CYCLANEUSMA (NAEMACYCLUS) NEEDLE-CAST OF PINUS RADIATA IN NEW ZEALAND

4: CHEMICAL CONTROL RESEARCH

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(Received for publication 12 April 1984: revision 25 July 1984)

ABSTRACT

Fungicides were screened for ability to control needle-cast of **Pinus radiata** D. Don caused by **Cyclaneusma minus** (Butin) DiCosmo **et al.** Undetached shoots were dipped in water-based suspensions at fortnightly intervals for 20 months from the time of flush. Significant improvements in levels of needle retention were achieved with dodine (86% retention), anilazine (76%), benomyl (74%), and dichlone (66%), in comparison with untreated controls (39%). Injections of acidified aqueous solutions of carbendazim into stems of 8- to 10-year-old trees resulted in reductions in foliage yellowing, needle loss, and numbers of **C. minus** colonies isolated from the foliage. Needle loss was reduced two- to three-fold on 0- to 1-year-old shoots after one season of injections; after 2 consecutive years of injections needle loss was reduced seven-fold on 1- to 2-year-old shoots. Yellowing and defoliation were also reduced by injections of the non-fungicidal compounds ortho-phenylenediamine and L-arginine monohydrochloride, implying that not all the effects of carbendazim are a consequence of its fungicidal properties. Aerial applications of benomyl (0.25 kg/ha) in an emulsion of water (6 litres/ha) and BP crop oil (4 litres/ha) in June and July failed to check the disease in a 10-year-old **P. radiata** plantation.

INTRODUCTION

The needle-infecting fungus, *Cyclaneusma minus,* causes premature defoliation of *Pinus radiata* in plantations throughout New Zealand (Gilmour 1966; Gadgil 1984). The disease has a widespread distribution and its impact on stand growth can be severe (van der Pas, Slater-Hayes, Gadgil & Bulman 1984; van der Pas, Bulman, & Slater-Hayes 1984). It was therefore considered desirable that a reliable method of control be developed. Exploratory trials were established to determine the effectiveness of different fungicides in controlling the disease. Three methods of application were tried. In one experiment a selection of fungicides was screened for ability to protect new foliage by dipping single shoots. In another, the systemic fungicide, carbendazim, was injected into tree stems to test its ability to prevent disease. The effects of injecting two non-fungicidal compounds were also evaluated during this trial. In the third experiment control of the disease was attempted in a stand of *P. radiata* by aerial applications of benomyl.

FUNGICIDE SCREENING TRIAL

Method

This experiment was conducted in a low-pruned 10-year-old *P. radiata* stand established from grafts of one clone (FRI 121) which is known to be particularly susceptible to the disease. The trees in this stand had deep crowns, as a result of early thinning to 500 stems/ha. Ten shoots located 2.5 to 3 m above the ground were selected from the bottom whorl of each of 10 trees. Selected shoots were evenly spaced around the tree to avoid cross-contamination of fungicides during treatment. Treatments were conducted at fortnightly intervals for 20 months, beginning in July at the onset of new shoot extension. Each shoot was dipped in one of nine aqueous fungicide suspensions, the tenth shoot being left untreated as a control (Table 1). Fungicide suspensions were mixed immediately prior to use, and care was taken to ensure complete needle coverage.

TABLE 1—Results of fungicide screening trial, in order of effectiveness

Treatment*	Concentration (g/l)	Needles retained† (%)
Dodine	0.65	$86 \pm 9_{a}$
Anilazine	1.0	$76 \pm 10_{ab}$
Benomyl	0.25	$74 \pm 13_{ab}$
Dichlone	0.5	$66 \pm 15_{ab}$
Maneb	1.6	$63 \pm 18_{b}$
Chlorothalonil	1.5	$61 \pm 23_{b}$
Captan	1.6	$60 \pm 23_{b}$
Untreated control	—	$37 \pm 18_{c}$
Oxycarboxin	0.75	$35 \pm 17_{c}$
Copper oxychloride	2.0	—

* 1 ml/l of Triton X-77 surfactant was added to all fungicide treatments.

† Means and 95% confidence limits are shown. Values linked by common letter subscripts are not significantly different ($p \geq 0.05$; Duncan's multiple range test).

Needle retention was evaluated as a measure of treatment effectiveness. Assessments were made 9 months (in April) and 18 months (in January) after treatments began. At each assessment a count was made of the number of needles still retained on the section of the stem produced and treated during the first season (distinguished from other shoot growth by marks). Numbers of needles counted in January were expressed as percentages of the numbers present the previous April to give needle retention level. On average, 110 (range 31–221) needles were counted per shoot in April, when foliage was still less than 1 year old.

Results

Disease symptoms were first observed in May, 10 months after treatments began, but did not intensify until August. Most diseased foliage fell in September and early October (spring) but needles still retained on treatment shoots the following January were green. Values of needle retention are ranked in Table 1 in order of fungicide effectiveness. Needle counts could not be made on shoots treated with copper oxychloride as regular dipping in this fungicide led to shoot death. There was considerable between-tree variation for different treatments, as indicated by the high confidence limits. Despite this, differences between treatments were significant. Greatest control of disease was achieved using dodine, anilazine, benomyl, and dichlone. Only oxycarboxin failed to give some control.

TREE INJECTION EXPERIMENT

Method

The fungicide carbendazim and two non-fungicidal compounds, *ortho*-phenylenediamine and L-arginine monohydrochloride, were injected into the stems of *P. radiata* trees, and levels of disease control evaluated. Twenty-one trees were selected in the stand previously described (FRI Clone 121). At age 10 years the treated trees averaged 14 m in height and 20 cm d.b.h. Treatments commenced when trees were 8.5 years old and continued for 3 years, the two non-fungicidal compounds being injected in the third year only (Table 2). Carbendazim (3.5 to 4 g/*l*), *ortho*-phenylenediamine (3 g/*l*), and L-arginine monohydrochloride (3 g/*l*), made up in 0.1N hydrochloric acid, were injected at a rate of approximately 4 *l*/tree at each injection. The treatment solution was introduced into the sap stream of each tree at five points around the stem just

TABLE 2—Programme of injection treatments. C = carbendazim, A = L-arginine monohydrochloride, I = ortho-phenylenediamine

Treatment	No. of trees	Growth season 1	Growth season 2		Growth season 3	
		Date of treatment: 26 Feb	2 Oct	31 Jan	26 Nov	9 Mar
1	3	C	—	—	C	C
2	2	C	—	C	C*	C*
3	2	—	C	—	C	C
4	1	—	—	C	C	C
5	1	—	—	C†	C	C
6	2	—	C	C	C	C
7	3	—	—	—	A‡	A§
8	2	—	—	—	O‡	O§
9	5	—	—	—	—	—

* 8 litres per injection in one large tree
† Half normal concentration
‡ Injected 13 December
§ Injected 12 March.

above ground level, and supply was maintained by gravity feed (*see* Hood & van der Pas (1979) for details of technique). Quantities normally used were such that each tree received 2.9 to 3.8 g of bound nitrogen per injection, regardless of the chemical employed.

Two evaluations of foliage retention were made during the experiment. Foliage was collected during November–December (early summer) 13 months after the first treatment in each of the second and third seasons. Eight side shoots were sampled per tree; they were cut at comparable positions from north- and south-facing main branches one-third and two-thirds up the tree stem. In the laboratory, counts of needle fascicles and fascicle scars were made from one or more spiral sets along the full length of each internode, in order to determine the percentages of different age-class foliage retained. Very short shoots occasionally found between internodes of more usual length were judged to be late-season secondary-shoot growth, and were ignored. Fungal isolations were carried out in October, 11 months after the first treatment in the third season. Isolations were made from needle segments plated on to 3% malt agar, after 3 min surface sterilisation in 10% hydrogen peroxide. Only one segment (1–1.5 cm long) was plated from each needle. All trees were sampled.

Results

Uptake times for 4 litres of the injected solutions varied from 3 hours to 4 days. No foliar symptoms of phytotoxicity were seen after treatment, but narrow longitudinal cankers eventually developed from injection sites on some trees after several years.

FIG. 1—The tree on the right was treated for two successive seasons with carbendazim (Treatment 2, second season), while that on the left is an untreated control tree (Treatment 9) of the same clone (FRI 121). Screens are 4.5 m tall, and extend nearly half the height of each crown.

Treatments had a marked effect on the colour and density of tree crowns (Fig. 1). Foliage of trees injected with carbendazim, L-arginine monohydrochloride, or *ortho*-phenylenediamine remained green during spring, whereas the foliage of untreated trees became chlorotic and was subsequently cast. Chlorosis reappeared on two trees when carbendazim was not injected again the following season (Treatment 1).

Between-tree differences in needle retention (within treatments) were highly significant ($p < 0.001$), but this did not mask the treatment effect. Carbendazim significantly increased retention of 1-year-old foliage (Tables 3 and 4), and when injected for two successive seasons caused an increase in retention of 2-year-old needle fascicles as well. Injections of the other two non-fungicidal compounds also increased 1-year-old foliage

TABLE 3—Mean percentage fascicle retention after tree injection, by treatments. Within each age-class, values linked by common letter subscripts are not significantly different ($p > 0.05$; Students range test)

Foliage age-class (years)	Treatment group	Growth season 2: Treatment No.*	Growth season 2: Mean	Growth season 3: Treatment No.*	Growth season 3: Mean
1	Injected with carbendazim (except where noted)			4	98_{a}
				3	97_{a}
				6	96_{a}
		4	66_{a}	8†	90_{a}
		2	60_{a}	1	87_{a}
		6	59_{a}	2	85_{a}
		5	43_{ab}	7‡	70_{a}
		3	41_{ab}	5	57_{ab}
	Not injected	1	22_{ab}	9	38_{b}
		7	17_{ab}		
		8	8_{b}		
		9§	–		
2	Injected with carbendazim in two successive growth seasons‖			6	56_{a}
				2	54_{a}
				4	45_{ab}
				5	28_{ab}
		2	23_{a}	3	24_{b}
	Not injected in two successive growth seasons‖	6	7_{a}	1	21_{b}
		8	5_{a}	7	3_{c}
		5	5_{a}	8	1_{c}
		3	2_{a}	9	1_{c}
		1	2_{a}		
		4	2_{a}		
		7	1_{a}		
		9§	–		

* Treatments outlined in Table 2
† Ortho-phenylenediamine
‡ L-arginine monohydrochloride
§ Not evaluated
‖ Including current season

TABLE 4—Mean percentage fascicle retention after tree injection, by groups

Foliage age (years)	Group	Growth season	
		2	3
1	Injected	54	85
	Not injected	17	38
	Significance of mean difference	***	***
2	Injected two successive growth seasons†	23	42
	Not injected two successive growth seasons†	3	6
	Significance of mean difference	**	***

*** Significant at p <0.001
** Significant at p <0.01
† Including current season.

retention. Cultures of *C. minus* were isolated from non-injected control trees (confirmed in five out of 60 needle segments from five trees) and from trees injected with the two non-fungicidal compounds (in 12 out of 54 needle segments from five trees). *Cyclaneusma minus* was not found in 118 needle segments from 11 trees injected with carbendazim.

AERIAL SPRAY TRIAL

Method

Disease levels were evaluated in a 10-year-old *P. radiata* plantation of trees susceptible to *C. minus,* after spraying with benomyl in an oil emulsion from the air. Oil was added to prevent evaporation of fine droplets while using Micronair spray equipment, and because it had been shown that oil enhances the effectiveness of benomyl in controlling *Dothistroma pini* Hulbary on nursery-grown *P. radiata* (Gilmour & Vanner 1972). The trial stand was divided into a treatment area of 25 ha and an adjacent unsprayed control area of 8.5 ha. Within each area, four assessment plots of 16 to 27 trees were established at random. Spraying was carried out twice, once on 14 June and again on 15 July. On each occasion benomyl was applied at a rate of 0.25 kg/ha suspended in an emulsion of 6 *l* water and 4 *l* BP crop oil/ha. Applications were made using a Hughes 300 helicopter, fitted with four Micronair AU 5000 spray units, and application rates were checked immediately before each spray application. Paper traps laid in stand openings confirmed that all the sprayed plots, and none of the unsprayed control plots, were treated. The crown disease level on each tree in the assessment plots was estimated to the nearest 5% on a scale of 0 to 100% of effective green crown remaining. Timing of peak symptom expression varies between trees, so five assessments of each tree were made over a 2.5-month period, beginning in mid-September. The maximum score for each tree was selected and results were compared by analysis of variance. In addition, at fortnightly intervals from the beginning of June needles were collected from four susceptible trees in the unsprayed area. Isolations were made from these needles as described in the previous section, in order to determine when the trees became infected by the fungus.

Results

Estimates of effective green crown retained are summarised in Table 5. There were no significant differences ($p > 0.05$) between means of sprayed and unsprayed areas. *Cyclaneusma minus* was isolated on and after 1 August, but was not found in needles sampled prior to that date.

TABLE 5—Mean percentage level of effective green crown retained* after aerial spraying with benomyl, by plots

	Plot No.			
	1	2	3	4
Sprayed	26 ± 12	49 ± 14	32 ± 11	46 ± 11
Unsprayed	37 ± 14	43 ± 14	27 ± 10	23 ± 11

* With 95% confidence limits. Evaluated using a 0–100% assessment scale.

DISCUSSION

Dipping of foliage was used to screen fungicides against Cyclaneusma needle-cast of *P. radiata* prior to undertaking the aerial spray trial. Treatments were applied in such a way that protection was continuous from time of budburst throughout the trial period. The most effective chemicals proved to be dodine, anilazine, benomyl, and dichlone. Kistler & Merrill (1978) and Merrill *et al.* (1980) have demonstrated fungicidal activity against *C. minus* by benomyl and mancozeb.

Injections of carbendazim gave good disease control in young pine trees belonging to one clone. Despite this, it is unlikely that this labour-intensive technique could be used on a management scale, even though the between-tree variation in symptom expression observed in plantations lends itself to a selective approach for fungicide applications. Injecting may have value in studies to elucidate the nature of the host : fungus : fungicide interaction. For instance, treatment with *ortho*-phenylenediamine and L-arginine monohydrochloride demonstrated that symptoms can be modified by chemicals that are not fungicidal. The disease symptoms have also been suppressed in smaller trees by heavy ground applications of urea fertiliser (G. M. Will, pers. comm.). All these compounds contain a significant proportion of nitrogen and so their use may correct a nitrogen imbalance induced by the fungus. Benomyl and other benzimidazole fungicides are known to have cytokinin-like properties which may explain their ability to influence foliage coloration (Skene 1972; Thomas 1974; Spencer 1977). Since carbendazim is derived from benomyl, it may also behave in a similar manner in *P. radiata* foliage.

Aerial spraying is now used routinely for the control of Dothistroma needle-cast in New Zealand pine forests (Kershaw *et al.* 1982). This method of application was therefore tried against *C. minus* in a stand of *P. radiata*. Benomyl was chosen because of the control achieved by this fungicide and carbendazim in the other two experiments.

However, in the aerial spray benomyl was unsuccessful in controlling the disease. Failure could have been caused by too low a fungicide application rate or, alternatively, by incorrect timing, since the fungicide may have given only short-term control. The lack of disease control cannot be a result of late spraying, since *C. minus* was not isolated from needles until after the second application. Life-cycle studies indicate that the infection period of *C. minus* is not consistent from year to year, and may begin as early as March (Gadgil 1984) or as late as July. The occurrence of an extended infection period in some years raises doubt about the suitability of aerial spraying as a practical option for disease control management. Magnani (1972) failed to achieve control with several different fungicides applied 11 times at 19-day intervals or 14 times at 24-day intervals. However, because the attainment of a practical chemical control method is highly desirable, further research is planned.

ACKNOWLEDGMENTS

Thanks are due to P. Allen, Yvonne Langridge, Lorraine O'Neale, C. J. Sandberg, D. Slater-Hayes, and Monique Williams, for assistance in the field and laboratory; I. A. Andrew helped with statistical analyses; and the text was reviewed by P. D. Gadgil, W. F. T. Hartill, and J. W. Ray.

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