1. Population (propagules/g dry soil) of Cylindrocladium floridanum in pruned and control plots at three different times, compartment A33, Midhurst Nursery

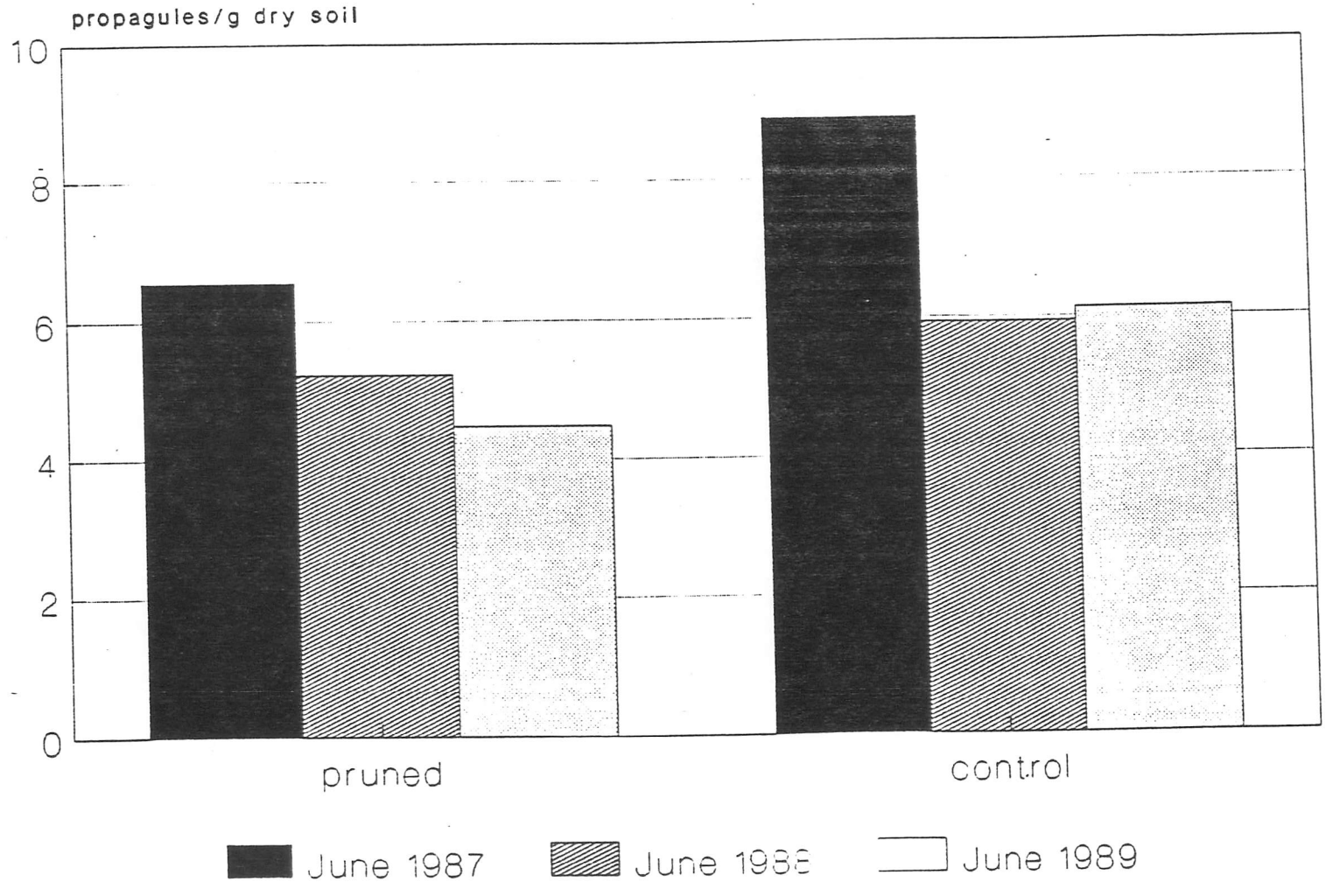

Table 5- Incidence of root rot (%) prior to pruning and wrenching, compartment A33, Midhurst Nursery, 1988.

treatment	Cylindrocladium	Cylindrocarpon	Fusarium
pruned	0.0	0.2	0.8
control	0.0	0.3	1.0

Table 6- Incidence of root rot (%) one month following pruning and wrenching compartment A33, Midhurst Nursery. 1988.

treatment	Cylindrocladium	Cylindrocarpon	Fusarium
pruned	0.3	1.3	2.3
control	0.2	0.2	0.7

Table 7- Incidence of root rot (%) ten months following pruning and wrenching, compartment A33, Midhurst Nursery, 1988.

treatment	Cylindrocladium	Cylindrocarpon	Fusarium
pruned	0.0 %	0.2 %	1.0 %
control	0.0 %	1.2 %	1.2 %

Table 8- Seedling density before and after pruning and wrenching treatment, compartment A33, Midhurst Nursery, 1988.

treatment	July initial tree stand .18 m^2	October final tree stand/ .18 m^2	final tree stand (%)
pruned	35.5	33.5	94.4
control	36.3	35.1	96.7

DISCUSSION

Thessalon:

Prior to treatment, root rot was low in all plots. Treatment did not effect disease incidence or tree stand. Although the soil inoculum level required to bring on root rot is not well defined, it is likely that levels recorded in July, 1988 were sufficiently high enough to be considered a hazard. The presence of Cylindrocarpon before and after treatment raises consideration to its role in root rots. Although the presence of Cylindrocarpon was recorded more often than Cylindrocladium, there was no obvious damage associated with it. It may be possible that disease expression cannot be monitored within three months of treatment.

Midhurst:

Root rot incidence was low prior to treatment, and remained low ten months after pruning and wrenching. Tree stand was not effected by pruning and wrenching. Cylindrocladium floridanum was present in all eight plots used to study treatment effect on jack pine.

Failure of pruning and wrenching to increase disease incidence has been previously reported (Petaisto, 1982). McGowan & Juzwik (1987) found that pruning and wrenching leads to higher incidence of root infection, yet the increase is not sufficient to justify a change in management practice.

Although increased disease incidence from pruning and wrenching has not yet been illustrated, the resulting hazard exists. Possibly, disease potential always exists in infested soils, but

the hazard is only realized under certain environmental conditions. Cylindrocladium root rot damage is greater during periods of moisture stress (Thies & Patton, 1970); if large roots are severed and placed in dry, infested soil, the potential for disease incidence seems high. From their work with Cylindrocarpon, Unestam et. al. (1989) suggest that root cutting, in combination with predisposing factors (light starvation, flooding) create a hazardous situation.

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Outplant Mortality of Black Spruce Seedlings Infected With *Cylindrocladium floridanum*

Progress Report

March, 1990

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INTRODUCTION

Cylindrocladium floridanum Sob. and Seymour has been confirmed in the ten bareroot nurseries in Ontario (Myren et. al., 1975, 1979; Juzwik et. al., 1987; Juzwik, pers. comm.). Losses of up to 33 % have been documented for six compartments in five provincial tree nurseries (Juzwik et. al. 1988). The number of cull seedlings in the compartments due to C. floridanum infection ranged from 225 to 176,000 seedlings (Juzwik et. al. 1987). At an average cost of $150/1000 trees, the monetary loss due to the fungus in the compartments at assessment time was estimated to be $65,000. Substantial losses due to cylindrocladium root rot are not uncommon. Thies and Patton (1971) report that Cylindrocladium caused a 60 - 90 % mortality of conifer transplants in three Wisconsin nurseries. At the nursery in Knife River, Minnesota, the fungus destroyed approximately 80 % of the black spruce seedlings, and was responsible for closure of the nursery (Menge and French, 1976).

Losses caused by C. floridanum are not restricted to culls and mortality in nursery beds since significant losses may occur after outplanting. Information on field performance of outplanted infected stock is very limited. To assess the ability of black spruce to recover from the disease, McGauley (1983) lifted seedlings from an infected nursery compartment, and accepted only those that passed a grading system using height, stem diameter, single leader, and colour. Four months after outplanting, mortality was recorded at 39.6 %.

Symptoms of cylindrocladium root rot on black spruce have been described by Anderson et. al. (1962): seedlings become chlorotic, and in an advanced stage of the disease, the current year's growth droops. The outer cortex dies, turns dark brown, and is easily peeled from the root.

This study was undertaken to determine the survival of black spruce seedlings infected with Cylindrocladium floridanum following field planting. Seedlings were rated according to foliar symptoms and/or root infection location upon lifting and prior to planting.

METHODS AND MATERIALS

At lifting time, April 28, 1988, 1 + 2 black spruce seedlings were selected from compartment 41 stock at the G. Howard Ferguson Nursery, Kemptville, Ontario. Seedlings were classified into four categories according to foliar or root condition: MRL - seedlings with a main root lesion but healthy appearing foliage; LRL - seedlings with a lateral root lesion but healthy appearing foliage, SYM -symptomatic seedlings exhibiting foliar symptoms of cylindrocladium root rot with or without visible root infections.; HEA - seedlings with an apparently healthy root and shoot system. On April 29, 1988, 350 (100 SYM, 100 HEA, and 99 MRL and 51 LRL) seedlings were selected and subsequently placed in small polybags lined with damp moss to keep roots moist while in cold storage (0.5 °C) until planting.

The second trial was conducted with 1.5 + 1.5 black spruce seedlings from compartment 21 at the provincial nursery at Thunder Bay, Ontario. On May, 20, 358 (100 SYM, 100 HEA, 89 MRL, 69 LRL) seedlings were selected and subsequently placed in small polybags lined with damp moss to keep roots moist while in cold storage (0.5 °C) until outplanting on May 27.

Since the Thunder Bay stock was lifted almost four weeks later than the Kemptville stock, particular attention was paid to the physiological state of the seedlings at the time of outplanting. At outplanting, symptomatic seedlings exhibited a chlorotic appearance from top down on the terminals, the roots were active with white tips up to 1 cm long. Terminal buds were dormant to

swollen, lateral buds were swollen, breaking, and beginning to flush. Approximately 20% of the needles had browning, approximately 10% had red tops.

The seedlings classified as HEA were a healthy green in appearance, roots were very active with white tips 1 to 2 cm long. Terminal buds were swollen to breaking, lateral buds were swollen to flushing. The needles had no browning, approximately 5% had red tops which is typical of winter damage in spruce.

LRL seedlings had active roots with white tips 1 to 2 cm long. Terminal buds were swollen,lateral buds were flushing. Needles had no browning, 10 -25% had red tops.

The MRL seedlings had active roots with white tips ranging from 1 to 2 cm. Terminal buds were swollen to breaking, lateral buds were flushing. Needles had approximately 2% browning, 5 - 10 % had red tops.

The two outplant trials were located at the Ontario Tree Improvement and Forest Biomass Institute Research Unit, in Midhurst, Ontario. The trial site had been sown down to rye in the fall of 1986, plowed and summer fallowed during 1987, plowed in the fall of 1987, and cultivated prior to planting in 1988. The soil type was clay loam, the planting method used was the modified wedge. Weed control was not used on the trial sites.

The trial designs were randomized complete blocks with four treatments (SYM, MRL, LRL, HEA) and four replications. Seedlings were spaced .76 X .76 m apart. Each block consisted of five rows, 20 spaces per row. The total area utilized per trial was 14.5 X

14.5 m. Monitoring of the Kemptville stock was performed weekly from May 6 to November 22. Monitoring of the Thunder Bay stock followed the same schedule but did not begin until June 7. Seedlings were monitored using 5 codes:

1: No apparent change from planting or last monitoring date.

2: Tree is yellowing or turning off colour.

3: Lateral or terminal branches drooping (new and old growth).

4: Seedling dead.

5: Seedling removed and sent to laboratory for isolations. Seedlings exhibited definite, well progressed chlorosis and drooping laterals or terminals.

Seedlings were kept in cold storage (2°C) in the laboratory until root isolations were performed. Roots were washed several times and examined for lesions. Root segments with necrotic lesions were aseptically removed and placed in 10% NaOCl for three minutes. After rinsing each segment with distilled water, four thinly sliced chips (approx. 5 mm in length) were aseptically removed from the root section, and plated onto a Cylindrocladium selective medium (Phipps et. al., 1976). After 10-14 days, the plates were examined for C. floridanum. If the fungus was recovered from any root section, the seedling was considered infected, and the fungus was considered the cause of seedling death.

The percent mortality (attributed to the fungus) was calculated for each category, and the percentages were transformed to arcsins for analysis. An analysis of variance with Duncan's multiple range test was conducted on the mortality data. As well,

a chi square analysis was performed, with differences being separated out using the Bonferroni-Zed statistic.

The following formula was used to calculate Bonferroni confidence intervals:

$$\bar{P} \pm \frac{Z_{\alpha}}{2} \sqrt{\frac{[P \quad X \quad (1 - P)]}{N}}$$

P = Proportion that died from C. floridanum

N = Sample Size

RESULTS AND DISCUSSION

With both the Kemptville and the Thunder Bay stock, mortality in the SYM category was severe after six months of outplanting. The SYM category consisted of seedlings exhibiting foliar symptoms of root rot at the time of lifting yet had passed the grading system used at the nursery. A modified grading system could cull SYM seedlings and thereby increase outplant survival.

In both trials, MRL seedlings had a severe mortality rate. Generally, seedlings chosen in this category displayed no top symptoms of root rot. Similar situations of seedlings afflicted with root rot but exhibiting no foliar symptoms have been reported (Trobaugh, J. et. al., 1987; Thies and Patton, 1970). Main root lesions are obvious to the naked eye, and could be graded out. The MRL seedlings used in this study, were selected from graded seedlings.

LRL seedlings had a substantial mortality rate, especially in the Kemptville stock. Generally mortality in LRL category was not as drastic as the MRL and SYM category.

The lowest mortality was recorded in the HEA category, yet a loss of 20 % occurred with the Thunder Bay stock. This high loss among healthy-appearing seedlings is consistent with the loss of 25% recorded by Thies and Patton (1971).

Chi square was used to test the independence of the classification system. With both the Thunder Bay and the Kemptville data, the null hypothesis was rejected ($p > .005$). The differences determined by Duncan's multiple range test were

differences determined by Duncan's multiple range test were supported by the Bonferroni confidence intervals. (Figures 3 & 4).

Several conclusions may be drawn from this study. Black spruce seedlings exhibiting shoot symptoms of cylindrocladium root rot, have a poor field performance. SYM seedlings could be graded out by foliar appearance, MRL seedlings could be graded out by examining the root collar area. If the nursery compartment is badly infected, further losses may be expected from seedlings exhibiting no obvious signs of root rot. This data is most useful in that it may be used to predict mortality rates of seedlings outplanted from infected nursery compartments (Table 3). The mortality will be followed into the second year of outplant.

TABLE 1: Mortality[1] of black spruce seedlings (Kemptville stock) 6 months after outplanting at the Midhurst Research Unit, 1988.

Treatment	Mortality[2] (%)[3]
Healthy	14 c
Symptomatic	37 a
Lateral Root Lesion	29 b
Main Root Lesion	34 ab

[1] Only mortality caused by C. floridanum is reported

[2] Means followed by the same letter are not significantly different (P = 0.05)

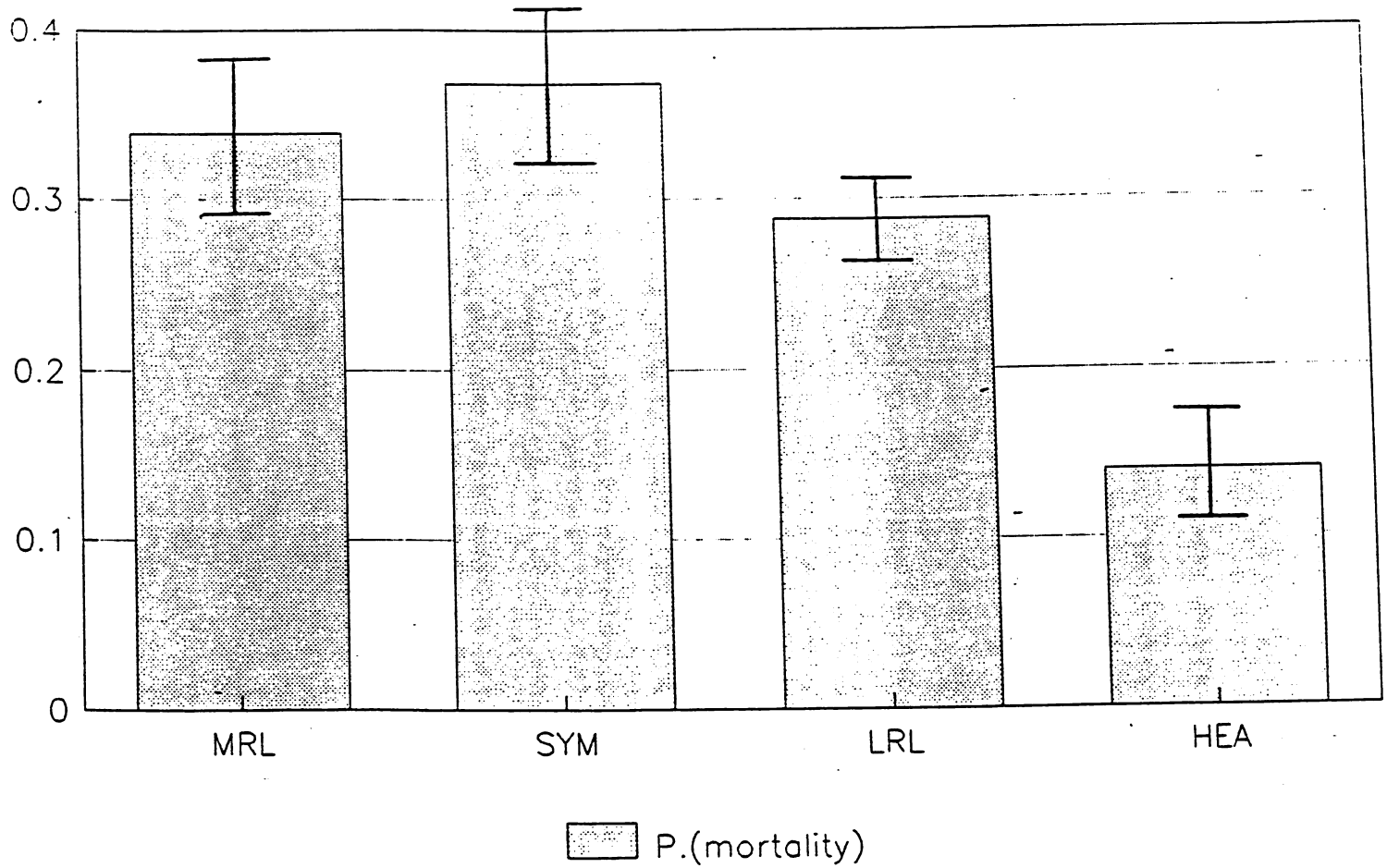
[3] Percentages subjected to arcsin transformation.

TABLE 2: Mortality[1] of black spruce seedlings (Thunder Bay stock) 6 months after outplanting at the Midhurst Research Unit, 1988.

Treatment	Mortality[2] (%)[3]
Healthy	20 b
Symptomatic	39 a
Lateral Root Lesion	23 b
Main Root Lesion	41 a

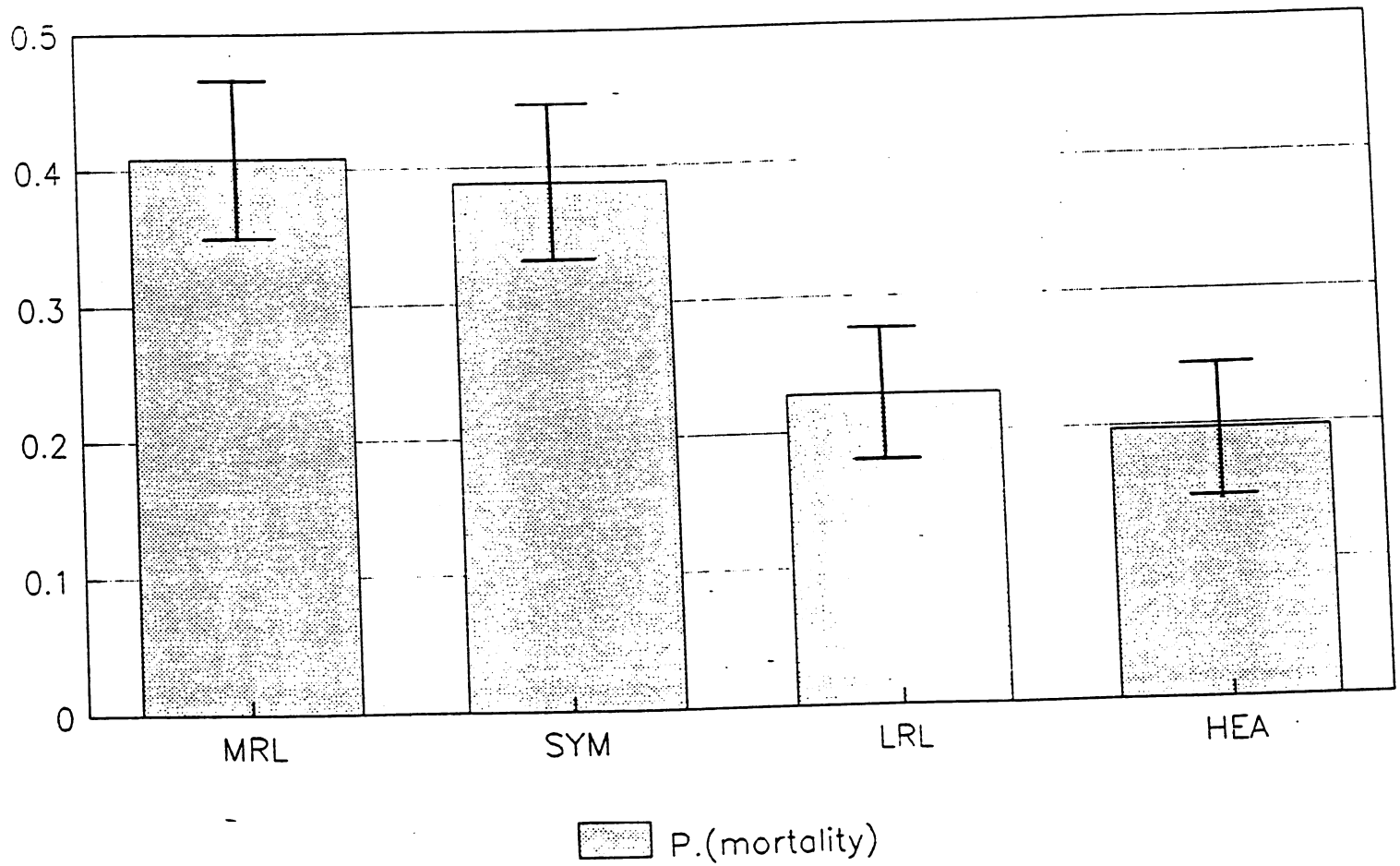
[1] See Table 1
[2] See Table 1
[3] See Table 1

Figure 1. Mortality of black spruce seedlings (Kemptville stock) 6 months after outplanting at the Midhurst Research Unit: Bonferroni Confidence Intervals.



MRL= Main Root Lesion / SYM= Symptomatic
HEA= Healthy / LRL= Later Root Lesion
P.= Proportion

Figure 2. Mortality of black spruce seedlings (Thunder Bay stock) 6 months after outplanting at the Midhurst Research Unit: Bonferroni Confidence Intervals.



MRL= Main Root Lesion / SYM= Symptomatic
HEA= Healthy / LRL= Lateral Root Lesion
P.= Proportion

TABLE 3. Mortality predictions for black spruce seedlings outplanted from infected nursery compartments

Category	Expected Loss after 6 months outplant (%)
Main Root Lesion	30 - 40
Symptomatic	30 - 40
Lateral Root Lesion	20 - 30
Healthy	10 - 20

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Effect of Basamid granular fumigation on populations of *Fusarium oxysporum* and *Cylindrocarpon destructans* at the bareroot nursery, Midhurst, Ontario

J.E. Saunders
J. Juzwik

PROGRESS REPORT - FEBRUARY, 1990

INTRODUCTION

Root rot diseases of seedlings in nurseries are caused mainly by pathogenic organisms; physiological causes are of relatively minor importance (Vaartaja, 1964). In the Pacific Northwest, soilborne diseases are the most numerous and economically important pest problems facing bareroot nurseries (McElroy, 1984). Likewise, in Ontario, soilborne pathogens are a significant concern to the nursery managers. It is believed that three fungi are responsible for the majority of losses in the provincial bareroot nurseries: Cylindrocladium floridanum, Cylindrocarpon destructans, and Fusarium oxysporum. While losses due to Cylindrocladium root rot have been reported (Juzwik et. al., 1988), impact data on root rot caused by F. oxysporum and C. destructans is presently being gathered. Although Fusarium has been the subject of much study, Cylindrocarpon root rot is not yet fully understood (Sutherland et. al., 1989; Unestam et. al., 1989). Both fungi have a variety of hosts including white spruce (Picea glauca (Moench) Voss) and white pine (Pinus strobus L.).

The most common means of controlling nursery diseases in seedbeds is by application of biocides. Broad spectrum biocides may be used as soil fumigants to control many plant pathogens, weeds, and nematodes. At the nurseries in Midhurst and St. Williams, Ontario, Basamid has been used in problem fields. Basamid granular (active ingredient: dazomet) has nematocidal, fungicidal, and herbicidal properties. When the active ingredient

3,5-dimethyl-1,3,5,-tetrahydrothiadiazine - thione -(2) comes into contact with moist soil, it breaks down into several compounds: methyl mustard oil (methylisothiocyanate)MITC, methyl amine, hydrogen sulphide, and formaldehyde. Methyl mustard oil is mainly responsible for the biological action of dazomet in the soil. Formaldehyde also has a biological effect. According to BASF (1975) basamid should be incorporated into the soil to at least 30 cm in order to achieve a reduction (not eradication) of infestation. The ability of fumigation to control root diseases is supported by various investigators (Thies and Patton, 1971; Smith and Bega, 1966).

Despite the many successes achieved with fumigation, the practice raises several concerns. Along with the pests, biocides also kill many organisms that are considered beneficial antagonists. Since the saprophytic activity of a pathogen is affected by the community of its microbial associates (Marois and Mitchell, 1981), the practice of fumigation which reduces microbial antagonism, could actually enhance the disease problem (Vaartaja, 1964; Kreutzer, 1960). There is also the possibility that fumigation may lead to stunted seedlings due to the killing of mycorrhizae (Menge, 1982).

Soil borne pathogens that grow poorly in untreated soil may grow readily as saprophytes in soil in which the microbial community has been upset by fumigation (Marois and Mitchell, 1981). The saprophytic potential of Fusarium, and its ability to reinvade fumigated soils is well documented (Hine, 1962; Marois

et. al., 1983).

Most of the fumigation studies found in the literature report on methyl bromide-chloropicrin (MBr-C). MBr-C is not used in Canada due to registration and economic considerations. Studies on the fungicidal capability of dazomet have only recently been considered. The purpose of the work reported here was to evaluate the effect of dazomet on the populations of Fusarium oxysporum and Cylindrocarpon destructans. The work was centred at the provincial tree nursery in Midhurst Ontario.

MATERIALS AND METHODS

Two independant trials were carried out at the Midhurst nursery between 1988 and 1989.

Compartment C1:

The trial in compartment C1 at the Midhurst nursery consisted of six basamid treated plots (each 4 m X 3 m) , and three controls. The areas used as controls were covered with plastic during fumigation. Prior to fumigation, the compartment was cultivated and irrigated to field capacity to insure rapid breakdown of the active ingredient in a uniformly moist environment. Basamid granular was spread evenly into the soil and then disced to a depth of approximately 20 cm. The application rate was 500 kg per hectare.

Populations of F. oxysporum and C. destructans were measured at three times: pre-treatment on August 09, 1988, post-treatment on September 28, 1988, and pre-sowing on June 15, 1989. All nine plots were sampled, and each sample consisted of ten soil cores taken at random with a 2 cm diameter Oakfield sampling tube. Soil samples were collected at 0-10 cm depth. The soil samples were kept in polyethylene bags and stored at room temperature until processing within four weeks.

Compartment C13:

A second trial was established in compartment C13 at the nursery in 1988. The trial consisted of 17 Basamid treated plots (each 4 m X 3m) and three controls. Populations of F. oxysporum and C. destructans were measured at three times: pretreatment in August

1988, post-treatment in September 1988, and presowing in June 1989. The areas used as controls were covered with plastic during fumigation. Field preparation and fumigation application was identical to that described for compartment C1.

Fungal populations:

Quantitative estimates of the relative populations of F. oxysporum and C. destructans in the soil were determined using dilutions. Each sample of soil was thoroughly mixed, three subsamples were removed from the sample, two 3 g subsamples were used in the isolations procedures, one approx. 10 g sample was dried at 105 °C to a constant weight for determination of moisture content.

Techniques differed slightly for Cylindrocarpon and Fusarium. A 10^{-1} dilution was made for Fusarium which is characterized by a high population density. Each 3 g subsample was put into an Oster Blender mini jar containing 250 ml of distilled water. The mixture was blended for 15 seconds, the suspension was allowed to settle for 15 seconds, the supernatant was decanted into a 500 ml beaker. 250 ml distilled water was added to the supernatant and stirred into suspension on a magmix (Precision Scientific Co.) Five drops of tween was added to the suspension.

F. oxysporum:

One ml of the suspension was added to 9 ml distilled water and mixed. 5 ml of the stock solution was added to a beaker containing 50 ml of melted and cooled (45 °C) selective media. After being mixed, the contents of the beaker were poured into

five petri plates.

C. destructans:

Five ml of the stock solution was added to a beaker containing 50 ml of melted and cooled (45 °C) media. After being mixed, the contents of the beaker was poured into five petri plates.

In ten days, the plates were checked for cultural morphology typical of F. oxysporum and C. destructans.

Data Analysis:

Pretreatment:Posttreatment and Presow:Posttreatment ratios were calculated using mean population values. The Wilcoxan Ranked Sum Test was used to compare treatment ratio values to control ratio values.

RESULTS

The mean population of *F. oxysporum* and *C. destructans* in control plots did not fluctuate greatly during the trial period (Figures 1-4). For both fungi, in both compartments, there was a sharp reduction in mean populations in soil immediately following fumigation. By comparing post-treatment to presow levels, the increase in populations of both fungi in both compartments is evident.

For both fungi, in both compartments, the mean ratio (pre-treatment population:post-treatment population) of the fumigated plots was significantly different from the mean ratio found for the control plots (Table 1). That is, fumigation resulted in significantly lower populations of the fungus when compared to populations in the controls.

In compartment C13, the mean ratio (presow:post-treatment) for the fumigated and the control plots were significantly different for both fungi. This result suggests that the fungus populations in the fumigated plots were still significantly lower than populations in the controls seven months following fumigation.

In compartment C1, with both fungi, the mean ratio (pre-sow:post-treatment) of the fumigated plots were not significantly different from the mean ratios of the controls. That is, the fungus populations in the fumigated plots were no longer significantly lower than populations in the controls seven months after treatment.

Figure 1. Mean populations of Fusarium oxysporum before and after fumigation with Basamid, compartment C13, Midhurst Ontario.

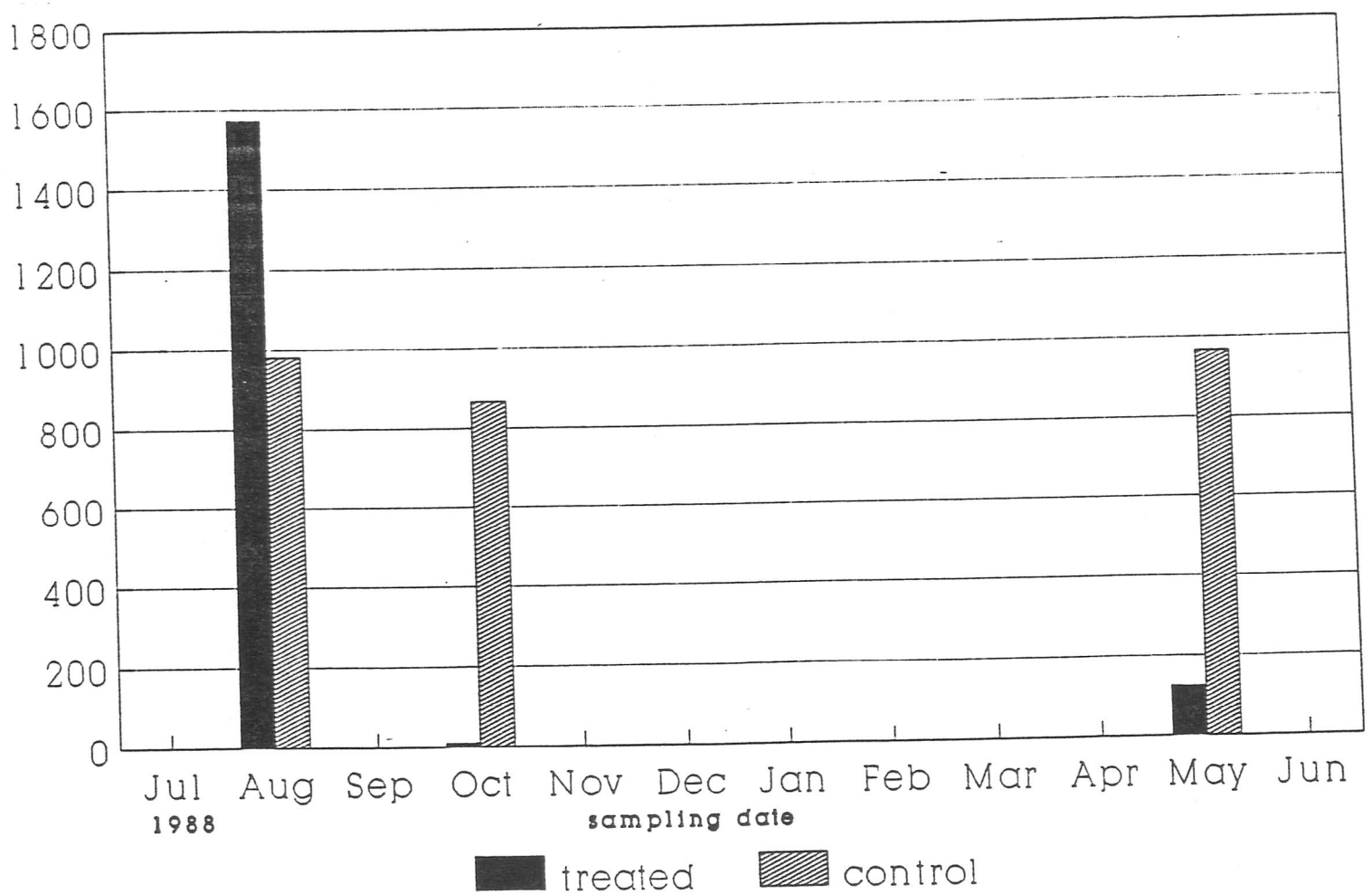


Figure 2. Mean populations of Cylindrocarpon destructans before and after fumigation with Basamid, compartment C13, Midhurst Ontario.

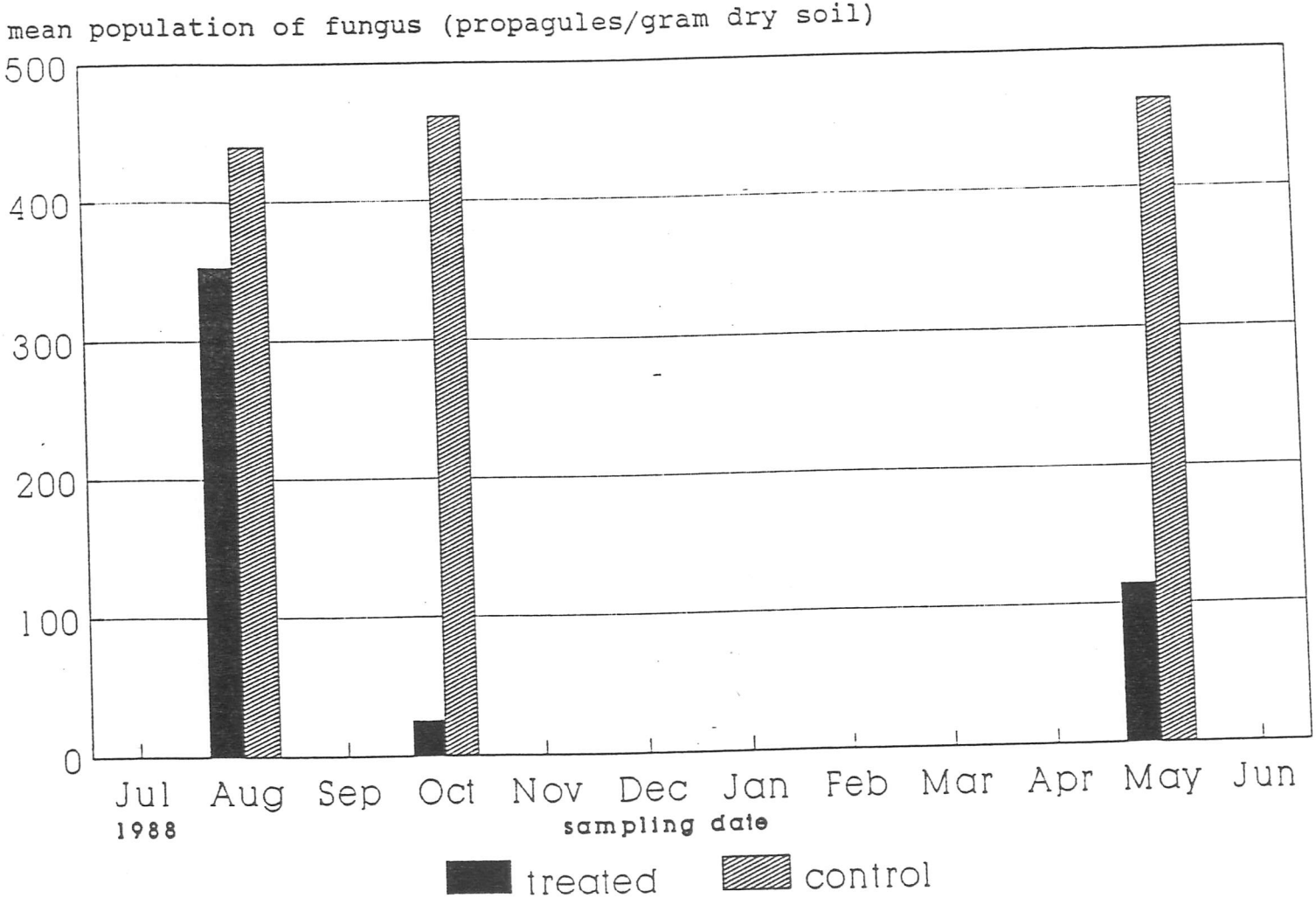


Figure 3. Mean populations of Fusarium oxysporum before and after fumigation with Basamid , compartment C1, Midhurst Ontario.

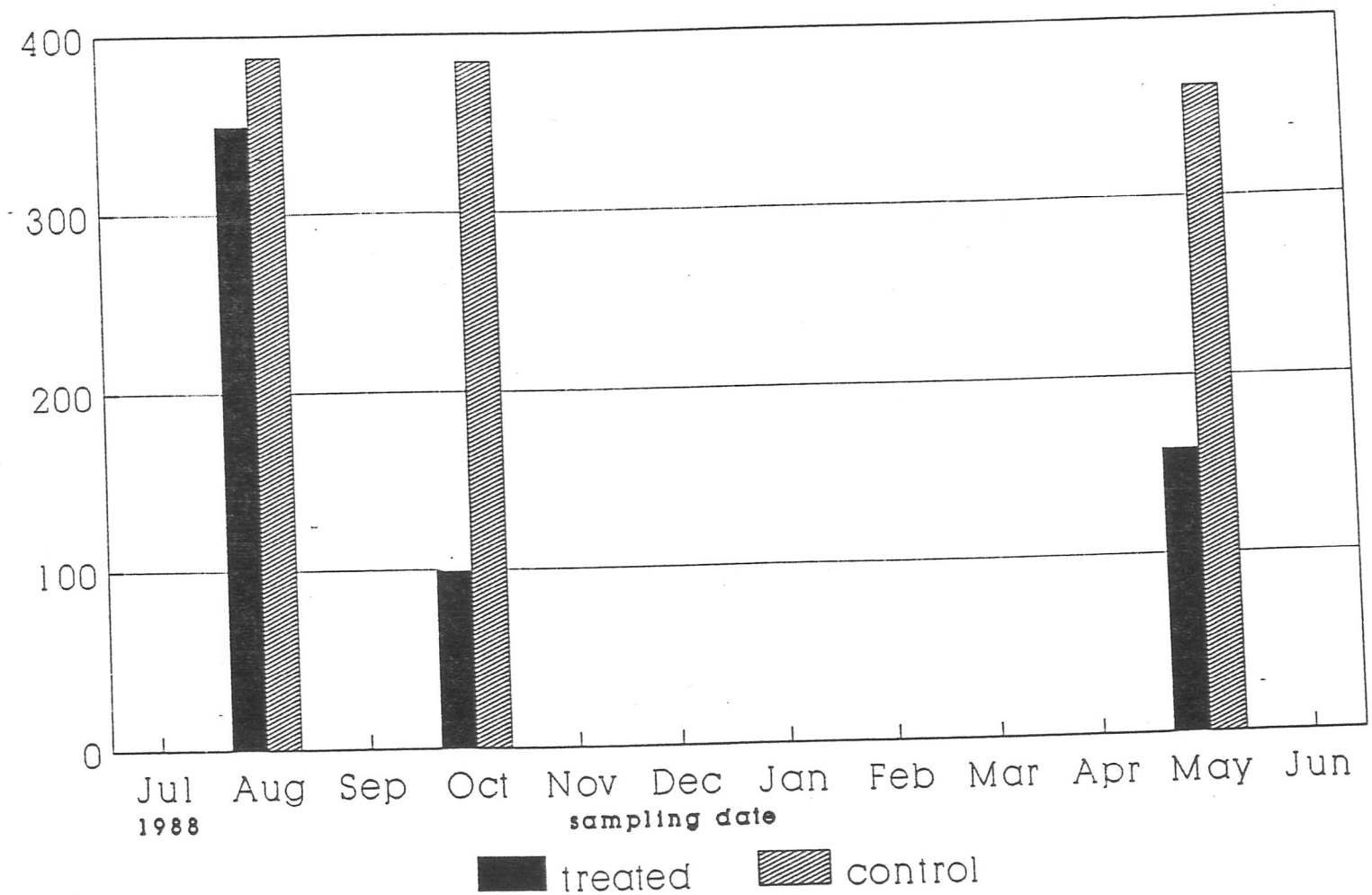
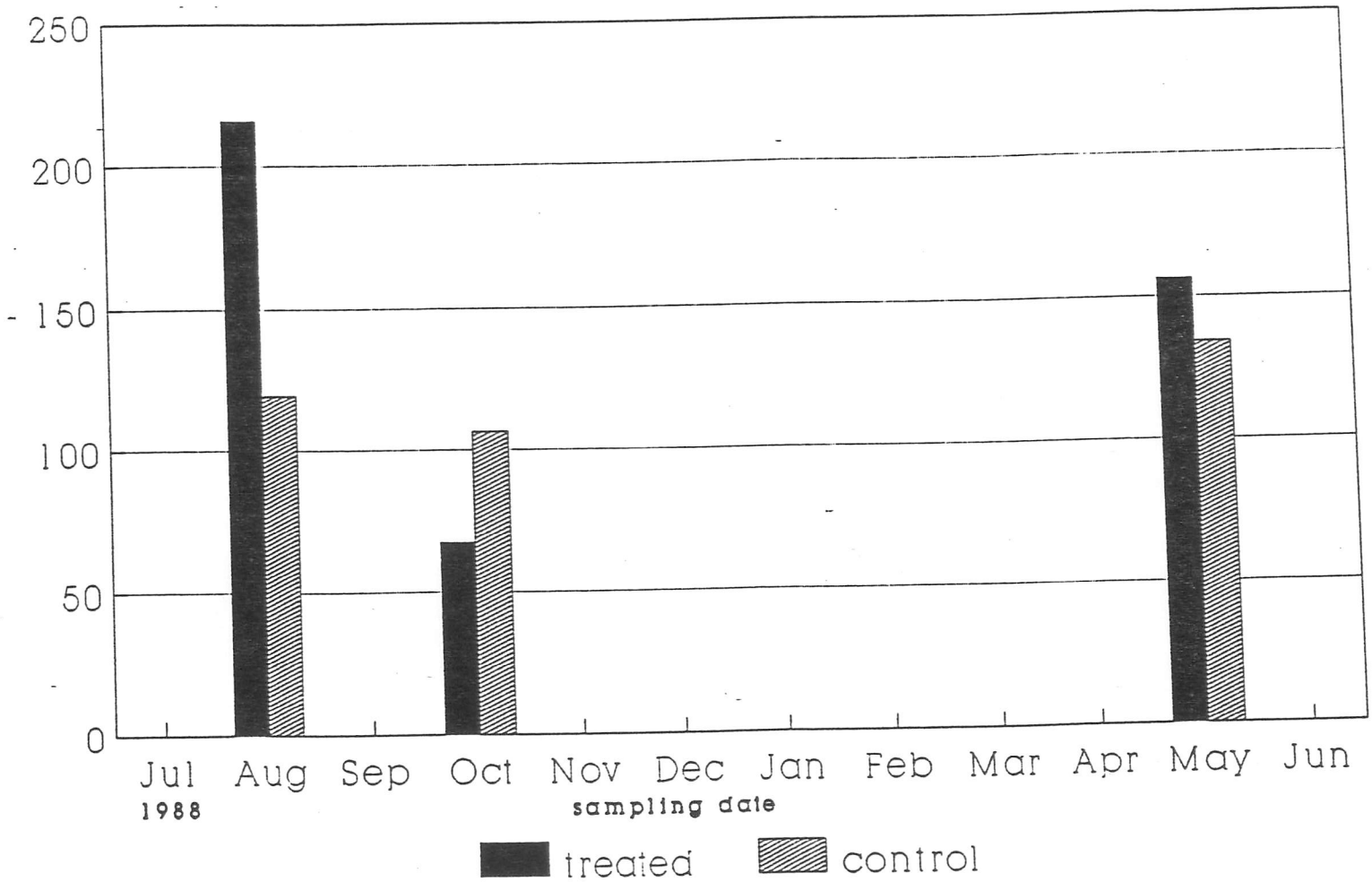


Figure 4. Mean populations of Cylindrocarpon destructans before and after fumigation with Basamid, compartment C1, Midhurst Ontario.



RESULTS

Table 1. Comparison of fungal populations (post-fumigation/pre-fumigation and presow/post-fumigation) using the Wilcoxan Ranked Sum Test, compartment C13, Midhurst, Ontario, 1989-1990.

Compartment	Fungus	Time Comparisons	Mean population Ratio	
C1	C. destructans	Oct/Aug	0.2654*	0.9928
	C. destructans	May/Aug	1.4242	1.3549
	F. oxysporum	Oct/Aug	0.1530*	0.9904
	F. oxysporum	May/Aug	0.2963	0.9324
C13	C. destructans	Oct/Aug	0.1490*	1.0483
	C. destructans	May/Aug	0.1671*	1.0562
	F. oxysporum	Oct/Aug	0.0073**	0.9297
	F. oxysporum	May/Aug	0.1145**	1.0012

* significant at .05% level
** significant at .01% level

DISCUSSION

Several studies have demonstrated that water sealed basamid is ineffective in reducing Fusarium propagules (Hildebrand & Dinkel, 1988; Campbell & Kelpas, 1988). The efficacy of fumigation would be significantly enhanced by tarping the treated soil with polyethylene (Thies & Patton, 1971; Sinclair et. al. 1975). Although effective, tarping fumigated soil is prohibitively expensive and thus is not a viable alternative. Hildebrand & Dinkel (1988) found that water sealed dazomet treatment was ineffective in controlling Fusarium spp. propagules, but polyethylene-sealed basamid could be an effective substitute for methyl bromide fumigation. Hansen et al (1990) found that fumigation with dazomet or methyl bromide, in combination with tarping, reduced populations of Fusarium spp and Pythium spp and the reduction was carried throughout the two year crop cycle. Enebak et. al. (1990) found that dazomet did significantly lower ($p=.05$) Fusarium levels, but nine months after treatment, soil populations in treated soils were not different from those in nontreated soils.

The limited success reported in this study may in part be due to the higher application rates used (approx. 440 lbs/acre as compared to 350 lbs/acre used in almost all other studies). Local soil conditions also must be considered.

Given that dazomet will only be effective if polyethylene tarping is added, alternative methods should be considered. Covercropping should be investigated as a means of inoculum

control.

In British Columbia, nurseries are run successfully without resorting to fumigation (Sutherland, 1984). At the end of their study, Hansen et. al (1990) found that population densities of Fusarium in unfumigated plots were comparable with those in cover-cropped, fumigated plots. Since the saprophytic activity of Fusarium oxysporum decreases due to competition in naturally recolonized and artificially augmented soils (Marois and Mitchell, 1981; Rowe and Farley, 1978) , the practice of fumigation which reduces competition must be critically evaluated.

Bloomberg (1976) reported that diseased roots of seedlings killed by Fusarium root rot in previous crops are likely the prime inoculum source for the disease. Fusarium oxysporum tends to be associated with large root segments (>4 mm). Thus removal of diseased seedlings, and thorough cultivation of diseased spots to encourage decomposition of diseased roots will assist in inoculum control. Removal of the sources of inoculum, along with fallow fields may offer an alternative to fumigation. Similarly, dead roots left in nursery soil after earlier harvests may act as reservoirs of Cylindrocarpon inoculum for long periods and pose a threat to new plants (Unestam et. al., 1989).

This study did not investigate the critical concern of the effect of fumigation on seedling survival and growth. Campbell and Kelpas (1988) found that although dazomet did not reduce Fusarium propagule counts in the soil, the fumigant performed as

well as methyl bromide-chloropicrin in terms of seedling survival and growth. Hansen et. al. (1990) reported that on fumigated plots, the trees had greater stem diameter and greater shoot to root ratio. Seedlings from unfumigated beds were smaller and more variable in size than those from fumigated beds.

The varied results reported are partially due to the various stages the propagules may be in at the time of treatment. In the present study, the successful control of Fusarium may be due to the propagules being in a germinating state.

The effect of dazomet application on Cylindrocarpon populations has not previously been reported on. Unestam et. al. (1989) found that fungicides seem to have little effect on Cylindrocarpon but strongly suppress its antagonists (eg- Trichoderma) and therefore can lead to an increase in the infection potential of the pathogen. This observation, accompanied with the findings of this study, infer that reinvasion is a possible danger of fumigation.

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EFFECT OF TELONE IIB, BENOMYL, AND EXPERIMENTAL CHEMICAL SN55B ON WHITE PINE DIEBACK AT THE ST WILLIAMS TREE NURSERY

PROGRESS REPORT

MARCH, 1990

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INTRODUCTION

Over the past few years, Ontario's southern tree nurseries have experienced substantial losses in their white pine compartments. White pine root rot has been reported previously (Riffle, 1959) in Michigan. The white pine 'dieback' disease in Ontario, similar to the situation described by Riffle (1959), appears to be a complex involving the stubby root nematode Paratrichodorous pachydermis and the fungi Fusarium oxysporum and Cylindrocarpon destructans.

Fusarium oxysporum has been associated with root rot of conifers in forest nurseries (Bloomberg, 1973; Riffle and Strong, 1960). The colonization of host roots by F. oxysporum can cause mortality but, often, root infections by Fusarium appear symptomless in foliage. F. oxysporum was isolated from the roots of over 80% of apparently healthy seedlings sampled from a forest nursery in British Columbia (Bloomberg, 1966). Cylindrocarpon destructans (Zins.) Scholten (=C. radicicola) has also been associated with root rot of nursery conifers (Unestam et. al. 1989, Booth, 1966; Vaartaja and Cram, 1956). It has been reported in association with root disease in Douglas-fir (Pseudotsuga menzisii (Mirb.) Franco) and in jack pine (Pinus banksiana Lamb.) seedlings. C. destructans however, is a fungus commonly found on many plant root surfaces as is F. oxysporum. Together, they have been isolated from nursery seedlings and are at times considered weak, endophytic pathogens. They are endemically found in many nursery soils in varying densities

(Juzwik, pers. comm.).

The stubby root nematode Paratrichodorus pachydermis, can be a pest of nursery grown conifers and is found in several compartments at the provincial tree nursery, St. Williams Ontario. The feeding of this nematode causes stubby root damage in seedlings: this damage can predispose roots to infection by the above mentioned fungi. Stubby root nematode damage has been observed on roots of white pine (Pinus strobus L.) from which F. oxysporum and C. destructans have also been isolated. A similar situation was suggested by Bloomberg and Orchard (1969) in the predisposition of Douglas-fir seedlings by the root nematode Paratylenchus to F. oxysporum. It was found that in the absence of Paratylenchus, growth responses in Douglas fir were related to reduced root rot (Bloomberg and Orchard, 1969).

Soil fumigation has been more successful against Fusarium diseases than other chemical treatments. Captan (Bloomberg and Lock, 1974) and Vorlex (Marois et. al., 1982) are among the selected chemicals for Fusarium control. Reinvasion of F. oxysporum to nursery soils after fumigation however, poses difficulty in controlling F. oxysporum. It was found that after 74 days, a population gradient of F. oxysporum was detected in a field treated with Vorlex and after 102 days, its distribution had returned to the pre-fumigation level (Marois et. al. 1982). Further, reduced proportions of F. oxysporum are not necessarily correlated to root disease incidence (Buxton et. al., 1962). Chemical fumigation, therefore, should not be considered a

complete but rather a partial control method for Fusarium root rot.

The objective of this fumigation study was to i) investigate the role of F. oxysporum, C. destructans and P. pachydermis in the decline of white pine at St. Williams Nursery and ii) evaluate the effectiveness of selected chemicals in controlling nematode and fungal populations.

METHODS

Compartment D6 at St. Williams Nursery was chosen for the fumigation trial after it was observed to have adequate populations of Cylindrocarpon and Fusarium and a high population of Paratrichodorus pachydermis. In the fall of 1985, the field was cultivated and 24 potential seedbeds 60 m X 1 m were delineated by soil stakes. The length of each alternate bed was divided into four 15 m plots allowing for 12 replications of four treatments in the compartment. The treatments included Benomyl (Benlate 50W) fungicide, Telone II B and SN55B nematicides and a control. The treatments were distributed as in a randomized complete block design.

Treatments:

On October 25, 1985, Telone II B and SN55B were applied at the rates of 93.5 litres per hectare (l/ha) and 233.8 l/ha respectively. A springtooth cultivator was used to apply the chemicals to a depth of approximately 20 cm. Benomyl was applied on April 14, 1986. A 50 ppm benomyl solution was prepared and applied. Benomyl was tank mixed with Tween and sprayed on the seedbed with a three-nozzle sprayer. The nozzles were 48 cm apart and each had a 60° swath angle. After application, drenching was achieved by irrigation.

Seeding:

On April 25,1986, seedbeds were formed according to standard nursery practice followed by an application of chlordane insecticide prior to seeding. Beds were evenly seeded with white

pine at a rate of 592 seeds per lineal metre. The seed was treated with captan and the seeding rate was targeted for the production of a 3-0 crop. The seeds were drill seeded with six drills per bed. The beds were then covered with mulch after seeding.

Soil Sampling:

Soil sampling of the entire trial area occurred in June 1985, in April 1986 and in June 1986 for the purpose of determining fungal and nematode population densities. Further samples were taken on April 25, 1986 only in the Benomyl-treated plots 11 days after the fumigant was applied. Soil sampling was done using a 2 cm diameter Oakfield sampling tube to a vertical depth of approximately 20 cm. Each plot was systematically sampled four times; sample locations were evenly spaced out at 4 m intervals along the longitudinal centerline of the plot. The four soil cores were placed in a polyethylene bag, hand mixed and divided roughly into halves; one half was processed for nematode population analysis and the other half for fungal population analysis. Bags were labelled and stored at room temperature until processed.

Stand Counts:

In June 1986, the seedlings were observed for symptoms of decline and for mortality. Four 9.29 dm^2 sample areas were located at even intervals near the centerline of each plot. All seedlings in the sample area were tallied as being healthy, symptomatic, or dead. Further stand counts were done in

September 1986, June 1987, and September 1987. For the statistical analysis of the stand count data, observed dead and symptomatic trees were totalled together and expressed as a percentage of the total trees counted in the plot. They were pooled since the cause of decline or death was not identified during the tally. The data sets were analyzed by ANOVA and treatment means were ranked by the method of Least Significant Differences (Steel & Torrie, 1980).

Soil Dilution Techniques:

Soil samples were processed using a quantitative soil dilution technique for the recovery of Cylindrocarpon and Fusarium. Each soil sample was thoroughly hand-mixed. Four subsamples were removed from the sample, two subsamples of approximately 10-g were used to gravimetrically determine moisture content; two 3-g subsamples were used in the dilution procedure. Each 3-g subsample was added to 250 ml of distilled water in an Oster Blender mini jar, the mixture was blended for 15 seconds. After allowing the suspension to settle for 15 seconds, the supernatant was decanted into a 500 ml beaker and diluted with an additional 250 ml of distilled water. The dilution was stirred into suspension on a magmixer, 5 ml of Tween 80 were added to the dilution. 5 ml of stock suspension were pipetted to each of two 100 ml beakers, one containing 50 ml of modified Komada's media (Komada, 1975) for Fusarium isolation and the other containing 50 ml of modified Phipps selective media (Phipps et. al., 1976) for Cylindrocarpon isolation. The solution was hand-swirled and

poured into five petri plates. The process was repeated for the second subsample.

The plates were incubated at room temperature for ten days. Fungal colony enumerations were then made using microscopic morphology for identification.

Statistical methods:

The total colonies counted for each subsample were corrected for dilution and for moisture content and reported as propagules per gram of dry weight soil. The following correction formula was used to describe the propagule density in each subsample:

$(f1 * 100)/W * (1-(m_1+m_2)/2)/100 = p/g$ where

f_1 = Fusarium subsample #1

w = weight of subsample (g)

m_1, m_2 = moisture content of 2 moisure determination subsamples, $1>m>0$.

p/g = propagules per one gram of dry weight soil

The two subsamples were averaged and for the purpose of analysis, a square root transformation was applied. The transformation was of the form:

$$\sqrt{p/g + 1 * 10^{-8}}$$

The resulting data set was analyzed using ANCOVA and ANOVA. Treatment means were ranked using the method of Least Significant Differences (Steel and Torrie, 1980).

Sampling and Isolation Techniques on Seedlings:

The stand count which was done in September, 1987 included seedling sampling. Five of the most symptomatic-looking

seedlings and five healthy seedlings were collected from each plot. The seedlings were placed in polyethylene bags and stored at 2°C until processed.

Fusarium and Cylindrocarpon selective media were prepared and poured into sterile disposable plates. Seedling roots were washed in distilled water then they were immersed in 10% solution of javex for 15 seconds for surface sterilization. The roots of each seedling were observed and isolations were made from i) the areas immediately adjacent to nematode damage, ii) the interface of healthy and dying root tissue iii) selected necrotic areas in that order of priority. Using sterile technique, two pieces of root tissue from each root were cut out and longitudinally sectioned; one half was plated onto Phipps selective media and the other half onto Komada's selective media. Records were kept as to which isolates originated from areas associated with nematode damage. The plates were incubated at room temperatures for ten days and then enumerated. The resulting data set was summarized and analyzed using ANOVA and treatment means were ranked using the method of Least Significant Differences (Steel and Torrie, 1980).

Grading of 3-0 Stock:

In November 1988, seedlings were assessed by i) root assessment for sparsity and density. Two subsamples of 15 seedlings each were collected within 1 m^2 plots located at the 5 m and 10 m locations within each plot. The seedlings were collected and taken back to the laboratory for analysis. Root systems were

given an overall rank ranging between 0(very sparse) to 5 (very dense). The grades were ranked, ranking order was analyzed with Friedman's Test for the Two-Way Classification. The test criterion is:

$$X_r^2 = 12/bt(t+1) * \sum_i r_i^2 - 3b(t+1)$$

where:

12 is a constant
3 is a constant
t = number of treatments
b = number of blocks

ii) diseased seedlings: Two subsamples of 15 seedlings each were collected within 1 m^2 plots located at the 5 m and 10 m locations within each plot. The seedlings were taken back to the laboratory and examined for infections. Any seedlings suspected of having root rot were prepared and isolation work as previously described was performed. iii) visual assessment of the foliar characteristics: patchiness, chlorosis, and stunting. iv) proportion of dead trees in each plot was calculated: Two 1 m^2 plots were chosen within each plot, and counts were made of healthy, dead, and dying seedlings. Dead and dying seedlings were totalled and expressed as a percentage of the total trees counted in the plot.

RESULTS

Direct Isolation By Dilution:

Results of the soil dilution tests are given in Tables 1 and 2. There was a general increase in recovered propagules between the samples of June, 1985 and April 1986 (Tables 1 and 2). There was an unscheduled time lapse between the collection date and processing date of the samples of June 1985 and April 1986. Those soil sets also yielded the lowest average propagule densities.

Treatment comparisons between dates were made using an ANCOVA procedure, however no significant correlation could be drawn between any combination of sampling dates $(p>F)>0.10$. ANOVA was, therefore, used to make all comparisons in the soil dilution results. No significant results were detected between treatments for Fusarium and Cylindrocarpon in either complete sets of April 1986 or June 1986 $(p>F)>0.10$.

Numbers of recovered Fusarium propagules from the Benomyl post-fumigation samples were greater than those from the pre-fumigation samples. Numbers of recovered Cylindrocarpon propagules from the benomyl post-fumigation samples, however, were significantly reduced in comparison to the pre-fumigated values $(p>F)>0.0001$.

By June 1986, one season after application, mean recovered Fusarium and Cylindrocarpon propagule densities in the Benomyl-treated plots were lower than but not statistically different from the control plots $(p>F)>0.10$. The nematicides showed no

evidence of fungicidal activity.

Table 1. Fusarium populations in soil[a] before and after treatment.

Collection Dates	Treatments[b]			
	Check	Telone II B	SN55B	Benomyl
June 1985 (pre)	20.98	17.41	18.66	16.27
April 1986 (post)	23.12	19.16	18.95	21.81
April 1986 (post benomyl)				25.76
June 1986 (post)	29.72	32.81	27.82	25.45

[a] propagules per gram of dry weight soil after squareroot transformation

[b] means of 12 transformed replications.

Table 2. *Cylindrocarpon* population in soil[a] before and after treatment

Collection Dates	Treatments[b]			
	Check	Telone IIB	SN55B	Benomyl
June 1985 (pre)	5.98	3.92	6.68	5.19
April 1986 (post)	10.30	9.73	9.36	13.06
April 1986 (post Benomyl)				1.14*
June 1986 (post)	22.20	27.17	21.70	19.94

[a] propagules per gram of dry weight soil after squareroot transformation.

[b] means of 12 transformed replications
* significant (p>F)= 0.0001

Stand Count Results:

There was a low frequency of dead and dying trees observed during the 1986 and 1987 growing season (Table 3). The total dead and dying trees tallied in the control plots during the 86 and '87 season averaged 1.83% and 1.94% respectively. Treatment differences within sampling dates were not statistically different (p>F)=0.13.

The Benomyl-treated plots displayed the lowest frequency of dead and dying seedlings in the September 1986 and in the September 1987 stand counts and the second lowest frequency of the same in both the June counts. The lowest and highest average frequency of dead and dying trees occurred during the first sampling of June 1986 and during the last sampling of September 1987 respectively. The difference in frequency of dead and dying trees between these two sample dates was significant (p>F)=0.02.

Seedling Isolation Results:

The results of the seedling isolations are given in Table 4. There were no treatment differences evident in the Fusarium isolation results (p>F)=0.54 but Fusarium was isolated from check trees more often than from trees in any other treatment. Treatment differences in the Cylindrocarpon were detected (p>F)=0.071 and when ranked using an LSD (alpha=0.05) the SN55B treatment resulted in the lowest percentage of isolated Cylindrocarpon. This isolation percentage, however, was not significantly different from that of the control.

Table 3. Total dead and dying trees[a] observed at four points in time after treatment.

Observation Dates	Treatments[b]			
	Check	Telone IIB	SN55B	Benomyl
June 1986	1.668	0.590	0.625	0.599
September 1986	1.993	2.212	1.765	1.103
June 1987	1.839	1.563	0.558	1.107
September 1987	2.038	2.089	1.642	1.565

[a] values are given as a percent of total observed seedlings in each sample

[b] means of 12 replications

Table 4. Number of Fusarium and Cylindrocarpon isolated from healthy and symptomatic white pine seedlings[a]

Treatments	Isolated Fungi[b]	
	Fusarium[c]	Cylindrocarpon[c]
Check	52.50 X	30.00 XY
Telone II B	47.50 X	35.83 X
SN55B	43.33 X	20.00 Y
Benomyl	43.33 X	29.17 XY

[a] percentage of trees from which the fungus was isolated
[b] means of 12 replications
[c] means with the same letter are not significantly different

Nematode Damage:

Results of observed nematode damage on seedling roots is summarized in Table 5. Treatment differences in the percent occurrence of nematode damage were detected (p>F)=0.76. An LSD ranking of the means showed that the SN55B treatment significantly reduced nematode damage on seedling roots in comparison to control (alpha = 0.05).

Data on nematode damaged areas associated with fungal isolations is summarized in Table 6. On average, Fusarium was associated with nematode damage sites more often than was Cylindrocarpon. Fusarium and Cylindrocarpon were associated with localized sites nematode damage less frequently in the control plots than in plots of any other treatment.

Table 5. Number of localized sites of nematode damage on the healthy and symptomatic white pine seedlings.

Treatment	Occurrence of nematode damage [ab]
Check	7.17 X
Telone II B	4.50 XY
SN55B	3.00 Y
Benomyl	4.50 XY

[a] means with the same letter are not significantly different
[b] means of 12 replications

Table 6. Percent recovery of *Fusarium* and *Cylindrocarpon* associated with nematode damaged seedlings[a].

Fungi	Treatments			
	Check	Telone II B	SN55B	Benomyl
Fusarium	29.41	50.00	37.50	35.71
Cylindrocarpon	26.92	26.92	30.00	41.67

[a] root isolations were made from areas immediately adjacent to nematode damage.

Performance of 3-0 stock:

Although not statistically significant (p>F)> 0.10, seedlings in the benomyl treated plots were rated as having the most dense root systems, the control plots were rated as having the most sparse root systems (Table 7). In a visual assessment of the above ground performance of the seedlings in the compartment, seedlings in the benomyl and SN55B plots were rated as exhibiting the least stunting, patchiness, and chlorosis (Table 8). Isolation of suspect seedlings did not reveal any treatment difference for either fungi (Table 9). Dead and dying seedlings were greatest in the check(4.02%) and telone plots(4.86%) and least in the SN55B(2.53%) and benomyl plots(1.56%).

Table 7. Ranked values of root density as determined with Freidman's test for the Two-way classification.

	Treatment			
	Control	Telone IIB	SN55B	Benomyl
Rank[a]	29.66	30.5	27.16	34.66

[a] each rank value is a summation of the 12 replicates in each treatment.

X^2 =7.448 with 3 df, X^2 = 7.81

Table 8: Foliar characteristics of 3-0 stock as determined through visual assessment.

Treatment	Stunted[a]	patchiness	chlorosis
check	9	7	4
telone IIB	8	5	5
SN55B	0	2	4
benomyl	2	2	4

[a] Values represent the total number of plots per treatment that exhibit the physical characteristic described.

Table 9. Number of Fusarium and Cylindrocarpon isolated from suspect seedlings lifted from 3-0 stock

	Cylindrocarpon	Fusarium
check	9	6
telone	8	6
SN55B	5	6
benomyl	10	8

DISCUSSION

With the exception of benomyl which reduced the population of Cylindrocarpon, none of the other treatments had any effect on population levels of Fusarium or Cylindrocarpon. The finding that Cylindrocarpon was lowered but Fusarium was not in the benomyl treatment suggested that Fusarium is not as sensitive to the treatment. Secondly, this finding may be an artifact of the sampling technique utilized. Soil was collected to a depth of 6" while the chemical and water drench likely penetrated only the upper inches of the soil. Therefore, the fungal population count may not reflect the treatment. Although statistically insignificant, by June 1986, the densities of Fusarium and Cylindrocarpon were lower in the benomyl treated plots than in the control plots.

Throughout 1986 and 1987, the frequency of dead and dying trees remained low with Benomyl among the lowest. In the third year, the percentage of dead and dying trees was low, and benomyl had the lowest percentage.

In the isolation work of 1986 and 1987, there were no treatment differences evident in the Fusarium isolation results, yet the fungus was isolated more often from check trees than the other treatments. SN55B had the lowest level of Cylindrocarpon. In 1988, there was no treatment differences evident for either fungi. This finding suggests that after their second year, the seedlings are less susceptible to the fungi.

Nematode damage seemed to be controlled best by SN55B, and

Fusarium seemed to be associated with these nematode sites more often than Cylindrocarpon, suggesting that Fusarium may be a more aggressive fungi.

The efficacy of benomyl is supported by the third year data collection. Seedlings in the benomyl plots had the most dense root systems, as well they exhibited the least stunting, patchiness, and chlorosis. SN55B produced good above ground characteristics, yet, the root systems were somewhat sparse. Telone IIB exhibited alot of stunted, patchy, and chlorotic growth. The stuntedness, chlorosis and patchiness may be due to one of two factors, or both of them in combination: phytotoxicity of Telone, and nematode feeding in the controls. Benomyl plots were rated as having the most dense root systems.

From consideration of the effect of the treatments on fungal populations, it seems that Benomyl produced the best 3 + 0 stock, followed by SN55B. Telone treatment resulted in low quality seedlings. Further conclusions may be evident when this data is combined with the nematode population counts being conducted by Dr. John Potter, Nematologist, Agriculture Canada, Vineland Research Station, Vineland Ontario.

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