THE EFFECTS OF DIET ON FATTY ACID COMPOSITION OF THE GREENBUG SCHIZAPHIS GRAMINUM (RONDANI)

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THE EFFECTS OF DIET OF FATTY ACID COMPOSITION OF

THE GREENBUG SCHIZAPHIS GRAMINUM (RONDANI)

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PREFACE

My interest in insect resistance in crop plants was sparked by Dr. Don C. Peters, Professor of Entomology at Oklahoma State University, when he informed me of the work being done with small grains, specifically wheat, and his work with the greenbug.

I wish to express my sincere gratitude to my major advisor, Dr. Don C. Peters, Professor, Department of Entomology, for his instruction, guidance and encouragement during my sojourn at Oklahoma State University. Sincere appreciation is expressed to Dr. Jack W. Dillwith, Assistant Professor, Department of Entomology, and committee member, for his criticisms, insightful suggestions and the use of his laboratory facilities without which I would not have been able to attempt this project. I am also grateful to my other committee member Dr. James A. Webster, USDA - ARS, Adjunct Professor, Department of Entomology, for his advisement and critiques of this manuscript. Special thanks to those professors in the Department of Entomology who passed on their wisdom and experience to me including: Dr. Raymond D. Eikenbary, Dr. John R. Sauer, Dr. Richard C. Berberet and Dr. Russell W. Wright.

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CHAPTER I

INTRODUCTION

The greenbug, Schizaphis graminum (Rondani), is a major pest of small grains and cereals in North America. The greenbug was described by Rondani in Italy in 1852. The greenbug population in the midwestern United States periodically reaches outbreak proportions. When this occurred in 1976, the damage and control costs to Oklahoma wheat producers was estimated by the Oklahoma Agricultural Extension Service to be $80 million (Starks and Burton 1977). Therefore, the greenbug warrants both study and concern.

One method of integrated control recommended for the greenbug is the use of resistant cultivars. Understanding what makes one plant resistant and another not resistant to the greenbug is very important when selecting plants for breeding. The early emphases in breeding for resistance were on whether resistance was inherited, genetically manageable, and reasonably stable. Because early workers in host plant resistance did not understand the biochemical basis of the resistance factor they were selecting for, they may have selected for one beneficial factor closely associated with other factors which were less beneficial and even harmful to the cultivar. Biochemical explanations of resistance were sought when further studies indicated more complex insect and plant interactions.

Recently the knowledge of the chemistry of plants has increased dramatically. In the middle 1960's, several key mechanisms were

explained. DIMBOA, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one, in Zea mays was shown to be a major repellent and feeding inhibitor of first-instar larvae of the European corn borer, Ostrinia nubilalis (Klun et al. 1967). The dimeric sesquiterpene, gossypol, proved to be a deterrent to several pests of cotton (Bottger et al. 1964). Decoding the chemical bases for plant-herbivore interactions is an important step in scientific inquiry. Some entomologists are using the greenbug as a model to study the inheritance of virulence to its host plants (Puterka and Peters 1989). Therefore, the research towards discovering the chemical basis of resistance of the greenbug's host plants is crucial in this process.

In a resistance screening program, the purpose is to characterize the insect/plant interactions. Therefore, it is important to have a large and consistent population of the insect to reduce the variance among observations. One question which researchers have pondered is whether differences within the host plant are causing differences in the insect? Many researchers have developed artificial diets for the insect they are rearing. The use of these diets eliminates the need to culture them on the host plant. A defined diet could effectively reduce the variance that researchers attribute to their host plants.

The initial goal of my research was to establish an artificial diet for the greenbug. Modifications of published diets were attempted with suggestions for the inclusion of lipids containing fatty acids added to the base diet by Dr. J.W. Dillwith, who suggested varying the sources of fatty acids from different lipid classes including phospholipid, free fatty acid and triglyceride sources. The greenbugs survived on the diet for nearly three weeks. They continued to molt but did not reach

reproductive maturity. Some of the greenbugs that had feed on artificial diet were dissected to look for developing embryos. No embryos were seen and the membranes appeared to contain emulsifications which were unlike the membranes of greenbugs that had been feeding on 'Triumph 64' wheat. Dr. Dillwith suggested that the artificial diet might be lacking some essential fatty acids affecting membrane formation and reproduction and other metabolic processes. This was a turning point from attempts to develop an artificial diet for the greenbug to research concentrating on the greenbug's fatty acid composition and how its diet affected it.

The research consisted of several objectives. The first was to characterize the fatty acid composition of greenbugs on a susceptible host. After establishment of this standard, the next objective was to examine the variation of greenbug fatty acids after feeding on several different host plants. The third objective was to investigate the variation of greenbug fatty acids on an artificial diet lacking any essential fatty acids, and finally to supplement that artificial diet with fatty acids and observe any changes in the greenbug's fatty acid profile.

CHAPTER II

LITERATURE REVIEW

Greenbug Biology

The greenbug which was the model used in this study is an aphid with facultative life cycle patterns of holocycly or anholocycly, depending on environmental conditions (Webster and Phillips 1912, Wadley 1931). Over 70 species of graminaceous plants (Michels 1986), serve as primary and secondary hosts of this oligophagous aphid. The greenbug population is not homogenous, but is comprised of various host races which are termed biotypes. Each biotype, although it is assumed that they are a composite of several different genotypes, is one distinct phenotype based on its ability to damage and utilize different host cultivars. Some phenotypic expressions may be based on a duplicate gene - modifier gene model for virulence (Puterka and Peters 1989). Greenbug biotypes are characterized by their ability to differentially damage five important field crops; barley, oats, rye, sorghum, and wheat. There are at least seven biotypes and a large number of sources of greenbug resistance (Puterka et al. 1988). By using biotype B in this study insight was gained into understanding its interactions with the host, be it plant or artificial diet. With this knowledge, further experiments on plants or the artificial diet across the range of biotypes will be more feasible.

Aphid Artificial Diets

Artificial diets for rearing insects are strictly a phenomenon of this century. The majority of work in development of many diets has been done since the 1950's. While studying the transmission of plant viruses (Hamilton 1935), the first attempts to feed aphids artificially using plant epidermis and a solution of dyes were made. Later, aphids were fed on an aseptic chemically defined diet via a rubber membrane (Maltais 1952). Another great contribution to the development of aphid artificial diets was the introduction of Parafilm® as a membrane via which aphids could feed (Bradley 1956). Another improvement was the sachet which is two Parafilm® membranes surrounding a droplet of the diet solution. This greatly simplified the rearing process because the sachets were capable of holding small volumes and maintaining diet sterility (Mittler and Dadd 1964). More than 20 years ago, the green peach aphid, Myzus persicae (Sulzer), was reared for over 20 generations on a chemically defined liquid diet of water, sucrose, amino acids, vitamins and trace metals (Dadd and Mittler 1966). The advantages of maintaining aphids on an artificial diet are two fold. The first advantage is to study the effects of the environment without interference by the host plant. The second is to study their nutrition separate from the uncertainties of the host plant (Mittler 1976).

Greenbug Artificial Diets

Auclair and Cartier (1965) developed an artificial diet for the pea aphid, Acyrthosiphon pisum (Harris), which was later improved by Akey and Beck (1971). Cress and Chada (1971) modified Auclair's pea aphid diet in attempts to rear the greenbug, but were not successful in

rearing the aphids for more than one generation. Ten years later, Dreyer et al. (1981) also tried to rear the greenbug using a modified Akey and Beck diet. More recently, Schmidt (1987) slightly modified the Dreyer diet while studying the physiology of greenbug feeding behavior, but he did not have any significant success in extending their longevity. In the search for a successful diet for the greenbug using a modification of Auclair's diet, it was observed that the internal membranes of greenbugs feeding on this diet appeared to contain emulsifications unlike the smooth, normal membranes seen in greenbugs which had been feeding on 'Triumph 64' wheat. This critical observation led to the hypothesis that the absence of some essential fatty acids from the artificial diet were affecting normal membrane formation. This observation was consistent with the hypothesis that polyunsaturated fatty acids, which had not been added to artificial diets, are essential for the greenbug.

Insect Lipids

It has been demonstrated that two major lipid classes, polyunsaturated fatty acids and sterols, are dietary requirements for insects (Rodriguez 1972, House 1973) with some exceptions to these requirements occuring in the family Aphididae. Currently, it has been theorized that the symbiotes of these aphids synthesize sterols (Dadd and Mittler 1966, Akey and Beck 1971, Srivastava and Auclair 1971, Campbell and Nes 1983). However, the results of Campbell and Nes (1983) showed that S. graminum and/or its symbiotes do not possess the complement of enzymes in the isopentenoid metabolic pathway that are required for the synthesis of cholesterol. These results were

inconsistent with the earlier work of Ehrhardt (1968) and Houk et al. (1976) who concluded that the symbiotes of Neomyzus circumflexus and Acyrthosiphon pisum respectively, synthesized sterols. Campbell and Nes (1983) concluded that the family Aphididae may be divided into two groups; those aphids which are capable of de novo synthesis of sterols via their symbiotes, and those that are not. Although this paradox remains, it may possibly be explained by the fact that individual aphids only require very small quantities of sterol in comparison to other insects (total sterol in adult S. graminum <18 ng). This very small quantity of sterol has been shown to be present in previously sterile artificial diets in which sterols were not an ingredient. This can be explained by the presence of fungal contaminants potentially present once the Parafilm membrane is pierced by the greenbug's stylets. Campbell and Nes (1983) cultured one species of fungus found in the artificial diet onto a potato dextrose agar while no fungal contaminants were found in eight control diets on which aphids did not feed (Campbell and Nes 1983).

Lipids, in particular fatty acids, are essential structural components of cell membranes. They also provide a rich source of metabolic energy for periods of sustained energy demand. They facilitate water conservation both by formation of an impermeable cuticular barrier and by yielding metabolic water upon oxidation. They also include several important hormones and pheromones important in regulatory physiology (Downer 1978, Stanley-Samuelson et al. 1988). Looking beyond the traditionally recognized roles, fatty acid metabolism and the alterations that move them from one area of biological significance to another are emerging in importance (Stanley-Samuelson et

al. 1988). A landmark paper on Homopteran fatty acids in 1963 by Strong demonstrated that although the percentages of fatty acids varied widely between aphids and leafhoppers and between species in these groups certain patterns were evident. Aphids have relatively large amounts of 14:0 myristic acid, from 33 to 87 percent of the total fatty acid content, and small amounts of the C_{18} series (Strong 1963).

Dietary Requirements

Studies of lipid biochemistry demonstrate that morphological and physiological symptoms associated with dietary insufficiency provide an indication that if the missing components are 18:2 or 18:3 fatty acids (18:2 and 18:3 = 18 carbon chain length with double bonds in the delta-9,12 and the delta- 6,9,12 positions, respectively) which accumulate in membrane phosphoglycerides, the results will be impaired membrane function. Although plants (Stumpf 1980), fungi (Weete 1980), and protozoa (Roughan and Slack 1982) were capable of synthesizing linoleic acid by inserting a double bond in the delta-12 position of oleic acid, nutritional (Dadd 1981) and radiotracer studies (Downer 1978) led to the conclusion that insects, like higher animals, were unable to synthesize linoleic acid.

Linoleate Synthesis

However, it has been reported by Cripps et al. (1986) that 8 of 32 insect species examined demonstrated *de novo* biosynthesis of 18:2 linoleic acid. More recently, Stanley-Samuelson et al. (1988) reported that 15 species from 4 orders can synthesize linoleic acid *de novo*. Of the 15 species capable of synthesizing linoleic acid, 4 were Aphididae;

black cherry aphid, woolly aphid, peach aphid, and pea aphid (Cripps et al. 1986). Cripps showed no correlation with the presence or absence of symbiotes and incorporation of [1-^{14}C]acetate into linoleic acid. However, all aphids which have been shown to be linoleate synthesizers have and require abundant symbiotes located in mycetocytes (Griffiths and Beck 1973). To be able to determine the absolute nature of dietary influence on the insect lipid composition, the biological availability of the lipids must be known. Most of the research in this area has concerned the digestion and absorption of neutral lipids; triglycerides and free fatty acids; and the need for comparable studies on dietary polar lipids has not been well recognized (Treherne 1958, Weintraub and Tietz 1973, Turunen 1973, Hoffman and Downer 1976). This study focused on the major fatty acids; saturated, mono-unsaturated, and polyunsaturated.

CHAPTER III

GREENBUG CULTURING, COLLECTION AND ANALYSIS OF FATTY ACIDS

Experimental Design

It was hypothesized that there would be no significant difference in the linoleic acid content of greenbugs that fed on several different host plants, but their linoleic acid content would be negatively correlated with the amount of time spent on the artificial diet. Therefore, the experiment was an analysis of biotype B greenbug on several plants ('Triumph 64' wheat, 'TAM 107' wheat, 'Pioneer 8300' sorghum, and 'Wintermalt' barley) as controls and the artificial diet, replicated three times through time. In this study, S. graminum biotype B were continuosly cultured on 'Triumph 64' wheat in the laboratory. These greenbugs thrive on this cultivar, reproduce abundantly, and appear robust and healthy. Therefore greenbugs cultured on 'Triumph 64' were chosen to best characterize the fatty acids of S. graminum biotype B. 'TAM 107' wheat was chosen because it was a resistant cultivar to this biotype. 'Pioneer 8300' sorghum was chosen because it is not a normal host and the host relationship is unique because its leaves became reddened by accumulation of pigments as the result of aphid feeding. The greenbugs were cultured on 'Wintermalt' barley because it is a susceptible cultivar of another species for comparison with 'Triumph 64' wheat.

Greenbug samples

Biotype B greenbugs were cultured on pots of 'Triumph 64' wheat in a growth chamber with a cycling temperature of 25°C:21°C (L:D) and a photoperiod of 13:11 (L:D). Biotype B was used because it was previously used by Cress (1971) in his work on artificial diets and also because it survived better than biotype C in some initial artificial diet studies. Adult greenbugs from cultures which had not been stressed for at least two previous generations were transferred to two-leaf seedlings of each of the host plants to be used; 'Triumph 64' wheat, 'TAM 107' wheat, 'Wintermalt' barley and 'Pioneer 8300' sorghum; and to sachets of the artificial diets. The artificial diet consists of the basic diet for greenbug developed by Auclair (1965) with slight modifications. The main ingredients in this liquid media were amino acids, trace metals, vitamins, sucrose and salts. The complete list of components can be found in the Appendix with preparation instructions. Greenbugs on the test plants were cultured in the same growth chamber under the same conditions described previously. Greenbugs on the artificial diet were cultured on the laboratory bench inside polystyrene crisper boxes to increase the humidity and reduce the air flow. The temperature was 24°C:21°C (L:D) and the photoperiod was 13:11 (L:D). The crisper boxes were misted with water daily to further maintain a higher humidity level. The nymphs born from these adults were the sample set and the adults were analyzed separately or discarded after they produced the required number of nymphs. The nymphs produced on the first two days were discarded to decrease the effects of transovarial nutrition and the adults were moved to another host plant or diet sachet of same variety. The diet sachets were changed every 3 days to provide

the greenbugs with fresh media.

Artificial Diet Sachets

The diet sachets were constructed of a 2.5 cm tall glass cylinder 2.5 cm diameter with a standard square of Parafilm® (5.0 cm x 5.0 cm) placed over the top with the overlapping amount smoothed down the side of the cylinder. A depression to contain the diet media was made by gently pressing a table tennis ball onto the Parafilm® membrane. After sterilization of the lower membranes in a Laminar flow hood with UV light for 1 hour, a 0.25 ml aliquot of the media was placed on the membrane. The upper membrane was made by cutting a standard Parafilm® square into fourths, and stretching the one quarter square so that it was the same size as the standard square. This membrane was carefully stretched over the diet droplet with the sterile side facing the media and the overlap was smoothed down the side of the cylinder. The diet sachets were then frozen and maintained at -10°C until they were needed. The greenbugs were caged on the diet sachet by means of a polypropylene stopper with the end cut out and covered with a piece of cotton mesh to allow for airflow.

The nymphs collected from the plants were harvested 6 days after the two-day discard period and the nymphs collected from the diet sachets were harvested 9 days after the two-day discard period. The three replications of samples of greenbugs were collected, weighed, and frozen until they could be processed in the laboratory.

Methods of Comparing Diets

The artificial diet was supplemented with three forms of fatty

acids and the greenbugs that fed on these three diets were compared by the above methods to observe any changes in their fatty acid profile. The composition of the first diet was supplemented with 1.8 mg of phosphatidyl choline per ml of diet solution with 1.0 mg/ ml of octylglucoside (OG) as a carrier. The second diet supplement contained 1.8 mg of trilinolein per ml of diet solution with 1.0 mg/ ml OG as a carrier. The third supplemented diet contained 0.9 mg of linoleic acid per ml of diet solution and the same amount of OG as a carrier. These supplements were added to the standard diet used in this study inside a laminar flow hood to maintain sterility of the diet solution.

Biochemical Laboratory Techniques

The Bligh and Dyer extraction, solid phase extraction, hydrolysis, methylation and purification were the techniques used to prepare the greenbug samples for analysis by gas chromatography.

Bligh and Dyer Extraction Technique

A total lipid extraction of each greenbug sample was done by the method described by Bligh and Dyer (1959). All of the samples were weighed before freezing. The greenbug sample was removed from the freezer and thawed at room temperature to minimize the condensation of moisture in the sample. The sample was placed in a ground glass homogenizer with 1 ml of chloroform, 2 ml of methanol and 0.8 ml water and homogenized. The contents were then transferred to a test tube and set aside for 5 min to allow the extraction to continue. Next, 1 ml of chloroform and 1 ml of water were added and vortexed for 30 seconds. Then, the homogenate was centrifuged at 3100 x G for 10 min. The bottom

chloroform layer was removed with a Pasteur pipet and saved in a vial to be combined with an identical fraction after 1 ml of chloroform was added to the test tube which was vortexed and centrifuged at the same parameters previously used. All the vials used in this study were pre-cleaned by a hexane rinse. After the 10 min centrifugation, the bottom layer was removed as previously described and combined with the lipid fraction in the vial. This was the total lipid extract. Then, the total lipid extract was filtered through a small pad of magnesium sulfate ca. 1 cm deep in a Pasteur pipet. The extract was dried in a sand-filled hot plate (60°C) under nitrogen gas and reconstituted in 200 µl of chloroform with butylated hydroxy toluene (BHT) at 5 mg/ 100 ml. An internal standard, 17:0 fatty acid, was added to the portion of the total lipid extract to be hydrolyzed and methylated for gas chromatography. The 17:0 fatty acid, heptadecanoic acid, was used because it was not detected in preliminary samples. The total lipid extract, the portion with the internal standard and the portion to be saved for further separation and analysis, were then stored in a cold box until they were needed.

Solid Phase Extraction (SPE)

The total lipid extract was separated into lipid classes using a modification of the technique developed by Kaluzny et al. (1985). This technique involved the usage of vacuum elution apparatus which was run at approximately 10 kPa. The solvents used in this procedure were as follows: A. chloroform:2-propanol (2:1); B. 2% acetic acid (glacial) in diethyl ether; C. methanol; D. hexane; E. 1% diethyl ether:10% methylene chloride:89% hexane; F. 5% ethyl acetate in hexane. The columns used in

this procedure were 500 mg Aminopropyl columns manufactured by Analytichem with a stainless steel frit. The first step in this procedure was to wash the three columns used for separation of one sample with 2 ml of hexane twice to clean out any impurities. It was important to release the vacuum before the packing dried. Then, 250 μl of total lipid extract was applied to the column. The lipid was bound to the packing by pulling the excess chloroform in the extract into a waste vial with care being taken to not dry out the packing. After inserting a clean vial, the first column was then washed with 8 ml of Solvent A and the eluate (neutral lipids) was saved. The column was washed with 6 ml of Solvent B and the eluate collected in a clean vial (free fatty acids). Next, the column was washed with 4 ml of Solvent C and the eluate collected in a clean vial also (phospholipids). The free fatty acid and phospholipid fractions were saved, but the neutral lipid fraction was dried and reconstituted in 100 μl of hexane and loaded onto a second fresh hexane-rinsed column in the same manner as the total lipid extract. The second column was washed with 4 ml of Solvent D and the eluate collected in a clean vial (cholesterol esters). Next, the second column was stacked on top of the third column by use of an adaptor and washed with 8 ml of Solvent E and the eluate collected in a clean vial and saved also (triglycerides). The columns were then separated and column #3 was washed with 7 ml of Solvent F which was collected and combined with the eluate from washing column #2 with 4 ml of Solvent F. This eluate was dried and combined with the triglyceride fraction to insure the inclusion of the whole triglyceride complex. The free fatty acid, phospholipid, sterol ester, and triglyceride fractions were then dried under N_2 in the sand-filled hot plate, combined with the

internal standard, reconstituted in 200 μl of chloroform and saved for further processing into fatty acid methyl esters (FAME). After completing this procedure, the aminopropyl columns were regenerated by washing with 4 mls of each of these solvents H, E, and D in the order listed. Solvent H was chloroform:methanol (2:1).

Thin Layer Chromatography

After SPE, the lipid fractions were checked for purity using thin layer chromatography (TLC). High Performance TLC plates from Analtech were used. After the plate was washed in the solvent tank with 70:30:1 Hexane:Diethyl ether:Acetic acid and allowed to dry for two minutes, two microliters of each lipid fraction were spotted one centimeter apart across the plate. Also, fatty acid standards were spotted in two lanes for comparison to the fractions. The plate was placed in the solvent tank until the solvent front reached one cm from the top of the plate. The plate was removed promptly from the tank and allowed to dry for four minutes before dipping in cupric acetate. After dipping, the plate was carefully charred on a hot plate to visualize the fatty acids.

Hydrolysis, Methylation and Purification of Fatty Acids

In further preparation of the total lipid extract and lipid classes for analysis by gas chromatography, they were hydrolyzed, methylated and purified. The total lipid extract and the lipid classes, except for the free fatty acid fraction, were hydrolyzed. One ml of 5% KOH in methanol was added to the vial containing the dried fraction or total extract and capped with a teflon-lined cap. The vial was heated in a heating block

at 60°C for 1 hour and then cooled. Next, 1 ml of 14% boron triflouride (BF_3) in methanol was added to all of the fractions, including the free fatty acid fraction, and they were capped with the teflon cap and heated for 30 minutes at 60°C and cooled. Next, 2 ml of water was added to each vial and the contents were poured into a test tube. This mixture was extracted 3 times with 2 ml of chloroform each time. After each extraction, the bottom chloroform layer was removed with a Pasteur pipet and saved in a clean vial and each subsequent extraction was combined with the previous one for that fraction. When the extraction was completed, the methylated fraction was filtered through a Pasteur pipet with glass wool and $MgSO_4$. This filtrate was dried with the same technique as previously used. After drying, 200 μl of hexane was added to the vial and it was capped and shaken to dissolve the contents. The vial's contents were loaded onto a Pasteur pipet with glass wool and activated Biosil A for purification and washed with 3 ml of hexane to remove hydrocarbons. The column was washed with 6 ml of 5% ether in hexane which eluted the FAME. This was dried down and the sample was ready for gas chromatography.

Gas Chromatography

Each of the fractions and total lipid extracts were reconstituted in 50 μl of chloroform and 1 μl was injected into a gas chromatograph. The HP 5890 gas chromatograph with the DB 225 column (J&W Scientific) was used. The composition of this column was 50% Cyanoprophenyl: 50% Methyl silicone. The heating program was as follows: 2 min at 120°C, then an increase of 10°C per min for 8 min until 200°C was reached, then an increase of 5°C per min until 225°C was reached and that temperature

was held for 4 min. After the program has been completed, approximately 4 min for cooling the column down to 120°C must elapse before another sample can be injected.

The traces obtained from the gas chromatograph were analyzed for amounts of fatty acids compared to the internal standard (17:0) and percent composition of total fatty acids in the sample. FAME structures were confirmed on three columns by comparison to a standard and by co-injection with an authentic standard. The two other columns of different polarity used were the DB - 5; 5% Phenyl: 95% Methyl silicone and the DB - 23; 50% Cyanopropyl: 50% Methyl silicone.

Statistical Methods

The data were analyzed statistically by calculating means and standard deviations for the amounts of fatty acids in major peaks and the percent compositions of the three replicates. The means were tested for significance ($P \leq 0.05$) by Duncan's Multiple Range Test (SAS Institute 1985).

CHAPTER IV

RESULTS AND DISCUSSION

Greenbugs on 'Triumph 64' Wheat

Figure 1a is a trace of the FAME standard that was used to identify fatty acids in greenbug FAME samples. Figure 1b is a gas chromatographic trace of the fatty acids found in one sample of S. graminum cultured on a plant. The fatty acids present in that sample were myristic (14:0), myristoleic (14:1), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3) and arachidic (20:0) acids. Fatty acid structures were confirmed by comparison to authentic standards and coinjection on three GC columns with different polarity; DB-225, DB-23, and DB-5.

The lipid classes present in the greenbug total lipid extract which correspond to authentic standards were phospholipids, free fatty acids, and sterol esters (Fig. 2). Note the absence of diglyceride and free sterol fractions. Two peaks that migrate close to the triglyceride standard were probably triglycerides. These two peaks and the unknown co-eluted in the solid phase extraction procedure at the position expected for a triglyceride. The triglyceride standard contains three eighteen carbon fatty acids, triolein. The triglyceride containing fraction from the greenbug, isolated by solid phase extraction contains predominantly fourteen and sixteen carbon fatty acids. This difference may account for lower relative mobility (Rm) as compared to the standard

Figure 1. Gas chromatographic trace of lipid standard and greenbugs cultured on plants.
a. A Fatty Acid Methyl Ester (FAME) standard was injected on the HP 5890 gas chromatograph with the DB-225 column. The oven program is inset. The standard retention times of the fatty acids present in the standard mixture were used to identify the fatty acids of unknown samples.
b. Typical chromatogram of total greenbug fatty acids of greenbugs reared on 'Triumph 64' wheat in which 14:0, 14:1, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3 and 20:0 are present.

Figure 1 a

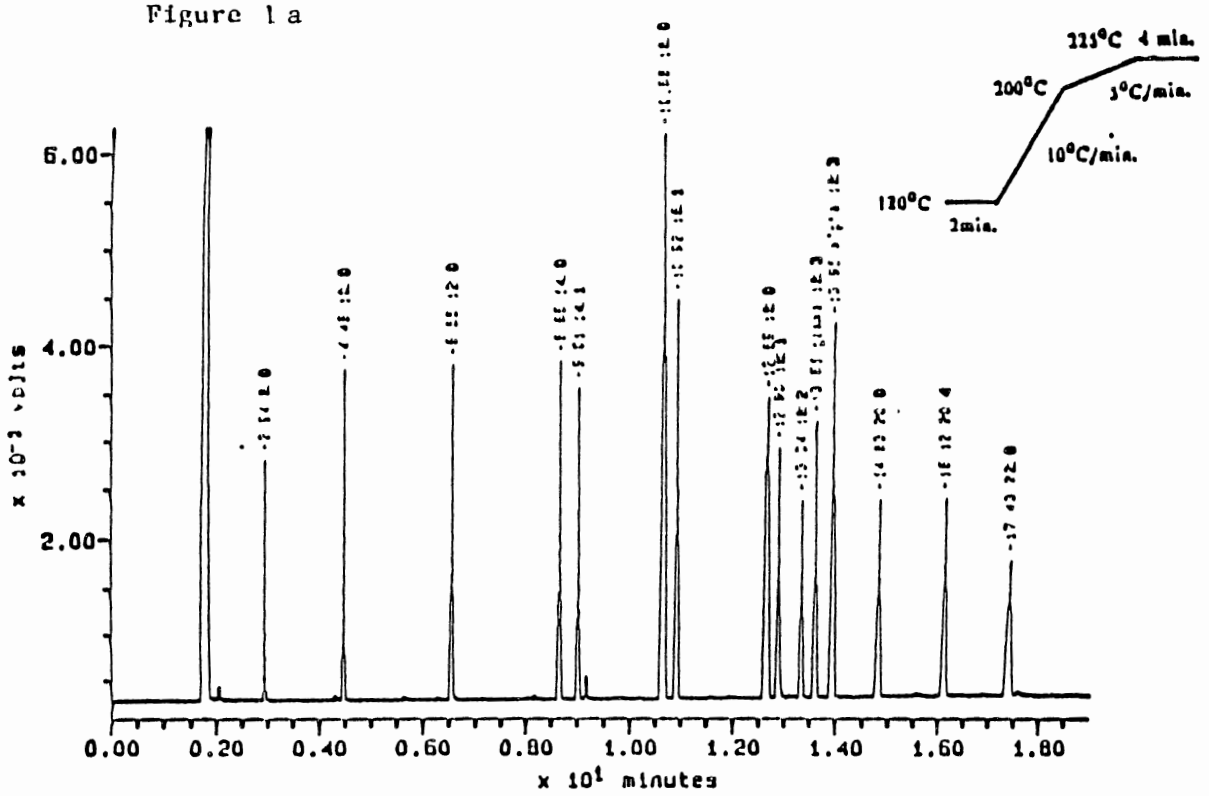


Figure 1b

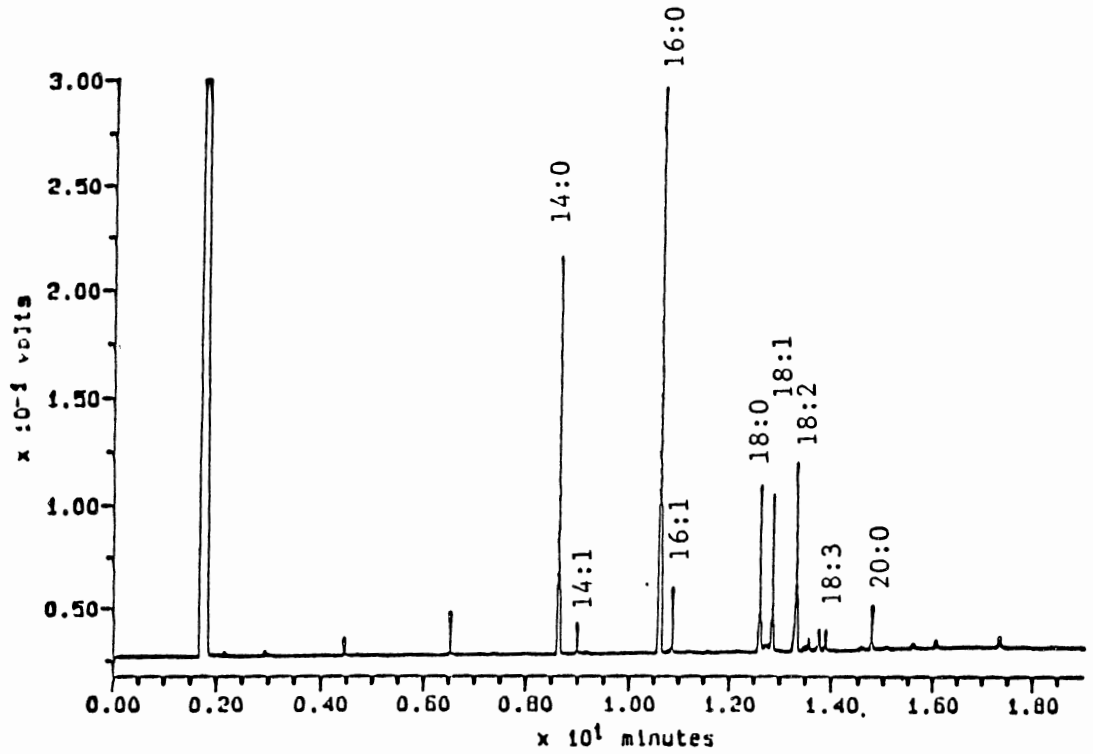
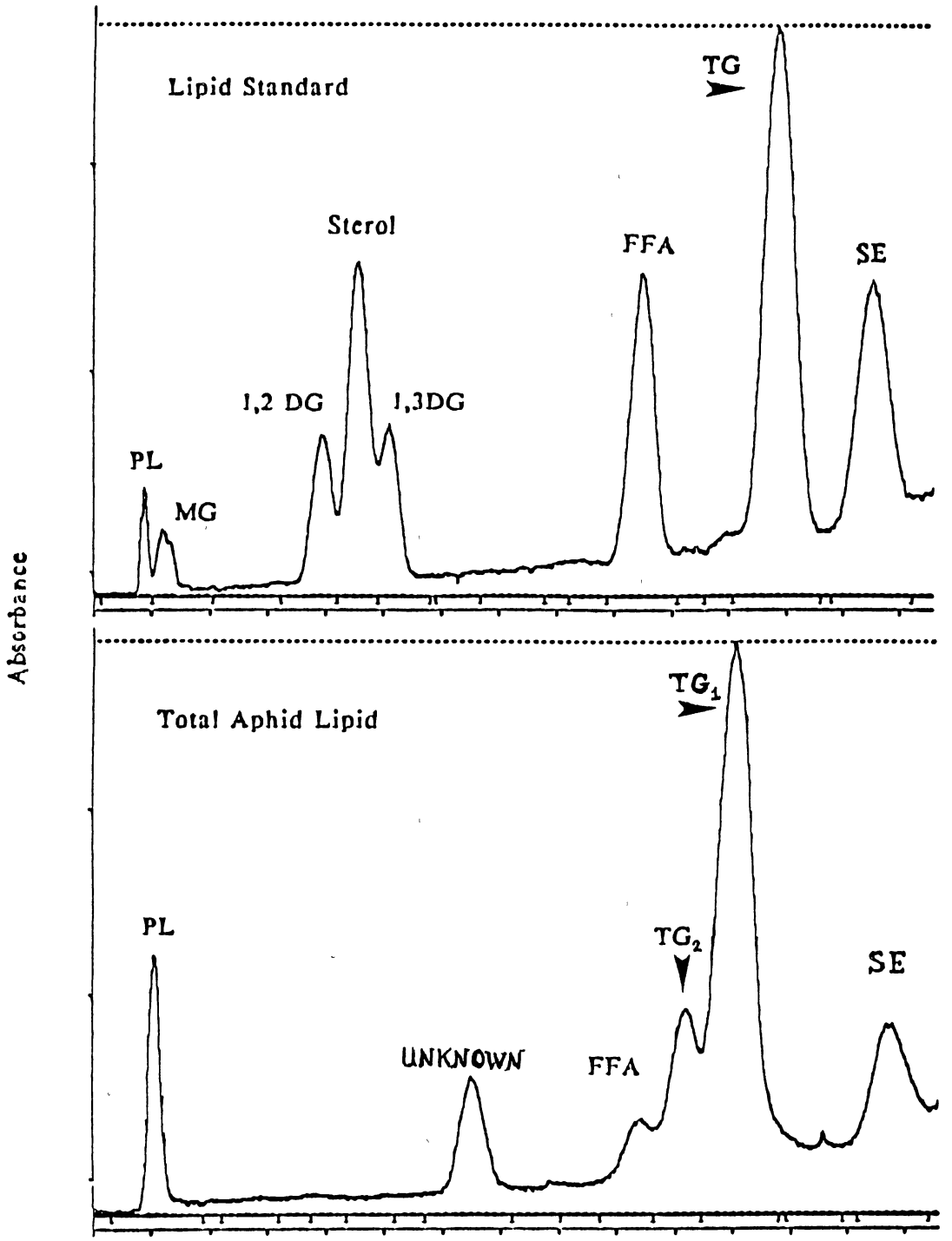


Figure 2. Absorbance trace of Greenbug Lipids on TLC. The lipid standard and total aphid lipid were spotted on a silica gel HPTLC plate and run concurrently in 70:30:1 (v/v) Hexane: Diethyl ether:Acetic Acid. After charring with 3% cupric acetate in 15% phosphoric acid, the plate was scanned with a laser densitometer in the absorbance mode. The lipids present in the lipid standard were Phospholipids (PL), Monoglycerides (MG), Diglycerides (DG), Cholesterol (Sterol), Free Fatty Acids (FFA), Triglycerides (TG), and Sterol Esters (SE). By comparison to the standard, the lipids present in the greenbug were identified as phospholipids (PL), Unknown, Free Fatty Acids (FFA), 2 Triglycerides (TG_1 and TG_2), and the Sterol Esters (SE).


Absorbance
Lipid Standard
TG
Sterol
FFA
SE
1,2 DG
1,3DG
PL
MG
Total Aphid Lipid
TG1
PL
TG2
SE
UNKNOWN
FFA

triglyceride. The peak labeled TG_2 also showed a quenching of flourescence when the plate was viewed under u.v. light. This suggests that this fraction contained sorbic acid which has been reported to occur in aphid triglycerides (Fallon and Shimizu 1977).

Four fractions were isolated by solid phase extraction. A phospholipid fraction, a free fatty acid fraction, a sterol ester fraction and a triglyceride fraction containing peaks TG_1, TG_2 and the unknown component. The predominant fatty acid in the FFA class was stearic acid which if combined with palmitic acid was more than 65% of the total (Table I). The PL class contained a large percentage of linoleic acid and oleic acid, The vast majority of fatty acids, ca. 87%, were in the C_{18} complex. The SE class was comprised of only saturated fatty acids with the majority being palmitic acid. The TG class was dominated by myristic and palmitic acid which were 92% of fatty acids in this lipid class. Of fatty acids present in these 4 lipid classes, the vast majority were in the TG class. The fatty acid profiles of the lipid classes correspond well with what was already known about these lipid classes. The large percentage of linoleic acid in the PL class reflects the requirement of linoleic acid for membrane formation in which phospholipids are known components (Stanley-Samuelson et al. 1988). Similar to animals and even humans, greenbugs also esterify only saturated fatty acids on sterols to form sterol esters. Triglycerides were the predominent lipid class in total body extracts. This was because the lipid storage system which stores potential metabolic energy as triglycerides contained the largest amount of lipids by nature of its storage function. This data coincided with the theory of the preference certain fatty acids, saturated and

TABLE I

PERCENT COMPOSITION OF FATTY ACIDS IN 4 LIPID CLASSES OF GREENBUGS CULTURED ON 'TRIUMPH 64' WHEAT[a]

Lipid Classes		Fatty acids									Totals[b]	
		12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	µg/mg aphid	% of total
FFA	$\bar{X}$	--	2.12	24.31	--	41.87	15.07	16.63	--	--	0.51	5.54
	S.D.		2.91	0.44		0.29	2.40	0.39			0.13	
PL	$\bar{X}$	--	--	6.43	2.99	14.46	23.46	43.06	5.67	3.92	1.07	13.34
	S.D.			0.36	0.11	0.11	0.04	0.14	0.02	0.06	0.07	
SE	$\bar{X}$	--	21.03	63.15	--	15.81	--	--	--	--	0.24	3.32
	S.D.		1.01	0.90		1.91					0.04	
TG	$\bar{X}$	0.25	36.54	55.50	--	5.67	1.40	0.49	--	0.13	8.34	77.80
	S.D.	0.01	1.38	1.06		0.43	0.05	0.02		0.16	3.63	

[a] Means and S.D. of percentages of fatty acids in free fatty acids (FFA), phospholipids (PL), sterol esters (SE) and triglycerides (TG) (n = 3).

[b] Totals represent the distribution of the fatty acids within the lipid classes.

unsaturated, have for particular lipid classes was biologically significant because of their movement from one lipid class to another during metabolism (Stanley-Samuelson et al. 1988).

Greenbugs on Four Host Plants

The fatty acid content of greenbug total lipid extracts from greenbugs reared on 4 host plants were compared (Table II, III). The fatty acid content of greenbugs cultured on 'Triumph 64' wheat was dominated by myristic and palmitic acids with a total amount of almost 40 μg of fatty acid/mg of aphid. Those greenbugs on 'TAM 107' wheat contained about 1/4 as much fatty acid/ mg of aphid. In addition there was a major change in the composition of fatty acids with a major reduction occurring in myristic acid. Greenbugs reared on the sorghum cultivar 'Pioneer 8300' had almost twice as much total fatty acid/mg of aphid as those on 'Triumph 64' wheat, however, the percent composition of fatty acids was similar. The greenbugs cultured on 'Wintermalt' barley had a very similar fatty acid profile and content to those on 'Triumph 64' wheat (Table II and III).

To obtain accurate profiles of the fatty acid content of greenbugs cultured on 'Triumph 64' wheat, careful attention was paid to the quality of the wheat the aphids were feeding on. Initially, greenbugs were cultured on wheat in various stages of infestation and some of the wheat was chlorotic and some was not. Greenbug populations on stressed wheat had low percentages, ca. 6%, of myristic acid, while greenbugs on unstressed wheat had ca. 50% myristic acid (Fig. 3). The standard deviations of the mean amounts of fatty acids for greenbugs on stressed wheat were large. Thereafter, careful attention was given to the

TABLE II

FATTY ACID CONTENT OF GREENBUGS CULTURED ON 4 HOST PLANTS[a]

Plants		μg of Fatty Acids/mg of aphid[b]						
		12:0	14:0	16:0	18:0	18:1	18:2	Total
Wheat								
'Triumph 64'[c]	$\bar{X}$	0.76ab	20.17b	17.47b	2.07b	0.61b	0.51b	39.80b
	S.D.	0.11	1.03	1.57	0.28	0.09	0.11	4.50
'TAM 107'	$\bar{X}$	--	1.48c	6.94c	1.17c	0.39c	0.47b	10.44c
	S.D.		1.96	1.74	0.15	0.04	0.02	3.78
Sorghum								
'Pioneer 8300'	$\bar{X}$	0.46b	34.14a	33.88a	4.28a	1.67a	2.41a	76.80a
	S.D.	0.53	7.71	5.28	0.65	0.07	0.07	13.92
Barley								
'Wintermalt'	$\bar{X}$	1.20a	18.53b	12.62b	1.82bc	0.65b	0.54b	35.66b
	S.D.	0.22	0.65	0.61	0.18	0.15	0.24	2.03

[a] Means and S.D. of amounts of fatty acids in greenbugs cultured on 4 host plants (n = 4).
[b] Trace amounts of 16:1, 18:3 and 20:0 were present in some samples.
[c] Means within columns followed by the same letter were not significantly different ($P \leq 0.05$) (Duncan's Multiple Range Test 1955).

TABLE III

PERCENT COMPOSITION OF FATTY ACIDS OF GREENBUGS CULTURED ON 4 HOST PLANTS[a]

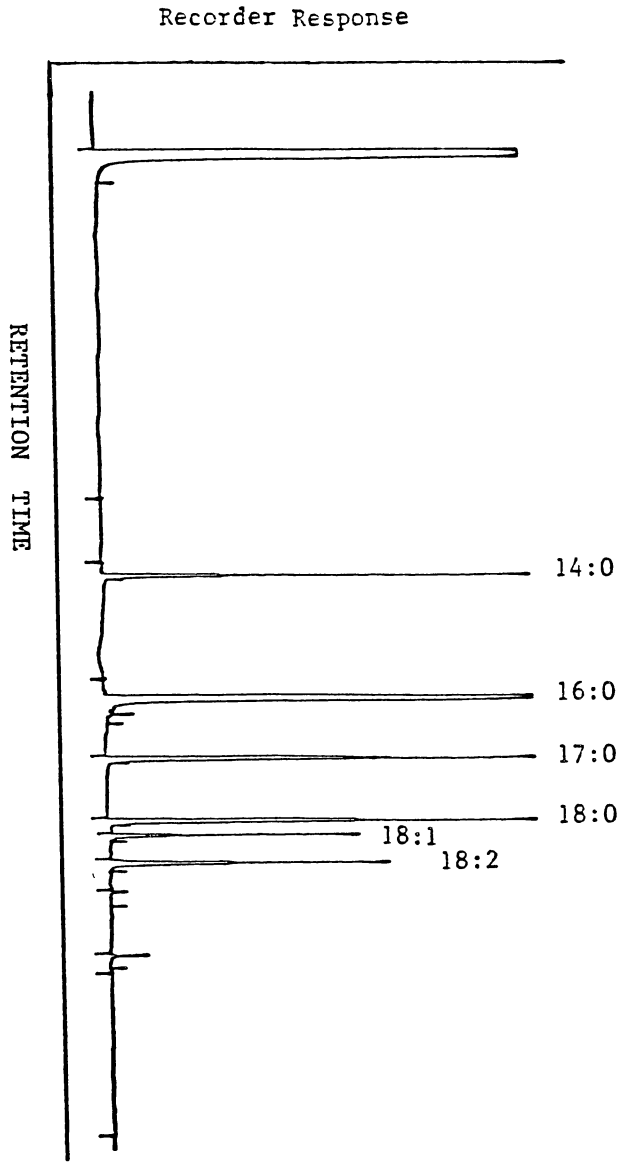
Plants		Fatty Acids[b] 14:0	16:0	18:0	18:1	18:2
Wheat						
'Triumph 64'[c]	$\bar{X}$	48.44a	41.75a	4.96a	1.43a	1.23a
	S.D.	2.30	1.71	0.43	0.16	0.23
'TAM 107'	$\bar{X}$	18.99b	63.66b	10.16b	3.28b	3.93b
	S.D.	19.31	11.19	4.22	1.56	2.37
Sorghum						
'Pioneer 8300'	$\bar{X}$	44.18a	44.28a	5.59a	2.21a	3.21a
	S.D.	2.45	2.21	0.15	0.32	0.58
Barley						
'Wintermalt'	$\bar{X}$	51.99a	35.40a	5.10a	1.80a	1.49a
	S.D.	1.29	0.39	0.26	0.32	0.57

[a] Means and S.D. of percentages of fatty acids in greenbugs cultured on plants (n = 4).

[b] Trace amounts of 12:0, 16:1, 18:3, and 20:0 fatty acids were present in some samples.

[c] Means within columns followed by the same letter were not significantly different ($P \leq 0.05$) (Duncan's Multiple Range Test 1955).

Figure 3. Gas chromatographic trace of fatty acid composition of greenbugs on "stressed" 'Triumph 64' wheat. Note the dramatic reduction of 14:0 Myristic Acid, ca. 6% compared to ca. 50% Myristic acid in greenbugs reared on "unstressed" wheat shown in Figure 1b.


Recorder Response
RETENTION TIME
14:0
16:0
17:0
18:0
18:1
18:2

quality of the wheat. The greenbugs were transferred to uninfested wheat and moved again before the wheat began to show signs of chlorosis and the population became stressed. Greenbugs reared under these conditions had fatty acid contents with small standard deviations. The greenbugs reared on a susceptible host, 'Triumph 64' wheat, were then compared with those on a resistant wheat cultivar, 'TAM 107'. Because of the resistance of 'TAM 107' to biotype B greenbugs, sample size was considerably smaller than the number of greenbugs on 'Triumph 64'. The greenbugs reared on the sorghum cultivar 'Pioneer 8300', which is not a normal host, were noticeably smaller than those on 'Triumph 64' wheat but had almost twice the concentration of fatty acids. Although, the percent composition of the major fatty acids were not statistically significant from those on 'Triumph 64' wheat. The greenbugs reared on 'Wintermalt' barley, a susceptible host, had a fatty acid profile very similar to those from 'Triumph 64' wheat. The total amount of fatty acids in greenbugs reared on 'Wintermalt' barley were also very similar to 'Triumph 64' greenbugs with a slight reduction in the amount of shorter saturated fatty acids and an increase in the longer unsaturated fatty acids. The greenbug's fatty acid profile may be revealing the health of the aphid. If the greenbug was imbibing nutrients it needed from the susceptible host plants, the resistant host plant may not have provided the essential nutrients it needed for proper fatty acid metabolism. This lack of essential nutrients may express itself in changes in the fatty acid profile or absolute amounts of fatty acids in the greenbug. The resistant plant might also have allelochemicals which were toxic to the aphid and caused its fatty acid profile to dramatically change. These conclusions were not consistent with those

of Strong (1963) who concluded that fatty acid composition of the aphids he studied were characteristic of each species not a manifestation of diet. Campbell and Nes (1983), however, concluded that the family Aphididae were divided into two groups, those capable of *de novo* biosynthesis and those that were not. The dichotomy between these results and Strongs may suggest that the fatty acid composition of some aphids with wide host ranges may not be influenced by diet, but monophagous, or oligophagous aphids like S. graminum, may be influenced by diet. In the future, this type of analysis may be used to quantitatively measure the effects of resistant host plants on the fatty acid composition of the greenbug.

Greenbug Nymphs on 'Triumph 64' Wheat

The fatty acid content of greenbug nymphs of different ages on 'Triumph 64' wheat were compared (Table IV and V). Greenbug nymphs born on 'Triumph 64' wheat were removed when they were 1-2 days old, 3-4 days old, and 5-6 days old. The fatty acid content of 1-2 day old nymphs was dominated by palmitic and myristic acid with smaller amounts of stearic, oleic, linoleic, and arachidic acids. This profile was nearly identical for the nymphs which were 3-4 and 5-6 days old except for almost 6 more μg of palmitic acid/mg of aphid than in the 1-2 day old nymphs.

The similarity of the fatty acid content of greenbug nymphs of different ages suggested that age was not a factor. These results may support those of Strong (1963) who said that various fat composition changes during life cycles were characteristic of certain species rather than aphids in general. However, apterae had higher percentages of myristic acid and lower percentages of C_{18} fatty acids.

TABLE IV

FATTY ACID CONTENT OF GREENBUG NYMPHS ON 'TRIUMPH 64' WHEAT[a]

Aphid Age (days)		μg of Fatty Acids/mg of aphid[b]							μg/aphid
		14:0	16:0	18:0	18:1	18:2	20.0	Total	
1-2[c]	$\bar{X}$	6.73a	12.96b	2.82a	1.11a	1.11a	0.13a	24.90b	.08
	S.D.	3.85	1.69	0.81	0.28	0.19	0.11	2.06	.01
3-4	$\bar{X}$	9.79a	19.97a	2.70a	1.36a	1.82a	0.33a	36.32a	.30
	S.D.	0.75	0.96	0.39	0.69	1.14	0.16	2.53	.01
5-6	$\bar{X}$	6.58a	19.11a	2.91a	1.51a	2.10a	0.30a	32.86a	.20
	S.D.	0.85	1.62	0.40	0.47	0.79	0.15	3.83	.04

[a] Means and S.D. of amounts of fatty acids from 3 groups of greenbug nymphs (n = 3).

[b] Trace amounts of 12:0, 16:1 and 18:3 were present in some samples.

[c] Means within columns followed by the same letter were not significantly different ($P \leq 0.05$) (Duncan's Multiple Range Test 1955).

TABLE V

PERCENT COMPOSITION OF FATTY ACIDS OF GREENBUG APTERAE AND NYMPHS ON 'TRIUMPH 64' WHEAT[a]

Aphid	Aphid Age (days)		Fatty Acids								
			12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Apterae[b]		$\bar{X}$	1.82	48.44	41.75	--	4.96	1.43	1.23	--	0.17
		S.D.	0.35	2.30	1.71		0.43	0.16	0.23		0.15
Nymphs	1-2[c]	$\bar{X}$	0.81	26.39	52.47	--	10.78	4.55	4.50	--	0.50
		S.D.	0.91	14.33	10.05		2.92	1.47	1.08		0.43
	3-4	$\bar{X}$	--	27.10	55.12	0.51	7.41	3.69	4.87	0.40	0.89
		S.D.		3.60	3.92	0.50	0.55	1.64	2.78	0.39	0.39
	5-6	$\bar{X}$	--	20.09	58.36	0.49	8.87	4.54	6.27	0.48	0.91
		S.D.		2.40	3.73	0.42	0.46	0.88	1.72	0.42	0.21

[a] Means and S.D. of percentages of fatty acids for each host age group (n = 3).
[b] Values coincide with data shown in Table III for greenbug apterae.
[c] Means within columns for the three nymph groups were not significantly different ($P \leq 0.05$) (Duncan's Multiple Range Test 1955).

TABLE VI

FATTY ACID CONTENT OF GREENBUGS REARED ON ARTIFICIAL DIET

Diet[b]		μg of Fatty Acid/mg of aphid[a]						Total
		12:0	14:0	16:0	18:0	18:1	18:2	μg/mg
BASE[d]	$\bar{X}$	9.76a	69.17a	52.89a	9.06a	1.38a	1.13a	144.09a
	S.D.	3.82	17.90	13.07	2.90	0.68	0.47	38.44
OCT	$\bar{X}$	3.81bc	49.86ab	40.87ab	6.65a	1.13a	0.75a	103.60ab
	S.D.	1.24	5.86	3.77	0.61	0.14	0.12	11.61
LIN	$\bar{X}$	1.46c	34.80b	29.69b	4.52a	1.23a	0.65a	72.85b
	S.D.	0.88	5.07	4.29	1.69	0.20	0.23	7.37
PC	$\bar{X}$	6.35ab	48.73ab	34.58b	6.10a	1.59a	1.51a	99.44ab
	S.D.	2.15	10.62	8.08	0.85	0.98	1.54	24.56
TL	$\bar{X}$	5.73abc	53.48ab	40.10ab	5.36a	0.79a	0.53a	106.37ab
	S.D.	0.33	5.97	3.66	0.25	0.05	0.09	10.15

[a] Means and S.D. of amounts of fatty acids of greenbugs cultured on 5 artificial diets (n = 3).

[b] Artificial diet alone (Base), Artificial diet with Octylglucoside (OCT)[c], Artificial diet with Octylglucoside and Linoleic Acid (LIN) or Phosphatidyl Choline (PC) or Trilinolein (TL).

[c] Octylglucoside is added to the Base diet to solubilize lipids.

[d] Means within columns followed by the same letter were not significantly different ($P \leq 0.05$) (Duncan's Multiple Range Test 1955).

TABLE VII

FATTY ACID COMPOSITION OF GREENBUGS REARED ON ARTIFICIAL DIET[a]

Diet[c]		Fatty Acids[b] % Composition						
		12:0	14:0	16:0	18:0	18:1	18:2	20:0
BASE[e]	$\bar{X}$	6.63a	48.07a	36.85a	6.23a	0.96b	0.79a	0.29a
	S.D.	0.88	1.70	1.43	0.35	0.22	0.29	0.04
OCT	$\bar{X}$	3.62b	48.07a	39.49a	6.43a	1.09a	0.72a	0.37a
	S.D.	0.89	0.39	1.21	0.18	0.05	0.05	0.09
LIN	$\bar{X}$	1.78bc	36.15a	47.44a	10.51a	2.04a	1.14a	0.52a
	S.D.	1.55	25.46	15.58	7.88	0.85	0.73	0.55
PC	$\bar{X}$	6.31a	49.18a	34.83a	6.23a	1.52ab	1.38a	0.44a
	S.D.	0.61	1.45	0.47	0.69	0.62	1.20	0.03
TL	$\bar{X}$	5.40ab	50.23a	37.72a	5.05a	0.74b	0.50a	0.20a
	S.D.	0.29	0.90	0.49	0.27	0.05	0.09	0.01

[a] Means and S.D. of percentages of fatty acids of greenbugs on 5 artificial diets (n = 3).

[b] Trace amounts of 16:1, and 18:3 were present in some samples.

[c] Artificial diet alone (Base), Artificial diet with Octylglucoside (OCT)[d], Artificial diet with Octylglucoside and Linoleic Acid (LIN) or Phosphatidyl Choline (PC) or Trilinolein (TL).

[d] Octylglucoside is added to the Base diet to solubilize lipids.

[e] Means within columns followed by the same letter were not significantly different ($P \leq 0.05$) (Duncan's Multiple Range Test 1955).

Greenbugs on Artificial Diet

The fatty acid content of greenbugs reared on artificial diet, base diet with and without lipid supplements, were compared (Table VI and VII). The fatty acid composition of S. graminum cultured on the Base diet consisted of a majority of saturated fatty acids and the largest amounts were myristic and palmitic acids. The same profile was consistent for greenbugs cultured on the Base diet with all the lipid supplements (Table VII). The point of interest should be that the amounts of fatty acids found in these greenbugs were much higher than for those cultured on plants. For example, greenbugs cultured on the diet had typically twice as much saturated fatty acids as greenbugs cultured on 'Triumph 64' wheat. However, the amounts of oleic and linoleic acids were comparable to what was found in greenbugs reared on 'Triumph 64' wheat. The unsaturated fatty acids of greenbugs cultured on Base diet supplemented with Trilinolein solubilized by octylglucoside closely resembled the amounts of unsaturated fatty acids in greenbugs on 'Triumph 64' wheat. Also, the appearance of greenbugs reared on diet with the Trilinolein supplement was healthier than on any other diet alone or with fatty acid supplement. Growth of aphids cultured on OCT, LIN, and PC was inhibited. This was possibly due to toxic levels of the dietary supplement. Therefore, the Base diet supplemented with Trilinolein and the Base diet alone were used in the next aspect of the study.

Greenbug Apterae and Nymphs on Artificial Diet

Greenbug apterae and nymphs were cultured on Base diet and Base supplemented with Trilinolein (TL) and their fatty acid contents were

compared (Table VIII and IX). Large amounts of saturated fatty acids were present in both apterae and nymphs on Base diet and TL. Nymphs 0-2 days old on both Base and TL had reductions in the amounts of saturated fatty acids. The percentage of their linoleic acid was larger than apterae or 3-6 day old nymphs. The fatty acid content of 3-6 day old nymphs was similar to that of apterae. There were no distinctive differences in the fatty acid compositions of greenbugs on Base diet and TL.

The lack of differences of greenbug fatty acid composition cultured on either Base diet or TL may be attributed to the point brought out by Campbell and Nes (1983). They stated that S. graminum received adequate sterols provided by fungal contaminants introduced by the aphid once its stylets pierced the Parafilm® membrane. If this were true, both Base diet and TL had sterols available for the greenbug which increased their similarities. The big difference that can be seen was the decrease in fatty acid content of 0-2 day old nymphs, but that was suspect because of the large standard deviations. The variance may be due to new born nymphs suffering from being introduced to an unfamiliar food source. Another possibility may be that many of the 0-2 day old nymphs feed on the diet only enough to maintain liquid balance and are using up stored fatty acids as a energy source and depleting their supply of saturated fatty acids which was reflected in their total fatty acid composition.

TABLE VIII

FATTY ACID CONTENT OF GREENBUGS REARED ON ARTIFICIAL ALONE (BASE) OR ARTIFICIAL DIET WITH OCTYLGLUCOSIDE[a] AND TRILINOLEIN (TL)[b]

Diet	Aphid	Aphid Age (days)		μg Fatty Acids/mg Aphid[c] 14:0	16:0	18:0	18:2	Total	μg/aphid
Base	Apterae[d]		$\bar{X}$ [e]	40.58ab	45.00a	5.27bc	1.76a	98.02a	0.76
			S.D.	7.70	7.99	0.87	0.72	18.48	0.24
	Nymph	0-2	$\bar{X}$	21.87bc	23.72c	5.94bc	2.31a	58.03b	0.12
			S.D.	9.58	7.76	1.55	0.85	19.34	0.06
		3-6	$\bar{X}$	40.58ab	41.62ab	8.37a	1.81a	98.49a	0.18
			S.D.	13.98	5.41	1.60	0.87	20.04	0.07
TL	Apterae		$\bar{X}$	27.21abc	31.65bc	4.23c	2.63a	70.17ab	0.68
			S.D.	4.73	4.92	0.61	1.63	12.02	0.13
	Nymph	0-2	$\bar{X}$	19.13c	23.22c	5.73bc	2.32a	53.99b	0.16
			S.D.	11.98	11.09	2.17	0.62	25.08	0.12
		3-6	$\bar{X}$	42.90a	41.03ab	7.33ab	1.88a	98.53a	0.21
			S.D.	16.99	7.11	0.76	0.87	24.00	0.08

[a] Octylglucoside is added to the Base diet to solubilze lipids.
[b] Means and S.D. are μg/mg of fatty acids for each diet age group(n=3).
[c] Trace amounts of 12:0, 18:1, and 20:0 were present in some samples.
[d] Apterae from 'Triumph 64' wheat on diet for 6 days.
[e] Means within columns followed by the same letter were not significantly different ($P \leq 0.05$) (Duncan's Multiple Range Test 1955).

TABLE IX

PERCENT COMPOSITION OF GREENBUGS REARED ON ARTIFICIAL DIET ALONE OR ARTIFICIAL DIET WITH OCTYLGLUCOSIDE[a] AND TRILINOLEIN[b]

Diet	Aphid	Aphid Age (days)		Fatty Acids[c] 12:0	14:0	16:0	16:1	18:0	18:1	18:2	20:0
Base	Apterae[d]		$\bar{X}$ [e]	2.26abc	41.41a	45.98a	0.49ab	5.39d	2.76ab	1.40b	0.24a
			S.D.	0.23	0.11	0.68	0.12	0.13	0.48	0.38	0.21
	Nymph	0-2	$\bar{X}$	1.90bc	35.66a	45.76a	0.45ab	11.28a	3.63a	4.15a	0.88a
			S.D.	1.25	4.97	11.16	0.18	1.75	1.25	2.07	0.30
		3-6	$\bar{X}$	3.42a	42.04a	41.76a	0.20b	8.31bc	1.77b	1.92b	0.51a
			S.D.	1.19	5.66	2.89	0.13	1.95	0.79	1.03	0.22
TL	Apterae		$\bar{X}$	0.95c	36.21a	47.88a	0.70a	6.66cd	4.38a	2.44b	0.57a
			S.D.	0.55	4.45	4.70	0.17	1.08	0.44	0.14	0.06
	Nymph	0-2	$\bar{X}$	1.40abc	34.06a	42.94a	0.33ab	10.86ab	4.13a	4.82a	0.85a
			S.D.	1.01	6.88	6.62	0.33	2.25	1.18	1.98	0.41
		3-6	$\bar{X}$	2.67ab	43.67a	42.19a	0.21b	7.34c	1.67b	1.69b	0.43a
			S.D.	1.30	6.23	4.47	0.12	1.38	0.75	0.79	0.16

[a] Octylglucoside is added to the Base diet to solubilize lipids.
[b] Means and S.D. of percentages of greenbug fatty acids for each age group (n = 3).
[c] Trace amounts of 18:3 were present in some of the samples. [d] Apterae from 'Triumph 64' wheat
[e] Means within columns followed by the same letter are not significantly different ($P \leq 0.05$) (Duncan's Multiple Range Test 1955).

CHAPTER V

SUMMARY AND CONCLUSIONS

Greenbugs were reared in growth chambers on 4 different plant cultivars; 'Triumph 64' and 'TAM 107' wheat, 'Pioneer 8300' sorghum, and 'Wintermalt' barley. Whole body lipid extracts were done to determine the percent composition and absolute amounts of fatty acids present. The total lipid extract of greenbugs cultured on 'Triumph 64' wheat was fractionated into 4 lipid classes; free fatty acid (FFA), phospholipid (PL), sterol ester (SE) and triglyceride (TG). The standard fatty acid profile of healthy, unstressed biotype B greenbugs on 'Triumph 64' wheat was established as; 12:0 (1.82%), 14:0 (48.44%), 16:0 (41.75%), 18:0 (4.96%), 18:1 (1.43%), 18:2 (1.23%) and 20:0 (0.17%). This standard was compared to the fatty acid profiles of greenbugs reared on the other host plants. The differences observed suggest that fatty acid composition of some aphids with wide host ranges may not be influenced by diet, but aphids, like S. graminum, with narrow host ranges may be influenced by diet. This type of analysis may be applied in the future to measure the effects of resistant host plants on aphids.

The lipid fractionation experiment resulted in differences among the lipid fractions that were easily distinguishable. The triglycerides were most prominent having 77.80% of the total lipid extract. The phospholipid fraction (13.34%), free fatty acid fraction (5.54%), and the sterol ester fraction (3.32%) comprised the remaining parts of the total lipid extract. Each fraction had a unique profile which was seen only because of the use of this technique, otherwise, the total lipid

extract was dominated by the triglycerides.

The base artificial diet which did not contain any fatty acids before exposure to the aphids did not significantly influence the greenbugs to produce a fatty acid profile different from the standard. The greenbugs feeding on artificial diets which contained fatty acids did have some significantly different fatty acids among themselves. In the future, several more samples would need to be collected to determine variation in fatty acids which were present in such small amounts. Observationally, the diet supplemented with the triglyceride, trilinolein, produced greenbugs which appeared healthiest on the artificial diet.

Although the greenbug age study on 'Triumph 64' wheat and artificial diet was inconclusive, trends of decreasing myristic acid and increasing of the other fatty acids could be seen in those nymphs cultured on 'Triumph 64' wheat. Even though there was no appreciable difference among the 2 diets used, a different trend was observed. The levels of palmitic and stearic acid in the nymphs were decreasing in accordance with longer time spent feeding on the diet, while the levels of myristic acid were increasing.

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APPENDIX

BASIC DIET FOR GREENBUG, *SCHIZAPHIS GRAMINUM* (RONDANI)

DEVELOPED BY AUCLAIR (1965) WITH SLIGHT MODIFICATIONS

PREPARATION OF DIET MEDIA:

Place given amounts of Amino Acids, sucrose, K_3PO_4, $MgCl_2 \cdot 6H_2O$, Salt Mixture No.2 in a 250 ml beaker and dissolve with 60 mls of distilled, deionized water. Check pH and adjust to 7.6 pH using 6M KOH (alkaline) and 6N H_3PO_4 (acid) trying not to exceed a total volume of 100 mls. Add 1 ml of Trace Metals 100x solution and 10 mls of Vitamins 10x solution. Continue to stir and add water until final volume reaches, but does not exceed 100 mls of solution. Then pipette 9 mls of this solution into individual Corning 15 ml centrifuge tubes, cap and freeze until needed.

L - Amino Acids and amides

	(in mg)
Alanine	100
Arginine	400
Asparagine	300
Aspartic Acid	100
Cysteine HCL	50
Cystine	5
Gamma amino butyric acid	20
Glutamic Acid	200
Glutamine	600
Glycine	20
Histadine - free base	200
DL - Homoserine	800
Isoleucine	200
Leucine	200
Lysine mono - HCL	200
Methionine	100
Phenylalanine	100
Proline	100
Serine	100
Threonine	200
Tryptophan	100
Tyrosine	20
Valine	200

TRACE METALS 100x SOLUTION

	(in mg)
Cu^{2+}	12
Fe^{3+}	92
Mn^{2+}	22
Na^{2+}	100
Zn^{2+}	40

VITAMINS 10x SOLUTION

	(in mg)
Ascorbic Acid	100
Biotin	1
Calcium pantothenate	50
Choline chloride	500
Folic Acid	10
Inositol	500
Nicotinic Acid	100
p-Amino benzoic acid	100
Pyridoxine HCL	25
Riboflavin	50
Thiamine HCL	25

OTHER ESSENTIALS:

Sucrose	35g
K_3PO_4	500mg
$MgCl_2 \cdot 6H_2O$	200mg
Salt Mixture No.2	5mg

VITA

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