GENETICS OF GREENBUG (HOMOPTERA: APHIDIDAE) VIRULENCE TO RESISTANCE IN WHEAT AND SORGHUM

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PREFACE

A series of studies were conducted to characterize greenbug virulence to resistance in sorghum and wheat, investigate how greenbug host races (biotypes) develop, and estimate how much diversity exists in the greenbug population. The results of this investigation are presented in four separate and complete manuscripts that have been published or have been submitted for publication. Part I is in press as a book chapter in "Aphid-Plant Genotype Interactions," R.K. Campbell and R.D. Eikenbary (Editors), Elsevier Scientific Publications. Part II is published in the December, 1988, issue of Journal of Economic Entomology. Part III is published in the February, 1989, issue of Genome. Part IV has been submitted to Theoretical and Applied Genetics.

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I dedicate this thesis to my deceased parents, Delores G. and Joseph E. Puterka, for they instilled in me an appreciation for honesty and hard work.

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PART 1

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INTRODUCTION

The greenbug, Schizaphis graminum (Rondani), is a monoecious aphid with over 70 species of graminaceous host plants (Michels 1986). The life cycle patterns vary between monoecious holocycly or anholocycly, depending on the environmental conditions (Webster and Phillips 1912, Wadley 1931). Several dozen resistance sources are available in the greenbug's five principle host crops; barley, oats, rye, sorghum, and wheat. Greenbug populations are comprised of distinct races that differ in the ability to damage the different resistance sources. These races are termed "biotypes" and each biotype is a phenotypic expression of an indefinite number of genotypes. The genotype of a particular biotype can vary because greenbug virulence to each source of plant resistance is 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 (Puterka and Peters 1989a). Biotypic 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 the resistance sources (Puterka and Peters 1989b).

The seven biotypes, countless other isolates

(unclassified biotypes), plus the large number of greenbug resistance sources available provide an ideal subject for the investigation of specific aphid-host interactions. The recent advances in laboratory breeding techniques (Puterka and Slosser 1983, 1986) have made the study of the genetics of these specific genetic interactions possible (Puterka and Peters 1989a,b). Our recent knowledge of inheritance of greenbug virulence, plus the numerous other greenbug studies on it's behavior, biology, and greenbug resistance sources, makes it the most complete model for phytotoxic Homoptera.

Herein, we present an overview of the greenbug's history, biotype concept, genetics and the role of the holocycle in creating genetic diversity in the greenbug population. The biology and genetics of the greenbug are considered in our hypothesis of population genetic structure and biotype evolution. The role and strategies of host plant resistance in greenbug management are discussed.

GREENBUG BIOTYPE HISTORY

Prehistory of Biotypes

Rondani (1852) first described the greenbug, Schizaphis graminum (Rondani), in Italy and found them infesting corn, bermuda grass and other grasses but made no reference to damage. In the United States, the greenbug is an important pest of wheat, sorghum, barley, oats, and rye. This pest was first recognized damaging oats in 1882 (Webster and Phillips 1912). It is not known if the greenbug was

introduced or migrated into the United States. Serious greenbug outbreaks in wheat soon followed with the first significant outbreak occurring in 1890. Webster and Phillips (1912), and Wadley (1931), gave detailed reviews of the greenbug outbreaks that have occurred in the United States from 1890 to 1926. The number of greenbug outbreaks since 1926 are too numerous to mention, but a review in Oklahoma found that outbreaks follow no set pattern and are erratic in both occurrence and duration. However, greenbug outbreaks generally followed a year with normal precipitation during the spring and summer, above normal temperatures during the fall, winter and spring, and below normal summer temperatures (Rogers et al. 1972). Nonetheless, damaging greenbug infestations that require insecticide applications occur annually at various locations throughout the midwestern United States.

Host plant resistance has been regarded as a welcome alternative to insecticides in managing insect pests (Maxwell and Jennings 1980). The first greenbug resistant wheat, Dickinson Selection 28A (DS28A), was reported by Painter and Peters (1956). However, DS28A was found to be susceptible to the greenbug population in 1959 while this source of resistance was being incorporated into wheat varieties. The race that had the ability to damage DS28A was designated biotype B (Wood 1961). This marked the beginning of biotype history in greenbugs.

Posthistory of Biotypes

Greenbug biotypes A to C (Wood 1961, Harvey and Hackerott 1969) and E to H (Porter et al. 1982, Kindler and Spomer 1986, Puterka et al. 1988) have been identified since 1960. The exception to designating biotypes by host damage was biotype D which was a biotype C population resistant to organophosphorus insecticides (Teetes et al. 1975), but reference to this biotype in the literature is rare because it does not follow the usual criteria for biotype classification.

Biotype A was the original greenbug population avirulent to DS28A and this population served as a reference point for biotype designations. Much effort has been expended since 1960 in an attempt to find lasting resistance to the greenbug. Biotype B was discovered damaging DS28A in greenhouses and was believed to be restricted to greenhouse environments, thus, it was called the "greenhouse strain" (Wood 1961). Although biotype A was considered to be the predominant biotype in the field up to 1965, no field surveys were conducted (Wood 1971). Biotype surveys in wheat during 1986 (Kerns et al. 1987) found that biotype B comprised up to 11% of the biotype complex in Oklahoma, therefore, biotype B is not restricted solely to a greenhouse environments.

Greenbugs were reported in light numbers on sorghum as early as 1916 (Hayes 1922) continuing up to the mid 1960's (Daniels 1975) but they were not considered to be sorghum

pests. However, decimating greenbug infestations in the mid-west during 1968 (Harvey and Hackerott 1969, Wood et al. 1969) marked an unexplained increase in the greenbug's virulence and fitness on sorghum. The greenbug population with the ability to feed on and damage sorghum was designated biotype C (Harvey and Hackerott 1969). Biotype C predominated the biotype complex in wheat and sorghum up to the mid 1980's (Puterka et al. 1982, Kindler et al. 1984, Dumas and Mueller 1986).

Biotype E was inadvertently discovered in the field, when biotype C resistance from 'Amigo' was being bred into wheat (Porter et al. 1982). Soon, 'Largo' was identified as a source of biotype E resistance (Porter et al. 1982), but was susceptible to biotype B (Webster et al. 1986) and to the new biotypes F and G that soon followed. In the process of characterizing greenbug isolates collected from various areas of the United States, Kindler and Spomer (1986) identified biotype F. Biotype F also had the ability to damage 'Amigo,' but not DS28A, and was later determined to be virulent to 'Largo' (Puterka and Peters 1988).

Biotype surveys initiated in Oklahoma (Kerns et al. 1987) and Texas (Bush et al. 1987) detected two greenbug isolates that were designated biotypes G and H, respectively (Puterka et al. 1988). Biotype G was virulent to all known sources of resistance in wheat, but was avirulent to sorghum and barley. Biotype H shared the same host plant relationships as biotype E on wheat, but was avirulent to

sorghum and was the first biotype reported to be virulent to greenbug resistant 'Post' barley. The biotypic diversity in greenbugs is far greater than first imagined and it appears that there are many new biotypes (isolates) to be discovered.

Current Status of Biotypes

Many recent reports of new greenbug isolates (undescribed biotypes) give testimony to the greenbug's genetic diversity (Porter et al. 1982, Kindler and Spomer 1986, Bush et al. 1987, Kerns et al. 1987). A list of biotypes and isolates we are currently maintaining are presented in Table 1. The virulence relationships of these biotypes to nine sources of greenbug resistance from five host crops are given. Biotypes A and B cannot be fully accounted for because the original colonies were not maintained after the appearance of biotype C. We obtained five additional greenbug isolates from across the United States with unique host plant relationships in contrast to the other biotypes. The virulence relationships of the biotypes and isolates are based on caged screening tests. We report biotypes B and F to be virulent on sorghum, however, there are no records of these biotypes being collected from sorghum in the field. Although biotypes B and F can be reared on various sorghum varieties for over five generations, progressive loss in body size was obvious. Evidently, biotypes B and F have the salivary components and

initial fitness to damage sorghum, but their fitness declines. Apparently, these biotypes have other host options during the summer after small grains have been harvested.

Greenbug biotypes superficially appear to be sequentially evolving because of the progressive letter designations (Table 1), but in reality, the designations are only a function of resistance gene deployment.

THE BIOTYPE CONCEPT IN RETROSPECT

The Biotype Concept in Aphids

Biotypes commonly occur in aphids and are most often characterized on the basis of differential host plant utilization within a species (Eastop 1973). In reference to host plant resistance, a biotype is regarded as an individual or population that differs from the rest of the population by criteria other than morphology, such as parasitic ability (Maxwell & Jennings 1980). Some researchers have chosen other criteria (e.g. insecticide resistance) to characterize biotypes, with little justification, which have caused overlaps in biotype designations within a species. As a result, several biotypes may be separated by one trait but may be grouped together under another biotype designation when considering another trait (van Emden et al. 1969). This inconsistency has confused the biotype concept to the point where its usage is regarded as having no distinct biological meaning

(Claridge and Den Hollander 1983) or as an ambiguous term that should be abandoned (Diehl and Bush 1984). However, the uses and the abuses of the biotype terminology in certain aphids is well entrenched in the literature. Therefore, to establish congruency in the study of some aphids it would be best to specifically define the criteria for determining a biotype.

Biotype designations have proven their agronomic utility in that they allow a broad range of specific aphid-host relationships to be described under a single letter designation. Although this may seem like 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. This knowledge is essential for entomologists and plant breeders involved in developing aphid-resistant crops. Biotype classifications are usually denoted by capital letters (i.e. biotype E). Similar classifications have also been utilized in plant pathogen races and strains (Flor 1971, Christ et al. 1987) and in Hessian fly, Mayetiola destructor (Say), races (Gallun 1972).

Biotypes: Phytotoxic Versus Nonphytotoxic Aphids

Precise use of biotypic terminology is only possible when information on an insect's genetics is available (Claridge and Den Hollander 1983). Recent advances in

greenbug genetics and studies on the resistance components (tolerance, antibiosis, antixenosis) in sources of greenbug resistance have made it possible to refine the definition of "greenbug biotype." Greenbug biotypes are characterized by virulence, the phytotoxic aphid's ability to damage a resistance source, and this is the primary reason biotypes are of great concern to researchers in host plant resistance.

Plant damage from greenbug feeding occurs as a response to an unknown substance in the saliva which the aphid injects while feeding. The salivary product influenced by the virulence genes interacts with the complementary gene products in the host plant to begin a cascade of physiological reactions in the plant that ultimately results in plant damage. 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). Plants fed on by radiolabeled (^{14}C) greenbugs had the recovered label concentrated at the feeding site and roots, thus confirming the injection and translocation of saliva (J. Burd, personal communication). Ultrastructural studies on susceptible wheat plants found necrosis and chlorosis at the feeding site that was characteristic of a phytotoxic response. Only white specks appear on leaves of resistant plants due to localized cell collapse, indicating no phytotoxic response (Al-Mousawi et al. 1983).

In sorghum, the phytotoxic damage manifests itself in quite a different manner. Greenbug damage on susceptible sorghum is exhibited as chlorosis, anthocyanosis, and necrosis which is a typical phytotoxic reaction. Methylated intercellular pectins have been implicated as one of the biochemical factors responsible for sorghum resistance to the greenbug saliva (Dreyer and Campbell 1984, Campbell and Dreyer 1985). The underlying implications of the biotype-host plant resistance correlation points to the effects of greenbug salivary components on the plant, regardless of whether the phytotoxic reaction increases fitness in greenbugs. Our definition of "virulence" considers the aphid's ability to evoke a phytotoxic response as the sole component of the greenbug biotype concept. Fitness ultimately determines which aphid genotypes, avirulent or virulent, will be successful in the ecosystem.

In contrast, non-phytotoxic aphids, like Acyrthosiphon pisum (Harris), have biotypes characterized by fitness parameters, such as fecundity or survival, (Muller 1985) that measure host utilization. Fitness is defined as the measure of a genotype's proportionate contribution of progeny to the next generation. This is where the biotype concept diverges between phytotoxic and non-phytotoxic aphids because our biotype fitness studies (Kerns et al. 1989) and reports by other researchers (Hackerott et al. 1969, Schuster and Starks 1973, Kindler and Spomer 1986, Beregovoy et al. 1988) have found that virulence does not

always increase the fitness of a biotype. The reason for this is that each resistance source may have several components of resistance (tolerance, antibiosis, or antixenosis) or only one of these components. Only antibiosis has a direct impact on the fitness parameters of the greenbug. As a result, these traits are probably governed by different genes that may not be linked to virulence genes.

Greenbug Biotypes: Does Virulence Equal Fitness?

There are many cases where virulence appears to be correlated with the fitness of a greenbug biotype in wheat. Greenbug fecundity (Kindler and Spomer 1986, Ryan et al. 1987, Niassy et al. 1987) and intrinsic rate of increase (r_m) (Kerns et al. 1989) of biotypes B, C, and E appears to be strongly correlated with greenbug resistance in wheat varieties, 'Amigo' ('TAM 107') and 'Largo' (5xL). Increased fitness may be related to host conditioning by greenbug saliva to make the plant a better food source (Dorschner et al. 1987) although there are exceptions. Fecundity and r_m of biotype F and G did not increase significantly on wheat varieties to which they were virulent compared with varieties to which they were avirulent (Kerns et al. 1989). Furthermore, the Idaho isolate (ID) in Table 1 has been found to be avirulent to 33 different wheat cultivars (D.C. Peters, unpublished data). Yet, we have reared this isolate continuously for over 2 years on ID resistant 'Triumph 64'

with no apparent loss in vigor and size. Thus, it does not seem essential for the greenbug to cause plant damage so it can obtain the necessary nutrients to carry out all necessary biological functions.

In sorghum, the connection between virulence and fitness is more obscure than in wheat. Tolerance is the main component of resistance although moderate amounts of antixenosis and antibiosis can be expressed (Hackerott et al. 1969, Wood 1971, Young and Teetes 1973). High populations of greenbugs occur on susceptible sorghum, but resistant sorghum can also support heavy greenbug populations and incur some injury although the growth of the plant is not perceptibly affected (Hackerott et al. 1969). Fecundity of a particular biotype on sorghum can be affected irrespective of a variety's resistance status (Hackerott et al. 1969, Schuster and Starks 1973). Comparisons of fecundity between biotypes has also shown that resistance did not always affect fecundity (Kindler and Spomer 1986, Beregovoy et al. 1988). Fitness of a biotype may not be accurately measured by fecundity because development time is not considered, whereas, the intrinsic rate of increase takes both development time and fecundity into account (Birch 1948). Even though the intrinsic rate of increase corresponds much closer to plant resistance, there are still exceptions among biotypes where virulence does not correspond with fitness (Kerns et al. 1989). The main reason for this lack of correlation is that each resistance

source differs in their antibiotic, antixenotic, and tolerance properties to a particular biotype. Furthermore, the resistance components in sorghum have been found to be independently inherited (Dixon 1988). Negative correlations between virulence and fitness have also been reported for biotypes B and C on greenbug resistant oats (Wilson et al. 1978). The use of fecundity to determine greenbug biotypes would certainly lead to biotype misclassifications. These examples support our argument that virulence and fitness are two separable components in the greenbug.

Identifying Greenbug Biotypes

Presently, the only reliable means of characterizing greenbug biotypes is through the damage response of the plant to greenbug feeding. Greenbug resistant germplasms have always been determined by tolerance, the plant's ability to withstand insect damage. Resistant plants exhibiting tolerance to phytotoxic aphid damage might suggest that the plant actually has a tolerance to the insect's saliva although plant resistance can be a manifestation of the interactions between tolerance, insect host preference (antixenosis) and antibiotic effects on the insect (Painter 1951). Tolerance has long been the measure of plant resistance and biotypic status of the greenbug because of the selection method employed by the plant breeders. By heavily infesting hundreds of plant selections, the breeder can identify resistant plants based

on a susceptible (dead)/resistant (live) relationship between plant selections (Painter 1951, Peterson 1985). When susceptible plants begin to die, a damage rating scale is used to aid the plant breeder in separating resistant from susceptible plants. If resistance is controlled by major genes, as in wheat (Tyler et al. 1987) and sorghum (Peterson 1985), the damage distribution would be discontinuous, however, variation in the expressivity of the resistance gene can make damage appear to be continuous (Russell 1975). This is the main reason a damage rating should be used in both the assessment of resistance and characterization of greenbug biotypes. Various methods for determining biotypes on a damage rating basis have been described (Wood et al. 1969, Starks and Burton 1977, Puterka and Peters 1988). A rapid method (Puterka and Peters 1988) has been developed for determining the virulence relationships for biotypes B, C, E, and F to greenbug resistance genes in wheat designated by Tyler et al. (1987) as Gb2 ('TAM 107') and Gb3 ('TAM105'5*/'Largo'). Using diagnostic feeding lesions, the greenbug virulence to Gb2 and Gb3 can be established within three days. The plant responses can be dichotomized allowing the scoring of a no damage (-) or damage (+) response.

Morphological, biochemical, and genetic markers for identifying greenbug biotypes have shown promise in biotype identification although these studies have not adequately addressed the variation within and between the many biotypes

that exist. Inayatullah et al. (1987) and Fargo et al. (1986) have found that alate and apterous greenbug biotypes form morphometrically distinct groups when multivariant analysis was applied to a large number of morphological measurements. Isozyme patterns (Abid et al. 1989) for biotypes B, F and the C/E group have distinctive patterns. Cuticular hydrocarbon profiles can be used to distinguish all but two of the six biotypes listed in Table 1 (Dillwith, Peters and Puterka, unpublished data). Measurements of total chromosome length in meiotic metaphase found Biotype A differed significantly from biotypes B and C, but biotypes B and C did not differ (Mayo and Starks 1972). However, a later report by Mayo et al. (1987) conflicts with the earlier report by finding significant differences between the biotype C/E group and the B/F group, but there were no significant differences between biotypes within a group. They did not address why these discrepancies occurred in their later paper, but they may have been due to differences in technique or data analysis. Comparisons of the mitochondrial DNA digested by restriction enzymes have found restriction fragment length polymorphisms between biotypes B, C, E, and F. Mitochondrial divergence has shown biotypes C and E are closely related, but diverged considerably from biotypes B and F (Powers et al. 1989). All of these studies have used only one clone of a biotype, therefore, the variation among clones within a biotype is not known. None of these studies can separate biotype C from E possibly

because they only differ in two virulence-host relationships. Continued efforts in these areas will hopefully provide a better understanding of the differences among biotypes.

The degree of characterizing greenbug virulence certainly depends on the type of study, but biotype designations should only be used by those willing to do a rigorous screening routine. The variety of host plant responses these biotypes and isolates have in common (Table 1) expresses the need to use as many host plants and varieties as possible when characterizing biotypes. We recommend that using at least 17 resistant and susceptible plant varieties to characterize biotypes as was done by Kindler et al. (1986) and Puterka et al. (1988). At least one susceptible plant entry should be used for each crop examined. The rapid lesion technique that was originally intended to distinguish biotypes B, C, E, and F (Puterka and Peters 1988), actually serves to evaluate greenbug virulence to Gb2 and Gb3 resistance genes in wheat. The discovery of biotype H (Bush et al. 1987) negated the lesion technique as an exclusive means of biotype identification because both biotypes E and biotype H have the same virulence relationships to Gb2 and Gb3.

Biotypes could be further subdivided as more resistance sources become available. Consequently, the biotypic measurement is dynamic and will increase in resolution and complexity as more host relationships are determined. Some

may ask, to what extent do these biotypes need to be characterized? Due to the enormity of the greenbug resistance sources that are becoming available in recent years it might be best to identify only those new biotypes capable of damaging significant sources of resistance that might be deployed in the field. Biotypes F (Kindler and Spomer 1986, Puterka and Peters 1988) and biotypes G and H (Puterka et al. 1988) were classified on this premise. This information is very important because it identifies a new virulence locus or loci in the greenbug population.

GREENBUG HOLOCYCLE IN THE UNITED STATES

The greenbug has two principle life cycle modes in the United States which gives it great adaptive flexibility to the environment. Monoecious anholocycly is the strict life cycle in southern regions of the United States because the temperate environment and photoperiodic threshold for induction of the sexual cycle is not met (Webster and Phillips 1912, Wadley 1931). In the northern latitudes, monoecious holocycly primarily occurs on bluegrass, Poa pratensis, (Webster and Phillips 1912). We have concentrated our study on the greenbug holocycle because it provides a mechanism for genetic recombination during the sexual phase to rapidly produce new biotypic diversity.

Washburn (1908a) first reported and described greenbug bisexual morphs (sexuals) in the United States. The males are alate and highly mobile while the females are apterous;

no exceptions have been reported. Webster and Phillips (1912) gave a detailed account of the greenbug holocycle and egg embryogenesis. Numerous reports of greenbug sexuals in greenhouses and insectaries have been made (Washburn 1908b, Luginbill and Beyer 1918, Tucker 1918, Wadley 1931, Daniels 1956, Mayo and Starks 1972). Field observations are scarce and no genuine effort to examine the greenbug holocycle has been made since Webster and Phillips (1912). The threshold of sexual reproduction was estimated to be north of the 35th parallel based on their biological data and surveys.

The greenbug holocycle was closely linked to bluegrass, although a few sexuals were found in grain fields. Greenbug sexuals have been reported in Indiana (Webster and Phillips 1912), Minnesota (Washburn 1908b), Kansas (Kelly 1917) and Oklahoma (Wood et al. 1969, Kerns et al. 1987). Special attention is directed toward recent reports of successful overwintering of eggs in Ohio (Niemczyk and Power 1982) and Kentucky (Potter 1982) on Kentucky bluegrass turf which lead to early damaging infestations. This is tangible proof that the greenbug holocycle is common in the north central United States and mainly associated with bluegrass.

LABORATORY BREEDING METHODS

Inducing Sexuals

Early reports on environmental effects on greenbug polymorphisms (Webster and Phillips 1912, Wadley 1931) provided a basis for the laboratory induction of sexual

forms and the hatching of eggs. The shift from parthenogenetic reproduction in the summer to sexual reproduction in the fall was in response to decreasing day length (Webster and Phillips 1912). Wadley (1931) reported that both photoperiod and temperature were factors and estimated the threshold photoperiod was near 12 hours in the field.

Puterka and Slosser (1983) described the optimal conditions to induce sexuals in the laboratory. A clone of biotype C greenbug was transferred from a 12-hour photoperiod to an 11-hour photoperiod under temperatures of 22°C which induced sexuals in about 30 days. Oviparae (oviparous females) appeared by the second generation, but males did not appear until the fourth generation. Differences in the threshold photoperiods for sexual production could affect mating between biotypes C and E (Eisenbach and Mittler 1987a). However, the 11-hour photoperiod is low enough to induce the sexual phase in biotypes C, E, and F (Inayatullah et al. 1987, Puterka and Peters 1989a) and biotype G and the KY isolate (Puterka, unpublished data).

Biotype B has not been induced into the sexual phase under laboratory conditions (Inayatullah et al. 1987) which is consistent with insectary and field observations (Wood et al. 1969). Furthermore, biotypes B and H could not be induced into the sexual phase, even under greatly reduced photoperiods of 8 hours (Puterka and Peters, unpublished

data). Apparently, biotypes B and H are anholocyclic forms of the greenbug.

Biotypes C, E and F mate readily under laboratory conditions (Puterka and Peters 1989a). We observed no differences in sexual attractiveness between biotypes even though differences have been reported in laboratory studies between biotypes C and E (Eisenbach and Mittler 1980). Biotypes have considerable overlap in suitable host plants, particularly during the fall. Therefore, it seems that some degree of biotype interbreeding is possible in the field.

Hatching Eggs

Hatching greenbug eggs became one of the great mysteries of greenbug biology. Numerous unsuccessful attempts have been made to hatch greenbug eggs (Wadley 1931, Tucker 1918, Wood 1971, Mayo and Starks 1972). Webster and Phillips (1912) determined that the eggs required exposure to freezing temperatures before they would hatch. The period from oviposition to egg hatch represents eudiapause, a form of diapause induced by photoperiod and terminated by chilling temperatures (Saunders 1982). Based on this observation, Puterka and Slosser (1986) used a series of environmental conditions and found that eggs from biotype C held at temperatures $\leq$ 1.7°C for $\geq$ 6 weeks duration would break egg diapause. After the cold treatments, the eggs hatched in about two weeks at 16°C. Humidity was also a critical factor in successful egg hatch (Hand 1983), so eggs

were held at 75% RH by saturated NaCl solutions during the cold treatments and incubation (Winston and Bates 1960). Egg hatch of 6 to 13% was obtained, depending on the host plant and temperature-duration regimen. Another study soon followed which presented a method to hatch greenbug eggs (Wipperfurth and Mittler 1986) based on breaking eudiapause. However, the experimental conditions and technique differed considerably between Wipperfurth and Mittler (1986) and Puterka and Slosser (1986). Higher percent egg hatch (19-45%) was reported by Wipperfurth and Mittler (1986), but the time required to hatch the eggs after cold treatments was considerably longer (71 days). The methods described by Puterka and Slosser (1986) reduced the time from oviposition to egg hatch by at least twenty days compared with Wipperfurth and Mittler (1986).

High relative humidities of 90% (Hand 1983) to 100% (Peterson 1917) can increase egg hatch in aphids. Increasing the humidity to 95% RH and other slight modifications in the techniques for hatching eggs (Puterka and Slosser 1986) improved egg hatch (19-26%, depending on biotype) and reduced egg hatch time (Puterka and Peters 1989a). Solving the mystery of egg hatch and refining egg handling techniques has opened an exciting new era of aphid research. Laboratory methods for breeding greenbugs have now been refined to the point that detailed studies can be done on aphid-host genetic interactions as well as other studies on greenbug genetics.

GREENBUG GENETICS

Inheritance of Virulence

Genetic studies on aphid virulence are rare, but suggest that virulence is a qualitative character conditioned by major genes. Virulence in the rubus aphid, Amphorophora rubi (Kaltenbach), followed a gene-for-gene relationship (Flor 1971) where virulence to two raspberry varieties was conditioned by single independent major genes, one being dominant and the other recessive (Briggs 1965). Muller (1985), through a series of insectary breeding experiments, crossed color biotypes within the pea aphid, Acyrthosiphon pisum (Harris), A. pelargonii (Kaltenbach), and Aphis fabae Scopoli, and found the progeny segregated into Mendelian ratios. Closely related aphid species in the Aphis fabae Scopoli group produced hybrids that also segregated by color into Mendelian ratios. Certain colored biotypes had distinct host preferences, but the linkage of color to host utilization was never established. Extra-nuclear inheritance has also been proposed for greenbug virulence to sorghum (Eisenbach and Mittler 1987b), but was based on very limited data (n = 3-5 progeny/cross).

The capability to induce sexuals, breed biotypes, and hatch the eggs in the laboratory has had a major impact on the study of specific aphid-plant genome interactions for the greenbug. Genetic studies have been further facilitated by the development of rapid methods for determining greenbug

virulence to the greenbug resistance genes Gb2 and Gb3 in wheat. The lesion technique has allowed the direct assessment of the phytotoxic saliva without the confounding effects of antibiosis and antixenosis. However, to determine greenbug virulence to Gb1 (DS28A), screening pots were heavily infested with ca. 8 greenbugs per plant which nullified the antibiotic and antixenotic plant effects on the greenbug. These three resistance sources and biotypes C, E and F provide an excellent differential to study the inheritance of virulence in wheat (Table 2).

Clones of biotypes C, E and F have been inbred and reciprocally crossed, and the resulting fundatrices evaluated on genes Gb2 and Gb3 using the lesion technique. Seven hundred and ten progeny were evaluated, including the testcrosses. The crosses between biotypes gave similar segregation ratios indicating that the virulence genes were allelic. Virulence to genes Gb2 and Gb3 was recessive and conditioned by duplicate dominant genes and a dominant modifier gene epistatic to one of the duplicate genes (Puterka and Peters 1989a). In another source of greenbug resistance, Gb1 (DS28A), virulence was inherited in the same manner as virulence to Gb2 and Gb3, however, it was dominantly inherited instead of recessively inherited.

Greenbug virulence to wheat is under polygenic influence where multiple genes in the aphid interact with a single corresponding gene in the host to establish a phytotoxic relationship. The genes in a parasite and host

can only be identified by a genetic interaction which leads to a phenotypic expression in the host. Once the interaction is established, a cascade of secondary changes can result in the host (Ellingboe 1979).

With the inheritance of resistance in the plant and the inheritance of virulence in the greenbug well characterized, the specific virulence gene-resistance gene interactions can be summarized (Table 3).

Resistance gene Gb1 in wheat has been shown to be recessively inherited while resistance genes Gb2 and Gb3 are dominantly inherited; all of these genes were independently inherited (Tyler et al. 1987). When resistance was dominantly inherited in the plant, virulence in the greenbug was recessively inherited and vice versa. This virulence gene-resistance gene relationships corresponded closely to the gene-for-gene relationship described for flax rust-host relationships (Flor 1971) although greenbug virulence was more complexly inherited. However, polygenic inheritance does not necessarily exclude the gene-for-gene hypothesis, particularly when specific parasite and host genes match (Christ et al. 1987). In principle, greenbug virulence could easily be regarded as a gene-for-gene relationship because the duplicate gene-modifier gene mode of inheritance still influences a single gene product in the aphid.

A strong linkage relationship existed between the genes conditioning virulence to Gb2 and Gb3 when heterozygous males were used in the testcrosses. When homozygous males

were used in the reciprocal crosses, 50% recombination occurred. This unusual linkage results from achiasmate spermatogenesis which has been documented in several invertebrates and aphids (Blackman 1985). Achiasmate spermatogenesis profoundly influences the linkage relationships of virulence genes within the greenbug population because genetic recombination is prevented. Evidently, the multiple genes that conditioned virulence to Gb2 and Gb3 reside on the same chromosomes, but were $\geq$ 50 map units apart. The greenbug's ability to preserve successful genotypes, yet adapt and respond to new selective pressures and the ever changing environment, may be greatly enhanced by this unusual linkage mechanism. The ramifications of this linkage mechanism to biotype evolution and to resistance gene deployment needs further investigation (Puterka and Peters 1989a).

The 710 progeny were also evaluated against the other resistance sources listed in Table 1, plus 4 susceptible small grain varieties. Over 20 clones with a combination of host relationships unique to any previously described biotypes were identified. We consider these laboratory clones to be recombinants and reserve the label "biotype" for recombinants that naturally occur in the field. Inheritance of virulence to 'Piper' and PI 264453 (Pioneer 8493) resistance in sorghum followed the same duplicate dominant gene-modifier gene model presented for wheat (Puterka and Peters 1989a). Virulence to SA 7536-1 (Pioneer

8515) sorghum and CI 1580 oats is simply inherited with virulence to 'SA 7536-1' being recessive and virulence to CI 1580 oats being dominant. 'Insave' and 'Post' resistance sources were resistant to all of the progeny (Puterka and Peters, unpublished data).

Our data did not support earlier conclusions that biotype inheritance for the greenbug on sorghum was extranuclear (Eisenbach and Mittler 1987b). Apart from their low progeny recovery from the crosses, the biotypic status of the progeny could have been misclassified because the classification was based on fecundity on IS 809 sorghum. Fecundity between biotypes C and E does not differ significantly on IS 809 (Beregovoy et al. 1988) and IS 809 also shows variability in resistance (Starks et al. 1983).

Population Genetics and Biotype Evolution

Genetic variation in aphid populations has primarily been built upon the knowledge of variation in aphid polymorphisms and life cycles (Blackman 1974, Dixon 1977, 1985), chromosome morphology (Blackman 1985), isozymes (Tomiuk and Wohrmann 1980, Loxdale et al. 1983), morphometrics (Singh and Cunningham 1981, Shaposhnikov 1984) and biological traits like fecundity (Weber 1985) or aphid establishment (Muller 1985).

Aphid parthenogenesis is ameiotic which usually does not allow genetic recombination, although there are other possible mechanisms (Blackman 1979a). Greenbug biotypes are

parthenogenetically stable, even after 2 years of heavy selection pressure by continuous rearing on resistant sorghum (Starks and Schuster 1976). We also have reared hundreds of unique clones on wheat in our biotype breeding operation with no loss of their integrity; therefore, the aphid-host relationships appear to be genetically stable.

Sex allows recombination and increases genetic variance (Williams and Milton 1973) which has been born out in cross breeding clones of greenbug biotypes (Puterka and Peters 1989a,b). Nevertheless, the role of parthenogenesis in generating genetic variation should not be underestimated for it allows the accumulation of new genetic mutations in the form of hidden genetic variance. In cyclic parthenogens, like the greenbug, large numbers of hidden mutations are accumulated during prolonged parthenogenetic cycles and are immediately converted to expressed genetic variance after the sexual cycle (Lynch and Gabriel 1983). The result is a sudden increase in an organism's ability to respond to selection. This situation seems applicable to the greenbug with it's ability to respond to a wide variety of environmental selection pressures and utilization of a broad range of hosts. Tremendous amounts of genotypic variability occurred in just one generation by crossing biotypes C, E and F (Puterka and Peters 1989a) which indicates that these biotypes were heterozygous for many virulence loci. Thus, there is a high degree of hidden variance in the form of recessive virulence genes. These

results clearly elucidate the role of sexual reproduction in expediting biotype evolution. Blackman (1979b) graphically illustrates how the genetic structure of holocyclic aphids fluctuates seasonally due to the ensuing action of selection pressures on the diverse genotypes produced by the sexual cycle. The large number of unique biotypes of unknown virulence and an even greater number of possible recombinant genotypes contribute to the unpredictability of biotype evolution. The numerous wild and cultivated hosts available to the new recombinants, plus the resistance genes being deployed, adds to this unpredictability. The greenbug's history is marked with these unexpected shifts in biotype composition. The shifts in biotype composition up to the appearance of biotype C can be explained as simply not recognizing the genetic diversity that already existed. However, the sudden and devastating appearance of biotype C on sorghum in 1968 (Harvey and Hackerott 1969) is significant and represents the greenbug's increased host range and virulence. Blackman (1979b) considers biotype C to be a new introduction into the United States a few years prior to 1968. If this is true, the greenbug threat will escalate as the sorghum virulence and fitness genes of biotype C are added to the greenbug gene pool.

Role of Host Plant Resistance

The role of host plant genetics in broadening the greenbug's host range in field crops is another possibility.

The continual change of the sorghum genome by breeding efforts for crop improvement could have unintentionally bred in greenbug susceptibility. Corn also seems a likely candidate for accidentally breeding in susceptibility since greenbugs have long been reported to occur in low numbers on corn (Wadley 1931) and are capable of reproducing on it (Michels et al. 1987). Selected Vica spp. cultivars with increasing domestication and degree of plant breeding were shown to decrease in resistance to three aphid species (Holt and Birch 1984). Several instances of breeding insect susceptibility into other crops (i.e. Frego bract cotton) are also known (Maxwell 1972).

Biotype surveys during the early 1980's indicated another dramatic shift from biotype C to E. In 1981, 90% of the greenbug population in wheat was determined to be biotype C in the Texas Panhandle area (Puterka et al. 1982). By 1985, approximately 90% of the greenbug population in Oklahoma (Kerns et al. 1987) and Texas Panhandle area (Bush et al. 1987) was biotype E in both wheat and sorghum. Biotype C resistant sorghum was available in 1975 and by 1980, 90% of the sorghum acreage in the Southern Plains was resistant to biotype C. Sorghum resistance has been shown to reduce the r_m of avirulent biotypes compared with virulent biotypes (Kerns et al. 1989). Sorghum is utilized by certain biotypes during the summer when selective pressures can be magnified by the parthenogenetic reproduction. The result of this scenario was a shift in

the predominant biotype from biotype C to E over approximately a 10-year period. Laboratory breeding of greenbug biotypes has produced clones with increased virulence capable of damaging biotype E resistant sorghum which is evidence that another biotype shift in the field is inevitable.

The primary goal of host plant resistance is to breed resistance that is durable in the field. Satisfactory success in breeding durable greenbug resistant grain sorghum was achieved by breeders despite the biotype shifts. Biotype C resistant sorghum, available in 1975 (Starks et al. 1983), had a durability of about 10 years. Approximately 38% of the greenbug resistant sorghum was biotype E resistant in 1986 (Kerns et al. 1987), therefore, greenbug resistance continues to be feasible in sorghum. Success of greenbug resistance in wheat was stifled by the appearance of biotypes. However, new sources of greenbug resistance in Triticum spp. show great promise because they are resistant to all the known biotypes, isolates, and laboratory clones tested. This new source of resistance may be the first horizontal resistance source to the greenbug but its performance in the field will be the true test. Laboratory breeding experiments have played a vital role in identifying holistic sources of greenbug resistance. Breeding greenbug biotypes has produced recombinants that have a broader range of virulence than their parents (Puterka and Peters 1989a,b). Through the laboratory

production of highly virulent biotypes we are able to screen potential sources for resistance to biotypes not yet recognized in the field. Furthermore, the pyramiding of wheat and sorghum virulence genes into one greenbug genotype has been accomplished in the laboratory. This gives breeders the opportunity to screen their resistance sources with one highly virulent clone, instead of requiring them to maintain and screen the potential plant germplasm against a series of biotypes. In point, this has been set into practice by some innovative breeders, where laboratory bred clones are currently used in a wheat germplasm improvement program, along with biotype G (Puterka et al. 1988). However, great caution is required when using these virulent clones to prevent their release into the environment. Biotype G has enormous pratical utility to wheat breeding programs, however, the biotypes and isolates collected up to this time should be maintained for future studies. These biotypes are a valuable resource which is needed if we are to understand how specific aphid-plant interactions evolve.

Deploying plant resistance genes exerts selection pressures on the pest population and will change the gene frequency in favor or more virulent genes. This is inherent to most programs aimed at managing insect populations. New tactics in applying and deploying greenbug resistant cultivars must be developed to prevent the untimely loss of these hard earned resistance sources. Plant tolerance,

without the expression of antibiosis, has long been recognized as the best form of resistance because it reduces the selection pressure for increased virulence (Schuster and Starks 1973). Unfortunately, most of the resistance sources in sorghum exhibit much higher degrees of antibiosis than was previously reported (Dixon 1988). If antibiosis, antixenosis, and tolerance are expressed in a plant, this would impose a selection pressure for a more fit and virulent greenbug providing that virulence enhances fitness. Theoretically, selection pressure for virulence would be reduced using a mixture of varieties (multiline resistance) with differential resistance to biotypes. Pyramiding resistance genes, multiline resistance, quantitative resistance, and tolerance (exclusively) are all well known concepts that could contribute to more efficient greenbug management with host plant resistance.

CONCLUSIONS

From our laboratory breeding experiments, we have demonstrated that genetic recombination during the sexual phase can generate the biotypic diversity that commonly occurs in the greenbug species. Inheritance of virulence in the greenbug usually conforms to a duplicate dominant gene-modifier gene inheritance model. The aphid-plant interactions for the greenbug follow a gene-for-gene relationship that is commonly associated with host-parasite relationships.

Laboratory methods for inducing sexuals, breeding biotypes, and hatching eggs have immense value in studying the many facets of aphid genetics. The ability to examine specific aphid-host interactions has added a new perspective on how aphids adapt and interact with their hosts. These capabilities make the greenbug one of the best defined phytotoxic aphid-host interaction models available.

Many questions concerning the linkage of virulence, fitness and host preference in the greenbug, as well as the linkage of tolerance, antibiosis, and antixenosis, still remain unanswered. Continued effort in these areas will eventually lead to a better understanding of how aphid-host interactions evolve and how we can manage the greenbug more effectively with host plant resistance.

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TABLE 1

Specific Virulence Relationships of Greenbug Biotypes and Isolates Collected throughout the United States to Nine Sources of Resistance based on Caged No Choice Tests; Avirulent (-) or Virulent (+) to a Resistance Source

Biotype	Wheat			Sorghum[a/]			Oats	Barley	Rye
(Isolate)	DS 28A	GB2	GB3	Piper	8515	8493	1580	Post	Insave
B[b,c/]	+	-	+	+	+	+	+	-	-
C[c/]	+	-	-	+	-	-	-	-	-
E[c,d/]	+	+	-	+	+	-	-	-	-
F[c,d/]	-	+	+	-	+	-	+	-	+
G[d/]	+	+	+	-	-	-	+	-	-
H[d/]	+	+	-	-	-	-	+	+	-
(AL)[e/]	+	+	+	-	-	+	+	-	-
(ID)[e/]	-	-	-	-	-	-	-	+	-
(KY1)[e/]	+	+	-	-	-	+	+	-	+
(KY2)[e/]	+	+	+	+	+	+	-	-	-
(OK3)[e/]	+	-	+	-	+	+	+	-	-

[a/] Pioneer hybrids 8515 (biotype C resistant SA 7536-1) and 8493 (biotype E resistant PI 264453).

[b/] Webster et al. 1986.

[c/] Kindler and Spomer (1986)

[d/] Puterka et al. (1988)

[e/] Puterka and Peters, unpublished data. Double letter designations are collection sites by state (AL= Alabama, ID=Idaho, KY=Kentucky, OK=Oklahoma).

TABLE 2

Virulence Relationship of Biotypes to Wheat Resistance Genes

Biotype	Resistance Gene[a/]		
	Gb1	Gb2	Gb3
C	+	-	-
E	+	+	-
F	-	+	+

a/ Avirulent (-), Virulent (+)

TABLE 3

Specific Virulence Gene-Resistance Gene Interactions in Wheat

Source	Plant Resistance	Aphid Virulence
Gb1	Recessive	Dominant
Gb2	Dominant	Recessive
Gb3	Dominant	Recessive

PART II

RAPID TECHNIQUE FOR DETERMINING GREENBUG, *SCHIZAPHIS GRAMINUM* (RONDANI) VIRULENCE TO RESISTANCE GENES *GB2* AND *GB3* IN WHEAT

ABSTRACT

A method for quickly determining greenbug virulence to resistance in wheat was developed. 'Largo' resistance in TAM 105 x 'Largo' (5XL), 'Triumph 64' (TR), 'TAM W-107' (107), and 'CI 9058' was evaluated against biotype F so these host relationships could be compared to the other biotypes. Mean damage ratings for 5XL did not differ significantly from the susceptible checks, TR and 107. Therefore, only TR, 107, and 5XL are needed to differentiate the biotypes B, C, E, and F. These cultivars were exposed for 1,2,4, and 6 h feeding exposure times to determine the time required for each biotype to make lesions. Feeding damage appeared as brown lesions on the leaves of susceptible plants. Lesions did not form on cultivars resistant to each biotype. Counts of lesions per leaf were made 24, 48, 72, and 96 h after infestation. All of the lesions that were to appear did so by 72 h after infestation. Some of the susceptible plants developed lesions at the 1- and 2-h feeding exposure. All of the susceptible plants formed lesions at the 4- and 6-h feeding times. Mean lesion numbers of 5.0 and 5.2 for 4 and 6 h, respectively, were not significantly different. Our technique determines greenbug virulence to 5XL and 107 within 3 days after feeding exposure. Details on the

construction of a clip cage, which is an integral part of the methodology, are provided.

INTRODUCTION

Six greenbug, Schizaphis graminum (Rondani), biotypes have been classified in the United States; however, only biotypes B, C, E, and F are currently found in the field. These biotypes have been characterized by the feeding damage they cause to small grain and sorghum cultivars. 'Amigo' is resistant to biotypes B and C and is susceptible to biotypes E (Porter et al. 1982) and F (Kindler and Spomer 1986). 'Dickinson Selection 28-A' and 'CI 9058' are resistant to biotype F and susceptible to biotypes B, C, and E (Kindler and Spomer 1986). 'Largo' is resistant to biotypes C and E (Porter et al. 1982) and is susceptible to B (Webster et al. 1986). The relationship of biotype F to greenbug resistance in 'Largo' has not been established. Screening greenbug resistant and susceptible plants in small pots has been the most common method of determining greenbug biotypes (Puterka et al. 1982, Kindler et al. 1984, Dumas and Mueller 1986). However, about 5 wk are required to make clone colonies from a single aphid sample, infest the plants in the pot, and wait for definite plant responses.

Feeding damage caused by biotype C to the susceptible wheat, 'TAM W-101' has been characterized by brown necrotic lesions (0.5 to 1.0 mm diameter) that appear on leaves 4-6 d after 1 h of feeding. However, lesions did not form on resistant wheat, 'TAM W-101' x 'Amigo' (Al-Mousawi et al.

1983). Using this criterion, greenbug virulence to 'Amigo' (gene Gb2) and 'Largo' (gene Gb3) can be quickly identified by the lesions they make on leaves of wheat plants.

Our objective was to determine if greenbug virulence to 'Amigo' and 'Largo' resistance in wheat can be evaluated by the lesions that result from greenbug feeding. Differences in the time required for greenbug biotypes B, C, E, and F to make lesions was also investigated. Plant resistance evaluations for 'TAM W-105' x 'Largo' (5XL) to biotype F feeding were conducted to see if 5XL could be utilized as a greenbug virulence differential.

MATERIALS AND METHODS

Biotype F Plant Resistance

An individual biotype F greenbug from the colony originally collected by Kindler and Spomer (1986) was cultured on 'Triumph 64' (TR) wheat 2 months prior to this experiment. Resistance of TR, 5XL, and 'TAM W-107' (107) with 'Amigo' resistance was compared to the biotype F resistant wheat, CI 9058, in a randomized complete block design with five replications. Clear plastic containers (16 cm wide x 30 cm long x 8 cm tall) with 8 mm diameter drainage holes placed 6 cm apart and sealed with fine mesh cloth screen lids, served as screening flats. The flats were filled 2 cm deep with sandy loam soil and four rows (13 cm long and 3 cm apart) were marked. One replication consisted of cultivars that were randomly assigned to the

rows and planted at a seeding rate of ten seeds per row. These were held in a growth chamber at 21°C, 13 h photophase. After 7 d the rows were thinned to six plants, the plants were cut to 3 cm in height. The plants were infested with about six greenbugs per plant at the two-leaf stage. All plants in the container were rated on a damage scale of 1 to 6 (1 = 0% damage, 2 = 1-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-99%, 6 = 100% [dead plant]) when all the plants within a particular susceptible cultivar rated a 6 (ca. 8 days). The 1 to 6 damage rating had been used by Porter et al. (1982) to evaluate 'Amigo' and 'Largo' resistance to greenbugs. Plant rating were analyzed by analysis of variance. Mean damage ratings were separated by Duncan's (1955) multiple range test (P = 0.05).

Clip-on Cages

The study of lesion formation on TR, 107, and 5XL caused by each biotype's feeding was facilitated by a clip-on cage. The clip-on cages were constructed from 6 mm diameter, clear plastic drinking straws, hair curl clips (Goody^{R}), and white felt. The clip arms were shortened to 12 mm, and 6 mm of the two center posts of the upper clip arm were bent at a 90° angle so that a 1-cm length of plastic straw could be hot glued to the center posts. One end of the straw piece was positioned flush with the bottom clip arm before gluing to the upper clip arm to form a good seal with an 0.8 cm x 0.8 cm piece of felt that was hot glued to the bottom clip arm. The completed clip cage can

be positioned on the leaf, infested from the top of the clip-on cage, and plugged with a foam cork.

Lesion Study

Experiments were conducted in a growth chamber maintained at 21°C, and with a photoperiod of 13:11 (L:D). All greenbug biotype stock cultures were maintained on TR wheat for ca. 2 months in the growth chamber before experiments began. The damage response of the wheat cultivars to biotypes B, C, E, and F was determined with a 4 (biotype) x 3 (cultivar) x 4 (feeding exposure) factorial analysis that had a split-plot design. TR, 107, and 5XL wheat seedlings were grown in styrofoam cups (8 cm diameter) filled with sandy soil. Six seeds of a cultivar were planted in a cup and thinned to four plants at the 2-leaf stage (ca. 7 d). Each of the plants randomly received a split treatment of one of four biotypes at one feeding exposure time to reduce chamber space requirements. Each seedling was infested with two apterous adults of a biotype in one clip-on cage fastened to the middle of leaf no. 1. Only adults that were actively reproducing were chosen. The cultivar/biotype treatments were assigned feeding exposure times of 1, 2, 4, and 6 h and arranged in a randomized complete block design with six replications. The clip-on cages and greenbugs were removed in the same order they were infested to keep exposure times as correct as possible. Lesions per leaf were counted a 24, 48, 72, and 96 h after

the greenbugs were removed. Only brown necrotic lesions $\geq$ 0.5 mm in diameter were counted.

Data were subjected to analysis of variance and means were compared (P = 0.05) by the least significant difference (LSD) method (SAS Institute 1985). Data from cultivars that were resistant to the biotypes did not form lesions and were not entered into the analyses. The analyses were limited to lesion numbers recorded at 72 h; the minimum time interval for all lesions to appear.

RESULTS AND DISCUSSION

Biotype F Plant Resistance

Damage ratings differed significantly between cultivars ($F = 133.6$; df = 3,12; $P < 0.01$). The damage rating of 2.3 for 'CI 9058' was significantly less than those of the other cultivars, which sustained high ratings of 5.5 to 6.0 (Table 1). Low damage ratings for 'CI 9058' reported by Kindler and Spomer (1986), and by this study confirm that 'CI 9058' has a high level of resistance to biotype F. Damage ratings for the two major greenbug resistance sources showed that TR, 107, and 5XL were equally susceptible to biotype F feeding. This is the first report that 'Largo' resistance in 5XL, effective against biotypes C and E (Porter et al. 1982), is not effective against biotype F. Because 5XL is susceptible to biotype F, only three wheat cultivars are needed to identify greenbug biotypes B, C, E, and F. These are 107 and 5XL, which are

differentially susceptible to the biotypes, and a universally susceptible check, TR.

Clip-on Cages

Clip-on cages were an integral part of the lesion study; they allowed greenbugs to be manipulated on plants quickly. The time the greenbugs used to locate and settle on the leaf was minimized by the small size of the clip-on cage. Lesions were easily located within the confined area of the cage. Eighty clip cages can be made in 4 h and a cage has a service life of 20 to 30 uses. The cages required warm water rinses after several uses to remove honeydew build-up that can trap greenbugs and prevent feeding.

Lesion Study

The susceptible relationship of TR, 107, and 5XL to each biotype is shown in Table 2. Cultivars resistant to specific biotypes did not form brown lesions, therefore, they are not represented in Table 2. However, all of the resistant cultivars exhibited some degree of white specking caused by mechanical damage to the leaf mesophyll cells by the greenbugs stylets (Al-Mousawi et al. 1983). The appearance of white specking was evidence that greenbugs had attempted to feed.

The lesions were characteristic brown necrotic spots surrounded by chlorotic halos 0.5-1.0 mm diameter, as

previously described by Al-Mousawi et al. (1983). Lesions became visible on leaves of some susceptible plants as early as 24 h after infestation. However, the minimum time for all plants to form lesions was 72 h after infestation, and no additional lesions appeared after that time. This minimum time differs from the minimum of 96 h reported by Al-Mousawi et al (1983). Different susceptible cultivars were used, and environmental conditions or experimental procedures may have differed. We report only 72-h observations (Table 2) because this is the shortest time interval for accurate lesion counts. At 1- and 2-h feeding exposures, not all susceptible plants formed lesions. These feeding exposure times were not sufficient for the greenbugs to consistently make lesions. At 1 h, the number of lesions on the cultivars between and within biotypes did not differ significantly. Significantly fewer lesions were made on TR and 107 by biotype F than by the other biotypes at the 2 h, but not at the 4- and 6-h feeding exposures. Biotype B made significantly more lesions on 5XL than on TR at 2 and at 4 h, but not when the feeding time was increased to 6 h. These differences suggest that there are differences in the ability of each biotype to lesion cultivars. However, all plants susceptible to a particular biotype formed lesions in six replications of the 4- and 6-h feeding exposures. Lesion numbers for all the biotypes were high enough at 4 and 6 h so that accurate determinations of virulence could be made. Mean lesion numbers were 5.0 and 5.2 for 4- and 6-

h, respectively, but they did not differ significantly (F = 0.26; df = 1,20; $P > 0.05$). There were few significant gains in lesion numbers for cultivars within biotypes by increasing feeding time from 4 to 6 h. Only TR exposed to feeding of biotype F produced significantly more lesions at 6 than 4 h. Overall, more large lesions (1.0 mm diameter) were made at 6 h, and were more visible, than at 4 h.

In preliminary evaluations of lesion formation on 'CI 9058' we found that this cultivar formed lesions on both resistant and susceptible host plant-biotype relationships. Therefore, 'CI 9058' could not be used in the lesion study because the resistance mechanism differed from those of 5XL and 107.

We have used the lesion method successfully to determine the virulence status of hundreds of greenbug samples. In all cases, the susceptible check, TR, developed lesions at the feeding durations from 6 to 24 h. Apterous greenbug adults that are actively reproducing should be used to insure that feeding times are not interrupted by the molting process of immatures or the tendency for alate forms to move from the plant. Up to 24 greenbug samples can be evaluated on eight seedlings of a single cultivar grown in an 8.0-cm-diameter styrofoam up. Our technique can determine the virulence status of a greenbug within 2 wk after collection compared with about 5 wk (Puterka et al. 1982) for the conventional screening method to determine virulence. Therefore, substantial savings in materials,

space, maintenance, and time could be realized using our technique.

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TABLE 1

Damage Ratings for Wheat Cultivars Infested with Biotype F Greenbugs

Variety	Damage Rating[a b/]
'Triumph 64'	5.7 a
'TAM W-107'	6.0 a
'TAM W-105' x 'Largo'	5.5 a
CI 9058	2.3 b

[a/] Means followed by the same letter are not significantly different ($P > 0.05$; Duncan's [1955] multiple range test). Mean of five replications. Damage rating on 1-6 scale, in which 1 represents no damage and 6 a dead plant.

TABLE 2

Mean Number of Lesions Per Plant and Number of Susceptible Plants with Lesions 72 h after 1, 2, 4, and 6 h Feeding Exposures

Biotype	Cultivar[a]	Lesion No. (No. Plants with Lesions)[b] 1 h	2 h	4 h	6 h
B	TR	0.50(2)a	2.00(6)bc	3.17(6)a	4.33(6)ab
	5XL	0.33(2)a	3.67(6)cd	6.67(6)b	4.67(6)ab
C	TR	0.17(1)a	2.00(5)bc	3.83(6)ab	6.33(6)ab
E	TR	0.17(1)a	3.50(6)cd	6.67(6)b	4.33(6)ab
	107	0.33(2)a	3.00(6)bcd	5.33(6)ab	5.50(6)ab
F	TR	0.00(0)a	0.83(4)ab	3.67(6)ab	6.50(6)b*
	107	0.33(2)a	0.50(3)a	5.67(6)ab	6.00(6)ab
	5XL	0.00(0)a	1.00(4)ab	4.67(6)ab	4.00(6)a
LSD		0.53	1.26	2.89	2.43

a/ TR = 'Triumph 64', 107 = 'TAM W-107', 5XL = 'TAM W-105' x 'Largo'. Cultivars not listed within each biotype are resistant and formed no lesions.

b/ Means within a column followed by different lower case letters are significantly different (P = 0.05; LSD). The mean for 6 h followed by an asterisk(*) significantly different from the mean for 4 h, for a cultivar within a biotype (LSD = 2.66; P = 0.05). Average of six replications.

PART III

INHERITANCE OF GREENBUG, *SCHIZAPHIS GRAMINUM* (RONDANI) VIRULENCE TO GENES *GB2* AND *GB3* IN WHEAT

ABSTRACT

The inheritance of greenbug, Schizaphis graminum (Rondani), virulence to wheat, Triticum aestivum L, was investigated. Clones of greenbug biotypes C, E, nd F were induced into the sexual cycle, reciprocally crossed and inbred. The resulting progeny were cloned via parthenogenetic reproduction, so their virulence to resistance genes Gb2 ('Amigo') and Gb3 ('Largo') could be established using diagnostic feeding lesions. The data for both resistance sources fit a duplicate gene-modifier gene inheritance model where avirulence was dominant and virulence was recessive. Virulence to genes Gb2 and Gb3 was conditioned by duplicate genes and a dominant modifier gene epistatic to one of the duplicate genes. Linkage was definite among the genes conditioning virulence to Gb2 and Gb3 when heterozygous males were used in crosses, due to achiasmic spermatogenesis. When homozygous males were used in reciprocal crosses, 50% recombination occurred. This unique linkage affinity suggests that the multiple genes conditioning virulence to Gb2 and Gb3 reside on the same chromosomes, but are $\geq$ 50 map units apart. Specific aphid-host genetic interactions did not fully conform to gene-for-gene inheritance hypothesis normally associated with host-parasite relationships. Nevertheless, polygenic inheritance

of greenbug virulence in wheat could easily be regarded as a gene-for-gene relationship because the duplicate gene-modifier gene mode of inheritance still influences a single gene product in the aphid.

INTRODUCTION

The greenbug, Schizaphis graminum (Rondani), population is a composite of biotypes. Each biotype is a phenotypic expression of many genotypes, depending on the mode of inheritance (Puterka and Peters 1989). Greenbug biotypes are usually characterized by their ability to differentially damage various sources of greenbug resistance in sorghum, (Sorghum bicolor [L.]), wheat, (Triticum aestivum L.), and other small grains. The plant damage occurs as a phytotoxic response to an unknown substance in the saliva which the greenbug injects while feeding. The response of the plant establishes virulence, the phytotoxic aphid's ability to damage the plant (Puterka and Peters 1988).

Since 1960, biotypes A-C (Wood 1961, Harvey and Hackerott 1969) and E-H (Porter et al. 1982, Kindler and Spomer 1986, Puterka et al. 1988) have been identified. The appearance of new biotypes (Porter et al. 1982, Puterka et al. 1988) and shifts in biotype composition has made the greenbug difficult to manage with host-plant resistance. Inheritance of host-plant relationships in greenbugs is fundamental to our understanding on how greenbugs nullify host plant resistance and maintain their host-plant diversity. With such knowledge, we could predict new

biotype recombinants and develop new resistance deployment strategies for optimum use of resistance sources.

Studies on the inheritance of virulence in aphid species are rare but have suggested that it is a qualitative character conditioned by major genes. Virulence in the rubus aphid, Amphorophora rubi (Kaltenbach), followed a gene-for-gene relationship (Flor 1971) where virulence to two raspberry varieties was conditioned by single independent major genes, one being dominant and the other recessive (Briggs 1965). Muller (1985), through a series of insectary breeding experiments, crossed color biotypes within the pea aphid, Acyrthosiphon pisum (Harris), A. pelargonii (Kaltenbach), and Aphis fabae Scopoli, and found the progeny segregated into Mendelian ratios. Closely related aphid species in the A. fabae group produced hybrids that also segregated by color into Mendelian ratios. Certain colored biotypes had distinct host preferences, but the linkage of color to host plant utilization was never established. Extra-nuclear inheritance has also been proposed for greenbug virulence to sorghum (Eisenbach and Mittler 1987), but was based on very limited data (n = 3-5 progeny).

Genetic studies on aphids have been hampered by the inability to conduct laboratory breeding experiments. Recently, laboratory methods were developed to induce sexually reproducing greenbugs (Puterka and Slosser 1983) and hatch the eggs produced (Puterka and Slosser 1986).

Using diagnostic feeding lesions (Puterka and Peters 1988), the virulence relationship of greenbugs to two important greenbug resistance genes, 'Amigo' (Gb2) and 'Largo' (Gb3) (Tyler et al. 1987), in wheat can be quickly established. Gene Gb2 confers resistance to biotype C, but not to biotypes E and F (Porter et al. 1982, Kindler and Spomer 1986). Gene Gb3 confers resistance to biotypes C and E (Porter et al. 1982), but not to biotype F (Puterka and Peters 1988). These two resistance genes and biotypes C, E, and F provide an excellent differential to study the inheritance of greenbug virulence.

In this experiment, clones of biotypes C, E, and F were induced into the sexual cycle and crossed to study the genetic interaction of virulence between these biotypes and the Gb2 and Gb3 resistance genes in wheat. In addition, a method for breeding greenbug biotypes in the laboratory is presented.

MATERIALS AND METHODS

The parental greenbug colonies were initiated from single parthenogenetic females of biotypes C, E, and F to produce homogeneous clones. The clones of each biotype were maintained parthenogenetically since spring, 1986, on caged pots of greenbug susceptible 'Triumph 64' wheat (Puterka and Peters 1988) in a growth chamber (13h light:11h dark; 25°C:20°C (light:dark).

Oviparous females and males (sexuals) of each clone

were induced in a growth chamber with a reduced photoperiod (11h light:13h dark; 21°C:18°C (light:dark) after Puterka and Slosser (1983). The sexual colonies were also maintained on caged pots of 'Triumph 64'. Two pots of each clone provided a source for the sexuals. When the sexuals began to appear in the colonies, virgin oviparous females (oviparae) were obtained by isolating five sexuparae (parthenogenetic females producing sexuals) per cup cage and allowing them to produce nymphs for 24 h. The cup cages consisted of 227 cm^3 Styrofoam cups with 2 to 3 'Triumph 64' wheat seedlings grown in sand and caged by 3.5 cm diameter by 15 cm tall clear plastic tubes. The newborn nymphs were left in the cup cages to mature. Nymphs developing wing pads (males or alate parthenogenetic females) were removed daily to prevent sib matings. Fourth instar or adult virgin oviparae were identified by their characteristic dark and enlarged hind tibia and removed from the cup cages for matings.

Sexuals of clones C, E and F were inbred and reciprocally crossed in a growth chamber with an 11h light:13h dark; 20°C:18°C (light:dark). The hypothesized inheritance models were tested by crossing sexuals of clones C and E with clone numbers 77 and 81 (recessive F_1 colonies obtained from inbreeding biotype C), and inbreeding sexuals of clones 77 and 81. Virgin females were contained with males in cup cages on 'Triumph 64' seedlings at ratios of 15 ♀♀:4 ♂♂ or 12 ♀♀ :3 ♂♂ ; depending on the availability of

sexuals. Eggs were collected by camel's-hair brush and 50 eggs/replicate were placed in small 3 cm wide by 3 cm long by 2 cm high clear snap-lid boxes. The total numbers of eggs collected per cross varied from n = 500 to 1100 in an attempt to reach a minimum sample size of 30 progeny evaluated per cross. However, this goal was unrealistic for some crosses due to poor survival of the progeny, so a smaller sample size was accepted.

The eggs were held at 0°C (no light) for 6 weeks in an air tight sandwich container at ca. 95% relative humidity that was 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 solution. After the cold treatment, the eggs were transferred to a long-day regimen (15h light:9h dark; 18°C) for incubation (Puterka and Slosser 1986). Egg hatch began within one week after incubation and continued for 4 to 6 days. The eggs were incubated for 2 weeks after egg hatch to insure egg hatch ceased. The resulting S_1 and F_1 progeny (stem mothers) were individually caged on 'Wintermalt' barley, Hordeum vulgare L., seedlings to establish homogeneous colonies (clones).

Virulence relationships of the clones were evaluated on resistance genes Gb2 and Gb3 by using the leaf lesion method (Puterka and Peters 1988). Greenbugs virulent (V) to a resistance gene caused necrotic brown spots with chlorotic halos on leaves, characteristic of a phytotoxic response, 3

days after 12 hours of feeding. Avirulent (A) greenbugs produced no visible evidence of a phytotoxic response although white specks were made by stylet penetration into the leaf. The following biotype-host plant relationships between the parental biotypes and the resistance genes have been established (Porter et al. 1982, Puterka and Peters 1988):

	Resistance Gene	
Biotype	Gb2	Gb3
C	A	A
E	V	A
F	V	V

The resistance sources used in the study were 'TAM 107' (gene Gb2) and 'TAM 105'5*/'Largo' (gene Gb3).

The data was analyzed by a two class (A:V) chi-square analysis ($P = 0.05$). Segregation ratios for one, two, and three gene models were investigated, but only the most probable models are presented. A linkage analysis to test both the model and linkage was made using the data obtained from the C x F reciprocal crosses (chi-square analysis, $P = 0.05$) . Linkage in the C x 81 reciprocal crosses was analyzed by a 2 x 2 contingency table to disregard the inheritance model since there were some deviations from the model. All data was transformed using Yates (1934)

correction for continuity for small sample sizes.

RESULTS AND DISCUSSION

Breeding Greenbug Biotypes

Sexuals of clones C, E, and F inbred and reciprocally crossed readily. All of the resulting progeny that survived were capable of parthenogenetic reproduction. Percent egg hatch between clones C, E, and F was 19.1 $\pm$ 1.16, 22.5 $\pm$ 1.67, and 26.8 $\pm$ 3.32 ($\bar{x}$ $\pm$ SEM), respectively, when inbred. No significant differences in egg hatch between the parental clones was apparent, hence, no lethal gene action that would modify the segregation ratios was suspected. The required handling of the eggs probably contributed the most in reducing egg hatch.

Percent survival of the offspring produced from inbreeding was significantly lower for clone F than for clones C and E (C = 25.65 $\pm$ 6.33, E =18.66 $\pm$ 5.15, F = 8.20 $\pm$ 2.42 [$\bar{x}$ $\pm$ SEM]). 'Wintermalt' may not have been the best host for establishing progeny from the F x F cross. However, preliminary studies found offspring produced from the various matings survived better on 'Wintermalt' barley than on 'Triumph 64'. Offspring mortalities were high because newborn nymphs were prone to injury while being transferred to plants, and many offspring also walked or fell from the plants and were lost. Egg hatch was about the same for crosses between clones, but progeny survival was generally higher than for the inbreds, possibly because of

hybrid vigor.

S_1 and F_1 Segregation Ratios

Inbreeding and crossing sexuals of clones C, E, and F produced S_1 and F_1 segregation ratios that followed either Mendelian or modified Mendelian phenotypic ratios on Gb2 (Table 1) and Gb3 (Table 2) resistance genes. Based on the segregation ratios, avirulence was determined to be dominant and virulence was recessive. Inbreeding or crossing sexuals of clones avirulent to a resistance source produced ratios that best fit a 15:1 (A:V) ratio which was typical of duplicate dominant genes in the heterozygous condition. Crossing sexuals of avirulent clones with virulent clones yielded ratios that fit an 1:1 (A:V) ratio, which suggested single gene inheritance, one parent being heterozygous and the other homozygous. Inbreeding or crossing sexuals of virulent clones produced ratios that fit a 0:1 (A:V) ratio which suggested genetic homogeneity. Most of the S_1 and F_1 segregation ratios followed this inheritance pattern and showed no reciprocal differences on either gene. However, reciprocal differences occurred in the C x E and C x F (female x male) crosses evaluated on gene Gb2 (Table 1). No reciprocal differences were found in the testcross data for gene Gb2 (Table 4) suggesting that some unknown extrinsic factor was affecting progeny recovery. Evidently, biotypes which shared the same virulence relationships also had virulence conditioned by the same genes at the same loci.

Furthermore, the chromosomes of clones C, E, and F had a high degree of homology.

The segregation ratios provided a basis to form a hypothesis on the genes conditioning virulence to the resistance sources. The majority of the data from both resistance sources fit a duplicate gene-modifier gene inheritance model (Table 3). The hypothesized genotypes for virulence in the parental clone to Gb2 and Gb3 were designated by different letters to denote nonallelic sets of genes. It was coincidental that all parental clones virulent to a resistance source had homozygous recessive duplicate genes and a dominant modifier gene. Whereas, all parental clones avirulent to a resistant source had heterozygous duplicate genes and homozygous recessive modifier gene.

In both inheritance models, the first two gene pairs represent duplicate genes while the third gene pair is a modifier gene (Table 3). The duplicate genes are located on independent loci, but are identical in function. The modifier gene is epistatic to one of the duplicate genes when dominant, so only one of the duplicate genes is expressed. Virulence ratios of 15:1, 1:1, or 0:1 (A:V) will result for Gb2 or Gb3 with this model, when crossing A x A, A x V or V x V greenbugs, respectively.

Testing the Hypothesized Model

The inheritance model was tested by using two of the

three recessive (virulent) progeny resulting from inbreeding sexuals of clone C (Table 1). Clone 77 was virulent to Gb2 and avirulent to Gb3 while clone 81 was virulent to both resistance sources. One important feature of these testers was the homozygous recessive modifier gene (Table 3) which contrasts them from the virulent parental clones that had a dominant modifier gene. Crossing a tester virulent to a resistance gene with an avirulent clone should result in a 3:1 ratio. This enabled us to test the hypothesized three gene model and further characterize the modifier gene.

Testcross data for virulence to Gb2 strongly supported the duplicate gene-modifier gene model (Table 4). Crossing sexuals of avirulent clone C with clone 81 resulted in F_1 segregation ratios that fit the expected 3:1 (A:V) ratio. Sexuals of virulent clone E crossed with the virulent testers (clones 77 and 81) produced offspring that segregated to the expected 0:1 (A:V) ratios. Inbreeding sexuals of clone 81 or crossing 77 x 81 also produced the expected 0:1 ratios. No significant reciprocal cross differences in A:V ratios occurred.

One deviation from the model was noted for 77 x C where a 1:1 (A:V) occurred (X^2 = 0.00, df = 1) instead of the expected 3:1 (A:V) ratio (Table 4). This indicated that the modifier gene was in action when predicted not to be. Further characterization of the modifier gene was not possible. Clone 77 had complete male sterility, thus, it could not be reciprocally crossed or inbred. Inbreeding

clone 81 sexuals indicated that the modifier gene was homozygous, but clone 81 was also homozygous for the virulence genes which made the dominance relationship of the modifier gene of no consequence.

Most of the data involving the testers and parental clones supported the duplicate gene-modifier gene model for virulence to Gb3 (Table 5). Support of the model came from crosses 77 x C, 77 x E, 77 x 81, and 81 x 81. The genotype of avirulent clone 77 differed from clones C and E avirulent to Gb3 (Table 3) because the segregation ratios fit a 7:1 (A:V) ratio better than a 15:1 (A:V) ratio (X^2 = 5.43 for 77 x C, X^2 = 2.28 for 77 x E; df = 1) that would result if both duplicate genes were heterozygous.

Some discrepancy from the model and reciprocal differences were noted in testcrosses C or E x 81 (Table 5). When C or E females were crossed with clone 81 males, the offspring segregated 1:1 instead of the expected 3:1 (A:V) ratio suggesting that the modifier gene was in action when hypothesized not to be. Crossing clone 81 females with parental C or E males showed significant reciprocal differences where offspring segregated 1:3 instead of the expected 3:1 (A:V). The reciprocal differences in both C x 81 and E x 81 testcross data for Gb3 were consistent which indicates that there could be a genetic basis for the differences. Most discrepancies from the model could be attributed to the modifier gene acting on the duplicate genes when hypothesized to be inactive. Therefore, it is

possible that the duplicate genes are influenced by more than one modifier gene, some heterozygosity exists at the modifier gene loci, or that there is incomplete penetrance of the modifier gene(s).

Greenbug chromosomes (2n = 8), like all aphids, are holocentric (Mayo and Starks 1971). Meiosis appears to be normal in the aphids (Blackman 1985), but some meiotic disturbance induced by structural, genetic or cytoplasmic incompatibilities could have produced the deviations from expected ratios. More breeding experiments and a detailed cytogenetic analysis of the parents and progeny are needed to reconcile the occasional discrepancies from the model.

Greenbug virulence to wheat is under polygenic influence, where multiple genes in the aphid interact with a single corresponding gene in the host to establish a phytotoxic relationship. Inheritance of virulence to wheat does not fully conform to gene-for-gene inheritance hypothesis normally associated with host-parasite relationships (Flor 1971). However, polygenic inheritance does not necessarily exclude the gene-for-gene hypothesis, particularly when specific parasite and host genes match (Christ et al. 1987). In principle, greenbug virulence could easily be regarded as a gene-for-gene relationship for the duplicate gene-modifier gene mode of inheritance still influences a single gene product in the aphid. The preponderance of data presented by this study and others (Puterka and Peters 1989) does not support earlier

conclusions that biotype inheritance for the greenbug on sorghum was extranuclear (Eisenbach & Mittler 1987). This conclusion was premature; little can be inferred from only 3-5 progeny recovered from the C x E matings. Inheritance of greenbug virulence to wheat is novel compared with the virulence relationships described in aphids (Briggs 1965) and other insects (Gallun 1972) thus far.

Linkage Between Virulence Genes

The linkage analyses using the expected ratios from the model and the observed phenotypic classes showed reciprocal cross differences in the C x F matings (Table 6). Linkage was definite between the genes conditioning virulence to Gb2 and Gb3 when heterozygous clone C males were used in crosses. However, when homozygous clone F males were used in reciprocal crosses, 50% recombination occurred.

Testcross data obtained from crossing sexuals of clone C with homozygous clone 81 already showed reciprocal cross differences and would bias the results of a standard linkage analysis. Therefore, a 2 x 2 contingency table was used to test for independence between the classes, but disregard the model. Linkage was apparent between the segregating classes (AL = 2, Al = 8, aL = 27, al = 1; X^2 = 19.4; df = 1 [see Table 6 for class terminology]) when sexuals of clone 81 males were crossed with clone C females. Crossing clone C females with clone 81 males resulted in independently

segregating classes (AL = 8, Al =5, aL = 16, al = 12 ; X^2 = 0; df = 1). These results supported the reciprocal cross differences in the C x F matings (Table 6).

The reciprocal differences are best explained by cytogenetic studies on aphids where spermatogenesis has been observed to be almost entirely achiasmate with crossovers being rare. However, cytogenetic examinations of aphid oogenesis found meiosis and crossovers occurred normally (Blackman 1985). Therefore, we have concluded that the genes conditioning virulence are located on the same chromosomes because there were distinct linkage relationships when the males were heterozygous. Nonetheless, the virulence genes were $\geq$ 50 map units apart because 50% recombination occurred when the males were homozygous.

Linkage between these virulence genes appears to be an exception in host-parasite relationships and contrasts with the rubus aphid study. Rubus aphid virulence to two resistance sources were conditioned by independent genes (Briggs 1965), although, linkage may have been detected had reciprocal crosses been made. A review of the genetics of plant pathogenicity by Christ et al. (1987) found that linkage between virulence genes in plant pathogenic systems are rare, especially when the resistance genes are independently inherited as is the case in wheat (Tyler et al. 1987).

The occurrence of sexually reproducing greenbugs has

been well documented (Webster and Phillips 1912), but its significance to biotype formation has been overlooked. Our breeding data and linkage analyses illustrates how new biotypes and rapid shifts in biotype composition can arise through genetic recombination during one sexual reproductive cycle. Recessive progeny from inbreeding sexuals of clone C (Table 1) represent new biotypes because they had virulence relationships unlike any of the previously described biotypes when evaluated on nine resistance sources from five crops. Furthermore, the linkage analyses indicates that a much high number of recombinant progeny were produced than the parental type classes (Table 6).

Achiasmate spermatogenesis profoundly influences the linkage relationships of virulence genes within the greenbug population. Greenbug's ability to adapt to new selective pressures and the ever-changing environment may be greatly enhanced by their unusual linkage mechanism. The ramifications of the greenbugs unique linkage mechanism to biotype evolution and to resistance gene deployment needs further investigation. Genetic recombination can account for the biotype-host plant relationships documented in greenbugs thus far. However, the fitness of the new recombinants will inevitably determine their economic importance.

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TABLE 1

Avirulent (A):Virulent (V) F_1 Segregation Ratios for Virulence to the Gb2 Resistance Gene

Cross ♀ x ♂	n	Observed A:V	Expected A:V	X2[a/]
C x C	32	29:3	15:1	0.13
E x E	36	0:36	0:1	0.00
F x F	28	0:28	0:1	0.00
C x E	45	30:15	1:1	4.36*
E x C	42	21:21	1:1	0.00
C x F	66	23:43	1:1	5.46*
F x C	53	29:24	1:1	0.30
E x F	48	0:48	0:1	0.00
F x E	89	0:89	0:1	0.00

[a/] Chi-square values marked with an asterisk (*) are significant at the P = 0.05 level.

TABLE 2

Avirulent (A):Virulent (V) F_1 Segregation Ratios for Virulence to the Gb3 Resistance Gene

Cross ♀ x ♂	n	Observed A:V	Expected A:V	$X^{2\,a/}$
C x C	32	30:2	15:1	0.00
E x E	36	36:0	15:1	1.45
F x F	28	0:28	0:1	0.00
C x E	45	43:2	15:1	0.34
E x C	42	37:5	15:1	1.48
C x F	66	33:33	1:1	0.00
F x C	53	21:32	1:1	1.89
E x F	48	22:26	1:1	0.19
F x E	89	38:51	1:1	1.62

Note: None of the segregation ratios deviated from the tested.

TABLE 3

Genotypes of the Parental Clones and Recessive Testers Obtained from C x C Crosses

Clone[a/b/]	Resistance Gene	
	Gb2	Gb3
C	AaBbrr	L1Mmss
E	aabbRR	L1Mmss
F	aabbRR	l1mmSS
77	aabbrr	L1mmss or l1Mmss
81	aabbrr	l1mmss

[a/] Genes A and B, and genes L and M, are duplicate genes. Genes R and S are modifier genes that are epistatic to one of the duplicate genes when dominant.

b/ Clones 77 and 81 are testers that were recessive progeny which resulted from the C x C cross.

TABLE 4

Avirulent (A):Virulent (V) Segregation Ratios for Offspring from Testcrosses Evaluated on the Gb2 Resistance Gene

Cross ♀ x ♂	n	Observed A:V	Expected A:V	X^{2} [a/]
C x 81	41	28:13	3:1	0.65
81 x C	38	28:10	3:1	0.00
E x 81	43	0:43	0:1	0.00
81 x E	34	0:34	0:1	0.00
77 x C	31	15:16	3:1	10.32*
77 x E	42	0:42	0:1	0.00
77 x 81	24	0:24	0:1	0.00
81 x 81	18	0:18	0:1	0.00

[a/] Chi-square value marked with an asterisk (*) was significant at the P = 0.05 level.

TABLE 5

Avirulent (A):Virulent (V) Segregation Ratios for Offspring from Testcrosses Evaluated on the Gb3 Resistance Gene

Cross ♀ x ♂	n	Observed A:V	Expected A:V	X^{2} [a/]
C x 81	41	17:24	3:1	22.83*
81 x C	38	9:29	3:1	50.66*
E x 81	43	21:22	3:1	14.33*
81 x E	34	8:26	3:1	45.33*
77 x C	31	27:4	7:1	0.05
77 x E	42	37:5	7:1	0.01
77 x 81	24	14:10	3:1	2.72
81 x 81	18	0:18	0:1	0.00

[a/] Chi-square values marked with an asterisk (*) are significantly different at the P = 0.05 level.

TABLE 6

Linkage Between Gb2 and Gb3 Virulence Genes

Cross	Classes[a]				X^2[b]
♀ x ♂	AL	Al	aL	al	(P=0.05)
C x F					
Observed	10.0	13.0	23.0	20.0	
Expected	16.5	16.5	16.5	16.5	
Ratio tested	1 :	1 :	1 :	1	5.45
F x C					
Observed	0.0	29.0	21.0	3.0	
Expected	13.3	13.3	13.3	13.3	
Ratio tested	1 :	1 :	1 :	1	40.81*

a/ Letter a = Gb2 , letter l = Gb3; Uppercase letters indicate virulence, lowercase letters indicate avirulence (AL = parental clone F, al = parental clone C).

b/ Chi-square value marked with an asterisk (*) was significant at the P = 0.05 level.

PART IV

INHERITANCE OF GREENBUG (HOMOPTERA: APHIDIDAE) VIRULENCE TO RESISTANCE IN SORGHUM

ABSTRACT

The inheritance of greenbug, Schizaphis graminum (Rondani), virulence to sorghum (Sorghum bicolor (L.) Moench) and 'Piper' sudangrass (Sorghum sudanense (Piper) Stapf.) was investigated. Biotypes C, E, and F of the greenbug were induced into the sexual phase and inbred, reciprocally crossed, and backcrossed. The progeny were cloned via parthenogenesis and each clone was evaluated on 'Piper' sudangrass, and SA 7536-1 and PI 264453 greenbug resistance in sorghum. Virulence to 'Piper' was dominant and governed by a duplicate dominant gene-modifier gene model. The modifier gene was epistatic to one of the duplicate genes when dominant. Virulence to PI 264453 was also controlled by a duplicate dominant gene-modifier gene, but virulence was recessive. The modifier gene had the same action as in virulence to 'Piper.' In SA 7536-1, virulence was recessive and simply inherited. Analysis of the F_1 phenotypic class frequencies for virulence to the three resistance genes from the reciprocal crosses of parental clones C and F, and C and E, indicated no reciprocal differences that would indicate linkage. The dominance relationships between greenbug virulence and resistance genes in the plant closely follow the gene-for-gene relationships common in parasite-host genetic interactions.

These aphid-host interactions demonstrate that although resistance in sorghum may be simply inherited, greenbug virulence to a resistance source usually requires a more complex interaction between several genes. From our inheritance studies, it is evident that genetic recombination during the sexual cycle can produce the biotypic diversity that commonly occurs in the greenbug species.

INTRODUCTION

The greenbug, Schizaphis graminum (Rondani), is a phytotoxic aphid capable of utilizing over 70 graminaceous hosts (Michels 1986) and is a major pest of sorghum and wheat. The greenbug species is comprised of various races termed "biotypes" that have the ability to differentially damage sources of resistance in barley, oats, rye, sorghum, and wheat. This resistance damaging ability is termed virulence and the damage is related to the phytotoxic saliva the aphid injects while feeding (Puterka and Peters 1989a). The history of greenbug biotypes began in 1960 when the first greenbug resistant wheat, 'DS28A,' was damaged by a race of greenbug called "biotype B" (Wood 1961). Since that time, 7 biotypes have been designated by sequential capital letters as new biotypes were identified. Although greenbug damage to sorghum was reported in Kansas in 1916 (Hays 1922), it was not until 1968 that the greenbug became a serious pest of sorghum. This greenbug population that first attacked sorghum in 1968 was designated biotype C (Harvey and Hackerott 1969) and it was the predominant biotype in the midwestern United States from 1968 to the mid-1980's (Puterka et al. 1982, Kindler et al. 1984). However, biotype E identified in 1980 (Porter et al. 1982) now predominates the biotype complex (Bush et al. 1987, Kerns et al. 1987).

The first aphid to be successfully induced into the sexual phase (Puterka and Slosser 1983), bred (Puterka and Slosser 1986), and to have progeny cloned under laboratory conditions was the greenbug. Overcoming this obstacle has facilitated studies on aphid-host genetic interactions in host plant resistance. Inheritance studies of greenbug virulence to resistance genes Gb2 and Gb3 in wheat have indicated that virulence is governed by polygenic major genes (Puterka and Peters 1989b). The biotypic diversity that resulted effectively demonstrated how shifts in biotype composition could be due to genetic recombination during the sexual phase of this cyclic parthenogenetic aphid (Puterka and Peters 1989a).

Laboratory breeding experiments provide a tool to comprehend and characterize the genetic diversity in the greenbug population so that resistance sources can be chosen more wisely. Although the inheritance of most sources of resistance in sorghum has been determined to be simply inherited (Peterson 1985), the inheritance of greenbug virulence to sorghum is not known. Herein, we report the inheritance of greenbug virulence to resistance sources, 'Piper' sudangrass (Sorghum sudanense (Piper) Stapf.), and SA 7536-1 and PI 264453 in sorghum (Sorghum bicolor (L) Moench) which was determined by various crossing procedures involving greenbug biotypes C, E, and F.

MATERIALS AND METHODS

The parental greenbug biotypes C, E, and F and their progeny were the same clones (Puterka and Peters 1989b) evaluated on genes Gb2 and Gb3 in wheat. Induction of greenbug sexuals, biotype breeding, egg collection and hatch, and cloning of the progeny were done under the procedures reported in that study.

The parental greenbug colonies were initiated in 1986 from single parthenogenetic females of biotypes C, E, and F to produce homogeneous clones. Each biotype population was maintained parthenogenetically on caged pots of greenbug susceptible 'Triumph 64' wheat (Puterka and Peters 1988) in a growth chamber (13:11 L:D, 25°C:20°C).

Oviparous females and males (sexuals) of each clone were induced in a growth chamber with a short-day photoperiod of 11:13 L:D with a temperature of 21°C:18°C (L:D) as outlined by Puterka and Slosser (1983). The sexual colonies were also maintained on caged pots of 'Triumph 64'. Sexuals of clones C, E, and F were isolated so that they could either be inbred, reciprocally crossed, and backcrossed in the short-day growth chamber. Clones C and E were backcrossed with clone numbers 77 and 81 (S_1 colonies obtained from inbreeding parental clone C), and clones 77 and 81 were inbred to serve as a progeny test. The eggs were held at 0°C (no light) for 6 wks in an air tight sandwich container with a 95% relative humidity maintained by a saturated K_2SO_4 salt solution. These conditions were

necessary to break diapause in the eggs (Puterka and Slosser 1986). After the cold treatment, the eggs were transferred to a long-day regimen (15:9 L:D, 18°C) for incubation.

The resulting progeny of the S_1, F_1, S_2, and BC_1 were cloned on 'Wintermalt' barley, Hordeum vulgare L., seedlings to establish homogeneous populations via parthenogenetic reproduction. The parental clones and resulting progeny were evaluated on three greenbug resistance sources, 'Piper' sudangrass, SA 7536-1 in 'Pioneer 8515' and PI 264453 in 'Pioneer 8493' sorghum. The virulence relationships of biotypes C, E, and F to these sources of resistance have been established (Harvey and Hackerott 1969, Wood 1971, Puterka et al. 1988) as follows:

Virulence Relationship of the Parental Biotypes to Resistance Sources in Sorghum Species

Biotype	Resistance Source		
	Piper	SA 7536-1	PI 264453
C	+	-	-
E	+	+	-
F	-	+	-

+ (Virulent), - (Avirulent)

These biotypes and sources of resistance provided the necessary differentials of avirulence (-) and virulence (+) to study the inheritance of virulence. Although none of the biotypes were virulent to PI 264453, this resistance source

was investigated to determine if the greenbug had the genetic capacity to become virulent to it. In addition, biotypes with the same virulence relationships to a resistance source could be compared to determine if the virulence genes among biotypes were allelic.

Virulence of the progeny was determined by using a 6-inch diameter pot that contained 3 plants of each entry planted in a 3 x 3 Latin-square arrangement. The plants were grown in sandy loam soil at a 13:11 L:D photophase with a cycling temperature of 25°C:22°C. Each pot was infested with 150 to 200 aphids from a clone when the plants reached a height of 15 to 25 mm, and then caged to evaluate the clone's virulence status. These heavy infestation levels were required to evaluate the phytotoxic effects of greenbug virulence without the confounding plant effects of antibiosis or antixenosis (aphid nonpreference) to the aphid. All of the plants were evaluated when at least two plants of one cultivar was severely damaged (5-7 days) to establish whether the clone was avirulent or virulent to each resistance source. Plant damage was scored by a 1 to 6 damage scale (1 = no damage to 6 = dead plant). The damage ratings of the parental biotypes provided a basis for determining the score range for virulence and avirulence. The damage ratings of the three plants/cultivar were summed and then converted to a dichotomous avirulent (score total $\leq$10) or virulent (score total >10) relationship. The score was adjusted to avirulent = $\leq$13 and virulent = >13 for

virulence evaluations of S_1 from clone F on PI 264453, based on parental clone F's performance on biotype F resistant PI 264453.

The observed S_1, S_2, F_1, and BC_1 segregation ratios were tested against hypothesized inheritance models with up to five independent loci (three loci regulating virulence and two loci epistatic to one or two virulence loci). The S_1, S_2, F_1, and BC_1 data were analyzed by a 2 class (avirulent:virulent) chi-square analysis ($\underline{P}$ = 0.05). Independence among the segregating classes for virulence to the three resistance genes was tested by 2 x 2 x 2 contingency tables ($\underline{P}$ = 0.05, Df = 1). All data were transformed using Yates (1934) correction for continuity for small sample sizes.

RESULTS AND DISCUSSION

Virulence to 'Piper'

Three distinct S_1 or F_1 segregation ratios of 1:1, 15:1 and 1:0 (avirulent(A):virulent(V)) were produced on 'Piper' demonstrating that virulence was dominant (Table 1). In S_1 progeny of virulent clones C and E, a 1:15 (A:V) ratios were obtained, therefore, when crossing these clones a 1:15 (A:V) in the F_1 was expected and was observed. All S_1 progeny of clone F were avirulent indicating homozygousity of the recessive avirulence genes. Crossing avirulent clone F with virulent clone C or E produced F_1 segregation ratios of 1:1 (A:V). The 1:15 (A:V) ratios are characteristic of

duplicate dominant gene action, but the 1:0 and 1:1 (A:V) ratios suggest single gene action. These ratios are best explained by the duplicate dominant gene-modifier gene model presented by Puterka and Peters (1989b) for greenbug virulence to wheat. Clones C and E were heterozygous for both duplicate virulence genes and homozygous recessive for the modifier gene. Clone F was homozygous recessive for the virulence genes and homozygous dominant for the modifier gene. The modifier gene was epistatic to one of the duplicate genes when dominant, hence, when avirulent clones are crossed with virulent clones one of the duplicate genes is inactivated to produce a single gene ratio of 1:1. The majority of the S_1 and F_1 data fit the duplicate dominant gene-modifier gene model. The only discrepancy from this model was the C♀ x F♂ cross. No reciprocal differences in the crosses were evident.

The progeny tests done by crossing clone 77 and 81 (S_1 colonies obtained from inbreeding parental clone C) with clones C and E and selfing clone 81 completely supported the duplicate gene-modifier gene model (Table 2). The BC_1 and S_2 ratios for 81 indicated that this virulent clone had the same genotype as it's parent, clone C. Clone 77 had complete male sterility, thus preventing inbreeding, but when crossed with clone 81, a 1:15 (A:V) S_2 ratio was obtained indicating that it was also the same genotype as clone 81. Segregation of progeny from the 77♀ x C♂ or E♂ crosses supported the hypothesized genotype of clone 77.

Virulence to SA 7536-1

The S_1 and F_1 ratios for virulence to SA 7536-1 revealed that virulence was recessive and simply inherited (Table 3). The S_1 ratio for avirulent clone C fit a 3:1 (A:V) ratio which suggested that it was heterozygous for the virulence gene and that virulence was recessive. Selfing virulent clones E and F and reciprocally crossing these clones produced S_1 and F_1 ratios of 0:1 (A:V) indicating that they were homozygous recessive for virulence. Therefore, when crossing an avirulent clone with a virulent clone, the expected segregation ratio would be 1:1 (A:V). The F_1 ratios from reciprocally crossing avirulent clone C with virulent clone E fit the expected 1:1 ratio. However, the F_1 ratios from reciprocally crossing avirulent clone C with virulent clone F fit a 1:3 ratio ($C♀ \times F♂$ $X^2 = 0.07$; $F♀ \times C♂$ $X^2 = 0.23$; $\underline{P} = 0.05$) instead of the expected 1:1 ratio. The consistency between the reciprocal ratios in the C x F matings suggest that there is a genetic basis for these results; i.e. clone F may have had different virulence alleles than clone E or a complex dominance hierarchy in virulence alleles. However, more breeding tests are needed to explain this discrepancy.

The progeny tests involving clones C, E, 77 and 81 support the hypothesis of recessive simply-inherited virulence to SA 7536-1 (Table 4). When virulent clone 81 was reciprocally crossed with avirulent clone C, the expected 1:1 (A:V) ratios were obtained. Reciprocally

crossing virulent clone 81 with virulent clone E, virulent clone 77♀ with clone E♂ or clone 81♂, and selfing clone 81 all produced the expected 0:1 (A:V) ratios. The only discrepancy in the progeny tests was the virulent clone 77♀ x E♂ cross which produced a 1:3 (A:V) ratio (X^2 = 0.56) instead of the expected 1:1 (A:V). Since clone 77 could not be inbred or reciprocally crossed with the other clones, we have no reasonable explanation for this large discrepancy from the expected ratio. However, the bulk of the breeding data leaves little doubt that virulence to SA 7536-1 was recessive and simply inherited.

Virulence to PI 264453

The S_1 and F_1 ratios that resulted from selfing and reciprocally crossing the parental clones produced three distinct ratios providing evidence that virulence is governed by a duplicate dominant gene-modifier gene model similar to the model presented for virulence to 'Piper' (Table 5). Selfing or crossing avirulent parental clones C and E produced segregation ratios of 15:1 (A:V) indicating that clone C and E had heterozygous duplicate genes and a recessive modifier gene. Avirulent clone F had a S_1 ratio of 1:0 (A:V), however when clone F was crossed with avirulent clones C or E, an F_1 ratio of 1:1 (A:V) resulted. This suggests that biotype F was homozygous dominant for one of the duplicate genes, homozygous recessive for the other duplicate gene, and had a homozygous dominant modifier gene.

When clone F was selfed, the dominant modifier gene was epistatic to the homozygous recessive locus which only allowed the expression of the homozygous dominant duplicate gene. But, when clone F was crossed with clone C or E, the heterozygous modifier gene became epistatic to the homozygous dominant duplicate gene locus, thus, F_1 ratios of 1:1 (A:V) would result.

The progeny tests did not completely support the duplicate gene-modifier gene model, but did provide evidence that it was the most feasible model for the data presented (Table 6). Support of the model came from the crosses involving avirulent clone 77 x avirulent clone C and E, selfing avirulent clone 81, and the 81♀ x E♂ cross. In these crosses, both clones 77 and 81 were determined to have the same genotypes as clone C (heterozygous duplicate dominant genes with homozygous recessive modifier gene), based on the 15:1 (A:V) ratios obtained in the S_2 of clone 81 and the BC_1 of the different clone crosses. The discrepancies from the model were greatest in the C♀ x 81♂, E♀ x 81♂, and 77♀ x 81♂ crosses (Table 6). No real pattern to the segregation ratios emerged which would aid in explaining these incongruities so more progeny tests are needed. Nevertheless, the breeding data indicate that virulence to PI 264453 is recessive and is under major polygenic control that best fit a duplicate dominant gene-modifier gene model.

Independent Inheritance of Virulence Traits

The F_1 phenotypic class frequencies for virulence to the three resistance genes from reciprocal crosses of parental clones C and F (Table 7), and C and E (Table 8) were used to determine linkage. No reciprocal differences resulted in either of the reciprocal crosses indicating that there was no genetic interaction that could be attributed to linkage between virulence genes. Therefore, we conclude that the genes regulating virulence to the three resistance genes are probably located on different chromosomes, otherwise, linkage would have been noticed, particularly in virulence governed by a single gene as was virulence to SA 7536-1. Strong linkage relationships between the genes influencing virulence to wheat have been established in greenbugs. Virulence genes located on the same chromosome, even at distances of $\geq$ 50 map units, will display linkage because no genetic recombination occurs in aphid spermatogenesis (Puterka and Peters 1989b). Achiasmate spermatogenesis has also been documented in cytogenetic studies of other aphids (Blackman 1985).

Since the inheritance of the sorghum virulence traits are free of reciprocal differences in the biotype intercrosses, the genes conditioning virulence to sorghum are allelic among the biotypes. The greenbug population should have the ability to capitalize on any combination of virulence traits, regardless of the mating direction or biotype mating combination. This would allow the free flow

of sorghum virulence genes in the greenbug population provided that there are no other reproductive isolating mechanisms.

Virulence Gene-Resistance Gene Interactions

With inheritance of resistance for most of the cultivars characterized (Peterson 1985), and through our inheritance studies on greenbug virulence to sorghum species, these specific virulence gene/resistance gene interactions can be summarized:

Virulence Gene/Resistance Gene Interactions in Sorghum Species

Resistance Source	Plant Resistance	Greenbug Virulence
'Piper'	Recessive?	Dominant
'SA 7536-1'	Incompletely Dominant	Recessive
'PI 264453'	Incompletely Dominant	Recessive

Although the inheritance of resistance in 'Piper' has not been characterized, we suspect it is recessive based on what has been previously reported on the greenbug-resistance gene dominance relationships in wheat (Puterka and Peters 1989b). The aphid-host interactions we presented show that although resistance in the plant may be simply inherited, the greenbugs adaptation to a resistance source requires a more complex interaction between several genes. The dominance relationships between greenbug virulence and

resistance genes in the plant closely follow the gene-for-gene relationships common in other parasite-host genetic interactions (Flor 1971).

Cyclic parthenogenesis allows the greenbug to rapidly respond to the gamut of selection pressures. As a result, the genetic structure of the greenbug population can fluctuate from year to year as greenbugs continue to adapt to selection pressures. Many studies indicate that virulence does not always increase the fitness of greenbug biotypes (Puterka and Peters 1989a). Yet, more virulent biotypes will continue to increase in frequency if virulence enhances fitness to a resistance source. Therefore, the identification and development of resistant germplasm that minimizes the impact on greenbug fitness should be pursued.

In 1986, a sorghum seed sales survey in Oklahoma (Kerns et al. 1987) showed that 91% of the sorghum seed sold was greenbug resistant and of this 53% was biotype C resistant and 38% was both biotype C and E resistant. Therefore, as the selection pressure exerted by biotype E resistance increases, our laboratory studies indicate that a shift to a biotype virulent to biotype E resistance will result.

CONCLUSIONS

From our inheritance studies, it is evident that genetic recombination during the sexual cycle can produce the biotypic diversity that commonly occurs in the greenbug species. Through genetic recombination, PI 264453 which was

resistant to all three parental biotypes, could be killed by some of their progeny. Furthermore, some progeny were virulent to all three sorghum entries as well as being virulent to the three major sources of resistance in wheat (Puterka and Peters 1989b). Breeding field biotypes in the laboratory, we have created over 25 new laboratory biotypes that represent various combinations of virulence genes. These laboratory biotypes are invaluable because they can be used to identify new sources of resistance and determine what recombinants are possible.

Laboratory breeding experiments can play a vital role in identifying new sources of greenbug resistance in the laboratory before a biotype shift occurs. Moreover, laboratory breeding experiments provide a tool to characterize the genetic diversity in the greenbug population so that plant resistance sources can be chosen more wisely.

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TABLE 1

Avirulent (A):Virulent(V) S_1 and F_1 Segregation Ratios for Virulence to 'Piper'

Cross ♀ x ♂	n	Observed A:V	Expected A:V	X^{2} [a/]
C x C	31	0:31	1:15	1.63
E x E	33	0:33	1:15	2.02
F x F	27	27:0	1:0	0.00
C x E	40	1:39	1:15	0.43
E x C	37	0:37	1:15	1.41
C x F	65	41:24	1:1	3.94*
F x C	52	27:25	1:1	0.02
E x F	48	32:16	1:1	4.68*
F x E	89	48:41	1:1	0.40

[a/] Chi-square value marked with an asterisk (*) is significant at $P < 0.05$.

TABLE 2

Avirulent (A):virulent (V) S_2 and BC_1 Segregation Ratios from Progeny Tests Evaluated on 'Piper'

Cross ♀ x ♂	n	Observed A:V	Expected A:V	X^2 [a/]
C x 81	37	1:36	1:15	0.30
81 x C	33	2:31	1:15	0.16
E x 81	39	1:38	1:15	0.61
81 x E	31	2:29	1:15	0.85
77 x C	29	1:28	1:15	0.05
77 x E	45	0:45	1:15	2.06
77 x 81	62	1:61	1:15	1.56
81 x 81	31	2:29	1:15	0.11

[a/] None of the segregation ratios deviated significantly from the tested ($P > 0.05$).

TABLE 3

Avirulent (A):Virulent (V) S_1 and F_1 Segregation Ratios for Virulence to Biotype C Resistant SA 7536-1

Cross ♀ x ♂	n	Observed A:V	Expected A:V	X^2 [a/]
C x C	31	24:7	3:1	0.01
E x E	33	0:33	0:1	0.00
F x F	27	0:27	0:1	0.00
C x E	40	21:19	1:1	0.03
E x C	37	13:24	1:1	2.70
C x F	65	15:50	1:1	17.78*
F x C	52	15:37	1:1	8.48*
E x F	48	0:48	0:1	0.00
F x E	89	0:89	0:1	0.00

[a/] Chi-square values marked with an asterisk (*) are significant at $P < 0.05$.

TABLE 4

Avirulent (A):Virulent (V) S_2 and BC_1 Segregation Ratios from Progeny Tests Evaluated on Biotype C Resistant SA 7536-1

Cross ♀ x ♂	n	Observed A:V	Expected A:V	X^2 [a/]
C x 81	37	19:18	1:1	0.00
81 x C	33	19:14	1:1	0.48
E x 81	39	0:39	0:1	0.00
81 x E	31	0:31	0:1	0.00
77 x C	29	5:24	1:1	11.77*
77 x E	45	0:45	0:1	0.00
77 x 81	62	0:62	0:1	0.00
81 x 81	31	0:31	0:1	0.00

[a/] Chi-square value marked with an asterisk (*) is significant at $P < 0.05$.

TABLE 5

Avirulent (A):Virulent (V) S_1 and F_1 Segregation Ratios for Virulence to Biotype C and E Resistant PI 264453

Cross ♀ x ♂	n	Observed A:V	Expected A:V	$X^{2a/}$
C x C	31	31:0	45:1	1.10
E x E	33	32:1	15:1	0.16
F x F	27	27:0	1:0	0.00
C x E	40	40:0	15:1	1.71
E x C	37	35:2	15:1	0.02
C x F	65	36:29	1:1	0.55
F x C	52	23:29	1:1	0.48
E x F	48	25:23	1:1	0.02
F x E	89	42:47	1:1	0.18

[a]/None of the segregation ratios deviated significantly from the tested ($P > 0.05$).

TABLE 6

Avirulent (A):Virulent (V) S_2 and BC_1 Segregation Ratios from Progeny Tests Evaluated on Biotype C and E Resistant PI 264453

Cross ♀ x ♂	n	Observed A:V	Expected A:V	X^2 [a/]
C x 81	37	18:19	15:1	121.66*
81 x C	33	26:7	15:1	9.83*
E x 81	39	27:12	15:1	36.76*
81 x E	31	27:4	15:1	1.43
77 x C	29	26:3	15:1	0.29
77 x E	45	44:1	15:1	0.64
77 x 81	62	47:15	15:1	30.71*
81 x 81	31	30:1	15:1	0.09

[a/] Chi-square values marked with an asterisk (*) are significant at $P < 0.05$.

TABLE 7

Contingency Table Test for Independent Inheritance of Virulence to 'Piper', SA 7536-1, and PI 264453 in the F_1 Progeny of Biotype C and F Reciprocal Crosses

Cross		Resistance Source: Virulence Relationship[a/]				
		PI 264453				
$C_{♀} \times F_{♂}$		A		V		
		SA 7536-1				
		A	V	A	V	
'Piper'	A	0	26	6	9	
	V	1	9	8	6	$X^2 = 0.28$
		PI 264453				
$F_{♀} \times C_{♂}$		A		V		
		SA 7536-1				
		A	V	A	V	
'Piper'	A	0	10	12	5	
	V	0	13	3	9	$X^2 = 0.01$

[a/] A = avirulent, V = virulent. None of the segregation ratios deviated from the tested ($P > 0.05$, Df = 1).

TABLE 8

Contingency Table Test for Independent Inheritance of Virulence to 'Piper', SA 7536-1, and PI 264453 of the F_1 Progeny of biotype C and E Reciprocal Crosses

Cross		Resistance Source: Virulence Relationship[a/]				
		PI 264453				
		A		V		
		SA 7536-1				
		A	V	A	V	
♀C x ♂E						
'Piper'	A	0	1	0	0	
	V	20	19	0	0	$X^2 = 0.25$
♀E x ♂C						
		PI 264453				
		A		V		
		SA 7536-1				
		A	V	A	V	
'Piper'	A	0	0	0	0	
	V	13	23	0	1	$X^2 = 0.12$

a/ A = avirulent, V = virulent. None of the segregation ratios deviated from the tested ($P > 0.05$, Df = 1).

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