

## FOURTH AND FIFTH GENERATION PROGRESS IN BREEDING WHITE CLOVER FOR RESISTANCE TO ROOT-KNOT NEMATODE

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### ABSTRACT

Root-knot nematode (*Meloidogyne trifoliophila* previously referred to as *M. hapla*) is widespread in New Zealand pasture and parasitises white clover. A current breeding programme aims at achieving economic control through the use of white clover cultivars resistant to the nematode. In the fourth and fifth generations of the programme, there were significant differences between resistant and susceptible lines in terms of number of galls and galls/g of root dry weight (the measure of resistance). The fifth generation had an improved level of resistance over earlier generations. The influence of plant size on the expression of resistance is discussed. Results showed that continued selection and crossing is likely to further enhance resistance.

**Keywords:** breeding, resistance, root-knot nematodes, *Trifolium repens*, white clover

### INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) have been studied in New Zealand pasture since the 1960s (Yeates 1977) and data on their distribution (Mercer and Woodfield 1986) and their injury to white clover (*Trifolium repens* L.) (Watson *et al.* 1994) have been published. The dominant root-knot nematode infecting clovers in New Zealand was incorrectly identified as *M. hapla* Chitwood but is now recognised as *M. trifoliophila* Bernard and Eisenback (Mercer *et al.* 1997).

In a breeding programme screening white clover for resistance to *M. trifoliophila*, early results on variation in the numbers of root galls caused by *M. trifoliophila*, and the calculations of heritability, indicated that breeding for resistance was possible (van den Bosch and Mercer 1989; 1996a). Later, this was confirmed in the early generations of the selection programme (van den Bosch and Mercer 1989; 1996b). This paper reports results of two further generations of selection for resistance.

### MATERIALS AND METHODS

The 132 lines screened in the fourth generation were 104 resistant lines, 20 susceptible lines, and eight 'benchmark lines' used as controls in this and previous screenings. In the fifth generation, of the 128 lines, 101 were resistant, 19 susceptible and again, eight were benchmark lines. Fourth generation (fifth generation) seed was sown on 23 March 1992 (14 April 1993) and inoculated with a suspension of *M. trifoliophila* eggs on 30 April 1992 (11 May 1993). The sowing procedure, soil mix, inoculum source and rate, and glasshouse conditions for the pots have been described previously (van den Bosch and Mercer 1996b). Three to four weeks after inoculation, roots were washed, galls counted, and roots dried and weighed. "Galls/g" of root was calculated by dividing the number of galls by the root dry weight for each genotype.

### RESULTS

There were significant differences between line groups for galls, root dry weight, and galls/g in the fourth generation and for galls and galls/g in the fifth generation (Table 1). Galls/g values for the resistant selections of the fourth generation averaged 69% of the

values for the susceptible selections; fifth generation resistant selections averaged 34%.

**TABLE 1: Mean number of *Meloidogyne trifoliophila* galls, root dry weight, and galls/g on *Trifolium repens* lines in two screenings for resistance.**

Selection group	No. of genotypes	No. of galls	Root dry weight (g)	Galls/g of root dry weight <sup>2</sup>	Mean - Range
Fourth generation					
Resistant	1017	16.9 a <sup>1</sup>	0.0205 a	920 a	233 - 2226
Susceptible	182	23.0 b	0.0201 a	1350 b	747 - 3116
Benchmark	75	17.0 a	0.0142 b	1280 b	541 - 1800
Significance	-	P<0.05	P<0.001	P<0.05	-
Fifth generation					
Resistant	968	27.6 a	0.0427	760 a	190 - 1880
Susceptible	184	80.7 c	0.0432	2230 c	1370 - 4520
Benchmark	76	68.3 b	0.0465	1700 b	1410 - 2180
Significance	-	P<0.05	NS	P<0.01	-

<sup>1</sup> Within a column, means followed by the same letter do not differ at the probability level stated.

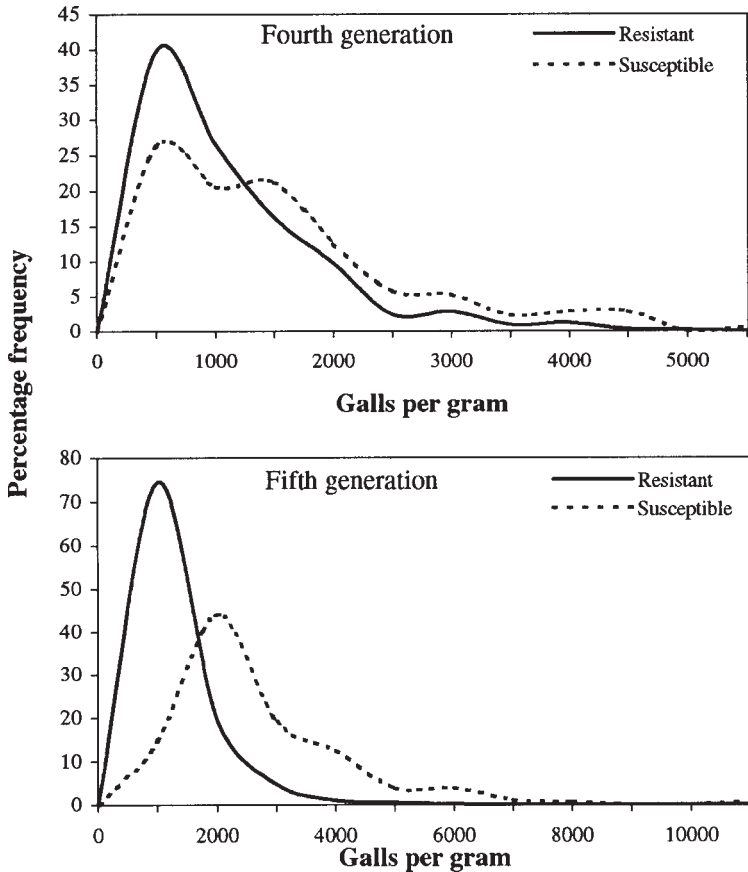
<sup>2</sup> "Mean" applies to the means of all the genotypes in each grouping and "range" applies to the means of lines.

Plots of frequency of galls/g, adjusted so the areas under the resistant and susceptible curves were equal, showed separate peaks for these two line groupings in each screening (Fig. 1).

## DISCUSSION

The recurrent selection programme is continuing to improve the level of resistance. The resistant selections in the third generation averaged 43% of the susceptible selections' galls/g (van den Bosch and Mercer 1996a) and the ratios were 69% and 34% for the fourth and fifth generations reported here. A possible explanation for the fourth generation's deviation from a steady downward trend is the small size of the plants in the fourth generation (benchmarks were a mean of 0.0142 g root dry weight) compared to the generation before (benchmarks' mean root dry weight of 0.033 g) and to the generation after (benchmarks' mean root dry weight of 0.0465 g). The poorer growth was possibly due to a toxin released from the soil during the pasteurisation. Eno and Popenoe (1964) measured large increases in N, NH<sub>4</sub><sup>+</sup>, P and S following steam treatment and it is possible that an accidental increase in temperature during the heating process resulted in an imbalance of nutrients. Heat treatment made soil toxic to white clover seedlings in other experiments in our laboratory and is no longer used to treat soil.

Improved resistance against root-knot nematodes has been reported for other selection programmes. Pederson and Windham (1995) reported development of a high level of resistance to *M. incognita* in white clover over four generations but direct comparisons with the present breeding programme are not possible because of the different methods of assessment of the level of parasitism. However, it seems that the level of resistance of Pederson and Windham's (1995) white clover line 'MSNR4' is greater than that of the fifth generation resistant group in the current programme as it is described as having 97% of the plants with no galling or just 1-10% of the root system galled. In the work reported here, 18% of the total number of genotypes in the resistant grouping were plants with ≤10% of the maximum galls/g value (1880 galls/g, resistant grouping line means). The reason for the more rapid progress made by MSNR4 is not known but perhaps white clover's resistance to *M. incognita* is under more simple genetic control.



**FIGURE 1: Frequency distribution of galls/g of root dry weight for resistant and susceptible selections after four and five generations of selection in white clover for resistance to the root-knot nematode *Meloidogyne trifoliophila*. Data have been adjusted to give equal areas under the two curves in each figure.**

In New Zealand, the misidentification of *M. trifoliophila* has led to some confusion in the literature on New Zealand nematodes but the root-knot nematode in the early screenings of this programme (van den Bosch *et al.* 1993) and in the first pathotype test (Mercer and Grant 1993) was certainly *M. trifoliophila*. Methods for distinguishing *M. trifoliophila* from *M. hapla* have been described by Mercer *et al.* (1997).

Bernard and Jennings (1997) discussed the possibility of misidentification of a Mississippi isolate of *M. graminicola* against which resistance has been reported in white clover by Pederson and Windham (1995). Bernard and Jennings (1997) reported that the putative *M. graminicola* used in the Pederson and Windham (1995) resistance screenings more closely resembled *M. trifoliophila* than *M. graminicola*. If the misidentification is confirmed, then MSNR4 will be included in the breeding programme and compared with New Zealand white clover resistant to *M. trifoliophila*.

Further improvement in resistance should be possible with additional recurrent selection cycles; crossing with superior agronomic material is desirable for the development of resistant cultivars.

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