

Pasture nematodes: the major scourge of white clover

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Abstract

This paper reviews current research within AgResearch to reduce the impact of plant-feeding nematodes. Plant-feeding nematodes reduce pasture production by around 15% annually, mainly through their effect on white clover. Nematicide application increases clover yields in pasture by an average 40% and N-fixation levels by over 50%. The problem occurs nationally. When pasture nematode burdens are reduced in established or newly sown ryegrass–white clover pasture, white clover can generally assume dominance during periods of active plant growth. The impact of clover nematodes in reducing nitrogen inputs and forage quality is estimated to exceed \$1 billion annually in lost production potential. A gain of 1% in clover performance applied nationally is estimated to be worth up to \$48 million. The research programme on pasture nematodes conducted within AgResearch has included evaluation of management practices that may reduce nematode impacts, selection of white clover seedlines for resistance or tolerance to nematodes, and identification of agents for biological control of nematodes within New Zealand pastures.

Keywords: clover nematodes, *Heterodera trifolii*, impacts, *Meloidogyne trifoliophila*, *M. hapla*, *Pratylenchus* spp., *Trifolium repens*

Nematodes in pasture

Nematodes are unsegmented unpigmented worms that are usually circular in cross section. They are the most numerous multicellular animals on earth and occupy almost every conceivable habitat. In agriculture they are more readily recognised in the form of roundworm parasites of farm animals (e.g., *Ostertagia*), than for their major depletion of pasture performance, as plant parasites. Most nematodes associated with pastures live in the soil and are either beneficial or neutral to man's interests. The majority of these are species that feed on bacteria or fungi (Yeates 1999). Other nematodes include those that predate small soil organisms, including other nematodes, or are parasites of larger

animals including insects, slugs and snails. All of these nematodes together commonly comprise 80–90% of nematodes in pasture soil.

Nematodes that feed on plant tissues usually attack the roots. In New Zealand pastures, most are less than 2mm long and barely visible to the naked eye. Typical pasture soils in New Zealand may contain up to six genera of plant-feeding nematodes, usually comprising exotic or pandemic species. Some nematode species feed only on grasses (e.g., the pin nematode species *Paratylenchus nanus*), some on clovers and not grasses (e.g., clover cyst nematode *Heterodera trifolii*, and the root-knot nematodes *Meloidogyne hapla* and *M. trifoliophila*), while others have mixed plant hosts (e.g., the lesion nematodes *Pratylenchus crenatus*, *P. penetrans*, and stubby root nematode, *Paratrichodorus minor*).

Other genera occur on a much more localised basis. These include four species of ectoparasitic nematode largely restricted to or first described from the Otago/Southland region: *Helicotylenchus canadensis*, *Subanguina radiculicola*, *Tylenchorhynchus maximus* and *Geocenamus nanus* (Mercer 1986; Yeates & Wouts 1992; Yeates 1992). Other localised genera include *Ditylenchus*, *Mesocriconema*, *Longidorus* and *Xiphinema*. Some of these less common species may be expected to gradually increase their distribution as soil is unintentionally moved around the country, adding to the total pest burden in pastures. Presently, the plant-feeding nematode fauna in New Zealand pasture is generally less diverse than in comparable situations elsewhere (Cook & Yeates 1993).

Of the nematodes feeding in clover roots, the clover cyst and lesion nematodes are present throughout New Zealand (Mercer & Woodfield 1986) but the clover root-knot nematode, *Meloidogyne trifoliophila*, is absent from southern regions of the South Island. *M. trifoliophila* appears to predominate in warmer and moister parts of the North Island, but in lighter volcanic ash soils, *M. hapla* predominates. Most regions of New Zealand do not contain cyst and root-knot nematode species that feed on grasses (Cook & Yeates 1993).

Nematode impacts in pasture

Debilitation of root function from plant-feeding nematodes occurs at several levels:

- Feeding by ectoparasitic nematodes leads to loss of root hairs, disruption of root elongation and nutrient uptake.
- Root invasion and movement within roots by endoparasitic nematodes causes root trauma, tissue leakage and entry sites for root diseases. Massed invasion of root tips can kill rootlets directly or prevent root elongation.
- Nematode feeding restricts the flow of water and nutrients needed in the leaves for photosynthesis.
- Root deformation as a result of feeding structures may also disrupt nutrient flow.
- Secondary effects include the disruption of both nodulation for N fixation and establishment of beneficial mycorrhizal fungi in roots that assist with P uptake.

Nematode damage resulting from loss of root efficiency is intensified when other stresses are acting such as summer drought or low soil nutrient levels. The effects of nematodes on pasture performance have been measured by applying nematicides. In a major comparison on 16 Waikato/Bay of Plenty sites, which included dairying and sheep/beef systems (Watson *et al.* 1985), nematicide treatments gave much greater herbage responses than treatments applied to control foliar or soil insects of pasture. Over all sites, the treatments gave a 13% increase in annual total herbage, a 40% increase in clover herbage yield and 55% increase in nitrogen fixation. In further evaluations on three dairy farms, responses ranged up to 35% for total pasture yield and 136% for annual levels of N-fixation, equivalent to 79–109 kg N/ha per annum (Watson *et al.* 1993; 1994). Typically, the responses were minimal in late winter and increased from spring through until autumn (Watson *et al.* 1993). Similar clover responses shown earlier in Southland (Risk 1975), Taranaki and Wairarapa (Yeates *et al.* 1975a; 1975b) indicate that the underperformance of clover is a national problem. Non-chemical means of reducing clover nematode numbers achieves similar responses to the field trials using nematicides e.g., sowing seed in seedtrays where soil freezing was used to exclude nematodes (Watson 1990), and when pasture is established after cropping with cereals or brassicas (e.g., Riley 1979; Yeates *et al.* 1975b). When nematode burdens were reduced chemically or non-chemically, pasture typically moved towards clover dominance indicating that in New Zealand pasture nematodes act differentially against the clover component – the component which largely underpins this country's international competitive advantage in pastoral agriculture (Caradus *et al.* 1996).

The direct benefit of white clover to the New Zealand economy in terms of N inputs into pasture, and animal

outputs from high quality forage, are estimated at \$1.49 billion and \$1.55 billion respectively (Caradus *et al.* 1996). Thus, based on 13% loss of forage production potential and 55% lost N input potential (as urea equivalent), the under-performance of white clover caused by nematodes can easily be estimated to exceed \$1 billion annually in lost input or production potential to New Zealand. A gain of 1% in clover performance applied nationally has been estimated to be worth up to \$48 million (Mercer 1994). This places the cost of pasture nematodes alongside that estimated for animal parasites in the sheep industry if anthelmintic drenching was not available (Leathwick & Vlassoff 1996).

Plant resistance and tolerance

A programme of classical plant selection for resistance to the clover cyst (*H. trifolii*) and clover root-knot (*M. trifoliophila*) nematodes has been conducted at Grasslands Research Centre, Palmerston North. The programme was based on selecting white clover plants that reduced nematode breeding success after inoculation with either of the nematodes. Plants supporting least nematode breeding success were then crossed and the progeny used successively in further cycles of selection. Finally, resulting progeny were crossed with commercial seedlines thereby incorporating nematode resistance into white clover genotypes that were suited to New Zealand pasture conditions (Mercer *et al.* 1999). A few susceptible lines were also selected and crossed for genetic studies.

A concurrent selection programme for tolerance to nematodes, in which white clover seedlines were grown in trays using a heavy natural infestation of nematodes, was conducted at Ruakura Research Centre, Hamilton. The most vigorous seedlings were then grown on in the field as spaced plants under plastic mulch. Plants were scored for vigour over 1 or 2 years with the best performing plants in large, medium and small leaf size classes poly-crossed to provide seed for the next cycle of selection. Seed in half-sib families were maintained for rescreening.

In 1998, material from both programmes was brought together for field screening by planting seedlings into ryegrass pasture in 12 trials and four sites nationally, under sheep or dairy grazing (Mercer *et al.* 1999). Replicated plots were treated with (split treatments using fenamiphos and oxamyl) (T) or without (U) nematicide application. Evaluations of vigour showed which seedlines had high inherent vigour (T), and those seedlines able to express high relative vigour in the presence of the pasture nematodes at each site (U). A low vigour response to nematicide (T/U) was also indicative of seedlines with the ability to withstand

nematode attack. Summarising across all sites, those lines selected for resistance were superior to the susceptible bred lines. The better performing of these lines, along with the top performing commercial cultivars, made up the top yielding 25% of lines. Assessment of nematode numbers in the roots, or cyst counts from soil of some of the seedlines, showed that in general, the breeding background was expressed, with reduced numbers of targeted nematodes in the resistance selections (Watson and Mercer, unpublished data). The trials have highlighted the need for clover to have resistance to both clover cyst and clover root-knot nematodes, and possibly also to the lesion nematode where these nematodes occur together, as cross-resistance is not expressed. A number of the nematode tolerant seedlines have expressed better field performance than commercial standards and these seedlines have been added to the AgResearch germplasm collection of high vigour seedlines. The tolerant seedlines do not express resistance to a particular nematode, but the total root burden was reduced in some seedlines (Watson, unpublished data). It is possible that tolerant material may express advantages for other growth limiting reasons, such as resistance to root rots that are secondary to nematode attack, or an inherent ability to better cope with nematode-induced stress.

Management of nematode impacts

Fertiliser

For both economic and environmental reasons, it is not tenable for farmers to apply pesticides to control nematodes. Management strategies to reduce nematode impacts may include methods which optimise production without affecting the nematodes directly. One way to compensate for a root system compromised by nematodes is to make nutrients more readily available. In fact, the high rates of phosphorus applied by New Zealand farmers to maintain clover in order to fix N is doing just that (Widdowson *et al.* 1973). Undoubtedly, if nematode impacts on clover could be reduced by other means this farm expense could be reduced substantially. Similarly, irrigation allows summer clover performance to be improved even though clover nematode numbers may be increased.

Deferred grazing

In the summer-dry coastal Bay of Plenty on light ash soils, loss of clover between mid November and mid February occurs frequently. Clover brown-off can occur when sun impacts directly onto dry soil in open pastures, creating surface temperatures that are lethal to clover stolons (Watson *et al.* 1994; Archer and Robinson 1989). The effect is exacerbated by the effect of nematodes on

stolon roots. Bay of Plenty farmers who deferred grazing over this period promoted a proliferation of well-rooted stolons under the rank pasture, which promoted good pasture quality once grazing had resumed (Watson *et al.* 1996). Without deferment, clover may take several years to recover from severe drought. The limitations to the method are that only a small area of the farm can be deferred in any season, and the summers in which significant advantages accrue cannot be predicted in advance. One adaptation is to avoid long rotations in late winter/spring, thereby maintaining a tighter sward and more closed ground cover into the summer.

Cropping and alternative species

Cropping during summer, particularly using non-hosts of clover nematodes such as graminaceous crops, can reduce nematode numbers sufficiently to ensure the vigorous establishment of clover in new pasture. In a Bay of Plenty study, however, 15 years of continuous maize cropping resulted in non-detectable levels of clover nematodes and exceptional clover vigour in the establishment year, but patches of weaker clover started to appear in the second year and clover cyst and root-knot nematode numbers peaked by the third year (Watson *et al.* 2000). Severe drought in the fourth year then almost eliminated white clover from the pasture, and clover nematodes again declined to a low proportion of total plant-feeding nematodes present. In the following autumn and spring, white clover was able to re-establish vigorously, largely from seed germination. This confirmed that nematodes can be a driving factor in observed natural cycles of white clover abundance, by suppressing clover vigour and competitive ability during normal seasons and enhancing the loss of clover during summer drought. Clover loss in turn suppresses populations of clover nematodes, which then facilitates a vigorous plant recovery.

In a 6-year Bay of Plenty trial, the use of caucasian clover (*Trifolium ambiguum*) as an alternative to white clover was investigated in order to improve clover persistence in a summer-dry dairying system. The rhizomatous habit of caucasian clover means that underground growing points are better protected from lethal surface temperatures and overgrazing during summer. Caucasian clover was slower to establish than white clover but from years 2 to 6, pasture yield and/or clover content was enhanced (Watson *et al.* 1997; 1998). Clover cyst nematode populations were substantially suppressed in caucasian clover and populations of lesion nematode were also reduced, but the root-knot nematode *M. hapla* occurred at higher levels than in white clover plots (Watson *et al.* 2000). Since clover root-knot nematodes are largely absent in southern parts of the South Island, this area appears to

be more favourable for the advantages of caucasian clover.

Biological control

Pasture soil contains a rich flora of fungal species, some of which are parasitic on nematodes. In a survey of 10 North Island pasture sites, Hay & Skipp (1993) found that 93% of clover cyst nematode cysts were colonised by one or more fungi, including known parasites (Hay 1995). Despite the high incidence of fungi potentially pathogenic to nematodes, the level of natural regulation they provide is not enough to bring population densities below the damage threshold. Many of these fungi are also common in clover roots as pathogens, which reduces the number of species that could be developed to enhance nematode suppression. Pathogenic bacteria can similarly regulate nematode populations, either by direct parasitism from the soil e.g., *Pasteuria penetrans*, or as endophytes within the plant, e.g., *Pseudomonas* spp. (Siddiqui & Mahmood 1999). Effective diseases of plant-feeding nematodes in some crops have been determined after the discovery of nematode-suppressive soils. These soils show a decline in numbers from former levels of a plant-feeding nematode despite the continued production of the crop. No pasture soils with these characteristics have been described in New Zealand.

Possibilities for exploiting micro-organisms for nematode control have been demonstrated in New Zealand laboratory studies (Hay *et al.* 1996). Amendments applied to soil can elicit beneficial organisms which lead to a reduction in plant parasitic nematodes e.g., chitin applied to a field soil produced a large response from bacteria and fungi capable of using this substance as a substrate (Sarithchandra *et al.* 1996), and *Paratrichodorus minor* abundance was reduced (Bell *et al.* 2000). Current work is directed at identifying the most effective micro-organisms presently active in soil, or as plant associates, and trying to develop these into effective means of nematode control. The search for more effective microbial organisms should also be conducted in areas that are endemic for white clover and its nematode parasites.

Conclusions

Plant-feeding nematodes are insidious but ubiquitous pasture pests, and a particular scourge of white clover in New Zealand. As such, they diminish a major competitive advantage for the pastoral farmer in New Zealand. Progress to ameliorate the nematode problem has progressed on several fronts, in particular in plant improvement. Small gains in clover performance,

applied nationally, have the potential to significantly enhance the productive sustainability and value of pasture, while reducing requirements for artificial N and P applications.

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