Progress towards clover cyst nematode resistant white clover

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Abstract

Clover cyst nematode *(Heterodera trifolii*) reduces growth and nutrient uptake of white clover (*Trifolium repens*) in New Zealand, and breeding resistant cultivars is the preferred control method for ecological and economic reasons. Resistant and susceptible selections were taken from a wide range of white clover seedlines. Selected plants were intercrossed and reselected over two cycles of selection. Cysts per gram of root dry weight (cysts/g) was the measure of resistance used, with low cysts/g plants being more resistant. The mean cysts/g value of the progenies from resistant parents was 69% of the mean value of the progenies from susceptible parents in the first generation, and 38% in the second generation. Root weights were similar, while the resistant selections had 58% and 34% (first and second generations respectively) of the number of cysts in the susceptible selections. There were no immune plants (0 cysts) in the first generation and only two in the second. Counts were made of cysts, eggs per cyst and eggs per plant on clones of two resistant and two susceptible genotypes. The resistant genotypes had lower counts than the susceptible genotypes in terms of cysts per plant (means of 6 cf. 108), eggs per cyst (11 cf. 21) and eggs per plant (35 cf. 1650). The differences in numbers of cysts between resistant and susceptible lines increased during two generations of selection, supporting earlier results that indicated that progress in breeding for resistance was possible. Furthermore, the resistance is also acting on clover cyst nematode egg production which would further reduce recruitment to later generations of clover cyst nematode.

Keywords: breeding, *Heterodera trifolii*, resistance, screening, selection, *Trifolium repens*

Introduction

The clover cyst nematode (*Heterodera trifolii* Goffart) occurs throughout New Zealand (Mercer & Woodfield 1986) and causes a general root malfunction and an accompanying depression of foliage growth in white clover (*Trifolium repens* L.). Reductions in growth, nitrogen content and efficiency of phosphorus utilisation have been measured in pot experiments of nematode infected white clover (Skipp & Gaynor 1987; Widdowson *et al.* 1973; Yeates 1977). Field trials in the USA measured dry weight reductions of 14% and 46% for two lines of white clover (Norton 1967). In New Zealand, pesticide treatment that reduced a number of nematode species including clover cyst nematode, was associated with increases in pasture yield (mean of 13%), clover yield (mean of 40%) and nitrogen fixation (mean of 57%) over 16 North Island sites (Watson *et al.* 1985). Greater responses were found on Bay of Plenty soils and a Hamilton site where nematode control gave better clover persistence during a summer drought resulting in a greater advantage in clover growth and N fixation in the following autumn and winter especially (Watson *et al.* 1994).

Nematicides for nematode control are not viable for economic and ecological reasons, and breeding resistant white clover cultivars is the most desirable alternative. Even partial resistance would be of benefit as an additional pressure on pest nematode populations. Resistant plants are ones on which nematode reproduction is prevented or reduced relative to susceptible controls. Some variation in resistance (relative or partial resistance), but no immunity (complete resistance) to the clover cyst nematode has been reported in white clover. Dijkstra (1971) used a range of material including apparently resistant New Zealand white clover and concluded that inheritance was most likely determined by more than one gene. However, Yeates *et al.* (1973) tested seven lines, including Grasslands Huia, and found all were susceptible. An increase in resistance in lines selected for seedling vigour in field soil was reported by Mercer *et al.* (1992).

The aim of the experiments reported here was to identify variation in resistance to clover cyst nematode in progenies from resistant white clover plants. Resistant and susceptible selections were taken from a wide range of white clover seedlines tested previously. Pair crosses were made within these resistant and susceptible selection groups to provide the first generation progeny (tested in Experiment 1 & van den Bosch *et al.* 1993a) ; the second generation was produced from five

polycrosses (three resistant and two susceptible, tested in Experiment 2). Previous results from this breeding programme have been published (van den Bosch *et al.* 1993a; van den Bosch *et al.* 1997).

Materials and methods

To test for resistance, 10 pre-germinated white clover genotypes per seedline were sown at one seedling per pot, in 6 cm diameter pots containing a pasteurised sand-soil mix (Manawatu silt loam, pH 6.1). The pots were placed in randomised blocks, in metal trays, which were rotated weekly within the glasshouse. Pots were kept in temperature-controlled glasshouses with soil temperature at 18–24°C, and total nutrients were applied fortnightly. Each pot was inoculated with approximately 2000 clover cyst nematode eggs in a 3 ml suspension placed in a hole near the roots and the hole filled in. The nematodes used were from a culture initiated from field-collected cysts at Palmerston North and maintained on white clover. At harvest, roots were washed in elutriation towers (Wood & Foot 1977) in which cysts and soil organic matter were washed up free of mineral particles that sank in the water flow. The cysts and organic matter were collected in fine sieves, and cysts counted. Roots were dried and weighed, and stolon tips kept to maintain all the plants for possible further selection and crossing. All selections were based on individual genotype data, not the seedline means.

Four "benchmark" seedlines sown in both Experiments 1 and 2 allow comparisons between experiments. The benchmark lines were a Grasslands Huia parent progeny, Ladino Gigante Lodigiano, Espanso, and a *Heterodera*-resistant seedline from SVP, Netherlands. Cysts per gram of root dry weight (referred to from here as cysts/g) was calculated by dividing the number of cysts by the root dry weight for each genotype. Means were compared by ANOVA, using GENSTAT.

Experiment 1: First generation progeny evaluation
The seedlines screened were 56 resistant and 12 susceptible first generation progeny sown in April 1990. Total nutrients were applied fortnightly: N 111 (as NH_4^+), P 40, K 238, S 60, Ca 127, Na 88, Cl 723, Mg 21, Fe 3, B 0.1, Zn 0.25, Mn 0.25, Cu 0.05, Mo 0.01 and Co 0.009 ppm. Plants were inoculated 3½ weeks after sowing, and harvested 6 weeks after inoculation. A shoot growth score (1 = smallest to 5 = largest) was visually assessed just before harvest time, to record plant performance, check experimental procedures and allow comparisons between shoot and root growth.

Experiment 2: Second generation progeny evaluation
The seedlines screened were 91 resistant and 18 susceptible second generation progeny sown in January 1992. 'Thrive' nutrient solution was applied at half-strength every 2 weeks. Plants were trimmed and repotted in March, inoculated after 2 weeks and then again 1 week later, and harvested 7 weeks after the first inoculation.

Experiment 3: Eggs per cyst experiment
Cuttings of two resistant and two susceptible genotypes from first generation progeny (van den Bosch *et al.* 1993a) were rooted for study of nematode egg production in June 1990. Five to nine copies (stolon tips) of each genotype were trimmed to standardise the leaf number and root mass, and inoculated as described above, a week later. Plants were maintained as before and 7 weeks after inoculation, cysts were extracted as described above and counted. Up to 10 cysts per plant were selected at random and crushed between a microscope slide and coverslip, and eggs counted. "Eggs per plant" values were the product of cysts per plant and the mean of the eggs per cyst values.

Results

There were highly significant differences (ANOVA F-test $P<0.001$) between individual seedlines in both generations for number of cysts, root dry weight, growth score (only scored in Experiment 1) and cysts/g. When individual seedlines were grouped on their selection criteria, the mean cysts/g value of the progenies from resistant parents was 69% of the mean value of the progenies from susceptible parents in the first generation (Table 1), and 38% in the second generation (Table 2). Root weights were similar for the three groups. The resistant selections had 58% and 34% (Experiments 1 and 2 respectively) of the number of cysts on the susceptible selections (Tables 1 and 2). There were no immune plants (0 cysts) in the first generation; however, two were identified in the second. The benchmark lines had fewer cysts and cysts/g than the susceptible selections and were not significantly different from the resistant selections in Experiment 1, but were intermediate between resistant and susceptible selections for cysts in Experiment 2.

The number of cysts (and females) on cuttings of two resistant and two susceptible genotypes reflected the genotypes' status in the screening. The trend in eggs per cyst was not significant at 5%, but there were significantly fewer eggs per plant on resistant genotypes (Table 3); the two resistant genotypes had a mean of 35

eggs/plant, compared with 1650 for the two susceptible genotypes.

Discussion

These experiments confirm predictions (van den Bosch *et al.* 1997) based on the variability and broad sense heritabilities of the parent material that progress in breeding for resistance was possible. The divergence, in terms of cysts/g, in these early cycles of selection is encouraging progress for the breeding programme. Similar results have been found in a parallel programme breeding for resistance to the root-knot nematode, *Meloidogyne* sp. (van den Bosch & Mercer 1996a, 1996b; van den Bosch *et al.* 1993b), though the rate of progress was slower.

Kuiper (1960) tested clones selected from nematode-infested fields, and found several were "virtually resistant", though none were "absolutely resistant". These clones were from a range of cultivars from many countries, with the resistant clones coming from six different countries (Kuiper 1960). The parent plants of the selections described in this paper also came from a wide range of overseas and New Zealand germplasm.

Genetic variation among New Zealand's clover cyst nematode populations may limit the usefulness of this resistance selected against one population. However, the survey of North Island populations by Mercer & Grant (1993) showed that six out of eight populations tested were sensitive to the resistant seedlines, and there was a seedline x nematode population interaction. Although a preliminary result, because of the moderate level of resistance in the plants used, their result indicated that the resistance being developed against a Palmerston North population of clover cyst nematode will probably have widespread applicability.

Differences in ability to host clover cyst nematode between genotypes are greater when the parasite population is measured as eggs/plant rather than the easier to measure cysts/plant. However, selecting for cysts/plant was effective in terms of both eggs/plant and cysts/plant after just one generation. Partial resistance, as seen in this white clover, can improve plant growth directly by reducing the debilitation owing to nematode feeding and also indirectly by lowering the number of nematodes in subsequent generations, thereby diminishing the soil population density of potential parasites. Our calculations of nematode egg production show that the reduced fecundity on resistant genotypes is likely to have a major effect on the numbers of clover cyst nematodes in pasture where these resistant plants are used.

Table 1 Mean number of *Heterodera trifolii* cysts, root dry weight, growth score, and cysts/g on groups of *T. repens* first generation progeny seedlines in Experiment 1 (10 genotypes/seedline).

Selection	Lines (no.)	Cysts (no.)	Root dry weight (g)	Growth score (1–5)	Cysts/g root DWt Mean	Cysts/g root DWt Range
Resistant	56	41 B	0.014	2.7	3744 B	726–7584
Susceptible	12	71 A	0.015	2.9	5441 A	4270–6941
Benchmark	4	31 B	0.011	2.4	2918 B	1848–4824
Significance (Selections)		***	n.s.	n.s.	**	
Significance (Lines)		***	***	***	***	
$LSD_{0.05}$						2347

Means in a column with the same letter are not significantly different.

Table 2 Mean number of *Heterodera trifolii* cysts, root dry weight, and cysts/g on three groups of *T. repens* second generation progeny seedlines in Experiment 2 (10 genotypes/seedline).

Selection	Lines (no.)	Cysts (no.)	Root dry weight (g)	Cysts/g root DWt Mean	Cysts/g root DWt Range
Resistant	91	82 C	0.283	341 B	104–879
Susceptible	18	242 A	0.304	895 A	551–1190
Benchmark	4	122 B	0.342	405 B	198–625
Significance (Selections)		***	n.s.	***	
Significance (Lines)		***	***	***	
$LSD_{0.05}$					277

Means in a column with the same letter are not significantly different.

Table 3 Effect on egg numbers in *Heterodera trifolii* cysts after one cycle of selection for reduced cyst numbers on white clover in Experiment 3.

Genotype	Number of copies	Mean females + cysts/plant	Mean eggs/cyst	Mean eggs/plant
84/12 (resistant)	6	8 A	16	61 A
88/15 (resistant)	5	4 A	6	9 A
90/7 (susceptible)	9	100 B	21	1560 B
82/15 (susceptible)	9	115 B	21	1740 B
Significance		***	n.s.	**

Means in a column with the same letter are not significantly different.

Our finding that resistance to clover cyst nematode can be improved by further selection raises issues for the long-term use of resistant cultivars (Young 1992): 1) nematode species unaffected by the resistance may increase in incidence, 2) resistance-breaking races of clover cyst nematodes may be selected for, and 3) the resistant seedlines may be more sensitive to parasitism than their parent material (Roberts 1992). The last named was not indicated in our work as all resistant seedlines grew well, but we plan to monitor yield in glasshouse and field trials. The other two issues would require field study over several seasons.

Conclusion

The differences in numbers of cysts between resistant and susceptible lines increased during two generations of selection, supporting earlier results that indicated that progress in breeding for resistance was possible. Furthermore, the resistance is acting on clover cyst nematode egg production which would further reduce recruitment to later generations of the clover cyst nematode. We believe that this breeding programme will result in a white clover cultivar with useful levels of resistance to a widespread pest.

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