Third generation progress in breeding white clover for resistance to root-knot nematode

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Abstract

Root-knot nematode (*Meloidogyne* sp.) reduces growth and nutrition of white clover (*Trifolium repens* L.) in New Zealand, and breeding resistant cultivars (with low galls per gram of root) is the preferred control method. Resistant and susceptible selections were bred from a wide range of white clover lines for three generations. In the third generation there were significant differences between seed lines from the selections for number of galls, root dry weight, visual growth score and galls/gram of root dry weight. Resistant selections had 43% of the susceptible selections' galls per gram, and 50% of the number of galls. Germplasm showing resistance to *Meloidogyne* spp. in the USA showed partial resistance to the local *Meloidogyne* sp. Two resistant and two susceptible genotypes were also compared for nematode egg production; resistant genotypes had a mean of 3,460 eggs/plant, compared to 25,030 for susceptible genotypes.

Keywords: breeding, *Meloidogyne* sp., resistance, root-knot nematode, screening, selection, *Trifolium repens*, white clover

Introduction

Root-knot nematode *(Meloidogyne* sp.), an undescribed species previously thought to be *Meloidogyne hapla* (Chitwood), has been found throughout most of New Zealand, except the east and south of the South Island (Mercer & Woodfield 1986; Skipp & Christensen 1983). Reductions in growth, nitrogen content and efficiency of phosphorus utilisation of nematode-infected white clover (*Trifolium repens* L.) have been measured in pot experiments (Skipp & Gaynor 1987; Yeates 1977). Results from field experiments are more variable, but reductions in nematode populations from nematicide application, including root-knot nematode were associated with increases in pasture yield (mean of 13% over 16 sites), clover yield (mean of 40%) and nitrogen fixation (mean of 57%) (Watson et al. 1985).

The use of nematicides to control nematodes is not viable for economic and ecological reasons. Breeding resistant white clover cultivars is the most desirable alternative, but white clover resistance to the undescribed *Meloidogyne* sp. has not been reported. Genetic variation in response to other root-knot nematodes in white clover has been reported previously (Bain 1959; Quesenberry et al. 1986; Windham & Pederson 1991). As cross resistance for *Meloidogyne* spp. has been found, germplasm showing resistance to other root-knot nematodes was included in this breeding programme.

The original wide range of seedlines tested for resistance, as well as the results of the first and second cycles of selection, have been reported already (van den Bosch & Mercer 1996; van den Bosch et al. 1993). All selections were based on individual genotype data, not the seedline means. Pair crosses and open pollinations were made within resistant and susceptible selection groups in the first generation; the second generation were all pair crosses, and the third generation, reported in this paper, were in two polycrosses (1 resistant, 1 susceptible).

Materials and methods

The 102 seedlines screened were 63 resistant and 23 susceptible third generation polycross progeny, eight lines of six different overseas germplasm selections, and eight 'benchmark' lines also used in previous experiments. The 'benchmark' lines were three Grasslands Kopu parent plant progenies, two Grasslands Tahora parent plant progenies, Ladino Gigante Lodigiano, Espanso, and Quin Zhen, an ecotype from China. The 'new' overseas lines were generally larger leaved ladino types. In April 1991, 10 pre-germinated white clover seeds per line were sown at one per pot, in 6 cm diameter pots containing a sterilised sand/soil mix (Manawatu silt loam pH 6.1). Five weeks after sowing, each pot was inoculated with approximately 2000 root-knot nematode eggs in a 3 ml suspension placed in a hole near the roots and the hole filled in.

Meloidogyne sp. inoculum was prepared from a culture maintained on white clover (cv. Grasslands Huia); root galls were crushed with a roller and extracted using the chlorine method of Hussey and Barker (1973). The pots were placed in randomised blocks, in metal trays, which were rotated weekly within the glasshouse. Soil temperature in pots was maintained at 18–24°C, and total nutrients were applied fortnightly. A visual

shoot growth score (1–5) was made at harvesting five weeks after inoculation when roots were washed, galls counted, and roots dried and weighed. "Galls/gram" was calculated by dividing the number of galls by the root dry weight for each genotype.

Cuttings were rooted from two resistant and two susceptible genotypes for study of root-knot nematode egg production. Seven copies 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 five weeks after inoculation, roots were cut off, blotted dry and weighed, then stained in Phloxine B and egg masses counted. Eggs were extracted in chlorine solution as described above and counted.

Results

There were highly significant differences between individual seedlines, and between the four seedline groupings, for number of galls, root dry weight, growth score and galls/gram (Table 1). Resistant selections averaged 43% of the susceptible selections' galls/gram, despite the large range of means (Figure 1, Table 1). Resistant selections had 50% of the susceptible selections' number of galls, and the plants were a similar size.

Germplasm showing resistance to *Meloidogyne* spp. in the USA, also showed partial resistance (low galls/gram); their mean root dry weights were about twice those of the other plants but the number of galls was also much higher. Their larger root weight was also reflected in their higher herbage growth score (Tables 1 and 2).

The number of galls on cuttings of the two selected resistant and two susceptible genotypes reflected the genotypes' status in the screening. The trend in egg mass numbers was as expected from the gall data though it was not significant at 5%, but there were significantly more eggs per plant on susceptible germplasm (Table 3). The two resistant genotypes had a mean of 3,460 eggs/plant, compared to 25,030 for the two susceptible genotypes.

Table 1: Mean number of *Meloidogyne* sp. galls, root dry weight, growth score, and galls/gram on four groups of *T. repens* seedlines in a screening for resistance (10 genotypes/seedline).

Selection	Lines (no.)	Galls (no.)	Root dry weight (g)	Growth score (1–5)	Galls/g root DM Mean	Galls/g root DM Range
Resistant	63	13 a[1]	0.049 a	2.9 ab	378 a	62–965
Susceptible	23	25 b	0.042 a	3.1 b	883 b	289–1,622
New	8	21 b	0.085 b	3.7 c	354 a	219–584
Benchmark	8	24 b	0.033 a	2.6 a	927 b	619–1,132
$LSD_{0.05}$		*	*	*	*	520

[1] means in a column with the same letter are not significantly different at P<0.05 level.

Table 2: Mean number of *Meloidogyne* sp. galls, root dry weight, growth score, and galls/gram on the eight new *T. repens* seedlines (10 genotypes/seedline).

Germplasm	Lines (no.)	Galls (no.)	Root dry weight (g)	Growth score (1–5)	Galls/g root DM
PI 350706	2	23	0.104	3.7	328
PI 291847	2	18	0.060	3.7	450
SRVR	1	20	0.067	3.7	481
Brown Loam	1	23	0.083	3.7	347
Will	1	22	0.113	3.7	219
N.C.5	1	19	0.086	3.6	242

Table 3: Comparison of *Meloidogyne* sp. reproduction on four genotypes of *T. repens.*

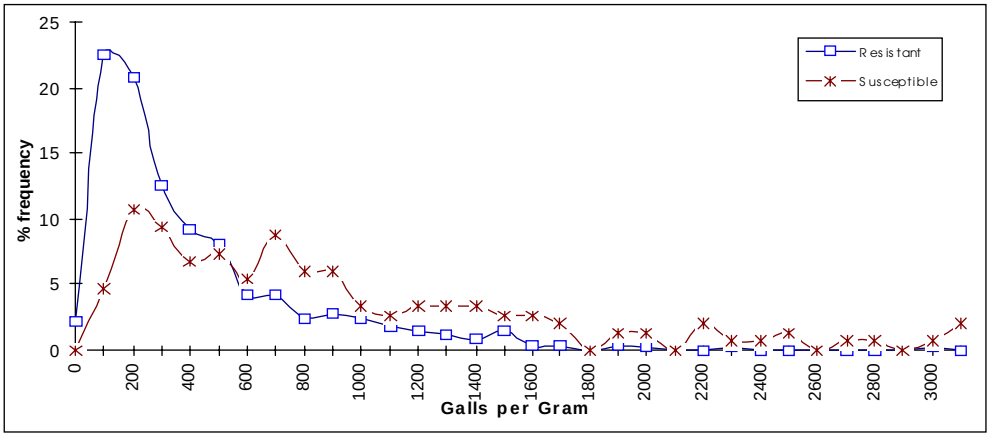
Genotype	Root fresh weight (g)	Galls (no.)	Egg masses (no.)	Eggs (no.)
412/7 (resistant)	4.1	79 a[1]	8	1,960 A[1]
426/9 (resistant)	3.9	79 a	27	4,960 A
444/5 (susceptible)	4.6	185 b	40	23,230 B
446/8 (susceptible)	4.2	115 b	41	26,830 B
P	NS	*	NS	**

[1] means in a column with the same letter are not significantly different at P<0.05(*) and P<0.01(**) levels.

Discussion

This experiment confirms earlier results (van den Bosch & Mercer 1989) where the variability found, and broad sense heritabilities calculated, on the parent material indicated that progress in breeding for resistance was possible. The divergence, in terms of galls per gram, of the resistant lines from the susceptible and benchmark lines is encouraging progress for the breeding programme. Also evident is the progress in galls/gram made over each generation when comparing resistant and susceptible lines: an average of 76% (mean of resistant/mean of susceptible lines) and 69% for the first two generations (van den Bosch et al. 1993) and 43% for the third generation reported here. Bain (1959) also made progress in breeding over two cycles, from genotypes showing some resistance to *M. incognita*. Estevez (1992) found significant variation in reaction of white clover parent plants and their progeny to a population of *M. hapla;* some progress in increasing resistance to *M. hapla* was made, and genotypic recurrent

Figure 1: Frequency distribution of galls/gram root DM for resistant and susceptible selections after three generations of selection in white clover for resistance to *Meloidogyne* sp.



selection was found to be more efficient than phenotypic recurrent selection in increasing host resistance. The autotetraploid nature of white clover was given as a reason for the low genetic progress (Estevez 1992).

Among the seedlines not tested previously, some SRVR genotypes had shown partial resistance to *M. incognita* (Windham & Pederson 1991) which was inherited into half-sib lines developed subsequently (Pederson & Windham 1992). Resistant plants selected by Windham and Pederson (1991) were used to initiate a recurrent selection programme for *M. incognita* resistance in white clover, similar to that described in this paper. The white clover seedline Brown Loam Synthetic No. 2 had moderate tolerance to *M. incognita* (Knight et al. 1988), though two other Brown Loam populations showed no resistance (Windham & Pederson 1991). The Brown Loam populations were selected for drought tolerance, suggesting their ability to withstand drought conditions is not related to *M. incognita* nematode resistance (Windham & Pederson 1991). The ecotypes P.I. 350706 (Algeria) and P.I. 291847 (probably Australia) had moderate gall scores when tested with *Meloidogyne* spp. (Quesenberry et al. 1990; Quesenberry pers. comm.). Resistance to one nematode conferring some resistance to a related species has been reported previously (Rebois et al. 1970). Will (formerly known as N.C.2) and N.C.5 are from North Carolina.

The differences between genotypes in ability to host root-knot nematode are much greater when the parasite population is measured as eggs rather than the indirect, but more easily assessed, number of galls. The difference in number of eggs may have been less in samples taken at a later time if the resistance works only to delay development rather than to arrest it; an experiment with regular samples from a population of inoculated genotypes would distinguish between these possibilities as reported for *Trifolium semipilosum* (Mercer & Grant 1994). Significant differences were not achieved in numbers of egg masses in the current work, possibly because they are difficult to stain and count. Unlike other *Meloidogyne* spp. in which the egg mass is prominent and easily stained early in its development, the egg mass of the local *Meloidogyne* sp. does not protrude from the epidermis of the root gall until the root degrades. In selections for resistance to other *Meloidogyne* spp., correlations in reductions of gall and egg mass numbers have been reported in red and white clover (Quesenberry et al. 1989; Pederson & Windham 1992). Mercer and Grant (1993) reported more egg masses per gall on resistant than susceptible white clover genotypes but that trend was not evident in these data.

Acknowledgements

Thanks to John Grant for technical assistance.

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