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Resistance Reactions to *Meloidogyne trifoliophila* in *Trifolium repens* and *T. semipilosum*

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Abstract: The predominant root-knot nematode in New Zealand pastures is *Meloidogyne trifoliophila,* and a recurrent selection program in *Trifolium repens* has developed resistance to this species. No data are available, however, on the mechanisms of resistance in *T. repens* or resistant genotypes of *T. semipilosum.* The development of *M. trifoliophila* in roots of *T. repens* and *T. semipilosum* was examined weekly after a 2-day inoculation with eggs. More second-stage juveniles (J2) were found in two resistant genotypes of *T. repens* than in two susceptible ones 1 week after inoculation. J2 did not develop further in resistant genotypes, but in susceptible plants development proceeded to the adult stage, visible at 4 weeks after inoculation. The mode of action of resistance to *M. trifoliophila* in *T. repens* and in *T. semipilosum* was compared after a 24-hour inoculation with J2. Numbers of J2 per root tip ranged from 0 to 12 with a median of one for each species. At 24 hours after inoculation (HAI), similar numbers of J2 were seen in the cortex oriented toward the root tip in both resistant and susceptible genotypes of both plant species. At 48 HAI, accumulations of J2 were seen in the meristem in both resistant and susceptible genotypes of both plant species. At 72 HAI, differences in nematode responses were evident between resistant and susceptible genotypes of both plant species; in susceptible roots, J2 heads were embedded in the developing stele. At this time, a browning reaction in resistant genotypes of both plant species indicated a hypersensitive response, and differences in the reaction were recorded between *T. repens* and *T. semipilosum.* More study is needed to determine whether the resistance reaction in *T. semipilosum* is suitable for introgression or insertion into *T. repens.*

Key words: hypersensitive response, invasion, Kenya white clover, *Meloidogyne trifoliophila,* penetration, resistance, root-knot nematode, *Trifolium repens, Trifolium semipilosum,* white clover.

The clover root-knot nematode (*Meloidogyne trifoliophila* Bernard and Eisenback) (CRKN) debilitates white clover (*Trifolium repens*) in New Zealand pasture (Watson and Mercer, 2000). Prior to 1997, reports of *Meloidogyne* spp. from New Zealand pasture wrongly cited *M. hapla* as the dominant species, but the resistance program and development studies cited below report research on *M. trifoliophila* (Bernard and Eisenback, 1997; Mercer et al., 1997).

Resistant or tolerant cultivars are the preferred control option due to the cost and environmental issues associated with chemical intervention. A recurrent selection program developed resistant (R) germplasm (Mercer et al., 1999) that is currently being evaluated in field trials. Genetic analysis of the CRKN resistance in *T. repens* indicates that it is multigenic and that the R alleles are recessive to most wild-type (S) alleles. Only homozygous R genotypes exhibit the R phenotype, making it difficult to retain stable expression of resistance in this open-pollinated species. Preferred scenarios for genetic resistance to CRKN would include incorporation of single-gene dominant resistance and (or) the integration of multiple sources of resistance (von Mende, 1997).

Introgression of resistance through hybridization or direct insertion of resistance genes offers more robust genetic resistance when complementary response mechanisms are available. A small number of species in the genus *Trifolium* can be hybridized with *T. repens* (Williams and Baker, 1987), but none are highly resistant to CRKN (Mercer and Miller, 1997). Kenya white clover (*Trifolium semipilosum* Fresen) has not been hybridized with *T. repens* but is an attractive model species for study of the resistance reaction in the genus, because resistance to CRKN is conferred by the single dominant locus *TRKR* (Barrett et al., 2002).

The development of CRKN (but described as *M. hapla*) in susceptible *T. repens* was reported from a preliminary experiment (Mercer, 1990) using roots constantly exposed to egg inoculum. The effects of resistance on the *T. repens* galling reaction and on CRKN egg production also have been described (Mercer et al., 2000; van den Bosch and Mercer, 1996), but the histology of the resistance reaction has not been described. Similarly, in *T. semipilosum,* comparisons of CRKN (but described as *M. hapla*) invasion rates and development in susceptible and resistant genotypes (from using egg inoculum), but not histology, have been reported (Mercer and Grant, 1994).

The objectives of this study were to more precisely describe the timing of the development of CRKN life stages in *T. repens* following a brief exposure to inoculum and to compare expression of resistance in *T. repens* and *T. semipilosum.* Such observations should indicate whether these are complementary sources of resistance.

Materials and Methods

Development of M. trifoliophila in T. repens: Four *T. repens* genotypes (Table 1) were cloned: two had exhibited a resistant reaction to CRKN, and two were susceptible (Mercer et al., 2000). To generate a population of clones, stolon tips were rooted in pasteurized peat-based potting mix in 300 × 450-mm trays and kept in a greenhouse at 20 °C to 25 °C. After 4 weeks, plants were lifted out of the potting mix and the roots rinsed in

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TABLE 1. Mean fresh weight, number of root tips and number of galls in resistant and susceptible *Trifolium repens* genotypes after inoculation with *Meloidogyne trifoliophila.*

Genotype	1 week after inoculation	2 weeks after inoculation		
	Weight[a] (g)	Weight (g)	Number of root tips	Number of root galls
Resistant C17530/3	0.55a	1.35a	1,240a	0a
Resistant C17515/2	0.44a	1.35a	1,415a	3a
Susceptible C17505/8	0.56a	1.78a	1,215a	169c
Susceptible C17508/4	0.64a	0.89a	1,215a	81b

[a] Values are means of five replicates. Means in columns with letters in common do not differ at $P < 0.05$.

water. The roots were laid on the surface of the growing medium in a 3/4-full 60-mm-diam. pot and a suspension of 3,000 *M. trifoliophila* eggs was poured over the roots, which were then covered with potting mix. Nematode eggs had been extracted with 1.0% NaOCl from a culture on *T. repens* (Hussey and Barker, 1973). After 2 days, roots were washed free of potting mix and plants re-potted, with roots placed vertically into fresh, pasteurized potting mix in 60-mm-diam. pots. Plants were maintained in a greenhouse as described previously.

At 1, 2, 3, and 4 weeks after inoculation (WAI), five clones of each genotype were selected at random, washed free of potting mix, and roots separated from the tops and weighed. Roots of the five clones were combined, stained in acid fuschin (Byrd et al., 1983), and cleared and stored in acidified glycerol. The staining process softened roots and, at 2 WAI and later, prevented the separation of the roots from individual clones; after 1 WAI, the root mass mix from the five genotype copies was spread out in a 150-mm-diam. petri dish and divided into five approximately equal-sized portions. One portion was chosen at random, and the selected and remaining portions blotted and weighed. Root tips were then counted in the chosen portion. Stained root tips (unswollen) and galls were squashed between microscope slides and examined using a compound microscope. Nematodes were assigned to development stages. The separation between slides of nematodes from plant tissue allowed distinction of third- and fourth-stage juveniles at this level of magnification. At 1 WAI, all tips were examined for nematodes, but in subsequent samples a randomly chosen sub-sample of tips (N = 30 to 90 tips) was examined. At 1 and 2 WAI, all galls were examined, but in subsequent samples a sub-sample (N = 10 to 80 galls) was examined. At each sample date, galls were counted in the five combined root systems. Root segments were designated as galls if they were two times wider than the adjacent unswollen root. The counts of tips and developmental stages presented in Figure 1 and Table 1 are expressed as counts per five plants (clones).

Mean root weights and numbers of J2 at 1 WAI were compared by analysis of variance, and means were separated by LSD (Minitab Inc., State College, PA). Counts were compared using the Chi-square statistic.

Nematode migration in roots: Roots (with tops attached) of four clones of genotypes C17515-2 and C17508-2 of *T. repens* and TSM 19 and TSM 4 of *T. semipilosum* were spread in a 12-cm-diam. petri dish and partially covered with silver sand; 5,000 freshly hatched J2 (Vrain, 1977) in 5 ml water were poured over the roots and then

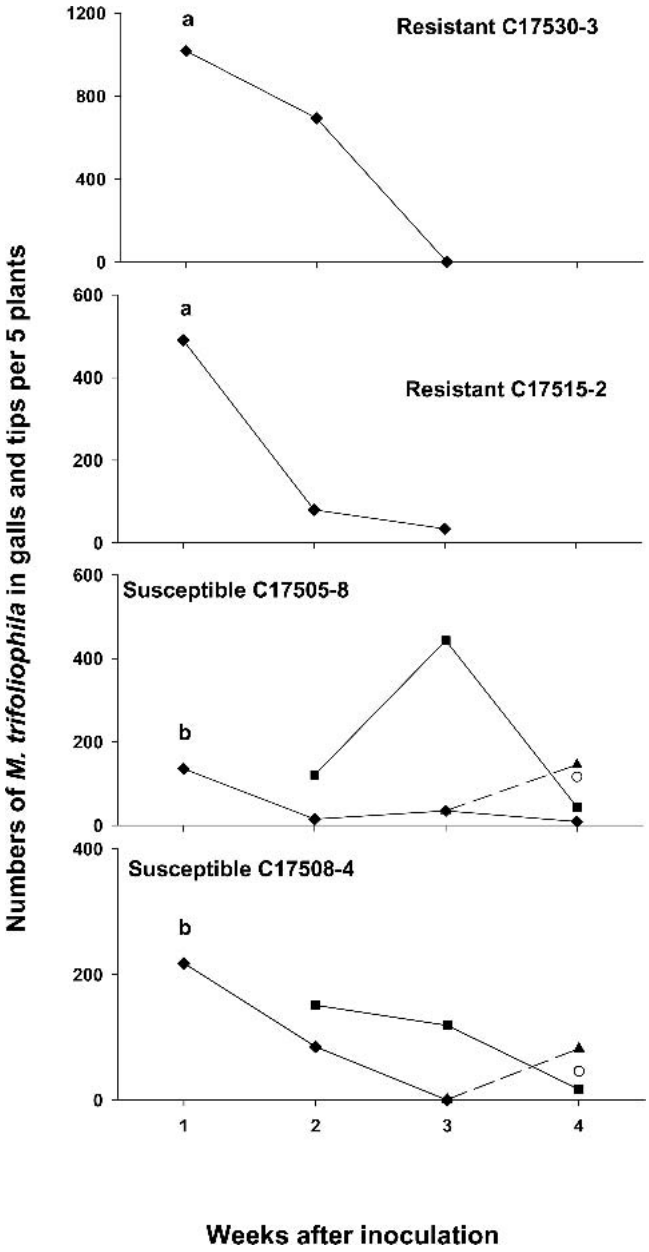

FIG. 1. Mean numbers of *Meloidogyne trifoliophila* life stages in roots of two resistant and two susceptible *Trifolium repens* genotypes at four weekly intervals following inoculation. Means of second-stage juvenile counts at week 1 with letters in common do not differ at $P < 0.05$. Stages are vermiform J2 (♦), J3 (■), J4 (▲), and adult (○).

more sand was added to just cover the roots. Petri dishes were left on a bench top for 24 hours at 18 °C to 22 °C and then sand was washed off and the roots suspended in a 500-ml beaker of continuously aerated tap water. In three experiments, at 24, 48, or 72 hours after inoculation (HAI), roots were cut off stolons, stained as described previously, and examined whole without squashing using a compound microscope. The number of J2, their position in the root, and the direction of orientation were recorded for each sample.

At 72 HAI, the root-gall width was recorded as a function of the unswollen root width (basipetally) and the galls assigned to one of three categories: (i) normal development with one or more hockey-stick-shaped J2 and with heads in the root stele, (ii) abnormal development with J2 straight or curved several times and J2 heads not embedded, (iii) with several J2 where shape and embedded heads could not be distinguished. Also at 72 HAI, browning of cells in galls was recorded. Mean relative gall widths were compared by analysis of variance and counts were compared using the Chi-square statistic.

Results

Development of M. trifoliophila in T. repens: At 2 WAI, the numbers of galls per five roots (Table 1) confirmed the differences between resistant and susceptible genotypes seen in previous experiments. There was no difference in fresh root weight among genotypes at 1 and 2 WAI (Table 1) or in later samples (data not shown). Numbers of root tips for each genotype were also similar at 2 WAI.

In stained roots at 1 WAI, the number of J2 in resistant genotypes exceeded the numbers in susceptible genotypes by factors of 2 to 7.5 (Fig. 1). Numbers of J2 in resistant genotypes fell steadily to nearly zero at 3 WAI and later nematode life stages were not seen in any samples from the resistant genotypes. In the roots of the susceptible genotypes, J3 were observed in galls at 2 WAI, J4 at 3 WAI, and adults at 4 WAI.

Nematode migration in roots: Counts of J2 in galls and root tips ranged from 0 to 11 (*T. repens*) or 0 to 12 (*T. semipilosum*) with a median of one for each species (Fig. 2).

At 24 HAI, J2 were seen in the cortex oriented distally in both resistant and susceptible genotypes of both plant species (Table 2) and were located between the meristem and the zone of root hair initiation. At 48 HAI, accumulations of J2 were seen in the meristem in resistant and susceptible genotypes of both plant species. At 72 HAI, differences in nematode responses were evident between resistant and susceptible genotypes in both plant species (Table 3). In the susceptible genotypes, J2 heads were embedded in the developing stele—J2 bodies were bent at a position about a third of the body length from the head with the tail pointed proximally. In contrast, in the resistant genotypes, J2 heads were rarely embedded in the developing vascular tissue and J2 bodies were often bent in two places (Fig. 3). Furthermore, in resistant *T. repens* genotypes, root galls differed from those developing in susceptible genotypes. The galls in resistant genotypes were nar-

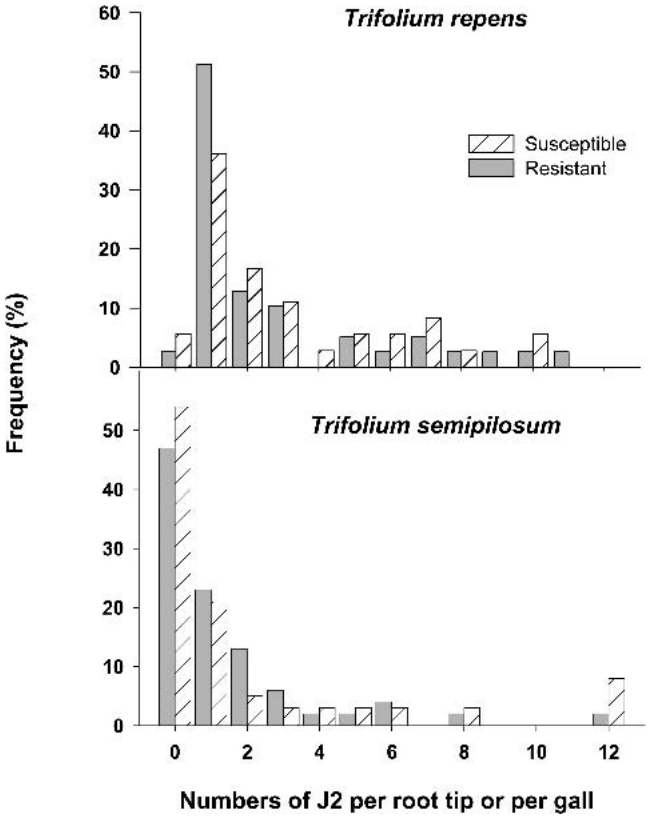

Fig. 2. Frequency (as a percentage) of *Meloidogyne trifoliophila* second-stage juveniles in galls and root tips of *Trifolium repens* and *T. semipilosum* 72 hours after inoculation with second-stage juveniles. In the single-juveniles-per-gall category, $n = 13$ for the resistant and $n = 20$ for the susceptible genotypes, respectively, for *T. repens;* $n = 8$ for the resistant and $n = 12$ for the susceptible genotypes, respectively, for *T. semipilosum.*

Table 2. Orientation of second-stage juveniles of *Meloidogyne trifoliophila* in root tips of resistant and susceptible *Trifolium repens* and *T. semipilosum* genotypes 24 and 48 hours after inoculation.

	Numbers of second-stage juveniles			
	24 hours after inoculation[a]		48 hours after inoculation[a]	
Genotype	Distal	Proximal	Distal	Proximal
T. repens				
Resistant	18a	0b	18a	2b
Susceptible	25a	1b	9a	0b
T. semipilosum				
Resistant	24a	1b	17a	1b
Susceptible	17a	2b	20a	4b

Plants were inoculated with second-stage juveniles. Data are combined within a plant species for the two resistant and the two susceptible genotypes.

[a] Within rows and within a date, means with letters in common do not differ at $P < 0.001$.

TABLE 3. Numbers of *Meloidogyne trifoliophila* second-stage juveniles in galls and gall characteristics in susceptible and resistant genotypes of *Trifolium repens* and *T. semipilosum* 72 hours after inoculation.

Genotype	Relative gall width[a]	Appearance of J2 in galls[b]		Occurrence of brown cells in galls[c]	
		In stele[d]	In cortex[e]	Present	Absent
T. repens					
Resistant C17515-2	2.00a	13a	15a	20a	18a
Susceptible C17508-2	4.38b	30a	2b	3a	32b
T. semipilosum					
Resistant TSM 19	1.89a	2a	48b	6a	6a
Susceptible TSM 4	4.22b	30a	12b	0a	8b

[a] Values are the ratio of the gall at its greatest width of the adjacent unswollen root. Means with letters in common do not differ at $P < 0.05$.
[b] Within rows, means with letters in common do not differ at $P < 0.01$.
[c] Within rows, means with letters in common do not differ at $P < 0.01$.
[d] J2 with their heads in the stele and their body in the cortex. The tails point proximally and the J2 is hockey-stick shaped.
[e] The J2 is positioned entirely in the cortex.

rower (Table 3) and were curved through 15° to 40° with a wavy surface on the inside curve (Fig. 3). Susceptible plant galls at 3 DAI were rarely bent beyond 10° and were smooth-surfaced. The galls at 3 DAI in *T. semipilosum* were also narrower in resistant genotypes but rarely curved.

There was generally more of a browning reaction in resistant genotypes of both plant species (Table 3), but differences in the browning reaction were recorded between *T. repens* and *T. semipilosum.* In *T. repens,* brown cells were seen in patches in the epidermis, cortex, and stele sometimes localized in developing cells around the J2 head (Fig. 3). In *T. semipilosum,* brown cells extended in a root-wide volume from the root cap to the zone of elongation.

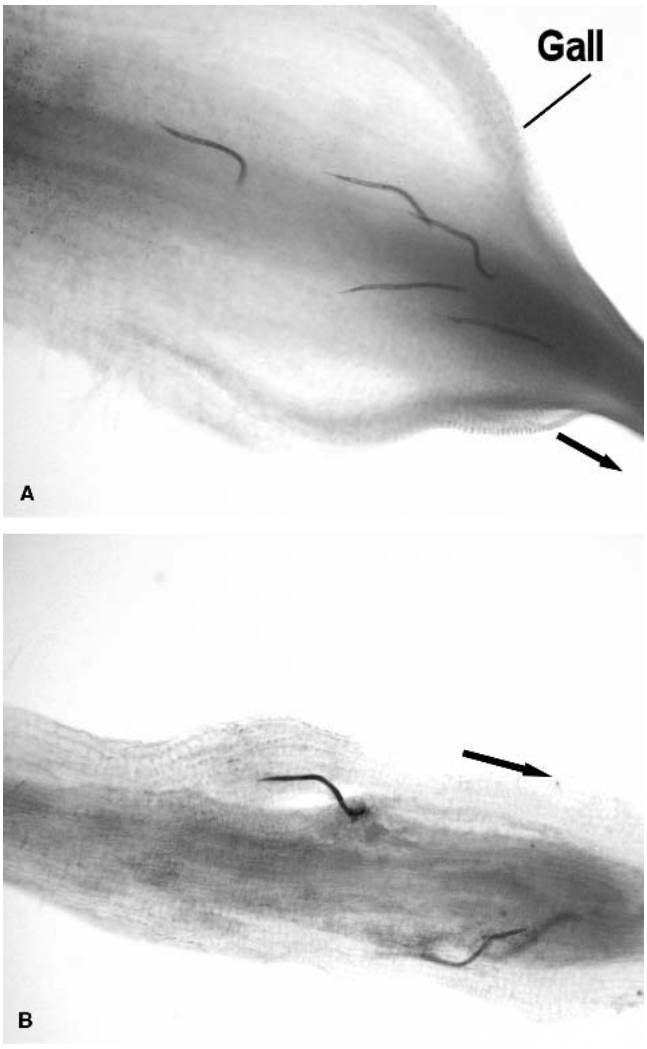

FIG. 3. *Trifolium repens* roots 48 hours after a 24-hour period of inoculation with *Meloidogyne trifoliophila* second-stage juveniles. Top panel: Second-stage juveniles with heads embedded in the root stele of a susceptible genotype. Bottom panel: Resistance reaction—at the top, a second-stage juvenile head is adjacent to a cell-browning reaction. Arrows indicate the direction of the root tip.

DISCUSSION

The weekly samples of *T. repens* in the development study confirmed earlier preliminary observations on dissected roots that there is no development of CRKN beyond the invasive J2 stage in resistant roots. The development rate in susceptible genotypes was similar to that reported by Mercer (1990) (but CRKN was described as *M. hapla*), where CRKN adults first appeared at 4 WAI, and to other species of root-knot nematode parasitizing legumes (Call et al., 1996; Call et al., 1997; Mercer and Grant, 1993) (the last reference referred to CRKN as *M. hapla*). Numbers of J2 in resistant genotypes declined probably because J2 emigrated from the root after failing to establish a feeding site. Numbers of J2 declined because of emigration in resistant genotypes of *T. semipilosum* infected by CRKN (Mercer and Grant, 1993), in peanut infected by *M. arenaria* (Bendezu and Starr, 2003), and in soybean infected by *M. incognita* (Herman et al., 1991). High sampling error was evident in randomly selecting five clones for staining; for example, the 443 J3 at 3 WAI in the susceptible genotype 505-8 (Fig. 1) could not have developed from the 136 J2 at 1 WAI.

The higher numbers of CRKN in resistant *T. repens* genotypes at 1 WAI suggest that those roots are more attractive or may be more easily penetrated by J2. Similar or greater invasion rates in roots of susceptible than resistant varieties are more common (Trudgill and Blok, 2001; von Mende, 1997). Greater invasion in resistant varieties has been reported for *M. incognita* in tobacco (Schneider, 1991) and for *M. incognita* in soybean (Herman et al., 1991). These two earlier studies and our own data suggest that genetic control of the invasion rate is independent of the subsequent expression of resistance. The greater invasion rate in one of the resistant *T. repens* genotypes was also seen in the migration study where there were more galls with one

J2 per gall or root tip in the resistant genotypes, but the difference was non-significant ($P > 0.2$). To what extent attractiveness is a component of the different invasion rate seen could be determined by hourly observation of root tips in water agar (Balhadere and Evans, 1994).

von Mende (1997) reported that, in general, the invasion of a root tip by one J2 will attract others that invade at the same point. Our frequency data on J2 per gall or root tip at 3 DAI do not reflect this because the most frequent category is the single J2. Exposure of roots to higher inoculum densities may increase the frequency of the higher numbers per gall or root tip. Perhaps the density used in the present experiment constrained the numbers available for invasion although 5,000 J2 per root system is a higher rate than generally cited for such inoculations.

Entry at the zone of elongation into the roots of both clovers, followed by distal migration through the cortex, matched the pattern of root invasion reported for other *Meloidogyne* spp. (Cook, 1991; von Mende, 1997). Migration duration could not be assessed with an accuracy better than 12 hours, as J2 may have entered the root at any time in the 24-hour inoculation period. However, at 48 HAI, accumulations of J2 were seen in the meristem, indicating a migration duration of 48 to 72 hours. This is longer than the *M. incognita* migration described in the comparatively thin *Arabidopsis thaliana* roots but is similar to reports for larger root sizes of pineapple, cowpea, and soybean (von Mende, 1997). More accurate determination of the migration time would require shorter inoculation periods.

Migration in both clovers stopped when J2 heads entered the zone of undifferentiated cells just proximal to the zone of division with the J2 tails still pointing proximally. Their heads then remained in the stele as the tissues of the vascular bundle developed around them (Fig. 3) so that from 72 HAI onward, J2 heads were embedded in the stele through the endodermis with their bodies in the cortex. This pattern of J2 behavior before the initiation of the giant cells contrasts with that described for *M. incognita* in *A. thaliana* (Wyss et al., 1992), which von Mende (1997) stated is the common behavior for root-knot nematode migration. In *A. thaliana, M. incognita* migrates distally through the cortex to the undifferentiated cells and then turns to face proximally and to lie within the advancing tube of endodermis. In contrast, in the clovers we studied, CRKN stopped migrating while still facing the root tip. They apparently identified the xylem cell initials and remained there while the endodermis formed around them.

The browning reaction in cells of roots invaded by nematodes may be due to mechanical damage from the nematode movement and stylet probing, or from a hypersensitive host response. Bendezu and Starr (2003) questioned the adequacy of the staining method used here to detect necrosis of a small number of host cells. However, the browning reaction was readily detected in roots of both clover species, and the browning reaction was localized around the J2 frequently enough to support the resistance reaction being a localized hypersensitive host response. This hypersensitive response to root-knot nematodes has been well characterized at the light microscope level for two cereals (Balhadere and Evans, 1994) and tomato (Bleve Zacheo et al., 1982).

Localized browning reactions were not seen around the J2 head in *T. semipilosum.* In contrast to *T. repens,* a more general reaction was seen in infected root tips obscuring any detail of individual cells. Although both clovers studied exhibited a hypersensitive response, they had different resistance reactions to CRKN J2. It cannot be concluded that different genetic mechanisms are involved without more detailed study. Observation of auto-fluorescence (Hutangura et al., 1999) and the use of immunolabelling (Gravato Nobre et al., 1995) may be useful techniques to study the hypersensitive response more precisely. Whether the more extensive browning reaction in *T. semipilosum* kills the root tip was not investigated, but an extreme sensitivity to CRKN infection is not an attractive character for introgression or direct insertion into *T. repens.*

Literature Cited

Balhadere, P., and A. A. F. Evans. 1994. Characterization of attractiveness of excised root tips of resistant and susceptible plants for *Meloidogyne naasi.* Fundamental and Applied Nematology 17:527–536.

Barrett, B. B., C. F. Mercer, K. K. Moore, and D. R. Woodfield. 2002. Microsatellite markers for a nematode resistance locus in *Trifolium.* Proceedings of the 12th Australasian Plant Breeding Conference:367–371.

Bendezu, I. F., and J. L. Starr. 2003. Mechanism of resistance to *Meloidogyne arenaria* in the peanut cultivar COAN. Journal of Nematology 35:115–118.

Bernard, E. C., and J. D. Eisenback. 1997. *Meloidogyne trifoliophila* n.sp. (Nemata: Meloidogynidae), a parasite of clover from Tennessee. Journal of Nematology 29:43–54.

Bleve Zacheo, T., G. Zacheo, M. T. Melillo, and F. Lamberti. 1982. Ultrastructural aspects of the hypersensitive reaction in tomato root cells resistant to *Meloidogyne incognita.* Nematologia Mediterranea 10: 81–90.

Byrd, D. W., Jr., T. Kirkpatrick, and K. R. Barker. 1983. An improved technique for clearing and staining root tissues for detection of nematodes. Journal of Nematology 15:142–143.

Call, N. M., K. H. Quesenberry, and R. A. Dunn. 1996. *Meloidogyne arenaria* development in three clover populations. Crop Science 36: 1393–1397.

Call, N. M., K. H. Quesenberry, and R. A. Dunn. 1997. *Meloidogyne javanica* development in three clover populations. Crop Science 37: 265–269.

Cook, R. 1991. Resistance in plants to cyst and root-knot nematodes. Agricultural Zoology Reviews 4:213–241.

Gravato Nobre, M. J., N. von Mende, L. Dolan, K. P. Schmidt, K. Evans, and B. Mulligan. 1995. Immunolabelling of cell surfaces of *Arabidopsis thaliana* roots following infection by *Meloidogyne incognita* (Nematoda). Journal of Experimental Botany 46:1711–1720.

Herman, M., R. S. Hussey, and H. R. Boerma. 1991. Penetration and development of *Meloidogyne incognita* on roots of resistant soybean genotypes. Journal of Nematology 23:155–161.

Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter 57:1025–1028.

Hutangura, P., U. Mathesius, M. G. K. Jones, and B. G. Rolfe. 1999. Auxin induction is a trigger for root gall formation caused by root-knot nematodes in white clover and is associated with the activation of the flavonoid pathway. Australian Journal of Plant Physiology 26: 221–231.

Johansen, D. A. 1940. Plant micro technique. McGraw-Hill Book Company.

Mercer, C. F. 1990. Development of the nematodes *Meloidogyne hapla* Chitwood and *Heterodera trifolii* Goffart in white clover. Nematologica 36:227–236.

Mercer, C. F., and J. L. Grant. 1993. Reproduction of *Meloidogyne hapla* and *Heterodera trifolii* from several sites in New Zealand on resistant and susceptible lines of white clover. Nematologica 39:312–321.

Mercer, C., and J. Grant. 1994. Development of *Meloidogyne hapla* in resistant and susceptible lines of *Trifolium semipilosum*. Proceedings of the 6th Australasian Conference on Grassland Invertebrate Ecology: 195–201.

Mercer, C. F., and K. J. Miller. 1997. Evaluation of 15 *Trifolium* spp. and of *Medicago sativa* as hosts of four *Meloidogyne* spp. found in New Zealand. Journal of Nematology 29:673–676.

Mercer, C. F., K. J. Miller, and J. van den Bosch. 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., J. L. Starr, and K. J. Miller. 1997. Host-parasite relationships of *Meloidogyne trifoliophila* isolates from New Zealand. Journal of Nematology 29:55–64.

Mercer, C. F., R. N. Watson, J. van den Bosch, K. H. Widdup, K. J. Miller, and C. J. Pennell. 1999. 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.

Schneider, S. M. 1991. Penetration of susceptible and resistant tobacco cultivars by *Meloidogyne* juveniles. Journal of Nematology 23: 225–228.

Trudgill, D. L., and V. C. Blok. 2001. Apomictic, polyphagous root-knot nematodes: Exceptionally successful and damaging biotrophic root pathogens. Annual Review of Phytopathology 39:53–77.

van den Bosch, J., and C. F. Mercer. 1996. Third generation progress in breeding white clover for resistance to root-knot nematode. Pp. 163–166 *in* D. R. Woodfield, ed. White clover: New Zealand's competitive edge. Lincoln, New Zealand: Agronomy Society of New Zealand and New Zealand Grassland Association.

von Mende, N. 1997. Invasion and migration behavior of sedentary nematodes. Pp. 51–64 *in* C. Fenoll, F. M. W. Grundler, and S. A. Ohl, eds. Cellular and molecular aspects of plant nematode interactions. Dordrecht, Netherlands: Kluwer Academic Publishers.

Vrain, T. C. 1977. A technique for the collection of *Meloidogyne* larvae and a comparison of eggs and larvae as inocula. Journal of Nematology 9:249–251.

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

Williams, W. M., and M. J. Baker. 1987. White clover taxonomy and biosystematics. Pp. 323–342 *in* M. J. Baker and W. M. Williams, eds. White clover. Wallingford, UK: CAB International.

Wyss, U., F. M. W. Grundler, and A. Munch. 1992. The parasitic behaviour of second-stage juveniles of *Meloidogyne incognita* in roots of *Arabidopsis thaliana*. Nematologica 38:98–111.