Breeding white clover for tolerance to nematodes: overview of process and progress

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Abstract
A base population of 80 cultivars or seedlines of white clover from around the world was used to develop nematode tolerant selections. Clover plants were grown in strips of black plastic, akin to strawberry growing, in order to increase soil temperature and thence nematode activity in clover roots. Clover growth was assessed visually and by dry matter determination in five rounds of selection from 1989–2001. After each round of selection the 20–30 best performing plants for each of three leaf sizes (large, medium and small) were polycrossed and the seed collected. The resulting selections have performed at least as well as, and in many cases better, than standard commercial cultivars at a range of field sites.
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Introduction

As early as the 1970s studies had shown that plant feeding nematodes had a large effect on white clover yield and nitrogen fixation (Yeates *et al.* 1977). Research conducted in the 1980s demonstrated that eliminating nematodes from pasture soil increased pasture production by 8-–20% (Watson *et al.* 1985). The bulk of this increase came from greater clover production (28–40%) and resulted in higher nitrogen fixation (49–76%) (Steele *et al.* 1985; Watson *et al.* 1985). This effect has been observed in virtually all pasture growing areas of New Zealand, and is due to any one or a combination of three plant-feeding nematodes: root knot (*Meloidogyne*), cyst (*Heterodera*) and lesion (*Pratylenchus*).

A breeding approach to overcome nematode effects on white clover by selecting plants based on vigour in a field-based situation was undertaken at AgResearch Ruakura Research Centre from 1989 to 2001. Aspects of white clover and nematode biology were taken into account when designing the selection process. Since white clover is an obligate outcrossing plant, selections were made within groups large enough to prevent inbreeding and associated loss of vigour. Thus the programme was developed to incorporate wide diversity from as many sources as possible in the initial stages, and selections made within three leaf size classes for agronomic reasons (e.g. large and medium leaf for dairy, small leaf for sheep).

Because the objective was fitness of white clover plants against nematodes, a field-based system using plastic mulch was designed to restrict the root system and create a warm environment which favoured nematodes (Watson *et al.* 1988). It was possible that root rots could also be favoured in such an environment, adding disease pressure to the roots and thereby adding to the fitness of plants surviving the selection process. Thus it was envisioned that the selected plants would be tolerant of nematodes and any of a wide range of other stresses present in the field (e.g. drought, insects, slugs, viruses). This paper outlines the selection process and the outcome of the process in terms of on-farm field trials.

Methods

For all selection rounds, ca. 350 seeds for each cultivar or seedline were planted into seed trays (500 × 300 × 90 mm) containing inverted turfs from nematode-infested pasture. All but the 20 most vigorous seedlings were weeded out after 2 months growth in the trays.

For all but the final selection round, two trial sites were established, one on a dairy farm and the second on a sheep farm, both on Ruakura Research Centre property in Hamilton, known to be infested with plant-parasitic nematodes. Selected clover plants from the seed trays were planted into 5 cm diameter holes in plastic mulch which was established using the method of Watson *et al* (1988). Both sites had nine strips of plastic mulch, each strip 1 m wide × ca. 23 m long. Every strip of mulch was planted with two rows of white clover, each of 55 plants.

Two replicates of each seed line were planted per site with each replicate comprising five plants in consecutive holes in the mulch. Both sites consisted of two blocks, each block containing one replicate of all the seedlines. Clover growth was assessed by visual scoring on six or seven dates for each site for all selection rounds. Seven categories were used to score growth (0 to 6) based on the diameter of the plant so that: 0 = plant dead; 1 = plant diameter < 5 cm; 2 = 5–10 cm; 3 = 10–20 cm; 4 = 20–30

Table 1 Country of origin of the white clover cultivars and seed lines used as the basis for nematode tolerant selections.

Country of origin	Cultivars/ seed lines
Australia	Irrigation, Haifa
Belgium	Merwi, Blanca
Denmark	Milka, Milkanova, Rivendel
France	Lustar
Germany	Gigant, Lirepa
Ireland	Aran, Ross
Italy	Ladino,
Netherlands	Tamar, Pronitro, Retor, Cultura
New Zealand	Kopu, Pitau, Huia, Tahora, 40 local ecotypes
Sweden	Nora, Sonja, Lena, Sandra, Kivi
UK	Alice, Olwen, Donna, Menna, Nesta, Gwenda, Kersey
Uruguay	Bayucua
USA	Osceola, Regal, SC-1, Louisiana
Former USSR	Jygeva, Pastevec

cm; 5 = > 30 cm; 6 = > 30 cm and free of any visible disease symptoms and pest damage. After scoring, the plants were trimmed back to a crown diameter of no more than ca 8 cm.

Selection round I

The origins of the white clover parent plants to start the recurrent selection programme are given in Table 1. Local ecotypes were from field sites around New Zealand including those sites of Watson *et al.* (1985). Other local ecotype material was collected from old pastures, often from large clonal patches within pasture, which was evidence of their competitive capacity and ability to survive under grazing.

Some cultivars already showed resistance or tolerance to nematodes: Alice, Donna, Gigant, Kopu and Regal for stem nematode; Ladino, Milkanova and Milka for clover cyst nematode and SC1 for root-knot nematode (Caradus 1986). Other cultivars showed resistance or tolerance to other pests and diseases: Blanca, Jygeva, Merwi, Nesta, Nora, Retor and Sonja to *Sclerotinia*; Cultura, Huia, Pitau and Irrigation to viruses; Cultura and Kivi to aphids (Caradus 1986).

The field sites for this selection round were planted in autumn 1989 and assessed until summer 1991. Stolon cuttings from the best plants were grown on and polycrossed using nucleus bee hives to produce the next generation of seed.

Selection rounds II to V

Seedlings of three leaf size classes (large, medium and small) from the previous round were planted into plastic strips in early autumn 1992, 1995, 1998 and 2000 and assessed until summer 1994, 1996, 1999 and 2001 respectively when stolon cuttings from the best plants were grown on and polycrossed as above. Included in each section from every site was a commercial standard cultivar appropriate to the leaf size class.

Field testing

Ten seedlings from F1 seed were planted out in 1 m rows in grazed sheep pasture at Ruakura Research Centre in autumn 1992 (Short 1995), with six replicate rows per seed line. Commercial cultivars were planted for comparison with each of three leaf size classes: small, medium and large. Relative vigour scores were visually assessed at least quarterly with the most vigorous row of plants being scored a 10 and all other rows scored in relation to it. Rows where all plants were dead scored 0.

F3 seed lines were planted out in field sites in the Waikato (Cambridge), Manawatu (Palmerston North) and Canterbury (Lincoln) regions in spring 1997 and assessed for vigour and reaction to nematicide application in comparison with the commercial cultivars Kopu, Prestige, Demand and Sustain (Mercer *et al.* 1999; Mercer *et al.* 2005).

F4 seedlings were planted out in a field trial investigating the effect of clover root weevil (*Sitona lepidus*) on white and red clover at Ruakura Research Centre in 2000–2001 (Watson *et al.* 2002). Clover root weevil feeding, larval populations and clover vigour were assessed (Watson *et al.* 2002).

In 2002, F5 seedlines were planted in four field trials in the Waikato (Waerenga) and Northland (Warkworth and Kerikeri) under dairy or sheep grazing and assessed for vigour in comparison with a number of other seed lines and commercial cultivars (Crush *et al.* 2005, 2004).

Table 2 Mean score and maternal parent of the top performing white clover plants from combined dairy and sheep farm sites for field screening from September 1989 to July 1990.

Mean score	Cultivar/ seedline
≥ 5.0	Regal, Merwi, Pronitro, 1 local ecotype
4.5–4.9	Alice, Aran, Bayucua, Blanca, Donna, Gwenda, Haifa, Irrigation, Kopu, Ladino, Louisiana, Milka, Nesta, Nora, Olwyn, Osceola, Ross, SC1, Sonja, Tamar, 8 local ecotypes
4.0–4.4	Gigant, Huia, Jygeva, Lustar, Milkanova, Pastevac, Pitau, Retor, 10 local ecotypes

Results and Discussion

Selection round I

Of the 40 cultivars which had seedlings planted in the first round of selection, 31 contributed to the top performing plants

Table 3 Mean (± standard error) plant dry weight (g) for 20 white clover plants per leaf size which scored 5 or 6 (from a range 0–6 with 6 being the best performing) for combined dairy and sheep farm sites.

	1992		1993			1994
Leaf size	Sept/Oct	Nov	Feb/Mar	June	Oct	Jan
Large	22.2 (1.8)	147.7 (8.6)	74.9 (9.5)	30.2 (4.3)	72.5 (9.0)	69.4 (6.1)
Medium	24.9 (3.7)	106.6 (6.2)	59.7 (6.1)	28.2 (6.3)	55.4 (4.8)	54.3 (7.2)
Small	19.0 (2.4)	91.0 (7.8)	45.7 (2.6)	18.9 (2.3)	40.3 (3.8)	42.0 (5.9)

(score > 4.0) from the first round of selection, and a further 19 top performing plants were sourced from local selections (Table 2). The cultivars with plants that performed well included all those with some nematode tolerance or resistance (Alice, Donna, Gigant, Kopu, Regal, Ladino, Milkanova, Milka and SC1), but not the two cultivars with aphid tolerance (Cultura and Kivi).

Selection round II

Of the 20 top performing plants (mean score > 5.0), in each leaf size class, a large proportion (27%) had a maternal background of nematode resistance or tolerance (large: Kopu, SC1, Regal, Ladino; medium: Alice; small: Milkanova) with the addition of backgrounds of resistance to *Sclerotinia* (medium: Merwi; small: Jygeva) (5%), or drought (large: Louisiana, Regal) (5%). Other cultivars and seed lines represented in the top performing plants included Osceola, Aran, Ross and three local ecotypes for the large leaf; Pitau, and 14 local ecotypes for medium leaf; and 17 local ecotypes for small leaf.

After establishment in the plastic mulch, all three leaf size classes had similar dry weights for those plants which had scored 5 or 6 (Table 3). For the remaining five sample times, the large leaf selections always had the highest dry weight, followed by the medium and small leaf selections.

Selection round III–IV

The top performing plants (mean score = 5.5) showed a predominance of nematode resistant/ tolerant maternal parents (27%), and local ecotypes (50%) which were especially dominant in the small and medium leaf size classes. For the large leaf selections, the original maternal parents were Regal, Ladino, Ross, SC1, Louisiana, Osceola, Bayucua and two local ecotypes; medium leaf Alice, Gigant, Blanca and four local ecotypes; small leaf Pronitro, Milkanova and five local ecotypes.

Selection round V

Although by the final selection round the origin of maternal lines was less important than earlier, it is still clear that material from cultivars which were originally resistant or tolerant to nematodes (SC1, Kopu in large leaf, Gigant in medium leaf and Milkanova in small leaf) (33%) or *Sclerotinia* (Blanca and Merwi in medium leaf) (13%) contributed a large proportion of the best performing plants (mean score >5.0, n = 69). Selections of local ecotypes also figured strongly (three in all leaf size classes), ensuring adaptation to local conditions was maintained.

Field testing

The four vigour scores in 1994, 2 years after sowing, for the best five F1 seedlines were 5.1 (± 0.4 SEM), 5.1 (±0.4), 4.9 (±0.5) and 4.6 (±0.3) compared to the commercial standards scores of 3.1 (±0.2), 3.4 (±0.3), 2.6 (±0.2) and 3.3 (±0.3) respectively. These scores showed that even the early selection process had started to make gains in vigour under field conditions. Nematode counts from roots of some of the tolerance selected plants showed reduced populations of root knot and lesion nematodes compared to commercial cultivars (Mercer & Watson 1996; Short 1995).

Early results showed that the F3 material had greater vigour than commercial cultivars at all three sites tested, with the increase being significant at the Palmerston North site (Mercer *et al.* 1999). Results pooled from all sites and assessments between 1998–2000 showed the medium leaf selections were significantly more vigorous than all the commercial standards and that large and small leaf selections performed as well or better than the standards, significantly so in comparison with Demand (Mercer *et al.* 2005).

F4 seed lines appeared to support lower populations of clover root weevil feeding and larvae than other seed lines and commercial cultivars while still being highly vigorous in a field situation (Watson *et al.* 2002).

The best lines from the F5 nematode selections were more vigorous than the commercial standards (relative vigour = 1.00) in grazed trials on a Waikato dairy (1.12) and sheep farm (1.13) and two Northland dairy farms (1.21, 1.22) (Crush *et al.* 2004). Indeed, the top performing nematode selection was more vigorous than any other of the 10 cultivars or 39 seed lines tested at the Waikato dairy and sheep farms from 2002–2005 (Crush *et al.* 2005).

In conclusion, the field-based selection process for white clover vigour under high nematode burdens has produced seedlines which are at least as vigorous as commercial cultivars and in some cases considerably more vigorous. This vigour advantage appears to be

expressed across a range of sites in the North and South Islands suggesting that these seed lines are suitable for incorporation into future cultivar development efforts. The nematode tolerance breeding programme seems to have borne out the idea that selecting for vigour in field-based situations produces germplasm able to be highly productive in field situations where multiple stressors are present.

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