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**Marker-Assisted Selection for Soybean Cyst Nematode Resistance and Accompanying  
Agronomic Traits**

**A THESIS  
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL  
OF THE UNIVERSITY OF MINNESOTA  
BY**

**Joann Mudge**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS  
FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY**

**James H. Orf, Adviser  
Nevin Dale Young, Adviser**

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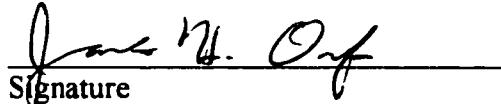
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and that any and all revisions required by the final  
examining committee have been made.

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GRADUATE SCHOOL

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## **Dedication**

I would like to dedicate this thesis to my husband, Simon Mudge, for the love and support of he has given me throughout. Although I cannot say he has done it without complaint, he has successfully braved the humid summers and frigid winters of Minnesota, including his first ice storm. He has always encouraged and supported me, and has helped me keep my perspective through many difficult periods. His presence and love have made this thesis less difficult.

## Abstract

The soybean cyst nematode [*Heterodera glycines* Ichinohe; SCN] causes extensive economic damage to soybean [*Glycine max* (L.) Merrill] through yield reduction. The use of resistant soybean cultivars is recognized as an integral part of control strategies. However, breeding for resistance is challenging. Phenotypic assays for resistance are laborious, time-consuming, and subject to environmental and experimental error. However, molecular markers linked to genes of interest have recently been used in place of phenotypic assays to indirectly select for traits. This process, termed marker-assisted selection (MAS), may decrease the labor and time involved in SCN resistance screening. In addition, markers are not affected by the environment as are phenotypic assays. Another challenge to SCN resistance breeding is that resistant lines often show lower yield than comparable susceptible lines. If a yield depression locus is linked to a resistance locus, molecular markers may be useful in separating the two. In this study, simple sequence repeats (SSRs) were mapped to the region surrounding *rhg1*, the main SCN resistance locus. These markers provide several highly polymorphic, codominant, easily-assayed markers for use in MAS. The SSRs, BARC-Satt309 and BARC-Sat\_168, were placed within 1 centimorgan of *rhg1* and can distinguish most commonly used susceptible parents from resistant parents. In total, nine SSR markers were placed in an 11 cM region surrounding *rhg1*. This study also located a yield depression locus linked to *rhg1* through quantitative trait locus (QTL) mapping analysis. The SSRs may be able to separate these two loci to produce resistant, high yielding lines. Because the MAS program developed here has focused on *rhg1*, the effect of alternate parental alleles at *rhg1* on SCN reproduction was examined using near-isogenic lines. Lines with a resistance allele at *rhg1* proved effective in limiting reproduction of SCN in the field. Finally, MAS was compared to greenhouse assays for SCN resistance. Markers eliminated susceptible lines efficiently, reducing line numbers by up to 65%. MAS can be used to efficiently breed for SCN resistance. Markers have been positioned near *rhg1* that can be easily assayed to routinely select for SCN resistance at *rhg1* and reduce linkage drag around this locus.

## Table of contents

Acknowledgments.....	i
Dedication.....	iii
Abstract.....	iv
Table of contents.....	v
List of tables.....	ix
List of figures.....	x
Literature review .....	1
Soybean cyst nematode damage.....	1
Economic impact .....	1
Symptoms.....	1
Environmental influences.....	2
Biology of soybean cyst nematode.....	2
Life cycle.....	2
Race divisions.....	3
Long distance movement .....	3
Host range .....	3
Control of soybean cyst nematode .....	4
Nematicides.....	4
Tolerance.....	4
Biocontrol.....	4
Crop rotation and resistance.....	5
Genetics of SCN resistance.....	6
Sources of resistance.....	6
Classical genetic studies.....	6
Molecular genetic studies.....	7
Traditional breeding for SCN resistance .....	7
Molecular markers in plant breeding.....	8
Marker-assisted selection.....	9
Simple sequence repeat markers.....	10

<b>References</b>	12
<b>Chapter 1 . Genetic analysis of yield depression associated with <i>rhg1</i> and accompanying agronomic traits</b>	20
<b>Abstract</b>	21
<b>Introduction</b>	22
<b>Material and methods</b>	24
<b>Plant materials and field trials</b>	24
<b>DNA genotyping and mapping</b>	25
<b>Statistical analysis of quantitative trait loci</b>	25
<b>Results and discussion</b>	28
<b>Yield locus near <i>rhg1</i></b>	28
<b>QTL for agronomic traits across the genome</b>	30
<b>Homoeologous regions</b>	32
<b>Summary</b>	32
<b>Perspectives</b>	32
<b>References</b>	35
<b>Chapter 2 . Effect of <i>rhg1</i> on SCN populations in the field and further characterization of a yield locus associated with <i>rhg1</i></b>	50
<b>Abstract</b>	51
<b>Introduction</b>	52
<b>Material and methods</b>	54
<b>Plant materials</b>	54
<b>Field trials</b>	55
<b>Agronomic data</b>	56
<b>Field SCN resistance assays</b>	56
<b>Greenhouse SCN resistance assays</b>	57
<b>DNA extraction</b>	58
<b>Genotyping</b>	58
<b>Statistical analysis</b>	59
<b>Results and discussion</b>	61

<b>NIL sets</b>	61
Effect of <i>rhg1</i> on SCN reproduction in the greenhouse	62
Effect of <i>rhg1</i> on SCN reproduction in the field	63
Yield locus linked to <i>rhg1</i>	65
Yield in SCN-infested fields	67
Summary	69
<b>References</b>	70
<b>Chapter 3 . Molecular marker discovery for use in marker-assisted selection</b>	100
<b>Abstract</b>	101
<b>Introduction</b>	102
<b>Material and methods</b>	104
Plant materials	104
Greenhouse assays for SCN resistance	104
DNA extraction and RFLP marker analysis	105
SSR marker development	105
SSR analysis	106
Mapping	107
<b>Results and discussion</b>	108
Early MAS results	108
Satt309 in MAS	109
Other SSRs	110
Summary	110
<b>References</b>	111
<b>Chapter 4 . Value of marker-assisted selection in selecting soybean lines resistant to soybean cyst nematode</b>	121
<b>Abstract</b>	122
<b>Introduction</b>	123
<b>Material and methods</b>	125
Plant materials	125
Greenhouse assays	125

<b>Genotyping</b> .....	126
<b>Statistical analysis</b> .....	127
<b>Results and discussion</b> .....	128
<b>Distribution of Satt309 genotype and SCN phenotype</b> .....	128
<b>Accuracy of MAS in predicting susceptibility to SCN</b> .....	130
<b>Accuracy of MAS in predicting resistance to SCN</b> .....	130
<b>Summary of data from populations 1, 2, and 3</b> .....	131
<b>Limitations of the greenhouse assay standard</b> .....	131
<b>Other limitations</b> .....	134
<b>Conclusions on MAS for SCN resistance</b> .....	135
<b>References</b> .....	136
<b>Summary and perspectives</b> .....	151
<b>Summary</b> .....	151
<b>Perspectives</b> .....	153
<b>Appendix</b> .....	156

## List of tables

Table 1. Soybean cyst nematode race classification system.....	19
Table 1-1. Summary of ANOVA data for yield.....	38
Table 3-1. SSRs near <i>rhg1</i> .....	113
Table 4-1. Breeding populations tested for reaction to SCN both in the greenhouse and indirectly using a linked molecular marker.....	138
Table A-1. Climatic and environmental data.....	157
Table A-2. Precipitation data for 1996, 1997, 1998, and averages for Waseca and Lamberton, MN.....	158
Table A-3. Planting and sampling dates for the RIL populations and the NIL sets .....	159
Table A-4. Summary of phenotypic data available for the RIL population and NIL sets .....	160
Table A-5. Genetic map used in the analysis of data in the RIL population.....	162
Table A-6. ANOVA table comparing Bng173Hae-III to yield in the RIL population ...	164
Table A-7. Planting information for NIL sets.....	165
Table A-8. Phenotypic data taken on the NIL sets.....	166
Table A-9. Cyst count data for control cultivars used in the greenhouse and field SCN resistance assays for the NIL-49 subset and NIL-79-23.....	197
Table A-10. Cyst count data for control cultivars used in greenhouse assays that were compared to marker-assisted selection for SCN resistance .....	198

## List of figures

Figure 1-1. Test statistic plots for yield.....	40
Figure 1-2. Yield of recombinant inbred lines grouped by their alleles at Bng173Hae-III .....	42
Figure 1-3. Test statistic plots for yield QTL x environment interaction.....	44
Figure 1-4. QGENE multiplot output for yield and lodging near <i>rhg1</i> .....	46
Figure 1-5. QGENE multiplots of agronomic traits in select linkage groups .....	48
Figure 2-1. Genotypes of R and S lines in the four NIL sets.....	72
Figure 2-2. NIL lines assayed with BARC-Satt163.....	74
Figure 2-3. Cyst counts for NIL-79-23 lines in the greenhouse SCN resistance assay ..	76
Figure 2-4. SCN cysts on the roots of NIL-79-23 lines .....	78
Figure 2-5. Spring Egg Counts .....	80
Figure 2-6. Cysts on the roots of NIL-49 subset lines.....	82
Figure 2-7. Reproduction factor for NIL-79-23 .....	84
Figure 2-8. Reproduction factor for the NIL-49 subset in 1998 .....	86
Figure 2-9. Reproduction factor for the NIL-49 subset in 1997 .....	88
Figure 2-10. Scattergram of reproduction factor for the NIL-49 subset in 1997 at Lamberton .....	90
Figure 2-11. Yield of NIL-49-29-R and NIL-49-29-S lines in non-infested and infested sites .....	92
Figure 2-12. Yield of NIL-79-23-R and NIL-79-23-S lines in non-infested and infested sites .....	94
Figure 2-13. Yield of NIL-49-R and NIL-49-S lines in non-infested and infested sites ..	96
Figure 2-14. Yield of NIL-79-R and NIL-79-S lines in non-infested and infested sites ..	98
Figure 3-1. Genetic map of the region surrounding <i>rhg1</i> .....	115
Figure 3-2. Amplification of BAC clones using SSR primers .....	117
Figure 3-3. BARC-Sat_168 alleles.....	119
Figure 4-1. Genotype of breeding lines at BARC-Satt309.....	139
Figure 4-2. SCN phenotype of breeding lines .....	141
Figure 4-3. SCN phenotype of lines predicted to be susceptible by BARC-Satt309.....	143

Figure 4-4. SCN reactions in the greenhouse for lines predicted to be resistant by BARC-Satt309 .....	145
Figure 4-5. Comparison of greenhouse assays and marker-assisted selection for soybean cyst nematode resistance in populations 1, 2, and 3.....	147
Figure 4-6. Comparison of SCN reactions across greenhouse runs.....	149
Figure A-1. Genotypes of R lines, S lines, and crossovers in the NIL sets.....	199

## Literature review

### Soybean cyst nematode damage

#### Economic impact

The soybean cyst nematode [SCN, *Heterodera glycines* Ichinohe] causes extensive economic damage to soybean [*Glycine max* (L.) Merrill] in the United States and around the world (Doupnik, 1993; Wrather *et al.*, 1997; Pratt and Wrather, 1998). In 1996 and 1997, it was estimated to cause an 8% yield loss in the United States, approximately equal to damage caused by all other diseases combined (Wrather, 1998). In Minnesota, the nematode has slowly been spreading north and economic damage is increasing as the area of nematode infestation increases. SCN has been reported to reduce Minnesota soybean yields up to 13 kg/hectare during warm, dry growing seasons (Stienstra and MacDonald, 1990).

#### Symptoms

The soybean cyst nematode was first described in Japan in 1915 by Hori (1915). In the United States SCN was first observed in 1954 in North Carolina (Winstead *et al.*, 1955). The above-ground symptoms of SCN damage include yellowing, stunting, and decreased yield, which all occur in a patchy distribution across an infested field (Sinclair and Backman, 1989). These symptoms are similar to symptoms of other diseases, as well as symptoms of some mineral deficiencies and abiotic stresses. Because above-ground symptoms are not unique to SCN damage, diagnosis is possible only by identifying the cyst stage on the root. It is important to look for cysts because yield losses can occur before SCN levels are high enough to cause detectable above-ground symptoms (Noel and Edwards, 1996).

## Environmental influences

Damage by SCN is influenced by several environmental factors, including soil type (Koenning *et al.*, 1988), soil moisture (Heatherly and Young, 1991; Sardanelli and Kenworthy, 1997), temperature, pH (Anand *et al.*, 1995), and soil disturbance (Tyler *et al.*, 1987; Young, 1987). Because of environmental influences, nematode damage may vary from year to year.

This makes field screening of resistance difficult. Even under controlled conditions in the greenhouse, environmental and experimental variation necessitate the use of several progeny replications. Controlling soil moisture in greenhouse assays has, however, helped reduce environmental variation (Sardanelli and Kenworthy, 1997).

## Biology of soybean cyst nematode

### Life cycle

Nematodes go through several molting stages during their life cycle. The first nematode molt occurs within the egg. Nematodes in the second juvenile (J2) stage hatch from eggs in response to hatching factors released from the roots of soybean plants. The nematodes must then swim to a root within a few centimeters and burrow into the root, causing mechanical damage. Inside the root, the nematode sets up a feeding site, or syncytium, by taking over a cell's machinery for its own use (Endo, 1964; Endo, 1971). The syncytium acts as a sink, allowing the nematode to tap into the plant's resources. The drain on the plant's resources is thought to reduce plant yield.

After additional molts, the males exit from the root and migrate to find a female with which to mate. The body of the female swells and bursts through the exterior of the root. Females produce eggs that are contained both inside and outside of their bodies. Afterward, the females die and their bodies harden to form a lemon-shaped cyst that can protect eggs in the soil for many years. The entire life cycle takes less than a month.

During a single growing season, the nematode can undergo several cycles of reproduction (Riggs and Wrather, 1992).

### Race divisions

SCN “races” are extremely variable and difficult to define precisely. Traditionally, nematode populations have been assigned races by testing them for pathogenicity on a set of soybean host differentials and classifying them according to their ability to reproduce on each (Table 1; Riggs and Schmitt, 1988). Increased virulence and even race shifts have been known to occur within an SCN population when challenged with a resistant soybean cultivar (Young, 1982, 1998; Luedders, 1985). Races 1 and 3 are the most common races in the northern United States (Kim *et al.*, 1997).

### Long distance movement

Nematodes can only travel short distances in the soil on their own. However, nematodes have spread over most of the soybean producing regions of the United States since their first detection in the 1950s. Long distance spread of SCN is accomplished through the bulk movement of soil, which can occur through movement of *Rhizobium* inoculum, plants (Riedel, 1988), farm equipment, birds (Epps, 1971), soil peds in seed (Epps, 1969), and water or wind erosion of soil.

### Host range

The soybean cyst nematode has a very broad host range. More than 100 different plant species, many but not all in the Fabaceae family, are known to be efficient hosts for SCN. More than 150 additional species can support nematode reproduction (summarized in Riggs, 1992). As more species are tested, this number will certainly increase. Most crop species, however, are considered non-hosts, including corn, which is often used in a rotation with soybean in Minnesota. Unfortunately, hosts of SCN can grow as weeds in

agricultural fields. This, combined with the longevity of eggs contained in cysts, makes it impractical to eliminate SCN once it is in a field by not rotating out of soybeans.

### Control of soybean cyst nematode

#### Nematicides

Once a field is infested with soybean cyst nematode, several control options exist. Nematicides have been effective against SCN (Endo and Sasser, 1958; Schmitt *et al.*, 1983; Zirakparvar, 1985). However, environmental concerns have led to the banning of several nematicides and the use of registered nematicides is often not economical on soybean crops (Johnson and Feldmesser, 1987). Also, nematicides protect soybean plants for only a short time at the beginning of the season. This early season protection allows more roots to form, which can support higher nematode reproduction later in the growing season. Therefore, although the nematicides provide enough protection to reduce SCN damage in the current season, the next crop can be attacked by a high number of nematodes (Anand and Koenning, 1986).

#### Tolerance

Lines that show tolerance to SCN allow the nematode to reproduce but show little yield loss (Radcliffe *et al.*, 1990). This minimizes selection pressure on SCN populations. However, because tolerant lines allow SCN reproduction, the inoculum load for the next season is high. Also, tolerance is thought not to hold up well under high stress conditions (Endo and Sasser, 1958).

#### Biocontrol

Researchers have looked at biocontrol as a method of controlling the *Heterodera* nematodes for more than 100 years (summarized in Tribe, 1977). Many studies have been

performed to look for potential biocontrol agents associated with SCN in the field (for example, Chen *et al.*, 1994). Few of these have been commercialized. One that has been utilized commercially was an application of nematode-trapping fungi, which was shown to be ineffective by Niblack and Hussey (1986). Several biocontrol agents currently under study show promise. For example, ARF-18, an unidentified fungal pathogen of SCN, can reduce SCN eggs 86-99% in sterile soils. It is effective across soybean cultivars and SCN races (Kim and Riggs, 1995) and is able to penetrate cysts and attack the eggs inside (Kim *et al.*, 1992). Promising results have similarly been found using strains of the fungus *Verticillium* alone (Meyer and Meyer, 1995; Stirling *et al.*, 1998) and in conjunction with sex pheromones and pheromone analogs to control SCN (Meyer and Huettel, 1996; Meyer *et al.*, 1997).

### Crop rotation and resistance

Crop rotation is currently an important strategy for managing SCN (Weaver *et al.*, 1988; Koenning *et al.*, 1993; Noel and Edwards, 1996). Farmers often plant two seasons of soybean followed by one season of corn, which is not a host for SCN (Young, 1998). At least one of the soybean lines in the rotation must be resistant (see below) in order to limit the reproduction of the nematode and keep soybean yield potential high (Macguidwin *et al.*, 1995; Wallace *et al.*, 1995; Senyu Chen *et al.*, University of Minnesota Southern Experiment Station, personal communication, 1998). Resistance, combined with crop rotation, is currently considered the best method of control (Stienstra and MacDonald, 1990; Caviness *et al.*, 1992; Macguidwin *et al.*, 1995; Young, 1998).

However, there are complications associated with planting resistant cultivars. Resistant cultivars cannot prevent root penetration and mechanical damage by the nematode. Under high nematode pressure, yield of resistant cultivars is still compromised, though not to the same extent as susceptible cultivars (Macguidwin *et al.*, 1995; Senyu Chen *et al.*, University of Minnesota Southern Experiment Station, personal communication, 1998). In addition, selection pressure imposed by resistant lines may cause race shifts (Young, 1982; Luedders, 1985; Young, 1998). Finally, the yield

potential of resistant lines may not be as high as susceptible lines when SCN damage is low or absent.

## Genetics of SCN resistance

### Sources of resistance

Several sources of SCN resistance have been found. 'Peking' and PI (plant introduction) 88788 have been the most extensively used sources of resistance in soybean breeding (Hartwig, 1985). PI 209332 is another source of resistance that has not been fully exploited (Anand and Brar, 1983; Anand and Gallo, 1984). PI 437654 is resistant to all of the common races of soybean cyst nematode (Anand and Gallo, 1984; Anand, 1986; Rao-Arelli *et al.*, 1991b). However, transfer of resistance has proven difficult and the agronomic qualities of PI 437654 are among the poorest of any of the sources of resistance. Recently, varieties with resistance from PI 209332, such as 'Faribault' (Orf and MacDonald, 1995) and from PI 437654, such as 'Hartwig' (Anand, 1992) have been released. Other sources of resistance have also been found, but few have been incorporated into varieties (Ross and Brim, 1957; Thomas *et al.*, 1975; Anand, 1982, 1986; Anand and Brar, 1983; Anand and Gallo, 1984; Anand *et al.*, 1985; Young, 1990, 1995).

### Classical genetic studies

Caldwell *et al.* (1960) published the first report on the genetics of SCN resistance. The authors suggested that resistance to SCN is controlled by three recessive genes, *rhg1*, *rhg2*, and *rhg3*. These three genes were shared in the three sources of resistance they examined, 'Peking', PI 90763, and PI 84751. They were likely testing resistance to race 1 of SCN (Rao-Arelli *et al.*, 1991a). Matson and Williams (1965) discovered another gene in Peking conferring resistance to what was probably race 3 (Rao-Arelli *et al.*, 1991a). This was a dominant gene that the authors named *Rhg4*. Mansur *et al.* (1993) also

investigated resistance to race 3 in several sources, claiming that no more than four genes were involved in resistance and that resistance is predominantly additive, although some dominance effects might be involved. Other classical studies have also found the genetic control of resistance to race 3 to be oligogenic (Rao-Arelli *et al.*, 1989; Rao-Arelli *et al.*, 1992; Rao-Arelli, 1994).

### Molecular genetic studies

Molecular genetic studies have also been conducted to further characterize resistance loci and map their genomic location. Again, resistance was found to be oligogenic. One locus, on molecular linkage group G (MLG-G; Shoemaker and Olson, 1993) is shared by the resistance sources Peking, PI 90763, PI 88788, PI 437654, and PI 209332. This locus, termed *rhg1* after Caldwell's major gene (Caldwell *et al.*, 1960), is effective across several races, including races 1, 3, and 6 (Concibido *et al.*, 1994, 1996a, 1996b, 1997; Mahalingam and Skorupska, 1995; Webb *et al.*, 1995; Qiu *et al.*, 1997). However, Vierling *et al.* (1996) found the major gene on MLG-B.

Other loci that affect SCN resistance have been uncovered, most notably *Rhg4* on MLG-A in Peking and PI 437654, and possibly PI 209332 (Concibido *et al.*, 1994; Mahalingam and Skorupska, 1995; Webb *et al.*, 1995). Other minor QTL have also been detected but their effects are small, and they have not always been consistently uncovered (Concibido *et al.*, 1994, 1996a, 1996b, 1997; Mahalingam and Skorupska, 1995; Webb *et al.*, 1995; Vierling *et al.*, 1996; Qiu *et al.*, 1997).

### Traditional breeding for SCN resistance

There are several challenges that must be faced when developing SCN resistant soybean lines. Breeding for resistant cultivars is complicated because screening for SCN resistance is difficult. Patchy field distribution of nematodes and environmental variation makes results from field screenings variable. Even under controlled conditions in the

greenhouse, experimental error is high (Riggs and Schmitt, 1991) although some progress has been made in this area by controlling soil moisture (Sardanelli and Kenworthy, 1997). In order to obtain significant results from SCN resistance screening, one must test several progeny replications. Assays are labor-intensive and results take approximately 30 days to obtain. The number of lines that can be carried through a breeding program is therefore limited by the number of SCN resistance assays that can be performed.

A second challenge in breeding SCN resistant lines is the that the yield potential of resistant cultivars is often not as high as that of susceptible cultivars (Thomas *et al.*, 1984). When nematode pressure is low or absent, susceptible lines may even yield more than resistant lines. While some commercial varieties seemed to have eliminated this yield depression (Cary *et al.*, 1998), the depression in yield potential that often accompanies resistant lines needs to be overcome so that high-yielding, resistant cultivars can be produced routinely.

### Molecular markers in plant breeding

Molecular markers have provided new tools to plant breeders. These markers, which are short, specific stretches of DNA, can tag chromosomes and provide landmarks to characterize specific chromosomal regions. The closer two or more markers are together on a chromosome, the more often the same parental alleles from each marker will be inherited together. By studying patterns of coinheritance, a framework map of molecular markers can be constructed. Several dense genetic maps have been constructed for soybean (Lark *et al.*, 1993; Shoemaker and Olson, 1993; Keim *et al.*, 1997; Cregan *et al.*, 1999).

Genetic loci controlling traits of interest can be positioned in relation to markers (Tanksley *et al.*, 1989). For qualitative traits controlled by a single locus, this is relatively straightforward. In a population segregating for the trait of interest, classes of the trait can be compared to parental alleles at each marker. The trait is close to those markers whose alleles exhibit the best correspondence with trait classes.

Genetic loci underlying polygenic traits are more complicated to identify. These traits are controlled by many loci (termed quantitative trait loci or QTL). Several loci segregating in a population make it more difficult to match classes of a trait to marker alleles. Nevertheless, mapping QTL for quantitative traits is important because most agriculturally important traits are polygenic and quantitative in nature.

A great deal of literature has been devoted to the theory and practice of QTL detection. Several methods have been proposed for detecting QTL. Marker alleles can be compared to phenotypic values of the trait of interest using simple ANOVA, t-tests, or regression (Niemann-Soressen and Robertson, 1961). Interval mapping based on maximum likelihood procedures has also been implemented for QTL detection (Jayaker, 1970; Lander and Botstein, 1989; Jansen, 1992). Interval mapping uses information from flanking markers to test for QTL in intervals between markers, rather than just at marker loci. A computationally simpler method of interval mapping based on regression gives results similar to those of maximum likelihood-based interval mapping (Haley and Knott, 1992; Martinez and Curnow, 1992). Because regression-based interval mapping is computationally simpler, it can be extended to larger problems, such as analysis across multiple environments (Tinker and Mather, 1995a, 1995b). Composite interval mapping (CIM) has also been implemented to take into consideration variation caused by other QTL in the genome (Jansen and Stam, 1994; Zeng, 1994).

Once QTL are identified, more precise mapping of QTL and separation of linked QTL can be accomplished by the use of substitution mapping (Paterson *et al.*, 1990). By comparing the phenotypes of lines, such as near isogenic lines (NILs), with different intervals of introgression in a common background, one can narrow down the region in which the QTL resides.

#### Marker-assisted selection

Markers linked to QTL can be exploited by breeders in selection. Rather than selecting directly for the desired allele at the genetic locus of interest, one can select the desired parent's allele at a nearby molecular marker. The closer a marker is to the locus

of interest, the more often progeny will inherit the alleles at each from the same parent. Therefore the alleles at a nearby marker can be used to predict the alleles at the gene of interest. This indirect selection for genetic loci of interest using linked molecular markers, is called marker-assisted selection (MAS). MAS can be used with both qualitative and quantitative trait loci. (Paterson *et al.*, 1988; Lander and Botstein, 1989; Tanksley *et al.*, 1989; Keim *et al.*, 1990; Lande and Thompson, 1990).

MAS is especially attractive for SCN resistance breeding. Often the cost of marker analysis is substantially higher than the cost of assaying the trait phenotypically. In the case of SCN resistance, however, greenhouse assays are labor intensive and require several replications, so they cost only slightly less than the use of molecular markers (Denny *et al.*, 1996). Moreover, molecular marker analysis is much faster than SCN greenhouse screening, which allows more lines to be assayed. Molecular markers are not subject to environmental variation as are the phenotypic assays for SCN resistance, which require several progeny replications (Paterson *et al.*, 1988; Paterson *et al.*, 1990; Dudley, 1993; Beavis, 1994). Finally, *rhg1* provides a good target for MAS. This locus can control more than 50% of the variation in SCN resistance (Concibido *et al.*, 1996a, 1997). Although other loci have an effect on this trait, *rhg1* is considered essential for resistance.

### Simple sequence repeat markers

Simple sequence repeat markers (SSRs or microsatellites; Tautz, 1989; Weber and May, 1989) are a type of molecular marker particularly suited to MAS. SSRs provide a high degree of polymorphism, meaning that there is a high degree of likelihood that any two parents' alleles at the SSR locus can be distinguished from each other. This is critical for a marker system in soybeans, because soybean has an extremely narrow genetic base and therefore shows limited polymorphism (Keim, 1989). Other marker systems, especially restriction fragment length polymorphism (RFLPs; Keim *et al.*, 1992), are often unable to distinguish parental alleles.

In addition, SSRs, because they are based on PCR (polymerase chain reaction; Mullis *et al.*, 1986), are much faster and easier to assay than RFLPs. But like RFLPs,

SSRs are codominant markers and highly repeatable. This gives an advantage over RAPDs (random amplified polymorphic DNA; Williams *et al.*, 1990), another PCR-based marker system. SSRs are also less complicated and time-consuming than AFLPs (amplified fragment length polymorphisms; Vos *et al.*, 1995). Frequently, the size polymorphism between parental alleles is large enough that SSRs can be assayed on high resolution agarose gels. This eliminates the need for polyacrylamide gels and radioactivity- or fluorescence-based detection of DNA products, making screening of a large number of breeding lines relatively straightforward and rapid. Because SSRs have a high rate of polymorphism, are codominant, repeatable, and can often be run on agarose gels, these markers are an excellent candidate for use in MAS.

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**Table 1. Soybean cyst nematode race classification system**

Sixteen races of soybean cyst nematode have been defined based on the reaction of four differentials to soybean cyst nematode (Riggs and Schmitt 1988). The differentials are Pickett, Peking, PI 88788, and PI 90763. The '+' signal represents reactions in which the differential line developed more than 10% of the number of cysts that the susceptible variety, Lee, developed. If fewer than 10% developed, the reaction was classified as '-'.

Race	Pickett	Peking	PI 88788	PI 90763
1	-	-	+	-
2	+	+	+	-
3	-	-	-	-
4	+	+	+	+
5	+	-	+	-
6	+	-	-	-
7	-	-	+	+
8	-	-	-	+
9	+	+	-	-
10	+	-	-	+
11	-	+	+	-
12	-	+	-	+
13	-	+	-	-
14	+	+	-	+
15	+	-	+	+
16	-	+	+	+

**Chapter 1. Genetic analysis of yield depression associated with *rhg1* and  
accompanying agronomic traits**

## Abstract

Damage from the soybean cyst nematode (SCN) causes yield reduction in soybean cultivars. Planting resistant cultivars along with crop rotation is considered the best method of control. However, when nematode pressure is low or absent, susceptible cultivars often yield more than resistant cultivars. It has not been shown whether this yield depression in resistant lines is due to a yield gene linked to a resistance gene, a pleiotropic effect of a resistance gene, or some other cause. Data from a recombinant inbred population segregating for SCN resistance from plant introduction 209332 were used to examine this putative yield locus and its relationship with SCN resistance. Yield was most strongly associated with the marker Bng173-HaeIII, which is approximately 6 cM from *rhg1*, a major locus for SCN resistance. Data analyzed across years, locations, and replications using regression-based interval analysis and single marker analysis of variance, gave significance levels just below and above an experiment-wide threshold of  $p = 0.05$ , respectively. Considerable differences between locations and years were found with ANOVA and regression analysis but not with the regression-based interval mapping. The allele for low yield came from the resistant, low yielding parent. Other agronomic traits, including maturity, lodging, height, protein, and oil were also examined.

## Introduction

The soybean cyst nematode causes significant economic damage to soybean in the United States (Doupnik, 1993; Wrather *et al.*, 1994; Pratt and Wrather, 1998). The use of soybean lines with resistance to the nematode is one of the best methods for controlling SCN (Stienstra and MacDonald, 1990; Macguidwin *et al.*, 1995; Young, 1998). However, there are several difficulties associated with breeding soybean cultivars for resistance.

One challenge in the use of resistant genotypes is that agronomic properties of resistance sources are poor. These lines often lodge, have low yield, colored seed, and other undesirable characteristics (Matson and Williams, 1965). Because resistance sources are poor agronomically, it may be easier for breeders to use lines derived from resistant sources rather than the resistant sources themselves. However, it may be desirable to breed directly from the source because all resistance and related genes are not always passed on to the progeny (Luedders, 1989).

Another challenge in breeding for SCN resistance is that resistant varieties often yield less than comparable susceptible varieties when nematode pressure is low or absent. This could be a pleiotropic effect of a gene or genes controlling resistance. Alternatively, a yield gene could be linked to a resistance gene and introduced into resistant varieties as a result of linkage drag. If this second alternative is true, the resistance locus and the yield depression locus should be separable.

Molecular marker technology has given breeders new tools to face challenges in breeding. Even poor agronomic parents, such as SCN resistant sources, can contribute genes to improve agronomically-important traits. Markers can be used to identify and direct alleles for desirable traits from the less desirable parent, potentially making wide crosses less problematic (deVicente and Tanksley, 1993, Eshed and Zamir, 1994). By mapping individual loci that affect a trait, markers near loci of interest can be found and used to monitor the alleles at these loci. Desirable loci can be retained and undesirable

loci discarded, even if the two locus types are linked. Markers can also be used to target large sections of the genome to obtain the agronomically-superior parent's alleles. Using markers, therefore, any chromosomal section with desirable alleles can be retained and sections with deleterious alleles discarded (Tanksley and Nelson, 1996).

In the case of SCN resistance, it may be possible to use molecular markers to ensure transfer of the resistance allele at *rhg1*, the main SCN resistance locus (Caldwell *et al.*, 1960; Concibido *et al.*, 1994, 1996a, 1996b, 1997; Mahalingam and Skorupska, 1995; Webb *et al.*, 1995; Qiu *et al.*, 1997). Simultaneously, crossovers on either side of *rhg1* could be sought. This would minimize linkage drag and, if there is indeed a linked yield locus, ensure this locus contains the high yield allele from the susceptible parent. The genome could also be monitored for other desirable alleles at specific loci or for the susceptible and agronomically superior parent's alleles in regions other than those conferring SCN resistance.

In this study, the presence of a yield locus associated with SCN resistance was confirmed and the genetic relationship between the yield locus, *rhg1*, and molecular markers was examined. QTL for agronomically-important traits across the genome, including yield, height, maturity, lodging, protein, and oil were found.

## Material and methods

### Plant materials and field trials

A recombinant inbred line (RIL) population was previously developed from the cross of a susceptible soybean cultivar, Evans (Lambert and Kennedy, 1975), by a resistant plant introduction, PI 209332 (Anand and Brar, 1983; Anand and Gallo, 1984). This population has been previously described (Concibido *et al.*, 1996a). One of the original 98 lines was dropped because of a change in seed coat color. Field trials were conducted on the F<sub>4:8</sub>, F<sub>4:9</sub>, and F<sub>4:10</sub> generations in 1996, 1997, and 1998, respectively. All trials were conducted at Lamberton and Waseca, MN in each year. In order to separate the effect of the yield depression locus from the effect of SCN pressure on yield, all trials were conducted in sites not infested with SCN. Lines were blocked into three maturity groups based on preliminary results from a field trial done in Waseca, MN in 1995. Lines that were not in this preliminary test were put in the latest maturity block. Information on climate, field conditions, and planting dates are given in the appendix (Tables A-1, A-1, and A-3).

Field trials were conducted in four row plots 3.7 m long with 25 cm spacing between rows. Plots were planted with 240 seed and were end-trimmed from 3.7 m to 2.5 m in length to avoid border effects. All four rows were harvested with a combine. Seed was weighed at 13% moisture and converted to kg per hectare to determine yield. Data were also taken on maturity, lodging, and height, which can affect yield. Maturity was recorded as the number of days before or after August 31 when 95% of the plants in each plot reached mature color. Lodging was recorded on a scale from 1 to 5 with 1 being erect and 5 being prone. Height, measured in centimeters, was taken on three plants from the middle to rows of each plot and averaged. Height was not recorded in Lamberton in 1997. Total protein and oil as a percentage of seed weight at 13% seed moisture were determined on only the first replication of each location in each year using near-infrared

technology (Shenk and Westerhaus, 1991). Equations to determine total protein and oil from NIR spectra were developed by Art Killam (University of Minnesota, St. Paul, MN, personal communication, 1999). Information on obtaining the data is contained in the appendix (Table A-4).

### DNA genotyping and mapping

The entire RIL population was previously genotyped in the F<sub>4:5</sub> generation with markers across the genome and a genetic map constructed (Concibido *et al.*, 1996a). The *rhg1* locus was scored as a quantitative trait and placed on the map (Concibido *et al.*, 1994, 1996a). In this study, simple sequence repeats (SSRs) were added to the map, especially in the molecular linkage group G (MLG-G; Shoemaker and Olson, 1993) region near *rhg1*. These were mapped either with the entire RIL population or with a subset of the population with crossovers near *rhg1* (see Chapter 3). A total of 142 markers were used (see appendix, Table A-5).

### Statistical analysis of quantitative trait loci

QTL were identified using several methods. Phenotypic data were analyzed across years and locations and compared to genotypic data at every marker using MQTL (Tinker and Mather, 1995a, 1995b). MQTL was specifically designed to analyze data from replicatable progeny, such as RILs, across years and locations without requiring homogeneous variances. And, unlike traditional single marker ANOVA or regression, MQTL uses interval mapping to search for QTL at and between markers. In addition, MQTL uses permutations of the data to derive an experiment-wide type I error threshold specific to the data used and uninfluenced by violations of the requirement of homogeneous variances of ANOVA analysis (Churchill and Doerge, 1994). The regression-based interval mapping described by Haley and Knott (1992) was used, which gives results similar to those of likelihood-based interval mapping but is simpler computationally. Because it is simpler computationally, more intense analyses, such as analyzing data across locations can be done. There are also some drawbacks to MQTL.

Data from replications within locations have to be averaged. The year and location variables are confounded because the same location in a different year must be treated as a different location. Also, MQTL assumes that locations are fixed and does not allow heterozygous progeny in RIL populations.

Because few significant QTL were found, only the simple interval mapping (SIM) function of MQTL was used rather than simplified composite interval mapping (sCIM) function. The latter takes into account the effect of other QTL. Experiment-wide threshold levels of the test statistics for a type I error rate of 0.05 were obtained empirically from the data analyzed by randomly rearranging the data. This effectively breaks any real correlation between markers and phenotypic data, so that any QTL found in the permuted data are not real but fall into the type I error category. The threshold for which only 5% of markers and phenotype are significant by chance can then be determined. This eliminates the need for homogeneous variances to combine data across locations. Permutation of the data was done a minimum of 1000 times. For yield data, 9,999 permutations were used because the QTL peak was near the experiment-wide threshold and therefore, a precise threshold was desired.

The genome was scanned at every marker and at every 1 cM between markers for yield or 5 cM for other traits. Both the main effect of the dependent variable and QTL x environment interaction were tested. Test statistics were graphed in Microsoft Excel (Microsoft Corporation). Bar graphs were created in Statview 5.0 (SAS Institute, Inc.).

The data were also analyzed using QGENE (Nelson, 1997). QGENE uses several different types of analyses but its strength is providing a graphical output that makes it easy to interpret the data. QGENE's multiplot function, which plots p values obtained from single-marker regression, was used. Data were analyzed for each replication separately. The output made it easy to see significance in individual replications and compare significance across replications. Heterozygous individuals were included in the QGENE analysis. An experiment-wise threshold of  $p = 0.05$  was sought. This corresponds with a per locus p value somewhere between  $p = 0.01$  and 0.001 (Lander and Botstein 1989). For this study, a per locus p value of 0.01 was considered significant.

Markers across the genome were tested. The main purpose of using QGENE was to identify differences between replications, locations, years.

Finally, ANOVA analysis, an analysis familiar to most researchers, was used to examine the yield peak near *rhg1*. Only Bng173HaeIII was tested for association with yield with this method. Repeated measure ANOVA analyses were conducted using Statview 5.0. The same plants were not measured in different replications, locations, and years, so a repeated measures analysis does not technically apply. However, the same genotypes are repeatedly measured and therefore plots of the same genotype are correlated, not independent. Using repeated measures may slightly overestimate p values, thereby underestimating significance. But because the data were correlated, a repeated measure ANOVA was the most accurate way to analyze the data. Replications were crossed with, rather than nested in locations due to limitations in the program. All data across years, locations, and replications were analyzed. In addition, data were broken down by year and/or location to see differences between years and locations. The ANOVA table for the comparison of Bng173HaeIII and yield is given in the appendix (Table A-6). A per locus threshold of  $p = 0.01$  was considered significant.

## Results and discussion

### Yield locus near *rhg1*

Molecular markers near *rhg1* showed association with yield in the MQTL analysis. This yield locus was most significantly associated with the marker Bng173HaeIII ( $p=0.07$ , Figure 1-1). This  $p$  value was an experiment-wide  $p$  value and just missed the threshold of  $p = 0.05$ . An experiment-wide  $p$  value of 0.05 corresponds to a per locus  $p$  value of 0.01 to 0.001, often used as a threshold in QTL studies (Lander and Botstein, 1989). Bng173HaeIII is approximately 6 cM on the telomeric side of *rhg1*. The  $R^2$  value for Bng173HaeIII's association with yield was 0.13 and those lines homozygous for the susceptible parent's allele at Bng173HaeIII yielded more (17.9 kg/hectare) than those homozygous for the resistant parent's allele (15.1 kg/hectare; Figure 1-2a). The same trend was seen in each year (Figure 1-2b) and each location (Figure 1-2c). No significant QTL  $\times$  E interactions were detected (Figure 1-3).

With QGENE, markers near *rhg1*, including Bng173HaeIII, also showed association with yield (Figure 1-4a). After analyzing the data from MQTL, it comes as a surprise that the locus showed environmental interaction in the QGENE analysis. Yield was significant in only 8 of the 18 replications ( $p \leq 0.01$ ). Five of these replications meet a more stringent threshold of  $p = 0.001$ . All three replications at a location showed significance only in Lamberton in 1996. In 1998, significance was only found in one of the six replications. Some markers centromeric to *rhg1* also showed some significance though this tends to occur only in locations where association of telomeric markers with yield was strong. It is likely that at least the locus telomeric to *rhg1* is real. Most QTL are not detected in all environments due to the power of QTL detection rather than the QTL not being real or not being present in all the environments tested (Beavis, 1994). However, the effects of the QTL detected is likely overestimated.

Although the yield peak found using MQTL does not meet the experiment-wise threshold for significance ( $p = 0.07$ ) and was not significant in every replication in the QGENE analysis, MQTL showed an  $R^2$  value of 0.13 and eight replications in QGENE showed significance. In order to further characterize Bng173HaeIII's association with yield, ANOVA was implemented with a per locus significance threshold of 0.01 (Table 1-1). In all replications, locations, and years, lines homozygous for the Evans (susceptible parent) allele at Bng173HaeIII yielded more (17.9 kg/hectare) than lines homozygous for the PI 209332 allele (15.1 kg/hectare). This difference was significant overall at  $p = 0.0006$ , which meets an experiment-wide threshold of  $p = 0.05$ . However, it was not significant at the per locus threshold of  $p = 0.01$  in 1998, 1996 Waseca, 1997 Lamberton, and 1998 Waseca.

In general, lines with alternate parental alleles at Bng173HaeIII showed smaller differences in 1998 than 1996 and 1997 and in Waseca than Lamberton. Significant QTL x environment interactions were found using ANOVA (Table 1-1). Year had a significant effect on yield in all analyses that included data from different years. Location was also significant, except in 1998 ( $p = 0.012$ ) when it just missed the threshold. Replications showed significance overall but when the data were broken down, only a few of the subsets showed a significant effect on yield from replications.

The results of yield associated with alleles at markers near *rhg1*, depended, to a certain extent, on the analysis used. MQTL appears to be the most conservative of the three analyses. Because experiment-wide  $p$  values were determined empirically based on the data at hand, these values are likely more accurate than in the other two analyses where per locus  $p$  values were found and conversion to experiment-wide values was difficult (Lander and Botstein, 1989). Also, it was expected that  $p$  values were somewhat inflated in the ANOVA analysis due to violations of the need for homogeneous variances to combine data across years, locations, and replications. Because  $p$  values are determined empirically, the MQTL analysis was not affected by homogeneous variances. The QGENE analysis was also unaffected because replications were analyzed individually. Some loss of power occurred in MQTL by averaging replications and because location and year were confounded. Indeed, based on simulation studies by

Beavis (1994), because of the small population size, this study would not be expected to detect all QTL in all environments if there are a large number of QTL with large effect for the trait in question. This is likely for yield. Therefore, combining data across years and locations may actually reduce power to detect QTL.

ANOVA and QGENE gave similar results, both showing a significant yield locus near *rhg1*. This locus showed QTL x environment interaction in both analyses which makes it surprising that no significant QTL x environment interactions were found with MQTL. Perhaps the confounding of the year and location variables affects the power to detect these interactions.

If this yield QTL is real, its position, especially with respect to *rhg1*, is important. However, it is difficult to pinpoint exactly the position of a QTL. In the MQTL analysis, the peak of the putative QTL was at Bng173Hae-III approximately 6 cM telomeric to *rhg1*. The *rhg1* locus itself, when compared to yield, had a p value of 0.81 in MQTL as compared to p = 0.07 for Bng173HaeIII (Figure 1-1). Based on this evidence, it does appear that if there is a yield locus, it is telomeric to and separate from *rhg1*. However, MQTL gave the most conservative results of the three analyses and though *rhg1* did not show significance with yield, neither did Bng173HaeIII (p = 0.07).

### QTL for agronomic traits across the genome

Data for maturity, lodging, height, protein and oil were also taken on the lines in the RIL population. These traits are agronomically important and several can have an effect on yield. QTL were sought for these traits and yield across the genome using MQTL and QGENE.

No other QTL for yield were identified using MQTL (Figure 1-1). However, some QTL for other agronomic traits were uncovered. Maturity and height were both associated with the unlinked marker, Bng037I, in MQTL. In other populations, Bng037I maps to MLG-C (Concibido *et al.*, unpublished data, 1995). Shoemaker *et al.*, 1997) map Bng037 to MLG-Y but used the restriction enzyme TaqI, rather than EcoRI. However, results with Bng037I should be interpreted with caution. Approximately one-third of the

lines did not have genotypic data for Bng037I which might bias the results. Because the marker was unlinked, missing marker genotypes could not be calculated based on flanking markers. No other loci passed the experiment-wide threshold for significance.

Significant QTL x E interactions were found only for protein and oil on MLG-A in MQTL (data not shown). However, a significant QTL was not found in this region when all environments were tested. When individual environments were tested in MQTL, a QTL for protein was found in this region of the genome but was only significant in Lamberton, MN in 1996. Oil QTL in this region were significant in Lamberton and Waseca, MN in 1996 and in Lamberton, MN in 1997. In Waseca, MN in 1997, this region on MLG-A just missed the threshold for significance with oil.

Some interesting points can be seen in the QGENE output. Several markers showed association with more than one trait. In some replications, lodging showed significance with markers near *rhg1* and the yield locus (Figure 1-4b). However it rarely met the threshold of  $p = 0.01$  and was not consistently placed. Maturity, and to a lesser extent, lodging and oil, showed association with markers on MLG-B in several locations (Figure 1-5a). Bng037I, as mentioned above, showed significance in some locations with maturity, lodging, height, and protein (Figure 1-5c).

MLG-P showed association in some replications with yield, lodging, and height (Figure 1-5b). Evans, the agronomically superior parent, contributes the allele for increased lodging, an undesirable trait, even though it lodges less than PI 209332. PI 209332 contributes the allele for increased height and surprisingly, yield. It has been shown before that markers can identify and track alleles to improve traits from the less desirable parent (deVicente and Tanksley, 1993, Eshed and Zamir, 1994). So, it was not unexpected that Evans, the least lodging parent, might contribute an allele for increased lodging. It also makes sense that an increased lodging effect from Evans might reduce yield. The PI 209332 allele, which comes from the taller parent, increased height. An increase in height can, if it doesn't cause lodging, increase yield. If so, the two regions on MLG-P associated with yield likely affect yield only indirectly by controlling lodging and height.

## Homoeologous regions

Soybean is an ancient diploidized tetraploid and therefore has several duplicated regions in its genome (Shoemaker *et al.*, 1996). The region near *rhg1* has been shown to be duplicated on MLG-K, MLG-L, and on MLG-D2 (Peñuela *et al.*, 1999). An SCN resistance gene has been found on a region of MLG-D2 known to be homoeologous to the region on the MLG-G near *rhg-1*. (Peñuela *et al.*, 1999). Yield loci were also sought in these duplicated regions. None of these regions showed significant association with yield. MLG-D2 did show some association to lodging in the region that is homoeologous to MLG-G but it was only in half of the replications and was rarely significant above a per locus value of  $p = 0.05$ . Data were not sufficient to claim QTL for any trait in these regions.

## Summary

An association between yield and alleles at markers near *rhg1* was found in all three analyses. It was significant across years and locations in the ANOVA analysis but just missed the threshold in the MQTL analysis. It was also significant individual replications, locations, and/or years in the ANOVA and QGENE analysis. Because QTL loci often are not detected in every environment (Beavis, 1994), analyses which combine data across years and locations may actually lose power. It has also been shown that QTL that are detected, even if only in a few environments are likely real (Beavis, 1994). Therefore, there does appear to be a yield locus near *rhg1*.

## Perspectives

Some aspects of this study should be interpreted with caution. These lines were organized into three maturity blocks nested in locations. This blocking was ignored in all analyses. The alleles at *rhg1* were not distributed evenly in each block. Maturity blocks were planted next to one another in the field so variation between blocks may be relatively low. If lines had not been blocked, added variation in yield would be

introduced into the data because early lines could not be harvested until all lines were mature and would likely shatter, reducing yield. Also, late lines, planted near early lines would not have competition late in the season and could therefore yield more than if they had been planted in a homogeneous field. Therefore, maturity blocking introduced some variation into the experiment that was not accounted for in the analyses used but also likely reduced some variation in yield. Results of this analysis are valuable, but should be interpreted with caution.

The wide range in maturity in this test may have also made it more difficult to detect maturity and yield loci. Yield tended to increase with maturity. However, the yield of the latest maturing lines dropped off to near zero because they did not mature by the end of the growing season. As the length of the growing season changed, so may have the yield of these lines. This complexity makes it more difficult to associate marker alleles with patterns in maturity and yield.

A further shortcoming of the MQTL analysis was that environments were assumed to be fixed. Therefore, the results of this analysis can only be applied to Waseca and Lamberton, MN in 1996, 1997, and 1998. No significant QTL x E effects were found for the yield QTL near *rhg1* for the environments tested (Figure 1-2). However, both QGENE (Figure 1-4a) and ANOVA analysis (Table 1-1) showed differences in years and locations. This may be due to QTL x environment interaction, but may also be due to a lack of detection rather than absence in those environments which showed no QTL (Beavis, 1994). Both locations were in the southern portion of the state, where most of the soybeans are grown and where the biggest problems with soybean cyst nematode occur.

These sites were not infested with SCN as are most of the fields surrounding them. One reason for planting them in non-infested sites was that SCN is known to affect yield and therefore any results obtained in an SCN-infested field would be confounded with the effect of the nematode. This was especially worrisome when testing for the presence of a yield QTL near *rhg1* because they both may have an effect on yield and their effects may not be distinguishable. Because these lines were grown at the University of Minnesota Experiment Stations, which are in agricultural communities and are

surrounded by fields infested with soybean cyst nematode, the results of this analysis should be considered by breeders and farmers dealing with soybean cyst nematode in southern Minnesota. However, further study is needed to confirm the yield locus near *rhg1*.

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**Table 1-1. Summary of ANOVA data for yield.**

ANOVA data comparing the effect of alternate alleles at Bng173HaeIII on yield are shown. The significance of year, location, and replication on yield are also listed. Data were analyzed in Statview 5.0 (SAS Institute, Inc.) using repeated measures ANOVA. The data were analyzed across years, locations, and replications and subsets of the data, by year and/or location, were also analyzed.

	p value for Bng173HaeIII	Bng173HaeIII	Year	Location	Replication
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All data	0.0006	**	**	**	*
1996	0.0027	*	N/A	*	ns
1997	0.0026	*	N/A	*	ns
1998	0.0188	ns	N/A	ns	ns
Lamberton	0.0002	**	**	N/A	ns
Waseca	0.0068	*	**	N/A	**
1996 Lamberton	0.0004	**	N/A	N/A	ns
1996 Waseca	0.0348	ns	N/A	N/A	ns
1997 Lamberton	0.0143	ns	N/A	N/A	**
1997 Waseca	0.0051	*	N/A	N/A	ns
1998 Lamberton	0.0086	*	N/A	N/A	ns
1998 Waseca	0.1207	ns	N/A	N/A	**

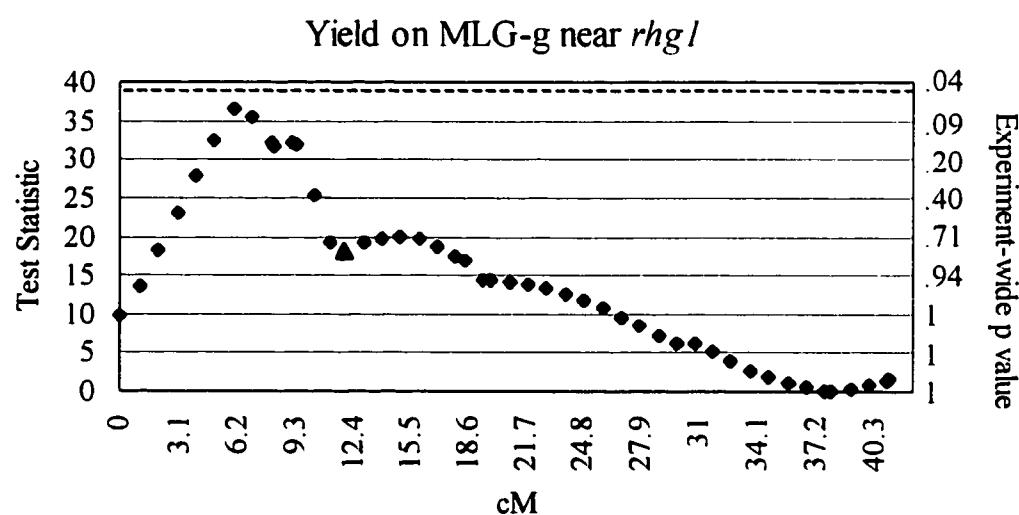
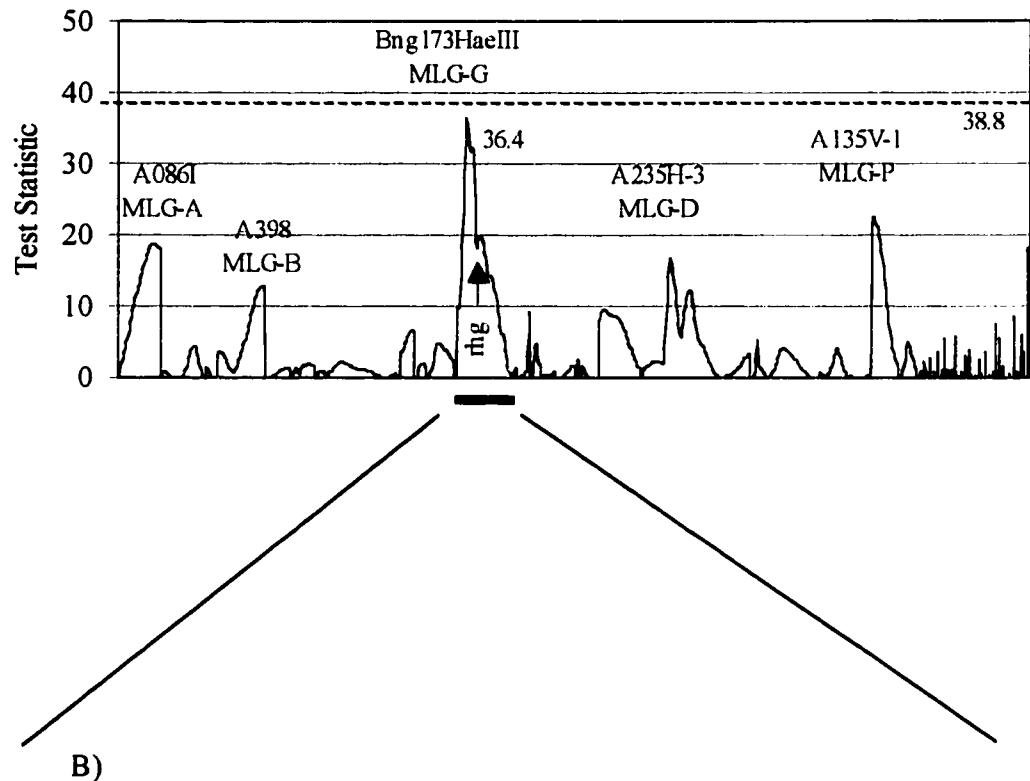
\* p = 0.01

\*\* p = 0.0001

**Figure 1-1. Test statistic plots for yield**

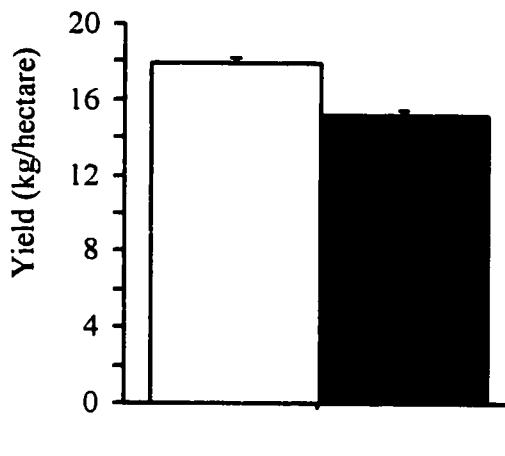
Test statistics for yield from the regression-based simple interval mapping algorithm in MQTL (Haley and Knott, 1992; Tinker and Mather, 1995a, 1995b) were plotted. The genome was tested at each marker and at 1 cM intervals. A) The x axis represents the position in the genome. The position of *rhg1*, a major gene for SCN resistance, is labeled (Caldwell *et al.*, 1960; Concibido *et al.*, 1994, 1996a, 1996b, 1997; Mahalingam and Skorupska, 1995; Webb *et al.*, 1995; Qiu *et al.*, 1997). The y axis shows the test statistics for association with yield. The threshold for the empirically-determined experiment-wise type I error rate of 0.05 is shown as a dotted line. Peaks are labeled with the marker name and molecular linkage group. B) Test statistics for yield on MLG-G near *rhg1*. The molecular marker, Bng173Hae-III is at the peak of the QTL on MLG-G. The *rhg1* locus is represented with a triangle. The broken line represents the threshold test statistic for an empirically-derived experiment-wide type I error rate of 0.05. The right-hand y axis lists p values.

A)

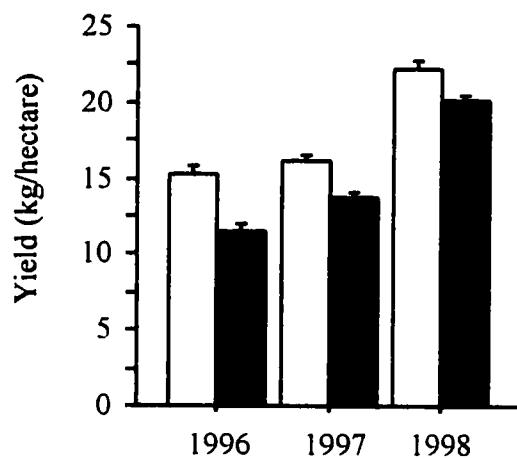


**Figure 1-2.** Yield of recombinant inbred lines grouped by their alleles at Bng173Hae-III  
Graphs of yield comparing lines homozygous for alternate parental alleles at  
Bng173HaeIII are shown. Those lines homozygous for the Evans (susceptible) allele are  
shown in white and the PI 209332 (resistant) allele in black. Error bars represent standard  
errors. Data are separated by year in B) and by location in C). Lamberton and Waseca  
represent two University of Minnesota experiment stations in southern Minnesota.

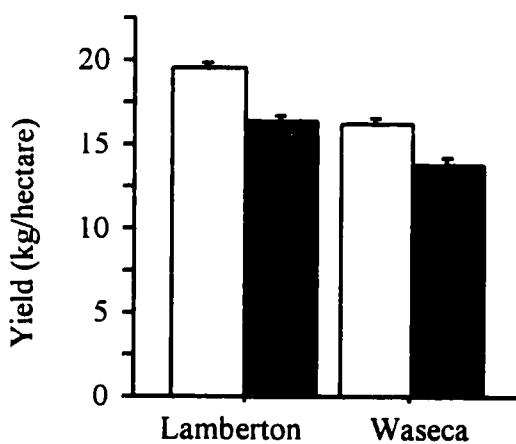
A)



B)



C)

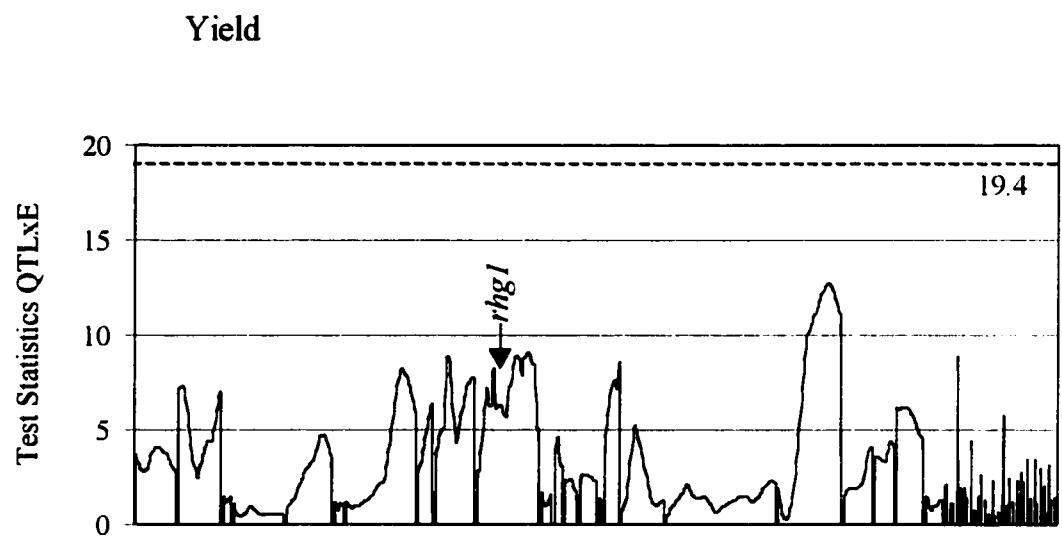


Lines with alleles at  
Bng173HaeIII inherited from:

- Evans  
(Susceptible parent)
- PI 209332  
(Resistant parent)

**Figure 1-3. Test statistic plots for yield QTL x environment interaction**

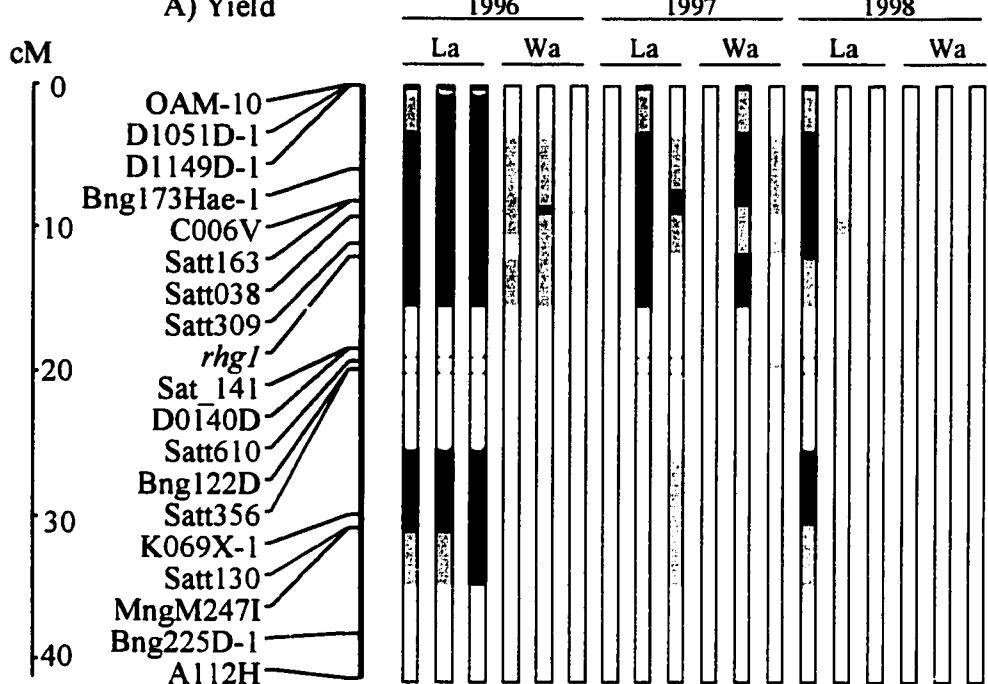
Test statistics for yield QTL x environment interaction were plotted against markers and intervals between markers across the genome. A test was done every one cM. The experiment-wise type I error threshold ( $p = 0.05$ ) is marked with a dotted line. The position of *rhg1*, a major gene for SCN resistance, is labeled.



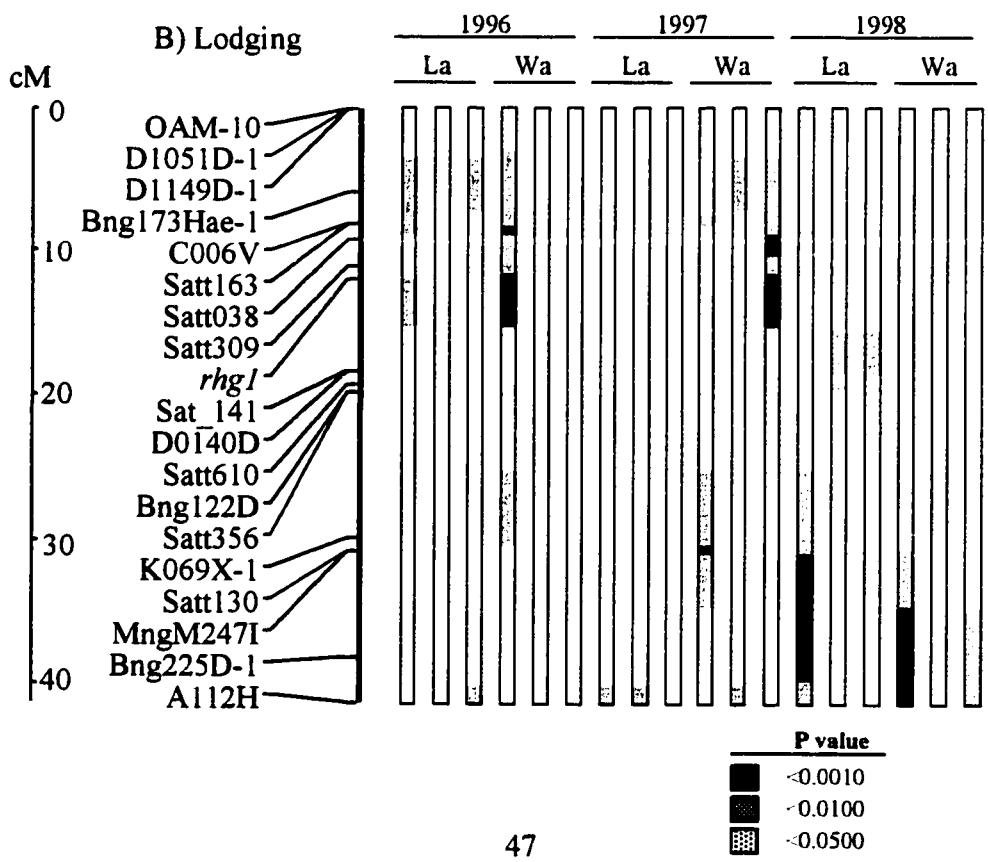
**Figure 1-4. QGENE multiplot output for yield and lodging near *rhg1***

A multiplot output from QGENE (Nelson, 1997) is shown for yield (A) and lodging (B) on molecular linkage group G (Shoemaker and Olson, 1993) near the main SCN resistance gene, *rhg1*. Each bar represents one replication. The first six bars represent the year 1996, the next six, 1997, and the last six, 1998. Within each year, the first three bars are three replications in Lamberton, MN and the second three are replications in Waseca, MN.

A) Yield

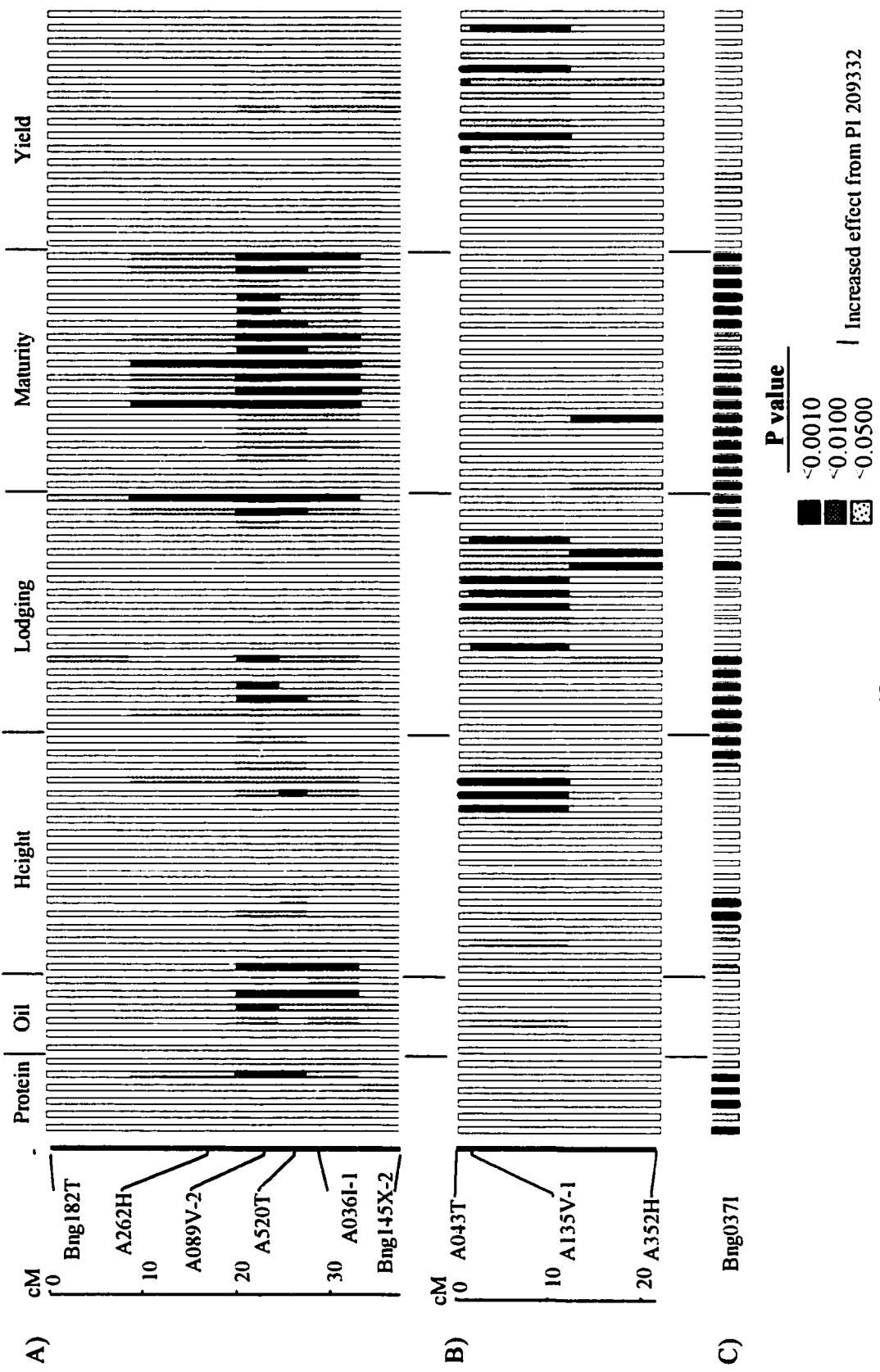


B) Lodging



**Figure 1-5. QGENE multiplots of agronomic traits in select linkage groups**

Plots show associations between phenotypic traits and markers on molecular linkage group B (A) and P (B) and to the unlinked marker Bng037I (C), which maps to linkage group C in other populations. Association was determined using simple regression with QGENE's multiplot function (Nelson, 1997). Protein, oil, height, lodging, maturity, and yield were analyzed. Each bar represents an individual replication. For protein and oil, each bar represents the first replication. The first two bars represent 1996 data, the next two, 1997, and the last two, 1998. The first bar in each location represents Lamberton, MN data and the second Waseca, MN. For the rest of the traits, three replications were assayed. The first six bars of each trait represent 1996 data, the second six, 1997 data, and the last six, 1998 data. Within each year, there are three replications at Lamberton, MN followed by three at Waseca, MN.



**Chapter 2. Effect of *rhg1* on SCN populations in the field and further characterization of a yield locus associated with *rhg1***

## Abstract

Keeping soybean cyst nematode (SCN) reproduction to a minimum is important for keeping yield potential of both resistant and susceptible soybean cultivars high. Resistant cultivars have been tested and found effective in controlling, or even reducing, SCN populations by limiting reproduction of the nematode. In this study, near-isogenic lines (NILs) of soybean segregating for the chromosomal segment surrounding the major SCN resistance locus, *rhg1*, were tested to determine the effect of alleles at this particular locus on SCN reproduction. The ability of *rhg1* to affect SCN reproduction will affect the feasibility of basing a marker-assisted selection program for SCN resistance on *rhg1*. Reproduction was measured in several ways. In the first method, reproduction factor was defined as the fall egg count, taken just before or after harvest, divided by the spring egg count, taken just after planting in each plot. In the second, female counts were determined by estimating the number of cysts on the roots approximately 40 days after planting. Cyst counts were also determined in the greenhouse. In general, plants with the resistance alleles at *rhg1* had significantly fewer number of cysts and a lower reproduction factor than did plants with the susceptible alleles. NILs were grown in non-infested sites as well, so the effect on yield of SCN and a putative yield locus linked to *rhg1* could be differentiated. A yield locus linked to *rhg1* was found to cause significant differences in yield in some sets of NILs. The low yield allele was inherited from the resistant parent. The yield locus appears to be separate from and telomeric to *rhg1*. In summary, the resistance allele at *rhg1* can significantly reduce SCN reproduction and, therefore, MAS based on *rhg1* should be valuable, especially if the low yield allele at the linked yield locus can be separated from the resistance allele at *rhg1*.

## Introduction

Soybean cyst nematode (SCN) causes substantial yield loss in soybean in the United States every year (Doupenik, 1993; Pratt and Wrather, 1998). Resistance to SCN, along with rotation out of soybean is considered the best method of control (Stienstra and MacDonald, 1990; Macquidwin *et al.*, 1995; Young, 1998). However, breeding soybean lines for resistance is challenging. Screening for resistance in the field or greenhouse is labor-intensive, time-consuming, and highly variable. An alternative to these tradition methods of screening for SCN resistance is marker-assisted selection (MAS; Tanksley *et al.*, 1989). In the case of SCN resistance, alleles at molecular markers could be used to predict alleles at nearby loci controlling SCN resistance. Because of the difficulties involved in traditional screening methods, MAS for SCN resistance is an attractive alternative to direct phenotypic screening.

Several loci have been found to affect resistance to SCN in soybean. However, one locus, *rhg1*, has been shown to contribute more than 50% of the variation in resistance and is required for resistance (Caldwell *et al.*, 1960, Concibido *et al.*, 1994, 1996a, 1996b, 1997; Mahalingam and Skorupska, 1995; Webb *et al.*, 1995; Qiu *et al.*, 1997). Using linked molecular markers to indirectly screen for the resistance allele at *rhg1* could make breeding for SCN resistance more effective.

The ability of resistant cultivars to reduce SCN reproduction in the field has been documented (Macquidwin *et al.*, 1995; Wallace *et al.*, 1995). However, little is known about the ability of *rhg1*, itself, to impact SCN reproduction. Because an increase in the number of SCN eggs in the field reduces the yield potential in both resistant and susceptible varieties (Macquidwin *et al.*, 1995; Senyu Chen, University of Minnesota Southern Experiment Station, Waseca, MN, personal communication, 1998), the ability of the resistance allele at *rhg1* to keep SCN reproduction in the field low is crucial. If *rhg1* can significantly affect SCN reproduction, MAS based on *rhg1* should be a valuable

tool in producing soybean lines that can limit SCN reproduction and keep soybean yield potential high.

Alternate alleles at *rhg1* can be compared in near-isogenic lines (NILs), which are lines that segregate in only one or a few regions of the genome. Comparing NILs with alternate parental alleles in the region of segregation allows one to compare the effects of the alleles in this region in a similar genetic background without the effects of other segregating loci confounding the results. Using NILs with an interval of segregation surrounding *rhg1*, the effect of alternate alleles at *rhg1* on reproduction of SCN can be determined. If the resistance allele at *rhg1* can significantly reduce SCN reproduction in comparison to the susceptible allele, MAS based on *rhg1* will be valuable.

Another critical aspect in understanding the value of MAS for SCN resistance is the possibility of separating a putative yield locus from *rhg1* using markers (see Chapter 1). By growing NILs in fields not infested by SCN, the effect on yield of this linked locus can be determined. However, if both the yield locus and *rhg1* are included in the interval of segregation, any effect on yield potential of *rhg1* itself cannot be separated from the effect of the yield locus.

In this research, four sets of NILs, all segregating in the region surrounding *rhg1*, were used to explore the ability of the resistant parent's allele at *rhg1* to control SCN reproduction. In addition, the effect of a yield depression locus linked to *rhg1* was examined. This information will help improve the efficiency and application of MAS for SCN resistance in soybeans.

## Material and methods

### Plant materials

Two recombinant inbred lines (RILs), lines 49 and 79, from the cross of ‘Evans’ x PI 209332 (SCN resistant; Anand and Brar, 1983; Anand and Gallo, 1984; Concibido *et al.* 1996a), were shown previously to be derived from a plant heterozygous in the region surrounding *rhg1* (Roxanne Denny *et al.*, University of Minnesota, unpublished data). Neither line was segregating in other regions of the genome known to be associated with SCN resistance or yield.  $F_{4:7}$  plants from each of these lines were planted in the greenhouse and genotyped across the interval of segregation near *rhg1* as described below. Approximately, ten plants homozygous for the resistant parent’s alleles and ten homozygous for the susceptible parent’s alleles were retained from each of the two lines, as well as several plants showing crossovers in the interval of segregation (Figure 2-1 and Appendix, Figure A-1). One  $F_{4:7}$  plant heterozygous across all or part of the interval of interest from each of the two lines was also retained (lines 49-29 and 79-23). These plants were selfed and the resulting  $F_{7:8}$  progeny were genotyped across the region of interest (see below). Again, approximately ten lines homozygous for either parental allele were retained (Figure 2-1, A-1). Each NIL set is identified by the letters ‘NIL-’ followed by its parent line (NIL-49, NIL-79, NIL-49-29, and NIL-79-23). Within each set, lines inheriting the resistant parent’s alleles across the region are referred to with “-R” at the end of their name (ie. NIL-49-R) and the susceptible parent’s with “-S”. Susceptible and resistant cultivars were also included in all experiments. For the tests involving NIL-49 and NIL-49-29, the cultivars included Parker (susceptible), Marcus 95 (susceptible), Faribault (resistance from PI 209332). For NIL-79 and NIL-79-23, the cultivars included Parker, Faribault, and Bell (resistance from PI 88788). Newton (Peking) and A91-607 (susceptible) were also included in the non-SCN plots for NIL-49 in 1997.

## Field trials

The F<sub>4</sub>-derived NIL sets, NIL-49 and NIL-79, were increased in Chile in the winter of 1996/97. In 1997, F<sub>4:9</sub> lines of these NIL sets were planted in four 25 cm row plots in three replications in non-infested sites at Waseca and Lamberton, MN. In 1998, F<sub>4:10</sub> lines of the same NIL sets were planted again in non-infested sites at Waseca and Lamberton, MN, as well as sites naturally infested with SCN at the same locations. The number of replications was increased to four in the infested site because of the large variability associated with SCN egg and cyst data. Plots were planted with 240 seed. Plots were end-trimmed from 3.7 m to 2.5 m. All four rows were harvested with a combine.

A subset of the lines in NIL-49 were also grown in SCN infested sites in three replications at Waseca and Lamberton, MN in 1997. Two lines with the resistant parent's alleles in the region of interest and three with the susceptible parent's alleles were planted. One of the susceptible lines had a crossover to the resistant parent's alleles on the centromeric end of the region of segregation. This crossover was somewhere between 8 and 19 cM from *rhg1* on the centromeric side (See Appendix, Figure A-1; NIL-49-x2). For all SCN reproduction data (see below), this line was treated as an S line. Lines were chosen for inclusion in the subset by whether or not they had enough seed in 1997 and therefore the selection of lines was biased. In 1998, this subset was not grown as a separate test but rather as part of the full NIL-49 test. However, only the subset lines were assayed for SCN reproduction.

The F<sub>7</sub>-derived NIL sets (NIL-49-29 and NIL-79-23) were increased at Rosemount, MN in 1997. Only two replications and smaller plots were planted because of seed constraints. Each plot was planted with 60 seed. The plots consisted of two 75 cm rows each 0.9 m long rather than the typical four 25 cm row, 2.5 m long plot used in this study to simulate yield in a homogeneous field. Plots were harvested by hand and plants run through a stationary thresher. The Rosemount data were used only to confirm data obtained in the following year. In 1998, these lines were grown in the F<sub>7:10</sub> generation at Waseca and Lamberton, MN. Two replications of the typical four 25 cm plots were grown at each location in non-infested fields and three replications in SCN-infested

fields. For more details on climate, planting dates, and a summary of the NIL plots, see the Appendix, Tables A-1, A-2, A-3, and A-7.

### Agronomic data

Agronomic data were taken for all NIL plots (see Appendix, Tables A-4 and A-8). Seed yield was determined for all plots by harvesting all rows, weighing the seed at 13% moisture, and converting the data to kg per hectare. In addition, plots were measured for traits that affect yield to ensure that any segregation in yield was due to yield genes, not genes for traits indirectly affecting yield. These traits include: stand counts (taken only at infested sites), maturity, lodging, and height. Stand counts were measured three times for each plot by counting the number of plants in one row along a one m stick placed at random in the plot. The three measurements were averaged to obtain an estimate of the number of plants per meter. From this estimate, the total number of plants per plot was extrapolated. Maturity was recorded as the number of days before and after August 31 when 95% of the plants in each plot reached mature color. Lodging was recorded on a scale from 1 to 5 with 1 being erect and 5 being prone. Lodging was not recorded in Waseca (infested site) in 1997 for NIL-49. Height was measured in cm on three plants from the middle two rows of each plot. Height was not taken at all locations (see appendix, Table A-4, A-8). Some of these traits, especially height and stand counts, can be affected by nematode pressure.

### Field SCN resistance assays

The number of cysts on the roots of the NILs were counted to determine the effect of the two alternate alleles at *rhg1* on nematode reproduction. Assays were done approximately 40 days after planting, which corresponds to cysts from the first SCN generation of the growing season (see Appendix, Table A-3). Ten to twelve plants were dug up from the ends of each plot and soaked in water. Cysts were knocked off the roots with mechanical rubbing and water pressure and collected on a 150 µm sieve. Dislodged nematodes were separated from other debris by centrifuging down the debris in 70%

sucrose and then recollecting the cysts on sieves. Cysts were counted under a dissecting microscope and the number of cysts per plant calculated. Cysts were collected from SCN-infested sites during 1997 and 1998 from the NIL-49 subset and during 1998 from all NIL-79-23 lines ( $F_{7,8}$ ). Susceptible and resistant cultivars were also tested (see Appendix, Table A-9).

Reproduction factor was determined for the same plots for which cyst counts were done. Ten soil cores were obtained at a depth of 6 inches from the middle two rows of each plot. Soil samples were sieved through a one quarter inch screen to break up and mix the soil. Cysts were separated from the soil using an elutriator (University of Georgia, Athens, GA). The cysts were broken by grinding to release the eggs. The mixture was then sieved to get rid of broken cysts and any other debris and the eggs counted for a portion of the sample under a microscope. The number of eggs per 100 cc of soil was calculated. For each plot, the reproduction factor was calculated by dividing the number of eggs in the fall by the number of eggs in the spring. Spring eggs were collected just after planting, in early June, and fall eggs just before or after harvest, (see Appendix, Table A-3). The reproduction of the nematode depends in part on the root mass available to it. However, measurement of the root mass and normalization of measure of SCN reproduction was not done in this study. Data from all reproduction studies done in the field are summarized in the Appendix, Table A-8.

#### Greenhouse SCN resistance assays

Greenhouse SCN resistance assays were conducted as described in Concibido *et al.* (1996a), except only four replications per line rather than 12 were assayed. Greenhouse assays were done on the same group of plants that were tested for cysts and reproduction factor (see Appendix, Table A-9 for a summary of the data). PI 209332, Faribault (PI 209332 resistance), and Parker (susceptible) were also included (see Appendix, Table A-9).

## DNA extraction

DNA was extracted in one of two ways. For DNA in the traditional liquid form, the modified method of Dellaporta *et al.* (1983) described in Concibido *et al.* (1996a) was used. For high throughput DNA extraction, DNA was extracted by the disk method described by Lange *et al.* (1998).

## Genotyping

Simple sequence repeats (SSRs) were assayed either with radioactivity on a polyacrylamide gel or without radioactivity on a high resolution agarose gel. In the polyacrylamide-based method, both forward and reverse primers were end-labeled with  $\gamma$ - $^{33}\text{P}$  according to the following protocol. The primer mixture contained 0.75  $\mu\text{M}$  each of forward and reverse primers, 1X kinase buffer (70 mM Tris-HCl at pH 7.6, 10 mM MgCl<sub>2</sub>, 5 mM DTT), 0.1  $\mu\text{L}$  of 3,000 Ci/mmol  $\gamma$ - $^{33}\text{PdATP}$  per sample, and 0.8 U T4 polynucleotide kinase (New England Biolabs, Beverly, MA) per sample. The primer mixture was incubated at 37°C for 1 hour, then at 70°C for 10 min. Each amplification reaction contained a single 3-mm DNA disk or 5 ng of DNA suspended in TE, 1 mM MgCl<sub>2</sub>, 100  $\mu\text{M}$  of each dNTP, 2  $\mu\text{L}$  of end-labeled primer mix (0.15  $\mu\text{M}$  of forward and reverse primers), 1X Buffer (20 mM Tris-HCl at pH 8.4, 50 mM KCl), and 1 unit *Taq* DNA Polymerase in a 10  $\mu\text{L}$  reaction volume. Samples were amplified in an MJ Research PTC-100 thermocycler in 96-well microplates. Each of 32 cycles consisted of 25 s at 94°C to denature the DNA, 25 s at 47°C to anneal the primers, and 25 s at 68°C to replicate the DNA. Samples were electrophoresed on a 6% polyacrylamide gel and visualized using autoradiography.

Alternatively, an agarose-based method was used when length polymorphisms between parental alleles were large enough. Reactions were amplified as above but in the absence of radiolabeled nucleotides and reaction volumes were scaled to 15  $\mu\text{l}$ . Products were electrophoresed on a 3.5% high-resolution agarose gel, such as Metaphor (FMC Bioproducts, Rockland ME) or Ameresco (Ameresco, Solon, OH), and visualized with

ethidium bromide. In each method, SSRs were scored by comparing the migration of each progeny's SSR fragment on the gel to that of the parents' to determine which parental alleles the progeny inherited.

All RFLPs were assayed as described in Concibido *et al.* (1996a). One RAPD marker, AM-10 (Operon Technologies, Alameda, CA) was assayed according to Williams *et al.* (1990).

### Statistical analysis

Lines were analyzed for differences in agronomic traits using a series of two-tailed t-tests in the computer program, Statview 5.0 [SAS Institute, Inc.]. The three assumptions of a t-test are independence of measurements, homogeneity of variances, and normal distribution of data. Measurements were not independent because the same genotypes were used in each replication in each location and year. Data were therefore organized as repeated measures to take into account correlations base on genotype, even though the measures were not taken repeatedly on the same plants. If there was a significant difference in variance between the two groups being compared in a t-test, transformations of the data were sought to correct this problem. Cysts per plant and reproduction factor always were transformed because susceptible lines showed much higher variances than resistant lines. Transformations were sought that reduced p values for the F test for homogeneous variances across locations and sometimes years; individual replications were not checked for homogeneity of variances. Either the log of the data was used (reproduction factors, cysts per plant in 1997 and reproduction factors in 1998) or the log of the log (cysts per plant 1998 for NIL-49) or the sixth root was taken (cysts per plant for NIL-79-23 in 1998, yield in non-infested sites for NIL-49 in 1998). Analysis of transformed data usually gave similar results to that of untransformed data. However, in one case, 1998 reproduction factor for NIL-49, the difference between NIL-49-R lines and NIL-49-S lines was not significant ( $p = 0.11$ ) with untransformed data, but was significant ( $p = 0.05$ ) with transformed data. Data for traits other than yield and SCN reproduction measurements were not transformed, even if they violated the assumption of

equal variances. Normality was checked only on transformed data. In general, yield, height, and maturity were normally distributed while lodging was not. Female counts and the reproduction factor tended to be bimodally distributed. No adjustments of the data were made to meet assumptions of normality. Replications were crossed with locations, rather than nested, because limitations of the statistical program. Data were combined across locations and, if the number of replications was consistent, across years.

A nonparametric test, the Mann-Whitney U test, was also used on a few comparisons. This test is resistant to outliers. However, similar results were obtained with the Mann-Whitney U test as with t-tests so the former was not pursued.

## Results and discussion

### NIL sets

The interval of segregation was characterized for each NIL set using molecular markers to assay both the parent line, which was heterozygous around *rhg1*, and the progeny that made up each set. Markers that showed homozygosity in parent lines were not always retested in the progeny because the progeny should be homozygous for the same allele as well. In cases in which these markers were redone, progeny were homozygous as expected. Lines were determined to be R or S lines by testing them with markers (Figure 2-2) that were heterozygous in their parent line to determine whether they had segregated to become homozygous resistant (R line) or homozygous susceptible (S lines) across the interval of segregation (Figure 2-1). Lines still heterozygous were not used.

Crossovers within the interval of segregation were also discovered by testing markers within the interval of segregation. Because the F<sub>7</sub>-derived NIL sets were tested after only one generation (F<sub>7:8</sub>) of selfing the heterozygous parent, the crossovers detected were homozygous on one side of the crossover and heterozygous on the other. Because these lines would segregate further upon selfing, they were not pursued. Among the crossovers in the F<sub>4</sub>-derived NIL sets, each line had a unique crossover point. Therefore few datapoints were available for each crossover and statistical power was low when comparing each crossover line with non-crossovers. Conclusions involving data from crossover lines were difficult to draw. Therefore the results will not be discussed.

Some of the last markers to be genotyped were assayed on bulks of R lines and S lines within NIL sets, rather than individual lines. Therefore, only two reactions per NIL set had to be assayed. If all lines in the R line bulk showed the resistance allele and all lines in the S line bulk, the susceptible, genotypes of the bulks appear as homozygous for their respective allele. If more than one allele is present in a bulk, a heterozygous

condition would be seen and the individual lines should be assayed. This was never the case in this study for the few markers that were assayed in this way.

Resolution of crossover points within or at the edge of the interval of segregation could only be narrowed down to the position between the two flanking markers. This resolution varied depending on the distance between the markers the crossover points fell between (see Figure 2-1, Figure 3-1, and Appendix, Figure A-1 for approximate genetic distances).

NIL-79-23, derived from an F<sub>7</sub> plant heterozygous around *rhg1* had the smallest interval of segregation of all the NIL sets tested (Figure 2-1). Within each of the F<sub>7</sub>-derived NIL sets (NIL-79-23 and NIL-49-29), lines were expected to have more than 98% of their alleles in common in all areas of the genome. Within each of the F<sub>4</sub>-derived NIL sets (NIL-79 and NIL-49), lines were expected to have more than 87% of their alleles in common.

#### Effect of *rhg1* on SCN reproduction in the greenhouse

Resistant and susceptible checks were included in the greenhouse assay to ensure that resistant plants showed resistance and susceptible plants showed susceptibility. The susceptible checks had an average of 340 cysts per plant and the resistant only 37 cysts per plant. This difference was significant at  $p < 0.0001$ .

NIL-79-23-S and NIL-79-23-R lines showed significant segregation in greenhouse tests for SCN resistance. NIL-79-23-S lines had more cysts on their roots than did NIL-79-23-R lines ( $p < 0.0001$ ; NIL-79-23-S averaged 253 cysts per plant; NIL-79-23-R, 115 cysts per plant; Figure 2-3).

NIL-49-S lines in the NIL-49 subset also had significantly more cysts on their roots in the greenhouse assay than did the NIL-49-R subset lines ( $p = 0.0073$ ; NIL-49-S lines averaged 246 cysts per plant and NIL-49-R lines, 154 cysts per plant). The presence of *rhg1* alone appears to be able to confer some resistance and limit nematode reproduction in the greenhouse.

## Effect of *rhg1* on SCN reproduction in the field

The NIL-79-23 set was tested in the field for cysts on the roots of the NILs. There were significantly fewer cysts from the first SCN generation on the roots of NIL-79-23-R lines than NIL-79-23-S lines ( $p < 0.0001$ ; NIL-79-23-S averaged 23 cysts per plant; NIL-79-23-R, 9 cysts per plant; Figure 2-4). This was true in each location as well, with both R and S lines showing higher female counts in Lamberton, where higher inoculum levels were recorded (Figure 2-5). In addition, two NIL-49 resistant and three susceptible near-isogenic lines, one of which had a crossover at least 8 cM from *rhg1* were tested for the number of cysts on the roots in 1997 and 1998. Again, there were significantly fewer cysts per plant for NIL-49-R lines than for NIL-49-S lines in both 1997 and 1998 (Figure 2-6,  $p = 0.02$ ,  $p = 0.01$ , respectively). NIL-49-S lines averaged 38 and 28 cysts per plant in 1997 and 1998, respectively. NIL-49-R lines averaged 9 cysts per plant in each year. Again, this trend was seen in each location. Again, cyst counts are higher in Lamberton in 1998, though the difference is slight. In 1997, Waseca had slightly higher inoculum levels than Lamberton (Figure 2-5) and also higher levels of cysts. Apparently, the presence of the resistance allele at *rhg1* significantly reduced the numbers of cysts on the roots 40 days after planting, both in 1997 and 1998.

The reproduction factor, defined as the fall egg count divided by the spring egg count, was also determined for NIL-79-23 at both locations in 1998. The reproduction factor for NIL-79-23-R lines was significantly less than that of NIL-79-23-S lines ( $p = 0.002$ ; NIL-79-23-S averaged 4.5; NIL-79-23-R, 1.5; Figure 2-7). In Waseca, egg counts increased in plots for both R lines and S lines over the growing season. However, the increase was slight for R lines. In Lamberton, the number of eggs increased slightly in S line plots but actually decreased in R line plots. The initial inoculum load at Lamberton was extremely high (Figure 2-5). It is possible that this high inoculum load accounts for the fact that the increase in egg counts in S line plots is small over the growing season and that egg counts in R line plots actually decrease. This is because when nematode numbers are high, root penetration by the nematode on both resistant and susceptible lines can damage the roots and compromise their ability to support SCN reproduction (Curt Reese, University of Minnesota Southern Experiment Station, Waseca, MN).

In 1998, NIL-49-R lines in the subset of NIL-49 also had a significantly lower reproduction factor than NIL-49-S lines ( $p = 0.05$ ; NIL-49-S averaged 42.0; NIL-49-R, 7.8; Figure 2-8). However, it should be noted that this  $p$  value is derived from analysis on a log transformation of the data. Analysis of the original data did not show significance ( $p = 0.11$ ) but the assumption of homogeneous variances was violated ( $p = 0.0001$ ). The transformed data do not violate this assumption ( $p = 0.83$ ) and therefore the transformed data will be used in this discussion. Egg numbers increased much more in Waseca than in Lamberton. This lower reproduction factor in Lamberton, as compared to Waseca, may be due to the high numbers of SCN eggs in the spring, which may have caused extensive root damage, compromising the plant's ability to support nematode reproduction (Figure 2-5). In Waseca, spring egg counts for NIL-49-S lines and NIL-49-R lines were comparable in the spring but increased much more for NIL-49-S lines than NIL-49-R lines. In Lamberton, egg numbers were also comparable in the spring but decreased in NIL-49-R lines plots and increased in NIL-49-S line plots.

In 1997, NIL-49-S lines of the NIL-49 subset had a lower reproduction factor than NIL-49-R lines, although the difference was not significant ( $p = 0.98$ ; NIL-49-S lines averaged 12; NIL-49-R, 7; Figure 2-9). When the data were broken down by location, R lines have the lower reproduction factor in Waseca. However, in Lamberton they have a much higher reproduction factor with a larger variance than do S lines. When spring and fall egg counts are averaged within R or S lines, rather than paired for each line, the increase in eggs is most noticeable for S lines at Waseca. When the data were examined, a datapoint for an R line was observed showing double the reproduction factor of any of the other datapoints. (Figure 2-10). Because there were so few datapoints in the study, any unusual datapoints will have a large effect on the means of the two groups being compared.

When the natural field races were tested in separate tests from those of this study, all of them were scored as race 3, except Lamberton in 1997, which scored as race 1 (Table 1). The difference between the two races is that PI 88788 is susceptible to race 1 but resistant to race 3. The level of susceptibility was close to the 10% cyst index, cutoff, however, and the same field was used in 1998 and tested as a race 3. The resistance

source used in the study, PI 209332, does show resistance to race 1, as well race 3. However, Concibido *et al.* (1996a) found that a marker near *rhg1*, C006V (see Figure 3-1), accounted for 50% of the variation in resistance to race 3 but only 35% of the variance to race 1. The lower efficacy of *rhg1* on race 1, however, does not explain the higher reproduction factor on NIL-49-R lines than on NIL-49-S lines in Lamberton in 1997.

One reason field studies involving SCN are so difficult to conduct is that obtaining comparable numbers of nematodes from plot to plot is difficult if not impossible. This was compensated for somewhat when analyzing the number of nematode eggs in a plot by dividing the fall egg count by the spring egg count to get the reproduction factor. The largest variation in spring egg numbers was not between plots, but between locations. Spring egg counts in Lamberton in 1998 were extremely high (Figure 2-5). Fields used in Lamberton in 1998 were planted with soybeans in 1997 and therefore, the high number of nematodes was not surprising.

#### Yield locus linked to *rhg1*

By planting the NIL sets in non-SCN-infested sites, the effect of alternate alleles at a putative yield locus linked to *rhg1* could be examined without the effect of the nematode on yield confounding the results. However, it cannot be assumed that *rhg1* itself has no effect on yield.

In the F<sub>7</sub>-derived NIL-49-29 set, there was a significant difference in yield between NIL-49-29-S and NIL-49-29-R lines ( $p = 0.02$ ; NIL-49-29-S lines averaged 22.8 kg/hectare; NIL-49-29-R, 20.2 kg/hectare; Figure 2-11a,b) in yield in non-infested sites between the NIL-49-29-S and NIL-49-29-R lines. The NIL-49-29-S lines yielded more than the NIL-49-29-R lines. In 1997, in preliminary trials at Rosemount, MN, similar results were obtained for yield ( $p = 0.02$ ; NIL-49-29-S lines averaged 25.7 kg/hectare; NIL-49-29-R, 18.3 kg/hectare).

On the other hand, the difference in yield in non-infested sites between NIL-79-23-S and NIL-79-23-R lines in the F<sub>7</sub>-derived NIL-79-23 set was not significant ( $p = 0.47$ ; NIL-79-23-S lines averaged 18.1 kg/hectare; NIL-79-23-R lines, 17.5 kg/hectare;

Figure 2-12a,b). Preliminary data from Rosemount, MN in 1997 also showed a difference in yield that was not significant ( $p = 0.75$ ; NIL-79-23-S lines averaged 33.0 kg/hectare; NIL-79-23-R lines, 33.8 kg/hectare). NIL-79-23 has a smaller interval of segregation than NIL-49-29 so it is possible that, although both sets are segregating for *rhg1*, only NIL-49-29 has an interval of segregation large enough to include the yield locus. This is supported by the fact that the NIL-79-23 interval of segregation does not include Bng173Hae-III, the marker most significantly associated with the yield locus near *rhg1* in studies using recombinant inbred lines (Figure 2-1, see Chapter 1).

However, there are other possible explanations for this difference between NIL-49-29 and NIL-79-23. Other loci may be segregating in each NIL set, though less than 2% of the genome is theoretically still segregating. These intervals could be different for each NIL set and may affect yield. In addition, all lines in the NIL-79-23 set tended to shatter. This may have affected the ability to distinguish between the yields of NIL-79-23-R and -S lines or may have even affected the yield of NIL-79-23-R and NIL-79-23-S lines differently.

Comparing the F<sub>4</sub>-derived NIL sets with the F<sub>7</sub>-derived NIL sets derived from the same heterozygous parent gave a more uniform comparison than comparing the two F<sub>7</sub>-derived NIL sets because more than 87% of the genome should be identical between the F<sub>4</sub>-derived and the F<sub>7</sub>-derived NIL sets. The F<sub>4</sub>-derived NILs will have more regions that are still segregating than the F<sub>7</sub>-derived NILs. However, almost 90% of the genome was expected to be fixed in the F<sub>4</sub>-derived lines and will be the same in the F<sub>7</sub>-derived lines. Because there are more loci segregating in an the F<sub>4</sub>-derived line, however, the statistical power will be reduced. Yield plots were analyzed for NIL-49 and NIL-79 in Lamberton and Waseca, MN in both 1997 and 1998.

The full NIL-49 set, like the NIL set derived from it (NIL-49-29), showed significantly higher yield in NIL-49-S lines than NIL-49-R lines in an analysis combined across 1997 and 1998 ( $p = 0.03$ ; NIL-49-S lines averaged 21.8 kg/hectare; NIL-49-R lines, 20.3 kg/hectare; Figure 2-13 a, b, and c). However, NIL-49-R lines were significantly more lodged than NIL-49-S lines ( $p = 0.0001$ ; NIL-49-S lines averaged 3.9, NIL-49-R lines averaged 4.4), which can reduce yield.

NIL-79 was also comparable to the NIL set derived from it, NIL-79-23. But it has an interval of segregation that was larger than that of NIL-79-23. The interval of segregation for NIL-79 includes the marker Bng173-HaeIII which was associated with yield (Figure 2-1; see Chapter 1). Despite this, this set, like NIL-79-23, does not segregate for yield in 1997 and 1998 ( $p = 0.52$ ; NIL-79-S lines averaged 16.8 kg/hectare; NIL-79-R lines, 16.3 kg/hectare; Figure 2-14 a, b, and c). However, in Lamberton in 1998 NIL-79-S lines do yield more than NIL-79-R lines ( $p = 0.0009$ ; NIL-79-S lines averaged 19.5 kg/hectare; NIL-79-R lines, 16.6 kg/hectare). Again, most NIL-79-R and NIL-79-S lines in this NIL set shattered, which may have affected the ability to distinguish differences between NIL-79-R and NIL-79-S lines.

The fact that NIL-79 segregates for yield in only one location in one year, and NIL-79-23 does not segregate for yield, but NIL-49 and NIL-49-29 do, may be due to differences in the genetic background of the line 49- and 79-derived sets, such as the shattering that occurs in the line 79-derived sets, rather than differences in the retention of the yield locus in the interval of segregation. Alternatively, NIL-79, which shows some segregation, may contain the yield locus in its interval of segregation, but the segregation is rarely detected because these lines are only F<sub>4</sub>-derived and therefore 12.5% of the genome is still segregating and could have affected yield. Nevertheless, segregation in the two line 49 sets indicates that there is a yield locus near *rhg1* with low yield accompanying resistance.

### Yield in SCN-infested fields

The soybean cyst nematode lowers yield in both susceptible and resistant cultivars, but yield is reduced less in resistant cultivars (Macguidwin *et al.*, 1995; Senyu Chen *et al.*, University of Minnesota Southern Experiment Station, Waseca, MN, personal communication, 1998). However, no data are available on the effect of alleles at *rhg1* on response to SCN. All NIL sets were tested in SCN-infested fields in 1998. In 1997, only the NIL-49 subset lines were in infested fields. In general, the nematode reduced yield more for -S lines than for -R lines (Figures 2-11, 2-12, 2-13, 2-14). This

reduced the difference seen between the two types of lines in non-infested fields. Often, R lines yielded more than -S lines, but this was only a significant difference for NIL-49 in 1997 ( $p < 0.0001$ ; NIL-49-S lines averaged 6.7 kg/hectare; NIL-49-R lines, 10.9 kg/hectare). In general, in 1998, yields tended to be lower in Lamberton than in Waseca for both R and S lines. The high numbers of eggs at Lamberton in the beginning of the planting season was likely responsible (Figure 2-5; Macquidwin *et al.*, 1995; Senyu Chen *et al.*, University of Minnesota Southern Experiment Station, personal communication, 1998).

Stand counts, a trait often affected by nematodes, were taken in SCN-infested sites for NIL-79-23 in 1998 and the NIL-49 subset in 1997 and 1998. There was no significant difference between stand counts for R and S lines, except for the NIL-49 subset in 1997. In this case, NIL-49-R lines had a significantly higher stand count ( $p = 0.003$ ; NIL-49-S lines averaged 69 plants per plot; NIL-49-R lines, 90), which likely influenced the significantly higher yield for these NIL-49-R lines as compared to NIL-49-S lines, as mentioned above. NIL-49-R lines also lodged significantly more than NIL-49-S lines ( $p = 0.003$ ; NIL-49-S lines averaged 3.7; NIL-49-R lines, 4.8; data only available at Lamberton). No data is available on height.

Significant differences between -R and -S lines in other traits could have influenced yield in SCN sites as well. The NIL-79-23-R were significantly taller than the NIL-79-23-S lines ( $p = 0.01$ ; NIL-79-23-S lines averaged 39.4 cm, NIL-79-23-R lines, 41.8 cm). Just as in 1997, R lines lodged significantly more than S lines in SCN sites in 1998 in the NIL sets, NIL-49 ( $p = 0.0002$ ; NIL-49-S lines averaged 3.1; NIL-49-R lines, 3.6), NIL-49-29 ( $p = 0.03$ ; NIL-49-29-S lines averaged 3.3; NIL-49-29-R lines, 3.8), and NIL-79-23 ( $p = 0.01$ ; NIL-79-23 S lines averaged 2.6, NIL-79-23-R lines, 2.9). In NIL-79, the difference just missed significance ( $p = 0.053$ ; NIL-79-S lines averaged 2.6; NIL-79-R lines, 2.8). R lines also lodged significantly more than S lines in NIL-49 ( $p = 0.0001$ , NIL-49-S lines averaged 3.9, NIL-49-S lines, 4.4) and NIL-79 ( $p = 0.003$ ; NIL-79-S lines averaged 2.8, NIL-79-R lines, 3.1) in noninfested sites in 1998. The segregation in lodging is not surprising because association was found between lodging and markers near *rhg1* in Chapter 1.

## Summary

Because SCN pressure can reduce yield potential of both resistant and susceptible soybean lines, it is important to limit reproduction of the nematode. The ability of the resistant allele at *rhg1*, specifically, to limit SCN reproduction is important in MAS programs based on *rhg1*. In general, the resistant allele at *rhg1* was able to significantly reduce the numbers of cysts that developed on the plant roots, both in the greenhouse and in the field, and also to significantly lower the reproduction factor. This is at odds with earlier findings by Wang *et al.* (1999), who found that the reproduction factor was not significantly different for R versus S lines. However, their studies involved a different resistance source, Peking, and utilized only two near-isogenic lines that were derived from a parent heterozygous at both *rhg1* and *Rhg4*. If the lines inherited different alleles at *Rhg4*, this would have influenced their results. The results in the present study indicate that MAS based on *rhg1* with PI 209332 resistance should be effective in breeding SCN resistant soybean lines.

NIL-49-R and NIL-49-29-R lines had a significantly lower yield potential than their respective -S lines in non-infested fields. The -S lines were more highly infested in nematode-infested fields, though, where they yielded comparably to or less than -R lines, though the difference was usually not significant. Wang *et al.* (1999) found that Peking-derived R lines yielded more than S lines in infested fields only when spring egg counts were low. In a MAS program for SCN resistance, it is important to minimize linkage drag around *rhg1* because of this linked yield depression.

The present study indicates that lines with the resistance allele at *rhg1* are a better choice for fields infested with SCN because they yield as well as susceptible lines in infested fields and can also reduce the reproduction of the nematode. Molecular markers, in addition to their use in selecting for SCN resistance at *rhg1* may also be useful in selecting for the susceptible parent's high yield allele at the linked yield locus, allowing efficient selection of high yielding, SCN resistant soybean lines.

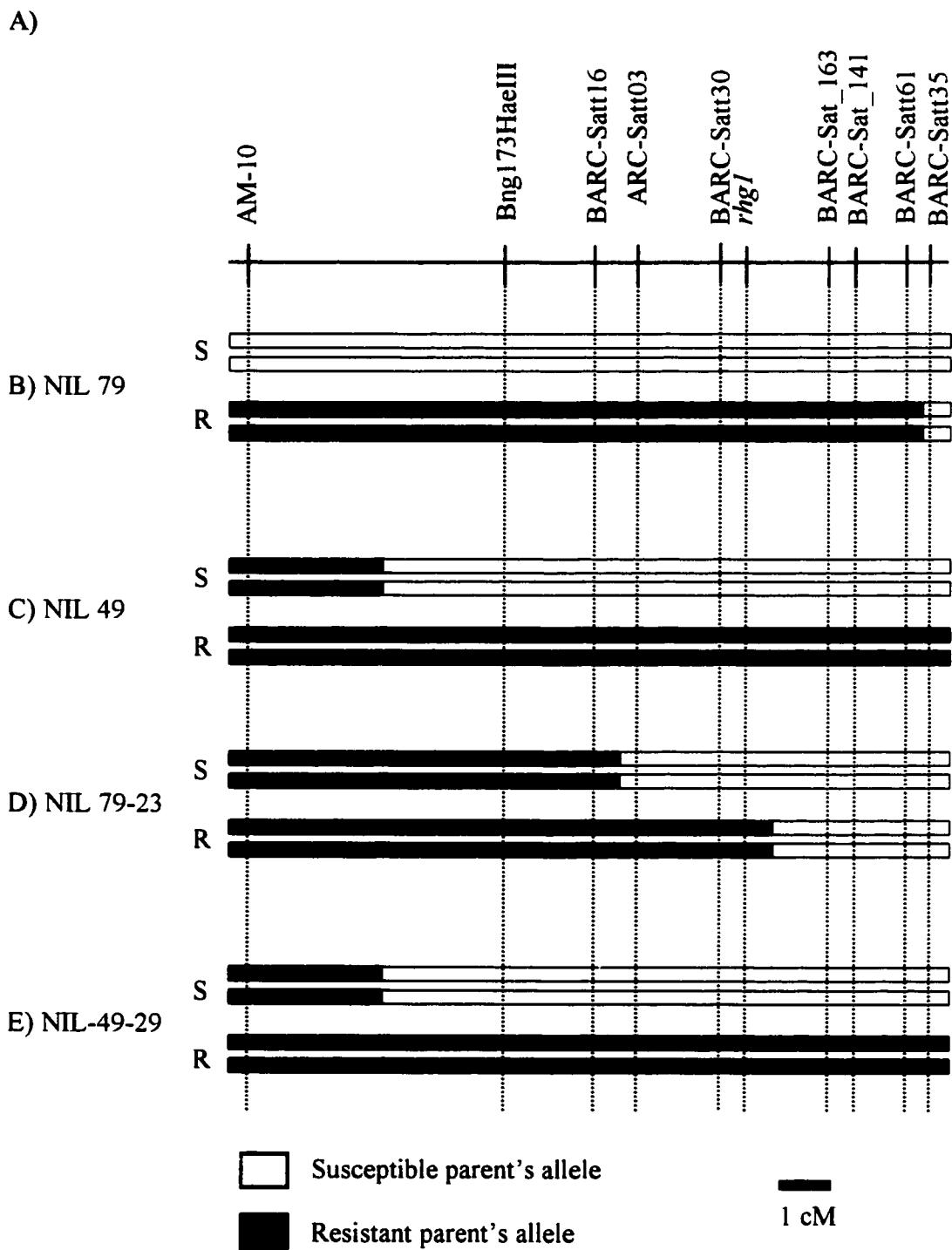
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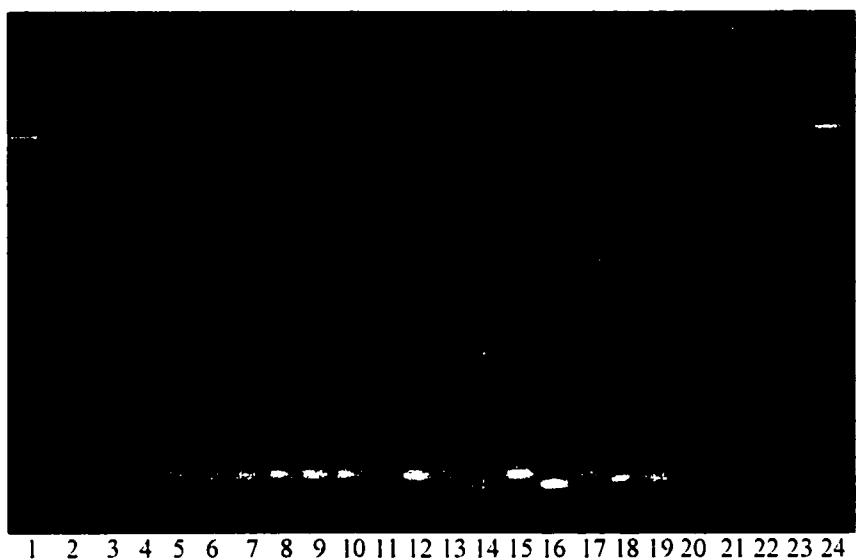
**Figure 2-1. Genotypes of R and S lines in the four NIL sets**

A) Genetic map of the region on MLG-G (Shoemaker and Olsen, 1993) near *rhg1*, a major gene for SCN resistance (Concibido *et al.*, 1994, 1996a, 1996b, 1997; Mahalingam and Skorupska, 1995; Webb *et al.*, 1995; Qiu *et al.*, 1997). B-E) Representations of the genotypes for the four NIL sets showing the interval of segregation for each. Crossover points within and at the boundaries of the intervals of segregation could not be determined exactly but have been narrowed down to an interval between markers. For some markers, genotypes for the lines were inferred from the genotype of the parent line.



**Figure 2-2. NIL lines assayed with BARC-Satt163**

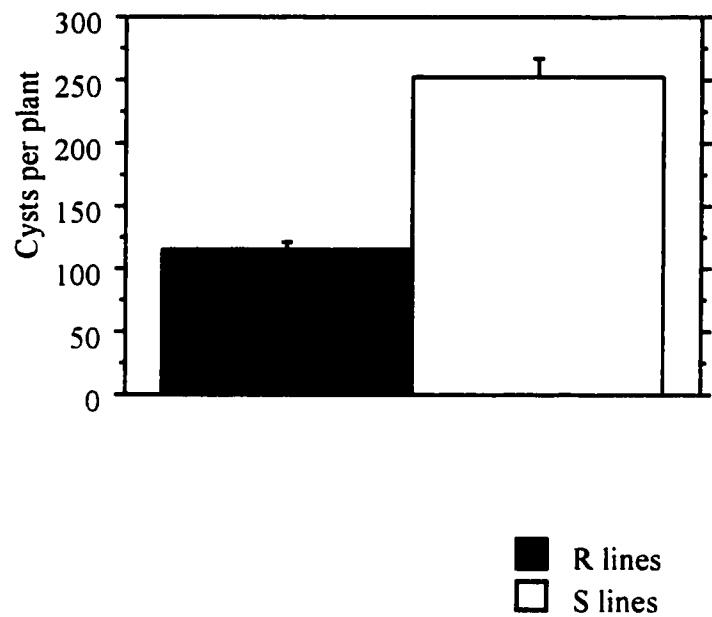
Lines from NIL-49 (lanes 2-11), NIL-79 (lanes 12-19), and NIL-79-23 (lane 20) along with the cultivar, Evans (lane 21), and the resistant plant introduction 209332 (lane 22) were assayed using BARC-Satt163 primers. Alleles were separated on an agarose gel. Lane 23 is a control reaction without DNA. Lanes 1 and 24 contain 1 kb molecular weight DNA ladder (Gibco/BRL).



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

**Figure 2-3. Cyst counts for NIL-79-23 lines in the greenhouse SCN resistance assay**  
Comparison of cyst counts on the roots of NIL-79-23-S and NIL-79-23-R lines. These data were obtained from greenhouse assays for SCN resistance. Error bars shown represent standard errors.

**NIL-79-23**  
**Greenhouse**

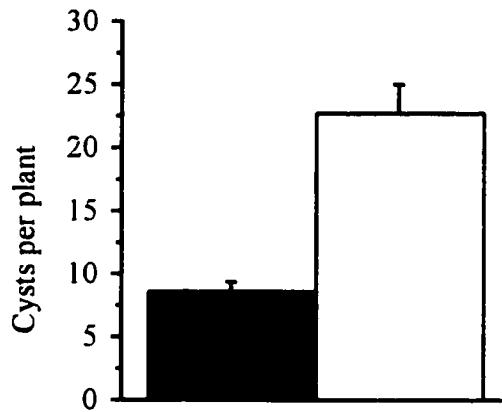


**Figure 2-4. SCN cysts on the roots of NIL-79-23 lines**

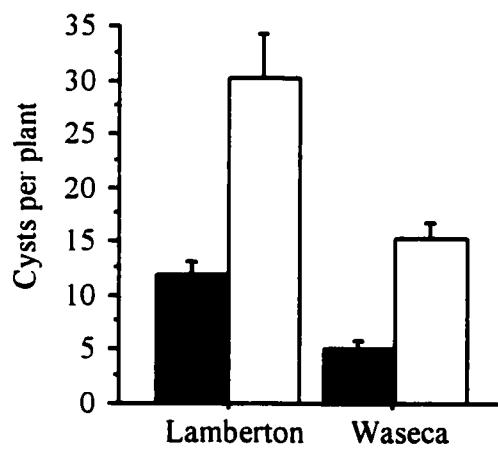
A) Comparison of female counts on roots approximately forty days after planting for NIL-79-23-R and NIL-79-23-S. Data are from 1998 in SCN-infested fields in Lamberton and Waseca, MN combined. B) Data for female counts broken down by location. Standard error bars are shown.

**NIL-79-23  
1998**

A



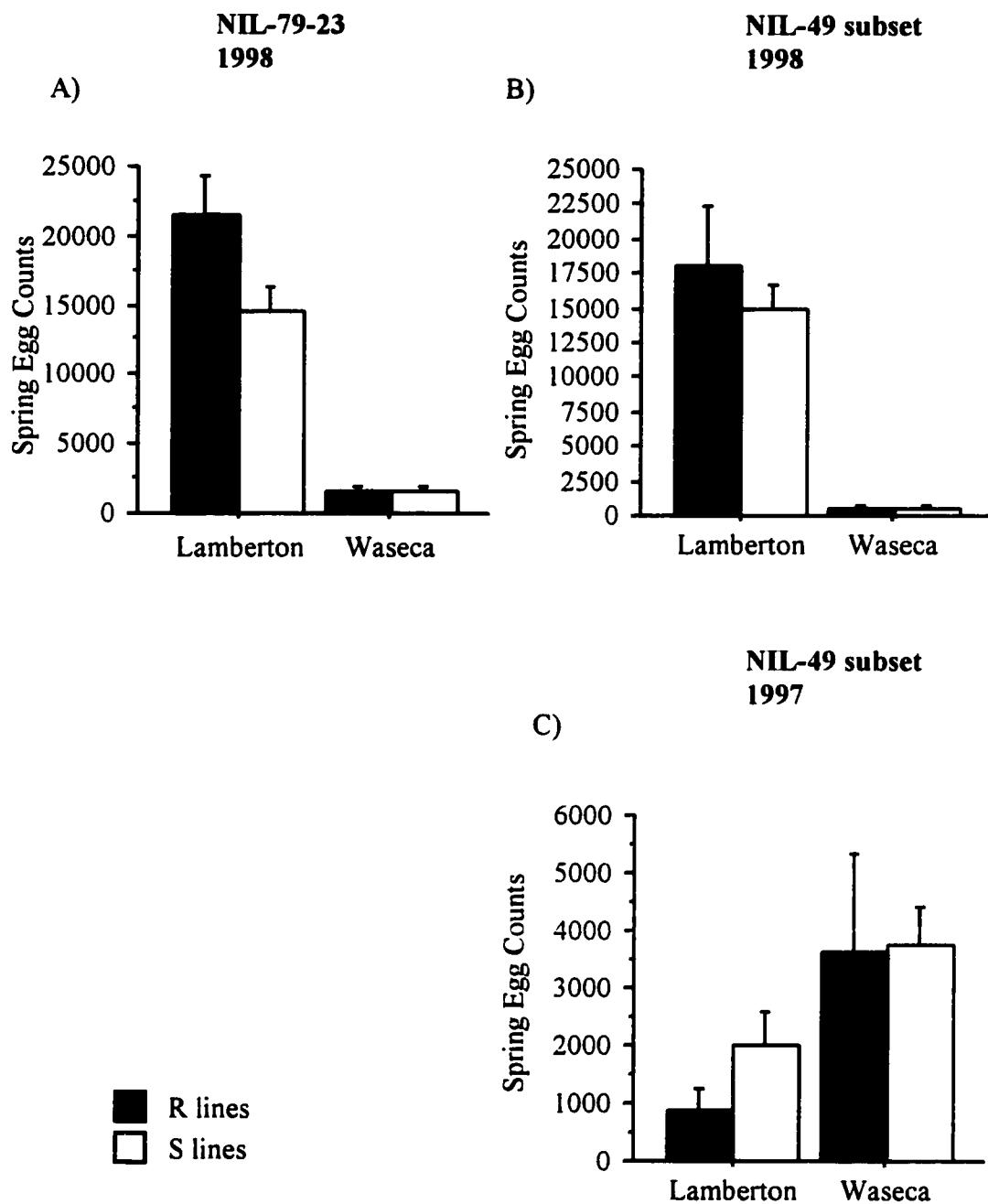
B)



■ R lines  
□ S lines

**Figure 2-5. Spring Egg Counts**

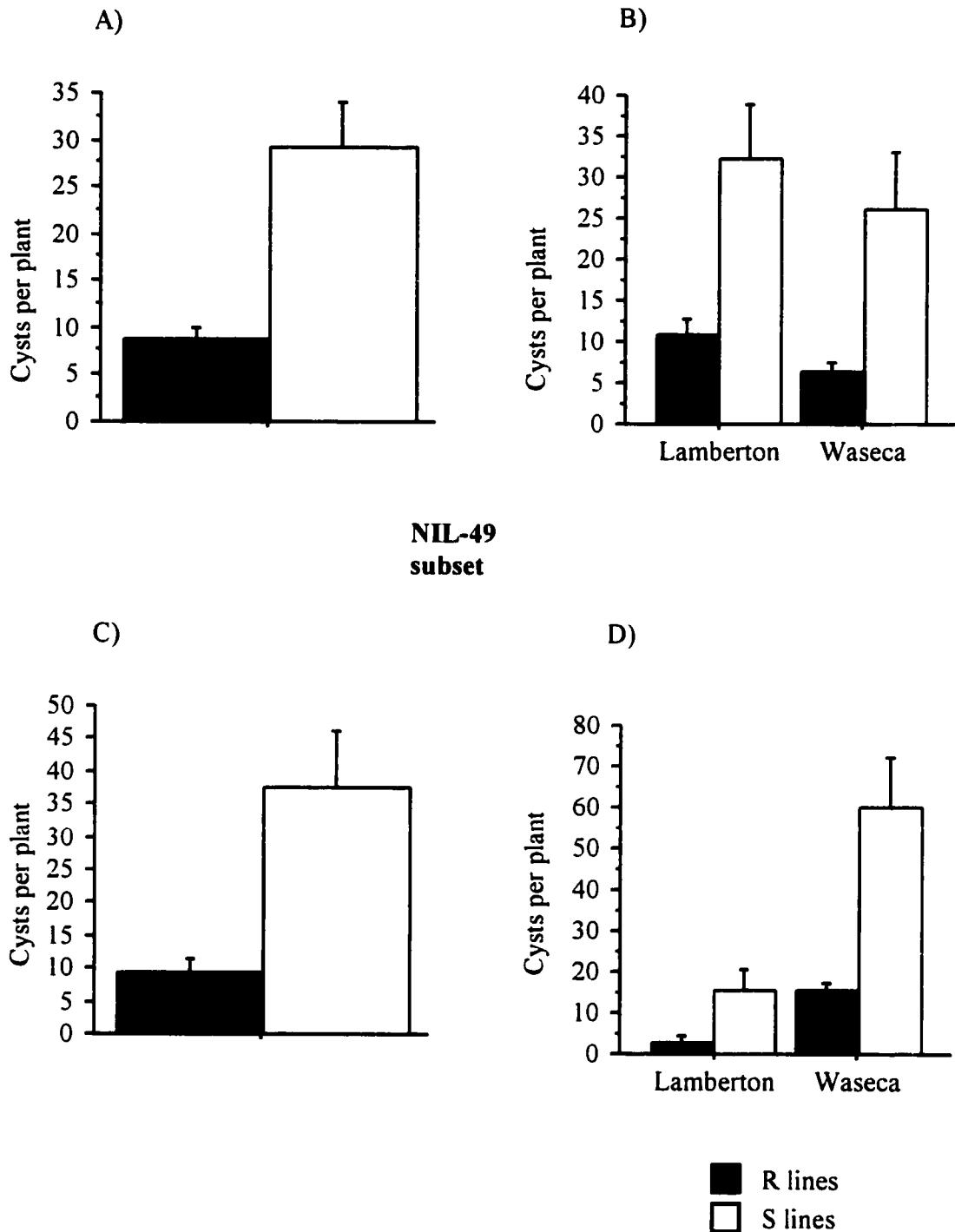
Spring egg counts are plotted for A) NIL-79-23 in 1998, B) NIL-49 subset in 1998, and C) NIL-49 subset in 1997. All plots show data for the University of Minnesota Experiment Stations at Lamberton and Waseca, MN split by genotype. Standard error bars are shown.



**Figure 2-6. Cysts on the roots of NIL-49 subset lines**

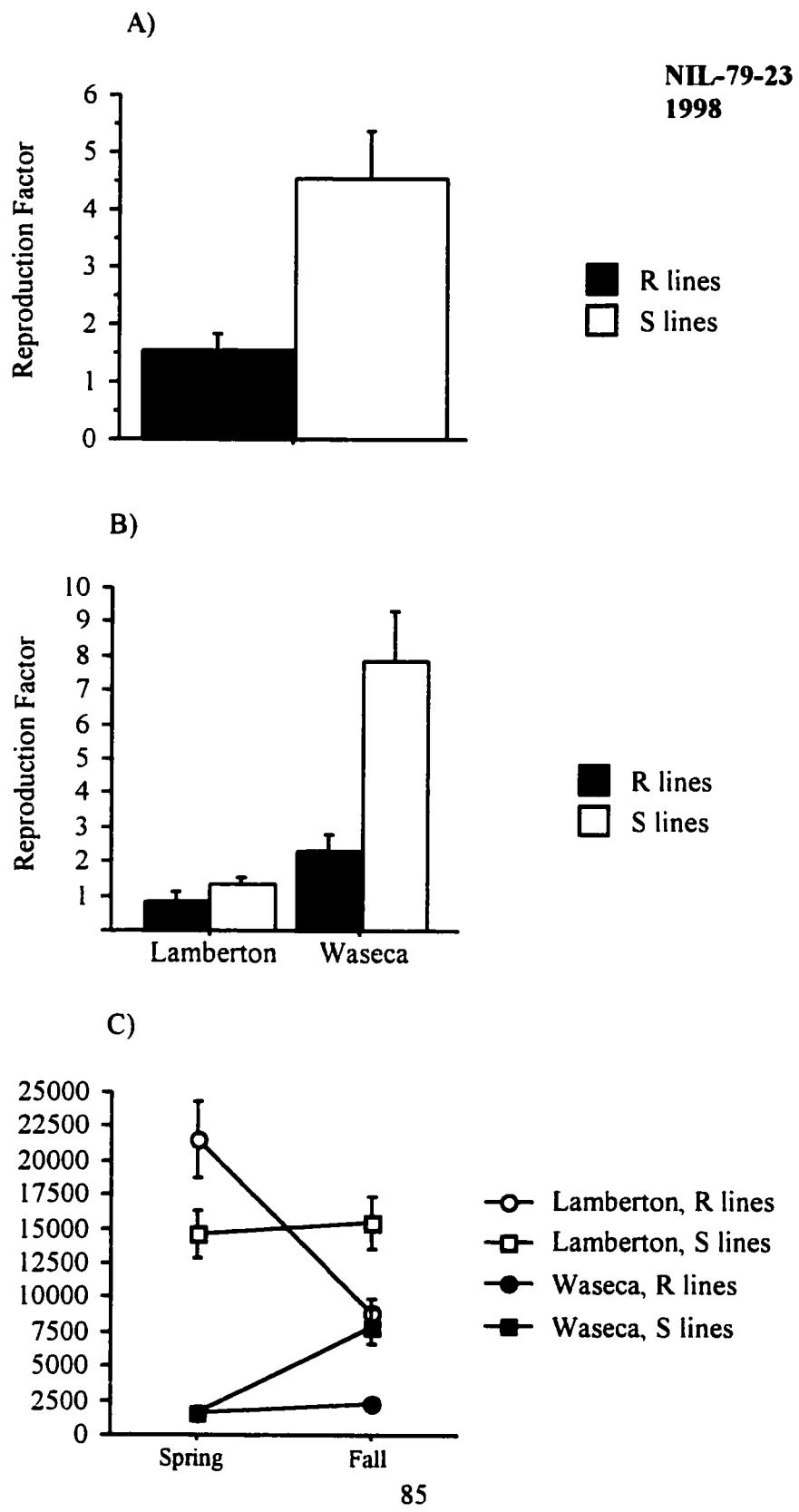
Plots of cysts on roots for the NIL-49 subset, which consisted of two R and three S lines, are shown. (A) and (B), 1998 data; (C) and (D), 1997 data; (A) and (C), Waseca and Lamberton data combined; (B) and (D), data broken down by location. Standard error bars are shown.

**NIL-49  
subset**



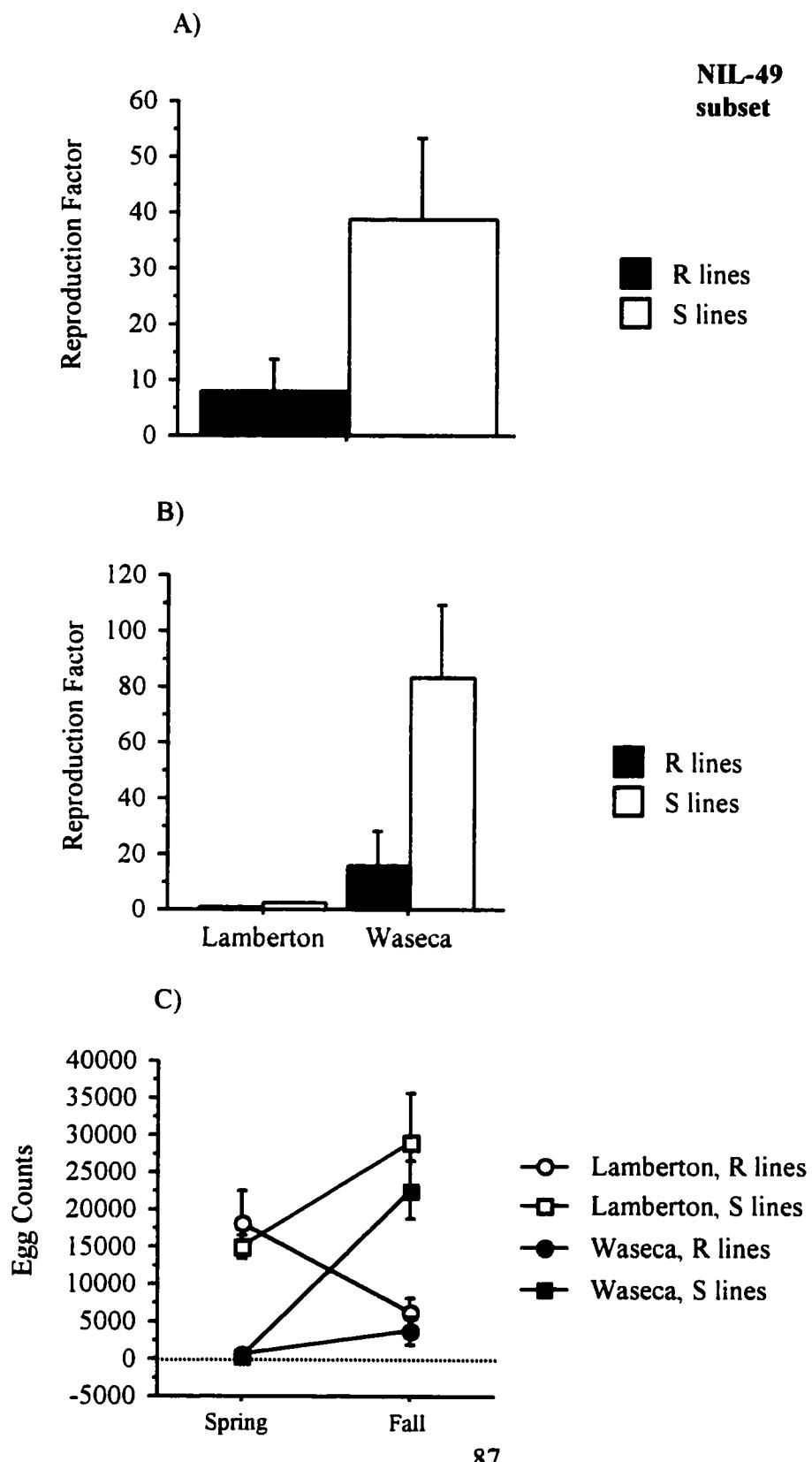
**Figure 2-7. Reproduction factor for NIL-79-23**

A) Comparison of reproduction factor of NIL-79-23-R and NIL-79-23-S lines. B) Broken down by location. Fall and spring egg counts are paired for each plot in the analysis for B). C) Spring and fall egg count means broken down by location and genotype. Standard error bars are shown.



**Figure 2-8. Reproduction factor for the NIL-49 subset in 1998**

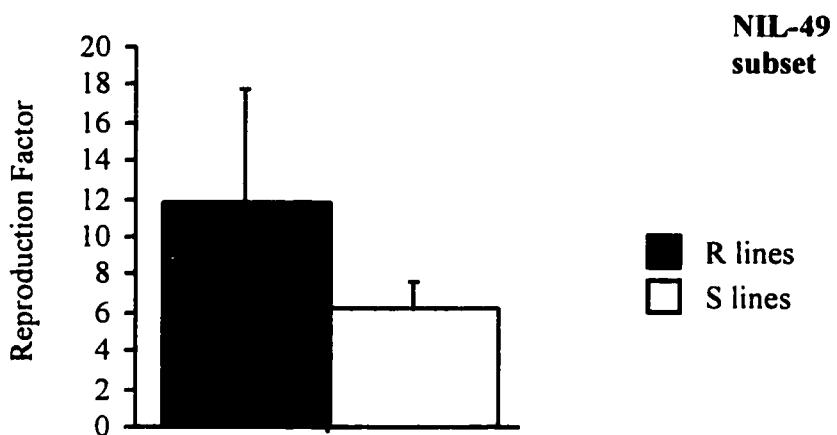
A) Comparison of reproduction factor of NIL-49-R and NIL-49-S lines in the NIL-49 subset in 1998. B) Broken down by location. C) Spring and fall egg count means broken down by location and genotype. Standard error bars are shown.



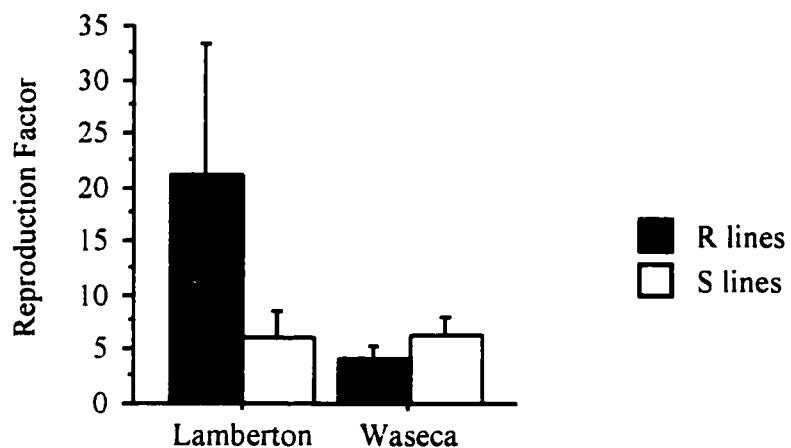
**Figure 2-9. Reproduction factor for the NIL-49 subset in 1997**

A) Reproduction factor in 1997 for the NIL-49-R and NIL-49-S lines in the NIL-49 subset. B) Broken down by location. C) Comparison of spring and fall egg counts broken down by location and genotype. Standard error bars are shown.

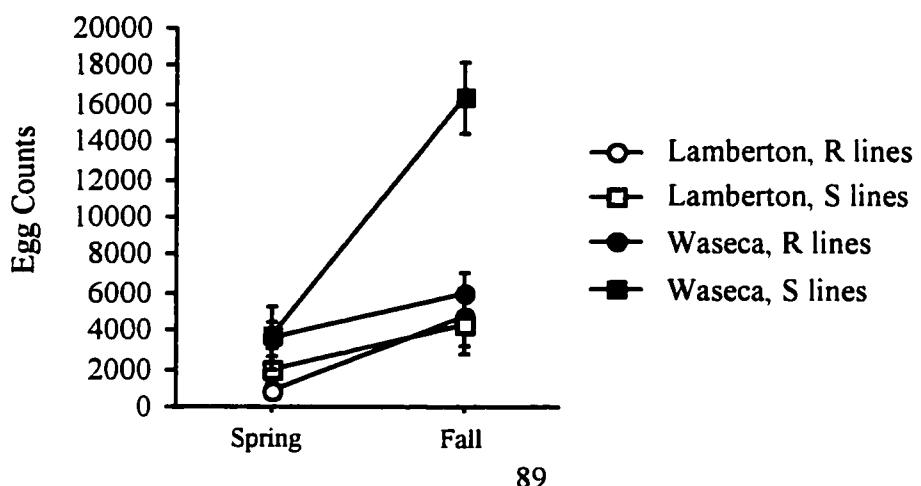
A)



B)



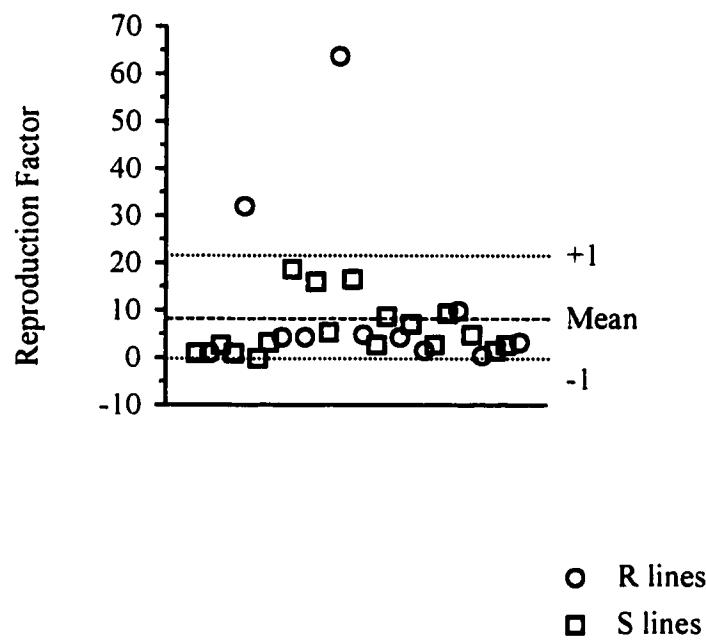
C)



**Figure 2-10. Scattergram of reproduction factor for the NIL-49 subset in 1997 at Lamberton**

Scattergram of reproduction factor datapoints obtained for NIL-49 subset lines in 1997 in infested sites at Lamberton, MN. Circles represent NIL-49-R lines and squares represent NIL-49-S lines. Mean and + and - one standard deviation lines are shown for each set of lines.

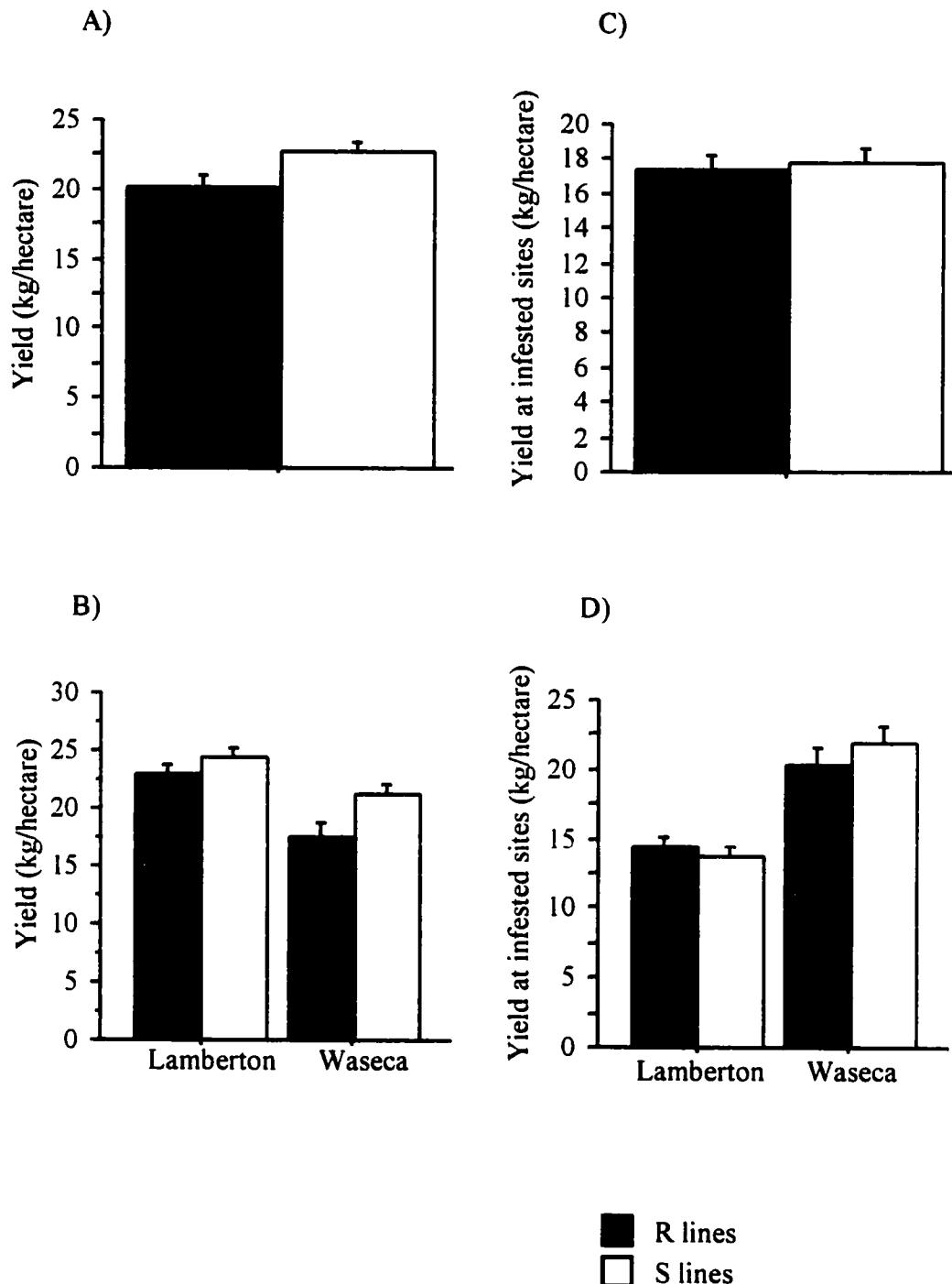
**NIL-49 subset  
1997**



**Figure 2-11. Yield of NIL-49-29-R and NIL-49-29-S lines in non-infested and infested sites**

Comparison of yield of NIL-49-29-R and NIL-49-29-S lines in 1998. (A) and (B), non-infested sites; (C) and (D), SCN-infested sites. (B) and (D) are broken down by location. Standard error bars are shown.

**NIL-49-29**  
**1998**

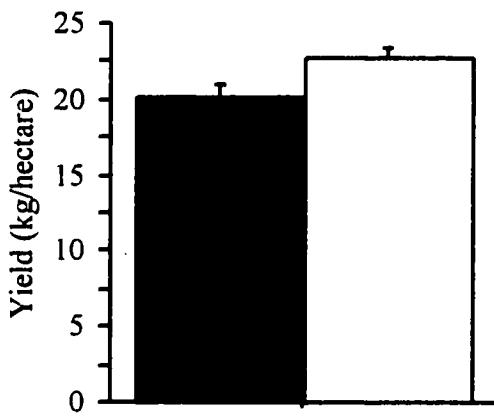


**Figure 2-12. Yield of NIL-79-23-R and NIL-79-23-S lines in non-infested and infested sites**

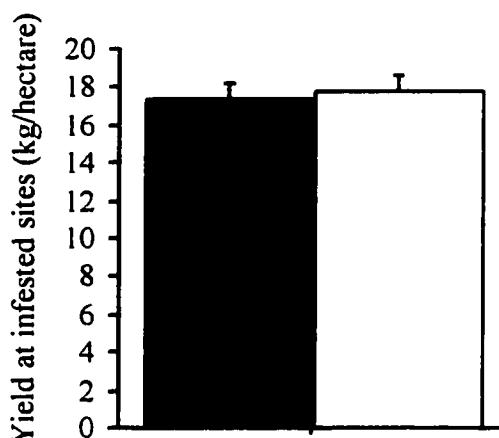
Comparison of yield of NIL-79-23-R and NIL-79-23-S lines in 1998. (A) and (B), non-infested sites; (C) and (D), SCN-infested sites. (B) and (D) are broken down by location. Standard error bars are shown.

**NIL-49-29  
1998**

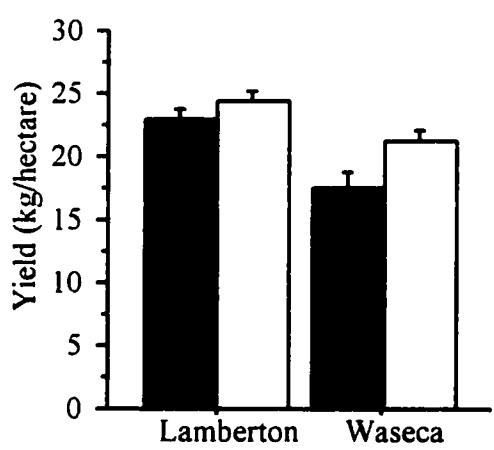
A)



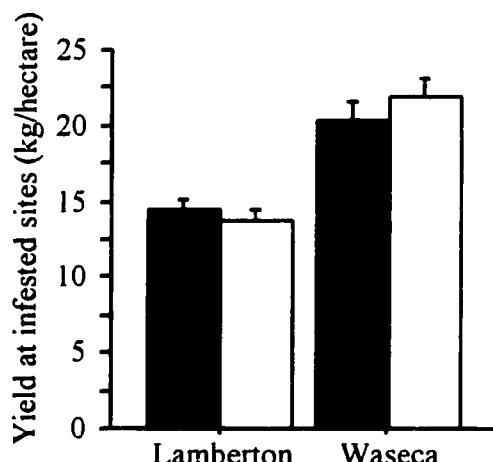
C)



B)

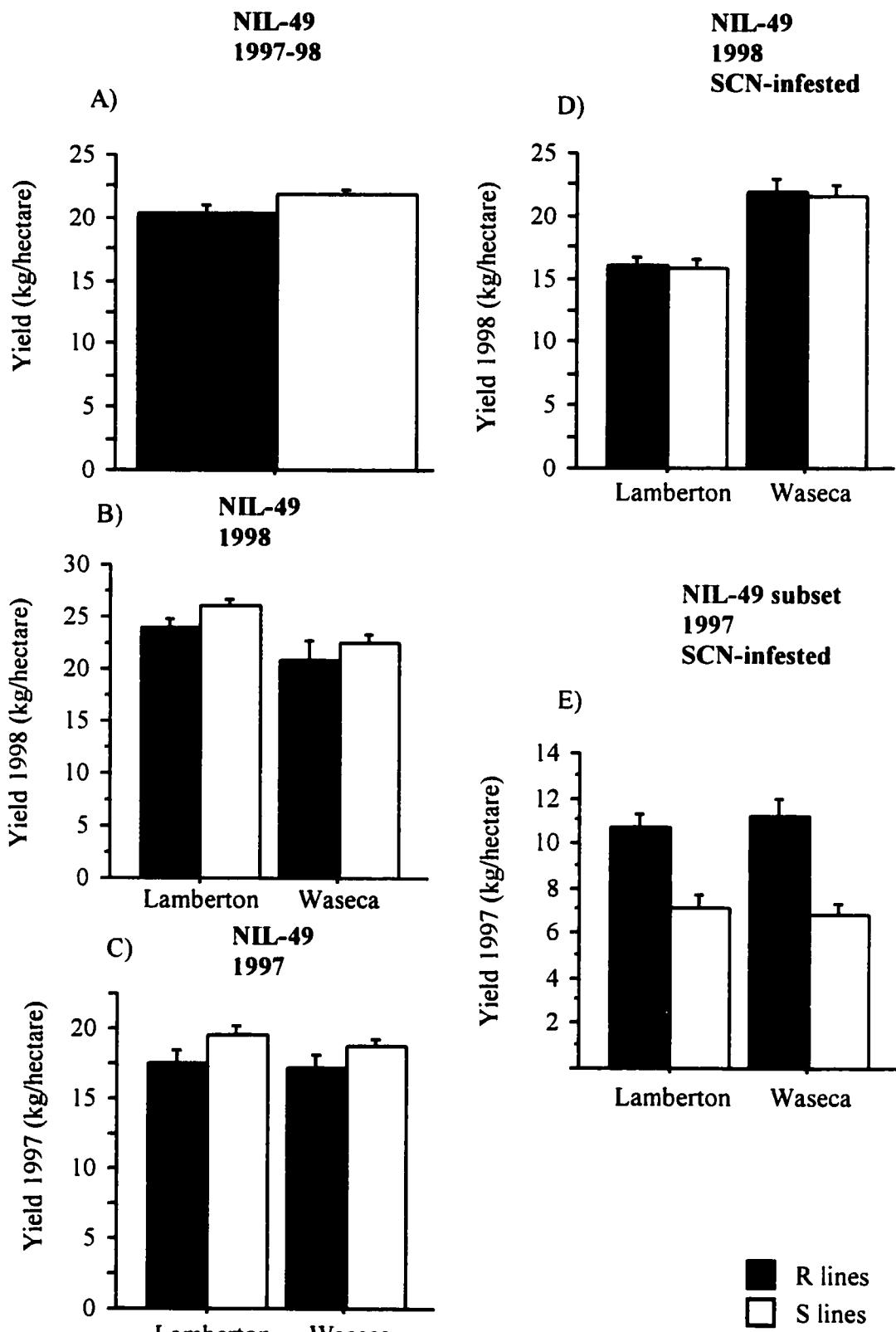


D)

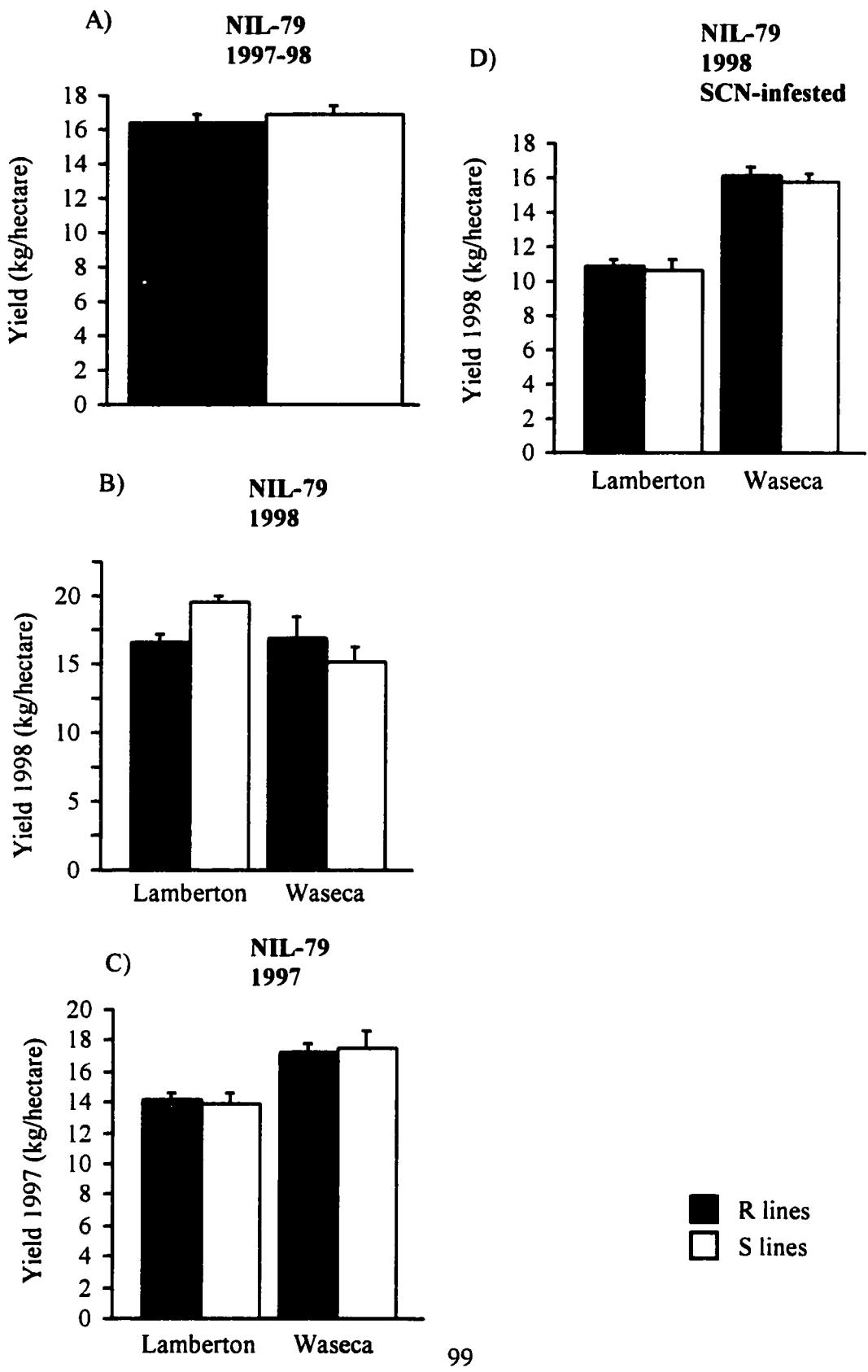


■ R lines  
□ S lines

**Figure 2-13. Yield of NIL-49-R and NIL-49-S lines in non-infested and infested sites**  
Comparison of yield of NIL-49-R and NIL-49-S lines. (A), (B), and (C), non-infested sites; (D) and (E), SCN-infested sites; (B), (C), (D), and (E) are broken down by location; (A), 1997 and 1998 data combined; (B) and (D), 1998 data; (C) and (E), 1997 data. Standard error bars are shown.



**Figure 2-14. Yield of NIL-79-R and NIL-79-S lines in non-infested and infested sites**  
Comparison of yield of NIL-79-R and NIL-79-S lines. (A), (B), and (C), non-infested sites; (D), SCN-infested sites. (B), (C) and (D) are broken down by location. (A), 1997 and 1998 data combined; (B) and (D), 1998 data; (C), 1997 data. Standard error bars are shown.



**Chapter 3. Molecular marker discovery for use in marker-assisted  
selection**

## Abstract

Molecular markers have given plant breeders new tools, including the ability to use molecular markers to select for traits indirectly. This procedure, termed marker-assisted selection (MAS), requires several qualities in the molecular markers used. Markers should be highly polymorphic, fast and simple to assay, and tightly-linked to the genetic locus controlling the trait of interest. SSRs (simple sequence repeats, also called microsatellites) are molecular markers that are highly polymorphic. Some can be assayed easily and rapidly using agarose gel electrophoresis. In this study, the density of SSRs in the region surrounding *rhg1*, a major gene for resistance to soybean cyst nematode (SCN), was increased in order to obtain markers tightly linked to *rhg1* for use in a marker-assisted selection program. Ten SSRs were placed in a 23 cM region surrounding *rhg1*. Nine of these SSRs were within an 11 cM region around *rhg1*. Two markers, BARC-Satt309 and BARC-Sat\_168 were placed within 1 cM of the *rhg1* locus and together they can distinguish most susceptible genotypes from SCN resistant sources. These markers can be assayed easily and rapidly on agarose gels. Several markers were placed on either side of *rhg1*, which can be used to reduce linkage drag when breeding for SCN resistance or as flanking markers to ensure retention of the resistance allele at *rhg1*.

## Introduction

Traditionally, plant breeders have selected for important traits based on their phenotypic expression. This has worked well for a variety of traits, especially those that can be scored easily, inexpensively, and accurately. Unfortunately, not all traits share these qualities. The advent of molecular marker technology has given plant breeders another option for monitoring and selecting desirable traits. Markers physically linked on the chromosome to genes or loci controlling a trait of interest can be used to indirectly select for the trait in a process called marker-assisted selection (MAS; Tanksley *et al.*, 1989).

MAS may be a good alternative to SCN resistance screening because the phenotypic assay is lengthy, labor-intensive and highly variable. Although several loci appear to be involved in SCN resistance there is one major gene, *rhg1*, that contributes more than 50% of the variation in SCN resistance (Concibido *et al.*, 1994, 1996a, 1996b, 1997; Mahalingam and Skorupska, 1995; Webb *et al.*, 1995; Qiu *et al.*, 1997). This locus, located on molecular linkage group G (MLG-G; Shoemaker and Olson, 1993), is essential in any resistant line and can be targeted for MAS.

The quality of molecular markers near the gene of interest greatly influences the efficacy of MAS. First, markers need to be tightly linked to the gene of interest to avoid crossovers between the marker and the gene. The closer the marker and the gene, the more often the progeny of a cross will inherit the same parent's allele and therefore the more often the marker allele will correctly predict the gene's allele.

Second, molecular markers must have a high degree of polymorphism. Cultivated soybean has a narrow genetic base and finding markers that can separate several parental alleles has been difficult (Keim, 1989; Keim *et al.*, 1992). SSR markers (simple sequence repeat markers, also known as microsatellite markers; Tautz, 1989, Weber and May, 1989) have one of the highest degrees of polymorphisms of any molecular marker

(Akkaya *et al.*, 1992, 1995; Rongwen *et al.*, 1995), making them a valuable target for use in MAS.

Third, molecular markers are needed that can be assayed and visualized rapidly and easily. Again, SSRs can fit this need because they are based on polymerase chain reaction (PCR; Mullis *et al.*, 1986), which is relatively fast and easy. Further, visualization of alleles at some SSRs can be done on agarose gels. In addition to increasing the throughput of the assay and simplifying it, agarose systems avoid the use of dangerous chemicals such as polyacrylamide and radioactivity, often used to visualize SSR markers. To work in an agarose system, SSR markers must be found for which the difference in length of parental alleles is large enough that they can be separated on agarose gels.

In this research I mapped SSRs in the region surrounding the *rhg1* locus in order to find highly polymorphic markers tightly linked to this locus. The first two SSRs characterized in the early stages of these studies, BARC-Satt038 and BARC-Satt130, were valuable in MAS. In addition, several other SSR markers have been placed near *rhg1*. Two of these markers, BARC-Satt309 and BARC-Sat\_168, were shown to be closer to *rhg1* than the original pair of SSR markers, and, unlike BARC-Satt038, were assayable on agarose gels. The increase in SSR marker density around *rhg1* will provide more marker options to be used in MAS for SCN resistance and help characterize the genomic region surrounding *rhg1*.

## Material and methods

### Plant materials

SSRs were mapped in at least one of the following populations. The first population consisted of 98  $F_{4:5}$  recombinant inbred lines (RILs) advanced by single-seed descent from a cross between the SCN-susceptible cultivar, Evans, and the resistant source, PI 209332 (Anand and Brar, 1983; Anand and Gallo, 1984). This population is described in detail by Concibido *et al.* (1996a) and will here after be referred to as the PI 209332 full population. A high resolution subset ( $F_{4:6}$ ) from this population was also used, consisting of lines with crossovers in the region surrounding *rhg1* and control lines homozygous for either parent's alleles across the region. This will be referred to as the PI 209332 subset A population. Markers near *rhg1* can be mapped in this subset population and placed in the region by comparing the parental alleles for this marker in each crossover line with those of other markers already mapped. In addition, a high resolution subset of another Evans x PI 209332 RIL population in the  $F_{4:6}$  generation was used (the PI 209332 subset B population). Another population consisted of 110  $F_{5:6}$  recombinant inbred lines from the cross Evans x the resistant source 'Peking' (the Peking full population). These lines were single-seed descent derivatives of the  $F_{2:3}$  lines described by Concibido *et al.* (1997). A high resolution subset ( $F_{5:7}$ ) was also used (the Peking subset population).

### Greenhouse assays for SCN resistance

Greenhouse assays were conducted previously (Concibido *et al.*, 1996a). Twelve progeny replications from each line were assayed. However, the only population with Peking resistance that was assayed was the Peking subset population. Cysts from three plants were combined so only four groups of cysts were counted per line. If the average

number of cysts recovered from the roots of the plants in each line was less than 30% of the susceptible check, Evans, lines were classified as resistant. Otherwise, they were classified as susceptible. This 30% cutoff is often used by breeders during the development of SCN resistant varieties (Schmitt and Shannon, 1992) and is biologically significant (Concibido *et al.*, 1996b).

#### DNA extraction and RFLP marker analysis

For each line, DNA was previously extracted following the method of Dellaporta *et al.* (1983) from bulked leaf tissue of approximately 30 plants. Restriction fragment length polymorphism (RFLP) genotyping of the PI 209332 and Peking full populations used in this study has been described (Concibido *et al.*, 1996a). DNA had previously been extracted from bacterial artificial chromosome (BAC) clones as described in Danesh *et al.* (1998).

#### SSR marker development

All SSR marker primers were developed in Dr. Perry Cregan's lab at the Beltsville Agricultural Research Center (BARC, Beltsville, MD). All SSR markers will subsequently be referred to without their 'BARC-' prefix. The markers were developed according to one of two methods. The first method is described in Cregan *et al.* (1994) and involves SSR discovery from genomic DNA. Briefly, this involved random cloning of genomic DNA. This was followed by screening the transformed bacterial colonies with either the (TAA)<sub>10</sub> or (AT)<sub>15</sub> oligomers to identify SSRs. Positive clones were sequenced and SSR primers flanking the repeat were developed and tested.

The second method of SSR development employed in Dr. Cregan's lab targeted SSR discovery to the chromosomal region surrounding *rhg1* using BAC clones known to be located in the region of interest (Cregan *et al.*, 1999c; Danesh *et al.*, 1998; Dawn Foster-Hartnett, University of Minnesota, St. Paul, MN, 1999). A BAC library of approximately 32,000 clones or three genome equivalents, which was made from DNA of

'Faribault' (PI 209332-derived resistance), was previously screened using RFLP molecular markers near *rhg1* (Danesh *et al.*, 1998). I also screened the library using primers from an SSR marker developed in Dr. Cregan's lab. BAC clones were pooled for SSR screening according to the method described in Green and Olsen (1990). Primers from Satt309 were used to amplify BAC clones to identify one carrying Satt309. Separation of SSR alleles was performed using the agarose-based method described below except only 15 pg of template were used. BAC clones containing the SSR or RFLP markers, were then sent to Dr. Perry Cregan (USDA-ARS, Beltsville, MD) for SSR marker development. SSR development was similar to that for finding SSRs in genomic DNA except that the BAC clone inserts were randomly cloned rather than the entire genome. In most cases, several BAC clones were combined for SSR marker development. Upon receipt of newly developed SSR primers, I matched each SSR with the BAC clone from which it was derived by amplifying all the BAC clones used in developing each set of SSR primers to find one BAC clone with the correct amplification product. The forward and reverse primer sequences of all SSRs are listed in Table 3-1.

### SSR analysis

The amplification of SSR alleles followed two alternative methods. In the first method, both forward and reverse primers were end-labeled with  $\gamma$ -<sup>33</sup>P according to the following protocol. The primer mixture contained 0.75  $\mu$ M each of forward and reverse primers, 1X kinase buffer (70 mM Tris-HCl at pH 7.6, 10 mM MgCl<sub>2</sub>, 5 mM DTT ), 0.1  $\mu$ L of 3,000 Ci/mmol  $\gamma$ -<sup>33</sup>PdATP per sample, and 0.8 U T4 polynucleotide kinase (New England Biolabs, Beverly, MA) per sample. The primer mixture was incubated at 37°C for 1 hour, then at 70°C for 10 min. Each amplification reaction contained a single 3-mm DNA disk or 5 ng of DNA suspended in TE, 1 mM MgCl<sub>2</sub>, 100  $\mu$ M of each dNTP, 2  $\mu$ L of end-labeled primer mix (0.15  $\mu$ M of forward and reverse primers), 1X Buffer (20 mM Tris-HCl at pH 8.4, 50 mM KCl), and 1 U *Taq* DNA Polymerase in a 10  $\mu$ L reaction volume. Samples were amplified in an MJ Research PTC-100 thermocycler in 96-well microplates. Each of 32 cycles consisted of 25 s at 94°C to denature the DNA, 25 s at 47°C

to anneal the primers, and 25 s at 68C to replicate the DNA. Samples were electrophoresed on a 6% polyacrylamide gel and visualized using autoradiography.

Alternatively, when length polymorphisms were large enough, an agarose-based approach was adopted. Reactions were amplified as above but in the absence of radiolabeled nucleotides and reaction volumes were scaled to 15 µl. Products were electrophoresed on a 3.5% high-resolution agarose gel and visualized with ethidium bromide.

### Mapping

SSRs assayed in the PI 209332 or Peking full mapping population were placed on the genetic linkage map using Mapmaker Macintosh Version 2.0 (Lander *et al.*, 1987). Linkage was determined using two-point analysis ('Group') with a LOD score threshold of 3.0. Markers were ordered using multipoint analysis to find the best order ('First Order') and the order confirmed by testing all possible marker orders within each set of three markers ('Ripple'). For SSRs mapped in subset populations, crossover positions were used to identify where the marker fit along the chromosome. Map distances were estimated by dividing the number of crossovers in an interval by the original number of lines in the full population and dividing by two to adjust for the fact that selfing for infinite generations gives opportunity for twice as much recombination as would be expected in a single generation. This method slightly underestimates the distance between markers the lines used are F<sub>4</sub>-derived and have not been selfed an infinite number of generations. Therefore, more recombination has occurred than would occur in a single generation, but not twice as much.

## Results and discussion

Ten SSR markers were mapped in the MLG-G region surrounding *rhg1* and their genetic relationship with *rhg1* defined (Figure 3-1; Cregan *et al.*, 1999c). These markers cover a 23 cM interval and nine of them are located within approximately 11 cM spanning *rhg1*. Several markers were placed on each side of *rhg1*. The closest markers, Satt309 and Sat\_168, both on the telomeric side, are less than 1 cM from *rhg1*.

### Early MAS results

Satt038 was one of the first SSR markers to be found near *rhg1* (Mudge *et al.*, 1997). It is approximately 3 cM from *rhg1* on the telomeric side. I compared the prediction of resistance based on Satt038 with actual resistance data obtained from greenhouse assays in the PI 209332 full population. Satt038 correctly predicted resistance in 95% of the lines. This was close to the value expected based on the distance of Satt038 from *rhg1* (97%). A second SSR discovered early in the work was Satt130. By also assaying Satt130 (Mudge *et al.*, 1997), which maps approximately 20 cM on the centromeric side of *rhg1*, prediction accuracy increased to 98%. Assaying the flanking markers, Satt038 and Satt130, both found by searching genomic DNA, should predict resistance incorrectly only in the rare case of a double crossover. This was expected in less than 1% of the lines and was close to the actual value of 2% obtained in the experiments.

Satt038, by itself or with Satt130, predicts SCN resistance with high accuracy but has one major drawback for use in MAS. Because the difference in the lengths of the alleles at Satt038 was so small, parental alleles must be separated on polyacrylamide gels, making assaying of Satt038 relatively slow and tedious. Satt130 can be assayed on agarose gels without radioactivity but was 20 cM from *rhg1* and accurately predicted resistance in only 74% of the lines.

## Satt309 in MAS

Another marker, Satt309, that was developed after the discovery of Satt038 and Satt130, proved more useful in MAS than Satt038 and Satt130 (Cregan *et al.*, 1999b). Satt309 was discovered in a search of genomic DNA by Dr. Cregan. I mapped it to a location less than 1 cM from *rhg1*. Satt309 is a marker that can be routinely assayed on high resolution agarose gels. One drawback of Satt309, discovered by Dr. Cregan, is that it is unable to distinguish between commonly used southern cultivars and some resistant sources, including PI 88788 and PI 209332 (Cregan *et al.*, 1999b).

In order to solve this problem, another SSR was sought near Satt309 (Cregan *et al.*, 1999b). I used Satt309 primers to amplify DNA pools from bacterial artificial chromosomes (BAC) clones to find a BAC clone that contained this SSR marker. BAC UMN-51-K4 yielded an amplified product the same size as that of the genomic DNA of Faribault, from which the BAC clone was derived, and of PI 209332, from which Faribault was derived (Figure 3-2). This BAC was sent to Perry Cregan to look for SSRs, with the assumption that any SSRs identified would be near Satt309. Sat\_168 was found from this BAC. Sat\_168 was able to amplify UMN-51-K4, producing the same fragment size as from Faribault, from which the BAC was derived, and from PI 209332, which contributed resistance to Faribault (data not shown). Sat\_168, as expected, was very near Satt309 and mapped to the same position as Satt309 in all the mapping populations tested. Unlike Satt309, Sat\_168 was able to distinguish the southern cultivars from PI 88788 and PI 209332 (Figure 3-3). Through the use of both Satt309 and Sat\_168, *rhg1* alleles from essentially all common susceptible parents and resistance sources can be differentiated. In addition to gaining a valuable marker, this is one of the first examples of targeting SSR markers to a specific region of the genome (Lightfoot *et al.*, Southern Illinois University, personal communication, 1998; Cregan *et al.*, 1999b, Cregan *et al.*, 1999c).

## Other SSRs

I have also determined the map positions of several more SSR markers near *rhg1*, including Sat\_141, Sat\_163, and Sat\_210, Satt163, Satt356, and Satt610 (Figure 3-1; Cregan *et al.*, 1999c). Of these, Sat\_141, Sat\_163, Sat\_210, and Satt610 were targeted to the region using BAC clones previously identified with RFLP markers in the region (Table 1; Figure 3-2; Danesh *et al.*, 1998; Dawn Foster-Hartnett, University of Minnesota, St. Paul, MN, 1999). Again, Dr. Cregan developed the SSRs.

Having several SSRs near *rhg1* is useful for several reasons. The SSRs have been used to characterize the region surrounding *rhg1* in the NIL populations (see Chapter 2). This has facilitated characterization of the *rhg1* and yield depression loci. Several SSR markers are available, making it likely that a polymorphic marker can be found for most, if not all, crosses. These markers can also be used to reduce linkage drag during MAS. This is done by using the markers closest to *rhg1* to select for the resistant parent's allele at *rhg1* and markers just outside of these to select for crossovers back to the susceptible, but agronomically superior parent's alleles. This may be extremely valuable in eliminating negative yield potential that often accompanies SCN resistance (see Chapters 1 and 2).

## Summary

Ten SSRs have been placed near *rhg1* on MLG-G. These SSRs will greatly aid MAS, increase the availability of simple, convenient, polymorphic markers, and provide options for using flanking markers or reducing linkage drag. This research also showed that SSR markers can be targeted to specific regions of the genome using BAC clones as intermediaries.

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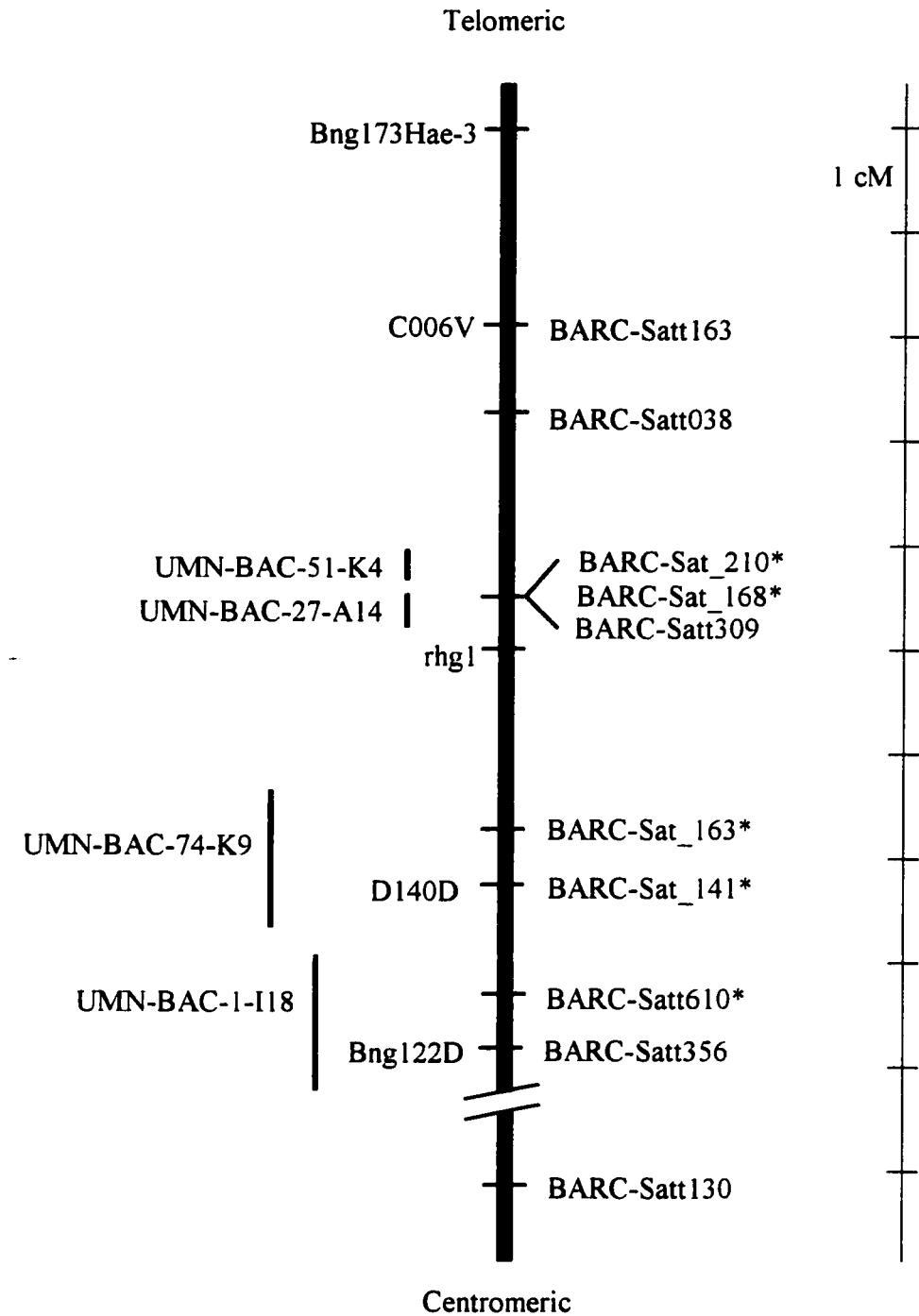
**Table 3-1. SSRs near *rhg1*.**

All SSRs and primer sequence data shown were obtained from Perry Cregan at the Beltsville Agricultural Research Center (BARC; Mudge *et al.*, 1997; Cregan *et al.*, 1999a, 1999b). Primer sequences are shown in the 5' to 3' orientation. SSRs that were targeted to the region surrounding *rhg1* using BAC clone intermediates are so noted, along with the BAC clone that was used in SSR marker development and the marker used to identify the BAC clone. All other SSRs were found as part of an effort to place SSRs across the genome (Cregan *et al.*, 1999a).

SSR	Primer Sequences: Forward	Placement	BAC clone
	Primer Sequences: Reverse	near <i>rhg1</i>	searched
		(Marker used to find BAC clone)	
BARC-Sat_141	CGC AAT CAA AGA CCT GTT GCC TTG GCT ATT TCC TTA	Targeted	MI_074_K09 (DI40D)
BARC-Sat_163	GCG GTA TAT ATG TTT GCA AGA CAT ATT GCG GAA TCT CGC CCA GGA GGA ACT T	Targeted	MI_074_K09 (DI40D)
BARC-Sat_168	TGT GGA TAA AAG AGC ATT CAA AAT G GCG ATC CTT GTT TAT CTC AAA AAA GTG T	Targeted	MI_051_K04 (BARC-Satt309)
BARC-Sat_210	GCGCCAGCAACAAAGTTCTGACAAA GCGCATGCAAATGAAATAATAA	Targeted	MI_027_A14 (MI_051_K04)
BARC-Satt038	GGG AAT CTT TTT TTC TTT CTA TTA AGT T GGG CAT TGA AAT GGT TTT AGT CA	Random	
BARC-Satt130	TGG TAG TGA AAG CAC GAG AT AAC ACT TTG AAT GGC TAA AAA C	Random	
BARC-Satt163	AATAGCACGAGAAAAGGAGAGA GTGTATGTGAAGGGGAAAAACTA	Random	
BARC-Satt309	GCGCCTCAAATTGGCGTCTT GCGCCTTAAATAAAACCCGAAACT	Random	
BARC-Satt356	CATGCCCTGGTCCATTTG TCAAGGCCACGATAACAGTA	Random	
BARC-Satt610	CCC TCC GCA AGC AAT AAT TAA TCT GCG GAA TGC TTC CAT TTT AT	Targeted	MI_001_I18 (BngI22D)

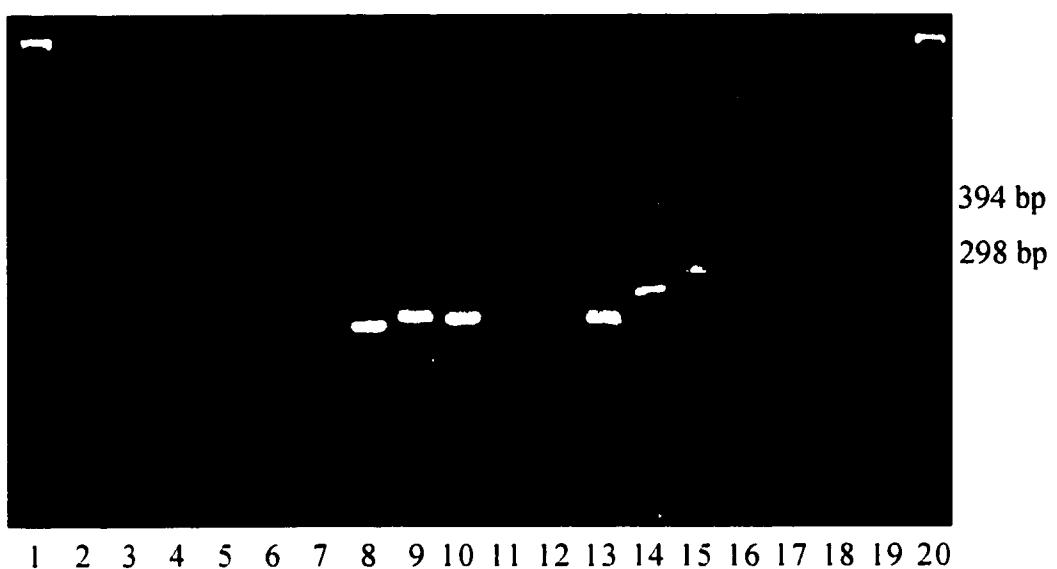
**Figure 3-1. Genetic map of the region surrounding *rhg1*.**

SSRs are identified with the “BARC”- prefix (Beltsville Agricultural Research Center). SSRs are starred that were targeted to this region using bacterial artificial chromosomes identified using other molecular markers in the region. All other markers are restriction fragment length polymorphisms previously identified (Concibido *et al.*, 1996b). BAC clones that were used to target SSRs to this region are shown on the left (Danesh *et al.*, 1998; Dawn Foster-Hartnett, University of Minnesota, St. Paul, MN, 1999). UMN-BAC-K4 and UMN-BAC-A14 are non-overlapping BAC clones whose relative position cannot be distinguished in the mapping populations used. BARC-Satt130 was approximately 20 cM away from *rhg1* on the centromeric side.



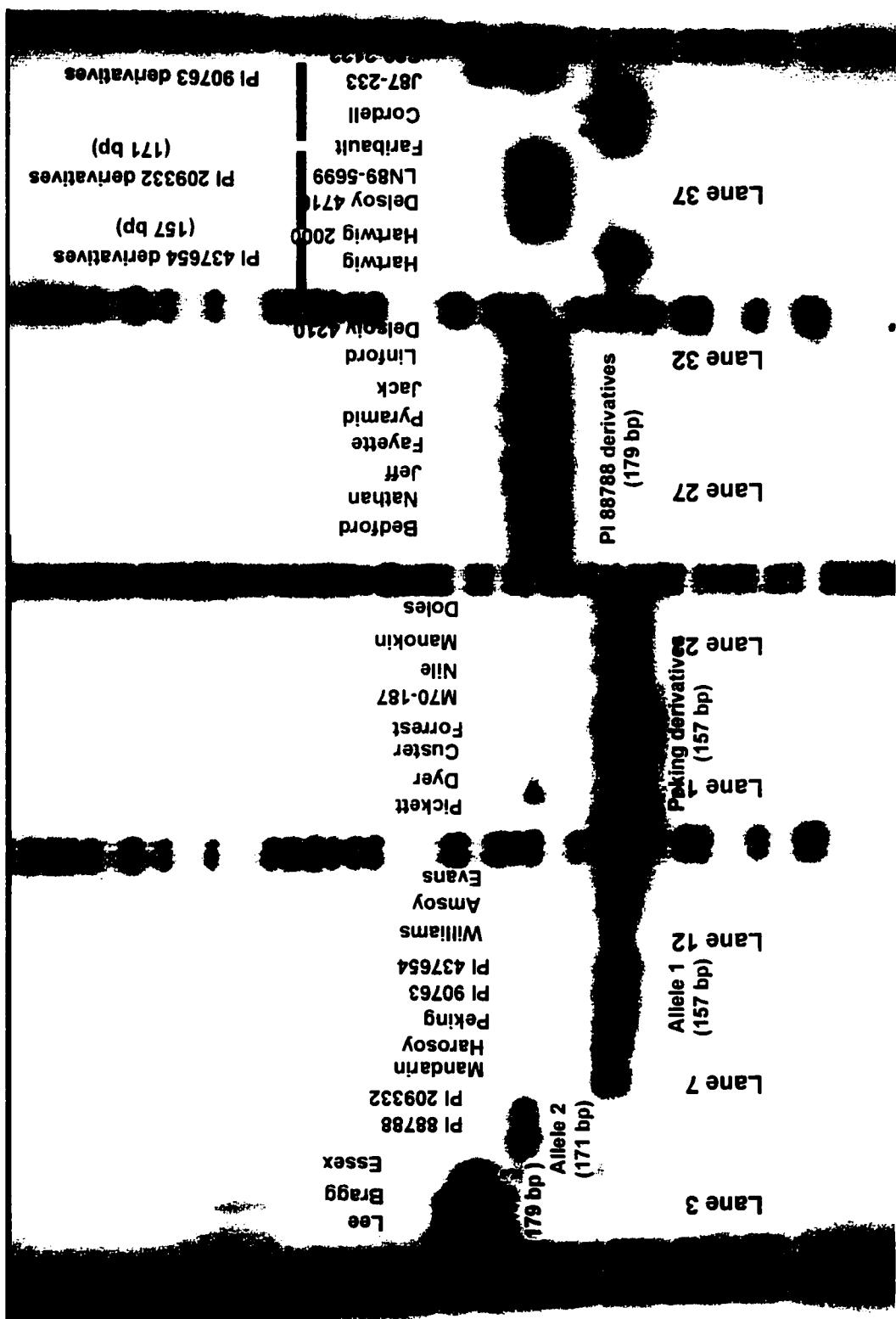
**Figure 3-2. Amplification of BAC clones using SSR primers**

Three SSR markers, discovered from BAC clones known to be near *rhg1*, are shown here. BARC-Satt309 (lanes 2-7) identified UMN-BAC-51-K4, which was subsequently searched to obtain Sat\_163. BARC-Satt610 (lanes 8-13) and BARC-Sat\_141 (lanes 14-19) were both discovered in a pool consisting of UMN-BAC-74-K9 and UMN-BAC-1-I18. Each set of SSR primers was used to amplify ‘Evans’ (lanes 2, 8, and 14), PI 209332 (lanes 3, 9, and 15), ‘Faribault’ (lanes, 4, 10, and 16), UMN-BAC-51-K4 (lanes 5, 11, 17), UMN-BAC-74-K9 (lanes 6, 12, and 18), and UMN-BAC-1-I18 (lanes 7, 13, and 19). Lanes 1 and 20 contain 1 kb molecular weight DNA ladder (Gibco/BRL). The molecular weight of selected bands are given on the right.



**Figure 3-3. BARC-Sat\_168 alleles**

BARC-Sat\_168 is assayed on several cultivars and sources of resistance. DNA molecular weight standards were derived from sequencing M13 single stranded DNA with either ddTTP, ddGTP, and ddCTP and combining the three reactions (lanes 1, 2, 25, and 43) or ddATP (lanes 16, 34). The standards split the gel into sections. The first section contains susceptible cultivars, including the commonly used southern susceptibles, Lee, Bragg, and Essex. The first section also contains sources of SCN resistance, including PI 88788, PI 209332, Peking, PI 90763, and PI 437654. The next section contains cultivars derived from Peking. The third section has cultivars derived from PI 88788 and the last section, cultivars from PI 437654, PI 209332, and PI 90763. This picture was obtained from Perry Cregan (*Cregan et al.*, 1999b).



**Chapter 4. Value of marker-assisted selection in selecting soybean lines  
resistant to soybean cyst nematode**

## Abstract

Because greenhouse and field screening of soybean lines for soybean cyst nematode (SCN) resistance is time-consuming, labor-intensive, and variable, marker-assisted selection (MAS) is an attractive alternative to traditional breeding strategies. In order for MAS to be a valuable option, it must efficiently predict reaction to SCN. Four breeding populations were used to compare MAS predictions of SCN resistance to resistance determined by standard greenhouse tests. These populations cover a variety of resistance sources, including Peking, PI 209332/PI 88788, PI 88788, and PI 437654. No resistant lines were found in the greenhouse for the PI 437654 population. However, in the other three populations, in which both resistant and susceptible lines were available, MAS proved to be very efficient at discarding susceptible lines and reducing overall population size. With the ability of MAS to efficiently reduce the number of lines that must be carried through an SCN breeding program, more lines can be tested and/or fewer resources used in producing soybean varieties resistant to SCN.

## Introduction

Molecular DNA markers have provided new tools to plant breeders. One application of markers to breeding programs is their use for selection in a process called marker-assisted selection (MAS; Tanksley *et al.*, 1989). In MAS, molecular markers linked to a locus controlling a trait of interest are used to indirectly select for the desired parent's allele at the trait locus. The closer the locus and marker are to each other on the chromosome, the more often they should contain the same parent's allele and therefore, the alleles at the marker can predict with high accuracy the alleles at the locus of interest.

However, MAS should not always be used in the place of traditional phenotypic selection. Often, phenotypic selection is straightforward and can be done quickly and accurately with little expense, making it much more practical than MAS. Scoring the trait directly may be more efficient than using markers linked to each locus. In addition, it may be possible to do phenotypic selection on several important agronomic traits at the same time, making it less practical to score a single phenotypic trait using markers.

In the case of SCN resistance scoring, greenhouse and field assays for resistance are labor intensive and require more than 30 days before results are available. In addition, phenotypic assays for resistance are highly sensitive to experimental and environmental error, although recent advances in greenhouse assays have reduced this error (Sardanelli and Kenworthy, 1997). From a practical standpoint, only one or two replications of a greenhouse assay are feasible on the first screening of a breeding population, making the large amount of experimental error a concern.

Using MAS instead of phenotypic assays for SCN resistance leads to results much more quickly, often in just a few days. This allows breeders to test material while it is in the field and to harvest only those lines that are predicted to be resistant to SCN. In addition, MAS reduces the labor involved and is estimated to cost only slightly more than phenotypic selection (Denny *et al.*, 1996). Although several loci have been shown to

influence SCN resistance, there is one major locus, *rhg1*, necessary for resistance that contributes over 50% of the variation in SCN resistance (Caldwell *et al.*, 1960, Concibido *et al.*, 1994, 1996a, 1996b, 1997; Mahalingam and Skorupska, 1995; Webb *et al.*, 1995; Qiu *et al.*, 1997). This locus is considered necessary for resistance and can be targeted in a MAS program. SCN resistance breeding based on *rhg1* is therefore an excellent candidate for MAS. In this study, two replications of the greenhouse assay, which are typical of early screening in the University of Minnesota soybean breeding program, are compared to MAS predictions of SCN reaction in four breeding populations.

## Material and methods

### Plant materials

Breeding lines from four different crosses (Table 4-1) were advanced by single-seed descent. Population 1 consisted of 271 F<sub>4.5</sub> progeny from the cross, 'Parker' x A92-526007. A92-526007 has SCN resistance derived from Peking (Ross and Brim, 1957). Population 2 consisted of 367 F<sub>4.5</sub> progeny from the cross, 'Lambert' x M92-1631. M92-1631 has resistance derived from both PI 88788 (Epps and Hartwig, 1972; Thomas *et al.*, 1975) and PI 209332 (Anand and Brar, 1983; Anand and Gallo, 1984). Population 3 consisted of 259 F<sub>4.5</sub> progeny from the cross, 'Stride' x M92-1731. M92-1731 has resistance from PI 88788. Population 4 consisted of 58 F<sub>3.4</sub> progeny from the cross, Lambert x M91-102011. M91-102011 has resistance from PI 437654 (Anand and Gallo, 1984; Nelson *et al.*, 1988). Some artificial selection did occur. Lines without enough seed for plots in the following year were discarded as were lines with poor seed quality, such as cracks in the seedcoat. No other intentional artificial selection was practiced.

### Greenhouse assays

Greenhouse assays were conducted as described in Concibido *et al.* (1996a). Briefly, this involved planting seed of the lines to be tested in sand. The containers were put into sand-filled buckets and placed in a waterbath to keep the temperature constant at 28°C. Plants were inoculated with 2,000 eggs one week after planting. After approximately 28 days, plants were removed from containers. The sand was washed off the roots and cysts were knocked off using high pressure water. The cysts were collected on a sieve and counted under a microscope.

Two plants for every line from each of the four populations were assayed. Assaying any more replications would not have been practical in this experiment because of the large number of lines involved. Cyst indices, defined as the number of cysts on the

roots of a plant divided by the number of cysts on the roots of the susceptible control, ‘Evans’, were calculated. Lines with an average cysts index below 30% were designated as resistant and above 30% as susceptible. This cutoff is biologically significant because resistance has been shown to be bimodally distributed with the range of resistant and susceptible plants overlapping slightly near a cyst index of 30% (Concibido *et al.*, 1994). If cyst counts exceeded 40% of the susceptible check, counting was stopped and the line designated susceptible. Lines were included in the analysis only if both greenhouse replications agreed. Because our greenhouse setup accommodated only about 400 plants (or 200 lines replicated twice), five separate greenhouse runs were used in this study. Several of the populations were split across two or more runs.

Six cultivars were also assayed in each greenhouse run, including the susceptible cultivar, ‘Evans’, and the resistant cultivars, PI 209332, PI 88788, ‘Peking’, PI 90763, and ‘Pickett’. The latter four are the differentials used to determine SCN race (see Literature Review, Table 1) and were included to ensure that inoculum conformed to race 3. Four to five plants of each cultivar were included in each greenhouse assay, as were the parents of any crosses included in the greenhouse run.

### Genotyping

DNA was extracted according to the disk method described in Lange *et al.* (1998). This method allowed the extraction of a large number of lines with relatively little time and labor, as is necessary in a breeding program. Briefly, it involved rubbing an imprint of a leaf onto a DNA collection card (Gentra Systems, Minneapolis, MN) and punching a 3 mm disk from the card using a hole punch. The disk was then washed three times for 15 minutes each with DNA purification solution (Gentra Systems). A final rinse was done with ethanol. The disks were dried overnight or at 60°C for 30 min, after which they are put directly into a simple sequence repeat (SSR) reaction. All SSR assays were done according to the agarose-based, non-radioactive method described in Cregan *et al* (1999a). Only the first greenhouse replication was genotyped. Plants were genotyped using the SSR primers from BARC-Satt309, which is within 1 cM of *rhg1* (hereafter

referred to as Satt309; Cregan *et al.*, 1999a). No attempt was made to genotype several plants per line in order to distinguish lines still segregating in the region of interest. Lines genotyped as heterozygous were not included in the analysis.

#### Statistical analysis

The cyst numbers of the susceptible and resistant cultivars, included in the SCN resistance assays in the greenhouse as checks, were compared using t-tests. A nonparametric test, the Mann-Whitney test, was also used because of the violation of the homogeneous variances assumption of t-tests. Both results gave the same p value. Normality of data was not met for the t-test. Also, datapoints were not independent because four replications per greenhouse run were done on each cultivar and the data was not grouped as repeated measures on identical units. Because cyst counts for the lines in the populations studied in this experiment were stopped after they reached 40% of the susceptible check, statistical analyses similar to those done on the resistant and susceptible cultivars were not possible. Lines were defined as resistant or susceptible based on the 30% cyst index cutoff.

## Results and discussion

### Distribution of Satt309 genotype and SCN phenotype

In the absence of segregation distortion, a 1:1 distribution of parental alleles is expected at Satt309. However, other studies have shown fewer lines with the resistant parent's alleles at markers near *rhg1* (Mudge *et al.*, 1997). The reason for this is unknown, although it is possible that resistant lines are less fit than susceptible lines due to deleterious genes near *rhg1* or the breeder may unconsciously select against resistant lines when advancing lines. The current study also showed fewer lines that were predicted to be resistant than susceptible (Figure 4-1). Populations 1, 2, and 3 had 39%, 41%, and 23% of the lines in this analysis that were homozygous for the resistant parent's allele at Satt309.

Even fewer lines showed resistance in both replications of the greenhouse assay (Figure 4-2). For populations 1, 2, and 3 these numbers were 13%, 35%, and 6%, respectively. The difference in the number of resistant lines in population 2 versus populations 1 and 3 may have been due to environmental variation in the greenhouse assays or an actual difference resulting from differences in the population.

The results for population 4, with resistance derived from PI 437654, were substantially different from the other three populations. The analysis that follows includes all lines in the population. None of the lines showed resistance in both replications of the greenhouse assay. In fact, only five of 58 lines showed resistance in one of the replications. Three of these were at the cyst index level of 27% or above, which was close to the 30% cutoff for resistance. Twelve of the 58 lines were predicted by Satt309 to have the resistance allele at *rhg1* and therefore would likely show resistance in the greenhouse at the 30% cutoff. However, only one line was both genotyped as resistant at Satt309 and showed resistance in one replication of the greenhouse (cyst index = 27%). In populations with resistance from PI 437654, earlier studies have found that resistant plants are rare

(Myers and Anand, 1991). The reasons for this are unknown. However, it has been shown that another locus, *Rhg4*, also has a large effect on resistance and the presence of both *rhg1* and *Rhg4* gives more resistance than the sum of the effects of each locus (Webb *et al.*, 1995). The *Rhg4* locus was not tested. Also, PI 437654 is extremely poor agronomically and it was possible that linkage drag associated with *rhg1* made resistant progeny of PI 437654 less fit. Although these plants have not undergone any intentional agronomic selection in the field, seed quantity and quality were selected before any MAS or greenhouse testing for resistance and this selection could have been biased against resistant plants. Plants yielding less than 30 seeds were generally eliminated. Plants with seed that was immature, diseased, showed growth cracks or a wrinkled seed coat, or showed hilum colors inconsistent with the hilum colors of the parents was discarded.

PI 437654 is an important source of resistance. It is the only source that shows resistance to all known SCN races (Anand and Gallo, 1984; Anand, 1986; Rao-Arelli *et al.*, 1991b; Rao-Arelli *et al.*, 1992). When breeding with PI 437654 resistance, no artificial selection for agronomic traits should be practiced in early generations. Rather, the first steps of selection should be based on *rhg1* or a linked marker. For PI 437654 it is important to keep heterozygous lines and any lines that show resistance or the resistant parent's allele at a molecular marker near *rhg1* regardless of agronomic performance. Agronomic selection should occur only when susceptible lines have been eliminated.

In each of the five greenhouse runs, the four differentials for determining SCN race, Pickett, Peking, PI 88788, and PI 90763, were resistant with average cyst indices below 10% as is expected for race 3 inoculum (see Literature Review, Table 1; Appendix, Table A-10). PI 209332 also gave a cyst index below 10% in each of the greenhouse runs. Also, there was a significant difference in cyst count numbers between the susceptible and resistant cultivars ( $p < 0.0001$ ; the susceptible cultivar, Evans, averaged 182 cysts per plant; the resistant cultivars, 5 cysts per plant).

## Accuracy of MAS in predicting susceptibility to SCN

The 61% of the lines in population 1 (Peking resistance) that had the susceptible parent's allele at Satt309 (Figure 4-1a) would have been discarded in a MAS-based breeding program because they were assumed to also have the susceptible parent's alleles at the nearby *rhg1* locus and to be susceptible to SCN. These lines were also tested in the greenhouse to determine if these lines showed susceptibility. Of the lines that were predicted to be susceptible to SCN, 99% showed susceptibility while only 1% showed resistance (Figure 4-3a). Therefore, MAS appears able to reduce population size while sacrificing only a small number of resistant lines.

In population 2 (PI 209332 and PI 88788 resistance), 57% of the lines were predicted to be susceptible based on Satt309 (Figure 4-1b). Of these, 81% were susceptible in the greenhouse and 19% were resistant (Figure 4-3b). MAS was not as efficient at discarding only susceptible lines as in population 1 in this case. However, there were twice as many resistant lines in the portion of the population that was selected using Satt309 than in the portion discarded using Satt309. Moreover, the percentage of resistant lines increased from 35% in the full population to 57% in the portion of the population selected using Satt309 (compare Figures 4-2 and 4-4).

Population 3 (PI 88788 resistance), had a larger percentage (77%) of the lines that would have been discarded in a MAS program based on their genotype at Satt309 (Figure 4-1c). Of these, 99% showed susceptibility in the greenhouse (Figure 4-3c). Only 1 of the 124 lines that showed the susceptible parent's alleles at Satt309 was resistant. MAS reduced the population size to one-quarter of its original size, while sacrificing only one line that showed resistance in both greenhouse replications.

## Accuracy of MAS in predicting resistance to SCN

The correlation between marker and greenhouse assays for SCN resistance is not as good in lines predicted to be resistant. Of the lines that were predicted to be resistant using Satt309, only 32%, 57%, and 21%, actually were resistant in populations 1, 2, and

3, respectively. As mentioned above, population 4 did not have any lines that showed resistance in both greenhouse replications.

Although these results appear disappointing, the data merit a closer look. Using marker-assisted selection, populations sizes were decreased to 23% to 41% of their original size (Figure 4-4). In addition, the proportion of resistant lines in the lines that were retained increased 2.5, 1.6, and 3.5 fold in populations 1, 2, and 3, respectively (compare Figures 4-2 and 4-4). Therefore, MAS was successful in reducing population sizes while increasing the proportion of resistant lines.

### Summary of data from populations 1, 2, and 3

Populations 1, 2, and 3, showed a combined percentage of 65% of lines that would be discarded in MAS because they had the susceptible parent's alleles at Satt309 (Figure 4-5b). Of these, 93% were confirmed as susceptible in the greenhouse (Figure 4-5c). Therefore, only 7% of that lines that would have been discarded based on MAS using Satt309 were found to be phenotypically resistant. Approximately one-third of the lines in the three populations were retained using MAS and 40% of these showed resistance (Figure 4-5d). This percentage increased from 18% in the original populations (Figure 4-5a). Overall, MAS reduced the number of lines to one-third of the original number while doubling the proportion of resistant lines.

### Limitations of the greenhouse assay standard

The largest source of error in this study was in the greenhouse test, which was our standard for comparison. This test is prone to environmental and experimental error (Riggs and Schmitt, 1991). In previous studies in which 12 plants per line were assayed, there were levels of agreement between the greenhouse and MAS tests that would be predicted based on the proximity of the molecular marker used to *rhg1*, both in predicting resistance and susceptibility (Mudge *et al.*, 1997; Cregan *et al.*, 1999a). However, in this study, lines predicted to be resistant were more often susceptible than resistant (Figure 4-5d). The five resistant cultivars tested, however, did show resistance in each of the

greenhouse runs (see Appendix, Table A-10). Four to five replications were used for these cultivars, while only two were used with the population lines. The large number of susceptible lines that were predicted to be resistant was likely due to the low number of replications used in the greenhouse assays.

If error associated with cyst index readings is assumed to be symmetric, namely that changes in environmental conditions or other error cause deviations from “true” cysts indices in equal distributions for resistant and susceptible lines, resistant lines will more often falsely appear susceptible in greenhouse assays than susceptible lines resistant. This is because the cyst index cutoff for resistance and susceptibility is not at the mean of all the cyst indices or at the center of the cyst index range, but rather at 30%. It is much more likely that resistant lines, with a cyst index range from 0-30% can erroneously show cyst indices higher than 30%, than for susceptible lines with cyst indices that generally range from 30% to more than 100%, to erroneously read below 30%. Therefore, if the error is symmetric, it is not surprising that more lines that should be resistant show susceptibility in the two greenhouse assays than the other way around.

The need for caution in interpreting the greenhouse data was also evident in the fact that 26% of the lines in populations 1, 2, and 3 showed resistance in one greenhouse replication and susceptibility in the other greenhouse replication. Because of the ambiguity in these lines, they were not included in the analysis. The discrepancy could be due to the fact that lines are still segregating for resistance, but this probably accounted for only a small portion of the lines tested (see below). Alternatively, the ambiguity reflected the large amount of experimental and environmental error in the greenhouse.

More evidence of the error involved in the greenhouse assays can be seen by looking at different greenhouse runs. Because the number of lines that can be tested at once was limited, several of the populations spanned greenhouse runs. The first three greenhouse runs used the same increase of SCN inoculum. However, the fourth and fifth greenhouse runs each used inoculum increased during the first and second greenhouse runs, respectively. All inoculum, although increased at different stages was from the same original inoculum source. During the first two greenhouse runs, the thermostat on

the water bath was not functioning properly, resulting in temperatures varying between 21°C and 32°C, rather than being held at 28°C.

There are clearly differences between greenhouse runs. However, the cyst indices are calculated with data from the susceptible check included in the particular greenhouse run, and therefore, some of the variation would have been adjusted for. Also, despite of the inconsistency between runs, the five resistant cultivars included in each greenhouse run always showed resistance (see Appendix, Table A-10).

Greenhouse runs two and three had the most lines excluded from the analysis. Lines were discarded due to ambiguity in the greenhouse test or heterozygosity at Satt309. These two runs also showed a much higher proportion of lines showing resistance in the greenhouse than any of the other runs (Figure 4-6 a, b), even considering the slightly larger percentage of lines predicted to be resistant in these runs (Figure 4-6 d). Greenhouse run 3 was composed entirely of population 2 and greenhouse runs 2 and 4 also contains some lines from population 2. Population 2 was unique in that 19% of the lines discarded based on Satt309 were resistant in the greenhouse verses 1% in populations 1 and 3 (Figure 4-3). The difference may be in the population and its sources of resistance. Another explanation may be that the fluctuation in water bath temperature in the second run may have resulted in less SCN growth and more lines appearing resistant that were not. If a large number of susceptible lines showed resistance in one or two replications, the large number of lines discarded and the relatively large proportion of resistance lines could be explained. It must be cautioned, however, that the first greenhouse run, containing population 1, also had temperature fluctuation in the water bath but did not show a comparable proportion of lines showing resistance in the greenhouse. Ideally, the populations should have been randomized throughout all five greenhouse runs.

It is evident that the greenhouse “standard” was flawed. However, this is the standard used to determine resistance and susceptibility in the University of Minnesota breeding programs because more replications would not be practical. MAS appears to be comparable to the current greenhouse standard in discarding susceptible lines. More lines were retained with MAS than with the greenhouse assay, but the populations were

nevertheless reduced to 23-41% of their original size (Figure 4-4) with less effort and time than greenhouse assays. Few resistant lines were eliminated and the percentage of resistant lines in the portion of the population retained was increased using MAS.

### Other limitations

This MAS program focuses only on *rhg1* for predicting SCN resistance. Phenotypic assays measure effects of all resistance and related genes. It is known that other loci affect SCN resistance, most notably *Rhg4* on MLG-A in Peking and PI 437654 (Webb *et al.*, 1995). Other minor QTL have also been detected for all the sources of resistance used in this study but these have not always been consistently uncovered (Concibido *et al.*, 1994, 1996a, 1996b, 1997; Mahalingam and Skorupska, 1995; Webb *et al.*, 1995; Vierling *et al.*, 1996; Qiu *et al.*, 1997). Expanding a MAS program to include *Rhg4* would be wise, especially if Peking and PI 437654 resistance are involved. SSR markers have been found tightly linked to *Rhg4* (Cregan *et al.*, 1999b) that could be assayed in addition to markers near *rhg1*. It should be possible to multiplex markers near the two loci by assaying them on the same gel.

It is also possible that resistance alleles at *rhg1*, itself, are different in the different sources of resistance. This may affect how well marker alleles and phenotypic resistance scores correspond for different sources of resistance.

Crossovers between the marker used for selection and the actual gene of interest are another source of error for MAS. However, in this case it accounts for very little of the error. Satt309 is less than one cM from *rhg1* and therefore crossovers are expected to produce different parental alleles at Satt309 than at *rhg1* in less than 1% of the lines.

Another source of error in this study is that only one of the two greenhouse replications was genotyped. Therefore, lines still segregating in the region of interest were not detected. Because these lines are F<sub>3</sub>- and F<sub>4</sub>-derived, it was probable that the lines from which they were derived had not reached homozygosity at all loci. An F<sub>3</sub> plant theoretically has 25% of its loci that are still heterozygous. Alternatively, 25% of the lines in our study should still be heterozygous in the region surrounding *rhg1* and a

population derived from these heterozygous lines would still be segregating in this region. Likewise, for the F<sub>4</sub>-derived lines (populations 1, 2, and 3), this percentage of lines still segregating near *rhg1* was expected to be 12.5% and therefore, less error was expected than in F<sub>3</sub>-derived lines (population 4). However, the contribution of the segregating lines to the overall error rate was mitigated by several factors. Theoretically, half of the segregating lines were excluded from the analysis, because any plant that was genotyped as heterozygous was not included. Some of the lines retained will by chance have the same genotype for both replications, and therefore will not contribute to the overall error rate. In addition, lines that showed resistance in one replication and susceptibility in another replication were thrown out of the analysis, reducing the amount of error from segregation of lines. Overall, the inability to detect all of the lines that are still segregating in the region of interest probably accounts for only a small portion of the error rate.

### Conclusions on MAS for SCN resistance

With the exception of the PI 437654 population, which had no lines that were resistant in the greenhouse, MAS gave promising results. Population sizes were reduced to 39%, 41%, and 23% in populations 1 (Peking resistance), 2 (PI 209332/PI88788 resistance), and 3 (PI 88788 resistance), respectively. Less than 1% of the lines discarded in populations 1 and 3 showed resistance in the greenhouse and 19% showed resistance in population 2 (Figure 4-3). The proportion of lines that showed greenhouse resistance increased in the populations selected by markers by up to three times the original populations (compare Figures 4-2 and 4-4).

Although most of the lines discarded using MAS were susceptible in greenhouse assays, fewer lines were discarded with MAS than would have been based on the two replications of the greenhouse study normally assayed in the breeding program (compare Figures 4-1 and 4-2). Even so, MAS was valuable in reducing population sizes by approximately two-thirds in all but the PI 437654-derived populations (Population 4), with less time and effort expended to obtain results.

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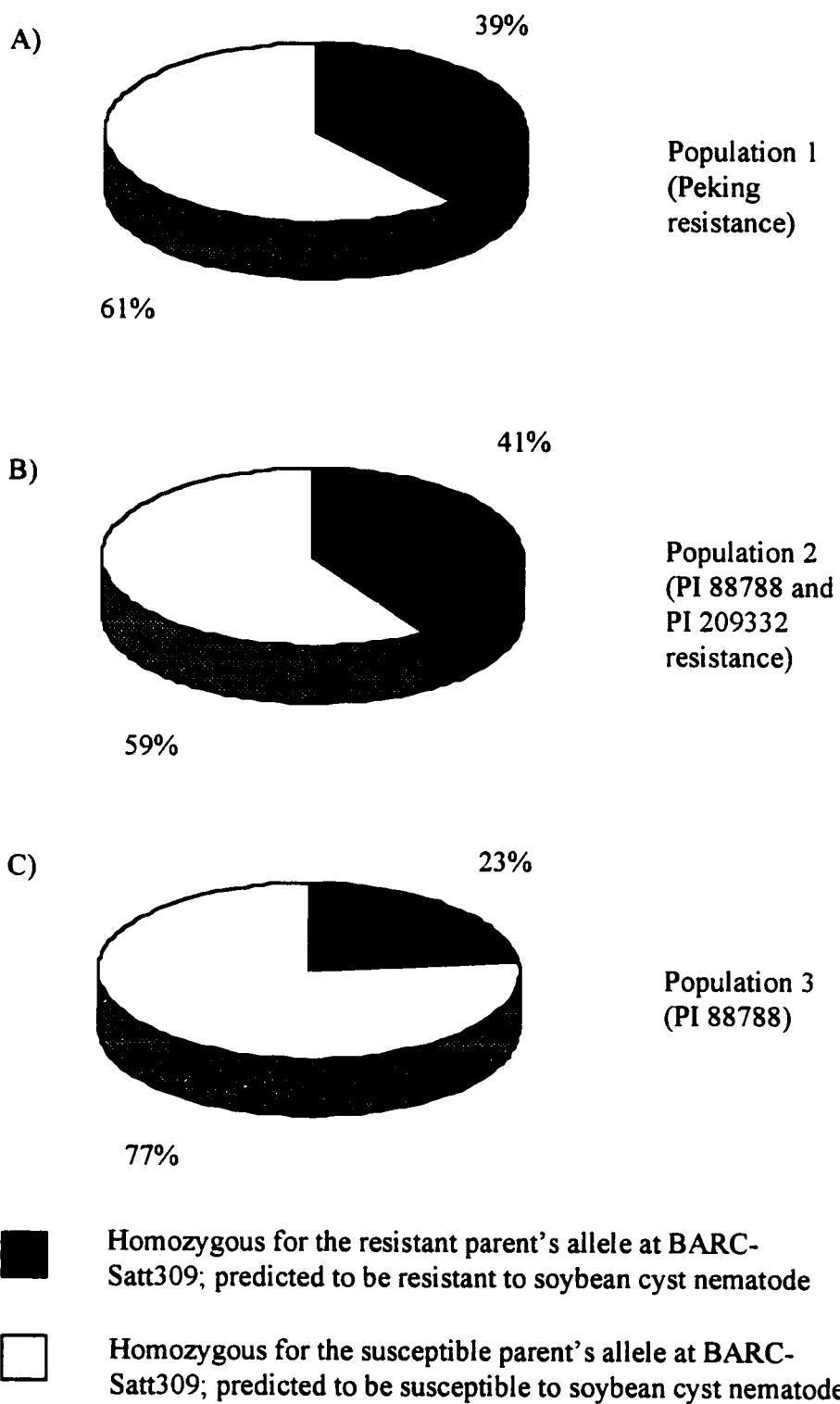
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**Table 4-1. Breeding populations tested for reaction to SCN both in the greenhouse and indirectly using a linked molecular marker**

Population	Parents	Resistance Source	Individuals	Generation
1	Parker x A92-526007	Peking	271	F <sub>4:5</sub>
2	Lambert x M92-1631	PI 88788 & PI 209332	367	F <sub>4:5</sub>
3	Stride x M92-1731	PI 88788	259	F <sub>4:5</sub>
4	Lambert M91-102011	PI 437654	58	F <sub>3:4</sub>

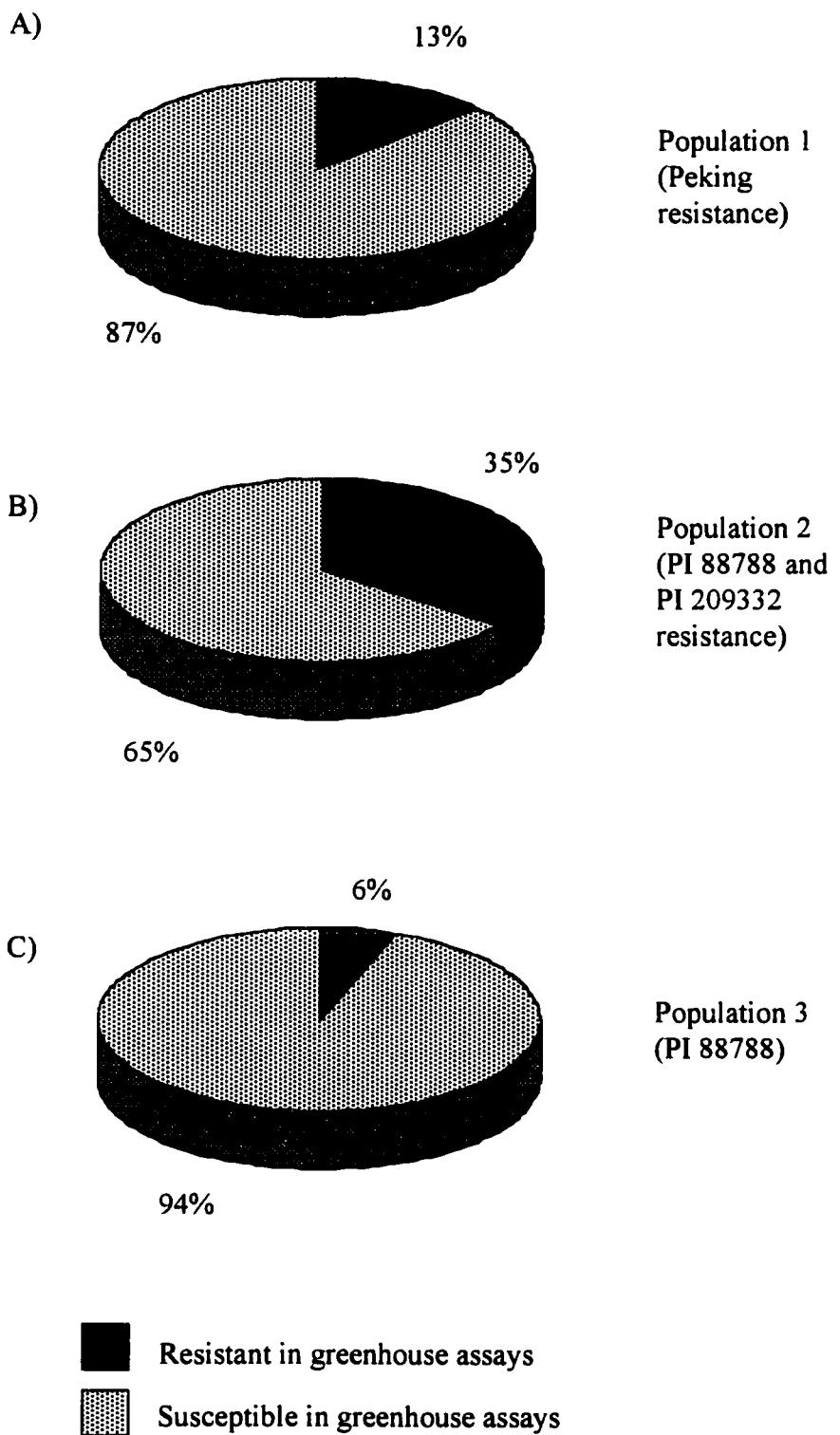
**Figure 4-1. Genotype of breeding lines at BARC-Satt309**

Lines in populations 1-3 are broken down according to whether they were homozygous for the resistant parent's allele (black) or the susceptible parent's (white) at BARC-Satt309 (Cregan *et al.*, 1999a), near *rhg1*. Those with the susceptible parent's alleles at BARC-Satt309 would be assumed to have the susceptible parent's allele at the nearby *rhg1* locus and would be discarded. A) Population 1 with resistance derived from Peking. B) Population 2 with resistance derived from PI 88788 and PI 209332. C) Population 3 with resistance derived from PI 88788.



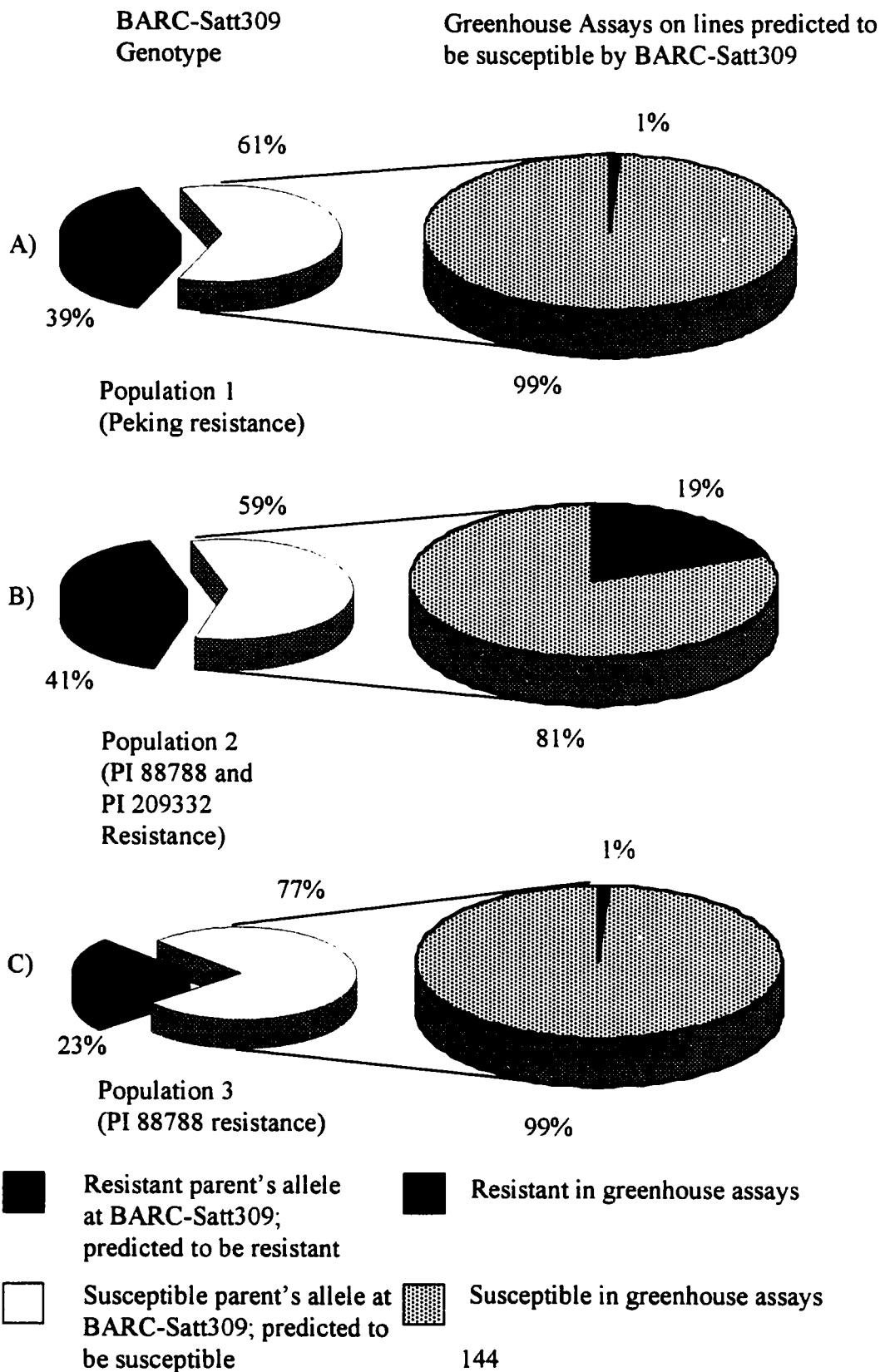
**Figure 4-2. SCN phenotype of breeding lines**

Lines in populations 1, 2, and 3 are broken down according to their phenotype in greenhouse assays for SCN resistance. Lines with fewer than 30% of the number of cysts of the susceptible check in both greenhouse replications were considered resistant (dotted black). Those with greater than 30% in both replications were considered susceptible (dotted white). A) Population 1 with resistance derived from Peking. B) Population 2 with resistance derived from PI 88788 and PI 209332. C) Population 3 with resistance derived from PI 88788.



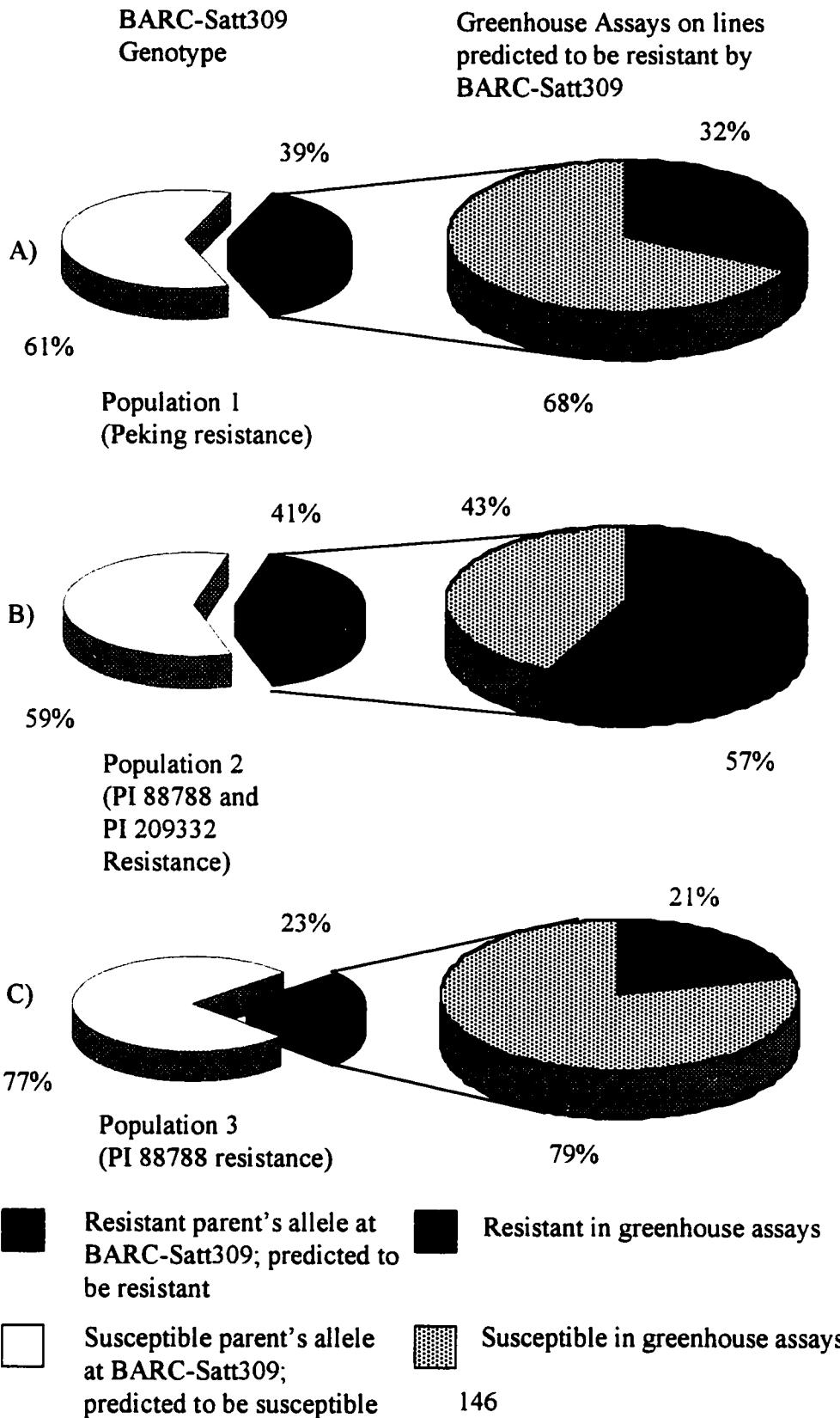
**Figure 4-3. SCN phenotype of lines predicted to be susceptible by BARC-Satt309**

Lines that were predicted to be susceptible using BARC-Satt309 (white) were tested in the greenhouse to confirm susceptibility. Lines with fewer than 30% of the number of cysts of the susceptible check in both greenhouse replications were considered resistant (dotted black). Those with greater than 30% in both replications were considered susceptible (dotted white). A) Population 1 with resistance derived from Peking, B) Population 2 with resistance derived from PI 209332 and PI 88788, and C) Population 3 with resistance derived from PI 88788.



**Figure 4-4. SCN reactions in the greenhouse for lines predicted to be resistant by BARC-Satt309**

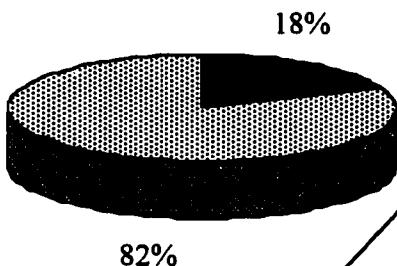
Lines that were predicted to be resistant using BARC-Satt309 (black) were tested in the greenhouse to confirm resistance. Lines with fewer than 30% of the number of cysts of the susceptible check in both greenhouse replications were considered resistant (dotted black). Those with greater than 30% in both replications were considered susceptible (dotted white). A) Population 1 with resistance derived from Peking, B) Population 2 with resistance derived from PI 209332 and PI 88788, and C) Population 3 with resistance derived from PI 88788.



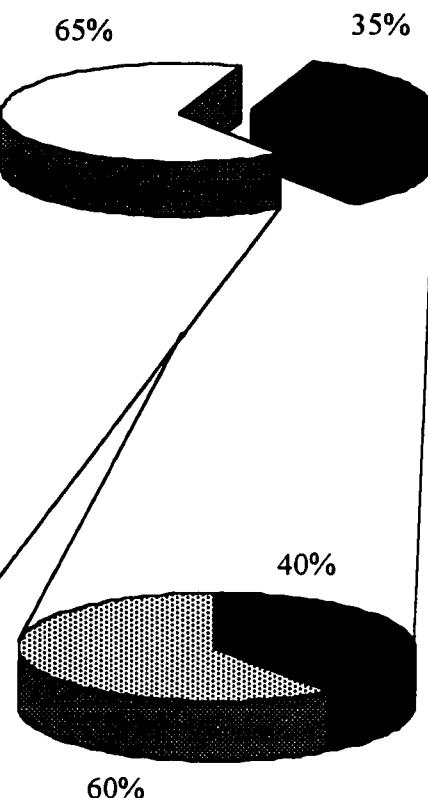
**Figure 4-5. Comparison of greenhouse assays and marker-assisted selection for soybean cyst nematode resistance in populations 1, 2, and 3.**

Combined data from populations 1 (Peking resistance), 2 (PI 209332 and PI 88788 resistance), and 3 (PI 88788 resistance) for greenhouse assays and marker-assisted selection for resistance to soybean cyst nematode. A) Greenhouse data showing the percentage of lines showing resistance (dotted black) versus susceptibility (dotted white) in greenhouse assays. B) Marker-assisted selection data showing the percentage of lines predicted to be resistant (black) versus susceptible (white) using BARC-Satt309. C) SCN reactions of soybean lines in greenhouse assays on lines that are predicted to be susceptible by BARC-Satt309. These lines are discarded in a MAS program. D) SCN reactions of soybean lines in greenhouse assays on lines that are predicted to be resistant by BARC-Satt309. These lines are retained in a MAS program.

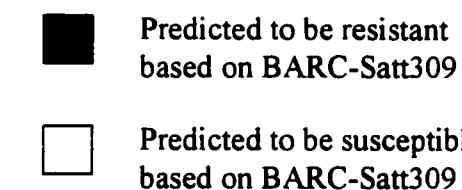
A) Greenhouse resistance in populations 1, 2, 3



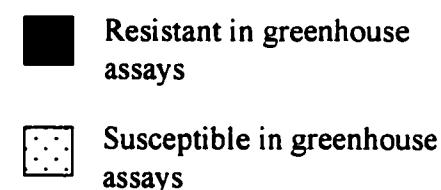
B) BARC-Satt309 predicted resistance in populations 1, 2, 3



C) Greenhouse resistance in lines predicted by BARC-Satt309 to be susceptible



D) Greenhouse resistance in lines predicted by BARC-Satt309 to be resistant



Predicted to be resistant based on BARC-Satt309



Resistant in greenhouse assays



Predicted to be susceptible based on BARC-Satt309

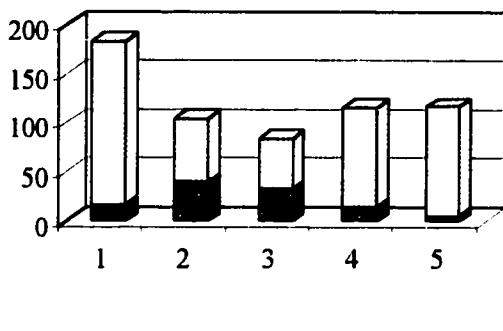


Susceptible in greenhouse assays

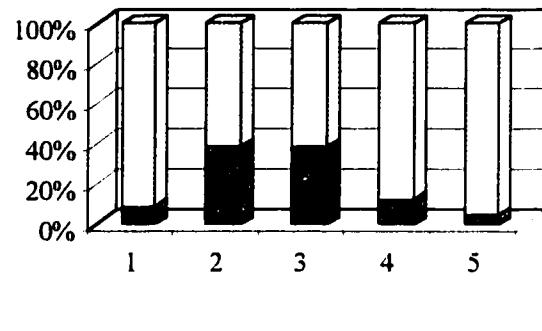
**Figure 4-6. Comparison of SCN reactions across greenhouse runs.**

A) Number of lines susceptible (dotted white) and resistant (dotted black) in greenhouse assays broken down by greenhouse run. B) Same data as A) presented as a percentage of lines. C) Number of lines predicted to be susceptible (white) and resistant (black) broken down by greenhouse run. D) Same as C) presented as a percentage of lines.

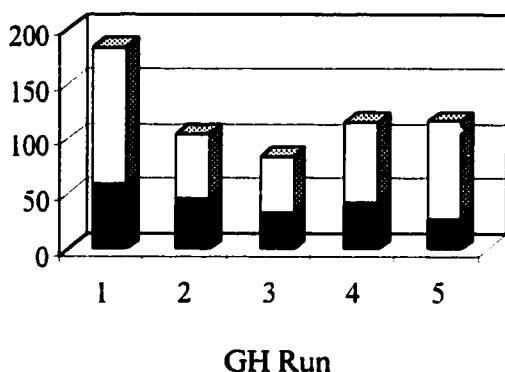
A)



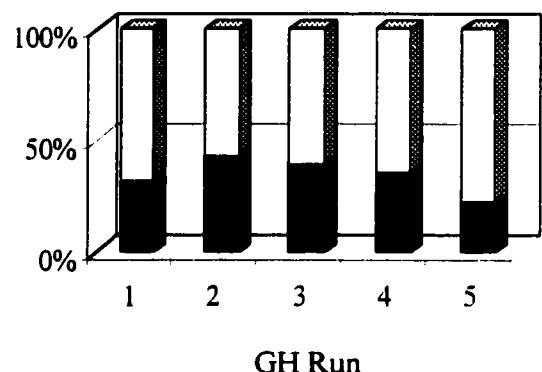
B)



C)



D)



Predicted to be  
resistant  
based on BARC-Satt309



Predicted to be  
susceptible based on  
BARC-Satt309



Resistant in  
greenhouse assays



Susceptible in  
greenhouse assays

## **Summary and perspectives**

### **Summary**

The use of resistant soybean lines is one of the best methods to control soybean cyst nematode damage. However, several challenges exist to breeding for SCN resistance, which may be addressed with molecular markers. There are two main areas where these markers will be useful. The first is to select directly for SCN resistance. Although resistance is oligogenic, the main resistance gene, *rhg1*, can account for more than 50% of the variation. Because of the large effect of this locus, a MAS program based on this locus should be valuable. Marker-assisted selection has some attractions that will be valuable in selecting for the resistance allele at *rhg1*. First, markers are not affected by the environmental effects which plague SCN resistance screening. Also, they can be assayed much more quickly than resistance assays.

MAS for the resistance allele at *rhg1* has shown some success. In comparing markers to greenhouse assays, markers were found to be effective identifying lines that would be discarded based on traditional greenhouse screening. Unfortunately, MAS did not do as well in retaining only resistant lines. Because only two replications were used in the greenhouse, one explanation for the discrepancy is error in the greenhouse replication rather than the marker. When rigorous mapping studies were done, markers and greenhouse results corresponded as much as would be expected based on the recombination distance between them. However, twelve replications in the greenhouse were used. Another explanation is that SCN resistance is controlled by several genes and this research focused only on *rhg1*. Lines having only *rhg1* may not always show resistance in the greenhouse. Nevertheless, it is encouraging that when discarding lines in early generations of SCN breeding, markers were comparable to the previously used greenhouse tests at discarding lines, with less effort.

A second area in which molecular markers can be useful in SCN resistance breeding is in dealing with the low yield potential that often accompanies resistance. Resistant soybean lines often have a lower yield potential than comparable susceptible

lines. This study has confirmed that there is a yield locus linked to the main SCN resistance allele, *rhg1*. Bng173HaeIII does meet the significance threshold in ANOVA analysis with a per locus p value of 0.0006 overall. Bng173HaeIII and nearby markers also showed significance in several locations in analyses using regression in QGENE and ANOVA. Based on my results, it is likely that this locus is on the telomeric side of *rhg1* and is separate from the resistance gene. In studies with recombinant inbred lines, the peak of the yield QTL was at Bng173HaeIII, 6 cM on the telomeric side of *rhg1*. This marker misses significance, however, with an experiment-wide p value of 0.07 for yield, while the *rhg1* locus, had a p value of 0.81. The estimated R<sup>2</sup> value for yield at Bng173HaeIII was 0.14. A locus that controls 14% of variation in yield linked to an important SCN resistance locus should be of concern to a breeder.

This yield locus was further examined in four sets of near-isogenic lines without the background caused by other loci segregating for yield. Only two sets segregated for yield in non-infested fields. In these two sets, susceptible lines yielded more than resistant lines by 4-16% in every location in every year. The two sets which did not segregate shattered extensively. This shattering may have masked differences between R and S lines. It is unlikely that they both do not segregate because they do not include the yield locus in the interval of segregation. Although one has an interval of segregation that does not include the most likely position of the yield locus, the other has an interval of segregation that does and would be expected to segregate for resistance.

Molecular markers were useful in characterizing the germplasm used to test for the yield locus linked to *rhg1*. They should also be useful in routinely separating the yield locus from *rhg1*. Two markers, such as Satt309 and Sat\_168 between the resistance and yield loci can be used. One should be used to select for SCN resistance and the other to select for a crossover to high yield. Linkage drag on the other side of *rhg1* could also be eliminated in the same way with markers such as Sat\_163 and Sat\_141.

Before a MAS program can be implemented, quality markers are required for MAS to be effective. Several SSR markers were placed near *rhg1* in this study. SSR markers in general work well in marker-assisted selection programs because of their high rate of polymorphism, potential for assay on agarose gels, codominance, and relative

ease. By mapping markers found through random searches of genomic DNA and also by targeting searches to the region of interest using bacterial artificial chromosomes, ten SSRs were added to the map. Several of these may be useful in marker assisted selection, not only in direct selection of *rhg1*, but to reduce linkage drag or as flanking markers to ensure transmission of the resistance allele at *rhg1*. Two of these, BARC-Satt309 and BARC-Sat\_168 are within one cM of *rhg1* and have proven effective in predicting resistance. As a pair, they can distinguish most commonly used susceptible parents from resistant sources. With the addition of these markers, marker-assisted selection for soybean cyst nematode resistance has gone from promise to reality.

## Perspectives

There are several weaknesses in this study that could be overcome if the experiments were performed again, a few of which I will mention here. I would begin development of near-isogenic lines sooner. I would look for more differences in interval length in NIL sets closely related to each other. NIL sets that do not contain Bng173Hae-III and do not segregate for yield, likely do not have the yield depression locus. However, stronger evidence needs to be obtained by showing that a closely related NIL set, derived from the same heterozygous parent but with a larger interval of segregation which includes the putative yield locus, does segregate for yield. This would allow the positions of the yield and *rhg1* loci to be further characterized. Earlier development would increase the amount of seed available and allow the same number of replications for each NIL set in infested and non-infested sites in each location in each year. This would make statistical analysis more powerful and straightforward.

Another area that could be improved is the comparison of greenhouse assays with DNA markers for predicting SCN resistance. Because only one or two replications of the greenhouse test are used in early generation screening for SCN resistance in the breeding program, it is valuable to compare marker screening with two greenhouse replications. This showed how MAS compares to currently implemented procedures. However, we know that the greenhouse assay is highly influenced by the environment. To ensure

assays for resistance actually reflected the presence or absence of the resistance allele at *rhg1*, previous mapping studies on different germplasm used twelve replications. Plants were grouped into threes for knocking the cysts off the roots and counting the cysts, so it is difficult to determine how often two replications give the same results as twelve. Although much work would be involved, it would be valuable to quantify, for at least a few breeding populations, how markers compare to more rigorous greenhouse studies.

Also, the maturity of PI 209332 is too late for growth in Minnesota (group 4). The progeny were, in general, earlier due to the cross with Evans, a group 0 variety. However, there was a wide range in maturity in the progeny, which affects yield. The later maturing lines were, the more they tended to yield. However, this relation changed in the latest maturing lines. These lines often did not mature by the end of the growing season and therefore gave little or no yield. Because the growing season fluctuated from year to year, some of these lines would yield well in some years and produced little or nothing in others. This complicated the relationship between yield and maturity, reducing the power of QTL detection. Undoubtedly, selection took place based on maturity against those lines that could not produce enough or any seed for the next generation during increase. In retrospect, crossing resistant sources with parents of similar maturity and growing the lines in the appropriate maturity zones would be better than maturity blocking of the germplasm. Segregation for maturity would, of course, still occur, but it would likely be less extreme.

More work needs to be done in several areas addressed by these studies. The exact placement of the yield gene with respect to the *rhg1* locus is still undetermined. Substitution mapping or further studies with other sets of NILs would give a better estimate of its position.

Although MAS for SCN resistance has been shown to be effective in these studies, the use of a second marker to eliminate the yield depression allele at the yield locus still has not been attempted. A marker needs to be used to select for *rhg1* and then a second to select for a crossover between *rhg1* and the putative position of the yield depression locus so that the resistance and, theoretically, high yield allele are retained. This line(s) must then be tested against comparable sister lines without the crossover to

determine if there is a difference in yield that would make selection with the second molecular marker worthwhile.

Most of the work done in this study utilized the resistance source PI 209332. Crosses involving other sources of resistance should be examined to determine if resistant lines show lower yield and if this yield depression is due to a locus near *rhg1*. Also, it is not known whether all resistance alleles at *rhg1* are the same. Repeating field resistance studies using NILs from other sources of resistance, especially those in which *Rhg4* has a large effect on SCN resistance, would be valuable. Testing NILs segregating both in the region around *rhg1* and in the region around *Rhg4* would allow the comparison of different combinations of resistant and susceptible alleles at each locus. In addition, other types of measurements related to SCN resistance, beyond SCN reproduction, would be valuable. Some of these traits are nematode penetration, root mass, total plant mass, and so on.

## **Appendix**

**Table A-1. Climatic and environmental data**

Climatic environmental data for Lamberton and Waseca, MN for the years, 1996, 1997, and 1998.

Location	Year	Frost Date <sup>†</sup>	Soil Type	P	K	pH	Herbicides
Lamberton, MN	1996	September 14	Normania Ves Webster	39 ppm	192 ppm	6.1	Treflan-Broadstrike, preplant
	1997	October 14	Normania Ves Webster	32 ppm	149 ppm	6.3	Treflan-Broadstrike, preplant incorporated
Waseca, MN	1998	October 1	Okoboji Canisteo Seaforth	†	†	†	Dual II, preplant incorporated; Pursuit, post-emergence
	1996	September 14	Webster Clay Loam	15 ppm	132 ppm	6.7	Treflan plus Pursuit, preplant incorporated
Waseca, MN SCN-infested	1997	October 14	Webster Clay Loam	20 ppm	129 ppm	6.5	Treflan plus Pursuit, preplant incorporated
	1998	October 13	Webster Clay Loam	20 ppm	192 ppm	6.9	Treflan plus Pursuit, preplant incorporated
Lamberton, MN SCN-infested	1997	October 14					
	1998	October 1					
Waseca, MN SCN-infested	1997	October 14	Webster Clay Loam				Lasso and Lorox, preemergence
	1998	October 13	Webster Clay Loam	11 ppm	177 ppm	7.8	Treflan, preplant incorporated; Poast, Basagran, crop oil

<sup>†</sup>The first occurrence of a 0° C temperature at the experiment station weather station.

<sup>‡</sup>Not available.

**Table A-2. Precipitation data for 1996, 1997, 1998, and averages for Waseca and Lamberton, MN**

Monthly precipitation data for Waseca and Lamberton, MN are shown for the years, 1996, 1997, 1998, and the average for 1961-1990.

Month	Year	Precipitation <sup>†</sup> (cm)			
		Lamberton	Lamberton 30 year average	Waseca	Waseca 30 year average
January	1998	1.52	1.57	5.77	2.49
February	1998	1.55	1.45	3.40	2.46
March	1998	8.46	3.86	10.03	5.79
April	1998	4.45	6.88	8.31	7.54
May	1998	3.56	7.92	10.69	9.27
June	1998	7.49	8.84	11.13	10.44
July	1998	13.72	9.42	9.63	10.69
August	1998	7.47	7.09	7.44	10.67
September	1998	3.00	7.65	4.75	9.04
October	1998	8.94	5.31	13.0	6.22
November	1998		2.92	3.23	4.37
December	1998	0.89	1.73	2.13	3.35
January	1997	2.57	1.57	6.10	2.49
February	1997	0.41	1.45	1.35	2.46
March	1997	3.40	3.86	4.98	5.79
April	1997	3.84	6.88	3.73	7.54
May	1997	3.76	7.92	9.98	9.27
June	1997	11.79	8.84	7.85	10.44
July	1997	12.45	9.42	18.08	10.69
August	1997	12.83	7.09	16.48	10.67
September	1997	4.32	7.65	5.54	9.04
October	1997	3.20	5.31	4.95	6.22
November	1997	1.65	2.92	3.38	4.37
December	1997	0.61	1.73	1.47	3.35
January	1996	4.83	1.57	8.28	2.49
February	1996	0.30	1.45	0.36	2.46
March	1996	3.02	3.86	8.36	5.79
April	1996	0.89	6.88	2.82	7.54
May	1996	11.25	7.92	8.69	9.27
June	1996	14.63	8.84	13.84	10.44
July	1996	14.12	9.42	4.57	10.69
August	1996	11.84	7.09	18.01	10.67
September	1996	5.66	7.65	4.60	9.04
October	1996	6.76	5.31	7.37	6.22
November	1996	12.09	2.92	10.57	4.37
December	1996	3.71	1.73	4.90	3.35

<sup>†</sup> 1996, 1997, and 1998 data obtained from <http://climate.umn.edu>, 30 year averages obtained from <http://mcc.sws.uiuc.edu/summary/data/>

**Table A-3. Planting and sampling dates for the RIL populations and the NIL sets**

	Location	Year	Date
Planting	Lamberton	1998	May 12
Planting	Waseca	1998	May 5
Planting	Lamberton SCN	1998	May 12
Planting	Waseca SCN	1998	May 11
Planting	Lamberton	1997	May 8
Planting	Waseca	1997	May 6
Planting	Lamberton SCN	1997	May 8
Planting	Waseca SCN	1997	May 6
Planting	Rosemount	1997	June 13
Planting	Lamberton	1996	May 21
Planting	Waseca	1996	May 30
Cysts per plant	Lamberton SCN	1998	June 30
Cysts per plant	Waseca SCN	1998	June 30
Cysts per plant	Lamberton SCN	1997	July 16
Cysts per plant	Waseca SCN	1997	July 11
Egg Counts	Lamberton SCN	1998 Spring	
Egg Counts	Waseca SCN	1998 Spring	May 11
Egg Counts	Lamberton SCN	1998 Fall	September 16
Egg Counts	Waseca SCN	1998 Fall	September 18
Egg Counts	Lamberton SCN	1997 Spring	
Egg Counts	Waseca SCN	1997 Spring	June 8
Egg Counts	Lamberton SCN	1997 Fall	June 10
Egg Counts	Waseca SCN	1997 Fall	
Stand Counts	Lamberton SCN	1998	June 30
Stand Counts	Waseca SCN	1998	June 29
Stand Counts	Lamberton SCN	1997	
Stand Counts	Waseca SCN	1997	Week of July 7

**Table A-4. Summary of phenotypic data available for the RIL population and NIL sets**

Phenotypic data, including fall egg count (eggs per 100 cc of soil), SCN cysts per plant, height (cm), lodging (scale of 1 to 5; 1 = erect, 5 = prone), maturity (days after August 31 when 95% of the plot has reached mature color), oil (total oil as a percentage of seed weight at 13% moisture), protein (total protein as a percentage of seed weight at 13% moisture), reproduction factor (fall egg count / spring egg count), spring egg count (eggs per 100 cc soil), stand count (plants per plot), and yield (kg/hectare) were gathered. These data were obtained on five different populations as noted in the table by check marks. These populations are RIL (recombinant inbred population; Evans x PI 209332) and four near-isogenic line populations (NIL) from the same two parents (see body of thesis for details). Because approximately 20,000 phenotypic datapoints were gathered in the studies reported in this thesis, it is impossible to include all of them here. However, summaries of the data are included in the appendix and the data are available in electronic tab-delimited form by contacting Nevin Young, 411 Borlaug Hall, 1991 Upper Buford Circle, University of Minnesota, St. Paul, MN 55108 ([neviny@tc.umn.edu](mailto:neviny@tc.umn.edu)).

	Fall Egg Count	SCN Cysts per plant	Height	Lodging	Maturity	Oil	Protein	Reproduction Factor	Spring Egg Count	Stand Count	Yield
RIL <sup>†</sup>			✓ <sup>#</sup>	✓	✓	✓ <sup>††</sup>	✓ <sup>††</sup>				✓
NIL-49 <sup>‡, §</sup>	✓ <sup>††</sup>	✓ <sup>††</sup>	✓ <sup>#</sup>	✓ <sup>§§</sup>	✓			✓ <sup>††</sup>	✓ <sup>††</sup>	✓ <sup>††</sup>	✓
NIL-79 <sup>‡</sup>			✓ <sup>#</sup>	✓	✓						✓
NIL-49-29 <sup>¶</sup>			✓ <sup>#</sup>	✓	✓						✓
NIL-79-23 <sup>¶</sup>	✓	✓	✓ <sup>#</sup>	✓	✓			✓	✓	✓	✓

<sup>†</sup> Data were obtained from three replications in Waseca and Lamberton, MN (non-SCN-infested sites), in 1996, 1997, and 1998.

<sup>‡</sup> Data were obtained from three replications in Waseca and Lamberton, MN (non-SCN-infested sites) in 1997 and 1998 and from four replications in Waseca and Lamberton, MN (SCN-infested sites) in 1998.

<sup>§</sup> Data were also obtained from three replications in Waseca and Lamberton, MN (SCN-infested sites) in 1997. Only a subset of NIL-49 was planted, including two NIL-49-R lines, two NIL-49-S lines, and one line with a crossover in the interval of segregation (NIL-49-39) showing the susceptible parent's alleles at markers surrounding *rhg1*, the main locus for SCN resistance.

<sup>¶</sup> Data are from two replications in Rosemount, MN in 1997, from two replications in Waseca and Lamberton, MN (non-SCN-infested sites) in 1998, and from three replications in Waseca and Lamberton, MN (SCN-infested sites) in 1998.

<sup>#</sup> Height was not taken for all replications. Specifically, it is missing in Lamberton in 1997 for the RIL population; Waseca and Lamberton, MN (both non-infested and infested sites) in 1997 for NIL-49; Waseca (non-infested) and both locations (infested) in 1997 for NIL-79; and Waseca (infested) in 1998 for NIL-49-29.

<sup>††</sup> Only obtained on the first replication in each location in each year.

<sup>‡‡</sup> Only obtained on the subset of NIL-49 described above and in the body of the thesis.

<sup>§§</sup> Lodging was not obtained in Waseca (infested site) in 1997 for NIL-49.

**Table A-5.** Genetic map used in the analysis of data in the RIL population.  
Genetic map used in the MQTL and QGENE analysis of data in the RIL population. The cM distance to the next marker is given after each marker. The end of a linkage group is denoted by 'end'.

Marker	cM	Marker	cM	Marker	cM
A085I	15.7	Sat_141	0	Satt006	11.2
K400V	17.4	D0140D	0.9	Bng095I-2	6.4
A486I	end	Satt610	0.5	A802T	end
A122V-3	0	Bng122D	0	A071I-1	8.3
A885H	14.6	Satt356	10	D1327T-1	4.1
Bng121H	9.3	K069X-1	0.9	A077V-2	1.6
A095I-2	0	Satt130	0	Satt009	end
A117H	0	MngM247I	7.3	A043T	1.6
A118Hae-2	6.9	Bng225D-1	3.1	A135V-1	19.8
ACCCAT-1	end	A112H	end	A352H	end
Bng205X	5.2	K286T	4.8	MngM287T	5.9
K401I	end	B151T-1	2.8	A955X	6.8
B219V-1	2.7	MngQ043Rsa	end	A947X	end
A333I	10.4	A378H	2.4	Satt082	1.1
A588I	10.8	A632V-2	2.2	Satt001	end
Bng119D-2	15.3	A586X	end	A806T	1.9
A398V-2	end	ACCCTT-1	4.8	Bng161T-2	end
Bng182T	16.8	MngM374H	4.4	Bng037I	end
A262H	6	A131I	end	103.15B	end
A089V-2	3.4	A102I-1	12.1	103.15e	end
A520T	2.6	Satt049	end	Bng070I	end
A036I-1	8.6	A584H-2	0.5	A810X	end
Bng145X-2	end	A644V	end	ACCCAG-1	end
A401T-2	4.4	B032V-1	6.9	ACCCAG-2	end
A257V	0.6	A724T	4	ACCCTT-2	end
A064I-2	end	A132T-2	0.8	ACCCTT-3	end
A069H-2	8.3	A199X-2	end	ACCCTT-4	end
A963I	16.8	Bng027Hae-2	8.9	ACCCTT-5	end
A086H	5.6	A315I	2	ACCCAT-2	end
A386Hae	14.9	MngM151V	23.3	ACCCAT-4	end
A427H-3	11.8	G15R	end	ACCCAT-5	end
ACCCAT-3	end	Bng224T	4.3	A450D	end
A111X-2	12	CwP238D-2	12.7	BLT024I	end
A711V	end	A668H	4.8	B151T-2	end
A708D	5.2	A235H-3	9.5	A060T	end
K644V-1	3.2	K387H	7.8	Bng063T	end
A186T	6.4	Bng062V-2	6.3	A136X-2	end
A517X-2	14.4	Bng007H	21.3	A162X	end
Bng118D	end	A489I-2	7.5	A338V	end
OAM-10	0	D1452Nsi-2	14.2	A463D	end
D1051D-1	0	D1149X-2	end	Bng035V	end
D1149D-1	6	A264X	3.3	A135V-2	end
Bng173Hae-3	2.1	A106V	0.8	Bng161T-3	end
C006V	0	D1862Rsa	0.7	A329H-2	end
Satt163	1.2	A023T	18.5	A083V	end
Satt038	1.8	D1327V-3	27.6	A141T	end
Satt309	0.8	B157T-2	end		
<i>rhg1</i>	6.5	A130Hae-3	3.8		

Table A-6. ANOVA table comparing Bng173Hae-III to yield in the RIL population

	Degrees of freedom	Sum of squares	Mean square	F-value	p-value
Bng173Hae-III	2	7194	3597	7.12	0.0014
Subject(Group) <sup>†</sup>	89	44962	505		
Year	2	27795	13897	87.97	<0.0001
Year * Bng173Hae-III	4	574	143	0.91	0.4605
Year * Subject(Group) <sup>†</sup>	178	28119	158		
Location	1	5081	5081	71.17	<0.0001
Location * Bng173Hae-III	2	152	79	1.07	0.3492
Location * Subject(Group) <sup>†</sup>	89	6355	71		
Replication	2	354	177	5.53	0.0047
Replication * Bng173Hae-III	4	31	8	0.24	0.9156
Replication * Subject (Group) <sup>†</sup>	178	5692	32		
Year * Location	2	3594	1797	34.03	<0.0001
Year * Location * Bng173Hae-III	4	365	91	1.73	0.1460
Year * Location * Subject(Group) <sup>†</sup>	178	9402	53		
Year * Replication	4	344	86	2.69	0.0310
Year * Replication * Bng173Hae-III	8	136	17	0.53	0.8317
Year * Replication * Subject(Group) <sup>†</sup>	356	11392	32		
Location * Replication	2	316	158	6.11	0.0027
Location * Replication * Bng173Hae-III	4	40	10	0.39	0.8173
Location * Replication * Subject(Group) <sup>†</sup>	178	4601	26		
Year * Location * Replication	4	1625	406	15.53	<0.0001
Year * Location * Replication * Bng173HaeIII	8	184	23	0.88	0.5344
Year * Location * Replication * Subject (Group) <sup>†</sup>	356	9315	26		
Within subject error					

<sup>†</sup> Within subject error

**Table A-7. Planting information for NIL sets**

Planting information for the NIL sets is shown below. The number of replications (reps) at each site are listed. Empty cells indicate that the NIL set was not planted at the corresponding location in the corresponding year. Plots at Rosemount were two 0.9 m long plots with 75 cm spacing, planted with 60 seed. These plots were harvested by hand and threshed with a stationary thresher. All others were four 3.7 m long plots with 25 cm spacing that were trimmed to 2.5 m. These plots were planted with 240 seed and all four rows harvested with a combine.

Year	Rosemount	Lamberton	Lamberton	Waseca	Waseca
	Non-SCN	Non-SCN	SCN	Non-SCN	SCN
NIL-49	1997		3 reps		3 reps
NIL-49	1998		3 reps	4 reps	3 reps
NIL-49 subset <sup>†</sup>	1997			3 reps	3 reps
NIL-79	1997		3 reps		3 reps
NIL-79	1998		3 reps	4 reps	3 reps
NIL-49-29	1997	2 reps			
NIL-49-29	1998		2 reps	3 reps	2 reps
NIL-79-23	1997	2 reps			
NIL-79-23	1998		2 reps	3 reps	2 reps
					3 reps

<sup>†</sup> The NIL-49 subset, consisting of 2 resistant, 2 susceptible, and one crossover line, was only planted separately from the rest of the NIL-49 set in 1997 in SCN-infested sites. These lines were also included with the NIL-49 set whenever it was planted.

**Table A-8. Phenotypic data taken on the NIL sets**  
The mean, standard deviation, standard error, count, minimum, and maximum for each phenotypic trait measurement taken on the NIL sets are shown in the table below. Data are given for each year, location, and site. In addition, data are split by group. R lines are lines homozygous for the resistant parent's (PI 209332) alleles in the interval of segregation. S lines are homozygous for the susceptible parent's (Evans) alleles in the interval of segregation. R cultivars are resistant cultivars included in the test as controls.

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49	Cysts per plant	1998	Greenhouse	SCN	R	153.88	56.24	19.89	8	79	234
NIL-49	Cysts per plant	1998	Greenhouse	SCN	S	258.13	78.42	27.73	8	99	362
NIL-49	Cysts per plant	1998	Greenhouse	SCN	x2	223	64.28	32.14	4	146	303
NIL-49	Cysts per plant	1998	Greenhouse	SCN	x4	248.33	77.78	44.91	3	165	319
NIL-49	Cysts per plant	1998	Greenhouse	SCN	R cultivars	36.88	25.85	9.14	8	7	71
NIL-49	Cysts per plant	1998	Greenhouse	SCN	S cultivars	339.75	59	29.5	4	276	413
NIL-49	Cysts per plant	1998	Lamberton	SCN	R	10.863	5.502	1.945	8	4.4	17.6
NIL-49	Cysts per plant	1998	Lamberton	SCN	R cultivars	12.025	8.796	4.398	4	4	21.5
NIL-49	Cysts per plant	1998	Lamberton	SCN	S	34.975	26.802	9.476	8	8.8	88.3
NIL-49	Cysts per plant	1998	Lamberton	SCN	S cultivars	28.2	21.763	10.881	4	12.2	60.3
NIL-49	Cysts per plant	1998	Lamberton	SCN	x2	26.85	14.271	7.136	4	7.4	40.7
NIL-49	Cysts per plant	1998	Waseca	SCN	R	6.412	3.434	1.214	8	2.1	11.5
NIL-49	Cysts per plant	1998	Waseca	SCN	R cultivars	2.075	1.323	0.661	4	0.7	3.8
NIL-49	Cysts per plant	1998	Waseca	SCN	S	21.613	18.5	6.541	8	2.2	56.4
NIL-49	Cysts per plant	1998	Waseca	SCN	S cultivars	31.15	2.073	1.036	4	28.9	33.7
NIL-49	Cysts per plant	1998	Waseca	SCN	x2	35.15	33.991	16.996	4	5.4	71.6
NIL-49	Cysts per plant	1997	Lamberton	SCN	NIL parent	22.307	19.399	11.2	3	3.92	42.58
NIL-49	Cysts per plant	1997	Lamberton	SCN	R	3.002	3.673	1.5	6	0.42	9.92
NIL-49	Cysts per plant	1997	Lamberton	SCN	R cultivars	3.167	2.466	1.424	3	1.5	6
NIL-49	Cysts per plant	1997	Lamberton	SCN	S	17.068	17.706	7.229	6	1.67	47.08
NIL-49	Cysts per plant	1997	Lamberton	SCN	S cultivars	32.947	47.268	19.297	6	2.92	126.67
NIL-49	Cysts per plant	1997	Lamberton	SCN	x2	12.693	6.236	3.601	3	5.5	16.58
NIL-49	Cysts per plant	1997	Waseca	SCN	NIL parent	47.777	13.325	7.693	3	32.5	57
NIL-49	Cysts per plant	1997	Waseca	SCN	R	15.43	4.554	1.859	6	10.42	21.92
NIL-49	Cysts per plant	1997	Waseca	SCN	R cultivars	9.777	4.749	2.742	3	6.75	15.25
NIL-49	Cysts per plant	1997	Waseca	SCN	S	58.333	47.636	19.447	6	18.67	124.33

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49	Cysts per plant	1997	Waseca	SCN	S cultivars	133.887	88.251	36.028	6	42.75	247.33
NIL-49	Cysts per plant	1997	Waseca	SCN	x2	62.527	12.945	7.474	3	53.33	77.33
NIL-49	Fall Egg Count	1998	Lamberton	SCN	R	6174.571	5370.48	2029.851	7	0	14667
NIL-49	Fall Egg Count	1998	Lamberton	SCN	R cultivars	5034.5	2357.967	1178.983	4	2933	8322
NIL-49	Fall Egg Count	1998	Lamberton	SCN	S	27981.429	17801.306	6728.261	7	8246	60000
NIL-49	Fall Egg Count	1998	Lamberton	SCN	S cultivars	17249.5	7254.977	3627.489	4	6772	22282
NIL-49	Fall Egg Count	1998	Lamberton	SCN	x2	31102.5	30815.785	15407.893	4	11442	76893
NIL-49	Fall Egg Count	1998	Waseca	SCN	R	8594.875	14282.509	5049.63	8	0	42229
NIL-49	Fall Egg Count	1998	Waseca	SCN	R cultivars	2206	1390.507	802.809	3	969	3711
NIL-49	Fall Egg Count	1998	Waseca	SCN	S	25699.625	13733.896	4855.666	8	4484	40404
NIL-49	Fall Egg Count	1998	Waseca	SCN	S cultivars	13335.5	10595.369	5297.684	4	4999	28788
NIL-49	Fall Egg Count	1998	Waseca	SCN	x2	14437	6154.165	3553.109	3	7669	19697
NIL-49	Fall Egg Counts	1997	Lamberton	SCN	NIL parent	50666.667	3780.653	2182.761	3	800	8000
NIL-49	Fall Egg Counts	1997	Lamberton	SCN	R	46666.667	3556.778	1452.048	6	1600	9600
NIL-49	Fall Egg Counts	1997	Lamberton	SCN	R cultivars	2346.667	1602.664	925.299	3	800	4000
NIL-49	Fall Egg Counts	1997	Lamberton	SCN	S	5280	5046.979	2257.078	5	0	11200
NIL-49	Fall Egg Counts	1997	Lamberton	SCN	S cultivars	4341.333	1318.876	538.429	6	3168	6720
NIL-49	Fall Egg Counts	1997	Lamberton	SCN	x2	26666.667	2571.64	1484.737	3	800	5600
NIL-49	Fall Egg Counts	1997	Waseca	SCN	NIL parent	15216.667	8259.893	4768.851	3	10200	24750
NIL-49	Fall Egg Counts	1997	Waseca	SCN	R	6000	2822.942	1152.461	6	3300	11050
NIL-49	Fall Egg Counts	1997	Waseca	SCN	R cultivars	3466.667	2180.787	1259.078	3	1200	5550
NIL-49	Fall Egg Counts	1997	Waseca	SCN	S	15341.667	5515.108	2251.533	6	9850	25400
NIL-49	Fall Egg Counts	1997	Waseca	SCN	S cultivars	19075	8543.053	3487.687	6	7500	28900
NIL-49	Fall Egg Counts	1997	Waseca	SCN	x2	18300	6882.768	3973.768	3	12900	26050
NIL-49	Height	1998	Lamberton	non-SCN	NIL parent	37.667	1.528	0.882	3	36	39
NIL-49	Height	1998	Lamberton	non-SCN	R	38.444	5.731	1.351	18	31	52
NIL-49	Height	1998	Lamberton	non-SCN	R cultivars	42.333	0.577	0.333	3	42	43
NIL-49	Height	1998	Lamberton	non-SCN	S	39.323	4.743	0.852	31	30	47
NIL-49	Height	1998	Lamberton	non-SCN	S cultivars	42.667	2.944	1.202	6	39	46
NIL-49	Height	1998	Lamberton	non-SCN	x1	36.333	0.577	0.333	3	36	37
NIL-49	Height	1998	Lamberton	non-SCN	x2	37.667	1.155	0.667	3	37	39

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49	Height	1998	Lamberton	non-SCN	x3	38	1.732	1	3	36	39
NIL-49	Height	1998	Lamberton	non-SCN	x4	41.333	4.933	2.848	3	38	47
NIL-49	Height	1998	Waseca	non-SCN	NIL parent	39	5.196	3	3	33	42
NIL-49	Height	1998	Waseca	non-SCN	R	38.111	7.661	1.806	18	28	58
NIL-49	Height	1998	Waseca	non-SCN	R cultivars	35.333	1.528	0.882	3	34	37
NIL-49	Height	1998	Waseca	non-SCN	S	38.129	7.464	1.341	31	28	56
NIL-49	Height	1998	Waseca	non-SCN	S cultivars	35.333	3.933	1.606	6	32	42
NIL-49	Height	1998	Waseca	non-SCN	x1	50	8.718	5.033	3	44	60
NIL-49	Height	1998	Waseca	non-SCN	x2	41.333	1.155	0.667	3	40	42
NIL-49	Height	1998	Waseca	non-SCN	x3	46	11.136	6.429	3	34	56
NIL-49	Height	1998	Waseca	non-SCN	x4	38	3.464	2	3	36	42
NIL-49	Height	1998	Lamberton	SCN	NIL parent	37.25	6.238	3.119	4	28	41
NIL-49	Height	1998	Lamberton	SCN	R	32.792	5.15	1.051	24	22	40
NIL-49	Height	1998	Lamberton	SCN	R cultivars	34	2.16	1.08	4	32	37
NIL-49	Height	1998	Lamberton	SCN	S	32.511	4.708	0.702	45	19	45
NIL-49	Height	1998	Lamberton	SCN	S cultivars	30.75	2.866	1.013	8	27	36
NIL-49	Height	1998	Lamberton	SCN	x1	37.25	3.096	1.548	4	33	40
NIL-49	Height	1998	Lamberton	SCN	x2	36.75	4.031	2.016	4	31	40
NIL-49	Height	1998	Lamberton	SCN	x3	33.25	3.304	1.652	4	30	37
NIL-49	Height	1998	Lamberton	SCN	x4	35.25	2.363	1.181	4	32	37
NIL-49	Height	1998	Lamberton	SCN	x5	37.25	1.708	0.854	4	35	39
NIL-49	Height	1998	Waseca	SCN	NIL parent	31			1	31	31
NIL-49	Height	1998	Waseca	SCN	R	36.7	5.253	1.175	20	25	45
NIL-49	Height	1998	Waseca	SCN	R cultivars	35.333	5.033	2.906	3	30	40
NIL-49	Height	1998	Waseca	SCN	S	33.968	6.264	1.125	31	24	48
NIL-49	Height	1998	Waseca	SCN	S cultivars	37.25	5.795	2.898	4	31	45
NIL-49	Height	1998	Waseca	SCN	x1	38			1	38	38
NIL-49	Height	1998	Waseca	SCN	x2	40			1	40	40
NIL-49	Height	1998	Waseca	SCN	x3				0		
NIL-49	Height	1998	Waseca	SCN	x4	32	11.314	8	2	24	40
NIL-49	Height	1998	Waseca	SCN	x5	40.5	6.364	4.5	2	36	45

NIL set	Trait		Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49	Lodging		1998	Lamberton	non-SCN	NIL parent	5	0	0	3	3	5
NIL-49	Lodging		1998	Lamberton	non-SCN	R	5	0	0	0	0	5
NIL-49	Lodging		1998	Lamberton	non-SCN	R cultivars	3.3333	0.577	0.3333	3	3	4
NIL-49	Lodging		1998	Lamberton	non-SCN	S	4.29	0.693	0.124	31	3	5
NIL-49	Lodging		1998	Lamberton	non-SCN	S cultivars	3.3333	0.816	0.3333	6	2	4
NIL-49	Lodging		1998	Lamberton	non-SCN	x1	5	0	0	0	0	5
NIL-49	Lodging		1998	Lamberton	non-SCN	x2	5	0	0	0	0	5
NIL-49	Lodging		1998	Lamberton	non-SCN	x3	5	0	0	0	0	5
NIL-49	Lodging		1998	Lamberton	non-SCN	x4	5	0	0	0	0	5
NIL-49	Lodging		1998	Lamberton	non-SCN	x5	4	0	0	0	0	5
NIL-49	Lodging		1998	Waseca	non-SCN	NIL parent	4	1	0.577	3	3	5
NIL-49	Lodging		1998	Waseca	non-SCN	R	3.889	0.963	0.227	18	1	5
NIL-49	Lodging		1998	Waseca	non-SCN	R cultivars	1.3333	0.577	0.3333	3	1	2
NIL-49	Lodging		1998	Waseca	non-SCN	S	3.548	0.81	0.145	31	1	5
NIL-49	Lodging		1998	Waseca	non-SCN	S cultivars	1.5	0.837	0.342	6	1	5
NIL-49	Lodging		1998	Waseca	non-SCN	x1	3.667	0.577	0.3333	3	3	4
NIL-49	Lodging		1998	Waseca	non-SCN	x2	3.667	0.577	0.3333	3	3	4
NIL-49	Lodging		1998	Waseca	non-SCN	x3	3.667	0.577	0.3333	3	3	4
NIL-49	Lodging		1998	Waseca	non-SCN	x4	4	0	0	0	0	4
NIL-49	Lodging		1998	Waseca	non-SCN	x5	2	1	0.577	3	1	3
NIL-49	Lodging		1998	Lamberton	SCN	NIL parent	3	0	0	4	3	3
NIL-49	Lodging		1998	Lamberton	SCN	R	3.292	0.55	0.112	24	2	4
NIL-49	Lodging		1998	Lamberton	SCN	R cultivars	2.25	0.5	0.25	4	2	3
NIL-49	Lodging		1998	Lamberton	SCN	S	2.533	0.661	0.098	45	2	4
NIL-49	Lodging		1998	Lamberton	SCN	S cultivars	2	0	0	8	2	2
NIL-49	Lodging		1998	Lamberton	SCN	x1	2.75	0.5	0.25	4	2	3
NIL-49	Lodging		1998	Lamberton	SCN	x2	3.5	0.577	0.289	4	3	4
NIL-49	Lodging		1998	Lamberton	SCN	x3	2.75	0.5	0.25	4	2	3
NIL-49	Lodging		1998	Lamberton	SCN	x4	3	0	0	4	3	3
NIL-49	Lodging		1998	Lamberton	SCN	x5	2.25	0.5	0.25	4	2	3
NIL-49	Lodging		1998	Waseca	SCN	NIL parent	3.25	1.5	0.75	4	1	4

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49	Lodging	1998	Waseca	SCN	R	3.958	0.606	0.124	24	2.5	5
NIL-49	Lodging	1998	Waseca	SCN	R cultivars	3.125	1.25	0.625	4	2.5	5
NIL-49	Lodging	1998	Waseca	SCN	S	3.633	0.643	0.096	45	2	5
NIL-49	Lodging	1998	Waseca	SCN	S cultivars	3.188	0.884	0.312	8	2	4
NIL-49	Lodging	1998	Waseca	SCN	x1	4.25	0.5	0.25	4	4	5
NIL-49	Lodging	1998	Waseca	SCN	x2	4.25	0.5	0.25	4	4	5
NIL-49	Lodging	1998	Waseca	SCN	x3	4.125	0.25	0.125	4	4	4.5
NIL-49	Lodging	1998	Waseca	SCN	x4	3.625	0.479	0.239	4	3	4
NIL-49	Lodging	1998	Waseca	SCN	x5	3.875	0.629	0.315	4	3	4.5
NIL-49	Lodging	1997	Lamberton	non-SCN	NIL parent	4	0	0	3	4	4
NIL-49	Lodging	1997	Lamberton	non-SCN	R	4.583	0.669	0.193	12	3	5
NIL-49	Lodging	1997	Lamberton	non-SCN	R cultivars	2.833	0.753	0.307	6	2	4
NIL-49	Lodging	1997	Lamberton	non-SCN	S	3.963	0.587	0.113	27	3	5
NIL-49	Lodging	1997	Lamberton	non-SCN	S cultivars	2.444	0.527	0.176	9	2	3
NIL-49	Lodging	1997	Lamberton	non-SCN	x1	4	1	1	4	4	4
NIL-49	Lodging	1997	Lamberton	non-SCN	x2	4.333	0.577	0.333	3	4	5
NIL-49	Lodging	1997	Lamberton	non-SCN	x3	4	1	0.577	3	3	5
NIL-49	Lodging	1997	Lamberton	non-SCN	x4	5	1	1	5	5	5
NIL-49	Lodging	1997	Waseca	non-SCN	NIL parent	4.667	0.577	0.333	3	4	5
NIL-49	Lodging	1997	Waseca	non-SCN	R	4.231	0.599	0.166	13	3	5
NIL-49	Lodging	1997	Waseca	non-SCN	R cultivars	3	0.632	0.258	6	2	4
NIL-49	Lodging	1997	Waseca	non-SCN	S	4	0.77	0.145	28	3	5
NIL-49	Lodging	1997	Waseca	non-SCN	S cultivars	2.778	0.411	0.147	9	2	3
NIL-49	Lodging	1997	Waseca	non-SCN	x1	4.667	0.577	0.333	3	4	5
NIL-49	Lodging	1997	Waseca	non-SCN	x2	4.667	0.577	0.333	3	4	5
NIL-49	Lodging	1997	Waseca	non-SCN	x3	4.667	0.577	0.333	3	4	5
NIL-49	Lodging	1997	Waseca	non-SCN	x4	5	1	1	5	5	5
NIL-49	Lodging	1997	Lamberton	SCN	NIL parent	3.667	0.577	0.333	3	3	4
NIL-49	Lodging	1997	Lamberton	SCN	R	4.833	0.408	0.167	6	4	5
NIL-49	Lodging	1997	Lamberton	SCN	R cultivars	2	1	0.577	3	1	3
NIL-49	Lodging	1997	Lamberton	SCN	S	3.167	0.983	0.401	6	2	5

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49	Lodging	1997	Lamberton	SCN	S cultivars	2.667	0.516	0.211	6	2	3
NIL-49	Lodging	1997	Lamberton	SCN	x2	4	1	0.577	3	3	5
NIL-49	Maturity	1998	Lamberton	non-SCN	NIL parent	27	2.646	1.528	3	24	29
NIL-49	Maturity	1998	Lamberton	non-SCN	R	21.889	2.632	0.62	18	18	28
NIL-49	Maturity	1998	Lamberton	non-SCN	R cultivars	19.667	0.577	0.333	3	19	20
NIL-49	Maturity	1998	Lamberton	non-SCN	S	21.935	3.777	0.678	31	15	29
NIL-49	Maturity	1998	Lamberton	non-SCN	S cultivars	20.667	5.645	2.305	6	15	28
NIL-49	Maturity	1998	Lamberton	non-SCN	x1	29	1	0.577	3	28	30
NIL-49	Maturity	1998	Lamberton	non-SCN	x2	28.667	1.155	0.667	3	28	30
NIL-49	Maturity	1998	Lamberton	non-SCN	x3	27.667	0.577	0.333	3	27	28
NIL-49	Maturity	1998	Lamberton	non-SCN	x4	28.333	0.577	0.333	3	28	29
NIL-49	Maturity	1998	Lamberton	non-SCN	x5	21.667	9.815	5.667	3	16	33
NIL-49	Maturity	1998	Waseca	non-SCN	NIL parent	21.333	1.155	0.667	3	20	22
NIL-49	Maturity	1998	Waseca	non-SCN	R	14.778	3.507	0.827	18	10	22
NIL-49	Maturity	1998	Waseca	non-SCN	R cultivars	14.667	0.577	0.333	3	14	15
NIL-49	Maturity	1998	Waseca	non-SCN	S	16.032	4.278	0.768	31	10	24
NIL-49	Maturity	1998	Waseca	non-SCN	S cultivars	16.833	1.329	0.543	6	15	19
NIL-49	Maturity	1998	Waseca	non-SCN	x1	22.667	1.155	0.667	3	22	24
NIL-49	Maturity	1998	Waseca	non-SCN	x2	22	0	0	3	22	22
NIL-49	Maturity	1998	Waseca	non-SCN	x3	22	2	1.155	3	20	24
NIL-49	Maturity	1998	Waseca	non-SCN	x4	21.333	1.155	0.667	3	20	22
NIL-49	Maturity	1998	Waseca	non-SCN	x5	18.667	6.429	3.712	3	14	26
NIL-49	Maturity	1998	Lamberton	SCN	NIL parent	28.75	0.5	0.25	4	28	29
NIL-49	Maturity	1998	Lamberton	SCN	R	18.875	4.59	0.937	24	14	28
NIL-49	Maturity	1998	Lamberton	SCN	R cultivars	18.75	0.5	0.25	4	18	19
NIL-49	Maturity	1998	Lamberton	SCN	S	19.244	6.467	0.964	45	6	29
NIL-49	Maturity	1998	Lamberton	SCN	S cultivars	21.25	7.086	2.505	8	12	30
NIL-49	Maturity	1998	Lamberton	SCN	x1	29	0.816	0.408	4	28	30
NIL-49	Maturity	1998	Lamberton	SCN	x2	29.5	0.577	0.289	4	29	30
NIL-49	Maturity	1998	Lamberton	SCN	x3	28.75	0.5	0.25	4	28	29
NIL-49	Maturity	1998	Lamberton	SCN	x4	28.5	1	0.5	4	27	29

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49	Maturity	1998	Lamberton	SCN	x5	24.25	10.372	5.186	4	15	39
NIL-49	Maturity	1998	Waseca	SCN	NIL parent	22.5	1.732	0.866	4	21	25
NIL-49	Maturity	1998	Waseca	SCN	R	13.708	3.593	0.733	24	9	22
NIL-49	Maturity	1998	Waseca	SCN	R cultivars	15.75	6.185	3.092	4	12	25
NIL-49	Maturity	1998	Waseca	SCN	S	15.267	5.425	0.809	45	9	25
NIL-49	Maturity	1998	Waseca	SCN	S cultivars	16.875	5.249	1.856	8	12	25
NIL-49	Maturity	1998	Waseca	SCN	x1	22.25	0.5	0.25	4	22	23
NIL-49	Maturity	1998	Waseca	SCN	x2	23	1.414	0.707	4	22	25
NIL-49	Maturity	1998	Waseca	SCN	x3	23	0.816	0.408	4	22	24
NIL-49	Maturity	1998	Waseca	SCN	x4	19.75	4.5	2.25	4	13	22
NIL-49	Maturity	1998	Waseca	SCN	x5	17.75	6.131	3.065	4	12	24
NIL-49	Maturity	1997	Lamberton	non-SCN	NIL parent	35.667	0.577	0.333	3	35	36
NIL-49	Maturity	1997	Lamberton	non-SCN	R	29.333	1.497	0.432	12	26	31
NIL-49	Maturity	1997	Lamberton	non-SCN	R cultivars	32.333	2.805	1.145	6	29	36
NIL-49	Maturity	1997	Lamberton	non-SCN	S	30.63	3.272	0.63	27	26	36
NIL-49	Maturity	1997	Lamberton	non-SCN	S cultivars	32.556	2.186	0.729	9	29	35
NIL-49	Maturity	1997	Lamberton	non-SCN	x1	37	.	.	1	37	37
NIL-49	Maturity	1997	Lamberton	non-SCN	x2	36.667	0.577	0.333	3	36	37
NIL-49	Maturity	1997	Lamberton	non-SCN	x3	35.333	0.577	0.333	3	35	36
NIL-49	Maturity	1997	Lamberton	non-SCN	x4	37	.	.	1	37	37
NIL-49	Maturity	1997	Waseca	non-SCN	NIL parent	32.333	0.577	0.333	3	32	33
NIL-49	Maturity	1997	Waseca	non-SCN	R	23.923	2.216	0.615	13	21	28
NIL-49	Maturity	1997	Waseca	non-SCN	R cultivars	26.5	6.156	2.513	6	21	34
NIL-49	Maturity	1997	Waseca	non-SCN	S	25.857	5.924	1.119	28	15	35
NIL-49	Maturity	1997	Waseca	non-SCN	S cultivars	25.556	5.615	1.872	9	19	32
NIL-49	Maturity	1997	Waseca	non-SCN	x1	30.333	5.686	3.283	3	24	35
NIL-49	Maturity	1997	Waseca	non-SCN	x2	33	1.732	1	3	32	35
NIL-49	Maturity	1997	Waseca	non-SCN	x3	32.667	0.577	0.333	3	32	33
NIL-49	Maturity	1997	Waseca	non-SCN	x4	32	.	.	1	32	32
NIL-49	Maturity	1997	Lamberton	SCN	NIL parent	34.333	0.577	0.333	3	34	35
NIL-49	Maturity	1997	Lamberton	SCN	R	30.167	1.169	0.477	6	29	32

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49	Maturity	1997	Lamberton	SCN	R cultivars	30	0	0	3	30	30
NIL-49	Maturity	1997	Lamberton	SCN	S	32.333	3.011	1.229	6	29	36
NIL-49	Maturity	1997	Lamberton	SCN	S cultivars	24.667	5.164	2.108	6	19	30
NIL-49	Maturity	1997	Lamberton	SCN	x2	33.667	0.577	0.333	3	33	34
NIL-49	Maturity	1997	Waseca	SCN	NIL parent	34	1	0.577	3	33	35
NIL-49	Maturity	1997	Waseca	SCN	R	29.667	0.516	0.211	6	29	30
NIL-49	Maturity	1997	Waseca	SCN	R cultivars	30.333	1.528	0.882	3	29	32
NIL-49	Maturity	1997	Waseca	SCN	S	31	2.966	1.211	6	28	34
NIL-49	Maturity	1997	Waseca	SCN	S cultivars	22.667	2.944	1.202	6	20	26
NIL-49	Maturity	1997	Waseca	SCN	x2	33.667	1.155	0.667	3	33	35
NIL-49	Reproduction Factor	1998	Lamberton	SCN	R	0.579	0.587	0.222	7	0	1.48
NIL-49	Reproduction Factor	1998	Lamberton	SCN	R cultivars	0.362	0.173	0.086	4	0.23	0.6
NIL-49	Reproduction Factor	1998	Lamberton	SCN	S	2.294	2.636	0.996	7	0.72	8.22
NIL-49	Reproduction Factor	1998	Lamberton	SCN	S cultivars	1.53	0.528	0.264	4	0.94	2.14
NIL-49	Reproduction Factor	1998	Lamberton	SCN	x2	2.07	1.185	0.592	4	1.11	3.58
NIL-49	Reproduction Factor	1998	Waseca	SCN	R	16.208	29.684	12.119	6	0	76.46
NIL-49	Reproduction Factor	1998	Waseca	SCN	R cultivars	2.29	1.671	0.965	3	1.01	4.18
NIL-49	Reproduction Factor	1998	Waseca	SCN	S	81.701	86.071	32.532	7	2.23	252.53
NIL-49	Reproduction Factor	1998	Waseca	SCN	S cultivars	16.602	11.756	5.878	4	3	28.11
NIL-49	Reproduction Factor	1998	Waseca	SCN	x2	88.915	50.424	35.655	2	53.26	124.57
NIL-49	Reproduction Factor	1997	Lamberton	SCN	NIL parent	3.207	1.553	0.897	3	2.29	5
NIL-49	Reproduction Factor	1997	Lamberton	SCN	R	21.152	27.029	12.088	5	1	64
NIL-49	Reproduction Factor	1997	Lamberton	SCN	R cultivars	4.28	3.824	2.208	3	0.36	8
NIL-49	Reproduction Factor	1997	Lamberton	SCN	S	7.71	8.904	3.982	5	0	18.67
NIL-49	Reproduction Factor	1997	Lamberton	SCN	S cultivars	2.494	0.868	0.388	5	1.73	3.73
NIL-49	Reproduction Factor	1997	Lamberton	SCN	x2	3.207	2.166	1.251	3	1	5.33
NIL-49	Reproduction Factor	1997	Waseca	SCN	NIL parent	6.217	4.762	2.749	3	2.28	11.51
NIL-49	Reproduction Factor	1997	Waseca	SCN	R	4.142	3.243	1.324	6	0.54	9.75
NIL-49	Reproduction Factor	1997	Waseca	SCN	R cultivars	0.707	0.489	0.282	3	0.28	1.24
NIL-49	Reproduction Factor	1997	Waseca	SCN	S	6.053	5.711	2.331	6	1.69	16.9
NIL-49	Reproduction Factor	1997	Waseca	SCN	S cultivars	9.687	11.38	4.646	6	1.11	32.11

NIL set	Trait	Reproduction Factor	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49	Spring Egg Count	1997 Waseca	SCN	x2	6.97	3.833	2.213	3	2.55	9.38	38688	
NIL-49	Spring Egg Count	1998 Lamberton	SCN	R	18027	12525.12	4428.299	8	7200	38688	38688	
NIL-49	Spring Egg Count	1998 Lamberton	SCN	R cultivars	14780	5805.426	2902.713	4	8448	22032	22032	
NIL-49	Spring Egg Count	1998 Lamberton	SCN	S	15422	5535.223	1956.997	8	7296	25760	25760	
NIL-49	Spring Egg Count	1998 Lamberton	SCN	S cultivars	12630	6947.254	3473.627	4	3848	19152	19152	
NIL-49	Spring Egg Count	1998 Lamberton	SCN	x2	14146	7258.701	3629.351	4	5936	21504	21504	
NIL-49	Spring Egg Count	1998 Waseca	SCN	R	558.857	323.132	122.133	7	184	1008	1008	
NIL-49	Spring Egg Count	1998 Waseca	SCN	R cultivars	1128	703.409	406.113	3	576	1920	1920	
NIL-49	Spring Egg Count	1998 Waseca	SCN	S	564	652.604	230.731	8	0	2048	2048	
NIL-49	Spring Egg Count	1998 Waseca	SCN	S cultivars	1028	543.745	271.873	4	336	1664	1664	
NIL-49	Spring Egg Count	1998 Waseca	SCN	x2	90.667	78.926	45.568	3	0	144	144	
NIL-49	Spring Egg Counts	1997 Lamberton	SCN	NIL parent	1566.667	1200.347	693.021	3	350	2750	2750	
NIL-49	Spring Egg Counts	1997 Lamberton	SCN	R	880	869.339	388.78	5	100	2000	2000	
NIL-49	Spring Egg Counts	1997 Lamberton	SCN	R cultivars	1066.667	981.495	566.667	3	500	2200	2200	
NIL-49	Spring Egg Counts	1997 Lamberton	SCN	S	2508.333	1954.333	797.853	6	50	5450	5450	
NIL-49	Spring Egg Counts	1997 Lamberton	SCN	S cultivars	1475	827.496	337.824	6	0	2300	2300	
NIL-49	Spring Egg Counts	1997 Lamberton	SCN	x2	933.333	709.46	409.607	3	300	1700	1700	
NIL-49	Spring Egg Counts	1997 Waseca	SCN	NIL parent	2983.333	1486.887	858.455	3	2100	4700	4700	
NIL-49	Spring Egg Counts	1997 Waseca	SCN	R	3625	4185.899	1708.886	6	400	10300	10300	
NIL-49	Spring Egg Counts	1997 Waseca	SCN	R cultivars	5500	3354.475	1936.707	3	2950	9300	9300	
NIL-49	Spring Egg Counts	1997 Waseca	SCN	S	4000	2238.08	913.692	6	1000	6750	6750	
NIL-49	Spring Egg Counts	1997 Waseca	SCN	S cultivars	3433.333	1915.898	782.162	6	900	6750	6750	
NIL-49	Spring Egg Counts	1997 Waseca	SCN	x2	3216.667	1697.302	979.938	3	1700	5050	5050	
NIL-49	Stand Count	1998 Lamberton	SCN	R	153.396	11.508	4.35	7	142.22	174.22	174.22	
NIL-49	Stand Count	1998 Lamberton	SCN	R cultivars	148.445	52.366	26.183	4	81.78	209.78	209.78	
NIL-49	Stand Count	1998 Lamberton	SCN	S	170.668	20.025	7.08	8	149.33	209.78	209.78	
NIL-49	Stand Count	1998 Lamberton	SCN	S cultivars	184.888	17.235	7.708	5	167.11	213.33	213.33	
NIL-49	Stand Count	1998 Lamberton	SCN	x2	166.225	25.203	12.601	4	131.56	192	192	
NIL-49	Stand Count	1998 Waseca	SCN	R	155.111	15.195	5.372	8	138.67	181.33	181.33	
NIL-49	Stand Count	1998 Waseca	SCN	R cultivars	139.555	20.804	10.402	4	113.78	156.44	156.44	
NIL-49	Stand Count	1998 Waseca	SCN	S	143.11	13.672	4.834	8	124.44	160	160	

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49	Stand Count	1998	Waseca	SCN	S cultivars	137.775	15.189	7.595	4	117.33	149.33
NIL-49	Stand Count	1998	Waseca	SCN	x2	136.002	22.929	11.465	4	113.78	163.56
NIL-49	Stand counts	1997	Lamberton	SCN	NIL parent	68.667	5.132	2.963	3	63	73
NIL-49	Stand counts	1997	Lamberton	SCN	R	89.5	10.895	4.448	6	75	108
NIL-49	Stand counts	1997	Lamberton	SCN	R cultivars	94.667	6.658	3.844	3	89	102
NIL-49	Stand counts	1997	Lamberton	SCN	S	68.667	7.607	3.106	6	57	76
NIL-49	Stand counts	1997	Lamberton	SCN	S cultivars	67.167	16.642	6.794	6	48	94
NIL-49	Stand counts	1997	Lamberton	SCN	x2	74	17.436	10.066	3	54	86
NIL-49	Yield	1998	Lamberton	non-SCN	NIL parent	1.984	1.145	0.3	25.48	29.03	0
NIL-49	Yield	1998	Lamberton	non-SCN	R	3.914	0.923	0.18	16.07	30.94	0
NIL-49	Yield	1998	Lamberton	non-SCN	R cultivars	6.907	3.988	0.3	29.51	42.47	6
NIL-49	Yield	1998	Lamberton	non-SCN	S	3.698	0.664	0.31	19.62	32.34	5
NIL-49	Yield	1998	Lamberton	non-SCN	S cultivars	6.8	2.776	0.6	34.76	50.48	6
NIL-49	Yield	1998	Lamberton	non-SCN	x1	3.199	1.847	0.3	23.14	29.43	0
NIL-49	Yield	1998	Lamberton	non-SCN	x2	2.816	1.626	0.3	20.21	25.43	0
NIL-49	Yield	1998	Lamberton	non-SCN	x3	2.704	1.561	0.3	25	30.16	0
NIL-49	Yield	1998	Lamberton	non-SCN	x4	3.331	1.923	0.3	19.17	25.83	0
NIL-49	Yield	1998	Lamberton	non-SCN	x5	12.97	7.488	0.3	19.35	45.1	0
NIL-49	Yield	1998	Waseca	non-SCN	NIL parent	1.881	1.086	0.3	18.63	22.39	0
NIL-49	Yield	1998	Waseca	non-SCN	R	8.405	1.981	0.18	12.26	51.18	0
NIL-49	Yield	1998	Waseca	non-SCN	R cultivars	12.174	7.029	0.3	12.69	36.1	6
NIL-49	Yield	1998	Waseca	non-SCN	S	4.441	0.798	0.31	12.85	32.71	5
NIL-49	Yield	1998	Waseca	non-SCN	S cultivars	11.414	4.66	0.6	32.36	61.69	6
NIL-49	Yield	1998	Waseca	non-SCN	x1	8.983	5.186	0.3	13.28	29.43	0
NIL-49	Yield	1998	Waseca	non-SCN	x2	3.019	1.743	0.3	18.28	24.19	0
NIL-49	Yield	1998	Waseca	non-SCN	x3	2.684	1.549	0.3	20.4	25.37	0
NIL-49	Yield	1998	Waseca	non-SCN	x4	6.602	3.811	0.3	13.12	25.64	0
NIL-49	Yield	1998	Waseca	non-SCN	x5	10.442	6.029	0.3	18.57	39.43	0
NIL-49	Yield	1998	Lamberton	SCN	NIL parent	18.708	2.377	0.189	4	15.32	20.62
NIL-49	Yield	1998	Lamberton	SCN	R	16.035	2.935	0.599	24	10.35	20.86
NIL-49	Yield	1998	Lamberton	SCN	R	10.91	2.361	0.394	36	7.123	15.16

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49	Yield	1998	Lamberton	SCN	R cultivars	29.963	5.375	2.688	4	22.01	33.49
NIL-49	Yield	1998	Lamberton	SCN	S	15.794	4.27	0.637	45	8.31	27.1
NIL-49	Yield	1998	Lamberton	SCN	S	10.6	3.444	0.651	28	-0.699	17.848
NIL-49	Yield	1998	Lamberton	SCN	S cultivars	20.255	2.82	0.997	8	15.97	25.35
NIL-49	Yield	1998	Lamberton	SCN	x1	13.86	0.324	0.162	4	13.63	14.33
NIL-49	Yield	1998	Lamberton	SCN	x2	15.012	1.545	0.772	4	12.96	16.61
NIL-49	Yield	1998	Lamberton	SCN	x3	16.45	2.912	1.456	4	12.34	18.92
NIL-49	Yield	1998	Lamberton	SCN	x4	16.155	2.298	1.149	4	13.92	18.47
NIL-49	Yield	1998	Lamberton	SCN	x5	16.598	8.055	4.027	4	4.7	21.8
NIL-49	Yield	1998	Waseca	SCN	NIL parent	27.127	9.926	4.963	4	19.43	41.66
NIL-49	Yield	1998	Waseca	SCN	R	21.907	5.261	1.074	24	14.25	37.55
NIL-49	Yield	1998	Waseca	SCN	R	16.135	2.885	0.481	36	11.075	23.251
NIL-49	Yield	1998	Waseca	SCN	R cultivars	29.523	6.485	3.242	4	23.17	35.7
NIL-49	Yield	1998	Waseca	SCN	S	21.478	5.891	0.888	44	0	36.37
NIL-49	Yield	1998	Waseca	SCN	S	15.702	3.088	0.584	28	11.693	25.213
NIL-49	Yield	1998	Waseca	SCN	S cultivars	35.611	3.595	1.271	8	29.59	38.92
NIL-49	Yield	1998	Waseca	SCN	x1	19.058	3.506	1.753	4	16.8	24.27
NIL-49	Yield	1998	Waseca	SCN	x2	32.568	18.87	9.435	4	20.4	60.35
NIL-49	Yield	1998	Waseca	SCN	x3	24.688	2.403	1.201	4	22.36	26.83
NIL-49	Yield	1998	Waseca	SCN	x4	21.5	2.104	1.052	4	19.6	24.49
NIL-49	Yield	1998	Waseca	SCN	x5	25.273	14.412	7.206	4	12.82	40.72
NIL-49	Yield	1997	Lamberton	non-SCN	NIL parent	1.796	1.037	0.3	13.06	16.48	0
NIL-49	Yield	1997	Lamberton	non-SCN	R	3.269	0.944	0.12	12.15	23.04	6
NIL-49	Yield	1997	Lamberton	non-SCN	R cultivars	4.475	1.827	0.6	19.81	32.98	3
NIL-49	Yield	1997	Lamberton	non-SCN	S	3.231	0.622	0.27	13.12	26.91	9
NIL-49	Yield	1997	Lamberton	non-SCN	S cultivars	3.017	1.006	0.9	23.76	33.52	3
NIL-49	Yield	1997	Lamberton	non-SCN	x1			1	17.61	17.61	2
NIL-49	Yield	1997	Lamberton	non-SCN	x2	3.043	1.757	0.3	12.39	18.36	0
NIL-49	Yield	1997	Lamberton	non-SCN	x3	1.282	0.74	0.3	14.54	17.1	0
NIL-49	Yield	1997	Lamberton	non-SCN	x4			1	11.42	11.42	2
NIL-49	Yield	1997	Waseca	non-SCN	NIL parent	1.24	0.716	0.3	17.66	20.13	0

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49	Yield	1997	Waseca	non-SCN	R	2.89	0.802	13	12.96	21.83	5
NIL-49	Yield	1997	Waseca	non-SCN	R cultivars	6.139	2.506	6	14.65	32.34	3
NIL-49	Yield	1997	Waseca	non-SCN	S	2.837	0.546	27	14.76	27.5	9
NIL-49	Yield	1997	Waseca	non-SCN	S cultivars	5.168	1.723	9	22.2	36.74	3
NIL-49	Yield	1997	Waseca	non-SCN	x1	8.292	4.788	3	13.79	29.22	0
NIL-49	Yield	1997	Waseca	non-SCN	x2	2.836	1.637	3	14.17	19.22	0
NIL-49	Yield	1997	Waseca	non-SCN	x3	4.08	2.355	3	12.18	20.05	0
NIL-49	Yield	1997	Waseca	non-SCN	x4			1	18.04	18.04	2
NIL-49	Yield	1997	Lamberton	SCN	NIL parent	9.273	1.101	6.636	3	8.01	10.03
NIL-49	Yield	1997	Lamberton	SCN	R	10.687	1.558	0.636	6	9	12.58
NIL-49	Yield	1997	Lamberton	SCN	R cultivars	17.54	2.676	1.545	3	15.7	20.61
NIL-49	Yield	1997	Lamberton	SCN	S	7.09	1.54	0.629	6	5.07	9.46
NIL-49	Yield	1997	Lamberton	SCN	S cultivars	11.915	1.56	0.637	6	10.3	14.86
NIL-49	Yield	1997	Lamberton	SCN	x2	7.443	1.304	0.753	3	5.99	8.51
NIL-49	Yield	1997	Waseca	SCN	NIL parent	6.83	1.26	0.728	3	5.45	7.92
NIL-49	Yield	1997	Waseca	SCN	R	11.138	1.896	0.774	6	8.94	13.92
NIL-49	Yield	1997	Waseca	SCN	R cultivars	21.69	1.182	0.683	3	20.59	22.94
NIL-49	Yield	1997	Waseca	SCN	S	6.827	1.25	0.51	6	5.38	8.94
NIL-49	Yield	1997	Waseca	SCN	S cultivars	13.933	5.782	2.361	6	5.54	21.31
NIL-49	Yield	1997	Waseca	SCN	x2	3.033	2.632	1.519	3	0	4.71
NIL-49-29	Height	1998	Lamberton	non-SCN	NIL parent	36	4.243	3	2	33	39
NIL-49-29	Height	1998	Lamberton	non-SCN	R	33.176	2.856	0.693	17	29	38
NIL-49-29	Height	1998	Lamberton	non-SCN	R cultivars	36.5	7.778	5.5	2	31	42
NIL-49-29	Height	1998	Lamberton	non-SCN	S	35	2.938	0.657	20	29	40
NIL-49-29	Height	1998	Lamberton	non-SCN	S cultivars	42	2.449	1.225	4	39	45
NIL-49-29	Height	1998	Lamberton	non-SCN	x1	33	2.828	2	2	31	35
NIL-49-29	Height	1998	Lamberton	non-SCN	x2	36.5	2.121	1.5	2	35	38
NIL-49-29	Height	1998	Lamberton	non-SCN	x3	30		1	30	30	30
NIL-49-29	Height	1998	Lamberton	non-SCN	x4	31	1.414	1	2	30	32
NIL-49-29	Height	1998	Waseca	non-SCN	NIL parent	48	2.828	2	2	46	50
NIL-49-29	Height	1998	Waseca	non-SCN	R	39.294	6.283	1.524	17	26	48

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49-29	Height	1998	Waseca	non-SCN	R cultivars	35	1.414	1	2	34	36
NIL-49-29	Height	1998	Waseca	non-SCN	S	40.9	5.447	1.218	20	30	48
NIL-49-29	Height	1998	Waseca	non-SCN	S cultivars	36.5	2.517	1.258	4	34	40
NIL-49-29	Height	1998	Waseca	non-SCN	x1	39	4.243	3	2	36	42
NIL-49-29	Height	1998	Waseca	non-SCN	x2	43	4.243	3	2	40	46
NIL-49-29	Height	1998	Waseca	non-SCN	x3	38	.	.	1	38	38
NIL-49-29	Height	1998	Waseca	non-SCN	x4	35	4.243	3	2	32	38
NIL-49-29	Height	1998	non-SCN	Total		37.288	5.391	0.529	104	26	50
NIL-49-29	Height	1998	Lamberton	SCN	NIL parent	33.667	6.028	3.48	3	28	40
NIL-49-29	Height	1998	Lamberton	SCN	R	33.04	3.889	0.778	25	24	42
NIL-49-29	Height	1998	Lamberton	SCN	R cultivars	32.333	3.215	1.856	3	30	36
NIL-49-29	Height	1998	Lamberton	SCN	S	34.379	5.716	1.061	29	24	48
NIL-49-29	Height	1998	Lamberton	SCN	S cultivars	31.833	3.189	1.302	6	28	36
NIL-49-29	Height	1998	Lamberton	SCN	Total	33.237	4.8	0.551	76	24	48
NIL-49-29	Height	1998	Lamberton	SCN	x1	31.333	2.517	1.453	3	29	34
NIL-49-29	Height	1998	Lamberton	SCN	x2	34	7.81	4.509	3	25	39
NIL-49-29	Height	1998	Lamberton	SCN	x3	26	.	.	1	26	26
NIL-49-29	Height	1998	Lamberton	SCN	x4	30.667	4.041	2.333	3	27	35
NIL-49-29	Height	1997	Rosemount	non-SCN	NIL parent	38	8.485	6	2	32	44
NIL-49-29	Height	1997	Rosemount	non-SCN	R	37.938	6.34	1.585	16	24	52
NIL-49-29	Height	1997	Rosemount	non-SCN	R cultivars	42	0	0	2	42	42
NIL-49-29	Height	1997	Rosemount	non-SCN	S	36.5	3.425	0.989	12	30	42
NIL-49-29	Height	1997	Rosemount	non-SCN	S cultivars	30.5	2.38	1.19	4	28	33
NIL-49-29	Height	1997	Rosemount	non-SCN	x1	51	.	.	1	51	51
NIL-49-29	Height	1997	Rosemount	non-SCN	x2	36.5	3.536	2.5	2	34	39
NIL-49-29	Height	1997	Rosemount	non-SCN	x3	34	.	.	1	34	34
NIL-49-29	Height	1997	Rosemount	non-SCN	x4	35	.	.	1	35	35
NIL-49-29	Lodging	1998	Lamberton	non-SCN	NIL parent	4	0	0	2	4	4
NIL-49-29	Lodging	1998	Lamberton	non-SCN	R	4	0	0	0	17	4
NIL-49-29	Lodging	1998	Lamberton	non-SCN	R cultivars	3	0	0	2	3	3
NIL-49-29	Lodging	1998	Lamberton	non-SCN	S	4	0	0	0	20	4

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49-29	Lodging	1998	Lamberton	non-SCN	S cultivars	3	0.816	0.408	4	2	4
NIL-49-29	Lodging	1998	Lamberton	non-SCN	x1	5	0	0	2	5	5
NIL-49-29	Lodging	1998	Lamberton	non-SCN	x2	4	0	0	2	4	4
NIL-49-29	Lodging	1998	Lamberton	non-SCN	x3	5			1	5	5
NIL-49-29	Lodging	1998	Lamberton	non-SCN	x4	4.5	0.707	0.5	2	4	5
NIL-49-29	Lodging	1998	Waseca	non-SCN	NIL parent	5	0	0	0	2	5
NIL-49-29	Lodging	1998	Waseca	non-SCN	R	5	0	0	0	17	5
NIL-49-29	Lodging	1998	Waseca	non-SCN	R cultivars	2.5	0.707	0.5	2	2	3
NIL-49-29	Lodging	1998	Waseca	non-SCN	S	5	0	0	0	20	5
NIL-49-29	Lodging	1998	Waseca	non-SCN	S cultivars	3	0.816	0.408	4	2	4
NIL-49-29	Lodging	1998	Waseca	non-SCN	x1	5	0	0	0	2	5
NIL-49-29	Lodging	1998	Waseca	non-SCN	x2	5	0	0	0	2	5
NIL-49-29	Lodging	1998	Waseca	non-SCN	x3	5	0	0	0	2	5
NIL-49-29	Lodging	1998	Waseca	non-SCN	x4	4.5	0.707	0.5	2	2	3
NIL-49-29	Lodging	1998	Lamberton	SCN	NIL parent	3	1	0.577	3	2	4
NIL-49-29	Lodging	1998	Lamberton	SCN	R	3.32	0.627	0.125	25	2	4
NIL-49-29	Lodging	1998	Lamberton	SCN	R cultivars	2	0	0	0	3	2
NIL-49-29	Lodging	1998	Lamberton	SCN	S	2.655	0.553	0.103	29	2	4
NIL-49-29	Lodging	1998	Lamberton	SCN	S cultivars	2	0	0	0	6	2
NIL-49-29	Lodging	1998	Lamberton	SCN	x1	3.667	0.577	0.333	3	3	4
NIL-49-29	Lodging	1998	Lamberton	SCN	x2	2.333	0.577	0.333	3	2	3
NIL-49-29	Lodging	1998	Lamberton	SCN	x3	2			1	2	2
NIL-49-29	Lodging	1998	Lamberton	SCN	x4	2.333	0.577	0.333	3	2	3
NIL-49-29	Lodging	1998	Waseca	SCN	NIL parent	4.667	0.577	0.333	3	4	5
NIL-49-29	Lodging	1998	Waseca	SCN	R	4.217	0.85	0.177	23	2	5
NIL-49-29	Lodging	1998	Waseca	SCN	R cultivars	2.5	0.866	0.5	3	2	3.5
NIL-49-29	Lodging	1998	Waseca	SCN	S	4.115	0.816	0.16	26	2	5
NIL-49-29	Lodging	1998	Waseca	SCN	S cultivars	3.083	1.497	0.611	6	2	5
NIL-49-29	Lodging	1998	Waseca	SCN	x1	4.667	0.577	0.333	3	4	5
NIL-49-29	Lodging	1998	Waseca	SCN	x2	4.333	0.577	0.333	3	4	5
NIL-49-29	Lodging	1998	Waseca	SCN	x3	3.5	0.707	0.5	2	3	4

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49-29	Lodging	1998	Waseca	SCN	x4	3.333	1.528	0.882	3	2	5
NIL-49-29	Lodging	1997	Rosemount	non-SCN	NIL parent	3	0	0	2	3	3
NIL-49-29	Lodging	1997	Rosemount	non-SCN	R	3.562	0.727	0.182	16	3	5
NIL-49-29	Lodging	1997	Rosemount	non-SCN	R cultivars	2	0	0	2	2	2
NIL-49-29	Lodging	1997	Rosemount	non-SCN	S	3.167	0.718	0.207	12	2	4
NIL-49-29	Lodging	1997	Rosemount	non-SCN	S cultivars	2.5	0.577	0.289	4	2	3
NIL-49-29	Lodging	1997	Rosemount	non-SCN	x1	5	.	.	1	5	5
NIL-49-29	Lodging	1997	Rosemount	non-SCN	x2	3	0	0	2	3	3
NIL-49-29	Lodging	1997	Rosemount	non-SCN	x3	3	.	.	1	3	3
NIL-49-29	Lodging	1997	Rosemount	non-SCN	x4	3	.	.	1	3	3
NIL-49-29	Maturity	1998	Lamberton	non-SCN	NIL parent	31	2.828	2	2	29	33
NIL-49-29	Maturity	1998	Lamberton	non-SCN	R	30	3.623	0.879	17	21	34
NIL-49-29	Maturity	1998	Lamberton	non-SCN	R cultivars	20	0	0	2	20	20
NIL-49-29	Maturity	1998	Lamberton	non-SCN	S	28.15	3.66	0.818	20	22	34
NIL-49-29	Maturity	1998	Lamberton	non-SCN	S cultivars	22	7.528	3.764	4	15	29
NIL-49-29	Maturity	1998	Lamberton	non-SCN	x1	33	0	0	2	33	33
NIL-49-29	Maturity	1998	Lamberton	non-SCN	x2	30	4.243	3	2	27	33
NIL-49-29	Maturity	1998	Lamberton	non-SCN	x3	25	.	.	1	25	25
NIL-49-29	Maturity	1998	Lamberton	non-SCN	x4	25	2.828	2	2	23	27
NIL-49-29	Maturity	1998	Waseca	non-SCN	NIL parent	26.5	0.707	0.5	2	26	27
NIL-49-29	Maturity	1998	Waseca	non-SCN	R	24	3	0.728	17	17	29
NIL-49-29	Maturity	1998	Waseca	non-SCN	R cultivars	16.5	0.707	0.5	2	16	17
NIL-49-29	Maturity	1998	Waseca	non-SCN	S	22.05	3.804	0.851	20	17	29
NIL-49-29	Maturity	1998	Waseca	non-SCN	S cultivars	17.75	3.096	1.548	4	15	22
NIL-49-29	Maturity	1998	Waseca	non-SCN	x1	24.5	2.121	1.5	2	23	26
NIL-49-29	Maturity	1998	Waseca	non-SCN	x2	26.5	0.707	0.5	2	26	27
NIL-49-29	Maturity	1998	Waseca	non-SCN	x3	21	.	.	1	21	21
NIL-49-29	Maturity	1998	Waseca	non-SCN	x4	20	0	0	2	20	20
NIL-49-29	Maturity	1998	Waseca	non-SCN	Total	25.317	5.113	0.501	104	15	34
NIL-49-29	Maturity	1998	Lamberton	SCN	NIL parent	30.333	1.528	0.882	3	29	32
NIL-49-29	Maturity	1998	Lamberton	SCN	R	30.64	4.966	0.993	25	18	39

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49-29	Maturity	1998	Lamberton	SCN	R cultivars	18.667	1.528	0.882	3	17	20
NIL-49-29	Maturity	1998	Lamberton	SCN	S	28	4.848	0.9	29	20	37
NIL-49-29	Maturity	1998	Lamberton	SCN	S cultivars	21.667	5.82	2.376	6	16	30
NIL-49-29	Maturity	1998	Lamberton	SCN	x1	32.667	3.055	1.764	3	30	36
NIL-49-29	Maturity	1998	Lamberton	SCN	x2	31.333	1.155	0.667	3	30	32
NIL-49-29	Maturity	1998	Lamberton	SCN	x3	21	.	.	1	21	21
NIL-49-29	Maturity	1998	Lamberton	SCN	x4	27	4.359	2.517	3	22	30
NIL-49-29	Maturity	1998	Waseca	SCN	NIL parent	23.667	5.859	3.383	3	17	28
NIL-49-29	Maturity	1998	Waseca	SCN	R	22.826	4.589	0.957	23	14	28
NIL-49-29	Maturity	1998	Waseca	SCN	R cultivars	18.667	3.512	2.028	3	15	22
NIL-49-29	Maturity	1998	Waseca	SCN	S	23.741	3.448	0.664	27	17	28
NIL-49-29	Maturity	1998	Waseca	SCN	S cultivars	21.833	3.71	1.515	6	18	28
NIL-49-29	Maturity	1998	Waseca	SCN	x1	28	0	0	3	28	28
NIL-49-29	Maturity	1998	Waseca	SCN	x2	19.333	5.774	3.333	3	16	26
NIL-49-29	Maturity	1998	Waseca	SCN	x3	21	9.899	7	2	14	28
NIL-49-29	Maturity	1998	Waseca	SCN	x4	21	3.606	2.082	3	17	24
NIL-49-29	Maturity	1998	Waseca	SCN	Total	25.638	5.663	0.464	149	14	39
NIL-49-29	Maturity	1997	Rosemount	non-SCN	NIL parent	37	4.243	3	2	34	40
NIL-49-29	Maturity	1997	Rosemount	non-SCN	R	36	5.441	1.36	16	25	40
NIL-49-29	Maturity	1997	Rosemount	non-SCN	R cultivars	34	0	0	2	34	34
NIL-49-29	Maturity	1997	Rosemount	non-SCN	S	33.417	5.696	1.644	12	25	40
NIL-49-29	Maturity	1997	Rosemount	non-SCN	S cultivars	31.5	1.732	0.866	4	29	33
NIL-49-29	Maturity	1997	Rosemount	non-SCN	x1	40	.	.	1	40	40
NIL-49-29	Maturity	1997	Rosemount	non-SCN	x2	37.5	3.536	2.5	2	35	40
NIL-49-29	Maturity	1997	Rosemount	non-SCN	x3	32	.	.	1	32	32
NIL-49-29	Maturity	1997	Rosemount	non-SCN	x4	35	.	.	1	35	35
NIL-49-29	Yield	1998	Lamberton	non-SCN	NIL parent	28.345	1.153	0.815	2	27.53	29.16
NIL-49-29	Yield	1998	Lamberton	non-SCN	R	22.935	3.662	0.888	17	17.66	29.73
NIL-49-29	Yield	1998	Lamberton	non-SCN	R cultivars	42.405	4.236	2.995	2	39.41	45.4
NIL-49-29	Yield	1998	Lamberton	non-SCN	S	24.354	3.855	0.862	20	15.43	30.97
NIL-49-29	Yield	1998	Lamberton	non-SCN	S cultivars	43.633	9.145	4.572	4	34.89	55.21

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49-29	Yield	1998	Lamberton	non-SCN	x1	20.32	6.307	4.46	2	15.86	24.78
NIL-49-29	Yield	1998	Lamberton	non-SCN	x2	23.67	1.428	1.01	2	22.66	24.68
NIL-49-29	Yield	1998	Lamberton	non-SCN	x3	17.23			1	17.23	17.23
NIL-49-29	Yield	1998	Lamberton	non-SCN	x4	22.255	0.346	0.245	2	22.01	22.5
NIL-49-29	Yield	1998	Waseca	non-SCN	NIL parent	27.04	3.196	2.26	2	24.78	29.3
NIL-49-29	Yield	1998	Waseca	non-SCN	R	17.499	4.938	1.198	17	9.49	25.99
NIL-49-29	Yield	1998	Waseca	non-SCN	R cultivars	38.57	6.845	4.84	2	33.73	43.41
NIL-49-29	Yield	1998	Waseca	non-SCN	S	21.274	3.961	0.886	20	14.09	30.72
NIL-49-29	Yield	1998	Waseca	non-SCN	S cultivars	44.492	7.087	3.544	4	35.59	52.6
NIL-49-29	Yield	1998	Waseca	non-SCN	x1	15.225	1.916	1.355	2	13.87	16.58
NIL-49-29	Yield	1998	Waseca	non-SCN	x2	17.595	2.227	1.575	2	16.02	19.17
NIL-49-29	Yield	1998	Waseca	non-SCN	x3	15.83			1	15.83	15.83
NIL-49-29	Yield	1998	Waseca	non-SCN	x4	20.32	4.214	2.98	2	17.34	23.3
NIL-49-29	Yield	1998	Lamberton	SCN	NIL parent	17.847	2.666	1.539	3	15.51	20.75
NIL-49-29	Yield	1998	Lamberton	SCN	R	14.394	3.124	0.625	25	8.92	20.67
NIL-49-29	Yield	1998	Lamberton	SCN	R cultivars	31.937	3.562	2.057	3	29.84	36.05
NIL-49-29	Yield	1998	Lamberton	SCN	S	13.782	3.288	0.6	30	7.85	21.5
NIL-49-29	Yield	1998	Lamberton	SCN	S cultivars	21.738	3.029	1.236	6	15.94	23.87
NIL-49-29	Yield	1998	Lamberton	SCN	x1	12.757	5.065	2.924	3	7.66	17.79
NIL-49-29	Yield	1998	Lamberton	SCN	x2	11.503	1.728	0.998	3	10.35	13.49
NIL-49-29	Yield	1998	Lamberton	SCN	x3	8.66			1	8.66	8.66
NIL-49-29	Yield	1998	Lamberton	SCN	x4	10.83	3.553	2.051	3	6.99	14
NIL-49-29	Yield	1998	Waseca	SCN	NIL parent	24.687	2.666	1.539	3	22.39	27.61
NIL-49-29	Yield	1998	Waseca	SCN	R	20.364	5.756	1.151	25	6.4	34.22
NIL-49-29	Yield	1998	Waseca	SCN	R cultivars	35.403	2.334	1.348	3	33.6	38.04
NIL-49-29	Yield	1998	Waseca	SCN	S	21.83	6.783	1.238	30	2.53	34.86
NIL-49-29	Yield	1998	Waseca	SCN	S cultivars	31.138	2.902	1.185	6	26.58	33.68
NIL-49-29	Yield	1998	Waseca	SCN	x1	19.36	2.724	1.573	3	16.45	21.85
NIL-49-29	Yield	1998	Waseca	SCN	x2	19.697	3.404	1.965	3	17.1	23.55
NIL-49-29	Yield	1998	Waseca	SCN	x3	17.885	3.401	2.405	2	15.48	20.29
NIL-49-29	Yield	1998	Waseca	SCN	x4	14.083	4.422	2.553	3	8.98	16.77

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-49-29	Yield	1998	SCN	Total	18.666	7.037	0.565	155	2.53	38.04	
NIL-49-29	Yield	1997	Rosemount	NIL parent	29.175	15.309	10.825	2	18.35	40	
NIL-49-29	Yield	1997	Rosemount	R non-SCN	18.288	7.277	1.819	16	7.39	30.46	
NIL-49-29	Yield	1997	Rosemount	R cultivars	58.455	4.306	3.045	2	55.41	61.5	
NIL-49-29	Yield	1997	Rosemount	S non-SCN	25.708	8.017	2.314	12	11.9	43.65	
NIL-49-29	Yield	1997	Rosemount	S non-SCN	50.285	9.75	4.875	4	39.57	59.78	
NIL-49-29	Yield	1997	Rosemount	S cultivars	32.61			1	32.61	32.61	
NIL-49-29	Yield	1997	Rosemount	x1							
NIL-49-29	Yield	1997	Rosemount	x2							
NIL-49-29	Yield	1997	Rosemount	x3							
NIL-49-29	Yield	1997	Rosemount	x4							
NIL-49-29	Height	1998	Lamberton	NIL parent	44.667	5.774	3.333	3	38	48	
NIL-79	Height	1998	Lamberton	non-SCN R	43.963	3.299	0.635	27	36	48	
NIL-79	Height	1998	Lamberton	non-SCN R cultivars	43	1.549	0.632	6	40	44	
NIL-79	Height	1998	Lamberton	non-SCN S	44.857	2.476	0.54	21	39	48	
NIL-79	Height	1998	Lamberton	non-SCN S cultivars	46.667	1.155	0.667	3	46	48	
NIL-79	Height	1998	Lamberton	x1	46.333	2.082	1.202	3	44	48	
NIL-79	Height	1998	Lamberton	x2	45.667	1.528	0.882	3	44	47	
NIL-79	Height	1998	Lamberton	x3	43	2.646	1.528	3	40	45	
NIL-79	Height	1998	Lamberton	x4	44.333	3.215	1.856	3	42	48	
NIL-79	Height	1998	Lamberton	x5	46	3.464	2	3	42	48	
NIL-79	Height	1998	Waseca	NIL parent	36.667	5.033	2.906	3	32	42	
NIL-79	Height	1998	Waseca	non-SCN R	40.519	4.527	0.871	27	30	48	
NIL-79	Height	1998	Waseca	non-SCN R cultivars	40	3.098	1.265	6	36	44	
NIL-79	Height	1998	Waseca	non-SCN S	41.5	3.035	0.679	20	36	48	
NIL-79	Height	1998	Waseca	non-SCN S cultivars	41.333	4.163	2.404	3	38	46	
NIL-79	Height	1998	Waseca	x1	38	2	1.155	3	36	40	
NIL-79	Height	1998	Waseca	x2	39	7.071	5	2	34	44	
NIL-79	Height	1998	Waseca	x3	43	2.646	1.528	3	41	46	
NIL-79	Height	1998	Waseca	x4	38	6	3.464	3	32	44	
NIL-79	Height	1998	Waseca	x5	45.333	2.309	1.333	3	44	48	
NIL-79	Height	1998	Lamberton	SCN	33.5	3.697	1.848	4	31	39	

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-79	Height	1998	Lamberton	SCN	R	34.056	3.189	0.531	36	29	41
NIL-79	Height	1998	Lamberton	SCN	R cultivars	31.875	2.357	0.833	8	28	35
NIL-79	Height	1998	Lamberton	SCN	S	32.714	3.43	0.648	28	25	38
NIL-79	Height	1998	Lamberton	SCN	S cultivars	32.5	3.697	1.848	4	29	37
NIL-79	Height	1998	Lamberton	SCN	Total	33.58	3.66	0.366	100	25	45
NIL-79	Height	1998	Lamberton	SCN	x1	35.25	1.893	0.946	4	34	38
NIL-79	Height	1998	Lamberton	SCN	x2	32.25	5.439	2.72	4	25	38
NIL-79	Height	1998	Lamberton	SCN	x3	31.75	2.986	1.493	4	28	35
NIL-79	Height	1998	Lamberton	SCN	x4	40.75	4.992	2.496	4	34	45
NIL-79	Height	1998	Lamberton	SCN	x5	34.25	3.403	1.702	4	31	39
NIL-79	Lodging	1998	Lamberton	non-SCN	H parent	3	0	0	3	3	3
NIL-79	Lodging	1998	Lamberton	non-SCN	R	3.111	0.641	0.123	27	1	4
NIL-79	Lodging	1998	Lamberton	non-SCN	R cultivars	2.833	0.408	0.167	6	2	3
NIL-79	Lodging	1998	Lamberton	non-SCN	S	3	0	0	21	3	3
NIL-79	Lodging	1998	Lamberton	non-SCN	S cultivars	3.667	0.577	0.333	3	3	4
NIL-79	Lodging	1998	Lamberton	non-SCN	X1	3	0	0	0	3	3
NIL-79	Lodging	1998	Lamberton	non-SCN	X2	3	0	0	0	3	3
NIL-79	Lodging	1998	Lamberton	non-SCN	X3	3	0	0	0	3	3
NIL-79	Lodging	1998	Lamberton	non-SCN	X4	3	0	0	0	3	3
NIL-79	Lodging	1998	Lamberton	non-SCN	X5	3	0	0	0	3	3
NIL-79	Lodging	1998	Waseca	non-SCN	H parent	2.667	1.155	0.667	3	2	4
NIL-79	Lodging	1998	Waseca	non-SCN	R	2.889	0.801	0.154	27	2	5
NIL-79	Lodging	1998	Waseca	non-SCN	R cultivars	1.167	0.408	0.167	6	1	2
NIL-79	Lodging	1998	Waseca	non-SCN	S	2.35	0.875	0.196	20	1	4
NIL-79	Lodging	1998	Waseca	non-SCN	S cultivars	3	0	0	0	3	3
NIL-79	Lodging	1998	Waseca	non-SCN	X1	2.333	0.577	0.333	3	2	3
NIL-79	Lodging	1998	Waseca	non-SCN	X2	3	1.414	1	2	2	4
NIL-79	Lodging	1998	Waseca	non-SCN	X3	2.667	0.577	0.333	3	2	3
NIL-79	Lodging	1998	Waseca	non-SCN	X4	2	0	0	3	2	2
NIL-79	Lodging	1998	Waseca	non-SCN	X5	1.333	0.577	0.333	3	1	2
NIL-79	Lodging	1998	Lamberton	SCN	NIL parent	2.25	0.5	0.25	4	2	3

NIL set	Trait		Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-79	Lodging		1998	Lamberton	SCN	R	2.472	0.506	0.084	36	2	3
NIL-79	Lodging		1998	Lamberton	SCN	R cultivars	2.25	0.463	0.164	8	2	3
NIL-79	Lodging		1998	Lamberton	SCN	S	2.179	0.39	0.074	28	2	3
NIL-79	Lodging		1998	Lamberton	SCN	S cultivars	2	0	0	4	2	2
NIL-79	Lodging		1998	Lamberton	SCN	X1	2	0	0	4	2	2
NIL-79	Lodging		1998	Lamberton	SCN	X2	2.25	0.5	0.25	4	2	2
NIL-79	Lodging		1998	Lamberton	SCN	X3	2	0	0	4	2	2
NIL-79	Lodging		1998	Lamberton	SCN	X4	3	0	0	4	3	3
NIL-79	Lodging		1998	Lamberton	SCN	x5	2	0	0	4	2	2
NIL-79	Lodging		1998	Waseca	SCN	NIL parent	3	0.577	0.289	4	2.5	3.5
NIL-79	Lodging		1998	Waseca	SCN	R	3.153	0.607	0.101	36	2	4.5
NIL-79	Lodging		1998	Waseca	SCN	R cultivars	3.125	0.231	0.082	8	3	3.5
NIL-79	Lodging		1998	Waseca	SCN	S	3.018	0.372	0.07	28	2.5	3.5
NIL-79	Lodging		1998	Waseca	SCN	S cultivars	3	0.408	0.204	4	2.5	3.5
NIL-79	Lodging		1998	Waseca	SCN	X1	2.875	0.479	0.239	4	2.5	3.5
NIL-79	Lodging		1998	Waseca	SCN	X2	3.25	0.5	0.25	4	3	4
NIL-79	Lodging		1998	Waseca	SCN	X3	3.125	0.479	0.239	4	2.5	3.5
NIL-79	Lodging		1998	Waseca	SCN	X4	3.25	0.354	0.25	2	3	3.5
NIL-79	Lodging		1998	Waseca	SCN	x5	3.25	0.645	0.323	4	2.5	4
NIL-79	Lodging		1997	Lamberton	non-SCN	H parent	2.667	0.577	0.333	3	2	3
NIL-79	Lodging		1997	Lamberton	non-SCN	R	3.211	0.631	0.145	19	2	4
NIL-79	Lodging		1997	Lamberton	non-SCN	R cultivars	2.333	0.516	0.211	6	2	3
NIL-79	Lodging		1997	Lamberton	non-SCN	S	3	0.471	0.149	10	2	4
NIL-79	Lodging		1997	Lamberton	non-SCN	S cultivars	2.667	1.155	0.667	3	2	4
NIL-79	Lodging		1997	Lamberton	non-SCN	X1	3	0	0	3	3	5
NIL-79	Lodging		1997	Lamberton	non-SCN	X2	3	0	0	3	3	3
NIL-79	Lodging		1997	Lamberton	non-SCN	X3	3	0	0	3	3	3
NIL-79	Lodging		1997	Lamberton	non-SCN	X4	4	1.114	1	2	3	5
NIL-79	Lodging		1997	Lamberton	non-SCN	X5	3.333	0.577	0.333	3	3	4
NIL-79	Lodging		1997	Waseca	non-SCN	H parent	3	0	0	3	3	3
NIL-79	Lodging		1997	Waseca	non-SCN	R	3.409	0.503	0.107	22	3	4

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-79	Lodging	1997	Waseca	non-SCN	R cultivars	3.5	0.548	0.224	6	3	4
NIL-79	Lodging	1997	Waseca	non-SCN	S	3.091	0.539	0.163	11	2	4
NIL-79	Lodging	1997	Waseca	non-SCN	S cultivars	3.667	0.577	0.333	3	3	4
NIL-79	Lodging	1997	Waseca	non-SCN	X1	3	0	0	3	3	3
NIL-79	Lodging	1997	Waseca	non-SCN	X2	3	0	0	3	3	3
NIL-79	Lodging	1997	Waseca	non-SCN	X3	3	0	0	3	3	3
NIL-79	Lodging	1997	Waseca	non-SCN	X4	5	0	0	2	5	5
NIL-79	Lodging	1997	Waseca	non-SCN	X5	3	0	0	3	3	3
NIL-79	Maturity	1998	Lamberton	non-SCN	H parent	19.333	1.528	0.882	3	18	21
NIL-79	Maturity	1998	Lamberton	non-SCN	R	18.852	2.214	0.426	27	15	23
NIL-79	Maturity	1998	Lamberton	non-SCN	R cultivars	20.333	0.516	0.211	6	20	21
NIL-79	Maturity	1998	Lamberton	non-SCN	S	19.048	1.91	0.417	21	17	23
NIL-79	Maturity	1998	Lamberton	non-SCN	S cultivars	15.667	0.577	0.333	3	15	16
NIL-79	Maturity	1998	Lamberton	non-SCN	x1	19	1	0.577	3	18	20
NIL-79	Maturity	1998	Lamberton	non-SCN	x2	18	1	0.577	3	17	19
NIL-79	Maturity	1998	Lamberton	non-SCN	x3	18.667	0.577	0.333	3	18	19
NIL-79	Maturity	1998	Lamberton	non-SCN	x4	36	0	0	3	36	36
NIL-79	Maturity	1998	Lamberton	non-SCN	x5	22.333	1.155	0.667	3	21	23
NIL-79	Maturity	1998	Waseca	non-SCN	H parent	13	0	0	3	13	13
NIL-79	Maturity	1998	Waseca	non-SCN	R	13.222	0.934	0.18	27	11	16
NIL-79	Maturity	1998	Waseca	non-SCN	R cultivars	16.833	0.753	0.307	6	16	18
NIL-79	Maturity	1998	Waseca	non-SCN	S	13.8	1.576	0.352	20	12	18
NIL-79	Maturity	1998	Waseca	non-SCN	S cultivars	16	0	0	3	16	16
NIL-79	Maturity	1998	Waseca	non-SCN	x1	14.333	1.528	0.882	3	13	16
NIL-79	Maturity	1998	Waseca	non-SCN	x2	12.5	0.707	0.5	2	12	13
NIL-79	Maturity	1998	Waseca	non-SCN	x3	13.333	0.577	0.333	3	13	14
NIL-79	Maturity	1998	Waseca	non-SCN	x4	30	0	0	3	30	30
NIL-79	Maturity	1998	Waseca	non-SCN	x5	14.333	1.155	0.667	3	13	15
NIL-79	Maturity	1998	Lamberton	SCN	NIL parent	22.25	0.957	0.479	4	21	23
NIL-79	Maturity	1998	Lamberton	SCN	R	21.444	2.833	0.472	36	16	27
NIL-79	Maturity	1998	Lamberton	SCN	R cultivars	20	2.507	0.886	8	15	23

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-79	Maturity	1998	Lamberton	SCN	S	21.5	2.95	0.558	28	16	30
NIL-79	Maturity	1998	Lamberton	SCN	S cultivars	18.75	3.403	1.702	4	16	23
NIL-79	Maturity	1998	Lamberton	SCN	x1	21	2.828	1.414	4	17	23
NIL-79	Maturity	1998	Lamberton	SCN	x2	21.75	2.363	1.181	4	20	25
NIL-79	Maturity	1998	Lamberton	SCN	x3	23.5	1.732	0.866	4	22	25
NIL-79	Maturity	1998	Lamberton	SCN	x4	39	0	0	4	39	39
NIL-79	Maturity	1998	Lamberton	SCN	x5	24	0.816	0.408	4	23	25
NIL-79	Maturity	1998	Waseca	SCN	NIL parent	12	1.633	0.816	4	10	14
NIL-79	Maturity	1998	Waseca	SCN	R	12.222	1.533	0.255	36	10	15
NIL-79	Maturity	1998	Waseca	SCN	R cultivars	14.75	0.463	0.164	8	14	15
NIL-79	Maturity	1998	Waseca	SCN	S	12.679	1.056	0.2	28	11	14
NIL-79	Maturity	1998	Waseca	SCN	S cultivars	13	1.155	0.577	4	12	14
NIL-79	Maturity	1998	Waseca	SCN	x1	12.25	1.708	0.854	4	10	14
NIL-79	Maturity	1998	Waseca	SCN	x2	12.75	1.5	0.75	4	11	14
NIL-79	Maturity	1998	Waseca	SCN	x3	12.75	1.708	0.854	4	11	15
NIL-79	Maturity	1998	Waseca	SCN	x4				0		
NIL-79	Maturity	1998	Waseca	SCN	x5	12.25	0.957	0.479	4	11	13
NIL-79	Maturity	1997	Lamberton	non-SCN	H parent	27.333	0.577	0.333	3	27	28
NIL-79	Maturity	1997	Lamberton	non-SCN	R	26.632	0.831	0.191	19	25	28
NIL-79	Maturity	1997	Lamberton	non-SCN	R cultivars	31	2.28	0.931	6	28	33
NIL-79	Maturity	1997	Lamberton	non-SCN	S	26.6	0.966	0.306	10	25	28
NIL-79	Maturity	1997	Lamberton	non-SCN	S cultivars	30.333	0.577	0.333	3	30	31
NIL-79	Maturity	1997	Lamberton	non-SCN	x1	28.333	0.577	0.333	3	28	29
NIL-79	Maturity	1997	Lamberton	non-SCN	x2	26.333	0.577	0.333	3	26	27
NIL-79	Maturity	1997	Lamberton	non-SCN	x3	26.333	1.155	0.667	3	25	27
NIL-79	Maturity	1997	Lamberton	non-SCN	x4	33	5.657	4	2	29	37
NIL-79	Maturity	1997	Lamberton	non-SCN	x5	29.667	0.577	0.333	3	29	30
NIL-79	Maturity	1997	Waseca	non-SCN	H parent	24.333	2.082	1.202	3	22	26
NIL-79	Maturity	1997	Waseca	non-SCN	R	23	1.852	0.395	22	20	27
NIL-79	Maturity	1997	Waseca	non-SCN	R cultivars	22.833	3.189	1.302	6	20	27
NIL-79	Maturity	1997	Waseca	non-SCN	S	23.909	2.343	0.707	11	22	28

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-79	Maturity	1997	Waseca	non-SCN	S cultivars	17.667	0.577	0.333	3	17	18
NIL-79	Maturity	1997	Waseca	non-SCN	x1	23	2.646	1.528	3	20	25
NIL-79	Maturity	1997	Waseca	non-SCN	x2	21.333	1.528	0.882	3	20	23
NIL-79	Maturity	1997	Waseca	non-SCN	x3	22	2	1.155	3	20	24
NIL-79	Maturity	1997	Waseca	non-SCN	x4	35	0	0	2	35	35
NIL-79	Maturity	1997	Waseca	non-SCN	x5	21	1	0.577	3	20	22
NIL-79	Yield	1998	Lamberton	non-SCN	H parent	17.363	1.235	0.713	3	16.13	18.6
NIL-79	Yield	1998	Lamberton	non-SCN	R	16.628	3.089	0.594	27	11.72	22.15
NIL-79	Yield	1998	Lamberton	non-SCN	R cultivars	35.382	4.55	1.857	6	26.88	39.43
NIL-79	Yield	1998	Lamberton	non-SCN	S	19.504	2.311	0.504	21	15.62	23.74
NIL-79	Yield	1998	Lamberton	non-SCN	S cultivars	34.173	0.693	0.4	3	33.71	34.97
NIL-79	Yield	1998	Lamberton	non-SCN	x1	18.807	1.073	0.619	3	17.58	19.57
NIL-79	Yield	1998	Lamberton	non-SCN	x2	18.063	3.047	1.759	3	15.43	21.4
NIL-79	Yield	1998	Lamberton	non-SCN	x3	17.847	1.359	0.785	3	16.34	18.98
NIL-79	Yield	1998	Lamberton	non-SCN	x4	20.583	4.915	2.838	3	16.67	26.1
NIL-79	Yield	1998	Lamberton	non-SCN	x5	17.13	1.253	0.723	3	15.83	18.33
NIL-79	Yield	1998	Waseca	non-SCN	H parent	13.923	1.219	0.704	3	12.63	15.05
NIL-79	Yield	1998	Waseca	non-SCN	R	16.819	8.155	1.569	27	9.25	41.83
NIL-79	Yield	1998	Waseca	non-SCN	R cultivars	38.107	11.234	4.586	6	18.04	49.22
NIL-79	Yield	1998	Waseca	non-SCN	S	15.164	5.176	1.129	21	0	21.77
NIL-79	Yield	1998	Waseca	non-SCN	S cultivars	28.53	13.548	7.822	3	13.44	39.65
NIL-79	Yield	1998	Waseca	non-SCN	x1	20.36	2.206	1.274	3	18.12	22.53
NIL-79	Yield	1998	Waseca	non-SCN	x2	6.453	6.844	3.951	3	0	13.63
NIL-79	Yield	1998	Waseca	non-SCN	x3	17.453	8.195	4.731	3	9.25	25.64
NIL-79	Yield	1998	Waseca	non-SCN	x4	17.543	7.111	4.105	3	9.35	22.1
NIL-79	Yield	1998	Waseca	non-SCN	x5	17.4	3.167	1.829	3	15	20.99
NIL-79	Yield	1998	Waseca	non-SCN	x2	6.453	6.844	3.951	3	0	13.63
NIL-79	Yield	1998	Waseca	non-SCN	x3	17.453	8.195	4.731	3	9.25	25.64
NIL-79	Yield	1998	Waseca	non-SCN	x4	17.543	7.111	4.105	3	9.35	22.1
NIL-79	Yield	1998	Waseca	non-SCN	x5	17.4	3.167	1.829	3	15	20.99
NIL-79	Yield	1998	Lamberton	SCN	NIL parent	12.022	2.222	1.111	4	9.247	14.488

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-79	Yield	1998	Lamberton	SCN	R	10.91	2.361	0.394	36	7.123	15.16
NIL-79	Yield	1998	Lamberton	SCN	R cultivars	29.434	3.42	1.209	8	23.251	32.82
NIL-79	Yield	1998	Lamberton	SCN	S	10.6	3.444	0.651	28	-0.699	17.848
NIL-79	Yield	1998	Lamberton	SCN	S cultivars	16.397	4.7	2.35	4	11.988	22.445
NIL-79	Yield	1998	Lamberton	SCN	x1	10.537	2.619	1.309	4	8.225	14.085
NIL-79	Yield	1998	Lamberton	SCN	x2	8.924	2.009	1.004	4	6.532	10.779
NIL-79	Yield	1998	Lamberton	SCN	x3	10.255	1.21	0.605	4	8.978	11.827
NIL-79	Yield	1998	Lamberton	SCN	x4	17.358	1.083	0.541	4	15.832	18.252
NIL-79	Yield	1998	Lamberton	SCN	x5	11.908	2.723	1.361	4	8.924	15.268
NIL-79	Yield	1998	Waseca	SCN	NIL parent	14.905	2.987	1.493	4	11.773	17.929
NIL-79	Yield	1998	Waseca	SCN	.R	16.135	2.885	0.481	36	11.075	23.251
NIL-79	Yield	1998	Waseca	SCN	R cultivars	33.872	3.137	1.109	8	28.439	38.385
NIL-79	Yield	1998	Waseca	SCN	S	15.702	3.088	0.584	28	11.693	25.213
NIL-79	Yield	1998	Waseca	SCN	S cultivars	30.327	3.36	1.68	4	27.74	35.267
NIL-79	Yield	1998	Waseca	SCN	x1	15.389	1.554	0.777	4	14.031	17.418
NIL-79	Yield	1998	Waseca	SCN	x2	15.947	2.987	1.494	4	13.548	20.079
NIL-79	Yield	1998	Waseca	SCN	x3	14.125	1.901	0.95	4	11.451	15.725
NIL-79	Yield	1998	Waseca	SCN	x4	26.255	2.906	1.453	4	22.337	29.138
NIL-79	Yield	1998	Waseca	SCN	x5	14.676	3.909	1.954	4	11.101	19.434
NIL-79	Yield	1997	Lamberton	non-SCN	H parent	14.903	0.444	0.256	3	14.41	15.27
NIL-79	Yield	1997	Lamberton	non-SCN	R	14.125	1.804	0.414	19	11.69	17.04
NIL-79	Yield	1997	Lamberton	non-SCN	R cultivars	19.385	4.701	1.919	6	10.64	24.7
NIL-79	Yield	1997	Lamberton	non-SCN	S	13.924	2.249	0.711	10	9.95	17.36
NIL-79	Yield	1997	Lamberton	non-SCN	S cultivars	25.643	1.074	0.62	3	24.94	26.88
NIL-79	Yield	1997	Lamberton	non-SCN	x1	15.383	0.648	0.374	3	14.84	16.1
NIL-79	Yield	1997	Lamberton	non-SCN	x2	13.88	0.932	0.538	3	12.82	14.57
NIL-79	Yield	1997	Lamberton	non-SCN	x3	13.923	2.472	1.427	3	11.1	15.7
NIL-79	Yield	1997	Lamberton	non-SCN	x4	19.315	6.866	4.855	2	14.46	24.17
NIL-79	Yield	1997	Lamberton	non-SCN	x5	14.8	0.462	0.267	3	14.3	15.21
NIL-79	Yield	1997	Waseca	non-SCN	H parent	18.823	3.154	1.821	3	15.24	21.18
NIL-79	Yield	1997	Waseca	non-SCN	R	17.286	2.579	0.55	22	13.41	22.39

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Err.	Count	Minimum	Maximum
NIL-79	Yield	1997	Waseca	non-SCN	R cultivars	24.867	5.386	2.199	6	18.52	33.98
NIL-79	Yield	1997	Waseca	non-SCN	S	17.523	3.754	1.132	11	11.77	25.08
NIL-79	Yield	1997	Waseca	non-SCN	S cultivars	30.033	2.516	1.453	3	27.42	32.44
NIL-79	Yield	1997	Waseca	non-SCN	x1	21.5	3.751	2.165	3	17.71	25.21
NIL-79	Yield	1997	Waseca	non-SCN	x2	19.523	3.242	1.872	3	15.89	22.12
NIL-79	Yield	1997	Waseca	non-SCN	x3	18	4.036	2.33	3	15.64	22.66
NIL-79	Yield	1997	Waseca	non-SCN	x4	13.14	1.824	1.29	2	11.85	14.43
NIL-79	Yield	1997	Waseca	non-SCN	x5	17.373	1.534	0.886	3	15.78	18.84
NIL-79-23	Cysts per plant	1998	Greenhouse	SCN	R	115.05	36.35	5.9	38	37	218
NIL-79-23	Cysts per plant	1998	Greenhouse	SCN	S	252.52	91.77	14.16	42	88	403
NIL-79-23	Cysts per plant	1998	Greenhouse	SCN	R cultivars	36.88	25.85	9.14	8	7	71
NIL-79-23	Cysts per plant	1998	Greenhouse	SCN	S cultivars	339.75	59	29.5	4	276	413
NIL-79-23	Cysts per plant	1998	Lamberton	SCN	H parent	21.467	16.75	9.671	3	3.1	35.9
NIL-79-23	Cysts per plant	1998	Lamberton	SCN	R	11.844	6.673	1.335	25	3.1	28.5
NIL-79-23	Cysts per plant	1998	Lamberton	SCN	R check	9.515	5.582	2.279	6	2.8	17.8
NIL-79-23	Cysts per plant	1998	Lamberton	SCN	S	30.189	21.307	4.027	28	5.9	93.8
NIL-79-23	Cysts per plant	1998	Lamberton	SCN	S check	31.767	21.28	12.286	3	7.2	44.5
NIL-79-23	Cysts per plant	1998	Lamberton	SCN	x	7.4	7.637	5.4	2	2	12.8
NIL-79-23	Cysts per plant	1998	Waseca	SCN	H parent	12.45	8.132	5.75	2	6.7	18.2
NIL-79-23	Cysts per plant	1998	Waseca	SCN	R	5.208	3.548	0.71	25	0.5	12.3
NIL-79-23	Cysts per plant	1998	Waseca	SCN	R check	3.02	2.744	1.227	5	0.8	7.7
NIL-79-23	Cysts per plant	1998	Waseca	SCN	S	15.261	8.098	1.53	28	4.8	35.9
NIL-79-23	Cysts per plant	1998	Waseca	SCN	S check	22.367	0.231	0.133	3	22.1	22.5
NIL-79-23	Cysts per plant	1998	Waseca	SCN	x	10.95	2.192	1.55	2	9.4	12.5
NIL-79-23	Fall Eggs	1998	Lamberton	SCN	H parent	15731	6786.151	3917.986	3	8322	21645
NIL-79-23	Fall Eggs	1998	Lamberton	SCN	R	8814.08	5353.609	1070.722	25	0	19764
NIL-79-23	Fall Eggs	1998	Lamberton	SCN	R check	8037.5	5028.597	2052.916	6	999	15645
NIL-79-23	Fall Eggs	1998	Lamberton	SCN	S	15405.733	10332.605	1886.467	30	0	44938
NIL-79-23	Fall Eggs	1998	Lamberton	SCN	S check	21803.333	14044.193	8108.418	3	7282	35316
NIL-79-23	Fall Eggs	1998	Lamberton	SCN	x	7202	540.459	312.034	3	6626	7698
NIL-79-23	Fall Eggs	1998	Waseca	SCN	H parent	7301	4354.525	2514.086	3	2938	11647

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-79-23	Fall Eggs	1998	Waseca	SCN	R	2301.692	1771.3	347.38	26	0	7339
NIL-79-23	Fall Eggs	1998	Waseca	SCN	R check	5976.5	7868.275	3212.21	6	0	20501
NIL-79-23	Fall Eggs	1998	Waseca	SCN	S	7876.667	6785.52	1238.861	30	479	35127
NIL-79-23	Fall Eggs	1998	Wascea	SCN	S check	17993	4364.967	2520.115	3	12997	21068
NIL-79-23	Fall Eggs	1998	Wascea	SCN	x	6096.5	2499.622	1767.5	2	4329	7864
NIL-79-23	Height	1998	Lamberton	SCN	H parent	34.667	2.309	1.333	3	32	36
NIL-79-23	Height	1998	Lamberton	SCN	R	40.37	4.797	0.923	27	30	48
NIL-79-23	Height	1998	Lamberton	SCN	R check	35.5	2.258	0.922	6	33	39
NIL-79-23	Height	1998	Lamberton	SCN	S	36	4.363	0.797	30	27	44
NIL-79-23	Height	1998	Lamberton	SCN	S check	36.667	5.033	2.906	3	32	42
NIL-79-23	Height	1998	Lamberton	SCN	x	37.667	3.215	1.856	3	34	40
NIL-79-23	Height	1998	Waseca	SCN	H parent	38	3.464	2	3	34	40
NIL-79-23	Height	1998	Waseca	SCN	R	43.259	4.129	0.795	27	35	49
NIL-79-23	Height	1998	Wascea	SCN	R check	37.833	4.119	1.682	6	32	44
NIL-79-23	Height	1998	Wascea	SCN	S	42.867	3.803	0.694	30	36	48
NIL-79-23	Height	1998	Waseca	SCN	S check	41	1.732	1	3	39	42
NIL-79-23	Height	1998	Waseca	SCN	x	42	6	3.464	3	36	48
NIL-79-23	Lodging	1998	Lamberton	SCN	H parent	2.333	0.577	0.333	3	2	3
NIL-79-23	Lodging	1998	Lamberton	SCN	R	2.556	0.506	0.097	27	2	3
NIL-79-23	Lodging	1998	Lamberton	SCN	R check	2.167	0.408	0.167	6	2	3
NIL-79-23	Lodging	1998	Lamberton	SCN	S	2.1	0.305	0.056	30	2	3
NIL-79-23	Lodging	1998	Lamberton	SCN	S check	2.333	0.577	0.333	3	2	3
NIL-79-23	Lodging	1998	Lamberton	SCN	x	2.333	0.577	0.333	3	2	3
NIL-79-23	Lodging	1998	Waseca	SCN	H parent	3.167	0.577	0.333	3	2	3
NIL-79-23	Lodging	1998	Waseca	SCN	R	3.315	0.557	0.107	27	2	45
NIL-79-23	Lodging	1998	Waseca	SCN	R check	3	0.632	0.258	6	2.5	4
NIL-79-23	Lodging	1998	Waseca	SCN	S	3.15	0.438	0.08	30	2.5	4
NIL-79-23	Lodging	1998	Waseca	SCN	S check	2.833	0.289	0.167	3	2.5	3
NIL-79-23	Lodging	1998	Waseca	SCN	x	3.167	0.764	0.441	3	2.5	4
NIL-79-23	Maturity	1998	Lamberton	SCN	H parent	23	1.732	1	3	22	25
NIL-79-23	Maturity	1998	Lamberton	SCN	R	25.963	3.18	0.612	27	20	30

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-79-23	Maturity	1998	Lamberton	SCN	R check	21.833	0.983	0.401	6	20	23
NIL-79-23	Maturity	1998	Lamberton	SCN	S	25.7	3.007	0.549	30	20	30
NIL-79-23	Maturity	1998	Lamberton	SCN	S check	17.333	2.309	1.333	3	16	20
NIL-79-23	Maturity	1998	Lamberton	SCN	X	24	2.646	1.528	3	22	27
NIL-79-23	Maturity	1998	Waseca	SCN	H parent	14	0	0	3	14	14
NIL-79-23	Maturity	1998	Waseca	SCN	R	14.074	0.267	0.051	27	14	15
NIL-79-23	Maturity	1998	Waseca	SCN	R check	14.667	0.816	0.333	6	14	16
NIL-79-23	Maturity	1998	Waseca	SCN	S	14.533	0.973	0.178	30	14	18
NIL-79-23	Maturity	1998	Waseca	SCN	S check	15.667	0.577	0.333	3	15	16
NIL-79-23	Maturity	1998	Waseca	SCN	X	14.333	0.577	0.333	3	14	15
NIL-79-23	Reproduction Factor	1998	Lamberton	SCN	H parent	0.937	0.342	0.197	3	0.64	1.31
NIL-79-23	Reproduction Factor	1998	Lamberton	SCN	R	0.816	1.393	0.279	25	0	7.04
NIL-79-23	Reproduction Factor	1998	Lamberton	SCN	R check	1.108	1.269	0.518	6	0.09	2.83
NIL-79-23	Reproduction Factor	1998	Lamberton	SCN	S	1.343	1.164	0.216	29	0	4.93
NIL-79-23	Reproduction Factor	1998	Lamberton	SCN	S check	1.51	0.888	0.513	3	0.5	2.17
NIL-79-23	Reproduction Factor	1998	Lamberton	SCN	X	0.47	0.197	0.114	3	0.31	0.69
NIL-79-23	Reproduction Factor	1998	Waseca	SCN	H parent	8.553	6.695	3.865	3	0.83	12.7
NIL-79-23	Reproduction Factor	1998	Waseca	SCN	R	2.279	2.521	0.504	25	0	11.17
NIL-79-23	Reproduction Factor	1998	Waseca	SCN	R check	1.822	1.895	0.774	6	0	4.16
NIL-79-23	Reproduction Factor	1998	Waseca	SCN	S	7.839	7.775	1.469	28	0.17	25.48
NIL-79-23	Reproduction Factor	1998	Waseca	SCN	S check	10.285	4.561	3.225	2	7.06	13.51
NIL-79-23	Reproduction Factor	1998	Waseca	SCN	X	3.7	0.028	0.02	2	3.68	3.72
NIL-79-23	Spring Eggs	1998	Lamberton	SCN	H parent	17104	6976.839	4028.08	3	13032	25160
NIL-79-23	Spring Eggs	1998	Lamberton	SCN	R	21528	14362.832	2816.783	26	2808	55264
NIL-79-23	Spring Eggs	1998	Lamberton	SCN	R check	18700	23582.178	9627.384	6	4352	66048
NIL-79-23	Spring Eggs	1998	Lamberton	SCN	S	14626.759	9471.942	1758.896	29	4000	43080
NIL-79-23	Spring Eggs	1998	Lamberton	SCN	S check	14672	4217.116	2434.753	3	10512	18944
NIL-79-23	Spring Eggs	1998	Lamberton	SCN	X	17160	7176.321	4143.251	3	10512	24768
NIL-79-23	Spring Eggs	1998	Waseca	SCN	H parent	1690.667	1609.598	929.302	3	576	3536
NIL-79-23	Spring Eggs	1998	Waseca	SCN	R	1576	1286.252	247.539	27	0	5304
NIL-79-23	Spring Eggs	1998	Waseca	SCN	R check	2241.333	1530.435	624.797	6	720	4928

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-79-23	Spring Eggs	1998	Waseca	SCN	S	1636	1243.174	230.852	29	0	5372
NIL-79-23	Spring Eggs	1998	Waseca	SCN	S check	1133.333	991.43	572.402	3	0	1840
NIL-79-23	Spring Eggs	1998	Waseca	SCN	x	1752	504	290.985	3	1176	2112
NIL-79-23	Stand count	1998	Lamberton	SCN	H parent	136.333	16.743	9.667	3	117	146
NIL-79-23	Stand count	1998	Lamberton	SCN	R	140.87	21.93	4.573	23	110	178
NIL-79-23	Stand count	1998	Lamberton	SCN	R check	147.833	24.02	9.806	6	117	178
NIL-79-23	Stand count	1998	Lamberton	SCN	S	148.267	20.621	3.765	30	117	188
NIL-79-23	Stand count	1998	Lamberton	SCN	S check	138.667	6.351	3.667	3	135	146
NIL-79-23	Stand count	1998	Lamberton	SCN	x	134	1.732	1	3	132	135
NIL-79-23	Stand count	1998	Waseca	SCN	H parent	146	0	0	3	146	146
NIL-79-23	Stand count	1998	Waseca	SCN	R	142.5	23.556	4.808	24	110	178
NIL-79-23	Stand count	1998	Waseca	SCN	R check	148	17.527	7.155	6	132	164
NIL-79-23	Stand count	1998	Waseca	SCN	S	147.2	22.398	4.089	30	117	188
NIL-79-23	Stand count	1998	Waseca	SCN	S check	135	0	0	3	135	135
NIL-79-23	Stand count	1998	Waseca	SCN	x	135	0	0	3	135	135
NIL-79-23	Yield	1998	Lamberton	SCN	H parent	9.703	1.759	1.015	3	7.69	10.94
NIL-79-23	Yield	1998	Lamberton	SCN	R	13.173	2.076	0.4	27	6.99	16.26
NIL-79-23	Yield	1998	Lamberton	SCN	R check	32.697	2.389	0.975	6	30.11	34.97
NIL-79-23	Yield	1998	Lamberton	SCN	S	11.81	2.177	0.397	30	7.37	16.4
NIL-79-23	Yield	1998	Lamberton	SCN	S check	22.347	4.535	2.618	3	18.14	27.15
NIL-79-23	Yield	1998	Lamberton	SCN	x	9.93	1.925	1.111	3	7.82	11.59
NIL-79-23	Yield	1998	Waseca	SCN	H parent	19.19	0.65	0.375	3	18.79	19.94
NIL-79-23	Yield	1998	Waseca	SCN	R	19.33	3.01	0.579	27	13.87	27.36
NIL-79-23	Yield	1998	Waseca	SCN	R check	38.307	5.81	2.372	6	32.98	49.73
NIL-79-23	Yield	1998	Waseca	SCN	S	18.252	2.753	0.503	30	13.22	27.26
NIL-79-23	Yield	1998	Waseca	SCN	S check	24.893	9.021	5.208	3	15.94	33.98
NIL-79-23	Yield	1998	Waseca	SCN	x	19.92	3.109	1.795	3	16.91	23.12
NIL-79-23	Height	1998	Lamberton	non-SCN	NIL parent	44.5	0.707	0.5	2	44	45
NIL-79-23	Height	1998	Lamberton	non-SCN	R	44.444	3.929	0.926	18	37	48
NIL-79-23	Height	1998	Lamberton	non-SCN	R cultivars	41.25	1.708	0.854	4	39	43
NIL-79-23	Height	1998	Lamberton	non-SCN	S	45.75	2.074	0.464	20	40	48

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-79-23	Height	1998	Lamberton	non-SCN	S cultivars	47.5	0.707	0.5	2	47	48
NIL-79-23	Height	1998	Lamberton	non-SCN	x	45.5	2.121	1.5	2	44	47
NIL-79-23	Height	1998	Waseca	non-SCN	NIL parent	43	1.414	1	2	42	44
NIL-79-23	Height	1998	Waseca	non-SCN	R	40.556	4.488	1.058	18	32	50
NIL-79-23	Height	1998	Waseca	non-SCN	R cultivars	36.5	1	0.5	4	36	38
NIL-79-23	Height	1998	Waseca	non-SCN	S	40.3	1.867	0.417	20	36	44
NIL-79-23	Height	1998	Waseca	non-SCN	S cultivars	39	1.414	1	2	38	40
NIL-79-23	Height	1998	Waseca	non-SCN	x	39	1.414	1	2	38	40
NIL-79-23	Height	1997	Rosemount	non-SCN	R	34.308	2.81	0.779	13	26	37
NIL-79-23	Height	1997	Rosemount	non-SCN	S	32.765	2.047	0.497	17	28	36
NIL-79-23	Lodging	1998	Lamberton	non-SCN	NIL parent	2.5	0.707	0.5	2	2	3
NIL-79-23	Lodging	1998	Lamberton	non-SCN	R	3.111	0.323	0.076	18	3	4
NIL-79-23	Lodging	1998	Lamberton	non-SCN	R cultivars	2.75	0.957	0.479	4	2	4
NIL-79-23	Lodging	1998	Lamberton	non-SCN	S	3	0	0	0	20	3
NIL-79-23	Lodging	1998	Lamberton	non-SCN	S cultivars	4	0	0	0	2	4
NIL-79-23	Lodging	1998	Lamberton	non-SCN	x	3	0	0	0	2	3
NIL-79-23	Lodging	1998	Waseca	non-SCN	NIL parent	3	0	0	0	2	3
NIL-79-23	Lodging	1998	Waseca	non-SCN	R	3	0	0	0	18	3
NIL-79-23	Lodging	1998	Waseca	non-SCN	R cultivars	2.5	0.577	0.289	4	2	3
NIL-79-23	Lodging	1998	Waseca	non-SCN	S	3	0	0	0	20	3
NIL-79-23	Lodging	1998	Waseca	non-SCN	S cultivars	3	0	0	0	2	3
NIL-79-23	Lodging	1998	Waseca	non-SCN	x	3	0	0	0	2	3
NIL-79-23	Lodging	1997	Rosemount	non-SCN	NIL parent	3.5	0.707	0.5	2	3	4
NIL-79-23	Lodging	1997	Rosemount	non-SCN	R	2.833	0.577	0.167	12	2	4
NIL-79-23	Lodging	1997	Rosemount	non-SCN	R cultivars	2.5	0.577	0.289	4	2	3
NIL-79-23	Lodging	1997	Rosemount	non-SCN	S	2.824	0.728	0.176	17	2	4
NIL-79-23	Lodging	1997	Rosemount	non-SCN	S cultivars	2.5	0.707	0.5	2	2	3
NIL-79-23	Lodging	1998	Lamberton	non-SCN	NIL parent	21.5	0.707	0.5	1	2	2
NIL-79-23	Maturity	1998	Lamberton	non-SCN	R	21.611	0.778	0.183	18	20	23
NIL-79-23	Maturity	1998	Lamberton	non-SCN	R cultivars	21	0.816	0.408	4	20	22

NIL set	Trait	Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-79-23	Maturity	1998	Lamberton	non-SCN	S	21.45	0.686	0.153	20	21	23
NIL-79-23	Maturity	1998	Lamberton	non-SCN	S cultivars	16	0	0	2	16	16
NIL-79-23	Maturity	1998	Lamberton	non-SCN	x	21	0	0	2	21	21
NIL-79-23	Maturity	1998	Waseca	non-SCN	NIL parent	13.5	0.707	0.5	2	13	14
NIL-79-23	Maturity	1998	Waseca	non-SCN	R	14	1.237	0.291	18	13	17
NIL-79-23	Maturity	1998	Waseca	non-SCN	R cultivars	15.25	0.957	0.479	4	14	16
NIL-79-23	Maturity	1998	Waseca	non-SCN	S	14	0.649	0.145	20	13	15
NIL-79-23	Maturity	1998	Waseca	non-SCN	S cultivars	15.5	0.707	0.5	2	15	16
NIL-79-23	Maturity	1998	Waseca	non-SCN	x	14	0	0	2	14	14
NIL-79-23	Maturity	1997	Rosemount	non-SCN	NIL parent	32	0	0	2	32	32
NIL-79-23	Maturity	1997	Rosemount	non-SCN	R	33.083	1.084	0.313	12	31	35
NIL-79-23	Maturity	1997	Rosemount	non-SCN	R cultivars	35.333	0.577	0.333	3	35	36
NIL-79-23	Maturity	1997	Rosemount	non-SCN	S	33.706	1.312	0.318	17	32	36
NIL-79-23	Maturity	1997	Rosemount	non-SCN	S cultivars	32	0	0	2	32	32
NIL-79-23	Maturity	1997	Rosemount	non-SCN	x	32	.	.	1	32	32
NIL-79-23	Yield	1998	Lamberton	non-SCN	NIL parent	15.7	0.495	0.35	2	15.35	16.05
NIL-79-23	Yield	1998	Lamberton	non-SCN	R	19.588	2.387	0.563	18	15.08	23.12
NIL-79-23	Yield	1998	Lamberton	non-SCN	R cultivars	36.685	3.99	1.995	4	34.35	42.63
NIL-79-23	Yield	1998	Lamberton	non-SCN	S	19.302	1.902	0.425	20	15.24	22.31
NIL-79-23	Yield	1998	Lamberton	non-SCN	S cultivars	33.075	3.741	2.645	2	30.43	35.72
NIL-79-23	Yield	1998	Lamberton	non-SCN	x	17.915	0.021	0.015	2	17.9	17.93
NIL-79-23	Yield	1998	Waseca	non-SCN	NIL parent	14.27	3.422	2.42	2	11.85	16.69
NIL-79-23	Yield	1998	Waseca	non-SCN	R	15.508	2.551	0.601	18	11.34	20.03
NIL-79-23	Yield	1998	Waseca	non-SCN	R cultivars	32.06	7.391	3.695	4	22.01	37.98
NIL-79-23	Yield	1998	Waseca	non-SCN	S	16.809	3.135	0.701	20	11.85	24.76
NIL-79-23	Yield	1998	Waseca	non-SCN	S cultivars	30.805	5.664	4.005	2	26.8	34.81
NIL-79-23	Yield	1998	Waseca	non-SCN	x	12.94	1.047	0.74	2	12.2	13.68
NIL-79-23	Yield	1997	Rosemount	non-SCN	NIL parent	29.205	17.996	12.725	2	16.48	41.93
NIL-79-23	Yield	1997	Rosemount	non-SCN	R	33.841	6.146	1.774	12	17.56	39.64
NIL-79-23	Yield	1997	Rosemount	non-SCN	R cultivars	48.885	6.572	3.286	4	43.58	57.63
NIL-79-23	Yield	1997	Rosemount	non-SCN	S	32.965	7.797	1.891	17	17.42	44.37

NIL set	Trait		Year	Location	Site	Group	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum
NIL-79-23	Yield		1997	Rosemount	non-SCN	S cultivars	58.525	8.973	6.345	2	52.18	64.87
NIL-79-23	Yield		1997	Rosemount	non-SCN	x	33.98	.	.	1	33.98	33.98

**Table A-9. Cyst count data for control cultivars used in the greenhouse and field SCN resistance assays for the NIL-49 subset and NIL-79-23**

Location	NIL set(s)	Cultivar	Reaction to SCN	Mean	Standard Deviation
Greenhouse	NIL-49 subset and NIL-79-23 <sup>†</sup>	Faribault	Resistant	60	12
Greenhouse	NIL-49 subset and NIL-79-23 <sup>†</sup>	Parker	Susceptible	340	59
Greenhouse	NIL-49 subset and NIL-79-23 <sup>†</sup>	PI 209332	Resistant	14	6
Field 1998	NIL-79-23	Bell	Resistant	8	7
Field 1998	NIL-79-23	Faribault	Resistant	5	3
Field 1998	NIL-79-23	Parker	Susceptible	27	14
Field 1997	NIL-49 subset	Evans	Susceptible	72	94
Field 1997	NIL-49 subset	Freeborn	Resistant	6	5
Field 1997	NIL-49 subset	Parker	Susceptible	95	84
Field 1998	NIL-49 subset	Faribault	Resistant	7	8
Field 1998	NIL-49 subset	Parker	Susceptible	30	14

<sup>†</sup> Both NIL sets were included in the assay along with one set of checks.

Table A-10. Cyst count data for control cultivars used in greenhouse assays that were compared to marker-assisted selection for SCN resistance

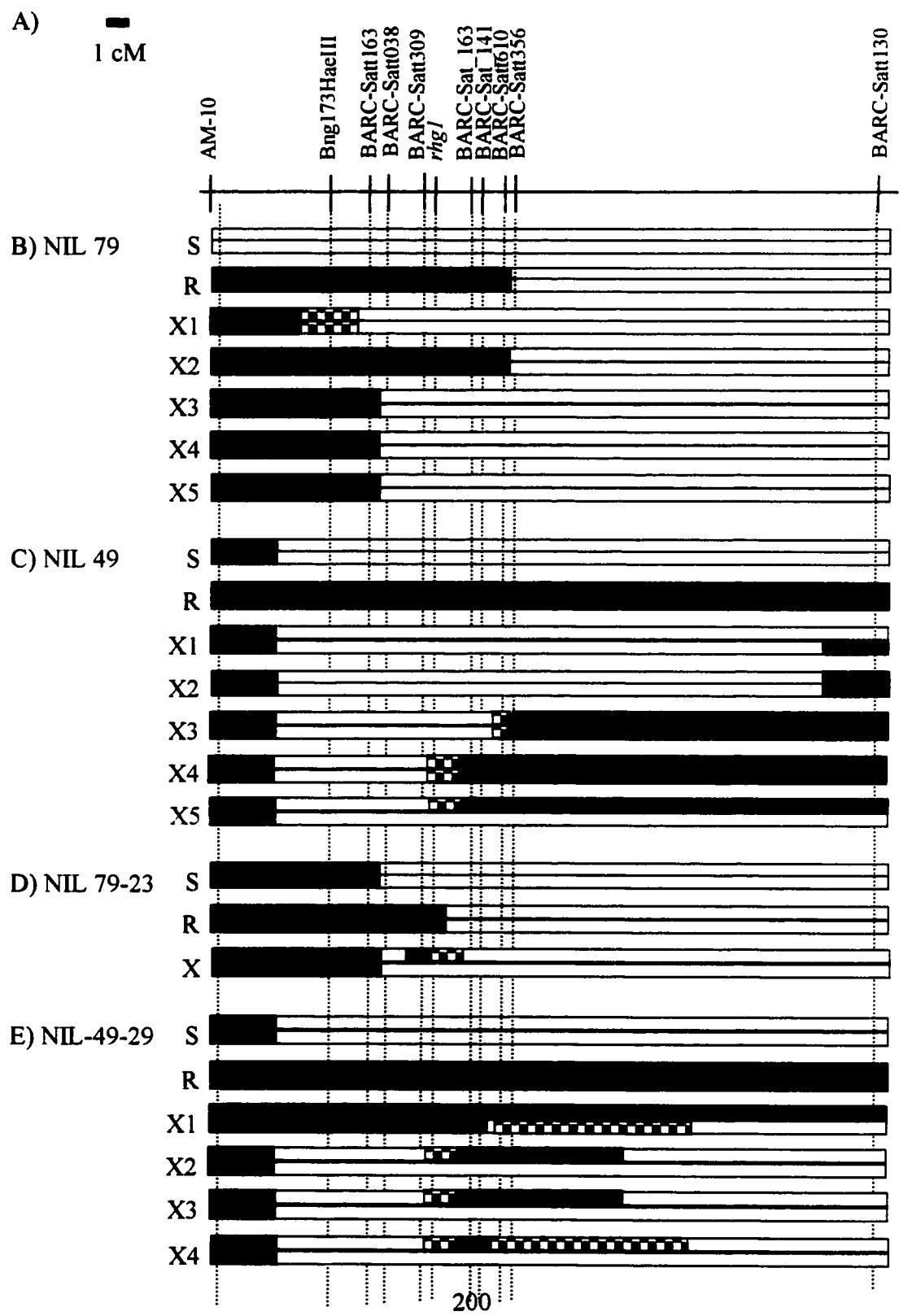
Cultivar	SCN reaction	Greenhouse run <sup>†</sup>	Cyst count:		Cyst index <sup>‡</sup>
			Mean	Standard deviation	
Evans	Susceptible	1	140.0	14.5	1.00
		2	116.4	15.5	1.00
		3	133.0	30.7	1.00
		4	305.2	13.8	1.00
		5	205.6	76.2	1.00
		Overall	181.7	80.4	1.00
PI 209332	Resistant	1	5.0	2.8	0.04
		2	5.5	3.1	0.05
		3	4.8	1.7	0.04
		4	11.5	5.4	0.04
		5	16.5	8.2	0.08
		Overall	8.7	6.4	0.05
Peking	Resistant	1	0	0	0.00
		2	0	0	0.00
		3	1.5	1.7	0.01
		4	2.0	3.4	0.01
		5	0.3	0.5	0.00
		Overall	0.8	1.7	0.00
PI 88788	Resistant	1	5.5	1.3	0.04
		2	8.5	2.4	0.07
		3	5.3	2.8	0.04
		4	18.8	12.1	0.06
		5	16.5	4.8	0.08
		Overall	10.9	7.9	0.06
PI 90763	Resistant	1	0.8	1.5	0.01
		2	0	0	0.00
		3	0.3	0.5	0.00
		4	0	0	0.00
		5	0	0	0.00
		Overall	0.2	0.7	0.00
Pickett	Resistant	1	5	4.3	0.04
		2	2.8	2.1	0.02
		3	7.0	4.2	0.05
		4	6.8	3.0	0.02
		5	3.8	1.9	0.02
		Overall	5.1	3.4	0.03

<sup>†</sup>The first three greenhouse runs used inoculum from the same increase. The fourth and fifth runs used inoculum increased during the first and second runs, respectively.

<sup>‡</sup>Average cyst count divided by the average cyst count of Evans.

**Figure A-1. Genotypes of R lines, S lines, and crossovers in the NIL sets**

A) Genetic map of the region on MLG-G near *rhg1*, a major gene for SCN resistance. B-E) Representations of the genotypes for the four NIL sets showing the genotype of R lines, S lines, and lines with crossovers within the interval of segregation (X). Crossover points are not known exactly but have usually been narrowed down to an interval between markers. In some cases, the crossover point has been narrowed down to the two intervals, shown with a checkered line. Genotypes at some of the markers were inferred from the parent line.



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Lange, D.A., S. Peñuela, R.L. Denny, J. Mudge, V.C. Concibido, J.H. Orf, N.D. Young. 1998. A plant DNA isolation protocol suitable for polymerase chain reaction based marker-assisted breeding. *Crop Science* 38:217-220.

Mudge, J., P.B. Cregan, J.P. Kenworthy, W.J. Kenworthy, J.H. Orf, and N.D. Young. 1997. Two microsatellite markers that flank the major soybean cyst nematode resistance locus. *Crop Science* 37:1611-1615.

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>resistance conditioned by the rhg1 locus. Crop Science. In press.  
>>  
>>Lange, D.A., S. Peñuela, R.L. Denny, J. Mudge, V.C. Concibido, J.H. Orf,  
>N.D. Young. 1998. A plant DNA isolation protocol suitable for polymerase  
>chain reaction based marker-assisted breeding. Crop Science 38:217-220.  
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>>Mudge, J., P.B. Cregan, J.P. Kenworthy, W.J. Kenworthy, J.H. Orf, and N.D.  
>Young. 1997. Two microsatellite markers that flank the major soybean cyst  
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> R.C.  
> Shoemaker, B.F. Matthews, T. Jarvik, and N.D. Young. Targeted isolation of

> simple sequence repeat markers through the use of bacterial artificial  
> chromosomes. Theoretical and Applied Genetics 98:919-928.  
>  
> Danesh, D., S. Peñuela, J. Mudge, R. Denny, H. Nordstrom, J.P. Martinez,  
> and N.D. Young. 1998. A bacterial artificial chromosome library for  
> soybean  
> and identification of clones near a major cyst nematode resistance gene.  
> Theoretical and Applied Genetics 96:196-202.

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