PHENOTYPIC DIFFERENCES IN ROOT-KNOT NEMATODE (*Meloidogyne* spp.) WHITE CLOVER (*Trifolium repens* L.) INTERACTIONS AND COMBINING ABILITY ANALYSIS OF RESISTANCE

By

ANANTA RAJ ACHARYA

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2009

To my late Grandparents,

ACKNOWLEDGMENTS

I would like to extend my appreciation to my committee chair Dr. Kenneth Quesenberry. His encouragements and insights into the subject matter always helped me throughout the research period. He always helped me with suggestions in graduate work, research and even some tiny day to day matters. He has been an excellent guardian and friend at the same time. I would also like to thank Dr Kevin Kenworthy for his valuable suggestions in quantitative procedures involved in the research. I also want to thank Dr. Yoana Newman for her support and suggestions in my research. I want to appreciate Dr. David Wofford for his research ideas and thought provoking questions and suggestions.

I want to thank technicians Eric Ostmark and Judith Dampier. Eric was the first to teach me every skill required to conduct the research and his wittiness and friendship always entertained during the laborious lab works. I want to thank Samantha, Kevin, Dylan, Jamie, Olubunmi, Carlos, Vivienne and Karen for their help with tedious and laborious lab works.

I also want to thank Subodh, Shweta and Prakash for a close friendship which often reminded me of my home. I would like to thank Smita for her encouragement, support and understanding.

Last but not the least, I would like to thank and congratulate my parents Hari Acharya and Radha Acharya whose dream, direction and support brought me to this stage. And I heartily want to thank my brother Kul Acharya and sister-in-law Sulochana for their unselfish support. My brother is always my source of inspiration and his support since childhood brought me here, even when he himself needed support and might have had other priorities. Finally I want to remember all my family members; brothers, sisters, niece, nephew and everyone.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

PHENOTYPIC DIFFERENCES IN ROOT-KNOT NEMATODE (*Meloidogyne* spp) AND WHITE CLOVER (*Trifolium repens* L.) INTERACTION AND COMBINING ABILITY ANALYSIS OF RESISTANCE

By

Ananta Raj Acharya

May, 2009

Chair: Kenneth H. Quesenberry
Major: Agronomy

White clover (*Trifolium repens* L.) is an important forage legume worldwide and also in the southeastern USA. Its higher crude protein and digestibility make it an important component in mixture with grasses to increase the overall nutritive value. Root-knot nematodes (RKN) (*Meloidogyne* spp.) can be a major factor limiting the production and persistence of white clover especially in the sandy soil condition of Florida. The purpose of this study was to compare the new cultivar UFWC5 released as tolerant to southern RKN with the commercial cultivar 'Osceola' for host-pathogen responses to different populations of RKN. A second objective was to estimate the magnitudes of general combining ability (GCA) and specific combining ability (SCA) for various RKN resistance responses in UFWC5 to better understand the genetics behind the RKN resistance responses.

Our study found that UFWC5 was resistant to all four races of *M. incognita* with gall scores and egg mass scores less than 2.0 compared to Osceola which had egg mass scores and gall scores higher than 3.0. Similarly, eggs per plant were reduced by ca. 50% when inoculated with *M. incognita* race 1 and ca. 80 to 90% when inoculated with other the three races of *M. incognita*. The egg mass score and gall score for UFWC5 roots inoculated with *M. arenaria* race

1 and *M. javanica* was above the level for it to be classified as resistant (more than 2.0) but still much reduced compared to Osceola roots (above 3.0 and above 4.0 respectively for *M. arenaria* race 1 and *M. javanica*). Egg production as assessed by eggs per plant was reduced by ca. 70% when inoculated with *M. arenaria* race 1 and by ca. 80% when inoculated with *M. javanica*. This study pointed out the differences in the virulence of different RKN populations. This may suggest the involvement of different genes for resistance to the different populations of RKN.

There were no significant differences between non-inoculated Osceola and UFWC5 for either root or shoot weights. This finding suggests that selecting for RKN resistance did not alter the yield potential of this newly selected white clover cultivar.

Based on three different diallel analysis studies involving three RKN populations *M. incognita* race 4, *M. arenaria* race 1 and *M. javanica*, additive genetic variance appeared to be the principal type of gene action involved in selection for RKN resistance in UFWC5. All these genetic studies showed that additive variance was more important than non-additive variance in the inheritance of resistance to RKN. The plants which were resistant to *M. incognita* race 4 were not necessarily resistant in the same degree to *M. arenaria* or *M. javanica* and the degree of susceptibility was also different in these three populations. One parent that showed resistance to *M. incognita* race 4 was susceptible to *M. javanica*, which suggests that there may be differences in the genes that confer resistance to different populations of RKN.

The importance of additive variance suggests that selection of a few superior parents for development of a synthetic variety would be the most appropriate breeding strategy. Based on our research, the clones R1, R4 and M3 would be superior parents for breeding resistance to *M. incognita* race 4. Only one parent in each case was outstanding for resistance to *M. arenaria* race 1 and *M. javanica* (R6 and R1, respectively).

CHAPTER 1
INTRODUCTION

White clover (*Trifolium repens* L.) is a major forage crop in many areas of the world including the southeastern USA. It is a cool season perennial legume but acts as a reseeding annual in Florida (Chambliss and Wofford, 2006). The warm season grasses that dominate Florida pastures generally have lower nutritive values. Thus, white clover can be an important component of Florida pasture. It generally has higher crude protein and digestibility than tropical grasses and when grown in a mixture with grasses will result in increased nutritive value of the overall diet. Despite the added benefit in terms of forage quality, under Florida conditions, there are many diseases and nematodes which decrease the persistence and yield of white clover. Root-knot nematodes (RKN, *Meloidogyne* spp.) may be one of the major problems of white clover in the southeastern region of the United States (UC SAREP, 2008).

There are many kinds of nematodes that damage plant roots, but root-knot nematodes (*Meloidogyne* spp.) cause about 75 percent of all nematode damage to landscape ornamentals and annual crops in warm climates (Dunn and Sydenham, 1992). Root-Knot nematodes have a very wide host range, are favored by sandy soils with moist and warm soil conditions. They are very small (0.25 mm to 3 mm long) and a transparent organism. They induce the formation of giant cells (hence, galls) and use these cells as feeding sites to parasitize the plant roots. Root-knot nematodes not only compete for nutrients but also open the door for other pathogens and pests to invade plant roots (Dunn and Sydenham, 1992).

There have been several attempts to manage RKN disease in white clover. These methods have included chemical., cultural., biological and resistance breeding (Dropkin, 1989). Due to many factors limiting utilization of other techniques, development and planting of cultivars resistant to RKN may be the best practical solution for RKN management. Some resistant white

clovers have been selected in an attempt to reduce the damage caused by RKN including SC-1 (Gibson, 1973), and MSNR4 (Pederson and Windham, 1995). A new cultivar 'UFWC5' was recently released as a result of five cycles of phenotypic recurrent selection for resistance to the southern RKN (*M. incognita*) from 'Osceola' (Baltensperger et al., 1984). This cultivar has shown reduction in root gall rating and egg mass rating against *M. incognita* (Wofford and Ostmark, 2005).

Depending on the species being evaluated, resistance to RKN has been shown to be monogenic, oligogenic or quantitative in nature. Regardless of the resistance mechanism in white clover, it is important to know the inheritance pattern of resistance to RKN. Partitioning of the genotypic variance into general and specific combining abilities will be even more important in breeding for resistance in synthetic varieties (Baker, 1978). Griffing (1956) gave the generalized model to estimate combining abilities using a diallel mating design that allows partitioning of total genetic variances into general and specific combining abilities.

One objective of this research was to compare the new cultivar UFWC5 and Osceola for resistance responses to four races of *M. incognita* and two other species, *M. arenaria* and *M. javanica*. A second objective was to estimate the magnitudes of general and specific combining ability for various RKN resistance responses in UFWC5 to better understand the genetics behind the RKN host-pathogen responses.

CHAPTER 2
LITERATURE REVIEW

White Clover

White clover (*Trifolim repens* L.) is known to be a well adapted perennial legume in temperate climates, but it is also adapted to humid, subtropical climates. White clover is grown throughout the humid eastern USA and also in drier areas of the western USA using irrigation. It grows well both on clay and silt soils in humid and irrigated areas and white clover prefers a soil pH range of 5.5 to 7.0 (USDA, 2002). Although thought to be native to Eurasia, white clover is widely distributed around the world (Williams, 1978a).

Although white clovers have been cultivated as ornamentals and cover crops, the main usage is as grazed forage. White clover is considered one of the most nutritious forages available and it is generally mixed with grasses to increase the nutritive value of the available forage. White clover has good persistence for grazing and is also suitable for hay, silage and green chop. White clover is also known as a very good nitrogen fixing crop when inoculated with appropriate symbiotic rhizobium bacteria. The amount of nitrogen fixation depends on the genotype, effective inoculation, growing season, and sward density (Gibson and Cope, 1985).

White clover cultivars have been classified as small, intermediate and large (ladino) types. Most commercially available cultivars are the ladino type including ‘Regal’ and ‘Osceola’ or the intermediate type such as ‘Lousiana S-1’, ‘Grasslands Huia’, and ‘Durana’. Intermediate types have more profuse and early flowering which results in sufficient seed production for reseeding (Gibson and Cope, 1985).

White clover is a tetraploid ($2n = 4x = 32$) plant. It has a gametophytic self incompatibility system based on multiple alleles at an S locus that has been suggested to have more than 30 alleles (Williams, 1987). This system is also known to have the presence of a self fertility (Sf)

allele partially dominant to the self incompatible alleles. Because of the high degree of outcrossing in white clover, individuals are highly heterozygous and populations are highly heterogenous in nature. White clover breeding programs have been conducted in different countries and different locations within the USA. The main goals of breeding have been for plant types, yield, seasonal yield, persistence and resistance to physical stresses, nematodes, insects, viruses, fungi and bacteria. There also have been emphases on improving forage quality and nodulation characteristics (Williams, 1987). The main breeding method used in these efforts has been phenotypic recurrent selection. A white clover breeding program generally can be a 10 to 12 year long process with this method. One reason for the length of breeding programs is that heritability for many traits is low, e.g. yield as reported by Suzuki et al. (1958).

Interspecific crosses with perennial and annual species related to white clover have been utilized to introduce desirable genes into the cultivated crops. *Trifolium ambiguum* a strong perennial species that has been hybridized with white clover has abundant rhizomes and resistance to many viruses. *Trifolium uniflorum* another perennial species that has been hybridized with white clover has larger seeds, shorter internodes and woody roots. The interspecific hybrids of {*T. repens* × *T. nigrescens* (an annual species)} × *T. repens*, *T. isthmocarpum* (an annual species) × *T. repens*, and *T. repens* × *T. uniflorum* were identified as good sources of southern RKN resistance (Pederson and Windham, 1989).

Several viral and fungal diseases, and nematodes can affect white clover stands. Pepper spot (casual organism, *Leptosphaerulina trifoli* (Rost.) Petr.) is one of the foliar disease prevalent in cool, wet weather. Sooty blotch (casual organism, *Cymadothea trifoli* (Pers.) Ex Fr.), cercospora leaf and stem spot (casual organism, *Cercospora zebrine* Pass.) are some other common foliar diseases that infest white clover. Root and stolon rot is also a profound problem

caused by *Fusarium, Rhizoctonia, Collectrotrichum, Mycoleptodiscus, Curvularia, Macrophomina, Scleretinia* and *Sclerotium* (Gibson and Cope, 1985). Root-knot nematodes (RKN, *Meloidogyne* spp.) can be one of the most important pathological problems of white clover persistence and production (UC SAREP, 2008).

Root-knot Nematode

Root-knot nematodes (RKN) were first identified by Goeldi (1887 as cited by Taylor and Sasser, 1978) and named *Meloidogyne exigua*. Later on, RKN was thought to be a species of *Heterodora* and synonimized with *H. radicicola* and *H. marioni*. Chitwood (1949) again described them as a different genus *Meloidogyne* and identified four species, *M. incognita* (Kofoid and White) Chitwood, *M. arenaria* (Neal) Chitwood, *M. javanica* (Treub) Chitwood and *M. hapla* Chitwood (Taylon and Sasser, 1978).

More than 100 species of the genus *Meloidogyne* have been reported. Among them, Southern RKN (*M. incognita*) accounts for 51% of the worldwide population, Javanese RKN (*M. javanica*) accounts for 31% and *M. arenaria* and *M. hapla* each contribute 8%. These four species together account for more than 95% of RKN populations worldwide (Sasser et al., 1983).

In some RKN species, there are host specific races that cannot be differentiated morphologically but only with host differentiation tests. These are known as physiological races. Four host races of *M. incognita* and two races of *M. arenaria* have been defined with their differential host specificity to a particular set of hosts (Sasser et al., 1983). When a large number of *M. incognita* populations were subjected to North Carolina Host Differential Test (Hartman and Sasser, 1985), *M. incognita* race 1 comprised about 72% of all *M. incognita* populations, whereas *M. incognita* race 2, *M. incognita* race 3 and *M. incognita* race 4 accounted for 13%, 13% and 2%, respectively. While in *M. arenaria,* 16% of populations were race 1 (peanut race)

and other 84% were race 2. *M. javanica* and *M. hapla* are not reported to show any host specificity (Sasser et al., 1983).

These nematodes have a very extensive host range. They have been shown to attack almost every crop of agronomic or horticultural importance. But the species and race pathogenicity may be different for different hosts. Their worldwide distribution appears to be affected by many ecological factors. Average minimum temperatures have been one important factor related to the distribution of all four major *Meloidogyne* species. *Meloidogyne incognita*, *M. arenaria* and *M. javanica* were not found in areas whose average temperature in cold months was below 3°C while *M. hapla* occurred in cooler climates with minimum temperatures as low as -15°C and average temperatures of about 24 to 27°C. *Meloidogyne javanica* is best adapted in areas with distinct dry and wet seasons while *M. incognita* is less adapted to these conditions (Sasser et al., 1983)

Root-knot nematodes are sexually dimorphic species. Adult females are pear shaped endoparasites measuring about 0.5 mm in length and 0.3 to 0.4 mm in width. They bear a 12 to 15 μm long stylet which is of tylenculous type with a prominent basal knob (Dropkin, 1989). The stylet has a continuous lumen from the tip to the basal knobs from which this lumen continues to the esophageal tube. They have an esophagus with a prominent spherical metacorpus. Muscles attached to the metacorpus serve as a pump for food intake (Taylor and Sasser, 1978). Uteri of two gonads join just anterior to the vulva. A distinct perennial pattern (striations surrounding vulva and anus) can be seen in RKNs which often serve as an identification tool for different species (Dropkin, 1989). The adult males are cylindrical., worm shaped, about 2 mm in length, and free living in the soil. Males possess a stylet, but the esophagus is not developed as they apparently do not feed on plants (Taylor and Sasser, 1978).The RKN eggs are elongated and oval

shaped. The embryogenesis of RKN is holoblastic (whole egg dividing) and determinate. The division of cells ultimately leads to the formation of the first stage juvenile (J1) inside the egg. This juvenile stage remains coiled inside the egg and molts to form a second stage juvenile (J2). The J2 hatches from the egg breaking it by its stylet (Thorne, 1961). The J2 may remain in the egg mass for some time and then it can move in search of plant roots. The J2 are elongate measuring 400 to 500 μm and are the infective stage which bears a stylet, esophagus and esophageal glands. When a root tip is encountered, the J2 penetrates the root just above the root cap and moves intercellularly. It reaches the cortex region and then pierces the cells in this region with its stylet and an esophageal secretion is injected. These secretions cause the formation of giant cells (synctia) both by large cell size (hypertrophy) and cell number due to intense cell multiplication (hyperplasia) (Thorne, 1961).

Each infected host cell enlarges and the large central vacuole is replaced by small vacuoles while the cytoplasm increases in volume and density. The cell wall is remodeled to form elaborate ingrowths which are the sites to meet the nutrient demand of nematodes (Hussey and Janssen, 2002). This process then may lead to the development of visible galls (Taylor and Sasser, 1978). Dropkin (1969) suggested that the cellular reaction of plant cells to RKN was not a passive reaction to enzymes but an active host participation to some controlling force of the parasite. After the J2 establishes itself in the plant root system, its width increases and the esophageal gland enlarges. The cell of genital primordium starts to differentiate either into a female form exhibiting fork shape or into a male form exhibiting cylindrical growth. With continuous feeding the second stage juvenile becomes flask shaped and molts twice to form third stage juvenile (J3) and fourth stage juvenile (J4). Male and female adults start to differentiate

with the third stage larvae. The female becomes pyriform shaped while the male remains eel shaped (Taylor and Sasser, 1978).

Except for some *M. hapla* populations (Triantaphyllou, 1966), all other species reproduce parthenogenetically. Oogonia are formed in female reproductive system and divide mitotically. The most advanced oogonia then stops dividing and becomes an oocyte which ultimately becomes an egg after one mitotic division and is deposited in a gelatinous egg mass surrounding the posterior end of the female. The number of eggs in an egg mass may vary with an average of 200-300 (Taylor and Sasser, 1978).

Larvae hatched from eggs move to nearby root cells within the same gall/root system. When there is complete destruction of cell tissues, the larvae move to nearby roots of the same plant or other plants. There are very low numbers of males in root galls and the number may vary according to the microclimatic conditions (Thorne, 1961).

Since RKN invade and damage fine roots, the RKN infected plants wilt easily, become stunted and may die. Symptoms of chlorosis may also be seen. The RKN damage in infected fields often is manifested as patches of dead plants indicating localized areas of high infection. The clear sign of root-knot nematodes is that the roots are swollen due to galling and have a knot like appearance. Young small seedlings may die without any clear sign of galling (Thorne, 1961). One reason for the spotty appearance of field damage is that RKN are sedentary parasites and do not move long distances laterally but move up and down according to the soil water table.

Root-knot Nematode Disease in White Clover

The association of root-knot nematodes and white cover is well documented. Root-knot nematodes on white clover have been found in a very wide geographic area including the USA (Cook and Yeates, 1993), Australia (Mcleish et al., 1997), New Zealand (Skip and Christensen, 1983), and Europe (Cook et al., 1992). The presence of RKN in white clover has been shown to

decrease yield and persistence of white clover. The economic loss due to infestation with *M. incognita* has been reported to limit persistence in white clover by 51 to 79% (Baxter and Gibson, 1959). As a forage crop, the whole top part of the plant is economically important. Brink and Windham (1990) reported a reduction in dry weight of stolons by 36 to 83% by infection of RKN in SC-1 and Regal white clover. The numbers of stolons also were decreased by 12 to 20% and yield was reduced by 6 to 17% in a study by Pederson et al., (1991) in Mississippi USA. About 58% of New Zealand white clover pastures were infected by *Meloidogyne* Spp (Skipp and Christensen, 1983), and about 77% of Australian pastures (Mcleish et al., 1997). *M. hapla* appears to be the predominant root-knot nematode species in cool regions. In England and Wales 4% of white clover pastures were infected by this species (Cook et al., 1992).

Root-knot nematodes have been shown to be one of the factors adversely affecting white clover growth, stolon density, persistence, seedling vigor, nitrogen fixing ability, and phosphorus utilization (Zahid et al., 2001). In addition to their direct invasion effects, RKN root penetration can create wounds for secondary pathogens attack. The secondary attack is caused due to the access to the root facilitated by nematodes, the change in rhizosphere, physiological changes and resistance break caused by interactions (Evans and Haydock, 1993). Nematodes interact with other pathogens including fungi, bacteria, viruses, and even other nematodes. *Fusarium Oxysporum*, and *Verticilium* spp are common wilt inducing parasites that interact with RKN (Francl and Wheeler, 1993). *Rhizoctonia solani, Pythium ultimum,* and *Fusarium oxysporum* are some other fungi that have been shown to interact with RKN to cause root rot (Evans and Haydock, 1993). The RKNs also interact with other ecto or sedentary parasites such as *Hoplolaimus galeatus*, *H. columbus*, *Tylenchorhynchus vulgaris*, *Scutellononema brachyrum* to stimulate their reproduction and penetrance (Eisenback, 1993). The interaction of RKN and

Mycorrhizae can reduce plant yield (Francl, 1993). Nematodes can interact with bacteria by creating wounds for bacterial entry and increase susceptibility by modifying plant cells, breaking resistance to bacteria, and sometimes act as a vector. *Pseudomonas solanacearum* and *Meloidogyne* spp interaction is one of the common bacterial-RKN interaction. The RKN also interact with *Corynebacterium flaccumfaciens, Xanthomonas phaseoli, Erwinia carotovora* (Sitaramaiah and Pathak, 1993). It has also been shown that RKN can reduce nodulation by interacting antagonistically with nodule inducing bacteria (*Rhizobium* spp.) (Abd-El-Samie and Taha, 1993).

When nematodes infect one part of a plant host, the entire physiological processes throughout the plant may be disrupted. These physiological anomalities then may affect host plant yield and persistence (Melakeberhan and Webster, 1993).

The control of RKN disease in white clover can be difficult. Although some nematicides have been shown to decrease RKN populations (Taylor and Sasser, 1978; Yeates et al., 1975), currently there are no registered nematicides for use in pasturelands. Crop rotations have been proposed to limit RKN infestation as juveniles generally move no more than 50 cm. Crop rotations including host and non host plants may help reduce the nematode populations in the field (Taylor and Sasser, 1978). Other cultural practices including sanitation, fallowing, dessication, and use of antagonistic plants have also been practiced (Taylor and Sasser, 1978; Sasser et al., 1983). Biological controls using *Paecilomyces* fungus (Sasser et al., 1983), *Catenaria anguillulae*, *Arthrobotrys*, *Dactylella* (Taylor and Sasser, 1978) had been utilized. Other predatory nematodes, arthropods, and worms can also be utilized but with little documented effect (Taylor and Sasser, 1978). Sasser et al. (1983) summarized that effective control would be the best combination of all available control measures, including resistant

cultivars, crop rotation, nematicides, and sanitary and cultural practices used to develop integrated crop protection systems. Thus development of resistant varieties is likely the best solution to have a persistent productive forage crop in the field.

Root-knot Nematode Resistance Breeding

Selections have been conducted for decades to achieve the RKN resistance in white clover. Bain (1959) evaluated lines of white clover seedlings and selected genotypes with tolerance to RKN. Gibson (1973) developed 'SC-1' white clover, which was reported to be tolerant to southern RKN. This population was a first generation recombination among 145 genetically diverse white clover clones selected for tolerance to southern RKN. Those clones were screened from thousands of plants from white clover cultivars and foreign introductions. Studies from Windham and Pederson (1989) showed that SC-1 was only moderately tolerant to two of eight populations of *M. incognita*. This different response compared to the results of Gibson may be due to the fact that different races were used by Windham and Pederson that overcame the resistance in SC1. These results show that there is a need for selection and evaluation using all the predominant races and populations of RKN. Mercer et al. (2000) gained some success in selecting white clover strains resistant to *M. trifolia* (Bernard & Eisenback), previously thought to be *M. hapla*. This *Meloidogyne* isolate failed to reproduce in tomato and other plants of the North Carolina Host Differential Test but reproduced in white clover and as a consequence was taxonomically described as a new species, *M. trifolia* (Zahid et al., 2001). Pederson and Windham (1995) have released 'MSNR4' after four cycles of recurrent selection from a wide pool of white clover germplasm. This population was shown to be resistant to *M. incognita* [percent root system galled (PRSG) score of 1.0, egg score of 2.3], *M. arenaria* (PRSG score of 0.9 and egg score of 2.2) and *M. graminicola* (Golden & Birchfield) (PRSG score of 0.9 and egg score of 1.9). The cultivar 'UFWC5' was recently developed by recurrent phenotypic selection

using ‘Osceola’ as the base population and southern root-knot nematode race 4 as the selective pathogen. This population was officially released by the University of Florida (Wofford and Ostmark, 2005). A standard greenhouse screening procedure as described by Quesenberry et al. (1993) was used for the selection process. The two week old seedlings were inoculated with ca. 1200 to 1500 eggs of *M. incognita* race 4. Eight weeks after inoculation, the seedlings were extracted from growth containers, washed and immersed in Phloxine-B to highlight egg masses. The root system of individual plants was evaluated for gall and egg masses using the scale; 0 = no gall and/or egg mass, 1 = 1 to 2 galls and/or egg masses, 2 = 3 to 10 galls and/or egg masses, 3 = 11 to 30 galls and/or egg masses, 4 = 31 to 100 galls and/or egg masses and 5 = more than 100 galls and/or egg masses. Only elite plants with the lowest gall and egg mass scores were selected. It was field tested later and showed resistance to Southern RKN (Wofford and Ostmark, 2005). Nevertheless it is important to screen any cultivar for all the predominant species/races of RKN because their resistance interaction may be different.

Other than recurrent selection, interspecific hybridization, genetic transfer, and somaclonal variations can be other possible sources for resistance breeding. The study from Pederson and Windham (1989) also showed that interspecific hybrids utilizing *T. nigrescens* could be utilized in resistance breeding. Quesenberry et al. (1997) and Koume et al. (1998) have identified several native North American *Trifolium* species resistant to RKN. Two annual species, *T. carolinianum* and *T. bejariense* were found to be resistant and two perennial species, *T. calccaricum* and *T. stoloniferum* were highly resistant. But their lack of sexual compatibility with cultivated clovers has been a constraint to gene transfer.

Although some sources of resistance have been found and incorporated, without a proper understanding of the inheritance patterns and genetics behind the resistance to RKN, it is difficult to achieve success in resistance breeding.

Mode of Resistance

There are many mechanisms related to how plants defend against RKN. One of the mechanisms is non-preference where a resistant plant allows entrance of *Meloidogyne* juveniles that subsequently leave the plant due to non-preference and seek an alternative host. Another mechanism of resistance is hypersensitivity where cell are penetrated by nematodes die quickly blocking further development of nematodes. Reduced juvenile growth rate is another mode of resistance and the inhibition of female growth is also another mechanism which causes an increased sex ratio of males to females and reduces egg production (Dropkin, 1989).

Genetics of Resistance

The nature of resistance to RKN has been described as varying from control by a single dominant gene to polygenic inheritance. Several dominant or semidominant resistance genes have been identified and mapped (Williamson and Hussy, 1996). Plum [*Prunus cerasifera* (Ehrh.), Salses et al., 1998], peach [*P. persica* (L.) Batsch, Claverie et al., 2004], tomato [*Solanum lycopersicom* (L.), Williamson, 1998], peach [*P. persica* (L.) Batsch] for *M. javanica* (Zhen-Xiang et al., 2000) are reported examples of a single dominant gene for resistance. Resistance in peach [*P. persica* (L.) Batsch] to *M. incognita* was described as controlled by two dominant genes (Zhen-Xiang et al., 2000); whereas resistance in blackeye-type cowpea [*Vigna unguiculata* (L.) Walp.] line H8-8R was controlled by a single recessive gene (Ehlers et al., 2000). Red clover [*Trifolium pratense* (L.), Quesenberry et al., 1989] is an example of a number of legumes that have shown polygenic inheritance of resistance to RKN. In some cases,

polygenic resistance has been resolved into major genes that are genetically dominant and minor genes that may modulate the response (Williamson and Hussey, 1996).

Barett et al. (2005) have identified a single dominant gene (designated TRKR) in *Trifolium semipilosum* which conferred resistant to clover root-knot nematode (*M. trifolia*) by screening with *T. repens* SSR markers. In tomato and some other crops, the Mi (Mi-1) gene was identified conferring resistance to *M. incognita*, *M. javanica* and *M. arenaria* (Hussey and Janssen, 2002). Mi-3 (Tomato), Mi-9 (Tomato), Ma (Plum), Me3 (Pepper, *Capsicum annuum* L.), Rmc1 (Potato, *Solanum tuberosum* L.) are other mapped genes that confer resistance to one or more species of RKN (Williamson and Kumar, 2006). The RKN resistance in soybean was identified as multigenic and quantitative and some Quantitative Trait Loci (QTL) have been identified. (Tamulonis et al., 1997).

The resistance mode of inheritance to *M. hapla* is not as straightforward and is always under oligogenic or polygenic control (Bunte et al., 1997). Experiments by Van De Bosch and Mercer (1996) showed the variability for resistance to an unidentified *Meloidogyne* species thought previously to be *M. hapla*, but more recently classified as *M. trifolia* had low repeatability (heritability). Broad-sense heritability estimates also showed that breeding for resistance is possible, but that progress could be slow.

Regardless of the number of genes involved in resistance, for breeding it is important to estimate the type of gene action involved. Partitioning of the variances to additive and non-additive sources of variation can be more important in the case of a quantitative mode of inheritance (Zhang et al., 2007). Partitioning variance components into General Combining Ability (GCA) and Specific Combining Ability (SCA) can be very useful in designing a breeding program. The GCA is defined as the average performance of a line in multiple hybrid

combinations while the SCA is defined as the performance of a specific cross. Sprague and Tatum (1942) defined SCA as the deviation expected from the sum of the GCA of both the parents. This information will be helpful in development of synthetic varieties (Baker, 1978). Many authors have looked at GCA and SCA effects. Studies on white clover (Pederson and Windham, 1992), corn [*Zea mays* L., Williams and Windham (1990)], cotton [*Gossypium hirsutum* L., Mcpherson et al. (1995); Zhang et al., (2007)], and red clover (Call et al., 1997) are some examples that have identified GCA as more important than SCA for resistance to RKN.

Pederson and Windham (1992) used three resistant and three susceptible plants for a diallel study and found that resistant parents produced progeny with the least *M. incognita* reproduction while susceptible parents produced susceptible progeny. Progeny from two crosses performed worse than expected from the GCA effects of the parents but no crosses performed significantly better than expected. Although non-additive gene actions such as dominance and epistasis might have been involved in some crosses, additive gene action was more significant.

A diallel analysis of four resistant, three intermediate, and two susceptible red clover parents performed by Call et al. (1997) also showed predominantly significant GCA effects and non significant SCA effects. The crosses involving a resistant parent (119) showed the least number of galls and egg masses while the crosses involving susceptible parents (N1, K4) produced the highest number of galls and egg masses. This study also suggested the importance of additive gene action in breeding for RKN resistance in red clover.

Statistics

According to Sprague and Tatum (1942), average mean performance of a cross between two lines is expressed as eqauation (Eq. 2-1)

$$\bar{X}_{ij} = GCA_i + GCA_j + SCA_{ij} \quad (2\text{-}1)$$

The differences due to GCA are due to additive genetic variance and additive × additive epistasis while the differences due to SCA are due to non-additive variances (dominance, and dominance × additive epistasis). The relative contribution of GCA and SCA would be determined by the magnitude of additive and non-additive variation.

Griffing (1956) has postulated a model for the estimation of GCA and SCA using diallel mating designs. He proposed eight different models according to the crosses included and fixed and random effects in the model. Griffing's analysis method 4, model I is based on fixed effects and crosses that do not include parents and reciprocals. So there are n (n-1)/2 entries where n is equal to the number of parents.

Statistically, the phenotypic variation is given by the equation. (Eq. 2-2)

$$\sigma^2_P = \sigma^2_G + \sigma^2_E \quad (2\text{-}2)$$

where,

σ^2_E = Environmental variation

σ^2_G = Total genotypic variation

this total genotypic variation is given by Eq. 2-3

$$\sigma^2_G = \sigma^2_A + \sigma^2_{NA} \quad (2\text{-}3)$$

where,

σ^2_A = additive variance

σ^2_{NA} = non-additive variance

In the absence of epistasis non-additive variance is equivalent to dominance variance (σ^2_D). In the case of completely inbred parents (F = 1), the additive and dominance variance are equivalent as given in Eq. 2-4 and Eq. 2-5 respectively.

$$\sigma^2_A = 2 \times \sigma^2_{GCA} \quad (2\text{-}4)$$

$$\sigma^2_D = \sigma^2_{SCA} \quad (2\text{-}5)$$

but in the absence of inbreeding (F = 0), the additive and dominance variance are equivalent as given in Eq. 2-6 and Eq. 2-7 respectively.

$$\sigma^2_A = 4 \times \sigma^2_{GCA} \quad (2\text{-}6)$$

$$\sigma^2_D = 4 \times \sigma^2_{SCA} \quad (2\text{-}7)$$

The phenotypic value of any cross is also composed of the GCA and SCA effects. Statistically, the phenotypic value of ij^{th} observation can be represented as equation 2-8.

$$x_{ij} = \mu + g_i + g_j + s_{ij} + \varepsilon \quad (2\text{-}8)$$

where,

μ = population mean

$g_{i(j)}$ = GCA effect of i^{th} (j^{th}) line

s_{ij} = SCA effect of cross of i^{th} and j^{th} line including reciprocals

ε = Environmental error

For the purpose of identifying the relative importance of GCA and SCA effects many authors have used the GCA:SCA variance ratio (Baker, 1978). The nearer the ratio is to unity, the greater will be the prediction of progeny based on a single parent.

Due to the cumbersome calculations needed to conduct a diallel analysis, many authors have reported the use of statistical analyses programs. One of the most popular programs for diallel analysis in crop species is DIALLEL-SAS written by Zhang and Kang (1997) and its successor DIALLEL-SAS05 (Zhang et al., 2005). Both of these programs are written in SAS utilizing the GLM procedure. Xiang and Li (2001) have also developed a program in SAS utilizing PROC MIXED. Some authors have also referenced a program written by Burrow and

Coors (1994). Magari and Kang (1994) also reported a program in BASIC for analysis of Griffing's models.

Availability of root-knot nematode resistant cultivars can be very helpful to farmers who wish to incorporate legumes in grass dominated pastureland. The availability of a new white clover cultivar showing tolerance to southern RKN can be advantageous for producers. The existence of multiple populations of RKN requires the screening of this new cultivar to all those economically important RKN populations. The understanding of the inheritance pattern of the resistance to RKN helps in the further breeding attempts. Thus the focus of this research was characterization of the response of UFWC5 to multiple RKN species/races, and study of the quantitative basis of inheritance of resistance to those populations.

CHAPTER 3
COMPARISON OF OSCEOLA AND UFWC5 FOR RESPONSE TO DIFFERENT SPECIES/RACES OF ROOT-KNOT NEMATODE

Abstract

White clover (*Trifolium repens* L.) is a major forage crop of the southeastern USA, including Florida. Although it is a cool season perennial legume it acts as an annual in Florida. White clover is one of the most nutritious forages available and is generally mixed with grasses to increase their nutritive value. There are many constraints to white clover production. Root-knot nematodes (*Meloidogyne* spp., RKN) can be a factor adversely affecting the white clover growth, stolon density, persistence, seedling vigor, nitrogen fixing ability, and phosphorus utilization. Root-knot nematodes are endoparasites that have a diverse host range. In addition to their direct invasion, they create wounds that can lead to infection by secondary pathogens. No nematicides are labeled for pastures but even if available, it is likely their use would be cost prohibitive. Thus, development of resistant varieties appears to be the best solution to enhance field production and persistence of white clover. The cultivar UFWC5 was developed by recurrent phenotypic selection for reduced RKN galling and was recently released primarily on the basis of improved tolerance to root-knot nematodes. This research compared UFWC5 and the commercial cultivar 'Osceola' for response to six different RKN species and/or races (herein after called RKN populations). Ninety-eight plants of UFWC5 and of Osceola were planted in Cone-tainers® (Steuwe and Sons, Inc., Tangent, OR) in a randomized complete block design to access response to each RKN population. Three weeks after germination, 98 plants of UFWC5 and of Osceola were inoculated with ca. 500 eggs (ca. 3 eggs cm^{-3} of soil) of each RKN population. Nine weeks after inoculation, data were collected for shoot growth, root growth, egg mass score, gall score and eggs per plant. Differences in response to all six RKN species/races were observed for egg mass score, gall score and eggs per plant with UFWC5 being lower than

Osceola for all comparisons. The largest reduction in gall score and egg mass score between Osceola and UFWC5 were observed in response to the four races of *M. incognita*, the species that was used in the selection process.

Introduction

'Osceola' is an established cultivar of white clover (*Trifolium repens* L.). Although it has been planted for over 20 years and has many useful traits it lacks resistance to root-knot nematodes (RKN). Root-knot nematodes (*Meloidogyne* spp.) may be a limiting factor to the growth and establishment of white clover in the southeastern USA. There have been previous selection efforts to breed for resistance to RKN in white clover. Bain (1959) evaluated lines of white clover seedlings and selected genotypes with tolerance to RKN. Gibson (1973) developed SC-1 white clover, which was reported to be tolerant to southern RKN. The SC-1 population was a first generation recombination among 145 genetically diverse white clover clones selected for tolerance to southern RKN. Those clones were screened from thousands of plants from white clover cultivars and foreign introductions. Studies from Windham and Pederson (1989) showed that SC-1 was only moderately tolerant to two of eight populations of *M. incognita*. This different response compared to the results of Gibson may be due to the fact that different races were used by Windham and Pederson that overcame the resistance in SC1. These results show that there is a need for selection and evaluation using all the predominant races and populations of RKN. UFWC5 is a new cultivar derived from Osceola through five cycles of phenotypic recurrent selection for resistance to Southern RKN (*M. incognita)* (Wofford and Ostmark, 2005).

Among more than 100 species of *Meloidogyne*, four species account for more than 95% of worldwide RKN population. Southern RKN (*M. incognita*) accounts for 51% of the worldwide population, Javanese RKN (*M. javanica*) accounts for 31% and *M. arenaria* and *M. hapla* each contribute 8% (Sasser et al., 1983). In some species, host specific races are found that cannot be

differentiated morphologically but can through host differentiation tests. These are known as physiological races. Four host races of *M. incognita* and two races of *M. arenaria* have been defined with their host specificity to a particular set of hosts (Sasser et al., 1983) when the populations were subjected to North Carolina Host Differential Test (Hartman and Sasser, 1985). *Meloidogyne incognita* race 1 comprised about 72% of all *M. incognita* populations whereas *M. incognita* race 2, *M. incognita* race 3 and *M. incognita* race 4 accounted for 13%, 13% and 2%, respectively (Sasser et al., 1983).

The plant response to the RKN can be assessed by the amount of galling and the host plant effects on the RKN lifecycle can be accessed by egg and egg mass production. Mani (as cited by Bird, 1979) described galls as the physiologically developed cells, tissues or organs of plants that mostly arise by hypertrophy and hyperplasia under the influence of a parasitic organism. After a RKN infective juvenile (J2) establishes inside the root, it will typically form galls. With normal life cycle progress, females reproduce by laying eggs in a gelatinous egg mass on the root surface (de Guiran and Ritter, 1979). Thus galling can be viewed as a measure of the response of the plant to RKN infection, and egg mass production can be viewed as a measure of RKN ability to reproduce on a given host. A single egg mass may contain 200 to 300 nematode eggs (Taylor and Sasser, 1978). Therefore, egg counts are more representative of RKN reproduction than egg mass score alone, but egg extraction for counting is a labor and time consuming variable to determine.

The cultivar UFWC5 was originally selected for resistance to *M. incognita* race 4. As discussed above regarding the existence of multiple economically important populations of RKN, it is necessary to screen any cultivar with as many of the RKN populations as possible. In this research, we tested the response of UFWC5 in comparison to Osceola to *M. arenaria* race 1

(peanut RKN), *M. javanica* (Javanese RKN) and 4 races of *M. incognita (M. incognita* race 1, *M. incognita* race 2, *M. incognita* race 3 and *M. incognita* race 4).

Materials and Methods

To test the response of white clover cultivars to each isolate of RKN, 98 seeds of Osceola and an equal number of UFWC5 were planted in Ray Leach Cone-tainers® (ca. 150 cm^3 soil volume) (Stuewe & Sons Inc., Tangent, OR) and placed in RL98 trays (Stuewe & Sons Inc., Tangent, OR) for support. Before planting, the seeds were gently scarified. The cone-tainers were filled with commercial building sand. Two weeks after germination of the seeds, the plants were inoculated with ca. 500 eggs of the appropriate *Meloidogyne* populations. Just prior to inoculation, the cultivars UFWC5 and Osceola were arranged in a randomized complete block design of 7 replications and 14 plants per replication. Each of the six different RKN populations used (*M. incognita* race 1, *M. incognita* race 2, *M. incognita* race 3, *M. incognita* race 4, *M. arenaria* race 1, and *M. javanica*) was treated as a separate randomized complete block experiment. Due to nematode containment issues, a second environment consisting of 7 replications of 7 plants each of Osceola and UFWC5 was planted at the same time and compared as a non-inoculated control for shoot and root weights only. An extra flat of Osceola was planted and inoculated at the same time and evaluated for nematode symptom progression to determine the appropriate time for termination of the experiment. The experiment was terminated when most of the check Osceola plants showed a root galling score and egg mass score between 3 and 5.

Nematode Egg Extraction and Inoculation

The six different RKN populations were maintained in a separate greenhouse from that used for the experiment. These nematodes were maintained on RKN susceptible 'Rutgers' tomato (*Solanum lycopersicom*). Nematodes were extracted from the plants which had been

inoculated on 9 to 10 weeks earlier. The tomato plant was uprooted gently and the root system was washed gently. After washing, the roots were cut into small pieces about 2 cm in length. Then roots were placed in a blender with 0.25% chlorine to break the proteinous gel of the egg mass and blended for 20 seconds. Sieves of 500, 200 and 50-mesh size were stacked together with 50-mesh on top and 500-mesh on bottom. The blended solution was poured through this sieve stack and washed for 2 to 3 minutes with tap water to remove most of the chlorine. The residue remaining on the 500-mesh sieve (primarily RKN eggs) was collected in a beaker and diluted. The concentration of the nematode eggs was determined using a hemocytometer slide (1 ml volume composed of 24 grids). Four random grids were counted at 40× on a compound microscope. This process was repeated three times and the counts were then averaged and multiplied by 24 to estimate the total nematode eggs in 1 ml solution. This number was then multiplied by the total volume of solution to obtain the total number of eggs extracted.

The extracted egg solution was then diluted so that a 3 ml injection contained ca. 500 eggs and this volume was injected into each cone-tainer containing two-week old seedlings. This solution was placed in a 3.5 L beaker with a magnetic stirrer inside it to keep the egg suspended in solution while inoculating. All the six races were inoculated by this procedure. The inoculum concentration of *M. incognita* race 1 was ca. 1000 eggs per plant. A tray with Osceola was also inoculated with the respective race of RKN as a susceptible control. Between any two subsequent extractions and inoculations, all the apparatus were cleaned using chlorine to kill the previous nematode eggs and prevent any cross inoculation.

Maintenance

These plants were regularly fertilized and irrigated until they were ready for data collection as determined by the root galling of the Osceola. The fertilizer used was Peters® 20:20:20 $N:P_2O_5:K_2O$. A diluted solution of 1.5 g L^{-1} of N, P_2O_5, K_2O was applied as irrigation weekly.

The plants were also treated for thrips, mites, aphids, white flies, army worms and other worms, and snail. We used tank mixture of Avid® (0.5 mL L^{-1}), Parmethrin® (0.5 mL L^{-1}), Conserve® (1.5 mL L^{-1}), Mavrick® (0.5 mL L^{-1}) for mite control. We used tank mixture of Enstar® (0.8 mL L^{-1}), Parmethrin® (0.5 mL L^{-1}), Conserve® (0.8 mL L^{-1}) for aphids and white fly control. We used tank mixture of Enstar® (0.8 mL L^{-1}), Parmethrin® (0.5 mL L^{-1}), Conserve® (1.5 mL L^{-1}), Mavrick® (0.5 mL L^{-1}) for thrips control. We also used Xentari® (2.5 mL L^{-1}) for controlling army worm. Ortho® (Bug-Geta bait) was used for snail and slug control.

Data Collection

Depending upon the nematode population, the plants were evaluated 8 to 10 weeks after inoculation. The root system of plants were carefully removed from container and washed. Roots were then immersed in a solution of 0.05% red food color (McCormik & Co®, Hunt Valley, MD). Although other researchers have used Phloxine-B to stain egg masses (Holbrook et al., 1983), we found the red food color to be equally effective with a reduced level of toxicity than that of Phloxine-B. Individual plants were given a score for egg mass and galls. They were scored as 0 = 0 galls/egg masses, 1 = 1 to 2 galls/egg masses, 2 = 3 to 10 galls/egg masses, 3 = 11 to 30 galls/egg masses, 4 = 31 to 100 galls/egg masses and 5 = more than 100 galls/egg masses (Taylor and Sasser, 1978). After scoring, the individual plants were separated into root and shoot. All shoots from a replication were placed in paper bag and dried at 50°C to constant weigh. Root parts of a replication were also collected in a plastic bag and processed further for egg extraction.

The root systems of a replication were cut into smaller pieces of about five centimeters and mixed with 0.5% chlorine solution. This was blended for 20 seconds and sieved through the stack of three sieves of 500, 200 and 50-mesh size. Residue from the bottom (500-mesh size)

sieve containing the nematode eggs was collected in a tube for counting. The macerated roots were collected in a paper bag and also dried at 50°C to constant weight.

The egg solution collected in the tube was brought to a fixed volume and counted using the same procedure described above for inoculation. The scoring of galls, egg masses and egg counting were done by different individuals who were always associated with replications.

Data were analyzed as a randomized complete block using the GLM procedure in SAS. The means were separated using Duncan's critical range (CR). We compared the shoot and root weights of the inoculated vs non-inoculated Osceola and UFWC5 using a model of entries nested within inoculation treatments.

Results and Discussion

An analysis of variance showed that there were significant ($P < 0.01$) differences in gall scores and egg mass scores between Osceola and UFWC5 for all RKN populations (Table 3-1). Osceola showed a higher degree of susceptibility for both variables. Although the differences were statistically significant ($P < 0.01$) in all races, there was a marked difference in the egg mass scores when these plants were inoculated with any of the *M. incognita* races while less marked for plants inoculated with *M. arenaria* race 1 and *M. javanica*. This is likely due to the fact that UFWC5 was originally selected using race 4 of *M. incognita.* For the gall scores, there was also marked difference between Osceola and UFWC5 for all races of *M. incognita* except race 1 although it was statistically significant ($P < 0.01$). There was also a marked difference in the means gall score of Osceola and UFWC5 when these plants were inoculated with *M. arenaria* race 1 and *M. javanica*. The plants with scores 0, 1 or 2 are categorized as resistant and 3, 4 and 5 are categorized as susceptible (Taylor and Sasser, 1978). If one follows this convention, UFWC5 would be categorized as resistant to all the *M. incognita* races. For *M. arenaria* race 1 and *M. javanica*, the mean scores are in between 2 and 3, so UFWC5 cannot be

categorized as resistant to these species but they show significantly lower scores of gall and egg mass than Osceola. There was a strong correlation between the egg mass score and gall score for Osceola ($r = 0.73$, $P < 0.001$) while there is less correlation between those two variables for UFWC5 ($r = 0.35$, $P < 0.001$) when inoculated with *M. incognita* race 1. This may signify for UFWC5 that although nematodes enter the plant root and initiate galling, they may not have matured to egg producing females. This difference between Osceola and UFWC5 was also found when plants were inoculated with *M. arenaria* race 1. While for the other races of *M. incognita* and for *M. javanica*, the correlations between the gall scores and egg mass scores were similar for both Osceola and UFWC5 (Table 3.2). The differences in *M. incognita* race 1 may be due to the fact that the plants were inoculated with ca.1000 eggs per plant (about double than other populations). A higher amount of inoculum might have lead to higher galling and reproduction in Osceola. Although the higher galling was achieved in UFWC5, the nematodes might not have completed their lifecycle to produce eggs. The similar result of *M. arenaria* race 1 could be due to the reported aggressiveness of this species.

The eggs plant^{-1} were significantly ($P < 0.001$) reduced in UFWC5 compared to Osceola when inoculated with any race. Eggs plant^{-1} were reduced by 50% in UFWC5 compared to Osceola when inoculated with *M. incognita* race 1 and was reduced by ca. 70% when inoculated with *M. arenaria* race 1. When inoculated with other four RKN populations reductions were ca. 80 to 90% (Table 3.1). Although the numbers of eggs per plant were still high enough to maintain the population in the soil, the reduced numbers on UFWC5 compared to Osceola should give improved stand persistence in UFWC5.

As expected there were no galls, egg masses and nematode eggs in the non-inoculated Osceola or UFWC5. The shoot weights were not significantly different between the inoculated

and non-inoculated plants of Osceola for all the races used except for *M. incognita* race 4 (P = 0.03) (Table 3.3). Similarly, the shoot weights in UFWC5 were not significantly different between the inoculated and non-inoculated for any RKN population used. But there was a significant difference in the root weights of Osceola with higher weights in non-inoculated roots in comparison to those when inoculated with *M. incognita* race 2 (P = 0.002) and *M. incognita* race 3 ($P < 0.001$). For UFWC5, the roots showed a significantly lower weight in the inoculated treatment when inoculated with *M. incognita* race 2 (P = 0.0002), *M. incognita* race 3 ($P <$ 0.001), *M. incognita* race 4 (P = 0.007) and *M. javanica* (P = 0.01). Except for these, there was no significant difference between inoculated and non-inoculated UFWC5 for the other RKN populations although the roots tended to weigh higher in the inoculated treatments (Table 3.3). The reason for higher root weight on non-inoculated treatments than inoculated is likely due to the decay of plant root system. The *M. incognita* race 2 and *M. incognita* race 3 were harvested at ten weeks versus eight weeks for other races. Thus there may have been an opportunity for additional disease development and decay of root system in the plants inoculated with these two races leading to the higher root weights in the inoculated treatment.

There were no significant differences between the shoot and root weights of non-inoculated Osceola vs UFWC5 (Table 3.3). This fact leads us to believe that yield and production characteristics were not altered by the selection for RKN resistance in Osceola.

UFWC5 showed resistance to the races of *M. incognita* studied under these greenhouse condition. Although UFWC5 cannot be classified as resistant (score of 2.0 or less) to *M. javanica* and *M. arenaria* race 1, it did demonstrate reduced galls and egg masses compared to Osceola for these nematode populations. UFWC5 can be utilized with a high level of confidence in southern root-knot nematode infested areas while there may be need for further cycles of

selection for resistance to peanut and northern root-knot nematode populations. With very short growth period in the greenhouse and with good irrigation and fertilization, the shoot and root growth was not observed to be impacted by root-knot nematodes, but with a longer growth period and exposure to moisture stress the root system will ultimately decay or become less functional for translocation of minerals and water; and thus, will likely effect yield and stand persistence.

Additional study of the mechanism(s) of reduction in root galling, egg mass production and egg number production would seem to be fruitful areas for research. Call (1997) showed pre-infectional or early post-infectional resistance indicated by lower penetration (as measured by gall score) and post-penetration resistance showed by delayed maturation, lower fecundity rates and fewer adult females (as measured by egg mass score) in red clover selected for resistance to RKN. Similar types of mechanisms may have been involved in expression of resistance RKN in UFWC5 white clover. Further research on the mechanism(s) of resistance should also be fruitful.

Table 3-1. Egg mass score, gall score and eggs plant^{-1} of Osceola and UFWC5 white clover when inoculated with six different root-knot nematode populations.

RKN population	Cultivar	Egg mass score	Gall score	Eggs plant^{-1}
M. incognita race 1	Osceola	3.5†a*	2.6a	17,600a
	UFWC5	0.9b	2.0b	9,200b
	CR‡	*0.3*	*0.3*	*5,500*
M. incognita race 2	Osceola	3.8a	3.8a	28,700a
	UFWC5	1.3b	1.7b	4,400b
	CR	*0.2*	*0.3*	*9,000*
M. incognita race 3	Osceola	3.7a	3.7a	22,300a
	UFWC5	0.6b	1.0b	2,300b
	CR	*0.3*	*0.3*	*3,000*
M. incognita race 4	Osceola	3.9a	3.3a	44,200a
	UFWC5	1.6b	1.0b	4,800b
	CR	*0.3*	*0.3*	*12,000*
M. arenaria race 1	Osceola	3.9a	3.5a	28,800a
	UFWC5	2.6b	2.4b	8,900b
	CR	*0.2*	*0.2*	*3,600*
M. javanica	Osceola	4.0a	4.2a	71,900a
	UFWC5	3.0b	2.6b	14,600b
	CR	*0.2*	*0.2*	*9,700*

* Means followed by different letters are different ($P = 0.05$).

† Egg masses and galls were rated on a 1 to 5 scale where 0 = 0 galls or egg masses, 1 = 1 to 2 galls or egg masses, 2 = 3 to 10 galls or egg masses, 3 = 11 to 30 galls or egg masses, 4 = 31 to 100 galls or egg masses and 5 = more than 100 galls or egg masses

‡ CR: Duncan's critical range ($P = 0.05$)

Table 3-2. Correlations between gall scores and egg mass scores of Osceola and UFWC5 white clover when inoculated with six RKN populations.

	Combined	Osceola	UFWC5
M. incognita race 1	0.56 ***	0.73***	0.35***
M. incognita race 2	0.87***	0.61***	0.69***
M. incognita race 3	0.86***	0.65***	0.69***
M. incognita race 4	0.89***	0.65***	0.67***
M. arenaria race 1	0.54***	0.87***	0.33***
M. javanica	0.69***	0.43***	0.46***

*** significance in < 0.001 probability level.

Table 3-3. Response in the shoot and root growth of Osceola and UFWC5 white clover when inoculated with six different populations of root-knot nematodes.

Nematode population	Varieties	Shoot			Root		
		Inoculated	**Non-inoculated**	***CR*†**	**Inoculated**	**Non-inoculated**	***CR***
		-g-	-g-		-g-	-g-	
M. incognita race 1	Osceola	1.05	1.15	*0.21*	0.40	0.31	*0.15*
	UFWC5	0.94	0.95	*0.01*	0.35	0.27	*0.15*
	CR‡	*0.16*	*0.14*		*0.14*	*0.15*	
M. incognita race 2	Osceola	1.36	1.26	*0.22*	0.44	0.80	*0.14*
	UFWC5	1.29	1.27	*0.19*	0.39	0.83	*0.01*
	CR	*0.17*	*0.3*		*0.10*	*0.13*	
M. incognita race 3	Osceola	1.22	1.26	*0.22*	0.40	0.80	*0.15*
	UFWC5	1.35	1.27	*0.43*	0.36	0.83	*0.19*
	CR	*0.26*	*0.30*		*0.16*	*0.13*	
M. incognita race 4	Osceola	0.99	1.17	*0.17*	0.36	0.45	*0.13*
	UFWC5	1.00	1.16	*0.24*	0.34	0.47	*0.08*
	CR	*0.18*	*0.23*		*0.06*	*0.16*	
M. arenaria race 1	Osceola	1.21	1.15	*0.14*	0.44	0.31	*0.12*
	UFWC5	0.91	0.95	*0.07*	0.33	0.27	*0.07*
	CR	*0.10*	*0.16*		*0.06*	*0.15*	
M. javanica	Osceola	1.02	1.17	*0.22*	0.39	0.45	*0.14*
	UFWC5	0.98	1.16	*0.19*	0.35	0.47	*0.08*
	CR	*0.19*	*0.22*		*0.08*	*0.16*	

† CR: Duncan's critical range ($P = 0.05$) between inoculated and non-inoculated plants

‡ CR: Duncan's critical range ($P = 0.05$) between UFWC5 and Osceola white clover plants

CHAPTER 4
QUANTITATIVE GENETIC BASIS OF INHERITANCE OF RESISTANCE IN WHITE CLOVER TO SOUTHERN ROOT-KNOT NEMATODE

Abstract

White clover (T*rifolium repens* L.) is an important forage crop. Root-knot nematodes (*Meloidogyne* spp.) can be a major factor limiting white clover production and persistence. This study was conducted to determine the genetic basis of inheritance of resistance to *M. incognita* race 4 on white clover. Eight parents composed of three resistant, two intermediate and three susceptible clones were crossed in partial diallel design and progeny of those 28 crosses were evaluated for egg mass score, gall score, eggs g^{-1} dry root weight, eggs $plant^{-1}$, shoot weight and root weight. Progeny from crosses were arranged in a randomized complete block design with 5 replications each consisting 14 individual plants. Two weeks after germination, plants were inoculated with ca. 500 *M. incognita* race 4 eggs. To serve as a non-inoculate control, cross progeny were arranged in another randomized complete block design with 3 replications each consisting 7 plants. The analysis of egg mass score, gall score, eggs per gram dry root weight and eggs per plant showed that both general combining ability (GCA) and specific combining ability (SCA) were significant in the expression of those variables. With a very high GCA:SCA ratio, additive effects were more important than non-additive effects for the inheritance of the above traits. The GCA effects were related with previously classified resistance reaction to the southern RKN. Root weights in inoculated and non inoculated plants were significantly different with inoculated roots being heavier. For root weights, both GCA and SCA were significant, with a lower GCA:SCA ratio indicating the reduced importance of additive effects. The GCA values of parents were not in the same direction as previously mentioned variables and also did not match the previously classified resistance reaction. This suggested that root weight is not a good

variable to select for resistance to RKN in early growth stages. The shoot weights did not show any significant differences between inoculated and non-inoculated white clover parents.

Introduction

White clover (*Trifolium repens* L.) is one of the major legume forage crops worldwide and also in the southeastern USA including Florida. Although it is a cool season perennial legume, it generally behaves as a reseeding annual in Florida. It is suitable for hay, silage, green chop and importantly for grazed pastures. It has a higher crude protein and digestibility than grasses and can be an important component of Florida pastures (Chambliss and Wofford, 2006).

Among several pathological problems that may hinder the production and persistence of white clover, root-knot nematodes (RKN, *Meloidogyne* spp.) can be an important factor, especially on light textured soils common in Florida. There are four predominant species of root-knot nematodes that account for more than 95% of the world distribution. They are *M. incognita* (Kofoid and White) Chitwood, *M. arenaria* (Neal) Chitwood, *M. javanica* (Treub) Chitwood and *M. hapla* Chitwood (Sasser et al., 1983).

In some RKN species, host specific races are found and *M. incognita* is one such species with four races. These races cannot be differentiated morphologically but can be through host differentiation tests. These are known as physiological races. Four host races of *M. incognita* have been defined with their differential host specificity when subjected to the North Carolina Host Differential Test (Hartman and Sasser, 1985) composed of a particular set of hosts. When a large number of *M. incognita* populations were subjected to the test, *M. incognita* race 1 comprised about 72% of all *M. incognita* populations whereas *M. incognita* race 2, *M. incognita* race 3 and *M. incognita* race 4 accounted for 13%, 13% and 2%, respectively (Sasser et al., 1983). *Meloidogyne incognita* race 4 is less aggressive than other races of this species which

could be utilized to select for resistance to southern RKN in white clover (Windham and Pederson, 1989).

Meloidogyne incognita is distributed worldwide in tropical and other warm regions. They also have a wide host range attacking nearly all cultivated plant species in warmer regions (Sasser et al., 1983).

Since RKN invade and damage fine roots, the RKN infected plants wilt easily, become stunted and may die. Symptoms of chlorosis may also be seen. The RKN damage in infected fields often is manifested as patches of dead plants indicating localized areas of high infection. A clear sign of root-knot nematodes is that the roots are swollen due to galling and have a knot like appearance (Thorne, 1961).

Control of RKN disease can be very difficult, nevertheless the most effective control will be the combination of all available control measures including resistant cultivars. Bain (1959) evaluated lines of white clover seedlings and selected for RKN tolerance. Gibson (1973) developed 'SC-1' from the selection of wide pool of white clover germplasms which was reported to be resistant to RKN. Mercer et al., (2000) gained some success in selecting white clover strains resistant to *M. trifolia* (previously identified as *M. hapla*). Pederson and Windham (1995) released 'MSNR4' after four cycles of recurrent selection from a wide genetic base of white clover germplasm. This population was shown to be resistant to *M. incognita, M. arenaria* and *M. graminicola.* The cultivar 'UFWC5', which was reported to be resistant to southern RKN, was also developed by recurrent phenotypic selection using 'Osceola' as the base population and southern root-knot nematode (*M. incognita* Race 4) as the selective pathogen (Wofford and Ostmark, 2005).

Understanding the inheritance pattern of RKN resistance and understanding the importance of additive and non-additive effects in inheritance of RKN resistance should improve progress from selection in a breeding program to enhance RKN resistance. Partitioning the genetic variability to General Combining Ability (GCA) and Specific Combining Ability (SCA) effects would help understand the genetics conditioning resistance. Such information should be helpful in development of synthetic varieties that are common in white clover (Baker, 1978). The GCA provides a measure of the additive variation and SCA provides a measure of the non-additive variations. Griffing (1956) has given a procedure to differentiate these combining abilities using diallel crosses. This procedure has been utilized in many crops to understand the inheritance pattern. The objective of this research was to estimate the GCA and SCA effects on expression of host-pathogen interaction responses using a set of white clover diallel cross progeny inoculated with *M. incognita* race 4.

Materials and Methods

Selection of Parents

Seeds of UFWC5 were planted in Cone-tainers® (Stuewe and Sons, Inc., Tangent, OR) filled with fine commercial building sand. Two weeks after germination, the seedling plants were inoculated with ca. 500 eggs of *M. incognita* race 4. Eight weeks later, these plants were carefully taken out from each container. The root systems were rinsed in water to remove sand and then immersed in a solution of 0.05% red food color (McCormik & Co.®, Hunt Valley, MD) to stain and highlight the egg masses. The number of egg masses and galls were counted and the plants were classified. The plants with 0 to 5 galls or egg masses were classified as resistant, plants with 6 to 30 as intermediate and those with more than 30 galls or egg masses as susceptible (Call et al., 1997). Eleven resistant, eleven susceptible and nine intermediate plants were selected. These plants were then planted in 15-cm diameter pots. Two to five clonal

cuttings of each plant were produced and planted to other 15-cm diameter pots. These pots were maintained in a pollinator free greenhouse.

Crossing

Flowers were not emasculated prior to making crosses, since white clover is known to be relatively self incompatible. Hand crosses were made with the aid of a toothpick and Emory paper glued to the flat surface of the toothpick as described by Taylor (1980). Attempts were made to complete all possible crosses within these 31 parents. As white clover is self incompatible, no selfs were made and attempts at selfing yielded only 6 seeds from about 100 flower heads, each head containing 30 to 40 flowers (ca. 3000 to 4000 total flowers). Under short day conditions, artificial light was used to extend the daylength to 16 hours in the greenhouse to ensure the flowering in white clover as it is known to be a long day flowering plant.

At 20 to 30 days after pollination, the flower heads were harvested and seeds were hand threshed. These seeds were collected in small paper bags, labeled by crosses and replications and stored. The seeds of reciprocal crosses were combined.

Although we attempted to complete all crosses among the 31 white clover clones, only progeny from eight clones were used for this diallel experiment. The availability of enough seeds from every cross for a half diallel design was the major factor determining the number of parents. We also chose to use a larger number of progeny of each cross in each replication, rather than attempting analysis with a large number of crosses.

Inoculation

Eight parents, consisting of three resistant (R1, R4, R7), two intermediate (M1, M3) and three susceptible (S1, S3, S7), were used in this diallel experiment. Ninety-one plants of each cross, from a total of 28 crosses, were planted in the cone-tainers. Prior to inoculation, plants were arranged in a randomized complete block design with 5 replications of 14 plants each for

inoculation. Due to nematode containment issues, non-inoculated controls were arranged in a different randomized complete block design with 3 replications of 7 plants each. We also planted and inoculated 98 Osceola plants as check plants to monitor the extent of galling and egg mass production on susceptible plants. After two weeks of seedling growth, the progeny plants of 28 crosses arranged for inoculation were inoculated with ca. 500 eggs of *M. incognita* race 4 with the aid of a continuous flow syringe as described previously in chapter three. The source inoculum was maintained in a separate greenhouse and eggs were extracted with the same method described in chapter three.

Data Collection and Analysis

The diallel experiment was terminated when most plants of check Osceola showed a gall score and egg mass score between 3 and 5. Variables evaluated were egg mass score, gall score, eggs g^{-1} dry root weight and eggs $plant^{-1}$. Individual plants were scored for egg mass and gall numbers. The scores used were 0 = 0 galls or egg masses, 1 = 1 to 2 galls or egg masses, 2 = 3 to 10 galls or egg masses, 3 = 11 to 30 galls or egg masses, 4 = 31 to 100 galls or egg masses and 5 = more than 100 galls or egg masses (Taylor and Sasser, 1978). All plants in a replication were pooled for egg extraction and the eggs were counted on a replication basis with the aid of a hemocytometer slide. Four grids on the hemocytometer slide were counted, and 3 sub-samples from each replication were counted and averaged to calculate total egg numbers extracted from each replication of each progeny. The egg counts were then divided by the dry root weight to obtain eggs g^{-1} dry root weight. Although the experiment was initiated with 14 plants in each replication, all did not survive. Thus at the time of termination and we divided the egg count by the number of surviving plants to obtain the eggs $plant^{-1}$ variable. The data collection procedure was as described in chapter three. Individuals were associated with replications for counting egg masses and gall numbers and for counting egg numbers with the microscope.

The data analysis was conducted based on Griffing's method 4 model I (Griffing, 1956) using the SAS code as described by Zhang et al. (2005).

Results and Discussions

Egg Mass Score

An analysis of variance for the variable egg mass score showed that there were significant ($P < 0.001$) differences both due to the replication and crosses (Table 4-1). The replication effects may be due to environmental effects inside the greenhouse or to differences in how individuals visualized and scored egg masses. Any effects due to individuals may also contribute to significant replication effects seen for other response variables. The cross effects were partitioned into the GCA effect and the SCA effect. Both effects were a significant source of variation with $P < 0.001$ (Table 4-1). The contribution due to GCA effect (or GCA:SCA ratio) was 0.9. A GCA:SCA ratio closer to unity signifies the higher importance of additive effects than SCA effects, additive effects were more important in the expression of egg mass production in white clover roots inoculated with *M. incognita* race 4.

All the parents had significant ($P < 0.001$) GCA effects and thirteen out of twenty eight SCA effects were significant (Table 4-2). Seven out of thirteen significant SCA effects were negative indicating those cross combinations reduced the egg masses from the expected from their GCA effects. The progeny from cross combination of resistant parent R1 (GCA = -0.7) with other resistant parent R7 (GCA = -0.6) produced higher egg masses (SCA = 0.2) than expected from GCA of those parents. The progeny from resistant parent R4 (GCA = -0.6) produced more egg masses (SCA = 0.2) when combined with a susceptible parent M1 (GCA = 0.2) while the same parent produced less egg masses (SCA = -0.3) when combined with other susceptible parent S1 (GCA = 0.3). Progeny from the most of the resistant by susceptible crosses (R4S1, R4S3, R7M1, M3S3, and M3S7) tended to produce less egg masses than expected from their

GCA. This result may suggest the dominance or partial dominance of resistance genes over the susceptible ones.

The average egg mass score from all the progeny of each resistant parent was less than 2.0 (Table 4-3) while the egg mass scores from all the progeny from two susceptible parents S3 and S7 produced an average of more than 3.0. Based on the averages from the progeny, the best individual cross combination was R1R4 (average egg score = 0.9) and the worst cross combination was S3S7 with average egg score 4-2.

Gall Score

An analysis of variance for the variable gall score showed that there were significant ($P < 0.001$) differences both due to the replication and crosses (Table 4-1). The cross effects were partitioned into the GCA effect and SCA effect. Both effects were significant source of variation with $P < 0.001$ (Table 4-1). The contribution due to GCA effect (or GCA:SCA ratio) was 0.88. The GCA:SCA ratio closer to unity signifies that the additive effects were more important in the expression of gall production.

The analysis for the individual GCA and SCA effects resulted in significant ($P < 0.001$) GCA effects of all eight parents (Table 4-4). Progeny from only six out of nine significant cross combinations produced significantly fewer galls. As we discussed for egg mass score, progeny from most of the resistant by susceptible cross combinations also produced fewer galls. No progeny of susceptible by susceptible or resistant by resistant crosses had the significant SCA effects. This suggests that the behavior of gall production in progeny can be well described with the GCA effects alone. This suggests the importance of additive effects in the inheritance of gall production in white clover.

The average gall scores of all the progeny from resistant parents R1 and R4 was 2.1 each, while the average gall scores of all the progeny from susceptible parents S3 and S7 was 3.3 each

(Table 4-5). The best cross combination producing the least gall scores was R1R4 (mean gall score = 1.4) while the cross combination producing the highest gall score (mean gall score = 4.3) was S3S7 which was also true based on the egg mass score variable.

Eggs g^{-1} Dry Root Weight

The variable eggs g^{-1} dry root weight was log transformed to meet the normality requirements for the analysis. Both the replication effect and cross effects were significant ($P < 0.001$) source of variation (Table 4-6). The partition of the cross effect variance resulted in significant GCA effects ($P < 0.001$) and significant SCA effects ($P < 0.001$). The GCA:SCA ratio was 0.8 signifying the relatively higher importance of additive effects in the inheritance of egg production in white clover.

All eight parents had significant GCA effects ($P < 0.001$) (Table 4-7). All three parents classified as resistant had negative GCA effects and all three parents classified as susceptible had positive GCA effects. One of the parents (M1) previously classified as intermediately resistant to *M. incognita* race 4 had positive GCA score (0.22) and another (M3) had negative GCA score (-0.39). Progeny from seventeen individual crosses showed the significant SCA score on which seven were on desirable direction (reduced egg). The progeny from the resistant parent R1 (GCA = -0.85) crossed with susceptible parent S1 (GCA = 0.46) reduced the egg production from the expected while the same parent R1 resulted in progeny with increased egg production when crossed with another susceptible parent S3 (GCA = 0.88). The progeny from cross combination R1R4 resulted in higher (SCA = 0.24) egg production than expected from the parents' GCA effects.

The mean eggs g^{-1} dry root weight for the progeny of cross R7M3 was least (2,700) while it was highest (60,000) for the progeny of S3S7 (Table 4-8). The average eggs g^{-1} dry root weight of all progeny from R1 crossed with other parents was least (6,900) showing highest level

of resistance while the average eggs g^{-1} dry root weight of all progeny from S7 crossed with other parents showed the highest (28,200) level of susceptibility.

Eggs Plant^{-1}

The analysis of variance showed that there was a significant difference in eggs plant^{-1} both in between the replications ($P < 0.0001$) and in between the crosses ($P < 0.0001$) (Table 4-6). The variance due to cross effect was again partitioned into GCA and SCA. Both the GCA and SCA showed their significance ($P < 0.0001$) in the expression of the resistance to RKN (Table 4-6). The higher GCA:SCA ratio was high (0.86) indicating the higher importance of additive effects in the inheritance of RKN egg production in white clover.

The effects due to GCA were significant for all parents while SCA were significant only for nineteen crosses (Table 4-9). Again the GCA effects were as expected from the previously classified resistance reaction, R1, R4 and R7 showing negative score suggesting decrease in eggs plant^{-1} and S1, S3 and S7 showing positive score suggesting increased eggs plant^{-1}. Ten out of those nineteen crosses with significant SCA had negative SCA effect indicating that these combinations produced less eggs plant^{-1} than expected from their GCA. The progeny from the cross of two resistant parents R1 (GCA = -0.87) and R7 (GCA = -0.75) produced a more resistant parent (SCA = -0.19) than the expected from GCA effects while the cross of R1 with resistant parent R4 (GCA = -0.5) produced higher eggs plant^{-1} (SCA = 0.25) than expected from GCA. The same resistant parent R1 produced fewer (SCA = -0.58) eggs plant^{-1} when crossed with one susceptible parent S1 (GCA = 0.38) while the same parent produced as higher (SCA = 0.66) eggs plant^{-1} when crossed with another susceptible parent S3 (SCA = 0.66). But, this susceptible parent S3 produced lower (SCA = -0.46) eggs plant^{-1} when crossed with another resistant parent R4 (GCA = -0.5). This difference might suggest that some epistatic effect is also

involved along with additive and dominance effect in the inheritance of RKN resistance in white clover as quantified by eggs plant^{-1}.

The progeny from cross combinations R1R4, R1R7 and R4R7 each produced a mean eggs plant^{-1} less than 1,000 while the cross S3S7 produced ca. 20,000 eggs plant^{-1} (Table 4-10). The best parents for resistance to RKN ware R1 and R7 as indicated by their mean eggs plant^{-1} from all crosses while the most susceptible parent was S7.

Root Weight

An analysis for mean separation between the inoculated and non-inoculated white clover suggested that there was significant difference in the root weights the inoculated plants weighing more (Table 4-11). The mean root weight of inoculated plants was 0.3 g and the mean root weight of non-inoculated plants was 0.26 g and the Duncan's critical difference for mean separation was 0.02. Although this was a statistically significant difference, it is questionable whether this translates to a biologically important difference.

Further analysis with only inoculated plants resulted in significant difference both due to replication and cross effects (Table 4-12). The cross effects were partitioned into GCA and SCA effects which were both significant. The GCA:SCA ratio was only 0.16 indicating that only the additive effects cannot predict the root weight of white clover plants inoculated with *M. incognita* race 4 but non-additive effects are also involved in the inheritance of expression of root weight.

All the parents except one resistant (R1) and one susceptible (S3) showed significant GCA effects (Table 4-13). One parent classified as resistant (R4) showed a negative GCA effect (-0.012) and another parent classified as resistant (R7) showed a positive GCA effect (0.017). Similarly, one parent classified as susceptible (S1) showed a negative GCA effect (-0.031) and another parent classified as susceptible (S7) showed a positive GCA effect (0.059). Both the

intermediate parents showed negative GCA effects. These effects are not consistent with their previous classification and are also not consistent with the results from other variables egg mass score, gall score, eggs g^{-1} dry root weight and eggs $plant^{-1}$. Our result suggested that root weight is not a good variable to select for resistance to *M. incognita* race 4 in white clover. The mean root weights shown in Table 4-14 reflect the above disparities. A likely cause is the fact that roots of susceptible plants with a high amount of galling may weigh more than roots from resistant plants that are mostly fine fibrous roots.

Shoot Weight

An analysis for mean separation between the inoculated and non-inoculated white clover suggested that there was no significant difference in the shoot weights (Table 4-11). Further analysis with only inoculated plants resulted in significant difference both due to replication and cross effects (Table 4-12). The cross effects were partitioned into GCA and SCA effects which were both significant. The GCA:SCA ratio was only 0.005 indicating that additive effect alone cannot predict the inheritance of shoot weight in inoculated white clover.

Five of eight parents showed significant GCA effects and twenty one out of twenty eight cross combinations showed significant SCA (Table 4-15). These GCA effects were not consistent with the previous classification and also not consistent with the results from other variables egg mass score, gall score, eggs g^{-1} dry root weight and eggs $plant^{-1}$. The mean shoot weights shown in Table 4-16 reflect the above disparities. These results suggested that along with root weight, shoot weight is also not a good variable to select for resistance to *M. incognita* race 4 in white clover.

Correlations

There was a high degree of correlation ($r = 0.82$, $P < 0.0001$) between the egg mass score and gall score. This signifies the interrelation of the galls and egg masses produced. There was

also intermediate correlation between egg mass score and eggs plant^{-1} (r = 0.55, $P < 0.0001$) and between gall score and eggs plant^{-1} (r = 0.45, $P < 0.0001$). The higher correlation with egg mass is obvious because the eggs are inside egg mass. There could be a higher correlation between eggs plant^{-1} and egg mass or gall scores if the actual numbers of egg masses or galls were counted instead of using the 0 to 5 scale that leads to subjective variability.

The overall results from this diallel study are similar to those of Pederson and Windham (1992) who found that selected resistant parents produced progeny with the least *M. incognita* reproduction in a diallel study of three resistant and three susceptible plants. Their study also found that additive genetic effects were of much greater importance in inheritance of RKN resistance in white clover than non-additive genetic effects although some degree of epistasis may be involved. A different diallel analysis by Call et al. (1997) using four resistant, three intermediate and two susceptible red clover parents also showed predominantly significant GCA effects and non-significant SCA effects. Some other diallel studies have also identified GCA effects as more important than SCA effects in resistance to RKN (Williams and Windham, 1990; Mcpherson et al., 1995; Zhang et al., 2007).

Table 4-1. Analysis of variance of combining abilities of the variables egg mass score and gall score of selected white clover clones inoculated with *M. incognita* race 4.

Source	DF	Egg Mass Score†	Gall Score
REP	4	7.83***	25.48***
Cross	27	47.17***	34.34***
GCA	7	168.13***	117.60***
SCA	20	3.89***	3.41***
Error		0.80	0.80

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

† Egg masses and galls were rated on a 1 to 5 scale where 0 = 0 galls or egg masses, 1 = 1 to 2 galls or egg masses, 2 = 3 to 10 galls or egg masses, 3 = 11 to 30 galls or egg masses, 4 = 31 to 100 galls or egg masses and 5 = more than 100 galls or egg masses

Table 4-2. General combining ability (GCA) and Specific combining ability (SCA) effects on egg mass scores of three resistant, two intermediate and three susceptible white clover clones inoculated with *M. incognita* Race 4.

	R1†	R4	R7	M1	M3	S1	S3	S7
R1	**-0.7*****‡§	-0.1	0.2*	-0.1	0.1	-0.2*	0.0	0.1
R4		**-0.6***	0.0	0.2*	0.3***	-0.3***	-0.2*	0.1
R7			**-0.6***	-0.5***	-0.2*	0.3***	0.1	0.2*
M1				**0.2***	0.3***	0.1	0.0	0.0
M3					**-0.3***	0.0	-0.2*	-0.3***
S1						**0.3***	0.2	-0.1
S3							**0.8***	0.1
S7								**1.0***

GCA effects are in bold on diagonal.

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

‡ Negative values of GCA and/or SCA indicate that this particular clone and/or cross decreased the egg mass score from the mean and the positive value means it increased.

§ Egg masses were rated on a 1 to 5 scale where 0 = 0 egg masses, 1 = 1 to 2 egg masses, 2 = 3 to 10 egg masses, 3 = 11 to 30 egg masses, 4 = 31 to 100 egg masses and 5 = more than 100 egg masses

Table 4-3. Mean egg mass score of roots of three resistant, two intermediate and thee susceptible white clover inoculated with *M. incognita* race 4.

	R1†	R4	R7	M1	M3	S1	S3	S7
R1	**1.7**‡	0.9	1.1	1.7	1.4	1.7	2.4	2.6
R4		**1.8**	1.1	2.2	1.7	1.7	2.4	2.8
R7			**1.8**	1.3	1.1	2.2	2.6	2.9
M1				**2.5**	2.5	2.9	3.4	3.5
M3					**2.0**	2.3	2.6	2.7
S1						**2.5**	3.6	3.5
S3							**3.0**	4.2
S7								**3.2**

The bold in the diagonal are means of that parent crossed with others.

† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

‡ Egg masses were rated on a 1 to 5 scale where 0 = 0 egg masses, 1 = 1 to 2 egg masses, 2 = 3 to 10 egg masses, 3 = 11 to 30 egg masses, 4 = 31 to 100 egg masses and 5 = more than 100 egg masses

Table 4-4. General combining ability (GCA) and Specific combining ability (SCA) effects on gall scores of three resistant, two intermediate and three susceptible white clover clones inoculated with *M. incognita* Race 4.

	R1†	R4	R7	M1	M3	S1	S3	S7
R1	**-0.7*****‡§	0.1	0.1	-0.3**	0.0	0.1	0.0	0.1
R4		**-0.6*** **	0.0	0.2*	0.1	-0.3***	-0.2*	0.1
R7			**-0.4*** **	-0.3***	-0.3***	0.3***	0.0	0.2*
M1				**0.3*** **	0.4	0.0	0.1	-0.1
M3					**-0.2*** **	0.1	0.0	-0.3***
S1						**0.1****	0.0	-0.1
S3							**0.8*** **	0.1
S7								**0.7*** **

GCA effects are in bold on diagonal.

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

‡ Negative value of GCA and/or SCA indicate that this particular clone and/or cross decreased the gall score from the mean and the positive value means it increased.

§ Galls were rated on a 1 to 5 scale where 0 = 0 galls, 1 = 1 to 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls and 5 = more than 100 galls

Table 4-5. Mean gall score of roots of three resistant, two intermediate and thee susceptible white clover inoculated with *M. incognita* race 4.

	R1†	R4	R7	M1	M3	S1	S3	S7
R1	**2.1**‡	1.4	1.7	2.0	1.8	2.2	2.7	2.8
R4		**2.1**	1.7	2.5	2.0	1.9	2.6	2.9
R7			**2.4**	2.2	1.9	2.8	3.1	3.2
M1				**2.9**	3.2	3.0	3.8	3.6
M3					**2.5**	2.7	3.3	2.9
S1						**2.8**	3.5	3.4
S3							**3.3**	4.3
S7								**3.3**

The bold in the diagonal are means of that parent crossed with others.

† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

‡ Galls were rated on a 1 to 5 scale where 0 = 0 galls, 1 = 1 to 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls and 5 = more than 100 galls

Table 4-6. Analysis of variances of combining abilities of the variables Eggs g^{-1} and Eggs $plant^{-1}$ of selected white clover clones inoculated with *M. incognita* race 4.

Source	DF	Eggs g^{-1}	Eggs $plant^{-1}$
REP	4	118.51***	62.50***
Cross	27	59.78***	63.05***
GCA	7	201.70***	220.64***
SCA	20	8.37***	5.79***
Error		0.21	0.17

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

Table 4-7. General combining ability (GCA) and Specific combining ability (SCA) effects on log transformed eggs g^{-1} dry root weight of three resistant, two intermediate and three susceptible white clover clones inoculated with *M. incognita* Race 4.

	R1†	R4	R7	M1	M3	S1	S3	S7
R1	**-0.85*** **	0.24**	-0.04	0.02	-0.09	-0.66***	0.66***	-0.14*
R4		**-0.47*** **	0.01	-0.12	0.19**	-0.23**	-0.52***	0.42***
R7			**-0.80*** **	-0.42***	-0.10	0.32***	0.09	0.14*
M1				**0.22*** **	0.24***	0.18*	-0.11	0.22**
M3					**-0.39*** **	0.43***	-0.15*	-0.52***
S1						**0.46*** **	0.06	-0.09
S3							**0.88*** **	-0.03
S7								**0.94*** **

GCA effects are in bold on diagonal.

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

‡ Negative value of GCA and/or SCA indicate that this particular clone and/or cross decreased the eggs g^{-1} dry root weight from the mean and the positive value means it increased.

Table 4-8. Mean eggs g^{-1} dry root weight of three resistant, two intermediate and thee susceptible white clover inoculated with *M. incognita* race 4.

	R1†	R4	R7	M1	M3	S1	S3	S7
R1	**6,900**	3,100	3,100	5,500	2,800	3,900	20,800	8,800
R4		**9,200**	3,100	7,600	5,100	8,400	12,800	24,200
R7			**7,200**	3,700	2,700	9,700	12,800	15,600
M1				**16,700**	10,700	21,900	26,300	41,300
M3					**9,300**	17,100	13,700	13,000
S1						**19,700**	42,900	34,000
S3							**27,100**	60,600
S7								**28,200**

The bold in the diagonal are means of that parent crossed with others.
† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

Table 4-9. General combining ability (GCA) and Specific combining ability (SCA) effects on log transformed eggs plant^{-1} of three resistant, two intermediate and three susceptible white clover clones inoculated with *M. incognita* Race 4.

	R1†	R4	R7	M1	M3	S1	S3	S7
R1	**-0.87*** **	0.25***	-0.19**	-0.13*	0.03	-0.58***	0.66***	-0.04
R4		**-0.50*** **	0.07	-0.14*	0.14*	-0.09	-0.46***	0.24***
R7			**-0.75*** **	-0.10	-0.26***	0.31***	0.02	0.16**
M1				**0.19*** **	0.28***	0.18**	-0.14*	0.05
M3					**-0.47*** **	0.24***	-0.14*	-0.28***
S1						**0.38*** **	0.08	-0.12*
S3							**0.89*** **	-0.01
S7								**1.12*** **

GCA effects are in bold on diagonal.

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

†R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

‡ Negative value of GCA and/or SCA indicate that this particular clone and/or cross decreased the eggs plant^{-1} from the mean and the positive value means it increased.

Table 4-10. Mean eggs plant^{-1} of three resistant, two intermediate and thee susceptible white clover inoculated with *M. incognita* race 4.

	R1†	R4	R7	M1	M3	S1	S3	S7
R1	**1,900**	800	700	1,300	700	1,000	5,600	3,100
R4		**2,400**	900	1,800	1,200	2,300	3,300	6,200
R7			**2,000**	1,500	600	2,600	3,300	4,800
M1				**4,400**	2,700	5,400	7,000	10,800
M3					**2,300**	3,100	3,700	4,300
S1						**5,100**	11,100	10,000
S3							**7,700**	19,900
S7								**8,500**

The bold in the diagonal are means of that parent crossed with others.
† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

Table 4-11. Analysis of variances of combining abilities of the variables root weight and shoot weight of selected white clover clones inoculated with *M. incognita* race 4 and non-inoculated clones.

Source	DF	Root weight	Shoot weight
REP	4	0.11***	0.03
Inoculation	1	0.29***	0.02
Error		0.01	0.05

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

Table 4-12. Analysis of variances of combining abilities of the variables egg mass score and gall score of selected white clover clones inoculated with *M. incognita* race 4.

Source	DF	Root weight	Shoot weight
REP	4	1.54***	0.86***
Cross	27	0.19***	0.77***
SCA	7	0.31***	0.79***
GCA	20	0.15***	0.77***
Error		0.01	0.02

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

Table 4-13. General combining ability (GCA) and specific combining ability (SCA) effects on root weights of three resistant, two intermediate and three susceptible white clover clones inoculated with *M. incognita* Race 4.

	R1†	R4	R7	M1	M3	S1	S3	S7
					-g-			
R1	**-0.004**	0.006	-0.049***	-0.041***	0.037***	0.017*	-0.009	0.039***
R4		**-0.012****	0.010	-0.004	-0.020*	0.041***	0.028***	-0.062***
R7			**0.017*****	0.103***	-0.056***	-0.004	-0.022**	0.018*
M1				**-0.011****	0.003	0.003	-0.001	-0.063***
M3					**-0.018*****	-0.046***	-0.003	0.085***
S1						**-0.031*****	0.007	-0.018*
S3							**0.000**	0.000
S7								**0.059*****

GCA effects are in bold on diagonal.

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

‡ Negative value of GCA and/or SCA indicate that this particular clone and/or cross decreased the root weight from the mean and the positive value means it increased.

Table 4-14. Mean root weights of three resistant, two intermediate and thee susceptible white clover inoculated with *M. incognita* race 4.

	R1†	R4	R7	M1	M3	S1	S3	S7
					-g-			
R1	**0.30**	0.29	0.26	0.24	0.31	0.28	0.29	0.39
R4		**0.29**	0.32	0.27	0.25	0.30	0.32	0.29
R7			**0.31**	0.41	0.24	0.28	0.29	0.39
M1				**0.29**	0.27	0.26	0.29	0.29
M3					**0.28**	0.20	0.28	0.43
S1						**0.27**	0.28	0.31
S3							**0.30**	0.36
S7								**0.35**

The bold in the diagonal are means of that parent crossed with others.

† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

Table 4-15. General combining ability (GCA) and Specific combining ability (SCA) effects on shoot weights of three resistant, two intermediate and three susceptible white clover clones inoculated with *M. incognita* Race 4.

	R1†	R4	R7	M1	M3	S1	S3	S7
					-g-			
R1	**-0.009**	0.012	-0.109***	-0.031*	-0.005	0.001	0.095***	0.036**
R4		**-0.003**	0.210***	-0.017	-0.073***	0.072***	-0.086***	-0.118***
R7			**-0.016****	0.061***	-0.079***	0.051***	-0.132***	-0.003
M1				**0.020****	0.062***	-0.037**	0.001	-0.040**
M3					**-0.082*****	-0.190***	0.113***	0.171***
S1						**0.028*****	0.079***	0.023
S3							**0.071*****	-0.070***
S7								**-0.010**

GCA effects are in bold on diagonal.

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

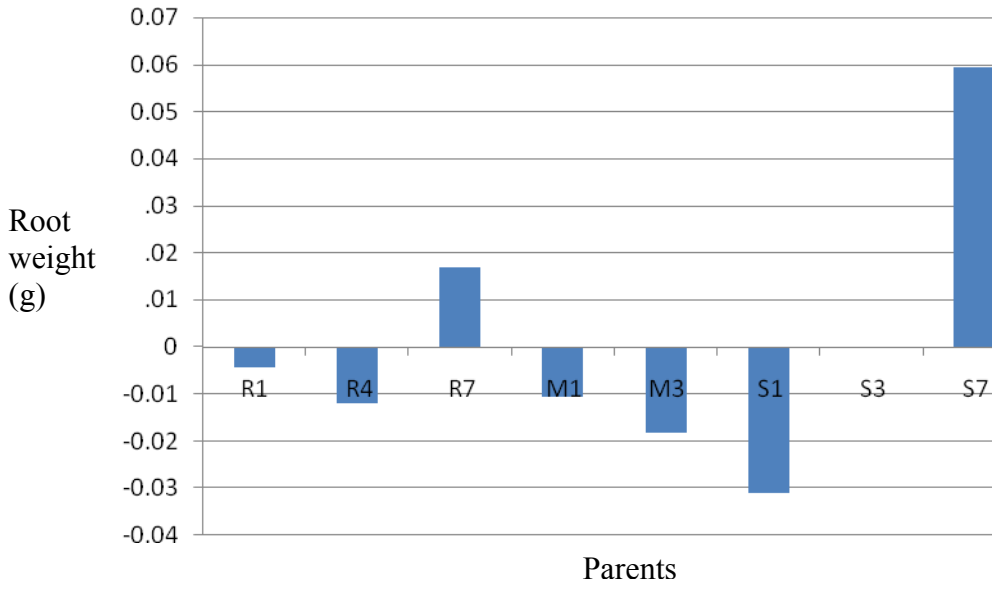
‡ Negative value of GCA and/or SCA indicate that this particular clone and/or cross decreased the shoot weight from the mean and the positive value means it increased.

Table 4-16. Mean shoot weights of three resistant, two intermediate and thee susceptible white clover inoculated with *M. incognita* race 4.

	R1†	R4	R7	M1	M3	S1	S3	S7
					-g-			
R1	**0.78**	0.79	0.65	0.77	0.69	0.81	0.94	0.80
R4		**0.78**	0.98	0.79	0.63	0.88	0.77	0.66
R7			**0.77**	0.85	0.61	0.85	0.71	0.76
M1				**0.80**	0.79	0.80	0.88	0.76
M3					**0.72**	0.54	0.89	0.87
S1						**0.81**	0.96	0.83
S3							**0.85**	0.78
S7								**0.78**

The bold in the diagonal are means of that parent crossed with others.
† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

Fig 4-1. General combining ability (GCA) effects on root weights of three resistant, two intermediate and three susceptible white clover clones inoculated with *M. incognita* race 4.



† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

‡ Negative value of GCA indicates that this particular clone decreased the root weight from the mean and the positive value means it increased.

CHAPTER 5
QUANTITATIVE GENETIC BASIS OF INHERITANCE OF RESISTANCE IN WHITE CLOVER TO PEANUT ROOT-KNOT NEMATODE

Abstract

White clover (T*rifolium repens* L.) is an important forage crop worldwide. It can also be an important component of pastures on light textured soil found in much of Florida. Root-knot nematodes (*Meloidogyne* spp.) can be a major limiting factor in white clover production and persistence especially on sandy soils. This study was conducted to determine the genetic basis of inheritance of resistance to *M. arenaria* race 1 in a selected group of white clover clones. Eight parents composed of three resistant, two intermediate and three susceptible clones were crossed in a partial diallel design and those 28 crosses were evaluated for percentage root system galled (PRSG), egg mass score, gall score, eggs per gram of dry root weight and eggs per plant. This original resistant/intermediate/susceptibility classification of parents was based on their reaction to *M. incognita* race 4. The progeny evaluation experiment was arranged in a randomized complete block design with 7 replications of 14 plants of each cross grown in a greenhouse. At two weeks after germination, seedlings were inoculated with ca. 500 eggs of *M. arenaria* race 1. Eight weeks after inoculation, the plant roots were washed and evaluated for the above mentioned variables. A diallel analysis (Griffing's method 4 model I) of the variables PRSG, gall score and egg mass score showed that both General Combining Ability (GCA) effects and Specific Combining Ability (SCA) effects were significant. An analysis for the variables eggs per gram of dry root and eggs per plant showed only GCA effects were significant. A high GCA:SCA ratio for all variables indicated that additive effects were more important than non-additive effects. The GCA effects were correlated with resistance reaction to the nematodes. The GCA effects of resistant clones varied in magnitude from each other and that was also true in the case of susceptible clones. Only a few of the SCA effects were significant. The cross of R6 with

S3 gave more resistance than expected from GCA effects of parents while the cross of R6 with other susceptible clones gave less resistance than predicted. This outcome suggests a more complicated inheritance of resistance to *M. arenaria* race 1 in white clover than for resistance to *M. incognita.*

Introduction

White clover (*Trifolium repens* L.) is one of the major forage legume crops of the southeastern USA. Although the species is considered perennial, individual plants usually persist for only one year in Florida; and thus it behaves as a reseeding annual. White clover generally has been shown to have high nutritive value which will increase the overall feed value of the diet when mixed with grasses. Although very well adapted for the southern USA, white clover suffers from many pathological problems, one of them being root-knot nematode (*Meloidogyne* spp) infestation. Root-knot nematodes (RKN) create galling, compete for food and reduce crop yield, vigor and persistence. Due to the lack of any registered nematicides for use on pasturelands, RKN resistant cultivars are the only and best solution.

Meloidogyne arenaria is one of the four major species of *Meloidogyne* contributing 8% of the worldwide population with the other three being *M. incognita, M. javanica* and *M. hapla*. *Meloidogyne arenaria* shows differential host specificity (races) that cannot be distinguished morphologically but can be differentiated physiologically and by host differential tests. Sixteen percent of the worldwide *M. arenaria* population is contributed by race 1 and the remaining percent by race 2 (Sasser et al., 1983).

The control of RKN disease can best be achieved through the combination of all available control measure including resistant cultivars, chemical and cultural practices. Bain (1959) first reported selection for tolerance and/or resistance to RKN in white clover by evaluating lines of white clover seedlings and selected for RKN tolerance. Gibson (1973) developed ‘SC-1’ white

clover which was reported to be resistant to RKN. Mercer et al., (2000) gained some success in selecting white clover strains resistant to *M. trifolia,* a previously undocumented species. Pederson and Windham (1995) released 'MSNR4' after four cycles of recurrent selection from a wide pool of white clover germplasm. This population was shown to be resistant to *M. incognita, M. arenaria* and *M. graminicola.* The cultivar 'UFWC5' was also reported to be tolerant to southern RKN which was developed by recurrent phenotypic selection using 'Osceola' as the base population and southern root-knot nematode (*M. incognita*) race 4 as the selective pathogen (Wofford and Ostmark, 2005).

An understanding of the inheritance pattern of resistance should improve the progress from selection in a breeding program to enhance RKN resistance. Partitioning the genetic variability to General Combining Ability (GCA) and Specific Combining Ability (SCA) would help understand the genetics behind resistance. The information will be very helpful in development of synthetic varieties such as in white clover (Baker, 1978). The GCA is an indication of the additive genetic variation of the trait while SCA is the measure of the non-additive variation. Griffing (1956) has given a procedure to differentiate these combining abilities using diallel crosses. This method has been utilized for several crops to understand the genetics of those crops.

The understanding of the relative importance of GCA and SCA would lead to selection of the most efficient plant breeding procedures. The recurrent selection program using a set of resistant parents (having higher GCA effect for the resistance) should be the most efficient method to improve the resistance if additive variation is of primary importance. Conversely, hybridization of specific parential combinations with large SCA effects would be more desirable for crops where the non-additive variance component is more important than the additive

component. This procedure has been utilized in many crops to understand the inheritance pattern. The objective of this research was to estimate the GCA and SCA effects on expression of host-pathogen interaction responses using a set of white clover diallel cross progeny inoculated with *M. arenaria* race 1.

Materials and Methods

Selection of Parents

Seeds of UFWC5 were planted in Cone-tainers® (Stuewe and Sons, Inc., Tangent, OR) filled with fine commercial building sand. Two weeks after germination, the seedling plants were inoculated with ca. 500 eggs of *M. incognita* race 4. Eight weeks later, these plants were carefully removed from Cone-tainers. The root systems were rinsed in water to remove the sand. Roots were then immersed in a solution of 0.05% red food color (McCormik & Co.®, Hunt Valley, MD). The number of egg masses and galls were counted and classified in three groups. The plants with 0 to 5 galls or egg masses were classified as resistant, plants with 6 to 30 as intermediate and more than 30 galls or egg masses as susceptible (Call et al., 1997). Eleven resistant, 11 susceptible and 9 intermediate plants were selected. These plants were then planted in 15-cm diameter pot. Three to five clones of each selected plant were produced and planted in additional 15-cm diameter pots. These pots were maintained in a pollinator free greenhouse.

Crossing

Since white clover is known to be relatively self incompatible, flowers were not emasculated prior to making crosses. Hand crosses were made with the aid of a toothpick with emery paper glued to the flat surface of the tooth pick as described by Taylor (1980). Attempts were made to complete all possible crosses within these 31 parents. Since white clover is self incompatible, no selfs were made and attempts at selfing yielded only 6 seeds from about 100 flower heads, each head containing 30 to 40 flowers (ca. 300 to 4000 florets). White clover is a

long day flowering plant. Under short day conditions, artificial light was used to extend the daylength to 16 hours in the greenhouse to stimulate flowering during winter months.

At 20 to 30 days after pollination, the flower heads were harvested and seeds were hand threshed. These seeds were collected in small paper bags and stored. The seeds of reciprocal crosses were combined. Although we attempted to complete all crosses among the 31 white clover clones, only eight clones were used for this diallel experiments. The availability of enough seeds from every cross for a half diallel design was a major factor determining the number of parents. Rather than attempting a diallel analysis with a larger number of parents, we chose to use only parents for which a high number of progeny plants per replication were available.

Inoculation

Eight parents consisting of three resistant (R5, R6, R11), two intermediate (M3, M4), and three susceptible (S3, S4, S7) were used in this diallel experiment. Ninety eight progeny plants of each cross, a total of 28 crosses, were germinated in Cone-tainers. These plants were arranged in a randomized complete block design with 7 replications of 14 plants each. At two weeks after germination, each plant was inoculated with ca. 500 eggs of *M. arenaria* race 1 with the aid of a continuous flow syringe as described in chapter three. An extra tray with 98 plants of 'Osceola' was also inoculated to provide plants for uprooting to monitor the progression of the disease symptoms on susceptible plants. The source inoculum was maintained in a separate greenhouse and eggs were extracted with the same method described in chapter three.

Data Collection and Analysis

The diallel experiment was terminated when most plants of the extra Osceola flat were showing a gall and egg mass score between 3 and 5. The data collection procedure was the same as that described in chapter three. Separate individuals were assigned by replications to rate egg masses, galls and also to count eggs with the microscope. The variables accessed were

percentage root system galled (PRSG), egg mass score and gall score, eggs g^{-1} of dry root weight and eggs $plant^{-1}$. The PRSG variable has been used as a 1 to 5 scale variable by some authors (Pederson and Windham, 1989; Pederson and Windham, 1992; Windham and Pederson, 1989) but we chose to use this variable as absolute percentage. The other variables were measured as described in chapter 3 and 4. The data analysis was conducted based on Griffings method 4 model I (Griffings, 1956) using the SAS code as described by Zhang et al. (2005).

Results and Discussion

Percentage Root System Galled (PRSG)

There was a significant difference both due to crosses and replications (Table 5-1). The replication effects may be due to environmental effects inside the greenhouse or to differences in how individuals visualized and scored PRSG. Any effects due to individuals may also contribute to significant replication effects seen for other response variables. Variation among crosses was separated into variation due to GCA effects and variation due to SCA effects. Both the GCA and SCA effects were significant ($P < 0.001$) (Table 5-1). The GCA:SCA ratio was 0.87. This is an indication that additive effects are more important than non-additive effects in the expression of white clover tolerance to peanut RKN based on the PRSG.

The individual GCA effects of all three resistant (R5, R6, R11), one intermediate (M4) and all three susceptible (S3, S4, S7) parents were significant ($P < 0.001$) (Table 5-2), but the GCA effect of one intermediate parent (M3) was not. Within the resistant clones, most crosses involving R6 reduced the mean PRSG value in the range of 40% less than other resistant parents. The susceptible clones, all increased the PRSG value by almost an equal amount while one intermediate (M4) increased the PRSG value by about 10% more than that of other susceptible clones.

Among the twenty eight SCA effects, only eleven were significant ($P < 0.05$) and only six produced a favorable response (reduced PRSG). The cross between the two resistant clones (R5 and R11) increased the PRSG value (SCA = 6.1) in opposite response than expected based on their GCA, while the cross between two susceptible clones (S4 and S7) decreased the value (SCA = -5.2), again an opposite response from expected based on their respective GCA effects. The cross of resistant parent (R5) with one susceptible parent (S3) had a significant negative SCA (-8.3) PRSG value from expected while the cross of the same parent with another susceptible parent (S4) showed an increased value of PRSG (SCA = 4.8). Both susceptible parents (S3 and S4) had about the same GCA effects. Although the above SCA effects were significant, the predominance of GCA effects suggests primarily additive effects contributed to reduced PRSG. The differences in such reactions suggest that the inheritance of resistance to *M. arenaria* race 1 is not easily explained. This complicated inheritance may be due to the fact that the clones used in this diallel study were not selected using *M. arenaria* race 1 but with *M. incognita* race 4. If *M. arenaria* had been used, these GCA effects might have been more consistent with the classification of the parents. Nevertheless, parental classification based on response to *M. incognita* race 4 did identify one parent (R6) that showed a highly resistant PRSG response to *M. arenaria* race 1 as quantified by its very large negative GCA value (-25.6).

The crosses M4S3 and M4S4 were the most susceptible based on the PRSG score which was also confirmed by the GCA and SCA values for PRSG added to the population mean of 61.7 (Table 5-3). The crosses R5R6, R6R11 gave the most resistance based on the GCA:SCA and their calculated mean PRSG. When the single cross PRSG means were evaluated, no cross combination stands out for reduced PRSG except for all crosses of resistant parent R6.

The higher GCA effect of intermediate (M4) than that of any susceptible parent is further evidence that classification of parental phenotypes using *M. incognita* race 4 may not be valid for their responses to *M. arenaria* race 1. This suggests that there may be different genes involved in the resistance to *M. arenaria* race 1 than in *M. incognita* race 4. All other susceptible-resistance classifications were also consistent between *M. arenaria* race 1 and *M. incognita* race 4.

Egg Mass Score

Cross effects were significant for egg mass score ($P < 0.001$) (Table 5-1). The crosses effects were then partitioned into variability due to GCA effects and due to SCA effects, both of which were significant (Table 5-1). The GCA:SCA ratio was 0.70. This higher ratio suggests again that additive effects were more important than non-additive effects and selections based on a parent's performance should lead to improved resistance in the progeny population.

The effects due to GCA effects were significant in only one resistant (R6) and two susceptible (S3 and S7) parents. The SCA effects were significant in only 5 of the 28 crosses (Table 5-4). The parent contributing most to reduced egg mass score was R6 (GCA = -0.2) whereas both S3 and S7 were equal in contributing to susceptibility with GCA = 0.3. The most favorable single cross combinations were R5M3, R5R6, R5R11, R6R11 and R6M1 all with egg mass scores below 3.0. Single cross combinations that increased egg mass scores were S3S7 and S4S7 (Table 5-5).

Gall Score

There were significant differences in variability both due to replications and due to crosses ($P < 0.001$) (Table 5-1). The variability within crosses was partitioned into the variability due to GCA effects and SCA effects, both of which were significant ($P < 0.001$) (Table 5-1). The GCA:SCA ratio was 0.77 indicating that additive effects are more important in the inheritance of white clover gall score in response to *M. arenaria* race 1.

The analysis of individual GCA and SCA effects showed that seven of the eight parents' GCA effects were significant with M3 being the only parent not showing a significant GCA effect (Table 5-6). The relative magnitudes of these GCA effects were similar to that of the PRSG variable with R6 being the parent contributing to reduced gall score and M4 being the parent that increased gall score. Twelve of the twenty eight combinations had significant SCA effects and six of them reduced the gall score. The most resistant combinations were R5R6 and R6R11 while the most susceptible combinations were M4S3, M4S4, M4S7, S3S4 and S3S7. These combinations suggest that progeny of the most resistance parent (R6) when combined with another resistant parent were resistant and progeny of susceptible parents (M4, S3) were susceptible when combined with other susceptible parents. However, the resistant by susceptible crosses produced progeny ranged from resistant (R5S3) to susceptible (R5S4, R5S7, R6M3) (Table 5-7).

Eggs g^{-1} Dry Root Weight

The variable eggs g^{-1} of dry root weight was tested for normality and then was log transformed to meet the normality assumptions. Crosses were a significant ($P < 0.01$) (Table 5-8) source of variation. The effects of the crosses were partitioned into GCA effects and SCA effects where only the GCA effects were significant ($P < 0.001$). The GCA:SCA ratio was 0.97 indicating a high level of importance of additive effects in the expression of eggs g^{-1} dry root weight when inoculated with *M. arenaria* race 1.

The individual GCA and SCA effect analysis identified that three of the eight clones have significant GCA effects and only one cross (M4S4) had significant SCA effects and it was toward resistance (reduced eggs g^{-1} dry root weight SCA effect). Only one resistant clone (R6) had a significant ($P < 0.001$) (Table 5-9) GCA effect and two susceptible parents (S3 and S7)

had significant GCA effects ($P < 0.01$). The best cross combination for reducing egg number was R5R6 and the crosses that produced the most eggs were S3S7 and S4S7 (Table 5-10).

Eggs Plant^{-1}

An analysis of variance for the variable eggs plant^{-1} showed similar results as obtained from eggs g^{-1} dry root weight. Crosses were significant ($P < 0.001$), and when partitioned into GCA and SCA effects, only the GCA effects were significant ($P < 0.001$) (Table 5-8). The GCA:SCA ratio was 0.73 indicating that non-additive effects are not as important as additive effects in the inheritance of eggs plant^{-1}.

The analysis of the individual GCA and SCA effects resulted in significant GCA effects for two resistant (R5, $P < 0.05$ and R6, $P < 0.001$) clones and two susceptible (S3 and S7, both $P < 0.001$) clones (Table 5-11). Only two crosses (R5R6 and R5S4) gave significant SCA effects. The cross of R5 with resistant R6 reduced (SCA = -0.29) the egg number more than expected from GCA effects while the cross of R5 with non-significant GCA effects parent S4 increased (SCA = 0.31) the egg number. The cross combination with the lowest eggs plant^{-1} was R5R6 and the combination with the highest eggs plant^{-1} was S3S7 (Table 5-12).

Correlation

The correlation between PRSG and gall rating was 0.71 ($P < 0.001$) (Table 5-13). This very high correlation is as expected because the number of galls and the percentage of galled roots are related variables. The correlation between the egg mass score and gall score was low ($r = 0.33$, $P < 0.001$). This may indicate that some juvenile *M. arenaria* race 1 successfully entered the root system and produced galls but could not reach maturity and produce eggs. This correlation suggests a post infection mechanism of tolerance in these white clover plants by inhibiting the juvenile maturation or depressing the number of females that matured to produce egg masses.

The utilization of very resistant parent (R6) and other resistant parent (R5 and R11) can be very helpful in a selection program to breed for RKN tolerance/resistance in white clover. As most white clover cultivars are developed by population improvement methods and synthetic cultivars are released rather than emphasizing an individual single crosses, findings from this research should be helpful for synthetic cultivar development.

The results of this diallel study with *M. arenaria* race 1 are similar to those of the previous chapters studying combining ability effects of white clover progeny when inoculated with *M. incognita* race 4. Nevertheless, there were some differences in magnitudes of GCA and SCA effects. Among both the resistant and susceptible parents, a wide range in magnitude of GCA effects may suggest the involvement of multiple genes in the inheritance of resistance to *M. arenaria* race 1. Some parents identified as intermediate in response to *M. incognita* race 4 produced progeny that were as or more susceptible than progeny from parents identified as susceptible. This result may suggest the involvement of different genes in resistance to different RKN populations. These findings are in agreement with the study by Windham and Pederson (1989) showing that SC-1, developed by Gibson (1973) as resistant to *M. incognita*, was only moderately tolerant to some RKN populations.

The predominance of GCA effects (additive genetic variation) in the inheritance of all discussed variables in this research is supported by other studies in white clover (Pederson and Windham, 1992) and red clover [*Trifolium pretense* (L.); Call et al., 1997]. Furthermore, studies on corn [*Zea mays* L., Williams and Windham, 1990], and cotton [*Gossypium hirsutum* (L.), McPherson et al., 1995; Zhang et al., 2007] also identified additive effect to be more important than non-additive effects in inheritance of resistance to RKN populations.

Table 5-1. Analysis of variance of combining abilities of the variables percentage root system galled (PRSG) egg mass score, and gall score of selected white clover clones inoculated with *M. arenaria* race 1.

Source	DF	PRSG	Egg Mass Score†	Gall Score
REP	6	7381***	11.97***	9.80***
Cross	27	20510***	5.37***	12.78***
GCA	7	72832***	33.61***	41.78***
SCA	20	2281***	3.13***	2.53***
Error		498	0.46	0.54

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

† Egg masses and galls were rated on a 1 to 5 scale where 0 = 0 galls or egg masses, 1 = 1 to 2 galls or egg masses, 2 = 3 to 10 galls or egg masses, 3 = 11 to 30 galls or egg masses, 4 = 31 to 100 galls or egg masses and 5 = more than 100 galls or egg masses

Table 5-2. General combining ability (GCA) and Specific combining ability (SCA) effects for percentage root system galled (PRSG) of three resistant, two intermediate and three susceptible white clover clones inoculated with *M arenaria* race 1.

	R5†	R6	R11	M3	M4	S3	S4	S7
R5	**-3.3*****‡	-2.3	6.1**	-6.0***	2.7	-8.3***	4.8*	3.0
R6		**-25.6*****	0.7	7.8***	4.9*	-3.0	-2.5	-5.5**
R11			**-4.4*****	0.0	-6.7**	2.8	-3.5	0.7
M3				**0.0**	-4.9*	-2.7	-1.1	7.1***
M4					**14.2*****	3.9	3.8	-3.7
S3						**6.2*****	3.7	3.7
S4							**5.5*****	-5.2*
S7								**7.3*****

GCA effects are in bold on diagonal.

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

† R5, R6 and R11 resistant; M3 and M4 intermediate and S3, S4 and S7 susceptible to *M. incognita* race 4

‡ Negative values of GCA and/or SCA indicate that this particular clone and/or cross decreased the PRSG from the mean and a positive value means it increased.

Table 5-3. Mean percentage root system galled (PRSG) of roots of three resistant, two intermediate and three susceptible white clover inoculated with *M. arenaria* race 1.

	R5†	R6	R11	M3	M4	S3	S4	S7
R5	**59**	31	60	52	75	56	69	69
R6		**39**	32	44	55	39	39	38
R11			**58**	57	65	66	59	65
M3				**62**	71	65	66	76
M4					**74**	86	85	79
S3						**67**	77	79
S4							**66**	69
S7								**68**

The bold on the diagonal are means of that parent.
† R5, R6 and R11 resistant; M3 and M4 intermediate and S3, S4 and S7 susceptible to *M. incognita* race 4

Table 5-4. General combining ability (GCA) and Specific combining ability (SCA) effects on egg mass score of three resistant, two intermediate and three susceptible white clover clones inoculated with *M arenaria* race 1.

	R5†	R6	R11	M3	M4	S3	S4	S7
R5	**-0.1** ‡§	-0.1	0.1	-0.3***	-0.1	0.1	0.2***	0.0
R6		**-0.2*** **	0.0	-0.1	0.2***	0.0	-0.0	-0.1
R11			**-0.1**	0.2**	-0.1	-0.1	-0.2**	0.1
M3				**-0.1**	0.0	0.1	-0.1	0.1
M4					**-0.1**	-0.1	0.1	-0.1
S3						**0.3*** **	-0.0	-0.0
S4							**0.0**	0.0
S7								**0.3*** **

GCA effects are in bold on the diagonal.

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

† R5, R6 and R11 resistant; M3 and M4 intermediate and S3, S4 and S7 susceptible to *M. incognita* race 4

‡ Negative values of GCA and/or SCA indicate that this particular clone and/or cross decreased the egg mass rating from the mean and the positive values means it increased.

§ Egg masses were rated on a 1 to 5 scale where 0 = 0 egg masses, 1 = 1 to 2 egg masses, 2 = 3 to 10 egg masses, 3 = 11 to 30 egg masses, 4 = 31 to 100 egg masses and 5 = more than 100 egg masses

Table 5-5. Mean egg mass scores of roots of three resistant, two intermediate and thee susceptible white clover inoculated with *M. arenaria* race 1.

	R5†	R6	R11	M3	M4	S3	S4	S7
R5	**3.0**‡	2.7	2.9	2.6	2.9	3.3	3.2	3.3
R6		**2.9**	2.8	2.7	3.1	3.2	2.9	3.1
R11			**3.0**	3.1	2.9	3.2	2.8	3.3
M3				**3.0**	3.0	3.4	3.0	3.3
M4					**3.1**	3.3	3.2	3.3
S3						**3.3**	3.4	3.7
S4							**3.1**	3.5
S7								**4.2**

The bold on the diagonal are means of that parent.

† R5, R6 and R11 resistant; M3 and M4 intermediate and S3, S4 and S7 susceptible to *M. incognita* race 4

‡ Egg masses were rated on a 1 to 5 scale where 0 = 0 egg masses, 1 = 1 to 2 egg masses, 2 = 3 to 10 egg masses, 3 = 11 to 30 egg masses, 4 = 31 to 100 egg masses and 5 = more than 100 egg masses

Table 5-6. General combining ability (GCA) and Specific combining ability (SCA) effects on gall score of three resistant, two intermediate and thee susceptible white clover clones inoculated with *M arenaria* race 1.

	R5†	R6	R11	M3	M4	S3	S4	S7
R5	**-0.1*****‡	-0.3***	0.1	-0.1	0.1	-0.2*	0.2*	0.2*
R6		**-0.6*** **	-0.2**	0.2**	0.3***	0.0	0.1	-0.1
R11			**-0.1****	0.0	-0.2**	0.0	-0.1	0.2**
M3				**0.0**	-0.2**	-0.1	0.0	0.0
M4					**0.3*** **	0.0	0.0	0.0
S3						**0.2*** **	0.2**	0.1
S4							**0.1****	-0.3***
S7								**0.1*** **

GCA effects are in bold on the diagonal.
*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively
† R5, R6 and R11 resistant; M3 and M4 intermediate and S3, S4 and S7 susceptible to *M. incognita* race 4
‡ the negative value of GCA and/or SCA indicate that this particular clone and/or cross decreased the gall rating from the mean and the positive value means it increased.
§ Galls were rated on a 1 to 5 scale where 0 = 0 galls, 1 = 1 to 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls and 5 = more than 100 galls

Table 5-7. Means of gall score of roots of three resistant, two intermediate and thee susceptible white clover inoculated with *M. arenaria* race 1.

	R5†	R6	R11	M3	M4	S3	S4	S7
R5	**4.0**‡	3.1	4.0	4.0	4.4	4.0	4.2	4.3
R6		**3.6**	3.3	3.8	4.1	3.7	3.7	3.5
R11			**4.0**	4.1	4.2	4.2	4.1	4.4
M3				**4.2**	4.3	4.3	4.2	4.3
M4					**4.4**	4.7	4.5	4.6
S3						**4.3**	4.6	4.6
S4							**4.2**	4.0
S7								**4.2**

The bold values on the diagonal are means of that parent.
† R5, R6 and R11 resistant; M3 and M4 intermediate and S3, S4 and S7 susceptible to *M. incognita* race 4
‡ Galls were rated on a 1 to 5 scale where 0 = 0 galls, 1 = 1 to 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls and 5 = more than 100 galls

Table 5-8. Analysis of variance of combining abilities for the variables eggs g^{-1} of dry root weight and egg $splant^{-1}$ of selected white clover clones inoculated with *M. arenaria* race 1.

Source	DF	Egg per gram	Egg per plant
REP	6	1.05***	2.22***
Cross	27	0.49**	0.72***
GCA	7	1.23***	2.15***
SCA	20	0.23	0.23
Error		0.24	0.12

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

Table 5-9. General combining ability (GCA) and specific combining ability (SCA) effects on eggs g^{-1} of dry root weight of three resistant, two intermediate and thee susceptible white clover clones inoculated with *M arenaria* race 1.

	R5†	R6	R11	M3	M4	S3	S4	S7
R5	**-0.09**‡	-0.22	-0.03	-0.07	0.11	-0.10	0.22	0.09
R6		**-0.28*** **	-0.03	-0.04	0.25	0.10	0.01	-0.07
R11			**-0.05**	0.21	0.08	0.07	0.00	-0.30
M3				**0.00**	0.10	-0.19	-0.18	0.16
M4					**-0.06**	-0.03	-0.36*	-0.15
S3						**0.23****	0.10	0.06
S4							**0.01**	0.22
S7								**0.25****

GCA values are in bold on the diagonal. The original data was log transformed to meet the requirements of analysis.

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

† R5, R6 and R11 resistant; M3 and M4 intermediate and S3, S4 and S7 susceptible to *M. incognita* race 4

‡ Negative values of GCA and/or SCA indicate that this particular clone and/or cross decreased the eggs g^{-1} from the mean and the positive value means it increased.

Table 5-10. Means of eggs g^{-1} of dry root weight of three resistant, two intermediate and three susceptible white clover inoculated with *M. arenaria* race 1.

	R5†	R6	R11	M3	M4	S3	S4	S7
R5	**130,000**	74,300	116,100	114,800	131,500	136,400	151,500	184,500
R6		**116,500**	97,600	115,900	125,600	156,700	108,900	136,500
R11			**132,300**	157,300	131,100	171,700	125,100	127,600
M3				**141,000**	141,100	140,400	111,300	202,500
M4					**139,700**	157,000	151,600	140,300
S3						**169,100**	191,400	224,300
S4							**150,700**	221,300
S7								**176,700**

The values in bold on the diagonal are means of that parent.
† R5, R6 and R11 resistant; M3 and M4 intermediate and S3, S4 and S7 susceptible to *M. incognita* race 4

Table 5-11. General combining ability (GCA) and specific combining ability (SCA) effects on eggs plant^{-1} of three resistant, two intermediate and thee susceptible white clover clones inoculated with *M arenaria* race 1.

	R5†	R6	R11	M3	M4	S3	S4	S7
R5	**-0.14*‡**	-0.29*	0.09	-0.23	0.01	-0.13	0.31*	0.24
R6		**-0.44*** **	0.16	0.09	0.21	-0.01	-0.03	-0.13
R11			**-0.07**	0.01	0.08	-0.10	-0.06	-0.18
M3				**0.02**	0.08	0.04	-0.15	0.16
M4					**0.05**	-0.20	-0.10	-0.07
S3						**0.27*** **	0.23	0.16
S4							**0.05**	-0.18
S7								**0.27*** **

GCA effects are in bold on the diagonal. The original data was log transformed to meet the requirements of analysis.

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

† R5, R6 and R11 resistant; M3 and M4 intermediate and S3, S4 and S7 susceptible to *M. incognita* race 4

‡ Negative values of GCA and/or SCA indicate that this particular clone and/or cross decreased the eggs plant^{-1} from the mean and positive values means it increased.

Table 5-12. Means of eggs plant^{-1} of three resistant, two intermediate and three susceptible white clover clones inoculated with *M. arenaria* race 1.

	R5†	R6	R11	M3	M4	S3	S4	S7
R5	**25,700**	11,700	24,300	19,500	25,300	25,800	33,100	39,200
R6		**20,200**	19,800	22,300	22,500	25,500	19,100	20,600
R11			**26,300**	26,100	29,500	31,400	25,000	27,800
M3				**29,400**	31,100	38,100	24,800	43,400
M4					**29,400**	33,500	28,700	35,000
S3						**36,700**	47,100	55,200
S4							**29,600**	31,900
S7								**36,100**

The bold values on the diagonal are means of that parent.
† R5, R6 and R11 resistant; M3 and M4 intermediate and S3, S4 and S7 susceptible to *M. incognita* race 4

Table 5-13. Correlations among egg mass score, gall score and PRSG of eight clones of white clover inoculated with *M. arenaria* race 1.

	Gall Score	PRSG
Egg Score	0.34***	0.33***
Gall Score		0.71***

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

CHAPTER 6
QUANTITATIVE GENETIC BASIS OF INHERITANCE OF RESISTANCE IN WHITE CLOVER TO JAVANESE ROOT-KNOT NEMATODE

Abstract

White clover (T*rifolium repens* L.) is an important forage legume of the southeastern USA including Florida. Root-knot nematodes (*Meloidogyne* spp.) can be one of the major limiting factors in white clover production and persistence in this region. This study was conducted to determine the relative importance of additive and non-additive variance in the inheritance of resistance to *M. javanica* in a selected group of white clover clones. Eight parents including three resistant, two intermediate and three susceptible clones were crossed in a partial diallel design and the progeny from these 28 crosses were evaluated for egg mass score, gall score, eggs per gram dry root weight and eggs per plant. The parent plant's resistance reaction was based on prior response to *M. incognita* race 4. Progeny of the 28 crosses were arranged in a randomized complete block design with 5 replications and 14 plants in each replication in a greenhouse. Two week old progeny seedlings were inoculated with ca. 500 eggs of *M. javanica*. Eight weeks after inoculation, the plant roots were washed and evaluated for the above variables. Analysis of the variables gall score, egg mass score and eggs per plant showed that both General Combining Ability (GCA) and Specific Combining Ability (SCA) effects were significant for these root response variables. The variable eggs per gram dry root weight showed that only GCA effects were significant. A high GCA:SCA ratio for every variable indicated that additive effects were more important than non-additive effects. The GCA effects of both resistant and susceptible clones varied in magnitude from other clones of the same resistance class. Only a small number of the SCA effects were significant. The clone R7 which was classified as a resistant parent based on its response to *M. incognita* race 4 was susceptible to *M. javanica*. This indicated the

involvement of different genes controlling the resistance response between *M. javanica* and *M. incognita*.

Introduction

White clover (*Trifolium repens* L.) is one of the major legume forage crops worldwide and also in the southeastern USA. Although it is a cool season perennial legume, it generally behaves as a reseeding annual in Florida. With higher crude protein and digestibility than grasses, it can be an important component of Florida pastures. It is suitable for hay, silage, green chop and importantly for grazed pastures.

Several pathological problems exist that may limit the production and persistence of white clover. Root-knot nematodes (*Meloidogyne* spp.) can be a factor, especially on light textured soils which are common in Florida. There are four predominant species of root-knot nematodes (RKN) that account for more than 95% of the world distribution (Sasser et al., 1983). They are *M. incognita* (Kofoid and White) Chitwood, *M. arenaria* (Neal) Chitwood, *M. javanica* (Treub) Chitwood and *M. hapla* Chitwood.

M. javanica is best adapted in the areas with distinct dry and wet seasons (Sasser et al., 1983). *M. javanica* does not have pathological races based on host specificity. *M. javanica* is found in warm regions of the world and often predominant in higher altitudes of warm climate. It is the most serious nematode pest in central Africa (Ferris, 1999).

Since RKN invade and damage fine roots, the RKN infected plants wilt easily, become stunted and eventually may die. Symptoms of chlorosis may also be seen. The RKN damage in infected fields often is manifested as patches of dead plants indicating localized areas of high infection. A clear sign of root-knot nematodes is that the roots are swollen due to galling and have a knot like appearance (Thorne, 1961).

Control of RKN disease is very difficult and the most effective control will be the combination of all available control measures including resistant cultivars, chemical and cultural practices. The first reported selection for tolerance and /or resistance to RKN in white clover dates back to Bain (1959). Bain evaluated lines of white clover seedlings and selected for RKN tolerance. Gibson (1973) developed 'SC-1' white clover which was reported to be resistant to RKN. Mercer et al., (2000) gained some success in selecting white clover strains resistant to *M. trifolia* (previously identified as *M. hapla*). Pederson and Windham (1995) released 'MSNR4' after four cycles of recurrent selection from a wide genetic base of white clover germplasm. This population was shown to be resistant to *M. incognita, M. arenaria* and *M. garaminicola.* The cultivar 'UFWC5' was also developed by recurrent phenotypic selection using 'Osceola' as the base population and southern root-knot nematode (*M. incognita*) as the selective pathogen (Wofford and Ostmark, 2005).

The progress from the selection in a breeding program should be improved with the understanding of the inheritance pattern of any trait such as RKN resistance. The information on the relative importance of additive and non-additive variations which gives the total genetic variation would help in understanding the genetics conditioning the resistance. Those variations can be related to General Combining Ability (GCA) and Specific Combining Ability (SCA) effects. Such information should be helpful in development of synthetic varieties that are common in white clover (Baker, 1978). The GCA provides a measure of the additive variation and SCA provides a measure of the non-additive variations. Griffing (1956) has given a procedure to differentiate these combining abilities using diallel crosses. This procedure has been utilized in many crops to understand the inheritance pattern. The objective of this research was

to estimate the GCA and SCA effects on expression of host-pathogen interaction responses using a set of white clover diallel cross progeny inoculated with *M. javanica*.

Pederson and Windham (1992) found that selected resistant parents produced progeny with the least *M. incognita* reproduction in a diallel study of three resistant and three susceptible plants. Their study found that additive gene action was of much greater importance in inheritance of RKN resistance in white clover than non-additive gene action. A different diallel analysis by Call et al. (1997) using four resistant, three intermediate and two susceptible red clover (*Trifolium repens* L.) parents also showed predominantly significant GCA effects and non-significant SCA effects. Some other diallel studies have also identified GCA effects as more important than SCA effects in resistance to RKN (Williams and Windham, 1990; Mcpherson et al., 1995; Zhang et al., 2007).

Materials and Methods

Selection of Parents

Seeds of UFWC5 were planted in Cone-tainers® (Stuewe and Sons, Inc., Tangent, OR) filled with fine sand. Two weeks after germination, the seedling plants were inoculated with ca. 500 eggs of *M. incognita* race 4. Eight weeks later, these plants were carefully taken out from each container. The root systems were rinsed in water to remove the sand. Roots were then immersed in a solution of 0.05% red food color (McCormik & Co.®, Hunt Valley, MD) to stain and highlight the egg masses. Other researchers had used Phloxine-B to stain the egg masses (Holbrook et al., 1983), but we found the red food color to be equally effective with a reduced level of toxicity than that of Phloxine-B. The number of egg masses and galls were counted and the plants were classified. The plants with 0 to 5 galls or egg masses were classified as resistant, plants with 6 to 30 as intermediate and those with more than 30 galls or egg masses as susceptible (Call et al., 1997). Eleven resistant, eleven susceptible and nine intermediate plants

were selected. These plants were then planted in 15-cm diameter pots. Two to five clonal cuttings of each plant were produced and planted to other 15cm diameter pots. These pots were maintained in a pollinator free greenhouse.

Crossing

Since white clover is known to be relatively self incompatible, flowers were not emasculated prior to making crosses. Hand crosses were made with the aid of a toothpick and emery paper glued to the flat surface of tooth pick as described by Taylor (1980). Attempts were made to complete all possible crosses within these 31 parents. As white clover is self incompatible, no selfs were made and attempts at selfing yielded only 6 seeds from about 100 flower heads, each head containing 30 to 40 flowers (ca. 3000 to 4000 total florets). White clover is a long day flowering plant. Under short day conditions, artificial light was used to extend the daylength to 16 hours in the greenhouse.

At 20-30 days after pollination, the flower heads were harvested and seeds were hand threshed. These seeds were collected in small paper bags, labeled by crosses and replications and stored. The seeds of reciprocal crosses were combined.

Although we attempted to complete all crosses among the 31 white clover clones, only progeny from eight clones were used for this diallel experiment. The availability of enough seeds from every cross for a half diallel design was the major factor determining the number of parents. Rather than attempting analysis with a large number of plants, we chose to use a larger number of progeny of each cross in each replication.

Inoculation

Eight parents consisting three resistant (R1, R4, R7), two medium (M1, M3) and three susceptible (S1, S3, S7) were used in this diallel experiment. Seventy plants of each cross, from a total of 28 crosses, were planted in the cone-tainers. Prior to inoculation, plants were arranged in

a randomized complete block design with 5 replications of 14 plants each for inoculation. We also included cuttings of the eight parents to compare their resistance reaction with the GCA given by Griffing's analysis. The cuttings were made at the same time as seeding of progeny. Two weeks after planting, 14 clones of each parent were selected and arranged in a randomized block design. After two weeks of seedling growth, both the progeny plants and parent clones were inoculated with ca. 500 eggs of *M. javanica* with the aid of a continuous flow syringe as described in chapter three. An extra tray with 98 plants of Osceola was also inoculated to provide plants for uprooting to monitor the progression of the disease symptoms on susceptible plants. The source inoculum was maintained in a separate greenhouse and eggs were extracted with the same method described in chapter three.

Data Collection and Analysis

The diallel experiment was terminated when most plants of Osceola were showing a gall and egg mass score between 3 and 5. Variables evaluated were egg mass score, gall score, eggs g^{-1} dry root weight and eggs $plant^{-1}$. Individual plants were scored for egg masses and galls. The scores used were 0 = 0 galls or egg masses, 1 = 1 to 2 galls or egg masses, 2 = 3 to 10 galls or egg masses, 3 = 11 to 30 galls or egg masses, 4 = 31 to 100 galls or egg masses and 5 = more than 100 galls or egg masses (Taylor and Sasser, 1978). All plants in a replication were pooled for egg extraction and the eggs were counted on a replication basis with the aid of a hemocytometer slide. Four grids on the hemocytometer slide were counted, and 3 sub-samples from each replication were counted and averaged to calculate total egg numbers extracted from each replication of each progeny. The egg counts were then divided by the dry root weight to obtain eggs g^{-1} of dry root weight. Although the experiment was initiated with 14 plants in each replication, all did not survive. Thus at the time of termination and we divided the egg count by the number of surviving plants to obtain the eggs $plant^{-1}$ variable. The data collection procedure

was the same as that of chapter three. Individuals were associated with replications for counting egg masses and gall numbers and for counting egg numbers with the microscope.

The data analysis was conducted based using Griffing's method 4 model I (Griffing, 1956) using the SAS code as described by Zhang et al., (2005).

Results and Discussions

Egg Mass Score

The analysis of variance for egg mass score showed that both replications and crosses were significant ($P < 0.001$) sources of variability (Table 6-1). The replication effects may be due to environmental effects inside the greenhouse or to differences in how individuals visualized and scored egg mass score. Any effects due to individuals may also contribute to significant replication effects seen for other response variables. The variation within crosses was partitioned into GCA and SCA effects. Both GCA and SCA effects were significant ($P < 0.001$) (Table 6-1). The GCA:SCA ratio was 0.51. Although this ratio is not as high as found for most variables in the previous two chapters, it still suggests that additive genetic variances were as important as non-additive genetic variances.

Analysis of the individual GCA effects of all parents showed five significant GCA effects including three resistant (R1, R4 and R7) and two susceptible (S3 and S7; Table 6-2). The GCA effects of both the intermediate parents and the susceptible parent S1 were not significant. The positive direction of the GCA effect of the resistant parent R7 showed that this parent actually increased the number of egg masses in the roots of white clover which is contrary to its expected reaction. Its magnitude (0.2) was the same as one susceptible S7 (0.2) and was similar to another susceptible (0.3) parent. The remaining two resistant clones conferred a negative GCA effect suggesting they had additive genetic effects for reducing the number of egg masses.

This aberrant positive GCA value of the resistant clone R7 may suggest that plants which are resistant to *M. incognita* may not be resistant to *M. javanica* and that there could be different genes conferring resistance to different populations of RKN.

Seven SCA effects out of twenty eight were significant (Table 6-2) and three were in the desirable direction (lower egg mass score). The combinations R1R7 and R1M1 were the most resistant crosses and R7S3 was the most susceptible cross (Table 6-3). From the overall means, it would seem that parent R1 was the most resistant parent which produced progeny with more resistance in each cross combination.

We also analyzed the egg mass scores of individual parent means obtained from inoculated rooted vegetative cuttings of each parent. The correlation between GCA effect and the mean of rooted cuttings of the parents themselves was r = 0.62 ($P < 0.05$).

The mean of the egg mass score from Osceola was 4.67 which was higher than any of the 28 crosses. But two parents S1 and S7 which were susceptible gave a higher score (5.0) than Osceola (Table 6-3).

Gall Score

The analysis of variance for gall score showed the significant cross effect ($P < 0.001$; Table 6-1). The GCA and SCA effects within the cross variation were also significant ($P < 0.001$ and $P < 0.01$ respectively). The GCA:SCA ratio was 0.57 indicating not so strong effect of additive variation in the inheritance of gall score in response to *M. javanica* in white clover.

The GCA effects of all the three resistant (R1, R4 and R7), one intermediate (M3) and one susceptible (S3) were significant (Table 6-4). The gall score also showed similar GCA effects as for egg mass score in the case of the resistant clones. The resistant clone (R7) had a positive GCA effect indicating its inclination towards susceptibility. The remaining resistant clones (R1 and R4) had negative GCA effects as expected from their original parental resistance reaction

classification. The significant GCA effects of the intermediate parent M3 and the susceptible parent S3 both were in a positive direction indicating they increased the number of galls in infected roots.

Only six out of twenty eight SCA effects were significant (Table 6-4) and three decreased the number of galls. The resistant parent R1 when crossed with resistant parent R4 gave a group of progeny that were more susceptible (SCA effect = 0.3) than expected from GCA effects. However, when it was crossed with susceptible (R7; although classified as resistant, the GCA value suggested it to be a susceptible parent), the progeny gall score was less (SCA = -0.4) than expected from GCA (Table 6-4). Another resistant clone (R4) when crossed with two clones having non-significant GCA effects (M1 and S1) showed SCA effects in contrasting directions; the cross R4M1 increased the gall score (SCA = 0.3) more than expected while the cross R4S1 decreased the gall score (SCA = - 0.3). This type of resistance reaction indicates the complexity of *M. javanica* resistance in white clover. The complexity may have originated due to the selection of parents based on response to *M. incognita* race 4 rather than *M. javanica*. There could be a higher GCA:SCA ratio as found with variables discussed in previous chapters if we have used the same pathogen (*M. javanica*) both for selection of parents and for this diallel study. The GCA effects of those parents could also be consistent with the resistance/susceptible classification of those parents. Still, by using a different RKN population in selection of parents and in the diallel study allowed us to identify the involvement of different genes in the resistance responses to different RKN population in white clover.

The most resistant cross was R1R7 and the most susceptible were R7S3 and M3S3 (Table 6-5). These results were in accordance with the results found from egg mass score. The

correlation between GCA effects and actual mean gall score of rooted cuttings of the parents was not significant.

The mean of the gall score from the Osceola was 4.2 which was higher than the mean of any of the 28 crosses. But inoculated rooted cuttings of the three parents M1, S1 and S7 which were susceptible (based on GCA effects of progeny) had higher mean gall score than Osceola (Table 6-5).

Eggs g^{-1} Dry Root Weight

The analysis of variance for eggs g^{-1} dry root weight showed crosses were significant ($P < 0.001$; Table 6-6). The within cross variance was further partitioned into GCA and SCA effects in which only GCA effects were significant ($P < 0.001$). The GCA:SCA ratio was 0.84 indicating the greater importance of additive variance than non-additive variance.

Only three of the eight clones had significant GCA effects (Table 6-7). Only one resistant clone (R1) showed a negative significant GCA effect and two susceptible clones (S3 and S7) had significant positive GCA effects. The only significant SCA effect was of resistant (R1) by resistance (R4) cross, but this SCA effect was in an undesirable direction (increased the number of eggs, SCA = 0.36) more than expected from the GCA of these parents.

The combinations with the lowest eggs g^{-1} of dry root weight were R1R7 and R1M3 while the combinations with the highest eggs g^{-1} of dry root weight were M1S7 and S3S7 (Table 6-8). As the additive effects appear to be more important, the best parent for use in production of a synthetic cultivar would be R1 which gave the least number of eggs and also had the most negative GCA effects (-0.69). The correlation between the GCA effects and mean of the parents from crosses was 0.99 ($P < 0.001$) while the correlation between the GCA effects and actual means from parent clones was 0.65 ($P < 0.05$).

The mean of the eggs g^{-1} dry root from the Osceola was 252,200 which was higher than any of the 28 crosses. But one susceptible parent (S7) also gave egg numbers of over 250,000 (Table 6-8).

Eggs Plant^{-1}

The analysis of variance for the RKN eggs plant^{-1} of white clover showed crosses were significant sources of variation (Table 6-6). The variation due to crosses was partitioned into GCA and SCA effects. The GCA effect was highly significant ($P < 0.001$) while SCA effect was significant ($P < 0.05$). The GCA:SCA ratio was 0.71. This higher ratio signifies that additive variation is more important than non-additive in the inheritance of eggs plant^{-1} in white clover.

Analysis of the individual GCA and SCA effects only showed significant GCA effects for two resistant parents (R1 and R7) and two susceptible parents (S3 and S7) (Table 6-9). The resistant parent R1 had negative GCA effects (-0.69) indicating a reduction in egg numbers while another resistant parent R7 had positive GCA effects (0.17). The findings were similar to egg mass score and gall score and support our previous statement that although R7 was identified as a parent resistant to *M. incognita, it was* not resistant for *M. javanica*. Four out of twenty eight SCA effects were significant and only one of these SCA effects (R1R7, -0.44) was negative indicating a reduction in egg number more than expected from GCA. The cross of resistant parent R1 with another resistant parent, R4, (having non-significant GCA effects) had a significantly more positive SCA effect (SCA = 0.43) for number of eggs than would have been estimated from GCA effects, This same resistant parent R1 yielded a lower egg number when crossed with another susceptible R7 (SCA = -0.44).

The cross combination with lowest egg number plant^{-1} was R1R7 and the cross with the highest egg number plant^{-1} was R7S3 (Table 6-10). The parent that resulted in the most overall reduction in egg number plant^{-1} was R1 both in terms of estimated GCA (-0.69) and mean eggs

plant^{-1}(14,300). The correlation between the GCA effects and actual mean number of eggs plant^{-1} from inoculation of rooted parent clones was 0.56 ($P < 0.05$).

The mean number of eggs plant^{-1} from inoculation of Osceola was 81,500 which was higher than the mean of any of the 28 crosses. However, rooted cuttings of one susceptible parent (S7) gave higher eggs plant^{-1} (128,700) than Osceola (Table 6-10).

Correlation

The correlation coefficient of egg mass score and gall score was 0.72 ($P < 0.01$) indicating that most nematodes that induced the formation of a gall also resulted in the production of an egg mass. Gall scores are indicative of the plant's response to the presence of invading RKN. If fewer egg masses were produced than galls, this would be an indicator that the plant is reducing fecundity of the RKN by reducing number of juveniles that mature to reproductive females and produce egg masses. Our results do not indicate that such a reduction occurred with these progeny.

The findings of this diallel study with *M. javanica* were similar to our findings with *M. incognita* race 4 and *M. arenaria* race 1. The higher GCA:SCA ratio of egg count variables (egg plant^{-1}, egg g^{-1} dry root weight) implies the higher importance of additive genetic effects which are also found by Pederson and Windham (1992). The egg mass score and gall score variable, however, gave the lower GCA:SCA ratio which implies non-additive genetic effects are also as much important as additive genetic effects. This finding was different from our previous findings for these variable with *M. incognita* race 4 and *M. arenaria* race 1. The reason behind this could be that the parents were selected utilizing *M. incognita* race 4 rather than *M. javanica*. This selection process even resulted in one parent (R7) to be susceptible to *M. javanica* which was classified as resistance to *M. incognita* race 4. This study identified the differences in genes that confer resistance to different populations of RKN. But all the parents resistant to *M. incognita*

race 4 were not susceptible to *M. javanica*, only one out of three resistant parents was susceptible.

Table 6-1. Analysis of variance of egg mass scores and gall scores combining ability of progeny from crosses of selected white clover parents inoculated with *M. javanica*.

Source	DF	Egg mass score†	Gall score
REP	4	26.51***	99.51***
Cross	27	7.20***	5.64***
GCA	7	18.42***	15.12***
SCA	20	3.52***	2.70**
Error		1.14	1.17

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

† Egg masses and galls were rated on a 1 to 5 scale where 0 = 0 galls or egg masses, 1 = 1 to 2 galls or egg masses, 2 = 3 to 10 galls or egg masses, 3 = 11 to 30 galls or egg masses, 4 = 31 to 100 galls or egg masses and 5 = more than 100 galls or egg masses

Table 6-2. General combining ability (GCA) and Specific combining ability (SCA) effects on egg mass score of three resistant, two intermediate and thee susceptible white clover clones inoculated with *M javanica*.

	R1†	R4	R7	M1	M3	S1	S3	S7
R1	**-0.5*****‡§	0.4**	-0.3*	-0.2	0.1	0.3*	-0.1	-0.1
R4		**-0.2***	-0.1	0.2	0.0	-0.3*	-0.2	0.0
R7			**0.2***	-0.2	0.0	0.1	0.5***	0.0
M1				**0.1**	0.2	0.1	-0.4	0.3*
M3					**0.0**	0.0	0.2	-0.3*
S1						**-0.1**	-0.1	0.0
S3							**0.3***	0.1
S7								**0.2****

GCA are in bold on diagonal.

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

‡ the negative value of GCA and/or SCA indicate that this particular clone and/or cross decreased the egg mass score from the mean and the positive value means it increased.

§ Egg masses were rated on a 1 to 5 scale where 0 = 0 egg masses, 1 = 1 to 2 egg masses, 2 = 3 to 10 egg masses, 3 = 11 to 30 egg masses, 4 = 31 to 100 egg masses and 5 = more than 100 egg masses

Table 6-3. Mean egg mass scores of roots of three resistant, two intermediate and thee susceptible white clover inoculated with *M. javanica*.

	R1†	R4	R7	M1	M3	S1	S3	S7	Parent mean
R1	**2.8**‡	3.0	2.6	2.6	2.8	2.9	3.0	2.8	**2.0**
R4		**3.1**	3.2	3.4	3.0	2.6	3.2	3.2	**3.9**
R7			**3.4**	3.3	3.4	3.4	4.2	3.6	**3.4**
M1				**3.3**	3.5	3.4	3.2	3.8	**4.4**
M3					**3.3**	3.1	3.7	3.1	**3.8**
S1						**3.1**	3.3	3.3	**5.0**
S3							**3.5**	3.8	**4.2**
S7								**3.4**	**5.0**

The bold values on the diagonal are means of that parent crossed with others. The last column is the mean obtained from inoculation of rooted cuttings of the parent clones.

† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

‡ Egg masses were rated on a 1 to 5 scale where 0 = 0 egg masses, 1 = 1 to 2 egg masses, 2 = 3 to 10 egg masses, 3 = 11 to 30 egg masses, 4 = 31 to 100 egg masses and 5 = more than 100 egg masses

Table 6-4. General combining ability (GCA) and Specific combining ability (SCA) effects on gall score of three resistant, two intermediate and thee susceptible white clover clones inoculated with *M javanica*.

	R1†	R4	R7	M1	M3	S1	S3	S7
R1	**-0.4*** ‡§**	0.3*	-0.4*	0.0	-0.3	0.4*	0.0	0.0
R4		**-0.2****	0.1	0.3*	0.1	-0.3*	-0.3	-0.2
R7			**0.2***	-0.1	0.1	0.2	0.2	0.0
M1				**0.0**	0.1	-0.1	-0.3*	0.1
M3					**0.2****	-0.1	0.2	-0.1
S1						**-0.1**	-0.1	0.0
S3							**0.3*****	0.2
S7								**0.1**

GCA values are in bold on the diagonal

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

‡ Negative values of GCA and/or SCA indicate that this particular clone and/or cross decreased the gall score from the mean and the positive value means it increased.

§ Galls were rated on a 1 to 5 scale where 0 = 0 galls, 1 = 1 to 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls and 5 = more than 100 galls

Table 6-5. Means of gall score of roots of three resistant, two intermediate and thee susceptible white clover inoculated with *M. javanica*.

	R1†	R4	R7	M1	M3	S1	S3	S7	Parent Mean
R1	**2.9**‡	2.9	2.6	2.8	2.8	3.1	3.2	2.9	**3.0**
R4		**3.1**	3.3	3.3	3.3	2.6	3.1	3.0	**3.8**
R7			**3.4**	3.2	3.6	3.5	3.9	3.4	**3.4**
M1				**3.2**	3.5	3.0	3.2	3.4	**4.4**
M3					**3.4**	3.3	3.9	3.4	**3.8**
S1						**3.1**	3.3	3.2	**4.5**
S3							**3.5**	3.8	**3.6**
S7								**3.3**	**4.5**

The bold values on the diagonal are means of that parent crossed with others. The last column is the mean obtained from inoculation of rooted cuttings of the parent clones.

† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

‡Galls were rated on a 1 to 5 scale where 0 = 0 galls, 1 = 1 to 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls and 5 = more than 100 galls

Table 6-6. Analysis of variance of eggs g^{-1} of dry root weight and eggs $plant^{-1}$ combining abilities of selected white clover parents inoculated with *M. javanica.*

Source	DF	Eggs g^{-1}	Eggs $plant^{-1}$
REP	6	0.84***	0.64**
Cross	27	0.92***	1.04***
GCA	7	2.99***	2.95***
SCA	20	0.20	0.37*
Error		0.16	0.19

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

Table 6-7. General combining ability (GCA) and specific combining ability (SCA) effects on eggs g^{-1} of dry root weight of three resistant, two intermediate and thee susceptible white clover clones inoculated with *M. javanica*.

	R1†	R4	R7	M1	M3	S1	S3	S7
R1	**-0.69*** ‡**	0.36*	-0.17	-0.21	-0.21	0.11	0.08	0.04
R4		**-0.01**	0.03	-0.18	-0.01	-0.17	-0.01	-0.01
R7			**0.03**	-0.16	0.11	-0.10	0.19	0.09
M1				**0.07**	0.23	0.30	-0.26	0.28
M3					**-0.05**	0.07	0.05	-0.24
S1						**-0.01**	-0.05	-0.16
S3							**0.33*** **	0.00
S7								**0.32*** **

GCA effects are in bold on the diagonal. The original data was log transformed to meet the normality requirements of analysis.

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

‡ Negative values of GCA and /or SCA indicate that this particular clone and/or cross decreased the eggs g^{-1} of dry root weight from the mean and the positive value means it increased.

Table 6-8. Means of eggs g^{-1} of dry root weight of three resistant, two intermediate and thee susceptible white clover inoculated with *M. javanica*.

	R1†	R4	R7	M1	M3	S1	S3	S7	Parent Mean
R1	**64,900**	75,500	48,900	56,800	49,000	58,000	88,100	78,100	**30,700**
R4		**110,600**	110,800	92,800	102,500	107,000	140,700	144,900	**74,700**
R7			**117,200**	96,900	118,900	97,600	181,900	165,700	**221,500**
M1				**125,600**	134,100	166,000	126,900	205,700	**141,200**
M3					**111,600**	106,100	156,500	114,400	**120,300**
S1						**114,700**	143,900	124,200	**208,600**
S3							**148,900**	204,300	**120,700**
S7								**148,200**	**273,900**

The bold values on the diagonal are means of that parent crossed with others. The last column is the mean obtained from inoculation of rooted cuttings of the parent clones.
† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

Table 6-9. General combining ability (GCA) and Specific combining ability (SCA) effects on eggs plant^{-1} of three resistant, two intermediate and thee susceptible white clover clones inoculated with *M. javanica.*

	R1†	R4	R7	M1	M3	S1	S3	S7
R1	**-0.69*‡**	0.43*	-0.44*	0.11	-0.16	0.22	-0.17	0.00
R4		**0.01**	-0.03	-0.25	0.12	-0.33	0.10	-0.03
R7			**0.17***	-0.25	0.11	-0.01	0.47**	0.14
M1				**0.00**	0.14	0.35*	-0.32	0.21
M3					**0.03**	0.09	0.03	-0.33
S1						**-0.10**	-0.22	-0.09
S3							**0.29***	0.11
S7								**0.29***

GCA values are in bold on the diagonal. The original data was log transformed to meet the normality requirements of analysis.

*, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

‡ Negative values of GCA and/or SCA indicate that this particular clone and/or cross decreased the eggs plant^{-1} from the mean and the positive value means it increased.

Table 6-10. Means of eggs plant^{-1}of roots of three resistant, two intermediate and thee susceptible white clover inoculated with *M. javanica*.

	R1†	R4	R7	M1	M3	S1	S3	S7	Parent Mean
R1	**14,300**	18,400	9,800	15,000	13,200	13,400	14,000	16,600	**8,800**
R4		**25,300**	27,600	19,000	28,200	17,300	34,800	32,000	**28,300**
R7			**32,000**	21,500	32,900	25,200	60,800	46,000	**52,400**
M1				**27,300**	28,800	41,100	25,500	40,400	**36,000**
M3					**26,800**	25,000	34,600	24,600	**74,600**
S1						**25,100**	24,100	29,400	**78,700**
S3							**34,300**	46,700	**38,800**
S7								**33,700**	**128,700**

The bold values on the diagonal are means of that parent crossed with others. The last column is the mean obtained from inoculation of rooted cuttings of the parent clones.
† R1, R4 and R7 resistant; M1 and M3 intermediate and S1, S3 and S7 susceptible to *M. incognita* race 4

CHAPTER 7
CONCLUSIONS

Root-knot nematodes (RKN) can be one of the major problems limiting production and persistence of forage legumes including white clover in light textured soils. Four major RKN species limiting the economic production of white clovers are *M. incognita, M. arenaria, M. javanica* and *M. hapla*. Southern RKN (*M. incognita)* has four physiological races all of which may attack white clover but race four is the less aggressive one (Windham and Pederson, 1989). *Meloidogyne hapla* is generally found in cooler regions and is not a significant problem for Florida. There have been attempts at various locations over a number of years to breed a white clover variety for RKN resistance (Bain, 1959; Gibson, 1973). The cultivar UFWC5, developed by five cycles recurrent selection from 'Osceola' using race 4 of *M. incognita*, was recently released from the University of Florida as having an improved level of RKN resistance (Wofford and Ostmark, 2005).

When this cultivar was evaluated for response to various RKN populations using the four races of *M. incognita*, *M. arenaria* race 1, and *M. javanica*, the resistance reaction of UFWC5 white clover to these different RKN populations was variable. UFWC5 produced significantly lower numbers of egg masses and galls when inoculated with the four races of *M. incognita*. Mean root egg mass and gall scores of UFWC5 plants inoculated with the *M. incognita* races were all below 2.0 signifying resistance to these populations. The roots of UFWC5 plants inoculated with *M. javanica* and *M. arenaria* race 1 also had reduced galling and egg mass production as compared to Osceola but were above the level (2.0) where they could be classified resistant (Call et al., 1997). This study pointed out the differences in the virulence of different RKN populations. This may suggest the involvement of different genes for resistance to the

different populations of RKN. There will likely be a need for multiple cycles of selection using the same RKN population for which resistance is desired.

There were no significant differences in both the root and shoot weights of non-inoculated Osceola and UFWC5. This leads us to the conclusion that selecting for RKN resistance did not hamper the yield potential of this selected white clover cultivar. The root weight of inoculated plants was higher than those of non-inoculated plants, likely because of the large galls instead of small fibrous roots.

Based on three different diallel analysis studies, additive genetic variance appeared to be the principal type of gene action involved in selection for RKN resistance in UFWC5. All three RKN populations used for genetic study showed that additive variance was more important than non-additive variance in the inheritance of resistance to RKN. The plants which were resistant to *M. incognita* race 4 were not necessarily resistant in the same degree to *M. arenaria* race 1 or *M. javanica* and the degree of susceptibility was also different in these three populations. One parent that showed resistance to *M. incognita* race 4 was susceptible to *M. javanica*. This observation suggests that there are differences in the genes that confer resistance to different populations of RKN.

The importance of additive variance suggests that selection of a few superior parents for development of a synthetic variety would be the most appropriate breeding strategy. Based on our research, the clones R1, R4 and M3 would be best parents for breeding resistance to *M. incognita*. For resistance to *M. arenaria* race 1 and *M. javanica,* only one parent in each case (R6 and R1 respectively) was outstanding.

In the future, it will be important to screen for response to specific RKN populations that are of interest if the target is resistance to those populations because of the differences in

virulence of different populations. In any such screening and breeding program, plant breeders have to focus on additive variance rather than non-additive. Thus, a search for superior sets of parents should be a major goal rather than identifying one or two superior hybrid combinations.

REFERENCES

Abd-El-Samie, and Y. Taha.1993. Nematode interactions with root-nodule bacteria. *In* Khan, M.W. (ed.) Nematode Interactions. Chapman & Hall. London.

Bain, D.C. 1959. Selection for resistance to root-knot of white and red clover. Plant Dis. Rep. 43:318-322.

Baker, R.J. 1978. Issues in diallel analysis. Crop Sci. 18:533-536.

Baltensperger, D.D., C.E. Dean, E.S. Horner. 1984. Registration of Osceola white clover. Crop Sci. 24:1211.

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

Baxter, L.W., and P.B. Gibson. 1959. Effect of root-knot nematodes on persistence of white clover. Agron. J. 10:603-604.

Bird, A.F. 1979. Histopathology and physiology of synctia. P. 155-172. *In* Lamberti, F. and C.E.Taylor (ed.). Root-knot nematodes (*Meloidogyne* Species) systematics, biology and control. Academic Press Inc. London.

Brink, G.E., and G.L. Windham. 1990. White clover response to nematode infestation and plant density. Crop Sci. 30:1295-1298.

Bunte', R., J. Muller' and W. Friedt. 1997. Genetic variation and response to selection for resistance to root-knot nematodes in oil radish (Raphanus sativus ssp. oleiferus). Plant Breed. 116:263-266.

Burow, M.D. and J.G. Coors, 1994. DIALLEL. A microcomputer program for the simulation and analysis of diallel crosses. Agron. J. 86:154-158.

Call, N.M., K.H. Quesenberry, D.S. Wofford, and R.A. Dunn. 1997. Combining ability analysis of resistance of southern root-knot nematode in red clover. Crop Sci. 37:121-124.

Chahal., G.S., and S.S. Gosal. 2002. Principles and procedures of plant breeding. Alpha Science Int'l Ltd, Oxford, UK.

Chambliss, C.G., and D.S. Wofford. 2006. White clover [Online]. Agronomy Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Available at http://edis.ifas.ufl.edu/AA198 (verified 10/01/2008).

Chitwood, B.G. 1949. Root-knot nematodes. I. A revision of the genus *Meloidogyne* Goeldi 1887. Proc. Helminthol. Soc. Wash. 16:90-104.

Claverie, M., N. Bosselut, A.C. Lecouls, R. Voisin, B. Lafargue, C. Poizat, M. Kleinhentz, F. Laigret, E. Dirlewanger and D. Esmenjaud. 2004. Location of independent root-knot nematode resistance genes in plum and peach. Theor. Appl. Genet. 108:765–773.

Cook, R., and G.W. Yeates, 1993. Nematode pests of grassland and forage crops. p. 305-350. *In* Evans, K., Trudgill, D.L., Webster, J.M. (Eds.). Plant parasitic nematodes in temperate agriculture. CAB International., Wallingford, UK

Cook, R., K.A., Mizen, R.A. Plowright and, P.A. York. 1992. Observations on the incidence of plant parasitic nematodes in grassland in England and Wales. Grass Forage Sci. 47:274-279.

De Guiran, G., and M. Ritter. 1979. Life cycle of *Meloidogyne* species and factors influencing their development. P. 173-192. *In* Lamberti F. and C.E.Taylor (eds). Root-knot nematodes (*Meloidogyne* Species) systematics, biology and control. Academic Press Inc. London.

Dropkin V.H. 1989. Introduction to plant nematology. John Wiley and Sons. NY

Dropkin, V.H. 1969. Cellular responses of plants to nematode infections. Annual review of phytopathology. 7:101-122

Dunn, R.A., and G.M. Sydenham. 2003. Plant nematode biology and root-knot nematode symptoms [Online]. Entomology and Nematology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Available at http://edis.ifas.ufl.edu/IN034 (verified 10/01/2008).

Ehlers, J.D., W.C. Matthews, Jr., A.E. Hall, and P.A. Roberts. 2000. Inheritance of a broad-based form of root-knot nematode resistance in cowpea. Crop Sci. 40:611-618.

Eisenback J.D. 1993. Interactions between nematodes in cohabitance. p. 134-174. *In* Khan, M.W. (ed.) Nematode interactions. Chapman & Hall. London.

Evans, K. and P.P.J. Haydock. 1993. Interactions of nematodes with root-rot fungi. p. 104-133. *In* Khan, M.W. (ed.) Nematode interactions. Chapman & Hall. London.

Ferris H. 1999. *Meloidogyne javanica*. Phylogenetic and functional classification of nematodes [Online]. Available at http://plpnemweb.ucdavis.edu/nemaplex/taxadata/G076S4.htm (verified on Nov 01, 2008).

Francl, L.J. and T.A. Wheeler. 1993. Interaction of plant-parasitic nematodes with wilt-inducing fungi. p. 79-103. *In* Khan, M.W. (ed.) Nematode interactions. Chapman & Hall. London

Gibson, P.B. and W.A. Cope. 1985. White clover. p. 471-490. *In* Taylor, N.L. (ed.). Clover science and Technology. ASA,CSSA, and SSSA. WI. USA.

Gibson, P.B. 1973. Registration of SC-1 white clover germplasm. Crop sci. 13:131.

Griffing, B. 1956a. Concept of general and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci. 9:463-493.

Griffing, B. 1956b. A generalized treatment of the use of diallel crosses in quantitative inheritance. Heredity. 23:31-50.

Hartman, K.M., and J.N. Sasser. 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. p. 69-77. *In* Barker K.R., C.C. Carter, and J.N. Sasser (eds.). An advanced treatise on *Meloidogyne*. Vol. 2. Methodology. North Carolina State University Graphics, Raleigh, NC.

Holbrook, C.C., D.A. Knauft, and D.W. Dickson. 1983. A technique for screening peanut for resistance to *Meloidogyne arenaria*. Plant Dis. 67:957-958.

Hussey, R.S., and G.J.W. Janssen. 2002. Root-knot nematodes: *Meloidogyne* Species .p. 43-70. *In* Starr, J.L., R.J. Cook, and J. Bridge (ed.). Plant resistance to parasitic nematodes. CABI Publishing, Egham, UK

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

Magari, R., and M.S. Kang. 1994. Interactive BASIC program for Griffing's diallel analyses. J. Hered. 85:336.

McLeish, L.J., G.N. Berg, J.M. Hinch, L.V. Nambiar, and M.R. Norton. 1997. Plant parasitic nematodes in white clover and soil from white clover pastures in Australia. Aust. J. Exp. Agric. 37:75-82.

McPherson, G.R., J.N. Jenkins, J.C. McCarty, and C.E. Watson. 1995. Combining ability analysis of root-knot nematode resistance in cotton. Crop Sci. 35:373-375.

Melakeberhan, H. and J.M. Webster. 1993. The phenology of plant-nematode interaction and yield loss. p. 26-41. *In* Khan, M.W. (ed.). Nematode interactions. Chapman & Hall. London.

Mercer, C.F., J. Van Den Bosch, K.J. Miller. 2000. Progress in recurrent selection and in crossing cultivars with white clover resistant to the clover root-knot nematode *Meloidogyne trifoliophila*. N.Z. J. Agric. Res. 43:1:41.

Pederson, G. A., and G.L. Windham. 1989. Resistance to *Meloidogyne incognita* in *Trifolium* interspecific hybrids and species related to white clover. Plant Dis. 73:567-569.

Pederson, G.A., G.L. Windham, M.M. Ellsbury, M.R. Mcloughlin, R.G. Pratt, and G.E. Brink. 1991. White clover yield and persistence as influenced by cypermethrin, benomyl, and root-knot nematode. Crop Sci. 31:1297-1302.

Pederson, G.A. and G.L. Windham. 1992. Diallel analysis of resistance in white clover to southern root-knot nematode. Crop Sci. 32:1160-1162.

Pederson, G.A, and G.L. Windham. 1995. Registration of MSNR4 root-knot nematode resistant white clover germplasm. Crop Sci. 35:1234-1235.

Quesenberry, K.H., D.D. Baltensperger, R.A. Dunn, C.J. Wilcox, and S.R. Hardy. 1989. Selection for tolerance to root-knot nematodes in red clover. Crop Sci. 29:62-65.

Quesenberry, K.H., N.M. Call, D.E. Moon, and R.A. Dunn. 1997. Response of native Eastern North American *Trifolium* spp. to root-knot nematodes. Crop Sci. 37:270-274.

Quesenberry, K.H., G.M. Prine, O.C. Ruelke, L.S. Dunavin, and P. Mislevy. 1993. Registration of 'Cherokee' red clover. Crop Sci. 33:208-209.

Salesses, G., E. Dirlewanger, D. Esmenjaud, and A.C. Lecouls. 1998. Root-knot nematode resistance in myrobalan plum inheritance and rootstock breeding perspectives using marker-assisted selection. Acta Hort. 478:45-52.

Sasser, J.N., J.D. Eisenback, C.C. Carter, and A.C. Triantaphyllou. 1983. The international meloidogyne project-its goals and accomplishments. Annu. Rev. Phytopathol. 21:271-288.

Sitaramaiah, K. and K.N. Pathak. 1993. Nematode bacterial disesae interactions. p. 232-250. *In* Khan, M.W. (ed.) Nematode interactions. Chapman & Hall. London.

Skipp, R.A., and, M.J Christensen. 1983. Invasion of white clover roots by fungi and other soil micro-organisms. Survey of root-invading fungi and nematodes in some New Zealand pastures. N.Z. J. Agric. Res. 26:151:5.

Sprague, G.F., and L.A. Tatum. 1942. General vs. specific combining ability in single crosses of corn. J. Am. Soc. Agron. 34:923-932.

Tamulonis, J.P., B.M. Luzzi, R.S. Hussey, W.A. Parrott, and H.R. Boerma. 1997. RFLP mapping of resistance to southern root-knot nematode in soybean. Crop Sci. 37:1903-1909.

Taylor, A.L., and J.N. Sasser. 1978. Biology, identification, and control of root-knot nematodes (*Meloidogyne* species). Dept. of Plant Pathology, North Carolina State University and the United States Agency for International Development.

Taylor, N.L. 1980. Clovers. *In* Fehr, W.R. and H.H. Hadley (eds). Hybridization of crop plants. ASA, CSSA, and SSSA. Wisconsin.

Thorne G. 1961. Principles of Nematology. McGraw Hill Book Company Inc. New York

Triantaphyllou, A.C. 1966. Polyploidy and reproductive patterns in the root-knot nematode Meloidogyne hapla. J. Morphol. 118:403-413.

UC SAREP. 2008. White Clover. UC SAREP Online Cover Crop Database [Online]. Available at http://www.sarep.ucdavis.edu/cgi-bin/ccrop.exe/show_crop_42 (verified on Nov 26, 2008).

USDA NRCS Plant Materials Program. 2002. White Clover. Plant fact sheet [Online]. Available at http://plants.usda.gov/factsheet/pdf/fs_trre3.pdf (verified on Oct 31, 2008),

Van Den Bosch, J., and C.F. Mercer. 1996. Variation in white clover for root-knot nematode resistance. N.Z. J. Agric. Res. 39:137-148.

Williams, W.M. 1987a. Adaptive variation. p. 299-322. *In* Baker M.J., and W.M. Williams (ed.) White Clover 1987. CAB international., Oxon, UK.

Williams, W.M. 1987b. Genetics and breeding. p. 343-420. *In* Baker M.J., and W.M. Williams (ed.) White Clover 1987. CAB international., Oxon, UK.

Williams, W.P., and G.L. Windham. 1990. Resistance of maize to *Meloidogyne arenaria* and *Meloidogyne javanica*. Theor. Appl. Genet. 80:810-812.

Williamson, V.M. 1998. Root-knot nematode resistance genes in tomato and their potential for future use. Annu. Rev. Phytopathol. 36:277-293.

Williamson, V.M. and R.S. Hussey. 1996. Nematode pathogenesis and resistance in plants. The Plant Cell. 8:1735-1745.

Williamson, V.M., and A. Kumar. 2006. Nematode resistance in plants: the battle underground. Trends in Genetics. 22:396-403.

Windham, G.L., and G.A. Pederson. 1989. Aggressiveness of *Meloidogyne incognita* host races on white clover. Nematropica 19:177-183

Wofford, D.S. and E. Ostmark. 2005. Effect of selection for root-knot nematode tolerance in white clover. Proc. Int. Annu. Meet. Nov. 6-10, 2005. ASA, CSSA, SSSA. Madison, WI.

Xiang, B., and B. Li. 2001. A new mixed analytical method for genetic analysis of diallel data. Can. J. For. Res. 31:2252-2259.

Yeates, G.W., W.B. Healy, J.P. Widdowson, N.A. Thompson, B.N. MacDiarmad. 1975. Influence of nematicides on growth of plots of white clover on a yellow brown loam. N.Z. J. Agric. Res. 18:411-416.

Zahid, M.I., J. Nobbs, G.M. Gurr, M. Hodda, A. Nikandrow, W.J. Fulkerson and H.I. Nicol. 2001. Effect of the clover root-knot nematode (*Meloidogyne trifoliophila*) on growth of white clover. Nematology 3:437-446.

Zhang, J.F., C. Waddell, C. Sengupta-Gopalan, C. Potenza, R.G. Cantrell. 2007. Diallel analysis of root-knot nematode resistance based on galling index in upland cotton. Plant Breed. 126:164-168.

Zhang, Y., and M.S. Kang. 1997. DIALLEL-SAS: A SAS program for Griffing's diallel analyses. Agron. J. 89:176-182.

Zhang, Y., M.S. Kang, and K.R. Lamkey. 2005. DIALLEL-SAS05: A comprehensive program for Griffing's and Gardner-Eberhart analyses. Agron. J. 97:1097-1106.

Zhen-Xiang, L.U., G.L. Reighard, A.P. Nyczepir, T.G. Beckman and D.W. Ramming. 2000. Inheritance of resistance to root-knot nematodes (*Meloidogyne* sp.) in Prunus rootstocks. HortScience 35:1344-1346.

BIOGRAPHICAL SKETCH

I was born in Chitwan, Nepal, 80 miles from the capital, Kathmandu. I was born to Mr. Hari Prasad Acharya and Mrs. Radha Devi Acharya as their youngest child. I completed my high school, always the first in my class. I received my Bachelor of Science degree in agriculture in 2005 from Institute of Agriculture and Animal Sciences (Tribhuvan University, Nepal) with a major in plant breeding. I joined The University of Florida in Spring 2007 for an M.S. in agronomy (genetics). I completed my M.S. in Fall 2008. I will join The University of Georgia for a Ph.D. degree from The Institute of Plant Breeding, Genetics and Genomics beginning in Spring 2009.