VARIABILITY IN GREENBUG BIOTYPES

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER I

GREENBUG AND HOST PLANT RESISTANCE

The greenbug, Schizaphis graminum (Rondani) (Hom.: Aphididae) is a cosmopolitan pest of many graminaceous crops. The Commonwealth Institute of Entomology (1963) has recorded its occurrence in about 42 countries. It has also been recorded in four additional countries, including Sweden (Ossiannilsson, 1948), Bulgaria (Kontev, 1976), Botswana (Flattery, 1982), and Mongolia (Fedosimov and Tsedev, 1970) (Fig. 1). The greenbug has been a major pest of small grains and/or sorghum, Sorghum bicolor L. Moench, in the USA (Starks and Burton, 1977), Canada (Twinn, 1932), Uruguay (Silveira and Conde, 1945), Argentina (Griot, 1944), Hungary, Italy (Wadley, 1931), Bulgaria (Kontev, 1976), Yugoslavia (Mitic-Muzina and Srdic, 1977), Romania (Barbulescu, 1976), Egypt (Ali and Rizk, 1979), Sudan (Muddathir, 1976), Kenya (Walker, 1954), South Africa (Brown, 1971), Botswana (Flattery, 1982), Pakistan (Hamid, 1983), India (Chaudhary et al., 1969), and in the USSR (Kushnerik, 1981). Besides small grains and sorghum, it is a damaging pest of Kentucky bluegrass, Poa pratensis L., in the USA (Potter, 1982) and of rice, Oryza sativa L., in the USSR (Myrzin and Shilovskii, 1983). However, in Australia it is a major pest of Pangola grass, Digitaria decumbens, (Franzmann, 1973), and in Sweden of meadow grasses, Phleum pratense L. (Ossiannilsson, 1948).

Detailed accounts on the biology of the greenbug in North America include those of Webster and Phillips (1912) and Wadley (1931). Walker et al. (1972) have compiled a bibliography on the greenbug. A generalized life-cycle of the greenbug and production of different morphs during the course of a year was compiled from the literature and is illustrated in Fig. 2. The life-cycle varies from one geographic zone to another, and will be discussed in detail in later sections. About 96 graminaceous plants are attacked by this aphid (Pettersson, 1971). Damage is caused by (i) extraction of plant sap, (ii) injection of toxic secretions while feeding, and (iii) transmission of viral diseases such as barley yellow dwarf (Plumb, 1983), maize dwarf mosaic (Nault et al., 1971), sugarcane mosaic (Komblas and Long, 1972), and abaca mosaic (Gavarra and Eloja, 1969). Of these diseases, barley yellow dwarf is of worldwide importance (Plumb, 1983).

In the USA, the most serious damage caused by the greenbug occurs in the Southwestern, Central, Northwestern, and Southeastern states (Starks and Burton, 1977). The first infestation of this pest in the USA was reported in Virginia in 1882. Since then at least 19 outbreaks have occurred. A serious one hit Texas and Oklahoma in 1942. More than 61 million bushels (1.66 million metric tons) of grains valued at $38 million were lost. Other serious outbreaks in Oklahoma occurred during 1901, 1903, 1906, 1907, 1916, 1922, 1933, 1934, 1939, 1949-51, 1961, 1968 and 1976 (Rogers et al., 1972; Starks and Burton, 1977). In 1976, damage and control costs on wheat, Triticum aestivum L., in Oklahoma alone exceeded $80 million (Starks and Burton, 1977).

The use of greenbug-resistant varieties is an effective control measure. Since the recognition of the greenbug as a damaging pest,

resistant varieties of both small grains and sorghum have been released. However, for reasons that are not understood, biotypes that overcome varietal resistance have developed and hindered the development of new resistant varieties (Porter et al., 1982; Starks et al., 1983). Biotype A of the greenbug predominated in the Great Plains of the USA until the early 1960's. Biotype B appeared in 1958 (Wood, 1961) and became dominant to biotype A by 1965. Biotypes A and B can be separated by the reaction of 'DS 28A' wheat, a hexaploid selection from the durum (Triticum durum Desf.) cultivar 'Dickinson No. 485' (CI 3707). DS 28A is resistant to biotype A but susceptible to biotype B. Another wheat, CI 9058, also reacts similarly (Curtis et al., 1960; Porter et al., 1982).

Biotype C was detected in 1968 in the USA, and has caused extensive damage to grain sorghum (Harvey and Hackerott, 1969). Later it became the predominant biotype in the Great Plains. Biotypes B and C can be separated by their reaction to 'Piper' sudangrass, Sorghum sudanense (Piper) Stapf, in the seedling stage. Piper is highly resistant to biotype B but susceptible to biotype C (Harvey and Hackerott, 1969).

Biotype D, having the same host plant reaction as biotype C, was first reported in the higher insecticide usage locations in the Edmonson, Texas, area in 1974 by Teetes et al. (1975). This biotype had a high level of resistance to organophosphate insecticides (Peters et al., 1975; Chang et al., 1980).

Following the development of 'Gaucho' (an 8X triticale, X Triticosecale Wittmack, involving 'Insave' rye, Secale cereale L.) (Wood et al., 1974), the wheat germplasm line 'Amigo' (CI 17609), which has a single dominant gene from Insave rye for resistance to biotypes A, B,

and C, was released (Sebesta and Wood, 1978). A new biotype, designated as E, appeared in Bushland, Texas, in 1980. An Insave rye selection, 'Will' barley, Hordeum vulgare L., and biotype C-resistant oats, Avena sativa L., (CI 1579, CI 1580, CI 13223, PI 251580, PI 251896, PI 251898, PI 258612, PI 258637 and PI 258644) were resistant to biotype E. Sorghum lines possessing biotype C resistance from PI 38108 tunis grass, Sorghum virgatum (Hack) Stapf, Amigo and Gaucho, also biotype C-resistant entries, were susceptible to this new biotype. The wheat cultivars 'Tam W-101' and 'OK 695157' were susceptible to both biotypes (Porter et al., 1982; Puterka et al., 1982). Contrary to Porter et al. (1982), who reported Will barley as resistant to biotype E, Will and Post barley were seriously damaged in some early Stillwater tests with biotype E greenbugs. Moreover, biotype E has been reported to be a more efficient vector of some isolates of maize dwarf mosaic virus than biotype C (Berger et al., 1983).

Puterka et al. (1982) made greenbug collections from wheat fields in 23 counties of the Texas Rolling Plains in 1981 to determine the prevalence and distribution of biotype E. Biotype E greenbugs were found in 17 counties. Fourteen counties contained both C and E biotypes in the same fields. Biotype C remained the predominant biotype, accounting for 75% of the greenbugs collected. Moffatt and Worrall (1983) followed up Puterka et al. (1982) to monitor any subsequent shifts in the biotype C to E ratio, and found a highly significant increase of biotype E in the Texas Rolling Plains: from 25% in 1981 to 48% in 1982. In a 1980 Texas-Oklahoma survey, all greenbugs found in the panhandles of these states were of biotype E. The percentage of biotype C in the field increased eastwardly with 100% biotype C in

central and southcentral Oklahoma. Biotype E is the predominant biotype in Nebraska and Kansas (Kindler et al., 1984).

Reasons for the development of greenbug biotypes are not yet fully understood. It is usually assumed that if the mechanism of resistance in a plant is tolerance, biotypes should not appear. On the other hand, if the mechanism of resistance is either antixenosis (nonpreference) (Kogan and Ortman, 1978) or antibiosis, then the probability of appearance of biotypes becomes higher (Gould, 1983). The three mechanisms of resistance interact and complement each other; thus all cases of resistance cannot be assigned to just one of these categories. Their interaction in Energy Circuit Language (Odum, 1983) is shown in Fig. 3.

According to Dobzhansky (1939) (see Smith, 1941) populations of insects are a store of concealed variability. This store contains some variants which are not useful under any set of conditions, other variants which might be useful under a set of circumstances which may never be realized in nature, and still other variants which are neutral or harmful at the time when they are produced but which will prove useful later on. Similarly, Smith (1941) stated that a shift in the characteristics of the population in the direction of greater fitness must occur if variants are present which are superior to the general population in their ability to persist and to reproduce in the presence of the changed conditions.

The development of greenbug biotypes seems to be due to the presence of high numbers of these genetic variants. In fact the insect-host plant and environmental interactions alter certain gene expressions for the better survival of the species (Blackman, 1979).

The rapid development and changes occurring in the relative abundance of different greenbug biotypes warrant studies to determine the amount and nature of variability present in this species. This was the prime objective of the present research. Efforts were also made to improve the efficiency and reliability of the existing techniques for testing the resistance components used in the detailed evaluation of germplasm. Specifically the objectives were:

1. To determine suitable statistical designs for antixenosis and antibiosis tests.
2. To develop a host plant resistance index for explaining the overall resistance level in a plant.
3. To determine the biological variation present within the greenbug cultures of biotypes B, C and E in the greenhouse.
4. To determine variation in clones of biotype E.
5. To determine the morphological variation within biotypes B, C and E, and to develop a discriminant function for their correct identification.
6. To determine the nature of resistance in a recently identified biotype C and E resistant source, Largo wheat, Triticum tauschii (Coss.) Schmal, to biotype B.

Figure 1. World distribution of the greenbug (modified from the map published by the Commonwealth Institute of Entomology, 1963).

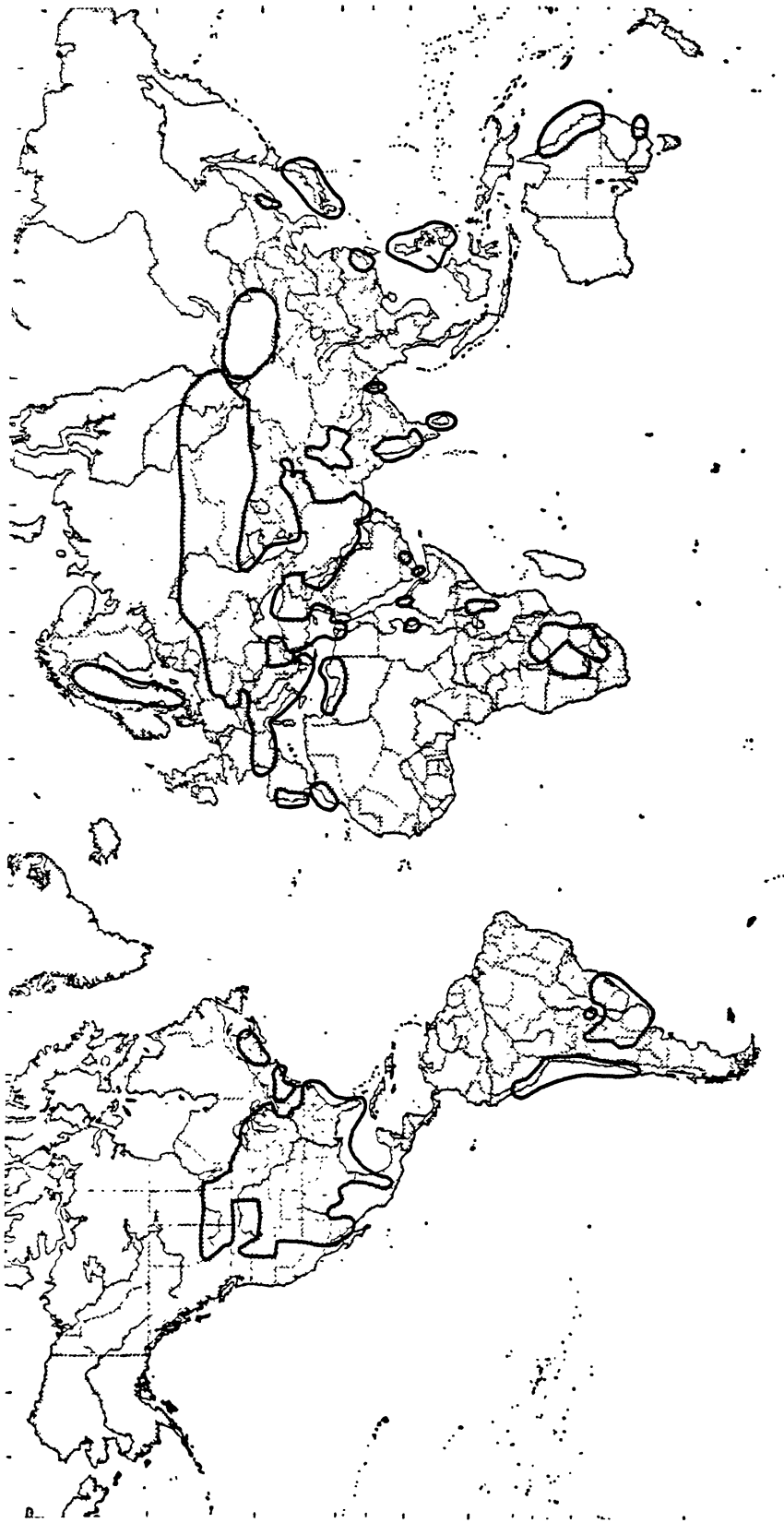
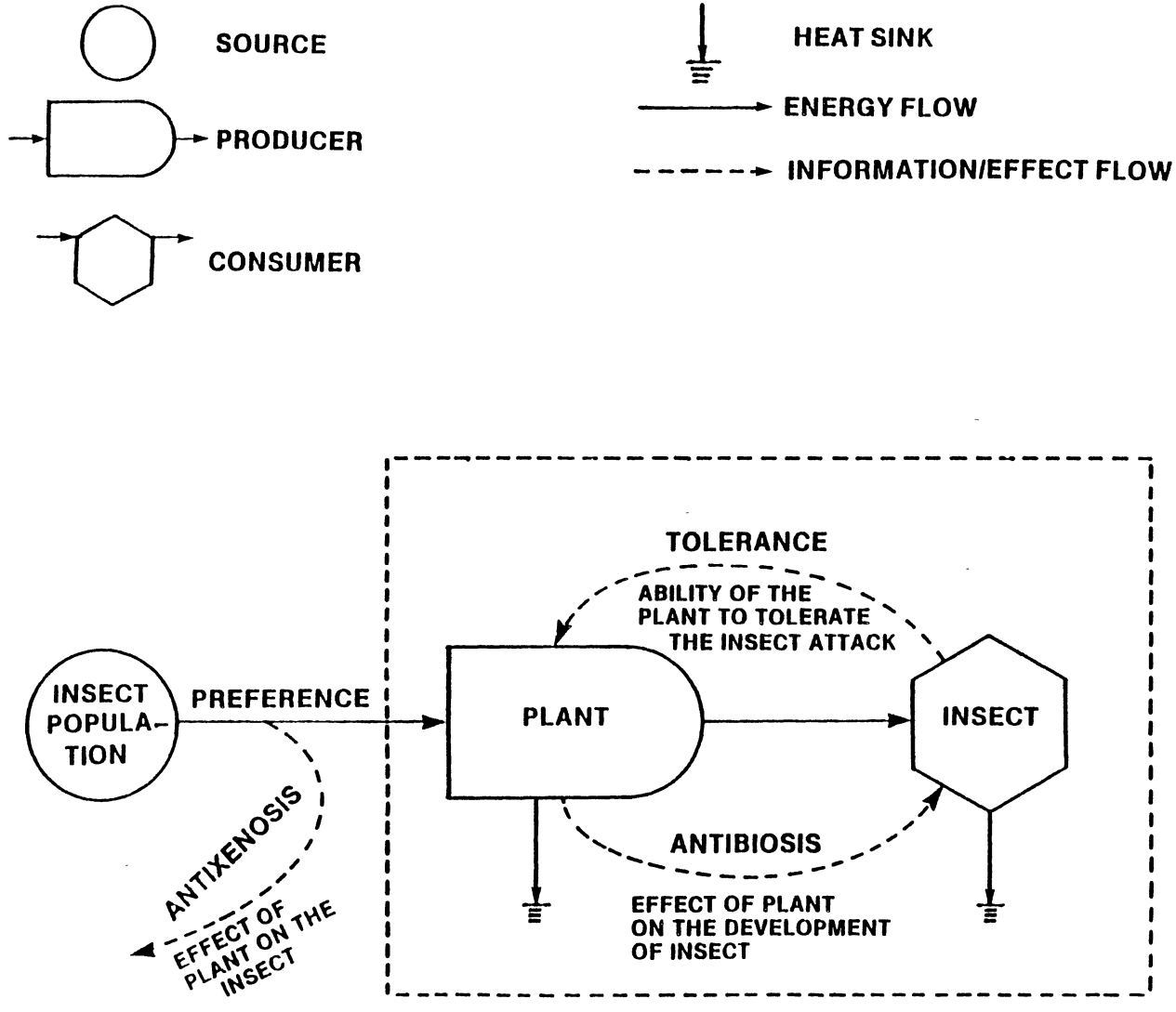


Figure 2. A generalized life cycle of the greenbug (plant and greenbug illustrations from Webster and Phillips, 1912).

SEXUAL CYCLE
ASEXUAL CYCLE
ASEXUAL CYCLE
VIVIPAROUS FEMALE
3RD INSTAR NYMPH
2ND INSTAR NYMPH
1ST INSTAR NYMPH
MALE
VIVIPAROUS FEMALE
MATING
FERTILIZED EGGS
UNFERTILIZED EGGS ?
EGGS
SEXUAL CYCLE
OVIPAROUS FEMALE
MALE
MATING
?
?

Figure 3. Components of resistance in energy circuit language.


SOURCE
PRODUCER
CONSUMER
HEAT SINK
ENERGY FLOW
INFORMATION/EFFECT FLOW
TOLERANCE
ABILITY OF THE PLANT TO TOLERATE THE INSECT ATTACK
INSECT POPULA-TION
PREFERENCE
PLANT
INSECT
ANTIXENOSIS
EFFECT OF PLANT ON THE INSECT
ANTIBIOSIS
EFFECT OF PLANT ON THE DEVELOPMENT OF INSECT

CHAPTER II

ASSESSMENT OF EXPERIMENTAL DESIGNS FOR TESTING ANTIXENOSIS

Introduction

Many workers including Dahms et al. (1955), Painter and Peters (1956), Weibel et al. (1972), Starks et al. (1972), Schuster and Starks (1973), Johnson et al. (1976), Starks and Merkle (1977), Webster and Starks (1984), and Webster and Inayatullah (1984) have worked on the isolation of antibiosis, antixenosis, and tolerance components of resistance to different greenbug biotypes. Wood et al. (1974) reported that in Gaucho triticale, antixenosis is the least important of the three components of greenbug resistance, but logically the degree of antixenosis of a host determines the initial infestation level on that host. Even a small degree of antixenosis can be of significant importance in the long run. Apablaza and Robinson (1967), Dixon (1971), Brown (1972), Schweissing and Wilde (1979a), Starks et al. (1973), Leather and Dixon (1982), Campbell et al. (1982), Kieckhefer and Stoner (1978), Kieckhefer (1983, 1984), Wright et al. (1984), and Lowe (1984) have studied host preference of the greenbug and other aphid species. A standard free-choice preference test has been adopted by many researchers. In this test, one plant of each entry to be tested is planted in a circular pattern near the edge of a pot and wingless

greenbugs are released in the center. The variability in this type of test is quite high (Webster and Inayatullah, 1984).

Batschelet (1981) reported that many insects show a bimodal or quadrimodal pattern of orientation with respect to the azimuth of the sun. Thus, measuring host preference by sowing plants in a circular pattern and releasing the insects in the center may not be a very precise test. Moreover, high variability in preference within a variety due to location in the circle makes the experimental error large, which in turn increases the chance of accepting the null hypothesis of no difference in preference among the test hosts. It also increases the standard error of difference between the means of two hosts, which may lead to nonsignificant differences between them. If the differences are significant, this high variability may also make the data difficult to interpret because of overlapping in multiple comparisons.

The objectives of the present research were to determine whether directional responses occur in the greenbug and to develop a better design with a smaller experimental error and coefficient of variation, while at the same time making the experiment easier to conduct. With these objectives in mind, the standard nonpreference test with apterous biotype E greenbugs using a Randomized Complete Block Design (RCBD), a Completely Randomized Design (CRD), and a CRD with Central Composite Arrangement (CRD-CCA) were evaluated.

Materials and Methods

Directional Response

The test was conducted in aluminum pans (20 x 20 x 4.5 cm). A 15-cm diam circle was drawn on the soil in the center of each pan, and

16 equidistant points were marked on the circumference of the circle. Two seeds of the test host were sown at each point and thinned to one plant after germination. Thus there were 16 plants of the same test host in a pan. The pans were kept on greenhouse benches. The temperature ranged from 22° to 36°C. When the plants were about 6-8 cm high, 160 greenbugs were released in the afternoon on the soil in the center of the pan to have a level of 10 greenbugs/plant. Each pan was covered with a 47- x 33- x 20-cm plastic cage with a muslin cloth top and having 10 side vents of 8 cm diam each.

Three separate tests with one pan per test were conducted in the greenhouse using the greenbug susceptible host Wintermalt and the resistant host Post barley. The same experiment was also conducted in a growth chamber programmed at 25°C during the day and 20°C at night, and at a 16:8 hr day:night regime. To eliminate possible effects of light and air flow in the greenhouse and in the chamber, a similar set of experiments was conducted in a chamber at 22°C but without light or air flow.

The plants were cut at the base, and the number of aphids on each plant was recorded 48 hr after release of the aphids in this as well as in the following experiments.

Standard Antixenosis Test--RCB Design

Four barley entries, PI 429365, PI 420491, CI 15811, and Wintermalt, having various levels of antixenosis to biotype E (Webster and Starks, 1984) were planted at random in a circular pattern about 1.5 cm from the edge of a 15-cm diam pot. There was one plant of each entry in each pot. When the plants were 6-8 cm tall, 40 greenbugs were

released on the soil in the center of the pot to have a level of 10 greenbugs per test plant. The plants and aphids were then covered with 12-cm diam x 30-cm high plastic cages with a cloth-covered top and two side vents (7.8 cm diam). The aphids were allowed 48 hr to select the plant of their choice, at which time the numbers on each plant were recorded. There were 10 replications (pots) in the test, and it was conducted in a greenhouse. Analysis of variance was performed on the data.

CRD and CRD-CCA

Individual tests for the CRD and the CRD-CCA were conducted using metal flats (51 x 35 x 9 cm) in a greenhouse. The four barley entries mentioned previously were randomized and sown in the flats. Randomization plans for each test are given in Fig. 4. For the CRD there were 24 experimental plants, six of each entry, with a plant-to-plant distance of 5 cm. On the borders, adjacent to each plant, there was a nonexperimental plant selected at random from one of the four barley entries. Thus there were a total of 48 plants in a flat, 24 experimental and 24 nonexperimental. The greenbugs were released on the soil in the center of each set of four plants. There were 35 release sites in the flat, and 14 greenbugs/site (about 10 greenbugs/plant in the flat) were released (Fig. 4A).

For the CRD-CCA, each of the four barley test entries were sown in the diamond-shaped patterns as shown in Fig. 4B. The plant-to-plant distance was 5 cm. There were 24 experimental (six of each entry) plants surrounded by 27 nonexperimental plants. There were 37 sites for

releasing greenbugs, and 14 greenbugs/site were released, also resulting in an approximate level of 10 greenbugs/plant in the flat.

Results

Directional Response

Tests conducted in a greenhouse using Wintermalt and Post barley revealed that the distribution of greenbugs among the plants of the same host variety was not uniform. On individual replications of Wintermalt, the number of greenbugs settled on individual plants ranged from 0 to 27; the range was 0 to 31 on Post. Chi-square tests performed separately on each replication of each host as well as on the means of the three replications for each host indicated that the distribution of greenbugs among plants of the same host variety was not random (Table I). Significantly ($P < 0.005$) more greenbugs settled on plants sown on NW-SW quadrants (Fig. 5). The distribution of greenbugs among the same host variety was also not random when the experiment was conducted in a growth chamber with light and air flow (Table I). In these tests, however, no trend of orientation in a particular direction could be discerned (Fig. 5).

Tests conducted in a growth chamber in the absence of light and air flow, and with Wintermalt barley as a host, indicated that the distribution of greenbugs settled/plant ranged from 1 to 11 and was uniform when each replication was examined separately (Fig. 5). The chi-square test performed on each replication separately, i.e., three chi-square tests, as well as on means of the three replications, rejected the hypothesis of nonuniform distribution of greenbugs (Table I). When a similar test was conducted in the dark with Post, the

chi-square test performed on two out of the three replications showed that the distribution of greenbugs among individual plants was not uniform. However, when the chi-square test was performed on the means of all three replications, the distribution of greenbugs on all the plants was found to be uniform (Fig. 5) ($P > 0.05$) (Table I).

RCB, CRD and, CRD-CCA Designs

A comparison of the experimental error, coefficient of variation (CV), and variance of treatment means using the conventional RCBD, the CRD, and the CRD-CCA is given in Table II. For the RCBD with 10 replications the experimental error was 14.87 and the CV was 42.49. When the same entries were tested using the CRD and the CRD-CCA with six replications for each entry, the experimental error and the CV were reduced by approximately one half (Table II). By using the CRD-CCA, the experimental error was a little less than the CRD (5.61 vs 7.92), but the CV was a little higher (28.00 vs 25.76) because of the lower overall entry mean (Table II).

Among the four test entries, PI 429365 was found to be the least preferred by the greenbug and was significantly different ($P < 0.05$) from the others in CRD and CRD-CCA designs (Table III). PI 420491 was the most preferred entry and was not significantly different from Wintermalt in the CRD test. However, it was significantly different from Wintermalt in the CRD-CCA and RCBD tests. CI 15811 was intermediate in preference. In the RCBD there were more overlappings in the multiple comparisons, making the data difficult to interpret (Table III).

Discussion

Tests conducted for determining directional response in the greenhouse (north-south) indicated that the greenbugs were distributed in a nonrandom pattern among plants of the same host variety. In these tests, a large number of greenbugs settled on the plants on the west side of the pans toward the direction of the afternoon sun. In the growth chamber, the distribution of greenbugs among plants of the same host was also not random; however, no particular directional trend in orientation could be observed. Tests conducted in the dark without air flow resulted in a uniform distribution of the greenbugs, which confirmed that the direction of the sun, and possibly air flow too, are responsible for the nonrandom distribution of greenbugs among plants of the same host variety in the greenhouse. Variation in light and air flow may also be responsible factors for nonrandom distribution of the greenbugs in growth chamber experiments.

Hisada (1972) studied the orientation of dragonflies of the genus Sympetrum with respect to the azimuth of the sun and reported that most of the dragonflies chose a direction of approximately 90° either to the right or left of sun rays, giving a bimodal distribution. Similarly, flies of several genera (Sarcophaga, Musca, Calliphora, Lucilla, Tubifera) are usually oriented in the NS or the EW axes of the magnetic field (magnetotaxis) when resting on a horizontal plane, leading to a quadrimodal distribution (Batschelet, 1981). Studies of alate greenbug orientation with respect to the azimuth have not been reported but would be interesting.

Antixenosis or host preference tests with alate greenbugs and other alate grain aphids have been conducted by Schweissing and Wilde

(1979a), Wright et al. (1984), and Brown (1972). In an antixenosis study, Schweissing and Wilde (1979a) found significant differences in the number of alates established on resistant and susceptible sorghum. They also used apterous greenbugs in the test. Their results with the apterous greenbugs closely followed the results of the alate forms even though the differences between hosts in the tests with the apterous forms were not significant. It would seem logical to use alate forms in preference studies since most often hosts are selected by the alates in the field. However, trials conducted with alates in the greenhouse may be adversely affected by extraneous factors.

It is suggested that if host plants are to be tested in a circular pattern by releasing the insects in the center, the experiment should be conducted in the dark. However, it is recognized that visual stimuli are important in many cases of host selection. In these situations the test insects could be released at night to minimize the directional response toward the sun. For this type of test the number of replications should be increased and the entries for different replications should be randomized in as many different ways as possible. Only the randomization and placement of pots in the greenhouse can minimize the directional response. Blocking measures only the variation in the number of insects from one pot to another and cannot minimize the directional response.

Another possibility is to use completely randomized designs in flats. In the present tests the experimental error and the CV were reduced using a CRD or a CRD-CCA with six replications as compared with tests using a RCBD with 10 replications. This reduction in experimental error and CV seems to be due to the following reasons: (i) all plants in

the CRD or CRD-CCA test were grown in the same soil and were covered with one cage, so there was no question of pot-to-pot variation, (ii) there was more than one plant of each entry in the flat, and the plants were influenced by each other simultaneously; thus if there was any effect of one entry on the other for selection by the greenbug, the effects were nullified, and (iii) since the greenbugs were released at many locations in the flat, they had a choice to move from one area of the flat to another while coming in contact with many plants of different entries and were, therefore, free to select any of the plants they encountered.

In the CRD-CCA all the test entries were present in a group and the greenbugs had an equal choice to select any of the four entries simultaneously. At the same time, each entry was influenced by all others. Such a design may become impossible to conduct if the number of entries exceeds more than five or six. By imposing the central composite arrangement on the CRD to have a CRD-CCA, there was not much reduction in the CV, so the CRD is a better design than the others.

TABLE I

NUMBER OF GREENBUGS ON WINTERMALT AND POST BARLEY SEEDLINGS SOWN IN A CIRCULAR PATTERN IN PANS, AND TESTED IN A GREENHOUSE AND GROWTH CHAMBER WITH AND WITHOUT LIGHT AND AIR FLOW

Host and location of the test	No. of greenbugs settled/plant		Chi-square value
	Range	Mean ± s.d.	
Greenhouse:			
Post	0-31	6.77 ± 6.13	83.25**
Wintermalt	0-27	9.18 ± 5.03	41.26**
Growth chamber with light and air flow:			
Post	0-19	4.08 ± 3.04	33.96**
Wintermalt	0-24	6.42 ± 5.72	76.34**
Growth chamber without light and air flow:			
Post	0-12	3.83 ± 1.41	7.77
Wintermalt	1-11	5.06 ± 1.83	9.91

**Number of greenbugs from plant to plant are significantly different at $P < 0.005$.

TABLE II

COMPARISON OF OVERALL ENTRY MEAN, EXPERIMENTAL ERROR, COEFFICIENT OF VARIATION (CV), AND VARIANCE OF THE TREATMENT MEAN FROM THREE ANTIXENOSIS TESTS CONDUCTED IN A GREENHOUSE WITH FOUR BARLEY ENTRIES

Design of test	Overall entry mean	Experimental error	CV(%)	Variance of the treatment mean
RCBD	9.07	14.87	42.49	1.48
CRD	10.92	7.92	25.76	1.32
CRD-CCA	8.46	5.61	28.00	0.93

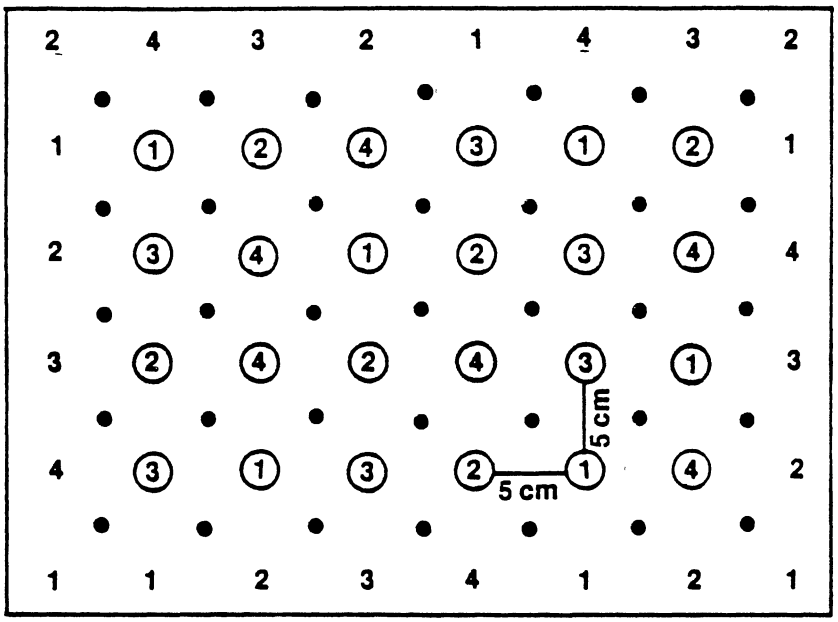
TABLE III

NUMBER OF GREENBUGS ON FOUR BARLEY ENTRIES TESTED IN THREE DIFFERENT EXPERIMENTAL DESIGNS IN A GREENHOUSE

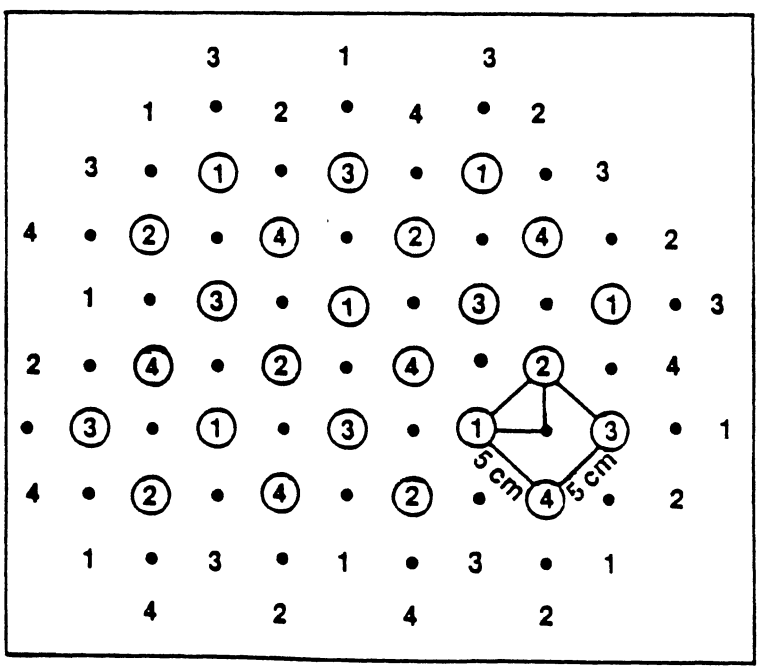
Design	Mean no. of greenbugs per plant of:			
	PI 429365	CI 15811	Wintermalt	PI 420491
RCBD	5.10a	7.80ab	9.60b	13.80c
CRD	7.67a	11.67b	12.17b	12.17b
CRD-CCA	3.00a	7.00b	9.66b	14.16c

Means followed by the same letters in a row are not significantly different at P = 0.05 by Duncan's New Multiple Range Test.

Figure 4. Randomization plans for antixenosis tests. (A) Completely Randomized Design, (B) Completely Randomized Design with Central Composite Arrangement. Numbers without circles indicate nonexperimental plants. 1 = PI 429365, 2 = CI 15811, 3 = PI 420491, 4 = Wintermalt, (●) = greenbug release site.

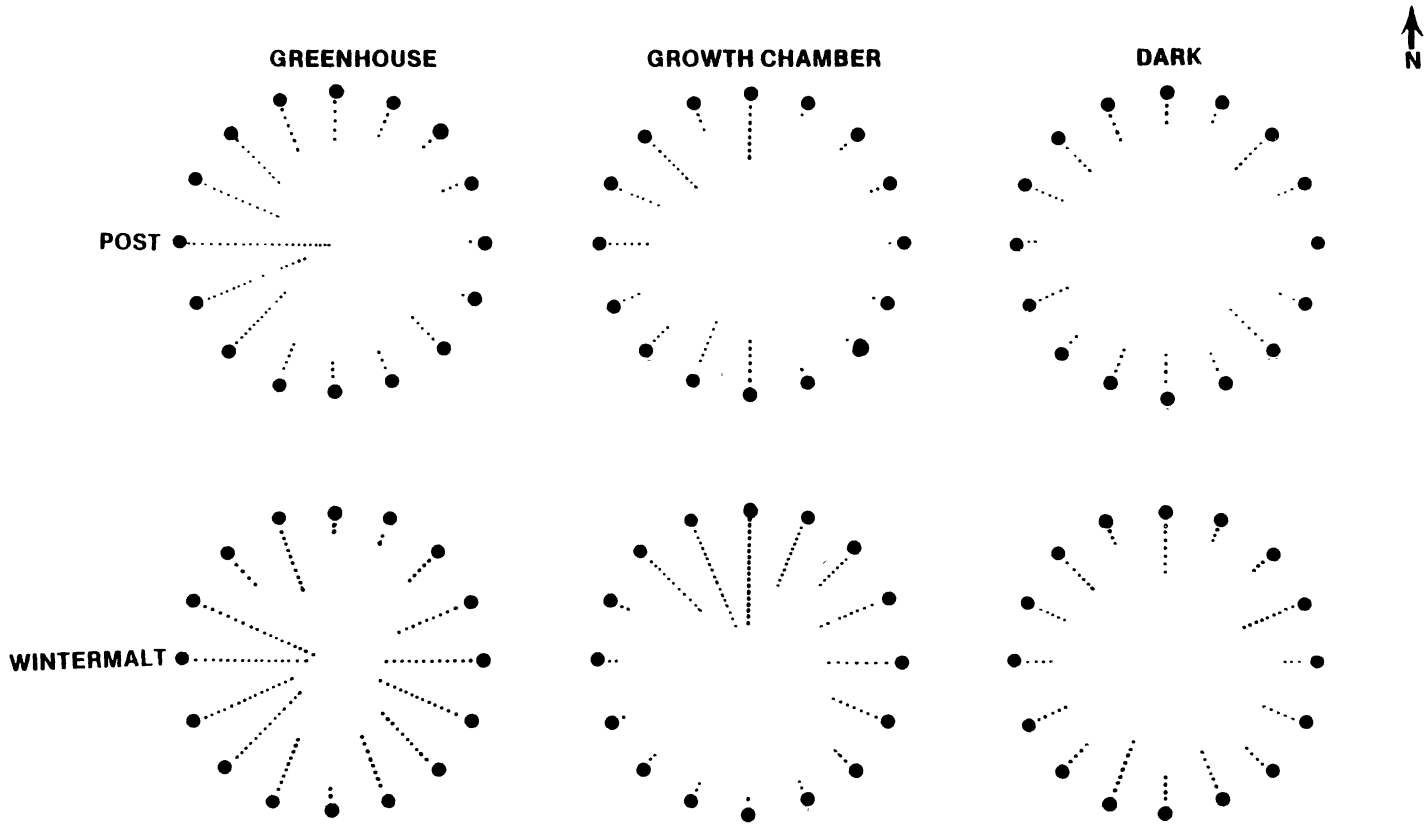


(A)



(B)

Figure 5. Mean number of greenbugs on Post and Wintermalt plants sown at different positions in pans, and tested under three environmental conditions. Each small dot (·) represents one greenbug on the test plant (●).


GREENHOUSE
GROWTH CHAMBER
DARK
N
POST
WINTERMALT

CHAPTER III

DESIGN OF EXPERIMENTS AND OPTIMUM SAMPLE SIZE FOR ANTIBIOSIS TESTS

Introduction

Because of the biotype problem in the greenbug, there has been increased interest in plant resistance research and related techniques. Refinements in techniques for evaluating plant resistance would increase the efficiency of developing resistant cultivars. Plant growth chambers are often used in host plant resistance studies, but recently Lee and Rawlings (1982) reported significant variation in tests conducted with plants in growth chambers. They suggested that proper experimental designs should be used to reduce the variation as much as possible to obtain more precise estimates of the parameters in question. One simple solution to this problem would be to increase the number of replications. However, the cost per experimental unit is often substantial, making the collection of unnecessarily large data sets impractical. The objectives of the present research were to determine suitable statistical designs and optimum sample sizes for greenbug antibiosis tests in a plant growth chamber.

Materials and Methods

Four barley entries (CI 15811, PI 415020, Wintermalt, and Post) having various levels of resistance (Webster and Starks, 1984) were infested with biotypes C and E at the rate of three adults/seedling. Individual plants in 7.6-cm diam pots were covered with clear plastic cages (6.0 cm diam and 30 cm high) having two muslin-covered side vents (6.5 cm diam) and a muslin-covered top.

The pots were kept on the ridges of the floor of a growth chamber (Conviron PGW-36) programmed at 25°C during day and 20°C at night and at a 16:8 hr day:night regime. The floor of the chamber was formed from aluminum channels (2.5 cm high and 4.0 cm wide). Small (about 1 cm diam) air holes were provided in the sides of the channels to minimize direct blasts of air on the plants. Thus, air flow in this chamber was upward through the floor at a velocity of less than 0.5 m/sec. The distance between the light source and plants was about 1.5 m. Under these temperature and photoperiod conditions, the greenbug reproduces parthenogenetically, and all progeny are females. After 2 to 3 days, the adults were removed, and about five nymphs were left on each seedling. About 2 to 3 days later, when the nymphs were about to mature, all except one were removed from each plant. Plants were then randomized in an 8 X 8 Latin square design, with biotypes and barley entries in a factorial arrangement and two subsamples (pots) in an experimental unit. Use of the Latin Square Design would enable the detection of row-to-row and column-to-column variation within the growth chamber. The subsamples were used to determine their role in increasing precision in the experiment. There were a total of 128 experimental barley plants, 32 of each entry, surrounded by a border of

nonexperimental plants covered with similar cages but without greenbugs. The plants were observed on alternate days, and the nymphs produced by each greenbug female were counted and removed. Experimental data were the total number of nymphs produced by each female during the reproductive period.

The optimum number of replications required to detect differences of a given size at a given level of significance and power (1-β) was determined by the following formula (Steel and Torrie, 1980):

$$r \geq \frac{2(Z\alpha + Z\beta)^2\sigma^2}{\delta^2}$$

where r is the number of replications required, Z is the normally distributed statistic available from statistical tables, α is the significance level for a one-tailed test, β is the type II error, δ is the size difference as a percentage that is to be detected, and σ^2 is the variance. The variance is equal to $\sigma_s^2 + \sigma_\varepsilon^2$ where σ_s^2 is the variance within experimental units, and σ_ε^2 is the variance among experimental units. This equation was also used to develop the power of the test curves at $\alpha = 0.01$ and 0.05 levels.

The variance of a treatment mean ($s_{\bar{x}}^2$) was calculated by the following formula (Helsel and Cowen 1983):

$$s_{\bar{x}}^2 = \frac{\sigma_\varepsilon^2}{r} + \frac{\sigma_s^2}{rp}$$

where p is the number of subsamples and r is the number of replications, 2 and 8, respectively, in this experiment. A coefficient of variation (CV) table was developed for various combinations of replications and subsamples by using the following formula (Gomez and Gomez, 1984):

$$CV = \frac{\sqrt{s_{\bar{x}}^2}}{\bar{x}} \times 100$$

where $\bar{x}$ is the overall treatment mean.

Results

The analysis of variance of the Latin Square Design indicated nonsignificant effects of rows, columns, biotypes, and the interactions between the biotypes and entries on the fecundity (Table IV). The treatment effect, consisting of the combination of entries and biotypes, was highly significant, but due mainly to differences in greenbug resistance among the entries (Table IV). The mean number of nymphs produced per female on Post barley (48.28) was significantly different ($P < 0.05$) than nymphal production on the other entries in the test. The greatest number of nymphs/female was produced on Wintermalt (77.22), followed by PI 415020 (72.59). There were no significant differences ($P < 0.05$) in nymphal production between these two entries. On CI 15811, the mean number of nymphs produced per female was 61.22, which was significantly different ($P < 0.05$) than nymphal production on the other entries.

Since the row-to-row and column-to-column effects were not significant (Table IV), the same data were analyzed as a Randomized Complete Block Design using the columns as blocks (Table V). The results were similar to those of the Latin Square Design. Variance within experimental units of the RCB design (σ_s^2) was 84.70, whereas variance among the experimental units (σ_ε^2) was 75.59. The variance associated with the treatment mean ($s_{\bar{x}}^2$) was 14.74 and the overall

treatment mean ($\bar{x}$) was 64.83 nymphs/female. The CV was calculated for various combinations of replications and subsamples, and it was found that it can be minimized by increasing the number of replications and subsamples. Based on the CV and the number of plants that could be efficiently tested in one experiment, a test should consist of a Randomized Complete Block Design with 8 to 10 replications and with two to four subsamples (Table VI).

The number of replications required to detect a difference (δ) of 5, 10, and 20% of the treatment mean at significance levels of 0.01 and 0.05 and at different levels of power ($1-\beta$) were also calculated (Fig. 6, 7). In developing these curves a one-tailed test was used since it was of interest to estimate the number of replications required to statistically separate the means of the hosts in the test. For example, an average of 77.22 nymphs were produced on Wintermalt while 72.59 were produced on PI 415020. These quantities were not significantly different with eight replications and two subsamples. It is of interest here to determine the number of replications required to separate the means of these two hosts at a given level of significance and power.

At the power $1-\beta = 0.9$ (90% assurance) and $\alpha = 0.01$, 167 replications were required for detecting a difference of 5% in the growth chamber (Fig. 6). Obviously it would be highly impractical to conduct such a test. For detecting a difference of 10% under the same conditions and at the same levels of α and β, 42 replications were required, and for detecting a difference of 20%, 11 replications were required.

Similarly, for $1-\beta = 0.9$ and $\alpha = 0.05$, 28 replications were required to detect a difference of 10%; 7 replications were required to

detect a difference of 20%; and 5 replications were the minimal acceptable number at α = 0.05, 1-β = 0.8, and δ=20% (Fig. 7). Figures 6 and 7 may be used for determining the required number of replications at given levels of α, β, and δ for future experiments.

Discussion

The nonsignificant effects of both rows and columns indicated no variation between rows or columns. Since the response variable in this experiment was the fecundity of the greenbug, which always remains in close proximity to the plant, it appears that the absence of variation is because of similarities in microclimate throughout the chamber. Schweissing and Wilde (1978) studied the influence of three temperature regimes on biotype C greenbug resistance to sorghum, rye, barley, and oats, and reported that in the growth chamber, temperature fluctuated as much as 4°C, but only ±1°C in the glass tube cages used to cover the seedlings. Thus the greenbug microclimates in their chamber were quite uniform. Temperatures in the corners and in the center of the growth chamber were almost uniform, differing by less than 0.5°C. The humidity, which varied from 32 to 70%, was highest during irrigation of the plants. Light quality measurements (taken 1.5 m from the light source) in the corners, near the walls, and in the center of the chamber averaged 360, 420, and 440 $\mu E/m^2/sec$, respectively. These differences may lead to gradients in the chamber as far as plant-related variables are concerned (Lee and Rawlings, 1982), but as far as fecundity of the greenbug is concerned, no gradient was detected. Schweissing and Wilde (1979) concluded that temperature made no significant difference in the fecundity of aphids on resistant sorghum plants ('KS-30') while numbers

on susceptible plants ('RS671') decreased as the temperature decreased. In addition, they found that temperature and nutrient fluctuations caused more variation in tolerance stability than in the antibiosis type of resistance, possibly because tolerance is strictly a plant-related attribute.

As there was no row-to-row or column-to-column variation in the greenbug fecundity test in the growth chamber, other statistical designs in addition to the Latin square may be used in antibiosis tests. There may be a gradient in some growth chambers if plant-related variables are considered. Thus, simple uniformity trials should be conducted in chambers to determine the direction of possible gradients before conducting the actual experiments.

If the primary objective of the experiment is to detect differences among biotypes, then using as many leaf cages as practical on a single plant would minimize the effect of plant-to-plant variation on the insect. However, if the main objective is to detect differences among entries, then individual plants should be used as subsamples. This could be accomplished by using 2 to 3 plants in a larger pot but caging them separately.

TABLE IV

ANALYSIS OF VARIANCE, USING A LATIN SOUARE DESIGN, OF FECUNDITY OF TWO BIOTYPES ON FOUR BARLEY ENTRIES IN A PLANT GROWTH CHAMBER

Source of variation	d.f.	Mean square	F value
Rows	7	217.57	0.91
Columns	7	224.42	0.94
Treatments	7	2633.39	11.02**
Entry	3	5340.36	22.35**
Biotype	1	750.78	3.14
Entry x Biotype	3	553.95	2.32
Experimental error	42	238.94	2.82**
Sampling error	64	84.70	

**Significant at P = 0.01.

TABLE V

ANALYSIS OF VARIANCE, USING A RANDOMIZED COMPLETE BLOCK DESIGN, OF FECUNDITY OF TWO BIOTYPES ON FOUR BARLEY ENTRIES IN A PLANT GROWTH CHAMBER

Source of variation	d.f	Mean square	F value
Replications	7	224.42	0.95
Treatments	7	2633.39	11.16**
Entry	3	5340.36	22.64**
Biotype	1	750.78	3.18
Entry x Biotype	3	553.95	2.35
Experimental error	49	235.89	2.78**
Sampling error	64	84.70	

**Significant at P = 0.01.

TABLE VI

THEORETICAL COEFFICIENT OF VARIATION (CV) FOR TREATMENT MEANS OF A GREENBUG FECUNDITY TEST ON BARLEY ENTRIES UNDER VARIOUS COMBINATIONS OF REPLICATIONS AND SUBSAMPLES

No. of Replications	CV (%) for number of subsamples				
	1	2	4	6	8
2	13.81	11.84	10.73	10.33	10.12
3	11.27	9.57	8.76	8.43	8.27
4	9.76	8.37	7.58	7.30	7.16
5	8.73	7.50	6.78	6.53	6.40
6	7.97	6.84	6.19	5.96	5.84
7	7.38	6.33	5.73	5.52	5.41
8	6.90	5.92	5.36	5.16	5.06
9	6.51	5.58	5.06	4.87	4.77
10	6.17	5.30	4.80	4.62	4.53
11	5.88	5.05	4.57	4.40	4.32
12	5.64	4.84	4.38	4.22	4.13

Figure 6. Number of replications required to detect a difference of 5, 10, and 20% among treatment means at $\alpha = 0.01$ level.

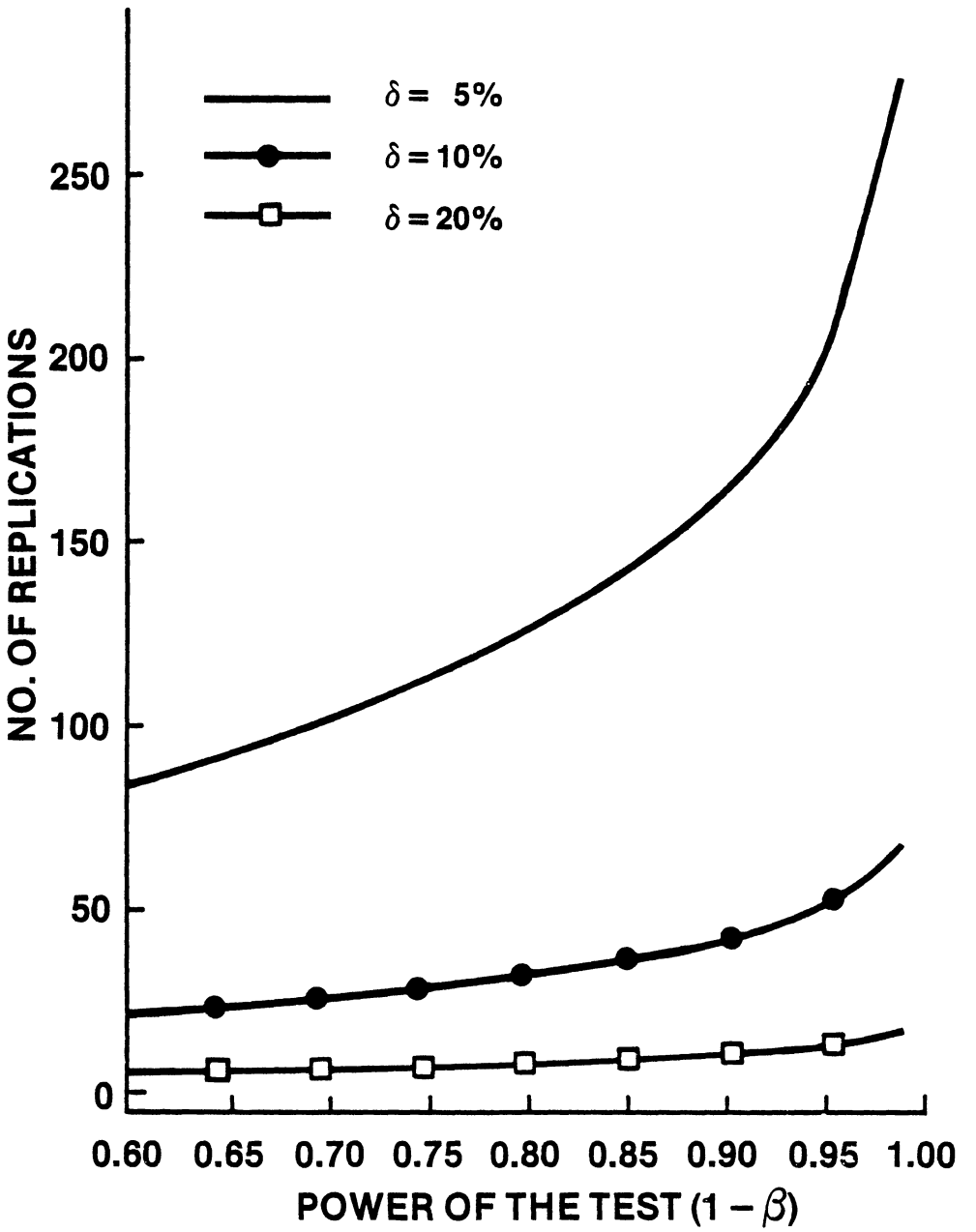
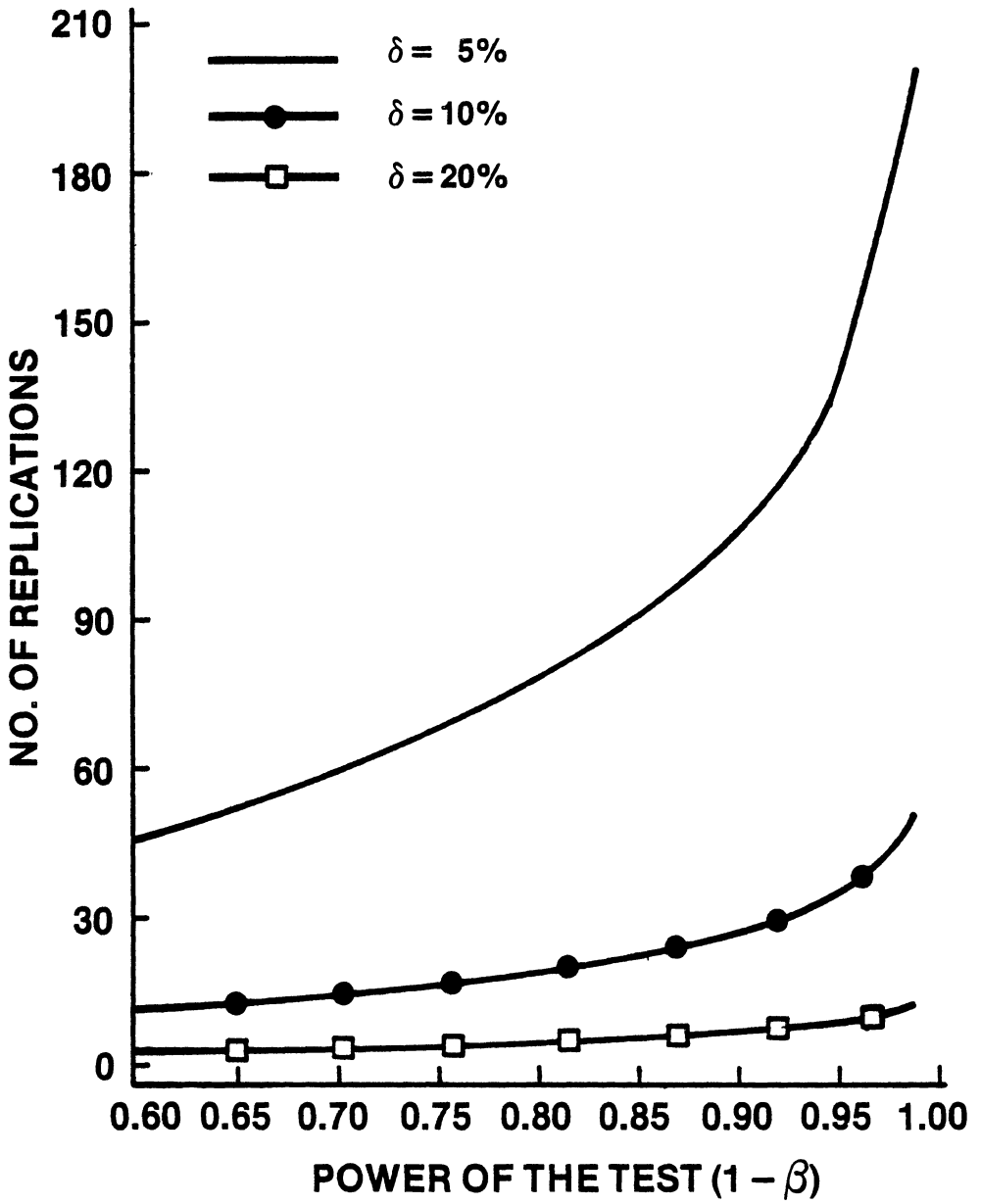

δ = 5%
δ = 10%
δ = 20%
NO. OF REPLICATIONS
250
200
150
100
50
0
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95
1.00
POWER OF THE TEST (1 – β)

Figure 7. Number of replications required to detect a difference of 5, 10, and 20% among treatment means at $\alpha = 0.05$ level.


δ = 5%
δ = 10%
δ = 20%
NO. OF REPLICATIONS
210
180
150
120
90
60
30
0
0.60 0.65 0.70 0.75 0.80 0.85 0.90 0.95 1.00
POWER OF THE TEST (1 – β)

CHAPTER IV

DEVELOPING A HOST PLANT RESISTANCE INDEX

Introduction

The establishment of a phytophagous insect on a particular host plant depends upon the suitability of the plant for feeding and oviposition (level of antixenosis), and the suitability for development and survival of the insect (level of antibiosis). Further, some hosts have the ability to withstand higher pest densities (level of tolerance) than other hosts. Antixenosis (nonpreference), antibiosis, and tolerance, as defined by Painter (1951), are the three major components of plant resistance.

Many workers, including Dahms et al. (1955), Weibel et al. (1972), Starks et al. (1972), Teetes et al. (1974), Starks and Merkle (1977), Starks and Weibel (1981), Webster and Inayatullah (1984), and Webster and Starks (1984), have worked on the isolation of the components of greenbug resistance in both small grains and sorghum. These researchers have described each resistance component separately because there has not been a model or standard procedure available to evaluate the three components simultaneously. A host could be resistant because of a high level of antixenosis, antibiosis, or tolerance, or a combination of various levels of all of these components. Thus, interpretation of each component separately becomes difficult when the overall resistance in a host plant is considered. This necessitates a mathematical model to

elucidate the overall resistance in a plant based on the components of resistance. Such types of models have not been available for plant resistance in relation to insects, but are available for disease resistance (Madden, 1980). Recently, Jeger (1980) presented multivariate models of the components of partial resistance of wheat to Septoria nodorum (Berk.) Berk. The objective of the present study was to develop a model for evaluating greenbug resistance in a host based on the antixenosis, antibiosis, and tolerance components of resistance.

Materials and Methods

Four barley entries, Wintermalt, PI 411025, PI 429365, and PI 426756, possessing various levels of resistance to biotype E of the greenbug (Webster and Starks, 1984), were used for the study. Two seeds of each entry were sown in a 7.6-cm diam pot, and after germination were thinned to one seedling/pot. When the seedlings were 5-6 cm high, they were infested with apterous biotype E greenbug females of the same age at the rate of 10 adults/seedling. The seedlings, along with the greenbugs, were covered with plastic cages (30 cm high x 6 cm diam) with cloth-covered tops and two side vents (6.5 cm diam). Five pots of each entry were arranged in a Randomized Complete Block Design (RCBD) in a plant growth chamber programmed at a 25:20°C and a 16:8 hr day:night regime.

The number of adults and nymphs per plant was recorded daily for 15 days. The damage done to each plant by the greenbug was also rated daily using a 0 to 9 scale (0 refers to no damage and 9 to a dead plant). The antibiosis and tolerance data were subjected to an analysis of variance. The results of this analysis, along with antixenosis data

from Webster and Starks (1984), were used in developing an Antibiosis Index (ABI), a Tolerance Index (TI), and an Antixenosis Index (AXI). These three component indices were then used to develop a Host Plant Resistance Index (HPRI).

To validate the HPRI, a test similar to that described by Starks and Burton (1977a) was conducted using the same entries in a metal flat (51 X 35 X 9 cm). There were two rows of each entry, and the position of each row was determined at random. Twenty seeds were sown in each row and thinned to 15 plants/row after germination. When the seedlings were about 5-6 cm high, they were infested with biotype E greenbugs by shaking the aphids from culture plants fairly uniformly over the flats. A density of approximately 10 greenbugs/plant was obtained. The plants and the greenbugs were covered with a plastic cage (47 X 33 X 20 cm), having a cloth-covered top and 10 side vents (8.0 cm diam). The plants were observed daily, and mortality was recorded. The experiment was conducted in a growth chamber under the same conditions as in the previous test.

Results

Antibiosis Index (ABI)

Since antibiosis is the adverse effect of the plant on the development and survival of an insect, the total number of greenbugs produced on a plant when the population has peaked is one method of measuring this component. When greenbugs encounter a susceptible host such as Wintermalt, the population increases geometrically. At the same time, the plant is unable to tolerate the increasing greenbug population and begins to die. Consequently, the greenbug population peaks and

crashes in a short period of time. On the other hand, if greenbugs develop on a resistant host such as PI 426756, both the greenbug population and the plants continue to grow, and the population may not peak during the test. Thus, the time required for the greenbug population to peak is a second measure of antibiosis. We have combined the two response variables, i.e., number of aphids at the peak population and time (days) required to peak, into one ratio as follows:

$$ABI = \frac{\text{No. of aphids at peak}}{\text{Time (days) required to peak}} \qquad (1)$$

ABI's for every experimental plant were determined using equation 1. Data for all the response variables were then subjected to an analysis of variance using a RCBD. A comparison of the experimental errors, the F values, and the coefficients of variation (CV) for the number of greenbugs at the population peak, the number of days to reach the population peak, and the corresponding ABI are shown in Table VII for counts of adults only and combined counts of adults and nymphs. There were significant differences between the barley entries in all of these variables. When comparing peak number of greenbugs, the CV was lower when all aphids were counted than when only adults were counted.

Table VIII shows the greenbug counts from the antibiosis test and the resulting ABI for the four barley entries. On Wintermalt, the greenbug population of adults, and adults and nymphs combined, peaked in fewer days when compared to the other entries, thus confirming its susceptibility. On one plant each of PI 429365 and PI 426756, the peak was obtained 13-14 days after infestation, but on the rest of these plants, the peak was never encountered. For those plants on which the

peak was not observed even up to the last day of the experiment (15th day after infestation), the 15th day was considered as the peak. The 15-day period was used because Starks and Burton (1977a) showed that if a plant were susceptible, it would die within this period.

On Wintermalt, the greenbug population peaked in 5-6 days. This made the denominator of the equation smaller, thus a higher ABI was obtained. On the other hand, with the resistant host PI 426756, at least 13-14 days elapsed before the population peaked, and in most cases it never peaked. Since the denominator for the ABI (number of days to peak) was higher for the resistant host, the ABI was lower than the ABI of Wintermalt. The differences among the entries in Table VIII were accentuated when the total number of aphids on individual plants were considered as compared to considering only the adults since there were more separations by the Duncan's New Multiple Range Test. Both types of indices are in close agreement with Webster and Starks' (1984) fecundity data on these entries.

Tolerance Index (TI)

Daily damage ratings for individual seedlings were regressed over time (days), and the slope of the regression line (damage/day) for each plant was determined. The data obtained on damage/day for each plant of all the test entries were analyzed as a RCBD. Damage/day on Wintermalt was significantly higher ($P < 0.05$) than that of the other entries. Damage/day was lowest on PI 426756, but it was not significantly different ($P > 0.05$) from PI 411025 and PI 429365 (Table IX, Fig. 8). The slope of the regression line is considered as the TI for developing the HPRI, which will be described later.

The intercepts of the fitted regression lines on the test entries were also analyzed by analysis of variance using a RCBD. The intercept of the regression line on PI 411025 was highest but not significantly different ($P > 0.05$) from that of Wintermalt. The intercepts of the regression lines of PI 429365 and PI 426756 were not significantly different ($P > 0.05$) from each other, but were significantly different ($P < 0.05$) from those of Wintermalt and PI 411025 (Fig. 8). Higher intercepts of the regression lines for both Wintermalt and PI 411025 show that these entries incur more damage than the other two entries in a shorter period of time which is another indication of susceptibility.

Antixenosis Index (AXI)

The level of antixenosis in a plant influences the number of greenbugs selecting it. In laboratory experiments an antixenosis index (AXI) can be calculated as:

$$AXI = \frac{\text{No. of aphids selecting the plant}}{\text{Total no. of aphids used in the test}} \qquad (2)$$

The value of the AXI according to this ratio varies from 0 to 1. If the host is rejected by all the test aphids, the value will be 0. If all of the test aphids select the host, the value will be 1. In the present studies data from Webster and Starks (1984) were used to calculate the AXI. Their data show that Wintermalt is highly preferred by the greenbug, followed by PI 411025, PI 429365, and PI 426756 (Table IX).

Host Plant Resistance Index (HPRI)

The HPRI is based on the three components of resistance. Since the three components of resistance are measured at different scales, they need to be normalized to a common scale free of units. A standard scale varying from 0 to 1 was chosen, with 0 refering to a high level of the component in question and 1 refering to a low level. The normalization is done by dividing each number with the respective highest number of a particular resistance component. To those not familiar with the host plant resistance literature, the AXI, ABI, and TI indices may be confusing at first since low values refer to high, or acceptable, levels of resistance. However, this is analogous to greenbug counts and damage ratings commonly found in plant resistance publications. Low greenbug numbers in antibiosis and antixenosis indicate good resistance levels, and low plant-damage ratings indicate good tolerance levels. Thus, the indices for the three components are consistent with existing data in the literature on antixenosis, antibiosis, and tolerance. However, since the HPRI refers to resistance levels, it would seem logical to designate low levels of resistance with low values and high levels with high values. The formula for the HPRI has been derived with this objective in mind. Based on the three components of resistance, the comparative HPRI can be calculated as follows:

$$HPRI = \frac{(1/TI)}{(AXI) \times (ABI)} \qquad (3)$$

For the numerator, the inverse of the TI is used because a resistant host will suffer less damage/day, and thus 1 divided by a small number close to 0 will give a larger numerator, resulting in a

higher HPRI. Alternatively, if the host is susceptible, then greater damage/day is expected. Thus, 1 divided by a number close to 1 will produce a small numerator and a corresponding small HPRI, indicating that the cultivar is susceptible.

The denominator for the HPRI is the product of AXI and ABI. If the values of these indices are close to 0, meaning that the cultivar has a high level of antibiosis or antixenosis, the HPRI will increase. When these indices are large, the HPRI will be low.

It should be noted that if any of the component indices are 0, the HPRI will approach infinity. This is possible theoretically but impossible practically, because no cereal grain entry is totally immune to the greenbug. Thus, for all practical purposes, none of the component indices of HPRI can be 0.

This method of approaching tolerance differs somewhat from Painter's definition of tolerance, which is "the ability of a plant to grow or repair injury by insect populations that could be harmful to other plants at the same insect densities." With this method, damage ratings obtained for a particular entry are based on the total number of aphids feeding on it, which could be higher than other entries because the entry in question may not possess appreciable antibiosis. In other words, the greenbug populations on the plants for determining the tolerance component were unequal. This necessitates adjustments in the damage/day value depending on the level of antibiosis in a particular entry. The HPRI equation makes this adjustment automatically. With the TI as the numerator and ABI as the denominator, when an entry has high level of antibiosis, its ABI will be close to 0, which in turn will

increase the HPRI. Thus, the bias in the TI due to the unequal number of greenbug populations on an entry is removed or adjusted.

A computer program was developed for calculating the HPRI in Standard Fortran using the three indices ranging from 0.1 to 1, which could be the possible practical values for the component indices. The HPRI ranged from 1 to 1000, depending upon the level of different indices. Tables of HPRI can be generated from the computer program to avoid laborious calculations.

Another computer program was developed using Statistical Analysis System (SAS) Graphics (SAS Institute, 1981) to observe the response surface of the HPRI (Fig. 9). The TI was held constant at 0.5, and AXI and ABI were varied from 0.1 to 1. Figure 9 shows how the changing levels of AXI and ABI affect the HPRI. When the TI was held constant over a range of 0.1 to 1, and the other two indices were varied from 0.1 to 1, the shape of the response surface was the same. Similarly, by holding any of the AXI or ABI constant and varying any one of the two remaining indices, the shape of the response surface observed was also the same. In each case only the scale of the HPRI changed.

The HPRI calculated for the barley entries is given in Table IX. The HPRI for PI 426756 was the highest, indicating greatest resistance, followed by PI 429365, PI 411025, and Wintermalt in descending order.

Validation of HPRI

When plants are evaluated for greenbug resistance with the standard test in greenhouse flats, all three components of resistance interact simultaneously. The greenbugs will usually avoid a host with a high level of antixenosis; but, if the plant they select possesses a high

level of antibiosis, then the rate of increase of the greenbug population will be adversely affected. If the plant has tolerance, it will sustain less damage compared to the other plants. In this type of test, depending upon the density of greenbugs in a flat, many plants may eventually be killed, but a longer period of time elapses before resistant plants are killed. In this test, Wintermalt plants died in a short time after infestation, followed by PI 411025, PI 429365, and PI 426756 (Fig. 10). These results validated our HPRI data.

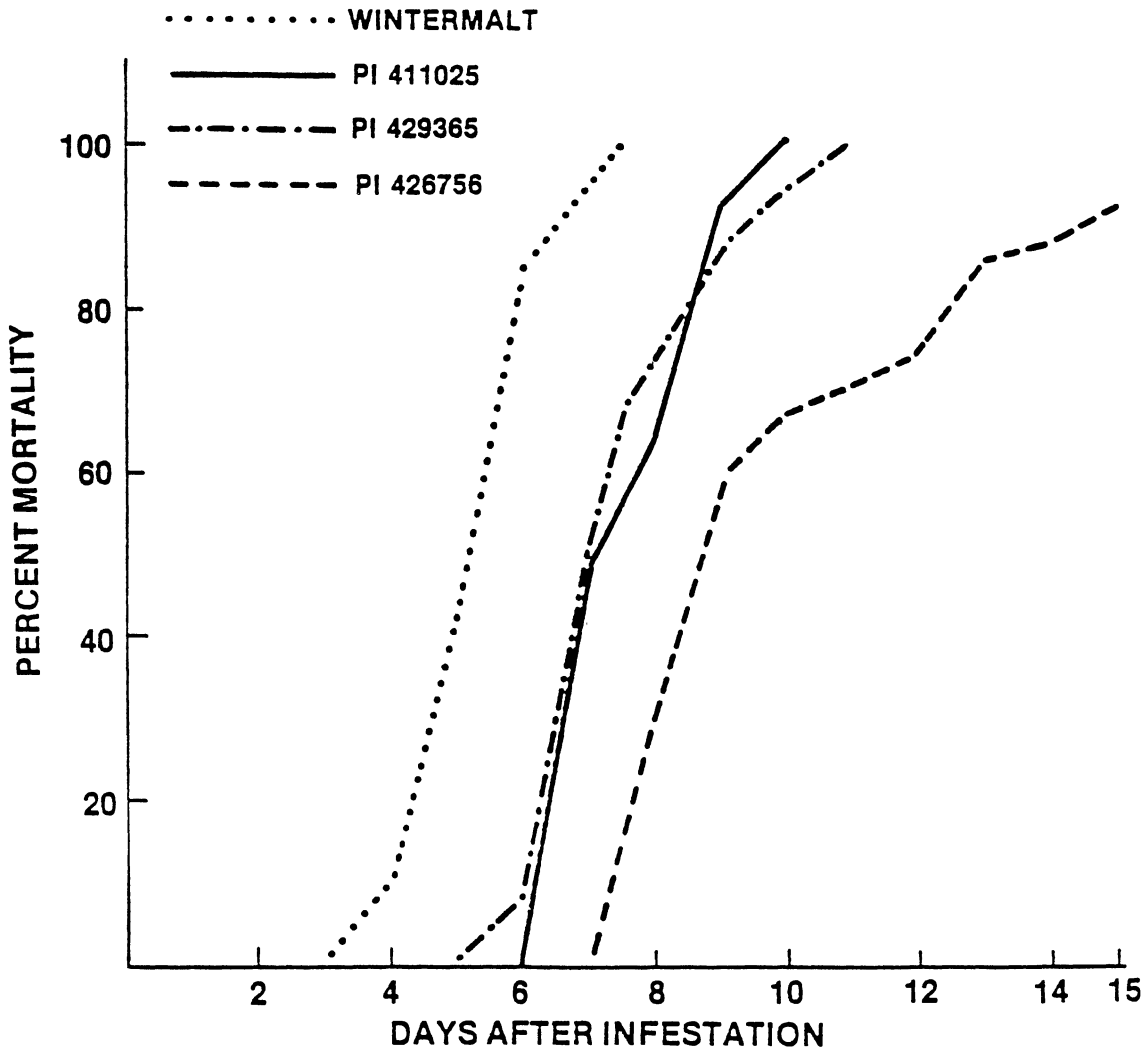
Discussion

The ABI was calculated as the ratio of total number of aphids at the peak population level and the time required to reach the peak. Laborious daily counting of all the aphids may be avoided by counting only the adults. This will lead to a higher coefficient of variation, but it is the trade-off between the efficiency of the test and the amount of time spent counting greenbugs. Another way to avoid the daily aphid counts would be to determine an aphid index (Rautapaa, 1966; Wratten et al., 1979), which is also a ratio between aphid counts and the number of days the test was run.

Lowe (1984a) made classes of antibiosis scores based on the density of aphids per wheat shoot. Lowe (1984b) also rated aphid densities on a plant with a 0-9 scoring scale based on a visual assessment. With this scale, 1 represented very low aphid densities and 9 exceptionally high densities. These scores are also an indication of the antibiosis level of a host and may be used for calculating the HPRI.

Further, in this HPRI model the denominators TI, AXI, and ABI were multiplied to give an equal weight to all resistance components. Wood

Figure 10. Mortality of plants of different barley entries infested by biotype E.


WINTERMALT
PI 411025
PI 429365
PI 426756
PERCENT MORTALITY
100
80
60
40
20
2
4
6
8
10
12
14
15
DAYS AFTER INFESTATION

CHAPTER V

BIOLOGICAL VARIATION WITHIN BIOTYPES AND THE RELATION TO THEIR DEVELOPMENT

Introduction

The occurrence of biotypes has been reported in many species of aphids (Eastop, 1973; Blakley, 1982; Claridge and Den Hollander, 1983). With the pea aphid, Acyrthosiphon pisum (Harris), it has been demonstrated that biotype characteristics vary from year to year (Frazer, 1972). Simultaneous occurrence of polymorphism, reproduction through parthenogenesis, as well as host alternation in most aphid species are examples of some unusual deviations from a normal insect life cycle. These factors probably contribute toward the presence of a large number of variants in aphids. These variants may differ in polymorphism, their morphology, behavior, biology, and virulence to the host plant.

Variation among individuals in a population is most often expressed in terms of the spread on either side of the mean, i.e., the standard deviation. This procedure assumes that the frequency distribution of the measured variable is essentially continuous, as in a normal distribution. Variation may also appear as more than one different form (polymorphism) or as scattered extremes or unusual forms differing greatly from the normal distribution. These cases are reflected in frequency distributions as modality, and as discontinuous or unusually

prolonged and often asymetrical "tails" to the distributions, which cannot be described by measures derived from their variance. When the main population is destroyed, the few extreme individuals gain disproportionate significance for they may produce a major fraction of the next generation. The adaptive value of extreme individuals is more important when they are relatively frequent and when their characters are closely connected to the genotype, e.g., parthenogenesis, or confer survival under heavy selection, e.g., insecticide resistance, industrial melanism. Thus the extreme individuals in a population cannot be regarded simply as a nuisance and dismissed as "noise" (Danks, 1983).

Frequency of occurrence of "super" or extreme individuals in parthenogenetic groups such as aphids, how they are maintained, how many are actually contributing at a given time to the adaptive fitness of the species, and the overall contribution of this pool of variation in long-term evolutionary processes are some factors to be considered in understanding the development of biotypes in aphids (Wills, 1981). Smith (1941) has discussed in detail the racial segregation in insect populations and its significance in applied entomology. Walters and Dixon (1983) also reported that within a clone some aphids may have significantly more or less ovarioles and are thus higher or lower in fecundity. Similarly Markkula and Roukka (1970) found variation in regard to fecundity in the grain aphid, Sitobion avenae (F.). Moran (1981, 1983) reported intraspecific variability in the aphid Uroleucon caligatum (Richards), and Lowe (1984) reported behavioral differences in host selection among the clones of pea aphid.

A great deal of research has also been published on the variability in host reaction to greenbug biotypes (Porter et al., 1982; Starks et al., 1983), but no information is available on variability within the greenbug biotypes. The objectives of the present research were to determine the variation in fecundity, overall virulence, and virulence independent of fecundity among greenhouse cultures of three greenbug biotypes.

Materials and Methods

Biotypes B, C, and E were used in the present studies. Since cultures of biotype A and D were not available, these could not be included.

Variation in Fecundity

Ten culture pots of greenbug-infested barley plants were selected at random from greenhouse cultures of each biotype. From each selected pot, three plants were then removed at random, and the plants with the greenbugs were mixed together. Next, 48 plants each of a susceptible host, Wintermalt, and a resistant host, Post, were infested with five females/plant. These plants, which were in individual 7.6-cm diam pots, were covered with clear plastic cages (6 cm diam and 30 cm high) and placed in a growth chamber programmed at a 25°C during the day and 20°C during the night and 16:8 hr day:night regime. The next day the adults were removed and the nymphs were thinned to 5-6/plant. After 2-3 days the plants were again observed, and only one nymph was left on each plant while all others were removed. The plants were observed on alternate days, and the nymphs produced per female were counted and

removed. This test determined the variation in fecundity of females reared on susceptible and resistant hosts.

Verification of the Fecundity Test

Another fecundity test was conducted with only the extreme high- and low-fecund individuals of biotype E isolated from both Wintermalt and Post. In this test, the fecundity of daughters of each low- and high-fecund parent isolated on Wintermalt was determined on Wintermalt and Post. Similar tests were performed with the progeny of high- and low-fecund females isolated from Post (Fig. 11). Each test was replicated 10 times, and the data were analyzed as a Randomized Complete Block Design (RCBD) with the hosts and four types of biotype E variants in a factorial arrangement.

Variation in Virulence

Separate cultures of some of the high- and low-fecund females of each biotype isolated both on Wintermalt and Post barley in the first experiment were established on Wintermalt. These will be referred to as greenbug isolates. A virulence charactertistic test was performed with the progeny of all types of the isolates. The hosts used in this test were Amigo, Largo, and Tam W-101 wheat, Post barley, and PI 264453 sorghum. Two seeds of each test host were randomly sown in a circular pattern about 2 cm from the edge of a 10-cm diam pot. After about 5 days, when the seedlings had emerged and were about 5-8 cm high, they were thinned to one seedling of each host per pot. Thus there was a total of five plants per pot. Each seedling was infested with 10 adults (50 adults/pot) of the same age from the progeny of each test isolate.

The seedlings and the greenbugs in each pot were covered with a clear plastic cage (9 cm in diam and 30 cm high).

The plants were evaluated daily for greenbug damage on a 0 to 9 scale. Based on the number of days required by a particular type of isolate to kill the hosts, the ability of the high-fecundity isolates to damage resistant hosts was compared with the low-fecundity isolates.

There were six separate tests, two with each biotype isolate from Wintermalt and Post. There were three replications for each test, and the pots were randomized in the growth chamber using a RCBD.

Variation in Virulence Independent of Fecundity

Since the virulence characteristic test involves both fecundity of the greenbug isolates and their ability to damage a host independent of their fecundity (virulence), a differential virulence test was designed to isolate these two variables.

Based on each virulence characteristic test conducted with each biotype isolate, two highly virulent and two less virulent isolates were selected for conducting the differential virulence test. Thus there were four isolates selected for conducting one differential virulence test with each type of greenbug biotype. For these tests, Wintermalt was used as the host. Seedling leaves were laid horizontally on the tops of petri dishes (9 cm diam and 1.5 cm high), which were covered with filter papers. The petri dishes were placed in aluminum pans (45.5 X 32.5 X 2.5 cm) filled with sand. Roots of the seedlings were buried in the sand. Glass rings (2.5 cm diam and 2.5 cm high) were used as cages to confine the aphids. One end of each glass ring was covered with a plastic cap (Fig. 12). Greenbug adults of the same age were

released near the tips of seedling leaves and covered with the cages. After 24 hrs, adults were removed from the cages, and three nymphs/cage were left as test greenbugs. Since only greenbug nymphs were used, this test measured virulence independent of fecundity because no reproduction occurred during the test. The seedlings were observed daily for a period of four days, and the damage to each leaf was recorded at 0 to 9 scale. There were three replications of each test randomized according to a Completely Randomized Design.

Results

Variation in Fecundity

Fecundity tests were initiated with 48 females for each biotype-host combination, but a few females died in each test. Therefore, the number of test females ranged from 35 to 46. On Wintermalt, the greatest number of nymphs were produced by biotype C, followed by those of E and B in descending order. All three means were significantly different from each other at $P < 0.05$ (Table X, Fig. 13). On Post, the greatest number of nymphs were produced by biotype E, followed by those of biotype C and B in descending order. The means of fecundity of biotypes B and C on this host were not significantly different ($P > 0.05$), but the two means were significantly lower ($P < 0.05$) than that of biotype E (Table X, Fig. 14).

Variances in fecundity of all biotypes reared on both hosts were almost equal, except in the case of biotype E on Post (Fig. 15). Variance in fecundity of biotype E reared on Post was about 1.3 times higher than the variances of the other biotypes reared on each host.

There was no significant difference (F-test, $P > 0.05$) among the variances.

The coefficient of variation (CV) was highest with biotype B on Post barley, followed by biotype C and E also on Post. The higher variance but lower CV of biotype E on Post as compared to that of other biotypes on both the hosts is because of its relatively greater mean. The CV of the three biotypes reared on Wintermalt ranged from 13.11 to 16.85 (Table X).

Fecundity distribution curves of the three biotypes on Wintermalt as well as that of biotype B on Post were normal. The Shapiro-Wilk statistics (w) for testing the normality distribution of biotype B on Wintermalt and Post were 0.97 ($P < w=0.47$) and 0.94 ($P < w=0.09$), respectively; w-normal statistics for biotype C and E reared on Wintermalt were 0.94 ($P < w=0.99$) and 0.98 ($P < w=0.84$), respectively. Skewness of biotype B on Post was positive, whereas that of biotypes B, C, and E reared on Wintermalt was negative, but all were close to 0, and all were not significant ($P > 0.05$) (Table X). Kurtosis of each frequency distribution curve was less than 3 (Table X), indicating that the curves were platykurtic (Fig. 13, 14).

In contrast to this, the frequency distribution curves of biotypes C and E on Post barley were not normal: w-normal statistics for biotype C and E were 0.90 ($P < w=0.01$) and 0.92 ($P < w=0.01$), respectively. Skewness of each biotype was positive and was significant ($P < 0.05$). Skewness of the frequency distribution curve of biotype C was higher than that of E. Moreover, in both distribution curves a hump on the positive side of the mean was evident. The hump in the frequency distribution curve of biotype E reared on Post was far away from that of

C, indicating the potential of this biotype to overcome resistance in Post barley in the future (Fig. 14).

Verification of the Fecundity Test

The objectives of the fecundity test conducted with the progeny of the extremely low- and high-fecund females of biotype E isolated from both Wintermalt and Post were to test: (i) whether the fecundity test was repeatable on the same host, (ii) whether the fecundity of daughters from an extremely high-fecundity parent isolated on Post was extremely high on Wintermalt, and (iii) whether the fecundity of daughters from a low-fecundity parent isolated on Wintermalt was extremely low on Post.

Analysis of variance indicated that the main effects of the host and isolates were significant at $P = 0.01$; however, the interaction among these two was also significant at $P = 0.05$ (Table XI). Overall, the fecundity of daughters on Wintermalt averaged 70.45 and that on Post averaged 47.80 (host main effects). The fecundity of daughters whose parent had high fecundity on Wintermalt was higher than those whose parent had low fecundity on this host (63.95 vs. 48.20). Similarly, the fecundity of daughters whose parent had high fecundity on Post was higher than those whose parent had low fecundity on this host (68.90 vs. 55.45). Duncan's New Multiple Range Test grouped all the daughters originating from high-fecundity parents into one group and those originating from low-fecundity parents into a significantly different ($P < 0.05$) group. This indicates that the fecundity of daughters is consistent with their parents.

When tested on Wintermalt, the increase in fecundity of the daughters of the high-fecundity parent on Post, and those of the

low-fecundity parent on Wintermalt was not as great as compared with the other types of the progeny. This is probably the main contributor toward the significant ($P < 0.05$) interaction illustrated in Fig. 16. Multiple comparisons among the progeny of the greenbug isolates on both Wintermalt and Post are also shown in Fig. 16. Conclusions of this experiment are: (i) the fecundity test is repeatable because the progeny of low-fecundity parents had low fecundity, and those of high-fecundity parents had high fecundity, (ii) the fecundity of daughters originating from the high-fecundity parent on Post increased when they fed on a susceptible host, but it was not the highest compared with the fecundity of daughters originating from the high-fecundity parent on Wintermalt, and (iii) the fecundity of daughters originating from the low-fecundity parent on Wintermalt declined to a greater extent when reared on Post, and was the lowest in the test.

Variation in Virulence

From the fecundity tests of each biotype on Wintermalt and Post, extremely low- and high-fecundity females of each biotype were selected for conducting virulence characteristic tests. The number of individuals selected and group means with their standard deviations are given in Table XII. In all cases, the low-group mean was significantly different ($P < 0.01$) from its respective high-group mean. Overall, the low-group means were two to three times smaller than the high-group means (Table XII).

Due to culture problems in colonies of the high-fecundity isolates of biotype E isolated from Post, the culture of three isolates was lost. Thus, the virulence characteristic test in this case was conducted with

the progeny of five low-fecundity isolates and the remaining two high-fecundity isolates. Analysis of variance conducted on all the six virulence characteristic tests indicated significant ($P < 0.01$) main effects of the isolates, except the one conducted with biotype B isolates from Post. For further analysis, contrasts between "low-fecund vs. high-fecund" isolates of each biotype for determining differences between their ability to kill the hosts were developed. The differences were significant ($P < 0.01$) in all tests, indicating that the high-fecund greenbugs of all biotypes were more efficient in killing both the susceptible and resistant hosts compared with low-fecund greenbugs. The mean number of days required by some of the isolates (to be used in differential virulence tests) to kill the test hosts are given in Table XIII.

In all virulence characteristic tests, the main effects of the host (longevity in days) were also significant ($P < 0.01$). In tests conducted with biotype C, Tam W-101 was the only susceptible host, which died after 5-6 days of infestation. With biotype E, Tam W-101 and Amigo were susceptible and died after 7-9 and 11-12 days of infestation, respectively. With biotype B, Tam W-101 died after 5-6 days of infestation. Largo, which is resistant to biotype C and E, died after 7-8 days of infestation with biotype B, and thus was almost as susceptible as Tam W-101 (Table XIV).

In all virulence characteristic tests, the interaction among hosts and isolates was also significant ($P < 0.05$). The significant interaction was mainly due to the differential longevity of the resistant hosts to a particular biotype. Some of the resistant plants

of such hosts died either very early or very late, and therefore contributed toward the significance of interaction.

Variation in Virulence Independent of Fecundity

From each virulence characteristic test, two high- and two low-virulent isolates were selected for a differential virulence test. The mean number of days for the selected isolates to kill the hosts in the virulence characteristic tests are given in Table XIII. It is evident that the isolates of all the biotypes which were originally selected as high-fecundity isolates were more virulent than the low-fecundity isolates. The differences in virulence could be due to differences in their fecundity. For the differential virulence test, two types of response variables were considered. One was the final damage ratings, i.e., ratings on the 4th day of infestation. The second response variable was the rate of damage/day (slope of the regression line). For calculating the rate of damage/day of each test seedling, daily damage ratings were regressed against time and the slope of the regression line was determined. Analysis of variance using a CRD was performed on the data on final damage ratings and slopes.

Whether considering damage per day (slope) or final damage ratings as the response variable, no significant ($P > 0.05$) differences were found among the greenbug isolates in all six differential virulence tests analyzed separately, indicating absence of variation among them. However, contrasts between low-fecundity isolates vs. high-fecundity isolates of biotype B isolated from Wintermalt and that of biotype C isolated from Post were significant. In both cases the high-fecundity isolates were more virulent than the low-fecundity isolates: damage

ratings for biotype B isolated from Wintermalt were 5.0 vs. 4.2, and those for biotype C isolated from Post were 5.7 vs. 4.5 for high-fecundity vs. low-fecundity isolates. When the slope of the regression line was considered as the response variable, the only significant ($P < 0.05$) contrast was between the low-fecundity isolates vs. high-fecundity isolates of biotype B isolated from Post. The high-fecundity isolates were also more virulent (damage/day = 1.16) as compared to low fecund isolates (damage/day = 0.89). On the other hand, when final damage ratings were considered as the response variable, this contrast was not significant.

All the data were also pooled to compare differences among the biotypes. But no significant ($P > 0.05$) differences could be detected among them, whether considering damage/day or the final damage ratings as the response variable. This test may lack precision because the grading of a small leaf area is difficult. Estimation of the amount of chlorophyll lost due to greenbug damage may be a more reliable estimate.

Discussion

The variation in fecundity of the three biotypes, as measured by the magnitude of their variances, was not significantly different, but the variance of biotype E on Post was about 1.3 times higher than the variances of the other biotypes on each host. The fecundity distribution curves of biotype C and E on Post were positively skewed and were not normal. The skewness was also significant. It illustrates that the resistance is not normally and bimodally distributed. Gould (1983) also made such types of conclusions based on the analysis of the CV, skewness, and kurtosis with the green peach aphid, Myzus persicae

(Sulz.), on 14 species of wild potato, Solanum spp. On Post, the mean fecundity and variance of biotype E were relatively higher. Further, there was a small hump on the positive side of the tail of the fecundity distribution, indicating potential in this biotype for overcoming resistance in Post barley.

According to Porter et al. (1982), Will and Post barley are resistant to biotype E, but in some early tests conducted at Stillwater with biotype E, both of these hosts were killed, which indicates probable change occurring in the host reaction of this biotype. Montllor et al. (1983) reported that biotype E has overcome resistance in two widely resistant sources, i.e., Sorghum bicolor and S. virgatum (Hack.). This biotype has also overcome resistance in Amigo wheat, which has a resistance gene derived from rye (Sebesta and Wood, 1978).

Variation in regard to host reaction among greenbug biotypes has been well documented by Porter et al. (1982), Starks et al. (1983), and Webster and Inayatullah (1984). Differences in their feeding habits have also been recorded. Biotype A greenbugs insert their stylets intercellularly and feed in the phloem tissue (Saxena and Chada, 1971); whereas, those of biotype B insert their stylets both intra- and intercellularly and feed in the mesophyll parenchyma of the leaf (Wood et al. 1969). Like biotype A, biotype C also feeds in the phloem tissue (Wood, 1971). Dreyer and Campbell (1984) reported that biotype E more efficiently depolymerized a biopolymer, pectin acting as an intercellular cement in a biotype C resistant sorghum entry (IS 809).

Berger et al. (1983) reported that biotype E is better adapted to cooler temperatures than biotype C, causes more damage by its toxin(s) to susceptible and resistant plants, and is a more efficient vector of

maize dwarf mosaic virus. Kvenberg and Jones (1974) reported that biotype C produces more alates as compared to biotype B. Development of large numbers of alates in biotype C indicates its greater propensity to migrate and become widespread in areas which may be more suitable for its development. Further studies are warranted to determine the associated changes occurring in the morphology and reproductive fitness of alates.

Wood and Starks (1972) reported that biotype C is better adapted than A or B to either temperature extremes. Mayo and Starks (1972a) compared the length of chromosomes of the greenbug biotypes, but they could not find any variation within biotypes A, B or C. However, total chromosomal length for biotype A was significantly different from that of biotypes B and C. Biotype B and C chromosomes did not significantly differ in length from each other. A recent study conducted by the same authors (Z B Mayo and K. J. Starks, USDA-ARS, Stillwater, Okla., pers. commun.) has revealed significant variation in chromosome length within biotype C. Similarly, Saxena and Barrion (1983) observed significant cytological variation among brown planthopper, Nilaparvata lugens (Stal), biotypes 1, 2, and 3.

Wood and Starks (1975) observed paedogenesis in biotype C of the greenbug, which is the first known case of paedogenesis in the family Aphididae and illustrates another unusual deviation in the greenbug life cycle which ensures maximum utilization of resources. Differences in biology, behavior, virulence, and cytology within biotype C are evidences that a new biotype could evolve from biotype C in the future. This may account for the development of biotype E. Unfortunately, such information on variability within biotype E is not complete. Isozyme

analysis of the greenbug biotypes should more precisely assess the genetic differences. Differences in isozymes have been reported in many species of aphids (Singh and Cunningham, 1981; Simon et al., 1982).

The occurrence of biotypes in aphid populations is aided because of their shorter life cycle, and reproduction by parthenogenesis as well as by sexual morphs. Parthenogenesis and paedogenesis are the strategies for maximum use of resources. Dahms (1972) reported that one alate greenbug could develop a colony of approximately four million aphids 50 days after the birth of first nymph. Great increases in numbers serve not only to offset the chances of extinction, but also enhance the prospects of mate-finding and promotion of genetic interchange when the sexual morph is produced (Clark, 1973). The occurrence of a sexual cycle in North America is unclear (Mayo and Starks, 1972, 1974; Daniels, 1981; Daniels and Chedester, 1980; Potter, 1982), but in other parts of the world the greenbug passes through asexual and sexual cycles of reproduction, depending upon the environment (Mitic-Muzina and Srdic, 1979; Barbulescu, 1980; Kushnerik, 1981). This geographic variation in the greenbug life cycle, like that of the green peach aphid (Blackman, 1974), is itself of great significance and illustrates the plasticity in this species to adjust to different environments. Essentially, the parthenogenetic reproduction also permits the pest to have an appropriate genetic match-up with its host and to spread rapidly without breaking its valuable gene complex that might be lost with sexual reproduction (Whitham et al., 1984). Thus, biotypes are expected to appear more frequently in areas where a few or no sexuales are produced. Also, in other insects the evolution of specialized pest races and gene-for-gene interactions with their hosts are often associated with

parthenogenetic reproduction or an asexual phase during the pest life cycle (see Gallun and Khush, 1980; Vanderplank, 1982; Whitham et al. 1984).

This research has demonstrated that within the three biotypes of the greenbug, superior gentoypes do exist which are more virulent than the others. These superior genotypes may lead to the occurrence of new biotypes in the future, if conditions for increasing their numbers become favorable. According to Dobzhansky (1937, see Smith, 1941), the pool of concealed potential variability contains: (i) variants which under no conditions are useful, (ii) some other variants which might be useful under a set of circumstances which may never be realized in practice, and (iii) still some more variants which were neutral or harmful at the time when they were produced, but which will prove useful later on. The individual variability is thus like a store of building material: the process of biotype formation consists of arranging the material in definite patterns. The nature of patterns depends upon the environment, and important modifications in the environment may be followed by changes in the patterns.

TABLE X

VARIATION IN FECUNDITY OF BIOTYPES REARED ON WINTERMALT AND POST BARLEY

Biotype	No. of females tested	Fecundity Range	Fecundity Mean	CV (%)	Skewness (g1)	Kurtosis (g2)
			Post			
B	35	4-43	18.37a	51.69	0.76	0.48
C	41	10-57	24.00a	41.19	1.35**	2.57
E	42	16-75	33.40b	34.65	1.27**	3.05
			Wintermalt			
B	46	33-82	54.85c	16.86	-0.10	1.10
C	40	52-99	75.77e	13.11	-0.08	0.08
E	45	47-93	69.31d	14.38	-0.08	-0.33

Means followed by the same letters in a column are not significantly different at P = 0.05 by t-test.

TABLE XI

ANALYSIS OF VARIANCE OF THE FECUNDITY OF LOW- AND HIGH-FECUND ISOLATES OF BIOTYPE E ON WINTERMALT AND POST BARLEY

Source of variation	d.f.	Mean square
Replications	9	95.14
Host	1	10260.45**
Greenbug isolate	3	1677.95**
Host X Greenbug isolate	3	459.48*
Experimental error	63	139.36
Coefficient of variation = 20%		

*Significant at P = 0.05

**Significant at P = 0.01

TABLE XII

MEAN FECUNDITY OF LOW- AND HIGH-FECUND GROUPS OF EACH BIOTYPE DEVELOPED ON WINTERMALT AND POST BARLEY FOR CONDUCTING THE VIRULENCE CHARACTERISTIC TESTS*

Biotype	Host used for isolation	n	Low fecund group Mean ± s.d.	High fecund group Mean ± s.d.
B	Wintermalt	4	37.50a ± 4.79	68.25b ± 9.32
	Post	4	7.00a ± 0.82	37.00b ± 5.83
C	Wintermalt	4	59.75a ± 5.56	91.50b ± 5.06
	Post	4	14.75a ± 3.30	44.75b ± 10.63
E	Wintermalt	5	55.60a ± 6.23	80.80b ± 2.80
	Post	5	22.00a ± 5.34	53.00b ± 15.21

*Groups were developed from the fecundity data of 35 to 46 females on each host.

Means followed by the different letters in a row are significantly different at $P < 0.01$ by t-test.

TABLE XIII

MEAN NUMBER OF DAYS REQUIRED TO KILL THE TEST HOSTS IN VIRULENCE CHARACTERISTIC TESTS BY DIFFERENT ISOLATES OF BIOTYPES, SELECTED ON THE BASIS OF THE FECUNDITY OF THEIR PARENTS

Biotype	Host used for isolation	Low fecund group isolates		High fecund group isolates	
		A	B	A	B
B	Wintermalt	17.33b	15.93b	13.33a	11.53a
	Post	15.33b	14.47b	13.06a	11.93a
C	Wintermalt	16.93b	16.40b	13.27a	12.47a
	Post	17.40b	17.13b	14.47a	13.13a
E	Wintermalt	16.33b	15.53b	9.87a	9.80a
	Post	16.87b	15.73b	12.46a	11.27a

Means followed by the same letters in a row are not significantly different at P = 0.05 by Duncan's New Multiple Range Test.

TABLE XIV

MEAN LONGEVITY OF DIFFERENT HOSTS IN VIRULENCE CHARACTERISTIC TESTS CONDUCTED WITH LOW- AND HIGH-FECUND INDIVIDUALS OF BIOTYPES, ISOLATED FROM WINTERMALT AND POST BARLEY

Biotype	Host used for isolation	Mean longevity (days) of:				
		Amigo	Largo	Tam W-101	Post	PI 264453
B	Wintermalt	18.00c	7.16b	5.62a	20.16d	20.67d
	Post	19.04c	7.12a	6.46a	17.08b	18.12bc
C	Wintermalt	17.58c	19.08d	5.54a	15.54b	16.00b
	Post	18.12c	19.25d	5.67a	17.12bc	16.50b
E	Wintermalt	11.96b	16.10d	8.30a	14.40c	14.56cd
	Post	11.85b	18.67e	7.47a	17.33d	14.14c

Means followed by the same letters in a row are not significantly different at P = 0.05 by Duncan's New Multiple Range Test.

Figure 11. Scheme for testing the performance of low- and high-fecund isolates of biotype E.

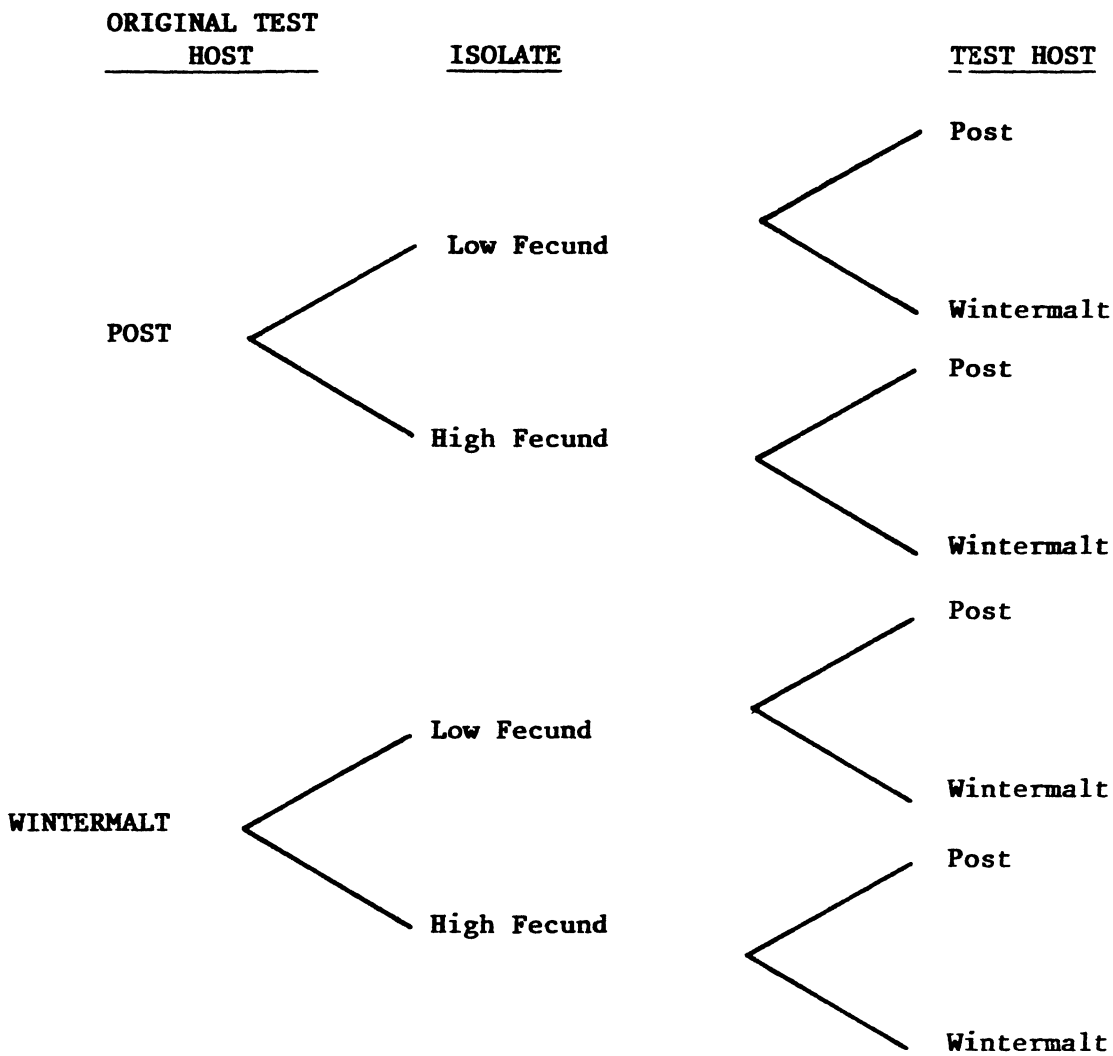

ORIGINAL TEST HOST
ISOLATE
TEST HOST
POST
Low Fecund
High Fecund
Post
Wintermalt
Post
Wintermalt
WINTERMALT
Low Fecund
High Fecund
Post
Wintermalt
Post
Wintermalt

Figure 12. Leaf cage used in the differential virulence test.

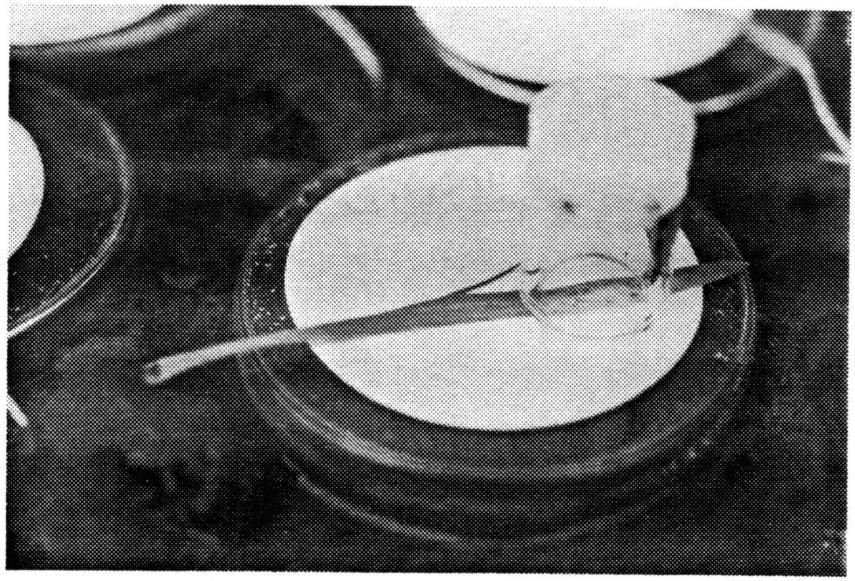


Figure 13. Fecundity distribution of biotypes on Wintermalt barley.

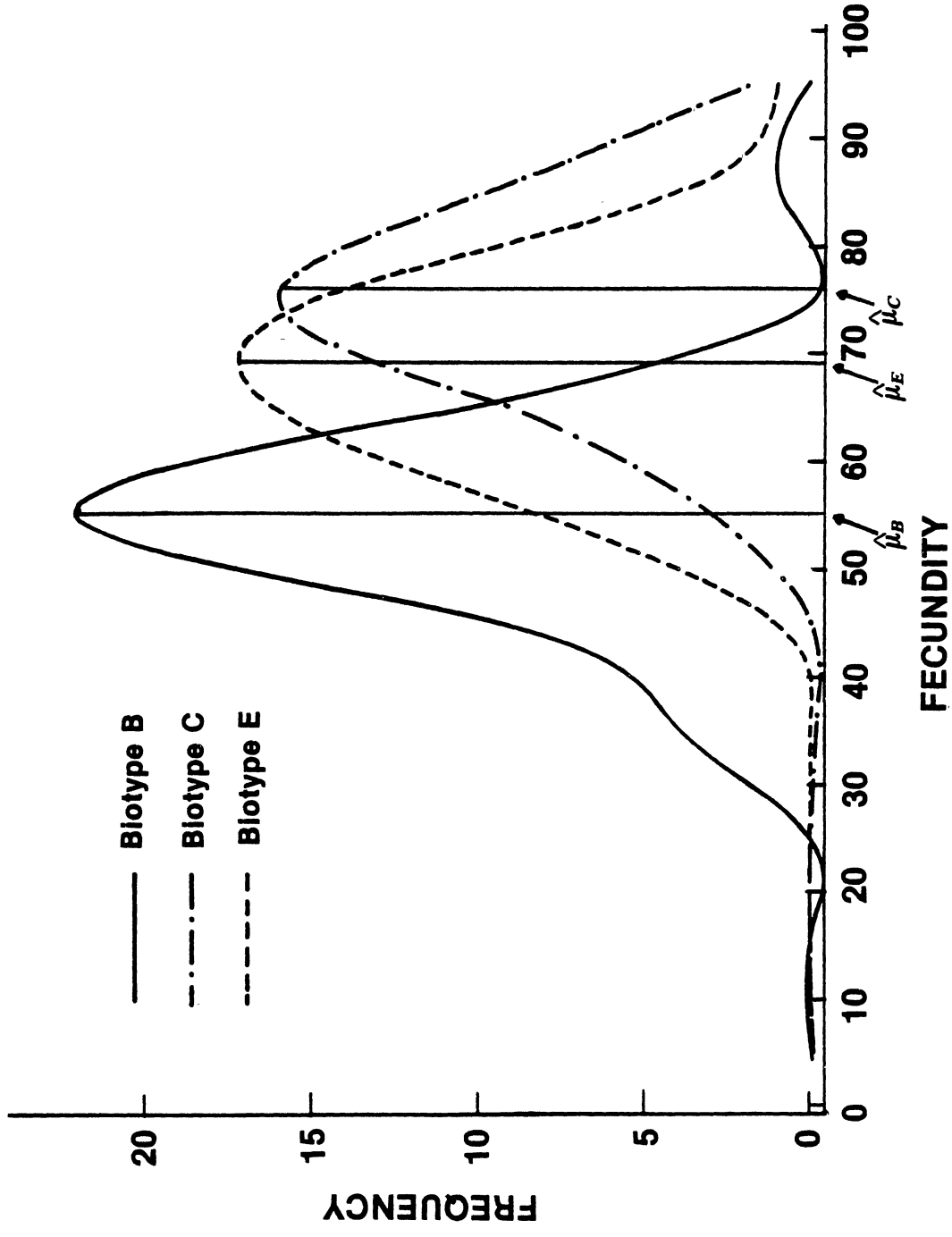

Biotype B
Biotype C
Biotype E
FREQUENCY
FECUNDITY
0
5
10
15
20
0
10
20
30
40
50
60
70
80
90
100
$\hat{\mu}_B$
$\hat{\mu}_E$
$\hat{\mu}_C$

Figure 14. Fecundity distribution of biotypes on Post barley.

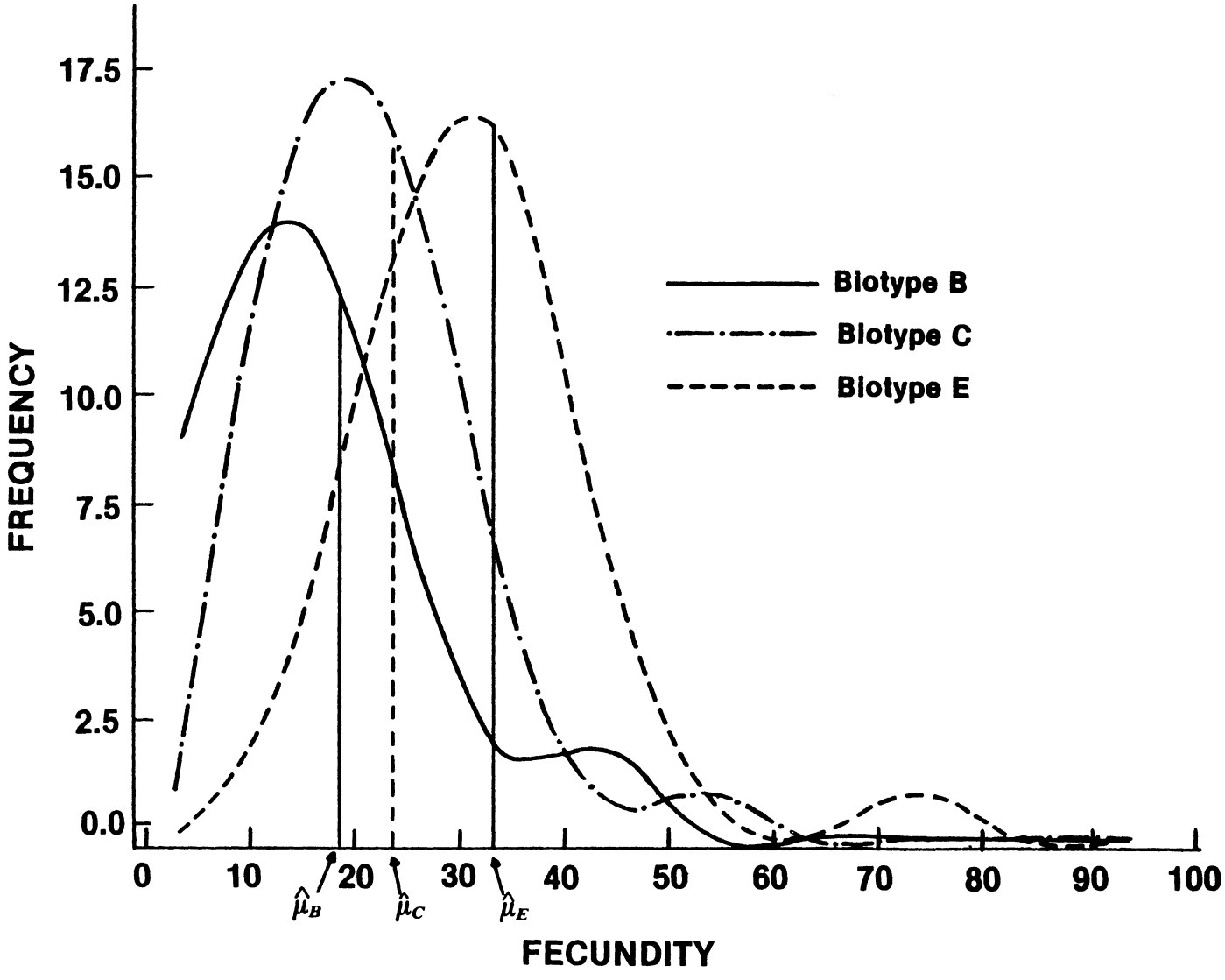

17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
FREQUENCY
0
10
20
30
40
50
60
70
80
90
100
FECUNDITY
$\hat{\mu}_B$
$\hat{\mu}_C$
$\hat{\mu}_E$
Biotype B
Biotype C
Biotype E

Figure 15. Variance in fecundity of biotypes reared on Wintermalt and Post barley. Vertical lines in the center of each block indicate the magnitude of variance at 95% confidence limits.

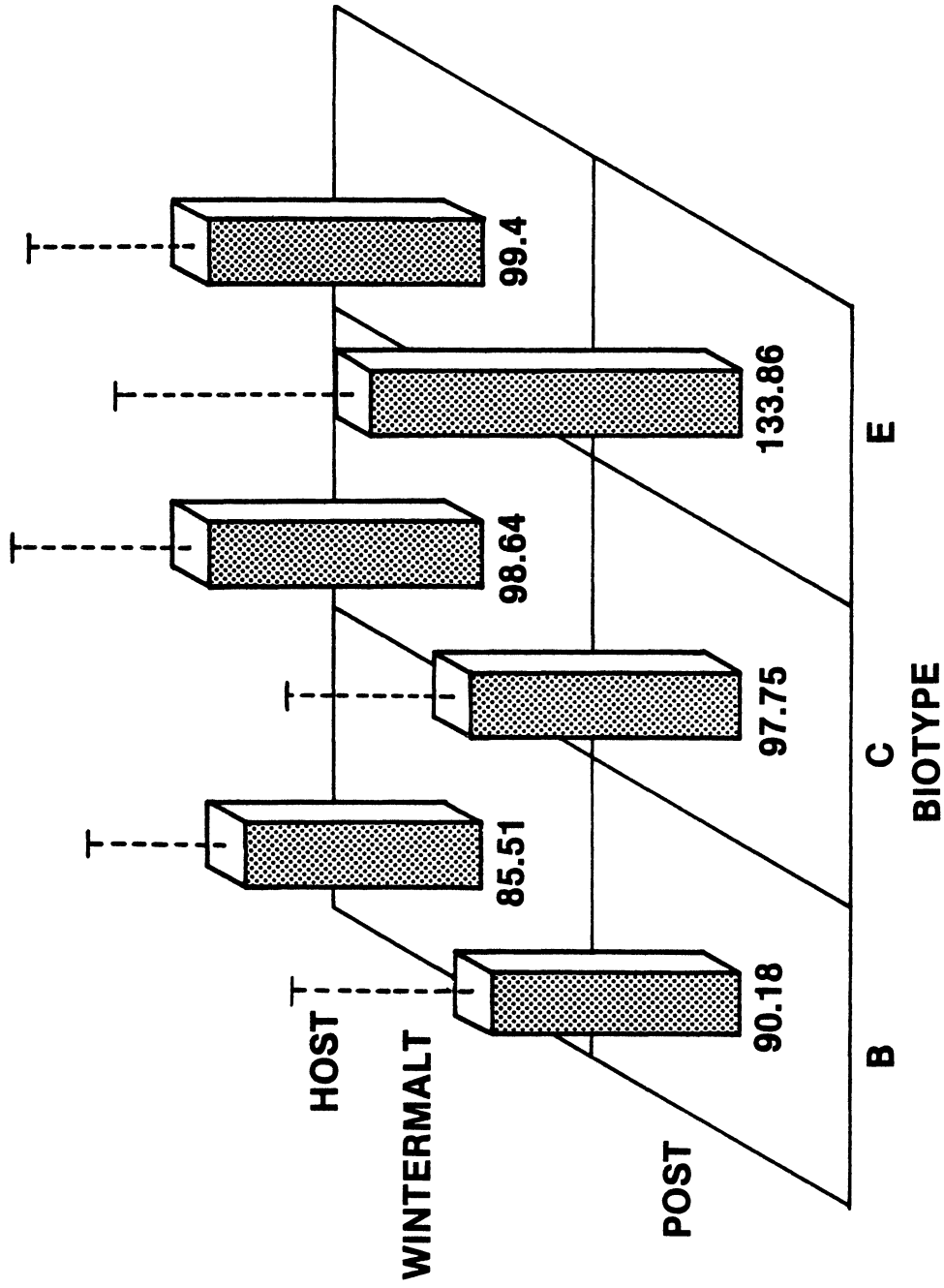
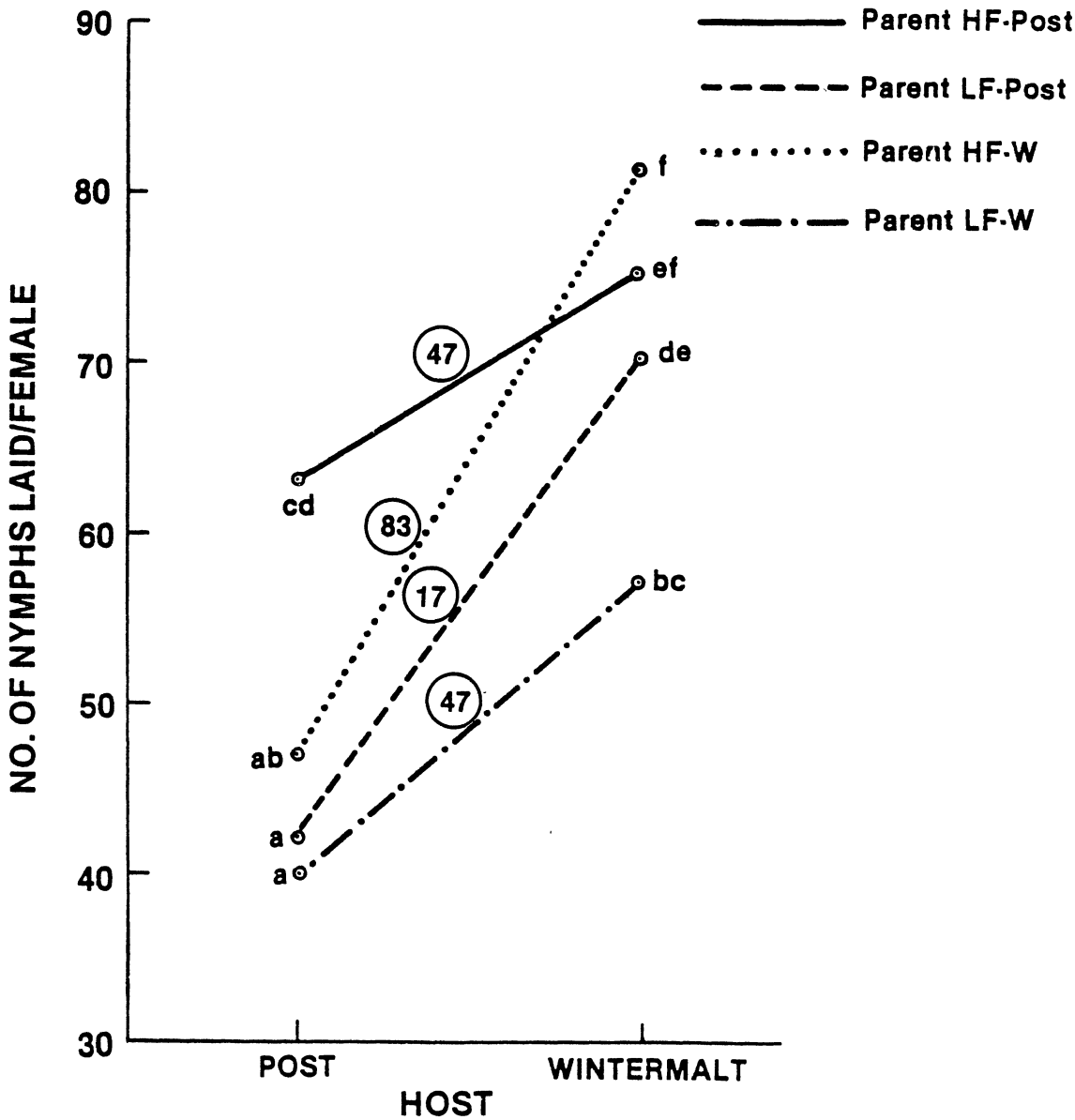

99.4
133.86
98.64
97.75
85.51
90.18
HOST
WINTERMALT
POST
B
C
E
BIOTYPE

Figure 16. Interaction among biotype E isolates and their hosts. Points at the end of each line indicate the mean fecundity of daughters on the respective host. Means followed by the same letters are not significantly different at P = 0.05 by Duncan's New Multiple Range Test. Encircled numbers indicate the fecundity of the respective parent. HF = extremely high fecundity; LF = extremely low fecundity; W = Wintermalt.


Parent HF-Post
Parent LF-Post
Parent HF-W
Parent LF-W
NO. OF NYMPHS LAID/FEMALE
90
80
70
60
50
40
30
POST
WINTERMALT
HOST
f
ef
de
bc
cd
ab
a
a
47
83
17
47

CHAPTER VI

VARIATION WITHIN CLONES AND THE RELATION TO GREENBUG LIFE HISTORY TACTICS

Introduction

Before the appearance of biotype C, the greenbug aestivated on wild grasses, especially wheat grass, Agropyron smithi (Rhyd.), but with the advent of biotype C in 1968, sorghum became its favorite summer host (Daniels and Chedester, 1980), and considerable damage occurred to this crop (Starks and Burton, 1977). This was an improvement in the life history tactics of the greenbug to oversummer effectively.

Kentucky bluegrass, like sorghum, has long been known as an incidental host of the greenbug in the USA and Canada (Webster and Phillips, 1912). Occasional damage had been reported by Webster and Phillips (1912), but in 1970, epidemic numbers of the greenbug caused a great deal of damage to this host (Street et al., 1978).

It is believed that in the USA, greenbugs overwinter in the adult and nymphal stages in the southern states (Lowe, 1952; Daniels and Chedester, 1980). The greenbug infestations in the north result from the dispersal of alates from Oklahoma and Texas on southerly winds (Lowe, 1952; Niemczyk, 1980). The adults and nymphs are more vulnerable to environmental hazards and also to natural enemies, thus the egg stage could be a better overwintering stage. The sexual cycle and overwintering in the egg stage in the USA has been suspected but not

confirmed (Mayo and Starks, 1972). Recently, Niemczyk and Power (1982) from Ohio and Potter (1982) from Kentucky reported that greenbugs overwinter in the egg stage on Kentucky bluegrass, with large numbers of eggs being found on bluegrass lawns. This is an improvement in the winter survival of the greenbug. Whether the greenbugs on Kentucky bluegrass are of the same biotype as those on small grains and sorghum is not clear, but it gives an insight about the adaptation and life history tactics occurring in greenbugs over time.

Stearns (1976) published a review on the life history tactics of different species. Similarly, Dixon and Dharma (1980), Ward and Dixon (1982), and Ward et al. (1983, 1983a) published on the reproductive investments of aphids based on the life history of the black bean aphid, Aphis fabae Scop., and of another aphid, Megoura viciae (Buckton). There appears to be no information on the reproductive investments of the greenbug in relation to its life history. Therefore, the objectives of the present research were to determine the key factors responsible for long-term evolutionary changes in the greenbug and its life history tactics, i.e., the variation within the greenbug and the genetic control of the parents over their progeny.

Materials and Methods

The greenbugs used in this experiment were biotype E from a greenhouse culture maintained on a mixture of 'Wheatland' sorghum and Wintermalt barley.

Formation of Clones

To develop clones, a single female was isolated from the colony and a culture of the progeny from this female was established on Wintermalt in a growth chamber programmed at 25°C during the day and 20°C at night with a 16-hr photophase. All tests were conducted in the same chamber under the same conditions. Under these environmental conditions, the greenbug reproduces parthenogenetically, and all progeny are females. When the culture was about two months old, five females were isolated from it, and their separate subcultures were established on Wintermalt. These females were designated as clones (A, B, C, D, and E, Fig. 17). From each subculture of clones, three nymphs were isolated. Each nymph was then placed on a Wintermalt seedling sown in a 7.6-cm diam pot. These nymphs are referred to as daughters (a1, a2, a3, ... e3, Fig. 17). The seedlings and nymphs were covered with clear plastic cages (6 cm diam and 30 cm high) with a cloth-covered top and two side vents (6.5 cm diam). The nymphs were observed daily to record the initiation of the reproductive phase, as their progeny were to be used in the following test.

Variation in Virulence

When each daughter began to reproduce (usually all on the same day) they were released on a Wintermalt seedling leaf laid horizontally on a filter paper lying on top of a petri dish (9 cm diam and 1.5 cm high), which was inverted in an aluminum pan (45.5 X 30.5 X 2.5 cm). Roots of seedlings in the aluminum pans were buried in sand. Glass rings (2.5 cm diam and 2.5 cm high) were used as cages to confine the aphids (Fig. 12). One end of each ring was covered with a plastic cap. After 24

hr (2nd day of the reproductive period), the daughters (a1, a2, ... e3) were removed from the leaf cages and placed individually back on their original host plants. The number of nymphs on Wintermalt seedlings from those daughters was reduced to four per cage. The leaf area enclosed within the cage was rated daily using a visual damage scale of 0 to 9, with 0 referring to no damage and 9 referring to necrosis of the caged leaf area. The experiment was continued for five days, at which time the nymphs had become adults and were used in the following fecundity test. This test would determine the virulence among the clones over time.

As mentioned previously, the daughters (a1, a2, ...) whose progeny were used in the virulence test were saved, and reared continuously. Nymphs produced by them were also removed on alternate days. Six days after the initiation of their reproductive phase, the daughters were again released on the experimental Wintermalt seedlings. After 24 hr, four nymphs from each daughter were then kept to repeat the test. The daughters were again transferred to a Wintermalt host plant. Eleven days after the initiation of the reproductive phase, the daughters were again used to produce four nymphs from each for another repetition of the virulence and fecundity tests. The sample of nymphs taken at different time intervals, i.e, 2nd, 7th and 12th day of the reproductive period of the parent, will hereafter be referred to as Phase I, Phase II, and Phase III, respectively. A flowchart of the tests is given in Fig. 17. The objective of the repetition over time was to determine whether the first-born individuals were more or less virulent than those born at the later stages of their mother's life.

Variation in Fecundity and Reproductive Period

Each maturing nymph from the previous experiment was released on a Wintermalt seedling in a 7.6-cm diam pot and covered with a clear plastic cage described previously. These aphids were reared until the end of their reproductive phase. The nymphs produced by each aphid were recorded and removed on alternate days. This test would determine the variation in fecundity among the clones over time (Fig. 17).

Results

Selection of Test Aphids

The progeny of each female was divided into three intervals over time, referred to as phases. This division was based upon a preliminary fecundity test conducted with 12 greenbugs. In this test, the nymphs produced by each female were removed and recorded daily. The average number of nymphs produced per day was calculated. The daily fecundity rate and the number of females contributing to daily reproduction are given in Fig. 18. It is evident that after the 12th day, the fecundity rate was less than four, and from the 8th to the 12th day of the reproductive phase, 1-3 females did not produce any nymphs. It is desirable to have a considerable number of offspring to simulate the group feeding in the virulence test (Dixon and Wratten, 1971). Further, greater number of offspring at this stage better represent the phases within the life of the parent and provide a legitimate error estimate for testing the differences between phases. Keeping in mind the reproductive period and the nymphs produced per day of the greenbug, a sample of four nymphs was selected at each phase. However, up until the

12th day, there is still a 25% chance that some daughters may not produce any nymphs (Fig. 18). This may decrease the reliability of the experiment because of the small sample size. If the number of nymphs selected for the virulence test is reduced, the number may be unsatisfactory for the subsequent fecundity test as the sample size becomes too small to represent the variation within the progeny of a female over time (phases). On the other hand, if the sample size is increased, then there is a need to decrease the interval between phases to have enough progeny per day. This may result in no significant differences among phases because of the short interval between them. Also there will be too many females to rear. To minimize the number of females, only three clones were used for determining the variation in fecundity, and four nymphs were selected at each phase from a female. In this way, there was a total of 108 aphids to be reared. Out of these, 14 were lost or died before the end of the test. However, for each phase within a female, there were at least three aphids remaining, thus providing a reasonable estimate of the fecundity of their progeny. The loss or death of aphids is an important point to be considered while deciding on the number of aphids to be reared for detecting the differences in phases.

Variation in Virulence

For testing variation in virulence, two types of response variables were used. One was the damage rating at the end of the experiment, and the other was the damage that occurred per day. For determining damage/day, daily damage ratings for each experimental unit were regressed against time (days) and the slope of the regression line was

analyzed as a response variable. Analysis of variance with a split plot arrangement (phases in subplots) was performed with both types of the response variables, but no significant differences ($P > 0.05$) could be found among the clones, daughters within the clones, phases, and phases within the clones.

Variation in Fecundity and Reproductive Period

The analysis of variance using a split-plot arrangement (phases in subplots) performed on the three response variables, i.e., fecundity, reproductive period, and daily fecundity rate, is given in Table XV. The analysis of variance performed on the fecundity of different clones could detect significant differences ($P < 0.05$) only among daughters within the clones [daughters (clones)] and among phases. There were no significant differences among the daughters of clone A (a1, a2, and a3) and those of clone B (b1, b2, and b3) where fecundity ranged from 82.6 to 89.09. However, in clone C, one daughter (c3) produced significantly ($P < 0.05$) fewer nymphs as compared to the other two (69 vs. 84-88, respectively). Thus the progeny of one female may vary in fecundity, but the chance is very low and was only 1 out of 9 in this experiment. The mean fecundity of offspring born in about the middle of the reproductive period of their mother (a1, a2, ... c3) was higher than that of those born in the beginning or near the termination of their reproductive period (Table XVI).

With the reproductive period, the only significant differences that could be detected were between the phases (Table XV). The nymphs born in the middle of the reproductive period of their mother had longer reproductive periods, followed by those born at about the termination of

their mother's reproductive period. The nymphs born in the beginning of their mother's reproductive period had the shortest reproductive periods. However, all three mean reproductive periods were significantly different ($P < 0.05$) from each other (Table XVI). By performing an analysis of variance on the response variable daily fecundity rate, significant differences could be detected among phases, phases within the clones [phase (clone)], and phase by daughter interaction within the clones [phase X daughter (clone)] (Table XV). Since the denominator (reproductive period) for daily fecundity rate was the lowest in phase I, this phase had the highest fecundity rate, which was significantly different ($P < 0.05$) from that of the other phases (Table XVI). Phases within the clones [phase (clone)] also indicated that in each clone the nymphs born in the beginning of the reproductive period of the parent (phase I) had a higher fecundity rate (Fig. 19).

The phase by daughter interaction within the clones for fecundity rate was significant at $P = 0.05$. Within clone A, the progeny of daughter a3 had a higher fecundity rate (6.8) in phase I, but it was not significantly different from those of a2 (5.53) and a1 (5.38) in phase I. However, the fecundity rate was significantly different from the progeny of all the daughters in phase II and III (fecundity rate ranged from 4.21 to 4.96). Within clone B, the progeny of daughter b3 had the highest fecundity rate (7.99) in phase I, and it was significantly different from those of all the other daughters in phase I (fecundity rate ranged from 5.52 to 6.56) and in phases II and III (fecundity rate ranged from 4.16 to 4.63). Within clone C, daughter c3 had the highest fecundity rate (6.28) in phase I, but the rate was not significantly different from that of c1 (5.25) or c2 (5.90). However, the rate was

significantly different from those of all the daughters in phase II and III (fecundity rate ranged from 3.88 to 4.88).

Discussion

With respect to virulence, no significant differences could be detected among the clones, daughters within the clones, phases, or phases within the clones, although the differences in damaged leaf areas were apparent. This may be because of the difficulty of grading the damage in the small leaf area. Estimation of chlorophyll reduction may yield some significant differences in future tests.

There were significant differences in fecundity, reproductive period, and the daily fecundity rate within the progeny of females when split over time. The nymphs born at the initiation of the reproductive phase of their mother were less fecund, had a shorter reproductive period, and as a result had a higher fecundity rate, as compared to those born in the middle or about the termination of the reproductive phase of their mother. The higher daily fecundity rate probably helps to increase the colony size in a shorter period of time. Group feeding and the presence of more nymphs may also enhance the fecundity of the parent. Dixon and Wratten (1971) demonstrated that black bean aphids reared singly on leaves were smaller and initially less fecund than those reared in small clusters because of the altered nutritional status of the leaves.

In the phase II greenbugs, the reproductive period was longer and the fecundity was also higher, but the fecundity rate was lower (because of the longer reproductive period) than those of phase I. Most likely, once the greenbug colony is established the progeny is spread over time

due to a longer reproductive period, which results in minimal competition and drainage of resources.

With the phase III greenbugs, the fecundity declined, but it was not significantly different from those of phase I. The reproductive period also declined slightly as compared to that of phase II (18.81 vs. 20.81), but it was significantly different from that of phase I greenbugs (18.81 vs. 13.26). At this time, it may be beneficial to the colony if the parent spreads its granddaughters over time and also decreases the fecundity to minimize the competition. By this time, the host plants are also maturing and becoming unsuitable for the greenbug, which is most destructive to the seedling stage (Starks and Burton, 1977a). Also, the parents may resorb the embryos as happens in another aphid, M. vicia (Ward and Dixon, 1982). With this aphid, it has been demonstrated that if the nutritional status of the mother becomes poor, the smallest embryos are resorbed, those of intermediate size cease to grow, and the largest ones continue to mature.

The change occurring in phases indicates the genetic control of the parent on the progeny of her daughters. Blackman (1979) reported that a parthenogenetic female aphid has a direct influence on the morphology of not only her daughters, but also on her granddaughters, some of which start their embryonic development even before their mothers are born. The morphology of an individual is the net product of the physiological processes going on within the body of that individual. Abebe (1983) determined the heritability of various reproductive variables and obtained significant heritability of offspring produced per day, indicating the genetic control of the parent over the fecundity of the daughters. He could not find any significant differences among the

phases with respect to prereproductive period, reproductive periods, fecundity, and the fecundity rate. Nonsignificant differences in fecundity between phases could be due to the small sample size, as he used only one aphid in each phase for a particular female. However, he found significant differences in the postreproductive period among phases. Offspring produced on the 14th day of the reproductive period of the mother lived longer than those produced on the 1st and 7th day of the mother's reproductive period. The significant postreproductive period also resulted in significant differences in longevity between phases. In the present studies, the postreproductive period was not observed, as this does not play a role in the life history tactics of the greenbug.

Leather (1982) reported that the apterous offspring of alate mothers of the cherry-oat aphid, Rhopalosiphum padi L., were more fecund, developed faster, and had higher mean relative growth rates than the apterous offspring of the apterous mothers. It is certain that the alates will spread their progeny more widely as compared to apterous aphids, thus the alate mothers should have higher fecundity and their progeny should possess a faster growth rate for successful colonization. Similarly, Dixon and Dharma (1980) reported that the offspring born to individuals of a particular morph or generation of the black bean aphid can vary in size, number, and distribution over time. Different morphs of most of the aphid species optimize rather than maximize their fecundity and rate of increase. To maximize its rate of increase, an aphid should develop fewer embryos but to a more advanced stage of development so that they can be born early in the mother's life. This occurs with gynoparae of the cherry-oat aphid. More specifically,

emigrants and gynoparae of Eriosoma, after settling on an appropriate host, produce all of their offspring in less than half an hour (see Dixon and Dharma, 1980).

The present studies have demonstrated tactical differences in the greenbug on a susceptible host. Since there is no selection pressure whatever, variability captured in this test is preprogrammed and under genetic control. This kind of study should be conducted with resistant hosts. Starks and Burton (1977b) reported that on resistant lines of barley, wheat, oats, and rye, the greenbugs were more mobile compared to their behavior on susceptible lines. The higher mobility on the resistant host suggests that the greenbug spreads its progeny in space, thus improving the chance of their survival by utilizing the nutritional variation within a plant. On the other hand, more aphids are produced in a short period of time on the susceptible host, but the mothers optimize the resources by controlling the fecundity and reproductive period of the daughters. The embryo count of the greenbugs reared on a resistant and susceptible host and the weight of the newly born nymphs should further clarify the life history tactics of the greenbug.

TABLE XV

ANALYSIS OF VARIANCE OF THE FECUNDITY, REPRODUCTIVE PERIOD, AND DAILY FECUNDITY RATE OF CLONES OVER TIME

Source of variation	d.f.	Mean squares		
		Fecundity	Reproductive period	Daily fecundity rate
Clones	2	277.95	1.36	1.72
Daughters (clones)	6	383.07*	10.56	0.83
Phases	2	1153.97**	475.66**	31.44**
Phases (clones)	4	261.19	25.23	1.48*
Phase X daughter(clones)	12	203.90	9.04	1.22*
Experimental error	67	134.98	10.21	0.51
Coefficient of variation		13.87%	18.09%	14.33%

*Significant at $P < 0.05$

**Significant at $P < 0.01$

TABLE XVI

MEAN FECUNDITY, REPRODUCTIVE PERIOD, AND DAILY FECUNDITY RATE OF THE GREENBUG PROGENY OVER TIME

	n	Fecundity	Reproductive period	Daily fecundity rate
		mean ± s.d.	mean ± s.d.	mean ± s.d.
Clone:*				
A	31	85.38 ± 9.75	17.87 ±3.65	4.92 ±0.91
B	32	85.59 ±13.34	17.65 ±5.27	5.22 ±1.48
C	31	80.32 ±17.10	17.45 ±4.65	4.75 ±0.99
Phase:				
I	31	78.42a ±9.69	13.26a ±3.08	6.13b ±1.17
II	32	90.59b ±11.40	20.81c ±3.79	4.42a ±0.56
III	31	82.13a ±16.72	18.81b ±2.70	4.36a ±0.64

*There were no significant differences ($P > 0.05$) among the clones as regards the three variables.

Means followed by the same letters in a column are not significantly different at $P = 0.05$.

Figure 17. Flowchart for testing the variability in virulence, fecundity, and reproductive period of clones over time.

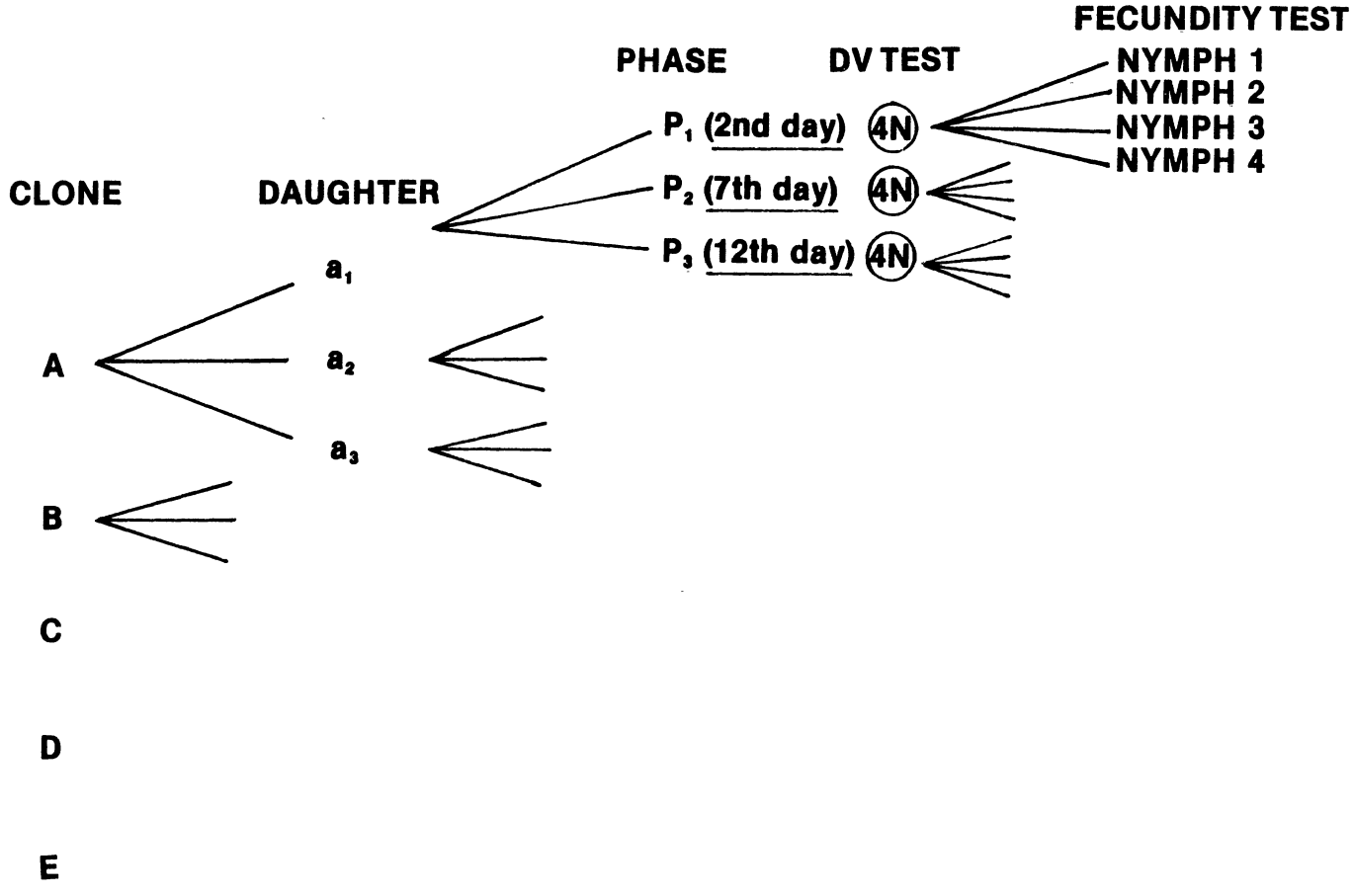

FECUNDITY TEST
PHASE
DV TEST
NYMPH 1
NYMPH 2
NYMPH 3
NYMPH 4
P1 (2nd day)
4N
P2 (7th day)
4N
P3 (12th day)
4N
CLONE
DAUGHTER
a1
a2
a3
A
B
C
D
E

Figure 18. Mean number of nymphs produced per day by the greenbug and the number of females contributing to reproduction over time.

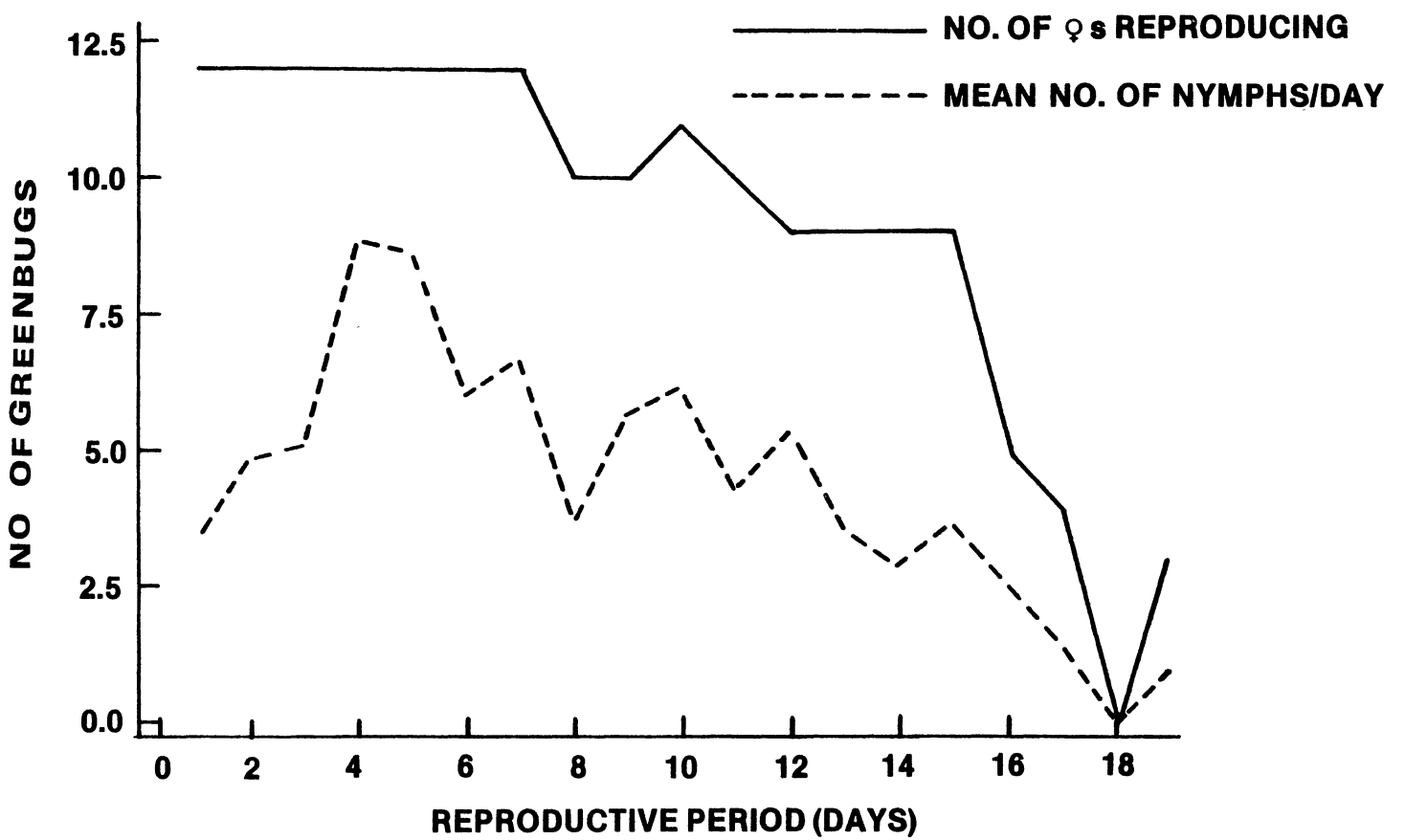
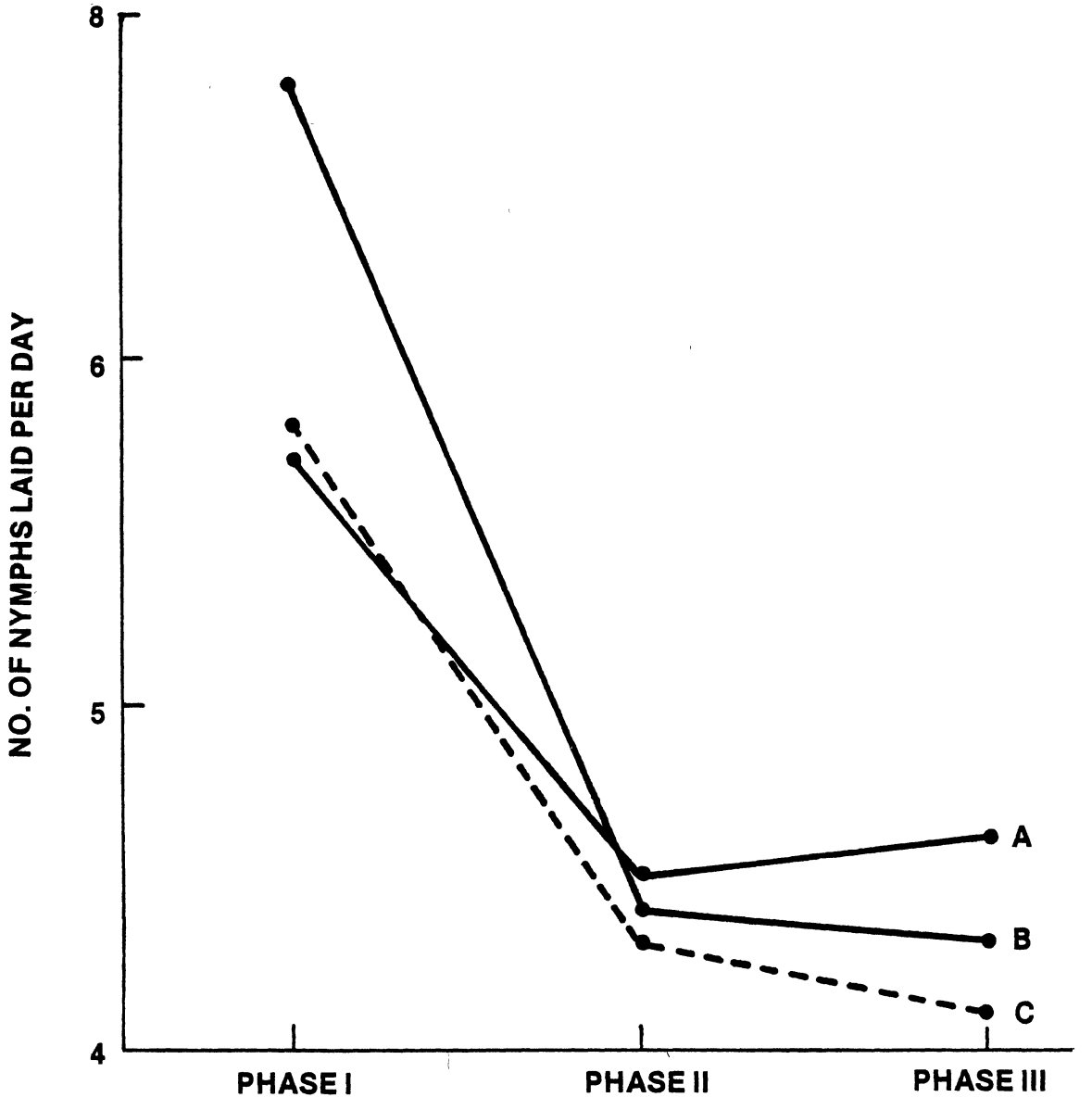

NO. OF ♀s REPRODUCING
MEAN NO. OF NYMPHS/DAY
NO OF GREENBUGS
12.5
10.0
7.5
5.0
2.5
0.0
0
2
4
6
8
10
12
14
16
18
REPRODUCTIVE PERIOD (DAYS)

Figure 19. Mean number of nymphs produced per day by clones at different stages of their life.


8
6
5
4
NO. OF NYMPHS LAID PER DAY
PHASE I
PHASE II
PHASE III
A
B
C

CHAPTER VII

MORPHOLOGICAL VARIATION WITHIN GREENBUG BIOTYPES AND THEIR EVOLUTION

Introduction

Of the five greenbug biotypes, biotype B is the only one which can be differentiated from the other biotypes. It has a dark body color and black-tipped cornicles. The identification of the remaining biotypes is based on either their ability to kill certain hosts or to withstand selected insecticides. Absence of any distinct morphological characteristics makes the identification of the different biotypes very difficult. In addition, determination of biotypes is often hampered by host plants dying due to extraneous factors, and there also may be substantial overlap in the reaction to different biotypes. It is clear that a morphological basis for determining greenbug biotypes is greatly needed.

Bey-Bienko (1958, see Saxena and Rueda, 1982) reported that in many organisms, changes in the ecological and physiological traits of the species are frequently followed by subtle changes in their morphological characteristics. Morphology is the end product of physiological activity and is initiated by the genome and modified by the environment (Eastop, 1973). Usually the morphological differences between recently evolved physiological groups are so minute that it is impossible to detect them. A biotype or group may not appear to be morphologically

different from the others if only one or just a few characters are considered singly; however, it may differ if a combination of characters in a multivariate space is considered. This concept has been used by Saxena and Rueda (1982) for the identification of biotypes of the brown planthopper and by Kim et al. (1967) for the identification of biotypes of the European corn borer, Ostrinia nubilalis (Hubner). This concept has also been used by Murdie (1969) for detecting size variation in the pea aphid, Acyrthosiphon pisum Harris; by Wool and Koach (1975) for detecting environmental variation in the gall-forming aphid, Geoica utricularia Passerni; and by Singh and Cunningham (1981) for detecting the morphological variation in seven species of aphids. Considerable research has been conducted on the morphology and instar identification of the greenbug (Saxena and Chada, 1971, 1971a, 1971b, 1971c; Sana and Schulz, 1967; Kirkland et al., 1981); however, no work has been done on the morphological differentiation of the greenbug biotypes.

The objectives of this study were to use multivariate analysis of variance and other related discriminatory techniques to differentiate between greenbug biotypes B, C, and E and to quantify the amount of variation present within each biotype to understand their pattern of evolution. Since cultures of biotypes A and D were not available, these were not included in this study.

Materials and Methods

The greenbugs used in this study were taken from greenhouse cultures maintained on Wintermalt barley. From the culture of each biotype, 40 apterous adults were isolated and released separately on Wintermalt seedlings in a 15-cm diam pot. The plants and the aphids

were enclosed in clear plastic cages (12 cm diam and 30 cm high) with cloth-covered tops and two side vents (7.8 cm diam). The pots were kept in a growth chamber programmed at 25°C during the day and 20°C at night with a 16:8 hr day:night regime. After about two hours or when about 40 nymphs of each biotype were produced, all the adults were removed. The nymphs were allowed to grow under the same temperature and photoperiod conditions for a period of five days. At this time, all the nymphs had matured and all were apterous females. The aphids were then killed in hot water (20°C) and left in Hoyer's mounting medium for a week and then mounted in the same medium. When the bodies of aphids clarified, their appendages, as listed in Table XVII, were measured with an ocular micrometer.

The data collected in this experiment were subjected to univariate analysis of variance for each response variable, multivariate analysis of variance (MANOVA), discriminant analysis, Fisher's canonical discrimination, and stepwise selection of variables. The computer analysis was performed using the Statistical Analysis System (SAS) (SAS Institute, 1982) and processed using an IBM 3081D computer.

Results

Simple Statistics

Standard deviations of all the characters (except head width) measured on biotype E greenbugs were higher than those of biotypes B and C. This resulted in higher coefficients of variation (CV) of all the characters of biotype E except head width and length of mesotibia (Fig. 20). The standard deviations of all morphological characteristics of biotype B (except length of two distal labial segments) were higher than

those of biotype C, thus coefficients of variation of all the characteristics (except length of the two distal labial segments) of biotype C were smaller when compared to the other biotypes (Fig. 20).

Pooled correlations among all the variables, except length of the two distal labial segments and cornicle width, were above 0.70 and were significant at $P < 0.01$. Correlations of length of two distal labial segments with the rest of the variables except cornicle width ranged from 0.37 to 0.46, and were also significant ($P < 0.01$). Correlations of cornicle width with the remaining variables were close to zero, and were not significant ($P > 0.05$). All correlations were positive, except those of cornicle width with lengths of mesotibia and metafemur, which were negative but near zero. Correlations between the characters within biotype B and E followed almost the same trend as the pooled correlations. Within biotype C, head width was not correlated with any other character, and a few other correlations also were not significant ($P > 0.05$).

Univariate Analysis

Analysis of variance using a Completely Randomized Design was performed on all the variables separately. Except cornicle width, all the characters showed significant differences ($P < 0.01$) among the biotypes. Duncan's New Multiple Range Test and Student-Newman-Keuls test were performed for multiple comparisons among the biotype means for each character. In all characters except cornicle width, the biotype E means were significantly different ($P < 0.01$) from those of biotypes B and C. Characters including the lengths of the first and fourth flagellum segments, profemur, mesofemur, mesotibia, metafemur, and

metatibia, separated the means of the three biotypes without any overlap and appear to be good discriminators (Table XVIII). With the exception of cornicle width, the remaining characters separated biotype E means from those of B and C, but could not distinguish between B and C (Table XVIII).

Multivariate Analysis of Variance

The MANOVA performed on all the 14 variables simultaneously indicated that the three biotypes are significantly different from each other. The MANOVA statistics with the significance probability are: Hotelling-Lawley trace, $F (28, 116) = 17.51$, $(P > F = 0.0001)$; Pillai's trace, F approximation $(28, 120) = 13.62$, $(P > F = 0.0001)$; Wilk's lambda, $F (28, 118) = 15.48$, $(P > F = 0.0001)$; and Roy's greatest root, $F (14, 60) = 28.14$, $(P > F = 0.0001)$. The first charactristic root was 6.56, and it explained 77.69% variation. The second characteristic root was 1.88, and it explained the remaining 22.31% variation. All the other characteristic roots were zero. The significant biotype effect illustrated in the MANOVA suggested that the data should be subjected to a discriminant analysis.

Discriminant Analysis

The discriminant model, also known as classification criterion, is based on a measure of generalized squared distance. The classification criterion is based either on the individual within-group covariance matrices or the pooled covariance matrix. In the present studies, the chi-square value for testing the homogeneity of variances was 405.24, and it was significant at the 0.1 level of probability. Thus

classification was based on within-group covariance matrices. Using this method, all the aphids were classified into their correct group, clearly indicating three different biotypes.

As a test of the above discriminant analysis, the classification of all the aphids was also tested by using the pooled covariance matrix, and again all the aphids of biotypes B and C were classified into their correct biotype group. Two individuals of biotype E were misclassified as biotype C. The misclassified individuals are shown in Fig. 21. The generalized squared distance from biotype B to C was 16.25, from biotype C to E 19.65, and from biotype B to E 39.99. The discrimination functions based on the pooled covariance matrix are given in Table XIX.

Canonical Discriminant Analysis

Canonical discriminant analysis is a dimension-reduction technique related to principal component analysis and canonical correlation. Given two or more groups of observations with measurements on several quantitative variables, canonical discriminant analysis derives a linear combination of the variables that has the highest possible multiple correlation with the groups. This maximal multiple correlation is designated as the first canonical correlation. The coefficients of the linear combination are designated as the canonical coefficients or canonical weights. The variable defined by the linear combination is the first canonical variable or canonical component. The second canonical correlation is obtained by finding the linear combination uncorrelated with the first canonical variable that has the highest possible multiple correlation with the groups. The number of canonical variables extracted equals the smaller value of the original variables

or the number of groups minus one (SAS Institute, 1982). In this research, there were two canonical variables (3 groups - 1).

The first adjusted canonical correlation was 0.91 (approximate standard error = 0.015), and it was highly significant [F statistic = 15.48, P > F (28, 118) < 0.0001)]. The second canonical correlation was 0.81 (approximate standard error = 0.04), and it was also significant [F statistic = 8.71, P > F (13, 60) < 0.0001)]. The characteristic roots and the amount of variability explained by each were already explained in the MANOVA section.

The biotype centeroids in the two-dimensional canonical variate are shown in Fig. 21. The 95% confidence intervals for the radii around the centeroids of biotypes B, C, and E were 0.33, 0.33, and 0.37, respectively. If circles were drawn around the centeroids, they would not overlap, which adds support to the significance of the three biotypes. The circles are not shown in Fig. 21 because of their extremely small radii. The Mahalanobis distance from biotype B to C was the shortest (4.03), followed by that from C to E (4.43). The distance from biotype B to E (6.32) was the greatest (Fig. 22). All these distances were significantly different from each other at P > Mahalanobis distance < 0.0001. A three-dimensional model of the Mahalanobis distance among the biotypes is illustrated in Fig. 23.

The standardized canonical coefficients are given in Table XX and shown graphically in Fig. 24. The first canonical variable gives maximum weight to the length of the metafemur, followed by that of mesotibia. The length of these two in Fig. 24 shows how they discriminate the biotypes, separating biotype E from the others. These two variables also separated the three biotypes in the univariate case

(Table XVIII). Lengths of the third flagellum segment, protibia, and cornicle contrasts to those of the metafemur and mesotibia and separated biotype B greenbugs from the others, but in univariate analyses they gave overlappings in biotype B and C means. Length of the metatibia is also a good discriminator of biotypes as it received a relatively higher weight in the second canonical variable. Lengths of the first and fourth flagellum segments received positive weights on the first canonical variable, but negative weights on the second canonical variable, and discriminate biotype C greenbugs from the others. Length of the mesofemur also discriminates biotype C. These three characteristics (lengths of the first and fourth flagellum segments, and mesofemur) produced no overlap in multiple comparisons in the univariate case. Relative weights of cornicle width in both the canonical variables are very low (Table XX), which is also shown by its short length in Fig. 24. This is the variable which did not detect any significant difference among biotypes in the univariate case.

The stepwise variable selection procedure (SAS Institute, 1982) also selected length of the mesotibia first, followed by second and fourth flagellum segments, metafemur, third flagellum segment, protibia, head width, and metatibia length, for discrimination among the biotypes. Of these, the lengths of mesotibia, fourth flagellum segment, metafemur, and metatibia did not result in any overlap in multiple comparisons in the univariate cases (Table XVIII), and it is evident in Fig. 24 how useful they are in separating the biotypes from one another.

Discussion

Qualitative morphological characters are convenient and useful for diagnostic purposes at the generic or specific levels. The availability of such characters becomes difficult when dealing with sibling or cryptic species, subspecies, host races, and biotypes (Saxena and Rueda, 1982). The variation of these taxa can be evaluated by the use of statistical techniques (Kim et al., 1967; Rochow and Eastop, 1966; Thottappilly et al., 1977; Saxena and Rueda, 1982).

This research has demonstrated that three biotypes of the greenbug represent morphologically distinct groups. Using discriminant functions based on within-group covariance matrices, all the aphids were correctly classified into their proper biotype group. However, when the pooled covariance matrix was used to develop the generalized squared distances, two biotype E individuals were misclassified into biotype C. The shortest Mahalanobis distance was between biotype B and C. This suggests that biotype B is more closely related to biotype C than it is to biotype E. This supports the hypothesis that biotype C evolved from B, and biotype E evolved from biotype C (B → C → E). Misclassification of biotype E greenbugs into the biotype C group (Fig. 21) is another indication that biotype E originated from biotype C.

Biotype B was first detected in 1958, followed by biotype C in 1968 and E in 1979. It is interesting to note that if these biotypes are plotted on a time scale based on the years elapsed between their detection, the shape of the plot is analogous to that based on the Mahalanobis distance (Fig. 22). Further, the ratio of the Mahalanobis distance between biotypes C and E (keeping the distance between biotype B and C as a unit), was the same as the ratio in the time scale between

these two biotypes (Fig. 22). The ratio of Mahalanobis distance between biotypes B and E is 1.57. This ratio in the time scale is 2.1. The difference in the two ratios between biotypes B and E is due to the fact that in the time scale, the ratio is based on one variable, i.e., time elapsed in the development, whereas the Mahalanobis distance is based on 14 variables. Moreover, there is no true record of the evolution of biotype E. It was first recorded in 1979 (Porter et al., 1982), but it may have been in existence in undetectable numbers in the field before its discovery. The analogous shapes of the two plots also suggest that biotype C evolved from biotype B, and E evolved from C. Kim et al. (1967) plotted the Mahalanobis distance between the four geographical (Missouri, Ohio, Iowa, and Montana) biotypes of the European corn borer, and the geographic distance between the localities. Interestingly, the shape of the plot based on the Mahalanobis distance and that of the geographical relation between the localities was the same, as in this case with the Mahalanobis distance and the greenbug biotype time scale.

The variance of all the morphological characteristics (except head width) of biotype E was higher than those of the other biotypes. Because of higher variance, the cluster of this biotype in the canonical variate plane was very loose (Fig. 21). The higher variability in this biotype suggests that if a new greenbug biotype were to occur, it would arise from this group. The cluster of biotype C in the canonical variate plane was comparatively tight due to low variability in the measured characteristics. The biological interpretation of this is difficult, but it could be that this biotype may perish in the future because of the low variability in it. Numbers of biotype A, B, and D are presumably undetectable in the field (Al-Mousawi et al., 1983), and

this may also occur with biotype C in the future. Kindler et al. (1984) reported that biotype E occurs in greater numbers than biotype C in the Great Plains. The areas of Texas where biotype E was originally detected and the panhandles of Oklahoma and Texas have virtually 100% biotype E. Clustering of biotype B is also loose when compared to that of biotype C, because of the comparatively higher variance in the characters. Nothing can be predicted about this biotype as it is no longer readily detectable in the field, and the aphids used in the present research were taken from a limited laboratory culture.

The discriminating function, based on the generalized squared distance using within-group covariance matrices, classified all the aphids into their correct biotype groups. This is possible because the lengths of first and second flagellum segments, profemur, mesofemur, mesotibia, metafemur, and metatibia differentiate the three biotypes in the univariate case. The method may be used for the classification of biotypes, but the size of the aphids is affected by many factors, especially density of the aphids on the host plant and the rearing temperature (Murdie, 1969). Dixon (1974) reported that in the case of large aphids such as the sycamore aphid, Drepanosiphum platanoides (Schr.), the appendages are larger and there are a greater number of rhinaria than in small aphids. In the seasonal succession of generations, aphids of the first generation have shorter appendages and fewer rhinaria than aphids of the same weight of the subsequent generations, even when reared at a constant temperature and on plants of the same age. Similar studies with alates of the greenbug may establish additional characters for the identification of biotypes. The role of

extraneous factors on the size of the aphid appendages should be taken into account while developing discriminatory functions.

TABLE XVII

MORPHOLOGICAL STRUCTURES MEASURED FOR EVALUATING DIFFERENCES AMONG APTEROUS ADULTS OF BIOTYPES

Structure
Head width
Length of first flagellum segment
Length of second flagellum segment
Length of third flagellum segment
Length of fourth flagellum segment
Length of two distal labial segments
Length of profemur
Length of protibia
Length of mesofemur
Length of mesotibia
Length of metafemur
Length of metatibia
Length of cornicle
Width of cornicle at the base

TABLE XVIII

ERROR MEAN SQUARES AND MEAN LENGTHS OF DIFFERENT MORPHOLOGICAL STRUCTURES OF APTEROUS ADULTS OF BIOTYPES

Structure (mm)	Error MS	Mean length of biotype:		
		B	C	E
Head (width)	0.0171	1.93a	2.01a	2.20b
First flagellum segment	0.1165	1.87a	2.16b	3.17c
Second flagellum segment	0.0435	1.34a	1.30a	1.94b
Third flagellum segment	0.0206	1.43a	1.38a	1.73b
Fourth flagellum segment	0.0934	4.27a	4.64b	5.16c
Two distal labial segments	0.0076	1.47a	1.45a	1.54b
Profemur	0.0732	2.51a	2.72b	3.46c
Protibia	0.1922	3.67a	3.92a	5.17b
Mesofemur	0.0621	2.43a	2.65b	3.39c
Mesotibia	0.1799	3.91a	4.31b	5.83c
Metafemur	0.1166	3.39a	3.70b	4.84c
Metatibia	0.4397	5.45a	5.99b	7.66c
Cornicle	0.0662	2.14a	2.17a	2.95b
Cornicle (width at the base)[1/]	0.0047	0.66	0.64	0.63

[1/] Not significant at P = 0.05.

Means followed by the same letters in a row are not significantly different at P = 0.01 by Duncan's New Multiple Range and Student-Newman-Keuls Tests.

TABLE XIX

LINEAR DISCRIMINANT FUNCTIONS FOR BIOTYPES, BASED ON THE POOLED COVARIANCE MATRIX

Character	Biotype		
	B	C	E
Constant	-349.48	-389.72	-423.15
Head (width)	119.49	128.64	134.27
First flagellum segment	- 38.24	- 31.55	- 28.63
Second flagellum segment	-110.86	-124.12	-120.02
Third flagellum segment	- 26.55	- 63.15	- 63.61
Fourth flagellum segment	53.60	67.49	60.59
Two distal labial segments	161.52	150.48	153.19
Profemur	- 5.27	- 9.41	- 16.55
Protibia	- 29.01	- 40.24	- 44.97
Mesofemur	- 36.78	- 34.27	- 42.94
Mesotibia	- 0.58	1.11	11.77
Metafemur	77.61	100.85	117.30
Metatibia	4.09	7.38	4.64
Cornicle	62.70	48.93	53.52
Cornicle (width at the base)	78.89	79.60	64.16

TABLE XX

STANDARDIZED CANONICAL COEFFICIENTS

Character	Canonical variable 1	Canonical variable 2
Head (width)	0.40	-0.14
First flagellum segment	0.97	-0.50
Second flagellum segment	-0.52	1.11
Third flagellum segment	-1.23	1.41
Fourth flagellum segment	0.55	-1.77
Two distal labial segments	-0.13	0.24
Profemur	-0.84	-0.19
Protibia	-1.95	1.04
Mesofemur	-0.44	-0.89
Mesotibia	1.73	1.30
Metafemur	4.34	-1.16
Metatibia	0.12	-1.21
Cornicle	-0.67	1.49
Cornicle (width at the base)	-0.16	-0.18

Figure 20. Coefficients of variation in body measurements of the biotypes.

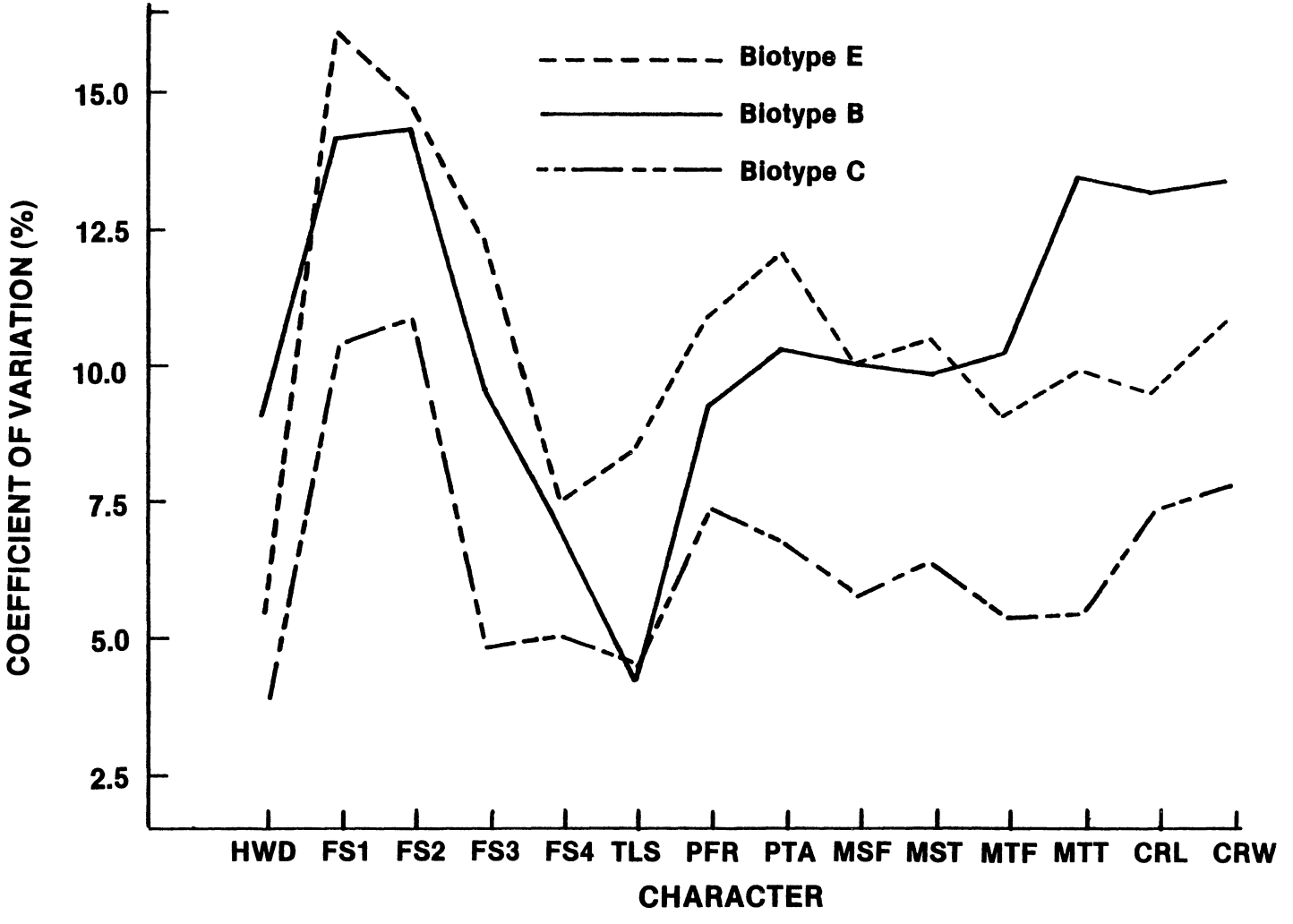

Biotype E
Biotype B
Biotype C
COEFFICIENT OF VARIATION (%)
15.0
12.5
10.0
7.5
5.0
2.5
HWD
FS1
FS2
FS3
FS4
TLS
PFR
PTA
MSF
MST
MTF
MTT
CRL
CRW
CHARACTER

Figure 21. Plot of individual greenbugs along the two axes of canonical discriminant space.

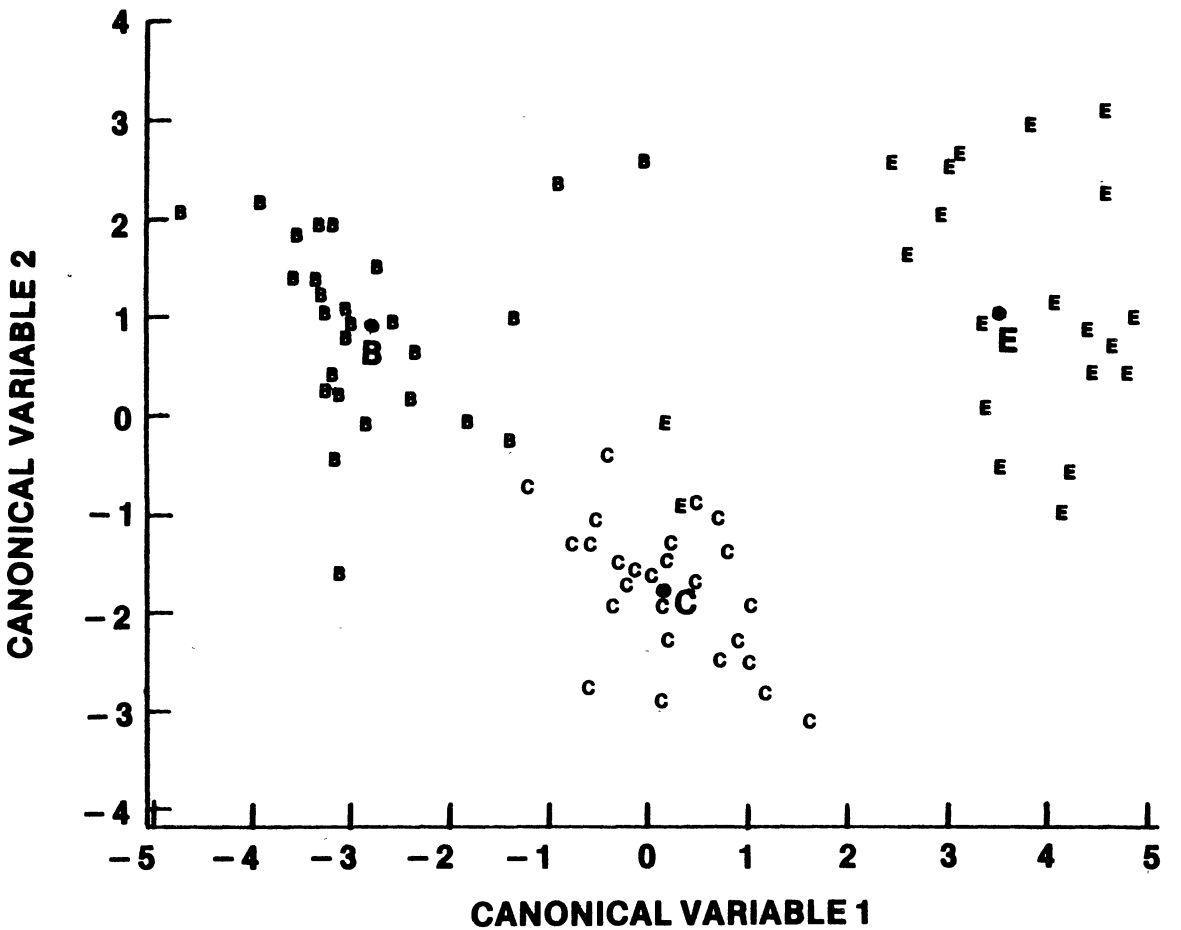
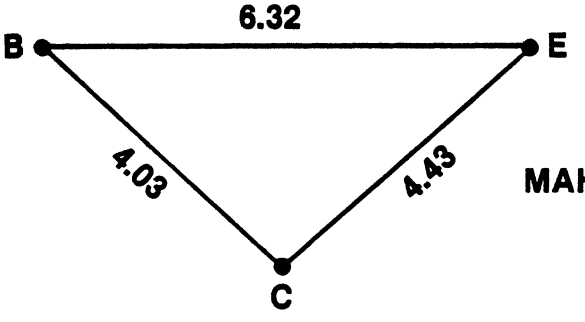
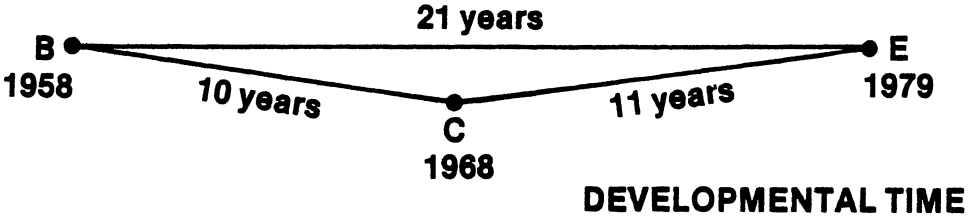

CANONICAL VARIABLE 2
CANONICAL VARIABLE 1
4
3
2
1
0
−1
−2
−3
−4
−5
−4
−3
−2
−1
0
1
2
3
4
5
B
E
C

Figure 22. Relationship between biotypes, based on the Mahalanobis distance and the time elapsed before their detection.



MAHALANOBIS DISTANCE



DEVELOPMENTAL TIME

Figure 23. Three-dimensional model of relationships of biotypes based on the Mahalanobis distance among them.

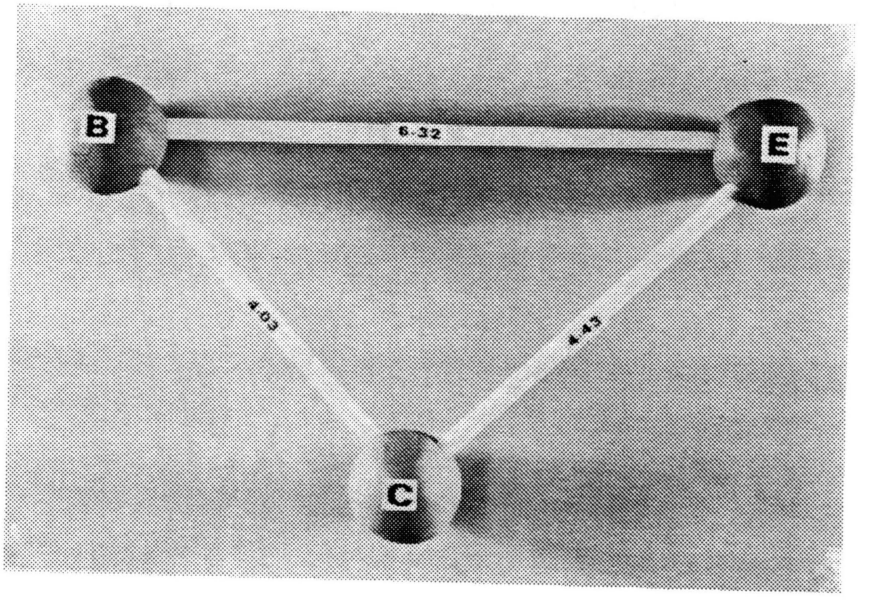
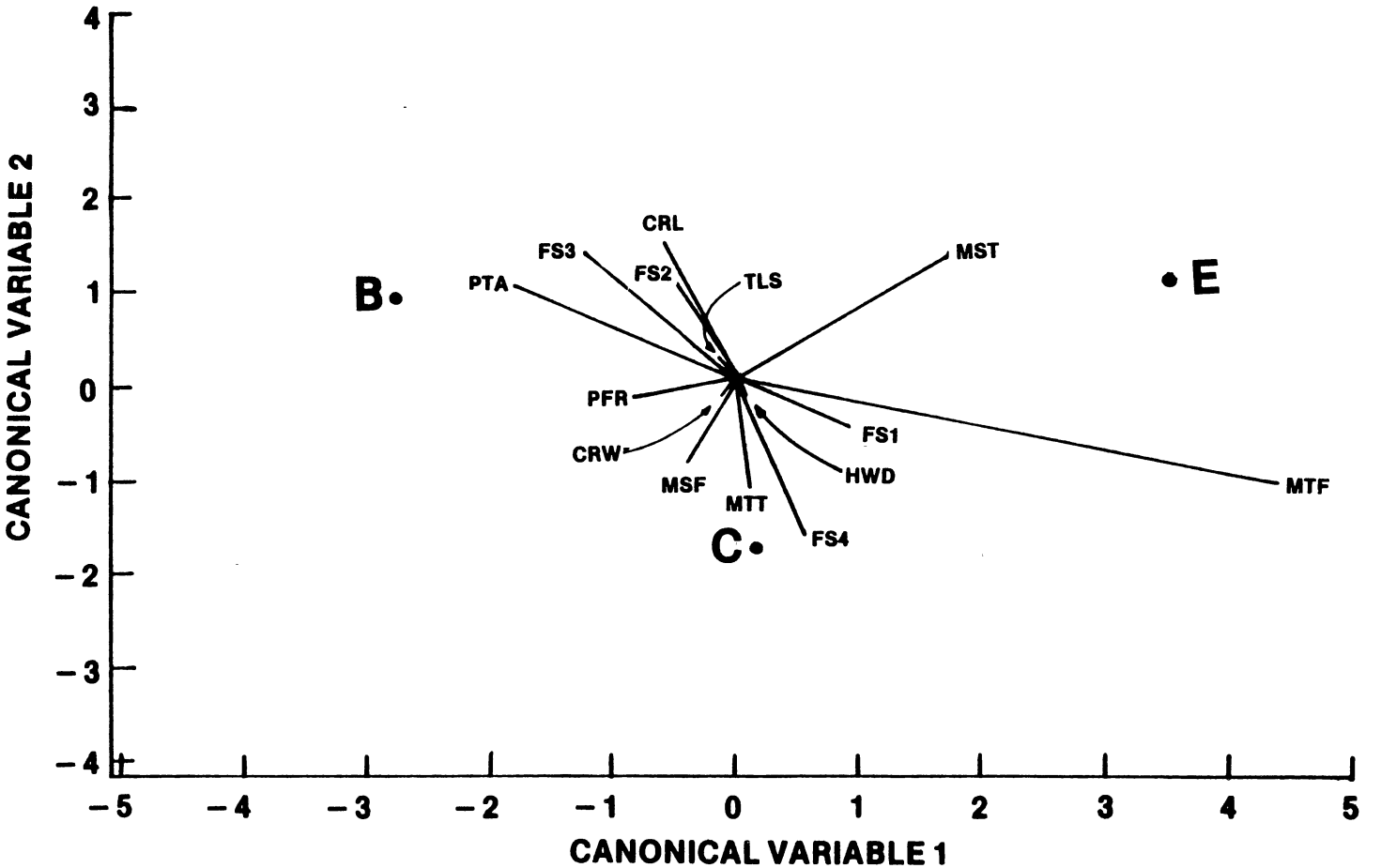

B
6.32
E
4.03
4.43
C

Figure 24. Two-dimensional canonical discriminant space showing group centeroids and vectors of variables.


CANONICAL VARIABLE 2
CANONICAL VARIABLE 1
4
3
2
1
0
−1
−2
−3
−4
−5
−4
−3
−2
−1
0
1
2
3
4
5
B
C
E
CRL
FS3
FS2
TLS
PTA
MST
PFR
CRW
MSF
MTT
FS4
FS1
HWD
MTF

CHAPTER VIII

COMPONENTS OF BIOTYPE B RESISTANCE IN LARGO, AMIGO, AND TAM W-101 WHEAT

Introduction

Following the occurrence of greenbug biotypes which damaged previously known sources of resistance in wheat, Sebesta and Wood (1978) transferred the resistance in Gaucho triticale (Wood et al., 1974) to wheat, which resulted in the resistant wheat germplasm line Amigo. Amigo was resistant to all known biotypes of the greenbug until Porter et al. (1982) detected biotype E.

At the same time, Joppa et al. (1980) developed another greenbug-resistant entry designated as Largo, an amphiploid between 'Langdon' (Triticum turgidum L., durum group, 2n = 28) and PI 268210 (T. taushii (Coss) Schmal, 2n = 14). This entry is resistant to the currently predominant greenbug biotypes C and E and is being utilized as a source of resistance in wheat (Porter et al., 1982). Harvey et al. (1980) also reported resistance to biotype C in T. tauschii entries.

Biotype B is no longer easily found in the field (Al-Mousawi et al., 1983; Kindler et al., 1984), although it was the predominant biotype in the late fifties and early sixties and killed the 'DS 28A' wheat selection which was resistant to biotype A. In tests with laboratory cultures of biotype B, Largo was killed, indicating that this new source of resistance is susceptible to this biotype.

The objectives of this research were to confirm the susceptibility of Largo to biotype B, and to study the nature of this susceptibility by comparing the resistance components of Largo with those of Amigo and Tam W-101.

Materials and Methods

For evaluating antixenosis, the three test entries, i.e., Largo, OK 78047--a wheat line with the Amigo gene, and Tam W-101, were randomized and planted in a circular pattern about 2 cm from the edge of a 10-cm diam pot. There was one plant of each entry in each pot. When the plants were about 5-8 cm tall, 30 apterous greenbugs were released on the soil in the center of each pot. The plants and the aphids were then covered with clear plastic cages (9.0 cm diam and 30 cm high) with a cloth-covered top and two side vents (6.5 cm diam). The aphids were allowed 48 hrs to select the plant of their choice, at which time the numbers on each plant were recorded.

There were 10 replications in this and the following tests, and the plants were randomized in a growth chamber programmed at 25°C during the day and 20°C during night and a 16:8 hr day:night regime using a Randomized Complete Block Design (RCBD).

For evaluating antibiosis, two seeds of the test entries were planted in 7.6-cm diam pots, and after germination thinned to one seedling per pot. Individual first-leaf stage plants were infested with 3-5 apterous adults of the greenbug. The plants were then covered with clear plastic cages (6 cm diam and 30 cm high) with a cloth-covered top and two side vents (6.5 cm diam). When reproduction began, the adults were removed, leaving five nymphs on each plant. The nymphs were

allowed to develop on the test plant until they matured and began to reproduce. At this time, all aphids except one were removed from the plants. The nymphs were removed from the plants on alternate days, and their numbers were recorded until the adults stopped reproducing.

For evaluating tolerance, seedlings, about 13 cm in height, were infested with 10 apterous aphids/plant. One set (10 seedlings of each entry with one seedling per pot) was infested, and another set was kept as control. All the plants were covered with plastic cages described earlier in the antibiosis section. The plants were observed daily to remove or add aphids to maintain 10 adults per plant. The plants were also visually rated daily using a damage scale of 0 to 9. The test was continued for eight days.

Another test was designed to simultaneously measure the role of the three components of resistance of the entries and to compare these entries with other standard host differentials of biotype B. The hosts used in this test in addition to Largo, the Amigo line, and Tam W-101, were DS 28A wheat, Post and Wintermalt barley, CI 1580 oats, PI 264453 sorghum, and 'Dex' and 'Piper' sudangrass. Thirty seeds of each test host were sown in a row in a metal flat (51 X 35 X 9 cm). Entries were assigned to a given row in the flat by randomization. When the seedlings were about 5-7 cm tall, greenbugs were uniformly distributed over them so there was an average density of about 10 greenbugs per plant. This test was conducted in a growth chamber programmed for the same conditions as the previous tests. The plants were observed daily for a period of 18 days, and the number of plants that died were recorded for each entry.

Results

The number of greenbugs on the Amigo plants in the antixenosis test ranged from 1 to 5, whereas those on Largo, and Tam W-101 ranged from 2 to 13 and 3 to 23, respectively. Because of this variation, the variances in antixenosis of the Amigo line, Largo, and Tam W-101 were 1.87, 20.62, and 45.43, respectively. The data were transformed by the square-root transformation to stabilize the variance and then subjected to analysis of variance using a RCBD. However, the analysis of original as well as the transformed data produced similar results. The mean number of greenbugs settled on Tam W-101 plants was highest, followed by Largo and Amigo. All of these means were significantly different ($P < 0.05$) from each other (Table XXI).

There were significantly ($P < 0.05$) fewer nymphs produced per female on the Amigo line as compared to Largo and Tam W-101. The numbers of nymphs produced on Largo and Tam W-101 were almost equal and not significantly different ($P < 0.05$) from each other (Table XXI).

The tolerance test also indicated that the Amigo line was resistant to biotype B. On Amigo, the final damage rating was the lowest and significantly different ($P < 0.05$) from that of Largo and Tam W-101. Largo and Tam W-101 did not significantly differ ($P > 0.05$) from each other (Table XXI). The antixenosis, antibiosis, and tolerance indices for the three cultivars are given in Table XXI.

These three tests demonstrated that Largo possesses a low level of antixenosis to biotype B as compared to the susceptible Tam W-101 which has virtually no antixenosis, antibiosis, or tolerance, and to the Amigo line which has a high level of all of these resistance components. The

Host Plant Resistance Index derived from the three components was also highest for Amigo (38.15), followed by Largo (2.25) and Tam W-101 (1.0).

In the flat test, in which all three components of resistance interact simultaneously, all plants of Tam W-101, Wintermalt, DS 28A, and CI 1580 oats died after 5-8 days of infestation. Plants of Largo started to die after the 5th day of infestation, and all had died by the 10th day. However, none of the Post barley, PI 264453 sorghum, Piper or Dex sudangrass plants died. In another similar flat test, CI 4888 oats was included to test it against biotype B. It was found to be resistant, with only 5 out of 15 plants dying. Wilson et al. (1978) also reported resistance to biotype B in CI 4888 oats.

Discussion

Mortality of CI 1580 oats and DS 28A wheat, and the black-tipped cornicles of the greenbugs used in the present tests indicated that the greenbugs were biotype B (Starks and Burton, 1977a). The survival of CI 4888 oats after being subjected to this biotype provided additional confirmation (Wilson et al., 1978). The laboratory culture of biotype B was established from greenbugs previously collected from Stillwater, Okla. However, it is not from the original colony that killed DS 28A wheat in 1958. Thus, it may not be the same "B" as reported by Wood (1961), although its appearance and host reaction are quite similar to his descriptions.

Recent surveys conducted in the Great Plains indicated that biotype A, B, and D are presently not readily detectable in the field (Al-Mousawi et al., 1983; Kindler et al., 1984). However, a critical and constant evaluation of the greenbug biotypes in the field is needed.

If biotype B again become dominant in the field populations, or any of the abundant biotypes, such as C or E, revert back to B, wheat lines possessing the Largo gene would be susceptible. A similar type of reversion occurred in the rice brown planthopper (Claridge and Den Hollander, 1982; Pathak and Heinrichs, 1982). However, a high level of resistance to biotype B is available in lines possessing the Amigo genes.

TABLE XXI

RESISTANCE RESPONSE OF THREE WHEAT CULTIVARS TO BIOTYPE B

Resistance component	Wheat cultivars			Error MS
	Largo	Tam W-101	Amigo	
Antixenosis				
Mean no. of adults/plant	7.20b	13.10c	1.90a	16.1
Index (normalized) (A)	0.55	1.0	0.14	
Antibiosis				
Mean no. of nymphs/female	54.80b	55.50b	26.70a	83.7
Index (normalized) (B)	0.99	1.0	0.48	
Tolerance				
Damage rating*	3.30b	4.10b	1.60a	0.9
Index (normalized) (C)	0.80	1.0	0.39	
Host Plant Resistance Index = 1/(ABC)	2.29	1.0	38.15	

*Visually rated at 0-9 scale; 0=healthy, 9=dead.

Means followed by the same letter in a row are not significantly different at $P < 0.01$ by Duncan's New Multiple Range Test.

CHAPTER IX

SUMMARY AND CONCLUSIONS

The greenbug, Schizaphis graminum (Rondani), is a cosmopolitan pest of small grains and sorghum. In the USA, extensive damage is caused by this pest every year. Principal control strategies for greenbugs involve the use of insecticides and resistant varieties. Unfortunately, both of these control measures have resulted in the development of biotypes in the greenbug. Since the recognition of the greenbug as a damaging pest in 1882, more than 19 outbreaks have occurred in the USA. A serious 1976 outbreak in Oklahoma caused a loss of about $80 million (Starks and Burton, 1977). Since then, work on greenbug control in Oklahoma has intensified. The present research was conducted to determine the relationships among the greenbug biotypes and reasons for their development. At the same time, efforts were also made to improve the efficiency and reliability of the existing techniques for testing the resistance components used not only in the detailed evaluation of plant germplasm, but also in determining how biotypes react when subjected to various germplasm lines.

Antixenosis tests are often conducted in the greenhouse. But in the greenhouse and even in the growth chamber, the distribution of the greenbugs on the plants of the same entries was found not to be random. The distribution may be affected by the direction of light and air flow. Tests conducted in the dark showed more uniform distribution of

greenbugs on the plants; therefore, it is recommended that the antixenosis tests be designed carefully to eliminate the extraneous factors.

The antibiosis tests are often conducted in growth chambers, and it was suspected that the variation in light, air flow, and temperature within the chambers might have an effect on the efficiency of the tests. This research indicated that in antibiosis tests there is no row-to-row or column-to-column variation within the chambers used in these tests. This was attributed to similarities in the microclimates of the greenbugs, which remain in close proximity to the plants throughout their lives. This permits the use of simple statistical designs for antibiosis tests in the growth chambers. Based on the different combinations of replications and subsamples, a coefficient of variation table and power of the test curves were developed for planning of future experiments.

The three components of resistance, i.e., antixenosis, antibiosis, and tolerance, are all important in plant resistance. These components are interrelated and complement each other. To develop a standard technique for estimating the degree of resistance in a variety, antixenosis, antibiosis, and tolerance indices were developed. Based on these indices, a host plant resistance index (HPRI) was derived. The HPRI is easier to interpret than interpreting the three components separately.

For determining the reasons for the development of biotypes, emphasis was placed on determining the nature and amount of variability present in the greenbug and the contribution of variability in the long-term evolutionary process. On a resistant host, Post barley,

biotype E was higher in fecundity than biotypes B and C: its variance in fecundity was about 1.3 times higher than the other biotypes. Its fecundity distribution curve was positively skewed, and there was a small hump on the positive side of the distribution tail. This suggests that this biotype may eventually overcome the resistance in Post.

Fecundity and virulence tests conducted with greenbug cultures of biotypes B, C, and E indicated that they possess certain genotypes which have high fecundity and are more virulent as compared to the others. The presence of such type(s) of variation in a parthenogenetic species is of utmost significance. The presence of high fecund and more virulent greenbug genotypes in the greenhouse cultures suggests that the field populations may be more variable than greenhouse cultures.

Prior to the development of biotype C in 1968, the greenbug aestivated on wild grasses, primarily wheatgrass, and to a lesser extent on volunteer small grains in the Great Plains. Biotype C adapted to an economic summer host (sorghum), and a great deal of damage began to occur to this host in 1968 (Starks and Burton, 1977; Daniels and Chedester, 1980). Prior to the early 1970's, the greenbug overwintered in the nymphal and adult stages. The greenbug is known to annually pass through the sexual and asexual cycles of reproduction in other parts of the world (Mitic-Muzina and Srdic, 1977; Barbulescu, 1980; Kushnerik, 1981), but in North America, its sexual cycle has been suspected but not confirmed (Mayo and Starks, 1972). The sexual forms of the greenbug were reported by Wadley (1931), but apparently no one has observed the hatching of greenbug eggs in the USA, so the importance of sexual reproduction in the life history of the greenbug remains unclear. Since 1970, a large number of greenbugs have been observed on Kentucky

bluegrass, and overwintering in Ohio and Kentucky has been reported in the egg stage (Niemczyk, 1980; Niemczyk and Power, 1982; Potter, 1982). These are the improvements in the life history tactics of the greenbug which have developed over time. Whether the greenbugs on Kentucky bluegrass are of the same biotypes as those on small grains and sorghum is not yet known. The variation in the life cycle in different geographic areas illustrates the plasticity that the greenbug possesses to survive under diverse climatic conditions.

The occurrence of biotypes in aphids is aided by their shorter life cycle, reproduction through parthenogenesis as well as through sexual morphs (Eastop, 1973). The sexual morphs provide new, and perhaps more virulent, gene combinations. On the other hand, parthenogenesis enhances the chance of survival of the species and also the mate finding because of the production of large numbers of aphids. Parthenogenesis also permits the pest to have an appropriate genetic match-up with its host and spread rapidly without disrupting the valuable gene complex with sexual reproduction (Whitham et al., 1984). Thus a single greenbug with virulent genes may develop into a new biotype.

In the USA, the greenbug is also known to attack sugarcane and transmit sugarcane virus (Ingram et al., 1939). In the USSR, in addition to small grains, the greenbug also severely damages rice (Myrzin and Shilovskii, 1983). A survey for the greenbug on rice, maize, and sugarcane in the USA is recommended. This may reveal some new biotypes or subspecies of the S. graminum complex. Further, the greenhouse cultures for evaluating resistant germplasm should be periodically replaced with field populations to keep the laboratory

testing of germplasm updated with the changes occurring in greenbug biotypes in the field.

The Mahalanobis distance, based on the body measurements of the greenbugs, was shortest between biotypes B and C. The distance between biotype B and E was the highest. This suggests that biotype E is closely related to biotype C, and that biotype C is closely related to biotype B. Biotype B was first reported in 1958, followed by C in 1968, and E in 1979. The plot of time elapsed between the development of biotypes is analogous to the Mahalanobis distance plot. This also suggests that biotype E evolved from C and that biotype C evolved from B. In terms of variation in the measured characters, biotype E was more variable compared to the other biotypes. Higher variation in the morphology, fecundity, and virulence in biotype E indicates that a new biotype may evolve from this biotype in the future.

LITERATURE CITED

Abebe, M. 1983. Parthenogenetic Reproduction and its Inheritance in Schizaphis graminum (Rondani). (M.S. thesis, Oklahoma State University.)

Ali, A. W. M., and M. M. Rizk. 1979. Studies on the abundance and dispersion dynamics of cereal aphids on wheat in the Arab Republic of Egypt. Arch. Phyto. Pflanz. 15: 323-329. (In German with English summary.)

Al-Mousawi, A. H., P. E. Richardson, and R. L. Burton. 1983. Ultrastructural studies of greenbug (Hemiptera: Aphididae) feeding damage to susceptible and resistant wheat cultivars. Ann. Entomol. Soc. Am. 76: 964-971.

Apablaza, J. U., and A. G. Robinson. 1967. Preference of the greenbug, the english grain aphid, and the corn leaf aphid among barley, wheat, or oats in a growth room. J. Econ. Entomol. 60: 1467-1468.

Barbulescu, A. 1976. Resistance of small-grain cereals and sorghum to the attack of cereal greenbug Schizaphis graminum Rond. Prob. Prot. Plant. 4: 63-85. (In Romanian with English summary.)

Barbulescu, A. 1980. The development of the morphological forms of the species Schizaphis graminum Rond. within the framework of its biological cycle. Anal. Inst. Cerc. Cereale Plante Tehn. 45: 391-397. (In Romanian with English summary.)

Batschelet, E. 1981. Circular statistics in biology. Academic Press, New York.

Berger, P. H., R. W. Toler, and K. F. Harris. 1983. Maize dwarf mosaic virus transmission by greenbug biotypes. Plant Dis. 67: 496-497.

Blackman, R. L. 1974. Life-cycle variation of Myzus persicae (Sulz.) (Hom., Aphididae) in different parts of the world, in relation to genotype and environment. Bull. Entomol. Res. 63: 595-607.

Blackman, R. L. 1979. Stability and variation in aphid clonal lineages. Biol. J. Linn. Soc. 11: 259-277.

Blakley, N. 1982. Biotic unpredictability and sexual reproduction: Do aphid genotype-host genotype interactions favor aphid sexuality. Oecologia 52: 396-399.

Brown, H. D. 1971. Distribution and importance of the wheat aphid, Schizaphis graminum (Rond.), in South Africa. J. Entomol. Soc. S. Afr. 34: 425-429.

Brown, H. D. 1972. The suitability of some crops to infestation by the wheat aphid, Schizaphis graminum (Homoptera: Aphididae). Entomol. Exp. Appl. 15: 128-138.

Campbell, B. C., D. L. Mclean, M. G. Kinsey, K. C. Jones, and D. L. Dreyer. 1982. Probing behavior of the greenbug (Schizaphis graminum, biotype C) on resistant and susceptible varieties of sorghum. Entomol. Exp. Appl. 31: 140-146.

Chang, R., C. R. Ward, and D. Ashdown. 1980. Spread and characterization of chemical resistance of biotype D greenbugs in Texas. J. Econ. Entomol. 73: 458-461.

Chaudhary, J. P., M. Ramzan, and A. S. Atwal. 1969. Preliminary studies on the biology of wheat aphids. Indian J. Agric. Sci. 39: 672-675.

Claridge, M. F., and J. Den Hollander. 1982. Virulence to rice cultivars and selection for virulence in populations of the brown planthopper, Nilaparvata lugens. Entomol. Exp. Appl. 32: 213-221.

Claridge, M. F., and J. Den Hollander. 1983. The biotype concept and its application to insect pests of agriculture. Crop Prot. 2: 85-95.

Clark, W. C. 1973. The ecological implications of parthenogenesis. In: A. D. Lowe (ed.) Perspectives in aphid biology. Bull. Entomol. Soc. N. Z. 2: 103-113.

Commonwealth Institute of Entomology. 1963. Distribution maps of pests. Series A (Agric.). Toxoptera graminum (Rondani), Map No. 173. Commonw. Inst. Entomol. Lond. 2 pp.

Curtis, B. C., A. M. Schlehuber, and E. A. Wood, Jr. 1960. Genetics of greenbug (Toxoptera graminum Rond.) resistance in two strains of common wheat. Agron. J. 52: 599-602.

Dahms, R. G. 1972. The role of host plant resistance in integrated insect control, pp 152-167. In: M. G. Jotwani and W. R. Young (eds.), Control of sorghum shoot fly. Oxford and IBH, New Delhi.

Dahms, R. G., T. H. Johnston, A. M. Schlehuber, and E. A. Wood, Jr. 1955. Reaction of small-grain varieties and hybrids to greenbug attack. Okla. Agric. Exp. Stn. Tech. Bull. T-55, 61 pp.

Daniels, N. E. 1981. Migration of greenbugs in the Texas Panhandle in relation to their biotypes. Texas Agric. Exp. Stn. MP-1487, 4 pp.

Daniels, N. E., and L. D. Chedester. 1980. Winter survival of greenbugs in the Texas Panhandle. Texas Agric. Exp. Stn. MP-1447, 4 pp.

Danks, H. V. 1983. Extreme individuals in natural populations. Bull. Entomol. Soc. Am. 29: 41-46.

Dixon, A. F. G. 1971. The life-cycle and host preferences of the bird cherry-oat aphid, Rhopalosiphum padi L., and their bearing on the theories of host alternation in aphids. Ann. Appl. Biol. 68: 135-147.

Dixon, A. F. G. 1974. Changes in the length of the appendages and the number of rhinaria in young clones of the sycamore aphid, Drepanosiphum platanoides. Entomol. Exp. Appl. 17: 1-8.

Dixon, A. F. G., and T. R. Dharma. 1980. 'Spreading of the risk' in developmental mortality: size, fecundity and reproductive rate in the black bean aphid. Entomol. Exp. Appl. 28: 301-312.

Dixon, A. F. G., and S. D. Wratten. 1971. Laboratory studies on aggregation size and fecundity in the black bean aphid, Aphis fabae Scop. Bull. Entomol. Res. 61: 97-111.

Dreyer, D. L., and B. C. Campbell. 1984. Association of the degree of methylation of intercellular pectin with plant resistance to aphids and with induction of aphid biotypes. Experentia 40: 224-226.

Eastop, V. F. 1973. Biotypes of aphids. In: A. D. Lowe (ed.), Perspectives in aphid biology. Bull. Entomol. Soc. N. Z. 2: 40-51.

Fedosimov, O. F., and D. Tsedev. 1970. The useful insects of Mongolia. Zash. Rast. 15: 51. (In Russian with English summary.)

Flattery, K. E. 1982. An assessment of pest damage of grain sorghum in Botswana. Exp. Agric. 18: 319-328.

Franzmann, B. A. 1973. Field studies of a Schizaphis on pangola grass in North Queensland. J. Agric. Anim. Sci. 30: 85-89.

Frazer, B. D. 1972. Population dynamics and recognition of biotypes in the pea aphid (Homoptera: Aphididae). Can. Entomol. 104: 1729-1733.

Gallun, R. L., and G. S. Khush. 1980. Genetic factors affecting expression and stability of resistance, pp. 63-85. In: F. G. Maxwell, and P. R. Jennings (eds.), Breeding plants resistant to insects. John Wiley, New York.

Gavarra, M. R., and A. L. Eloja. 1969. Further studies on the aphid vectors of the abaca mosaic virus: II. Experimental transmission of the abaca mosaic virus by Schizaphis cyperi (van der Gott) and S. graminum Rondani. Philip. J. Pl. Indus. 34: 89-96.

Gomez, K. A., and A. A. Gomez. 1984. Statistical procedures for agricultural research, 2nd ed. John Wiley & Sons, New York.

Gould, F. 1983. Genetics of plant-herbivore systems: interactions between applied and basic study, pp 599-653. In: R. F. Denno, and M. S. McClure (eds.), Variable plants and herbivores in natural and managed systems. Academic Press, New York.

Grafius, J. E. 1964. A geometry for plant breeding. Crop Sci. 4: 241-246.

Griot, M. 1944. Observaciones sobre el pulgon verde de los cereales y su parasite Aphidius platensis en Santa Fe. Rev. Argent. Agron. 11: 309-319.

Hamid, S. 1983. Natural balance of graminicolous aphids in Pakistan. Agronomie 3: 665-673.

Harvey, T. L., and H. L. Hackerott. 1969. Recognition of a greenbug biotype injurious to sorghum. J. Econ. Entomol. 62: 776-779.

Harvey, T. L., T. J. Martin, and R. W. Livers. 1980. Resistance in synthetic hexaploid wheats derived from Triticum taushii. J. Econ. Entomol. 73: 387-389.

Hatchett, J. H., and B. S. Gill. 1981. D-genome sources of resistance in Triticum taushii to hessian fly. J. Heredity 27: 126-127.

Haynes, D. L., and S. H. Gage. 1981. The cereal leaf beetle in North America. Ann. Rev. Entomol. 26: 259-287.

Helsel, D. B., and N. M. Cowen. 1983. Optimum sample size for measurement of leaf area in oats (Avena sativa L.). Cereal Res. Commun. 11: 43-49.

Hisada, M. 1972. Azimuth orientation of the dragonfly (Sympetrum), pp 511-512. In: S. R. Galler, K. Schmidt-Koenig, G. J. Jacobs, R. E. Belleville (eds.), Animal orientation and navigation. U.S. Govt. Print. Office, Washington, D.C.

Ingram, J. W., W. E. Haley, and L. J. Charpentier. 1939. Insect vectors of sugarcane mosaic in continental United States. Proc. Int. Soc. Sug. Tech. 6: 483-494.

Jeger, M. J. 1980. Multivariate models of the components of partial resistance. Prot. Ecol. 2: 265-269.

Johnson, J. W., G. L. Teetes, and C. A. Schaefer. 1976. Greenhouse and field techniques for evaluating resistance of sorghum cultivars to the greenbug. Southw. Entomol. 1: 150-154.

Joppa, L. R., R. G. Timian, and N. D. Williams. 1980. Inheritance of resistance to greenbug toxicity in an amphiploid of Triticum turgidum/T. taushii. Crop Sci. 20: 343-344.

Kieckhefer, R. W. 1983. Host preferences and reproduction of four cereal aphids (Hemiptera: Aphididae) on certain wheatgrasses, Agropyron spp. Environ. Entomol. 12: 442-445.

Kieckhefer, R. W. 1984. Cereal aphid (Homoptera: Aphididae) preferences for and reproduction on some warm-season grasses. Environ. Entomol. 13: 888-891.

Kieckhefer, R. W., and W. N. Stoner. 1978. Preferences of four cereal aphids for certain range grasses. Environ. Entomol. 7: 617-618.

Kim, K. C., H. C. Chiang, B. W. Brown, Jr. 1967. Morphometric differences among four biotypes of Ostrinia nubilalis (Lepidoptera: Pyralidae). Ann. Entomol. Soc. Am. 60: 796-801.

Kindler, S. D., S. M. Spomer, T. L. Harvey, R. L. Burton, and K. J. Starks. 1984. Status of biotype-E greenbugs (Homoptera: Aphididae) in Kansas, Nebraska, Oklahoma, and Northern Texas during 1980-1981. J. Kansas Entomol. Soc. 57: 155-158.

Kirkland, R. L., I. D. Peries, and G. C. Hamilton. 1981. Differentiation and developmental rate of nymphal instars of greenbug reared on sorghum. J. Kansas Entomol. Soc. 54: 743-747.

Kogan, M., and E. F. Ortman. 1978. Antixenosis--a new term proposed to define Painter's "Nonpreference" modality of resistance. Bull. Entomol. Soc. Am. 24: 175-176.

Komblas, K. N., and W. H. Long. 1972. Field studies of aphid vectors of sugarcane mosaic. J. Econ. Entomol. 65: 439-445.

Kontev., C. 1976. Principal enemies of wheat and their control in People's Republic of Bulgaria. Prob. Prot. Plant. 4: 153-159.

Kushnerik, V. M. 1981. Biology and injuriousness of cereal aphids. Zash. Rast. 10: 43. (In Russian.)

Kvenberg, J. E., and P. A. Jones. 1974. Comparison of alate offspring produced by two biotypes of the greenbug. Environ. Entomol. 3: 407-408.

Leather, S. R. 1982. Preliminary studies on the effect of host age and aphid generation on the reproduction and survival of the bird cherry-oat aphid, Rhopalosiphum padi (L.). Ann. Agric. Fenn. 21: 13-19.

Leather, S. R., and A. F. G. Dixon. 1982. Secondary host preferences and reproductive activity of the bird cherry-oat aphid, Rhopalosiphum padi. Ann. Appl. Biol. 101: 219-228.

Lee, C., and J. O. Rawlings. 1982. Design of experiments in growth chambers--uniformity trials in the North Carolina State University Phytotron. Crop Sci. 22: 551-558.

Lowe, A. E. 1952. Evidence of the overwintering of greenbugs in southwestern Kansas. J. Kansas Entomol. Soc. 25: 3-4.

Lowe, H. J. B. 1984. A behavioural difference amongst clones of the grain aphid Sitobion avenae. Ecol. Entomol. 9: 119-122.

Lowe, H. J. B. 1984a. Development and practice of a greenhouse screening technique for resistance of wheat to the aphid Sitobion avenae. Ann. Appl. Biol. 104: 297-305.

Lowe, H. J. B. 1984b. The assessment of populations of the aphid Sitobion avenae in field trials. J. Agric. Sci. Camb. 102: 487-497.

Madden, L. V. 1980. Quantification of disease progression. Prot. Ecol. 2: 159-176.

Markkula, M., and K. Roukka. 1970. Resistance of plants to the pea aphid Acyrthosiphon pisum Harris (Hom., Aphididae). I. Fecundity of the biotypes on different host plants. Ann. Agric. Fenn. 9: 127-132.

Mayo, Z B Jr., and K. J. Starks. 1972. Sexuality of the greenbug, Schizaphis graminum, in Oklahoma. Ann. Entomol. Soc. Am. 65: 671-675.

Mayo, Z B Jr., and K. J. Starks. 1972a. Chromosome comparisons of biotypes of Schizaphis graminum to one another and to Rhopalosiphum maidis, R. padi, and Sipha flava. Ann. Entomol. Soc. Am. 65: 925-928.

Mayo, Z B Jr., and K. J. Starks. 1974. Temperature influences on alary polymorphism in Schizaphis graminum. Ann. Entomol. Soc. Am. 67: 421-423.

Mitic-Muzina, N., and Z. Srdic. 1977. Distribution, food-plants and injuriousness of the greenbug (Schizaphis graminum Rond., Homoptera, Aphidoidea) in Yugoslavia. Zast. Bilja 28: 389-401. (In Croat with English summary.)

Moffatt, J. M., and W. D. Worrall. 1983. Greenbug biotype distribution in the Texas Rolling Plains. Proc. 16th Hard Red Winter Wheat Workers Conf.: 23-24.

Montllor, C. B., Campbell, B. C., and T. E. Mittler. 1983. Natural and induced differences in probing behavior of two biotypes of the greenbug, Schizaphis graminum, in relation to resistance in sorghum. Entomol. Exp. Appl. 34: 99-106.

Moran, N. 1981. Intraspecific variability in herbivore performance and host quality: a field study of Uroleucon caligatum (Homoptera: Aphididae) and its Solidago hosts (Asteraceae). Ecol. Entomol. 6: 301-306.

Moran, N. 1983. Seasonal shifts in host usage in Uroleucon gravicorne (Homoptera: Aphididae) and implications for the evolution of host alternation in aphids. Ecol. Entomol. 8: 371-382.

Muddathir, K. 1976. Studies on the biology of wheat aphids in the Gezira (D. R. Sudan). Beitr. Entomol. 26: 465-470.

Murdie, G. 1969. Some causes of size variation in the pea aphid, Acyrthosiphon pisum Harris. Trans. R. Entomol. Soc. Lond. 121: 423-442.

Myrzin, A. S., and V. N. Shilovskii. 1983. The reaction of the varieties to infestation by aphids. Zash. Rast. 12: 32-33. (In Russian.)

Nault, L. R., H. J. Harlan, and W. R. Findley. 1971. Comparative susceptibility of corn to aphid and mechanical inoculation of maize dwarf mosaic virus. J. Econ. Entomol. 64: 21-23.

Niemczyk, H. D. 1980. New evidence indicates greenbug overwinters in North. Weeds Trees Turf 19: 64, 80.

Niemczyk, H. D., and K. T. Power. 1982. Greenbug buildup in Ohio is linked to overwintering. Weeds Trees Turf. 21: 36.

Odum, H. T. 1983. Systems ecology: an introduction. John Wiley & Sons, New York.

Ossiannilsson, F. 1948. Ett for vart land nytt skadedjur pa gras. Vaxtskyddsnotiser 5: 79-80.

Painter, R. H. 1951. Insect resistance in crop plants. The Macmillan Co., New York.

Painter, R. H., and D. C. Peters. 1956. Screening wheat varieties and hybrids for resistance to the greenbug. J. Econ. Entomol. 49: 546-548.

Pathak, P. K., and E. A. Heinrichs. 1982. Selection of biotype populations 2 and 3 of Nilaparvata lugens by exposure to resistant rice varieties. Environ. Entomol. 11: 85-90.

Peters, D. C., E. A. Wood, Jr., and K. J. Starks. 1975. Insecticide resistance in selections of the greenbug. J. Econ. Entomol. 68: 339-340.

Pettersson, J. 1971. Studies on four grass-inhabiting species of Schizaphis (Hom.: Aph.). III. (a) Host plants. Swedish J. Agric. Res. 1: 133-138.

Plumb, R. T. 1983. Barley yellow dwarf virus--a global problem, pp 185-198. In: R. T. Plumb, and J. M. Thresh (eds.) Plant virus epidemiology: the spread and control of insect-born viruses. Blackwell Sci. Pub., Oxford.

Porter, K. B., G. L. Peterson, and O. Vise. 1982. A new greenbug biotype. Crop Sci. 22: 847-850.

Potter, D. A. 1982. Greenbugs on turfgrass: an informative update. Am. Lawn Appl. 3: 20-25.

Puterka, G. J., J. E. Slosser, and E. C. Gilmore. 1982. Biotype C and E greenbugs: distribution in the Texas Rolling Plains and damage to four small grain varieties. Southw. Entomol. 7: 4-8.

Rautapaa, J. 1966. The effect of the english grain aphid Macrosiphum avenae (F.) (Hom., Aphididae) on the yield and quality of wheat. Ann. Agric. Fenn. 5: 334-341.

Rochow, W. F., and V. F. Eastop. 1966. Variation within Rhopalosiphum padi and transmission of barley yellow dwarf virus by clones of four aphid species. Virology 30: 286-296.

Rogers, C. E., R. D. Eikenbary, and K. J. Starks. 1972. A review of greenbug outbreaks and climatological deviations in Oklahoma. Environ. Entomol. 1: 664-668.

Sana, D. L., and J. T. Schulz. 1967. Identity of instars of apterous viviparae of Rhopalosiphum fitchii (Sanderson) and Toxoptera graminum (Rondani), (Aphididae, Homoptera). Pak. J. Sci. Res. 19: 108-112.

SAS Institute. 1981. SAS/Graph user's guide, 1981 ed. SAS Institute, Inc., Cary, North Carolina.

SAS Institute. 1982. SAS user's guide: statistics, 1982 ed. SAS Institute, Inc., Cary, North Carolina.

Saxena, P. N., and H. L. Chada. 1971. The greenbug, Schizaphis graminum. 1. Mouth parts and feeding habits. Ann. Entomol. Soc. Am. 64: 897-904.

Saxena, P. N., and H. L. Chada. 1971a. The greenbug, Schizaphis graminum. 2. The salivary gland complex. Ann. Entomol. Soc. Am. 64: 904-912.

Saxena, P. N., and H. L. Chada. 1971b. The greenbug, Schizaphis graminum. 3. Digestive system. Ann. Entomol. Soc. Am. 64: 1031-1038.

Saxena, P. N., and H. L. Chada. 1971c. The greenbug, Schizaphis graminum. 4. Central and stomatogastric nervous systems. Ann. Entomol. Soc. Am. 64: 1038-1044.

Saxena, R. C., and A. A. Barrion. 1983. Cytological variation among brown planthopper biotypes 1, 2, and 3. Int. Rice Res. Newsl. 8: 14-15.

Saxena, R. C., and L. M. Rueda. 1982. Morphological variation among three biotypes of the brown planthopper Nilaparvata lugens in the Philippines. Insect Sci. Appl. 3: 193-210.

Schuster, D. J., and K. J. Starks. 1973. Greenbugs: components of host-plant resistance in sorghum. J. Econ. Entomol. 66: 1131-1134.

Schweissing, F. C., and G. Wilde. 1978. Temperature influence on greenbug resistance of crops in the seedling stage. J. Econ. Entomol. 71: 831-834.

Schweissing, F. C., and G. Wilde. 1979. Temperature and plant nutrient effects on resistance of seedling sorghum to greenbug. J. Econ. Entomol. 72: 20-23.

Schweissing, F. C., and G. Wilde. 1979a. Predisposition and nonpreference of greenbug for certain host cultivars. Environ. Entomol. 8: 1070-1072.

Sebesta, E. E., and E. A. Wood, Jr. 1978. Transfer of greenbug resistance from rye to wheat with x-rays. Agron. Abstr. 70: 61-62.

Silveira, G. A., and J. E. Conde. 1945. El pulgon verde de los cereales on al Uruguay [Toxoptera (=Schizaphis) graminum Rondani]. Rev. Fac. Agron. Univ. Montevideo, No. 41, 54 pp.

Simon, J. P., M. A. Parent, and J. L. Auclair. 1982. Isozyme analysis of biotypes and field populations of the pea aphid, Acyrthosiphon pisum. Entomol. Exp. Appl. 32: 186-192.

Singh, S. M., and T. K. Cunningham. 1981. Morphological and genetic differentiation in aphids (Aphididae). Can. Entomol. 113: 539-550.

Smith, H. S. 1941. Racial segregation in insect populations and its significance in applied entomology. J. Econ. Entomol. 34: 1-13.

Starks, K. J., and R. L. Burton. 1977. Preventing greenbug outbreaks. USDA, ARS Leaflet No. 309, 11 pp.

Starks, K. J., and R. L. Burton. 1977a. Greenbugs: determining biotypes, culturing, and screening for plant resistance with notes on rearing parasitoids. USDA, ARS, Tech. Bull. No. 1556, 12 pp.

Starks, K. J., and R. L. Burton. 1977b. Greenbugs: a comparison of mobility on resistant and susceptible varieties of four small grains. Environ. Entomol. 6: 331-332.

Starks, K. J., R. L. Burton, and O. G. Merkle. 1983. Greenbugs (Homoptera: Aphididae) plant resistance in small grains and sorghum to biotype E. J. Econ. Entomol. 76: 877-880.

Starks, K. J., and O. G. Merkle. 1977. Low level resistance in wheat to greenbug. J. Econ. Entomol. 70: 305-306.

Starks, K. J., and D. E. Weibel. 1981. Resistance in bloomless and sparse-bloom sorghum to greenbugs. Environ. Entomol. 10: 963-965.

Starks, K. J., E. A. Wood, Jr., and G. L. Teetes. 1973. Effects of temperature on the preference of two greenbug biotypes for sorghum selections. Environ. Entomol. 2: 351-354.

Starks, K. J., E. A. Wood, Jr., and D. E. Weibel. 1972. Nonpreference of a biotype of the greenbug for a broomcorn cultivar. J. Econ. Entomol. 65: 623-624.

Stearns, S. C. 1976. Life history tactics: a review of the ideas. Quart. Rev. Biol. 51: 3-47.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics, a biometrical approach, 2nd ed. McGraw-Hill Book Co., New York.

Street, J. R., R. Randell, and G. Clayton. 1978. Greenbug damage found on Kentucky bluegrass. Weeds Trees Turf 17: 26.

Teetes, G. L. 1980. Breeding sorghums resistant to insects, pp 457-485. In: F. G. Maxwell, and P. R. Jennings (eds.), Breeding plants resistant to insects. John Wiley & Sons, New York.

Teetes, G. L., C. A. Schaefer, J. R. Gipson, R. C. McIntrye, and E. E. Latham. 1975. Greenbug resistance to organophosphorus insecticides on the Texas High Plains. J. Econ. Entomol. 68: 214-216.

Teetes, G. L., C. A. Schaefer, and J. W. Johnson. 1974. Resistance in sorghums to greenbug; laboratory determination of mechanisms of resistance. J. Econ. Entomol. 67: 393-396.

Thottappilly, G., V. F. Eastop, and J. E. Bath. 1977. Morphological variation within Acyrthosiphon pisum and inability to transmit broad bean severe chlorosis virus. Entomol. Exp. Appl. 22: 29-34.

Twinn, C. R. 1932. Summary of insect conditions in Canada, in 1932. 23rd-24th Ann. Rep. Quebec Soc. Prot. Pl. 1930-32: 149-168.

Vanderplank, J. E. 1982. Host-pathogen interactions in plant disease. Academic Press, New York.

Wadley, F. M. 1931. Ecology of Toxoptera graminum, especially as to factors affecting importance in the northern United States. Ann. Entomol. Soc. Am. 24: 325-395.

Walker, A. L., D. G. Bottrell, and J. R. Cate, Jr. 1972. Bibliography on the greenbug, Schizaphis graminum (Rondani). Bull. Entomol. Soc. Am. 18: 161-173.

Walker, P. T. 1954. The influence of climate on an outbreak of wheat aphid in Kenya. Emp. J. Exp. Agric. 22: 293-304.

Walters, K. F. A., and A. F. G. Dixon. 1983. Migratory urge and reproductive investment in aphids: variation within clones. Oecologia 58: 70-75.

Ward, S. A., and A. F. G. Dixon. 1982. Selective resorption of aphid embryos and habitat changes relative to life-span. J. Anim. Ecol. 51: 859-864.

Ward, S. A., P. W. Wellings, and A. F. G. Dixon. 1983. The effect of reproductive investment on pre-reproductive mortality in aphids. J. Anim. Ecol. 52: 305-313.

Ward, S. A., A. F. G. Dixon, and P. W. Wellings. 1983a. The relation between fecundity and reproductive investment in aphids. J. Anim. Ecol. 52: 451-461.

Webster, F. M., and W. J. Phillips. 1912. The spring grain-aphid or greenbug. U.S. Bur. Entomol. Bull. 110, 153 pp.

Webster, J. A., and C. Inayatullah. 1984. Greenbug (Homoptera: Aphididae) resistance in triticale. Environ. Entomol. 13: 444-447.

Webster, J. A., and K. J. Starks. 1984. Sources of resistance in barley to two biotypes of the greenbug Schizaphis graminum (Rondani), Homoptera: Aphididae. Prot. Ecol. 6: 51-55.

Weibel, D. E., K. J. Starks, E. A. Wood, Jr., and R. D. Morrison. 1972. Sorghum cultivars and progenies rated for resistance to greenbugs. Crop Sci. 12: 334-336.

Whitham, T. G., A. G. Williams, and A. M. Robinson. 1984. The variation principle: individual plants as temporal and spatial mosaics of resistance to rapidly evolving pests, pp. 15-51. In: P. W. Price, C. N. Stebodehlkoff, and W. S. Gaud (eds.), A new ecology: new approaches to interactive systems. John Wiley, New York.

Wills, C. 1981. Genetic variability. Clarendon Press, Oxford.

Wilson, R. L., K. J. Starks, H. Pass, and E. A. Wood. 1978. Resistance in four oat lines to two biotypes of the greenbug. J. Econ. Entomol. 71: 886-887.

Wood, E. A. Jr. 1961. Biological studies of a new greenbug biotype. J. Econ. Entomol. 54: 1171-1173.

Wood, E. A. Jr. 1971. Designation and reaction of three biotypes of the greenbug cultured on resistant and susceptible species of sorghum. J. Econ. Entomol. 64: 183-185.

Wood, E. A. Jr., H. L. Chada, and P. N. Saxena. 1969. Reaction of small grains and grain sorghum to three greenbug biotypes. Okla. Agric. Exp. Stn. Prog. Rep. P-618, 7 pp.

Wood, E. A. Jr., E. E. Sebesta, and K. J. Starks. 1974. Resistance of 'Gaucho' triticale to Schizaphis graminum. Environ. Entomol. 3: 720-721.

Wood, E. A. Jr., and K. J. Starks. 1972. Effect of temperature and host plant interaction on the biology of three biotypes of the greenbug. Environ. Entomol. 1: 230-234.

Wood, E. A. Jr., and K. J. Starks. 1975. Incidence of paedogenesis in the greenbug. Environ. Entomol. 4: 1001-1002.

Wool, D., and K. Koach. 1975. Morphological variation of the gall-forming aphid, Geoica utricularia (Homoptera) in relation to environmental variation, pp 239-272. In: S. Karlin, and E. Nero (eds.), Population genetics and ecology. Academic Press, New York.

Wratten, S. D., G. Lee, and D. J. Stevens. 1979. Duration of cereal aphid populations and the effects on wheat yield and quality. Proc. 1979 British Crop Prot. Conf.--Pest and Diseases 1: 1-8.

Wright, M. A., B. D. Smith, and D. A. Kendall. 1984. Screening grass species for susceptibility to aphids. Tests of Agrochemicals and Cultivars (Ann. Appl. Biol., 104, supplement): 116-117.

VITA 2

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