FUNGICIDAL CONTROL OF *PHAEOCRYPTOPUS GAEUMANNII* INFECTION IN A 19-YEAR-OLD DOUGLAS FIR STAND

I. A. HOOD and J. B. VAN DER PAS

Forest Research Institute, New Zealand Forest Service, Rotorua

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ABSTRACT

Sixty-eight trees in a stand of unhealthy 19-year-old Douglas fir (**Pseudotsuga menziesii** (Mirb.) Franco) were treated with fungicides either by spraying twice-yearly for 2 years, or by injection three times during 4 years. Handspraying crown foliage to beyond run-off point during the growing season with separate applications of 0.05% copper (as copper oxychloride; 0.1% Multifilm X-77 added) and/or 0.02% triforine reduced plot mean current-needle infection by **Phaeocryptopus gaeumannii** (Rohde) Petrak from $\geq$ 99% to 42% or less. Helicopter spraying with these chemicals at about 2240 litres/ha/application was ineffective (mean infection $\geq$ 96%) and cannot yet be recommended for the control of **P. gaeumannii** in forest management. Trunk injections of 0.35% carbendazim in N/10 HCl at a rate of 3-4 litres/tree/injection reduced plot mean infection to 13% or less after two seasons. Positive responses in growth (all injection plots) and needle retention (one plot) were indicated 2 and 4 years, respectively, after injection plots were first treated. Most trees handsprayed with copper and several injected trees developed phytotoxicity symptoms after treatment.

INTRODUCTION

The ascomycetous fungus *Phaeocryptopus gaeumannii* (Rohde) Petrak is believed to be the main cause of Swiss needle cast disease of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) (e.g., Peace, 1962). Although infection has been reduced on current foliage of seedlings or small trees by fungicidal dipping, handspraying, or mistblowing during the spring-summer growing period (e.g., Morton, 1975; Hood, 1976; Morton and Miller, 1976), attempts at control by means of low-volume aerial spraying have been only moderately effective (Hood, 1978; J. W. Ray, pers. comm.). This paper describes an experiment designed to test three different methods of fungicidal control of infection in one Douglas fir stand. Aerial spraying was used to see if infection could be reduced using high-volume applications; handspraying was carried out at approximately the same time using even greater volumes than applied by air; and fungicide was injected into tree stems, a technique that has been effective in dealing with fungal infections in other tree species (reviewed, Clifford *et al.*, 1977; also Gregory *et al.*, 1973; Jaynes and Van Alfen, 1977; Jones *et al.*, 1973). For the spraying trial, the fungicides chosen

had been tested earlier in seedling experiments (Vanner and Hood, 1974). In other experiments, plants nearly free of infection were found to retain significantly more older foliage than did plants heavily infected with *P. gaeumannii* (Strittmatter, 1974; Hood, 1977).

METHOD

Site and Plot Layout

An unthinned stand of 19-year-old Douglas fir planted at 1.8 × 1.8 m on gently undulating land in Whakarewarewa State Forest near Rotorua was chosen for the trial. Trees were heavily infected by *P. gaeumannii* and showed symptoms of needle casting and chlorosis. Viewed from a distance the stand appeared a mottled, pale, yellow-green colour, especially prior to flush. Three treatment plots alternating with three control plots, each plot consisting of nine tagged trees, were set up in a portion of the stand set aside for aerial spraying. Three flight lines 80-100 m long were marked out with metal drums, in such a way that one line passed over each treatment plot. One swath width was sufficient to fully cover a treatment plot, but was at least 20 m from an untreated control plot. Eight more nine-tree plots were established in an adjacent part of the same stand. Of these eight plots, two were randomly selected for handspraying, three for injecting, and three as no-treatment controls. These plots were distributed irregularly, at least 15 m from each other. All plots consisted of groups of mainly dominant or codominant trees, suppressed trees being avoided as much as possible during selection. Two-thirds of the trees in the injection plots and their untreated controls had green crowns of either 10 or 11 complete whorls in 1977.

TABLE 1—Details of fungicide spraying trials

Trial	Year	Application dates	Interval between applications (weeks)	Active ingredient	Concentration (% a.i.)	Approximate rate per application (kg a.i./ha)
Aerial spray	1974-75	6 Dec./ 31 Jan.	8	Triforine*	0.04	0.9
	1975-76	9 Dec./ 3 Feb.	8	Copper†	0.05-0.06	1.1-1.5
Hand spray	1974-75	13-19 Dec./ 21-28 Jan.‡	5½-6	Triforine	0.02	17-30
	1975-76	3-8 Dec./ 21-22 Jan.	6½-7	Copper Triforine	0.03-0.05 0.02	44-70 26-30

* Applied as Cela W524 (20% w/v emulsifiable concentrate). A small quantity of dye (crystal violet or Sprayrite marker dye) was added as a pad marker for aerial applications.

† Applied as copper oxychloride (50% w/w Cu, wettable powder; 0.1% Multifilm X-77 was added as a surfactant).

‡ Rainfall in the periods 0-8 and 8-24 hours after handspraying plot 7 were nil and 1.9 cm respectively; part of plot 7 was also sprayed 5 days earlier (0.02% a.i. triforine, rate 24 kg a.i./ha) but very heavy rain fell immediately after.

Spraying

Aerial spraying was carried out over two successive growing seasons, and there were two applications per season of either triforine or copper oxychloride (Table 1). A helicopter fitted with boom and nozzle equipment sprayed fungicide suspension at a rate of 560 litres/ha (N.Z. Forest Service, 1976, p. 33). Because multiple passes were made, each spray plot usually received approximately 2240 litres/ha on each application. In order to protect most new foliage, the first spray each season was not applied until buds on nearly all trees had burst. Because of the variation in flushing time, shoots in upper crowns of some trees were up to 40 cm long when first sprayed while others had just flushed. Trees were sprayed under conditions of little or no wind, and, at most, there was only a trace of rainfall within 12 hours of each application. Blank paper pads placed in open tracks across flight paths were used to monitor coverage during spraying. Because of the high volumes applied some pads were completely saturated during the sprays in the first year. In the second year the greatest pad-surface-area coverage during the first spray was only about 20%, possibly because the aircraft flew further above tree-top level than during other aerial spray applications. Coverage was high during the second spray in the same season, however.

Trees in handspray plots were sprayed at comparable times to those of aerial applications (Table 1). Foliage was sprayed to beyond run-off point using a motorised pump and spray tank, with a pressure hose leading to a nozzle held by a person climbing within tree crowns. Spray quantities of 250-500 litres/plot were used on each application. This large variation was due partly to differences in crown volumes. With one exception (second spray of plot 7 in 1974-75, Table 1) no rain fell within 24 hours of spraying.

Injecting

Trees were injected with the fungicide carbendazim dissolved in diluted hydrochloric acid. The carbendazim used was either a commercial product (99.6% a.i.) or a preparation made in the laboratory by acidifying benomyl, heating, and then reprecipitating with dilute ammonia. The solution was introduced into the transpiration stream of each tree through five holes spaced around the circumference of the stem near the base, to ensure that fungicide reached as much of the crown as possible. Conducting tissue of Douglas fir sapwood follows the "sectorial winding" type of ascent as described by Vité and Rudinsky (1959) and therefore fluids injected at a single point are not sufficiently distributed to reach the full crown. Supply was maintained by gravity feed. When uptake was completed holes were plugged with corks. A concentration of 0.34% a.i. carbendazim in N/10 HCl was used in the first 2 years, but this was reduced to a half-strength mixture of both chemicals in the next 2 years because of the appearance of damage on a few of the smaller trees. For most trees during the first 3 years the quantity was 3-4 litres/tree/injection. In the fourth year larger trees were given proportionately greater amounts up to a maximum of 14 litres/tree in order to increase the effectiveness of control. Trees were injected annually between December and August (Table 3). Individual trees took from several days to 4 weeks to absorb 3-4 litres, although some trees took longer to absorb the same quantity in the fourth year.

Evaluation

In the sprayed plots mean percentage infection was examined over 3 years; growth and needle retention were recorded as well in the injected plots, for 5 years. Results were evaluated annually between August and November, usually within a 6-week period each year. At the start of the trial, d.b.h. measurements were taken, and heights were recorded by climbing with a pole and measuring tape. Foliage samples were detached for laboratory counts of infection and needle retention (Fig. 1). Side shoots (secondary

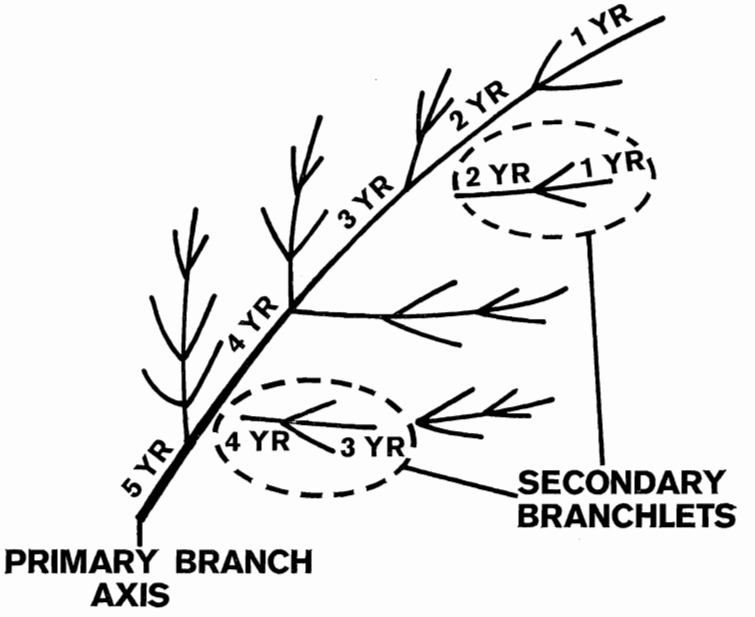

FIG. 1—Method of sampling shoots.

branchlets) were taken from two, dominant, primary branches pointing approximately north and south in the fifth whorl. In 1976-78, however, a change was made, and samples were taken from one randomly orientated primary branch in each of several whorls. Foliage was stored for brief periods at 4°C before examination.

In order to provide an index of infection, 50 current (nearly 1-year-old) needles per shoot were examined under a stereomicroscope and the percentage of needles bearing pseudothecia of *P. gaeumannii* was determined. A microscope was used to ensure that any small, immature pseudothecia (Rohde, 1937) were not overlooked. Needle retention levels of injection plot trees were evaluated from the third trial year by counting the numbers of needles and needle scars of one or two spiral sets running the full length of a year's shoot growth and calculating the percentage retained.

At the end of the experiment injected and untreated trees were felled for growth analysis. Final over-bark tree volumes were determined from diameter measurements made at 100-cm intervals along each stem, and annual basal areas that had accrued during the trial period were calculated from radial measurements made on discs cut at the breast height position on each felled tree. Annual volume increments were

obtained from these results, using a linear regression ($Y = b_0 + b_1X$) of volume (Y) against basal area (X). Regressions ($Y_1 = C_0 + C_1X_1$) of initial volume (Y_1) against annual volume increment (X_1) of treated and untreated groups were then compared for each year using analysis of covariance.

RESULTS

Graphical plotting of results indicated no difference in either plot needle retention or infection between north- and south-facing branches on the same whorl. Values for each pair of branches were therefore meaned to give a single-tree value prior to analyses. All percentage data were analysed in transformed (inverse sine) form.

Besides *P. gaeumannii,* ascocarps of two micropeltaceous fungi were sometimes observed on needles under the stereomicroscope. They were found to fit best into *Clypeolum* Speg. and *Clypeolinopsis* Batista. Photomicrographs and herbarium material are held at NZFRI.

Infection (spray plots)

Aerial spraying was unsuccessful in controlling infection by *P. gaeumannii* and mean plot infection did not fall below 96% after fungicide applications (Table 2). By contrast, thorough handspraying of tree crowns to beyond run-off point resulted in a reduction of mean infection to 42% or less. The first hand-application of copper oxychloride in the second year was followed by pronounced casting of older needles.

TABLE 2—Percentage of current foliage infected in the spray trials. The percentage infection figures are the means of counts from pairs* of fifth whorl, second-order branchlets, together with 95% confidence limits

Year	Plot No.	Treatment date	Evaluation date	Infection percentage Mean	95% conf. limits
(a) Aerial spray trial					
1973-74	9, 10, 11	—	Aug, Oct '74	100	99-100
	12, 13, 14	—	Aug, Oct '74	99	98-100
1974-75	9, 10, 11	Dec/Jan	Oct, Nov '75	100	100
	12, 13, 14	—	Nov '75	100	100
1975-76	9, 10, 11	Dec/Feb	Oct '76	98	96-100
	12, 13, 14	—	Oct, Nov '76	100	98-100
(b) Hand spray trial					
1973-74	7	—	Sept '74	100	99-100
	8	—	Oct '74	100	99-100
	4, 5, 6	—	Oct '74	100	99-100
1974-75	7	Dec/Jan	Nov '75	42	20-67
	8	Dec/Jan	Nov '75	10	4-17
	4, 5, 6	—	Nov '75	100	100
1975-76	7	Dec/Jan	Oct '76	24	8-47
	8	Dec/Jan	Oct '76	13	0-38
	4, 5, 6	—	Sept '76	100	100

* One branch pair per tree, 8 or 9 trees per plot. For untreated plots, group means are shown for all trees combined.

Infection (injection plots)

Mean plot infection was reduced to 13% or less after two consecutive periods of injecting (Table 3). Further treatment, even at the halved concentration, maintained the low level of current infection. Current infection was also low in eighth whorl foliage when it was evaluated in the fourth year. Current needles with low percentage-infection levels in 1975 and 1976 were found to be still low in infection when the same foliage sets were inspected a year later. Although infection in some plots appeared to rise in non-treatment years, increases were not found to be significant in the first year after injection ceased (t tests; $P > 0.05$). Foliage discolouration or stem injury was found on several trees after they had been injected, but most tree crowns showed no ill effects.

TABLE 3—Percentage of current foliage infected in the injection trial. The percentage infection figures are the means of counts from pairs* of second-order branches, together with 95% confidence limits

Year	Plot No.	Treatment date	Evaluation date	Infection percentage Mean	95% conf. limits
(a) Fifth-whorl branches					
1973-74	1	Mar-May '74	Aug '74	98	90-100
	2	—	Oct '74	99	96-100
	3	—	Oct '74	100	99-100
	4, 5, 6	—	Oct '74	100	99-100
1974-75	1	Dec-Jan	Oct '75	8	4-13
	2	Jan-Feb '75	Nov '75	41	15-70
	3	Feb '75	Nov '75	93	72-100
	4, 5, 6	—	Nov '75	100	100
1975-76	1	—	Sep '76	42	6-84
	2	Dec-Jan	Sep '76	9	0-30
	3	Jan-Feb '76	Sep '76	13	1-38
	4, 5, 6	—	Sep '76	100	100
1976-77	1	Dec-Apr	Oct '77	19	0-73
	2	Jan-Apr '77	Oct '77	1	0-4
	3	Mar-Aug '77	Oct '77	11	0-40
	4, 5, 6	—	Oct '77	99	97-100
1977-78	1	—	Sep '78	27	0-77
	2	—	Oct '78	21	0-61
	3	—	Oct '78	4	0-35
	4, 5, 6	—	Oct '78	93	84-98
(b) Eighth whorl branches					
1976-77	1	Dec-Apr	Oct '77	1	0-11
	2	Jan-Apr '77	Oct '77	0	0-1
	3	Mar-Aug '77	Oct '77	10	0-51
	4, 5, 6	—	Oct '77	88†	66-99

* Counts were from single branches in 1976-78; one branch or branch pair was sampled per tree, 6-9 trees per plot. For untreated plots, group means are shown for all trees combined.

† From counts of 16 single branches.

Needle Retention (injection plots)

One of the three injection plots showed significantly greater mean retention of one needle set relative to the other treated and untreated plots (Table 4: 1977-78, plot 1). This needle set also showed very low infection (8%) when it was current in 1974-75 (Table 3). In order to conserve space, sixth and eighth whorl needle-retention percentages are not given. At the sixth whorl, the same plot 1 needle set was also retained to a significantly greater degree both in 1976-77 (2- to 3-year-old needles) and 1977-78 (3- to 4-year-old needles), but at the eighth whorl this trend did not appear. In neither of the other two treatment plots was there a significant increase in needle retention at any whorl. Plot 1 tree crowns began to appear greener than those of surrounding untreated trees from the 1975-76 survey onwards. Secondary branchlets of all injected and untreated control trees showed significantly higher overall mean needle retention values than primary branches for 1- to 2-year-old and 2- to 3-year-old foliage in the 2 years evaluated (t tests, $P \leqq 0.001$).

TABLE 4—Percentage of foliage retained at the fifth whorl (injection plots)

Year	Group	Plot No.	Evaluation date	Foliage age (yr) 0-1	1-2	2-3	3-4
1975-76	Treat.	1	Sep '76	86	49	13	0
		2	Sep '76	94	28	14	2
		3	Sep '76	84	18	7	1
	Untreat.	4	Sep '76	91	23	39	< 1
		5	Sep '76	90	22	38	2
		6	Sep '76	92	14	20	1
1976-77	Treat	1	Oct '77	86	36	56	1
		2	Oct '77	96	52	27	2
			Oct '77	80	48	30	1
		3					
	Untreat.	4	Oct '77	92	35	16	< 1
		5	Oct '77	88	29	23	1
		6	Oct '77	89	48	3	< 1
1977-78	Treat.	1	Aug, Sep '78	65	59	51 53	14 25
		2	Aug, Oct '78	84	73	55 52	1 1
		3	Aug, Oct '78	70	60	39 42	< 1 0
	Untreat.	4	Aug, Oct '78	48	63	15 24	< 1 < 1
		5	Aug, Oct '78	48	37	26 21	< 1 0
		6	Aug, Oct '78	56	48	16 16	0 0

Values are those of mean percentage retention from counts from 7-9 second-order branches per plot. Mean plot values not significantly different at the 0.05 level are linked by a common bar (Scheffé's test). Two surveys were made in 1977-78 which concorded statistically; means of both are given.

Growth

At the beginning of the trial the mean height and d.b.h. values for all sprayed, injected, and control trees were 13.6 m and 13.2 cm, respectively. There were no significant between-plot differences for d.b.h., but some mean plot heights were significantly greater than others ($P \leq 0.05$; plot means ranged from 11.5 m to 14.8 m). The regressions of volume (Y) against basal area (X) calculated from stem analysis measurements were not substantially different between injected and control groups, so the combined regression was used to calculate tree volumes for each trial year. These figures were then used to determine plot volume increments (Fig. 2). A growth increase was indicated 2 years after first injections in each treatment plot. The volume (Y_1) against volume increment (X_1) regression lines differed significantly between treatment and control groups even before treatments were begun ($P \leq 0.05$). To overcome this difficulty comparisons were instead made between 12 selected pairs of treated and control trees of approximately equal volume in 1972 (Table 5). Significant differences between treatment and control groups developed in the last 2 years.

TABLE 5—Analysis of covariance for annual increment volume differences between 12 selected pairs of treated and untreated trees

Year	Treated			Untreated			F ratio	
	C_0	C_1	r^2	C_0	C_1	r^2	Difference in slope	Difference in level
1972-73	—1.28	0.13	0.71	0.67	0.07	0.39	1.2 NS	2.3 NS
1973-74	—2.84	0.14	0.85	0.57	0.06	0.53	4.0 NS	3.4 NS
1974-75	—3.42	0.14	0.83	—1.60	0.09	0.59	1.6 NS	1.7 NS
1975-76	—4.49	0.13	0.62	—1.26	0.07	0.44	0.9 NS	0.7 NS
1976-77	—5.68	0.17	0.80	—1.28	0.07	0.68	1.3 NS	9.5 **
1977-78	—3.18	0.16	0.87	—1.06	0.07	0.80	14.8 **	14.9 **

Regression formula: $Y_1 = C_0 + C_1X_1$

where Y_1 = ann. vol. increment,

X_1 = corresponding initial vol. for same growth period,

C_0, C_1 = regression coefficients,

r^2 = coefficient of determination.

** indicates treatment and control coefficients significantly different ($P \leq 0.01$)

NS = Not significant.

DISCUSSION

High-volume aerial spraying using conventional boom and nozzle equipment was even less effective in controlling *P. gaeumannii* infection than some of the earlier, low-

volume, aerial spraying which had reduced infection to between 55% and 93% of untreated control trees (Hood, 1978). However, handspraying gave good control. The dates of aerial spraying and handspraying were close enough to suggest that incorrect timing is not the explanation for the ineffectiveness of the aerial spraying. (Infection

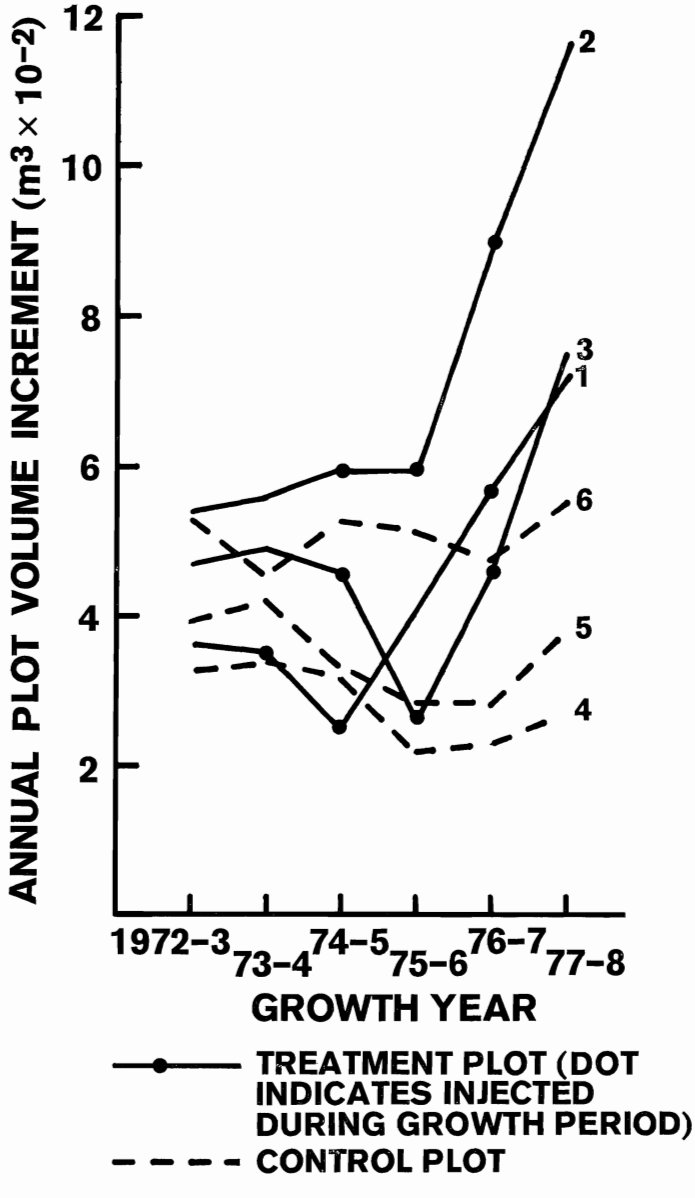

FIG. 2—Annual volume increments for injection plots and untreated controls.

on potted seedlings had also been reduced by spraying with a copper fungicide at times comparable to those used in these aerial trials — Hood, 1976). The possible explanation may be that insufficient chemical was used during aerial spraying, even though high volumes were applied. Handsprayed plots were sprayed with even higher volumes (40-80 times more per hectare) than were applied to aerial plots, and foliage was wetted thoroughly to beyond run-off point. This meant that, although comparable concentrations were used, handsprayed trees received greater amounts of fungicides (Table 1). Fungicide rates (2 or 13 kg a.i./ha/season) were also greater in some of the earlier aerial trials even though trees were then sprayed with lower volumes. At this stage, aerial spraying shows little promise as an effective means of controlling *P. gaeumannii* in the management of Douglas fir plantations.

Although the first handspray in the second year using copper oxychloride stimulated casting of older needles, most current foliage remained on the affected trees for evaluation of infection. Spraying of seedlings and young, unhealthy trees with copper fungicides has apparently sometimes caused defoliation in other experiments (Hood, unpubl. rep.; A. L. Vanner, pers. comm.). Neither aerial spraying nor handspraying with triforine produced any noticeable foliage damage in these trials.

In the fourth year the volumes injected per tree were proportionately adjusted to diameter size, because earlier in the trial foliage infection of several larger trees was apparently little affected by treatment. Had this been done earlier, plot infection means might have been further reduced. Needle retention was evaluated only for injection plots and controls, because of the ineffectiveness of aerial spraying and the foliage damage to handsprayed trees. One injection plot showed significantly improved needle retention associated with reduced infection. There is a suggestion that the other two treatment plots might also have demonstrated increased retention of 3- to 4-year-old foliage had the trial been continued for another year, since values of 2- to 3-year-old needle retention tended to be higher in treatment plots in 1977-78 (Table 4). Differences between treatment and control groups will show up only when sufficient time has elapsed for infected foliage to fall, as the variation present between trees in plots may tend to mask such differences.

It is also possible that some unknown site factor may have contributed to the ill health of this stand. Needle retention levels in the trial stand were particularly low, and even heavily infected trees of similar age surveyed in Kaingaroa State Forest still retained a mean level of 18% of 3- to 4-year-old needles on fifth whorl, secondary branchlets (cf. Table 4). There was an indication of a growth response by trees in all plots injected with carbendazim. In addition to reducing the level of *P. gaeumannii* infection parasitising current foliage, there are other ways in which carbendazim may have benefited trees in this trial. Spencer (1977) noted that when a number of crop plants are treated with the closely related compound, benomyl, greener foliage colour occurs, and treated plants may be heavier than untreated ones. He suggested that such effects could be associated with the known cytokinin-like behaviour of these compounds (Thomas, 1974). Carbendazim may also supplement host nitrogen requirements since the active ingredient consists of 22% by weight of this element. The response of Douglas fir to nitrogenous fertiliser applications has been studied by Gessel *et al.* (1969), Miller and Reukema (1977), and others. Over the full period of the injection trial,

trees received a total of between 6 and 10 g nitrogen each (average 7 g; approximately equivalent to 5 kg N/ha/year, over 4 years) through treatment with carbendazim. Although this is not a great quantity when compared to estimates of nitrogen being taken up annually in Douglas fir (e.g., > 62 kg/ha/year — Dice, 1970), it is still a contribution. These facts limit the value of tree injecting as a tool for determining pathogenicity in the field, but the method does provide a means of controlling the disease in individual trees.

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