DEVELOPMENT OF RED CLOVER WITH HIGH LEVELS OF RESISTANCE TO ROOT-KNOT NEMATODES

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ABSTRACT
Red clover (*Trifolium pratense* L.), is grown in many areas of the world, but production in subtropical regions may be limited by susceptibility to root-knot nematodes (*Meloidogyne* spp.) (RKN). Selection for early vigor in RKN infested soils resulted in moderate RKN tolerance in 'Cherokee'. However, improved RKN resistance in red clover was needed. Seven additional cycles of greenhouse selection using Cherokee as a base population developed a population (FLMR7) with high RKN resistance. When FLMR7 was infested with *M. arenaria*, *M. hapla*, *M. incognita*, or *M. javanica*, numbers of galls and egg masses were lower than on Cherokee or an earlier cycle of selection (FLMR6). The resistance mechanisms appeared to delay and reduce RKN maturation at all life stages, including reduction in number of egg masses per plant. Preliminary evidence suggests that resistance may interact with higher soil temperatures.

KEYWORDS
Trifolium pratense, red clover, *Meloidogyne*, root-knot nematode

INTRODUCTION
Red clover, a short-lived perennial legume with high forage quality is grown in diverse regions worldwide. Nematode parasitism of clovers in the USA has been estimated to cause 6% annual dry matter yield losses valued at $33 million (Hague, 1980), and root-knot species were among the most important parasitic nematodes. Susceptibility to RKN is a factor limiting the production and persistence of red clover in the southeastern United States (Quesenberry et al., 1989). Clover roots infected with RKN may be stunted or heavily galled, and can deteriorate rapidly from secondary pathogen infection. 'Cherokee' red clover, developed at the University of Florida using recurrent selection for early spring vigor in fields known to have a high RKN population, has moderate resistance to RKN compared to other available red clover cultivars (Quesenberry, et al., 1993). Nevertheless, RKN reproduction on Cherokee is at a level which would not likely suppress field RKN populations. The objectives of this research were to develop a red clover population with increased RKN resistance and to study the effects of this improved resistance on nematode life cycles.

METHODS
Using Cherokee as the base population, additional selection for improved RKN resistance was conducted in the greenhouse over a period of six years. Two cycles of recurrent half-sib family selection followed by five cycles of recurrent mass selection were conducted to develop a population designated FLMR6. One additional cycle was conducted where parents highly resistant to either *M. arenaria* race 1, *M. incognita*, race 1, or *M. javanica* were identified and then reselected by evaluation of rooted cuttings. Eighty-one selected parents were then intercrossed to produce FLMR7. For the population development research and the two experiments described below, seeds were germinated in petri dishes, transplanted into 150 cm^3 "Cone-tainers", and infested with 1500 nematode eggs per plant at about 21 days after transplanting (Quesenberry, et al., 1989).

Response to infestation with *M. incognita* race 3 of FLMR6, Cherokee, the base population from which Cherokee was selected (FLCY0), and Kenstar was investigated in Exp. 1 using a randomized complete block design of four replications of 12 plants from each of the four entries. At 4, 7, 14, 21, 28, and 42 days after infestation (DAI) two plants were removed from each replication and gently washed free of soil. Using a technique for clearing roots and staining nematodes in the roots described by Call et al. (1996b), the number of J2, J3/4, adult nematodes, and egg masses were determined.

Experiment 2 evaluated the response of Kenstar, Cherokee, FLMR6, and FLMR7 to infestation with *M. arenaria* race 1, *M. hapla*, *M. incognita* race 3, and *M. javanica*. There was a separate randomized complete block design experiment of two replications of seven plants for each nematode species. Mean number of egg masses were determined at 48 to 56 DAI as previously reported (Call et al., 1996b). The plants were infested with nematodes on 9 and 10 May 1996 and evaluated for number of egg masses during the period of 24 to 28 June when mean greenhouse daytime air temperatures were often at least 30° C.

RESULTS AND DISCUSSION
In Exp. 1, at 21, 28 and 42 DAI, fewer *M. incognita* adults in roots and fewer egg masses were found on FLMR6 and Cherokee than on FLCY0 and Kenstar (Table 1). At 42 DAI numbers of adults in roots of Kenstar, FLCY0, Cherokee, and FLMR6 were 90, 100, 35, and 9, respectively. Numbers of egg masses on roots of the same four populations at 42 DAI were 70, 88, 20, and 3, respectively. Results from similar research with *M. arenaria* and *M. javanica* (Call, et al., 1996b, 1997) supported these findings and suggested that FLMR6 impacts nematode development by reducing numbers of J2 stage nematodes maturing to later juvenile stages and the adult stage and also by reducing the number of adults producing egg masses.

Reproduction of the various RKN species on the different red clover populations in Exp. 2 was similar to that observed in Exp. 1. Rankings of mean egg mass numbers among the clovers for all four RKN species were Kenstar ž Cherokee > FLMR6 ž FLMR7 (Table 2). The greatest reduction in egg mass number on FLMR7 was when infested with *M. incognita* with a mean of eight egg masses per plant. Mean egg mass numbers on FLMR6 when infested with all nematodes in this experiment tended to be greater than observed in Exp. 1 or those reported by Call et al. (1996a, 1996b). This variance in results may be due to temperature interactions with resistance mechanisms since Exp. 1 and the reports by Call et al. (1996b, 1997) were carried out in mid to late winter when greenhouse temperatures were generally less than 24 C, whereas Exp. 2 was conducted when temperatures were 6 to 8 degrees higher. Egg mass number of all nematodes on FLMR7 were numerically lower than on FLMR6, but were only different ($P<0.05$) from FLMR6 with *M. arenaria* (Table 2).

Other recent research has shown that FLMR6 grown in microplots with RKN infestation was not different in dry matter yield from non-RKN infested plots, whereas RKN infested Kenstar microplots yielded significantly less than non-infested plots (Call, et al., 1996a). Our unpublished data also show that dry matter yields of Cherokee, FLMR6, and FLMR7 were not different when grown in soils with no RKN infestation.

The results of this research and previous reports demonstrate that recurrent phenotypic selection for low gall and egg mass scores, using screening procedures developed at the University of Florida has resulted in development of a red clover population with high levels of RKN resistance. This improved resistance has resulted in improved yield and persistence in RKN infested soils. Preliminary evidence suggests that the resistance may interact with increased temperatures. This population is currently being evaluated for cultivar release.

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Table 1
Number of *Meloidgoyne incognita* race 3 adults and egg masses in roots of four red clovers at various days after infestation (DAI).

	Number of Adults			Number of Egg Masses		
Clover	Days After Infestation			Days After Infestation		
Population	21	28	42	21	28	42
Kenstar	28a†	50a	90a	7a	13a	70a
FLCY0	17b	49a	100a	4ab	20a	88a
Cherokee	5a	17b	35b	1b	3b	20b
FLMR6	4c	9b	9b	1b	2b	3b

† Means within a column followed by different letters are significatly different (P<0.05, Lsd).

Table 2
Number of egg masses on various clover populations at 48 to 56 days after infestation with four root-knot nematodes.

Clover	Number of Egg Masses			
Population	Ma†	Mh	Mi	Mj
Kenstar	150a‡	101a	84a	156a
Cherokee	139a	61a	59a	107a
FLMR6	59b	34ab	13b	54ab
FLMR7	14c	27b	8b	23b

† Ma = *Meloidogyne arenaria* race 1, Mh = *M. hapla*, Mi = *M. incognita* race 3, and Mj = *M. javanica*.
‡ Means within a column followed by different letters are significantly different (P<0.05, lsd).