SEED TREATMENT FOR PROTECTION OF SEEDLING WHITE CLOVER FROM NEMATODES

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SUMMARY

Pesticides formulated as seed coatings were shown in pot experiments to be capable of providing seedling protection and enhancing plant growth in white clover (*Trifolium repens* L.) exposed to root-invading nematodes *Meloidogyne hapla* Chitwood and *Heterodera trifolii* Goffart. However, the level of nematode control and response in plant growth varied between experiments. The results are discussed in relation to pesticide application rate and levels of nematode infestation.
Keywords: white clover, *Trifolium repens*, seed treatment, nematodes, *Meloidogyne hapla*, *Heterodera trifolii*

INTRODUCTION

It has long been recognised that forage grasses are capable of suppressing the growth of white clover seedlings when these species are sown together (Haynes 1980; Harris 1987). These effects of competition from companion grasses are exacerbated by pests and diseases. In many areas of New Zealand endoparasitic nematodes can invade and establish in the roots of clover seedlings within several days of seed germination (Skipp and Christensen 1981; Skipp and Watson 1987). Their effect is to reduce seedling vigour, directly through tissue destruction and indirectly through disruption of nodulation and allowing infection by fungal pathogens (Skipp and Gaynor 1987).

Our research suggests that the vigour of seedlings during the first few weeks from germination determines the fate of white clover plants in mixed species swards (Watson *et al.* 1989). High vigour seedlings are able to compete effectively with high vigour companion grasses and contribute to the yield and nitrogen economy of the pasture. Low vigour clovers, such as those resulting from nematode infection, compete poorly and are lost from the swards.

Formulation of pesticides as a seed treatment, in order to reduce the nematode burden in roots, may maintain the high clover seedling vigour during the first weeks which are critical to successful establishment. This approach has been successful for lucerne establishment in nematode infested soils (Townshend and Chiba 1987). Several experiments evaluating the efficacy of seed treatments in white clover are described in this paper.

MATERIALS AND METHODS

Experiment 1

Bruntwood silt loam turves (100 mm deep) were dug from pasture at Ruakura Agricultural Centre on 15 October 1990. These were sieved through 4 mm mesh and the soil placed in plastic pots 170 x 100 mm x 100 mm deep. The pots were held at 4°C until 27 October when four replicate pots per treatment were sown with 20 white clover cv. 'Grasslands Pitau' seeds using untreated seed (methyl cellulose and talc powder only) or seed treated with one of 10 pesticides (Table 1) applied at the rate of 1 g ai/ 100 g seed using talc powder carrier and methyl cellulose binder. The seeds were sown as a single row at approximate spacings of 7.5 mm and 5 mm depth. On 2 November the emergent seedlings were counted before being washed free of soil, cleared, and stained in analine blue. Nematodes occurring as endoparasites in the root tissues were counted and identified to genera with the aid of a stereomicroscope.

Experiment 2

Plastic pots were prepared as above on 2 January 1991. One half of the soil-filled pots were held at -20^{0}C for 10 days to kill nematodes (Watson 1990), while the remainder were held at 4^{0}C. For both 'non-frozen' and 'frozen' pre-treated soils, four replicate pots per treatment were sown on 16 January with 20 white clover cv. 'Grasslands Pitau' seeds using untreated seed (methyl cellulose and talc powder only) or with seed treated with oxamyl, carbofuran or ethoprophos at rates varying from 1 to 10 g/100 g seed (Table 2) using talc powder carrier and methyl cellulose binder. The seedlings were counted and harvested after 21 days. Ten seedlings from each replicate pot were prepared for counting of nematodes in the roots. The remaining seedlings were sectioned into shoot and root tissues and dried at 90^{0}C for weight determinations.

Experiment 3

Plastic pots were prepared as above on 16 November 1991. The pots were held at 4^{0}C until 24 November when eight replicate pots were sown with 20 white clover cv. 'Grasslands Pitau' seeds as a single row. The seeds were subject to the following nematicide placement treatments: 1) untreated (no nematicide); 2) seed coating with oxamyl (as Vydate L 24EC) at the rate of 1 g/100 g seed with talc carrier and methyl cellulose binder; 3) seed coated with oxamyl at 1 g/100 g seed with talc carrier and polyvinylpyrrolidone binder; 4) seed soaked in 4.8% oxamyl solution for 20 minutes to give 1 g/100 g seed; or 5) 5 ml of 1% oxamyl solution applied as a band in the seed row, equivalent to 19.6 kg/ha. On each of 30 November and 14 December, seedlings from four replicate pots of each treatment were counted and then washed free of soil. Ten seedlings from each pot were prepared for counts of nematodes in the roots and measurement of root length, while the remaining seedlings were used for determination of shoot and root dry weights.

Statistical analyses

Seedlings established as a proportion of seed sown were analysed by generalised linear regression using a binomial distribution with a logit link. Numbers of nematodes, and seedling dry weights, root lengths, and nodule numbers were subjected to an analysis of variance.

RESULTS

In all three experiments *Meloidogyne hapla* and *Heterodera trifolii* were the principal nematodes recorded as endoparasites in the roots of white clover seedlings. *Pratylenchus* species occurred in very low numbers and have been excluded from the analyses.

In Experiment 1 only ethoprophos was effective in reducing numbers of juvenile *Meloidogyne* and *Heterodera* in roots in the first 6 days of seedling growth (Table 1). Root infections by nematodes were increased by several of the seed coat treatments. The pesticides applied as 1% seed coatings did not adversely affect seedling emergence.

The reduction in root infestations by nematodes with a 1% ethoprophos seed coat ($P<0.001$) was confirmed in Experiment 2 (Table 2). Ethoprophos applied at this rate resulted in seedling emergence rates and growth similar to that occurring in soil freed from nematodes by the freezing treatment (<1 nematode/10 seedlings). Increases in the ethoprophos content of the seed coat increased the level of nematode control (rate effect $P<0.01$) but tended to retard seedling emergence (rate effect $P<0.05$) and growth (shoot and root weights: rate effect $P<0.01$ and $P<0.05$, respectively) (Table 2).

Oxamyl and carbofuran applied to seed at 1% rate in Experiment 2 were effective in reducing nematode numbers in seedling roots (Table 2), despite failing to do so in Experiment 1. The effectiveness of carbofuran in reducing nematode infections increased with the content of the pesticide in the seed coat (rate effect $P<0.01$) (Table 2). A seed coating of 2% carbofuran was required to ensure seedling growth similar to that in nematode-free soil. The 5 and 10% rates of carbofuran were associated with retarded seedling emergence ($P<0.01$) and shoot growth ($P<0.05$) (Table 2).

TABLE 1: Seedling emergence and numbers of nematodes in root tissues for white clover seedlings grown from seed treated with various pesticides (Experiment 1).

Pesticide	Trade name & formulation	Percent seedling emergence	*Meloidogyne*/ seedling	*Heterodera*/ seedling
Untreated		82	9.2	25.9
Ethoprophos	Technical 98EC	83	4.4	12.7
Ivermectin	Eqvalan 1.87Paste	88	7.6	32.0
Oxamyl	Vydate L 24EC	89	9.4	21.0
Thiodicarb	Man. Conc. 90P	85	9.6	34.5
Methomyl	Lannate 20EC	84	15.4	39.2
Isazophos	Miral 50EC	80	12.2	25.4
Oftanol	Man. Conc. 40WP	87	11.2	23.0
Carbofuran	Furadan 75WP	88	9.9	27.7
Diazinon	Gesapon 80EC	82	13.6	40.3
LSD P<0.05		6.5	4.60	6.07

In Experiment 2, oxamyl 1% and 2% seed coatings were equally effective in reducing nematode numbers in seedling roots (P<0.001) and increased seedling growth to a level recorded in nematode-free soil (Table 2).

TABLE 2: Emergence, shoot and root weights and nematode infestation in roots of seedling white clover grown from nematicide treated seed (Experiment 2).

Treatment		Percent seedling emergence		Shoot DW/ seedling (mg)		Root DW/ seedling (mg)		Nematodes/ seedling
		UT	F	UT	F	UT	F	UT
Untreated		86	88	1.86	2.19	0.28	0.50	43.2
Oxamyl	1%	88	88	1.97	2.15	0.48	0.43	16.1
	2%	88	92	2.19	2.03	0.58	0.45	14.8
Carbofuran	1%	90	89	1.71	2.43	0.32	0.38	32.3
	2%	82	81	2.25	2.50	0.50	0.51	27.4
	5%	71	52	1.56	2.22	0.20	0.40	15.3
	10%	60	52	1.55	1.80	0.26	0.38	13.5
Ethoprophos	1%	82	88	1.90	1.97	0.54	0.45	28.4
	2%	84	83	1.73	1.80	0.47	0.43	25.4
	5%	46	81	1.18	1.18	0.30	0.33	7.3
	10%	68	56	1.21	1.30	0.23	0.30	12.3
LSD P<0.05		8.1		0.53		0.25		4.02

UT = Untreated soil
F = Soil frozen at -20°C prior to sowing

In Experiment 3 the results achieved in the 1% oxamyl seed coating with methyl cellulose binder were similar to that with polyvinylpyrrolidone, so the data from these treatments have been pooled in Tables 3 and 4. Oxamyl applied as a 1% seed coat reduced nematode infection in roots at day 6 of seedling growth (P<0.05) but failed to prevent ingress and development of nematodes in the roots during the following 14 day period (Table 3). This oxamyl seed coat treatment increased seedling root length during the first 6 days (P<0.05) but this was not associated with seedling weight increases during that period or subsequently (Table 4). Soaking of seeds in oxamyl solution prior to sowing failed to provide seedling protection from nematodes (Table 3) nor did it enhance seedling growth (Table 4). The application of oxamyl as an aqueous suspension to the soil in the furrow with the seed provided complete protection of roots from nematodes during the first 6 days of seedling growth and greatly reduced subsequent ingress of *Heterodera* (P<0.01) and the development of both *Heterodera* and *Meloidogyne* (P<0.001) (Table 3). This in-furrow treatment increased seedling root length (days 6 & 20: P<0.05), nodulation by rhizobia (day 20: P<0.001), and seedling weights (day 20: shoot weights P<0.05; root weights P<0.01) (Table 4).

TABLE 3: Nematode infestations in roots of white clover seedlings as influenced by placement of oxamyl at sowing (Experiment 3).

	Day 6	Day 20			
Treatment	Nematodes/ seedling	*Meloidogyne* juveniles/ seedling	Root knots/ seedling	*Heterodera* juveniles/ seedling	*Heterodera* females/ seedling
Untreated	41.0	17.0	5.7	33.6	27.9
Seed Coat	29.2	26.1	6.0	51.3	35.0
Seed Soak	34.5	30.0	5.7	32.7	19.0
In Furrow	0.0	11.0	1.2	9.0	3.2
LSD P<0.0	10.10	7.30	2.05	16.37	12.47

TABLE 4: Emergence, root and shoot growth and nodulation of white clover seedlings as influenced by placement of oxamyl at sowing (Experiment 3).

Treatment	Percent seedling emergence	Root length/ seedling (mm)	Shoot DW/ seedling (mg)	Root DW/ seedling (mg)	Nodules/ seedling
Day 6					
Untreated	93	17.8	1.64	0.88	0.77
Seed Coat	90	22.8	1.54	0.57	1.63
Seed Soak	95	20.2	1.50	0.28	1.65
In Furrow	91	26.3	1.04	0.27	1.65
LSD P<0.05	4.3	2.46	0.56	0.62	0.89
Day 20					
Untreated		30.9	1.50	0.68	2.50
Seed Coat		31.1	1.74	0.75	3.28
Seed Soak		33.4	1.43	0.59	1.83
In Furrow		91.6	2.45	1.89	7.83
LSD P<0.05		11.51	0.89	0.27	1.24

DISCUSSION

Seed treatment with nematicide is more target oriented and, because of reduced chemical rate per treated area, is potentially more cost effective and environmentally sound than granular or spray formulations. However, to be adopted by farmers, the seed treatment must produce consistent benefits for clover establishment. In the present work, while seed treatments were shown to be capable of providing seedling protection and increasing plant growth, the effect was inconsistent. The marked improvement in seedling performance with the in-furrow nematicide treatment in Experiment 3 indicated that the variable results of the seed treatments were due, at least in part, to the low quantities of nematicide delivered by 1-2% seed coatings. This deficiency is not readily overcome by simply increasing the quantity of active ingredient applied to the seed because phytotoxicity is induced at the higher application rates, as evident in Experiment 2. A further factor was the high level of root invasion by nematodes in these experiments. The reductions in nematode numbers by the seed treatments were apparently, in many instances, not sufficient to alleviate the debilitating effects of root infection by nematodes.

Nematicide seed treatments need to be tested in the field. There is a need to confirm that the temporary protection from nematodes afforded by seed treatments results in improved clover establishment in mixed swards, as has been demonstrated for other nematicidal treatments (Watson, 1990; Watson and Barker, unpubl.).

REFERENCES

Harris, W., 1987. Population dynamics and competition. Pp. 203-297. *In*: White Clover. Baker, M.J. and Williams, W.M. (eds.), C.A.B. International.

Haynes, R.J., 1980. Competitive aspects of the grass-legume association. *Advances in Agronomy 33:* 227-261.

Skipp, R.A. and Gaynor, D.L., 1987. Pests - Nematodes. Pp. 493-512 *In*: White Clover. Baker, M.J. and Williams, W.M. (eds.), C.A.B. International.

Skipp, R.A. and Christensen, M.J., 1981. Invasion of white clover roots by fungi and other soil microorganisms. I. Surface colonisation and invasion of roots growing in sieved pasture soil in the glasshouse. *N.Z. J. Agric. Res. 24:* 235-241.

Skipp, R.A. and Watson, R.N., 1987. Pot experiments with pasture soils to detect soilborne pathogens of white clover and lucerne, and effects of field application of fungicides. *N.Z. J. Agric. Res. 30:* 85-93.

Townshend, J.L. and Chiba, M., 1987. Control of *Pratylenchus penetrans* and *Meloidogyne hapla* and yield response of alfalfa due to oxamyl seed treatments. *J. Nematol. 19:* 454-458.

Watson, R.N., 1990. Effects of plant nematodes and *Acremonium* endophyte on white clover establishment with ryegrass or tall fescue. *Proc. 43rd N.Z. Weed and Pest Control Conf.:* 347-351.

Watson, R.N., Skipp, R.A. and Barratt, B.I.P., 1989. Initiatives in pest and disease control in New Zealand towards improving legume production and persistence. Pp. 441-464, *In*: Persistence of Forage Legumes. Marten, G.C., Matches, A.G., Barnes, R.F., Brougham, R.W., Clements, R.J. and Sheath, G.W. (eds.), Proceedings of a trilateral workshop, Honolulu, Hawaii, 18-22 July 1988.