GENETIC DIVERSITY OF GREENBUGS, *SCHIZAPHIS GRAMINUM* (RONDANI), IN SEXUAL REPRODUCTION AND VIRULENCE TO HOST PLANTS

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CHAPTER I

INTRODUCTION

Although greenbugs, *Schizaphis graminum* (Rondani), have been managed with natural resistance in sorghum (*Sorghum bicolor* L. [Moench]) and barley, (*Hordeum vulgare* L.) the occurrence of biotypes that overcome plant resistance has made it difficult to develop long-lasting, resistant wheat (*Triticum aestivum* L.) cultivars. These biotypes are comprised of distinct races that differ in the ability to damage the different resistance sources. While each biotype is a phenotypic expression of a specific series of host/cultivar relationships it may be composed of an indefinite number of genotypes at other loci (Puterka and Peters, 1989).

A critical prerequisite to resistance management is anticipation of the occurrence of insect resistance before control actually fails. This involves field monitoring and laboratory tests of the field collected insect samples.

When a new cultivar is introduced and is widely utilized, heavy pressure is placed on the pest population for the selection of genotypes with the corresponding virulence. Because several resistance sources are available in the greenbug's five principle hosts: barley, oat (*Avena sativa* L.), rye (*Secale cereale* L.), sorghum, and wheat; selection for new biotypes should be expected. Greenbug populations are comprised of various biotypes. The genotype within a biotype also varies because greenbug virulence to each source of plant resistance is believed to be regulated by a

specific gene or set of genes which can be heterozygous. As a result, a biotype can be a composite of different clones (genotypes) which would make each biotype heterogeneous. Biotype diversity can also be extensive because genetic recombination during the greenbug's sexual phase can produce many different combinations of virulence to any number of resistance sources (Puterka, 1989).

My research was conducted with the following objectives:

1. To identify the greenbug biotypes in Oklahoma. Evaluating the greenbug population in the field is important for monitoring biotype development and diversity. Knowing the field situation of biotype composition will help both producers and breeders. Harvey *et al.* (1991) identified a new greenbug which damaged resistant sorghums in Kansas as biotype I. Whether such biotype development has taken place in Oklahoma and how virulent new biotypes may be to present sources of wheat and sorghum resistance is important to plant breeders.

2. To determine sexual reproduction capabilities of greenbug biotypes. All the field collected as well as some laboratory reared biotypes and clones were tested for their abilities to produce sexual morphs. Observations on time intervals for entering into the sexual phase, cold treatment effects on egg hatching, and rearing of the fundatrices to the production of nymphs, were made and recorded for all major biotypes and important clones.

3. To determine mode of inheritance of virulence to resistant wheat sources. Biotypes C, E, F, I, a unique biotype C progeny of sib-mating (CC81), a sample from Georgia (GAB), and field collected Z18, Z35, and Z44 were sib-mated. Using Puterka and Peters' (1988) lesion techniques and a star-pot test, progenies of these

matings were tested for segregation on *Gb1* ('Dickinson Selection 28A'), *Gb2* ('Amigo'), *Gb3* ('Largo') and GRS1201 (a new resistant wheat source).

4. To determine mode of inheritance of virulences to selected sorghum sources. The same progeny of sib-mated biotypes and clones were tested for virulence to four sorghum sources using the star-pot technique.

I have prepared Chapter II as a general review of literature and Chapters III, IV, V and VI to address the specific objectives. References for literature cited are given at the end of each of these chapters. To assist the reader and facilitate preparation of manuscripts for journal publications, the figures and tables are also presented at the end of each chapter.

CHAPTER II

LITERATURE REVIEW

Biotype concept

Field populations of aphids are diverse and should not be categorized by the characteristics exhibited by a single clone from that population (MacKay, 1989). This may be especially true for an aphid species that disperses over large distances and shows considerable variability in biological traits (Mittler and Gorder, 1991).

The theory of natural selection predicts, for example, that given genetic variation in host plant adaptation within a pest population, planting pure stands of a resistant variety that reduces pest fitness can lead to evolution of a pest adapted to the variety (Gould, 1983). Strategies for deploying resistant genes to prolong their usefulness require an understanding of the pattern of genetic variation in pest populations (Wilhoit and Mittler, 1991).

Genetic variations in herbivore pest populations, the gene for gene concept, and host/parasitic compatibility and competence, are some of the factors considered in biotype development (Gallun and Khush, 1980). Biotypes may have arisen from chance mutation or through genetic recombination, or existing polymorphism may have been favored through selection pressure from introduced resistant plant cultivars which disrupt the genetic balance in the population (Blackman, 1979).

Eastop (1973) defined biotypes as those individuals of an insect species able to feed and grow significantly better on a resistant crop variety than other genotypes of the same species. Gallun and Khush (1980) defined biotypes as populations within an insect species that vary in their ability to utilize a crop plant. Biotype designations have proven their agronomic utility in that they allow a broad range of specific aphid-host relationships to be described by single letter designations. This may seem a convenient and simplistic means of subdividing a pest species. Biotype classifications have provided entomologists with a means to understand which arrangements of specific aphid-host relationships are successful and why they predominate in the field (Puterka, 1989). Smith (1989) reported that 14 insect species exhibit biotypes with the ability to overcome genetic plant resistance to insects. Nine of the 14 are aphid species in which parthenogenetic reproduction contributes greatly to the successful development of resistance breaking biotypes.

Greenbug biotypes are characterized by virulence, the aphid's ability to damage a resistance source (Puterka and Peters, 1990). Plant damage from greenbug feeding appears to occur as a response to an unknown phytotoxic substance in the saliva which the aphid injects while feeding. Electronically monitoring the feeding activity of the greenbug has shown that salivation was mandatory during the feeding process (Ryan *et al.*, 1987; Niassy *et al.*, 1987). In ultrastructural studies on susceptible wheat plants, Al-Mousawi *et al.* (1983) found necrosis and chlorosis at the feeding site that was characteristic of a phytotoxic response. Only white specks appeared on leaves of resistant plants due to localized cell collapse, indicating no phytotoxic response.

Greenbug History

The greenbug is an economically important pest of small grains and sorghum in the United States. The pest was first recorded in 1847 in Italy (Rondani, 1852). In the United States, the first greenbug infestation was reported on oat in Virginia in 1882 (Webster and Phillips, 1912). Intermittent severe outbreaks have occurred since that time (Rogers *et al.,* 1972). The greenbug occurs in all wheat-growing areas of the United States, but damage is usually most severe in the Southern and Central Great Plains (Joppa *et al.*, 1980). Oklahoma first experienced a serious greenbug outbreak in 1890 (Glenn, 1909). The 1907 and 1951 outbreaks each resulted in about $50 million in losses in Oklahoma, Texas, and Kansas (Walton, 1921; Dahms *et al.,* 1955). In 1976, in Oklahoma alone, losses due to greenbugs were estimated at $80 million (Starks and Burton, 1977).

Chemical control and use of resistant cultivars have been the two major approaches for the control of the greenbug. However, with increasing pesticide prices, coupled by decreasing sorghum and wheat prices and environmental concern, producers are looking toward host plant resistance as a more economically feasible means for control of the greenbug (Kerns, 1987).

Wood (1961) reported the first biotype in greenbugs when a wheat line, 'Dickinson Selection 28A' (DS28A), lost its resistance to a greenhouse strain of the greenbug. This strain was termed biotype B to differentiate it from the field population (biotype A). In 1968, biotype C was reported by Harvey and Hackerott (1969) for greenbugs which had extended their host range to include sorghum. In

1975, biotype D was reported (Teetes *et al.*, 1975) as an insecticide resistant strain of biotype C.

In the late 1970s, biotype E, capable of damaging the biotype C-resistant wheat, 'Amigo' (CI 7609), and several resistance sources of sorghum, developed in the High Plains of Texas (Porter *et al.*, 1982). Kindler and Spomer (1986) reported a sixth biotype, F, isolated from turfgrass in Ohio, which differed from biotype E in its ability to kill 'Reubens' Canada bluegrass, *Poa compressa* L., and its lack of a dorsal dark green stripe. Biotype G and H were identified by Puterka *et al.* (1988). Biotype G could damage the known sources of greenbug resistance in wheat, but was slow in damaging 'Wintermalt' barley, which is normally susceptible to greenbugs. Biotype H has the same effect on wheat cultivars as biotype E, but is relatively avirulent to all sorghums. Also, biotype H damages 'Post' barley, which had been resistant to all previous greenbug biotypes. Harvey *et al.* (1991) described biotype I as capable of damaging a large group of sorghum entries resistant to biotype E, but it is similar to biotype E on wheat and barley lines. Biotype I did not damage PI 266965 sorghum.

Field Survey for Greenbug Biotypes

Several surveys of greenbugs have been conducted to determine which biotypes are prevalent in the field. Puterka *et al.* (1982) conducted a greenbug survey in Texas, to determine biotype C and E distribution. Biotype E greenbugs were found in 17 of the 23 counties sampled; 14 of these 17 counties had both biotypes in the same wheat field. Biotype C was the predominant greenbug biotype (75%) in collections taken from wheat in the Texas Rolling Plains. Kindler *et al.* (1984)

determined the distribution of biotype E in Kansas, Nebraska, Oklahoma, and northern Texas during 1980-1981. In Kansas during 1980, biotype E was present in 5 of 13 counties, but by 1981 it was found in 23 of 24 counties sampled. During 1981, 40 of 42 counties sampled in Nebraska were infested with biotype E. The 1980 Oklahoma-Texas collections indicated that biotype E was present in 23 of 27 counties, with the highest concentration of biotype E in the "Panhandle" regions of both Oklahoma and Texas.

Dumas and Mueller (1986) made surveys to determine the distribution of greenbug, biotypes C and E on wheat in Arkansas. Results indicated that biotype C was the predominant biotype. Biotype E was also widely distributed throughout the wheat producing areas.

Kerns *et al.* (1987) conducted greenbug surveys in Oklahoma during the spring, summer and fall of 1986. Plant resistant sources, CI 1580 oat, 'Piper' sudangrass and 'TAM 107' (containing the 'Amigo' source), 'TAM 105' X LARGO, and CI 9058 wheat were used in testing collections so that described and undescribed biotypes could be identified. In the spring survey on wheat the biotype composition consisted of 83% biotype E, 11% biotype B, 4% biotype C, and 2% an undescribed biotype. The summer survey from sorghum and sorghum relatives resulted in 94% biotype E and 6% biotype C. The biotype composition for the fall survey on wheat was 97% biotype E, 2% biotype C and 1% biotype B. Bush *et al.* (1987) conducted a similar survey of greenbug biotypes in Texas from the fall of 1985 through the summer of 1986 to determine if the percentage of biotypes C and E had changed since 1981 and to determine if biotypes A, B, F or any new biotype existed in Texas.

Among their fall collected greenbugs, 11% were biotype C and 89% were biotype E; greenbugs collected in the spring and summer were 13.5% biotype C, 82% biotype E and 4.5% an unknown biotype. No biotype A, B or F were found in Texas.

Biotype E is considered the most common biotype in the Oklahoma wheat and sorghum fields (Kerns *et al.*, 1987). Biotypes F, G and H are not expected to displace biotype E in the field and are not considered to be a threat to wheat and sorghum (Kerns *et al.*, 1989). However, it is evident from laboratory inheritance studies (Puterka and Peters, 1990) that genetic recombination during the sexual cycle can result in some greenbug progeny being virulent to biotype E resistance sources in wheat and sorghum. Depending on the acreage planted to resistant cultivars, virulent biotypes would replace biotype E if they have superior fitness on the resistant plants (Harvey *et al.*, 1991).

Sexual Reproduction and Egg Hatching in Aphids

Greenbugs

Sexual morphs of the greenbug were first described by Washburn (1908). Webster and Phillips (1912) determined that greenbugs may overwinter as eggs north of 35° N latitude. In the northern states, sexual greenbug morphs occurred during October and November and deposited eggs mainly on bluegrass. The eggs passed through the winter and began to hatch in late March.

Mayo and Starks (1972) reported sexual morphs of the greenbug in greenhouses in Oklahoma. Males were found in biotype C colonies periodically from November to April, averaging about 17% of the alate population. Oviparae were found

consistently throughout the sexual season and averaged 15 to 25% of the apterous population. Mating was observed but no premating behavior was detected. Oviposition studies showed 2.5 to 3.5 eggs per female. None of the eggs hatched when held at 2, 4, 18, 21, and 27°C for 5 weeks.

Puterka and Slosser (1983) determined that sexual morphs of biotype C can be induced in environmental chambers at temperatures between 12 and 20°C with a 11:13 (L:D) h photoperiod. Eclosion was achieved in environmental chambers after eggs were held at temperatures <2.2°C for 6 to 12 weeks duration (Puterka and Slosser, 1986).

Puterka and Slosser (1986) reported on techniques to induce greenbugs into a sexual cycle and achieve egg hatching, thereby making it possible to sib-mate and cross different greenbug biotypes in the laboratory. Breeding greenbug biotypes enabled Puterka and Peters (1989) to pursue the mode of inheritance of virulence. Through the laboratory breeding program, it is now possible to screen plant resistance sources for potential biotypes not yet recognized in the field.

Eisenbach and Mittler (1987) compared biotype C and E on the basis of sexual morph production under different photoperiodic regimes. The threshold (minimal) scotophase for the induction of males in biotype C was 11 h, whereas biotype E required a scotophase (night length) between 11 h 14 min and 11 h 32 min. Oviparae production by both biotypes required a scotophase >11 h 32 min.

Mittler and Gorder (1991) reported variation in local California greenbug clones and biotype C and E (from Oklahoma and Nebraska, respectively) in response to length of scotophase. It was concluded from their findings that a scotophase of 11 h

and 15 min or more is required to get oviparae and males from these clones and biotypes in the growth chamber.

Other Aphids

Leather and Lehti (1981) sampled eggs of the bird cherry-oat aphid, *Rhopalosiphum padi* L., from 87 sites throughout southern Finland. Egg mortality was found to be 3 to 6% per week throughout the winter depending on locality. Egg abundance was significantly correlated with longitude, the highest egg densities being found on the western coast of Finland. This was attributed to the prevailing wind in August. Similarly the number of fundatrices hatching in the spring was significantly correlated with the number of eggs found in the January. Simon *et al.* (1991) tested 70 clones of *Rhopalosiphum padi* L. at two temperatures (10°C and 15°C) in the laboratory. Three of the clones, originating from the spring population on the primary host, bird cherry (*Prunus padus* L.), were holocyclic, producing gynoparae and then males in the second generation. Of the other clones, all originating from Gramineae, 78.5% were anholocyclic and 21% androcyclic (male-producing) at 15°C, but at 10°C only 12.7% were anholocyclic, and 4.7% showed an intermediate response (i.e., some of the alate females produced both sexual and parthenogenetic offspring). Considerable interclonial and inter-individual variation in the number of males produced by the androcyclic clones was reported.

Müller (1985) reviewed biotype formation, specialization, and hybridization in Aphidinae. Hybridization has been observed in *Acyrthosiphon pelargonii* (Kaltenbach) where crossbreeding the subspecies *A. p. pelargonii* Theobald and *A. p.*

geranii Kaltenbach in an insectary was successful although no hybrids were found in the field. Crossbreeding of aphid species has also been successful in the genus *Aphis* (Muller, 1985). The four species involved in the breeding experiment were *Aphis fabae* Scopoli, *A. cirsiiacanthoides* Scopoli, *A. solanella* Theobald, and *A. euonymi* Fab. All of these aphids were holocyclic and overwinter on spindle bush, *Euonymus europaea* L. Seven of the 12 crossing combinations resulted in fertile eggs and fundatrices. Viable F_2 cycle progeny were also obtained and revealed a typical Mendelian segregation ratio, 1:2:1, based on color.

Wegorek and Dedryver (1987) compared the sexual morph production in *Sitobion avenae* F., collected from three locations in France. Differences were found among the clones in their ability to produce gynoparae and oviparae at given temperature-photoperiod regimes. It was concluded that the three clones were holocyclic and probably able to produce some sexual morphs outside normal autumn conditions.

MacKay (1987) monitored production of sexual and viviparous morphs of two clones of pea aphid, *Acyrthosiphon pisum* (Harris), under photoperiods ranging from 18:6 to 10:14 (L:D) h at 20° C. Under long photophases only viviparae were produced. At intermediate photophases families were produced in which the oldest progeny were females while the youngest progeny were males. Similarly, photoperiodic responses of two clones of *A. pisum* (Harris) collected on the same day in the same field were compared by MacKay (1989). These clones differed not only in the photoperiod at which sexuals first appeared, but also in the rate of transition from production of one morph to another.

Via (1992) induced sexual morphs in six clones of pea aphids in the laboratory.

Eggs were successfully hatched by creating decreasing light conditions that closely resembled those found in the field situation. Eggs were exposed to a daily cycle of freezing and thawing in an incubator under a short day photoperiod. Eclosion averaged 60% but was as high as 89%.

Aphid Hybridization and Host Feeding

Puterka and Peters (1989) investigated inheritance of virulence in greenbugs to wheat. Clones of biotype C, E, and F were successfully induced into sexual cycle, sib-mated, and reciprocally crossed. The resulting progenies were tested against *Gb2* ('Amigo') and *Gb3* (LARGO). Based on their findings, a duplicate gene-modifier gene inheritance model was proposed where avirulence was dominant and virulence was recessive. Virulence to genes *Gb2* and *Gb3* appeared to be conditioned by duplicate genes and a modifier gene epistatic to one of the duplicate genes.

Sources of Plant Resistance to Greenbugs

Wheat (*Triticum aestivum* L.)

Screening of small grains germplasm for resistance to the greenbug goes back to Atkins and Dahms (1945) who found barley, wheat, and oat cultivars in field test plots in Oklahoma and Texas with different levels of susceptibility to greenbugs. Painter and Peters (1956), using a greenbug-resistant selection of 'Dickinson No. 485' in crosses with the 'Pawnee', 'Concho', and 'Bison' wheat, found a single gene difference between resistant and susceptible strains with susceptibility being dominant

to resistance. Similar results were obtained by Daniels and Porter (1958). Curtis *et al.* (1960) reported that 'Dickinson Selection 28A,' CI 13833, is a hexaploid selection from a durum wheat germplasm line 'Dickinson No. 485' (CI 3707), and has a recessive gene, designated *gbgb* (*Gb1*), that confers greenbug resistance. Their results indicated that CI 9058 appeared to have the same recessive gene.

Wood *et al.* (1974) developed 'Gaucho' (CI 15323) an octaploid triticale from a cross between susceptible 'Chinese Spring' wheat and an Argentine rye, 'Insave F. A.' which is resistant to greenbugs. The resistant gene (actually the short arm of 1R, but often referred to as a single dominant gene) from 'Insave F. A.' rye was incorporated into a winter wheat germplasm line, 'Amigo' (CI 17609), using X-ray techniques (Sebesta and Wood, 1978). Tyler *et al.* (1987) designated this gene as *Gb2*. 'Amigo' is resistant to biotypes A, B and C, but not to biotype E (Porter *et al.*, 1982). A single dominant gene (*Gb3*) derived from *Triticum tauschii* (Coss.) Schmal. has been identified in an amphiploid of *T. turgidum/T. tauschii* and is called 'Largo' (CI 17895) (Joppa *et al.*, 1980). *Gb4* is a dominant gene in CI 17959, another amphiploid from crossing *T. durum/T. tauschii* (Martin *et al.*, 1982). Both *Gb3* and *Gb4* provide resistance against biotypes C and E. *Gb5* which also provides resistance against biotype E was identified in CI 17882, a hexaploid wheat germplasm derived from *Triticum speltoides* (Tausch) Gren. ex Richter (Tyler *et al.*, 1987). Biotype G is capable of damaging all the above sources of resistance in wheat (Puterka *et al.*, 1988).

Porter *et al.* (1991) investigated a series of lines, (GRS1201-1205) developed by pollinating hard-red winter wheat females with X-ray irradiated pollen of

wheat/'Insave F. A.' rye. The GRS series are resistant to biotypes B, C, E and G. Resistance in the GRS series originated from 'Insave F. A.' rye but the response of the GRS series to biotype E and G in comparison to 'Amigo', suggests that this source of resistance is different from *Gb2*. Starks *et al.* (1983) suggested that 'Insave F. A.' rye carried more than one gene for resistance, but the one effective for biotype E was not transferred to 'Amigo'. This additional gene appears to be the source of resistance in the GRS series termed *Gb6* (Porter, personal communication).

Barley (*Hordeum vulgare* L.)

Atkins and Dahms (1945) reported high levels of resistance to greenbugs among several barley cultivars. Smith *et al.* (1962) tested four entries of winter barley 'Omugi', 'Dobaku', 'Kearney', and CI 5087 and concluded that there is at least one common dominant factor controlling greenbug resistance in these entries. 'Post' barley, CI 15695, originated as an F_3 head selection from the cross, 'Harrison'/'Will' (Edwards *et al.*, 1985). 'Post' was resistant to all known biotypes of greenbug until the appearance of biotype H (Puterka *et al.*, 1988). Webster and Starks (1984) identified 15 barley lines to be resistant to biotype E in a greenhouse screening tests. These lines were further tested for the components of resistance (antibiosis, antixenosis, and tolerance) to biotypes C and E. 'Post' had the highest level of antibiosis to both biotypes, however, PI 426756 appeared to have greater resistance when the combined resistance components were examined.

Rye (*Secale cereale* L.)

Several rye cultivars have been found to have greenbug resistance. Wood *et al.*

(1969) tested 'AR-4' against biotypes A, B, and C and found it to be resistant to all three biotypes.

'Insave F. A.' rye from Argentina was reported to be resistant to biotype A, B, and C (Sebesta and Wood, 1978), and biotype E (Porter *et al.*, 1982). *Gb2* in 'Amigo' wheat was derived from 'Insave F. A.' (Sebesta and Wood, 1978). Starks *et al.* (1983) reported moderate resistance in 'Insave F. A.' to greenbug biotype E. Starks *et al.* (1983) found 'Okema', rye which was also developed from 'Insave F. A.,' was resistant to biotypes A, B, and C.

Sorghum (*Sorghum bicolor* L.)

The occurrence of biotype C greenbugs on sorghum in 1968 (Harvey and Hackerott, 1969) led to a concerted effort to screen sorghum germplasm for resistance and to transfer this resistance to adapted germplasm (Schuster and Starks, 1973). A total of 18 sources of sorghum resistance to biotype C greenbugs were reported for the seedling and the adult plant stages by Hackerott *et al.* (1969) and Teetes *et al.* (1974a, b). By 1980, about 90% of the sorghum acreage in the United States was planted to biotype C resistant hybrids. These hybrids were derived mainly from SA 7536-1 and 'KS 30'. Both of these sources derived their resistance from *S. virgatum* (Hackel) Stapf (Starks *et al.*, 1983). After the detection of greenbug biotype E in a wheat field near Bushland, Texas in November, 1979 (Porter *et al.*, 1982), this biotype quickly replaced biotype C. Only four of the 18 sorghum sources of resistance to biotype C maintained their resistance when attacked by biotype E. These were PI 264453, PI 220248, PI 229828 and 'Capbam' (sarvasi) (Porter *et al.*,

1982; Starks *et al.*, 1983). The results of comparative screening (Starks *et al.*, 1983) for resistance to biotype E in nine sources of resistance to biotype C indicated that 'Capbam' and PI 264453 maintained their resistance at about the same level to the new biotype, but the resistance of PI 220248 was reduced. Hackerott *et al.* (1983) reported on efforts to screen 9,000 additional germplasm accessions for resistance to biotype E and found eight additional biotype E resistant sources.

Hackerott *et al.* (1969) reported that tolerance was the major component of resistance in accessions of *S. virgatum* although antibiosis and/or non-preference were also important in confinement tests. Schuster and Starks (1973) studied the components of host plant resistance to greenbug biotype C using 11 sorghum entries including 'BOK 8' as the susceptible check. They concluded that PI 229828, PI 302178, PI 226096, IS-809, and SA 7536-1 possessed high degrees of all three mechanisms of resistance. Tolerance was the main component of resistance in PI 264453, but PI 302231 and PI 220248 showed relatively low levels of tolerance. Teetes *et al.* (1974a) in a similar study found that PI 264453, SA 7536-1 and 'KS 30' all showed definite non-preference and antibiosis. Dixon *et al.* (1990) reported that out of 12 varieties tested for biotype E resistance, PI 229828 had the highest level of tolerance and the least tolerant was PI 302136, with all remaining entries showing intermediate tolerance.

Resistance of lines derived from *S. virgatum* was reported to be conferred by dominant genes at more than one locus (Hackerott *et al.*, 1969). Resistance to biotype C derived from IS 809, SA 7536-1, PI 220248, and PI 302236 was incompletely dominant but simply inherited (Johnson, 1971; Johnson and Teetes,

1972). Biotype E resistance derived from PI 220248, 'Capbam', and 'TAM BK42', (a derivative of PI 264453), was not inherited as a recessive characteristic according to Johnson *et al.* (1981).

Greenbug biotypes F, G, and H are relatively avirulent to sorghum (Puterka *et al.*, 1988). Sorghum hybrids resistant to greenbug biotype E lost their resistance to a greenbug isolate from Stevens County, Kansas in 1990. Since most resistant sorghum hybrids have similar sources of resistance, none of the 60 sorghum hybrids observed were resistant to the Stevens County greenbug isolate in the seedling stage in the greenhouse. This isolate was named, biotype I by Harvey *et al.* (1991). When additional sources of resistance to biotype E greenbugs were tested against biotype I, only PI 266965 was found resistant. 'Cargill 607E' and 'Cargill 797' have also been reported to withstand field infestations of biotype I (Harvey *et al.*, 1993).

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CHAPTER III

GREENBUG BIOTYPE SURVEY

Introduction

The greenbug, *Schizaphis graminum* (Rondani), is a versatile pest of small grains and sorghum which has developed new biotypes to the imposed plant resistance in both wheat and sorghum. Until now, eight host virulent biotypes have been identified. The greenbug enhanced its status as a serious pest in 1968 when the grain sorghum crop on the High Plains was severely attacked (Harvey and Hackerott, 1969). Biotype E developed on the High Plains of Texas in the late 1970s (Porter *et al.*, 1982) and was capable of damaging the biotype C-resistant wheat 'Amigo' and several resistant sources of sorghum.

Tracking the biotype composition of greenbug populations in the field is important for pest management. With the development of new greenbug biotypes and release of new sources of resistance, several biotype surveys have been conducted to determine which biotypes were most prevalent in the field populations. A greenbug biotype survey was conducted in the Texas Rolling Plains by Puterka *et al.* (1982). They concluded that biotype C was the most prevalent. Kindler *et al.* (1984) determined the distribution of biotype E in Kansas, Nebraska, Oklahoma, and northern Texas during 1980-81. In Kansas during 1980, biotype E was present in 5

of 13 counties, but by 1981 it was found in 23 of 24 counties sampled. During 1981, 40 of 42 counties sampled in Nebraska were infested with biotype E. The 1980 collections in Oklahoma and Texas indicated that biotype E was present in 23 of 27 counties, with the highest concentration in the "Panhandle" regions of Oklahoma and Texas.

Kerns *et al.* (1987) conducted three greenbug surveys in Oklahoma during 1986. Plant resistant sources, CI 1580 oat, 'Piper' sudangrass, and 'TAM 107', 'TAM 105' X 'Largo', and CI 9058 wheat were used to identify described and undescribed biotypes. In the spring survey on wheat, the biotype composition consisted of 83% biotype E, 11% biotype B, 4% biotype C, and 2% an undescribed biotype. The summer survey from sorghum and sorghum relatives resulted in 94% biotype E and 6% biotype C. The biotype composition for the fall survey on wheat was 97% biotype E, 2% biotype C, and 1% biotype B.

Bush *et al.* (1987) conducted a similar survey of greenbug biotypes in Texas from fall of 1985 through the summer of 1986 to determine if the percentage of biotypes C and E had changed since 1981 and to determine if the biotypes A, B, and F or any new biotype existed in Texas. Of the 1985 fall collected greenbugs, 89% were biotype E and 11% biotype C. Greenbugs collected in the spring and summer of 1986, were 82% biotype E, 13.5% biotype C, and 4.5% were undescribed. Biotypes A, B, or F were not found in Texas. From all these surveys it was concluded that biotype E was the most common biotype in the field.

Harvey *et al.* (1991) reported a new biotype 'I' from Stevens County, Kansas,

capable of damaging biotype E resistant sorghum. This finding was added justification for surveys of biotypes in Oklahoma.

Materials and Methods

General Materials and Methods

Two greenbug biotype surveys were conducted: spring (March) 1991, on wheat, and late summer (August-November) 1992, mostly on sorghum. The spring 1991 survey included 20 counties in western Oklahoma, while the late summer 1992 survey included Payne County and selected counties in southwestern and northwestern Oklahoma. A single forage sorghum (DeKalb FS25E) field near Stillwater was intensively sampled in May 1992 to determine within field variability in biotypes present.

A single sample of greenbugs was collected from each field at intervals of 10-15 miles along state and federal highways when such locations were available. In the spring survey, greenbugs were collected from wheat fields. Greenbugs were found mainly on the lower side of the lower leaves. Depending on their population density, five to 10 greenbugs were collected per sample. Greenbugs on portions of a leaf were placed in a 100 by 15 mm clear plastic petri dish. Before collection, a round piece of paper towel was cut to fit in the petri dish. Several drops of water were added to each sample to prevent desiccation. It was important to moisten the paper towel but avoid any free water in the dish. Petri dishes were held shut with masking tape, labeled and transported to laboratory.

As soon as samples arrived in the laboratory, a single greenbug from each sample

was transferred to a single-leaf stage seedling of 'Triumph 64' (TR64) wheat growing in a 9 cm high by 8 cm diameter styrofoam cup filled with sandy soil. Each selected greenbug was caged with a 17 cm high by 3.5 cm diameter transparent plastic tube with a top covered by a fine mesh cloth. The remainder of each sample was refrigerated at 6°C until the colonies were established. Any greenbug that died before establishing a colony was replaced by a greenbug from the same field sample. After colonies were established the field samples were discarded. The colonies were maintained in a controlled environment chamber with temperature regimes of 15°C and a photoperiod of 15:9 (L:D) h.

Biotypes were distinguished using various combinations of wheat, barley, rye, and sorghum cultivars. The study of lesion formation caused by each biotype's feeding on TR64, 'TAM 107'. 'TAM 105' X 'Largo' (LARGO), 'Dickinson Selection 28A' (DS28A), CI 17959, CI 9058 and GRS1201 was facilitated by clip-on cages (Puterka and Peters, 1988). Each entry was planted (4 to 5 seeds) in sandy soil in 9 cm high by 8 cm diameter styrofoam cups and covered before seedling emergence with a 17 cm high by 3.5 cm diameter plastic cage with the top end enclosed by a fine mesh cloth to prevent any possible aphid feeding. All such cups were placed either in a culture room or growth chamber at 22:20°C temperature and a 15:9 (L:D) h photoperiod.

At the two leaf stage (ca. 5 d after emergence), plants in a cup were thinned to three and three clip-on cages were mounted on one leaf per plant, totaling nine cages per cup. Cages were marked and numbered from the leaf base to the tip. For each clone, two apterous adults or large nymphs were placed into a clip-on cage for each

selected cultivar. Each clip-on cage was plugged with a foam stopper. All cups were placed in a growth chamber maintained at 18°C or 20°C, and with a photoperiod of 13:11 (L:D) h. The cages and aphids were removed after 24 h. Plants were placed back into the growth chamber for another three days and then each leaf was observed to determine if any lesions had formed (chlorosis and necrosis) in response to the greenbug feeding. Data were recorded as 'lesion' or 'no lesion'. This technique enabled us to determine biotypes B, C, E, F, G, and H. Using this method we were not able to differentiate between biotypes E and I, and some field isolates. Furthermore, to test the sorghum and barley sources of resistance, a modified star-pot technique (Wood *et al.*, 1969) was adapted for further biotype isolation and characterization. Selections within one pot configuration (wheat) consisted of TR64, DS28A, 'TAM 107', LARGO, GRS1201, and CI 9058 or 'Post' barley. A second configuration (sorghum) included 'Post' barley or 'Elbon' rye, 'Piper' sudangrass, and sorghum hybrids: 'Pioneer 8493', 'Pioneer 8515', and 'Cargill 607E', with TR64 as a control. The 'Post' barley was intended as a resistant standard, but at the 25°C temperature used for sorghum testing, the resistance occasionally broke down when infested by some clones.

The pots were 15 cm diameter by 15 cm deep and filled sandy soil. A row-marker was pressed into the soil in each pot, leaving six symmetrically radiating row depressions in a pie-shaped design. Seeds (4 to 6) of one entry were planted in one row and covered with about 1 to 2 cm soil. Each pot was watered from the base. To avoid unwanted infestation, the soil within pots was covered with cages 33 cm high by 12.5 cm diameter with the top and two side holes of 7 cm diameter covered with a

fine mesh cloth. Plants were watered as needed and a 25% concentration of Hoagland's solution was used once a week. When wheat seedlings were 5 to 8 cm in height or sorghum seedlings in 1 to 2 leaf stage or 6 to 9 d after planting, the plants in each row (entry) were thinned to 3 or 4 plants. The plants in each pot were then infested with 100 to 200 aphids from a clone. This level of infestation was used to ensure that mostly tolerance would be manifested and the confounding effects of plant antibiosis and antixenosis would be reduced (Puterka *et al.*, 1988). In case clone multiplication was not synchronized with plant emergence, as few as 75 aphids were used to infest the 2 to 5 d old plants rather than delay infestation and allow plant age to become a factor in the response to aphid feeding. For confirmation such under-infested experiments were often repeated. After infestation, the pots were placed in growth chambers at 15:9 (L:D) h photoperiod and temperature regimen of 20:18°C for wheat or 25°C and the same photoperiod for sorghum.

All of the entries in the pot were evaluated when one of the susceptible entries showed clear signs of severe damage (complete chlorosis or dead plants). This usually occurred about 14 days after infestation. Plants were visually rated for damage using a 1 to 6 scale: 1 = no visual damage; 2 = 1-25% damage (chlorosis, necrotic spots and stunting); 3 = 26-50% damage; 4 = 51-75% damage; 5 = 76-99% damage; and 6 = dead plant. Ratings of 1 to 3 were regarded as resistant while 4 to 6 were regarded as susceptible. Results for three plants in each row were recorded.

Materials and Methods for Spring 1991 Survey (March) on Wheat

Seventy-five samples were collected during this survey from wheat fields in 20 counties in western Oklahoma (Table 1). Aphids in 12 samples were lost to parasitization, leaving 63 samples for evaluation. Biotype determination was conducted using only the clip-on cage technique. TR64, 'Amigo', LARGO, CI 17959 and DS28A were used in this experiment. Virulence classification based on lesion tests of the respective biotypes are given in Table 2. Using these sources of resistance we were unable to differentiate between biotype E and I, so a subset of 33 clones was submitted to Dr. Dillwith's Laboratory (OSU, Entomology Department, Stillwater) for the determination of possible differences in cuticular hydrocarbons. A subset of 12 samples was sent to Roxanne Shufran at Kansas State University, Manhattan, KS, to be tested for possible resistance to a organophosphorus insecticides.

Materials and Methods for Late Summer 1992 Survey

This survey consisted of two sets. The first set of 10 samples was collected on sorghum from the Agronomy Farm near Perkins, Oklahoma (Payne County), and a second set of 24 samples was collected from selected counties (Table 4) in southwestern (sorghum) and northwestern (wheat) Oklahoma. Many additional fields were visited during this period but no greenbugs were found. Star-pots of wheat and sorghum were used for all samples along with the clip-on cages for further confirmation of biotype identification.

Materials and Methods for Intensive Sampling of a Sorghum Field

Extensive sampling was conducted in a forage sorghum field near Stillwater, Oklahoma (Payne County) using a stratified sampling plan with 30 m between samples. Samples were collected from the lower sides of the lower leaves of sorghum plants 40 to 80 cm in extended height, using a 10 x 10 grid method of sampling. Aphids in eight (7.8%) of the 102 samples collected were lost to parasitization. The samples were designated as #1 through #94.

Biotype determination tests were conducted using the clip-on cage method. After cloning and establishment of colonies each sample was tested for its capabilities to make lesions (chlorotic and necrotic lesions) on the selected wheat entries. A single greenbug from a sample was placed on the first leaf of TR64, 'Amigo', DS28A, LARGO, PI 9058, and GRS1201 (expected responses are given in Table 2) and confined using a clip-on cage. Sequence of the entries used was randomly assigned to a greenbug to prevent any possible plant conditioning response. Each greenbug was allowed to feed for 24 h. After 24 h the greenbugs and clip-on cages were removed and the plants were returned to the growth chamber. After an additional three days, the plants were observed for the presence (virulent) or absence (avirulent) of necrotic lesions on the test cultivars.

Star-pots of wheat and sorghum were used for a few selected samples in conjunction with the clip-on cage technique to insure soundness in the methodology. The wheat star-pot configuration consisted of TR64, 'Amigo', LARGO, GRS1201, DS28A, and 'Post' barley. A sorghum star-pot consisted of TR64 wheat, 'Piper' sudangrass, 'Elbon' rye, 'Cargill 607E', 'Pioneer 8515', and 'Pioneer 8493'

sorghum. Response of these wheat and sorghum entries should distinguish among all described biotypes, including the new biotype I.

RESULTS AND DISCUSSION

Spring Survey

On the basis of the lesion responses, the survey samples were assigned to biotypes B, C, E, F, and G (Table 3). Biotype B made lesions on DS28A, LARGO and CI 17959. 'Amigo' is reported as resistant to biotype B (Porter *et al.*, 1982). A field collected sample (Z23) caused lesions on DS28A, CI 17959, and LARGO but did not cause lesions on 'Amigo.' This sample was identified as biotype B and was later found to be virulent to the Syrian source of resistance in sorghum (PI 550610). When laboratory reared biotype B was tested against this source, it was as virulent as the field collected biotype B.

Biotype C is reported as virulent to DS28A, but avirulent to 'Amigo' (Porter *et al.*, 1982), LARGO (Webster *et al.*, 1986), and CI 17959 (Martin *et al.*, 1982). Three of the field collected clones gave the same pattern of responses and were considered as biotype C. Biotypes E and H are reported as virulent to DS28A and 'Amigo' (Porter *et al.*, 1982), and avirulent to LARGO (Webster *et al.*, 1986); using these criteria, 55 of the clones were considered as biotypes E or H. In unpublished tests, I found biotype H generally caused lesions on CI 17959 but biotype E did not. Twenty-four of the 55 clones were virulent to CI 17959 while the rest of the 32 clones did not make any lesions on CI 17959. When a subset of 33 clones were

tested for cuticular hydrocarbon peaks they gave the same pattern as biotype E. I interpreted this to indicate that not only is the field population of greenbugs heterogeneous, but variability also exists within biotype E. The subset of 12 clones tested for possible pesticide resistance showed negative results.

Based on their avirulence to DS28A and virulence to 'Amigo', LARGO, and CI 17959 as reported by Kindler and Spomer (1986), two of the samples were identified as biotype F. Two clones were virulent to DS28A, 'Amigo', LARGO, and CI 17959 and were considered as biotype G as reported by Puterka *et al.* (1988).

Biotype E was the dominant biotype comprising 87.3% of the samples and was collected from 19 of the 20 counties, followed by biotype C (4.75%) from Blaine, Major and Stephens Counties. The two samples of biotype F were collected from Garfield and Lincoln Counties, and the two samples of biotype G were collected from Jackson and Murray Counties. A single sample of biotype B was collected from Tillman County.

Late Summer Survey

Results for the summer 1992 survey are presented in Table 4. About 40 additional sorghum fields were visited and while most were infested with corn leaf aphids, *Rhopalosiphum maidis* (Fitch), no greenbugs were found. One (PK3 in Table 5) of the 10 samples collected from Perkins was virulent to Pioneer 8493 (biotype E resistant sorghum) and was considered as biotype I. Of the 24 samples collected from the selected counties, two clones (S26 and S27 in Table 5) were virulent to Pioneer 8493. These clones were also considered to belong to biotype I. One clone (S24 in Table 6) was virulent to 'Amigo', DS28A, and LARGO and was therefore considered to be biotype G.

Intensive Sampling

These samples were designated as #1 to #94. The clip-on cages results for this intensive sampling experiment are presented in Table 7. Using this technique, all of the clones were virulent to TR64, 'Amigo', DS28A, and PI 9058, but none to GRS1201. Results of testing laboratory reared biotypes and clones on the wheat star-pot are given in Table 8. These were used for characterizing the responses of some field collected aphids. Two samples (#49 and #51 in Table 6) were found making lesions on LARGO but these clones were not virulent to 'Post', and were considered as biotype G as described by Puterka *et al.* (1988). Using clip-on cage and wheat star-pot we were not able to differentiate between biotype E and I, so some selected clones were tested using sorghum star-pot. One of the samples (#57) was virulent to 'Pioneer 8493' (Table 5) and was considered biotype I as reported by Harvey *et al.* (1991). Ninety-one samples out of 94 were considered to be biotype E.

Biotype E is still the dominant biotype. However, the spreading distribution of biotype I suggests that a shift from biotype E to I has begun. The overall results of the two surveys and extensive field sample are given in Table 9. The presence of biotype I was detected in Oklahoma from these surveys. I did not obtain any biotype I samples from the field survey of March 1991. It is possible that biotype I was present in these samples. Only the clip-on cage technique was used for the March 1991 survey because it works best for wheat cultivars, but for biotype E and I, sorghums must be used as the differential hosts (Harvey *et al.*, 1991). None of the 1991 and 1992 field samples were virulent to GRS1201, the most recently reported (Porter *et al.*, 1991) wheat source with resistance to greenbugs.

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TABLE 1

GREENBUG SURVEY FROM WHEAT FIELDS FROM SELECTED COUNTIES IN OKLAHOMA, MARCH 1991

Crop Reporting District Number	County	Sample Number Assigned	Total Samples/County
2	Blaine	Z42, 43, 44	3
	Washita	Z75, 76	2
	Jackson	Z21, 27, 28, 80	4
	Greer	Z71, 72, 73	3
	Kiowa	Z14, 26	2
	Caddo	Z77, 78, 79	3
		Samples in District	17
4	Noble	Z31, 32, 33, 34	4
	Garfield	Z35, 36, 37, 38	4
	Major	Z40, 41	2
	Tillman	Z22, 23, 24, 25	4
		Samples in District	14
5	Kingfisher	Z45	1
	Payne	Z46, 51, 52	3
	Lincoln	Z53, 60, 61, 62, 63	5
	Pottawatomie	Z54, 55, 57, 58	4
	Grady	Z02, 03, 04	3
		Samples in District	16
6	Garvin	Z05, 06, 07, 10	4
	Carter	Z09, 16	2
	Stephens	Z01, 11, 17, 18, 19	5
	Jefferson	Z12, 13, 14, 15	4
	Murray	Z08	1
		Samples in District	16
Total Samples:			63

TABLE 2

VIRULENCE CLASSIFICATION OF GREENBUG TO WHEAT RESISTANCE SOURCES

Biotype	TR64	'Amigo'	LARGO	CI 17959	DS28A	PI 9058	GRS1201
B	V	A	V	V	V	V	A
C	V	A	A	A	V	V	A
E	V	V	A	A	V	V	A
F	V	V	V	V	A	A	V
G	V	V	V	V	V	V	A
H	V	V	A	V	V	V	V

V = virulent A = avirulent

TABLE 3

GREENBUG BIOTYPE COMPOSITION FOR WHEAT SURVEY IN OKLAHOMA, SPRING 1991

Biotype	Number of samples	Percentage	Sample Numbers
B	1	1.6%	Z23
C	3	4.8%	Z18, Z40, Z42
E	55	87.3%	All others
F	2	3.2%	Z38, Z53
G	2	3.2%	Z08, Z21

TABLE 4

GREENBUG SURVEY FROM GRAIN SORGHUM IN PERKINS AND SELECTED COUNTIES IN OKLAHOMA, (AUGUST-NOVEMBER) 1992

Oklahoma County	Sample Number	Biotype E	G	I
Stephens	S1, 2, 6	3	0	0
Garvin	S3, 4, 5	3	0	0
Jefferson	S7	1	0	0
Cotton	S8, 9	2	0	0
Commanche	S10	1	0	0
Grady	S11,12, 13, 14	4	0	0
Cleveland	S15	1	0	0
McClain	S16,17,18	3	0	0
Texas	S22,23,24,25,26,27	3	1	2
Payne (Perkins)	Pk01,1,...Pk9	9	0	1
Total		30	1	3

TABLE 5

DAMAGE RATINGS CAUSED BY FIELD COLLECTED GREENBUG SAMPLES TO THE SIX ENTRIES USED IN THE SORGHUM STAR-POT

	Field Collected Sample Designation			
Cultivars	#57	PK3	S26	S27
TR64	*6.0a	6.0a**	5.7a	6.0a
'Piper'	6.0a	5.7a	5.7a	5.7ab
'Elbon'	6.0a	4.3a	5.3a	6.0a
'Pioneer 8515'	5.7a	6.0a	5.0a	6.0a
'Cargill 607E'	5.3a	4.0a	5.0a	5.3ab
'Pioneer 8493'	5.0a	5.7a	5.0a	4.6b

* Mean damage rating (1 = 0 and 6 = 100% visual rating of leaf damage).

** Within columns means followed by the same letter are not significantly different. (Scheffe's, $P > 0.05$, SAS Institute, 1988).

TABLE 6

DAMAGE RATINGS CAUSED BY FIELD COLLECTED GREENBUG SAMPLES TO THE SIX ENTRIES USED IN WHEAT STAR-POT

Cultivars	Field Collected Sample Designation		
	#49	#51	S24
TR64	*6.0a	6.0a**	6.0a
DS28A	6.0a	6.0a	6.0a
Amigo'	6.0a	6.0a	6.0a
LARGO	5.0b	5.0b	6.0a
'Post'	2.0c	2.0c	3.7c
GRS1201	1.3c	1.0d	1.7c

* Mean damage rating (1 = 0 and 6 = 100% visual rating of leaf damage).

** Within columns means followed by the same letter are not significantly different. (Scheffe's, $P > 0.05$, SAS Institute, 1988).

TABLE 7

BIOTYPIC VARIATION IN GREENBUG SAMPLES COLLECTED FROM A SINGLE SORGHUM FIELD MAY 1992

Biotype	Number of samples	Percentage	Sample Number
E	91	97	All others
I	1	1	# 57
G	2	2	# 49, 51

TABLE 8

DAMAGE RATINGS CAUSED BY GREENBUG BIOTYPES TO CONFIGURATIONS OF THE WHEAT STAR-POT TEST*

Cultivars	B	C	E_1	E_2	F	G	H	I
TR64	6.0a**	6.0a	5.3a	6.0a	5.3ab	5.7a	5.0a	5.7a
'Amigo'	1.0b	1.0c	5.7a	6.0a	6.0a	6.0a	5.7a	6.0a
LARGO	6.0a	2.3b	1.7b	2.0c	4.0abc	6.0a	2.0b	2.0b
DS28A	5.7a	6.0a	6.0	6.0a	2.7bc	5.7a	6.0a	5.7a
PI 9058	5.7a	6.0a	5.7	-	2.0a	6.0a	6.0a	6.0a
'Post'	2.0b	2.0b	2.0b	4.0b	-	-	-	-
GRS1201	-	-	-	2.0c	4.3abc	1.0b	5.0a	2.0b

* Either 'Post' or GRS1201 were used except in the case of E1, where PI 9058 was substitute for GRS1201.

** Within columns means followed by the same letter are not significantly different (Scheffe, $P > 0.05$, SAS Institute, 1988).

TABLE 9

GREENBUG BIOTYPE COMPOSITION FOR THE TWO SURVEYS AND INTENSIVE FIELD SAMPLING IN OKLAHOMA, 1991-1992.

Biotype	March 1991 Number	(%)	May 1992 Number	(%)	Aug-Nov 1992 Number	(%)
B	1	(1.6)	0	(0)	0	(0)
C	3	(4.8)	0	(0)	0	(0)
E	55	(87.3)	91	(97)	30	(88)
F	2	(3.2)	0	(0)	0	(0)
G	2	(3.2)	2	(2)	1	(3)
I	0	(0.0)	1	(1)	3	(9)

CHAPTER IV

SEXUAL REPRODUCTION CAPABILITIES OF BIOTYPES AND SELECTED CLONES OF GREENBUGS

Introduction

Greenbug, *Schizaphis graminum* (Rondani), populations are usually composed of several biotypes. Greenbug biotypes are usually characterized by their ability to differentially damage various sources of resistance in small grains and sorghum. The capability of the greenbug to develop new biotypes, which overcome the resistance sources, is a continuing problem in the process of breeding for resistance against greenbugs. Each biotype is a phenotypic expression of an indefinite number of genotypes (Puterka and Peters, 1990). These genotypes may be changed either by mutation or by recombination during sexual reproduction. Understanding the sexual reproduction capabilities of various biotypes/clones of greenbugs is important in tracing biotype shifts in the field.

Sexual morphs of the greenbug were first documented by Washburn (1908). Webster and Phillips (1912) determined that greenbug egg production occurred mainly north of the 35th parallel where greenbugs overwintered as eggs. Tucker (1918) and Daniels (1956) reported egg production in greenhouses south of the 35th parallel in Texas.

Wood (1971) documented biotype C males from the field in Oklahoma. Field

reports of holocyclic greenbug morphs are few from grain fields, but common from bluegrass. Overwintering eggs were reported from Ohio (Niemczyk and Power, 1982) and Kentucky (Potter, 1982) on Kentucky bluegrass turf; these lead to early damaging infestations by the newly hatched greenbugs and their progeny.

Puterka and Slosser (1983) were successful in inducing sexual morphs in biotype C of greenbug using various temperature-photoperiod regimes. Later, Puterka and Peters (1989) were able to get eggs to hatch from greenbug biotypes C, E, and F.

Understanding the sexual reproductive capabilities of greenbug biotypes and clones will not only help in managing it's population in the field but also could be used in genetic studies and screening of resistance sources for future uses.

MATERIALS AND METHODS

Experiment to Produce Sexual Morphs Capable of Producing Eggs

In this experiment all major biotypes, a series of laboratory reared clones, and field collected samples were tested for sexual reproduction capabilities. Selected clones were transferred to TR64 wheat seedlings grown in 8 cm diameter styrofoam cups. To avoid contamination from other colonies, each cup was covered with a 5 cm diameter and 15 cm tall clear plastic cylinder with a fine mesh cloth top covering for ventilation. All such cups were placed in a controlled environmental chamber with a regime of 20:18°C and 11:13 (L:D) h photoperiod. Clones were transferred to fresh plants as needed until the clones went into the sexual phase or until the experiment was terminated after 10 weeks or more. Observations were made with 24 to 48 hr intervals for males, females, and egg production. No eggs were collected during this experiment.

Since I was more interested in getting uniform colonies, the number of aphids transferred to each cup was reduced to five for the first two generations. During the third and fourth generation, the greenbugs were allowed to reproduce to their maximum capacity and transferred to the fresh plants as needed. Females were identified on the basis of their thickened, dark hind tibia which could be observed as early as the second instar, while males were identified as small winged adults with developed (black) genitalia. Males could also be identified earlier by their wing pads (Puterka and Peters, 1989), but this criteria was not used.

Hatching Experiments

Based on the sexual reproduction data, four biotypes (C, E, F, and I), two laboratory reared clones (CC81 and XX101), and four field collected clones (GAB, Z18, Z35, and Z44) were selected for this experiment. The experiment was replicated four times. Some of the biotypes and clones tested had been reared on TR64 at 15°C, and a 15:9 (L:D) h photoperiod for more than 12 months while others, for example, the field collected clones, were either given little or no time for conditioning. A temperature regimen of 20:18°C with a 11:13 (L:D) h photoperiod was maintained throughout the experiment.

Five adults from each selected biotype/clone were transferred to first-leaf stage TR64 plants, grown in 8 cm diameter styrofoam cups and enclosed by 5 cm diameter and 15 cm tall clear plastic cages with the top covered with a fine mesh cloth for ventilation.

The adults were removed from the cups after producing nymphs for 24 h. The

number of nymphs was reduced to five per cup after each generation except the third and fourth generation where males and females were produced. Data were recorded for variation in sexual morph production sequences of the different biotypes and clones.

Collecting eggs with a camel's hair brush caused damage, and therefore, to facilitate egg collection without damaging them, eggs were collected either on pieces of leaves or on pieces of cloth used as substrates for oviposition. To collect eggs on pieces of cloth, a modified clear plastic cage of 5 cm in diameter and 15 cm tall was used. The top of the cage was covered with a 10 by 10 cm muslin cloth held tight with a rubber band. When enough eggs were laid, this muslin cloth was replaced with a new one. The muslin cloth was cut so that only those parts on which eggs were laid were kept in the snap boxes. This is an easy and quick method of egg collection, but a great number of unfertilized eggs (green and brown eggs) were included. This not only reduced the egg hatch percentage in terms of number but these eggs usually invited pathogens (fungal growth) which may have reduced the number of eggs hatched.

The leaves or cloth patches were placed in a 3 by 3 by 3 cm clear plastic snap-lid box. Depending on the availability of eggs, about 50 to 100 eggs were placed in each box. The snap-lid boxes with eggs were placed in a sandwich box at 95% relative humidity, maintained by a saturated K_2SO_4 salt solution (Winston and Bates, 1960). The container had a plastic grid in the bottom to keep the egg boxes suspended above the salt solution.

All sandwich boxes were held at 0°C with no light for 7 to 14 weeks. Cold

treatment durations were different for a variety of reasons associated with maintaining the various clones, but all were observed for any possible effects on egg hatching and survival of the progenies. After the cold treatment, eggs were transferred to a long-day regime, 15:9 (L:D) h with 15°C temperature for incubation (Puterka and Peters, 1988). Eclosion began within one week after incubation and continued for up to two weeks. Data were recorded for incubation period, hatching duration and time duration until the stem mother started reproducing, percent hatchability; and percent survival of the nymphs (stem mothers).

The newly hatched nymphs, (stem mothers) were individually caged on 'Wintermalt' barley seedlings (Puterka and Peters, 1988). Clip-on cages were used to give protection to the newly hatched nymphs. Puterka (1989) considered 'Wintermalt' barley a poor host for raising hatchlings of biotype F, so TR64 wheat was used as a host for progeny of this mating. Once the stem mothers were big enough and ready to reproduce, each was transferred to TR64 in 8 cm diameter styrofoam cups and covered with clear plastic cages. Each clone was named and numbered for identification. Clones were held at 15°C temperature and 15:9 (L:D) h photoperiod until needed for further studies.

RESULTS AND DISCUSSION

Production of Sexual Morphs Capable of Producing Eggs

Laboratory Reared Biotypes

The first set of experiments was conducted to identify the clones which could potentially be used in the insect breeding programs. A total of 279 clones of

greenbugs were tested for their sexual reproduction capabilities (Table 1). Five biotypes (C, E, F, G, and I) and a Georgia collection (GAB listed with "others" in Table 1) of greenbugs were successfully induced into the sexual cycle. Biotype B did not produce sexual morphs, which is consistent with results reported by Wood *et al.* (1969) and Inayatullah *et al.* (1987). Puterka (1989) proposed that biotypes B and H are anholocyclic forms of the greenbug. Biotype J was tested repeatedly for sexual morph production but did not produce any sexual morphs and continued to reproduce parthenogenetically even under prolonged scotophases of 13 h. It appears to be anholocyclic. Biotype G damages all known sources of resistance in wheat (Puterka *et al.*, 1988) except *Gb6* (Porter *et al.*, 1991). It was tested for sexual reproduction capabilities with the idea that its progenies could be used in studying the inheritance of virulence, but because of the low number of eggs produced, biotype G was found to be a poor candidate for sexual reproduction studies. Puterka and Peters (unpublished data) were successful in getting some hatching from biotype G. Biotype G either lost its sexual reproduction capabilities or the tested temperature/photoperiod regimes are not ideal. There are reports of clonal lines that have lost the ability to produce sexuals (Ossiannilsson, 1959), have become adapted to new plant hosts within 10 and 14 generation (Shaposhnikov, 1965; 1986; and Wilhoit and Mittler, 1991), and there are differences in biotypes for threshold scotophases for induction of males (Eisenbach and Mittler, 1987).

Laboratory Maintained Clones

Among the 91 clones, 36 were biotype C progenies including seven sib-matings

(CC) and 18 F_2 sib-matings (XX) of biotype C. Of the seven CC, only one clone proved to be a good candidate and was used for egg collection. Seventeen of the 18 XX clones produced males and females. Eggs were produced by 13 clones and collected from three clones. Seven of the nine E and F biotype sib-mated progenies were able to produce both sexes and eggs. Eggs were collected from six clones for hatching. Egg hatchings were observed in four clones. Four of the nine progenies of C by E crosses were found capable of producing males, females and eggs. Hatching was observed in three clones. Eleven progenies of biotype C by F crosses were tested for sexual reproduction capabilities and six progenies produced both sexes and eggs. When eggs from these colonies were tested for eggs hatching, eggs from all of the six clones hatched. Nineteen progenies of biotype F by E crosses were tested for sexual reproduction capabilities and 11 clones were capable of producing both sexes and eggs. Eggs were not collected from two clones because of low numbers. A set of seven clones (two sib-mated F_1, progeny of biotype G; K2 and K4 collected in Kentucky; GAB, a field collection from Georgia; and Ty I and Ty II, representing insecticide resistant greenbugs from Nebraska) were tested for sexual reproduction capabilities. Three clones, K4, GAB and Ty I, were capable of producing males, females and eggs. Eggs were collected from Ty I and GAB. GAB eggs resulted in an excellent hatching and were used in further studies.

Field Collected Clones

Of the 63 clones from spring survey (Table 1, March 1991) collection, 51 were capable of producing males, 43 produced females but only 13 clones were able to

produce enough healthy black eggs to justify collection. Twenty-five clones out of 43 were poor candidates for eggs production. They produced either green eggs or very few black eggs with a lot of brown eggs. The rest of the clones did not produce any eggs. Among the spring survey clones: Z18, Z35, and Z44 showed the best potential for sexual reproduction capabilities and were used for further studies.

None of the 94 clones collected from the sorghum field during May 1992 survey (Table 1), produced any sexual form when tested immediately after collection. It was speculated that since these samples were collected in late spring, during the lengthening day period, they might need more conditioning. After conditioning for seven months, three clones were retested and produced sexual morphs and eggs.

Only 23 out of 34 clones from the August to November survey 1992 (Table 1) were tested for sexual reproduction capabilities. One clone from Perkins and one from selected counties were capable of producing viable eggs.

Data on time duration for sexual morph production for the selected biotypes and clones are presented in Table 2. Time duration for female development ranged from 25.75 $\pm$ 2.21 days for CC81 to 30.50 $\pm$ 4.43 days for GAB. The overall average time for female development was 28.34 $\pm$ 1.75 days. When the experiment was repeated for biotype C, E, F, G, I, and CC81 (Table 3), the overall average dropped to 27.25 $\pm$ 2.24 days. CC81 took the least time of 23.25 $\pm$ 2.06 days among the tested biotypes/clones.

Time duration for male development ranged from 28.75 $\pm$ 0.96 for Z44 to 35.00 $\pm$ 4.97 days for GAB, with overall average of 31.77 $\pm$ 1.87 days. In the repeated experiment the overall average remained almost the same (31.11 $\pm$ 2.32 days) with

the longest of 32.75 ± 1.50 for biotype G and the least (26.50 ± 3.10 days) for CC81.

The overall time duration for egg production averaged 36.1 ± 2.33 (Table 2) and 35.38 ± 2.93 days (Table 3) in experiments one and two, respectively.

The data indicated that males were developed in more colonies than females (Table 1) and that females typically appear first in the colony (Tables 1 and 2). Males developing first was reported by Wilhoit and Mittler (1991). Differences in the methods used to identify the presence of the morphs is a probable explanation. Mittler and Gorder (1991) reported on the possible explanation of females being first; the first oocyte ovulated by each ovarial is female, even under long scotophases. It is possible that the first oocyte in each ovarial is chromosomally committed to develop as a female. Also, hormonal conditions leading to production of males (through a reduction in juvenile hormone level, and loss of an X chromosome during oocyte formation (Mittler et al., 1979; Hales and Mittler, 1983; Mittler, 1991) may not be established early enough in embryonic development of aphids. Almost all greenbugs in a colony under this extended scotophase turned into the sexual phase.

Hatching Experiment

Biotypes C, E, F, I, and clones GAB, CC81, XX101, Z18, Z35, and Z44 produced sexual morphs under tested conditions and were selected for the hatching experiments to provide clones for the genetic studies. Data for the cold treatment duration, incubation period, hatching duration, and time duration for stem mother to start establishing colony are presented in Table 4. When cold treatment duration was

variable, the incubation period and hatching duration ranged from 8 to 11, and 6 to 10 days, respectively. It took the stem mothers 6 to 15 days to begin parthenogenetic reproduction. Eggs from biotype I were given the maximum cold treatment of 93 days, but it did not affect the incubation period, hatching duration, or time duration for stem mothers to start reproduction. No detrimental effects of such prolonged cold treatment were observed on hatching or nymphal survival.

Biotypes I and F, and clone GAB were exposed a second time and given the same treatment of 51 days (second section of Table 4). It took biotype F eggs 13 days to start hatching, but this duration was only 7 days for biotype I eggs. My data confirms the cold treatment threshold of 6 weeks to break the diapause (Puterka and Slosser, 1986; Puterka and Peters, 1988).

Data regarding percent hatching of eggs and percent survival of the stem mother are given in Table 5. Egg hatch percentage ranged from 17.43 $\pm$ 2.25% of clone XX101 to 23.13 $\pm$ 1.53% of clone GAB. When the experiment was repeated for biotype I, F, and GAB clone, egg hatch averaged as high as 25.66 $\pm$ 4.24% in case of biotype I, while this average dropped to 17.20 $\pm$ 1.67% for biotype F. Puterka and Slosser (1986) obtained 6 to 13% hatching, depending on the host plant and temperature-duration regimen but with increasing relative humidity egg hatch increased to 19 to 26%, depending on biotype. Higher egg hatching percentages (19 to 45%) were reported by Wipperfurth and Mittler (1986). The reason for not getting such a high percentage of egg hatch may be due to differences in methodology. Since eggs were collected directly on leaves or cloth, nonviable eggs might have been included. The eggs were not given the preconditioning treatment proposed by Puterka

and Slosser (1986) because of the differences in techniques in egg collection. It seems that preconditioning eggs for about seven days in a refrigerator set at 6.0 ± 2°C with no light may be important for the progress of embryonic development towards diapause condition as described by Webster and Phillips (1912).

Survival of stem mothers ranged from 5.3% for biotype F to 51.2% for clone Z44. Percent survival of stem mothers in the second experiment increased for biotype F reared on TR64 to 9.3%, but was still far below the average of 52.0% for clone GAB.

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TABLE 1

SEXUAL MORPH PRODUCTION AND REPRODUCTION CAPABILITIES OF BIOTYPES AND CLONES TESTED

Grouping	Count	Males	Females	Eggs	Eggs not Collected	Hatching
Biotypes	8	5	5	5	-	4
C progenies	36	31	27	21[1]	9	7
E and F progenies	9	7	7	7	1	4
C by E progenies	9	4	4	4	-	3
C by F progenies	11	6	6	6	-	6
E by F progenies	19	11	11	11	2	7
Others, including GAB	7	3	3	3	1	2
May 1991	63	51	43	13[2]	8	5
May 1992	94	0	0[3]	-	-	-
Aug.-Nov. 1992	23	2	2	2	2	-
Total	279	123	111	75	23	38

[1] Four others produced only brown eggs.
[2] Twenty-five others produced only brown eggs.
[3] Results when tested within one month of collection.

TABLE 2

AVERAGE NUMBER OF DAYS AT A PHOTOPERIOD OF 11:13 (L:D) H AND 20:18°C TEMPERATURE FOR SEXUAL MORPH DEVELOPMENT AND EGG PRODUCTION IN VARIOUS BIOTYPES/CLONES OF GREENBUGS

Biotype/Clone	Female	Male	Eggs
C	29.50 ± 2.65	32.50 ± 2.38	37.00 ± 1.83
E	27.75 ± 2.99	31.25 ± 3.20	35.00 ± 3.37
F	30.00 ± 1.41	33.50 ± 1.91	38.00 ± 1.83
G	26.75 ± 1.50	30.75 ± 2.21	36.25 ± 4.03
I	29.50 ± 2.50	32.25 ± 3.40	37.25 ± 2.50
GAB	30.50 ± 4.43	35.00 ± 4.97	39.50 ± 3.70
Z18	28.25 ± 1.50	32.50 ± 1.82	37.75 ± 2.50
Z35	27.00 ± 0.82	30.25 ± 0.95	33.75 ± 1.50
Z44	26.25 ± 0.96	28.75 ± 0.96	32.50 ± 1.29
CC81	25.75 ± 2.21	29.50 ± 2.38	32.75 ± 2.36
XX101	30.50 ± 2.08	33.25 ± 2.06	38.00 ± 1.41
Overall Averages	28.34 ± 1.75	31.77 ± 1.87	36.16 ± 2.33

TABLE 3

AVERAGE NUMBER OF DAYS AT A PHOTOPERIOD OF 11:13 (L:D) H AND 20:18°C TEMPERATURE FOR SEXUAL MORPH DEVELOPMENT AND EGG PRODUCTION IN SELECTED BIOTYPES/CLONES OF GREENBUGS

Biotype/Clone	Female	Male	Eggs
C	28.50 ± 1.73	32.50 ± 1.29	36.50 ± 1.91
E	27.00 ± 0.82	31.50 ± 1.30	34.75 ± 1.71
F	28.25 ± 0.96	32.00 ± 0.82	35.50 ± 1.30
G	29.75 ± 0.95	32.75 ± 1.50	38.33 ± 3.51
I	26.75 ± 1.71	31.33 ± 1.53	37.25 ± 6.70
CC81	23.25 ± 2.06	26.50 ± 3.10	30.00 ± 3.98
Overall Averages	27.25 ± 2.24	31.10 ± 2.32	35.38 ± 2.93

TABLE 4

DAYS OF COLD TREATMENT DURATION OF EGGS AND TIME TO HATCHING, HATCHING DURATION, AND TIME REQUIRED FOR STEM MOTHER TO START REPRODUCTION IN SELECTED BIOTYPES AND CLONES

Biotype or clone	Cold Treat.	First Hatching	Hatching duration	Time till stem mother reproduced
		Experiment 1		
C	60	10	10	7-13
E	61	9	6	7-9
I	93	8	6	7-14
F	71	9	6	8-15
GAB	62	9	6	6-12
CC81	51	8	9	7-10
XX101	71	8	7	6-13
Z18	63	8	9	7-9
Z35	65	10	8	6-12
Z44	56	8	7	6-9
		Experiment 2		
I	51	7	8	6-13
F	51	13	10	9-15
GAB	51	9	9	7-12

TABLE 5

TOTAL NUMBER OF EGGS COLLECTED, EGGS HATCHING, AND SURVIVAL OF THE STEM-MOTHERS IN SELECTED BIOTYPES AND CLONES OF GREENBUGS

BIO./CLONE	TOTAl # EGGS	HATCHING #	(%)	SURVIVAL #	(%)
		Experiment 1			
C	396	87	21.97 ± 6.97	24	27.6
I	285	63	22.11 ± 3.02	32	50.8
F	400	75	18.75 ± 1.40	4	5.3
GAB	160	37	23.13 ± 1.53	18	48.7
E	300	63	21.00 ± 2.08	27	42.9
CC81	450	104	23.11 ± 1.94	32	31.7
XX101	350	61	17.43 ± 2.25	17	27.9
Z18	250	49	19.60 ± 4.32	18	37.7
Z35	160	30	18.75 ± 4.36	12	40.0
Z44	800	157	19.63 ± 2.06	81	51.2
		Experiment 2			
I	300	77	25.66 ± 4.27	37	48.0
F	250	43	17.20 ± 1.67	4	9.3
GAB	400	98	24.50 ± 3.05	51	52.0

CHAPTER V

INHERITANCE OF VIRULENCE OF GREENBUGS (*Schizaphis graminum*) RONDANI (HOMOPTERA: APHIDIDAE) TO *Gb1, Gb2, Gb3* AND *Gb6* IN WHEAT

Introduction

Aphids offer an advantage in studies of genetic variation because their parthenogenetic reproduction permits rearing many generations of genetically identical individuals (clones), while sexual reproduction can be induced to allow genetic crosses to be made (Mittler and Gorder, 1991). Genetic recombination during the sexual phase is thought to be an important source of variability within cyclically parthenogenetic populations (Via, 1992). Lynch and Gabriel (1983) also reported on the accumulation of hidden mutations during prolonged parthenogenetic cycles and immediate expression of genetic variance after a sexual cycle. This situation seems to be true for the greenbug with its ability to respond to a wide variety of environmental selection pressures and utilization of a broad range of hosts (Puterka and Peters, 1990).

Studies of the inheritance of virulence to raspberries in the rubus aphid, *Amphophora rubi* (Kalt.) suggested that it was a qualitative character conditioned by major genes. Virulence followed a gene-for-gene relationship as expounded by Flor (1971) for plant disease resistance. Virulence to two raspberry varieties was

conditioned by a single set of independent major genes, one being dominant and the other recessive (Briggs, 1965).

In the greenbug, variability has been particularly evident because of marked differences in the injury inflicted by various strains of the insect on their agricultural host plants, and in specific performance features of the aphid on different cultivars of these plant (Mittler and Gorder, 1991). During the past three decades, such differences have provided the basis for labeling as specific biotypes, a succession of strains that established themselves on crop varieties that were previously resistant to the aphid (Mittler and Gorder, 1991). Greenbug biotypes are parthenogenetically stable, even after two years of intense selection pressure by continuous rearing on resistance sorghum (Starks and Schuster, 1976). But the role of parthenogenesis in generating genetic variation should not be underestimated for it may also allow the accumulation of new genetic mutations in the form of hidden genetic variability (Puterka and Peters, 1989).

Inducing greenbugs into the sexual phase, getting eggs and making crosses between biotypes, was a breakthrough in the study of greenbug genetics (Puterka and Slosser, 1983, 1986; Puterka and Peters, 1988). Great amounts of genetic variability occurred in just one generation by crossing biotypes C, E and F (Puterka and Peters, 1989) which indicates that these biotypes were heterozygous for many virulence loci. There appears to be a high degree of hidden variability in the form of recessive virulence genes. Puterka and Peters (1989) studied greenbug crosses on the wheat resistance sources *Gb2* and *Gb3*, and proposed a modified Mendelian ratio for a

duplicate gene-modifier gene inheritance model where avirulence was dominant and virulence was recessive.

These results elucidate the role of sexual reproduction in expediting biotype evolution. Large numbers of unique biotypes of unknown virulence and an even greater number of possible recombinant genotypes contribute to the unpredictability of greenbug evolution. The numerous wild and cultivated hosts available to the new recombinant, plus the resistance genes being deployed, add to this unpredictability (Puterka and Peters, 1990).

In this study the duplicate gene-modifier gene model was further tested by using additional biotypes and clones. The tested biotypes and clones were sib-mated with the idea that virulent clones determined by recessive genes as proposed by Puterka and Peters (1989) should breed true. Understanding the mode of inheritance of virulence in greenbugs is important not only for the genetic study, but also for general understanding of the origin of agriculturally important variation in the destructive potential of the pest species.

Materials and Methods

Biotypes C, E, F, and I; a laboratory reared clone (CC81); plus clones from field collected samples GAB (from Georgia), 18Z, 35Z, and 44Z (from Oklahoma) were used for testing the duplicate gene-modifier gene model proposed by Puterka and Peters (1989). Clones of biotypes C, E, F, GAB and CC81 have been maintained parthenogenetically since 1988, on caged plants of TR64 in a growth chamber at 17° or 15°C temperature and a 15:9 (L:D) h photoperiod. Biotype I and clones 18Z,

35Z, and 44Z were maintained for at least six months on caged TR64 in a growth chamber at 15°C and 15:9 (L:D) h photoperiod.

A single greenbug from each of the above mentioned biotypes/clones was transferred to single leaf stage TR64 seedling, a susceptible wheat source to initiate pure colonies. Once the colonies were established, they were transferred to a growth chamber maintained at 11:13 (L:D) h photoperiod and a temperature of 20:18°C as suggested by Puterka and Peters (1990).

Sexual morphs appeared in about one month (Chapter IV). Only sib-matings for each biotype/clone were attempted. The males were allowed to mate with the ovaparae on the caged plants. Eggs were collected and held at 0°C with no light as described in Chapter IV. Handling newly hatched nymphs and stem mothers is also described in Chapter IV. Each clone was named and numbered for identification. These clones were held at 15°C temperature and 15:9 (L:D) h photoperiod until used in tests.

DS28A (*Gb1*), 'Amigo' (*Gb2*), LARGO (*Gb3*), and GRS1201 (*Gb6*) were grown along with the susceptible check, TR64. 'Post' barley or PI 9058 (reported as another source of *Gb1* [Curtiss *et al.*, 1960]) were the other entries in the wheat star-pot. These small grain entries were planted in a 15 cm diameter pot containing sandy soil. A marker was designed to fit into the pot, leaving 6 symmetrically radiating row depressions in a pie-shaped design. Seeds (4 to 6) from each entry were planted in a row and covered with about 1 to 2 cm soil. Each pot was watered from the base and to avoid any possible contamination (infestation), the pot was covered with a 33 cm high by 12.5 cm diameter transparent plastic cage with the top, and two side holes

of 7 cm diameter, covered with a fine mesh cloth. These pots were either kept in a culture room or growth chamber at 15:9 (L:D) h photoperiod with cycling temperature of 20:18°C. Plants were watered as needed and with a 25% concentration of Hoagland's solution once a week. After seedlings were 5 to 10 cm in height (1 to 2 leaf stage) or 2 to 5 days old, the plants in each row (entry) were thinned to 3 or 4 plants. Then the plants in each pot were infested with 100 to 200 aphids from a clone. In case clone multiplication was not synchronized with plant emergence, as few as 75 aphids were used to infest the 2 to 5 day-old plants rather than delay infestation and allow plant age to become a factor in the response to aphid feeding. If the damage progression was delayed or appeared questionable such under-infested experiments were most often repeated. The infested pots were maintained at a 15:9 (L:D) h photoperiod and temperature regimen of 20:18°C.

All of the rows in the pot were evaluated when one of the susceptible cultivars showed clear sign of severe damage (complete chlorosis or dead plants). This usually occurred about 14 days after infestation. Three plants in each row were visually rated for damage using a 1 to 6 scale, where 1 = 0% damage or apparently healthy plant; 2 = 1-25% damage (chlorosis, necrosis, and stunting); 3 = 26-50% damage; 4 = 51-75% damage; 5 = 76-99% damage; and 6 = dead plant. Ratings of 1 to 3 were regarded as resistant while 4 to 6 were regarded as susceptible. Results for each plant were recorded and segregates were identified based on responses from the differential host cultivars. I was looking for possible variation from the pattern established by the parents. The data were analyzed using PROC GLM and the mean damage rating of cultivars were compared using Scheffe's Multiple Range Test ($P > 0.05$) (SAS

Institute, 1988). Scheffe's procedure of multiple range comparison test was used for comparing damage rating means of wheat cultivars in response to various biotypes and also among the clones for a cultivar. This is considered not to be the most sensitive procedure for multiple mean comparison (Montgomery, 1991). Steel and Torrie (1980) also pointed out its large critical value. This resulted in ignoring minor differences when comparing a large number of means. It provided mean separations useful in virulence vs avirulence comparisons in greenbug clones and resistance vs susceptibility in the host plant. Furthermore, host plants considered resistant through this procedure should hold true in future uses.

Resistance in each of the six genotypes of wheat is considered to be controlled by a single gene, for example *Gb1, Gb2, Gb3, Gb4,* and *Gb5* that conditions greenbug resistance in DS28A, 'Amigo', LARGO, CI 17959, and CI 17882, respectively (Tyler *et al*, 1987) and *Gb6* that conditions resistance in the GRS series (David Porter, personal communication). Therefore, mean damage ratings, as explained in Chapter III, of the tested wheat genotypes were divided into two broad categories of resistant and susceptible.

Resistance classification criteria were based on:

Resistance = mean damage rating less than or equal to 3.67

Susceptible = mean damage rating more than 3.67

RESULTS AND DISCUSSION

The average damage ratings for parental clones in the wheat star-pot are given in Table 1. Full sib-mating clones C, E, F, I, GAB, 18Z, 35Z, 44Z, and CC81 produced F_1 segregation ratios that I attempted to fit into Mendelian phenotypic ratios.

Dickinson 28A (*Gb1*)

DS28A and PI 9058 have been considered as interchangeable (Curtiss *et al.*, 1960) and will be discussed as such in this section. The parental clones of biotypes C and E were virulent to DS28A (*Gb1*) (Table 1). All of their progenies were also virulent. Because there was no segregation, a ratio of 0:1 was tested for both the biotypes on *Gb1*.

The parental clone of biotype F was avirulent to *Gb1* (Table 1). Four of 10 progenies were virulent to this source (Table 2). A ratio of 3:1 was the best fit tested for the segregation of this limited population. Puterka (unpublished data) had a 19A:1V ratio for sib-mated biotype F; he also observed that C X F resulted in 22A:42V, F X C in 26A:31V; E X F in 20A:27V; and F X E resulted in 40A:46V segregation ratios. These results could be interpreted as indicating virulence in the biotype F to *Gb1* as simply inherited.

Biotype I and field collected clones GAB and 44Z were virulent to *Gb1* (Table 1). Segregation ratios of 3A:66V, 3A:74V, and 3A:78V, respectively, were found on *Gb1* (Table 2). When two or three gene models (1:15, 1:63) were tested, none of the tested ratios deviated from the expected one. There seems to be a second system observed when sib-mating virulent parents, where avirulence is the recessive product of two or three genes.

Both 18Z, a field collected biotype C, and 35Z, a field collected biotype E, were virulent to *Gb1* (Table 1). Segregation ratios of 1A:15V for 18Z progeny and 0A:10V for 35Z progeny were found on *Gb1* (Table 2). These progenies are too small to be effectively tested for the two gene model but the proposed hypothesis

would not be rejected. The CC81 progeny of sib-mating segregated into a 28A:4V ratio for *Gb1* (Table 2). Since the parental clone was avirulent to *Gb1*, a 15:1 ratio was proposed. The chi square results were in the acceptable range. Though CC81 may be an excellent tester for *Gb2* and *Gb3* (Table 3 and 4), it showed a heterozygous response to *Gb1*. Puterka (1989) reported a 22A:8V segregation ratio for CC81 progeny on *Gb1* which fit into a 3:1 segregation ratio ($X^2 = 0.044$).

'Amigo' (*Gb2*)

Puterka and Peters (1988) tested inheritance of virulence to 'Amigo' in greenbugs using the criterion of presence or absence of necrotic lesions. Beregovoy and Peters (1993) used lesions and plant death as combined criteria, which resulted in a more complex relationship than those for lesions only. I used damage rating criteria in these experiments to categorize virulence/avirulence relationships and found variations from those proposed by Puterka and Peters (1989).

Progenies of biotypes C, E, and 18Z fit into the Puterka and Peters (1989) model and so did CC81 (Table 3). Progenies of biotypes I, GAB, 35Z and 44Z fit into a two and three gene model for a secondary recessive avirulence as an extension of the previous model. This seems the most logical explanation if the Puterka (1989) model is accepted which assumes virulence to 'Amigo' was recessive.

Biotype F progeny segregated into 2A:8V ratio on *Gb2*, while Puterka and Peters (1988) reported a 0A:28V ratio based on lesion data. The discrepancies in the results are either due to the difference in methods or the clone tested was different from that tested by Puterka and Peters (1988). Beregovoy and Peters (1993) reported that biotype F made lesions but did not kill 'Amigo' under the time limits set in their

experiments. The poor hatchability and survival of biotype F limited my efforts in addressing this problem.

LARGO (*Gb3*)

Most parental clones were avirulent to *Gb3* except biotypes F and CC81. Responses of progenies of biotypes C, E, I, and clones GAB, 35Z and 44Z (Table 4) fit the Puterka and Peters (1989) scheme of a two gene-modifier gene model. CC81 also fit into the Puterka and Peters (1988) proposed tested scheme.

The 18Z parental clone was avirulent to *Gb3*, but among its 16 progenies, three were virulent to *Gb3*. When a segregation ratio of two genes (15:1) was tested, a significant chi-square was obtained. A one gene segregation ratio (3:1) was a better fit. The two gene hypothesis should not be rejected because of the small sample size.

Biotype F progenies segregated into 2:8 (A:V). This is again contradictory to sib-mating results of Puterka and Peters (1989). Beregovoy and Peters (1993) reported that biotype F caused lesions on LARGO but did not kill the plants under their experimental conditions. The poor hatchability and survival of biotype F did not allow me the opportunity to resolve this problem.

GRS1201 (*Gb6*)

This new source of resistance (Porter *et al.*, 1991) proved to be stable to all progenies of C, E, I, GAB, 18Z, and 44Z (Table 5). GRS1201 virulence was found in only one 35Z progeny and the clone was lost before it could be retested.

The biotype F parental clone was virulent to this new source of resistant (Table 1) and its progenies segregated into a 1:3 ratio (Table 5). Although the sample size

was small, based on its segregation on the other greenbug resistance genes in wheat, simple dominant inheritance was proposed.

The parent, CC81, was avirulent to GRS1201 but its progenies segregated into 13A:19V ratio (Table 5). A two gene model of incomplete dominance (7A:9V) for virulence was the best fit tested, but the parents were avirulent. Segregation of CC81 progenies on GRS1201 remains a challenge. At this point, any additional explanation will require another round of matings.

Within Clone Responses

The parental clone of biotype C was virulent to *Gb1* and avirulent to *Gb2, Gb3* and *Gb6* (Table 1). The 20 progenies segregated into 0:20, 17:3, 19:1, and 20:0 ratios for the four genes, respectively. Based on these segregations, ratios of 0:1 for *Gb1*, 15:1 for *Gb2* and *Gb3*, and 1:0 for *Gb6* were tested. None of the segregation ratios deviated from the tested.

Biotype E was virulent to *Gb1* and *Gb2* and avirulent to *Gb3* and *Gb6*. None of its 18 progenies segregated on *Gb1, Gb2* and *Gb6*, while these progenies were found virulent to *Gb3*. When a 15:1 ratio was tested, a non-significant chi square value of 3.33 was obtained. These results were consistent with those of Puterka (1989). Although both values were below the tabulated ($P = 0.05$) value of 3.84, a 3:1 ratio was a better fit (second part of Table 3).

Progenies of biotype F segregated 3:1 (A:V) ratio on *Gb1*, and 1:3 (A:V) on *Gb2, Gb3* and *Gb6*. This might suggest that only one gene was responsible for the virulence/avirulence relationships for these progenies.

Puterka (1989) proposed 0:1 segregation ratio for biotype F after finding no segregation (0:28) on *Gb2* and *Gb3* and assigned the respective genotypes aabbRR and llmmss to the parental clone. But my results were contradictory to those of Puterka. I found segregation on all the four genes tested. None of the observed ratios deviated from the tested one. The dilemma is how Puterka did not get segregation with 28 progenies while I observed segregation in only 10 progenies. Biotype F is virulent to *Gb2, Gb3* and *Gb6* and avirulent to *Gb1* (Table 1). Puterka reported tests for biotype F progenies only on two gene sources (*Gb2* and *Gb3*). Based on the segregation ratios on these four genes, a single gene model might be considered for the virulence/ avirulence relationship in biotype F. Reduced reproductive potential and growth of biotype F progenies which resulted in reduced damage ratios must also be considered.

Of the 69 progenies of biotype I, three were avirulent and 66 were virulent to *Gb1*. When a 1:15 ratio was tested, it fit perfectly into the two gene model while the three gene model resulted in a higher chi-square value. On *Gb2* the segregation would also fit both the two and three gene model. This segregation in what the Puterka model considered a homozygous recessive condition, must be considered as an extension of the genetic relationship in determining host damaging ability. Progenies of biotype I and clones GAB and 44Z produced segregation ratios which could fit 1:63 (A:V) on *Gb1* and *Gb2* suggesting that an additional three genes to the Puterka model were involved. Virulence to *Gb3* in biotype I progenies fit a two recessive gene model. No segregation for biotype I was recorded on *Gb6*.

CC81 was used as an assumed homozygous recessive progeny of sib-mating

biotype C tested by Puterka (1989). I also found no segregation of its progenies on *Gb2* and *Gb3*. However, four of the 32 were virulent to *Gb1* while the parental clone was not virulent. This indicates that virulence was recessively inherited to *Gb1*. The segregation ratio on *Gb6* was confusing.

GAB was virulent to *Gb1* and *Gb2* and avirulent to *Gb3* and *Gb6* (Table 1). As stated above, the GAB progenies on *Gb1* and *Gb2* included a few avirulent clones which require an extension of the Puterka (1989) model. None of the proposed ratios for the two and three gene models deviated from the tested ratios. Recessive virulence on *Gb3* and no segregation on *Gb6* was recorded for the 77 progenies of GAB.

The parental clone, 18Z, was virulent to *Gb1* and its progenies segregated into 1:15 ratio. This indicates that virulence to DS28A (*Gb1*) might be inherited as a dominant trait. The virulence relationship was opposite to *Gb2* and *Gb3*. The progenies segregated into 15:1 ratio, which suggests recessive virulence for these two genes (*Gb2* and *Gb3*). No segregation was obtained on *Gb6*.

Ten progenies were obtained from the sib-mating of 35Z. Nine of them were virulent to *Gb2*, suggesting a dominant inheritance of virulence. For *Gb3* and *Gb6* this relationship was absolutely opposite. Nine of the 10 progenies were avirulent and one was virulent suggesting that virulence was recessively inherited for *Gb3* and *Gb6*. All these ratios might fit into the two gene model.

Progenies of 44Z were tested for the two and three gene model. Neither of the ratios deviated from the tested ratio on *Gb1* and *Gb2*. The parent, 44Z, was virulent to *Gb1* and *Gb2*. Seventy-eight out of 81 were virulent to *Gb1,* and 79 out of 81

were virulent to *Gb2*. These ratios indicate dominant inheritance of virulence to these two genes. When 1:15 (two gene model) and 1:63 (three gene model) were tested, no significant chi square results were found. The parental clone of 44Z was avirulent to *Gb3* so its progenies segregated into 74 avirulent and 7 virulent, suggesting virulence being recessively inherited for *Gb3*. No deviation was found from the 15:1 ratio for a two gene model. The parent clone, 44Z, was avirulent to *Gb6* and no virulent clone was obtained from the 81 progenies.

The duplicate gene-modifier gene model was acceptable in most cases, but there were deviations from the two gene models. So one gene and three gene models were tested. Moreover, the assumption of virulence being recessive and avirulence being dominant as reported by Puterka and Peters (1990), was not true for most of the virulent clones/plant host relationships tested. Rather, a three dominant gene model of virulence was proposed in the case of *Gb1* and *Gb2*.

In conclusion, virulence was inherited dominantly when the parental clone was virulent and in a heterozygous condition at other loci. Virulence is inherited recessively in the case of an avirulent parent (assuming the parental clone was in heterozygous condition). In most cases, the two gene model was acceptable. For the large series clones, the three gene model generally was acceptable. Biotype F segregated either into 3:1 or 1:3 ratio on all of the four tested plant genotypes. It seems that only one gene is involved in the inheritance of virulence in biotype F.

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TABLE 1

AVERAGE DAMAGE RATING OF PARENTAL CLONES ON THE WHEAT STAR-POT

	Resistance	Biotypes				Parental Clones				
Entry	Gene	C	E	F	I	CC81	GAB	18Z	35Z	44Z
TR64		6.0a	5.3a	5.3ab	5.7a	5.7a	5.3a	6.0a	6.0a	6.0a
DS28A	*Gb1*	6.0a	6.0a	2.7bc	5.7a	1.3b	6.0a	5.7a	5.3a	6.0a
PI 9058	*Gb1*	6.0a	5.7a	2.0c	6.0a	1.0b	--	6.0a	6.0a	--
TAM 107	*Gb2*	1.0c	5.7a	6.0a	6.0a	5.7a	5.3a	1.3b	6.0a	6.0a
LARGO	*Gb3*	2.3b	1.7b	4.0abc	2.0b	5.7a	1.7b	1.3b	1.0b	2.0b
GRS1201	*Gb6*	--	--	4.3abc	2.3b	1.0b	1.3b	1.0b	1.0b	1.0c
'Post' barley		2.0b	2.0b	--	--	--	2.0b	--	--	2.3b

Values followed by the same letter in each column are not significantly different (Scheffe's multiple range test, $P < 0.05$).

TABLE 2

AVIRULENT (A) : VIRULENT (V) F_1 SEGREGATION RATIOS FOR VIRULENCE TO DICKINSON SELECTION 28A (*Gb1* RESISTANCE GENE)

Mating	Number Observed	Observed A : V	Tested A : V	X^2
C x C	20	0 : 20	0 : 1	0.00
E x E	18	0 : 18	0 : 1	0.00
F x F	10	6 : 4	3 : 1	1.20
I x I	69	3 : 66	1 : 15	0.43
I x I	69	3 : 66	1 : 63	3.48
GAB x GAB	77	3 : 74	1 : 15	1.75
GAB x GAB	77	3 : 74	1 : 63	2.73
18Z x 18Z	16	1 : 15	1 : 15	0.00
35Z x 35Z	10	0 : 10	1 : 15	0.00
44Z x 44Z	81	3 : 78	1 : 15	0.86
44Z x 44Z	81	3 : 78	1 : 63	2.81
CC81 x CC81	32	28 : 4	15 : 1	2.13

TABLE 3

AVIRULENT (A) : VIRULENT (V) F_1 SEGREGATION RATIOS FOR VIRULENCE TO 'AMIGO' (*Gb2* RESISTANCE GENE)

Mating	Number Observed	Observed A : V	Tested A : V	X^2
C x C	20	17 : 3	15 : 1	3.600
E x E	18	0 : 18	0 : 1	0.000
F x F	10	2 : 8	1 : 3	0.130
I x I	69	1 : 68	1 : 15	2.530
I x I	69	1 : 68	1 : 63	0.005
GAB x GAB	77	3 : 74	1 : 15	0.730
GAB x GAB	77	3 : 74	1 : 63	1.000
18Z x 18Z	16	15 : 1	15 : 1	0.000
35Z x 35Z	10	1 : 9	1 : 15	0.240
44Z x 44Z	81	2 : 79	1 : 15	2.190
44Z x 44Z	81	2 : 79	1 : 63	0.740
CC81 x CC81	32	0 : 32	0 : 1	0.000

TABLE 4

AVIRULENT (A) : VIRULENT (V) F_1 SEGREGATION RATIOS FOR VIRULENCE TO LARGO (*Gb3* RESISTANCE GENE)

Mating	Number Observed	Observed A : V	Tested A : V	X^2
C x C	20	19 : 1	15 : 1	0.05
E x E	18	15 : 3	15 : 1	3.33
E x E	18	15 : 3	3 : 1	0.67
F x F	10	2 : 8	1 : 15	3.22
F x F	10	2 : 8	1 : 3	0.13
I x I	69	61 : 8	15 : 1	3.36
GAB x GAB	77	68 : 9	15 : 1	3.83
18Z x 18Z	16	13 : 3	15 : 1	4.23*
18Z x 18Z	16	13 : 3	3 : 1	0.33
35Z x 35Z	10	9 : 1	15 : 1	0.24
44Z x 44Z	81	74 : 7	15 : 1	0.79
CC81 x CC81	32	0 : 32	0 : 1	0.00

* Chi - square values marked by asterisk (*) are significant at the $P = 0.05$

TABLE 5

AVIRULENT (A) : VIRULENT (V) F_1 SEGREGATION RATIOS FOR VIRULENCE TO GRS1201 (*Gb6* RESISTANCE GENE)

Mating	Number Observed	Observed A : V	Tested A : V	X^2
C x C	20	20 : 0	1 : 0	0.00
E x E	18	18 : 0	1 : 0	0.00
F x F	10	3 : 7	1 : 3	0.13
I x I	69	69 : 0	1 : 0	0.00
GAB x GAB	77	77 : 0	1 : 0	0.00
18Z x 18Z	16	16 : 0	1 : 0	0.00
35Z x 35Z	10	9 : 1	15 : 1	0.24
44Z x 44Z	81	81 : 0	1 : 0	0.00
CC81 x CC81	32	13 : 19	1 : 3	4.25*
CC81 x CC81	32	13 : 19	7 : 9	0.13

* Chi - square values marked by asterisk (*) are significant at the $P = 0.05$

CHAPTER VI

INHERITANCE OF VIRULENCE OF GREENBUGS TO SORGHUM

Introduction

Greenbugs, *Schizaphis graminum* (Rondani), are major pests of sorghum, *Sorghum bicolor* (L.) Moench. They have been destructive insect pests of sorghum since the appearance of biotype C in 1968 (Harvey and Hackerott, 1969). Since that time, biotype E and biotype I have arisen (Harvey *et al.*, 1991). These biotypes have overcome the majority of the sources of resistant germplasm within sorghum.

'SA7536-1' was the resistant source of choice by plant breeders to biotype C (Schuster and Starks, 1973). By 1980, at least 90% of the sorghum acreage in the United States was planted to resistant hybrids derived mainly from 'SA7536-1' and 'KS30'. Both of these sources derived their resistance from *S. virgatum* (Hack) Staph. (Starks *et al.*, 1983). Porter *et al.* (1982) reported the collection of a new greenbug biotype (E) in a wheat field near Bushland, Texas. Hackerott *et al.* (1983) reported that by 1981 biotype E had largely replaced biotype C in the Great Plains. Only four sources of resistance to biotype C were resistant to biotype E. These were: PI 264453, PI 220248, PI 229828, and 'Capbam' (Sarvasi) (Johnson, 1981; Porter *et al.*, 1982; Hackerott *et al.*, 1983; Starks *et al.*, 1983).

The most recently identified biotype, named "I" by Harvey *et al.* (1991), was collected from Stevens County, Kansas in August, 1990. The first sorghum found to be resistant to biotype I was PI 266965 (Harvey *et al.*, 1991). However, because it is a tetraploid (2N=40), its commercial use may be limited (Harvey *et al.*, 1993). The first reported commercial sorghum hybrid with resistance to biotype I was 'Cargill 607E' (Kofoid *et al.*, 1991). Andrews *et al.* (1993) identified at least two sources of resistance to biotype I, PI 550607 and PI 550610, from among 110 Russian plant introductions; eight other plant introductions were found with intermediate levels of resistance. Harvey *et al.* (1994) reported resistance in 'Cargill 607E' is temperature sensitive and is more effective against biotype I at 28°C than at 22°C.

Because almost all biotype E resistant hybrids are susceptible to biotype I, Wilde *et al.* (1993) collected greenbug samples from Kansas, Texas, Colorado, Nebraska and Oklahoma for biotype I distribution and found it in all but Oklahoma. During my 1991-92 survey, I did find biotype I in Oklahoma (Chapter III). This widespread distribution shows the adaptability of the biotype and lack of resistance in the currently used sorghum hybrids. The competitive displacement of biotype C by biotype E (Hackerott *et al.*, 1983) suggests that biotype E might be replaced by biotype I in the near future. The continuing challenge of developing resistant cultivars against the increasing number of greenbug biotypes is of great concern to the breeders as well as to entomologists. An understanding of the genetic mechanisms controlling greenbug biotypic diversity and plant resistance to biotypes is central to developing stable biological control methods.

The inheritance of most sources of greenbug resistance has been determined to be incompletely dominant but considered to be simply inherited (Peterson, 1985). Laboratory studies showed virulence being recessive and avirulence dominant to *Gb2* and *Gb3* in wheat (Puterka and Peters, 1989). Puterka and Peters (1990) reported that virulence was dominantly inherited to 'Piper' and recessively to 'Pioneer 8493' and was governed by a duplicate gene-modifier gene system.

In this study, the inheritance of greenbug virulence to 'Piper' sudangrass, 'Pioneer 8515', 'Pioneer 8493' and 'Cargill 607E' was investigated. Biotype I and field collected clones GAB and 44Z (considered biotype E) were sib-mated and their F_1 progenies were evaluated on the four sorghum cultivars to obtain frequency distributions of the damage caused by the progenies for testing hypotheses of the inheritance of virulence against sorghum.

MATERIALS AND METHODS

The parents, biotype I plus clones GAB and 44Z, and their progenies were the same colonies tested on wheat resistant sources (Chapter V). Biotype I was a sample from the original colony characterized by Harvey *et al.* (1991), and GAB and 44Z were field collections from Georgia and Oklahoma, respectively. The GAB clone had been maintained parthenogenetically since 1988 on caged pots of TR64, a susceptible wheat cultivar, in a growth chamber. For at least six months before the experiment began, all clones were maintained parthenogenetically on caged TR64 in a growth chamber at 15:9 (L:D) h photoperiod and 15°C temperature regimen.

A single greenbug from each of the above mentioned biotypes/clones was

transferred to a single leaf stage TR64 seedling to initiate pure colonies. Once the colonies were established, they were transferred to a growth chamber maintained at 11:13 (L:D) h photoperiod and a 20:18°C (L:D) temperature.

Induction of greenbug sexuals, sib-mating, egg collections, egg hatchings, and maintenance of stem mothers from each clone were reported in Chapter IV. Sexual morphs appeared within one month. Sib-mating was at random among greenbugs of the same clone. Eggs were given a cold treatment of 0°C with no light for a minimum of seven weeks in clear plastic snap-lid boxes placed in an air tight sandwich container with a 95% relative humidity maintained by a saturated solution salt of K_2SO_4 (Winston and Bates, 1960). After the cold treatment, eggs were transferred to a long-day regimen of 15:9 (L:D) h photoperiod with 15°C temperature for hatching. Egg hatch began within one week after incubation and continued for up to two weeks. Each newly hatched stem mother was individually caged on 'Wintermalt' barley seedlings. Clip-on cages were used to keep the newly hatched nymphs from wandering off the plants. Once these stem mothers were ready to reproduce, each was transferred to TR64 wheat seedlings grown in 8 cm diameter styrofoam cups and covered with clear plastic cages. Each clone was named and numbered for identification. These clones were held at 15°C temperature and 15:9 (L:D) h photoperiod for colony establishment and maintenance until used in experiments.

'Piper' sudangrass, 'Pioneer 8515', 'Pioneer 8493' and 'Cargill 607E', along with TR64 and 'Post' barley, were grown in the same configuration for the sorghum star-pots reported in Chapter III. These sorghum and small grain cultivars were

planted in a 15 cm diameter pot containing sandy soil. A marker was designed to fit into the pot, leaving six symmetrically radiating row depressions in a pie-shaped design. Seeds (4 to 6) from each cultivar were planted in a row and covered with about 1 to 2 cm of sand. Each pot was watered from the base. To avoid any possible contamination (infestation), the pot was covered with a 33 cm high by 12.5 cm diameter transparent plastic cage with the top and two side holes of 7 cm diameter covered with a fine mesh cotton cloth. These pots were either kept in a plant culture room with ambient temperatures of 25 $\pm$ 5°C or in a growth chamber at 15:9 (L:D) h photoperiod at 25°C temperature. Plants were watered as needed and a 25% concentration of Hoagland's solution was added once a week. After germination, at 4 to 8 cm in height (1 to 2 leaf stage) or 2 to 5 day old, plants in each row were thinned to 3 to 4 plants. The plants in each pot were infested with 100 to 200 aphids from a clone to evaluate the damage. In case clone multiplication was not synchronized with plant emergence, as few as 75 aphids were used to infest the 4 to 5 day old plants rather than delay infestations and allow plant age to become a factor in the response to aphids feeding. For confirmation such under infested experiments were often repeated. Infested pots were held at 15:9 (L:D) h photoperiod and a temperature regimen of 25°C.

All of the plants in the pot were evaluated when one of the susceptible cultivars showed clear signs of severe damage (complete chlorosis or dead plants). Plants were visually rated for damage using a 1 to 6 scale where 1 = 0% damage or an apparently healthy plant; 2 = 1-25% damage (chlorosis, necrosis and stunting); 3 = 26-50% damage; 4 = 51-75% damage; 5 = 76-99% damage; and 6 = dead plant.

Results for each plant were recorded and segregates were identified based on responses from the differential host cultivars. I was looking for possible variations from the pattern established by the parents. The data were analyzed using PROC GLM and the mean damage ratings for clones and cultivars were compared using Scheffe's Multiple Range Test ($P > 0.05$) (SAS Institute, 1988). Mean damage rating data were plotted in bar diagrams for frequency distribution of F_1 progeny of each clone tested on the sorghum entries. Resistance classification criteria was based on:

Resistance = mean damage rating less than or equal to 3.0.

Intermediate = mean damage rating above 3.0 but less than 4.8.

Susceptible = mean damage rating of 4.8 and above.

RESULTS AND DISCUSSION

Mean damage ratings of the parental clones and pooled progeny ratings for biotype I and clones GAB and 44Z are presented in Table 1. The damage ratings obtained from sorghum star-pots for clone GAB and 44Z (considered versions of biotype E) closely followed those reported previously on entries 'Piper', 'Pioneer 8515' and 'Pioneer 8493' (Table 1). 'Cargill 607E' is considered resistant to biotype I (Harvey *et al.*, 1991). My laboratory results showed an intermediate response (mean damage rating of 4.67). This might be attributed to the temperature used because they found the best expression of 'Cargill 607E' resistance at 28°C or above.

Frequency distribution for the progenies of the three parental clones on the sorghum cultivars: 'Piper' sudangrass, 'Pioneer 8515', 'Pioneer 8493' and 'Cargill 607E' are presented in Table 2 and bar diagrams (Figs. 1-6). Mean damage ratings

for 70 progenies of biotype I on 'Piper' sudangrass (Table 2) resulted in 36 virulent, 30 intermediate and only 4 avirulent clones. Progenies of biotype I distributed in a similar manner on 'Pioneer 8515' as they did on 'Piper,' but in this case the data were skewed comparatively more towards the susceptible side, with 53 clones being virulent, only 14 intermediate and 3 clones avirulent. Mean damage ratings for the 70 F_1 clones of biotype I on 'Pioneer 8493' were not different from those of 'Piper', but were different from 'Pioneer 8515.' A greater number of clones (12) were avirulent to this source. The skewness of the data shows that virulence in biotype I has been increased toward the resistance contained in 'Pioneer 8515' (SA 7536-1) and 'Pioneer 8493' (PI 264453). The great number of progeny with intermediate responses would appear to be due to the polygenic nature of resistance in these lines. Of the 70 F_1 clones of biotype I, only 15 were virulent to 'Cargill 607E.' The greater number of avirulent (34), and intermediate (21) clones indicates that virulence is probably inherited as a multigenic trait in biotype I to 'Cargill 607E'. The distorted dome shape distribution (Fig. 2B) may be due to the fact that these progenies were tested under temperature regimes of 25°C, which might be low for activation of the resistance mechanism (Harvey *et al.*, 1994). Moreover, if a greater number of progenies were available, I might have seen a smoother distribution pattern.

Since the parental clone of GAB was virulent to 'Piper' sudangrass and 'Pioneer 8515', its progenies gave a typical distribution pattern on 'Piper' sudangrass and 'Pioneer 8515' (Figs. 3A and 3B). Frequency distribution of GAB progeny on 'Pioneer 8493' and 'Cargill 607E' was opposite to those of 'Piper' and 'Pioneer

8515.' The basis for this difference in responses may be that the parental clone of GAB was avirulent to these resistant sources (mean damage rating 2.33 on both 'Pioneer 8493' and 'Cargill 607E'). Out of 77 F_1 progenies, only 7 clones were virulent to 'Pioneer 8493', while 58 were avirulent and 12 clones had intermediate responses. Frequency distribution of these clones on 'Cargill 607E' was 16 virulent, 29 intermediate and 32 with avirulent responses. The difference in the mode of inheritance of virulence in GAB on 'Pioneer 8493' and 'Cargill 607E' may be due to temperature sensitivity of the 'Cargill 607E' (Harvey *et al.*, 1994). The GAB progeny would appear to represent the response to 'Pioneer 8493' expected before selection pressure occurs.

The parental clone, 44Z, was virulent to 'Piper' sudangrass and 'Pioneer 8515' (mean damage rating of 4.67 and 4.34), and avirulent to 'Pioneer 8493' and 'Cargill 607E' (mean damage rating of 2.00 for both). When 81 F_1 progenies of 44Z were tested against 'Piper' and 'Pioneer 8515', four and two respectively were avirulent, 14 and 9 produced intermediate responses, and 63 and 70 were found virulent to these two sources, respectively. This lack of segregation suggests that virulence to these two resistant sources was inherited dominantly in 44Z. Results from 'Pioneer 8493' and 'Cargill 607E' showed an opposite trend in segregation; for example, virulence being inherited recessively, but probably multigenic in nature.

A large number of progenies produced an intermediate response on all the four resistant sources being tested. This intermediate response indicates that quantitative characters determine resistance to damage in these cultivars. This quantitative difference in the cultivar genotype resulted in the variable response to the greenbug

infestation. An obvious gradation was observed in the virulence — avirulence relationship. These quantitative variations were difficult to analyze genetically, because their response range appeared to be continuous and not similar to those found in small grains, particularly in wheat varieties as reported in Chapter V. This could be attributed to the distinct genes responsible for cultivar resistance.

If it is assumed that resistance is polygenic in the plant, we might theoretically assume that virulence in the aphid is also polygenic. Polygenes are genes with a small effect on a particular character that can supplement each other to produce observable quantitative changes (Mather, 1943). Some of these quantitative effects can be considered additive if they can be added together to produce phenotypes which are the sum total of the negative and positive effects of individual polygenes (resistance/virulence). Since not all quantitative characters are caused by genes with small additive effects, nor all gene effects are independent of each other, a distribution curve can appear asymmetric or "skewed," with a much greater frequency of phenotypes at one end of the curve than at the other. In case of dominance between alleles that effect a quantitative trait, a skewed distribution will occur in the F_1 progenies. Selection pressures, such as growing resistant cultivars may cause a skewed frequency of either dominant or recessive genotypes. Skewed patterns were very obvious in these experiments (Figs. 1-6).

From the frequency distribution shown in Figures 1-6 and Tables 1 and 2, it was concluded that in case of parental clones being virulent to a particular source in sorghum, virulence in its progenies were inherited dominantly, assuming that the parental clone was heterozygous. When a parental clone was avirulent to a certain

source, segregation on that source showed resistance was recessively inherited. This phenomenon was very obvious in wheat cultivars (Chapter V), where the resistance was controlled by a single major gene. In such situations, a clear cut answer of kill or no-kill response with no or low intermediate distribution was of great help in drawing conclusions, but still a line can be drawn between virulence and avirulence in the case of sorghum, though resistance in sorghum is polygenic and many of the progenies produced intermediate responses. These results are partially supported by the findings of Puterka (1989) where virulence was reported dominantly inherited to 'Piper' and recessively to 'Pioneer 8493'.

Puterka (1989) also reported a duplicate gene-modifier gene inheritance model in the greenbug on sorghum. I did not confirm this assumption because in his experiment, data were adjusted to fit the model, ignoring the intermediate responses. In nature, plants could be resistant, moderately resistant, or susceptible to insects or diseases. No matter how skeptical one is about the use of moderate resistance in breeding for resistance programs, its importance can not be ignored, particularly when resistance genes are scarce. I did not pursue the nature of resistance in these sorghum genotypes to the segregates in this experiment, but it is suggested that tolerant varieties and varieties with moderate resistance should be used in the cropping scheme to minimize the selection pressure from highly resistant cultivars. It would discourage the development of newer and virulent biotypes.

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TABLE 1

MEAN DAMAGE RATING OF PARENTAL CLONES AND POOLED PROGENIES ON SELECTED SORGHUM CULTIVARS

Cultivar	Biotype I		GAB		44Z	
	Parent	Pooled Progeny	Parent	Pooled Progeny	Parent	Pooled Progeny
TR64	4.67a	--	6.00a	--	6.00a	--
'Piper' sudangrass	6.00a	4.72ab	4.34b	5.01a	4.67b	5.26a
'Pioneer 8515'	6.00a	5.03a	5.00ab	5.12a	4.34b	5.39a
'Pioneer 8493'	5.33a	4.51b	2.33c	3.65b	2.00c	4.02b
'Cargill 607E'	4.67a	3.59c	2.33c	2.76c	2.00c	3.71c

* Values followed by the same letter in a column are not significantly different ($P >$ 0.05, Scheffe's, SAS Institute 1988).

Mean damage ratings based on rating scheme of 1 = 0% damage, 6 = 100% damage.

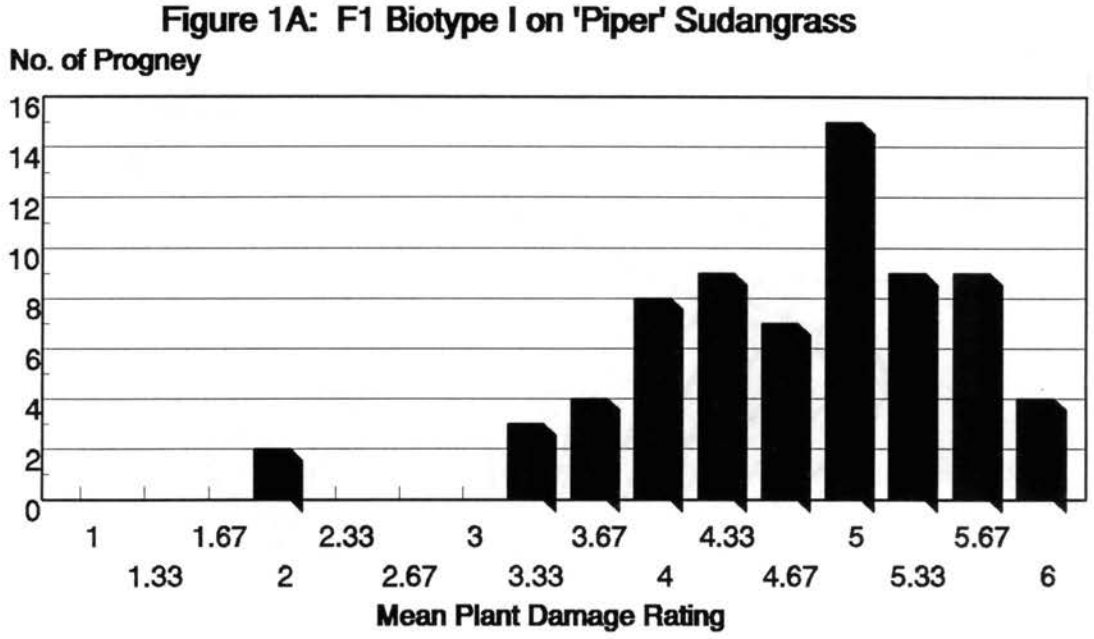
TABLE 2

VIRULENCE RELATIONSHIPS OF PROGENIES OF GREENBUG BIOTYPE I AND FIELD COLLECTED GAB AND 44Z TO FOUR SOURCES OF RESISTANCE IN SORGHUM BASED ON STAR-POT TESTS

Sorghum genotypes	Parental Clones								
	I			GAB			44Z		
	A[1]	I	V	A	I	V	A	I	V
'Piper' sudangrass	4[2]	30	36	5	21	51	4	14	63
'Pioneer 8515'	3	14	53	4	9	64	2	9	70
'Pioneer 8493'	12	20	38	58	12	7	35	29	17
'Cargill 607E'	34	21	15	32	29	16	25	34	22
Total Progeny	70			77			81		

[1] A = Avirulent (mean damage rating of 1-3); I = intermediate response (mean damage rating > 3.0 and < 4.80); V = virulent (mean damage rating >4.80). Plants were rated 1-6: 1 = no (0%) damage and 6 = dead plant (100% damage).

[2] Number of progenies from sib-mating assigned to classification.



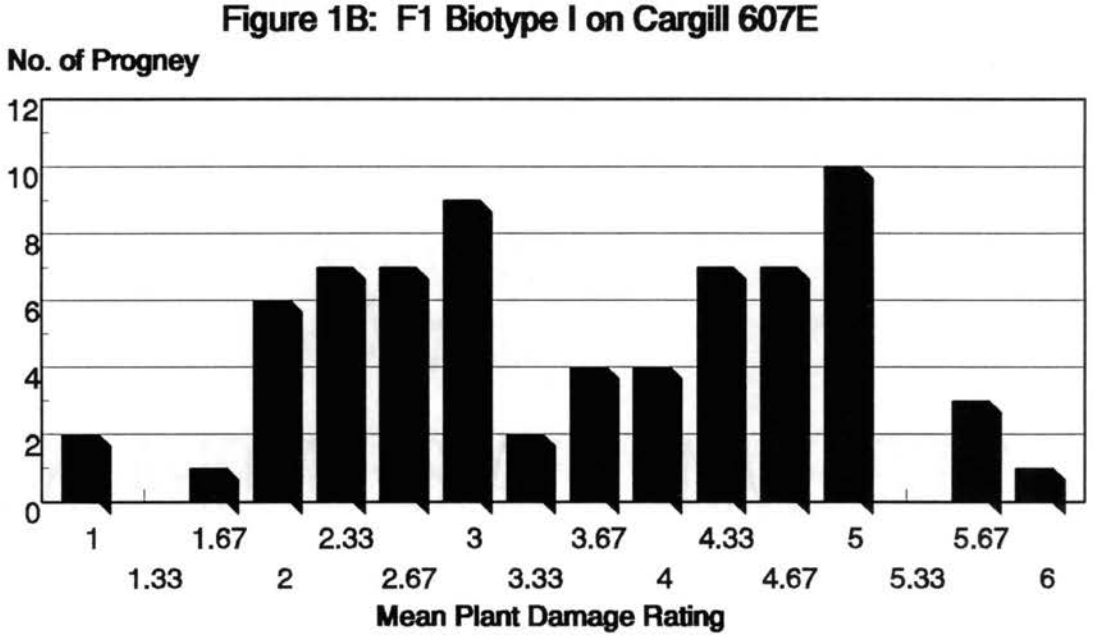
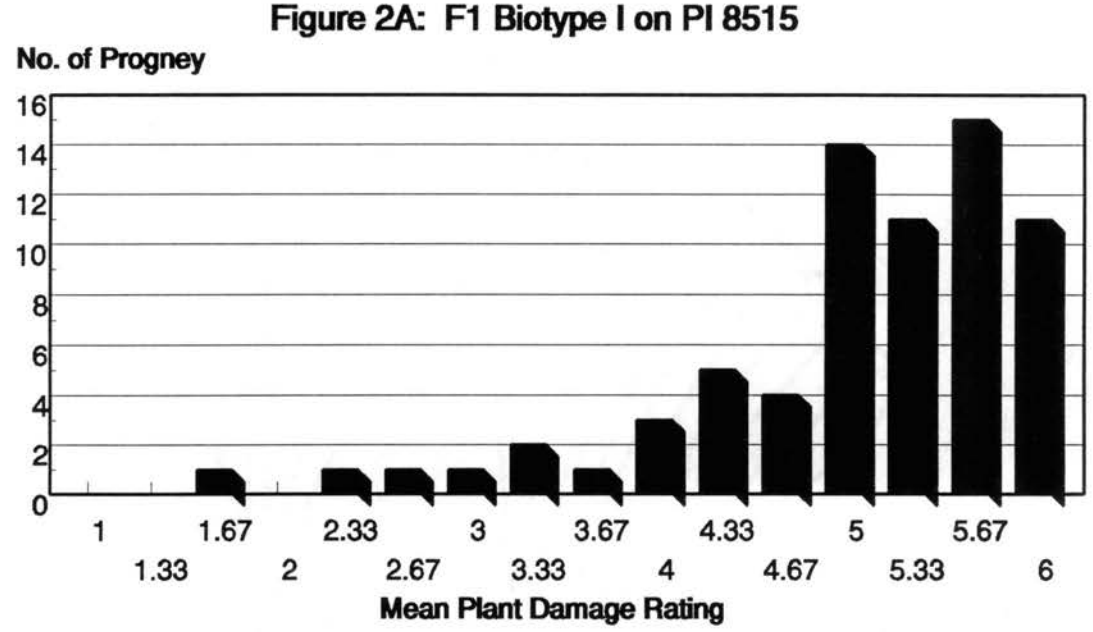


Figure 1: Frequency distribution of mean plant damage ratings for F1 progney of Biotype I on 'Piper' Sudangrass (Fig. 1A), and Cargill 607E (Fig. 1B).



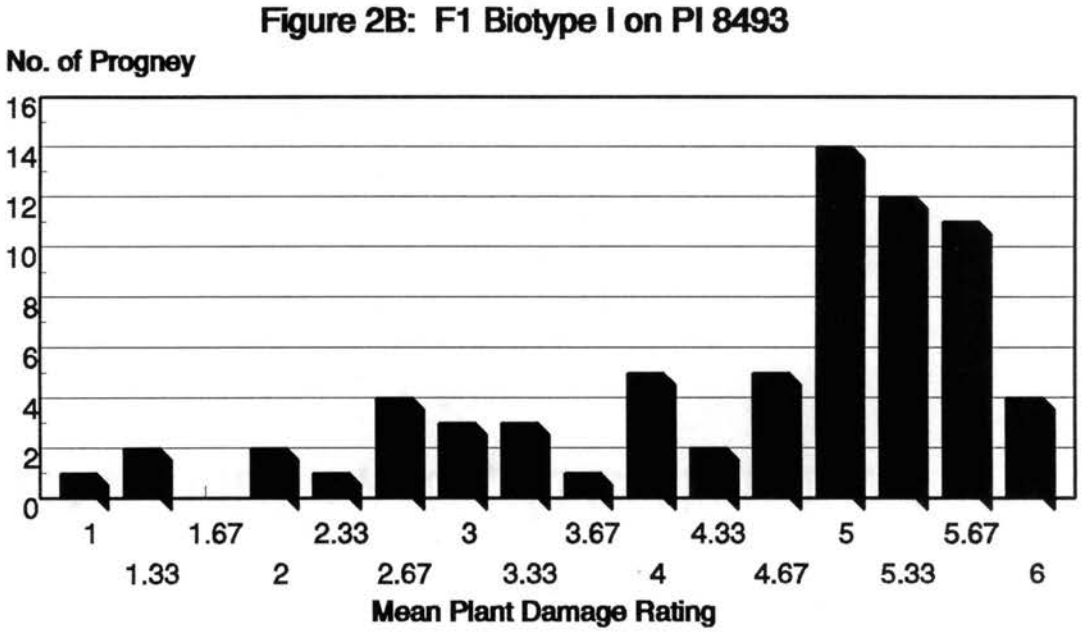
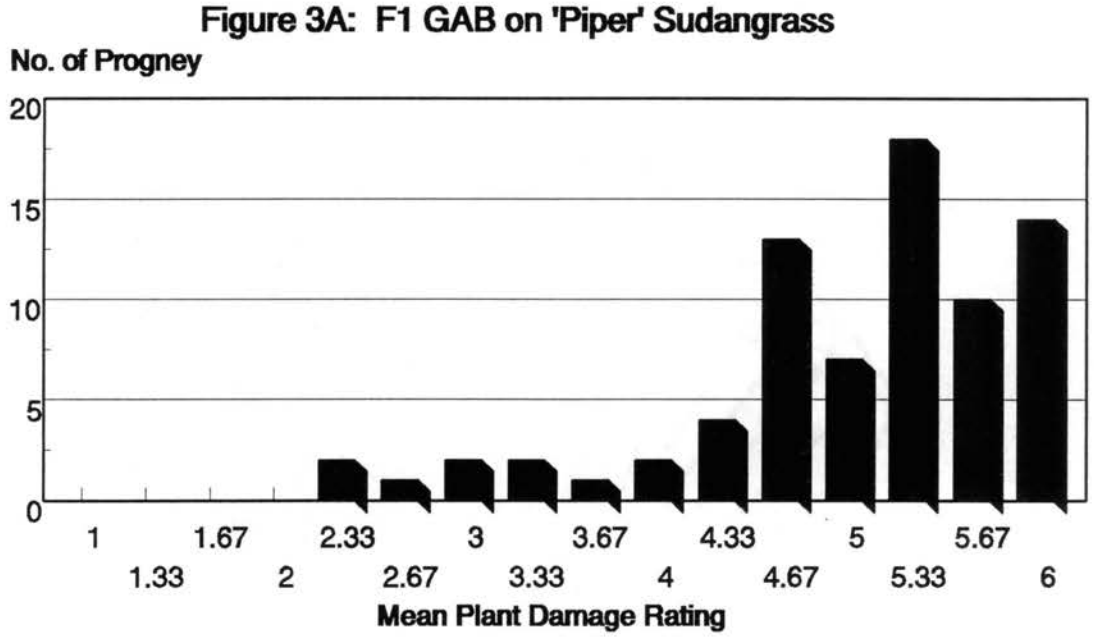


Figure 2: Frequency distribution of mean plant damage ratings for the F1 progney of Biotype I on Pioneer 8515 (Fig. 2A), and Pioneer 8493 (Fig. 2B).



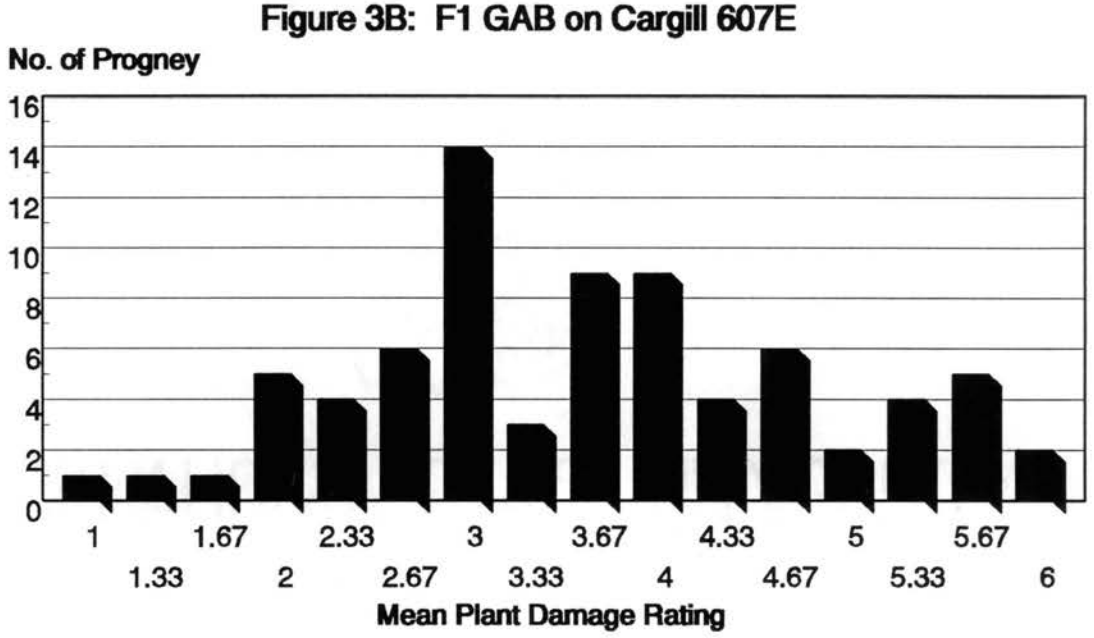
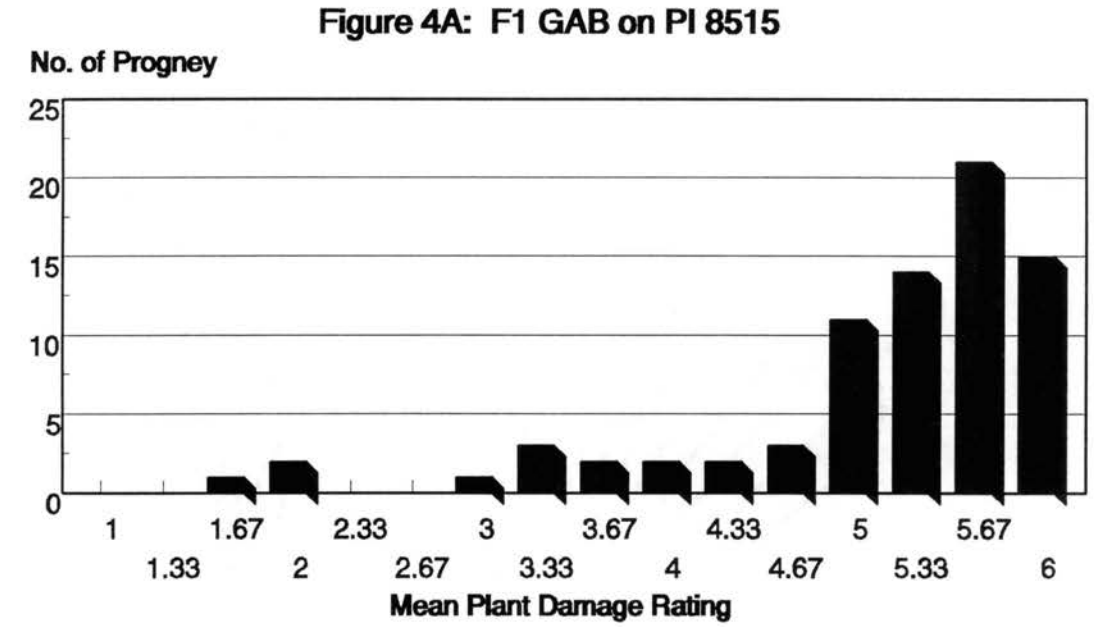


Figure 3: Frequency distribution of mean plant damage ratings for the F1 progney of GAB clone on 'Piper' Sudangrass (Fig. 3A), and Cargill 607E (Fig. 3B).



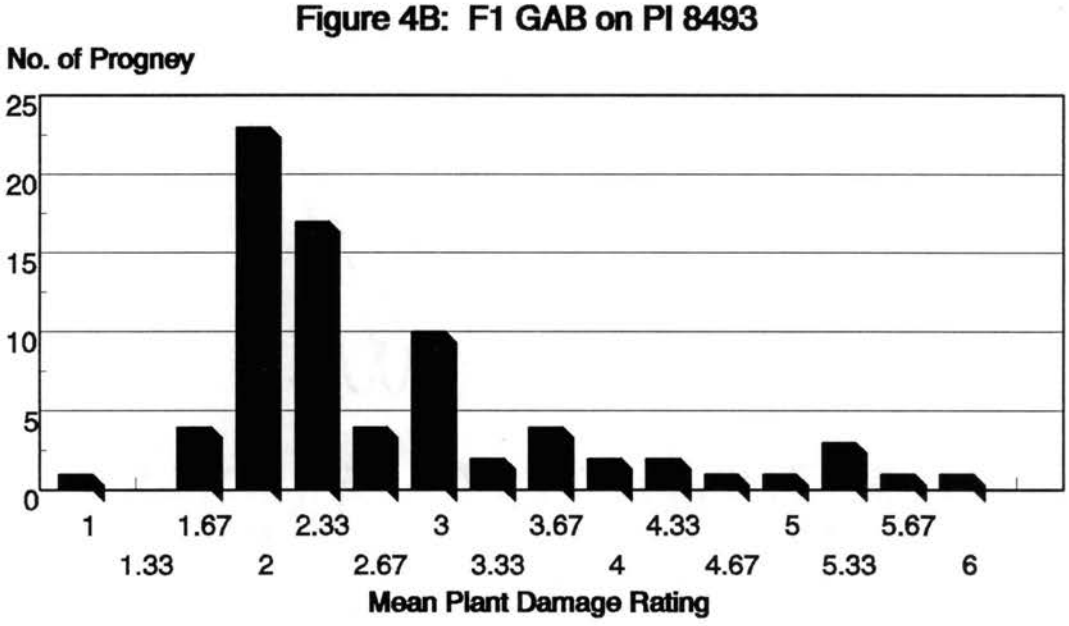
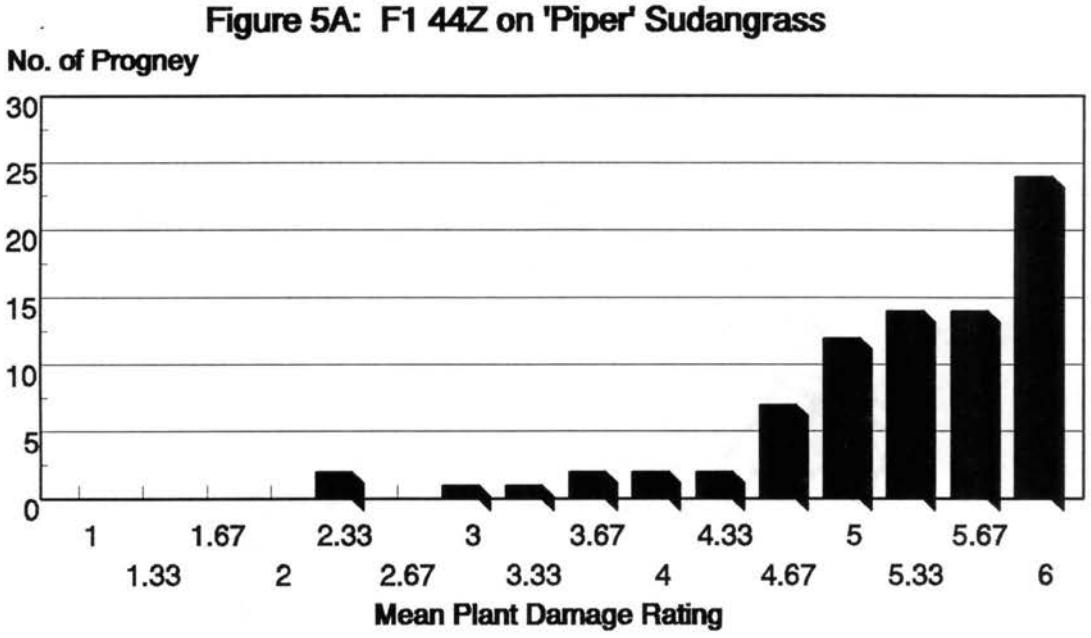


Figure 4: Frequency distribution of mean plant damage ratings for the F1 progney of GAB clone on Pioneer 8515 (Fig. 4A), and Pioneer 8493 (Fig. 4B).



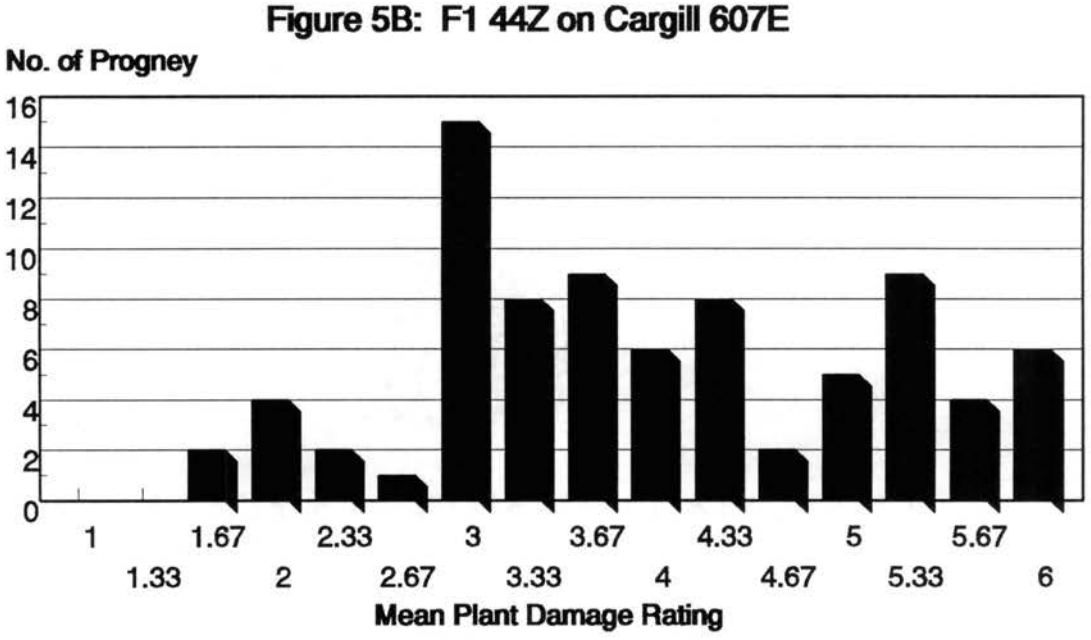
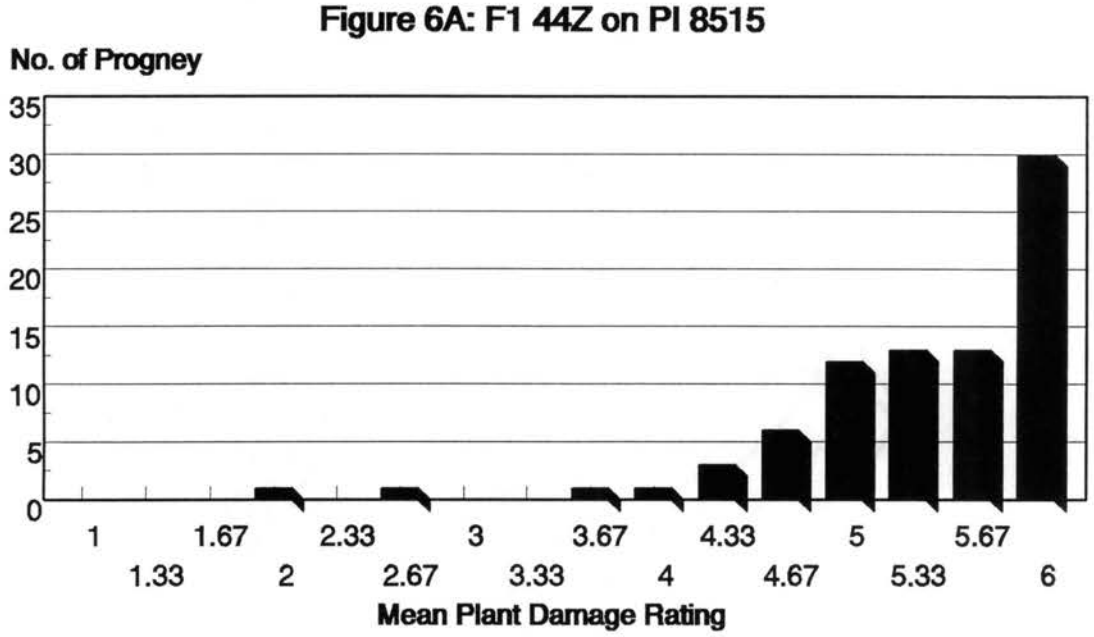


Figure 5: Frequency distribution of mean plant damage ratings for the F1 progney of 44Z on 'Piper' Sudangrass (Fig. 5A), and Cargill 607E (Fig. 5B).



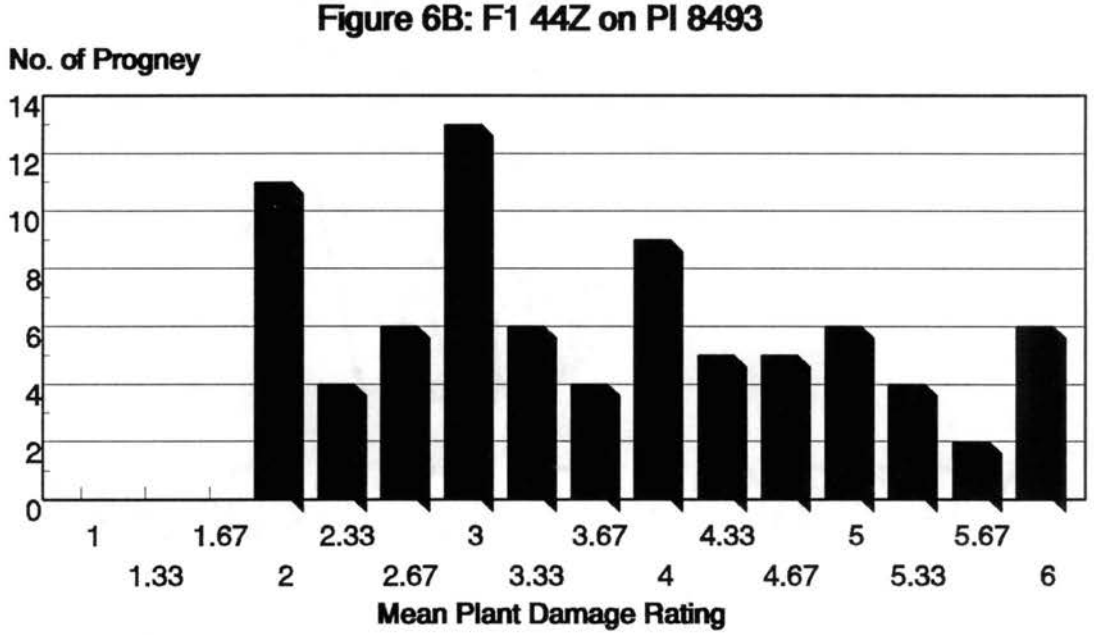


Figure 6: Frequency distribution of mean plant damage ratings for the F1 progney of 44Z on Pioneer 8515 (Fig. 6A), and Pioneer 8493 (Fig. 6B).

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