Journal of Nematology 39(4):298–304. 2007.


# Effects of Nematicides and Plant Resistance on White Clover Performance and Seasonal Populations of Nematodes Parasitizing White Clover in Grazed Pasture

C. F. Mercer,[1] R. N. Watson[2]

*Abstract:* Root-infecting nematodes are a major cause of white clover, *Trifolium repens*, not reaching its potential in New Zealand pastures. Resistance and/or tolerance are the preferred control options. Greenhouse-based, recurrent selection programs have developed resistance to *Meloidogyne trifoliophila* and *Heterodera trifolii*, and a field-based program has developed tolerance. Lines from these programs were compared with commercial cultivars as controls in a series of field trials at four sites over 4 years. Resistant lines from the CCN program performed better than susceptible lines and as well as most cultivars, reflecting the high level of resistance developed in this greenhouse-based program. In stained root from Cambridge, numbers of CCN were lower in resistant lines than in cultivars; numbers in susceptible lines were intermediate. CCN resistance was also reflected to a lesser extent in the number of cysts counted in soil under resistant lines in Palmerston North. The root-knot nematode-resistant material performed better than the susceptible and as well as most cultivars. In one trial of CRKN-resistant lines, resistant and susceptible lines had similar numbers of CRKN which were both lower than the numbers in the cultivars; in the second trial, there were fewer CRKN in resistant than in susceptible lines or cultivars. The tolerant selections, developed under field conditions, performed as well as or better than the cultivars. The selections from the breeding programmes have exhibited strong agronomic potential across locations and years, and the best material has been crossed; progeny are being assessed in current field trials.
*Key words:* *Heterodera trifolii*, *Meloidogyne trifoliophila*, nematode, New Zealand, pasture, resistance, tolerance, *Trifolium repens*, white clover.

White clover is an important component of New Zealand pastures because of its contributions to N fixation, feed quality and complementary growth patterns (Caradus et al., 1995). White clover rarely reaches its potential yield because of the various abiotic (particularly drought) and biotic restraints. Chief among the nematode parasites are clover root-knot nematode (*Meloidogyne trifoliophila*—CRKN) and clover cyst nematode (*Heterodera trifolii*—CCN) (Watson and Mercer, 2000). Other constraints include viruses (Guy and Forster, 1996), several insect pests (Goldson et al., 2002; Jackson et al., 2002) and fungi (Skipp and Hampton, 1996). Reductions in any component of the total stress load on white clover will increase plant survival and maintain viable populations.

Nematode numbers in NZ pastures can be reduced by nematicides (but impractical and costly), biological control and crop rotation (Watson and Mercer, 2000), and by growing plants bred for either resistance or tolerance (Mercer and Watson, 1996). Resistance reduces the number of pest individuals through abiosis, whereas tolerance allows plants to endure the presence of the pests.

Recurrent selection has identified and improved resistance to CRKN and CCN. Seven generations of selection for resistance to the CRKN lowered galls/plant in resistant material to 19% of that in susceptible material (Mercer et al., 2000). However, this level of resistance was approximately halved when resistant lines were crossed with elite cultivars to improve their agronomic characteristics. The difficulty of delivering this CRKN resistance is its apparently recessive genetic control (Barrett et al., 2005).

In contrast to CRKN, four generations of breeding resistance to CCN reduced the mean number of cysts per plant on resistant material to 4% of that observed on susceptible material. Furthermore, CRKN resistance held up in the first cross with cultivars (to improve their agronomic characteristics) with only a modest increase in cyst numbers on resistant material to 20% of that on the crosses with susceptible lines (Mercer et al., 1999a).

Field assessment of breeders' lines under grazing is a major part of the development of forage-plant cultivars in New Zealand. White clover lines are planted as seedlings in 1-m rows 1 m apart and scored visually for vigor and other agronomic traits before grazing. Typically, these trials are repeated at two to four sites, and each line is replicated four or more times, randomly in blocks, at each site. We modified this procedure by applying nematicide to half the blocks of plots to release the clover lines from nematode parasitism and allow their potential to be expressed. The ratio of the nematicide-treated to untreated vigor scores should be one for a resistant line unaffected by nematode parasitism and greater than one for a susceptible line whose vigor is reduced by nematode parasitism. Suitable nematicides and application protocols had previously been established for Waikato pasture (Watson et al., 1985).

Nematode population sampling was optimized based on known life cycles of CCN, CRKN and *Pratylenchus* sp. from a number of NZ locations and soil types (Yeates,

Received for publication April 30, 2007
[1] AgResearch, Grasslands Research Centre, Private Bag 11008, Palmerston North, 4442, New Zealand.
[2] AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton, 3240, New Zealand.
J. van den Bosch was involved in the breeding of the resistant and susceptible lines and in setting up the field trials. The authors thank John Koolard for statistical analyses, Bruce Cooper, Keith Widdup, Chris Pennell, Kylie Miller, Kenyon Moore, the late Frank Neville and many others for technical support. The authors thank Dexcel Ltd for pasture yield data.
E-mail: chris.mercer@agresearch.co.nz
This paper was edited by Brent Sipes.

1973b; Yeates and Risk, 1976; Yeates et al., 1986), with samples collected in early September for CCN invasion and early December for CRKN invasion.

The objective of these trials was to determine the influence of nematicide application and host plant resistance on CCN, CRKN and *Pratylenchus* sp. populations parasitizing white clover in field trials established to assess white clover lines bred for resistance and tolerance.

## Material and Methods

*Plant material:* A recurrent selection program for resistance to CCN and CRKN in white clover was conducted in greenhouses at AgResearch, Grasslands Research Centre, Palmerston North. Plants supporting the fewest (resistant) and most nematodes (susceptible) were polycrossed, and the progeny subjected to further cycles of crossing and selection. The most resistant progeny were finally crossed with commercial cultivars to improve general adaptation to NZ farming systems (Mercer et al., 1999b, 2000). The CCN and CRKN lines evaluated were bulks of four to seven half-sib families from crosses with five elite cultivars (Sustain, Demand, Prestige, Kopu and Huia).

A concurrent selection program for tolerance to nematodes was conducted at AgResearch, Ruakura Research Centre, Hamilton. The best-performing lines from nematode-infested trays were grown in the field under plastic mulch. Plants were scored for vigor, and the best plants were polycrossed to provide seed for the next cycle of selection (Watson et al., 2007). The tolerant lines evaluated were the result of four cycles of selection.

Four commercial white clover cultivars (Kopu, Sustain, Demand and Prestige) were included in the trials as benchmarks for agronomic performance and level of nematode susceptibility.

*Field trials: CCN program:* Twenty resistant and four susceptible lines were evaluated at sites at Lincoln, Palmerston North, Kerikeri and Cambridge from 1997 to 2000. Plants were first grown in trays of potting mix and transplanted at 12-wk-old. At each site, plant material was inserted into plots, blocks of which were either untreated (U) or treated (T) with nematicide. There were four replicates. The nematicide oxamyl was applied as a foliar spray (3 kg a.i./ha) and followed a month later by fenamiphos (5 kg a.i./ha), both before rain or irrigation. The nematicide-treated areas were treated again each spring and autumn to prevent recovery of nematode populations. The plants destined for the untreated (U) plots were inoculated in trays at 5-wk-old with CCN eggs at 1,000 eggs/plant. Ten plants of each line were planted into 1-m rows, 1 m apart, into a grass sward previously cleared of clovers by herbicide applications (at 2 liters Dicamba/ha applied 4 mon before the trial started). The integrity of the plots was maintained with Dicamba applications in between plots to prevent plots intermingling. The plots were established once. Clover root weevil was controlled where necessary by regular spraying with the synthetic pyrethroid decamethrin.

*Field trials: CRKN program:* Sixteen resistant and four susceptible lines were evaluated at sites at Lincoln, Palmerston North and Cambridge from 1998 to 2000. Plant raising, site preparation, replication, nematicide treatments and plot size were as above, except that plants for the U plots were inoculated with CRKN eggs at 5-wk-old at 1,000 eggs/plant.

*Field trials: Tolerance program:* Seven tolerant lines, five CCN-resistant lines and four CRKN-resistant lines were evaluated at sites at Palmerston North, Cambridge and Kerikeri from 1998 to 2000. Plant raising, site preparation, nematicide treatments and plot size were as above, except that plants for the U plots were inoculated with CCN and CRKN at 5-wk-old at the rates above.

The materials from the breeding programs are referred to as "lines" and the commercial controls as "cultivars." When referring to the line/cultivar effects and the interaction between sites and lines/cultivars in results, the term "entries" is used to include lines and cultivars.

*Field trials' grazing and plant vigour assessments:* Plant vigor was scored visually on a 1–9 scale before each grazing. The response to nematicide treatment of the plots was calculated by dividing the mean vigor in the treated plots (T) by the values for the untreated plots (U) on each date. Lower values indicate less response to nematicide due to resistance, and higher values indicate the greater response of susceptible material. Plots were irrigated by hand as necessary to facilitate survival of the summer droughts.

*Site descriptions:* Cambridge: The CCN and CRKN-7-gen trials were on a Bruntwood silt loam, and the CRKN and Tolerance trials were on a Horotiu sandy loam. All four trials were grazed by dairy cattle. Kerikeri: The CCN trial was on an Okaihau gravely clay loam and was grazed by sheep. Palmerston North: The CCN trial was on a Manawatu silt loam and was grazed by sheep. Lincoln: The CCN trial was on a Templeton silt loam and was grazed by sheep.

*Nematode sampling:* Cambridge: In four trials, high-vigor lines were sampled on either three or four occasions. Nematode samples were taken from a second CRKN trial (CRKN-7-gen), but vigor data are not reported here. Two 50 × 50 mm cores per plot were collected adjacent to clover plants. Roots were washed out and stained, and numbers of nematodes per gram of stained root calculated. Such samples were taken at the beginning of the trials (1998) and at the end (2000). However, two of the trials (CRKN and Tolerance) were terminated early because of poor white clover survival after drought and insect predation. Stages of CCN counted in roots were virtually all vermiform and swollen second-stage juveniles (J2), as developing females are easily dislodged when roots are washed. *Pratylenchus* sp. stages were not discriminated. Palmerston North: Three matching pairs of lines, i.e., resistant and susceptible lines from the same generation of the breeding program, were chosen from the CCN program. The resistant lines were the best-performing ones in the spring of 1999 that also had a matching and poor-performing susceptible line. In early-January 2000, four 25 × 75 mm cores were taken from each plot and bulked. Cores were taken at random, but adjacent to clover plants and distributed along the plot. Total stolon length per sample was measured as a measure of standing crop. Cysts were extracted and counted in two categories, new (full of eggs) and old (empty).

Yield data from pasture on a Dexcel farm (near Hamilton, 10 km from our trials) of improved species under irrigation are given solely to allow nematode invasion of white clover roots to be related to the pattern of herbage growth in this region. Similarly, daily maximum air temperatures from Ruakura, Hamilton, are presented to allow comparison with other studies.

*Statistical analysis:* Analysis of variance was performed on the vigor scores and the Palmerston North nematode count data with log transformation where necessary. Pair-wise comparisons were made using LSD. For the vigor data from the trials of the tolerance program selections and for the Cambridge nematode count data, residual maximum likelihood was used because of uneven replication.

## Results

*Vigor scores: CCN program:* Site ($P < 0.001$) and entry ($P < 0.001$) effects were significant. The absence of a significant interaction between site and entry ($P = 0.12$) suggested the relative performance of the entries was similar across sites. The resistant lines had significantly ($P < 0.05$) higher mean vigor scores than their respective susceptible lines (Table 1). The best-performing resistant lines did not have significantly higher mean vigor than the top-performing cultivars (Table 1). The T/U values differed between sites ($P < 0.001$) and entries ($P < 0.05$), but there was no interaction between site and entry ($P = 0.6$). The line with the highest T/U ratio was susceptible $BC_2$, with a ratio of 1.77, significantly higher than the resistant lines (Table 1). However, resistant lines did not differ in T/U values from cultivars.

*Vigor scores: CRKN program:* Site ($P < 0.001$) and entry ($P < 0.001$) effects were significant, but there was no evidence of interaction between site and entry ($P = 0.18$). The resistant lines outperformed the susceptible lines (Table 2) except for the $BC_2F_2$ comparison. Sustain had the highest overall vigour, however, the resistant lines were as good as the other commercial cultivars. The T/U values differed between sites ($P < 0.01$) but not between entries ($P = 0.08$); there was no significant interaction between site and entry ($P = 0.23$). The T/U values for the resistant lines were significantly lower than for the cultivars Demand and Kopu, but there was not a trend when comparing T/U values between cultivars and lines (Table 2).

*Vigor scores: Tolerance program:* The site ($P < 0.001$) and entry ($P < 0.001$) effects were significant, as was the

TABLE 1. Mean vigor scores and responses to nematicide treatment (T/U) in a four-site field comparison of white clover lines from the CNN resistance breeding program. Data are overall means of 71 assessments from 1997 to 2000. Vigor data are from plots not treated with nematicide.

| Line | Number of lines | Vigor relative to demand = 100 | T/U (log values) |
|---|---|---|---|
| Resistant $BC_1$ | 5 | 90 | 1.24 (0.219) |
| Susceptible $BC_1$ | 1 | 75 | 1.32 (0.280) |
| Resistant $BC_2$ | 5 | 93 | 1.24 (0.214) |
| Susceptible $BC_2$ | 1 | 68 | 1.77 (0.573) |
| Resistant $BC_1F_2$ | 5 | 86 | 1.30 (0.263) |
| Susceptible $BC_1F_2$ | 1 | 70 | 1.26 (0.230) |
| Resistant $BC_2F_2$ | 5 | 90 | 1.25 (0.226) |
| Susceptible $BC_2F_2$ | 1 | 77 | 1.47 (0.388) |
| Sustain | 1 | 100 | 1.22 (0.197) |
| Prestige | 1 | 99 | 1.06 (0.060) |
| Huia | 1 | 83 | 1.42 (0.348) |
| Kopu | 1 | 80 | 1.47 (0.387) |
| Demand | 1 | 100 | 1.18 (0.169) |
| lsd 5% for comparisons of breeding lines | — | 5 | (0.117) |
| lsd 5% for comparisons of cultivars | — | 12 | (0.262) |
| lsd 5% for comparisons of breeding lines and cultivars | — | 9 | (0.203) |

TABLE 2. Mean vigor scores and responses to nematicide treatment (T/U) in a three-site field comparison of white clover lines from the CRKN resistance breeding program. Data are overall means of 33 assessments from 1998 to 2000. Vigor data are from plots not treated with nematicide.

| Line | Number of lines | Vigor relative to demand = 100 | T/U (log values) |
|---|---|---|---|
| Resistant $BC_1$ | 4 | 109 | 1.168 (0.156) |
| Susceptible $BC_1$ | 1 | 92 | 1.379 (0.321) |
| Resistant $BC_1F_1$ | 4 | 98 | 1.198 (0.180) |
| Susceptible $BC_1F_1$ | 1 | 84 | 1.231 (0.208) |
| Resistant $BC_1F_2$ | 4 | 98 | 1.178 (0.164) |
| Susceptible $BC_1F_2$ | 1 | 76 | 1.525 (0.422) |
| Resistant $BC_2F_2$ | 4 | 98 | 1.220 (0.199) |
| Susceptible $BC_2F_2$ | 1 | 94 | 1.269 (0.238) |
| Sustain | 1 | 117 | 1.171 (0.158) |
| Prestige | 1 | 103 | 1.085 (0.082) |
| Demand | 1 | 100 | 1.508 (0.411) |
| Kopu | 1 | 96 | 1.516 (0.416) |
| lsd 5% for comparisons of breeding lines | — | 8 | (0.143) |
| lsd 5% for comparisons of cultivars | — | 16 | (0.286) |
| lsd 5% for comparisons of breeding lines and cultivars | — | 12 | (0.226) |

interaction between site and entry ($P < 0.001$), indicating inconsistent performance across sites. The medium-leaf tolerant line had a significantly ($P < 0.05$) higher mean than all other lines and the three poorer-performing cultivars (Table 3). There were no significant differences between the cultivars, even though Prestige had 8–15% higher vigor than the others. The T/U values differed between sites ($P < 0.01$) but not entries ($P = 0.55$), and there was no interaction between site and entry ($P = 0.76$) (Table 3).

*Nematode counts: Cambridge:* Numbers of CCN were consistent between trials, falling dramatically between September and December 1998 samplings but varying less between the two samplings in 1999–2000 (Fig. 1a). Stages of CRKN counted in roots included J2, J3/4 and adults. Numbers varied between the four trials, but populations in all trials increased between the first and second samples in each season (Fig. 1b). Numbers of *Pratylenchus* sp. (stages were not discriminated) fell between September and December 1998 in three of four trials (Fig. 1c). In 1999–2000, the CCN trial showed an increase in *Pratylenchus* sp. during the season, but numbers in the CRKN-7-gen trial remained constant.

Over all trials, counts of CCN per gram varied with entry ($P < 0.05$) but not with nematicide treatment ($P = 0.7$); the interaction was not significant ($P = 0.8$). There was a significant effect of entry ($P < 0.05$) and nematicide treatment ($P < 0.001$) on numbers of CRKN per gram of stained root; the interaction between entry and nematicide treatment was also significant ($P < 0.05$). Counts of *Pratylenchus* sp. per gram varied with nematicide treatment ($P < 0.001$) but not with entry ($P = 0.11$); the interaction was not significant ($P = 0.7$).

In the trial of lines from the CCN resistance breeding program, there was an entry effect ($P < 0.001$) but not an effect for nematicide treatment ($P = 0.85$) or for the interaction ($P = 0.9$). Numbers of CCN were lower in resistant lines than in cultivars; numbers in susceptible lines were intermediate (Table 4).

In the first trial of lines from the CRKN resistance breeding program, there were entry ($P < 0.05$) and nematicide treatment ($P < 0.001$) effects; there was no interaction effect ($P = 0.11$). Nematicide treatment reduced CRKN numbers, but resistant and susceptible lines had similar numbers of CRKN and were both lower than the numbers in the cultivars (Table 5).

In the second trial of lines from the CRKN resistance breeding program (CRKN-7-gen), there were entry ($P <$ 0.01) and nematicide treatment ($P < 0.001$) effects on

TABLE 3. Mean vigor scores and responses to nematicide treatment (T/U) in a three-site field comparison of white clover lines from the tolerance breeding program. Data are overall means of 43 assessments from 1998 to 2000. Vigor data are from plots not treated with nematicide.

| Line | Number of lines | Vigor relative to domain = 100 | T/U (log values) |
|---|---|---|---|
| Tolerant small leaf | 2 | 112 | 1.20 (0.184) |
| Tolerant medium leaf | 2 | 123 | 1.18 (0.168) |
| Tolerant large leaf | 2 | 113 | 1.25 (0.225) |
| RKN resistant | 3 | 99 | 1.18 (0.162) |
| RKN resistant $BC_1F_2$ | 4 | 98 | 1.23 (0.206) |
| CCN resistant $BC_1$ | 5 | 111 | 1.29 (0.252) |
| Prestige | 1 | 115 | 1.24 (0.212) |
| Kopu | 1 | 107 | 1.20 (0.180) |
| Sustain | 1 | 106 | 1.27 (0.236) |
| Demand | 1 | 100 | 1.45 (0.375) |
| lsd 5% for comparisons of breeding lines | — | 7 | (0.126) |
| lsd 5% for comparisons of cultivars | — | 15 | (0.264) |
| lsd 5% for comparisons of breeding lines and cultivars | — | 12 | (0.203) |

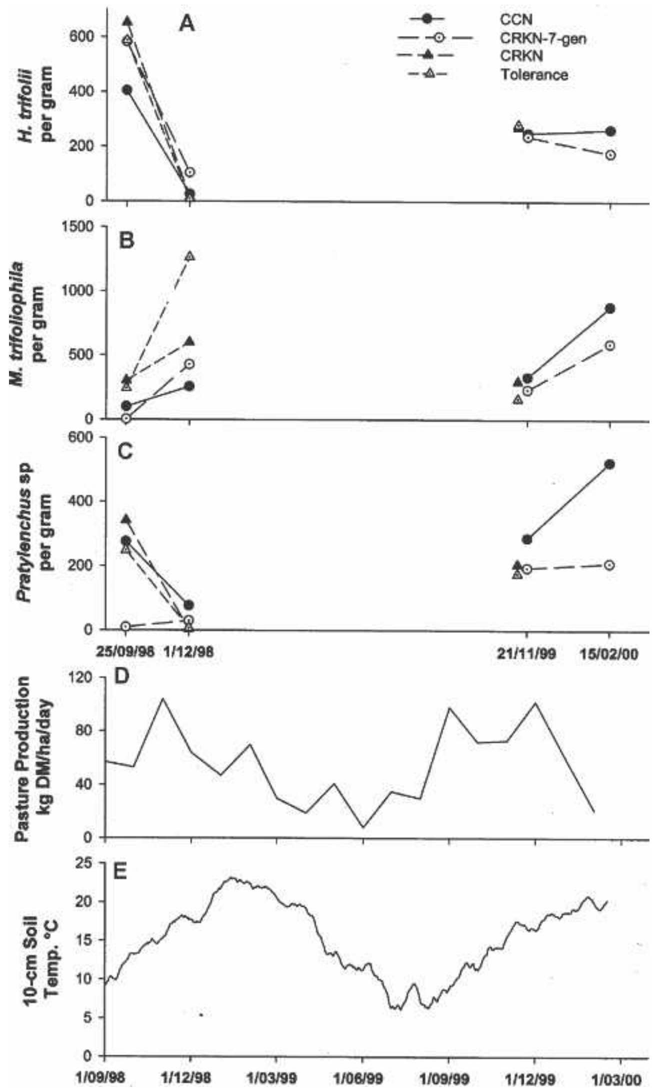


FIG. 1. A,B,C) Populations of three species of nematodes in roots of white clover (per gram stained root) from four trials in grazed pasture at Cambridge; plots not treated with nematicide. D) Pasture yield and E) 10-cm soil temperature from Ruakura 10 km from the trial sites. Means for populations on 21Nov 1999 are offset for clarity. Temperature data are calculated from a 2-weekly running mean of daily maxima.

TABLE 4. Effect of nematicide application and resistance on numbers of CCN in white clover roots from the CNN trial in grazed pasture in Cambridge. Means are numbers of all life stages per gram of stained root. Data are from four samplings and five lines per sampling.

| Treatment | Resistant[a] | Susceptible | Cultivar |
|---|---|---|---|
| T | 60a | 95ab | 193bc |
| U | 55a | 104ab | 162bc |

[a] Means with a letter in common do not differ ($P < 0.05$). REML conducted on LOG(x + 2) transformed data but back-transformed means are presented.
U = untreated controls. T = treated with nematicide.

counts of CRKN; there was no interaction effect ($P = 0.18$). In the untreated blocks, there were fewer CRKN in resistant than susceptible lines or cultivars, but nematicide treatment reduced CRKN numbers to similar levels among the lines (Table 5).

Alternate weed hosts for CCN and CRKN were rare at all sites, but grasses and forb weeds would have been alternate hosts for *Pratylenchus* sp.

*Palmerston North:* Nematicide treatment reduced numbers of new and old cysts per mm (Table 6). There was no effect for line or for the interaction between nematicide treatment and line. However, specific comparisons showed the resistant line $CCRKBC_1$ to have fewer old cysts per mm (back-transformed mean = 0.127, data from both T and U) than the susceptible line $CCSBC_1$ (back-transformed mean = 0.248) ($P < 0.05$); the comparison of new cysts per mm for the same pair of lines was not significant ($P = 0.098$).

## DISCUSSION

Our field evaluations showed that CCN- and CRKN-resistant selections from the greenhouse-based breeding program and tolerant selections from the field-based program exhibited strong agronomic potential across locations and years. Furthermore, selection for resistance under controlled environmental conditions in a greenhouse (Mercer et al., 2000) produced germplasm with better agronomic performance in the field than their susceptible counterparts.

TABLE 5. Effect of nematicide application and resistance on numbers of CRKN in white clover roots from the CRKN and CRKN-7-gen trials in grazed pasture in Cambridge. Means are numbers of all life stages per gram of stained root.

| Treatment | Resistant[a] | Susceptible | Cultivar |
|---|---|---|---|
| CRKN | | | |
| T | 9a | 14a | 14a |
| U | 28a | 203b | 110b |
| CRKN-7-gen | | | |
| T | 8ab | 4a | 57c |
| U | 24bc | 59c | 196d |

[a] Within a trial, means with a letter in common do not differ ($P < 0.05$). REML conducted on LOG(x + 2) transformed data but back-transformed means are presented.
U = untreated controls. T = treated with nematicide.

TABLE 6. Effect of nematicide application on numbers of cysts per mm of white clover stolon length at Palmerston North. Data are combined from samplings in late December 1999 and early January 2000. Means are for 48 bulks of four cores (three pairs of lines, two samplings, two categories of lines (resistant or susceptible) and four reps).

| Cyst category | Treatment T | Treatment U |
|---|---|---|
| New cysts per mm of stolon length[a] | 0.153a | 0.247b |
| Old cysts per mm of stolon length | 0.094a | 0.208b |

[a] Within a cyst category, means with a letter in common do not differ ($P < 0.01$). Analysis of variance conducted on LOG(x + 1) transformed data but back-transformed means are presented.
T = treated with nematicide. U = untreated controls.

The resistant and tolerant selections also performed favorably in comparison to the commercial cultivars. In particular, the CCN-resistant lines and the medium-leaved tolerant selection performed as well as the control(s) and warrant inclusion in commercial breeding programs. In comparison, the field performance of the CRKN-resistant selections was slightly poorer than the best cultivar. This poorer performance reflects a balance between (i) the positive effects from the resistance/cultivar's contribution, and (ii) the negative effects of some years of breeding solely in an artificial environment. The resistant and tolerant material with good agronomic performance in these trials has now been backcrossed with elite cultivars, and further testing for resistance and field performance is ongoing. We expect the nematode resistance to have a positive effect when incorporated in cultivars with better adaptation to NZ grazing systems.

There have been several previous reports of white clover with resistance to a number of root-knot nematode species under greenhouse conditions (Windham and Pederson, 1992; Pederson and Quesenberry, 1995; Kouame et al., 1998). However, there are no published reports of field evaluations of these resistant populations. The same is true for CCN, Cook and Mizen (1989) having reported resistance but with no field evaluation to date.

Nematode counts in roots broadly reflected the resistance in the white clover lines selected for resistance. Factors contributing to the high levels of variation included resistance to CCN and CRKN being under polygenic control (Barrett et al., 2005) and the material planted being untested progeny from polycrosses, so that each plot contained a range of resistance levels. Another factor is the aggregated distribution of nematodes in soil and roots, reflecting the reproductive success of the previous generation, the laying of eggs in batches (in CCN cysts and in CRKN egg masses) and the discrete distribution of clover roots in the soil.

We are not able to correlate the dynamics of nematode populations with variations in plant performance. Ideally, seasonal or monthly data on plant performance and nematode parasitism would have been collected on a site-by-site basis to facilitate a better understanding of the epidemiology of the pest/host interaction. Clearly, the best time to test resistance is during high pest population pressures, but these cannot be predicted in place and time. Our strategy of using four sites over 4 years incorporated some pest outbreaks, but ideally we would have identified these with regular sampling of roots. We expected a bigger site-by-line interaction reflecting the contrasting importance of nematodes at the sites, but the patchy nature of outbreaks was masked in the averaging of a large amount of data. The principal function of the field evaluations was to serve as a performance test, and the best material has been sequestered.

The variable nematicide effects were surprising as we followed the successful method of Watson et al. (1985). Nematicide application was technically challenging and required rain or irrigation, and sometimes there were conflicts of interest with the farm managers over application dates because of the requirements for the stock withholding periods. Accelerated degradation of the nematicides through adaptation of the soil microflora following repeated application probably did not contribute to the variation, as the treatment success rate was low over the first three sampling dates. The poorer control of CCN compared to the other two nematodes species is curious: control of these three species was achieved more or less similarly in the same district in an earlier series of trials (Watson et al., 1985). Field trials using oxamyl applications to white clover/ryegrass swards (Yeates and Barker, 1986) did not significantly reduce numbers of the three species counted here, but again this applied to all three species. The variable nematicide effects reduced the reliability of the ratios of the nematicide-treated to untreated vigor data; nematicide treatment, as used here, is not used now in field assessments of white clover.

The fall in numbers of CCN (J2 and a few females) in white clover roots at Cambridge, Waikato, from the late-September to early December-1998 samplings reflects that reported in a Wairarapa yellow-grey earth (Yeates, 1973a), a Taranaki yellow-brown loam (Yeates, 1973b) and in a Rukuhia, Waikato, soil (G.W. Yeates, personal communication) about 10 km from our sites. Our data confirm the published pattern of a peak of J2 and developing female numbers in clover roots in September, presumably resulting from a hatch of eggs and invasion of J2 as soil warms and root growth accelerates. Our September 1998 numbers of CCN per gram of stained root were six to ten times higher than those in the reports above. This may reflect the difference between our grass/white clover swards and pasture established for a longer time.

The populations of all stages of CRKN increased about four-fold in roots at Cambridge between the two spring-1998 samples, reflecting the spring hatch of eggs and invasion of J2. The increases are similar to those previously reported for two other Waikato soil types (Yeates et al., 1985). The three-fold increase from November 1999 to March 2000 is puzzling, as pasture production declined rapidly in the 2000 summer drought (Fig. 1d); in spite of the heat, the limited irrigation applied to the plots must have been sufficient to allow CRKN invasion and development. The population increase can also be explained in part by the reduction in root dry weight (data not shown) and the use of numbers per gram to express population density. The CRKN population densities varied similarly in the roots of the CCN and CRKN-7-gen trials in spite of the lines' contrasting histories. This may be explained by the low level of resistance in the CRKN-7-gen lines (Mercer et al., 2000) and by the breakdown of CRKN resistance at higher temperatures (Mercer, 2005).

The September 1998 *Pratylenchus* sp. mean of 218 individuals is close to that for a September 1983 sample from white clover in an Otorohanga silt loam site (Yeates, 1988). However, numbers of this nematode vary widely, as seen in the 10-fold drop in numbers in 1998 in three of our four trials and in the 3-weekly samples of Yeates (1988), where densities in roots ranged from 0 to 1,500 nematodes/g of stained root. The variation in our 1998 *Pratylenchus* sp. data is the more remarkable, as the means are for all stages of a population—the drop does not reflect recruitment of one stage to the next as seen in the CCN decrease over the same period.

These field trials were an essential component of the recurrent program which set out to improve resistance and tolerance to the most important root-infecting nematodes of NZ pasture. Prolonged breeding in an artificial environment like a greenhouse can result in a population which yields less than the parent germplasm; but such a focused program is essential to understanding the genetics of and improvement of a trait like nematode resistance. The study reported here has demonstrated that our back-crossing program and field trials have resulted in improved germplasm capable of tolerating nematode parasitism and animal grazing.

## Literature Cited


Barrett, B., Mercer, C. F., and Woodfield, D. R. 2005. Genetic mapping of a root-knot nematode resistance locus in *Trifolium.* Euphytica 143:85–92.

Caradus, J. R., Woodfield, D. R., and Stewart, A. V. 1995. Overview and vision for white clover. Pp. 1–6. *in* D. Woodfield, ed. White clover: New Zealand's competitive edge. Joint symposium Agronomy Society of New Zealand Special Publication No. 11, Grassland Research and Practice Series No. 6, New Zealand: Lincoln University.

Cook, R., and Mizen, K. A. 1989. Resistance to plant parasitic nematodes in ryegrasses and white clover. 16th International Grassland Congress; Nice, France. Pp. 737–738.

Goldson, S. L., Barlow, N. D., Gerard, P. J., Jackson, T. A., Phillips, C. B., Watson, R., and Rahman, A. 2002. Biosecurity for New Zealand's forage production systems. Proceedings of the New Zealand Grassland Association 64:135–138.

Guy, P. L., and Forster, R. L. S. 1996. Viruses of New Zealand pasture grasses and legumes. Pp. 289–302. *in* S. Chakraborty, ed. Pasture and Forage Crop Pathology, Madison: ASA, CSSA, SSSA.

Jackson, T. A., Popay, A. J., and Goldson, S. L. 2002. Bioprotection—getting the most out of biological control in pasture pest management. Proceedings of the New Zealand Grassland Association 64: 139–142.

Kouame, C. N., Quesenberry, K. H., Wofford, D. S., and Dunn, R. A. 1998. Genetic diversity for root-knot nematode resistance in white clover and related species. Genetic Resources and Crop Evolution 45:1–8.

Mercer, C. F. 2005. Resistance in *Trifolium repens* and *T. semipilosum* to *Meloidogyne trifoliophila* (Nematoda: Tylenchida) is temperature sensitive. New Zealand Journal of Agricultural Research 48:339–343.

Mercer, C. F., Miller, K. J., and van den Bosch, J. 2000. Progress in recurrent selection and in crossing cultivars with white clover resistant to the clover root-knot nematode *Meloidogyne trifoliophila*. New Zealand Journal of Agricultural Research 43:41–48.

Mercer, C. F., van den Bosch, J., and Miller, K. J. 1999a. Effectiveness of recurrent selection of white clover (*Trifolium repens*) for resistance to New Zealand populations of clover cyst nematode (*Heterodera trifolii*). Nematology 1:449–455.

Mercer, C. F., and Watson, R. N. 1996. Nematode pathogens of New Zealand pastures. Pp. 241–256. *in* S. Chakraborty, ed. Pasture and Forage Crop Pathology, Madison: ASA, CSSA, SSSA.

Mercer, C. F., Watson, R. N., van den Bosch, J., Widdup, K. H., Miller, K. J., and Pennell, C. J. 1999b. Resistance in white clover to the nematodes *Heterodera trifolii* and *Meloidogyne trifoliophila*—progress in breeding and early results from field tests. Proceedings of the 7th Australasian Conference on Grassland Invertebrate Ecology. 272–278.

Pederson, G. A., and Quesenberry, K. H. 1995. Registration of MSNR4 root-knot nematode resistant white clover germplasm. Crop Science 35:1234–1235.

Skipp, R. A., and Hampton, J. G. 1996. Fungal and bacterial diseases of pasture plants in New Zealand pastures. Pp. 213–236. *in* S. Chakraborty, ed. Pasture and Forage Crop Pathology, Madison: ASA, CSSA, SSSA.

Watson, R. N., Bell, N. L., Neville, F. J., Aalders, L. T., and Rohan, T. C. 2007. Breeding white clover for tolerance to nematodes: overview of process and progress. Proceedings of the New Zealand Grassland Association 69:193–196.

Watson, R. N., and Mercer, C. F. 2000. Pasture nematodes: The major scourge of white clover. Proceedings of the New Zealand Grassland Association 62:195–199.

Watson, R. N., Yeates, G. W., Littler, R. A., and Steele, K. W. 1985. Responses in nitrogen fixation and herbage production following pesticide applications on temperate pastures. Proceedings of the 4th Australasian Conference on Grassland Invertebrate Ecology 103–113.

Windham, G. L., and Pederson, G. A. 1992. Comparison of reproduction by *Meloidogyne graminicola* and *M. incognita* on *Trifolium* species. Journal of Nematology 24:257–261.

Yeates, G. W. 1973a. Annual cycle of root nematodes on white clover in pasture. I. *Heterodera trifolii* in a yellow-grey earth. New Zealand Journal of Agricultural Research 16:569–574.

Yeates, G. W. 1973b. Annual cycle of root nematodes on white clover in pasture. II. *Meloidogyne hapla* and *Heterodera trifolii* in a yellow-brown loam. New Zealand Journal of Agricultural Research 16:575–578.

Yeates, G. W. 1988. Distribution and impact of root lesion nematode (*Pratylenchus*) in New Zealand pastures. Proceedings of the 5th Australasian Conference of Grassland Invertebrate Ecology. 332–340.

Yeates, G. W., and Barker, G. M. 1986. Influence of repeated oxamyl application on nematode populations beneath 4 ryegrass cultivars on a yellow-brown loam. New Zealand Journal of Agricultural Research 29:501–515.

Yeates, G. W., and Risk, W. H. 1976. Annual cycle of root nematodes on white clover in pasture. III *Heterodera trifolii* in a yellow-brown earth. New Zealand Journal of Agricultural Research 19:393–396.

Yeates, G. W., Watson, R., and Steele, K. 1986. Complementary distribution of *Meloidogyne, Heterodera,* and *Pratylenchus* (Nematoda: Tylenchida) in roots of white clover. Proceedings of the 4th Australasian Conference of Grassland Invertebrate Ecology 71–79.