HESSIAN FLY RESISTANCE OF TRITICUM DURUM DERIVIVED SOFT WINTER WHEAT

by

KATHRYN HARMAN BOST

(Under the Direction of Jerry Johnson)

ABSTRACT

Hessian fly (*Mayetiola destructor* Say) is one of the most destructive pests of wheat (*Triticum aestivum* L.) worldwide. The objectives are to characterize Hessian fly resistance from IN97219-A3-5 and identify linked molecular markers to facilitate marker assisted selection. IN97219-A3-5 derived biotype L resistance from *Triticum durum*, PI 323440, and was crossed to AGS 2000, susceptible to biotype L. The $F_{2:3}$ progeny demonstrated a ratio of 3 Resistant/Segregating : 1 Susceptible (67R/Seg:24S). Hessian fly resistance segregated as a single dominant gene in this population. IN97219 is flanked by simple sequence repeat (SSR) markers *Xgwm234* at 8.7 cM and *Xwmc149* at 19.5 cM on the 5BS chromosome. A planting date study was conducted at two locations (Griffin and Plains, GA) and three dates. A growth chamber study was conducted at three temperatures (15°C, 18°C, and 23°C). Results from these studies revealed that IN97219 resistance is controlled by a temperature sensitive gene.

INDEX WORDS: *Triticum aestivum*, wheat, *Mayetiola destructor*, Hessian fly, *Triticum durum*, marker assisted selection, simple sequence repeats, SSR, temperature sensitive

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DEDICATION

I would wish to dedicate this work to my parents, Claude and Mary Anne Harman, my sister, Merriman Harman. Y'all have given so much support, encouragement, prayers, and love all my life. I would not be where I am today without you. I would also dedicate this work to my friend and husband, Chuck. Thank you for your patience and support. I would also like to thank all of the teachers, friends, and family who have believed in me.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LITERATURE REVIEW

Hessian fly (*Mayetiola destructor* Say) is one of the most destructive pest of wheat (*Triticum aestivum* L.) worldwide (Liu et al., 2005) in such parts as North America, north Africa and southern Europe (El-Bouhssini et al., 1996). It followed wheat to southern Europe as wheat was brought from its original habitat of southwest Asia (Briggle et al., 1982). During the American Revolution, Hessian fly is believed to have entered the United States through the Hessian soldiers' straw bedding. In 1779 it was recorded infesting wheat on Long Island, New York (Gallun, 1977; Naber et al., 2000). Hessian fly has spread to all major wheat growing areas from the Atlantic Coast to the Great Plains. Outbreaks of Hessian fly are sporadic and typically cause more local or regional damage even though pervasive outbreaks have occurred (Berzonsky et al., 2003).

The Hessian fly is capable of infesting and injuring all classes of wheat. Less preferred hosts include barley (*Hordeum vulgare*), triticale (*Triticale hexaploide*), and wild grasses such as *Aegilops* sp., *Agropyron repens* (L.), *A. smithii* Rybd., and *Elmus virginicus* L. (Jones, 1936). Yield losses due to Hessian fly were 36% in near isogenic lines of bread wheat and 42% when using the Furadan 5G insecticide as a control (El-Bouhssini et al., 1996). In durum wheat (*T. turgidum* L. var. *durum*) the losses have been reported at 32% in Morocco (Liu et al., 2005; Zaharieva et al., 2001), while farmers in the U.S. usually sustain 5% to 10% annual losses of wheat production (Buntin, 1999; Liu et al., 2005). In a single year in the United States, the damage caused by the Hessian fly has been estimated as great as $100 million

(Cartwright and Jones, 1953). In Georgia losses were estimated at $4 million in 1986 and $28 million in 1989 (Hudson et al., 1991; Hudson et al., 1988).

The Hessian fly is a gall midge insect whose lifecycle begins in winter wheat areas with the fall emergence of adults from infested wheat or wheat stubble. After the non-feeding adults mate, the female lays 200 to 300 eggs on the upper leaf surface of young wheat plants. Within a few hours of hatching from eggs, the larvae crawl down the leaf blade to the crown. The larvae, which cause all the feeding injury, feed on the lower surface of the youngest leaf sheaths. The Hessian fly has three instar larval stages but only the first instar is mobile. The first instar larvae use specialized mandibles to attack plant cells (Hatchett et al., 1990). Nutritive tissue is created at the base of the leaf and is associated with nuclear breakdown, degradation of cytoplasmic organelles, and an increase in the number and size of vacuoles. As a result of the breakdown of plant cell contents, the larvae are able to harvest cells in the nutritive tissue by sucking the contents which move from surrounding cells through now permeable cell walls (Harris et al., 2006). Before the onset of cold weather, most larvae are fully grown. By the end of the feeding and growth of the second instar larva, a protective puparium forms where the third instar and pupa develop. The Hessian fly overwinters in the puparium. In the following spring the pupa completes development and emerges as an adult. The adults infest the wheat plants as they begin to joint. The spring generation larvae are found just above the nodes between the leaf sheaths and stems (Gallun, 1977).

Since a portion of puparia of each generation does not emerge for several months or years, Hessian fly generations are more accurately termed broods (Buntin and Chapin, 1990). The number of broods that emerge can vary from regions within a state. In northern Georgia (Piedmont), Hessian fly has two to three broods in the fall with a single spring brood. The

southern region of Georgia (Coastal Plain) also has the two to three fall broods with a winter brood and one to two broods in the spring (Buntin et al., 1990).

The damage of the Hessian fly reduces both quantity and quality of wheat grain (Berzonsky et al., 2003). Younger leaves, internal to the leaf directly attacked by the Hessian fly, display abnormal development of stunting or sink effects (Harris et al., 2006). In some wheat plants the infested leaves are shorter and darker green. Susceptible wheat plants turn dark green due to chloroplast buildup in the leaves (Gallun, 1977). Infested plants also show a reduction in root and stem number and in foliar and root weights (Wellso et al., 1989). Hessian fly damage is diagnosed in seedlings with these morphological differences by peeling the leaves down to its attachment to the stem and observing larvae resembling flaxseed (dark reddish-brown). Further yield loss occurs in the winter because Hessian fly feeding reduces the winter hardiness of the plants that survive the fall infestation (Berzonsky et al., 2003). In the spring as the adults emerge and re-infest plants that survived the fall infestation, larvae move down the leaf to the node of the stem and begin feeding. Feeding at the node weakens the stem above the node resulting in lodging, smaller heads, shriveled kernels, and less kernels per spike (Gallun, 1977). It is probable that the stem will break before harvest due to the weakening at the node of the stem resulting in a thinner crop stand (Harris et al., 2004).

A gene-for-gene interaction exists between wheat and Hessian fly resulting in either a compatible or incompatible interaction (El-Bouhssini et al., 1996; Subramanyam et al., 2006). When the interaction is compatible, the wheat plant is susceptible to Hessian fly and the symptoms become irreversible after 4-5 days of virulent larval feeding. The incompatible interaction is where the wheat plant is resistant resulting in the death of larvae within 3-5 days of hatching (Subramanyam et al., 2006). Berzonsky et al. (2003) summarized the gene-for-gene

interaction by stating that "a biotype can only be virulent to a wheat cultivar if it is homozygous for recessive virulence genes at loci corresponding to loci at which the wheat plant has resistance genes."

A population within a species that differ in the ability to feed on the host plant is referred to as a biotype (Gallun and Khush, 1980). The 16 biotypes of Hessian fly are identified on the basis of virulence or avirulence to four wheat differentials with resistance genes H3, H5, H6, or H7H8 combination (Gallun, 1977; Gallun et al., 1961). The biotypes are the Great Plains (GP) and A through O; GP biotype is the least virulent and the L biotype is the most virulent (Gallun et al., 1961; Sosa, 1978). Table 0.1 describes the biotype reactions to the four differentials and a cultivar with no resistance gene.

Table 0.1—Reaction of differential cultivars to Hessian fly biotypes. (Sosa, 1978)
R = resistant; S = susceptible

	Wheat cultivars and genes for Hessian fly resistance				
Biotypes of Hessian fly	Blueboy (None)	Seneca H7H8	Monon H3	Knox 62 H6	Abe H5
GP	S	R	R	R	R
A	S	S	R	R	R
B	S	S	S	R	R
C	S	S	R	S	R
D	S	S	S	S	R
E	S	R	S	R	R
F	S	R	R	S	R
G	S	R	S	S	R
H	S	R	R	R	S
I	S	S	R	R	S
J	S	S	S	R	S
K	S	S	R	S	S
L	S	S	S	S	S
M	S	R	S	R	S
N	S	R	R	S	S
O	S	R	S	S	S

Saltzmann et al. (2008) discusses three different possibilities for susceptibility of wheat to Hessian fly. The first possibility is that the wheat plant does not initiate a defense response against the virulent Hessian fly larvae. Alternately, only a partial defense response is initiated. The last explanation is that the wheat defensive mechanisms are actively suppressed by the virulent feeding of the Hessian fly larvae (Saltzmann et al., 2008).

Wheat resistance to Hessian fly feeding is mostly controlled by dominant alleles but sometimes by partially dominant alleles (El-Bouhssini et al., 2001; Harris et al., 2006). Since the first instar larvae of avirulent biotypes die after feeding on resistant plants, the resistance mechanism in wheat is antibiosis (El-Bouhssini et al., 1996; Hatchett and Gallun, 1970; Subramanyam et al., 2006). This mechanism results in shortened infestation or the reduction in growth and/or development of the insect (Acquaah, 2007). There is support for the hypothesis that the phenotypic basis for resistance is hypersensitivity involving "recognition" of a Hessian fly avirulent gene product or process (Berzonsky et al., 2003). In 2007, Giovanini et al. characterized *Hfr-3*, a novel gene encoding a lectin-like protein which is associated with resistance against Hessian fly. Resistance is suggested to be localized instead of systemic. The evident starvation of first-instar larvae may be caused by anti-nutritional proteins such as the lectin encoded by the *Hfr-3* gene (Giovanini et al., 2007). Hessian fly resistance may also exhibit antixenosis (Berzonsky et al., 2003), the non-preference mechanism in which chemical or morphological characteristics make the host unattractive to the insect for ovipostion, feeding or shelter (Acquaah, 2007). There is an observed association between resistance and leaf pubescence (very small hair-like projections). Leaf pubescence reduces oviposition by females, egg hatching and larval establishment compared to glabrous leaves (Roberts et al., 1979).

Resistance is mostly controlled by dominant alleles; however, several examples exist of resistance genes and cultivars that are affected by temperature (Bouhssini et al., 1999; Cartwright et al., 1946; Maas et al., 1987; Obanni et al., 1989; Sosa and Foster, 1976; Tyler and Hatchett, 1983). Cartwright et al. reported in 1946 that the resistant line W38 had higher percentages of infestations at higher temperatures. W38 had a mean percent infestation of 35.8% at the warm temperature range of 24-27°C and 4.8% infestation at the cool range of 16-18°C (Cartwright et al., 1946). Sosa and Foster (1976) demonstrated that cultivars with different cultivars respond differently at different temperatures to specific biotypes of Hessian fly. 'Arthur 71' with the *H5* gene has higher percent infestation at higher temperatures for Biotypes GP and D although it is the same at all temperatures against Biotype C (Sosa and Foster, 1976). Tyler and Hatchett (1983) confirmed Sosa and Foster's report that 'Arthur 71' was not affected at 18°C but had reduced resistance at the higher temperatures of 23, 28, and 31°C. They also showed that even cultivars that were not affected at 23°C were affected at 31°C. 'Elva' (*H9H10*) had 100.0% resistant plants at 23°C and the significantly lower 89.2% resistant at 31°C (Tyler and Hatchett, 1983). Therefore, all resistance is reduced at high temperatures. 'Marquillo' is an effectively used cultivar with a temperature sensitive gene (*H18*) that is effective at 16°C but is completely susceptible at 20°C (Maas et al., 1987). Cultivars with *H1H1H2H2* and *H7H7H8H8* have higher percent of resistant plants with live larvae at higher temperatures (Bouhssini et al., 1999).

Since brief periods of above-normal temperatures are common in the fall and spring in the southern Great Plains, cultivars with temperature sensitive genes for Hessian fly resistance could be a potential hazard (Tyler and Hatchett, 1983). Obanni et al. (1989) also stated that sources of resistance that are stable at high temperatures may be more valuable in areas where Hessian fly is a serious pest. Alternatively temperature-sensitive genes may also provide a more

durable source of resistance (Bouhssini et al., 1999; Maas et al., 1987). Temperature-sensitivity may reduce selection for virulent biotypes and therefore maintain avirulent Hessian flies (Maas et al., 1987). The virulence within the Hessian fly population will be diluted by the avirulent flies which may will reduce the rate of virulent biotype development (Bouhssini et al., 1999).

In North America wheat is not intensively managed. In 1996, only 3 percent of spring and winter wheat production areas were treated with insecticides (Berzonsky et al., 2003). Since insecticides are expensive and not normally used on wheat, the most practical control method is resistant cultivars (El-Bouhssini et al., 1996). Thirty-two Hessian fly-resistance genes have been identified from wheat and its relatives and have been designated *H1-H32* (Subramanyam et al., 2006). These resistance genes have come from common wheat, *Aegilops tsauschii*, *Triticum turgidum* ssp. *durum*, *Ae. ventricosa*, *Ae. triuncialis*, *T. turgidum* ssp. *dicoccum*, and rye. The resistance genes derived from *T. turgidum* ssp. *durum* are *H6, H9, H10, H11, H14, H15, H16, H17, H18, H19, H20, H28, H29, H31* (Liu et al., 2005). New sources of Hessian fly resistance in different species are actively being researched. Zaharieva et al. (2001) found resistance in *Aegilops geniculata* Roth, a wild wheat relative. From the 17 accessions of *A. geniculata* tested, seven were resistant to the Hessian fly. The seven resistant accessions are now being used in CIMMYT/ICARDA Durum Wheat program (Zaharieva et al., 2001). The accessions must be tested to determine if and how the resistance is inherited. Then the resistance must be transferred into wheat through breeding or through genetic manipulation.

In breeding for resistance in wheat to the Hessian fly, the gene-for-gene interaction must be understood to create a durable resistance in wheat. Most resistance genes have been released as cultivars with only one resistance gene (Berzonsky et al., 2003). Since the use of single resistance genes with high levels of resistance exerts strong selection pressure on the Hessian fly

population, major shifts in biotype composition occur. Consequently the biotype population which survives is virulent to the resistant wheat (Berzonsky et al., 2003; El-Bouhssini et al., 1996). A shift in biotype composition has been reported in the eastern United States from the mid-1980s to the mid-1990s to a majority of the biotype L which is virulent to the *H3*, *H5*, *H6* genes and the *H7H8* combination. With the exception of populations from southern Georgia and South Carolina and northern Florida, the southeast is predominantly biotype L (Ratcliffe et al., 2000).

Several reasons exist to be concerned that single-gene resistance is not sustainable long term. The sources of resistance are probably limited and some genes have linkage drag and are unable to be used because of close association with unwanted agronomic traits. Other sources of resistance may not be useful because they do not confer 100% resistance to the wheat (do not kill 100% of avirulent larvae). The number of years between identifying effective Hessian fly resistance genes and releasing a cultivar with the gene to farmers is also a concern. The years involved in cultivar development may experience decreased funding of agricultural research (Harris et al., 2004).

To prevent the shift to virulent Hessian fly biotypes resulting in the "break-down" of a resistant gene, some breeders use the method of rotating wheat cultivars with different resistance genes or the sequential release of cultivars with different resistance genes (Liu et al., 2005). As a cultivar with resistance to a specific biotype begins to become less effective and the fly population shifts to a virulent biotype, another cultivar is released with a different resistance gene to prevent an explosion of the new virulent biotype population (Berzonsky et al., 2003). The Hessian fly biotypes must be closely monitored to determine if a shift in biotype occurs, so

that farmers know when to rotate to a different resistant cultivar. This requires close and effective communication between breeders and farmers.

Gene pyramiding may increase resistance in level, breadth, and duration (Berzonsky et al., 2003; Liu et al., 2005). Gene pyramiding is simply having multiple resistance genes in one cultivar with the assumption that the genes are not allelic to each other. The different resistance genes will increase the level and breadth of resistance since the cultivar would be damaged only by the very rare virulent biotype. A single virulence gene is able to become dominant within the population when a cultivar with a single resistance gene is used. Although for a pyramided cultivar to be susceptible to Hessian fly, individual insects must have multiple virulent genes. The resistance of pyramided wheat could be extended compared to a cultivar with a single resistance gene.

Gallun (1977) does not believe that pyramiding resistance genes is a good practice. By having several resistant genes in one cultivar, the cultivar may become genetically vulnerable and result in a Hessian fly epidemic. By using cultivar with a single resistance gene, sources of resistance can be changed once populations of specific biotypes begin to increase in the field. Epidemics can be prevented by sustaining a reserve of different Hessian fly resistance in advanced breeding lines which can be released as cultivars resistant to biotypes forming in the field (Gallun, 1977).

Gene pyramiding is very difficult, time consuming, and costly using conventional breeding methods based on phenotypic selection. It is very difficult to determine if more than one resistance gene is in a cultivar when the phenotype is the only basis of determining resistance (Liu et al., 2005). Since a cultivar with one resistance gene is crossed to another cultivar with another gene; it is difficult determine whether the new resistance gene is captured

in the first cultivar because most of the progeny will be resistant with at least one gene. Several rounds of crossing and evaluations must be conducted to conclude that the line would have both resistant genes. Phenotypic field evaluations can be inconclusive because of insufficient infestation levels, seeding delays, insufficient germination, and environment influences such as early freezes. Greenhouse and growth chamber tests can be used to control many of these factors and test Hessian fly resistance (Foster et al., 1988).

Molecular markers have increased the ability and efficiency of identifying different resistant genes (Liu et al., 2005) for gene pyramiding. Thirty-two Hessian fly genes have been identified using molecular markers (Subramanyam et al., 2006). Using marker-assisted selection (MAS) greatly accelerates a resistance breeding program. MAS is able to detect resistant genotypes in early generations which be difficult in the traditional phenotypic selection method. The types of markers linked to Hessian fly resistance are restriction fragment length polymorphism (RFLP), random amplified polymorphic DNAs (RAPD), sequence tagged site (STS), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR). MAS will increase the efficiency of a breeding program by removing unwanted susceptible lines and allowing only resistant lines through the program (Liu et al., 2005). For marker data to be useful in a breeding program, it must be correlated with greenhouse and field data. Greenhouse data is a good predictor and simulation of seedling Hessian fly resistance but may not have a direct connection to field performance or adult resistance or tolerance. Before markers can be completely depended on to make early generation selection without testing, a strong correlation between the markers and phenotypic data in both the greenhouse and field must be established by reducing the genotype by environment interaction.

Another breeding strategy for insect resistance is to have multiple resistance genes blended with susceptible genotypes. The released cultivar is a blend of near-isogenic genotypes with at least two resistance genes and one susceptible genotype. The resistant genotypes can be created from isogenic lines from progressive backcrossing to create lines with the same genotype except for the different resistant genes. Isogenic lines must be used when the resistance genes are allelic to each other; therefore gene pyramiding is impossible (Berzonsky et al., 2003). Alternately gene pyramiding can be used with a proportion of similar phenotypic susceptible plants. The susceptible plants would act as a "reserve" for the Hessian flies and should compose 20 to 50 percent of the plants in the cultivar. The lower selection pressure on the flies prolongs the shift to a virulent population (Gould, 1986).

Some cultural practices observed by farmers increase the survival of Hessian fly populations which affect the efficiency of breeding programs. Although reduced tillage controls soil erosion, it also increases Hessian fly survival and thereby increasing the fly population. When wheat is double-cropped with soybeans and planted into the wheat stubble, the volunteer wheat that grows with the soybeans act as a "reservoir" for the Hessian flies. By destroying volunteer wheat, the oversummering Hessian fly puparium are also destroyed decreasing the chance of increasing the fly population (Berzonsky et al., 2003).

The most commonly followed cultural practice of delaying planting helps control the Hessian fly by preventing attack. By delaying the fall planting date, the crop will escape the peak emergence of adult Hessian flies and reduce the damage sustained. Planting is delayed until after the "fly free" date which is adjusted based on the growing area and temperature. The "fly free" date is mid-September for the northeast and upper midwestern United States and in late October in the southeast (Berzonsky et al., 2003). Since oviposition and larval feeding

occurs throughout the winter in the southeastern United States, late planting in this area is a poor control method (Buntin and Chapin, 1990). Therefore the southeastern United States depends on resistant cultivars to control Hessian fly outbreaks.

Planting date studies have shown differences between two regions of Georgia (Coastal Plain and Piedmont). In the northern region of Georgia (Piedmont), higher control of Hessian fly was experienced by late planting due to more predictable cold weather to limit the winter activity of the insect. In the southern region (Coastal Plain), the fall and winter Hessian fly infestations were avoided but damage still occurred by spring infestations (Buntin et al., 1990).

Breeders have a difficult task in breeding wheat for Hessian fly resistance because of their close interaction. Although the Hessian fly is readily able to overcome the resistance developed and breed into wheat, there are promising methods to control populations and minimize damage. The most important key to resistance is having the possibility of heritable resistance. Wheat has inherent resistance to Hessian fly which may not be fully exhausted by breeders. Breeders have also identified resistance genes in some wheat relatives. New sources of resistance may be available in more distant relatives such as in *Aegilops geniculata* Roth. Once the resistance genes have been identified, they must be transferred into wheat either through backcrossing or through gene transformation. The genes can be pyramided into cultivars with various numbers of genes and in various combinations. Combining cultural practices such as late planting, removal of volunteer wheat, and the use of resistant cultivars will effectively control the Hessian fly population and damage. Berzonsky et al. (2003) summarizes the breeding efforts by stating, "The success that has been achieved in breeding wheat for Hessian fly resistance is underscored by the fact that the fly has been successfully managed in many areas of the United States after resistant cultivars have been grown for several years."

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CHAPTER 1

SSR MAPPING OF DURUM DERIVIVED HESSIAN FLY RESISTANCE IN IN97219-A3-5

ABSTRACT

Hessian fly (*Mayetiola destructor* Say) is one of the most destructive pests of wheat (*Triticum aestivum* L.) worldwide. The objectives of this study were to characterize Hessian fly resistance from IN97219-A3-5 and identify linked molecular markers to facilitate marker assisted selection. IN97219-A3-5 with the pedigree Len*3/3/Knox*2//D6647/PI 323440 derived Hessian fly resistance from the tetraploid, *Triticum durum*, PI 323440. IN97219-A3-5 was crossed to AGS 2000, a Georgia line susceptible to biotype L. The $F_{2:3}$ progeny showed a ratio of 3 Resistant/Segregating : 1 Susceptible (64R/Seg:22S). The Chi-Square value was 0.0916 with a P-value of 0.762 and indicated that resistance was controlled by a single dominant gene. IN97219 is flanked by simple sequence repeats (SSR) markers *Xgwm234* at 8.7 cM and *Xwmc149* at 19.5 cM. The markers will facilitate pyramiding of biotype L Hessian fly resistance.

INDEX WORDS: *Triticum aestivum*, wheat, *Mayetiola destructor*, Hessian fly, *Triticum durum*, marker assisted selection, simple sequence repeats, and SSR

INTRODUCTION

Hessian fly (*Mayetiola destructor* Say) is one of the most destructive pest of wheat (*Triticum aestivum* L.) worldwide (Liu et al., 2005) being present in wheat growing areas from North America, north Africa and southern Europe (El-Bouhssini et al., 1996). In the United States Hessian fly has spread to all major wheat growing areas from the Atlantic Coast to the Great Plains (Berzonsky et al., 2003). In durum wheat (*T. turgidum* L. var. *durum*) the losses have been reported at 32% in Morocco (Liu et al., 2005; Zaharieva et al., 2001), while farmers in the U.S. usually sustain 5% to 10% annual losses of wheat production (Buntin, 1999; Liu et al., 2005). In a single year in the United States, the damage caused by the Hessian fly has been estimated as great as $100 million (Cartwright and Jones, 1953). In Georgia losses were estimated at $4 million in 1986 and $28 million in 1989 (Hudson et al., 1991; Hudson et al., 1988).

A gene-for-gene interaction exists between wheat resistance genes and Hessian fly virulence genes resulting in either a compatible or incompatible interaction (El-Bouhssini et al., 1996; Subramanyam et al., 2006). When the interaction is compatible, the wheat plant is susceptible to Hessian fly and the symptoms become irreversible after 4-5 days of virulent larval feeding. The incompatible interaction occurs when the wheat plant is resistant resulting in the death of larvae within 3-5 days of hatching (Subramanyam et al., 2006).

Thirty-two Hessian fly (Hf) resistance genes have been identified from wheat and its relatives and have been designated *H1-H32* (Subramanyam et al., 2006). These resistance genes have come from common wheat, *Aegilops tsauschii*, *Triticum turgidum* ssp. *durum*, *Ae. ventricosa*, *Ae. triuncialis*, *T. turgidum* ssp. *dicoccum*, and rye (Liu et al., 2005). The resistance

genes derived from *T. turgidum* ssp. *durum* are *H6, H9, H10, H11, H14, H15, H16, H17, H18, H19, H20, H28, H29, H31* (Liu et al., 2005). *H31* was derived from CI3984 (durum wheat, *Triticum turgidum* Desf., accession) and exhibits biotype L resistance. *H31* is reported to be stable under the standard test conditions of 18°C with 14 hours of light (Williams et al., 2003).

The objectives of this research were to characterize the phenotypic expression of a new source of resistance to Hessian fly biotype L, to determine the chromosomal location of the gene, and to find markers that will be useful in marker assisted selection. The new source of resistance was from the durum germplasm line PI 323440. In a study of durum germplasm lines, PI 323440 was found to have a single gene for resistance to biotype L. The plant introduction line had 100% mortality of larvae on resistant plants (Ratcliffe et al., 2002). The resistance was transferred into the common wheat line IN97219-A3-5 (Len*3/3/Knox*2//D6647/PI 323440).

MATERIALS AND METHODS

Population Development

IN97219-A3-5 derived Hessian fly (Hf) biotype L resistance from the tetraploid, *Triticum durum*, PI 323440. IN97219-A3-5, hereafter shortened to IN97219, was crossed with the Hf biotype L susceptible cultivar AGS 2000. AGS 2000 is resistant to the Hessian fly biotypes E, G, and O (Johnson et al., 2002). The IN97219/AGS 2000 F_1 hybrid was selfed to produce F_2 seeds in the greenhouse. F_2 plants were grown in 4-inch pots in the greenhouse without selection to produce the $F_{2:3}$ lines. The $F_{2:3}$ families were used in the phenotypic screening against Hessian fly biotype L (Dweikat et al., 1997).

$F_{2:3}$ Generation Evaluations

Ninety-two $F_{2:3}$ families were evaluated for reaction to Hessian fly biotype L in 2008. Conetainers (RLC-3; Stuewe and Sons, Corvallis, OR) with a top cylinder of 2.5 cm and a length of 12 cm were filled with Sunshine SB400 (Sun Gro Horticultural Distribution Inc., Marysville, OH) and supplemented with 10-10-10 (N-P-K) fertilizer and Micromax (Scotts Inc., Marysville, OH). Fourteen $F_{2:3}$ seed of each line were planted into individual conetainers (2 rows on the rack). Two conetainers of each of the resistant parent (IN97219), the susceptible parent (AGS 2000), and USG3209 (susceptible check) were included on every rack. To promote uniform germination, the conetainers were lightly watered and placed into cold storage at 4°C for 10 days.

The racks of conetainers were placed into growth chambers that had 12 hours of light and watered daily to maintain a high relative humidity. Although the temperature in the growth chambers was set at 18 ± 1°C, the temperature raised to 21°C in the first evaluation. Moist organic material with the biotype L Hessian fly larvae was placed on trays to allow the adults to emerge and oviposition on the first leaf stages of the wheat plants. The larvae were obtained from Crop Production and Pest Control Research Unit in West Lafayette, Indiana. Twenty days after infestation seedlings were evaluated as resistant or susceptible. Susceptible plants had dark green leaves and live larvae or pupae present at the base of the sheath. Resistant plants had normal growth and the absence of live larvae or pupae. The F_2 families were classified as resistant if all seedlings within the family were resistant, as segregating if the family had both resistant and susceptible seedlings, and susceptible if all seedlings within the family were susceptible. The deviation of the observed data from theoretically expected segregating ratio of 3 Resistant/Segregating: 1 Susceptible was tested using Chi-Squared (χ^2) tests for goodness-of-fit.

Microsatellite Marker Analysis

Genomic DNA was extracted from leaf tissue samples of $F_{2:3}$ plants following the procedure described by Stein et al. (2001). Leaf samples from the fourteen plants per F_2 family were bulked to perform the DNA extractions.

Wheat microsatellite primers evenly distributed across the A and B genomes were synthesized according to the sequences published in the GrainGenes database (http://wheat.pw.usda.gov), with all forward primers modified to include the M13 sequence (CACGACGTTGTAAAACGAC-) at the 5' end for labeling purposes (Rampling et al., 2001; Schuelke, 2000).

The PCR reactions were conducted in a total volume of 12 µL containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 200 µM of each dNTP, 80 nM of forward primer, 400 nM of reverse primer, 400 nM of M13 labeled primer (6-FAM Blue label, PE-ABI , Foster City, CA), 0.9 U *Taq* DNA polymerase, and 100 ng of genomic DNA. Amplifications were performed using the following conditions: 94°C for 4 min; 35 cycles of 94°C for 1 min, annealing temp of primer from GrainGenes database (http://wheat.pw.usda.gov) for 1 min, and 72°C for 1 min; 10 cycles of 94°C for 1 min, 53°C for 1 min, and 72°C for 1 min; and a final extension step at 72°C for 7 min. The reaction was performed in a MJ Research PTC-225 thermal cycler (MJ Research, Inc., Reno, NV). PCR products were mixed 1:1 with loading dye (2 µL deionized formamide, 0.2 µL Genescan ROX-500 internal size standard (ABI, Foster City, CA), and 0.075 µL loading buffer) and denatured at 95°C for 3 min. The PCR products were analyzed on an ABI-Prism 377 DNA sequencer (PE-ABI) with a 4.8% acrylamide to bisacrylamide (19:1) gel at 750 V for 2 h. The marker fragments were analyzed using GeneScan software v.3.0.

The PCR reactions were conducted in a total volume of 12 μL containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.0 mM $MgCl_2$, 200 μM of each dNTP, 20 nM of forward primer, 100 nM of reverse primer, 100 nM of M13 labeled primer (IRD700 or IRD800 label, LI-COR Biosciences, San Diego, CA), 0.75 U *Taq* DNA polymerase, and 50 ng of genomic DNA. The amplification conditions used for the LI-COR used the following conditions: 94°C for 4 min; 35 cycles of 94°C for 30 seconds, annealing temp of primer from GrainGenes database (http://wheat.pw.usda.gov) for 30 seconds, and 72°C for 30 seconds; 10 cycles of 94°C for 30 seconds, 53°C for 30 seconds, and 72°C for 30 seconds; and a final extension step at 72°C for 7 min. PCR products, mixed 1:1 with loading buffer (95% formamide, 20 mM EDTA, and 0.08% bromo-phenol blue), were denatured at 95°C for 3 min. The PCR products were analyzed on a LI-COR sequencer (Model 4300) with a 6.5% polyacrylamide gel (KB Plus gel matrix, LI-COR Biosciences) at 42 W and 1500 V for 2.5 h. Photoshop was used to optimize and view gel images.

Primer pairs that were polymorphic between IN97219 and AGS 2000 were used on eight resistant and eight susceptible lines. Primer pairs that were polymorphic between the resistant and susceptible groups were used to genotype the F_2 population.

Linkage analysis was performed using MAPMAKER/Exp version 3.0b (Lincoln et al., 1993). Map distances were determined using the Kosambi mapping function (Kosambi, 1944) and loci were ordered using the 'sequence' and 'compare' commands, with an LOD threshold score ≥ 3.0.

RESULTS AND DISCUSSION

$F_{2:3}$ Generation Evaluations

Although the temperature in the growth chambers was set at 18 ± 1°C, the temperature reached up to 21°C in the first evaluation. Consequently, twelve of the 24 resistant IN97219 checks were susceptible; the other 12 were resistant. Majority of the $F_{2:3}$ plants were observed to be susceptible with only a few resistant plants. The temperature was lowered to 16°C and some of the seedlings recovered and grew more tillers; thus it was decided to repeat the evaluation at a lower temperature. It was concluded that the IN97219 had a Hf resistant gene which was temperature sensitive.

The experiment was repeated in a different chamber at 16°C. Only one of sixteen IN97219 plants was susceptible while all the AGS 2000 were susceptible. The $F_{2:3}$ progeny showed a segregating ratio of 3 R/Seg : 1 S (67 R/Seg : 24 S). The Chi-Square value was 0.0916 with a P-value of 0.762 (Preacher, 2001). The Yates Correction for one degree of freedom provided a Chi-Square value of 0.0330 and a P-value less than 0.9 and greater than 0.5. The P-values are both greater than 0.05 with or without the correction, i.e. no significant difference between the observed and expected numbers. Therefore, the deviation observed between the expected and observed numbers for the IN97219 progeny are due to chance. The Chi-Square test indicated that Hf resistance in IN97219 was controlled by a single dominant gene.

Microsatellite Marker Analysis

Among the 200 primer pairs tested, 80 were polymorphic between the parents. *Xwmc149* yielded amplification products that were polymorphic between the parents as well as the resistant and susceptible groups. Figure 1.1 shows the polymorphism between the parents; the faint upper

band in IN97219 amplifys the 1B locus as determined using the GrainGenes database (http://wheat.pw.usda.gov). The *Xwmc149* primer pair amplified a 195bp band on AGS 2000 and a 196bp fragment on IN97219. Due to the small band size separation, the population was scored considering *Xwmc149* as a dominant marker: individuals were scored "D" if they were homozygous for the IN97219 allele or heterozygous and "B" if they were homozygous for the AGS 2000 allele. Deviations of *Xwmc149* from the expected segregating ratio of 3 D : 1 B was tested using Chi-Squared (χ^2) tests for goodness-of-fit. The observed ratio was 74 D : 15 B. *Xwmc149* had a Chi-Square value of 3.15 and a P-value of 0.0759 (Preacher, 2001). The Yates Correction for one degree of freedom provided a Chi-Square value of 2.73 and a P-value less than 0.1 and greater than 0.05. The P-values are both greater than 0.05 with or without the correction, i.e. there is no significant difference between the observed and expected numbers.

Since *Xwmc149* mapped to chromosome 5BS (http://wheat.pw.usda.gov), twenty-two additional 5BS primer pairs were screened among the parents. One marker, *Xgwm234*, proved to be polymorphic and was tested in the population. This primer pair yielded a 264 bp fragment on AGS 2000 and a 274 bp fragment on IN97219. Deviations of *Xgwm234* from the expected segregating ratio of 1 A (IN97219 fragment) : 2 H (heterozygous) : 1 B (AGS 2000 fragment) was tested using Chi-Squared ($\chi 2$) tests for goodness-of-fit. The observed ratio was 19 A : 40 H : 17 B. *Xgwm234* had a Chi-Square value of 0.316 and a P-value of 0.854 (Preacher, 2001).

A genetic linkage map for IN97219 Hessian fly resistance gene and the two linked SSR markers (*Xwmc149* and *Xgwm234*) was constructed using MAPMAKER. IN97219 was flanked by markers *Xwmc149* and *Xgwm234* at distances of 19.5 cM with and 8.7 cM, respectively (Figure 1.3).

H31 is the first Hessian fly resistance gene mapped to 5BS (Williams et al., 2003). *H31* was haplotyped using both markers and was compared to the parents. The fragment size for *Xwmc149* for *H31* was 196 bp which is the same as the IN97219 fragment (Figure 1.1). The *H31* fragment with *Xgwm234* was 270 bp which is different than both the AGS 2000 and IN97219 fragments (Figure 1.2).

Although *H31* was mapped to the terminus end of chromosome 5BS, several factors seem to indicate that IN97219 and *H31* are not the same gene. The durum accession that are resistance donors for *H31* and IN97219 were collected in different geographic locations. The durum resistance of *H31* was derived from CI 3984, collected in Tunisia. The durum PI 323440 in IN9719 was obtained from Austria (Ratcliffe et al., 2002). Although both IN97219 and *H31* are linked to the SSR marker *Xgwm234*, the fragment sizes were different (274bp and 270bp, respectively). Further research must be conducted to determine if the Hf gene in IN97219 is not an allele of *H31* through an allelism test. The temperature sensitive nature of IN97219 must be further studied to understand the implications for cultivar development. The temperature sensitivity may also be a method to distinguish IN97219 and *H31*, which is stable under standard test conditions and is expected to be robust under field conditions (Williams et al., 2003).

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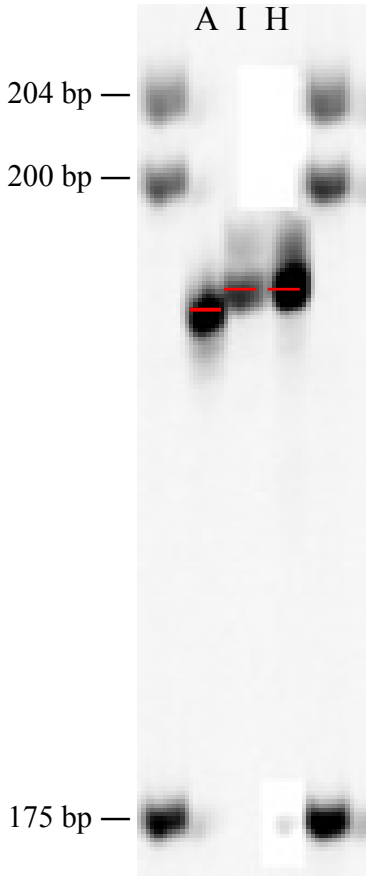

Figure 1.1 SSR *Xwmc149* fragments. The size standard flank AGS 2000 (A), IN97219 (I), and *H31* (H). The red lines highlight the middle of the band that was scored for fragment size. AGS 2000 has 195 bp, IN97219 has 196 bp, and *H31* has 196 bp. The faint band at 198 bp is amplifying the locus of 1B (GrainGenes database (http://wheat.pw.usda.gov)).

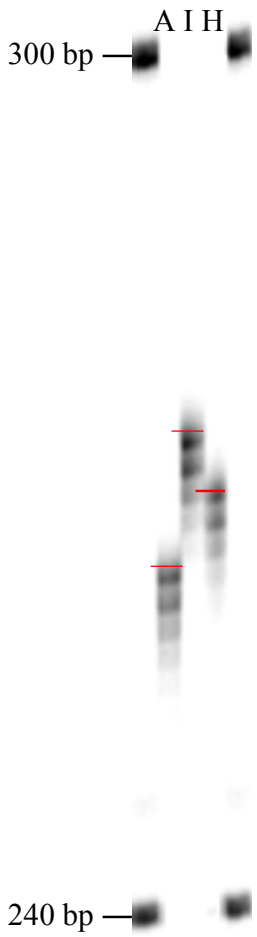

Figure 1.2 SSR *Xgwm234* fragments. The size standard flank AGS 2000 (A), IN97219 (I), and *H31* (H). The red lines highlight the middle of the band that was scored for fragment size. AGS 2000 has 264 bp, IN97219 has 274 bp, and *H31* has 270 bp.

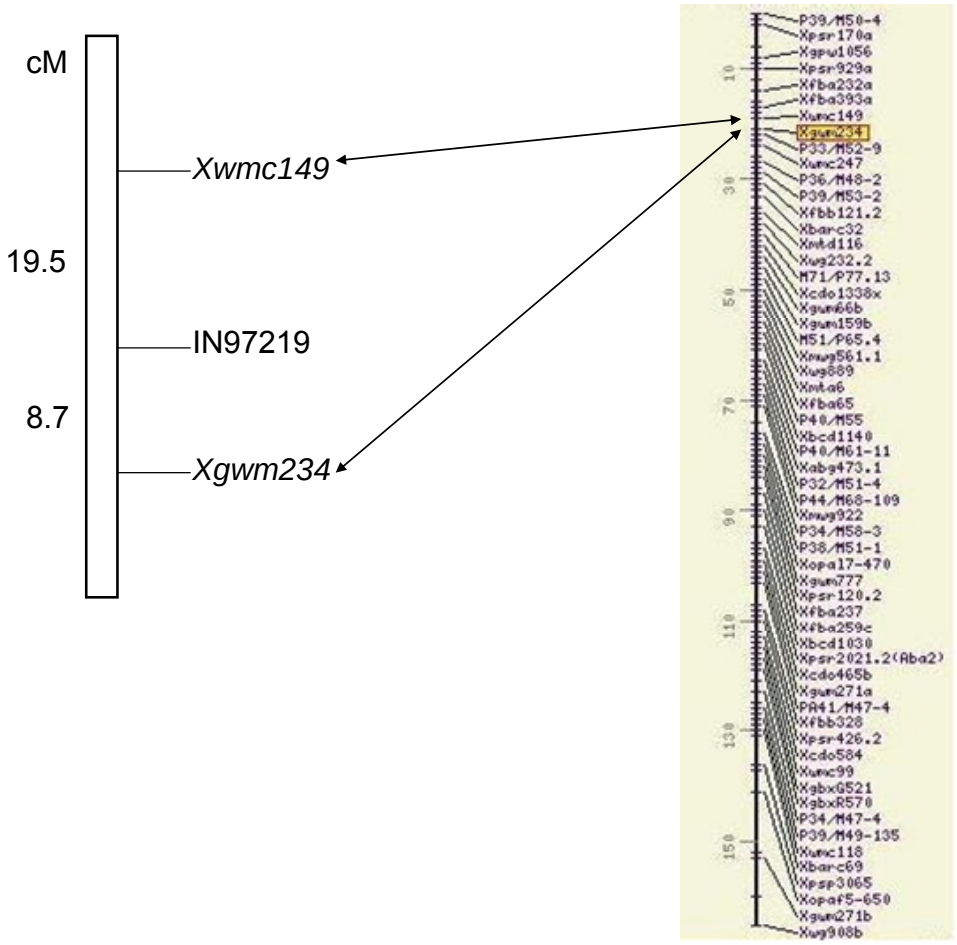

Figure 1.3 Comparison of IN97219 linkage map and 5B chromosome composite map. Linkage map of IN97219 (left) and Composite map of 5B chromosome (right) obtained from the GrainGenes database (http://wheat.pw.usda.gov) to display orientation of the SSR markers.

CHAPTER 2

TEMPERATURE CHARACTERIZATION OF HESSIAN FLY RESISTANCE IN DURUM DERIVIVED LINE IN97219-A3-5

ABSTRACT

Hessian fly (*Mayetiola destructor* Say) is one of the most destructive pests of wheat (*Triticum aestivum* L.) worldwide. The objectives of this study were to characterize Hessian fly (Hf) resistance from IN97219-A3-5 as affected by temperature in growth chambers and field planting dates. IN97219-A3-5 with the pedigree Len*3/3/Knox*2//D6647/PI 323440 derived Hf resistance from the tetraploid, *Triticum durum*, PI 323440. The planting date study was conducted at two locations (Griffin and Plains, GA) and at three dates. A growth chamber study was also conducted at three temperatures (15°C, 18°C, and 23°C). Results from these studies revealed that IN97219A3-5 resistance is controlled by a temperature sensitive gene.

INDEX WORDS: *Triticum aestivum*, wheat, *Mayetiola destructor*, Hessian fly, *Triticum durum*, planting date, and temperature sensitive gene

INTRODUCTION

Hessian fly (*Mayetiola destructor* Say) is one of the most destructive pest of wheat (*Triticum aestivum* L.) worldwide (Liu et al., 2005) being present in wheat growing areas from North America, north Africa and southern Europe (El-Bouhssini et al., 1996). Hessian fly has spread to all major wheat growing areas from the Atlantic Coast to the Great Plains. Outbreaks of Hessian fly are sporadic and typically cause more local or regional damage even though pervasive outbreaks have occurred (Berzonsky et al., 2003). In durum wheat (*T. turgidum* L. var. *durum*) the losses have been reported at 32% in Morocco (Liu et al., 2005; Zaharieva et al., 2001), while farmers in the U.S. usually sustain 5% to 10% annual losses of wheat production (Buntin, 1999; Liu et al., 2005). In a single year in the United States, the damage caused by the Hessian fly has been estimated as great as $100 million (Cartwright and Jones, 1953). In Georgia losses were estimated at $4 million in 1986 and $28 million in 1989 (Hudson et al., 1991; Hudson et al., 1988).

Resistance is mostly controlled by dominant alleles; however, several examples exist of resistance genes and cultivars that are affected by temperature (Bouhssini et al., 1999; Cartwright et al., 1946; Maas et al., 1987; Obanni et al., 1989; Sosa and Foster, 1976; Tyler and Hatchett, 1983). Elva (*H9H10*) has 100.0% resistant plants at 23°C and significantly fewer 89.2% resistant plants at 31°C (Tyler and Hatchett, 1983). Marquillo (*H18*) is an effective temperature-sensitive cultivar that is effective at 16°C but is completely susceptible at 20°C (Maas et al., 1987).

Since brief periods of above-normal temperatures are common in the fall and spring in the southern Great Plains, cultivars with temperature sensitivity could be a potential hazard

(Tyler and Hatchett, 1983). Obanni et al. (1989) also stated that sources of resistance that are stable at high temperatures may be more valuable in areas where Hessian fly is a serious pest. Alternatively temperature-sensitive genes may also provide a more durable source of resistance (Bouhssini et al., 1999; Maas et al., 1987). Temperature-sensitivity may reduce selection for virulent biotypes and therefore maintain avirulent Hessian flies (Maas et al., 1987). The virulence within the Hessian fly population is diluted by the avirulent flies which may reduce the rate of virulent biotype development (Bouhssini et al., 1999).

Planting date studies have shown differences between two regions of Georgia (Coastal Plain and Piedmont). In the northern region of Georgia (Piedmont), greater control of Hessian fly was experienced by late planting due to more predictable cold weather to limit the winter activity of the insect. In the southern region (Coastal Plain), the fall and winter Hessian fly infestations were avoided but damage still occurred by spring infestations (Buntin et al., 1990).

The objective of this experiment was to study the temperature-sensitivity of IN97219-A3-5, hereafter shortened to IN97219. IN97219, with the pedigree Len*3/3/Knox*2//D6647/PI 323440, derived biotype L resistance from the tetraploid, *Triticum durum*, PI 323440. The test for the optimal temperature for the expression of resistance was conducted in growth chambers. A planting date study was conducted to test if the different temperatures experienced at different planting dates would affect the level of resistance.

MATERIALS AND METHODS

Temperature-Sensitive Experiment

Conetainers (RLC-3; Stuewe and Sons, Corvallis, OR) with a top cylinder of 2.5 cm and a length of 12 cm were filled with Sunshine SB400 (Sun Gro Horticultural Distribution Inc., Marysville, OH) and supplemented with 10-10-10 (N-P-K) fertilizer and Micromax (Scotts Inc., Marysville, OH). To promote uniform germination, the conetainers were lightly watered and placed into cold storage at 4°C for 10 days. The racks of conetainers were placed into growth chambers that had 12 hours of light and watered daily to maintain a high relative humidity.

Three temperatures were used: low (15°C), medium (18°C), and high (23°C). Four cultivars were included in the experiment. AGS 2000 was included as the biotype L susceptible check; AGS 2000 is resistant to the Hessian fly biotypes E, G, and O (Johnson et al., 2002). IN97219-A3-5 (Len*3/3/Knox*2//D6647/PI 323440) derived Hessian fly (Hf) biotype L resistance from the tetraploid, *Triticum durum*, PI 323440. The cultivar 'Marquillo' (*H18*) was included as known temperature sensitive gene; it is completely susceptible at 20°C (Maas et al., 1987). 'Marquillo' is resistant to biotype L (Ratcliffe et al., 1996). *H31* was used as a non-temperature-sensitive check. *H31* is resistant to biotype L and is stable under standard conditions of 18°C (Williams et al., 2003). Twenty plants of each line were placed in individual cones to constitute a replication. Four replications were within each temperature. The experiment was arranged in the split plot design. The whole plot was the three temperatures with the cultivars as the sub-plot. Data was analyzed with an analysis of variance (ANOVA) for a split-plot design (Institute, 1982; Steel and Torrie, 1960).

Moist organic material with the biotype L Hessian fly larvae was placed on trays to allow the adults to emerge and oviposition on the first leaf stages of the wheat. The larvae were obtained from Crop Production and Pest Control Research Unit in West Lafayette, Indiana. Each seedling was classified twenty days after infestation as resistant or susceptible. Susceptible plants had dark green leaves and live larvae or pupae present at the base of the sheath. Resistant plants had normal growth and the absence of live larvae or pupae.

Planting Date Experiment

Eight cultivars were included in the planting date experiment. The resistant check IN97219 was included. AGS 2000 was included as the biotype L susceptible check. AGS 2000 is resistant to the Hessian fly biotypes E, G, and O (Johnson et al., 2002). 'Joy' (*H10),* is a durum-derived Hessian fly resistant cultivar (El-Bouhssini et al., 1998). *H10* was 92.8% resistant to biotype L (Ratcliffe et al., 1996). 'Lola' (*H12*) is a resistant cultivar derived from the common wheat *Triticum aestivum* L. (El-Bouhssini et al., 1998). *H12* was reported as 98% resistant to biotype B and 89.3% resistant to biotype L (Ratcliffe et al., 1996). 'Marquillo' (*H18*) is also a durum-derived resistant cultivar (El-Bouhssini et al., 1998). *H18* was reported as 100% resistant to biotype L (Ratcliffe et al., 1996). 'Jori' (*H20*) is a durum-derived cultivar which is resistant to biotype D (Amri et al., 1990). The cultivars *H10*, *H18*, and *H20* were included due to their temperature-sensitivity (Williams et al., 2003). The Georgia cultivar AGS 2031 was included as a susceptible Hessian fly check (Day et al., 2008). 'Kawvale' was included due to its history of Hessian fly resistance. 'Kawvale' was released in 1931 as a resistant cultivar and is believed to have more than one genetic factor responsible for the Hessian fly resistance (Painter et al., 1931).

A split-plot experimental design was used at two locations with three planting dates as whole plots arranged in a randomized complete block design with three replications per planting date. Cultivars were hand planted in subplots of one-foot rows. The first date of the Griffin location was planted on October 23, 2008 and the following two dates were a week apart, October 31 and November 7. The first planting date for Plains was November 18, 2008 with the following dates a week apart, November 25 and December 3. Data was analyzed with an analysis of variance (ANOVA) for a split-plot design. Cultivar and planting date means were compared by Fisher's least significant difference (LSD) (Institute, 1982; Steel and Torrie, 1960).

The whole row was collected in mid-February in Plains and the number of Hessian fly larvae and pupae were counted on 50 tillers. The count was conducted before the wheat had headed to provide a count of the fall and winter infestations. 'Lola' (*H12*) was dropped from analyses due to poor stand emergence.

RESULTS AND DISSCUSION

Temperature-Sensitive Experiment

The split plot design was analyzed with SAS program for the percent resistant plants. Using the replications by temperature as the error term, the replication was not significantly different; therefore, no significant variation between replications existed in this experiment. In Table 2.1, the replications had an *F* value of 1.44. The temperature was significant at the 0.0001 level with an *F* value of 135.10 (Table 2.1). Table 2.1 shows that the entry and the interaction of between entry and temperature were significant at the 0.0001 level. Entry had the significant

F value of 622.46 which indicates that the entries displayed a difference in percent resistance. With the significant *F* value of 69.79, the interaction between entry and temperature reveals that the percent resistance was affected by the temperature of the experiment.

AGS 2000 was the only entry that was completely susceptible to biotype L Hessian fly (Table 2.2). *H18*, the temperature sensitive cultivar, showed that its resistance was reduced as the temperature was increased. *H18* was 98.8%, 80.2%, and 17.5% resistant at 15°C, 18°C, and 23°C, respectively (Table 2.2). IN97219 exhibited similar resistance to *H18* and was also temperature sensitive. IN97219 was 97.4%, 51.0%, and 14.4% resistant at 15°C, 18°C, and 23°C, respectively (Table 2.3). IN97219 was reduced more dramatically at 18°C than *H18* but were similar at the highest temperature. *H31* was more stable across the temperatures. *H31* was 100.0%, 96.0%, and 65.1% resistant at 15°C, 18°C, and 23°C, respectively (Table 2.2). Although *H31* was only 65% resistant at 23°C, its resistance was three times greater than IN97219 and *H18* at this temperature. Therefore it can be concluded that *H31* is stable across temperatures whereas the level of resistance in IN97219 was reduced at high temperatures, i.e. temperature sensitive.

Planting Date Experiment

The split plot design was analyzed with SAS program for percent infestation. In Griffin the infestation level was low and inconsistent over the plots; consequently, the data was not analyzed. In Plains using replications by planting date as the error term, the replications and the planting dates were not significant at the 0.05 level. In Table 2.3, the replications had an *F* value of 0.39 and a probably of 0.7032 which is not significant at the 0.05 level.

For the Type III ANOVA (Analysis of Variance) for the percent infestation, the entries were significant at the 0.0001 level (Table 2.3). Entries were compared by Fisher's least significant difference (LSD) in Table 2.4 with the mean across replications and planting dates. AGS 2031 was significantly different than all other entries as it was susceptible to Hessian fly with a mean of 18.67 percent infestation. *H18* and *H20* were not significantly different with 0.0 percent infestation for both. *H10* and IN97219 were not significantly different from *H18* and *H20* with percent infestations of 1.78 and 2.75, respectively. Although, IN97219 was grouped with very resistant cultivars of *H10* and *H20*; it was not significantly different from 'Kawvale' and AGS 2000 with percent infestations of 6.44 and 6.25, respectively.

Planting dates (PD) were not significant different with an *F* value of 5.99 and a probability of 0.063 (Table 2.3). The Hessian fly infestation levels at the three planting dates are presented in Table 2.5. PD 1 (November 18), PD 2 (November 25), and PD 3 (December 3) had infestations of 7.62%, 4.30% and 3.40%, respectively. The planting date by entry interaction was not significant with an *F* value of 0.89 and a probability of 0.569 (Table 2.3). Since the planting dates and the planting date by entry interaction were not significant, planting the cultivars at different dates did not affect the expression of Hessian fly resistance. Low levels of infestation were detected with the susceptible check (AGS 2031) only having 18.6 percent infestation. The low level of Hessian fly infestation recorded in this experiment may have affected the expression of resistance.

IN97219 has a similar level of resistance when compared to *H10*, *H18*, and *H20* and significantly better than the susceptible cultivar AGS 2031. IN97219's low levels of infestation (2.75%) may provide more durable resistance by allowing some avirulent flies to survive which reduces selection pressure to overcome the resistance gene (Maas et al., 1987). Although the

resistance of IN97219 was greatly reduced at higher growth chamber temperatures, good resistance was sustained in field conditions at various planting dates.

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Table 2.1 — ANOVA for the temperature-sensitive experiment for percent resistance.

Source	DF	Mean Square	*F* Value
Rep[+]	3	105.05	1.44
Temp[+]	2	9852.11	135.10***
Error A	6	72.93	—
Entry	3	15833.94	622.46***
Temp*Entry	6	1775.28	69.79***
Error B	26	25.44	—

*** indicates significant *F* value at *P* = .0001 levels for Type III error, respectively.

[+] indicates use of Type III MS for Rep*Temp as the error term.

Table 2.2 — Mean percent resistant plants to Hessian fly for each cultivar at three constant temperatures.

Entry	Temperature	Mean Percent Resistant	Standard Variation
AGS 2000	15°C	0.0	0.00
AGS 2000	18°C	0.0	0.00
AGS 2000	23°C	0.0	0.00
H18	15°C	98.8	2.50
H18	18°C	80.2	12.17
H18	23°C	17.5	6.46
IN97219	15°C	97.4	5.25
IN97219	18°C	51.0	11.47
IN97219	23°C	14.4	5.93
H31	15°C	100.0	0.00
H31	18°C	96.0	4.93
H31	23°C	65.1	8.26

Table 2.3 — ANOVA for percent infestation using a split plot design in Plains, GA.

Source	DF	Mean Square	*F* Value
Rep[+]	2	6.57	0.39
Date[+]	2	102.26	5.99
Error A	4	17.07	—
Entry	6	380.97	17.41***
Date*Entry	12	19.39	0.89
Error B	34	721.89	—

*** indicates significant *F* value at *P* = .0001 levels for Type III error, respectively.

[+] indicates use of Type III MS for Rep*Temp as the error term.

Table 2.4 — Entry t Tests (LSD) for the mean percent infestation in Plains, GA. The means with the same letter are not significantly different.

Entry	Mean Percent Infestation	t Grouping		
AGS 2031	18.67		A	
Kawvale	6.44		B	
AGS 2000	6.25	C	B	
IN97219	2.75	C	B	D
H10	1.78	C		D
H18	0.00			D
H20	0.00			D

Table 2.5 — Planting date t Tests (LSD) for the mean percent infestation in Plains, GA. The means with the same letter are not significantly different.

Planting Date	Mean Percent Infestation	t Grouping
PD 1 (Nov 18)	7.62	A
PD 2 (Nov 25)	4.30	A
PD 3 (Dec 3)	3.40	A

CHAPTER 3

CONCLUSIONS

IN97219 was found to be controlled by a single dominant gene as the $F_{2:3}$ progeny showed a segregating ratio of 3 R/Seg : 1 S (67 R/Seg : 24 S). The Chi-Square value was 0.0916 with a P value of 0.762. Through marker analysis the gene was located on the short arm of chromosome 5B. *H31* is the only other Hessian fly resistance gene located on 5BS (Williams et al., 2003). IN97219 was flanked by the SSR markers at distances of 19.5 cM with *Xwmc149* and 8.7 cM with *Xgwm234*. The markers will facilitate pyramiding of biotype L Hessian fly resistance.

Although *H31* was mapped to the terminus end of chromosome 5BS (Williams et al., 2003), several factors lead to the conclusion that IN97219 and *H31* are not the same gene. Although both IN97219 and *H31* were mapped using the SSR marker *Xgwm234*, the fragment sizes were different (274 bp and 270 bp, respectively). The durum resistances of *H31* and IN97219 were collected in different geographic locations. The durum resistance of *H31* was derived from CI 3984, collected in Tunisia. The durum PI 323440 in IN9719 was obtained from Austria (Ratcliffe et al., 2002).

The temperature-sensitive experiment in the growth chamber revealed a significant interaction between entry and temperature for percent resistance. IN97219 and 'Marquillo' (*H18*) show a significant reduction in percent resistance at higher temperatures. IN97219 showed reduced resistance from 97% to 14% at 15°C and 23°C, respectively. Since IN97219

exhibited similar resistance to the temperature sensitive ‘Marquillo’ (*H18*), IN97219 was classified as temperature sensitive. *H31* was not as affected by temperature as much as IN97219 was affected. *H31* was 100% resistant at 15°C and 65% at 23°C (three times greater than IN97219).

Since the planting dates and the planting date by entry interaction were not significant in the planting date experiment, planting the cultivars at different dates did not affect the expression of Hessian fly resistance in this experiment. Low levels of infestation were detected with the susceptible check (AGS 2031) only having 18.6 percent infestation. The low level of Hessian fly infestation recorded in this experiment may have affected the expression of resistance. IN97219’s low levels of infestation (2.75%) may provide more durable resistance by allowing some avirulent flies to survive which reduces selection pressure to overcome the resistance gene (Maas et al., 1987).

Combining the marker data and the temperature sensitivity, it can be concluded that IN97219 probably has a different resistance gene than *H31*. Although further research must be conducted to further prove that IN97219 is not an allele of *H31* through an allelism test.

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