DEVELOPMENT AND UTILIZATION OF A SYNTHETIC DIET FOR THE GREENBUG, SCHIZAPHIS GRAMINUM (ROND.), FOR USE IN DETERMINING THE FACTOR OR FACTORS RESPONSIBLE FOR RESISTANCE IN BARLEY AND WHEAT

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PREFACE

My interest in insect resistance in crop plants was aroused by Dr. Harvey L. Chada, Professor of Entomology at Oklahoma State University, and Investigations Leader, Entomology Research Division, United States Department of Agriculture, when he informed me of the possibilities for the development of varieties of small grains resistant to the greenbug.

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INTRODUCTION

The greenbug, Schizaphis graminum (Rondani), is one of the most serious pests of small grains in the United States. It causes some damage yearly, and in serious outbreaks the losses have been estimated at more than 50 million bushels of grain.

The greenbug can reproduce well at temperatures between 40 and 80 F, whereas most of their insect enemies reproduce slowly at temperatures below 65 F (Daniels, et al. 1956). Thus, long, cool periods in the spring allow the greenbug population to increase rapidly. This increase, for the most part, is uninhibited by their natural enemies. Chemicals are the primary means of greenbug control. However, they are not always dependable in the cool spring temperatures, and the cost of application is relatively high when compared to the cash value of the crop. Thus, other means of control are being sought.

One of the most promising means of control for the greenbug has been the development of resistant varieties of small grains. However, only limited progress has been made (Chada et al. 1961). More definite advances in the development of resistant varieties may be made when the factor or factors responsible for the observed plant resistance are elucidated.

A review of the literature revealed little published work on the nutritional requirements of Aphididae, and no literature was available pertaining specifically to the nutritional requirements of the greenbug. Thus, techniques and diets developed for other aphid species were

modified and utilized for rearing the greenbug.

The present study concerns the development of a synthetic diet for the greenbug. Such a diet would make it possible to bioassay specific chemicals isolated from the various resistant and susceptible small grain varieties. The ultimate aim is to determine which chemicals in the plants are directly involved in plant resistance. With this knowledge, it may be possible to breed plants with such chemical characteristics which would be resistant to the greenbug.

The Departments of Biochemistry and Botany supplied a total of 39 isolates extracted from Rogers and Will barleys. Some of these were incorporated into the diet as purified chemicals and others were incorporated as the gross extracts.

REVIEW OF LITERATURE

General Ecology

Host Plant Preference - Apablaza and Robinson (1967a) conducted host preference tests with the greenbug, Schizaphis graminum (Rondani). The hosts were Swan and Parkland barleys, Selkirk wheat, and Rodney oats. A statistical analysis showed no preference among the four host plants and no significant differences between preferences by winged and wingless morphs.

Wadley (1931) reported that over 60 plant species are fed on by the greenbug. All of the food plants, with rare exception, are in the grass family. They include the common small grains, rice, corn, sorghum, millet, and many annual and perennial grasses. Little is known as to the degree of infestation of the different species. By far the majority of the serious infestations recorded have been on oats and wheat and to a lesser extent on rye and barley.

Wadley (1931) classified the hosts as preferred, second choice, and temporary food plants. Reduced fecundity and increased migration were encountered on other than preferred food plants. Wheat and oats were found unsatisfactory as food after the heading stage.

Light Quality and Quantity Preference - Auclair (1967) determined the effects of light on the cotton aphid, Aphis gossypii Glover, feeding on synthetic diets. It was found that aphids selected and colonized diets receiving no or only weak light of 5 to 50 ft-c. Survival was reduced on diets exposed to intense light of 600 ft-c.

When the quality of light reaching the diets was modified with appropriate filters, aphids preferred diets lighted in the colors yellow and orange (570 and 595 mu) and, in general, grew, reproduced, and survived best on diets lighted in the 525 to 595 mu spectral range. Violet and blue light (420 and 485 mu) had a repellent effect and, in general, poorest growth, reproduction, and survival occurred on those diets lighted in the range of 420 to 485 mu. Although nymphs could discriminate between different colors, they were less sensitive than adults to color changes once they had settled on the preferred diets. The intermediate wavelengths (mostly in the green) had little influence on aphid behavior, with results similar to those obtained in the absence of light.

Cartier and Auclair (1964) conducted color preference tests with biotypes of the pea aphid on a synthetic diet. Preferences were indicated for orange or yellow or both (615 to 590 mu). Aphids of biotype R1 on diets back-lighted blue or white survived only for a few days, whereas they lived longer and grew rapidly on diets lighted orange or yellow.

Overwintering, Migration, and Damage - Markkula and Pulliainen (1965) found that at 12 C the prereproductive period, reproductive period, and the total lifetime of the English grain aphid, Macrosiphum avenae (Fabricius), were two to four times longer than at 28 C. However, at 28 C there were fewer progeny than at either 20 or 12 C.

According to Wadley (1931) wintering of the greenbug in the egg stage is of little economic importance, owing to the delay in producing migrants in the spring. Nymphs and adults winter in southern states at mean temperatures of 30 F with only brief approaches to 0 F. A large

proportion of the outbreaks occur where this wintering takes place. Migration to the north from wintering places appears to occur in many seasons. Usually only part of the migration is at one time; the remainder is by later generations. Considerable injury may result if the infestation becomes established well in advance of summer conditions. Usually, infestation by migration is too late to become very important. In the field the population may reach from one to several thousand per square foot before killing the grain.

Wadley (1931) further stated that the greenbug is much more injurious in proportion to its numbers than other grain aphids. Its feeding causes a peculiar and characteristic injury to all its host plants. Pale spots with red centers develop around feeding punctures. Paleness was apparently due to the destruction of chlorophyll, while the red spots were caused by reddening of the leaf-cell nuclei.

Apablaza and Robinson (1967b) made comparisons of the injury of the greenbug, the English grain aphid, and the corn leaf aphid feeding on Parkland barley, Selkirk wheat, and Rodney oats at various stages of plant growth. The corn leaf aphid did not establish large populations on wheat or oats, but most seedlings of barley infested prior to heading were killed. After barley plants had headed out there were no reductions in yield of harvested grain. The greenbug and English grain aphid severely injured or killed seedlings of barley, wheat, and oats and caused reductions in kernel weight of harvested grain, even when placed on plants of advanced growth.

Greenbug Growth and Development - According to Wadley (1931) the wingless parthenogenetic female greenbug is typically about 1.8 mm in length and 0.8 to 0.9 mm in the greatest width. The winged females are

somewhat smaller, the oviparous females are a little larger, and the males are smallest of all.

The nymphs pass through four instars; the total time required is about one week with summer temperatures, but it varies widely. Advanced embryos occur in late fourth-instar nymphs (Wadley, 1931).

Wadley (1931) conducted extensive studies with controlled temperatures, both moderate and extreme. It was found that the greenbug developed from 7 to 33 C, the optimum being 30 C. However, the maximum rate of reproduction came at 22 C. It was quickly killed by temperatures as low as -15 C or as high as 42 C. At temperatures less extreme, but still above and below the range of development, killing was slower. Inactivity due to cold was fatal in a few weeks at most.

Wing Formation in Aphids - Many workers have conducted various experiments in an effort to elucidate the mechanism of wing formation in the various aphid species. Wadley (1931) concluded that all forms of curtailed nutrition in the greenbug produce a high percentage of alates and that the effect is seen in the succeeding generation. Curtailed nutrition, if severe, may be effective in wing production as late as the day before birth but is not effective after birth. In addition to nutritional effects, variable temperatures and day lengths were found to have an influence on the production of alate forms. Furthermore, winged adults produced nearly all wingless progeny under all conditions.

Johnson and Birks (1960) proposed a theory of the nature of the developmental processes resulting in wing polymorphism in aphids. In this theory, it was suggested that all aphids begin development as presumptive alate and that apterous and intermediates are forms which

have become irreversibly diverted from the alate course during development. Experimental evidence with Aphis craccivora Koch indicated that diversion resulting in the production of apterae could occur at any time over a protracted period extending from the late embryonic stages until the second larval molt.

Mittler and Dadd (1966) stated that Myzus persicae (Sulzer) involved in nutritional experiments, in most cases, became predominantly alate. It was found that with either isoleucine or histidine omitted from the standard diet, or supplied at very low levels, a majority of the aphids became apterous. While these experiments did not determine whether these amino acids exerted their effects only on nymphs after birth, indirectly via the mothers before birth, or in both ways, they did show unequivocally that the nature of the food influenced the extent to which wings developed in a population of aphids.

These authors further stated that it should not be construed that, in focusing attention on food as a specific factor involved in morph determination, the validity of other factors such as population density, microenvironmental differences, and parental age that have been implicated by other workers are of no importance. These may well be of overriding importance, given a different set of environmental circumstances, especially so when other species of aphids are concerned.

Rate of Reproduction by Greenbugs - Wadley (1931) found that greenbug population increase was difficult to calculate because of the overlapping generations. The developmental rate and reproduction early in adult life were found to be important factors in population increase. The potential increase at several temperatures was calculated and it was suggested that a population increase of thirteen times per

generation could be used as the reproductive capacity of this species. The wingless parthenogenetic females begin reproduction within a few hours after the last molt, while winged females do not reproduce until 24 to 48 hours after the last molt.

Rearing records from greenbugs feeding on oats in the greenhouse showed an average of 22.7 days for the reproductive period with an average of 63.1 progeny per female for the wingless parents. For winged parents the corresponding figures were 16.8 and 39.5, respectively. An average of 3.5 young per day for the first ten days of reproduction was obtained from 31 wingless females. Four young per day appeared to be about the maximum rate which could be sustained several days by young wingless females, although as many as ten were produced on individual days. A temperature of 79.7 F was optimum for reproduction by wingless females, while the optimum temperature for winged forms was 73.4 F (Wadley 1931).

Daniels et al. (1956) stated that greenbugs can reproduce well at temperatures between 40 and 80 F, whereas most of their insect enemies reproduce slowly at temperatures below 65 F. Thus, long periods of cool weather permit the greenbug to increase rapidly. It was found that most females begin reproduction 6 to 30 days after birth and continue to produce two or three progeny per day for 20 to 30 days.

Singh and Wood (1963) found that reproduction varied with both the temperature and the host plant. There was an increase in fecundity with each 5-degree rise in temperature above 35 F for both greenbug strains until the optimum for reproduction was reached. Fecundity of the field strain on resistant Dickinson Selection 28A wheat and susceptible Ward barley was not affected at the lower temperatures, but at

higher temperatures, fecundity and survival were greatly retarded on the resistant plants. Furthermore, fecundity of the field strain on DS28A was much lower at optimum temperatures than that of the greenhouse strain. Fecundity of the greenhouse strain on DS28A wheat was comparable to reproduction of the field strain on Ward barley at all temperatures.

Belvett, Sun, and Robinson (1965) reared the greenbug on Swan barley in a growth cabinet at a constant temperature of 70 F. The average length of the reproductive period was 16.8 days with an average of 84.2 progeny per female.

Aphid Mouth Parts

Auclair (1964), in summing up some of the recent advances in the feeding and nutrition of aphids, stated that, in aphids, the mouth parts have evolved into two slender chitinous bristles, the stylets, which are used to penetrate plant tissues in search of nutritious liquids. The food is imbibed via the stylet food canal or duct formed within the interlocked maxillary stylets. This food canal may be several hundred microns in length, but its lumen, especially at the distal end, is remarkably small. Mittler (1957) reported estimates on the diameter of the lumen ranging from fractions of a micron to over one micron. Auclair (1964) further stated that large particles such as tissular cells in general, blood cells, and many of the larger bacteria could not be ingested. In fact, the natural diet of many aphids, which is represented by the sieve-tube sap of plants, would be mostly in the form of solutes dissolved in water.

Aphid Feeding Sites and Natural Food - According to Mittler (1957), the fact that Tuberolachnus salignus (Gmelin) and other aphid species

insert their stylet tips into the phloem sieve-tubes of their host plant suggests that the insects ingest sieve-tube sap in preference to other plant sap. The high nutrient value of the sieve-tube sap has generally been supposed to be the basis for the aphid's choice.

Chatters and Schlehuber (1951) concluded that the stylets of the greenbug are highly selective organs and do not enter the tissue haphazardly, but are directed with marked precision. It was not determined whether the sensitivity of the stylets was due to the pH or carbohydrate content of the desired cells, whether it was due to nerve control of the greenbug, or whether the sensitivity was due to other factors.

Lowe (1967) observed that the aphids Megaura viciae, Aphis fabae Scopoli, Acyrthosiphon pisum (Harris), Aulacorthum solani, Myzus persicae, and Myzus ornatus settled on the undersurface of bean leaves in characteristic patterns. Some species settled wholly on veins, while other species settled away from veins, especially on the leaf margin. It was suggested that the phloem is the principal food source for most of these aphids but when the population reaches a certain level, then the mesophyll serves as an alternate food source for them.

Davidson (1923) and Lowe (1967) suggested that the physiology of the host plant is very important in the biology and physiology of the aphid in reference to the specific tissue used as a food source by the aphid.

According to Auclair (1964) plant sap may contain from 5 to 25% sucrose in addition to traces of some other carbohydrates such as raffinose. The sap also contains nitrogen, usually less than 1%, in the form of free amino acids and amides. As reported by Auclair (1964),

Ziegler and Ziegler (in Flora, Jena. 152:257-278. 1962.) indicated the presence of many of the water-soluble vitamins in sieve-tube sap, but that little is known concerning the presence in sap of other insect nutrients such as cholesterol and fatty acids.

Aphid Probing in Relation to Feeding - Mittler and Dadd (1965) allowed M. persicae to probe and insert its stylets through a parafilm membrane enclosing various test fluids. They found marked differences in the duration of initial probes. With a hard substrate or N HCl behind the membrane the typical duration of these probes was brief, less than one-fourth minute; with air or distilled water it was normal, i. e., as on a host plant, one-fourth to one-half minute; with a solution of six amino acids it was only slightly longer, but with a 20% sucrose solution it was appreciably longer, over one minute. With 20% sucrose containing the six amino acids, or all the constituents of the complex diet, the typical duration of initial probes was markedly extended to over four minutes.

According to Miles (1958), before beginning to feed, the milkweed bug, Oncopeltus fasciatus (Dallas), and some other Heteroptera secrete saliva onto the substrate. The saliva is then drawn up the stylet food canal where it is brought into contact with gustatory sensillae. It was suggested that sampling of this type may be a factor in determination of the point at which plant bugs begin to feed.

Aphid Salivation and Stylet Sheaths - Mittler and Dadd (1963a) allowed M. persicae to penetrate a parafilm membrane into water or sucrose solutions containing 0.1% neutral red stain. The neutral red stained the stylet sheaths as they were being formed. There were two types of stylet sheaths observed. The first type was similar in size

and shape to those normally formed in plant tissue when penetration was to some depth. The second type were termed "blobs" and were formed when the aphids just barely penetrated the membrane.

According to Miles (1959) the aphid, A. craccivora, was observed secreting two different types of saliva. The first type solidified almost immediately as it left the stylets and was evidently sheath material. The second type was a watery secretion and was drawn in and out of the stylet food canal.

McLean and Kinsey (1965), while working with pea aphids, observed that many of the aphids began secreting visible sheath saliva as soon as the stylets entered the sucrose substrate. Distinct diverticula in the form of bulges were readily seen as the sheath was extended. Close examination of the formation of these diverticula showed that the aphid secreted a small amount of sheath and then expanded a portion of this sheath into a bulge. As the bulge was expanding, a liquid material could be seen to swirl rapidly within the sheath creating the diverticula.

Aphid Excretion

Yust and Fulton (1943) observed exudation from the broken off rostalis of California red scale feeding on lemons, squash, and citron melons. Mittler (1953) successfully collected phloem fluid from the severed stylets of T. salignus feeding on two- to four-year-old Salix spp. stems. Chemical analysis of honeydew obtained from aphids feeding on the same stems at the same time showed no differential absorption of amino acids by the aphids. These results suggested that the nitrogenous matter ingested by an aphid is in the form of free amino acids and amides, which are usually in excess of the aphid's requirements.

Mittler (1958) reported that the amino acid and amide quality was always the same in the willow sap and aphid honeydew, however, the quantity was less in the honeydew. The relative reduction in each amino acid and amide appeared to be proportionate. Therefore, the amino acid and amide composition of the honeydew invariably reflected that of the stylet sap.

Peel and Weatherley (1959) found, as did Mittler (1958), that the amino acid fraction of the sieve-tube sap varied with the time of year. Moreover, they found that the only three amino acids present year around were aspartic and glutamic acids and asparagine.

Peel and Weatherley (1959) obtained sieve-tube sap from the willow, Salix viminalis. They found that apart from sucrose, the only other carbohydrates present were raffinose and starchyose. The former was found in quantities up to 15% of the total carbohydrate present, the latter in traces only. No methylated or phosphorylated sugars were detected.

Zimmermann (1957) analyzed the sugar content of the sieve-tube exudate of 11 families including 16 species of trees. Only non-reducing oligosaccharides of the raffinose family were found. Sucrose was the major transport sugar, but in some tree species most (White ash) or part (elm, linden) of the sieve-tube sugars were in the form of higher oligosaccharides, raffinose, and starchyose.

Maxwell and Painter (1959) investigated various factors affecting the rate of honeydew deposition by the greenbug. There were usually significant increases in the number of honeydew droplets excreted with each 5-degree increase in temperature above 35 F with both resistant and susceptible wheat and barley varieties. The rate of excretion was

higher on barley than on wheat. The highest average rate of excretion on barley was at 75 F and on wheat at 85 F. There was a significant decline in the rate of excretion of honeydew from aphids on barley above 75 F. Significantly lower rates of excretion were seen on Dickinson than on Pawnee, Ponca, and Bison wheats. When feeding on barley, more honeydew droplets were seen on Reno than on Dicktoo or MO-B475.

Reduced light intensities significantly reduced the excretion of honeydew on Pawnee and Dickinson wheats and Reno barley. This occurred whether the entire plant was darkened or only the part on which the greenbug was feeding.

Reduced moisture to the host plant resulted in significant differences in the rate of honeydew excretion. Greenbugs feeding on plants in vigorous growing conditions, where the soil was moist or wet, had a higher rate of excretion than when feeding on plants where the soil was dry enough to induce incipient plasmolysis. No significant differences were seen in the rate of excretion of the plants maintained at field capacity and ones maintained at the point of saturation.

Significantly greater amounts of honeydew were produced by greenbugs on the wheat leaf than on the stem. Similarly, more honeydew was produced on green leaves than on leaves yellowed by age or greenbug feeding. More honeydew was produced by greenbugs feeding at the base of the leaf than at the tip. Significant differences were recorded between the middle areas of the leaf and the tip area but not between the middle and the base portion.

Molting and reproduction both were found to reduce the rate of honeydew excretion.

It was suggested that the reduction in honeydew above 85 F in wheat and 75 F in barley was related to the effect of high temperature on the biology of the greenbug in that it tended to reduce the metabolic activity. Another possibility was that the high temperature may have been responsible for physiological changes in the plant which reduced the plant sap availability to the aphid.

The rate of excretion varied considerably among the different varieties of wheat and barley used. The rate of deposition of honeydew was correlated very closely with the known degree of resistance of the varieties to the greenbug.

Auclair (1959) measured the amount of honeydew excreted by pea aphids feeding on susceptible and resistant varieties of peas. It was found that excretion was interrupted at molting for mean periods of 12 to 16 hours and between molts and in the adult stage for mean periods of 3 to 15 hours, although in many cases the aphids remained in the feeding position. Droplet volume, frequency, and rate of excretion were generally proportional to the susceptibility of the host variety on which the aphids were feeding. Honeydew from aphids feeding on susceptible varieties usually contained a slightly higher concentration of free amino acids and amides than honeydew from aphids feeding on resistant varieties.

According to Auclair (1958), the average droplet volume and the mean frequency and rate of excretion increased from the first-instar nymphs to the adult stage of the pea aphid and then decreased slightly during the parthenogenetic reproduction. The decreased rate of excretion observed in bearing adults suggests that the would be excreted free amino acids were utilized in the production of the unborn progeny.

Auclair (1958) reported that quantitative chromatographic analysis of pea aphid honeydew revealed 19 free amino acids and amides that were also present in the pea plant and aphid blood. The total concentration of these compounds in the honeydew was similar to that in aphid blood, but was 2 to 10 times as high as that in pea plant juice.

Maltais and Auclair (1952) reported that the honeydew of the crescent-marked lily aphid consisted of: total nitrogen, 2.44%; amino nitrogen, 23.31 mg in 100 ml; and sugars, 35.7%. There were 22 free amino acids and amides found. Thus, the honeydew was composed of carbohydrates and a considerable amount of nitrogenous constituents such as amino acids and amides.

Paper chromatography analysis of honeydew of scale insects found on citrus in California was reported by Wolf and Ewart (1955) and Ewart and Metcalf (1956). The honeydew of these insects was found to consist of basically the same compounds as honeydew from Aphididae as reported by many other workers.

Gray and Fraenkel (1954) suggested that honeydew should be regarded as a digestive product rather than merely a mixture of excess carbohydrates and water. As early as 1923, Davidson concluded that the composition of the honeydew of aphids depends on the species of plant and aphid concerned and is in close relationship with the composition of the cell sap of the plant and the digestive processes of the aphid.

Cages for Rearing Aphids on Synthetic Diets

Attempts to rear plant-sucking insects apart from their host have resulted in a wide array of cages. Maltais (1952) stated that the basic requirements for cages used in aphid nutritional studies must (1) be made of materials easily and effectively sterilized by steam

heat, (2) be relatively small for economy of materials and convenient handling under the low power binocular microscope, (3) be bacteria-proof for prevention of food contamination, (4) be constructed with interchangeable parts for convenient and rapid assembly, and (5) provide adequate conditions for free development of the insects.

Mittler and Dadd (1963a) constructed cages from two sections of a test tube. A parafilm membrane was stretched over the end of one section and abutted to the end of the other section. The diet was contained in the upper section and the aphids were contained in the lower section. The entire cage was supported on a cork. Mittler and Dadd (1964a) modified their cage by containing the diet between two membranes in a sachet. This eliminated one piece of glass tubing and made it possible to prepare and store frozen a large number of cage assemblies in advance.

Auclair (1965) independently developed a sachet type of feeding device similar to the one of Mittler and Dadd (1964a). The sachet was supported on the upper end of a glass ring. The ring was then inverted and supported in a modified number five polyethylene stopper. The stopper was modified by replacing the bottom with organdy. Auclair and Raulston (1966) used similar cages in the rearing of lygus bug.

Types of Membranes Used in Synthetic Diet Studies with Aphids - Attempts to study the specific nutritional requirements of plant-sucking insects were hampered, if not effectively prevented, by the lack of a satisfactory membrane. Hamilton (1930) kept M. persicae alive for six to seven days feeding on various plant extracts through a fish skin membrane. Hamilton (1935) later removed the leaf epidermis and used it as a membrane. Pletsch (1937) attempted to feed aphids

through membranes of fish skin, gold-beaters' skin, and capping skin. Maltais (1952) used stretched rubber balloons as membranes to feed aphids artificially. The attempts to rear aphids by these workers were largely unsuccessful because, as they pointed out, the membranes either did not meet the physical and/or chemical requirements of the insects or the membrane would rupture easily or leak after being punctured.

Use of Parafilm M for Membranes in Aphid Feeding - Bradley (1956), in a study involving the ability of M. persicae to transmit potato virus Y, stretched parafilm over a tobacco leaf in such a way that Myzus was made to puncture the parafilm membrane before penetrating the leaf. The parafilm was stretched to a thickness of 10 to 20 microns, which was less than the thickness of the epidermis of the leaf. This was the first usage of parafilm as a membrane for aphids and its potential use in the artificial feeding of aphids was not exploited by this researcher.

Mittler and Dadd (1962) reported that M. persicae was maintained for 16 to 17 days feeding through a parafilm membrane. Parafilm has since been exhaustively used as the membrane in feeding aphids and other plant-sucking insects as exemplified by the works of Mittler and Dadd (1963a,b, 1964a,b), McLean and Kinsey (1965), Auclair (1965, 1967a,b), and Auclair and Raulston (1966).

Aphid Survival When Fed Via Parafilm M Membranes - Mittler and Dadd (1963a) described a method for feeding the green peach aphid on liquids accessible via an artificial membrane of stretched parafilm. Mittler and Dadd (1963b) found that M. persicae fed only water survived longer and gave birth to more nymphs than starved individuals. Survival was extended and larviposition increased by the ingestion of sucrose

solutions of various concentrations. Likewise, survival and larviposition was greater on a complex synthetic diet.

Auclair (1965) maintained the pea aphid longer than two months and through almost three generations on a synthetic diet. Through the first generation, aphid survival on the diet and on susceptible pea plants were similar. During the second generation, more mortality occurred and ultimately in the third generation all aphids died.

Auclair (1967b) reported that the survival of A. gossypii was reduced when any sugar other than sucrose was used, with the exception of a mixture of 20% sucrose and 10% maltose, which was superior to either 20% or 30% sucrose alone. A synergic effect was suggested by this mixture.

Nutritional Requirements

Hinton (1959), in detailed nutritional studies with genetically different strains of Drosophila, concluded that specific nutritional requirements are determined by the individual organism's genetic constitution. Moreover, the possibility exists that in metazoan organisms the genetic control of metabolic steps is complicated by modifiers, multiple genes, and other complex genetic systems.

Development of Synthetic Diets for Aphids

A complex synthetic diet containing 20 amino acids, 10 vitamins, sucrose, potassium phosphate, magnesium chloride, cholesterol, and water was made for M. persicae (Mittler and Dadd, 1962). The results showed a 50% survival time of 3.5 days on water only, 6.5 days on 18% sucrose only, and 13 days on the completely synthetic diet. The average number of nymphs produced per adult was 5 on water, 9 on

sucrose, and 19 on the diet. Over half of the nymphs survived two weeks, in which time they molted at least twice. Of 40 which survived 16 to 17 days, 11, 14, and 7 had developed into diminutive fourth-instar apterous and alatiform nymphs and apterous adults, respectively.

Auclair and Cartier (1963) reported on the development of a diet for the pea aphid based on the average concentration of various amino acids and amides in pea plants and pea aphid blood and honeydew. The diet consisted of 23 amino acids and amides, 11 water soluble vitamins, 35% sucrose, salts, and cholesterol. Aphids grew and developed during the first generation almost as well as those grown on pea plants. When pea aphids were reared by Auclair and Cartier (1963) on the diet developed for M. persicae by Mittler and Dadd (1962), there was little growth and death of all nymphs within one week.

Auclair (1965) modified the Auclair and Cartier (1963) pea aphid diet and maintained pea aphids longer than two months and through almost three generations. The diet contained, in aqueous solution, 40.17% dry matter consisting of 23 amino acids, 4.135%; 11 water soluble vitamins, 0.146%; sucrose, 35%; mineral salts, 0.705%; and cholesterol benzoate, 0.0025%. Variations in pH, or in the salt, sucrose, or total amino acid concentrations usually resulted in reduced aphid growth, reproduction, and survival.

Amino Acids Associated with Aphid Nutrition - Auclair, Maltais, and Cartier (1957) listed the amino acids arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine as being the ten amino acids essential to insects.

Strong and Sakamoto (1963) fed M. persicae on a solution of

glucose-U-C^{14}. The results indicated that this aphid was able to synthesize the following amino acids from glucose: aspartic acid, asparagine, serine, glutamic acid, glycine, alanine, cystine, and two unidentified compounds which reacted with ninhydrin. Amino acids which had very low levels of activity but were considered to be nutritionally essential included threonine, valine, methionine, isoleucine, leucine, lysine, histidine, arginine, tyrosine, and phenylalanine.

Mittler (1967a) tested fluid uptake by M. persicae in a series of diets ranging from 0 to 4.8% total amino acid concentration with 15% sucrose common to the series. It was found that uptake increased with dietary concentrations of amino acids up to 3% and then decreased slightly. Mittler (1967b) further reported that diets with less than 1% total amino acid concentrations were fed on minimally and supported poor or no growth. While uptake decreased at concentrations above 3%, the growth rate continued to increase beyond that level to 4.8%, the highest concentration tested.

Methionine, as the only amino acid in the diet, did not provide the degree of phagostimulation given by the mixture of all 20 amino acids, including methionine. The data indicated that the amount by which uptake was reduced by the omission of methionine was very close to the amount of uptake attributed to the phagostimulant effect of methionine alone (Mittler 1967b).

Dadd and Krieger (1968) reported that all 20 amino acids in the diet of M. persicae were tolerated over a wide range of concentrations.

According to Banks and Macauley (1965), adult A. fabae feeding on two varieties of field beans assimilated 70% of the nitrogen ingested; the nymphs were relatively inefficient and assimilated only 50% of the

nitrogen ingested. Mittler (1958) showed that T. salignus absorbed at least 55% of the nitrogenous matter they ingested.

Sugars Associated with Aphid Nutrition - Mittler (1958) reported that while willow trees were bearing leaves, sucrose was the only sugar in the stylet sap. Honeydew, however, was composed of roughly equal amounts of sucrose, glucose, fructose, and melezitose. These sugars were derived from sucrose, the only sugar ingested. An analysis (Yust and Fulton, 1943) of the exudation from broken rostilis of the California red scale feeding on lemon revealed practically pure sucrose. Tests for glycogen and reducing sugars were negative.

Mittler and Dadd (1964b) found that sucrose was a phagostimulant as it made water more attractive. Furthermore, when a mixture of six amino acids was added to the sucrose solution, there was a greater uptake than with the sucrose solution alone. Yet in the absence of sucrose, the amino acids did not show the phagostimulant effect.

According to Mittler (1967a) fluid uptake was poor or non-existent on diets having less than 5% sucrose. The optimal range of 10 to 20% was indicated with a progressive decline in fluid uptake with concentrations above 20%.

Auclair (1967a) reported that sucrose is a necessary phagostimulant for both the pea aphid and the cotton aphid and must be present in the chemical diet in fairly high concentrations (20-35%) to ensure feeding, growth, and reproduction. Sucrose is an important constituent of the natural diet of aphids and represents a key substance in the successful maintenance of aphids on artificial diets.

In other experiments, Auclair (1967b), found that on diets for the cotton aphid in which sucrose was replaced by glucose and fructose, or

in which sucrose was replaced in part or totally by cellibiose, fructose, galactose, glucose, lactose, melezitose, raffinose, ribose, sorbose, or trehalose, aphid survival was drastically reduced. This low survival rate may have been due to lack of palatability of these sugars or to their poor nutritive value.

Vitamins Associated with Insect Nutrition - Experiments involving the effects of the B-vitamin complex on Tenebrio molitor Linnaeus and Tribolium spp. and to a lesser extent other species by Fraenkel and Blewett (1942, 1943a, 1947) led Fraenkel et al. (1948) to the observation that the B-vitamins have all-important functions in the metabolism of all forms of life, from bacteria to man, and there has scarcely been a case of a new B-factor which was not ultimately proved to be of general significance.

Thorsteinson (1960) stated that the vitamins are a chemically heterogeneous group but may be considered together for convenience, inasmuch as their chemotactic significance has hardly been investigated. However, the few data at hand are in harmony with the concept that many of the substances which chemotactically influence feeding behavior are nutritionally important to insects.

Friend and Patton (1956) developed a chemically defined diet for the onion maggot, Hylemya antiqua (Meigen). This was the first chemically defined diet that would support growth and development of a phytophagous insect under aseptic conditions. The nine B-vitamins, coenzyme A, and thioctic acid were tested by the deletion method for their effects on the onion maggot.

Of the nine vitamins tested, i. e., biotin, pantothenic acid, choline, folic acid, pyridoxine, riboflavin, nicin, thiamine, and

vitamin B_{12}, only vitamin B_{12} was not considered essential to the onion maggot. The omission of vitamin B_{12}, thioctic acid, and coenzyme A slowed larval development slightly but did not result in death of the larvae.

In experiments conducted by Dadd et al. (1967) to determine the essentiality of the B-vitamins for M. persicae, it was found that in all cases the omission of thiamine, nicotinic acid, or pantothenate severely retarded growth. Without nicotinic acid no nymphs survived to become adult. Without thiamine or pantothenate only a few nymphs became adults, all of which were small and none produced any second-generation nymphs. Second-generation nymphs on diets deficient in pyridoxine, folic acid, inositol, or choline died within ten days without growth. Growth and survival were poor, in most cases, with second-generation nymphs on biotin- and riboflavin-deficient diets, however, some third-generation larvae were produced. Thus, it was concluded that thiamine, nicotinic acid, and pantothenate are essential for growth and maturation in the first generation; pyridoxine, folic acid, inositol, and choline are essential for continued growth in the second generation; and biotin and riboflavin are most probably essential for continued growth in the third generation.

Vanderzant (1959) found that larvae of the boll weevil, Anthonomus grandis Boheman, reared in the absence of inositol died in the first instar or soon after the first molt. Dadd (1961) found that the lack of inositol in the diet of the locust, Schistocerca gregaria (Forsk.), not only impaired growth but also caused irregularities in the development and pigmentation. In the same work, Dadd (1961) found that, in addition to inositol, thiamine, riboflavin, nicotinic acid, pyridoxine,

folic acid, calcium pantothenate, biotin, and calcium chloride were necessary for proper growth of S. gregaria.

Hinton et al. (1951) showed that Drosophila tolerated biotin over a wide range of concentrations. High concentrations of pyridoxine inhibited the development while vitamin B_{12} consistently raised the percentage of larvae to pupate. Inositol and p-aminobenzoic acid were somewhat inhibitory to Drosophila.

Subbarrow and Trager (1940) reported that the vitamins pantothenic acid and B_6 are essential for Aedes aegypti (Linnaeus) larvae. Later, Trager (1948) found that if biotin was omitted from the diet of A. aegypti, larval growth was very slow and metamorphosis to the adult stage did not occur.

Noland et al. (1949) reported on the B-vitamin requirements of Blattella germanica (Linnaeus). The omission of choline, pantothenic acid, and nicotinic acid resulted in extremely slow growth and death before maturity. The omission of pyridoxine, thiamine, and riboflavin resulted in retarded but significant growth with some reaching maturity. Diets lacking inositol, p-aminobenzoic acid or biotin showed no adverse effect upon growth rate or survival. Moreover, omission of folic acid appeared to stimulate growth somewhat.

House (1954) reared Pseudosarcophaga affinis (Fall.), a dipteran parasite of the spruce budworm, on a chemically defined diet. Thiamine, riboflavin, calcium pantothenate, nicotinic acid, choline chloride, and biotin were found to be essential for larval growth and development, but not B_{12}, pyridoxine, folic acid, p-aminobenzoic acid, or inositol. A slightly beneficial effect on pupation was given by B_{12} but omission of p-aminobenzoic acid slightly stimulated growth and development.

According to Pierre (1962) the American cockroach synthesized vitamin C. It was suggested that symbionts in the fat bodies were responsible for the synthesis of this vitamin. Dadd et al. (1967) found that the mean weights were greater for aphids reared on diets containing ascorbic acid than without. Hence, it is apparent that a dietary source of ascorbic acid must be supplied to M. persicae for an optimal rate of growth if reserves derived from the mother are depleted.

Minerals and Minor Elements Used in Synthetic Diets for Aphids - Retnakaran and Beck (1967) found, in studies on the mineral nutrition of the pea aphid, that diets containing mineral mixtures approximating the inorganic content of the host plant (Vicia faba L.) allowed good nymphal growth, but the resulting adults failed to reproduce. An imbalance in the calcium and phosphate ions was demonstrated to be the cause of the lack of reproduction. A calcium citrate:potassium phosphate ratio of 0.010:0.250 was found to improve growth without inhibiting reproduction. The pea aphid was found to require magnesium and, moreover, the concentration of magnesium depended on the phosphate concentration. The greatest growth and reproduction rates were obtained with a potassium:magnesium ratio of either 0.250:0.200 or 0.500:0.100.

Dadd and Krieger (1968) found that omission of cystine from the diet of M. persicae halted growth after one generation but this could be corrected by addition of inorganic sulphate or extra methionine, showing that cystine was needed only as a source of sulphur.

Dadd and Mittler (1966) and Dadd (1967) reported on the requirements of M. persicae for the trace elements of zinc, iron, manganese, copper, calcium, and sodium. The requirements for iron, zinc, and manganese were demonstrated individually. Deficiencies for iron and

zinc became apparent in the first generation of larval growth. Manganese deficiency became apparent in the second generation. Requirement for copper appeared in the third generation. Calcium was supplied as the vitamin, calcium pantothenate, at a routine level and deficiencies were not demonstrated. Sodium essentiality was not demonstrated as it may be supplied at sufficient levels as impurities in other dietary compounds. With the addition of the first four metals, the diet supported M. persicae through 20 generations and was still in progress.

Ehrhardt (1968a) reported on the trace element requirements of Neomyzus circumflexus (Buckton). It was found that reproduction in first generation aphids was halted when reared on diets devoid of iron, zinc, manganese, copper, and calcium. Furthermore, second generation aphids were sterile when reared on such diets. Individually these elements gave optimal growth of first generation nymphs at concentrations of 460 ug iron, 220 ug manganese, 190 ug zinc, 850 ug calcium, and 100 ug copper. These concentrations were based on 100 ml of diet. When all five trace elements were included in the diet, first generation aphid growth and reproduction was equal to aphids reared on host plants, Vicia faba. However, growth and reproduction was reduced to 90% in the second generation, but remained constant in succeeding generations.

Sterol Requirements in Insects - According to Fraenkel and Blewett (1943b), without cholesterol, growth of the beetles Tribolium, Lasioderma, and Silvanus became slightly, Ptinus appreciably, and Sitodrepa and Ephestia severely impaired. Pant and Fraenkel (1950) reported that symbiotic yeasts synthesized the necessary sterol for the

beetles, Lasioderma serricorne and Stegobium paniceum.

Dadd and Mittler (1966) did not include a sterol in their diet for M. persicae but suggested that it is provided by symbionts. Ehrhardt (1968b) reared N. circumflexus through more than 11 generations on a chemically defined diet which contained no sterol or any other lipid. Through eight generations, the sterol content of the aphids remained constant. Evidence was obtained that symbionts synthesize the required cholesterol.

Hydrogen Ion Concentration in Synthetic Diets for Aphids - Swingle (1931) determined the hydrogen ion concentration of the gut contents of about 40 species of insects. The pH varied from pH 4.0 to 9.5 in the various species. It was concluded that in the majority of insects, the pH increases from the mouth through the foregut and midgut and decreases from posterior part of the midgut through the hindgut. In general the pH is slightly acid. No correlation was found between acidity and the type of food eaten.

Day and Irzykiewicz (1953), working with M. persicae, found no difference in uptake of artificial diets adjusted to pH ranging from 5.3 to 8.0. Mittler and Dadd (1962), however, reported that diets for M. persicae were adjusted to pH 7.0 with phosphoric acid.

Auclair (1965) reported that the diet for the pea aphid was adjusted to 7.6. It was shown that the pea aphid can discriminate between as little as 0.3 to 0.6 of a pH unit.

Auclair (1967b) reared the cotton aphid through almost two generations on the diet constructed for the pea aphid (Auclair 1965). It was found that in uncontaminated diets, whether fed on or not by the cotton aphid, the pH decreased by about 0.2 to 0.4 of a pH unit during four

days at room temperature. Hence, this pH decrease apparently resulted from interactions between nutrients rather than from aphid feeding. It was found, in the case of contaminated diets, that the pH slightly increased as a result of microorganisms.

The cotton aphid was found to be not as sensitive to pH as the pea aphid, the optimum pH being 7.4 to 7.8.

MATERIALS AND METHODS

Source of Greenbugs Used in Diet Studies

The greenbugs, Schizaphis graminum (Rondani), used in this study were collected January 25, 1967, from a natural population occurring on Triumph wheat. They were taken to the laboratory where they were placed on Rogers barley. After 24 hours, all but one of the greenbugs were removed from the plants. Thus, the culture consisted of the progeny of a single greenbug. This culture was determined to be "biotype B" and was used in all diet tests unless otherwise noted.

Greenbugs determined as "biotype A" were obtained from the U. S. Department of Agriculture at Stillwater, Oklahoma. This culture was also maintained on Rogers barley.

Differences in greenbug populations based on reaction of small grains and sorghums to feeding have been apparent for some time. As the result of studies by U. S. Department of Agriculture and Oklahoma State University Entomologists on the differential plant reactions, biotype designations have been developed, quoted as follows:

> Biotype A - The "original" greenbug to which resistance has been developed in wheat, barley, and oats, but which does not occur in Southwestern small grain fields now. Dickinson Sel 28A wheat and wheat hybrids containing this germ plasm are resistant to biotype A, but all wheats are susceptible to biotype B. However, Will barley maintains resistance to biotype A. This greenbug is not morphologically or ecologically different from biotype B. Feeding is in the phloem sieve-tube of the leaf vascular bundle.
>
> Biotype B - This greenbug currently infests small grains in the Southwestern U. S. Dickinson Sel 28A and all other wheats and wheat hybrids are susceptible, but Will barley

maintains resistance to it. This greenbug is not morphologically or ecologically different from biotype A. Feeding is in the parenchyma of the leaf, in contrast to the phloem feeding by biotype A.

Maintenance of Stock Greenbug Cultures

The stock greenbug cultures were maintained on Rogers barley seeded at the rate of 10 to 12 seeds in four-inch plastic pots. The cultures were standardized by transferring about 50 greenbugs to fresh barley seedlings when the infested plants first began to show damage symptoms. Thus, the standardized stock culture consisted only of apterous aphids on seedling plants (Fig. 1).

Greenbugs Reared on Rogers and Will Barley

To establish a basis for comparison of greenbugs feeding on the various diets, it was necessary to obtain similar information from greenbugs feeding on plants. The plants used were susceptible Rogers and resistant Will barleys. Based on the results of this experiment, all dietary experiments were conducted over a period of 20 days, unless complete mortality was incurred earlier.

Twenty-five 4-inch plastic pots were seeded to Rogers barley. When the resulting plants were about two inches high, they were thinned to one plant per pot and a single adult apterous greenbug was placed on each plant. After 24 hours, the adult and all except one of the progeny were removed from each pot. The pots were then fitted with a clear plastic cage which prevented aphid escape or other aphids from getting on the plants. The plants were then placed in a plant growth chamber (Percival Refrigeration Co., Des Moines, Iowa).

Information from this experiment was compiled at 24-hour intervals. The information included (1) daily rate of growth and development,

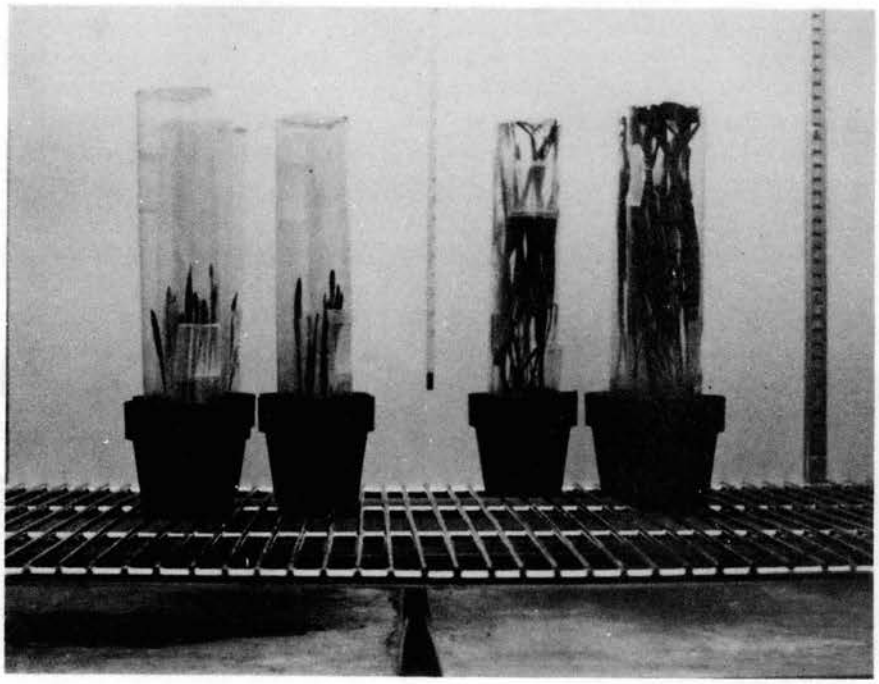

Fig. 1. Stock culture of greenbugs on potted seedling Rogers barley plants. L - Uninfested plants. R - Infested plants showing greenbug damage symptoms.

(2) number of days in the prereproductive, reproductive, and post-reproductive stages, (3) number of progeny per female per day, (4) total number of progeny per female, and (5) total length of life span. This information was then averaged for the 25 aphids to give, for example, the average daily rate of growth.

The average daily rate of growth and development is defined as the average weight gained in milligrams by a given number of aphids of the same age in any 24-hour interval. The aphids on Will and Rogers barley plants were individually weighed in terms of milligrams on a precision balance, Model LG (Federal Pacific Electric Co., Newark, New Jersey) at 24-hour intervals (Fig. 2). The average number of days in the pre-reproductive stage is defined as the average number of days from birth until reproduction commences by a given number of aphids of the same age. The average number of days in the reproductive stage is defined as the average number of days from the start of reproduction until reproduction ceases by a given number of aphids of the same age. The average number of days in the postreproductive stage is defined as the average number of days from the time reproduction ceases until all aphids die. The average number of progeny per female per day is defined as the total number of progeny produced in any 24-hour interval, divided by the total number of adult aphids. The average length of life span is defined as the total number of days survived by a given number of aphids of the same age divided by the total number of aphids.

The same information was obtained from greenbugs feeding on Will barley. Greenbugs were transferred from Rogers to Will and allowed to complete one generation on the latter before initiating the experiment. This was done to deplete the food reserves carried over in the mothers

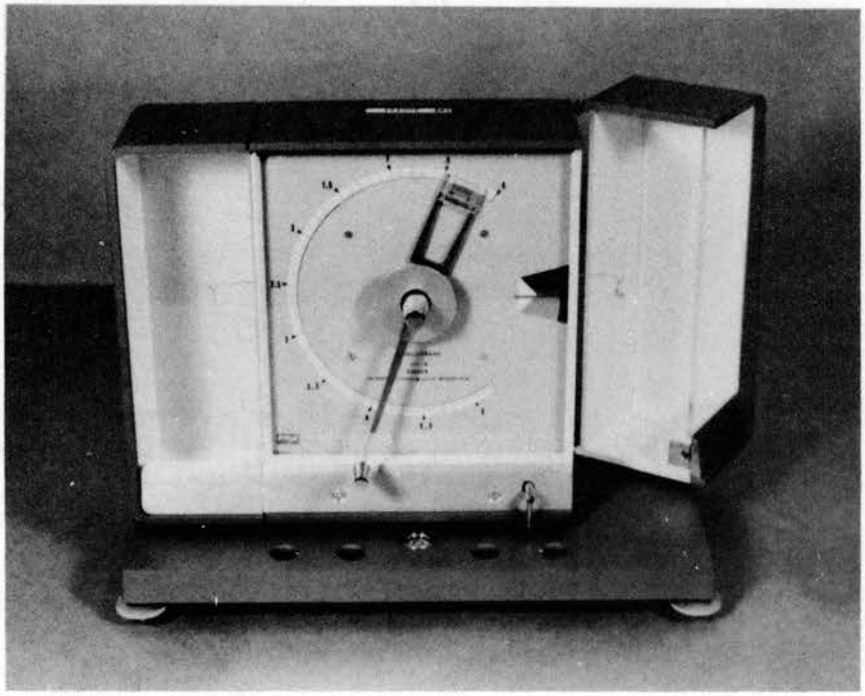

Fig. 2. Precision balance (5 mg capacity) used to weigh greenbugs and minor elements.

feeding on susceptible Rogers. This second generation, thus, represented a more reliable estimate of the nutritional adequacy of the resistant Will barley.

Diet Formulation and Mixing Technique

The diet formulation and mixing technique originally used in this study was adapted from Auclair (1965) as a starting point. Certain modifications were made to improve the technique in relation to the greenbug. The source of chemicals for this study was Nutritional Biochemical Corporation (Nutritional Biochemical Corp., Cleveland 28, Ohio), with the exception of the minor elements, zinc, iron, and manganese, which were obtained from Geigy Agricultural Chemicals (Geigy Chemical Corp., Ardsley, New York).

The desired amount of each amino acid, vitamin, salt, and cholesterol was weighed on a Mettler balance, Type H15. The sucrose was weighed on a Mettler balance, Type K5 (Mettler Instrument Corp., Highston, New Jersey) (Fig. 3). The minor elements were weighed on the precision balance.

Each amino acid was usually weighed at four times the amount required for 100 ml of diet. They were bulked together and placed in a Micromill (Chemical Rubber Co., Cleveland, Ohio) for five minutes to be reduced to a very fine homogeneous powder (Fig. 4). They were then placed in a dry bottle and stored in the dark under refrigeration until needed.

The vitamins were individually weighed at ten times the amount required for 100 ml of diet. They were bulked together and dissolved in 100 ml of distilled water and stored frozen in the dark at -20 F until needed. In experiments involving various combinations of

Fig. 3. Mettler balances used to weigh the various dietary components. L - Type K5. R - Type H15.

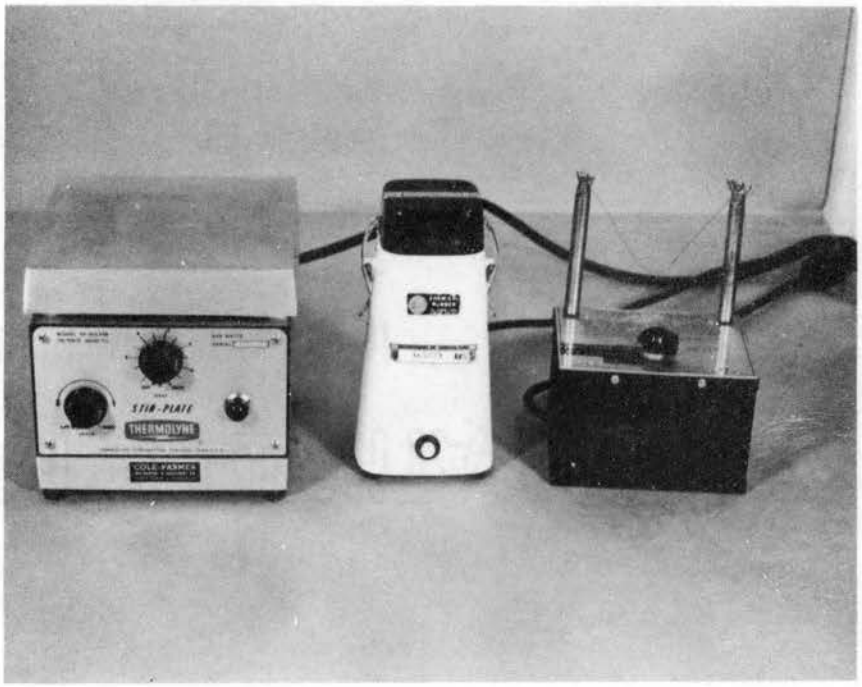

Fig. 4. Laboratory equipment used in preparation of dietary media and sachets. L - Thermolyne stir-plate. C - Micromill. R - Hot wire.

vitamins, only the vitamins being tested were omitted from the stock solution, but they were added to the diet individually as it was prepared.

Sucrose and magnesium chloride were prepared in a stock solution at twice the required concentration in 100 ml of diet and stored frozen until needed. This was achieved by dissolving 70 g of sucrose and 400 mg of magnesium chloride in enough distilled water to make a total of 100 ml.

The desired amounts of potassium phosphate, salt mixture no. 2, cholesterol benzoate, and minor elements were individually weighed as they were required in the diet.

To prepare 100 ml of diet, 4.315 g of the amino acid mixture was dissolved in about 25 ml of distilled water in a 100 ml beaker. To this was added 10 ml of vitamin concentrate, about 5 ml of cholesterol benzoate saturated water, the required weighed amounts of potassium phosphate and salt mixture no. 2, 50 ml of sucrose and magnesium chloride solution, and the required weighed amounts of the minor elements. Enough distilled water was added to bring the total volume to 100 ml. The diet was then stirred on a Thermolyne stir-plate, Model SP-A1025B (Thermolyne Corp., Dubuque, Iowa) for one-half hour to assure that the dietary components were dissolved (Fig. 4). Dietary media so prepared were allowed to stand for about 4 hours before adjusting the pH to the desired level with either concentrated phosphoric acid (85.8%) or saturated sodium hydroxide. Hydrogen ion concentrations were determined by means of a Beckman Model G pH meter (Beckman Instruments, Inc., Fullerton, California) fitted with a one-drop capacity glass electrode assembly (Fig. 5).

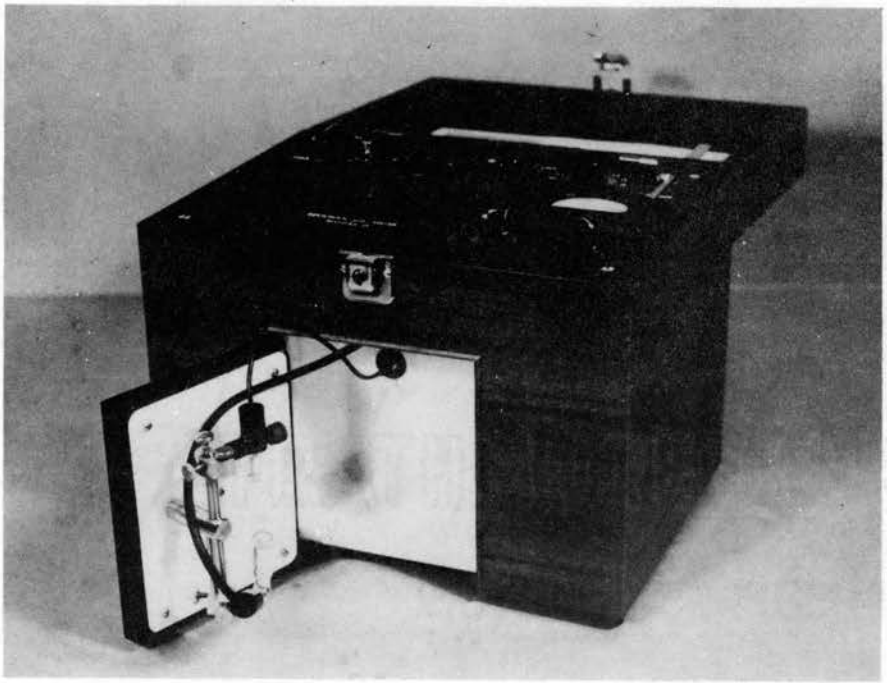

Fig. 5. Beckman Model G pH meter showing the one-drop capacity glass electrode assembly.

Preparation and Sterilization of Sachet Membranes

The various diets were presented to the greenbugs in sachets. The sachets were constructed from two 2-inch-square pieces of Parafilm M membrane (American Can Co., Neenah, Wisconsin) placed over the end of a piece of glass tubing measuring 1 inch long by 1 inch in diameter. In preparation, one piece of the parafilm was placed over the end of the tube and folded down tightly against the outside of the tube. A ping pong ball was pressed against the membrane to make it concave. This served as the lower membrane, and it, along with the second piece of parafilm, the upper membrane, was placed under an ultraviolet light for one hour to achieve sterilization (Fig. 6).

Asepsis of Diet Media

Asepsis of the diet media was achieved through the use of autoclaved B-D Multifit Leur-Lok 10 cc Interchangeable Syringes (Becton, Dickinson and Co., Rutherford, New Jersey) fitted with Swinny Filter Holders (Millipore Corp., Bedford, Massachusetts). The Millipore filters had a mean pore size of 0.22 ± 0.02 micron and were capable of filtering out the microorganism contaminants (Fig. 7).

Construction of Diet Sachet

To prepare a sachet, dietary media were drawn into the syringe and the filter holder fitted in place. The sterilized membranes were removed from under the UV light and 0.3 to 0.4 ml diet was immediately filtered directly onto the concave lower membrane. Then, care being taken not to touch the central area, the second piece of parafilm was stretched to its maximum length in two directions and placed with the sterilized surface over the diet in such a way as to exclude all air

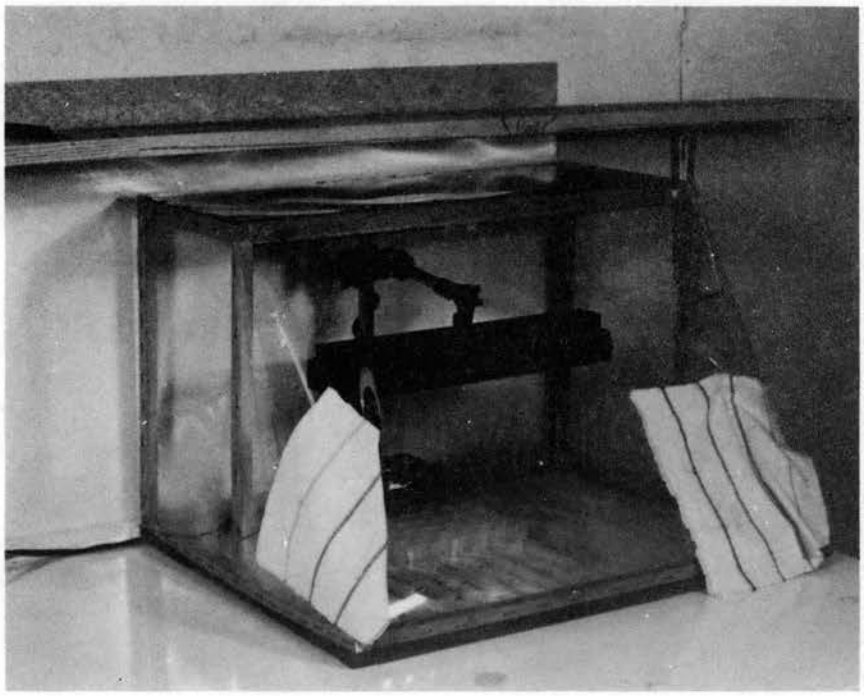

Fig. 6. The upper and lower Parafilm M membranes being sterilized under UV light.

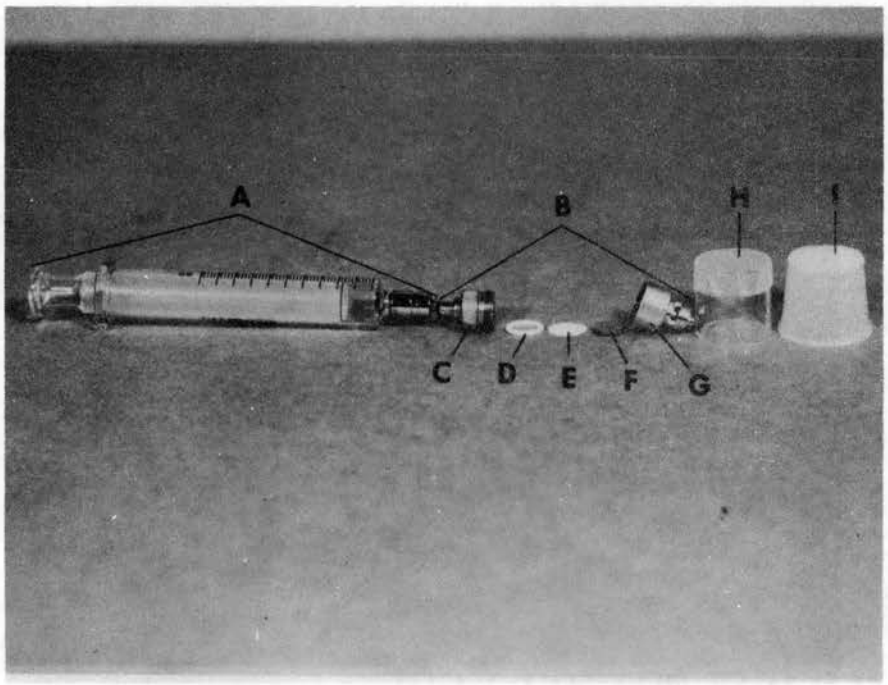

Fig. 7. Component parts of the B-D Multifit Leur-Lok 10 cc Interchangeable Syringe, Swinny Filter Holder along with sachet and polyethylene stopper. A - Syringe. B - Swinny Filter Holder. C - Base. D - Gasket. E. - Millipore Filter. F - Filter Supporter. G - Cap of Swinny Filter Holder. H - Diet sachet. I - Modified polyethylene stopper.

and folded down tightly against the sides of the tube (Fig. 8). The edges of the membranes were evenly cut off with a hot wire (Fig. 4) about one-half inch from the upper membrane. This simultaneously melted the membrane edges together, thus, sealing the diet media in the sachet.

Usually, 10 extra sachets of each diet combination were prepared in advance and stored frozen along with their respective stock at -20 F until required. This made it necessary to thaw the diet only one time to prepare the required number of sachets of each diet combination to complete the experiment. Fresh sachets were supplied at 3- to 4-day intervals throughout the 20-day experiments.

Environmental Conditions of Experiments

The stock aphid cultures and diet experiments were maintained in separate growth chambers. Both chambers were adjusted to 72 to 75 F with a relative humidity of 30 to 50 percent. The light intensity, as measured by a General Electric Light Meter, Type 213, was adjusted to 60 ft-c at the feeding membrane surface.

Initiation of Diet Studies

The various diet studies were initiated with five first-instar nymphs which were less than 24 hours old. This was accomplished by transferring adult apterous greenbugs to uninfested seedling plants 24 hours in advance of the test initiation. Nymphs were transferred directly from the plants onto the diet membranes. Hollow no. 5 polyethylene stoppers, modified by replacing the bottoms with broadcloth, were placed over the sachets in such a way as to retain the aphids on the membranes. These nymphs were termed the first generation

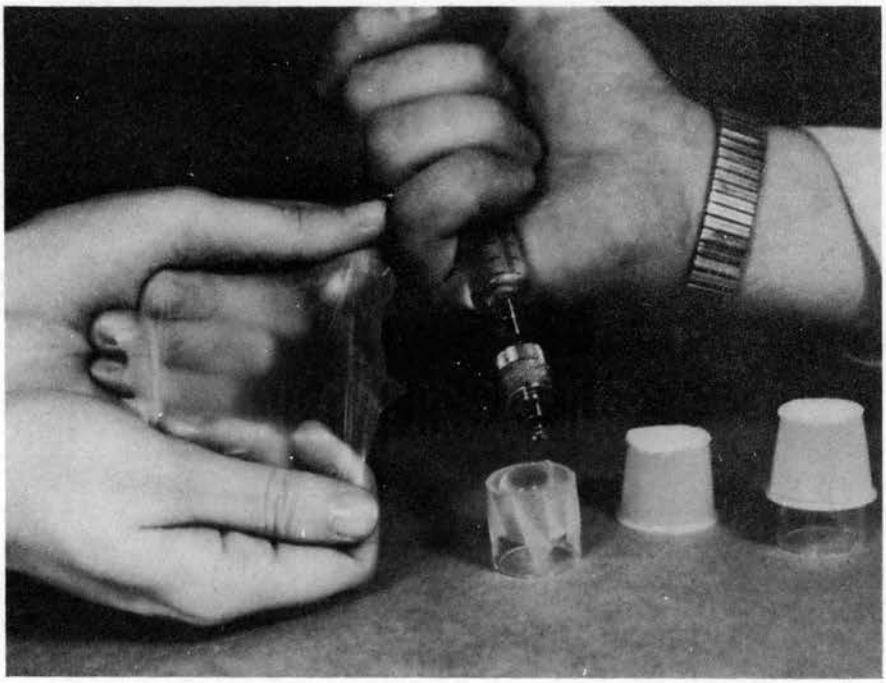

Fig. 8. Filtering diet onto concave lower membrane to be covered by stretched upper membrane.

and their progeny was the second generation. The second generation nymphs were transferred onto diets identical to those of their parents (Fig. 9).

Criteria of Greenbug Performance on Synthetic Diets

The criteria for success of the various nutrient solutions utilized in this study were (1) aphid survival, (2) aphid growth and development, and (3) aphid reproduction. Readings were made at 24-hour intervals throughout the experiments. The readings consisted of (1) number of aphids alive, (2) number on the membrane, (3) number of exuviae, (4) number live progeny, (5) number stillborn progeny, and (6) total progeny.

Weights of aphids reared on the complex diets were obtained by weighing those on two of the five sachets, at random, from each of the diets used in the experiment. These weighings were made at three- to four-day intervals, and then fresh sachets were supplied.

Statistical Analysis of the Data

Two statistical designs were used in the analysis of the data of the various experiments. The designs were (1) randomized block and (2) split plot in days with the main plots being the treatments in a randomized block design. Also, a split plot design in days with the main plots having three minor elements, zinc, iron, and manganese, each at four levels, along with a check treatment, was used.

The first design was used to analyze biotype B greenbugs feeding on the pea aphid diet and biotype A greenbugs feeding on the standard biotype B greenbug diet. The second design was used to run two experiments, each having 2^2 factorial arrangement of two factors: vitamins B_{12}

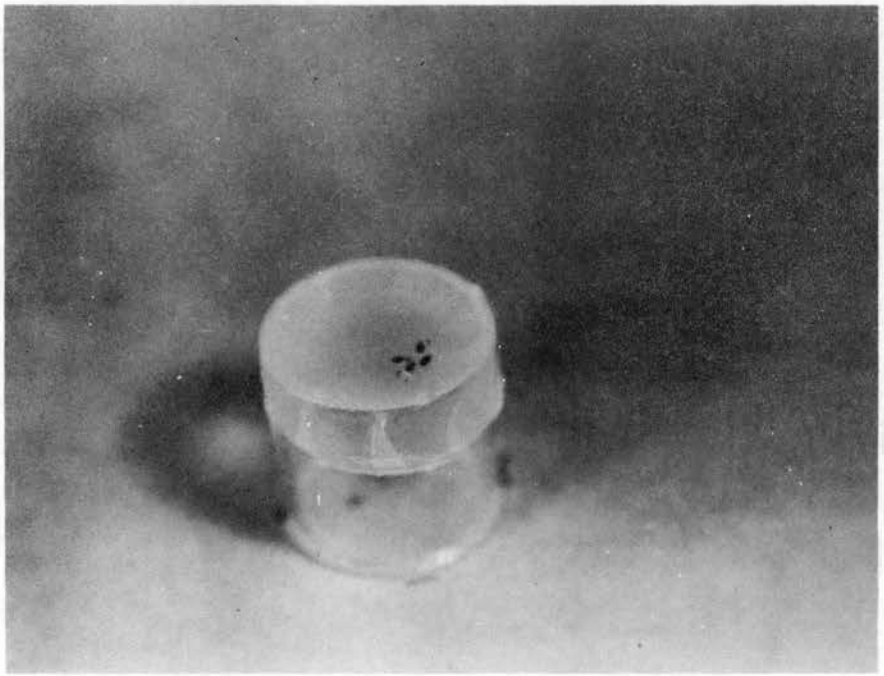

Fig. 9. Biotype B greenbugs feeding on the standard biotype B greenbug diet.

and E. The second design was also used to analyze experiments with five combinations of the minor elements, zinc, iron, and manganese. These combinations were as follows: (1) Zn-1, Fe-1 Mn-1, (2) Zn-2 Fe-2 Mn-3, (3) Zn-1 Fe-2 Mn-4, (4) Zn-3 Fe-3 Mn-3, and (5) Zn-4 Fe-4 Mn-4. The second design was also used to analyze an experiment with 13 treatments which consisted of four levels each of zinc, iron, manganese, and the check containing no minor elements.

The experiments analyzed by these statistical designs were replicated five times with five greenbugs per plot.

Order of Experiments in Study

The following experiments were conducted in the present study and will be dealt with in this order: (1) pH level, (2) sucrose concentration, (3) chamber uniformity, (4) light quality, (5) pea aphid diet, (6) the reduction in vitamin concentration, (7) minor elements, (8) vitamins B_{12} and E, (9) biotype A, and (10) plant extracts.

Determination of Hydrogen Ion Concentration

The pH to be used in the complex diets of this study was determined from a series of four pH levels ranging from 5.6 through 8.6 at 1-unit intervals. Two tests were conducted: one involved preference of the greenbug; the other involved greenbug development over a 20-day period. The complex pea aphid diet was common to these series. The pH was adjusted to the desired level through the use of either concentrated phosphoric acid (85.8%) or saturated sodium hydroxide.

Determination of Sucrose Concentration

Sucrose concentration to be included in the synthetic diet was determined by greenbug preference tests. A series of eight sucrose

solutions ranging from 10 to 45% at 5% intervals in distilled water was used. The pH in this series was adjusted to 7.6, which was found to be the optimum pH for greenbug development.

Construction of Preference Cages

The cages used in both the pH concentration and sucrose concentration determination experiments allowed the aphids to select from four sachets at once from the respective series. These cages, termed preference cages, were constructed from modified one-half-gallon ice cream cartons (Fig. 10). The walls of the carton were cut off at 2½ inches in height. Legs were constructed from wooden garden stakes and glued on the outside of the carton. Four holes, slightly less than 1 inch in diameter, were cut equidistant from each other and from the center of the bottom of the carton. The outside margin of the holes was one-half inch in from the wall of the carton. The central portion of the lid was replaced with white broadcloth which allowed light to enter the cage and yet retain the aphids. The sachets were supported on thick-walled glass tubing measuring 1 inch in outside diameter by 1 inch in length. The sachet membranes were pressed tightly against the end of the tube and carefully trimmed off evenly around the outside rim. The tubes were then inserted through the holes in the bottom of the carton in such a way as to position the upper membrane flush with the floor of the carton. A total of 20 aphids were placed in the center of the floor of the cage and allowed to move in any direction. The number of aphids on each sachet were counted at 1, 2, 6, 12, 24, 48, 72, and 96 hours after the test was initiated.

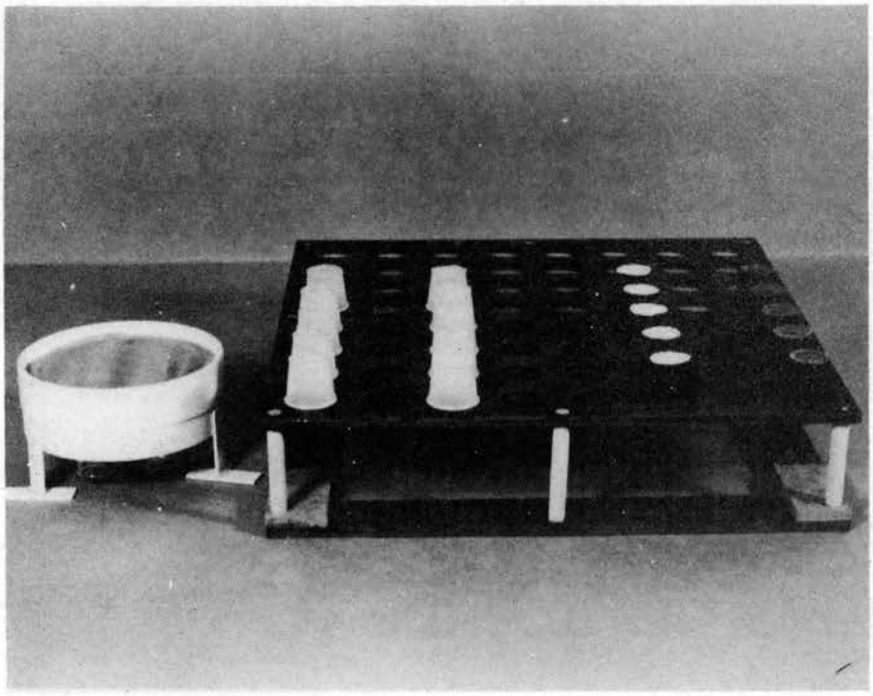

Fig. 10. Preference cage used to determine the pH and sucrose concentration (L) and sachet-supporting rack used in light quality and intrachamber uniformity experiments (R).

Chamber Uniformity Test

A uniformity test, designed to elucidate any intrachamber variation, was conducted in the growth chamber in which all subsequent diet testing took place. Two racks, supporting a combined total of 128 sachets, were used in this test. Two second- or third-instar greenbugs were placed on each sachet, which was then covered with an opaque stopper, the bottom of which was covered with yellow cloth. The diet used was the pea aphid diet. Fresh sachets were supplied at 3- and 4-day intervals.

Determination of Light Quality on Diet Experiments

The quality of light reaching the sachet membrane was initially determined through the use of various colors of loosely woven cloth mounted on translucent no. 5 polyethylene stoppers. The cloth was mounted onto the stopper by first cutting off the bottom of the stopper and then, with the cloth in place, pressing it against a hot plate of sufficient temperature to melt the polyethylene and hold the cloth.

In other experiments, the stoppers were painted with flat black enamel. This made the stopper opaque, thus, the only light reaching the sachet membrane passed through the colored cloth.

In these experiments, there were five first-instar greenbugs placed on each of five sachets and covered with the colored cloth stoppers. These were supported on a 14-inch square rack and placed in the growth chamber. There was a total of 64 holes in the rack, thus allowing simultaneous testing of seven colors, each replicated five times (Fig. 10). The colors used were red, blue, green, orange, yellow, purple, and white.

Kodak Wratten filters (Eastman Kodak Co., Rochester, New York), 3 inches square, were mounted in the bottom of one-half-gallon ice cream cartons. Five holes, slightly over 1 inch in diameter, were evenly spaced in a circle in the lid of the carton to support the sachets. Three legs, constructed from wooden garden stakes, were glued on the outside of the lid to hold the cage just high enough so the ends of the glass tubes supporting the sachets would not rest on the floor of the growth chamber (Fig. 11). The inside of the carton being white, it was assumed that the light was reflected and, thus, equal on all sachets in the carton. White cloth and translucent stoppers were used in these cages. Three colors, green 11 X1, yellow 15 G, and orange 22, were tested.

Complex Diet Experiments

Pea Aphid Diet - The pea aphid diet (Auclair, 1965) (Appendix. Diet 1) and mixing technique, as described above, was used as a starting point for the development of a synthetic diet for the greenbug.

Reduction of Vitamin Concentration - The concentration of all vitamins was reduced to 0.1 the concentration of the pea aphid diet (Appendix. Diet 2). This was accomplished by adding only 1 ml of the stock vitamin solution.

Minor Elements - The minor elements, zinc, iron, and manganese, were added to the diet containing 0.1 the vitamin concentration of the pea aphid diet. The minor elements were added as sodium sequestrenes in the EDTA form. There were four uniform levels of each element tested, both individually and in combination with each other (Appendix. Diets 3-19). The levels chosen for testing were based on the results of Dadd and Mittler (1966) working with the green peach aphid. Also,

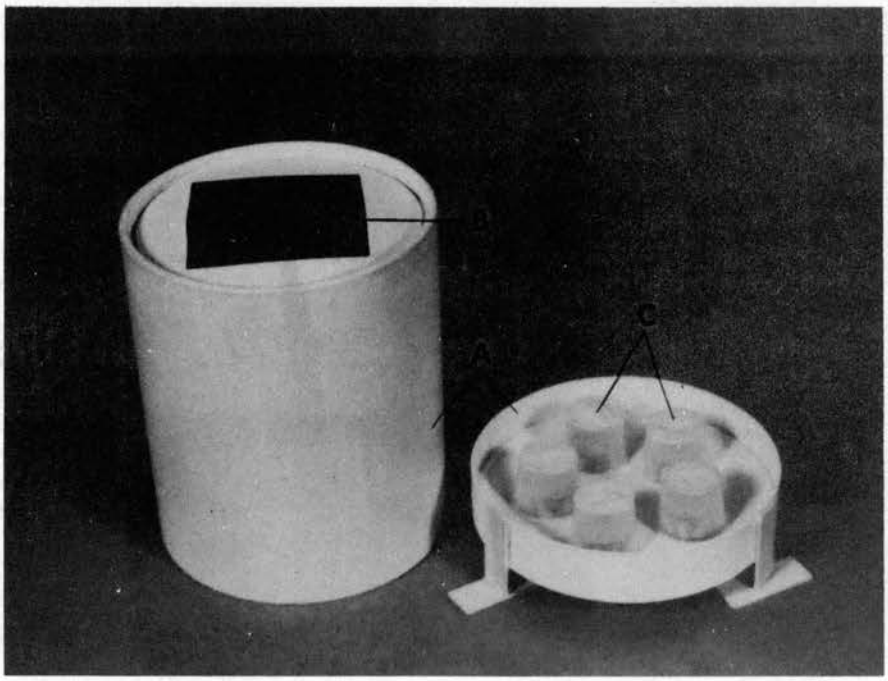

Fig. 11. Details of construction of the cage used in the various diet experiments. A - One-half-gallon ice cream carton. B - Kodak Wratten filter. C - Diet sachets.

on the basis of the results of Dadd and Mittler, copper was not included in the greenbug diet. It was the best of these diets (in terms of greenbug survival, growth, and reproduction) containing all three of the minor elements, that was designated as the standard biotype B greenbug diet (Appendix. Diet 19). Subsequent vitamin studies were modifications of the standard biotype B greenbug diet.

Vitamins B_{12} and E - The vitamins B_{12} (cyancobalamin) and E (DL-alpha tocopherol) were tested in combination with each other in the standard biotype B greenbug diet. Eight uniform levels of each of these vitamins were tested. They were not included in the stock vitamin solution, but rather the desired amounts were weighed and added to the diet as a final step in preparation (Appendix. Diets 20-27).

Biotype A Greenbugs Reared on Standard Biotype B Greenbug Diet

An experiment was conducted to compare the nutrient requirements of greenbug biotypes A and B. The standard biotype B greenbug diet was used in this experiment.

Bioassay of Plant Extracts

Plant extracts and, in some cases, purified chemicals, representing identified chemicals from the plant extracts, were incorporated in the standard biotype B greenbug diet at concentrations equivalent to their occurrence in Will and Rogers barleys. These compounds were added as the final step in preparation of the diet. There were 19 purified chemicals provided by the Department of Botany; all were individually incorporated into the diet at their concentration found in Will barley.

The Department of Biochemistry provided compounds for two

experiments. In the first experiment, the various extracts were individually incorporated into the standard biotype B greenbug diet at equivalent concentrations (one exception) found in both Will and Rogers barleys. These were gross extracts and were in the groupings of volatiles, amino acids, sugars, indole compounds, and total extracts. The volatiles were also tested at one half the concentration found in the plants. The second experiment involved purified chemicals and was based on the results of the first experiment. As such, four components of the volatile group and six components of the indole compound group were individually incorporated into the standard biotype B greenbug diet at the concentration found in Rogers barley.

RESULTS AND DISCUSSION

Greenbugs Reared on Rogers and Will Barley

The results obtained from greenbugs feeding on susceptible Rogers and resistant Will barleys are shown in Fig. 12 and Table 1. These results represent an average for 25 greenbugs. On Rogers the rate of weight gain was very rapid, slowing only slightly on the 7th day, until the 10th day when a plateau was reached. On Will, the rate of weight gain was much lower, with a slight reduction on the 7th day, and reached a plateau on the 15th day. It is shown in Table 1 that reproduction started on the 7th and 10th day on Rogers and Will, respectively, and continued to the 24th and 28th day, respectively. Thus, the rate of weight gain on both Rogers and Will was slightly reduced on about the initial day of reproduction and plateaued throughout the greater portion of the reproductive period. During the 13-day postreproductive period on Rogers, the aphids gained additional weight before dying. On Will, the postreproductive period lasted 4.68 days with no weight gained (Fig. 12).

It was assumed that greenbug survival, growth, and reproduction would be best on Rogers barley. Thus, greenbug performance on the various diets was compared to that on Rogers barley. On Rogers barley, reproduction ended when the aphids were 24 days old. From the standpoint of perpetuating the species, the parent is of no value once the reproductive period has been completed. It was noted that in the latter few days of the reproductive period, there were very few progeny

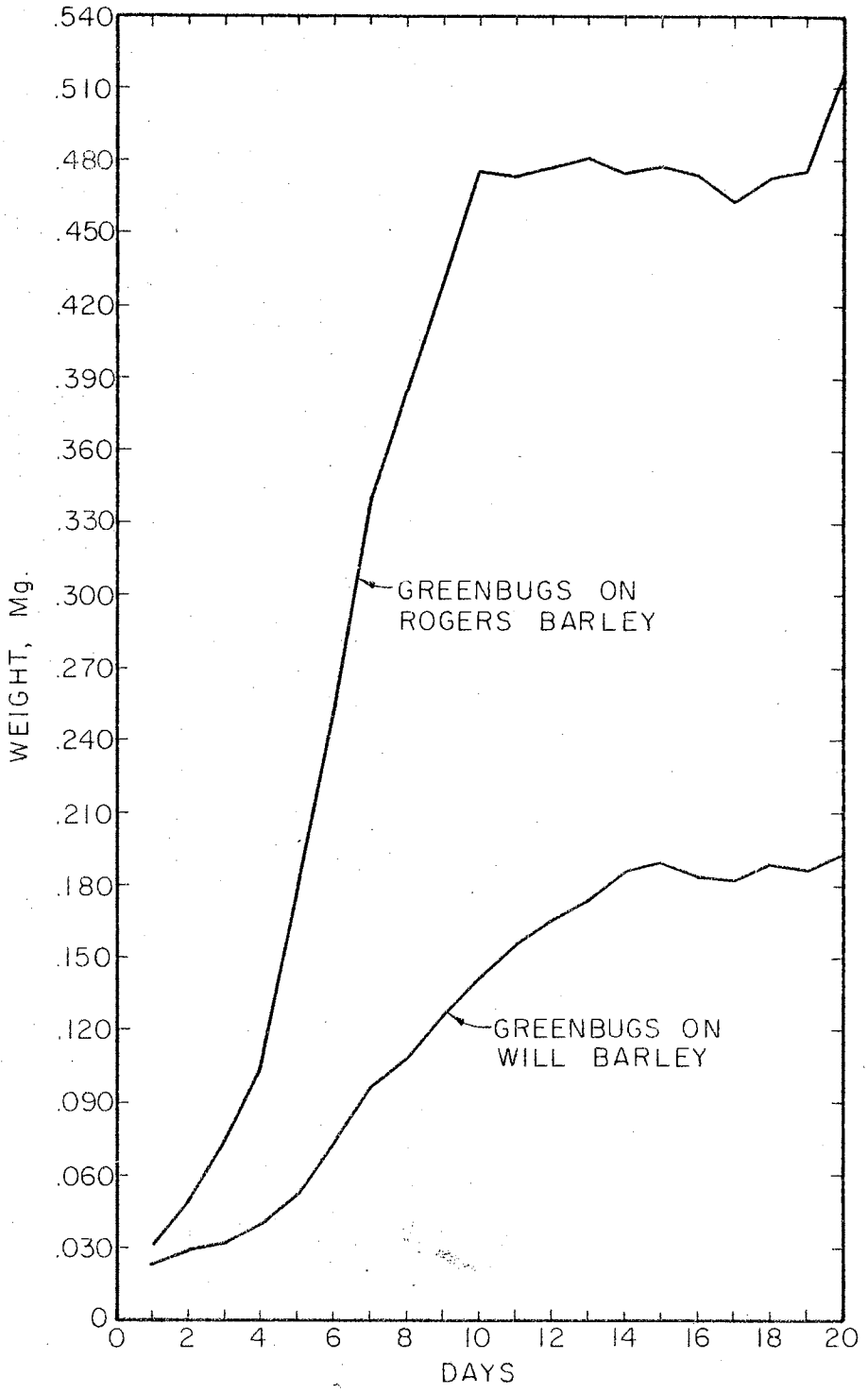

Fig. 12. Biotype B greenbug development, based on average weights, on Rogers and Will barley.

Table 1. Average duration of the various periods in the life cycle of greenbugs feeding on susceptible Rogers and resistant Will barleys.

	Average Number Days				Av. No. Progeny	
Barley	Pre reproductive Period	Reproductive Period	Post reproductive Period	Life Span	Per Female	Per female per day
Rogers	7.20	17.24	13.08	37.52	54.88	3.18
Will	10.20	18.44	4.68	33.32	20.40	1.10

produced on Rogers barley. Therefore, it was decided to terminate all diet tests at 20 days. With this information in mind, it is appropriate to turn attention to the various diet experiments.

Greenbug Preference Cage Design and Validity

A preference cage was designed which allowed the greenbugs a choice between any of four solutions behind a parafilm membrane. To determine whether greenbugs could or would choose one solution over another, the preference cage was equipped with three sucrose solutions, 15, 25, and 35%, and the pea aphid diet.

In this experiment, as in all other preference experiments, a mixture of 20 second- and third-instar nymphs was placed in the center of the cage. The number of aphids on each membrane was counted after 1, 2, 6, 12, 24, 48, 72, and 96 hours. The total number of aphids on each of the four solutions was calculated on the basis of the 96-hour count. The four totals were combined into a grand total or population total. The percent of the total population on each test solution was determined by dividing the total on each solution by the population total.

Eighty percent of the total population in the test preferred the pea aphid diet over the three sucrose solutions. These results show that the greenbug does prefer one solution over another and the design of the preference cage was such as to allow the preference to take place.

Greenbug Preference for pH Concentration

The pH to be used in greenbug diets was determined through preference tests and through rearing greenbugs on a complex diet. In these experiments duplicate series of 5.6, 6.6, 7.6, and 8.6 pH

concentrations were tested in the pea aphid diet. Preference of greenbugs in percentages for the various pH concentrations was as follows: 5.6 - 3.3%; 6.6 - 5.6%; 7.6 - 51.9%; and 8.6 - 39.1%. It was evident that the pH 7.6 was optimum for greenbug development.

The results of greenbug development when reared over a period of 20 days on diets of several pH concentrations are shown in Table 2. This experiment had five replications, each with five aphids. The "aphid days" is based on aphid survival. It is the cumulative total of aphids alive over the 20-day test period. Thus, for example, if all 25 aphids lived for three days and on the fourth day two died, it would be 25 + 25 + 25 + 23 = 98 aphid days. In this experiment, a maximum of 500 aphid days was possible if all aphids lived throughout the 20-day test period.

The pH 7.6 was best in terms of both survival and reproduction. This strongly confirms the results of pH 7.6 being preferred in the preference test.

Greenbug Preference for Sucrose Concentration

The concentration of sucrose to be used in the greenbug diet was determined through preference tests. Two series, each with four concentrations of sucrose in distilled water, were used. The pH 7.6 was common to both series. The first series contained 10, 20, 30, and 40% sucrose, while the second series contained 15, 25, 35, and 45% sucrose. There were two replications.

The results indicated that the greenbug requires a sucrose range between 30 to 40%. Within this range, 35% was greatly preferred over 30% but only slightly over 40%. In general, there was an increase in preference from the lowest concentration to the optimum of 35%. There

Table 2. Greenbug survival and reproduction when reared for 20 days on diets of various pH concentrations.

pH	Aphid days (Survival)	Total reproductions
5.6	72	0
6.6	298	56
7.6	379	76
8.6	199	14

was a marked decrease in preference from 40 to 45%.

On the basis of these results, 35% sucrose was used in all synthetic diets for the greenbug.

Intrachamber Uniformity Test

A uniformity test was conducted in the growth chamber in which all subsequent diet testing took place to determine whether there was any variation in aphid performance (survival, development, and reproduction) attributable to intrachamber variation. Two 14-inch square racks, supporting a combined total of 128 sachets were placed end to end in the growth chamber. This almost filled the chamber to capacity, thus, the whole area in the chamber was tested. The sachets all contained the pea aphid diet. A mixture of second- and third-instar nymphs was placed on the sachets (two per sachet) and covered with opaque stoppers supporting yellow cloth.

Readings of the number alive, number on the membrane, number of exuviae, and number of progeny were taken daily. Care was always taken to replace each rack in its original position in the chamber. Fresh sachets were supplied at 3- and 4-day intervals throughout the 20-day test period.

There was no apparent difference in greenbug development from side to side in the chamber. However, the aphids on the four sachets in the center front of the chamber (the door area) all died. This appeared to be due to sudden changes in the environmental conditions when the door was opened.

Based on the results of this uniformity test, no diet experiment cages were placed in the center of the chamber corresponding to the width of the door from the front to the rear. Moreover, as a

precautionary measure, dietary test cages were placed as far to the rear corners of the growth chamber as possible, thus leaving the front and center vacant. In most experiments, a hygrothermograph was placed in the central vacant area with its sensitive apparatus to the rear of the growth chamber (Fig. 13).

Light Quality Used in Dietary Studies

Kodak Wratten filters were used to test the quality of light preferred by the greenbug. The results are shown in Table 3. It is shown that in every case, with the exception of number alive, green was better than either orange or yellow light. This is also reflected in total progeny developing - 128 for green, as compared to 87 for both orange and yellow. Based on these results, green 11 X1 Kodak Wratten filters were used in all diet experiments.

Greenbugs Reared on Pea Aphid Diet

The pea aphid diet (Auclair, 1965) (Appendix. Diet 1) was used as the starting point for the development of a synthetic diet for the greenbug. Data in this regard are presented in Fig. 14 and Fig. 15. Greenbug performance refers to the six variables used for statistical analysis. They were (1) number alive, (2) number on the membrane, (3) number of exuviae, (4) number of live progeny, (5) number of still-born progeny, and (6) total progeny. Each of these variables measured a different response of the aphids to the particular diet. Thus, variable 1 measured aphid survival, variable 2 measured the palatability of the diet, variable 3 measured growth and development, variables 4, 5, and 6 measured reproduction.

The data were analyzed in a randomized block design. There were

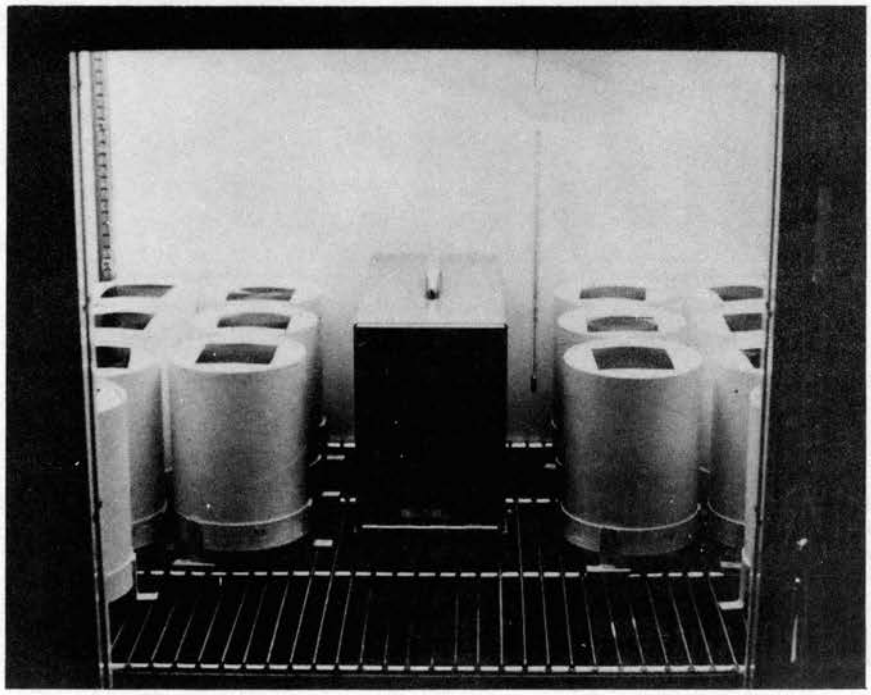

Fig. 13. Arrangement of cages within the growth chamber in accordance with the results of the intrachamber uniformity test.

Table 3. Results of greenbugs feeding under yellow, orange and green Kodak Wratten filters.

Color	Rep.	No. alive	No. on membrane	No. exuviae	Total progeny
Yellow 15G	1	89	82	17	7
	2	91	76	19	10
	3	86	79	16	21
	4	89	75	16	16
	5	99	94	17	33
	Totals	454	406	85	87
Orange 22	1	100	92	17	28
	2	100	77	15	23
	3	95	87	18	20
	4	87	78	16	9
	5	87	75	15	7
	Totals	469	409	81	87
Green 11 X1	1	93	86	19	21
	2	80	76	16	24
	3	87	79	19	21
	4	100	98	16	37
	5	77	72	17	25
	Totals	437	411	87	128

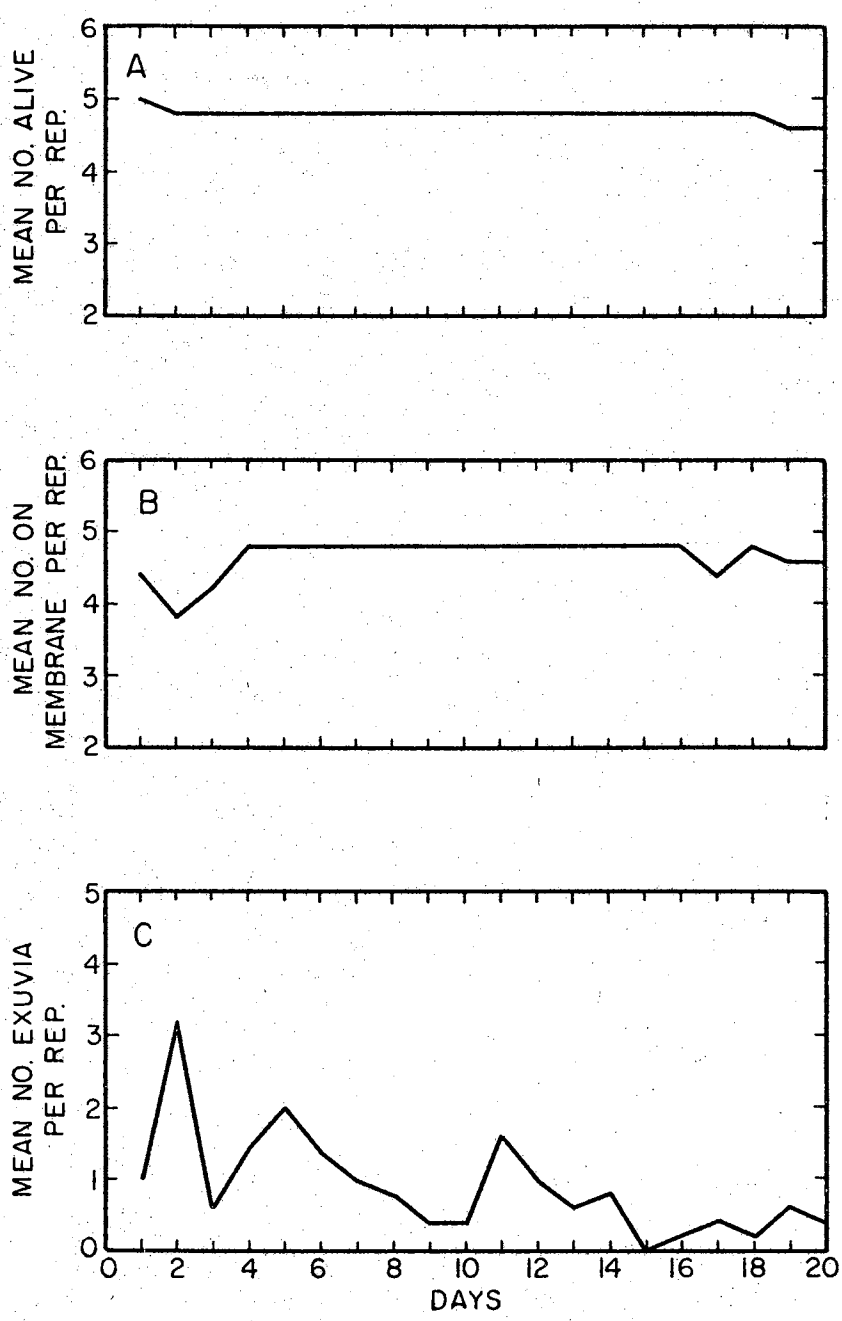

Fig. 14. Biotype B greenbug survival, growth and development when reared on pea aphid diet.

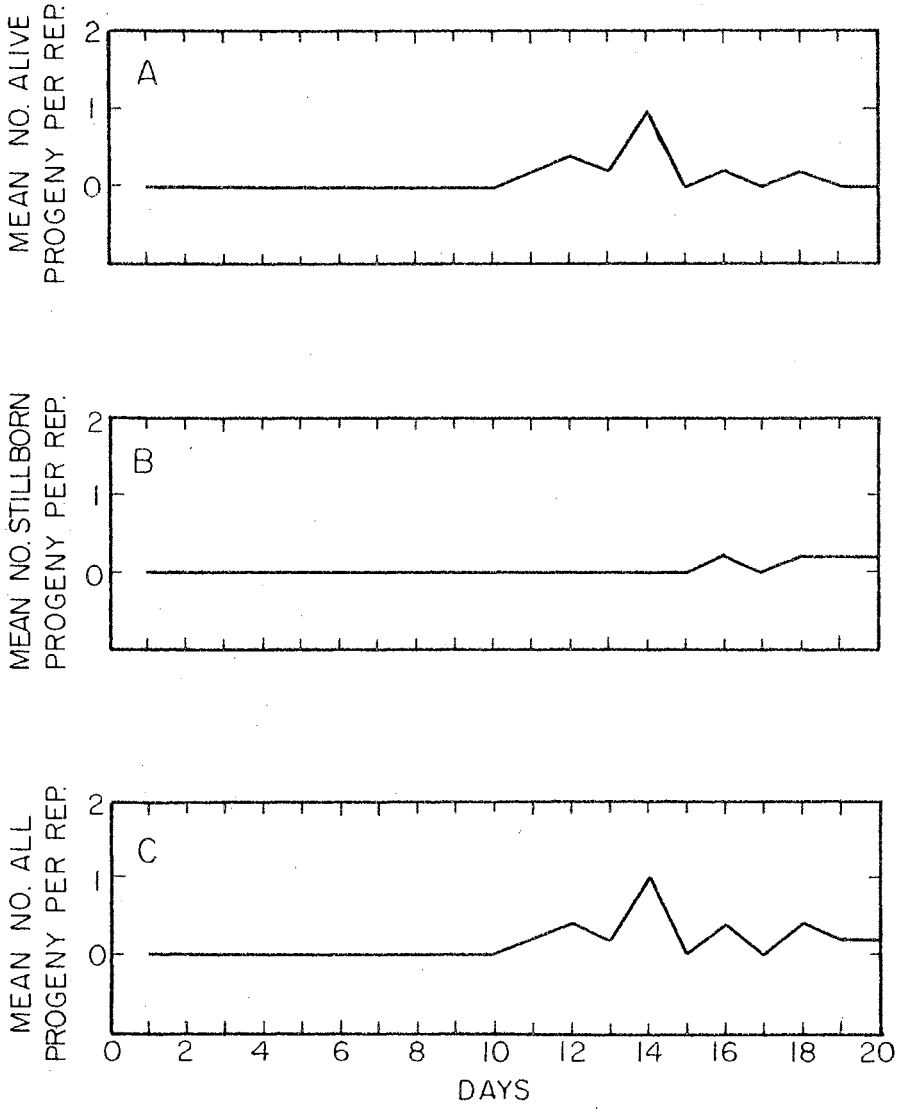

Fig. 15. Biotype B greenbug reproduction when reared on pea aphid diet.

five replications, each initiated with five aphids less than 24 hours old. The aphids were also weighed, usually at 3- to 4-day intervals. The weights were not statistically analyzed.

Variable 1 (Fig. 14, line A) shows the mean number of aphids alive per replication over the 20-day test period. There was no significant difference indicated over the 20 days.

Variable 2 (Fig. 14, line B) shows the mean number of aphids on the membrane over the 20-day test period. There was a highly significant difference (1% level) indicated over the 20 days. This difference was seen on days 1, 2, and 3 when there was a mean of about 4.4, 3.8 and 4.2 aphids on the membrane, respectively. It was suggested that these differences in the early days of the test were a result of the aphids being on an unnatural surface but not necessarily an unpalatable diet. The aphids remained on the membrane, almost without fail, from the fourth day throughout the remainder of the test.

Variable 3 (Fig. 14, line C) shows the mean number of exuviae per replication. There was a highly significant difference (1% level) over the 20 days. Such differences were not unexpected as all aphids do not molt daily nor on the same day. Moreover, the growth and development should occur in the early to mid stages of the test. The more important point was that ecdysis continued throughout the 20 days. This suggested that some essential dietary component was lacking, or that there was an imbalance in the dietary components which greatly retarded the rate of development. The latter was the most plausible explanation, because the aphids were able to survive with no significant mortality over the 20 days. In the case of lacking essential dietary components, a high rate of mortality would have been seen. Hence, survival was

adequate, but with a low rate of growth and development.

Variable 4 (Fig. 15, line A) shows the mean number of live progeny per replication. There was a highly significant difference (1% level) in the mean number of progeny over the 20 days. This was to be expected because no progeny could be born until the potential parents became adults. As evidenced by the continuous ecdysis (Fig. 14, line C), not all of the aphids reached the adult stage within the 20 days. This was offered as one reason for the low rate of reproduction. The second reason being, again, an imbalance in the dietary components.

Variable 5 (Fig. 15, line B) shows the mean number of stillborn progeny per replication. There was no significant difference over the 20 days. The cause of these stillborn progeny was attributed to some imbalance in the dietary components. Stillborn progeny were observed, to a greater or lesser extent, on all diets tested throughout the present study. Whether the imbalance responsible for the stillborn progeny was the same as, or was complicated by, the imbalance responsible for the reduced rates of growth and development was not determined. An interesting parallel was that greenbugs feeding on resistant Will barley were also seen to have stillborn progeny and reduced rates of growth and development. The number of stillborn progeny produced on Will barley was not determined, because the aphids were not restricted in their feeding site on the plant. Also, it was suspected that many of the stillborn nymphs fell to the soil or leaf axis, unnoticed, when the cage was removed from the pot.

Variable 6 (Fig. 15, line C) was a combination of variables 4 and 5. As such, it was the mean number of both live and stillborn progeny per replication. There was a significant difference (5% level) in the

mean number of progeny over the 20 days. This difference was carried over from variable 4. The more important point was that the maximum mean number of progeny per replication was one and this occurred on the 14th day. This rate of reproduction was markedly reduced when compared to Rogers and Will barley.

The rate of growth on the pea aphid diet, as measured by weight gain, is shown in Fig. 16. Starting at .031 mg (average weight of 100 nymphs less than 24 hours old) the weight increased to its maximum of .217 mg on the 18th day. This was a 5.75 fold increase. Then on the 19th and 20th days, there was a slight decrease in weight. There was a striking similarity between the general pattern of this curve and the comparable curve obtained for greenbugs feeding on Rogers barley (Fig. 12). There was a more rapid increase in weight starting on the fourth day in both cases, followed by a slight reduction in rate on the 7th and 11th days on the plant and diet, respectively. There was a plateau in the rate of weight gain on the 10th and 18th days, for the plant and diet, respectively. The changes in the rate of growth approximate the beginning and, later, the maximum rate of reproduction.

Greenbugs Reared on a Diet Containing Reduced Vitamin Concentration

The first modification in the pea aphid diet was the reduction of all vitamins to 0.1 their concentrations (Appendix. Diet 2). This reduction was made because the vitamins for insects, in general, are required in relatively small amounts, and the diet was originally formulated for the much larger pea aphid. The amino acid content was not reduced on the assumption that, as is the case with other aphids, excess amounts, unless greatly so, are excreted in the honeydew with no deleterious effects on the aphid (Mittler, 1958, and Auclair, 1958).

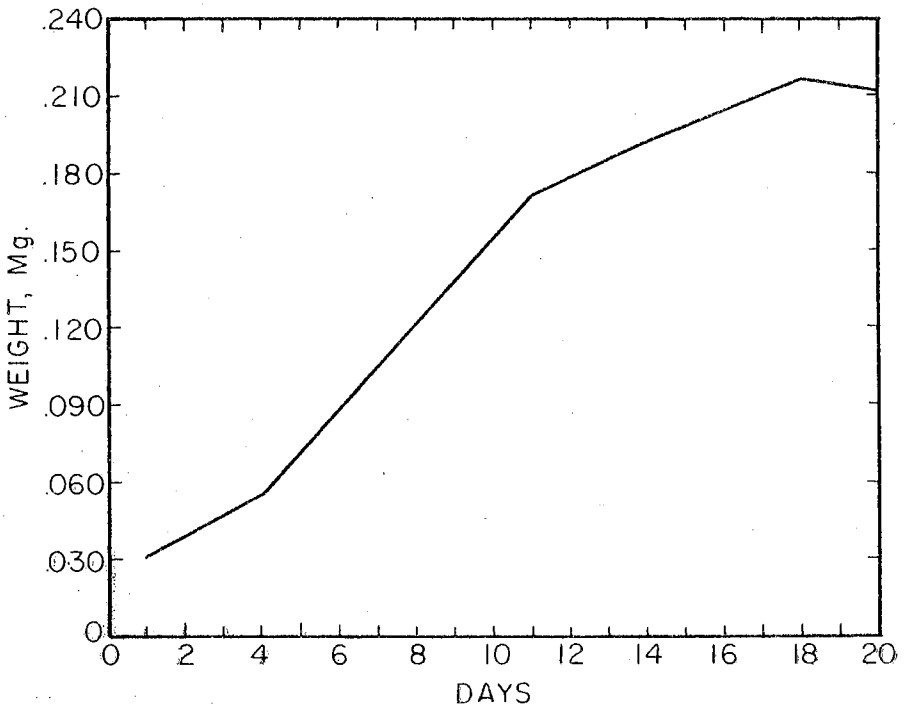

Fig. 16. Mean weight gained by biotype B greenbugs reared on pea aphid diet.

To this author's knowledge, excess quantities of vitamins are not excreted in the honeydew, and therefore, may readily become toxic when their concentration is in excess.

The diet containing the reduced vitamin concentrations served as the check diet in an experiment with 12 treatments of minor elements. Results of this experiment were statistically analyzed as a split plot in days with the main plots having three minor elements, zinc, iron, and manganese, each at four levels. There were five replications, each initiated with five aphids less than 24 hours old. Although this diet served as the check diet, it may be abstracted from the day by treatment contrast in the analysis of variance and compared to the pea aphid diet in a general way. It must be borne in mind that the two diets, per se, were not compared statistically but rather on a day-to-day basis within themselves.

The performance of the aphids feeding on the diet containing the reduced vitamin concentrations may be seen in Fig. 17 and Fig. 18.

Variable 1 (Fig. 17, line A) shows the mean number of greenbugs alive for each day of the experiment. There was no significant difference in the mean number alive from day to day. It may be noted that from the 11th day through the 18th day, there was a more or less steady decline of about two aphids per day in the mean number alive. It was quite possible that, had the statistical analysis been designed to compare the mean number alive at the beginning with the mean number alive at the end of the experiment, there would have been a significant difference.

Although there was greater mortality of aphids reared on the diet containing the reduced vitamin concentrations when compared to aphids

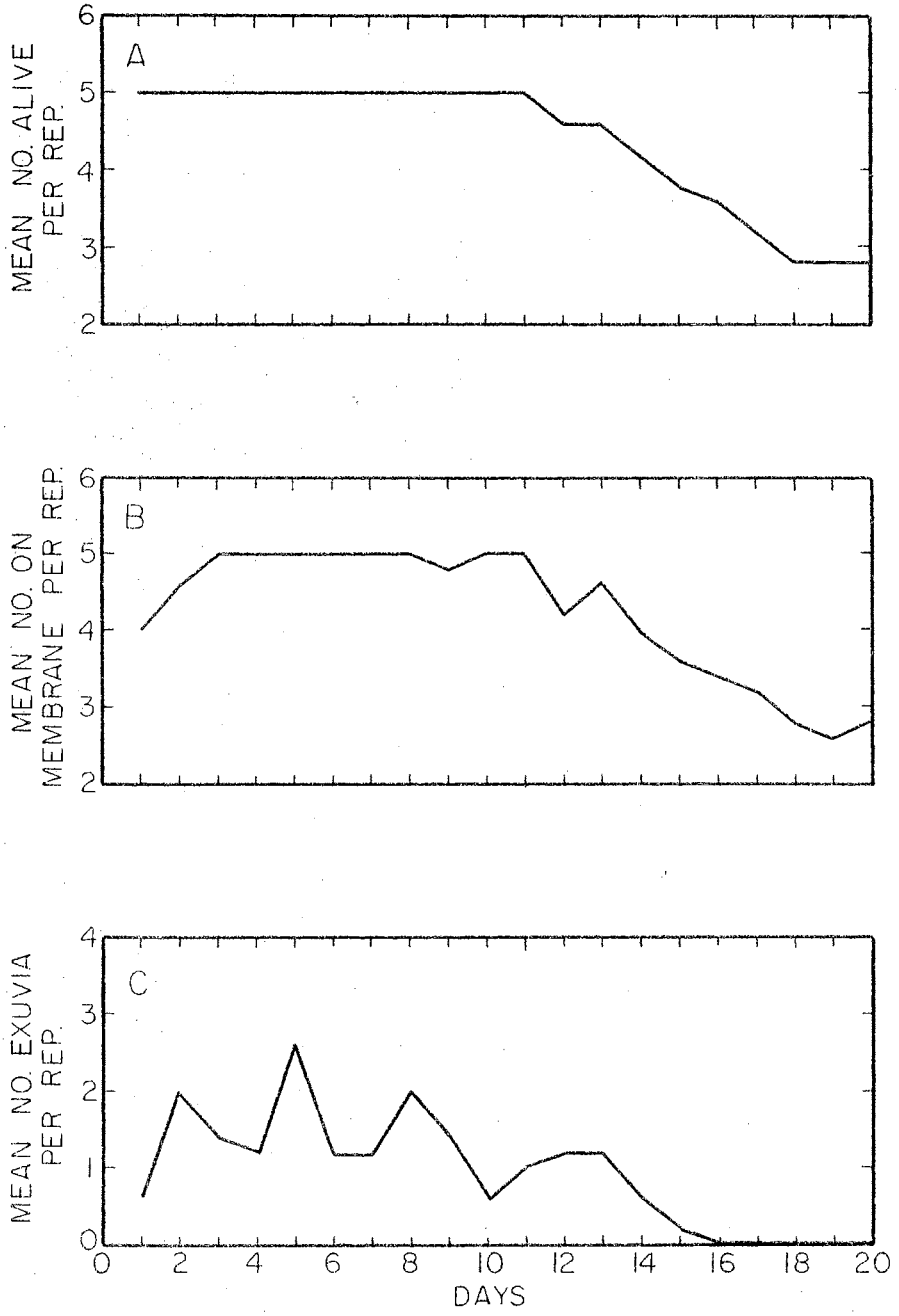

Fig. 17. Biotype B greenbug survival, growth and development when reared on a diet containing 0.1 the vitamin concentrations of the pea aphid diet.

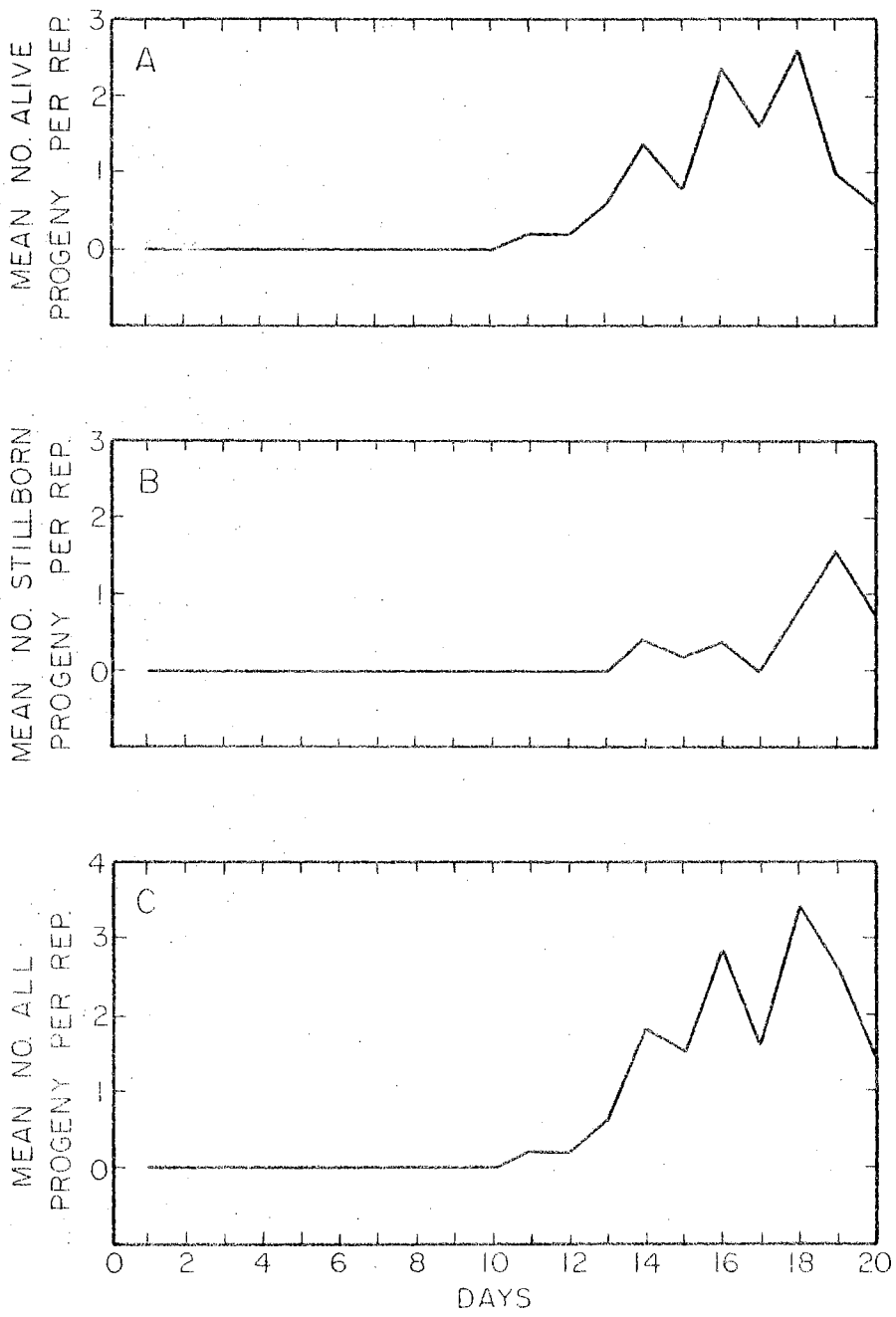

Fig. 18. Biotype B greenbug reproduction when reared on a diet containing 0.1 the vitamin concentrations of the pea aphid diet.

reared on the pea aphid diet, the analysis does not suggest any significant day-to-day difference on either diet.

Variable 2 (Fig 17, line B) shows the mean number of aphids on the membrane for each day of the experiment. The analysis showed a highly significant difference (1% level) from one day to the next. The analysis did not indicate on which day or days the difference occurred. However, it may be seen that between the first and second and second and third days there was a mean of 4 and 4.6 aphids on the membrane, respectively. On the 12th day there was a mean of 0.8 aphids apparently off the membrane. Of these, 50% were dead and 50% were alive. In the statistical analysis, there was no distinction between a dead aphid and a live aphid off the membrane.

In the case of the first two days, it was not unexpected that a few aphids would be off the membrane because this was an unnatural surface, and a certain amount of restlessness would be expected. Even if the difference in the number on the membrane between the 11th and 12th days was highly significant, it would not appear to be the result of the diet's being unpalatable, because the aphids returned to the membrane on the 13th day, and those that survived through the remainder of the experiment were on the membrane almost without fail.

Variable 3 (Fig. 17, line C) shows the mean number of exuviae per replication. Although there was variation, the analysis showed no significant difference from day to day. In general, the mean number of exuviae from day to day was higher on this diet (with the reduced vitamin concentrations) than the mean number of exuviae from day to day for the pea aphid diet. As a result of the higher rate of ecdysis, all aphids reached the adult stage by the 15th day. This may be contrasted

with the aphids feeding on the pea aphid diet (Fig. 14, line C), in which ecdysis continued throughout the 20 days.

Variable 4 (Fig. 18, line A) shows the mean number of live progeny per replication. The analysis showed a highly significant difference (1% level) in the mean number of live progeny from day to day. This difference was highly favorable because, when compared to the aphids feeding on the pea aphid diet (Fig. 15, line A), it was apparent that there was about a 2.5-fold increase in the rate of reproduction over the pea aphid diet. This increased rate of reproduction was attributed to two factors. First, the reduction in the vitamin concentrations in the diet was more favorable for the greenbug. Secondly, the aphids were better able to metabolize this diet, with the result being that the adult stage was reached sooner, and the metabolites could then be converted into progeny, rather than in growth and development.

Variable 5 (Fig. 18, line B) shows the mean number of stillborn progeny per replication. The analysis showed no significant difference from day to day. However, the observed variation was, for the most part, an increase in the mean number of stillborn progeny per day. Although these progeny were stillborn, they were evidence that the dietary metabolites were going into the production of progeny, rather than survival and growth of the parents.

Variable 6 (Fig. 18, line C) was the combination of variables 4 and 5 and as such showed the mean number of both live and stillborn progeny per replication. There was no statistical analysis of this variable, because the experiment in which this diet was used did not include this variable. However, by adding the mean number of live and stillborn progeny from day to day, it was possible to draw a curve for

general comparison. It may be seen that there was approximately a 3-fold increase in the rate of reproduction on this diet as compared to the rate of reproduction seen on the pea aphid diet (Fig. 15, line C).

The rate of growth as measured by the gain in weight of greenbugs feeding on the diet containing the reduced vitamin concentrations is shown in Fig. 19. It showed the same general characteristic shape as the comparable graphs for aphids reared on Rogers barley (Fig. 12) and the pea aphid diet (Fig. 16). There was a maximum weight of .304 mg on the 20th day. This was an 8.8-fold increase in weight during the 20-day period. Although the weight was markedly reduced when compared to the rate of weight gained by greenbugs reared on Rogers barley, it was a considerable improvement over the rate of weight gained by greenbugs reared on the pea aphid diet. It was also better than greenbugs reared on Will barley (Fig. 12).

These results indicate that the diet containing the reduced vitamin concentrations was superior to the pea aphid diet for growth and reproduction of the greenbug. Even though there was a slightly higher rate of mortality on the diet containing the reduced vitamin concentrations, it was equal to the pea aphid diet in the average number of aphids on the membrane from day to day. As a result of these findings, the diet containing the reduced vitamin concentrations was used as the basic and check diet for the testing of minor elements.

Greenbug Response to Minor Elements Individually Added to the Diet

The minor elements, zinc (Zn), iron (Fe), and manganese (Mn) were added to the diet containing the reduced vitamin concentrations (Appendix. Diets 3-14). The four levels of each element were based on levels used by Mittler and Dadd (1966) in the green peach aphid diet.

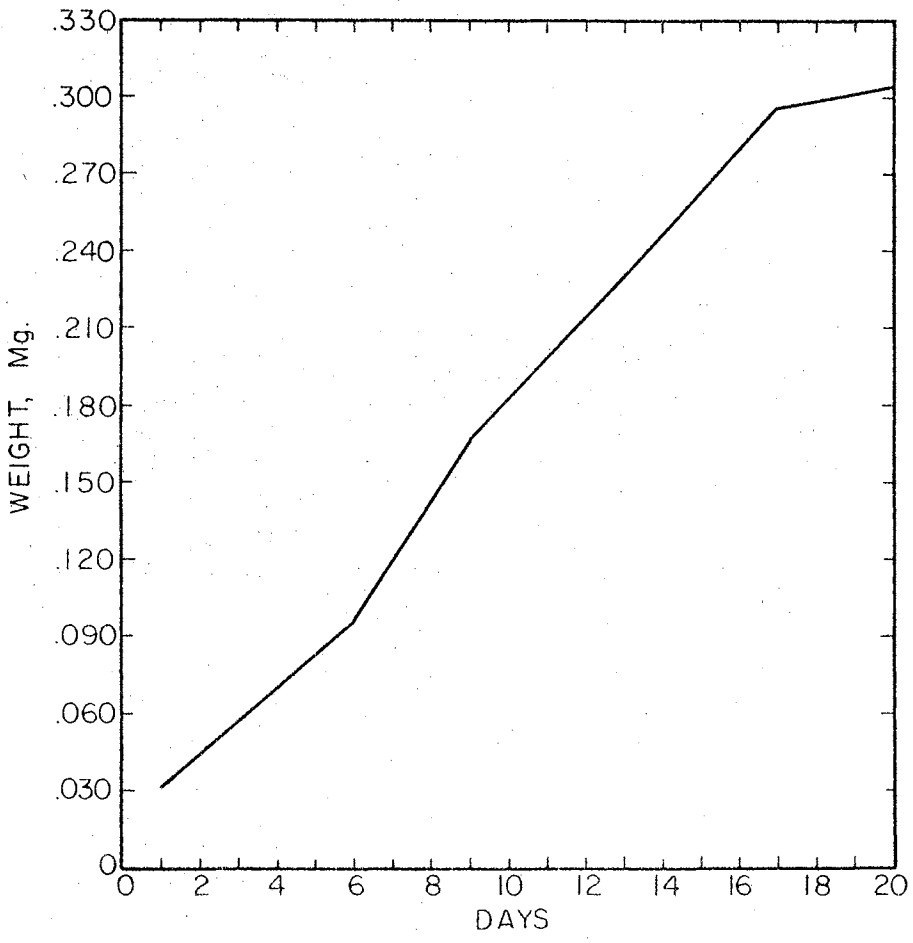

Fig. 19. Mean weight gained by biotype B greenbugs reared on a diet containing 0.1 the vitamin concentrations of the pea aphid diet.

The lowest level was denoted as 1 and the highest level as 4. Thus, the low level of zinc was Zn-1 and the high level was Zn-4. Also, on the basis of the finding of Mittler and Dadd (1966), copper was not included in the greenbug diet, because it was reported to be toxic.

This experiment was statistically analyzed as a split plot in days with the main plots being Zn, Fe, and Mn, each at four levels, along with the check diet. This made a total of 13 treatments in the experiment. Only the contrasts in the analysis of variance which showed significant or highly significant differences are presented and discussed, as there is little value in discussing a contrast in which there was no significant difference.

Variable 1 (Fig. 20, line A) concerned the number of aphids alive from day to day throughout the experiment. There was a highly significant difference (1% level) indicated for the day means and the day by element interaction (Fig. 20, lines B). The day means, as shown, were the mean number of live aphids per replication for all treatments plotted daily over the 20 days. This showed the mean rate of survival throughout the experiment. The mean rate of survival for each of the three elements was indicated over the 20 days in the day by element interaction. In this graph, the number of aphids alive on all four levels of each element were averaged over the 20 days. Thus, it was seen that iron had a lower rate of survival than zinc and manganese. The check diet, which contained no minor elements, was not involved in this particular interaction.

Variable 2 (Fig. 21, lines A and B and Fig. 22, lines A, B, and C) concerned the number of aphids on the membrane. The analysis indicated highly significant differences (1% level) for the day means, the day by

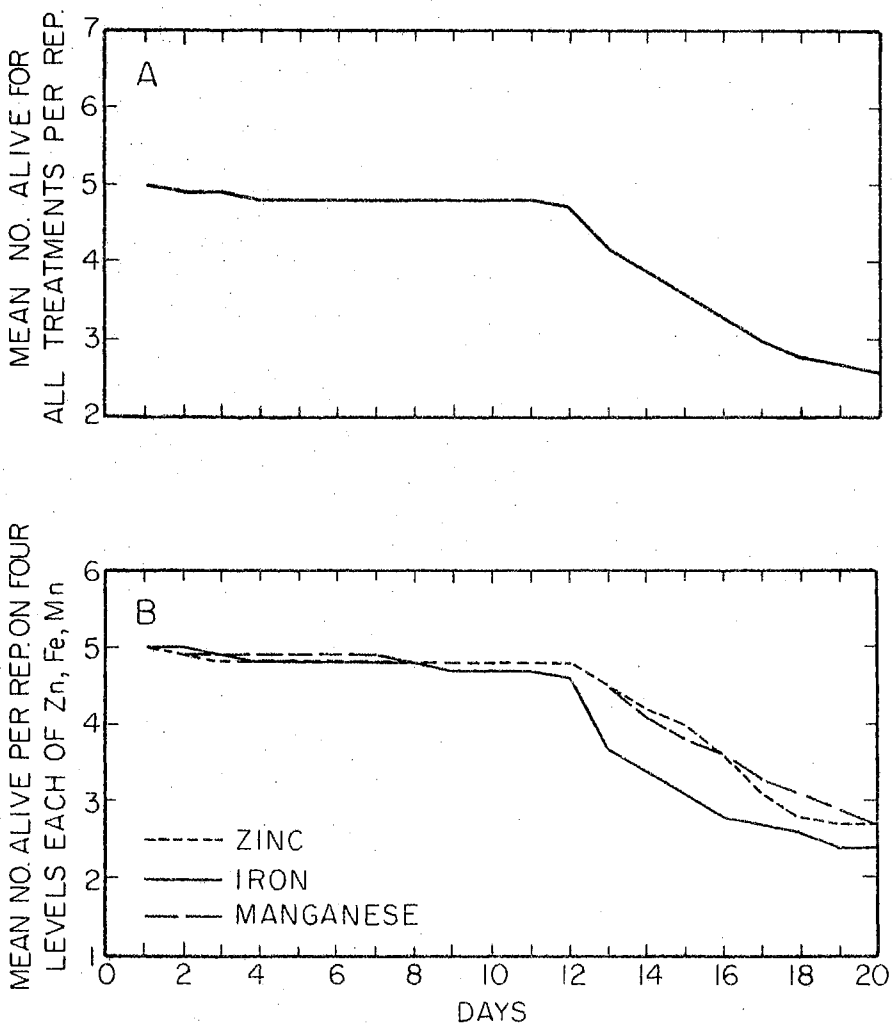

Fig. 20. Mean number of biotype B greenbugs alive feeding on diets containing Zn, Fe, and Mn individually at four levels. A - Day means. B - Day by element means.

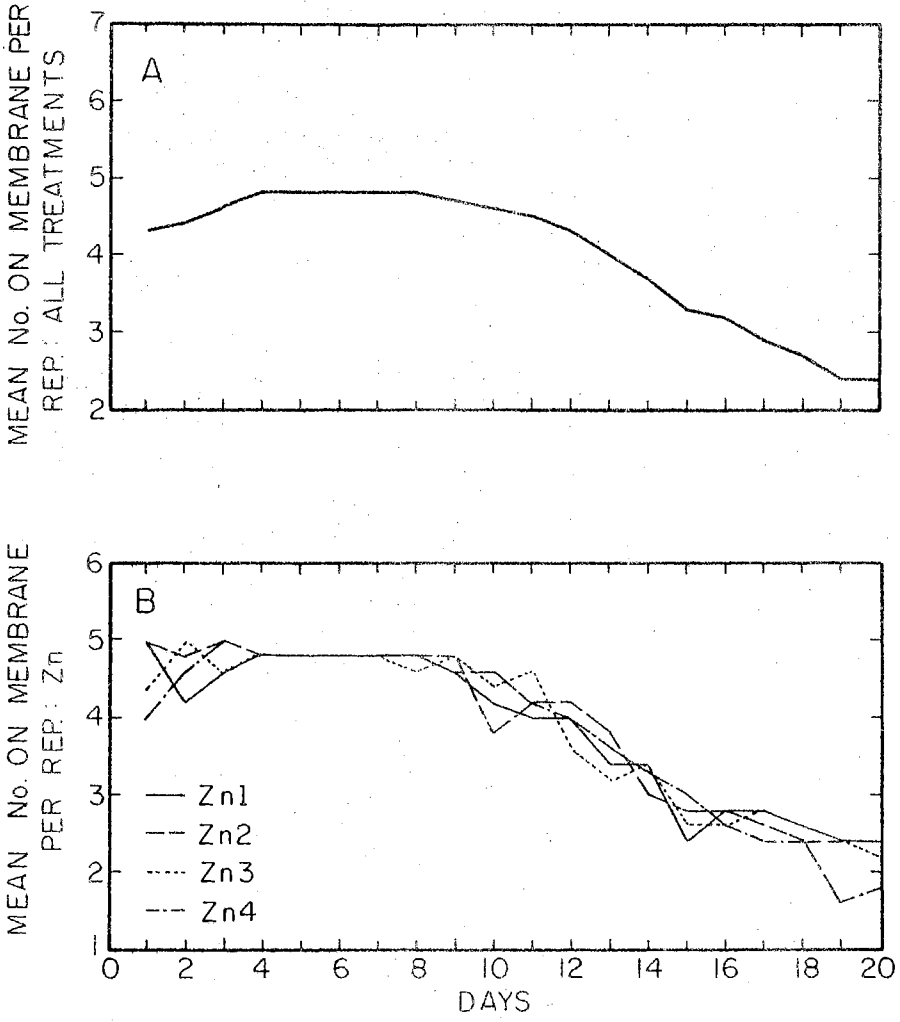

Fig. 21. Mean number of biotype B greenbugs on the membrane feeding on diets containing Zn, Fe and Mn individually at four levels. A - Day means. B - Day by treatment means for Zn.

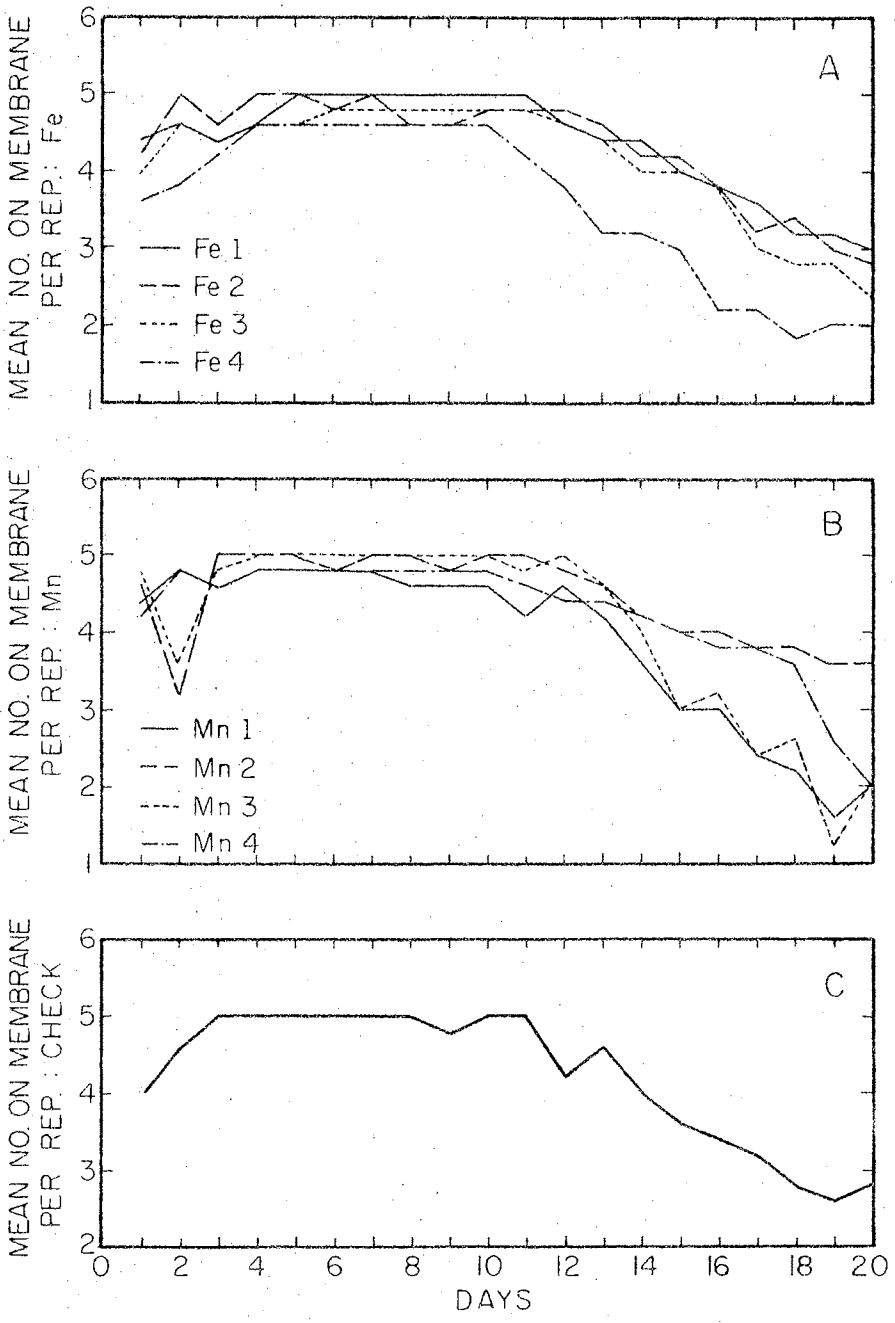

Fig. 22. Mean number of biotype B greenbugs on the membrane feeding on diets containing Zn, Fe and Mn individually at four levels. A - Day by treatment means for Fe. B - Day by treatment means for Mn. C - Day by treatment means for Check.

element interaction and the day by treatment interaction. The day means are shown in Fig. 21, line A. This is the mean number of aphids per replication for all treatments plotted daily over the 20 days. Thus, it was a general indication of the number of aphids on the membrane throughout the experiment. Progressively, more specific information was gained in the day by element and day by treatment interactions, respectively. This was because there were four treatments of each element. Hence, because more specific information was afforded in the day by treatment interaction than in the day by element interaction, only the day by treatment interaction will be discussed. The mean number of aphids on the membrane on each of the four levels of zinc, iron, manganese, and the check are shown in Fig. 21 and Fig. 22.

Concerning zinc (Fig. 21, lines B) it is shown that, in general, the mean number of aphids on the membrane on all four levels was about the same; that is, no one level was either consistently better nor poorer than any of the other levels. When iron (Fig. 22, lines A) was considered, it was seen that levels 1, 2, and 3 were similar, but they were considerably better than level 4. The four levels of manganese (Fig. 22, lines B) followed the same general pattern as the other two elements. However, on the last five days of the experiment, levels 2 and 4 appeared to be slightly better than levels 1 and 3. Since the mean number of aphids on the membrane of the check diet (Fig. 22, line C) followed the same trend as the various levels of the three elements, it was suggested that, although there was a significant difference between the treatments, no treatment was so unpalatable as to render it completely unacceptable to the greenbugs.

Variable 3 (Fig. 23) concerned the number of exuviae per day. The

analysis indicated a highly significant difference (1% level) for the day means and a significant difference (5% level) for the day by element interaction. The day means are shown in Fig. 23, line A. This was the mean number of exuviae for all treatments plotted daily. These differences in day effects were not unexpected, as all aphids do not molt daily nor on the same day. This accounted for the difference in the number of exuviae from day to day. The day by element interaction is shown in Fig. 23, lines B. This showed the mean number of exuviae for each element plotted daily. It was shown that aphids feeding on the diets containing zinc had a higher mean number of exuviae on the 2nd, 5th, 7th, 8th, 11th, and 12th days than did aphids feeding on either iron or manganese. Thus, the aphids feeding on diets containing zinc all reached the adult stage by the 14th day, whereas, the aphids feeding on diets containing iron and manganese continued to molt almost throughout the 20 days. It was noted that all aphids on the check diet (containing no minor elements and, thus, not considered in this interaction) reached the adult stage on the 15th day. These results suggest that, at the levels tested, iron and manganese significantly delayed the rate of development.

Variable 4 (Fig. 24 and Fig. 25) concerned the mean number of progeny born alive. The analysis showed a highly significant difference (1% level) for the main-effect means of elements, treatments, and days. There was also a highly significant difference (1% level) indicated for the day by element and day by treatment interactions.

The mean number of progeny born alive for the elements were 0.1, 0.3, and 0.1 for zinc, iron, and manganese, respectively. Thus, in general, aphids feeding on diets containing zinc and manganese had only

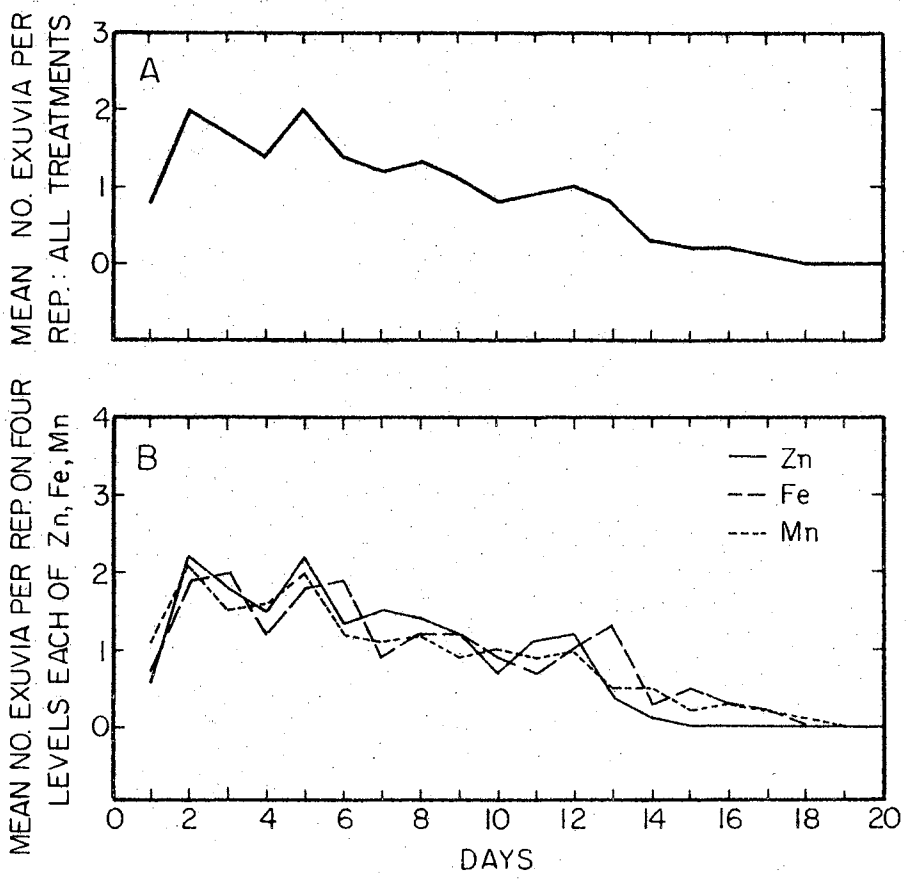

Fig. 23. Mean number of exuviae from biotype B greenbugs reared on diets containing Zn, Fe and Mn individually at four levels. A - Day means. B - Day by element means.

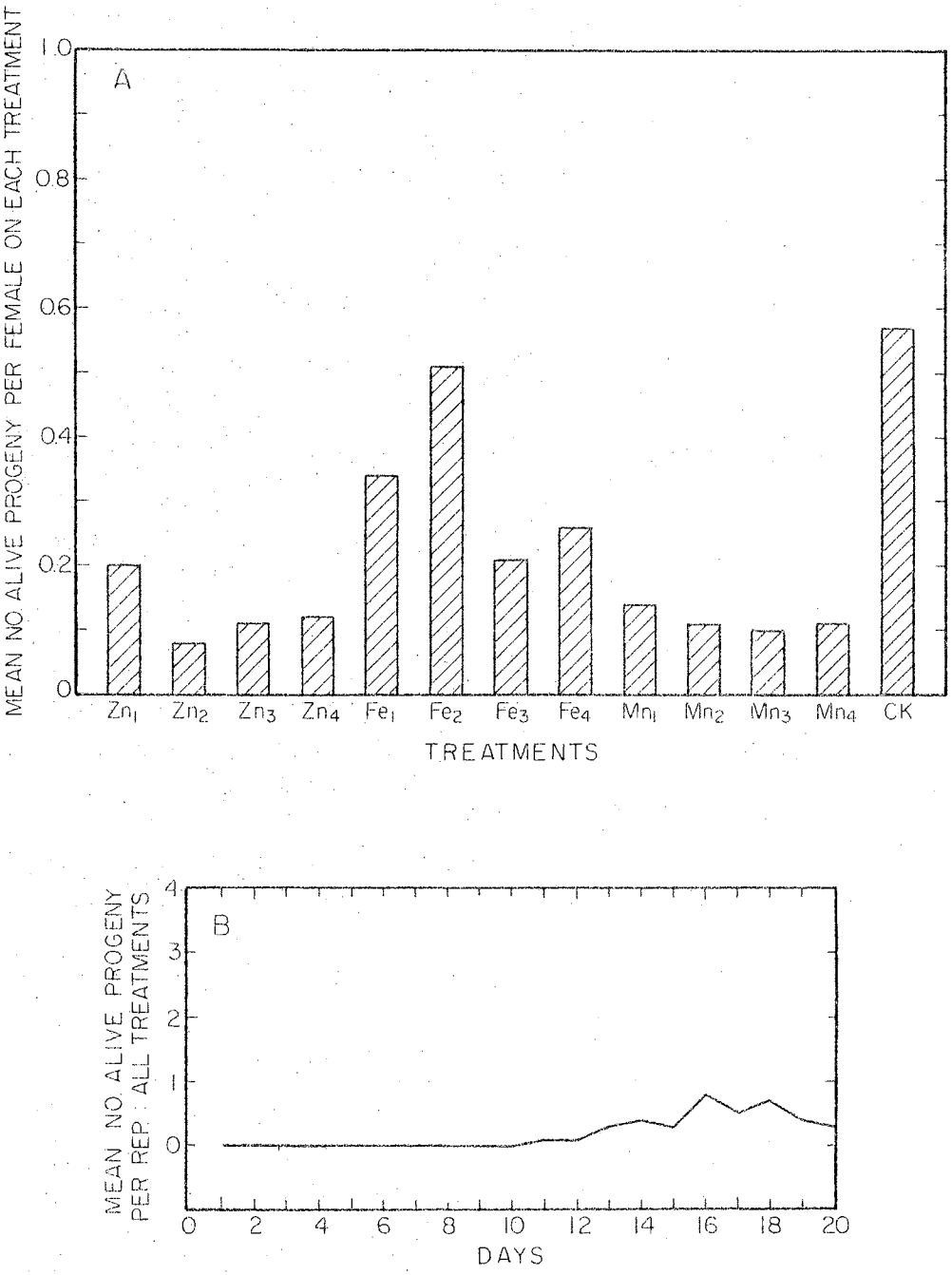

Fig. 24. Mean number of live progeny produced by biotype B greenbugs reared on diets containing Zn, Fe and Mn individually at four levels. A - Treatment means. B - Day means.

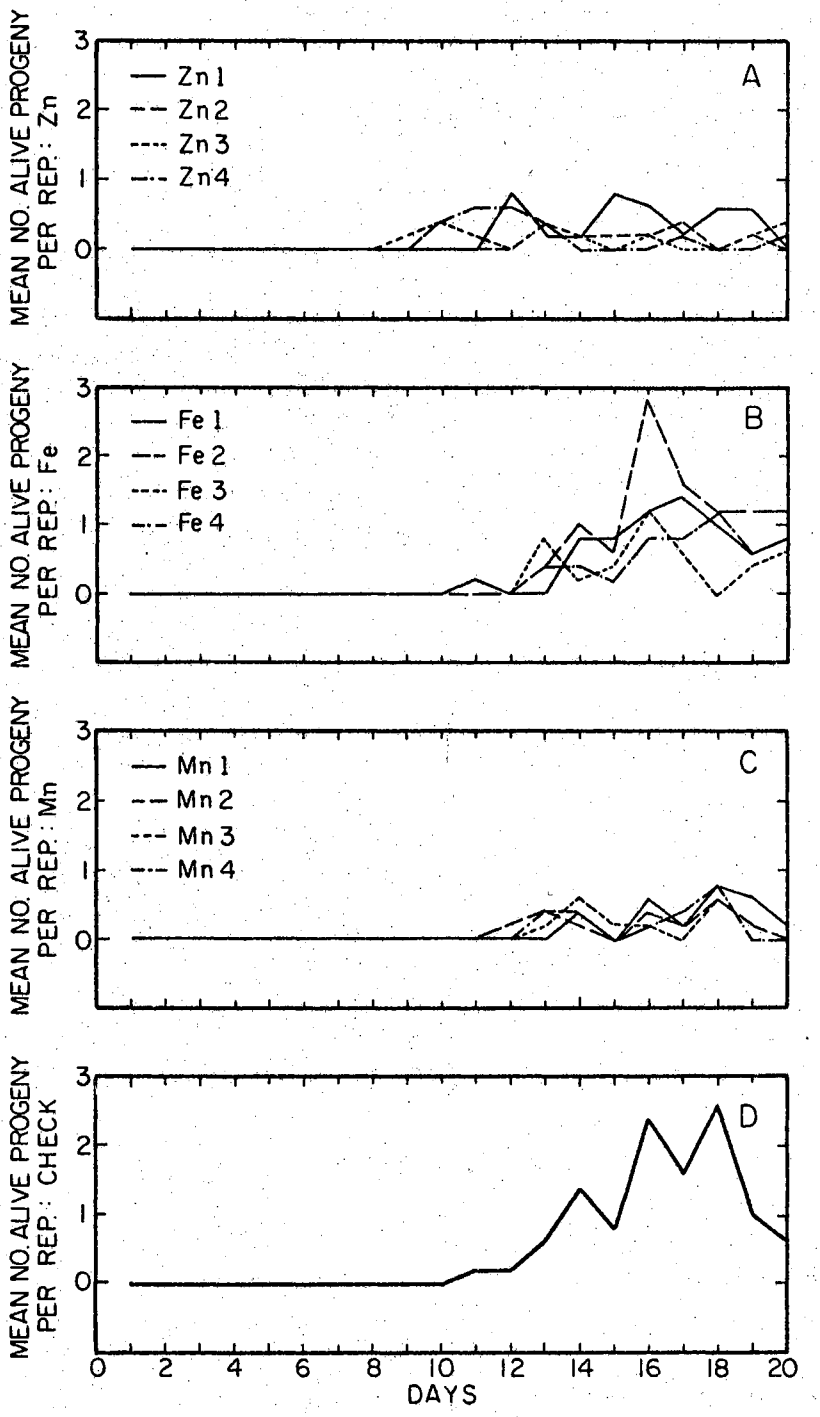

Fig. 25. Mean number of live progeny born to biotype B greenbugs reared on diets containing Zn, Fe, and Mn individually at four levels. A, B, C, D - Day by treatment means for Zn, Fe, Mn and the check, respectively.

about one third as many live progeny as aphids feeding on diets containing iron.

The treatment means for each element at all 4 levels are shown in Fig. 24, line A. This represented the mean number of progeny born alive per female for each of the treatments. It is shown that aphids feeding on diets containing iron produced more progeny than aphids feeding on diets containing either zinc or manganese. Aphids feeding on the diet containing Fe-2 had the greatest mean number of progeny per female, save for the check.

The day means are shown in Fig. 24, line B. This was the mean number of live progeny per replication produced on all treatments plotted daily. Reproduction began on the 11th day and, in general, the rate increased to the maximum of 0.8 on the 16th day. The rate then declined over the remaining days.

Since more specific information was obtained in the day by treatment interaction than in the day by element interaction, only the former will be presented (Fig. 25). This represented the mean number of progeny produced per replicate per treatment. It was seen that all treatments of zinc (Fig. 25, lines A) and manganese (Fig. 25, lines C) were about the same, while the treatments of iron (Fig. 25, lines B), particularly Fe-2, had a higher mean number of progeny per replication. The Fe-2 was the only treatment similar to the check (Fig. 25, line D).

Variable 5 (Fig. 26) concerns the number of stillborn progeny. The analysis indicated highly significant differences (1% level) for the day means and both day by element and day by treatment interactions. The day means are shown in Fig. 26, line A. Stillborn progeny were first born on the 12th day, and their birth rate increased rather

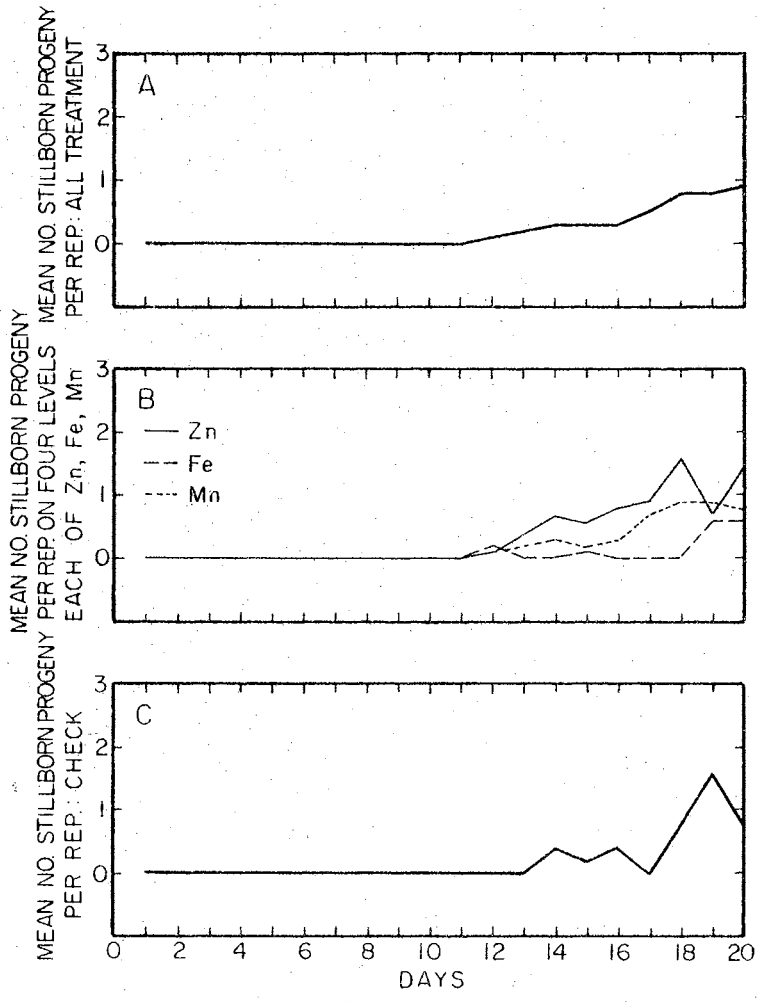

Fig. 26. Mean number of stillborn progeny born to biotype B greenbugs reared on diets containing Zn, Fe and Mn individually at four levels. A - Day means. B - Day by element means. C - Day by treatment means, check.

steadily throughout the remaining nine days of the experiment.

It may be seen in the day by element interaction (Fig. 26, lines B) that aphids feeding on the diets containing iron produced the lowest mean number of stillborn progeny per replication. Aphids feeding on diets containing zinc produced the highest mean number of stillborn progeny. Aphids feeding on diets containing manganese were about midway between zinc and iron. The mean number of progeny born per replication on the check diet is shown in Fig. 26, line C.

Variable 6 was not analyzed until subsequent experiments.

The mean rate of weight gained by aphids feeding on each of the 4 levels of zinc, iron, and manganese may be seen in Figs. 27, 28, and 29, respectively. The weights were not statistically analyzed.

Based on the assumption that the level of each element which supported the most continuous rate of increase was best, it was suggested that Zn-1, Fe-2, and Mn-4 were best. It was noted that, in general, the aphids on all levels of zinc weighed more than aphids on the check diet. The opposite was noted for iron and manganese.

To get an indication of which level of each element resulted in the best aphid development, the level by element means were totaled for each variable that showed a highly significant difference for the day by element and day by treatment interactions. Variables 2, 4, and 5 showed such differences. These means are shown in Table 4. The means for each level of each element were totaled. It was assumed that the level of each element with the highest total mean was the best level for aphid performance for each element as measured by variables 2, 4, and 5. Thus, Zn-1, Fe-2, and Mn-4 were indicated as being the best.

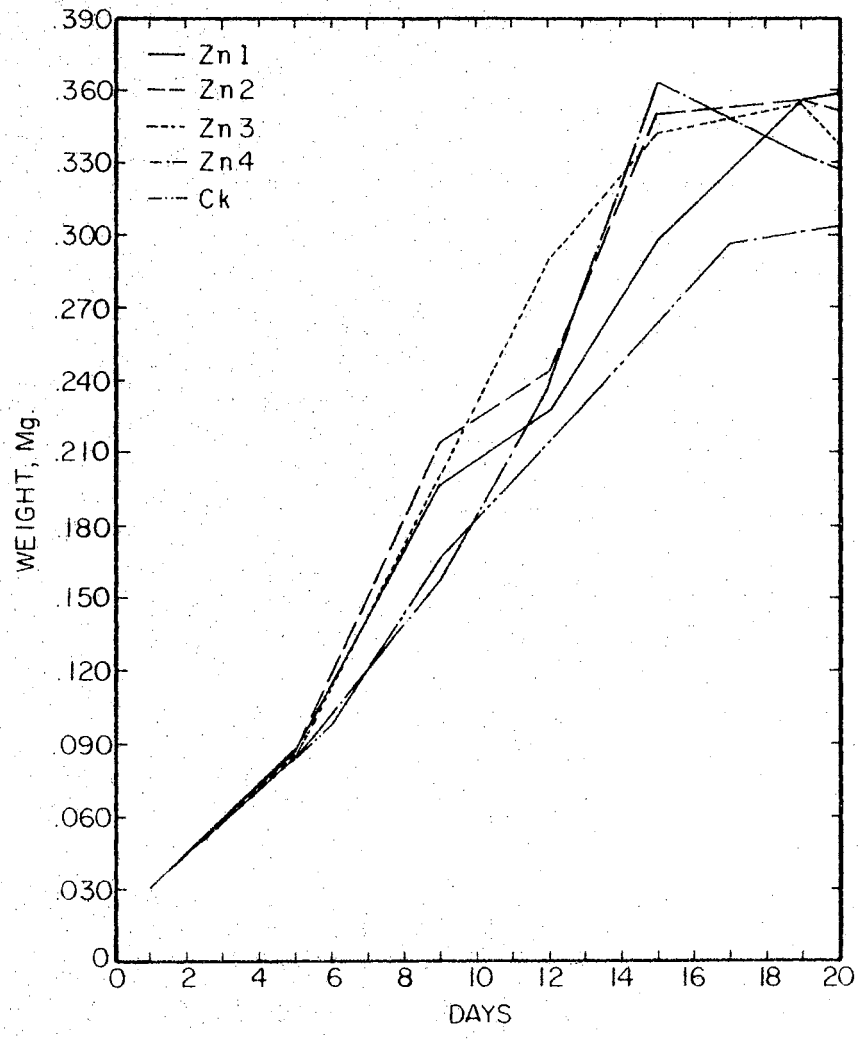

Fig. 27. Mean weight gained by biotype B greenbugs reared on diets containing four levels of zinc.

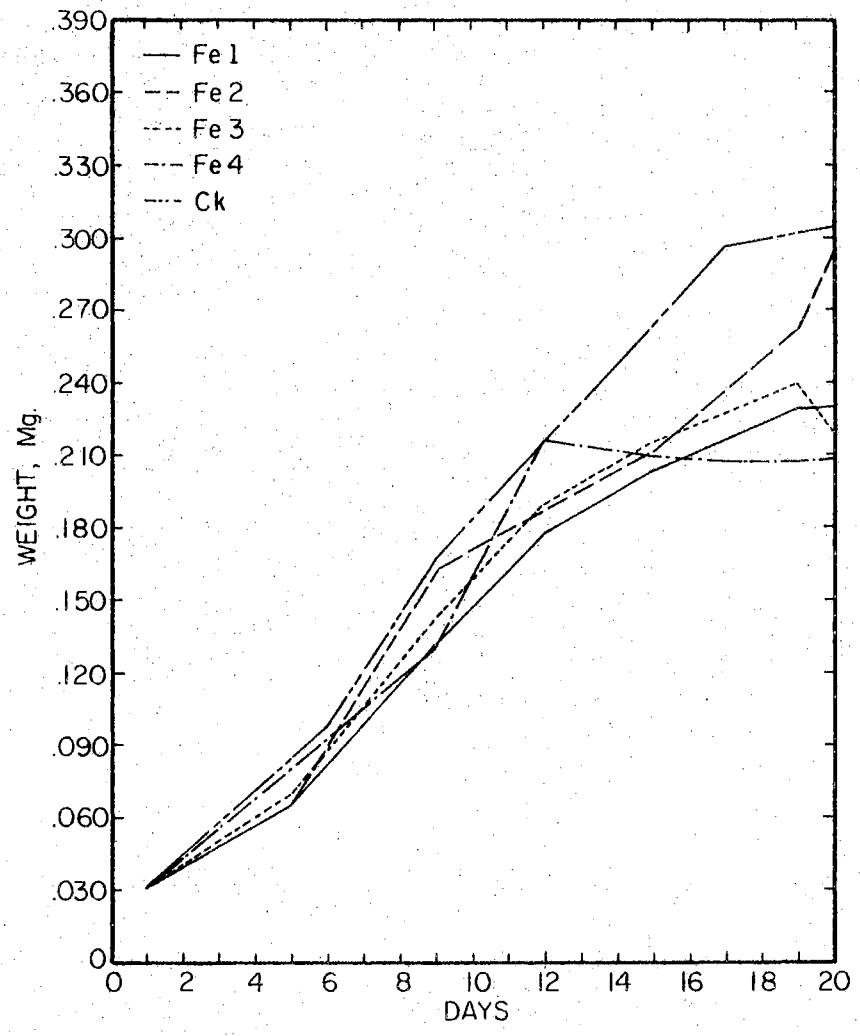

Fig. 28. Mean weight gained by biotype B greenbugs reared on diets containing four levels of iron.

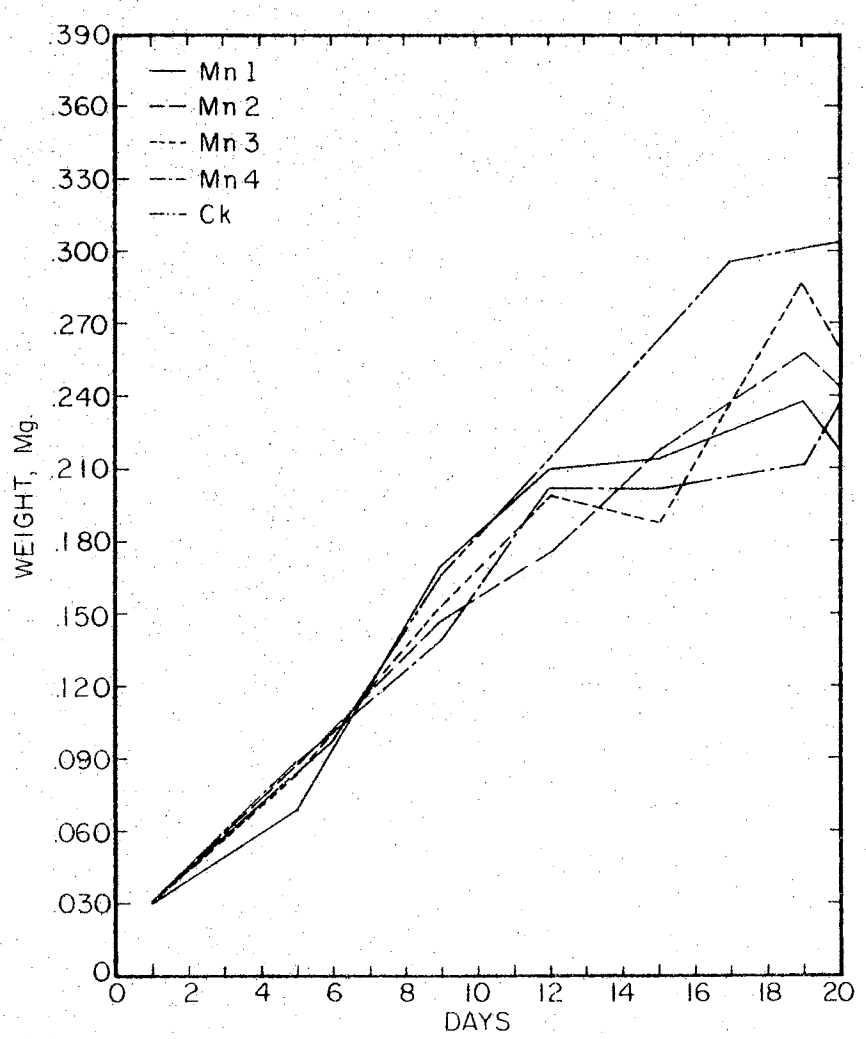

Fig. 29. Mean weight gained by biotype B greenbugs reared on diets containing four levels of manganese.

Table 4. Mean number of greenbugs on diets containing zinc, iron, and manganese.

Element	Level	Variable 1/ 2	4	5	Total
Zinc	1	3.84	0.20	0.46	4.50
	2	3.90	0.08	0.36	4.34
	3	3.85	0.11	0.39	4.35
	4	3.76	0.12	0.30	4.18
Iron	1	4.36	0.34	0.10	4.80
	2	4.32	0.51	0.10	5.03
	3	4.14	0.21	0.11	4.46
	4	3.57	0.26	0.03	3.86
Manganese	1	3.88	0.14	0.20	4.22
	2	4.32	0.11	0.20	4.63
	3	4.05	0.10	0.24	4.39
	4	4.23	0.11	0.30	4.64

1/ Variable 2 - mean number of greenbugs on the membrane.

Variable 4 - mean number of progeny born alive.

Variable 5 - mean number of progeny stillborn.

Greenbug Response to Minor Elements Added to the Diet in Combination

This experiment involved combining the four levels of zinc, iron, and manganese discussed in the previous experiment. The five combinations were (1) Zn-1, Fe-1, Mn-1, (2) Zn-2, Fe-2, Mn-2, (3) Zn-1, Fe-2, Mn-4, (4) Zn-3, Fe-3, Mn-3, and (5) Zn-4, Fe-4, Mn-4. In the following discussion, these combinations will be referred to as (1), (2), (3), (4), and (5). Each combination represents one of the five treatments in this experiment. Each combination was incorporated into the diet containing the reduced vitamin concentrations (Appendix. Diets 15-19).

This experiment was analyzed as a split plot in days with the main plots being the treatments in a randomized block design.

Considering variable 1 (Fig. 30), the analysis showed a highly significant difference (1% level) for the day means. These means are graphed in Fig. 30, line A. The rate of survival on all treatments was acceptable, even though the difference between the numbers alive from day to day was highly significant.

The analysis of variable 2 (Fig. 30) showed a highly significant difference (1% level) for the day means. These means are shown in Fig. 30, line B. These means approximated the means for variable 1. This was to be expected, because to remain alive, the aphids must remain on the membrane to feed.

The analysis for variable 3 (Fig. 30) showed a highly significant difference (1% level) for the day means. These means are shown in Fig. 30, line C. This represented the mean number of exuviae for all treatments per replication. In general, there was great variation from day to day, especially in the first nine days. It was also noted that all aphids had reached the adult stage by the 13th day.

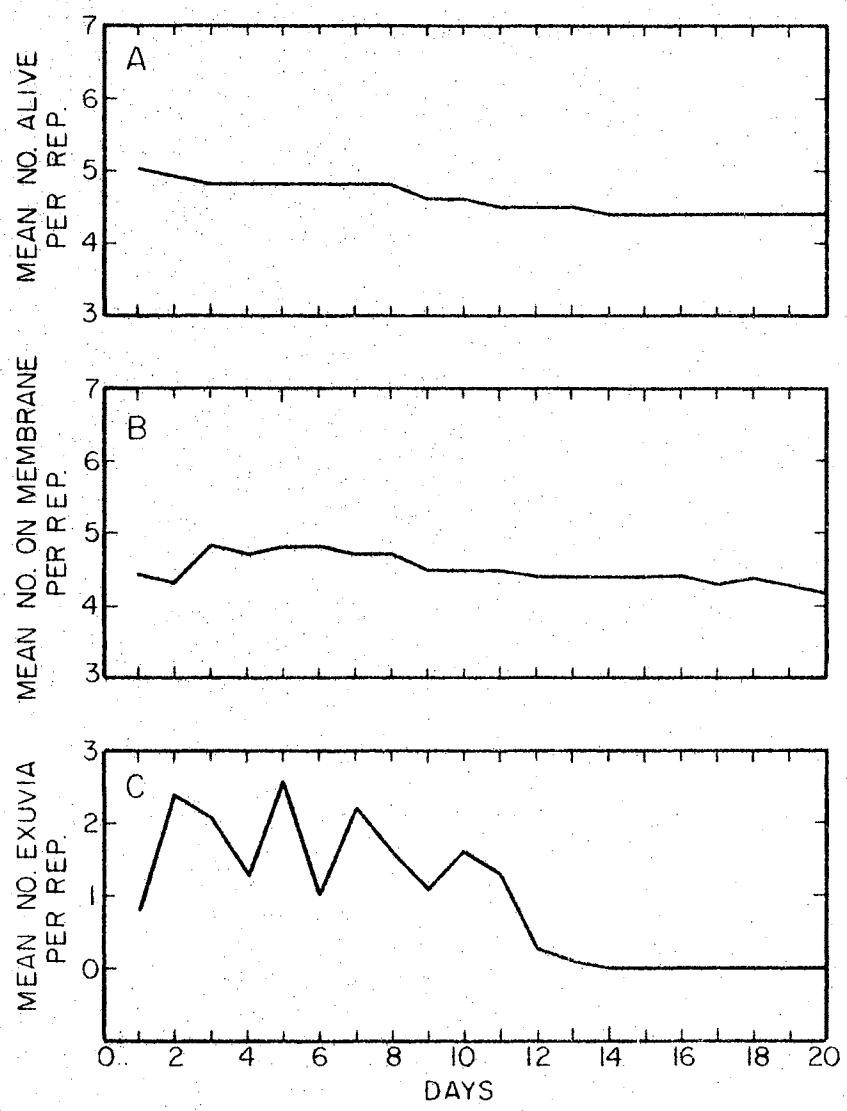

Fig. 30. Biotype B greenbug survival, growth and development when reared on diets containing Zn, Fe and Mn in five combinations.

The analysis of variable 4 (Fig. 31) indicated a highly significant difference (1% level) for the treatment means, day means, and the day by treatment interaction.

The treatment means were (1) 2.11, (2) 2.10, (3) 1.62, (4) 1.39, and (5) 2.69. Thus, treatment (5) had the greatest mean number of live progeny per female while treatment (4) had the lowest mean number of live progeny per female.

The day means are graphed in Fig. 31, line A. It was seen that on the average, reproduction of live progeny began on the 9th day, and the rate increased rapidly to its maximum on the 12th day, when it declined rather rapidly.

The day by treatment means are shown in Fig. 31, lines B. It was readily apparent that treatment (5) was superior to the other four treatments in the rate of reproduction and the mean number of live progeny produced per replication.

In general, these progeny survived an average of 10 days with very little or no growth, the second instar being reached by some individuals.

The analysis of variable 5 (Fig. 32) showed a significant difference (5% level) for the treatment means and a highly significant difference (1% level) for the day means and day by treatment interaction. The treatment means for the number of stillborn progeny were (1) 0.29, (2) 0.87, (3) 0.35, (4) 0.82, and (5) 0.74. Treatment (2) had the greatest mean number of stillborn progeny and treatment (1) had the least. The day means are graphed in Fig. 32, line A. Stillborn reproduction began on the 12th day. The rate of stillborn progeny reproduction was rather variable but, in general, it increased to its

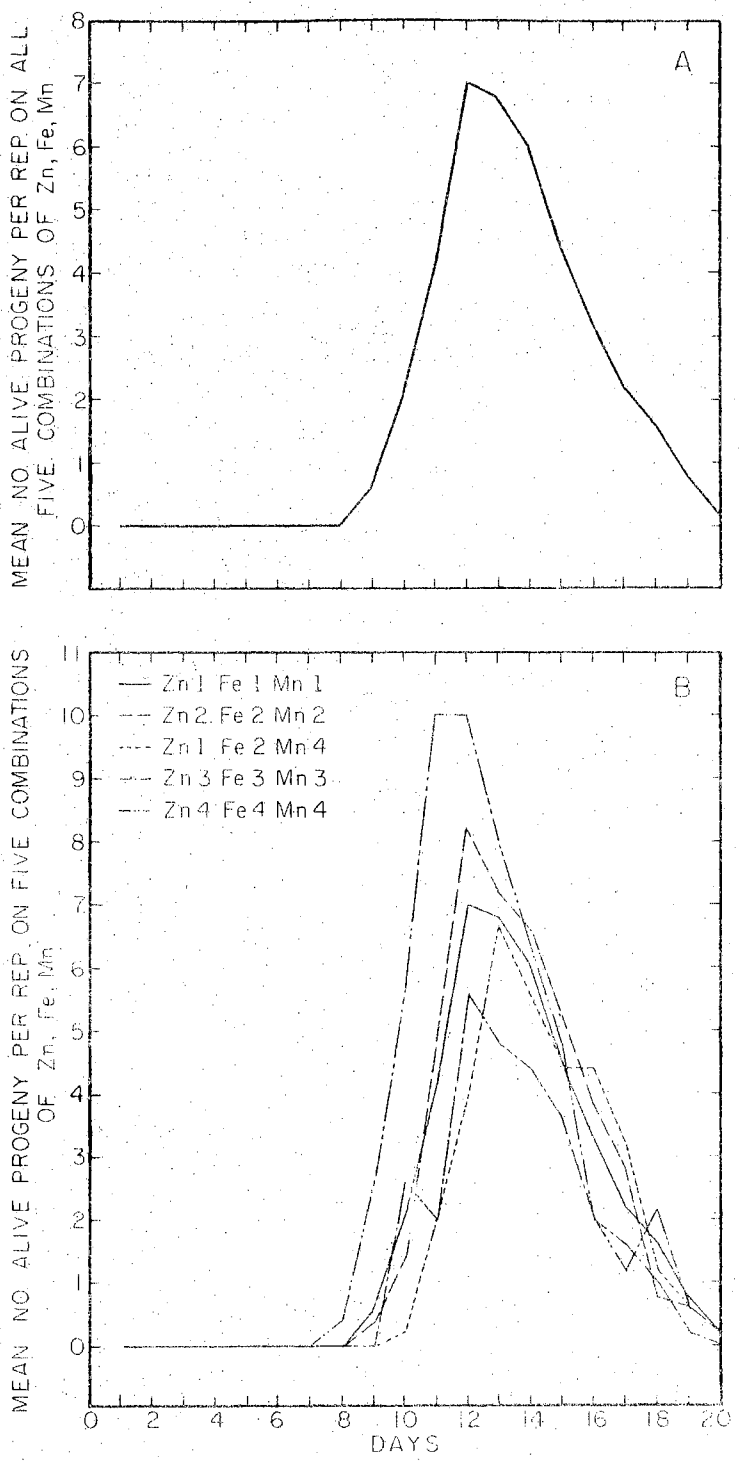

Fig. 31. Mean number of live progeny born to biotype B greenbugs reared on diets containing Zn, Fe and Mn in five combinations. A - Day means. B - Day by treatment means.

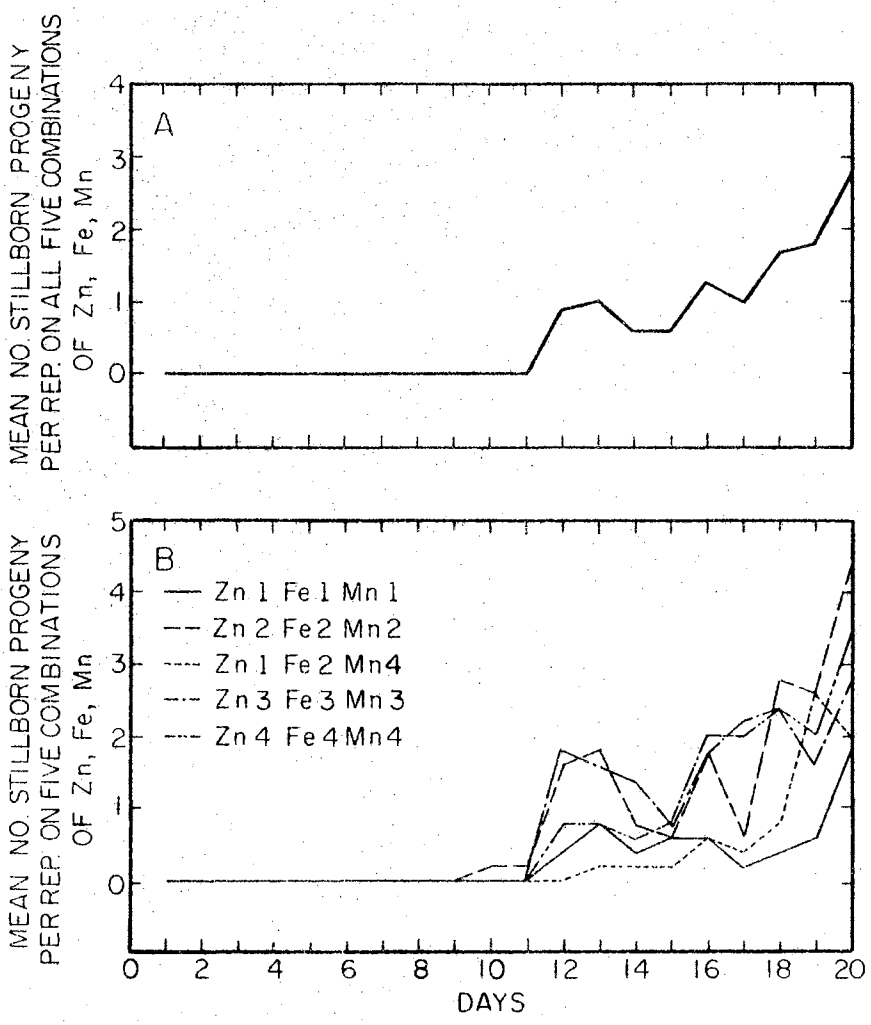

Fig. 32. Mean number of stillborn progeny born to biotype B greenbugs reared on diets containing Zn, Fe and Mn in five combinations. A - Day means. B - Day by treatment means.

maximum on the 20th day.

The day by treatment means are shown in Fig. 32, lines B. These means are rather variable but, in general, they increase to the 20th day. This suggested that assimilation of diet material into progeny was taking place but that some dietary components were deficient.

Variable 6 (Fig. 33) is a combination of variables 4 and 5. As such, it reflected the results of those variables. Therefore, it was not unexpected when the analysis showed highly significant differences (1% level) for the treatment means, day means, and day by treatment interaction. Because variable 6 is a combination of variables 4 and 5, only the means for the day by treatment interaction are shown (Fig. 33). It was readily seen, as in variable 4, that treatment (5) was superior to all other treatments in both the rate of reproduction and mean number of progeny per replicate.

The mean rate of weight gain by aphids feeding on these five treatments is shown in Fig. 34. The comparable curve for Rogers barley is also shown. The aphids feeding on treatment (5) had a rate of weight gain very similar to the aphids feeding on Rogers barley. Aphids feeding on all treatments, except treatment (3), had a more favorable rate of weight gain, which ultimately resulted in heavier aphids at the end of the experiment, than did aphids feeding on diets containing the individual elements at the various levels.

These results indicated that treatment (5) was equal or superior to all other treatments, as measured by the various statistically analyzed variables. In addition, it was superior to all other treatments in both rate of weight gain and total weight gained. On the basis of these results, treatment (5) was termed "the standard biotype

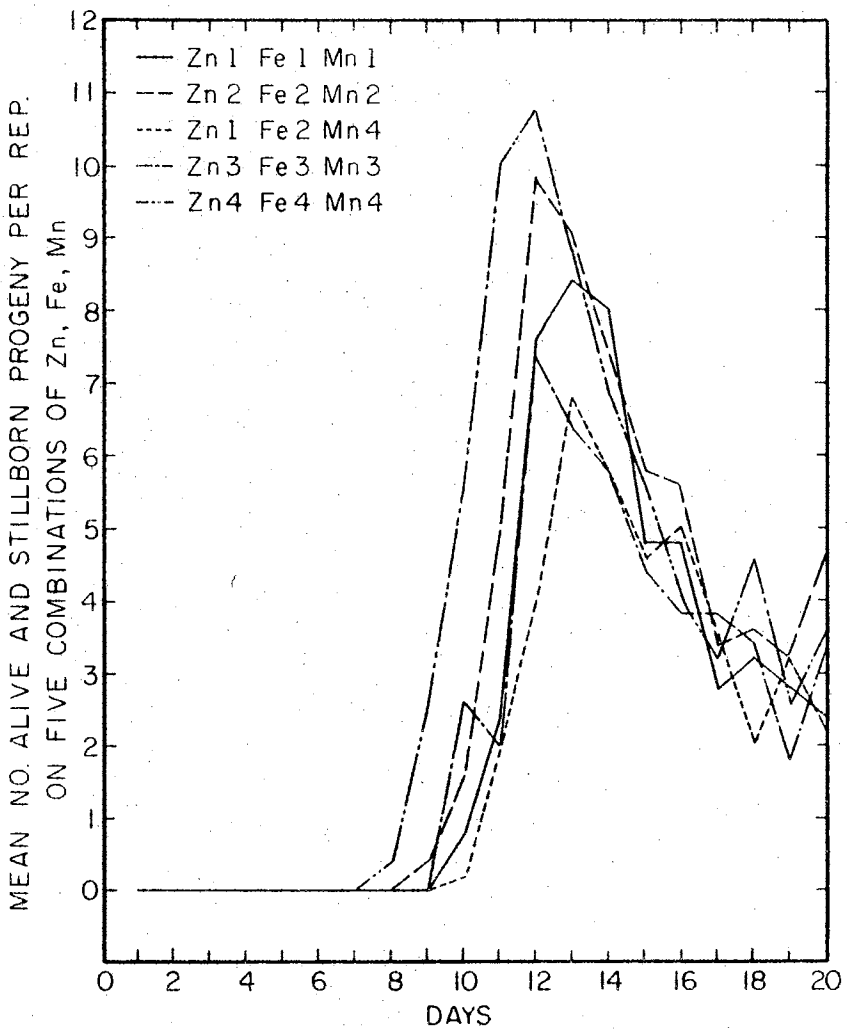

Fig. 33. Mean number of all live and stillborn progeny born to biotype B greenbugs reared on diets containing Zn, Fe and Mn in five combinations.

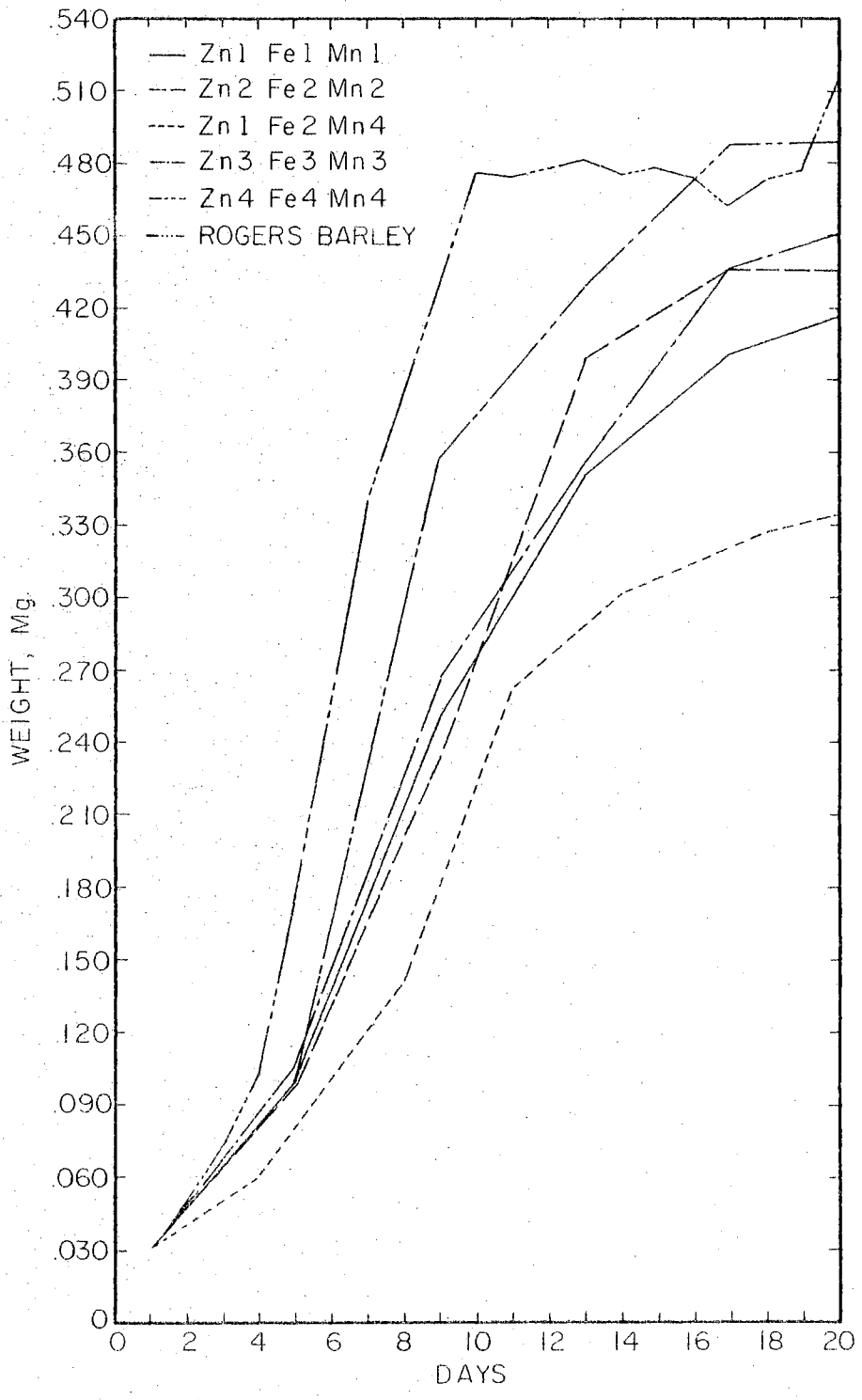

Fig. 34. Mean weight gained by biotype B greenbugs reared on diets containing Zn, Fe and Mn in five combinations.

B greenbug diet."

Greenbug Response to Vitamins B_{12} and E Added to the Standard Biotype B Greenbug Diet

Two experiments were conducted in which the vitamins B_{12} (Cyancobalamin) and E (DL-alpha tocopherol) were added to the standard biotype B greenbug diet in an attempt to reduce the number of stillborn progeny and increase the rate of reproduction. These two experiments are discussed individually.

In experiment 1, the vitamins B_{12} and E were incorporated at four high levels. There were 4 treatment combinations tested. They were numbered (1), (2), (3), and (4), according to vitamin level. These diets may be seen in Appendix, Diets 20-23.

The analysis of variables 1, 2, and 3 (Fig. 35, lines A, B, and C) showed a highly significant difference (1% level) for the day means. When these day means were compared to the day means for variables 1, 2, and 3 of the standard biotype B greenbug diet (Fig. 30, lines A, B, and C), it was suggested that vitamins B_{12} and E had little apparent effect on greenbug survival, diet palatability, or development.

The analysis of variable 4 (Figs. 36 and 37) indicated a highly significant difference (1% level) for the day means and day by treatment interaction. The day means (Fig. 36) reached a maximum mean of 8.1 live progeny per replication on the 13th day. The day by treatment means (Fig. 37) reached a maximum mean of 8.8 live progeny per day on the 13th day. This was seen for treatment (4). Aphids feeding on the standard biotype B greenbug diet showed a maximum day by treatment mean of 10.0 live progeny per replication on the 11th and 12th days (Fig. 31, lines B).

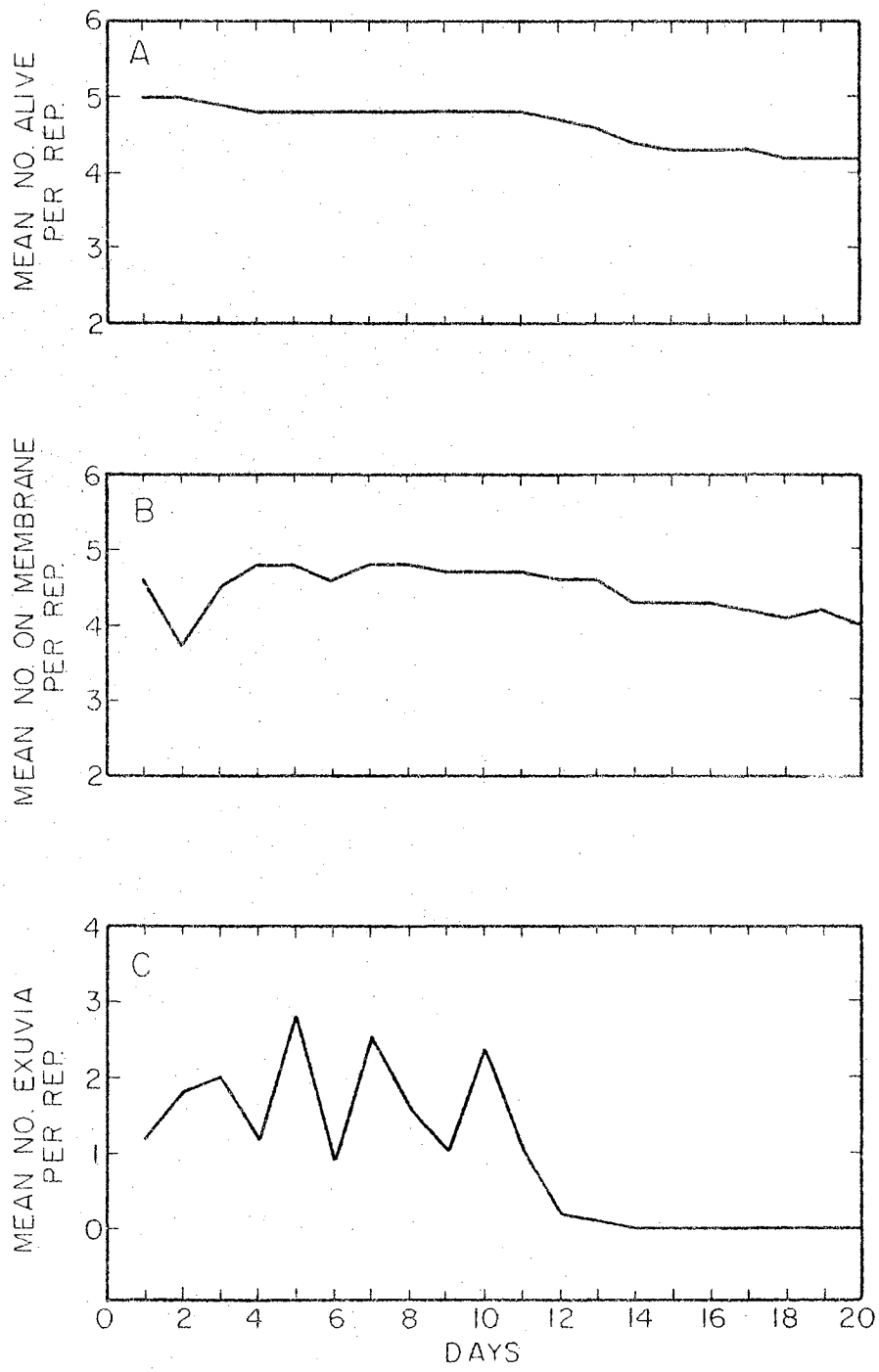

Fig. 35. Biotype B greenbug survival, growth and development when reared on diets containing all four high levels of vitamins B_{12} and E in combination.

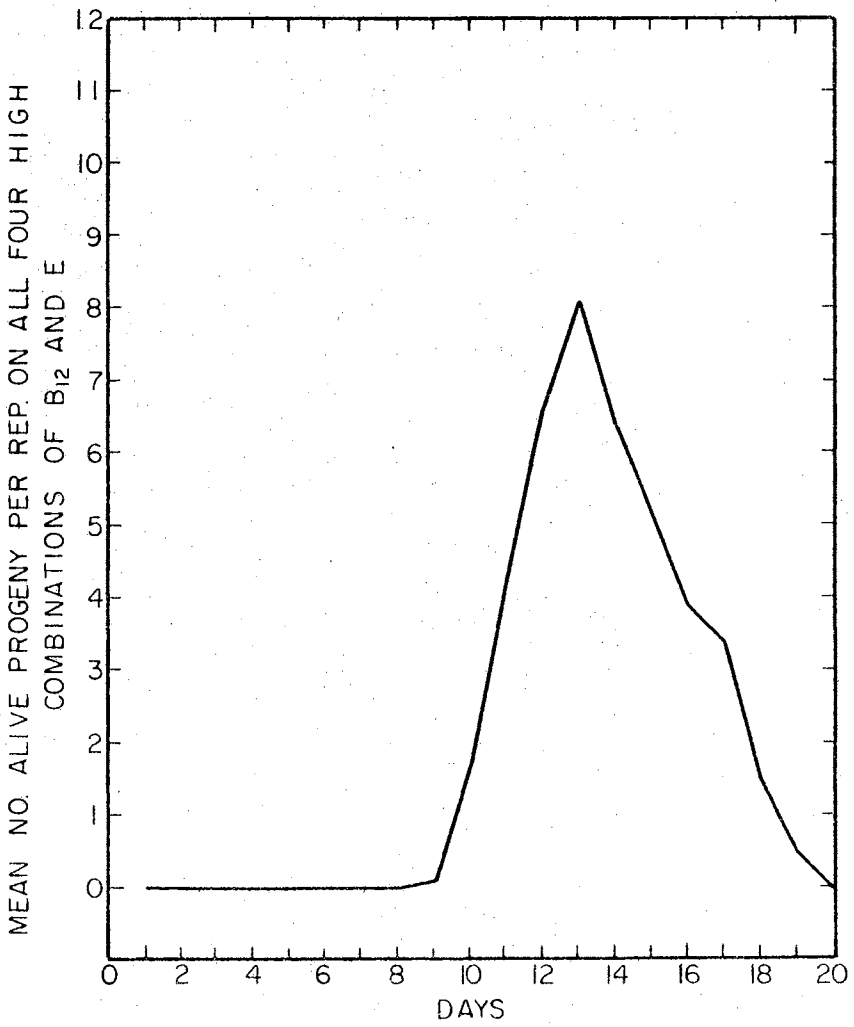

Fig. 36. Mean number of live progeny born to biotype B greenbugs reared on diets containing all four high combinations of vitamins B_{12} and E.

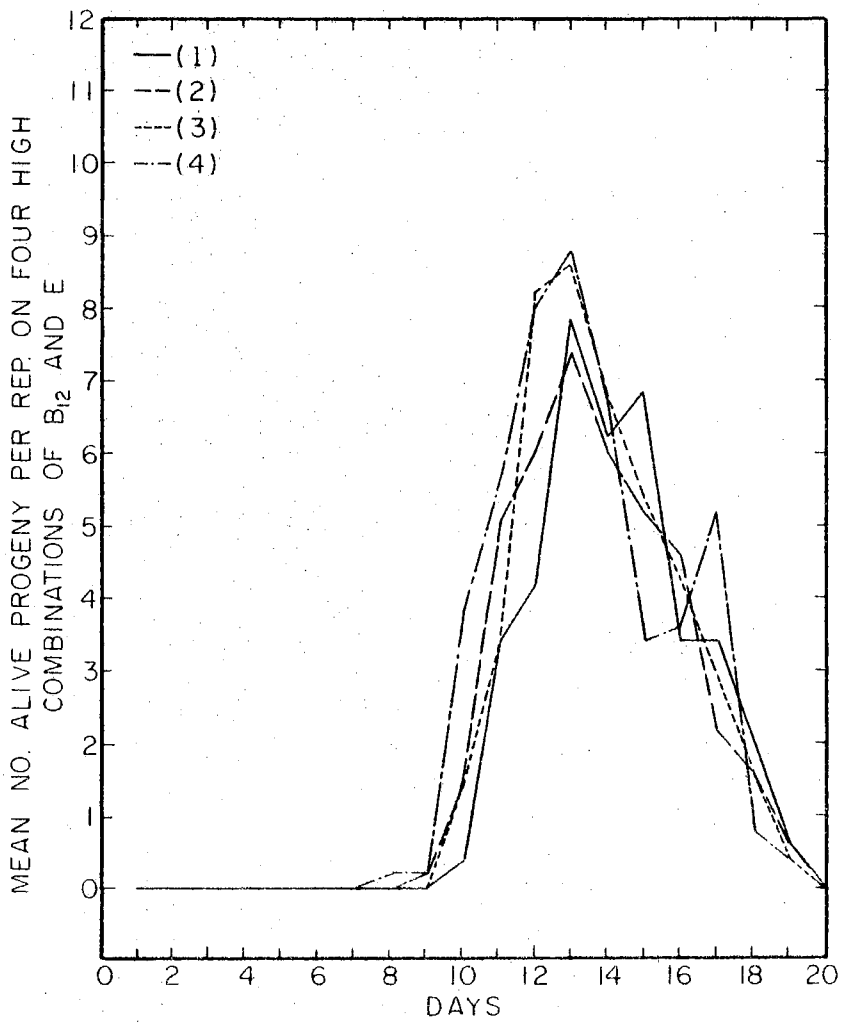

Fig. 37. Mean number of live progeny born to biotype B greenbugs reared on diets containing each of four high combinations of vitamins B_{12} and E.

The analysis for variable 5 (Fig. 38, line A) showed a highly significant difference (1% level) for the day means and day by treatment means. These means are, in general, comparable to the day means for variable 5 on the standard biotype B greenbug diet (Fig. 32, line A). However, the day by treatment means were so highly variable that no valid conclusions could be drawn.

The analysis of variable 6 showed a highly significant difference (1% level) for the day means as shown in Fig. 38, line B. Although the day by treatment means were not significant, it was noted that treatment (1) reached the maximum of 10.2 progeny per replication on the 13th day. This was superior to the other three treatments, but when compared to the maximum of 10.8 reached on the 12th day by aphids feeding on the standard biotype B greenbug diet (Fig. 33) it was suggested that these four treatments of vitamins B_{12} and E neither improved nor greatly hindered reproduction of aphids feeding on the standard biotype B greenbug diet. However, since the number of progeny from aphids feeding on treatment (1) almost equalled the number of progeny from aphids feeding on the standard biotype B greenbug diet, it was decided to run four more combinations at four lower levels.

In experiment 2, vitamins B_{12} and E were incorporated into the standard greenbug diet at four low level treatment combinations. The treatment combinations were numbered (1), (2), (3), and (4) with the lowest level being (1) and the highest level being (4). These diets may be seen in Appendix, Diets 24-27.

The analysis of variables 1, 2, and 3 (Fig. 39, lines A, B, and C) showed a highly significant difference (1% level) for the day means. These means, when compared to the day means for variables 1, 2, and 3

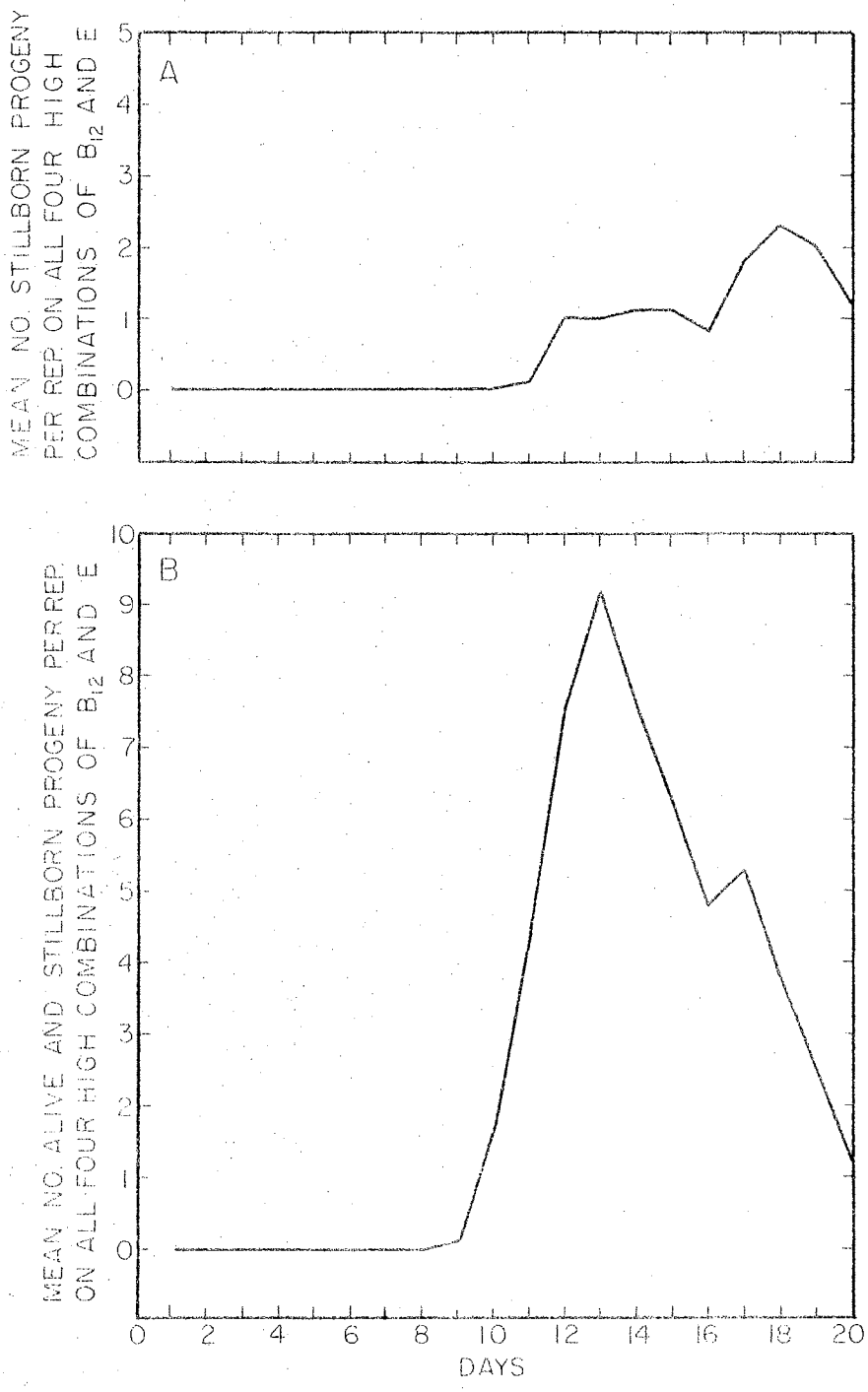

Fig. 38. Mean biotype B greenbug reproduction when reared on diets containing all four high combinations of vitamins B_{12} and E. A - Day means for stillborn progeny. B - Day means for all live and stillborn progeny.

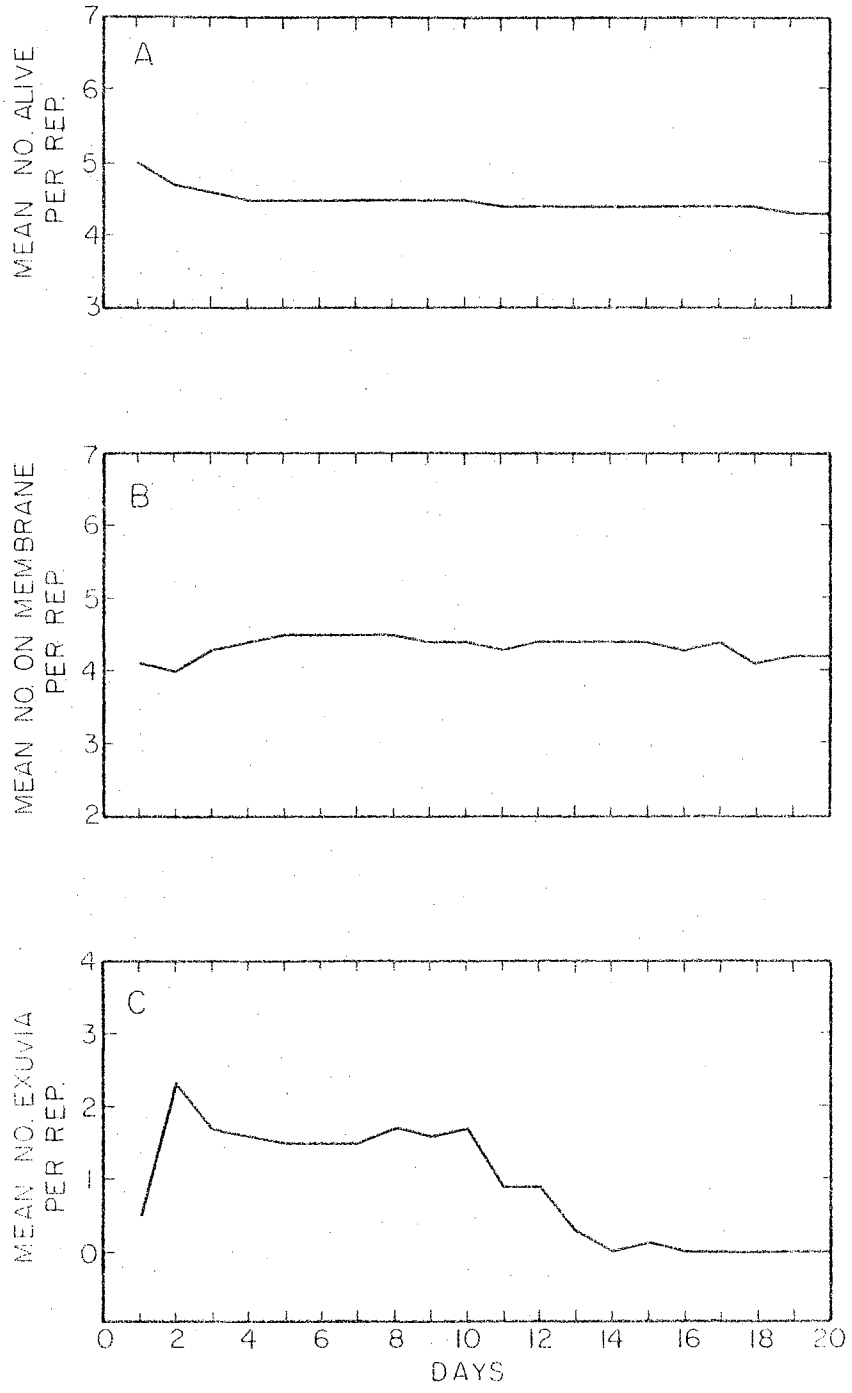

Fig. 39. Biotype B greenbug survival, growth and development when reared on all four low combinations of vitamins B_{12} and E.

of the standard biotype B greenbug diet (Fig. 30, lines A, B, and C), do not indicate any improvement in the greenbug survival, diet palatability, or development.

The analysis of variable 4 (Fig. 40) showed a highly significant difference (1% level) for the treatment means, day means, and day by treatment interaction.

The treatment means were (1), 2.1, (2) 2.3, (3) 1.6, and (4) 2.0 live progeny per female. Greenbugs feeding on the standard biotype B greenbug diet had a treatment mean of 2.6 live progeny per female.

The day by treatment means are shown in Fig. 40. A maximum mean of 8.2 live progeny per replication was reached on the 12th day by treatments (2) and (4). In comparison, the standard biotype B greenbug diet had a maximum mean of 10.0 on the 11th and 12th days (Fig. 31, lines B). These results indicate that the standard biotype B greenbug diet was superior to these four treatment combinations in the rate of assimilation of live progeny.

The analysis of variable 5 showed a significant difference (5% level) for the treatment means and day by treatment interaction. The day means were highly significant (1% level).

The treatment means were (1) 0.2, (2) 0.2, (3) 0.3, and (4) 0.4 stillborn progeny per female. Treatment (4) was the highest and when it was compared to the treatment mean of 0.7 for the standard biotype B greenbug diet, it was suggested that the rate of assimilation into progeny, at its best, was greatly reduced on these four combinations of vitamin B_{12} and E. This was in keeping with variable 4. The day means and day by treatment means were highly variable as suggested by the analysis and because the rate of assimilation has already been shown to

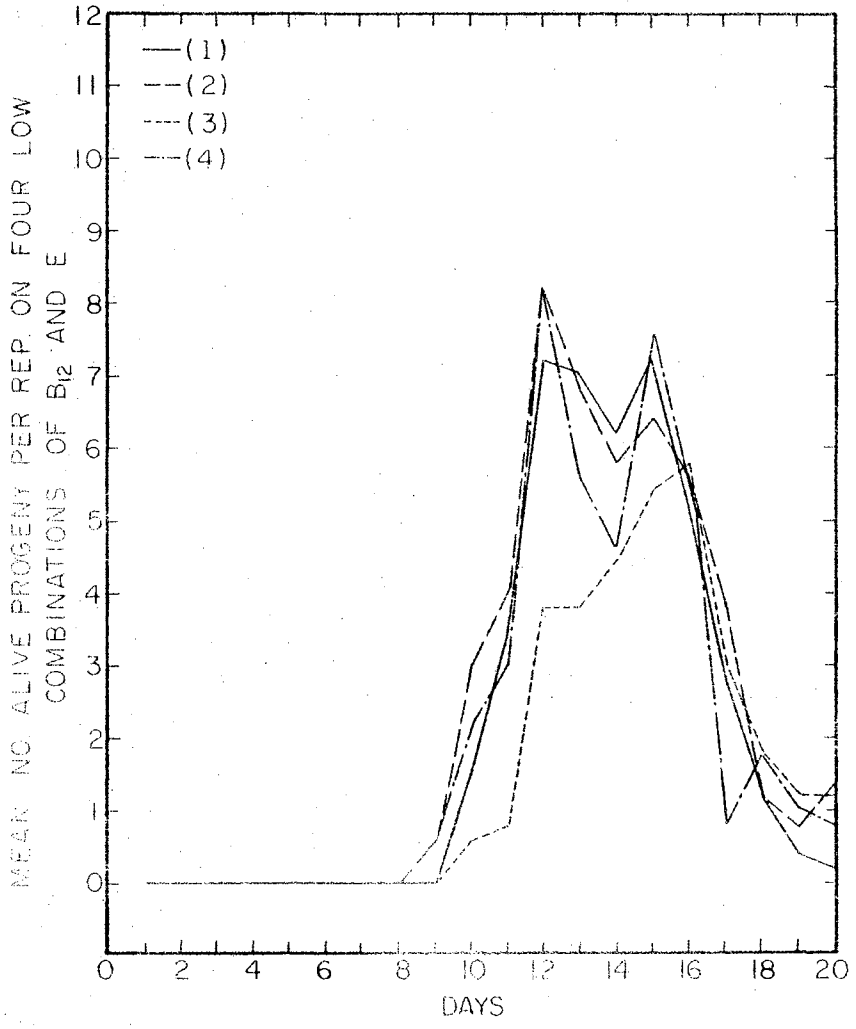

Fig. 40. Mean number of live progeny born to biotype B greenbugs feeding on diets containing each of four low combinations of vitamins B_{12} and E.

be reduced in comparison to the standard biotype B greenbug diet, these means need not be shown.

The analysis for variable 6 (Fig. 41) showed a highly significant difference for the treatment means, day means, and day by treatment interaction. The day by treatment means show the reduced rate of assimilation seen in variables 4 and 5 when compared to the standard biotype B greenbug diet (Fig. 33).

The results of these experiments suggest that vitamins B_{12} and E did not improve the standard biotype B greenbug diet, and in some cases reduced the rate of reproductive assimilation. Therefore, they were omitted from the diet.

Biotype A Greenbugs Reared on Standard Biotype B Greenbug Diet

This experiment was conducted to compare biotype A with biotype B greenbugs when reared on the standard biotype B greenbug diet. The rearing results for biotype B were presented above, therefore, the present discussion concerns the results for biotype A with comparisons to biotype B.

The analysis of variable 1 (Fig. 42, lines A) indicated a highly significant difference (1% level) for the day means. These results indicated that the rate of survival was acceptable, even though there was a highly significant difference between the number of greenbugs alive at the beginning and the end of the experiment. To compare these results with the results of biotype B feeding on the standard biotype B greenbug diet, it was necessary to use the day by treatment means for variable 1 in the experiment involving five combinations of minor elements. These means are also shown in Fig. 42, lines A. Such a comparison is, at best, conjectural, but as the only comparison it suggested

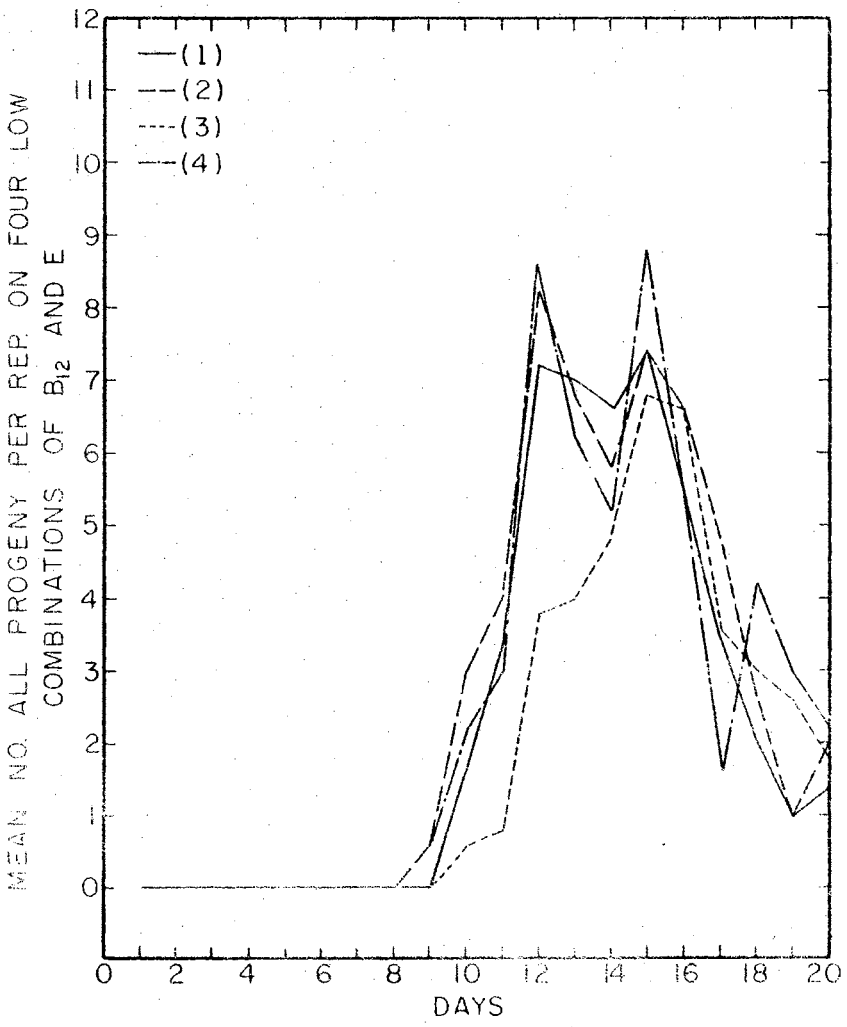

Fig. 41. Mean biotype B greenbug reproduction when reared on diets containing each of four low combinations of vitamins B_{12} and E.

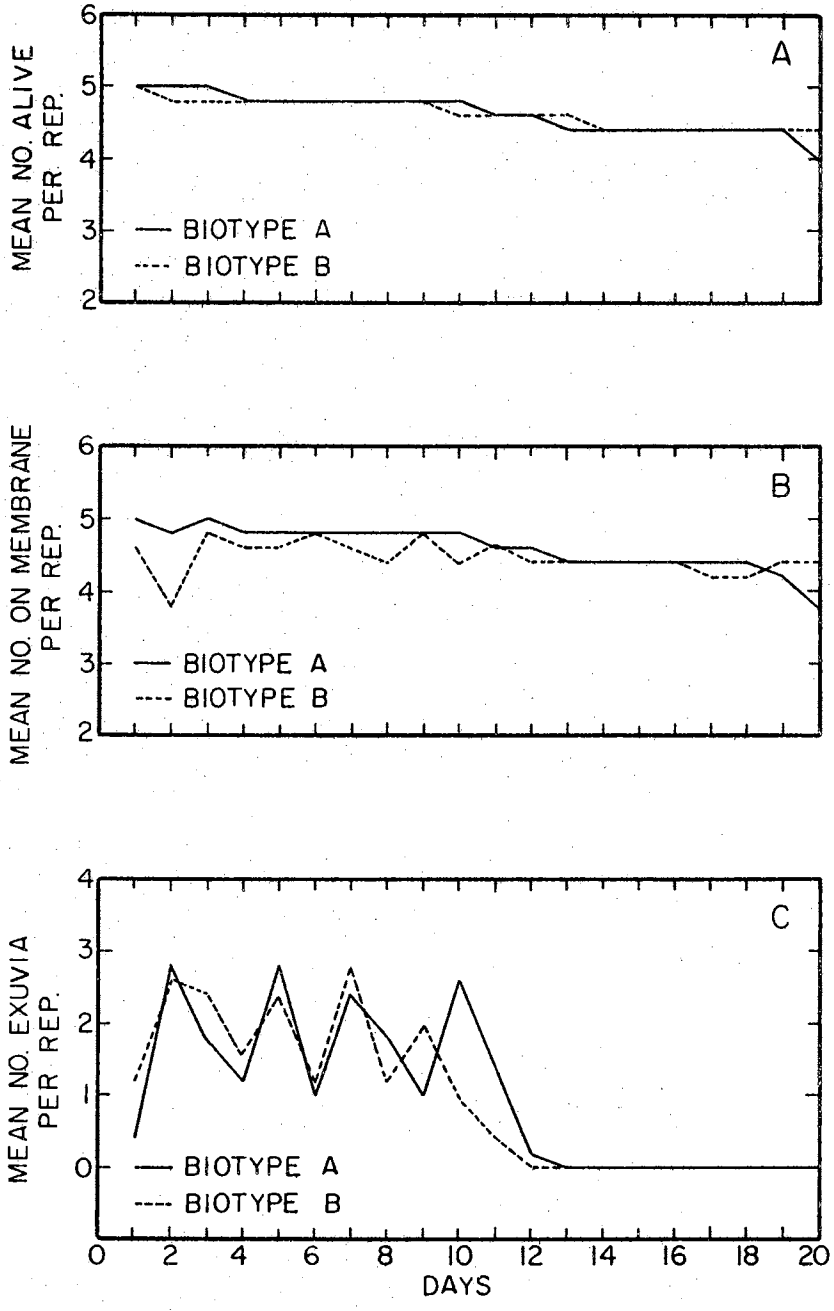

Fig. 42. Biotype A greenbug survival, growth and development when reared on the standard biotype B greenbug diet.

that there were only slight differences between the two biotypes concerning survival on the standard biotype B greenbug diet.

The analysis of variable 2 (Fig. 42, lines B) indicated a highly significant difference (1% level) for the day means. It was seen that the mean number of aphids on the membrane was very similar to the mean number alive. This was expected since, to remain alive over a period of several days, the aphids must remain on the membrane to feed. Also, since the rate of survival and number on the membrane were acceptable, it was suggested that the standard biotype B greenbug diet was palatable for biotype A greenbugs.

To compare these survival and feeding results to those for biotype B, it was necessary to make the same type of general comparison as in variable 1. The biotype B results may also be seen in Fig. 42, lines B. This comparison indicated only slight differences between biotype A and biotype B concerning the mean number on the membrane.

The analysis of variable 3 (Fig. 42, lines C) indicated no significant differences for the mean number of exuviae per day. However, as this was the only experiment with biotype A greenbugs, these means are shown in Fig. 42, lines C. It may be seen that there was considerable variation from day to day, but it was noted that all aphids had developed to the adult stage by the 12th day. By the same general type of comparison as above, it was noted that biotype B greenbugs (Fig. 42, lines C) had less variation from day to day and had all developed to the adult stage by the 11th day.

The analysis of variable 4 (Fig. 43, line A) showed a highly significant difference (1% level) for the day means. It was seen that reproduction started on the 10th day and the rate increased rapidly on

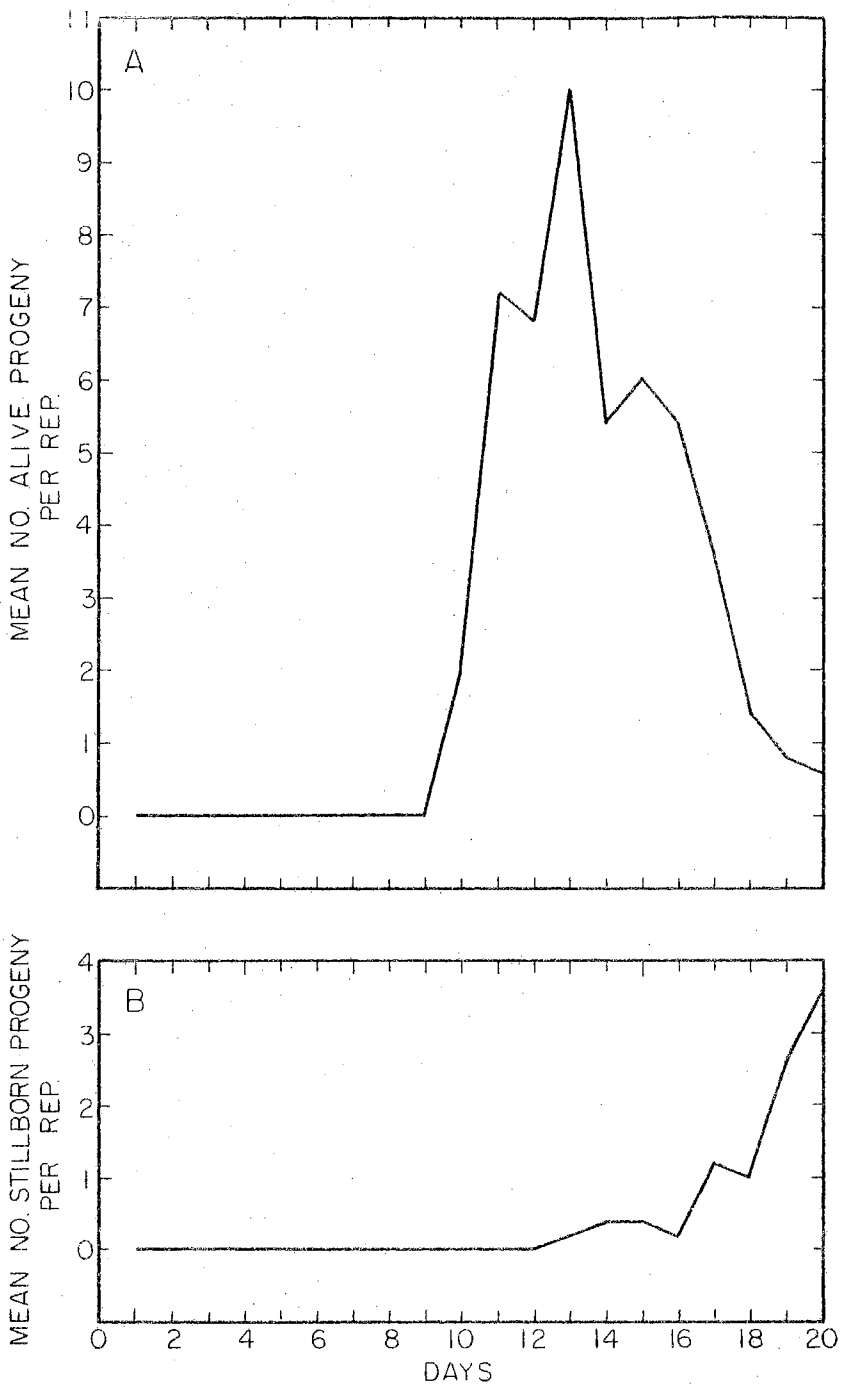

Fig. 43. Mean number progeny born to biotype A greenbugs reared on the standard biotype B greenbug diet. A - Live progeny. B - Stillborn progeny.

the 11th day. After a slight reduction on the 12th day, the maximum rate of 10.0 live progeny per replication was reached on the 13th day. As shown in Fig. 31, lines B, biotype B greenbugs began reproduction on the 8th day and reached the maximum rate of 10.0 live progeny per replication on the 11th and 12th days.

The analysis of variable 5 (Fig. 43, line B) indicated a highly significant difference (1% level) for the day means. It was seen that stillborn progeny production for biotype A was somewhat lower than for biotype B greenbugs (Fig. 32, lines B) for the first few days but ultimately there were more.

The analysis of variable 6 (Fig. 44) indicated a highly significant difference (1% level) for the day means. These means represent the mean number of both live and stillborn progeny per replicate. Thus, it was seen that the maximum of 10.3 progeny was reached on the 13th day. This compares to the maximum of 10.8 progeny on the 12th day for biotype B greenbugs reared on the standard biotype B greenbug diet (Fig. 33).

The weight gained by biotype A greenbugs is shown in Fig. 45. It was noted that the rate of weight gain was considerably reduced when compared to biotype B (Fig. 45), but the general shape of the curve was almost identical.

These results indicate that when biotype A greenbugs were reared on the standard biotype B greenbug diet, survival, diet palatability, and development were similar to those for biotype B. However, biotype A rate of reproduction, and especially the rate of weight gain and maximum weight gained, was somewhat reduced when compared to biotype B.

As a result of the studies on the development of a chemically

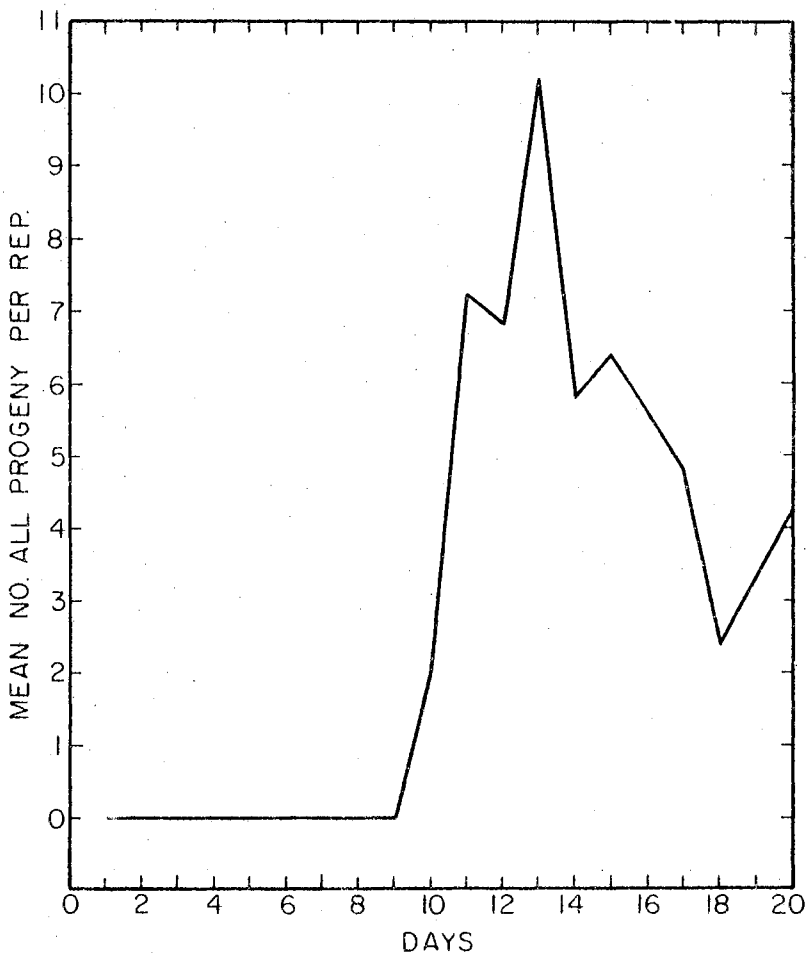

Fig. 44. Mean number all progeny born to biotype A greenbugs reared on the standard biotype B greenbug diet.

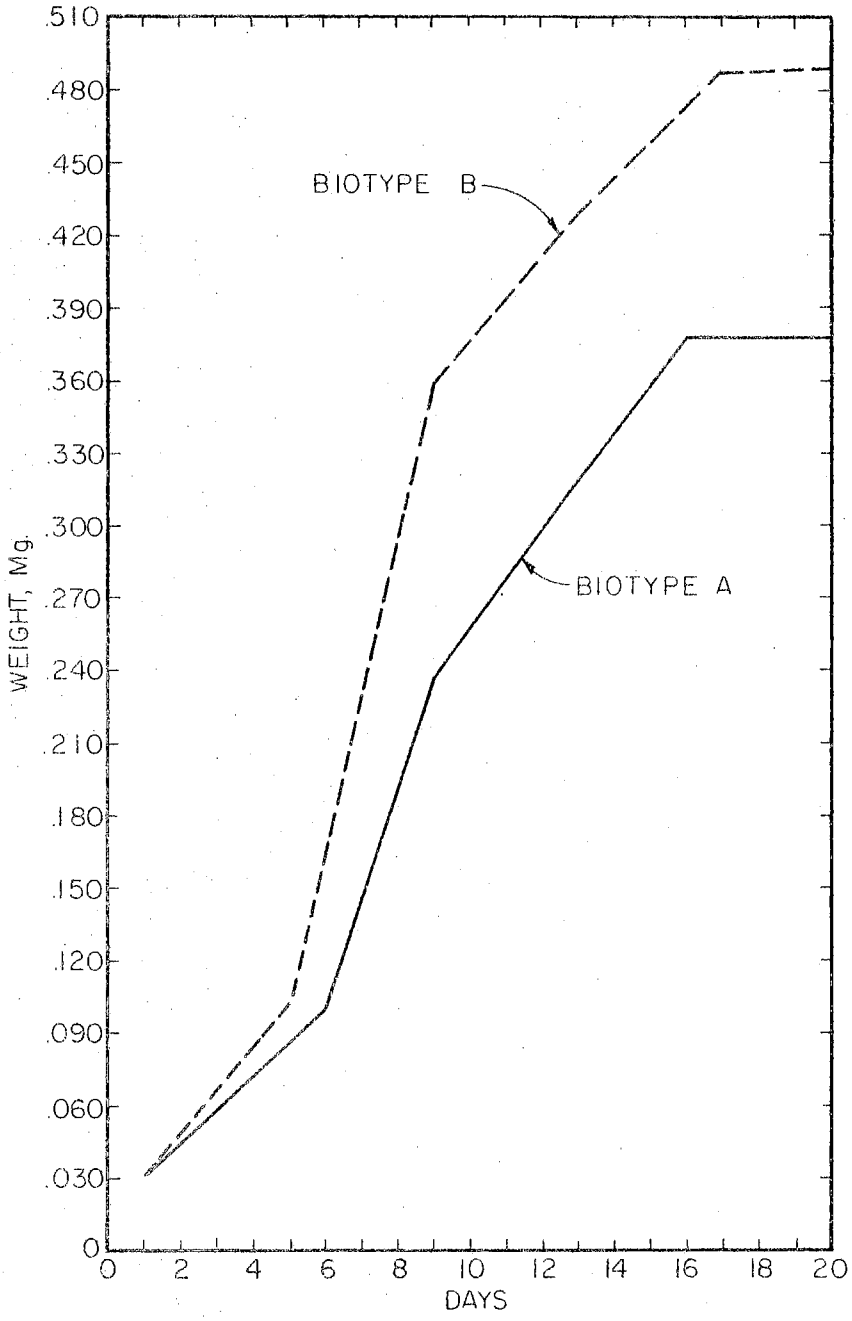

Fig. 45. Mean weight gained by biotype A and biotype B greenbugs reared on the standard biotype B greenbug diet.

defined diet for rearing greenbugs, the above data have shown that the biotype B greenbug diet was the most satisfactory in terms of greenbug development. Therefore, it was adopted for all subsequent greenbug rearing bioassay studies. This is now referred to as the "standard" biotype B greenbug diet (Appendix. Diet 19).

Plant Extracts Incorporated into the Standard Biotype B Greenbug Diet

The cooperating Departments of Botany and Plant Pathology and Biochemistry supplied various chemical compounds isolated from greenbug resistant and susceptible barley to be incorporated into the standard biotype B greenbug diet. These chemicals were tested in an effort to determine the factor or factors responsible for the observed plant resistance or susceptibility to the greenbug.

All bioassaying experiments were replicated five times with five biotype B greenbugs per replicate.

The Botany and Plant Pathology Department supplied 19 chemicals, which were used in two experiments. The chemicals were all purified but they were representative of chemicals isolated from resistant Will barley. These purified chemicals were incorporated into the diet at the same concentration they were found in the plants. The 19 chemicals, along with the concentration in 100 gm dry weight of Will barley and the amount incorporated into the diet are shown in Table 5.

The data were not statistically analyzed, as this was a preliminary testing process. The data for the first 10 chemicals and the check are shown as totals in Table 6. The comparable data for the second nine chemicals and the check are shown in Table 7.

In the first experiment, it was noted that for the variables 1, 2,

Table 5. The 19 purified chemicals and concentrations supplied by the Botany and Plant Pathology Department for bioassay in the standard biotype B greenbug diet.

Purified chemical	Conc./100 gm dry wt. Will barley (ug)	Amt. incorp. 100 ml diet (mg)
1. p-hydroxybenzoic acid	5175.0	5.1750
2. caffeic acid	6750.0	6.7500
3. sinapic acid	8400.0	8.4000
4. chlorogenic acid	13050.0	13.0500
5. syringic acid	7425.0	7.4250
6. salicylic acid	5175.0	5.1750
7. ferulic acid	7275.0	7.2750
8. protocatechuic acid	5775.0	5.7750
9. vanillic acid	6300.0	6.3000
10. gentisic acid (Na salt)	8625.0	8.6250
11. sulfanilic acid	6487.5	6.4875
12. pyrocatechol	4125.0	4.1250
13. phoroglucinol	6075.0	6.0750
14. vanillin	5700.0	5.7000
15. quercetin	11325.0	11.3250
16. syringealdehyde	6825.0	6.8250
17. scopoletin (p)	7275.0	7.2750
18. hydrocoumarin	5475.0	5.4750
19. farnesol (p)	8000.0	8.0000

Table 6. Summary of data on reactions of greenbugs feeding on diets containing 10 chemicals supplied by the Botany and Plant Pathology Department for bioassay in the standard biotype B greenbug diet.

	Variables					
	(1)	(2)	(3)	(4)	(5)	(6)
Compound[a]	No. alive	No. on membrane	No. exuviae	Live progeny	Stillborn progeny	Total progeny
1.	454	448	95	28	98	126
2.	211	188	41	1	8	9
3.	413	402	87	5	78	83
4.	426	409	87	0	37	37
5.	434	429	93	7	41	48
6.	422	413	87	16	73	89
7.	448	424	90	19	101	120
8.	433	421	89	1	44	45
9.	463	459	95	5	96	101
10.	396	358	84	1	28	29
Check	431	417	90	3	65	68

[a]Compound names may be seen in Table 5.

Table 7. Summary of data on reactions of greenbugs feeding on diets containing nine chemicals supplied by the Botany and Plant Pathology Department for bioassay in the standard biotype B greenbug diet.

	Variables					
	(1)	(2)	(3)	(4)	(5)	(6)
Compound[a]	No. alive	No. on membrane	No. exuviae	Live progeny	Stillborn progeny	Total progeny
11.	401	384	93	28	82	110
12.	331	311	78	0	3	3
13.	451	441	93	2	71	73
14.	437	432	85	43	132	175
15	402	388	84	0	26	26
16.	427	426	90	20	143	163
17.	385	370	86	9	49	58
18.	406	383	86	6	84	90
19.	433	421	91	21	99	120
Check	449	443	95	17	156	173

[a]Compound names may be seen in Table 5.

and 3, there was little difference, except for caffeic acid (2) and gentisic acid (10). These two chemicals appeared to be feeding repellents. This would account for the increased mortality, reduced number on the membrane, and reduced number of exuviae. For variables 4, 5, and 6, it was noted that aphids feeding on diets containing p-hydroxybenzoic acid (1), ferulic acid (7), and vanillic acid (9) had considerably increased rates of reproduction compared to aphids feeding on all other chemicals and the check. It was equally interesting to note that aphids feeding on diets containing chlorogenic acid (4) had no live progeny, and their rate of reproduction was greatly reduced compared to the check. The apparent repellency of caffeic acid (2) and gentisic acid (10) would also account for the reduced rate of reproduction by aphids feeding on these diets.

The weight of greenbugs reared on diets containing these various chemicals, in general, reflected the results of the six variables, i. e., where aphid survival, growth, and reproduction were high, aphid weights were high; the contrary was also observed.

In the second experiment, the variables 1, 2, and 3, in general, showed little difference, except for aphids feeding on pyrocatechol (12) and scopoletin (p) (17). These two compounds appeared to be repellent when compared to aphids feeding on diets containing the other chemicals and to the check. For variables 4, 5, and 6, it was noted that aphids feeding on diets containing the various chemicals all had a reduced rate of reproduction, save for vanillin (14), when compared to the check. Aphids feeding on diets containing vanillin had over 2.5 times as many live progeny as aphids feeding on the check. It may be noted that aphids feeding on diets containing pyrocatechol (12) and

quercetin (15) had no live progeny. The aphid weights were reduced on all chemicals when compared to the check. It was also noted that aphids feeding on diets containing pyrocatechol (12) and quercetin (15) weighed the least and, moreover, it was these aphids that had no live progeny. At the present time no explanation for these differences can be given. Further investigation may reveal some of the chemical factors involved in the resistance or susceptibility of these plants to greenbugs.

The Biochemistry Department supplied material for two experiments. The first experiment involved gross extracts under the headings: volatile compounds, sugars, indole compounds, total extract, and amino acids. These extracts were from both Will and Rogers barleys and were incorporated into the standard biotype B greenbug diet. The second experiment was based on the results of the first and involved selected purified chemicals representative of those found in the Rogers barley volatile and indole compound extracts. These were also incorporated into the standard biotype B greenbug diet.

The gross extracts used in the first experiment are shown in Table 8 along with the data on the results. These compounds were all incorporated into the diet at approximately the equivalent concentrations found in the plants. In addition, the volatiles were also used at approximately 50% of the concentration found in the plants.

The most outstanding observation was the very early mortality of all greenbugs feeding on diets containing the indole compounds. Complete greenbug mortality was observed at two and three days when fed on these compounds from Will and Rogers barleys, respectively.

Concerning the volatile compounds, it was noted that aphids

Table 8. Gross extracts supplied by the Biochemistry Department and summary of the data obtained in their bioassay in the standard biotype B greenbug diet.

	Variables					
	(1)	(2)	(3)	(4)	(5)	(6)
Gross extracts	No. alive	No. on membrane	No. exuviae	Live progeny	Stillborn progeny	Total progeny
Volatile Will 100%	422	410	86	80	97	177
Volatile Rogers 100%	485	480	97	129	170	299
Volatile Will 50%	470	464	96	112	149	261
Volatile Rogers 50%	408	387	83	66	58	124
Sugar Will	424	405	95	90	88	178
Sugar Rogers	448	399	94	84	41	125
Indole compounds Will	43	23	-	-	-	-
Indole compounds Rogers	45	21	-	-	-	-
Total extract Will	372	350	84	23	83	106
Total extract Rogers	445	432	92	44	88	132
Amino acids Will	438	336	96	2	22	24
Amino acids Rogers	406	337	55	13	14	27
Check	407	376	84	15	39	54

feeding on diets containing the Rogers 100% and Will 50% performed better in all six variables than did aphids feeding on Will 100% or Rogers 50%. Moreover, out of all these extracts being tested, aphid performance appeared best on diets containing Rogers 100% volatiles.

Aphid development appeared better on diets containing Will sugar than on diets containing Rogers sugar, but the reverse appeared in the cases of total extract and amino acids. It was noted that there were only about half as many total progeny on both Will and Rogers amino acid extracts as on the check. However, aphids feeding on diets containing the volatile Rogers 100% had a total progeny about six times that of the check.

The aphid weights reflected, in general, the trends as were pointed out in the discussion of the six variables.

The second experiment involved purified chemicals representative of those in the Rogers barley extracts. There were four volatiles and six indole compounds chosen. The volatiles were: (1) ethyl formate, (2) ethyl acetate, (3) ethanol, and (4) n-butanol. The indole compounds were: (5) 3-indole acetonitrile (IAN), (6) 3-indole aldehyde (I_3H), (7) 3-indole butyric acid (IBA), (8) 3-indole proprionic acid (IPA), (9) 3-indole acetic acid (IAA), and (10) 3-indole pyruvic acid (IpyA). These compounds, along with the data, are shown in Table 9.

It was interesting to note that, of the volatiles, ethyl formate showed the best aphid development and yet, in terms of total progeny, it had only about one third as many as the two best indole compounds, i. e., IPA and IAA. Also, aphids feeding on diets containing IPA and IAA had over twice the number of progeny as aphids feeding on the check.

Table 9. The purified chemicals supplied by the Biochemistry Department and summary of the data obtained in their bioassay in the standard biotype B greenbug diet.

Purified chemical	Variables (1) No. alive	(2) No. on membrane	(3) No. exuviae	(4) Live progeny	(5) Stillborn progeny	(6) Total progeny
Volatiles						
1. ethyl formate	427	399	96	8	33	41
2. ethyl acetate	389	361	89	17	20	37
3. ethanol	393	377	96	34	37	71
4. n-butanol	382	359	88	27	42	69
Indole compounds						
5. 3-indole acetonitrile (IAN)	436	412	94	30	63	93
6. 3-indole aldehyde (I_3H)	358	342	93	25	46	71
7. 3-indole butyric acid (IBA)	422	404	100	34	43	77
8. 3-indole proprionic acid (IPA)	444	425	96	41	84	125
9. 3-indole acetic acid (IAA)	419	401	96	71	40	111
10. 3-indole pyruvic acid (IpyA)	364	342	88	26	31	57
Check	387	359	93	23	27	50

The aphids' weights, in general, reflected the general trends of aphid development seen in the table.

Because chemical isolates from greenbug resistant Will and susceptible Rogers barleys were not supplied by the Botany and Plant Pathology and the Biochemistry Departments until a date late in the conduct of these experiments, the above bioassay studies were more or less preliminary in nature. However, on the basis of the limited studies, definite indications of differences in reaction of greenbugs to some of the chemical isolates incorporated in the chemically defined greenbug diets would indicate the possibility that they may be factors involved in greenbug resistance or susceptibility of barley plants. Further investigations in this regard beyond the scope of the present studies are warranted.

SUMMARY AND CONCLUSIONS

Greenbugs were reared on susceptible Rogers and resistant Will barley to determine the average duration of the prereproductive, reproductive, postreproductive periods, and life span. Also, the average number of progeny per female, average number of progeny per female per day, and average rate of weight gain were determined.

Greenbugs displayed definite preference among various diets. Based on the results obtained from preference diet tests with the pH concentration ranging from 5.6 through 8.6 at one-unit intervals, it was concluded that the optimum for the greenbug is pH 7.6. In similar preference tests with sucrose concentrations ranging from 10 to 45% at 5% intervals, it was concluded that the greenbug prefers a sucrose range of 30 to 40%, with 35% being the optimum.

Three colors of Kodak Wratten filters, yellow 15G, orange 22, and green 11 X1, mounted in one-half-gallon ice cream cartons were tested. Greenbugs feeding on diets lighted green developed better than those feeding under orange or yellow light.

Greenbug development on the pea aphid diet was not as good as it was on susceptible Rogers barley, but it was similar to that on resistant Will barley.

Greenbugs reared on a synthetic diet containing 0.1 the vitamin concentrations of the pea aphid diet had a slightly higher rate of mortality, but the rates of growth, development, and reproduction were much greater than greenbugs reared on the pea aphid diet. It was

concluded that the diet containing 0.1 the vitamin concentration of the pea aphid diet was more satisfactory for the greenbug.

The chemically defined greenbug diets were improved by the addition of very small amounts of zinc, iron, and manganese in the form of EDTA sodium sequestrenes. When these elements were added to diets individually, no beneficial effects were observed. When all 3 elements in combinations at varying levels were incorporated into the diet, greenbug growth and development rates increased and were comparable to that observed for Rogers barley.

It was suggested that additions of various vitamins would improve greenbug growth and development. The incorporation of vitamins B_{12} and E had no apparent beneficial effect.

When greenbug biotypes A and B were reared on the standard biotype B diet, both fed and reproduced, but the rates of reproduction and weight gain for biotype A were reduced. This indicates that, although there are no morphological differences, the nutritional requirements of the two greenbug biotypes are different. Since greenbug biotype B has replaced biotype A in the small grain infested areas, the diet used in all tests was that developed for greenbug biotype B.

The cooperating Departments of Botany and Plant Pathology and Biochemistry supplied a total of 39 isolates from Rogers and Will barleys which were bioassayed in the standard biotype B greenbug diet. Some of the compounds were incorporated into the diet as purified chemicals, while others were incorporated as gross extracts. These bioassays were of a preliminary screening nature and showed only wide differences in greenbug responses. The differences ranged from complete mortality and greatly reduced rates of growth, development and reproduction to

increased rates of survival, growth, development, and reproduction. The results indicated a definite need for future investigations in this area in order to determine factors associated with greenbug resistance or susceptibility.

LITERATURE CITED

Apablaza, J. U., and A. G. Robinson. 1967a. 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.

Apablaza, J. U., and A. G. Robinson. 1967b. Effects of three species of aphids on barley, wheat, or oats at various stages of plant growth. Can. J. plant Sci. 47:367-373.

Auclair, J. L. 1958. Honeydew excretion in the pea aphid, Acyrthosiphon pisum (Harr.). J. Insect Physiol. 2:330-337.

Auclair, J. L. 1959. Feeding and excretion by the pea aphid, Acyrthosiphon pisum (Harr.), reared on different varieties of peas. Entomol. Exp. Appl. 2:279-286.

Auclair, J. L. 1964. Recent advances in the feeding and nutrition of aphids. Can. Entomol. 96:241-250.

Auclair, J. L. 1965. Feeding and nutrition of the pea aphid, Acyrthosiphon pisum (Homoptera:Aphididae), on chemically defined diets of various pH and nutrient levels. Ann. Entomol. Soc. Amer. 58: 855-875.

Auclair, J. L. 1967a. Effects of pH and sucrose on rearing the cotton aphid, Aphis gossypii, on a germ free and holidic diet. J. Insect Physiol. 13:431-446.

Auclair, J. L. 1967b. Effects of light and sugar on rearing the cotton aphid, Aphis gossypii, on a germ free and holidic diet. J. Insect Physiol. 13:1247-1268.

Auclair, J. L., and J. J. Cartier. 1963. Pea aphid: rearing on a chemically defined diet. Science. 142:1068-1069.

Auclair, J. L., and J. R. Raulston. 1966. Feeding of Lygus hesperus (Hemiptera:Miridae) on a chemically defined diet. Ann. Entomol. Soc. Amer. 59:1016-1017.

Auclair, J. L., J. B. Maltais and J. J. Cartier. 1957. Factors in resistance of peas to the pea aphid, Acyrthosiphon pisum (Harr.). II. Amino acids. Can. Entomol. 89:457-464.

Banks, C. J., and E. D. M. Macauley. 1965. The ingestion of nitrogen and solid matter from Vicia faba by Aphis fabae Scop. Ann. Appl. Biol. 55:207-218.

Belvett, V. B., Y. R. Sun and A. G. Robinson. 1965. Observations on laboratory rearing of grain aphids (Homoptera:Aphididae). Can. J. Zool. 43:619-622.

Bradley, R. H. E. 1956. Effects of the depth of stylet penetration on aphid transmission of potato virus Y. Can. J. Microbiol. 2:539-547.

Chada, H. L., I. M. Atkins, J. H. Gardenhire, and D. E. Weibel. 1961. Greenbug resistance studies with small grains. Tex. Agri. Exp. Sta. Bull. 982:1-18.

Cartier, J. J., and J. L. Auclair. 1964. Pea aphid behavior: Colour preference on a chemical diet. Can. Entomol. 96:1240-1243.

Chatters, R. M., and A. M. Schlehuber. 1951. Mechanics of feeding of the greenbug (Toxoptera graminum (Rond.)) on Hordeum, Avena, and Triticum. Okla. Agr. Exp. Sta. Bull. T-40. 18 pp.

Dadd, R. H. 1961. The nutritional requirements of locusts. IV. Requirements for vitamins of the B-complex. J. Insect Physiol. 6:1-12.

Dadd, R. H. 1967. Improvement of synthetic diet for the aphid, Myzus persicae, using plant juices, nucleic acids, or trace metals. J. Insect Physiol. 13:763-778.

Dadd, R. H., and D. L. Krieger. 1968. Dietary amino acid requirements of the aphid, Myzus persicae. J. Insect Physiol. 14:741-764.

Dadd, R. H., and T. E. Mittler. 1966. Permanent culture of an aphid on a totally synthetic diet. Experientia. 22:832-833.

Dadd, R. H., D. L. Krieger and T. E. Mittler. 1967. Studies on the artificial feeding of the aphid, Myzus persicae (Sulzer). IV. Requirements for water-soluble vitamins and ascorbic acid. J. Insect Physiol. 13:249-272.

Daniels, N. E., H. L. Chada, D. Ashdown and E. A. Cleveland. 1956. Greenbugs and some other pests of small grains. Tex. Agr. Exp. Sta. Bull. 845. 14 pp.

Davidson, J. 1923. Biological studies of Aphis rumicis L. The penetration of plant tissues and the source of food supply of aphids. Ann. Appl. Biol. 10:35-54.

Day, M. F., and H. Irzykiewicz. 1953. Feeding behavior of the aphids, Myzus persicae and Brevicoryne brassicae, studied with radio-phosphorus. Austral. J. Biol. Sci. 6:98-108.

Ehrhardt, P. 1968a. Die Wirkung verschiedener Spurenelemente auf Wachstum, Reproduktion und Symbioten von Neomyzus circumflexus Buckt. (Aphidae, Homoptera, Insecta). Z. vergl. Physiol. 58:47-75. (Abstract only).

Ehrhardt, P. 1968b. Nachweis einer durch symbiontische Mikroorganismen bewirkten Sterinsynthese in künstlich ernäthrten Aphiden (Homoptera, Rhynchota, Insecta). Experientia. 24:82-83.

Ewart, W. H., and R. L. Metcalf. 1956. Preliminary studies of sugars and amino acids in the honeydews of five species of coccids feeding on citrus in California. Ann. Entomol. Soc. Amer. 49:441-447.

Fraenkel, G., and M. Blewett. 1942. Biotin, B_1, riboflavin, nicotinic acid, B_6, and pantothenic acid as growth factors for insects. Nature. 150:177-178.

Fraenkel, G., and M. Blewett. 1943a. Vitamins of the B-group required by insects. Nature. 151:703-704.

Fraenkel, G., and M. Blewett. 1943b. The sterol requirements of several insects. Biochem. J. 37:692-695.

Fraenkel, G., and M. Blewett. 1947. The importance of folic acid and unidentified members of the vitamin B-complex in the nutrition of certain insects. Biochem. J. 41:469-475.

Fraenkel, G., M. Blewett and M. Coles. 1948. B_T, a new vitamin of the B-group and its relation to the folic acid group and other anti-anaemia factors. Nature. 161:981-983.

Friend, W. G., and R. L. Patton. 1956. Studies on vitamin requirements of larvae of the onion maggot, Hylemya antiqua (Mg.), under aseptic conditions. Can. J. Zool. 34:152-162.

Gray, H. E., and G. Fraenkel. 1954. The carbohydrate components of honeydew. Physiol. Zool. 27:56-65.

Hamilton, M. A. 1930. Notes on the culturing of insects for virus work. Ann. Appl. Biol. 17:487-492.

Hamilton, M. A. 1935. Further experiments on the artificial feeding of Myzus persicae (Sulz.). Ann. Appl. Biol. 22:243-258.

Hinton, T. 1959. Miscellaneous nutritional variations, environmental and genetic, in Drosophila. Ann. N. Y. Acad. Sci. 77:366-372.

Hinton, T., D. T. Noyes and J. Ellis. 1951. Amino acids and growth factors in a chemically defined diet for Drosophila. Physiol. Zool. 24:335-353.

House, H. L. 1954. Nutritional studies with Pseudosarcophaga affinis (Fall.), a dipterous parasite of the spruce budworm, Choristoneura fumiferana (Clem.). II. Effects of eleven vitamins on growth. Can. J. Zool. 32:342-350.

Johnson, B., and P. R. Birks. 1960. Studies on wing polymorphism in aphids the developmental process involved in the production of the different forms. Entomol. Exp. Appl. 3:327-339.

Lowe, H. J. B. 1967. Interspecific differences in the biology of aphids (Homoptera:Aphididae) on leaves on Vicia faba. I. Feeding behaviour. Entomol. Exp. Appl. 10:347-357.

Maltais, J. B. 1952. A simple apparatus for feeding aphids aseptically on chemically defined diets. Can. Entomol. 84:291-294.

Maltais, J. B., and J. L. Auclair. 1952. Occurrence of amino acids in the honeydew of the crescent-marked lily aphid, Myzus circumflexus (Buck.). Can. J. Zool. 30:191-193.

Markkula, M., and E. Pulliainen. 1965. The effect of temperature on the lengths of the life periods of the English grain aphid, Macrosiphum avenae (F.) (Homoptera:Aphididae) and the number and colour of its progeny. Ann. Ent. Fenn. 31:39-45.

Maxwell, F. G., and R. H. Painter. 1959. Factors affecting rate of honeydew deposition by Therioaphis maculata (Buck.) and Toxoptera graminum (Rond.). J. Econ. Entomol. 52:368-373.

Miles, P. W. 1958. Contact chemoreception in some Heteroptera, including chemoreception internal to the stylet food canal. J. Insect Physiol. 2:338-347.

Miles, P. W. 1959. Secretion of two types of saliva by an aphid. Nature. 183:756.

Mittler, T. E. 1953. Amino acids in phloem sap and their excretion by aphids. Nature. 172:207.

Mittler, T. E. 1957. Studies on the feeding and nutrition of Tuberolachnus salignus (Gmelin). I. The uptake of phloem sap. J. Exp. Biol. 34:334-341.

Mittler, T. E. 1958. Studies on the feeding and nutrition of Tuberlachnus salignus (Gmelin). II. The nitrogen and sugar composition of ingested phloem sap and excreted honeydew. J. Exp. Biol. 35:74-84.

Mittler, T. E. 1967a. Effect of amino acid and sugar concentrations on the food uptake of the aphid, Myzus persicae. Entomol. Exp. Appl. 10:39-51.

Mittler, T. E. 1967b. Effect on aphid feeding of dietary methionine. Nature. 214:386.

Mittler, T. E., and R. H. Dadd. 1962. Artificial feeding and rearing of the aphid, Myzus persicae (Sulzer), on a completely defined synthetic diet. Nature. 195:404.

Mittler, T. E., and R. H. Dadd. 1963a. Studies on the artificial feeding of the aphid, Myzus persicae (Sulzer). I. Relative uptake of water and sucrose solutions. J. Insect Physiol. 9:623-645.

Mittler, T. E., and R. H. Dadd. 1963b. Studies on the artificial feeding of the aphid, Myzus persicae (Sulzer). II. Relative survival, development, and larviposition on different diets. J. Insect Physiol. 9:741-757.

Mittler, T. E., and R. H. Dadd. 1964a. An improved method for feeding aphids on artificial diets. Ann. Entomol. Soc. Amer. 57:139-140.

Mittler, T. E., and R. H. Dadd. 1964b. Gustatory discrimination between liquids by the aphid, Myzus persicae (Sulzer). Entomol. Exp. Appl. 7:315-328.

Mittler, T. E., and R. H. Dadd. 1965. Differences in the probing responses of Myzus persicae (Sulzer) elicited by different feeding solutions behind a parafilm membrane. Entomol. Exp. Appl. 8:107-122.

Mittler, T. E., and R. H. Dadd. 1966. Food and wing determination in Myzus persicae (Homoptera:Aphidae). Ann. Entomol. Soc. Amer. 59:1162-1166.

McLean, D. L., and M. G. Kinsey. 1965. Identification of electrically recorded curve patterns associated with salivation and ingestion. Nature. 205:1130-1131.

Noland, J. E., J. H. Lilly and C. A. Baumann. 1949. Vitamin requirements of the cockroach, Blattella germanica (L.). Ann. Entomol. Soc. Amer. 42:154-164.

Pant, N. C., and G. Fraenkel. 1950. The function of the symbiotic yeasts of two insect species, Lasioderma serricorne F. and Stegobium (Sitodrepa) paniceum L. Science. 112:498-500.

Peel, A. J., and P. E. Weatherley. 1959. Composition of sieve-tube sap. Nature. 184:1955-1956.

Pierre, L. L. 1962. Synthesis of ascorbic acid by the normal fat body of the cockroach, Leucophaea maderae (F.) and its symbionts. Nature. 193:904-905.

Pletsch, D. J. 1937. Improved device for artificial feeding of aphids. J. Econ. Entomol. 30:211-212.

Retankaran, A., and S. D. Beck. 1967. Aspects of mineral nutrition of the pea aphid, Acyrthosiphon pisum (Harris). J. Nutrition. 92:43-52.

Singh, S. R., and E. A. Wood. 1963. Effect of temperature on fecundity of two strains of the greenbug. J. Econ. Entomol. 56:109-110.

Strong, F. E., and S. S. Sakamoto. 1963. Some amino acid requirements of the green peach aphid, Myzus persicae (Sulzer), determined with glucose-U-C^{14}. J. Insect Physiol. 9:875-879.

Subbarow, Y., and W. Trager. 1940. The chemical nature of growth factors required by mosquito larvae. II. Pantothenic acid and vitamin B_6. J. Gen. Physiol. 23:561-568.

Swingle, M. C. 1931. Hydrogen ion concentration within the digestive tract of certain insects. Ann. Entomol. Soc. Amer. 24:489-495.

Thorsteinson, A. J. 1960. Host selection in phytophagous insects. Ann. Rev. Entomol. 5:193-218.

Trager, W. 1948. Biotin and fat-soluble materials with biotin activity in the nutrition of mosquito larvae. J. Biol. Chem. 176:1211-1223.

Vanderzant, E. S. 1959. Inositol: an indispensable dietary requirement for the boll weevil. J. Econ. Entomol. 52:1018-1019.

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

Wolf, J. P. III, and W. H. Ewart. 1955. Carbohydrate composition of the honeydew of Coccus hesperidum L.: Evidence for existence of two oligosaccharides. Arch. Biochem. Biophys. 58:365-372.

Yust, H. R., and R. A. Fulton. 1943. The exudation associated with the feeding location of the California red scale. J. Econ. Entomol. 36:346-347.

Zimmermann, M. H. 1957. Translocation of organic substances in trees. I. The nature of the sugars in the sieve-tube exudate of trees. Plant Physiol. 32:288-291.

APPENDIX

On the following pages in this Appendix, Diet number 1 is the 1965 Auclair pea aphid Diet (Auclair, 1965). Other Diets are modifications of this Diet. The ditto marks indicate the same amounts of chemicals as are indicated in the column to the left. Where figures are shown in the column, modifications have been made. For example, in Diet number 2, the modification was a reduction in the vitamin amounts. In Diet number 3, 0.71 mg of zinc was added. Diet number 19 is the Standard Biotype B Greenbug Diet.

	Diet Number			
	1	2	3	4
L-Amino acids and amides (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	"	"	"
Histidine	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	"	"	"
Vitamins (mg)				
Ascorbic acid	10.0	1.00	"	"
Biotin	0.1	0.01	"	"
Calcium pantothenate	5.0	0.50	"	"
Choline chloride	50.0	5.00	"	"
Folic acid	1.0	0.10	"	"
i-Inositol (meso)	50.0	5.00	"	"
Nicotinic acid	10.0	1.00	"	"
p-Aminobenzoic acid	10.0	1.00	"	"
Pyridoxine HCl	2.5	0.25	"	"
Riboflavin	5.0	0.50	"	"
Thiamine HCl	2.5	0.25	"	"
Cyancobalamin				
DL-alpha tocopherol				
Minor elements (mg)				
Zinc (EDTA)			0.71	1.42
Iron (EDTA)				
Manganese (EDTA)				
Others				
Cholesterol benzoate (mg)	2.5	"	"	"
K_3PO_4 (mg)	500.0	"	"	"
$MgCl_2 \cdot 6H_2O$ (mg)	200.0	"	"	"
Salt mixture no. 2 (mg)	5.0	"	"	"
Sucrose (g)	35.0	"	"	"
Water to make (ml)	100.0	"	"	"

	Diet Number			
	5	6	7	8
L-Amino acids and amides (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	"	"	"
Histidine	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	"	"	"
Vitamins (mg)				
Ascorbic acid	1.00	"	"	"
Biotin	0.01	"	"	"
Calcium pantothenate	0.50	"	"	"
Choline chloride	5.00	"	"	"
Folic acid	0.10	"	"	"
i-Inositol (meso)	5.00	"	"	"
Nicotinic acid	1.00	"	"	"
p-Aminobenzoic acid	1.00	"	"	"
Pyridoxine HCl	0.25	"	"	"
Riboflavin	0.50	"	"	"
Thiamine HCl	0.25	"	"	"
Cyancobalamin				
DL-alpha tocopherol				
Minor elements (mg)				
Zinc (EDTA)	2.13	2.84		
Iron (EDTA			1.75	2.25
Manganese (EDTA)				
Others				
Cholesterol benzoate (mg)	2.5	"	"	"
K_3PO_4 (mg)	500.0	"	"	"
$MgCl_2 \cdot 6H_2O$ (mg)	200.0	"	"	"
Salt mixture no. 2 (mg)	5.0	"	"	"
Sucrose (g)	35.0	"	"	"
Water to make (ml)	100.0	"	"	"

	Diet Number			
	9	10	11	12
L-Amino acids and amides (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	"	"	"
Histidine	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	"	"	"
Vitamins (mg)				
Ascorbic acid	1.00	"	"	"
Biotin	0.01	"	"	"
Calcium pantothenate	0.50	"	"	"
Choline chloride	5.00	"	"	"
Folic acid	0.10	"	"	"
i-Inositol (meso)	5.00	"	"	"
Nicotinic acid	1.00	"	"	"
p-Aminobenzoic acid	1.00	"	"	"
Pyridoxine HCl	0.25	"	"	"
Riboflavin	0.50	"	"	"
Thiamine HCl	0.25	"	"	"
Cyancobalamin				
DL-alpha tocopherol				
Minor elements (mg)				
Zinc (EDTA)				
Iron (EDTA)	2.75	3.25		
Manganese (EDTA)			0.60	1.20
Others				
Cholesterol benzoate (mg)	2.5	"	"	"
K_3PO_4 (mg)	500.0	"	"	"
$MgCl_2 \cdot 6H_2O$ (mg)	200.0	"	"	"
Salt mixture no. 2 (mg)	5.0	"	"	"
Sucrose (g)	35.0	"	"	"
Water to make (ml)	100.0	"	"	"

	Diet Number			
	13	14	15	16
L-Amino acids and amides (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	"	"	"
Histidine	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	"	"	"
Vitamins (mg)				
Ascorbic acid	1.00	"	"	"
Biotin	0.01	"	"	"
Calcium pantothenate	0.50	"	"	"
Choline chloride	5.00	"	"	"
Folic acid	0.10	"	"	"
i-Inositol (meso)	5.00	"	"	"
Nicotinic acid	1.00	"	"	"
p-Aminobenzoic acid	1.00	"	"	"
Pyridoxine HCl	0.25	"	"	"
Riboflavin	0.50	"	"	"
Thiamine HCl	0.25	"	"	"
Cyancobalamin				
DL-alpha tocopherol				
Minor elements (mg)				
Zinc (EDTA)			0.71	1.42
Iron (EDTA)			1.75	2.25
Manganese (EDTA)	1.80	2.40	0.60	1.20
Others				
Cholesterol benzoate (mg)	2.5	"	"	"
K_3PO_4 (mg)	500.0	"	"	"
$MgCl_2 \cdot 6H_2O$ (mg)	200.0	"	"	"
Salt mixture no. 2 (mg)	5.0	"	"	"
Sucrose (g)	35.0	"	"	"
Water to make (ml)	100.0	"	"	"

	Diet Number			
	17	18	19	20
L-Amino acids and amides (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	"	"	"
Histidine	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	"	"	"
Vitamins (mg)				
Ascorbic acid	1.00	"	"	"
Biotin	0.01	"	"	"
Calcium pantothenate	0.50	"	"	"
Choline chloride	5.00	"	"	"
Folic acid	0.10	"	"	"
i-Inositol (meso)	5.00	"	"	"
Nicotinic acid	1.00	"	"	"
p-Aminobenzoic acid	1.00	"	"	"
Pyridoxine HCl	0.25	"	"	"
Riboflavin	0.50	"	"	"
Thiamine HCl	0.25	"	"	"
Cyancobalamin				0.50
DL-alpha tocopherol				0.50
Minor elements (mg)				
Zinc (EDTA)	0.71	2.13	2.84	"
Iron (EDTA)	2.25	2.75	3.25	"
Manganese (EDTA)	2.40	1.80	2.40	"
Others				
Cholesterol benzoate (mg)	2.5	"	"	"
K_3PO_4 (mg)	500.0	"	"	"
$MgCl_2 \cdot 6H_2O$ (mg)	200.0	"	"	"
Salt mixture no. 2 (mg)	5.0	"	"	"
Sucrose (g)	35.0	"	"	"
Water to make (ml)	100.0	"	"	"

	Diet Number			
	21	22	23	24
L-Amino acids and amides (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	"	"	"
Histidine	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	"	"	"
Vitamins (mg)				
Ascorbic acid	1.00	"	"	"
Biotin	0.01	"	"	"
Calcium pantothenate	0.50	"	"	"
Choline chloride	5.00	"	"	"
Folic acid	0.10	"	"	"
i-Inositol (meso)	5.00	"	"	"
Nicotinic acid	1.00	"	"	"
p-Aminobenzoic acid	1.00	"	"	"
Pyridoxine HCl	0.25	"	"	"
Riboflavin	0.50	"	"	"
Thiamine HCl	0.25	"	"	"
Cyancobalamin	1.00	1.50	2.00	0.05
DL-alpha tocopherol	1.50	2.50	3.50	0.05
Minor elements (mg)				
Zinc (EDTA)	2.84	"	"	"
Iron (EDTA)	3.25	"	"	"
Manganese (EDTA)	2.40	"	"	"
Others				
Cholesterol benzoate (mg)	2.5	"	"	"
K_3PO_4 (mg)	500.0	"	"	"
$MgCl_2 \cdot 6H_2O$ (mg)	200.0	"	"	"
Salt mixture no. 2 (mg)	5.0	"	"	"
Sucrose (g)	35.0	"	"	"
Water to make (ml)	100.0	"	"	"

	Diet Number		
	25	26	27
L-Amino acids and amides (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	"	"
Histidine	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	"	"
Vitamins (mg)			
Ascorbic acid	1.00	"	"
Biotin	0.01	"	"
Calcium pantothenate	0.50	"	"
Choline chloride	5.00	"	"
Folic acid	0.10	"	"
i-Inositol (meso)	5.00	"	"
Nicotinic acid	1.00	"	"
p-Aminobenzoic acid	1.00	"	"
Pyridoxine HCl	0.25	"	"
Riboflavin	0.50	"	"
Thiamine HCl	0.25	"	"
Cyancobalamin	0.15	0.25	0.35
DL-alpha tocopherol	0.15	0.25	0.35
Minor elements (mg)			
Zinc (EDTA)	2.84	"	"
Iron (EDTA)	3.25	"	"
Manganese (EDTA)	2.40	"	"
Others			
Cholesterol benzoate (mg)	2.5	"	"
K_3PO_4 (mg)	500.0	"	"
$MgCl_2 \cdot 6H_2O$ (mg)	200.0	"	"
Salt mixture no. 2 (mg)	5.0	"	"
Sucrose (g)	35.0	"	"
Water to make (ml)	100.0	"	"

VITA

Donald C. Cress

Candidate for the Degree of

Doctor of Philosophy

Thesis: DEVELOPMENT AND UTILIZATION OF A SYNTHETIC DIET FOR THE GREENBUG, SCHIZAPHIS GRAMINUM (ROND.), FOR USE IN DETERMINING THE FACTOR OR FACTORS RESPONSIBLE FOR RESISTANCE IN BARLEY AND WHEAT

Major Field: Entomology

Biographical:

Personal Data: Born in Canon City, Colorado, July 6, 1941, the son of Earl E. and Dorotha I. Cress.

Education: Graduated from Custer County High School, Westcliffe, Colorado, May 1959. Attended Southern Colorado State College, Pueblo, Colorado, from September 1959 to May 1961. Received Bachelor of Science degree from Colorado State University, Fort Collins, Colorado, with a major in Entomology in May 1964. Received the Master of Science degree from the University of Wyoming, Laramie, Wyoming, with a major in Entomology in January 1967. Completed requirements for the Doctor of Philosophy degree in August 1969.

Professional Experience: Recreational Maintenance, United States Forest Service, Boulder, Colorado, summers 1960 and 1961. Mosquito Taxonomist, United States Public Health Service, Greeley, Colorado, summer 1962. Research Technician, Department of Entomology, Colorado State University, Fort Collins, Colorado, summers 1963 and 1964. Research Assistant, Department of Entomology, University of Wyoming, Laramie, Wyoming, September 1964 to October 1966. Research Assistant, Department of Entomology, Oklahoma State University, Stillwater, Oklahoma, November 1967 to present.

Organizations: Sigma Xi, Phi Sigma, Alpha Zeta, Entomological Society of America, and Kansas Entomological Society.