

## SUPPRESSION OF PLANT PARASITIC NEMATODES IN PASTORAL SOILS AMENDED WITH CHITIN

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### ABSTRACT

White clover and perennial ryegrass plants were grown in pots containing a sandy soil amended with chitin. Soil nematode numbers were examined after 6 weeks of plant growth. Amendment with chitin significantly reduced the abundance of *Heterodera trifolii* in white clover roots and *Pratylenchus* sp. in perennial ryegrass roots. Abundance of *Paratrichodorus minor* was significantly reduced by chitin amendment, compared to unamended controls. The total nematode fauna responded to chitin amendment with large increases in abundance.

**Keywords:** *Paratrichodorus minor*, *Heterodera trifolii*, *Meloidogyne* sp., *Pratylenchus* sp., ryegrass, white clover.

### INTRODUCTION

Many nematode species reduce growth of plants. The major root-parasitic nematodes causing significant damage in New Zealand pastures are the sedentary endoparasites *Heterodera trifolii* (clover cyst nematode) and *Meloidogyne* spp. (root-knot nematodes) (Yeates *et al.* 1977; Watson *et al.* 1985). The migratory endoparasites *Pratylenchus* spp. (lesion nematodes) are found in the roots of both ryegrass (*Lolium perenne*) and clovers (*Trifolium* spp.) in New Zealand pastures and may cause plant growth depression (Yeates 1977). The ectoparasite *Paratrichodorus minor* (stubby root nematode) has a worldwide distribution and is extremely polyphagous (Decraemer 1991). As well as crops, some pasture plants are hosts to this pathogen including white clover (*Trifolium repens*) and perennial ryegrass (Rohde and Jenkins 1957; Bell 1999). *Paratrichodorus minor* has also been implicated in reducing the yield of white clover in New Zealand (Yeates and Prestidge 1986).

For environmental and economic reasons, control of root-parasitic nematodes using chemicals is not a viable option. Modern integrated control strategies include the use of amendments, resistant or tolerant plants or a combination of these and other management practices. Chitin, the most commonly occurring nitrogen-containing polysaccharide in nature (Alexander 1977), has been used as a soil amendment to control root-parasitic nematodes (e.g. Gooday 1990). In this paper, we report the results of two pot experiments showing the effects of chitin amendment of a sandy soil on plant parasitic and total nematode populations, which typically contain bacterial and fungal feeding genera.

### METHODS

#### Experiment 1

Experiments were conducted as described previously (Sarithchandra *et al.* 1996). Five soil samples of Paengaroa shallow sand (Aquic Hapludand) were collected from a farm in the Bay of Plenty, sieved carefully (<4 µm) and mixed (1:1 by weight) with pumice. Pots were set up using fresh soil/ pumice mix with (C+) and without (C-) chitin. Crabshell-chitin (Sigma Chemicals) was sieved (<150 µm) and mixed (1% by dry weight) with soil/ pumice mix to obtain C+ pots. Each treatment was replicated four times for each soil sample. The forty pots were divided into four batches. The first batch was planted with three seeds each of white clover cv. Grasslands Kopu and

perennial ryegrass cv. Yatsyn and placed in a controlled environment (CE) room at 20°C. The second and third batches were stored at 4°C and planted with seeds 1 and 2 weeks later respectively. The fourth batch was transferred to a CE room after 3 weeks and left fallow. After 6 weeks incubation, pots were destructively sampled and nematode populations were determined by a variant of the Whitehead and Hemming (1965) tray method (Bell 1999). The effect of chitin on root nematodes was analysed by ANOVA after  $\log_e$  transformation of the data, while soil nematode abundance effects were estimated using a sign test.

### Experiment 2

Details of this experiment are described in Brown *et al.* (1995). Crabshell-chitin was mixed with Paengaroa shallow sand soil as for Experiment 1 to obtain rates of 0, 0.5 and 1.0% chitin by weight. Soil amended with each chitin rate was incubated for 0, 3, 6 and 9 weeks (i.e. 12 treatments) in a CE room at 20°C before planting with seeds of white clover or perennial ryegrass as in Experiment 1. Seven weeks after sowing three replicate pots per treatment were assessed for nematode abundance as in Experiment 1. Effects of chitin addition were assessed by an exact 2 × 2 binomial test for plant parasitic nematodes and by ANOVA for the total nematode fauna.

## RESULTS

### Experiment 1

The addition of 1% chitin to soil reduced the abundance of *Heterodera trifolii* ( $P < 0.01$ ) and *Pratylenchus* sp. ( $P < 0.05$ ) in white clover and ryegrass roots respectively (Table 1). In soil, only *Paratrichodorus minor* was reduced ( $P < 0.001$ ) by chitin amendment (Table 2). In contrast to the results for some plant parasitic nematodes, the abundance of the total nematode fauna was increased ( $P < 0.001$ ) by the addition of chitin (Table 2).

**TABLE 1: Mean abundance ( $\log_e$ ) of plant parasitic nematodes in roots of white clover and ryegrass plants grown in soil with (C+) or without (C-) chitin amendment (figures in parentheses are backtransformed from log data).**

Nematode	No. nematodes/plant		SED <sup>3</sup>
	C+	C-	
<i>Heterodera trifolii</i> <sup>1</sup>	1.6 (4.7)	2.6 (13.6)	0.34
<i>Meloidogyne</i> sp. <sup>1</sup>	1.3 (3.5)	1.6 (5.0)	0.32
<i>Pratylenchus</i> sp. <sup>1</sup>	1.2 (3.2)	1.1 (3.0)	0.33
<i>Pratylenchus</i> sp. <sup>2</sup>	1.9 (6.7)	2.4 (11.2)	0.25

<sup>1</sup>In white clover roots.

<sup>2</sup>In perennial ryegrass roots.

<sup>3</sup>Standard error of the difference.

**TABLE 2: Mean abundance of plant parasitic and total nematodes in soil with (C+) or without (C-) chitin amendment (figures in parentheses are standard errors of the means).**

Nematode	No. nematodes/100 g soil	
	C+	C-
<i>Heterodera trifolii</i>	0.4 (0.29)	0.9 (0.45)
<i>Meloidogyne</i> sp.	0.4 (0.17)	0.5 (0.19)
<i>Pratylenchus</i> sp.	3.4 (0.97)	1.9 (0.61)
<i>Paratrichodorus minor</i>	0.1 (0.06)	37.7 (8.32)
Total nematodes	1689.0 (241.60)	183.4 (24.82)

## Experiment 2

There were no effects ( $P > 0.10$ ) of the 0.5 or 1.0% chitin rates on the abundance of plant parasitic nematodes compared to no chitin, but there were fewer *P. minor* in soil amended with the 1.0% rate compared to the combined nil and 0.5% rates ( $P < 0.10$ ) (Table 3). Chitin amendment of soil at both the 0.5 and 1.0% rates increased the abundance of the total nematode fauna ( $P < 0.01$ ) (Table 3).

**TABLE 3: Mean abundance of plant parasitic and total nematodes in soil with nil, 0.5 or 1.0% chitin amendments, pooled for four incubation times (figures in parentheses are standard errors of the means).**

Nematode	No. nematodes /100g soil		
	0	0.5	1.0
<i>Heterodera trifolii</i>	10.5 (6.3)	10.7 (5.4)	0.0 (0.0)
<i>Meloidogyne</i> sp.	12.6 (7.7)	2.9 (1.5)	1.1 (1.1)
<i>Pratylenchus</i> sp.	0.0 (0.0)	5.1 (3.5)	1.1 (1.1)
<i>Paratrichodorus minor</i>	24.7 (13.1)	11.7 (6.9)	0.9 (0.9)
Total nematodes	515.2 (85.8)	2257.4 (388.2)	3378.0 (704.6)

## DISCUSSION

Although the mode of action of chitin in controlling plant parasitic nematodes is not well understood, the suggested mechanisms include increased microbial chitinase activity which may damage chitin-containing egg shells and the nematocidal activity of increased ammonia levels released by chitin hydrolysis (Mian *et al.* 1982). In the present work, chitin amendment markedly reduced the populations of *P. minor* in soil and *H. trifolii* in both soil and white clover roots, but increased the abundance of the total nematode fauna, suggesting that the mode of action of chitin amendment is not one of direct nematode toxicity. Fungal and bacterial feeding nematodes (i.e. part of the total nematode fauna) probably responded to a rapid increase in chitinolytic microbes resulting from chitin addition to the soil (Sarathchandra *et al.* 1996).

The results of Experiment 1 are consistent with those reported by Sarathchandra *et al.* (1996) for two contrasting soils. The effects of chitin amendment on soil abundance of nematodes observed in Experiment 2 are similar to those for root abundance data reported by Brown *et al.* (1995).

In the experiment reported by Sarathchandra *et al.* (1995) (of which the data in Experiment 1 here forms a part) two soils were used, a shallow sand and a silt loam. *Paratrichodorus minor* was found only in the shallow sand soil, which is similar to the observation of Thomason (1959) who reported that *P. minor* populations increased more rapidly and to a higher level in sandy loam than in silt clay loam or loam. The association of *P. minor* with sandy soils applies generally to all trichodorids (Decraemer 1995).

The levels of reduction of *P. minor* in soil were far greater than that of root-knot (*Meloidogyne* sp.) and lesion (*Pratylenchus* sp.) nematodes in the studies reported here. *P. minor* appears to be sensitive to low levels of the insecticide oxamyl (Yeates and Prestidge 1985) and other nematicides (Whitehead 1978), suggesting that this species is particularly sensitive to chemical amendments.

Both direct feeding damage and indirect damage by virus transmission have been attributed to Trichodoridae (Lamberti *et al.* 1975). The direct feeding of Trichodorid nematodes results in "stubby-root" symptoms, first reported by Christie and Perry (1951) in Florida where *P. minor* was the species responsible. Perhaps the best known example of these symptoms is the so-called Docking disorder of sugarbeet (*Beta* sp.) which occurs on sandy soils in the UK (see Cooke 1993).

Further work on the effect and the mode of action of chitin on plant parasitic nematodes from soil under pasture is warranted.

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## REFERENCES

- Alexander, M., 1977. Introduction to soil microbiology, 2nd edn. Wiley, New York.
- Bell, N.L., 1999. The biology of the plant parasitic nematodes *Paratylenchus nanus* and *Paratrichodorus minor* in soil under pasture. PhD thesis, Massey University, 163 pp.
- Brown, J. A., Neville, F. J., Sarathchandra, S. U., Watson, R. N., Cox, N. R. and Popay, A. J., 1995. Effects of chitin amendment on plant growth, microbial populations and nematodes in soil. *Proc. 48th N.Z. Plant Prot. Conf.*: 208-212.
- Christie, J. R. and Perry, V. G., 1951. A root disease of plants caused by a nematode of the genus *Trichodorus*. *Sci. 113*: 491-493.
- Cooke, D., 1993. Nematode parasites of sugarbeet. Pp 133-169 *In*: Plant parasitic nematodes in temperate agriculture, K. Evans, D. L. Trudgill and J. M. Webster (Eds); Wallingford, UK, CAB International.
- Decraemer, W., 1991. Stubby root and virus vector nematodes: *Trichodorus*, *Paratrichodorus*, *Allotrichodorus* and *Monotricodorus*. Pp 587-625 *In*: Manual of Agricultural Nematology, W.R. Nickle (Ed.); Marcel Dekker Inc., New York.
- Decraemer, W., 1995. *The Family Trichodoridae: stubby root and virus vector nematodes*. Dordrecht, Kluwer Academic Publishers, 360 pp.
- Goody, G.W., 1990. The ecology of chitin degradation. *Adv. Micro. Ecol. 11*: 387-419.
- Lamberti, F., Taylor, C.E., and Seinhorst, J.W. (Eds), 1975. Nematode vectors of plant viruses. Plenum, London. 460 pp.
- Mian, J.H., Godoy, G., Shelby, R.A., Rodriguez-Kabana, R. and Morgan-Jones, G., 1982. Chitin amendments for control of *Meloidogyne arenaria* in infested soil. *Nematopica 12*: 71-84.
- Rohde, R.A. and Jenkins, W.R., 1957. Host range of a species of *Trichodorus* and its host-parasite relationship on tomato. *Phytopathol. 48*: 295-298.
- Sarathchandra, S.U., Watson, R.N., Cox, N.R., Di Menna M.E., Brown, J.A., Burch, G. and Neville, F.J., 1996. Effects of chitin amendment of soil on microorganisms, nematodes and growth of white clover (*Trifolium repens* L) and perennial ryegrass (*Lolium perenne* L). *Biol. Fert. Soils 22*: 221-226.
- Thomason, I.J., 1959. Influence of soil texture on development of the stubby-root nematode. *Phytopathol. 49*: 552.
- Watson, R.N., Yeates, G.W., Littler, R.A. and Steele, K.W., 1985. Responses in nitrogen fixation and herbage production following pesticide application on temperate pastures. *Proc. 4th Australasian Conf. Grassland Invert. Ecol.*: 103-113.
- Whitehead, A.G., 1978. Chemical control. (A) Soil treatment. Pp 238-296 *In*: Plant Nematology, J.S. Southerby (Ed.); HMSO, London.
- Whitehead, A.G. and Hemming, J.R., 1965. A comparison of some quantitative methods of extracting vermiform nematodes from soil. *Ann. App. Biol. 55*: 25-38.
- Yeates, G. W., 1977. Soil nematodes in New Zealand pastures. *Soil Sci. 123*: 415-422.
- Yeates, G.W. and Prestidge, R.A., 1986. Nematode populations and their effects on herbage production in a volcanic plateau pasture. *N.Z. J. Ag. Res. 29*: 517-523.
- Yeates, G. W., Ross, D. J., Bridger, B. A. and Visser, T. A., 1977. Influence of the nematodes *Heterodera trifolii* and *Meloidogyne hapla* on nitrogen fixation by white clover under glasshouse conditions. *N.Z. J. Ag. Res. 20*: 401-413.