M-X-89
ISSN 0704-769X

EVALUATION OF PARTIAL STERILANTS FOR FOREST NURSERY SOILS

by

R.E. WALL

MARITIMES

MARITIMES FOREST RESEARCH CENTRE

The Maritimes Forest Research Centre (MFRC) is one of six regional establishments of the Canadian Forestry Service, within Environment Canada. The Centre conducts a program of work directed toward the solution of major forestry problems and the development of more effective forest management techniques for use in the Maritime Provinces.

The program consists of two major elements - research and development, and technical and information services. Most research and development work is undertaken in direct response to the needs of forest management agencies, with the aim of improving the protection, growth, and value of the region's forest resource for a variety of consumptive and non-consumptive uses; studies are often carried out jointly with provincial governments and industry. The Centre's technical and information services are designed to bring research results to the attention of potential users, to demonstrate new and improved forest managment techniques, to assist management agencies in solving day-to-day problems, and to keep the public fully informed on the work of the Maritimes Forest Research Centre.

EVALUATION OF PARTIAL STERILANTS FOR FOREST NURSERY SOILS

by

R. E. Wall

Maritimes Forest Research Centre
Fredericton, New Brunswick

Information Report M-X-89

Canadian Forestry Service
Department of Fisheries and the Environment

1978

ABSTRACT

Forest nursery soils were treated with two rates each of Mylone, Bunema, Busan, anhydrous ammonia, or potassium azide and sown 3-5 weeks later with jack pine and black spruce. None of the treatments appreciably affected emergence under greenhouse conditions but postemergence mortality was high on some soils treated with Busan, anhydrous ammonia, or potassium azide. Seedling survival was generally highest on soils treated with recommended dosages of Mylone but results were seldom significantly higher than in untreated checks. Seedling dry weights were increased by high rates of Mylone, unaffected by Bunema treatment, and depressed by the Busan treatments. Weed counts were suppressed by Mylone and Bunema in two of the three tests. Of the materials evaluated, Mylone appeared to be the most useful soil sterilant.

RESUME

On a traité des sols de pépinière forestière avec deux doses de chacun des composés suivants: Mylone, Bunema, Busan, ammoniaque anhydre, ou azide de potassium, puis on les a ensemencés 3 à 5 semaines plus tard de Pin gris (***Pinus banksiana***) et d'Epinette noire (***Picea mariana***). Aucun des traitements n'a affecté de façon appréciable l'émergence en serre mais la mortalité qui suivit l'émergence fut élevée dans certains sols traités au Busan, à l'ammoniaque anhydre ou à l'azide de potassium. La survie des semis fut généralement la plus élévee dans les sols traités selon les doses recommandées de Mylone mais les résultats furent rarement significativement meilleurs que chez les témoins non traités. Le poids anhydre des semis fut augmenté avec de fortes doses de Mylone, il fut insensible au traitement de Bunema et fut amoindri par le traitement au Busan. Dans deux des trois tests, le Mylone et le Bunema ont supprimé quantité d'herbes nuisibles. Parmi les composés evalués, il semble que le Mylone stérilisa mieux le sol.

INTRODUCTION

Sterilization or fumigation of soils prior to planting is recommended in forest nurseries where serious weed, root rot, or nematode problems exist. Favorable responses to fumigation in conifer seedbeds have been reported by Bloomberg (1965), Clifford and Massello (1966), Henry (1953), Hill (1955), Morris (1960), Reddy (1962), Ruehle et al. (1966), Smith and Bega (1966), van den Driessche (1963), Wright (1964), and Wycoff (1952). In most of these studies, an improvement in seedling development has been noted and this has, in some cases, been attributed to a reduction in pathogenic soil fungi. Peterson (1970) reported a reduction of damping-off and nematode injury in ponderosa pine seedbeds treated with methyl bromide. Warcup (1952) reduced ***Pythium*** populations and improved seedling stand by partial sterilization of soils with steam or formalin. Vaartaja(1958) and Low (1974) found dazomet to be an acceptable alternative to formalin for disease control, with the added benefit of weed control. Theis and Patton (1971) found that complete sterilization of nursery soils with methyl bromide gas was necessary to eliminate microsclerotia of the destructive root rot fungus, ***Cylindrocladium***, whereas partial sterilants were less effective. The fungicidal activity of anhydrous ammonia and potassium azide in soils was noted by Smiley et al. (1970) and Kelley and Rodriguez-Kabana (1975) respectively.

The distinction between 'partial' and 'complete' sterilants is important in considering nursery soil treatment. While no sterilization is likely to be complete under normal operational conditions, chemicals such as methyl bromide can eliminate most of the microflora in the rooting zone and thus render the soil more vulnerable to reinvasion by certain pathogens which are normally held in check by antagonistic fungi and bacteria. Vaartaja (1967) noted the reinvasion of Trizone[1]-treated seedbeds by ***Pythium, Fusarium,*** and ***Rhizoctonia***, 10 months after treatment. Reinvasion resulted from splashing rainwater, dust, and soil carried by man. The most serious damping-off in Maritime forest nurseries has occurred on seedbeds previously treated with vapam, methyl bromide, or Vorlex (Wall 1974). Hence, partial sterilants i.e. materials which reduce part of the microbial population are probably preferable to the more 'complete' sterilants, provided that the former reduce populations of target pathogens to safe levels.

In the following study, several partial sterilants, namely Mylone (dazomet), Bunema, Busan, anhydrous ammonia, and potassium azide, were evaluated in the greenhouse under conditions as close as possible to those in the field. Before seeding, the treated soils were deliberately contaminated with small quantities of untreated soil to simulate natural recontamination with soil from walkways or subsoil. Seedlings were grown under comparable conditions and monitored for natural mortality and dry weight increase. In addition, periodic isolations from dying seedlings and from the soil gave a rough measure of the pathogens present. No consideration was given to the effects of these chemicals on soil nutrient relations or to the effects of sterilization on mycorrhizal development as studied by Campagna and White (1973), Palmer and Hackskaylo (1958), and others. Instead, the study was restricted to early survival and development of conifer seedlings in partially sterilized soils.

MATERIALS AND METHODS

Three experiments were conducted with different soils, at different times, using slightly different methods and rates. Each is described separately. The chemistry and sources of the materials tested are listed in the Appendix.

I. Inoculated greenhouse soil

A greenhouse compost consisting of soils previously used in the culture of conifer seedlings was mixed 2:1 with an equal mixture of cornmeal-sand cultures of four pathogenic isolates of each of ***Fusarium oxysporum, Rhizoctonia solani,*** and ***Pythium irregulare***. The resulting mixture (pH 5.2) was placed in 25x32x6-cm wooden flats and treated as follows:

- Mylone 50D (Dazomet) - 0.83 g/L of soil = 1109 lb product/acre = 554 lb a.i./acre[2].
- Mylone 50D - 0.08 g/L = 111 lb product/acre = 55 lb a.i./acre.
- Bunema (40% WS) - 0.42 mL/L = 60 Imp. gal/acre = ca. 240 lb a.i./acre.
- Bunema - 0.08 mL/L = 12 gal/acre = ca. 48 lb a.i./acre.
- Busan (30% EC) - 0.27 mL/L = 36 gal/acre = ca. 108 lb a.i./acre.
- Busan - 0.06 mL/L = 8 gal/acre = ca. 32 lb a.i./acre.

[1] Mention of a commercial product does not imply its endorsement by the Department of the Environment.

[2] Based on a 15-cm (6-inch) furrow slice.

Each formulation was thoroughly mixed with the soil, the liquid formulations being diluted in 17 mL of water prior to addition. Four replicate flats of each treatment plus four untreated checks were watered to field capacity, covered with waxed paper, and placed in a fume hood for 10 days. The waxed paper was then removed and the flats were placed in the greenhouse for an additional 28 days, during which time they were watered periodically. The flats were then sown with 30 seeds of red pine, jack pine, and black spruce, each in predetermined parts of the flat. At the time of seeding, a small quantity (0.1-0.5 mL) of untreated soil was sprinkled over the surface of the treated flats to effect recontamination.

The test was conducted during the winter months in a greenhouse at 20-25°C, with the light supplemented by 300-watt incandescant lamps on a 16-h photoperiod. After emergence, a continuous record was kept of the number of healthy and dead seedlings. Dead seedlings were surface sterilized in 0.6% sodium hypochlorite and placed on 2% malt agar to isolate damping-off fungi. Five months after seeding, all remaining seedlings were washed free of soil, divided into roots and shoots, oven dried, and weighed. At the same time, weeds were counted, oven-dried, and weighed.

At different times during the experiment, soil samples were collected for microbiological assay. Using a calibrated cork borer, 1-cc samples (1.19 g dry weight) of packed soil were suspended in 100 mL of 0.1% water agar. A 1:9 dilution of this suspension was pipetted onto the surface of 9-cm Petri dishes of two different media[3]. The Petri dish cultures were incubated under diffuse light in the laboratory for 5-7 days, then colonies of different fungal and bacterial groups were counted.

II. Charlottetown nursery soil

Recently cultivated soil (pH 5.2) was collected in October 1975 from the Beach Grove Nursery of the Prince Edward Island Department of Agriculture and Forestry. The soils were stored at 4°C in plastic bags until March 1976, when they were treated. In this test, the soils were treated in plastic bags as follows:

- Mylone 50D - 0.37 g/L = 500 lb/acre = 250 lb a.i./acre.
- Mylone 50D - 0.08 g/L = 100 lb/acre = 50 lb a.i./acre.
- Bunema - 0.3 mL/L = 40 gal/acre = ca. 160 lb a.i./acre.
- Bunema - 0.06 mL/L = 8 gal/acre = ca. 32 lb a.i./acre.
- Busan - 0.007 mL/L = 1 gal/acre = ca. 3 lb a.i./acre.
- Busan - 0.0015 mL/L = 0.2 gal/acre = ca. 0.6 lb a.i./acre.
- Bunema + Busan - 0.3 mL + 0.007 mL/L.
- Bunema + Busan - 0.06 mL + 0.0015 mL/L.
- Anhydrous ammonia - ca. 1 g/L = 1000-1500 lb/acre acidified prior to seeding with 1 g/L of 85% phosphoric acid.
- Anhydrous ammonia - ca. 0.1 g/L = 100-150 lb/acre.

Ammonia was applied through a plastic hose from a 1-lb cylinder to the centre of the soil mass and the plastic bag was closed immediately. The quantity applied was determined by weight loss of the cylinder. The plastic bags of treated soil were kept closed for 7 days, then opened and allowed to aerate for 20 days. The soils were then placed in steamed 1-L clay pots (13 cm top diameter) and seeded with jack pine and black spruce. Numbers of weeds were recorded and removed periodically. Otherwise the procedures were the same as in experiment I with four replicates per treatment.

III. Paradise nursery soil

Soils (pH 4.5-5.8) were collected in November 1976 from a recently cultivated site at Paradise, near Lawrencetown, Nova Scotia. This site had formerly been farmland with some woodland and was being converted to nursery seedbeds. Soils were stored in plastic bags in an unheated shed for about three weeks. They were then brought into the greenhouse, the bags were opened, and the soil was allowed to air-dry for a few days before treatment. The methods were the same as in experiment II, except that ammonia was applied at lower rates, two potassium azide treatments were added (0.075 and 0.025 g/L of 100% a.i. mixed with the soil as a dry powder) and the lower rates of Mylone and Bunema were changed to 0.15 and 0.1 mL/L of

---

[3] Modified Martin's peptone agar. Peptone - 15 g, $MgSO_4.7H_2O$ - 0.5 g, KH_2PO_4 - 1.0 g, oxgall - 0.5 g, rose bengal - 30 mg, agar - 25 g, distilled water - 1000 mL. After autoclaving, 0.5 g taurocholic acid, 50 mg streptomycin, and 0.5 mL of 85% lactic acid were added.

Modified cornmeal agar. Difco Bacto cornmeal agar - 17 g, distilled water - 1000 mL. After autoclaving 100,000 units of nystatin, 10 mg of benomyl, 10 mg of streptomycin, and 0.5 mL of 85% lactic acid were added.

soil, respectively. Busan was applied only at the higher rate. The treated soils were kept in closed plastic bags for 7 days and then aerated for 20 days before seeding to jack pine and black spruce.

RESULTS

I. Inoculated greenhouse soil

The effects of the different treatments on total microbial populations depended on the medium used for assay (Fig. 1). Since both media contained several antibacterial substances, the numbers of bacteria recorded cannot be considered representative of the true populations. Also, many species of fungi may have been suppressed since the media, Martin's peptone agar and cornmeal agar, were developed to be selective for ***Fusarium*** spp. and ***Pythium*** spp., respectively. However, it is interesting to note that on Martin's agar there was an upsurge in the total fungal counts during the first 40 days after treatment with Mylone and Bunema and a simultaneous suppression of ***Fusarium*** (Fig. 2). ***Fusarium*** populations were not suppressed in Busan-treated soils. After planting to conifers, fungal populations tended to rise or fall toward original levels.

Seedling emergence differed significantly among treatments only in black spruce (Fig. 1, Table 1). Severe mortality of all species occurred in soils treated with the higher rate of Busan. The effects of Busan resembled typical damping-off as evidenced by rotting of root and stem tissues and the isolation of ***Pythium, Fusarium, Cylindrocarpon,*** and ***Rhizoctonia*** (Table 1). Survival was lowest on soils treated with the higher rate of Busan and highest on soils treated with the upper rate of Mylone, for all three species. However, seedling survival was not significantly higher than the check in any of the treatments.

Seedling dry weights were significantly higher than the check in the 0.8 g/L Mylone treatment (Table 2, Fig. 1). Numbers of weeds counted at the end of the experiment were not noticeably different from the check but the total weight of weeds was higher on treated soils, indicating a beneficial effect of sterilants on growth (Table 2).

II. Charlottetown nursery soil

Pythium spp. were not recovered from this soil until 80 days after treatment, although some of the damped-off seedlings yielded ***Pythium*** (Fig. 3, Table 3). ***Fusarium*** populations were stimulated by the Busan and Busan + Bunema treatments and suppressed by Mylone, the higher rate of Bunema, and ammonia (Fig. 4). Seedling emergence was not significantly affected by any of the treatments (Table 3). Greatest mortality occurred on ammonia- and Busan-treated soils, the dead seedlings yielding mainly ***Fusarium*** in culture. In no instance was survival significantly greater than in the untreated checks but survival of jack pine was reduced by the higher rate of ammonia and that of black spruce by Busan (Fig. 3, Table 4).

None of the treatments significantly improved seedling dry weight, although the higher rate of Mylone did produce visibly larger and greener seedlings. Weed counts were reduced by Mylone and the higher rate of Bunema (Table 4).

III. Paradise nursery soil

Mylone and Bunema stimulated bacterial populations during the first 3 weeks after treatment (Fig. 5). Otherwise, the changes in soil microflora were similar to the other tests. Populations of ***Fusarium*** and ***Pythium*** were suppressed by Mylone, Bunema (at the higher rate), and potassium azide. Seedling emergence was highest at the higher rate of Bunema for jack pine and with Busan for black spruce, while potassium azide at the higher rate caused reduced emergence in both species. Considerable postemergence mortality occurred on soils treated with potassium azide and with the lower rates of Mylone and Bunema (Table 5, Fig. 5). ***Fusarium oxysporum*** was the dominant fungus associated with mortality although the frequency of miscellaneous isolates from seedlings killed in potassium azide-treated soil suggests that direct chemical injury may have been the primary cause in that instance. None of the treatments significantly increased seedling survival; potassium azide generally decreased both final numbers and dry weights (Table 6). The only significant increase in dry weight occurred in jack pine treated with the lower rate of ammonia. Mylone and Bunema decreased numbers of weeds.

DISCUSSION

Although the foregoing experiments did not attempt to evaluate the long-term effects of partial sterilants under field conditions, certain trends should be noted. These trends generally agreed with one another throughout the three experiments, regardless of variations in soils, methods, and timing. With the exception of the first experiment (inoculated greenhouse soil) higher rates of partial sterilants closely approximated those that would be used in forest nurseries as preplanting treatments. The lower rates were generally less

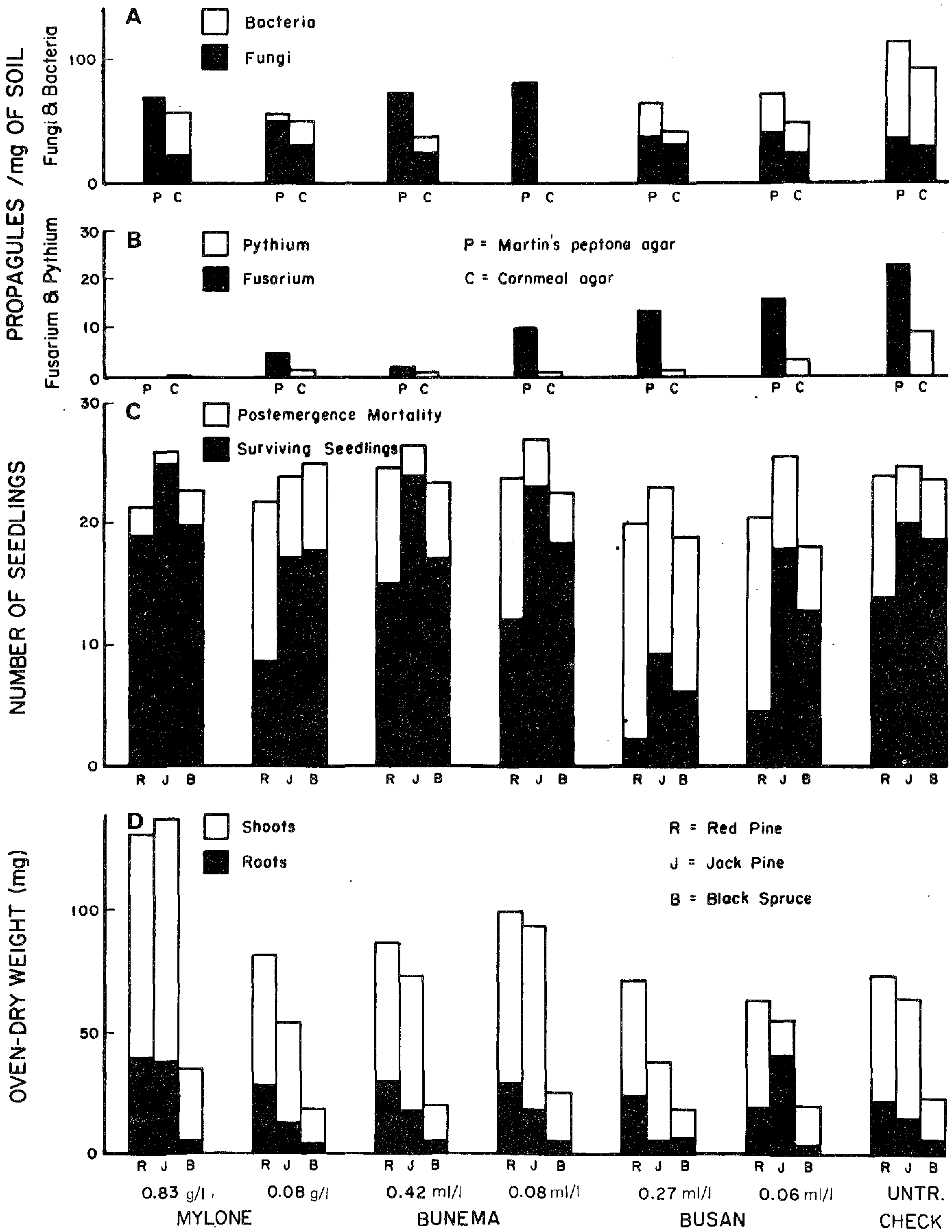

Figure 1. Populations of (A) fungi and bacteria, (B) Fusarium and Pythium in inoculated greenhouse soil, 40 days after treatment. (C) Emergence, mortality, and survival of seedlings. (D) Dry weight of seedlings after 5 months growth.

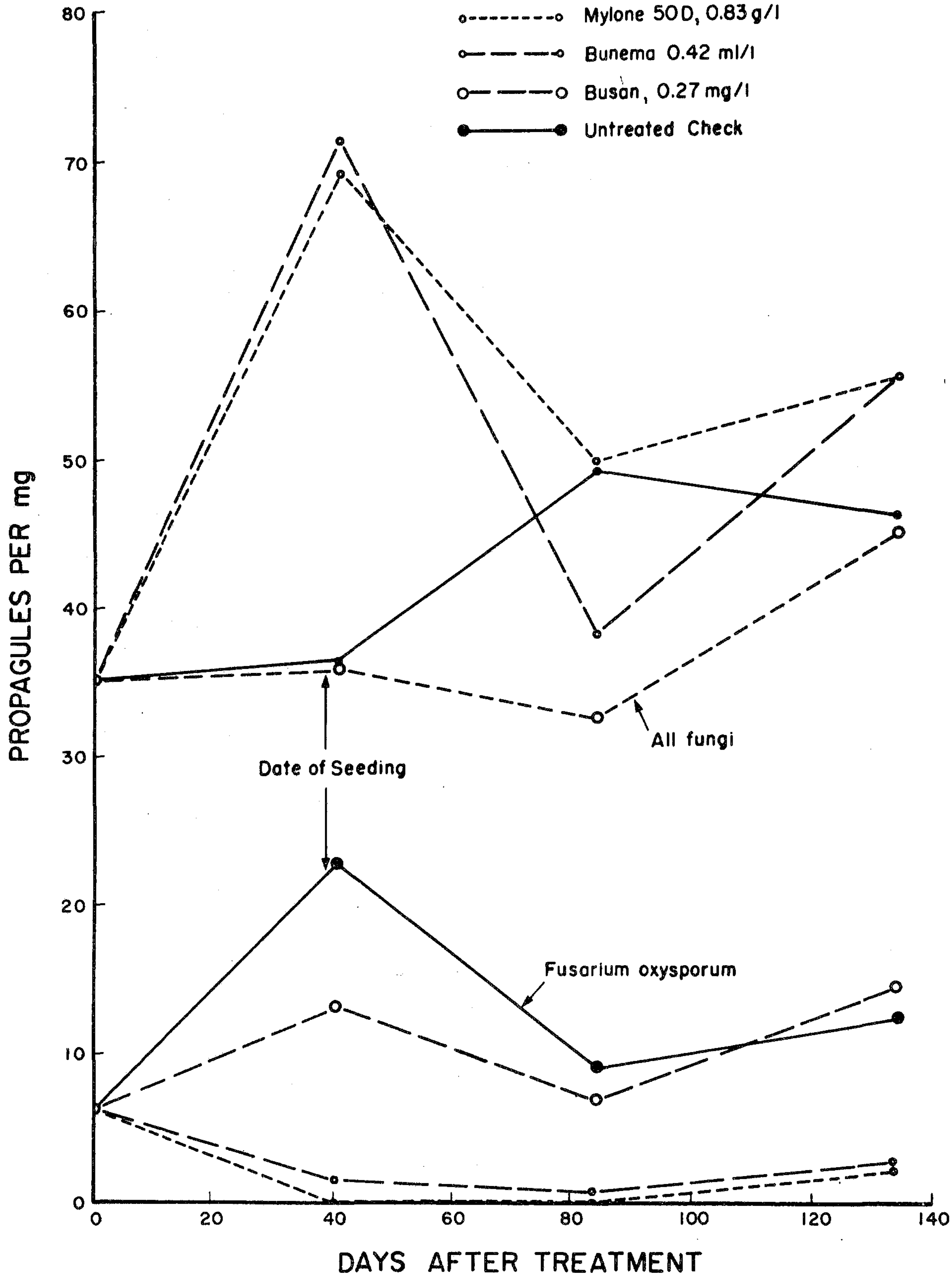

Figure 2. Changes in the recovery of fungi on Martin's peptone agar inoculated with greenhouse soil treated with partial sterilants and seeded with conifers.

Table 1. Emergence, mortality, and damping-off fungi in pine and spruce seedlings grown in inoculated greenhouse soil treated before seeding with partial sterilants

Species, treatment, and rate[1]	Seedling emergence (%)	Postemergence mortality (%)	Fungi isolated[2]				
			Pythium	*Fusarium*	*Cylindrocarpon*	*Rhizoctonia*	Other
Red pine							
Mylone 50D, 0.83 g	71a[3]	13a[3]	6	5	1	2	6
Mylone 50D, 0.08 g	73a	68bc	30	16	32	1	8
Bunema, 0.42 mL	82a	41ab	5	10	13		11
Bunema, 0.08 mL	78a	47ab	20	24	10	1	11
Busan, 0.27 mL	66a	96c	47	18	32	11	2
Busan, 0.06 mL	69a	82bc	33	21	33	2	11
Untreated check	79a	48ab	14	15	32		8
Jack pine							
Mylone 50D, 0.83 g	87a[3]	7a	2				3
Mylone 50D, 0.08 g	79a	35a	23	20	11	1	
Bunema, 0.42 mL	88a	13a	4	11	3	1	7
Bunema, 0.08 mL	90a	15a	14	8	3		1
Busan, 0.27 mL	78a	80b	5	12	9	8	4
Busan, 0.06 mL	85a	33a	26	15	6	2	2
Untreated check	83a	20a	16	8	6	1	7
Black spruce							
Mylone, 50D, 0.83 g	76ab[3]	17a[3]	4	4	2		4
Mylone 50D, 0.08 g	83b	24a	14	17	5	1	1
Bunema, 0.42 mL	78ab	31a	5	13	3		7
Bunema, 0.08 mL	75ab	16a	8	2	3	2	3
Busan, 0.27 mL	63a	84b	33	10	21	3	1
Busan, 0.06 mL	60a	35a	10	4	12		5
Untreated check	78ab	21a	13	8	7	1	5

[1] Amount of formulated product per litre of loosely packed soil.

[2] Number of cultures isolated from dead and dying seedlings on 2% malt agar.

[3] Numbers within a column followed by the same letter not significantly different at $P = 0.05$ using Duncan's multiple-range test.

Table 2. Seedling survival and dry weight plus incidence of weeds in inoculated greenhouse soil treated with partial sterilants

Species, treatment, and rate[1]	Seedling survival (%)	Seedling dry weight		Weeds[2]	
		Shoots (mg)	Roots (mg)	Number	Dry weight (mg)
Red pine					
Mylone 50D, 0.83 g	60c[3]	92b[3]	40b[3]	49	4620
Mylone 50D, 0.08 g	28ab	54a	28ab	43	450
Bunema, 0.42 mL	50c	58a	29ab	56	2670
Bunema, 0.08 mL	40abc	70ab	29ab	47	1000
Busan, 0.27 mL	7a	48a	24ab	57	1340
Busan, 0.06 mL	14ab	44a	19a	43	1650
Untreated check	46	51a	22a	47	330
Jack pine					
Mylone 50D, 0.83 g	83b[3]	99b[3]	39b[3]	–	–
Mylone 50D, 0.08 g	58ab	42a	12a	–	–
Bunema, 0.42 mL	79b	56ab	18a	–	–
Bunema, 0.08 mL	77b	75ab	18a	–	–
Busan, 0.27 mL	30a	33a	6a	–	–
Busan, 0.06 mL	59ab	40a	14a	–	–
Untreated check	67b	50ab	14a	–	–
Black spruce					
Mylone 50D, 0.83 g	66b[3]	30b[3]	6a[3]	–	–
Mylone 50D, 0.08 g	59b	14a	4a	–	–
Bunema, 0.42 mL	57b	15a	6a	–	–
Bunema, 0.08 mL	61b	20ab	5a	–	–
Busan, 0.27 mL	20a	12a	6a	–	–
Busan, 0.06 mL	42b	17a	4a	–	–
Untreated check	62b	17a	6a	–	–

[1] Amount of formulated product per litre of loosely packed soil.

[2] Weeds per 0.2 m^2 of soil surface at final harvest date.

[3] Numbers within a column followed by the same letter not significantly different at $P = 0.05$ using Duncan's multiple-range test.

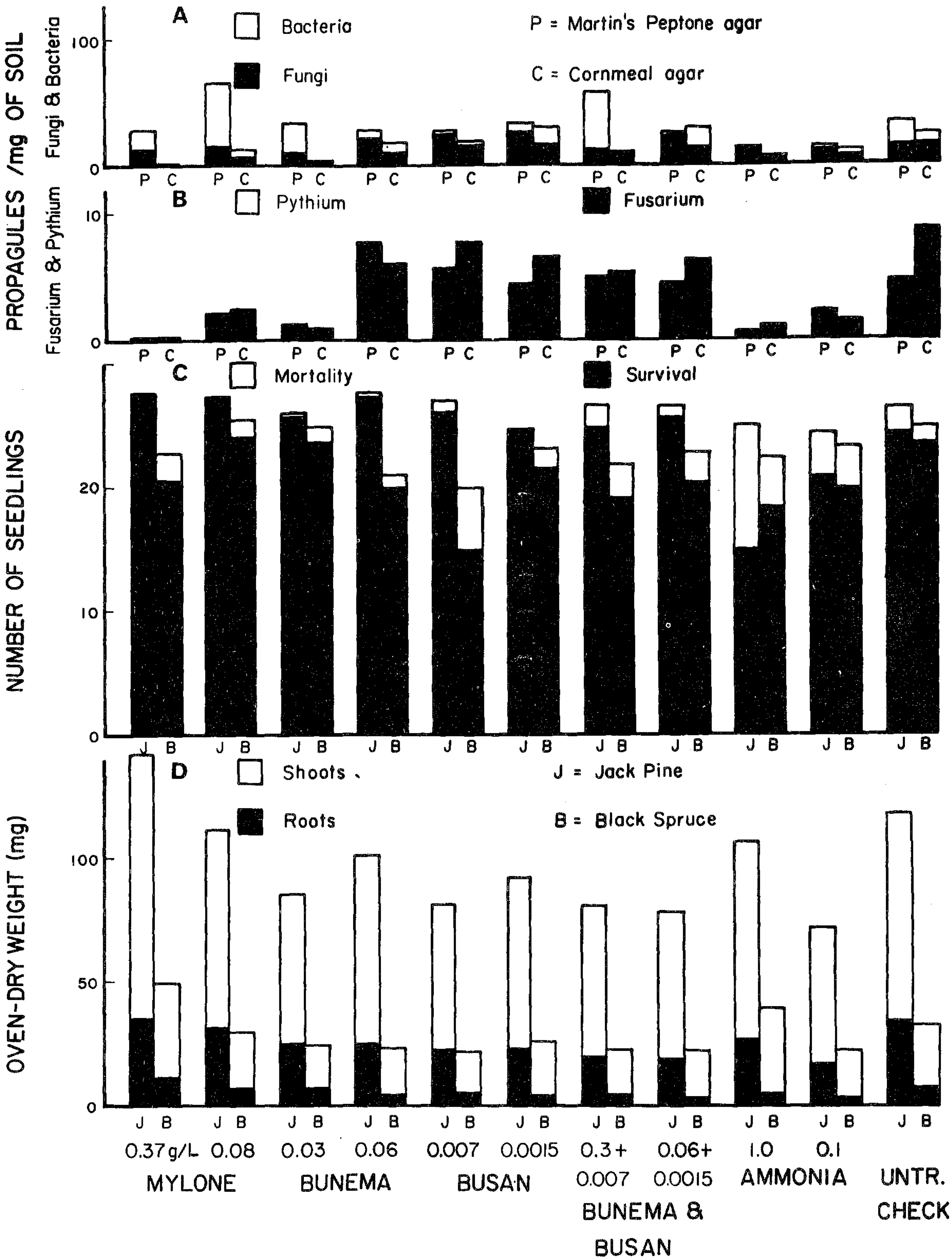

Figure 3. Populations of (A) fungi and bacteria, (B) Fusarium and Pythium spp., in Charlottetown soil. (C) Emergence, mortality, and survival of seedlings. (D) Dry weight of seedlings after 5 months.

Table 3. Emergence, mortality, and associated fungi in jack pine and black spruce seedlings grown in Charlottetown nursery soil pretreated with partial sterilants

Species, treatment, and rate[1]	Seedling emergence (%)	Postemergence mortality (%)	Fungi isolated[2]			
			Pythium	***Fusarium***	***Rhizoctonia***	Other
Jack pine						
Mylone 50D, 0.37 g	93a[3]	0a[3]				
Mylone 50D, 0.08 g	92a	0a				
Bunema 0.3 mL	87a	0a				
Bunema, 0.06 mL	93a	0a				
Busan, 0.007 mL	90a	2ab	2	2		
Busan, 0.0015 mL	83a	2ab	2	3		
Bunema, 0.3 mL + Busan, 0.007 mL	89a	6ab	5	5		
Bunema, 0.06 mL + Busan, 0.0015 mL	89a	2ab	2	4		
Ammonia, 1 g	84a	40c		35		5
Ammonia, 0.1 g	82a	14b	9	13		1
Untreated check	88a	6ab		4	6	
Black spruce						
Mylone 50D, 0.37 g	76a[3]	7a[3]	3	5		2
Mylone 50D, 0.08 g	85a	4a		2		
Bunema, 0.3 mL	83a	3a		2		1
Bunema, 0.06 mL	70a	3a		1		
Busan, 0.007 mL	67a	26a	2	12	9	1
Busan, 0.0015 mL	78a	5a	2			1
Bunema, 0.3 mL + Busan, 0.007 mL	73a	9a		9		
Bunema, 0.06 mL + Busan, 0.0015 mL	76a	7a		8		1
Ammonia, 1 g	75a	15a	6	10		2
Ammonia, 0.1 g	78a	12a	3	6	2	3
Untreated check	83a	3a		3		

[1] Amount of formulated product per litre of loosely packed soil.

[2] Number of cultures recovered from dead seedlings on 2% malt agar.

[3] Numbers within a column followed by the same letter not significantly different at $P = 0.05$ using Duncan's multiple-range test.

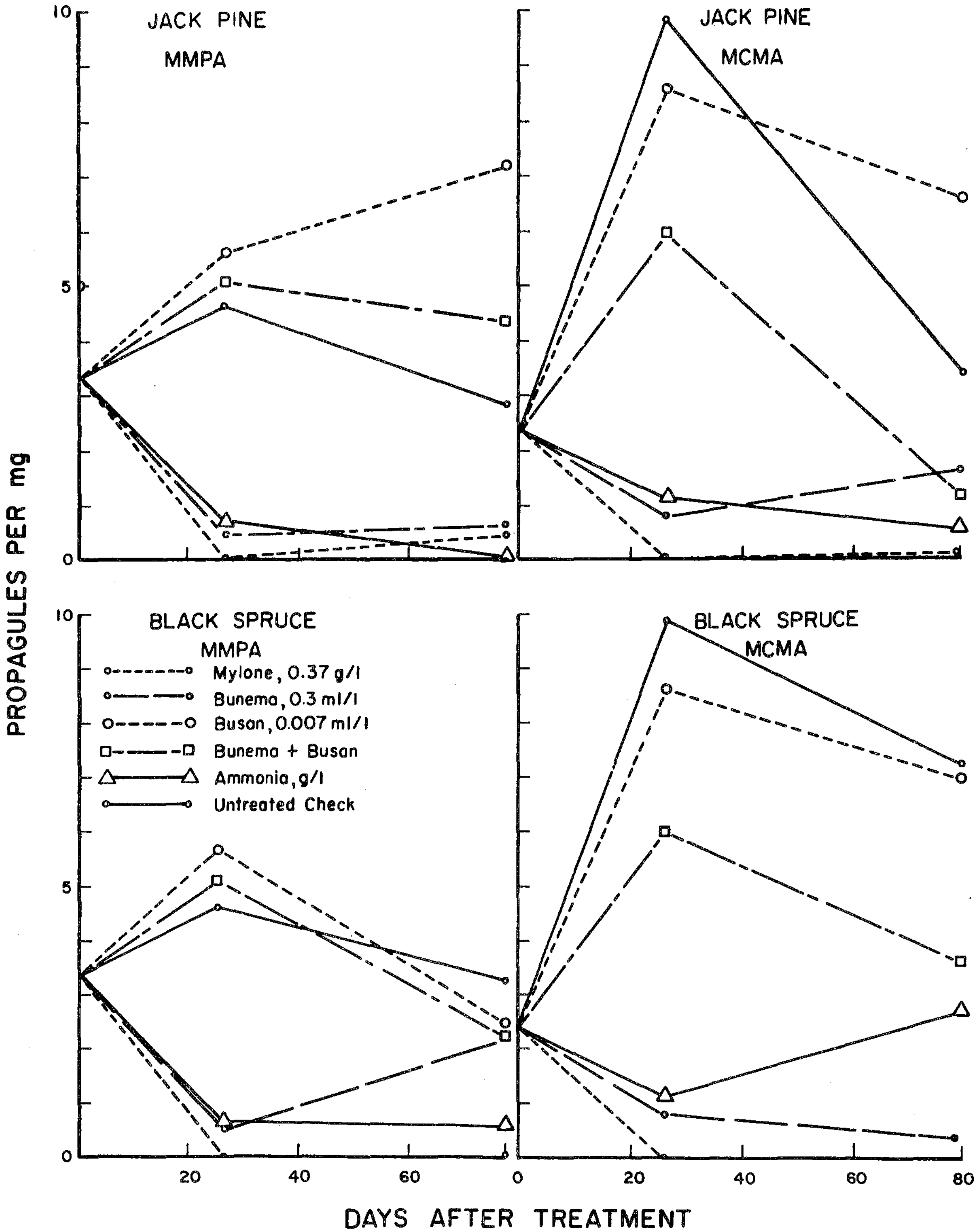

Figure 4. Changes in the recovery of Fusarium oxysporum on modified Martin's peptone (MMPA) and corn meal (MCMA) agars from Charlottetown nursery soil seeded to jack pine and black spruce after pretreatment with partial sterilants.

Table 4. Seedling survival and dry weight plus incidence of weeds in Charlottetown nursery soil treated with partial sterilants

Species, treatment, and rate[1]	Seedling survival (%)	Seedling dry weight: Shoots (mg)	Seedling dry weight: Roots (mg)	Number of weeds[2]: Broadleaved	Number of weeds[2]: Grassy
Jack pine					
Mylone 50D, 0.37 g	93c[3]	105d[3]	37d[3]	4	0
Mylone 50D, 0.08 g	92c	80bc	32bc	2	2
Bunema, 0.3 mL	86bc	61abc	25abcd	2	0
Bunema, 0.06 mL	92c	77abc	25abcd	5	22
Busan, 0.007 mL	87bc	58ab	23abc	14	25
Busan, 0.0015 mL	79bc	69abc	23abc	5	39
Bunema, 0.3 mL + Busan, 0.007 mL	83bc	61abc	20ab	3	0
Bunema, 0.06 mL + Busan, 0.0015 mL	86bc	60abc	20ab	3	10
Ammonia, 1 g	50a	79bc	28abcd	5	2
Ammonia, 0.1 g	70b	54a	18a	15	6
Untreated check	81bc	84cd	35cd	8	9
Black spruce					
Mylone 50D, 0.37 g	68ab[3]	36b[3]	12e	0	0
Mylone 50D, 0.08 g	80b	24ab	7cd	0	3
Bunema, 0.3 mL	78b	17a	8d	2	1
Bunema, 0.06 mL	67ab	19a	5abc	3	10
Busan, 0.007 mL	50a	15a	7bc	14	23
Busan, 0.0015 mL	73ab	22ab	4abc	6	34
Bunema, 0.3 mL + Busan 0.007 mL	64ab	17a	5abc	2	2
Bunema, 0.06 mL + Busan 0.0015 mL	68ab	19a	4abc	2	9
Ammonia, 1 g	61ab	35ab	5abc	4	5
Ammonia, 0.1 g	67ab	19a	4abc	8	9
Untreated check	79b	25ab	8d	6	9

[1] Amount of formulated product per litre of loosely packed soil.

[2] Number per 506 cm^2 of soil surface.

[3] Numbers within a column followed by the same letter not significantly different at $P = 0.05$ using Duncan's multiple-range test.

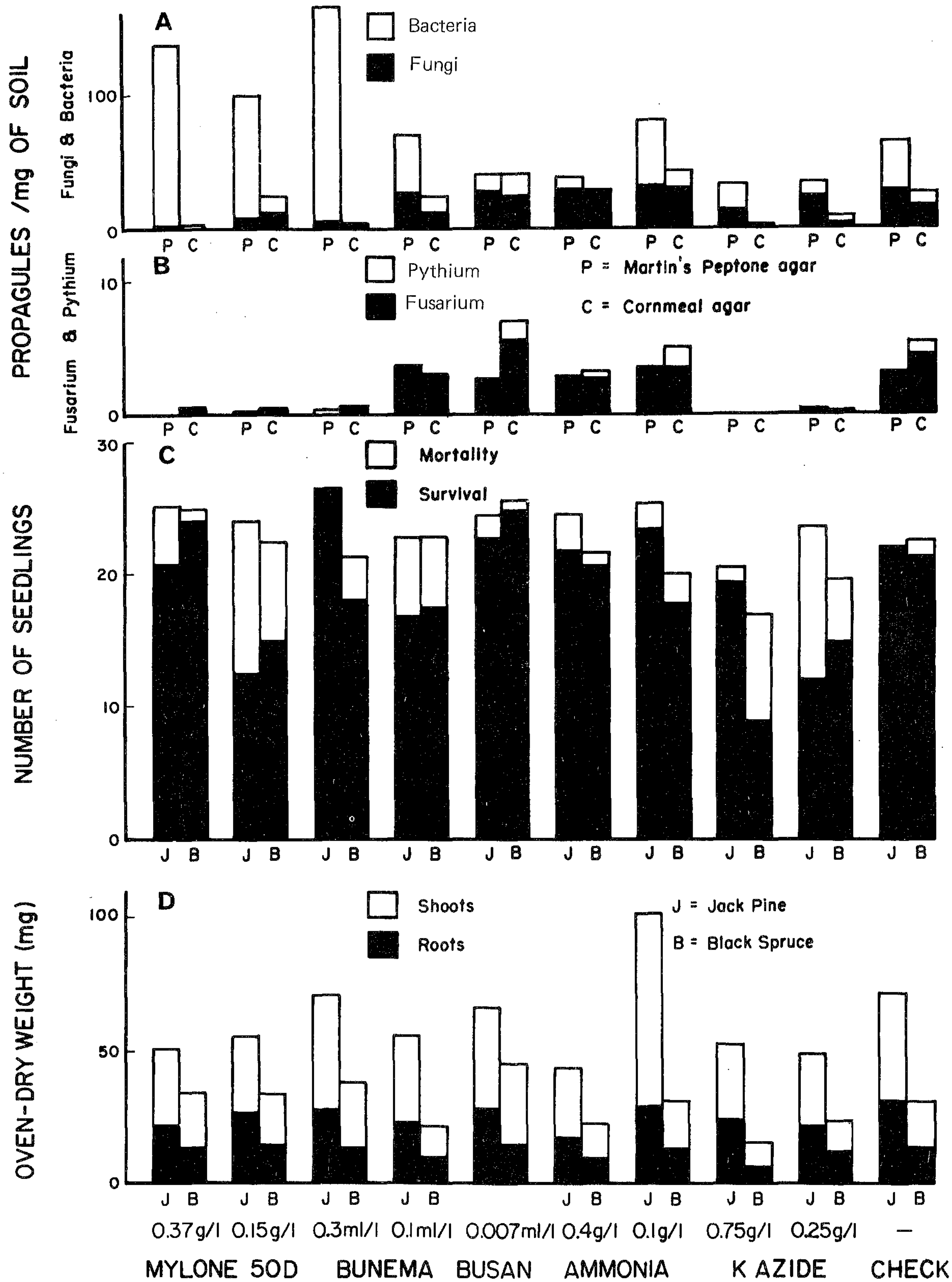

Figure 5. Populations of (A) fungi and bacteria and (B) Fusarium and Pythium spp. in Paradise soil 27 days after treatment (C) Emergence, mortality and survival of seedlings. (D) Dry weights of seedlings after 5 months.

Table 5. Emergence, mortality, and associated fungi in jack pine and black spruce seedlings grown for five months in Paradise nursery soils pretreated with partial sterilants

Species, treatment, and rate[1]	Seedling emergence (%)	Postemergence mortality (%)	Fungi isolated[2]			
			Pythium	*Fusarium*	*Rhizoctonia*	Other
Jack pine						
Mylone 50D, 0.37 g	84ab[3]	6ab[3]		4		9
Mylone 50D, 0.15 g	80ab	46bc		12		20
Bunema 0.3 mL	88b	0a				
Bunema 0.1 mL	76ab	36bc		12		3
Busan 0.007 mL	81ab	6abc		5	2	
Anhydrous ammonia, 0.4 g	82ab	11abc	1	9	1	
Anhydrous ammonia, 0.1 g	84ab	5ab		5		
Potassium azide, 0.075 g	68a	3ab	1			2
Potassium azide, 0.025 g	78ab	49c	3	10		30
Untreated check	73ab	1a				1
Black spruce						
Mylone 50D, 0.37 g	83b[3]	2ab[3]				1
Mylone 50D, 0.15 g	74ab	20ab		18		6
Bunema, 0.3 mL	71ab	12ab		5		4
Bunema, 0.1 mL	77ab	18ab		9		7
Busan, 0.007 mL	84b	1a	1	1		
Anhydrous ammonia, 0.4 g	73ab	4ab		1		1
Anhydrous ammonia, 0.1 g	67ab	11ab		2		2
Potassium azide, 0.075 g	57a	55b	1	1	1	18
Potassium azide, 0.025 g	65ab	16ab	1	5		4
Untreated check	75ab	3ab	1	2	2	3

[1] Amount of formulation per litre of loosely packed soil.

[2] Fungi growing from damped-off and dead seedlings after surface sterilization and placing on 2% malt agar.

[3] Numbers with a column followed by the same letter not significantly different at $P = 0.05$ using Duncan's multiple-range test.

Table 6. Seedling survival and dry weight and number of weeds in Paradise nursery soil treated with partial sterilants

Species, treatment, and rate[1]	Seedling survival (%)	Seedling dry weight		Number of weeds[2]	
		Shoots (mg)	Roots (mg)	Broadleaved	Grassy
Jack pine					
Mylone 50D, 0.37 g	69abc[3]	29a[3]	22a[3]	3	3
Mylone 50D, 0.15 g	41ab	28a	28a	0	12
Bunema, 0.3 mL	88c	43a	28a	0	3
Bunema, 0.1 mL	56abc	33a	22a	6	3
Busan, 0.007 mL	75abc	39a	28a	4	12
Ammonia, 0.4 g	73abc	26a	17a	0	11
Ammonia, 0.1 g	78bc	72b	29a	6	27
Potassium azide, 0.075 g	64abc	28a	24a	3	19
Potassium azide, 0.025 g	40a	28a	21a	2	17
Untreated check	73abc	41a	32a	7	15
Black spruce					
Mylone 50D, 0.37 g	80b[3]	21a[3]	14ab[3]	4	3
Mylone 50D, 0.15 g	49ab	18a	15b	0	6
Bunema, 0.3 mL	60ab	30a	12ab	0	5
Bunema, 0.1 mL	58ab	11a	10ab	4	8
Busan, 0.007 mL	83b	31a	14b	7	20
Ammonia, 0.4 g	69ab	13a	10ab	10	16
Ammonia, 0.1 g	59ab	20a	11ab	2	52
Potassium azide, 0.075 g	29a	10a	7a	0	19
Potassium azide, 0.025 g	49ab	12b	12ab	2	18
Untreated check	71b	18b	13ab	6	21

[1] Amount of formulated product per litre of loosely packed soil.

[2] Number per 506 cm^2 of soil surface.

[3] Figures within a column followed by the same letter not significantly different at $P = 0.05$ using Duncan's multiple-range test.

effective in reducing populations of pathogens or viable weed seeds.

Mylone (dazomet) was consistently the most beneficial material and is unlikely to be replaced by any of the newer products tested. At levels as low as 0.08 g/L (100 lb product or 50 lb a.i./acre) it suppressed recoverable populations of ***Fusarium*** and ***Pythium***. While it did not significantly increase seedling emergence or survival, the excessive rate (0.83 g/L = 1109 lb product/acre) used in the first experiment did enhance the growth of both conifer seedlings and weeds. In the second and third experiments (Charlottetown and Paradise soils) Mylone suppressed weed emergence, confirming its reputation as an effective preplant herbicide.

Bunema, recently recommended for control of fungi, bacteria, and nematodes as a preplant in-furrow or foliar treatment, appeared to hold some promise as a preplant treatment in the foregoing tests. In previous studies (Wall and Cormier 1976), this material was found to be phytotoxic at rates above 4 lb a.i. (1 gal product) per acre when applied to seedbeds at the time of sowing. This phytotoxic effect was not evident when used as a presowing treatment, even at the excessively high rates used in the first experiment. Although it did not enhance seedling survival and growth rates, it suppressed recoverable populations of ***Pythium*** and ***Fusarium*** and suppressed weed emergence at rates of 0.3 mL/L (40 gal product/acre). Its observed effects on bacterial populations were inconsistent, probably because antibacterial culture media were used. Its possible nematicidal properties, not evaluated in these experiments, are potentially useful in forest nursery practice.

Busan is recommended as a seed or soil treatment for cotton and other agricultural crops[4]. Its apparent phytotoxicity to germinating conifer seedlings (McDonald et al. 1973, Wall and Cormier 1976) prompted its inclusion in these tests as a preplant fungicide. As such, it appears to have no application since it enhanced, rather than suppressed, populations of ***Fusarium***, even at the excessively high rates used in the first experiment. In addition, it caused some mortality of black spruce at 0.06 mL/L (8 gal product/acre) in the Charlottetown soil and its effects on seedlings were completely devastating at the excessive rates used in the inoculated greenhouse soil. Whether its deleterious effects resulted from direct phytotoxicity or enhanced damping-off cannot readily be determined from the available evidence, but its use on conifer seedbeds must at present, be discouraged. When combined with Bunema, there was an increase in ***Fusarium*** populations and no measurable beneficial effects on seedlings.

Anhydrous ammonia was evaluated in only two tests and at varying and uncertain levels. However, it caused considerable postemergence mortality in one instance and therefore cannot be recommended as a preplant treatment for conifers. In the same soil in which it caused mortality, it also brought about a sharp reduction in levels of ***Fusarium***. Therefore, it holds some promise as a soil fungicide that would also raise nitrogen levels. In forest nursery practice, it might be useful if applied prior to planting a cover crop.

Potassium azide, at both 0.075 g/L and 0.025 g/L (100 and 33 lb/acre, respectively) reduced the levels of fungal pathogens but caused some postemergence mortality. Because of the latter effect, it cannot be recommended as a preplant treatment, but like ammonia, might have beneficial results if applied prior to the planting of a cover crop. For effective suppression of pathogenic fungi, rates of about 30-40 lb/acre appear adequate.

Since none of the materials evaluated showed any superiority over Mylone as preplanting treatments, there appears to be little value in checking the above results in the field. Mylone has been used sucessfully in many forest nurseries as a partial sterilant. However, in the event of shortages of this material or the discovery of adverse side effects, other approaches to nursery soil improvement should be explored. One such approach is the possibility of reducing fungal pathogens through the use of azides or ammonia followed by cover crops.

[4] Pulido, M.L. Buckman Laboratories, Inc., Memphis, Tenn. Personal correspondence.

REFERENCES

Bloomberg, W.J. 1965. The effect of chemical sterilization on the fungus population of soil in relation to root disease of Douglas-fir seedlings. For. Chron. 41: 182-187.

Campagna, J.P. and D.P. White. 1973. Nursery soil fumigation affects growth and phosphorus nutrition of pine and spruce seedlings. For. Chron. 49: 219-223.

Clifford, E.D. and J.W. Massello. 1966. Effect of soil fumigants on seedling development. Tree Planters' Notes 76: 9-12.

Henry, B.W. 1953. A root rot of southern pine nursery seedlings and its control by soil fumigation. Phytopathology 43: 81-88.

Hill, J.A. 1955. Methyl bromide gas controls weeds, nematodes and rots in seedbeds. Tree Planters' Notes 21: 221-227.

Kelley, W.D. and R. Rodriguez-Kabana. 1975. Effects of potassium azide on soil microbial populations and soil enzymatic activities. Can. J. Microbiol. 21: 565-570.

Low, A.J. 1974. The use of dazomet for partial sterilization of forest nursery soils. Forestry 47: 31-43.

McDonald, S.E., J.A. Jackson, B.E. Fisher, and R.M. Raymond. 1973. Post-planting fungicide application to reduce losses to damping-off may be detrimental. Tree Planters' Notes 24(2): 14-15.

Morris, C.L. 1960. Soil fumigation evaluations in white pine seedbeds and other nursery evaluations. Tree Planters' Notes 41: 17-21.

Palmer, J.G. and E. Hackskaylo. 1958. Additional findings as to the effects of several biocides on growth of seedling pines and incidence of mycorrhizae in field plots. Plant Dis. Reptr. 42: 536-537.

Peterson, G.W. 1970. Response of ponderosa pine seedlings to soil fumigants. Plant Dis. Reptr. 54: 572-575.

Reddy, M.A.R. 1962. A study of the effects of disease control measures on the soil microflora of sitka spruce (***Picea sitchensis***) in forest nurseries. In: For. Comm. Rept. Forest Res., London. p. 89-90.

Ruehle, J.L., J.T. May, and S.J. Rowan. 1966. Nursery fumigation with Vorlex. Tree Planters' Notes 76: 4.

Smiley, R.W., R.J. Cook, and R.I. Pappendick. 1970. Anhydrous ammonia as a soil fungicide against ***Fusarium*** and fungicidal activity in the ammonia retention zone. Phytopathology 60: 1227-1232.

Smith, R.S. and R.V. Bega. 1966. Root disease control by fumigation in forest nurseries. Plant Dis. Reptr. 50: 245-248.

Theis, W.G. and R.F. Patton. 1971. Evaluation of ***Cylindrocladium*** root rot control by fumigation. Forest Sci. 17: 323-331.

Vaartaja, O. 1958. Treating seedbeds with the new sterilizer mylone. Canada Dept. Agr., For. Biol. Div., Bi-mon. Progr. Rept. 14(2): 3.

Vaartaja, O. 1967. Reinfestation of sterilized nursery seedbeds by fungi. Can. J. Microbiol. 13: 771-776.

van den Driessche, R. 1963. Partial sterilization of Douglas-fir seedbeds with formalin and chloropicrin. For. Sci. 9: 330-334.

Wall, R.E. 1974. Recent conifer disease problems in forest nurseries in the Maritime provinces. Can. Plant Dis. Survey 54: 116-118.

Wall, R.E. and J.R. Cormier. 1976. Fungicidal drench trials for the control of damping-off in conifer seedbeds. Canada Dept. Env., Marit. For. Res. Cent., Inf. Rept. M-X-61.

Warcup, J.H. 1952. Effect of partial sterilization by steam or formalin on damping-off of Sitka spruce. Trans. Brit. Mycol. Soc. 35: 248-262.

Wright, E. 1964. Effect of fumigation with Trizone on microbial properties of soil and growth of seedlings. Down to Earth 20: 13-15.

Wycoff, H.B. 1952. Methyl bromide controls soil organisms which cause mortality in eastern white pine seedlings. Tree Planters' Notes 12: 11-14.

APPENDIX

(Sources and chemistry of products used)

Anhydrous Ammonia.	Canadian Liquid Air Ltd., Montreal, Quebec.
Bunema.	40% potassium N-hydroxymethyl-N-methyl dithiocarbamate in liquid water-soluble solution. Buckman Laboratories Inc., Memphis Tenn.
Busan.	30% liquid emulsifiable concentrate of 2-(Thiocyanomethylthio) benzothiazole. Buckman Laboratories Inc., Memphis, Tenn.
Mylone 50D.	(Dazomet) 3,5-dimethyl tetrahydro-2-thio 1,3,5-thiadiazime in 50% granular formulation. Hopkins Agricultural Chemical Co., Madison, Wisc.
Potassium azide.	100% active ingredient. Eastman Kodak Co., Rochester, N.Y.