EFFECTS OF CHITIN AMENDMENT ON PLANT GROWTH, MICROBIAL POPULATIONS AND NEMATODES IN SOIL

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ABSTRACT

A pot trial was carried out to determine the effects of soil amendment with three rates of chitin (0, 0.5 and 1.0% by weight) and pre-incubation of soil with chitin for 0, 3, 6, and 9 weeks on the growth of white clover (*Trifolium repens* L) and perennial ryegrass (*Lolium perenne* L) and on populations of soil bacteria, fungi and plant-parasitic nematodes. Ryegrass plant yield increased in 0.5 and 1% chitin amended pots compared to the control. White clover yield decreased with increasing chitin content, probably due to phytotoxic effects of chitin. Pre-incubation with chitin increased ryegrass yield but had no effect on white clover. Populations of micro-organisms increased in chitin treatments. Bacterial counts were greatest after 9 weeks pre-incubation but the fungal counts were unaffected by pre-incubation. The numbers of plant-parasitic nematodes, *Meloidogyne* sp. and *Heterodera trifolii* were reduced by chitin. Pre-incubation had no effect on nematode numbers.

Keywords: chitin amendment, bacteria, fungi, plant-parasitic nematodes, perennial ryegrass, white clover

INTRODUCTION

Chitin (poly- β -1,4-N-acetylglucosamine), is the most abundant naturally occurring nitrogen (N) containing polysaccharide (Alexander 1977). Mineralisation of chitin provides a source of carbon (C) and N for microbial growth in soil (Ueno *et al.* 1991). Chitin has been successfully used to control fungal pathogens and root-parasitic nematodes in soil (Gooday 1990). The increased chitinolytic microflora and chitinase activity resulting from chitin amendment can enhance nematode egg parasitism, and the increased ammonia concentration is known to have a nematode-deterrent effect (Spiegel *et al.* 1987).

Nitrogen supply in New Zealand pastures depends on biological N fixation in white clover (*Trifolium repens* L). The control of plant parasitic nematodes in white clover, in particular the root-knot nematode (*Meloidogyne* spp.) and the clover cyst nematode (*Heterodera trifolii* Goffart), can result in a 50% increase in N-fixation levels in dairy pastures in New Zealand (Watson *et al.* 1994). Tissue damage caused by nematodes can be compounded by the entry of fungal and bacterial pathogens into the root through wounds caused by nematodes (Skepp and Christensen 1989; Halpin, 1963). Therefore, practical means of controlling or reducing the plant parasitic nematode burden in white clover will lead to major benefits for the pastoral industry in New Zealand.

In previous work, chitin was found to have an anti-nematode effect but chitin was also phytotoxic to white clover (Sarathchandra *et al.* 1995). The work reported in this paper was conducted to determine the effects of soil amendment with three rates of chitin, and the effect of allowing the chitin to decompose for varying lengths of time before planting, on the growth of white clover and ryegrass (*Lolium perenne* L), and on nematode populations and soil microbial populations.

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MATERIALS AND METHODS

Soil (Paengaroa Shallow Sand - a Typic Hapudand) was collected (bulked 10 cm cores) and sieved to remove plant matter and debris. This was combined with pumice in an approximately 50% dry weight mix and the water holding capacity was adjusted to 75%. All manipulations of soil were done carefully to minimise nematode mortality.

Crabshell-chitin (Sigma Chemicals) was sieved to obtain particles < 150 µm before adding to the soil mix. The experiment contained three rates of chitin (0, 0.5% and 1.0% chitin by weight) pre-incubated for four time periods (0, 3, 6 and 9 weeks) to give a total of 12 treatments. The 0 week sample was planted immediately and separate quantities of each soil treatment were set aside in beakers for incubation at 20°C for periods of 3, 6 and 9 weeks before being divided into pots and planted. Moisture loss from stored soil was replenished weekly.

The soil was dispensed into three pots per treatment (approximately 138 g soil:pumice mix per pot) and were planted with three white clover (*T. repens* cv. Grasslands 'Kopu') and three ryegrass (*L. perenne* cv 'Yatsyn') seeds (surface sterilised and pre-germinated on water agar), and transferred to a constant environment room at 20°C with 16 h light (110 µE/m²/s) and 8 h dark periods. Ten days after planting, the seedlings were thinned to two of white clover and one of ryegrass. Each pot received 5 ml of quarter strength nutrient solution (Smith *et al.* 1983), at weekly intervals and all the pots were maintained at approximately 75% water holding capacity.

Plants were harvested after 7 weeks. Soil was carefully removed from the roots and shoot and root wet weights were recorded. The remaining soil was retained for microbiological and nematological analysis.

To determine microbe numbers, serial dilutions of soil were prepared and bacteria and fungi counted on 1/2 strength tryptic soy agar and 1/4 strength potato dextrose-aureomycin agar as described previously (Sarathchandra *et al.* 1995). Cultures for bacterial counts were grown at 25°C and for fungal counts at 20°C. Both were counted after 7 days. For determination of nematodes, white clover roots were washed, cleared with diluted commercial bleach (1 part bleach to 3 parts water) and stained with aniline blue (Byrd *et al.* 1983). The plant-parasitic nematodes in the roots were then identified and counted.

The effects of chitin and the effects of pre-incubation of soil with chitin on each variable were analysed using standard analysis of variance methods. All the variables except plant data were log₁₀ transformed prior to analysis. The zero chitin rate was omitted from the analysis of the effects of pre-incubation on the variables. For nematode populations, the back transformed means are given in the text.

RESULTS AND DISCUSSION

Increase in chitin decreased ($P < 0.001$) the yield of white clover and increased ($P < 0.001$) that of ryegrass (Fig 1A) confirming our earlier results (Sarathchandra *et al.* 1995) in which we found chitin to be phytotoxic to white clover but beneficial to ryegrass growth. Amendment with 0.5% and 1.0% chitin would supply an additional 34.5 and 69.0 mg of N per pot respectively. Chitin amendments at rates of 0.8% (Godoy *et al.* 1983) and 1.0% (Mian *et al.* 1982) have been reported to be phytotoxic to squash and it is likely that the release of ammonium from the degradation of chitin may reach levels damaging to the plant (Mian *et al.* 1982).

The pre-incubation of chitin had a significant effect ($P < 0.001$) on ryegrass growth with improved growth occurring after 3 weeks pre-incubation with chitin (Fig 1B). Microbial mineralisation of chitin and soil organic matter during pre-incubation may have resulted in greater N availability for ryegrass growth. Alternatively chitin may be phytotoxic to ryegrass (masked by the response to N) and that this toxicity was reduced by pre-incubation perhaps by the conversion of ammonia to nitrate as proposed by Spiegel *et al.* (1987). However, pre-incubation had no significant effect on clover growth suggesting that chitin remained phytotoxic even when allowed to decompose for up to 9 weeks.

FIGURE 1: Effect of different rates of chitin (A) and pre-incubation of chitin amended soil (B) on the wet weight of white clover and ryegrass. Error bars show the standard error of the mean.

The total bacterial and fungal populations increased ($P < 0.001$) with chitin amendment (Fig 2A) possibly due to increased C and N supply from chitin. Bacteria and fungi with chitinolytic activity and the soil chitinase activity also increased in chitin amended soil (results not shown). Numbers of bacteria were greatest ($P < 0.05$) after 9 weeks pre-incubation (Fig 2B). These results are consistent with previous findings (Sarathchandra *et al.* 1995)

FIGURE 2: Effect of different rates of chitin (A) and pre-incubation of chitin amended soil (B) on numbers of bacteria and fungi in the soil. Numbers expressed per g dry weight soil: pumice mix.

The populations of *Meloidogyne* sp. decreased from 8/plant in untreated soil to 3.4 and 1.7 in chitin treated soil ($P < 0.05$). The equivalent numbers of *H. trifolii* were 4.9, 3.9 and 1.7 ($P < 0.01$) (Fig 3A). Pre-incubation of the soil caused an overall linear reduction ($P < 0.05$) in numbers of *Meloidogyne* sp. after 9 weeks but had no effect on *H. trifolii* numbers (Fig 3B). The reduction in nematode numbers resulting from chitin amendment has been attributed to increased chitinolytic microflora and chitinase activity, which can enhance nematode egg parasitism, and increased ammonia concentration, which is also known to have a nematocidal effect (Spiegel *et al.* 1987).

Even in the presence of fewer plant-parasitic nematodes, white clover yield was reduced confirming the phytotoxicity of chitin to white clover.

FIGURE 3: Effect of different rates of chitin (A) and pre-incubation of chitin amended soil (B) on numbers of plant-parasitic nematodes per plant.

These results are consistent with other reports of the effects of chitin amendment on nematodes numbers. Chitin treatments of 1% resulted in significant reduction of gall formation in squash (*Cucurbita pepo* L.) caused by the nematode *Meloidogyne arenaria*, when the soil was pre-incubated for 3 weeks before planting (Mian *et al.* 1982). When the incubation time was increased to 10 weeks, reduction of galls was seen with only 0.4% chitin (Godoy *et al.* 1983).

While the numbers of plant-parasitic nematodes were reduced as a result of soil amendment with chitin, any resulting increase in yield was nullified by the phytotoxic effect of chitin on white clover. This result may mean that the commercial use of chitin, perhaps as a seed treatment to control early nematode invasion in white clover seedlings may not be feasible.

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