SEEDLING GROWTH OF NINE WHITE CLOVER CULTIVARS AFTER INVASION BY CLOVER NEMATODES

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ABSTRACT

Seedlings of nine white clover cultivars were grown in seed trays containing a field soil to compare growth effects in the presence and absence of clover cyst and root-knot nematodes. Freezing of soil prior to planting was used to eliminate plant-parasitic nematodes. The cultivar Prop contained about half the number of root-knot nematodes as two other small-leafed cultivars after 2 and 4 weeks growth but numbers of cyst nematodes were similar in the three cultivars. Seedling growth of Prop was least affected among nine cultivars by the presence of nematodes 9 weeks after planting. Partial resistance to one nematode species may therefore confer establishment advantages to clover where infestations include other clover feeding nematode species.

Keywords: *Trifolium repens* cultivars, seedling invasion, nematodes, *Heterodera trifolii*, *Meloidogyne* sp.

INTRODUCTION

Plant-parasitic nematodes cause a large reduction in the productivity and persistence of white clover (*Trifolium repens* L.) in New Zealand pasture (Watson *et al.* 1994). The main damaging species are the clover cyst (*Heterodera trifolii*) and root-knot (*Meloidogyne* spp.) nematodes. Equivalent grass-feeding species are generally not present in pasture so that a competitive imbalance between grass and clover arises. This reduces the ability of clover to adequately exploit periods of pasture N deficiency, and ultimately impairs the productive potential of pastures, which depend on symbiotic N-fixation from white clover as a nitrogen source. Seedling vigour is also greatly affected by nematode invasion which can affect the establishment success of sown or naturally regenerating plants (Watson 1990; Sarathchandra *et al.* 1995). Seedlines of nine white clover cultivars, most commercially available or developed within New Zealand, were screened for their seedling performance in a field soil which contained both clover cyst and root knot nematodes. The cultivars were compared on the basis of leaf size *viz.* large leaf: Aran, Grasslands G49, Grasslands Kopu; medium leaf: Grasslands Huia, Grasslands Demand, Grasslands Pitau; small leaf: Prop, Grasslands Tahora, Grasslands Prestige. This paper largely reports results from testing the small-leaf cultivars, with a more general comparison of seedling growth for all the seedlines.

METHOD

Seed trays (30 x 50 x 15 cm) were filled with inverted pasture turf of Bruntwood silt loam soil in late spring 1993. Two rows of each of the three clover cultivars in each leaf class were sown into trays at 2 cm spacing of seeds within rows. Half of the seed trays were frozen before planting (-20^{0}C) in order to reduce numbers of plant-parasitic nematodes (Watson 1990). There were two replicate trays of the frozen and non-frozen soils and individual seed rows were also treated as replicates. Trays were maintained in a nursery with automatic daily overhead watering. After two weeks, 10 whole seedlings per cultivar were eased from the ground and were washed, stained in aniline blue and stored in acid glycerol before nematodes in roots were counted. Plant measurements included root length, numbers of branch-roots, leaves, stolons and rhizobial nodules, and number of galls from root knot nematode. Shoots and roots bulked from each replicate were blotted and weighed to obtain a "stained weight". The

process was repeated after four weeks. Plants remaining at 9 weeks were cut at ground level and the numbers of stolons, stolon roots and leaves, and flowering status, were assessed before dry matter determination.

Plant performance was compared from combined and separate analyses of the data from seedlines within each leaf class x soil treatment to look for significant effects in the presence and absence of nematodes.

RESULTS

Soil freezing prior to planting eliminated clover cyst and root-knot nematodes in the soil and hence their invasion of clover roots. Among small-leaf clovers in non-frozen soil, the numbers of root knot nematode in Prop were approximately half those in the other two cultivars ($P<0.05$, Table 1). There was no effect of clover cultivar on clover cyst nematode invasion (average 36 and 44 nematodes/ seedling 2 and 4 weeks after sowing respectively). When adult cyst nematodes develop they become exposed on the root surface and some may have been dislodged before counting at week 4. The severity of the nematode infestation in seedlings can be better appreciated when expressed on the basis of numbers/g root, giving combined infestations of 28, 31 and 35 thousand/g in two week old roots of Prop, Tahora and Prestige respectively. Although overall the plants appeared to be growing out of the nematode infection 4 weeks after sowing, evidenced by the reduced nematode numbers/g root, some seedlings failed to recover as a result of root debilitation, nodulation failure or establishment of secondary pathogens. Low numbers of *Pratylenchus* sp. (^{3} 0.15/plant after 4 weeks) were also present in clover roots. Numbers of *Pratylenchus* were reduced 87% by soil freezing ($P<0.05$) and were not affected by clover cultivar.

TABLE 1: Number of vermiform and total nematodes of *Meloidogyne* and *Heterodera* in seedling roots of three small-leafed white clover cultivars and total nematodes/g root.

	Meloidogyne			*Heterodera*		
	Vermiform[1]	Total[2]	Total no./g	Vermiform	Total	Total no./g
Week 2						
Prop	6.0	7.2	4168	33.1	37.3	23618
Tahora	14.9	18.5	9763	34.4	38.6	21554
Prestige	18.9	24.8	14039	29.8	33.2	21075
SED	4.3	5.4		2.3	2.2	
Week 4						
Prop	9.2	14.3	1544	42.2	47.9	5717
Tahora	16.1	28.6	4622	35.5	41.1	7060
Prestige	15.7	24.5	4213	37.1	44.1	8906
SED	4.3	6.3		11.1	12.7	

[1]Vermiform nematodes: post-invasive juveniles before permanent feeding site establised.
[2]Total nematodes: includes juveniles and adults which have established feeding sites in roots.

Plant growth was reduced in the presence of nematodes (Table 2). Root weight was initially increased in seedling roots because of the effect of galling by root knot nematode adding to root mass. Root:shoot ratio was also increased by nematode parasitism, signifying a less efficient root system. Plant growth was similar for the three cultivars when grown in frozen soil (except that root weight was slightly reduced in Prop after 2 weeks growth; $P<0.05$). In non-frozen soil, Prop had consistent growth advantages over Tahora and Prestige (Table 2). At week 2 differences were significant for branch root number, with a significant cultivar x soil interaction occurring for

nodule numbers (P<0.05). At week 4 there were significant differences for leaf (P<0.01), stolon and nodule (P<0.05) numbers.

TABLE 2: Seedling performance of three small-leaf white clover cultivars in a Bruntwood silt loam soil 2 and 4 weeks after sowing in the presence (-F) and absence (+F) of plant-parasitic nematodes obtained by freezing soil.

Cultivar	No. trifoliate leaves		No. branch roots/ stolons[1]		Root length (mm)		No. nodules		Shoot weight (mgm)		Root weight (mgm)		Root: shoot ratio	
2 weeks	+F	-F	+F	-F	+F	-F	+F	-F	+F	-F	+F	-F	+F	-F
Prop	0.60	0.23	6.5	4.2	39.0	23.0	6.2	2.9	8.2	5.9	1.55	1.65	0.19	0.28
Tahora	0.63	0.15	6.4	3.1	39.6	21.3	7.5	2.5	8.4	4.8	1.70	1.85	0.21	0.43
Prestige	0.55	0.10	6.9	3.0	39.4	21.1	7.0	2.5	9.0	4.9	1.73	1.68	0.20	0.34
SED	0.10	0.08	0.4	0.4	1.8	1.8	0.4	0.4	1.2	1.2	0.03	0.09	0.05	0.05
4 weeks														
Prop	5.9	2.87	0.50	0.43	53.0	36.7	8.9	6.7	111	46	19.2	9.0	0.17	0.20
Tahora	5.1	2.28	0.28	0.10	54.2	36.0	8.5	4.3	105	21	16.0	6.9	0.15	0.33
Prestige	4.7	1.87	0.45	0.13	54.2	34.5	7.8	3.4	109	20	17.0	6.2	0.17	0.32
SED	0.8	0.14	0.22	0.08	5.2	2.5	1.9	1.1	23	11	3.4	1.6	0.04	0.04

[1] 2 weeks= no. branch roots; 4 weeks= no. stolons

Among all nine cultivars Prop showed unique levels of seedling growth advantage in the presence of nematodes, as illustrated by comparing the growth of 9 week old seedlings in frozen compared with non-frozen soil (Table 3). The mean seedling performance in non-frozen soil, averaged over the four growth characteristics used, was 80% of the growth in frozen soil for Prop, compared with Tahora (58%), G49 (57%), Kopu (52%), Pitau (47%), Prestige (34%), Huia (33%), Demand (32%) and Aran (27%). In frozen soil, Kopu had the best overall seedling growth performance ranking at 9 weeks, with Prop ranking fifth (data not shown). In the non-frozen soil Prop had the highest seedling performance ranking. Among the large leaf clovers, Aran seedlings were most affected by nematodes, with two of the replicates in non-frozen soil containing no plants by the 9 week harvest. Huia had the poorest growth ranking of seedling performance in both soil treatments. Flower development was also suppressed by nematodes, with a small proportion of plants in only two cultivars, Prop and Kopu, able to initiate flowers after 9 weeks (Table 4).

TABLE 3: Seedling growth after 9 weeks for nine white clover cultivars in the presence of clover nematodes (as % of growth in nematode free plants after soil freezing).

Leaf size	Cultivar	Stolon no.	Stolon root no.	Trifol. leaf no.	Dry matter (g)
Small	Prop	88	90	80	61
	Tahora	55	62	71	44
	Prestige	38	34	43	23
Medium	Huia	32	20	53	27
	Pitau	48	38	65	36
	Demand	30	32	38	28
Large	Kopu	65	40	61	44
	G49	53	58	72	44
	Aran	23	32	31	22

TABLE 4: Percent of plants with flower initiation in white clover cultivars after nine weeks growth in soil with (+F) and without freezing (-F) for nematode removal.

Soil	Prop	Tahora	Prestige	Demand	Huia	Pitau	Aran	G49	Kopu
+F	32.5	45.0	18.1	24.7	7.5	25.0	26.7	15.3	61.4
-F	5.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.1

DISCUSSION

Not all vermiform nematodes entering the plant go on to successfully establish a permanent feeding site in the root for continued development. The root invasion process can itself, however, cause considerable trauma to seedling roots and is associated with enhanced entry of root fungi and suppression of nodulation (Sarathchandra *et al.* 1995). Early seedling root invasion can therefore greatly influence the future growth course of plants. Prop clover seedlings showed partial resistance to root-knot nematode which provided some advantage to seedling growth even in the presence of a community of nematodes dominated by the clover cyst nematode. Resistance to root-knot nematode in Prop seems to have occurred at the point of entry into roots since a similar proportion of post-vermiform nematodes occurred in the three small-leafed clovers (Table 1). A recurrent selection programme for nematode resistance in white clover at AgResearch, Palmerston North has produced genotypes with levels of resistance to both root knot and clover cyst nematodes (van den Bosch *et al.* 1993a,b). Resistance in the clover cyst nematode is conferred at the time of formation of the feeding site, after invasion of roots (Grant 1995). Thus Prop may be resisting this root-knot nematode at a different site to that reported for the same species in *Trifolium semipilosum* (Mercer and Grant 1994) and to that reported in white clover for clover cyst nematode (Grant 1995).

The root knot nematode species present in this experiment has been wrongly identified in the past as *M. hapla*, the common temperate species which also occurs in NZ pasture. The new species is currently being described (E.C. Bernard, USA, pers. comm.) and comparison of geographic range and host/ pathogen relationships are underway to clarify its position in NZ pasture (C.F. Mercer pers. comm.).

The general populations from which the parental plants of Prop were selected were located on steep inter-track areas on north to west facing slopes in the Raglan county area, Waikato (G.W. Sheath pers. comm.). The main criteria for selection were for a dense stoloniferous habit, high flowering prolificacy, and early flowering and seeding. The object was to gain persistence in hard hill country through prolific re-seeding ability. The environment from which Prop was developed may have had the nematode pressure, and plant generation turnover, to have facilitated an advantage to seedlings which have a level of resistance to this species of root-knot nematode. The partial resistance in Prop seedlings requires confirmation in mature clover to determine if reduced infection by root knot nematode is not ultimately compensated by an increase in the fecundity of surviving females (Mercer and Grant 1993), or by infection from other species such as the clover cyst nematode.

CONCLUSIONS

Partial resistance to a new species of clover root-knot nematode conferred advantages to seedling growth of Prop white clover in the presence of other plant-parasitic nematodes. There may be no advantage for seedling growth of Prop where this species of root-knot nematode does not occur, such as the southern half of the South Island, or after long term cropping with cereals which are not hosts for this species. White clover seedling performance, even in Prop, was markedly reduced by the presence of plant-parasitic nematodes.

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