SYNTHETIC DIETS AND TECHNIQUES UTILIZED FOR DEVELOPMENT OF THE CONVERGENT LADY BEETLE, *HIPPODAMIA CONVERGENS* GUER.

By

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PREFACE

The Master of Science program has provided me with a period to metabolize all I have learned during the undergraduate program, and direct this knowledge toward an ultimate goal. Concomitant with this period of applying previously acquired knowledge, the doors have also opened into a universe of infinite ignorance. If you will, a realization of those subjects not learned and those areas of science clouded by a preponderence of conflicting theories and ideals. Each theory attempting to satisfy the unending quest for an answer to each ambiguity.

The philosophy of knowledge is best left to the epistemologists and the passion I have acquired for science best conveyed by Sir Isaac Newton. "Even now, we are as little children playing at the edge of a great ocean."

I wish to acknowledge and thank Dr. Raymond Eikenbary, my major adviser, Dr. Robert Burton, and Dr. John Sauer, committee members, for their considerable time and effort spent in guidance and assistance both in and out of the laboratory. I also wish to thank Dr. Ron McNew, Department of Statistics, for his valuable assistance in analysis of data, and serving on my graduate committee.

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I am especially grateful to those individuals of the Entomology graduate student body for nurturing an atmosphere both conducive to

learning and most pleasant to work in. I also wish to thank my good friend, Mike Holland, for sharing his ambitions and constant inspirations.

My most sincere appreciation is reserved for my wife, Eileen, who has given of her patience and has become as much a part of this learning experience as I, my parents who have always instilled a deep respect for learning and scholarship within me, and my sister and brother Wanda and Gary, for their continuing encouragement. In essence this degree was earned by my family, for without their constant influence, sacrifices and encouragement it would certainly not have been accomplished.

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CHAPTER I

INTRODUCTION

Social and political concerns for environmental quality, as well as an increasing arsenal of dangerous insecticides and increasing resistance of numerous pestilent insects to chemical insecticides, has initiated investigation into several alternative methods for achieving pest control with minimal danger to the ecosystem. It is expected that concomitant use of a variety of control methods will provide an effective integrative pest management program.

The area of particular interest, to which the ultimate purpose of this paper is devoted, is that of biological control of a pestilent insect. The insect of immediate concern is the greenbug, Schizaphis graminum (Rondani), and one of its agents of control and naturally occurring predator, the convergent lady beetle, Hippodamia convergens Guer.

The greenbug is considered a prominent pest of wheat, barley, oats, rye, and sorghum in the Midwest (Starks and Burton 1977). Three biotypes of the greenbug A, B and C have been designated. Biotype C being very destructive to small grains and the most predominant biotype found in the field since 1968 (Wood and Starks 1972).

A variety of predatory and parasitic insects which prey upon the greenbug already exist under natural conditions. Hymenopterous parasites and predators, sirphid fly larvae, larvae of various chrysopid species, several species of coccinellids and a few predatory hemipterans. The

convergent lady beetle proves to be a most specific and prominent aphidophagous coccinellid, locally present among field populations of greenbug. Although, convergents are voracious predators of greenbug their usefulness is made less advantageous due to a common predator-prey lag phase. In other words, greenbug populations reach a density causing tremendous crop damage before the corresponding predator population increases substantially enough to repress the pest population.

The significance of predation by lady beetles could be increased by mass rearing the insects and releasing them at the onset of greenbug population buildups. However, such a program would require extensive research, entailing the development of economically feasible methods for mass rearing the lady beetles.

A primary consideration of a mass rearing program is the development of a pragmatic synthetic diet. The diet should provide adequate nutrition for growth and development without loss of vigor or modification of behavior within laboratory populations of the biological control agent. Numerous reviews discussing mass rearing techniques and problems involved therein are: Smith 1966; Mackauer 1972, 1976; Debach 1965; Lewontin 1965; Boller 1972; and Flanders 1964. Daniels (1972), Hagan (1965), and Nielson (1960) discuss laboratory biology and rearing methods for the convergent lady beetle on natural prey.

Generally, research on human and animal nutrition is directed toward the improvement of health and well being of man and his animals by improving their nutrition. However, work in insect nutrition is done predominantly to determine particular nutrient requirements or to determine qualitative and/or quantitative nutritive requirements necessary in formulating a diet suitable for rearing uniform, experimental insects con-

tinually available, for research concerned with pest control.

The majority of previous studies relevant to synthetic diets have dealt predominantly with phytophagous pestilent insects. Historically, research investigating the various applications of synthetic diets to predatory and carnivorous insects are and remain severely deficient. However, synthetic diets have been developed for the following predatory and carnivorous species: Coleomegilla maculata De Geer (Atallah and Newsone 1966); the larvae of Chrysopa carnea Stephens (Vanderzant 1969 and 1973); both which are predators of the greenbug; Dermestes maculatus (DeG.) (Levinson et al. 1967); and Xylocoris flavipes (Reuter) (Mukherjee and Som Choudhury 1971).

Several aphidophagous coccinellids have been reared with either insect bodies or components, or other animal or plant components. Hawks (1920) attempted raising Adalia bipunctata (Linnaeus), larvae upon cooked and raw hen's eggs and upon pounded dates. Smith (1965a) succeeded in rearing Anatis mali Auct., Adalia bipunctata, H. tridecimpunctata (Linnaeus) and C. maculata on dried aphids, but failed with Coccinella species. However, feeding quick frozen aphids allows growth and development in Coccinella septempunctata Linnaeus, similar to that when fed live aphids (Shands et al. 1966). Similar results are found with H. convergens (Hagan 1962). Although Haug (1938) found that laboratory colonies of H. convergens could be maintained throughout the year on various species of frozen aphids.

Several authors have succeeded in rearing various coccinellids and Chrysopa spp. on dried materials other than aphids. Smith (1961) raised C. maculata from first instar larvae to adult on pollens collected from various plants. Sundby (1968) was unable to do so with C. septempunctata

Sundby (1966) reared C. septempunctata on eggs of Ephestia kuehniella Zeller, and Kesten (1969) alternated Musca larvae with bananas daily and often achieved completion of larval development and pupation in Anatis ocellata (Linnaeus).

Of greater significance is the success of Okada (1973) and Matsuka (1972) in rearing Harmonia axyridis Pallas, on pulverized drone honeybee brood for sixteen successive generations. Hukusima (1976) also succeeded in rearing H. axyridis on drone honeybee powder, as well as Propylea japonica (Thunberg), Menochilus sexmaculatus quadriplagiatus (Fabricius), and C. septempunctata bruckii Mulsant. The utilizability of drone honeybee brood as a diet for a variety of aphidophagous insects is reviewed by Matsuka (1972). Okada (1974) successfully reared Chrysopa septempunctata Wesmael, on pulverized drone honeybee brood and Matsuka (1977) has succeeded in raising H. convergens for at least two generations on the drone honeybee diet.

In preliminary studies drone powder was fractionated and an effective substance(s) was found to be in the hot water soluble fraction and is cationic in nature (Matsuka 1975). However, the methods applied in this report were too primitive to produce conclusive results.

Smirnoff (1958) had effective results in rearing numerous species of coccinellids on an artificial medium of honey, cane sugar, agar, and royal jelly. Hippodamia species were not included. Suzumkowski (1952) used raw meat which alone was not enough to sustain the growth and development of C. maculata larvae. When larvae were supplemented with fresh liver and vitamin C, successful growth and development was attained. Smith (1965b) elicited an egg laying response from H. convergens with a fresh minced pork liver diet; a fresh banana diet; and a dry

mix containing forty percent casein, ten percent yeast, and fifty percent sucrose. However, no data pertaining to the quantity of eggs laid or fertility is available.

Atallah and Newsom (1966) developed a synthetic diet by which C. maculata may be successfully maintained under laboratory conditions. However, it should be noted that out of sixteen diets attempted the most successful contained an ether extract from fifty cotton leaves. The diet failed to support Coccinella novemnatata Herbst, Cycloneda species or H. convergens. Tanaka and Maeta (1965) also failed attempts at rearing Coccinella species on the synthetic diet of C. maculata.

Hagan (1962) has developed a fat free diet which induces ovigenesis in H. convergens that have overwintered. Addition of cholesterol and choline chloride improves the fecundity, though not sufficiently to equal that obtained from aphids. The diet used contains water and fructose solution, B-vitamin solution, commercial salt mixture and an enzymatic protein hydrolysate of yeast. However, fat soluble factors are suspected of being required, in addition, to produce egg production.

Presently Hagan (1978) is exploring several possibilities dealing with presentation techniques similar to those used for C. carnea (Hagan 1965). However, to date, there has been no significant progress toward development of a synthetic diet providing adequate nutrients for the growth and development of convergent lady beetle larvae or adults.

Most qualitative nutritional requirements are known for many insect species; and many essential nutrients are provided by microbial symbionts. However, a brief review of the general nutritional requirements of insects is provided herein for the benefit of the reader.

Dougherty (1959) introduced terminology specifying degree of chemi-

cal definition of synthetic diets: oligidic- consisting principly of crude natural materials; meridic- composed mainly of defined chemicals, but with one or more crude, natural, or undefined components (proteins, natural oils, plant extracts), holidic- consisting wholly of pure chemicals.

For purposes of economic feasability, an oligidic or meridic synthetic diet is most practical when considering mass rearing programs. The simple reduction of nutrient components by using more complex, natural ingredients within a synthetic diet also lessens the time required for diet formulation and development of various methods for the storage of stock solutions and mixtures.

In reviewing the general nutrient requirements for insect development the classical period in insect nutrition owes much to G. Frankel who contributed a great deal of information pertaining to the B-vitamin requirements of insects. This group comprises thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, choline, inositol, p-amino benzoic acid, biotin and cyanocobalamin (B_{12}). The requirements of these accessory nutrients for insects and vertebrates are surprisingly similar. Nicotinic acid, pyridoxine and pantothenic acid are indispensable for growth. A review of this vitamin group and their value in insect diets is further elaborated on in Frankel and Blewett (1943b).

Minor nutrients such as choline and inositol may be stored to permit proper egg development during adulthood as in Anthomomus (Coleoptera). Choline may be synthesized by many insects or may be required in large amounts as in Blattella, Schistocerca, Locusta, and Acheta (Orthoptera) (Chapman 1976). In several experiments both choline and inositol function in a supportive role during growth and development (Vanderzant

1959 and Gordon 1959) and are also important components of insect phospholipids concerned with mobilization and transport of fats (Wigglesworth 1972). Ascorbic acid may be required by most phytophagous insects (Dadd 1963) and is widely distributed in predatory insect tissues indicating that it is synthesized and not an essential vitamin for predatory insects.

A necessity for the lipid factor, vitamin A, in insect diets for proper pigmentation, vision, growth, and vigor during the larval stages has been elucidated by Dadd (1961) and House (1966 and 1965). Another lipid factor, vitamin E, in the form of alpha and beta tocopherols, is required by several species described by House (1972) and also including: Anthomomus grandis Boheman (Vanderzant et al. 1959); Agria affinis Fallen (House 1966d); Acheta domesticus (Linnaeus), (Meikle and McFarlane 1965); and, Cryptolaemus montrouzieri Mulsant, (Chumakova 1962). It also has been found to increase the frequency of copulation between pairs of C. maculata (Atallah and Newsom 1966) and improve growth of Ephestia larvae. In addition to the necessity of alpha tocopherol in various synthetic insect diets for developmental requirements it has a beneficial effect upon the physical properties of the diet itself. This may be ascribed to its generalized antioxidative protection of unsaturated fatty acids within the diet (Mittler 1972; Tappel 1962; Dadd 1973 and Fridovich 1977).

Although, inorganic salts may be an extremely important factor worthy of investigation where an optimal synthetic diet is desired, it becomes an extremely difficult task to delineate, conclusively, the requirements of insects for various minerals. It has generally been found that insects require much less calcium and iron in their diets, than other animals but a higher proportion of potassium to sodium than is

found in mammalian salt mixtures. Numerous inorganic salt mixtures formulated especially for insect diets are commercially available. House (1972) and Dadd (1970 and 1973) review the general literature pertaining to the mineral requirements of various insects. Mittler (1976), Akey and Beck (1972) and Retnakaron and Beck (1967) have carried out extensive investigations pertaining to the mineral requirements of the pea aphid, Acyrthosiphon pisum (Harris), and Cress and Chada (1971) with respect to the greenbug.

The essential amino acids required for the growth and development of insects are the same as those required for the laboratory rat, Rattus norvegicus: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (House 1972). Of course there are numerous examples of additional amino acids being required for the proper growth and development of various insects. These exceptions are discussed by House (1972) and Dadd (1970 and 1973).

In preparing oligidic or meridic diets, the use of crude protein sources such as raw meats, protein hydrolysates or several of the available commercial forms of casein are commonly utilized and often preferable for simplicity of diet formulation. When growth and development of the insect is not complete or impaired to any degree it may become necessary to revert to amino acids and a holidic diet determining the proper ratios of nutrients using deletion techniques.

Carbohydrates are a major and an essential component of most insect diets except with several dipteran larvae having low carbohydrate requirements (Dadd 1970). Generally the carbohydrate requirements of insects may be met by a variety of polysaccarides (starch, glycogen, etc.), oligosaccarides (melezitose, raffinose, sucrose, galactose, etc.) and by

sugar alcohols (sorbitol). However, the carbohydrate requirement seems best served by the common hexoses, glucose or fructose. The sugars utilized will vary according to the insects specific requirements, as well as its physiological state. To exemplify this, it has been generally found that excess sugar is detrimental to growth in insect larvae (House 1972).

Lipid requirements for insects may be divided into the categories of fatty acids and sterols. Fatty acids are usually synthesized by the insects from protein and carbohydrates. However, several polyunsaturated fatty acids have been found to be of significant importance. These are linoleic, linolenic, and oleic acids. Omission of these fatty acids predominantly effects larval development, pupation, wing development, and emergence of various species (Chen 1971; Dadd 1970; and House 1972).

Essentially all insects require a source of sterol in their diets due to their inability to synthesize the sterol nucleus. Exceptions to the rule are those insects possessing intracellular symbiotes which are capable of synthesizing the sterol nucleous (Ehrhardt 1968; Pant and Kapoor 1963; Dadd and Mittler 1966).

Generally cholesterol and cholesterol derivitives, as well as a variety of plant sterols are acceptable. However, quantitative and qualitative differences exist between species. Dadd (1970, 1973), House (1972), Robbins (1971), and Svoboda (1975) have extensive coverage on this subject.

Generally synthetic diets are aseptically treated to content with microbial contamination. Sterilization has proven effective for liquid diets if the diets are changed on a daily basis. However, specific

additives prove more practical for solid diets (Kishaba et al. 1968). In addition to the diet itself it is considered good practice to clense all surfaces and tools with a ten percent clorox-water solution. Edwards et al. (1965) reviews several control barrier systems and decontaminant agents useful when dealing with storage and prolonged presentation of synthetic diet formulations.

Qualitatively requirements of developing larvae for amino acids, carbohydrates, fats, vitamins and minerals are much more uniform then for the adult. For nearly all larvae cholesterol or its derivatives, as well as the ten essential amino acids of insects are required.

Many of the nutrients required by larvae do not always serve the immediate purpose of growth, but some are stored in larval tissue and later used for adult development (Chen 1971).

CHAPTER II

METHODS AND MATERIALS

Maintenance of Greenbug Cultures

Culturing techniques utilized in establishment and maintenance of greenbug colonies follows the procedure described by Starks and Burton (1977), and is briefly summarized as follows with minor changes:

1) A soil mixture 3:1:1 of soil, sand, and peat is sterilized, in a Soil King 400 Model Deluxe Soil Sterilizer, at 170° F for 3½ hours. After cooling, the desired number of 8 inch plastic pots are filled to surface with soil. The central area of soil is then depressed to a depth of 1 inch, then seeded and covered with a thin layer of sand.
2) A mixture of 2:1 Rodgers barley and B-Wheatland sorghum (both susceptible varieties) are used as culture plants during the cooler periods of the year. At ambient temperatures above 80° F, sorghum is used solely as the culture plant commensurate with its ability to grow well and yield adequate populations of greenbug at higher temperatures than barley.
3) The pots are then fertilized with an indoor plant supplement, Hyponex, at 1 ounce:5 gal. of water until the soil is saturated. This is done only during the first watering.
4) Culture plants are grown in the greenhouse with a temperature range of 68-78° F during the late fall, winter, and early

spring. Two weeks after planting new cultures are infested by placing, among the new plantings, two or three plants infested with aphids from previous cultures. The new culture pots are then covered by cylindrical plastic cages of nitrocellulose to exclude extraneous insects and to confine the greenbugs (Figure 1). Within two-three weeks after infestation the cultures should have a maximum population of greenbugs. The plants are then harvested and placed within a ½ gal. cardboard canister and refrigerated at 45° F until needed. Aphids die after 1-1½ weeks in refrigerated storage. A small percentage of pots are set aside from each culture to infuse new cultures.

5) Plants were watered twice weekly or at the first indication of wilting. Excessive watering promotes mildews and a variety of diseases which can kill plants.

6) Several hymenopterous parasitoids may infest cultures causing serious damage if left unchecked. The small braconid, Lysiphelbus testaceipes (Cresson), is the most common occurring in our greenhouses and oviposits in most instars of the greenbug, later producing straw colored mummies. Another less frequent hymenopterous parasitoid, Aphelinus nigritus Howard, forms a black mummy. In event of an infestation all infested pots were immediately discarded and new clean cultures started.

Maintenance of the Convergent Lady Bettle Colony

A stock colony of convergent lady beetles was established April, 1977, from numerous field collections in local alfalfa fields infested

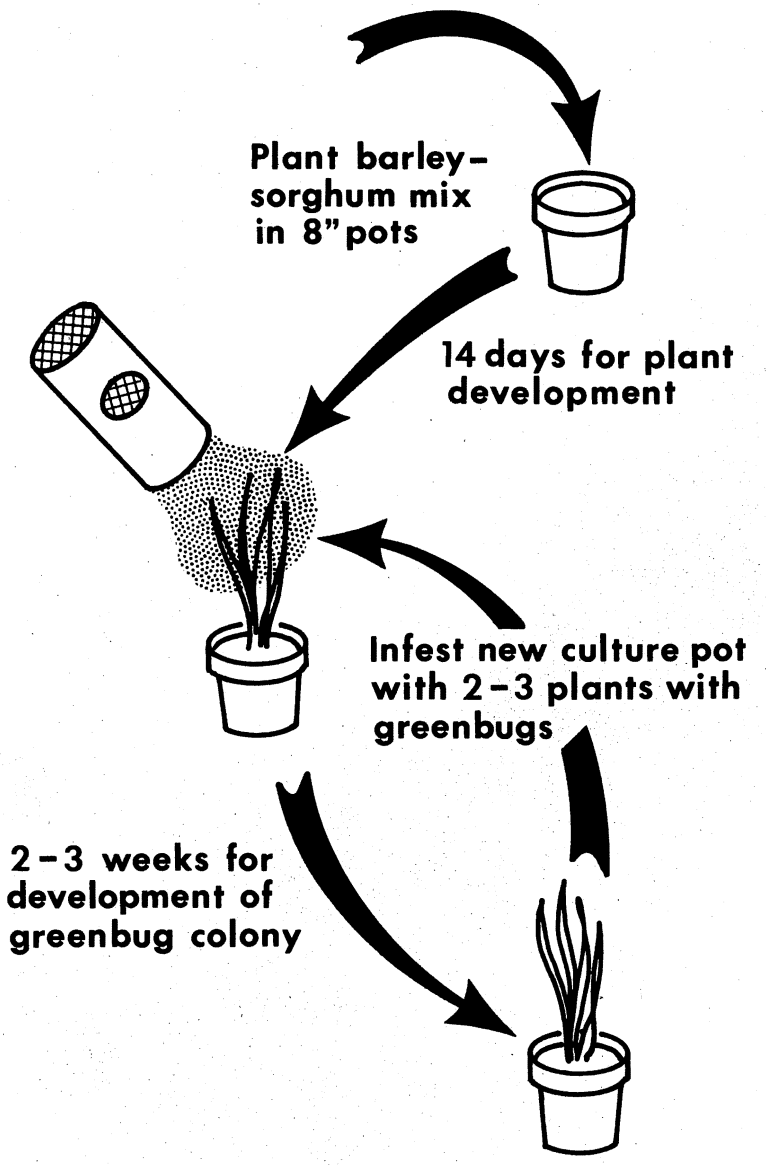

Figure 1. Planting and culture technique for establishing and maintaining a greenbug colony (after Starks and Burton 1977)

with pea aphids. The colony was maintained in the laboratory under a 16:8 LD photoperiod regime, a temperature varying between 23.3 - 25.6° C, and relative humidity of 30 - 40%. An increase to 78% relative humidity facilitated oviposition during the winter. The colony was housed in either a controlled rearing room or growth chambers, as space permitted, and according to the afore mentioned physical parameters.

The major portion of the following rearing techniques were developed by Farris (1976) while rearing the coccinellid M. sexmaculatus from Pakistan. However, due to minor differences in ecological and behavioral parameters between the two organisms, some procedural adjustments were necessary.

Sex determination of the convergent lady beetle may be accomplished by either one of two methods. The first and simplest is to observe a copulating pair in a large storage culture and remove the pair to an isolated cup. Second, if a storage culture is not available sex determinations may be carried out rather simply under a microscope. Touch a rolled piece of masking tape several times to the palm of the hand reducing its adhesiveness, thus preventing unnecessary damage to the elytra. Apply the tape to the elytra and place under the microscope with the insects' abdomen facing up. The sixth visible abdominal sternite of males is sharply emarginated at the middle of its hind margin. Females show little or no emargination of this sternite (Figures 2 and 3). When finished, gently pry the elytra from the tape with a number one camel hair artist's brush. Sex determinations may also be accomplished, though less accurately, by differences in body size and pigmentation of the elytra. The females were slightly larger and possessed a more deeply pigmented elytra.

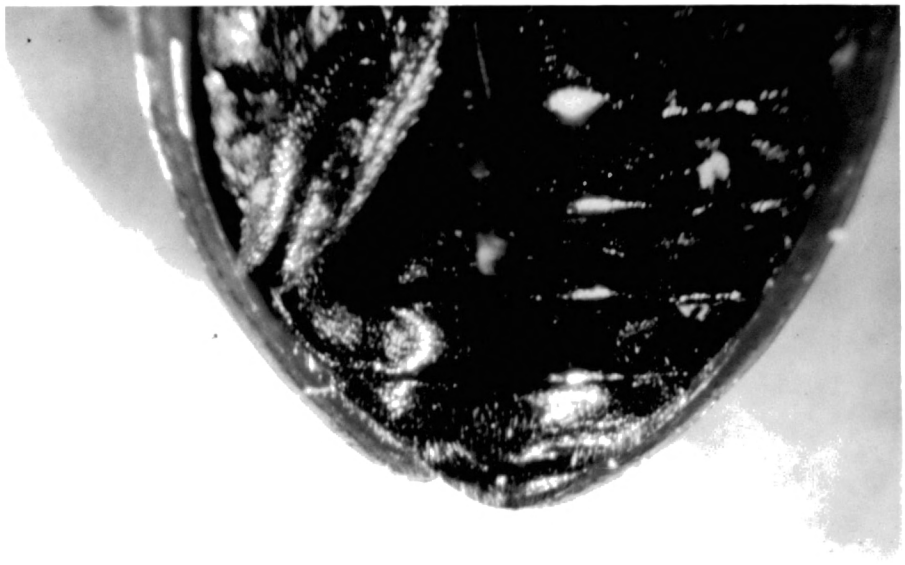

Figure 2. The sixth visible abdominal sternite of female lady beetles showed little or no emargination

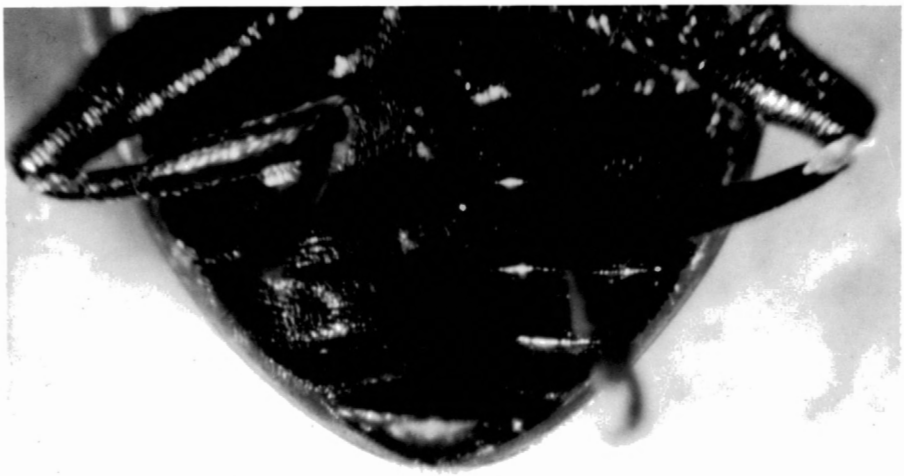

Figure 3. The sharply emarginated sixth visible abdominal sternite of male lady beetles was successfully used to differentiate between the sexes

One ounce plastic cups with white cardboard insert lids were used as oviposition chambers for mated adults and isolation chambers for larvae during development.

The bettles were fed, both adult and larvae, by first thrashing greenbug from barley or sorghum clippings taken from refrigeration. A wooden dowel, of 1 cm. diameter was utilized in thrashing aphids from plant clippings. Aphids were swept from the table top onto a folded piece of paper and brushed or tapped lightly into a common container. Using a number one camel hair bursh large numbers of aphids could be distributed among several hundred cups, containing lady beetles, in an hour or less.

Mated pairs were fed in excess of 100 greenbugs daily. Two small drops of a 50:50 honey-water solution were affixed to the inner sides of the cups and a small ball of absorbent cotton saturated with water was placed at the bottom of the cup. The cotton ball serves to provide drinking water and to increase relative humidity within the cup. Cups were replaced daily transferring adults from the previous days cups.

The lady beetles were never touched by hand. They were transferred either by an aspirator or number one camel hair brush. Aspirating proved quite practical during transfer of adults.

Eggs were usually laid in clusters of 30, though the number varies considerably, to the cups lid or to the cotton ball if dry. Less frequently, eggs were laid along the sides and bottom of the cup. After oviposition adults were removed from the cup, cups containing eggs were placed aside for 2-3 days until they hatched. One extra day was required before larvae detached from the ruptured eggs. To avoid excessive cannibalism, as is common among predatory coccinellids (Hodek 1973),

larvae were removed and placed in individual cups subsequent to detachment. Larvae were always transferred using a number one camel hair brush.

The first instar larvae were fed 20 aphids daily, the second instar 40 aphids daily, and the third and fourth instars between 75-100 aphids daily. Upon pupation the cups were set aside until emergence. During larval development the cups were brushed free of debris and replaced daily before feeding.

Newly emerged adults were fed approximately 75 aphids for a 24 hour period and then either mated or placed in storage until needed. If adults were not needed for laboratory experiments, or excessive numbers emerged, they were stored in a growth chamber set at a 8:16 LD photoperiod and temperature of 15.6^{o} C. About 30-35 adults were placed in one pint cardboard cartons filled with excelsor. Several small pieces of absorbent cotton saturated with water and a small petri dish (35 x 10 mm) containing a standard maintenance diet developed by Burton (1976) (Table 1) were included in the container. The cotton balls and standard diet were replaced daily. The stored beetles were fed every third day by brushing 400 aphids into the carton. These cartons were cleaned once weekly removing debris and dead beetles.

Preparation of Greenbug for the Lowry and Anthrone Assays

The Folin-Lowry Method of protein assay was utilized for determination of the percent total protein in greenbug supernatants and homogenates. The procedures followed and reagents used were similar to the methods outlined by Lowry et al. (1951), Plummer (1971), and Layne

Table 1. Quantities and composition of nutrient components utilized in the formulation of the Standard Maintenance Diet.

Nutrients	Amount
Agar	5.2 g.
Distilled water	200.0 ml.
Sucrose	32.0 g.
Honey	12.0 g.
Gelatin	10.0 g.
Torula yeast	10.0 g.
CSM	10.0 g.
Ascorbic acid	1.0 g.
Sorbic acid	0.1 g.
Methylparaben	0.1 g.

(1955).

The Anthrone reaction (Spiro 1971) was used to determine the percent of total free hexose in greenbug supernatants. Preparations of the greenbug for the Lowry and Anthrone assays is as follows:

1) Weigh out 1.5 g of live greenbug on a Mettler analytical balance.
2) Place greenbug in a 50 ml centrifuge tube with distilled water and gently vortex for about one minute. Most of the debris will settle to the bottom while the greenbugs remain in the upper third of the tube. Vacuum filter the upper half layer of water and greenbugs from the centrifuge tube.
3) Weigh out 1.0 g of the cleaned greenbug and add 2 ml of distilled water, grinding with a mortor and pistol.
4) Centrifuge for 10 minutes at 10,000 rpm 3^{o} C. A Sorvall superspeed RC2-B centrifuge with a refrigerated head was used. When doing a total homogenate (exoskeleton, hemolymph, and viscera) of greenbug, skip this step.
5) Determine the total volume of the supernatant. Isolate the supernatant and the lipid layer. Vortex gently for one minute. Dilute the supernatant with two equal volumes of distilled water and vortex again. If using a greenbug homogenate determine the total volume of the homogenate. Dilute with 3 equal volumes of distilled water and vortex.
6) Use 0.1 ml aliquots of the diluted supernatant or homogenate for the experiment.

Preparation of Stock Nutrients and Nutrient Components

The natural food of H. convergens consists of the host tissues of the greenbug, that is, the viscera and body fluids as well as chitinous portions of the exoskeleton. The ability of most predatory aphidophagous coccinellids to consume the entire host progresses with the development of stronger mandibles as the insect becomes older.

Young coccinellids pierce and suck the contents from their prey, while latter instars develope a chewing mechanism enabling them to consume the entire host (Hagan 1962). In view of the earlier instars propensity toward a fluid diet, it seemed reasonable to begin with a liquid diet. However, agar based diets proved more successful than did the liquid diet. Stock nutrient solutions and mixtures were prepared in bulk prior to the formulation of all diets. Preparation of stock nutrients and a brief summary of the nutrient content of all major experimental diets will be further elaborated upon under the headings of the major nutrient groups.

Vitamins

The vitamin mixture (Table 2) is exactly as that developed by Vanderzant (1969). Vitamins were weighed out on a Mettler analytical balance and disolved first followed by the remainder of vitamins and the solution was diluted to 500 ml. The solution was frozen at -10° C and thawed prior to use. This solution was renewed once a month to guarantee freshness.

Table 2. Composition and quantities of vitamin components utilized in preparation of a stock vitamin B solution (after Vanderzant 1969).

Vitamins	mg/ml	mg/100 ml	g/100 ml	g/500 ml
Nicatinamide	1.000	100.0	0.1000	0.500
Calcium pantothenate	1.000	100.0	0.1000	0.500
Thiamine hydrochloride	0.250	025.0	0.0250	0.100
Riboflavin	0.500	050.0	0.0500	0.200
Pyridoxine hydrochloride	0.250	025.0	0.0250	0.100
Folic acid	0.250	025.0	0.0250	0.100
Biotin	0.020	002.0	0.0020	0.010
Cyanocobalamine	0.002	000.2	0.0002	0.001

Add 2 ml. of the 500 ml. stock solution per 120 ml. of V1 diet (Table 3) and 5 ml. stock solution to all diets listed in Table 6.

Lipids

A stock suspension of soybean lecithin, soybean oil, and cholesterol were prepared by first heating 25.0 g of soybean lecithin and 25.0 g of soybean oil, mixing until smooth. Second, 5.0 g of cholesterol were added and mixed over heat until all the particles were suspended. For diets V2 and V3 (Table 3), 1 ml of Tween 80 (polyoxyethylene sorbitan monooleate) was added to ensure adequate suspension of cholesterol (Thompson 1975) (Table 4). The suspension was stored in an autoclaved 250 ml beaker covered with two layers of aluminum foil and two more layers of parafilm at 45° F. The aluminum foil prevented the absorption of lipids from the stock solution into the parafilm.

Wheat germ oil, contains the following: linoleic, linolenic, oleic acid and other saturated fatty acids; active tocopherols; sistosterols; and phospholipids. It was used in the majority of diets tested. However, no special preparation was required except that it be refrigerated at 45° F. when not in use.

Inorganic Salts

To overcome problems with precipitation, the salts were prepared as separate stock solutions. These solutions were prepared by dissolving each salt into deionized water (Table 5). The solutions were filter sterilized through 0.20 micron plain membrane (Nalgene Labware Division) and then stored in the refrigerator at 45° F. in sterilized 250 ml beakers sealed with parafilm. Generally, 5 ml of stock solution was utilized per 100 ml of diet.

Table 3. Quantities and composition of nutrient components utilized in the formulation of Vanderzant's liquid diet (Vanderzant 1969) and two modifications of the diet.

Nutrients	Amounts Diet numbers V1[1]	V2[1]	V3[1]	
Agar	-----	7.0	"	g.
Distilled water	360[2]	210.0	"	ml.
Sucrose	45.0	"	"	g.
K_2HPO_4	15.0	"	"	ml.
$NaH_2PO_4H_2O$	15.0	"	"	ml.
$MgSO_4 \cdot 7H_2O$	15.0	"	"	ml.
$FeSO_4 \cdot 7H_2O$	15.0	"	"	ml.
Soybean lecithin Soybean oil Stock	1.65	"	"	g.
Cholesterol Tween 80	-----	-----[3]	"	g.
Casein, vitamin free	3.0	5.0	"	g.
Casein hydroslysate, E	15.0	"	"	g.
Soy hydrolysate, E	15.0	"	"	g.
Yeast hydrolysate, E	6.0	"	"	g.
Liver "S"	-----	------	10.0	g.
Vitamin B- solution	6.0	"	"	ml.
Ascorbic acid	0.3	1.5	"	g.
Choline	1.5	"	"	g.
Inositol	0.6	"	"	g.
Sorbic acid	-----	0.15	"	g.
Methylparaben	-----	0.15	"	g.

[1] V1 = Vanderzant's liquid diet, V2 = Vanderzant's modified diet and V3 = Vanderzant's modified and liver "S" diet.

[2] In preparation of V1 use distilled water to make up 360 ml total liquid diet.

[3] 1 ml Tween 80 was added to the 100-fold lipid stock solution (Table 4).

Table 4. Quantities and components utilized in the preparation of a stock lipid suspension.

Nutrient	g/120 ml diet	100-fold stock (g.)[1]
Soybean lecithin	0.25	25.0
Soybean oil	0.25	25.0
Cholesterol	0.25	5.0

[1]Add 0.55 g of stock solution per 120 ml. diet for diet V1 (Table 3).

1 ml. of Tween 80 was added to the stock solution for diets V2 and V3.

Table 5. Quantities and components utilized in preparation of stock inorganic salt solutions.

Inorganic salt solutions[1]	g/250 ml H_2O
K_2HPO_4	37.0
$Na_2HPO_4H_2O$	5.00
$MgSO_4 \cdot 7H_2O$	12.0
$FeSO_4 \cdot 7H_2O$	2.0

[1]Inorganic salts were purchased from United States Biochemicals.

Protein

Protein nitrogen was supplied in the diets by several enzymatic hydrolysates and bulk forms. Soy and casein enzymatic hydrolysates provided the chief sources of protein in all but the standard maintenance diet (Table 1). Torula yeast, noted for a high vitamin B complex content (Elias and Bressani 1970) was used in most experimental diets. CSM (corn-soy-milk blend) a high protein food supplement for children of developing nations was also used in many experimental diets. CSM contains 20% protein of a higher protein efficiency ratio than casein, 1650 calories per pound, and added vitamins and minerals (Tollefson 1967). Burton (1970) has successfully utilized CSM as a nutritive component in a synthetic diet for the corn earworm, Heliothis zea (Boddie). Powdered milk (Carnation instant) provided not only a major supply of complete protein, but numerous additional nutrients such as inorganic salts in trace amounts, lipids, and vitamins. Yeast enzymatic hydrolysate and vitamin free casein were also used as minor sources of protein in diets V1, V2, and V3 (Table 3). Gelatine, although an incomplete protein, was also included as an additional source of amino acids in all agar based diets.

Two amino acids, cystine and tryptophan, although included already in most of the above sources of proteins were utilized in excess due to their particular nutritive and attractive qualities. Cystine, as noted in the discussion, was shown to be an essential requirement of several insects for normal growth, pupation, and emergence. Tryptophan, in addition to its nutritive qualities was found to be an attractant or arrestant of C. carnea when sprayed in alfalfa fields (Hagan 1976). Saad and Bishop (1976) have significantly increased numbers of Hippodamia spp.

utilizing artificial honeydew sprays and suspect tryptophan as one of the bioactive attractive components. Excess amounts of tryptophan were added to the experimental diets to increase their desirability for convergent larvae.

Various liver extracts, L, 2, S and concentrate 1:20 (United States Biochemical Corp.) (Table 6), provided sources of protein, glycogen, vitamins and unidentified active components. All protein sources are weighed out on a Mettler top loading balance into a common container and used shortly thereafter in preparation of the medium.

Carbohydrates

Sucrose, D-glucose, and honey were the carbohydrates utilized in the majority of diets. Sucose was the sole source of carbohydrate in the V1, V2 and V3 diets (Table 3). Honey and sucrose in the standard maintenance diet (Table 1), D-glucose in the high lipid diet (Table 7), and all three in combination were used for the nonliver and liver diets NL1 through L-10 (Table 6). No special preparation or storage was necessary.

Miscellaneous Water Soluble Vitamins

Ascorbic acid was added in small quantities to all the diets tested, possibly for nutritive value, but a more probable use is its antioxidative role in protection of lipids within the artificial diets.

Inositol and choline chloride were present in all but several of the preliminary diets and the standard maintenance diet (Table 1). Both inositol and choline chloride were kept under refrigeration at 45° F. and powdered ascorbic acid was stored in the freezer at -10°C. No special

Table 6. Quantities and composition of nutrient components utilized in the formulation of the artificial test diets.

Nutrients	Amounts Diet number NL1	L2	L3	L4	
Agar	7.0	"	"	"	g.
Distilled water	250.0	"	"	"	ml.
Sucrose	12.0	"	"	"	g.
Honey	20.0	"	"	"	g.
Torula yeast	10.0	"	"	"	g.
CSM	10.0	"	"	"	g.
Powdered milk	10.0	"	"	"	g.
Casein hydrolysate, E.	10.0	"	"	"	g.
Soy hydrolysate, E.	10.0	"	"	"	g.
L-Cystine	2.0	"	"	"	g.
DL-Tryptophan	2.0	"	"	"	g.
Gelatin	5.0	"	"	"	g.
Liver "L"	-----	10.0	----	----	g.
Liver "2"	-----	----	10.0	----	g.
Liver "S"	-----	----	----	10.0	g.
Liver conc. (1:20)	-----	----	----	----	g.
Wheat germ oil	3.0	"	"	"	g.
Vitamin B-solution	5.0	"	"	"	ml.
Ascorbic acid	1.0	"	"	"	g.
Choline	1.6	"	"	"	g.
Inositol	0.6	"	"	"	g.
Sorbic acid	0.12	"	"	"	g.
Methylparaben	0.12	"	"	"	g.

	L5	L6	L7	
Agar	7.0	"	"	g.
Distilled water	250.0	"	"	ml.
Sucrose	12.0	"	"	g.
Dextrose	20.0	"	"	g.
Honey	12.0	"	"	g.
Torula yeast	10.0	"	"	g.
CSM	10.0	"	----	g.
Powdered milk	10.0	"	----	g.
Casein hydrolysate, E.	10.0	"	"	g.
Soy hydrolysate, E.	10.0	"	"	g.
L-Cystine	2.0	----	2.0	g.
DL-Tryptophan	2.0	----	2.0	g.
Gelatin	5.0	"	"	g.
Liver "L"	-----	----	----	g.
Liver "2"	5.0	"	"	g.
Liver "S"	-----	----	----	g.
Liver conc. (1:20)	5.0	"	"	g.
Wheat germ oil	3.0	"	"	g.
Vitamin B-solution	5.0	"	"	ml.
Ascorbic acid	1.0	"	"	g.

Table 6 (Continued)

Nutrients	Amounts Diet number			
	L5	L6	L7	
Choline	1.6	"	"	g.
Inositol	0.6	"	"	g.
Sorbic acid	0.12	"	"	g.
Methylparaben	0.12	"	"	g.
	L8	L9	L10	
Agar	7.0	"	"	g.
Distilled water	250.0	"	"	ml.
Sucrose	12.0	"	"	g.
Dextrose	20.0	40.0	20.0	g.
Honey	12.0	"	"	g.
Torula yeast	10.0	"	"	g.
CSM	10.0	"	"	g.
Powdered milk	10.0	"	"	g.
Casein hydrolysate, E.	20.0	"	30.0	g.
Soy hydrolysate, E.	10.0	"	"	g.
L-Cystine	2.0	"	"	g.
DL-Tryptophan	2.0	"	"	g.
Gelatin	5.0	"	"	g.
Liver "L"	-----	----	----	g.
Liver "2"	-----	----	----	g.
Liver "S"	10.0	"	"	g.
Liver conc. (1:20)	-----	----	----	g.
Wheat germ oil	3.0	"	"	g.
Vitamin B-solution	5.0	"	"	ml.
Absorbic acid	1.0	"	"	g.
Choline	1.6	"	"	g.
Inositol	0.6	"	"	g.
Sorbic acid	0.12	"	"	g.
Methylparaben	0.12	"	"	g.

The majority of the nutrient components as well as liver extracts were purchased from either United States Biochemicals or Sigma Biochemicals.

Table 7. Quantities and composition of nutrient compounds utilized in the formulation of a synthetic medium rich in fatty acids and sterols.

Nutrients	Amounts
Agar	6.0 g.
Distilled water	220.0 ml.
Dextrose	30.0 g.
Torula yeast	10.0 g.
CSM	10.0 g.
Gelatin	10.0 g.
Casein hydrolysate, E	10.0 g.
Soy hydrolysate, E	10.0 g.
L-Cystine	2.0 g.
DL-Tryptophan	2.0 g.
Wheatgerm oil	3.0 g.
Soybean oil	3.0 g.
Soybean lechithin	2.0 g.
Cholesterol	3.0 g.
Vitamin B-solution	2.0 g.
Ascorbic acid	1.0 g.
Sorbic acid	0.1 g.
Methylparaben	0.1 g.

preparation was required.

Mold Inhibitors

Sorbic acid (2,4-Hexadienoic acid) a mold and yeast inhibitor was used in combination with methylparaben (p-Hydroxybenzoic acid methyl ester), also a mold and yeast inhibitor. These chemicals were carefully weighed in small quantities and added to all agar based diets.

Preparation of Media

Liquid Media

Preparation of the liquid media was as follows:

1) Dry nutrient components were weighed out on Mettler analytical and top loading balances then poured into a waring blender. Nutrient components were mixed only immediately prior to use in preparation of the synthetic media. In this way unnecessary chemical reactions between nutritive components may be avoided (Mittler 1972).
2) The vitamin B stock solution was thawed several hours previous to media preparation. The appropriate quantities were pipetted into the blender (Table 3).
3) The stock lipid suspension was warmed over a low flame and mixed ensuring proper suspension of cholesterol. The appropriate quantity was weighed out into a one ounce plastic cup on an analytical balance and poured into the blender.
4) The components were then mixed at high speed for 2-3 minutes until the mixture was smooth.
5) The diet was then poured into a 500 ml beaker and made up to

the required volume, 360 ml, with distilled water. The diet was then returned to a clean blender and mixed at high speed for another minute.

6) The diet was poured into two 250 ml Erlenmeyer flasks stopped with cotton and autoclaved at 15 psi, 120°C. for 30 minutes.

7) The diets were cooled to room temperature then poured into a series of 10 ml plastic bottles and frozen at -10° C. until needed.

Solid Media

Preparation of the solid media was as follows:

1) Agar and gelatin were weighed out on a Mettler top loading balance and placed into the desired amount of distilled water.

2) The mixture was heated to 90°C. on a gas range stirring constantly with a kitchen spatula or spoon to avoid burning and sticking of the agar.

3) The hot agar mix was poured into a blender and allowed to cool to 40-45° C.

4) While the agar was cooling the sources of protein, carbohydrate and miscellaneous water soluble vitamins were weighed out on either the top loading or analytical balance into a common container and placed aside.

5) Honey and wheat germ oil were weighed out into separate one ounce plastic containers and placed aside. If the stock lipid solution was being used, prepare as described in preparation for liquid media. The vitamin B solution was thawed just prior to use.

6) When the agar had cooled it was poured into the dry components and stirred with a spatula or spoon, followed with the honey and source of lipid. The desired aliquot of the vitamin B solution was pipetted into the blender and the contents were mixed at high speed for 2-3 minutes until smooth.

7) The mixed diet was then poured to a depth of 0.5 - 1.0 cm in one oz. plastic cups. These were allowed to solidify under a clean air hood in 25 cup capacity trays.

8) After hardening, the diet cups were wrapped in plastic bags and refrigerated at 45° C. They were frozen for longer storage, although the water content of the agar media was substantially decreased when thawed. If refrigerated diet was not kept for more than five days, apparently the amino acids react with the sugar in the diet causing a darkening and deterioration of nutritional quality.

Development of Feeding Procedures

Pieces of cellulose sponge, 0.5 cm x 0.5 cm x 0.5 cm, were cut and placed in the bottoms of one ounce plastic cups. The cellulose cubes were then saturated with a liquid diet V1 (Table 3). The cubes were renewed twice daily (Vanderzant 1969 and Hagan 1965).

A technique of paraffin encapsulation of synthetic diets was successfully utilized in rearing the green lacewing (Hagan 1965). This method of presentation was duplicated during preliminary experiments with convergent lady beetles. One gram of paraffin was melted atop 20 ml. of diet in a 30 ml. beaker until a layer 1-2 mm thick lay atop the diet. The melting point of paraffin wax is 43 - 47° C. The V1 diet

(Table 3) was utilized in place of Hagan's diet for this experiment. A glass applicator rod of 5 mm diameter was drawn out at the tip to a diameter of 2-1 mm over a 30 mm length. The end was burnished to form a solid tip. The rod was then dipped into the hot mixture, lifted out and the tip gently touched to a small piece of parafilm covering the bottom of one ounce plastic diet cups. A droplet would immediately form, encasing the diet. About 20-30 of the droplets were placed within a cup depending on the size of the larvae. The deeper the glass rod was plunged into the hot mixture the larger the droplet would be. Large droplets were utilized for third and fourth instars as well as adults.

In preparation for an experiment the agar diets were taken from the refrigerator and allowed to warm to room temperature. Once warmed the surface of each diet was crumpled with forceps to provide smaller particles and more edge area upon which the larvae could feed. A small ball of absorbent cotton, saturated with distilled water, was placed within the cup to increase the humidity and provide drinking water for later instars. One larvae was placed within each cup and sealed with a lid. The experimental groups were then placed either in a controlled rearing room or growth chamber under the regime prescribed for the stock colony. All experimental diets were replaced daily.

Collection of Eggs for Experiments

The eggs were removed from the plastic cups or lids surface using a number one camel hair paint brush. The bristles were thinned until 5-8 remained then dipped into distilled water. The egg cluster's base was moistened with the wet brush for a period of one minute, then the eggs could be gently rocked free, one at a time, from the cluster.

Each egg was placed in an individual 1 oz. plastic cup atop filter paper moistened with distilled water. The filter paper was moistened daily for a 2-3 day period until the eggs hatched. When the first instar larvae were from 12-24 hours old they abandoned the ruptured egg case and were promptly collected and placed on the appropriate experimental treatments.

Description of Preliminary Diet Experiments

Several preliminary synthetic diet experiments were carried out in an attempt to discern a logical progression for experimentation. They were as follows:

1) Banana diet. A fresh banana diet was made up (Table 8) (Smith 1965b), by simply mixing the nutritive components in a beaker and placing 36 portions directly onto the bottom of each diet cup for the first treatment. Raw pork liver (100 g) was pureed in a waring blender at high speed for four minutes and 3 g portions were placed onto the bottom of each diet cup for the second treatment. Ten pairs, male and female, were chosen for each of the above treatments. Each pair was isolated within individual diet cups. Two control treatments were also set up. 10 mated pairs were placed on the standard maintenance diet (Table 1) and 10 mated pair were fed an excess of live greenbug. All adults were reared in the laboratory and emerged on the same day. The adults were fed greenbug for one day after emergence then they were mated and placed on their respective diets the second day after emergence. The mated pairs were observed six times daily at two hour intervals noting frequency of copu-

Table 8. Composition and quantities of components utilized in the preparation of a fresh banana diet (after Smith 1965b).

Nutrients	Amount		
Fresh banana	90.0[1]	90.0[2]	g.
Vitamin free casein	9.0	9.0	g.
Wheat germ oil	0.9	0.9	g.
Vitamin B-solution	0.1	0.5	ml.
Ascorbic acid	----	2.0	g.

[1]As found in Smith (1965b).

[2]Minor modification of diet with supplementary nutrients and ascorbic acid slowing the oxidative process.

lation among pairs and oviposition among females.

2) Pea aphid diet, sterol diet, and diet V1. A second preliminary experiment was set up testing the effects of various diets on first and second instar larvae. On treatment 1 the aphid diet developed by Akey and Beck (1972) for the pea aphid (Acrpthosiphon pisum) was solidified by adding agar (1.9% of the total liquid diet) to the prepared liquid diet. Treatment 2 was composed of excess sources of fatty acids and sterols (Table 7). Preparation of this diet follows that described for all agar based diets. Treatment 3 involved diet V1 (Table 3) utilizing saturated cellulose cubes covered with cheese cloth. The standard maintenance diet and a live greenbug diet served as the two controls. Ten first instar larvae were placed in individual cups, 12-24 hours after they emerged, on each treatment. A total of 50 larvae were used. The larvae were checked several times daily for a period of three weeks recording frequency of molt, pupation, and emergence. The experiment was repeated using second instar larvae, which had previously been fed live greenbug.

D-glucose vs. Sucrose

This experiment was developed to ascertain whether or not nutrients fed to the larvae during their first 24 hour feeding period, since hatching, would illicit a noticeable difference in weight gain, molt, or increased mortality over a six day period on the standard maintenance diet (Table 1).

The experiment consisted of five treatments. The live greenbug

diet, fed in excess, and the standard maintenance diet served as the two controls. The experimental diets were: one, agar and sucrose; two, D-glucose and agar; and three; plain agar (Table 9). Only first instar larvae were used. The larvae remained on the experimental diets and controls for a 24-hour period, after which, all larvae were placed in separate cups containing the standard maintenance diet for the remainder of the experiment. All larvae were weighed three days after being switched to the standard maintenance diets and again on the sixth day. Larval mortality and frequency of molting were also recorded.

Description of Experiment on a Supplementary Water Source

The basis of this experiment is to determine if, infact, a water requirement could be a critical factor in the insects early development. First instar larvae were placed onto their respective treatments 12-24 hours after they hatched. Twelve individuals were used per treatment, each placed in an individual diet cup. The treatments were as follows:

1) Larvae were placed in empty cups without a diet or source of water.
2) Larvae were placed in empty cups containing a cotton ball saturated with distilled water.
3) Larvae were placed in cups containing only the standard maintenance diet.
4) Larvae were placed in cups containing the standard maintenance diet and a cotton ball saturated with distilled water.
5) Larvae were placed in a cup and fed an excess of live greenbug only.

Table 9. Composition and quantities of components utilized in preparation of simple agar-based test diets.

Components		Amounts		
		Agar-sucrose	agar-dextrose	Agar
Distilled water	ml.	100.000	100.000	100.000
Agar	g.	2.300	2.300	1.900
Sorbic acid	g.	0.046	0.046	0.037
Methylparaben	g.	0.046	0.046	0.037
Sucrose	g.	25.000	-------	-------
Dextrose	g.	-------	25.000	-------

6) Larvae were placed in a cup and fed an excess of live greenbug and offered a cotton ball saturated with distilled water.

All larvae were weighed as they were placed upon their treatments. Those larvae contained within the first four treatments were weighed every five days over a period of fifteen days at which time the experiment was discontinued. Those larvae in treatments five and six were weighed after the first, second, and third molts, pupation and emergence. Mortality was recorded for all treatments. All diets and cotton balls were replaced daily.

Description of Experiment on Cannibalism

This experiment was designed to illucidate the contribution of cannibalism to increased weight gains and molting of the first instar larvae. Larvae, 12-24 hour old, were placed on the following treatments for a period of 24 hours. The treatments are as follows:

1) Larvae were allowed to cannibalize within their egg clusters.
2) An excess of live greenbug was placed in the cup with the cannibalizing larvae.
3) Larvae which hatched from isolated eggs were placed into separate cups preventing cannibalism and fed an excess of live greenbug.
4) Larvae were isolated as in treatment three and immediately placed on standard maintenance diets.

After the larvae remained on the above treatments for 24 hours they were all switched to the standard maintenance diet. All larvae were weighed as they were placed upon the new diets and again at 4, 8, and 15

days, at which time the experiment was terminated. Mortality and frequency of molts were recorded. All diets were replaced daily.

Description of Liver Extract Diet Experiments

In all of the following experiments where only second instar larvae were used the first instars were fed live greenbug until a second molt was achieved. The larvae were then block randomized by weight, light to heavy, and placed on their respective diets. If the first instar larvae were utilized they were placed directly onto the diet 12-24 hours after they hatched, unless randomization was specifically infered. All larvae, pupa and adults were weighed on a precision balance of 5.0 mg capacity. All larvae were placed in individual cups, with 10-15 individuals per treatment (diet).

Liver L Extract Diet vs. Non-Liver Diet

In this experiment liver extract L containing the alcohol insoluble portion of the liver was incorporated into a combination of the V1 (Table 3) and standard maintenance diets (Table 1) and is denoted as diet L2 (Table 6). It was then compared with three control diets. The experimental treatments are as follows:

1) The control consisted of exclusion of the liver L extract, and is referred to as the non-liver diet or NL1 (Table 6).
2) Larvae in this group were fed the standard maintenance diet.
3) Larvae in this group were fed an excess of live greenbug.
4) This group received the liver L extract within the diet.

Second instar larvae, previously fed live greenbug, were utilized. Fifteen individuals were placed upon each treatment for a total of 60

larvae. Larvae were block randomized by weight, light to heavy, before being placed on the treatments. Weights were recorded daily from placement on the diet to emergence. Dates for the first, second and third molts, pupation and emergence were recorded along with mortality. Upon emergence the larvae were maintained for a 48-hour period of observation after which, the experiment was terminated. All diets were replaced daily.

Nutrient Deletion Experiments

This experiment was designed to serve as the first of a series of experiments to delete extraneous nutrient sources from the L2 diet (Table 6), thereby determining specific dietary requirements and establishing a more defined synthetic medium. Second instar larvae, previously fed live greenbug, were utilized placing eleven individuals per treatment for a total of 44 larvae. The experimental treatments are as follows:

1) Larvae were fed an excess of live greenbug.
2) Larvae received diet L5 (Table 6), replacing liver extract L with a combination of liver extract 2 and liver concentrate (1:20).
3) Larvae received diet L6 (Table 6), with a deletion of the amino acids tryptophan and cystine.
4) Larvae received diet L7 (Table 6), with a deletion of CSM (corn-soy-milk blend) and powdered milk.

Weights were recorded for the second and third molts, pupation, and emergence. Dates for the afore mentioned developmental stages were also recorded along with mortality. Emerging adults were maintained for observation, after which the experiment was terminated. All diets were

changed daily.

Liver L Extract Diet vs. Modified Vanderzant Diets

This experiment constrasted several experimental diets as to their ability to enable larvae to attain adequate growth and development in contrast to a control diet. Second instar larvae were used placing twelve individuals per treatment for a total of 48 larvae. The diet cups were replaced and data recorded as described in the previous experiment. The experimental treatments are as follows:

1) A control group of larvae were fed an excess of live greenbug.
2) A control group of larvae were fed diet L4 (Table 6) containing liver extract S, a completely solubilized liver component.
3) Larvae were fed diet V2 based on a modification of diet V1 (Table 3).
4) Larvae were fed diet V3 based on a modification of diet V1 (Table 3), with addition of liver extract S.

Comparison of Liver Extracts L, 2, and S

Three liver extracts L, 2, and S were each incorporated into separate diets without variation in the basal nutritive components. Second larval instars were used placing twelve individuals per treatment for a total of 48 larvae. The diet cups were replaced and data recorded as described for the previous experiment. The experimental treatments were as follows:

1) A control group fed an excess of live greenbugs.
2) Larvae receiving diet L2 (Table 6), containing liver extract L

or the alcohol in soluble portion of the liver.

3) Larvae receiving diet L3 (Table 6), containing the liver extract 2 or all but the water soluble portion of the liver.

4) Larvae receiving diet L4 (Table 6), containing the entirely solubilized liver S extract.

Development of First Instar Larvae on Liver Extract L

The object of this experiment was to determine the ability of first instar larvae to grow and develope, without prior feeding upon live greenbug or any other nutritive source, except the remaining egg yolk, on a liver diet L2 (Table 6). Twelve 12-24 hour old larvae were block randomized by weight, light to heavy, and placed directly onto their respective diets. A total of 36 larvae were utilized. The diet cups were replaced and data recorded as described in the previous experiment. However, a record of the date of the first molt and weight immediately after is also recorded. The experimental treatments are as follows:

1) A control group of larvae were fed an excess of live greenbug.

2) A control group of larvae were placed upon the standard maintenance diet.

3) Larvae received diet L2 (Table 6), containing the liver L extract.

High Protein Diet vs. High Carbohydrate Diet

The protein components of one experimental diet and the carbohydrate components of a second experimental diet were increased. Both diets were evaluated as to their ability to stimulate growth and development of

first instar larvae. Eleven 12-24 hour old larvae were placed on their respective diets for a total of 44 larvae. Larvae were weighed after being on the diets for three days and again on the sixth day of the experiment. The dates of all molts and mortalities were also recorded. The experimental treatments were as follows:

1) A control group fed an excess of live greenbug.
2) Larvae receiving diet L9 (Table 6), with an increase from 20 g to 40 g of D-glucose.
3) Larvae receiving diet L10 (Table 6), with an increase from 20 g to 30 g of enzymatic casein hydrolysate.
4) A control group of larvae receiving diet L4 (Table 6), containing liver extract S.

Adult Observations

After termination of all experiments involving liver diets, the surviving adults were mated and placed on a live greenbug diet as described in the section "Maintenance of the Convergent Lady Beetle Colony". Preliminary observations of frequency of copulation, oviposition, and fecundity were recorded. Similar preliminary experiments were run by maintaining the mated pairs on diet L2 (Table 6).

CHAPTER III

RESULTS AND DISCUSSION

Protein and Hexose Levels

The percent total protein within the supernatant was determined to be 5.05%/1 g of greenbug. The total protein within a total homogenate was approximately 12%/1 g of greenbug.

When the anthrone assay was run for the supernatant only, the percent of free hexose was found to be 0.97% within the supernatant/1 g of greenbug. It was hoped that estimates for total protein and free hexose would provide an estimate for formulation of protein: carbohydrate ratios in synthetic diets. Unfortunately, preliminary tests utilizing the above ratios did not promote the growth or development of H. convergens larvae.

Effects of Various Diet Presentation Techniques

The cellulose cubes proved unsuccessful as a means of synthetic diet presentation to the convergent lady beetle larvae. The first and second instar larvae became entrapped within the crevices of cellulose and drowned. Latter instars became supersaturated and most died within two days.

The cellulose cubes were later wrapped in fine cheese cloth, before saturation with diet, to prevent early instars from drowning. A hole was also cut into the cups lid and covered with fine screening to allow

aeration and prevent supersaturation of larvae. However, the earlier instar larvae continued to become entrapped within the cheese cloth and drown. 70% of the third and fourth instars became supersaturated and died. Those pupating became supersaturated and did not emerge.

The paraffin capsules were not successful in maintaining the diet utilized. Apparently, the fatty acids and sterols were able to penetrate the paraffin and parafilm resulting in leakage and drying of the diet. Coccinellids would feed on the capsules, however, very few molts were achieved (Hagan 1965), and many early instars drowned within the leaking diet.

The simple agar based diets proved most successful of all the techniques examined. All larval instars walked unencumbered upon the media and about the cup. Saturated cotton balls could also be placed atop the agar media without producing a sticky surface endangering the larvae. However, the diet must be replaced daily to prevent general deterioration of nutritional quality and build up of toxic products from lipid oxidation (Mittler 1972).

Effects of Preliminary Diet Experiments

Effect of Fresh Banana Diet on Oviposition

Those individuals placed on the live greenbug control oviposited fertile eggs throughout the experiment as expected. Those adults placed on the standard maintenance diet (Table 1) laid eggs during the first two days of the experiment and ceased to do so afterwards. However, they did feed on the diet. This was confirmed by dissecting individuals at the termination of the experiment and observing the gut contents. The beetles placed upon the fresh banana diet also oviposited fertile eggs

but only during the first two days of the experiment (Table 8). All pairs continued copulating and feeding throughout the experiment inspite of their diets.

The inability of H. convergens to oviposit when fed a fresh banana diet in the present experiments conflict with Smith's results (1965b). However, Smith provided no information pertaining to the number of eggs deposited or the frequency of oviposition throughout the experiment.

Effect of Miscellaneous Diets on Larvae

The first instar larvae placed upon the synthetic diet developed by Akey and Beck (1972) for the pea aphid, A. pisum, did not molt during the experiment, nor was there an increase in weight. However, such a diet provided adequate nutrition to sustain 35% of the individuals for two weeks. All larvae died on this treatment before the third week. Second instar larvae placed upon the synthetic pea aphid diet molted once and 50% survived the experiment. The one molt achieved by the second instar larvae is probably due to nutrients acquired during the prior instar, when these individuals were maintained on a live greenbug diet.

The standard maintenance diet surpasses the pea aphid diet in its ability to provide adequate sustanence to allow increased survival for a longer duration. The extended time spent in the larval period was not observed to affect later development severely when first or second instar larvae were placed upon a live greenbug diet.

First and second instar larvae placed on a synthetic diet rich in sterols and fatty acids (Table 7) suffered a 100% mortality within two days of placement on the diet. It is quite feasible that either the

high levels were toxic or toxic products from the oxidation of lipids collected within the diet and resulted in the observed high mortality. Mittler (1972) mentions the oxidation of lipids within diets as a common form of toxicity and a parameter to safe guard against when developing synthetic diets.

Apparently, when first instar larvae were placed upon the V1 diet (Table 3) 15% drowned due to entrapment in the diet. The remaining larvae achieved one molt before becoming supersaturated or also drowned. On the same diet, 50% of the second instar larvae molted twice and 10% pupated. However, the pupae becamse saturated with the diet and no emergence was observed.

Both first and second instar larvae placed upon the live greenbug control diets molted, pupated and emerged within the first two weeks of the experiment.

First instar larvae placed upon the standard maintenance diet (Table 1) did not molt during the experiment. However, 65% of the larvae survived the experiment. When these larvae were placed upon a live greenbug diet, 50% molted and reached maturity. The second instar larvae placed on the stardard maintenance diet achieved one molt and 80% survived the experiment. When the surviving second instar larvae were placed upon a live greenbug diet 70% attained maturity.

The difficulty involved in presenting a liquid diet to insects is also mentioned by Hagan (1950) and Vanderzant (1969). It is evident that one or more nutrient components within Vanderzant's diet V1 (Table 3) stimulate growth and development, to a slight degree, in H. convergens. However, a more practical method of presenting the diet must be developed to observe the full extent of the diet's stimulatory effect upon the

growth and development of larvae.

Effect of D-glucose vs. Sucrose on Larvae

A prior 24 hour feeding period, by newly hatched larvae, upon agar, agar and D-glucose, agar and sucrose, and a standard maintenance diet control (Table 1) did not illict a significant difference in molting frequency when all larvae were placed on a standard maintenance diet.

During preliminary experiments the standard maintenance diet proved capable of sustaining the larvae for over a one week period. However, it did not promote growth or induce molting.

Table 10 shows a total of zero molts observed for each treatment. Larvae provided with live greenbug during the prior 24 hour feeding period achieved a total of twelve molts.

At weigh dates one and two, there was a significantly greater weight gain for those larvae feeding upon live greenbug compared to the remaining diets (Table 11) (Figure 4). There was no significant difference in mean growth rate or mean death rate observed between the other five diets analyzed.

It is evident that the nutrient ratio and composition of live greenbug are adequate to provide a significantly greater weight gain during the 24 hour feeding period. However, once moved to the standard maintenance diet the growth rate slowed to that observed for the remaining four diets.

Effects of Supplemental Water on the Larvae

In numerous instances a supplemental supply of water enhances the development of insects (Edney 1957). The importance of drinking water

Table 10. Molting frequency of larvae as affected by a 24-hour period of feeding on live greenbugs, agar, various nutrients in an agar base, and a standard maintenance diet.

Treatment	Did not molt	Molted only once	Molted twice	Total molts[1]
Agar	12	0	0	0
Dextrose-agar	12	0	0	0
Sucrose-agar	12	0	0	0
Standard diet	12	0	0	0
Live greenbug	0	10	2	12

[1]Refers to the total number of individual larvae molting at least once per treatment.

Table 11. Mean weight, death rate, and growth rate of larvae after a 24-hour period of feeding on live greenbugs, agar, various nutrients in an agar base, and a standard maintenance diet.

Treatment	$\bar{X}$ Weight one (mg)[1,2]	$\bar{X}$ Weight two (mg)[1,2]	Days to death[1,3]	$\bar{X}$ Growth w_2-w_1 (mg)[1]
Agar	0.211 a	0.208 a	8 a	0.012 b
Dextrose-agar	0.234 a	0.255 a	9 a	0.021 b
Sucrose-agar	0.217 a	0.216 a	8 a	0.013 b
Standard diet	0.201 a	0.212 a	10 a,b	0.015 b
Live greenbug	0.639 b	0.706 b	12 b	0.065 b
Standard deviation	0.137	0.160	4.401	0.069

[1]Means followed by the same letter, within a column, are not significantly different at the 5 % level (Duncan).

[2]Weights taken on the third and sixth days after placement on the standard diet.

[3]Counted from the day of placement on the above experimental treatments.

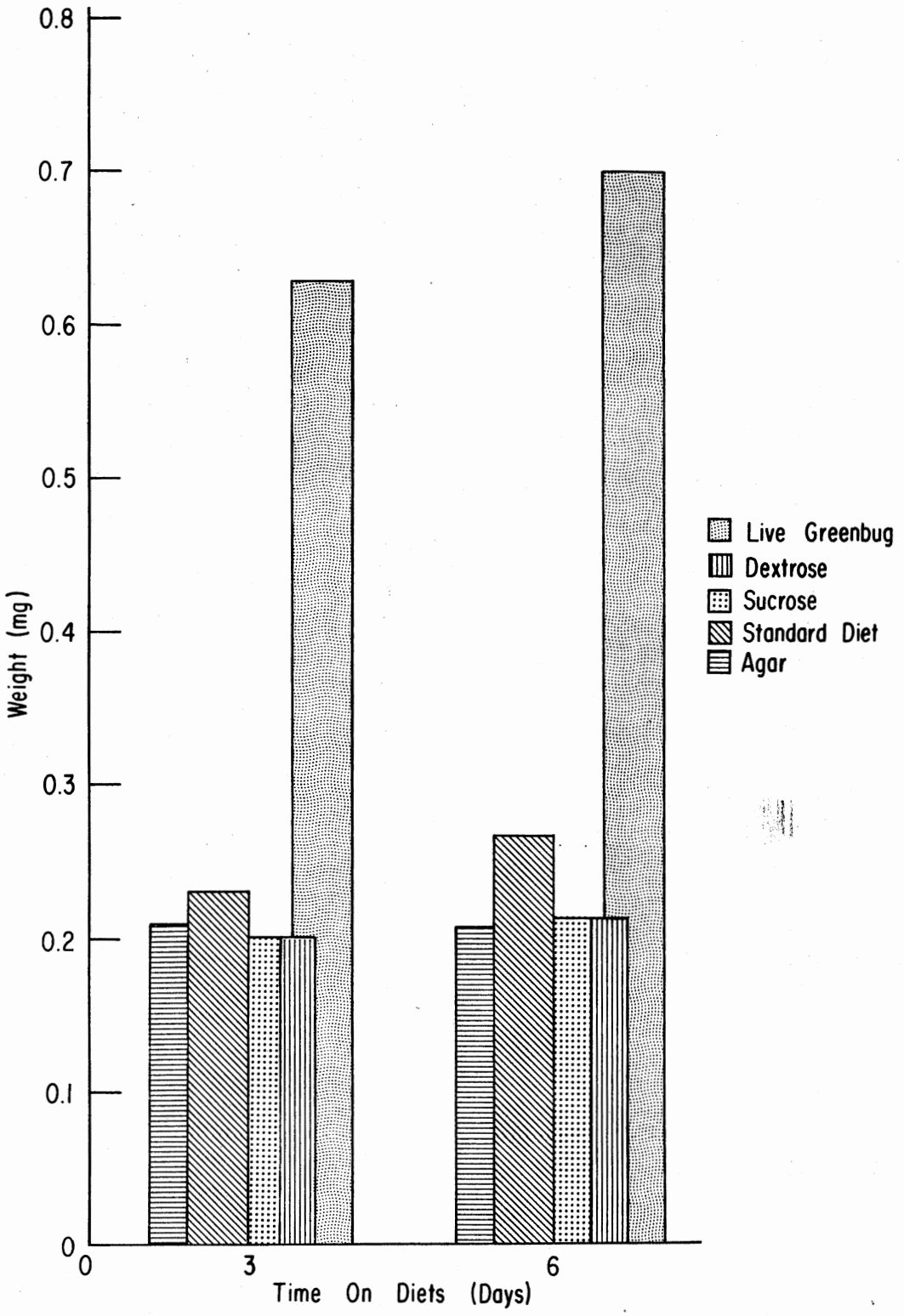

Figure 4. Mean weight of larvae after a 24-hour period of feeding on live greenbug, agar, various nutrients in an agar base, and a standard maintenance diet

to larvae has also been discussed by Mellanby and French (1958). However the majority of their work deals with insects other than predators. Smith (1965a) studied the effects of drinking water on the adult lady beetle Coccinella trifasciata, showing significant differences between individuals with a supplemental water source and those without water. Both adult males and females showed increased weight gains with a supplemental source of water. Up to this point very little data has been available pertaining to the water requirements of predatory coccinellid larvae.

Larvae placed on the treatment without food or water and the treatment with only water suffered 100% mortality within the first two days of the experiment. When testing food and water and food and no water, there was no significant difference in weights recorded on the fifth, tenth, or fifteenth days for those individuals on the standard maintenance diets with water or without water (Table 12) (Figure 5).

Weights recorded for the first, second and third molts of larvae placed on live greenbug with water and live greenbug without water show no significant difference (Table 13) (Figure 6). However, the mean weight recorded at pupation for those larvae feeding on live greenbug with a supplemental source of water was observed to be significantly different. There was no significant difference between the mean weights of the two treatments at emergence. Neither was there an observed significant difference in the time required for larval development between the two treatments (Table 14) (Figure 7).

It is probable that an adequate supply of water was present within the standard maintenance diet, thereby, excluding the necessity of an extraneous source of water. However, this does not preclude the possi-

Table 12. The effects of a standard maintenance diet with and without a supplementary source of water over a 15 day period upon mean larval weights throughout various periods of development.

Treatment	Initial weight (mg)[1]	After 5 days (mg)[1]	After 10 days (mg)[1]	After 15 days (mg)[1]
Standard diet with water	0.168 a	0.201 a	0.381 a	0.367 a
Standard diet without water	0.157 a	0.194 a	0.364 a	0.284 a
Standard deviation	0.041	0.039	0.084	0.111

[1]Means followed by the same letter, within a column, are not significantly different at the 5 % level (LSD).

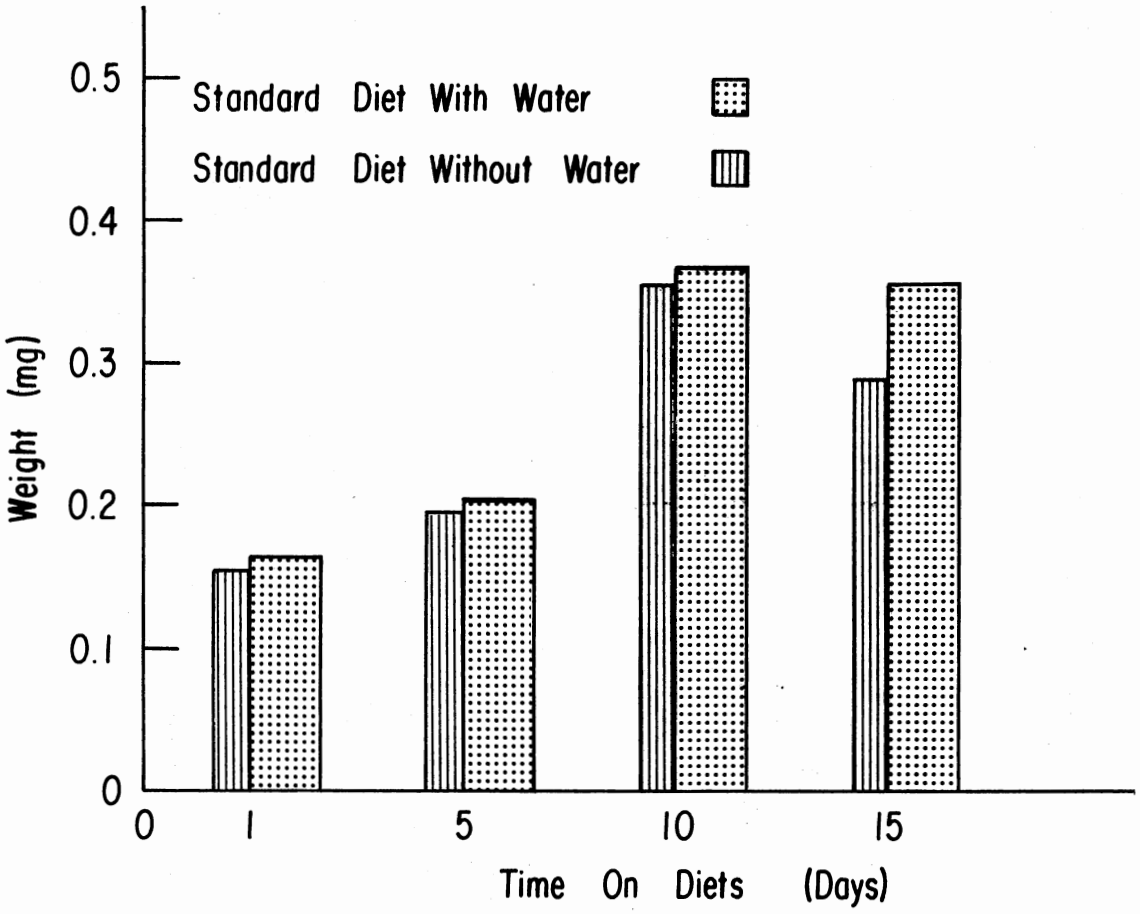

Figure 5. The effects of a standard maintenance diet with and without a supplementary source of water over a 15 day period upon mean larval weights throughout various periods of development

Table 13. The effects of live greenbugs with and without a supplementary source of water upon mean larval weights throughout various periods of development.

Treatment	1st Molt (mg)[1]	2nd Molt (mg)[1]	3rd molt (mg)[1]	Pupation (mg)[1]	Emergence (mg)[1]
Live greenbug with water	0.156 a	1.175 a	6.534 a	21.415 a	17.721 a
Live greenbug without water	0.172 a	0.894 a	5.985 a	20.008 b	16.188 a
Standard deviation	0.035	0.277	1.447	3.017	2.812

[1]Means followed by the same letter, within a column, are not significantly different at the 5 % level (LSD).

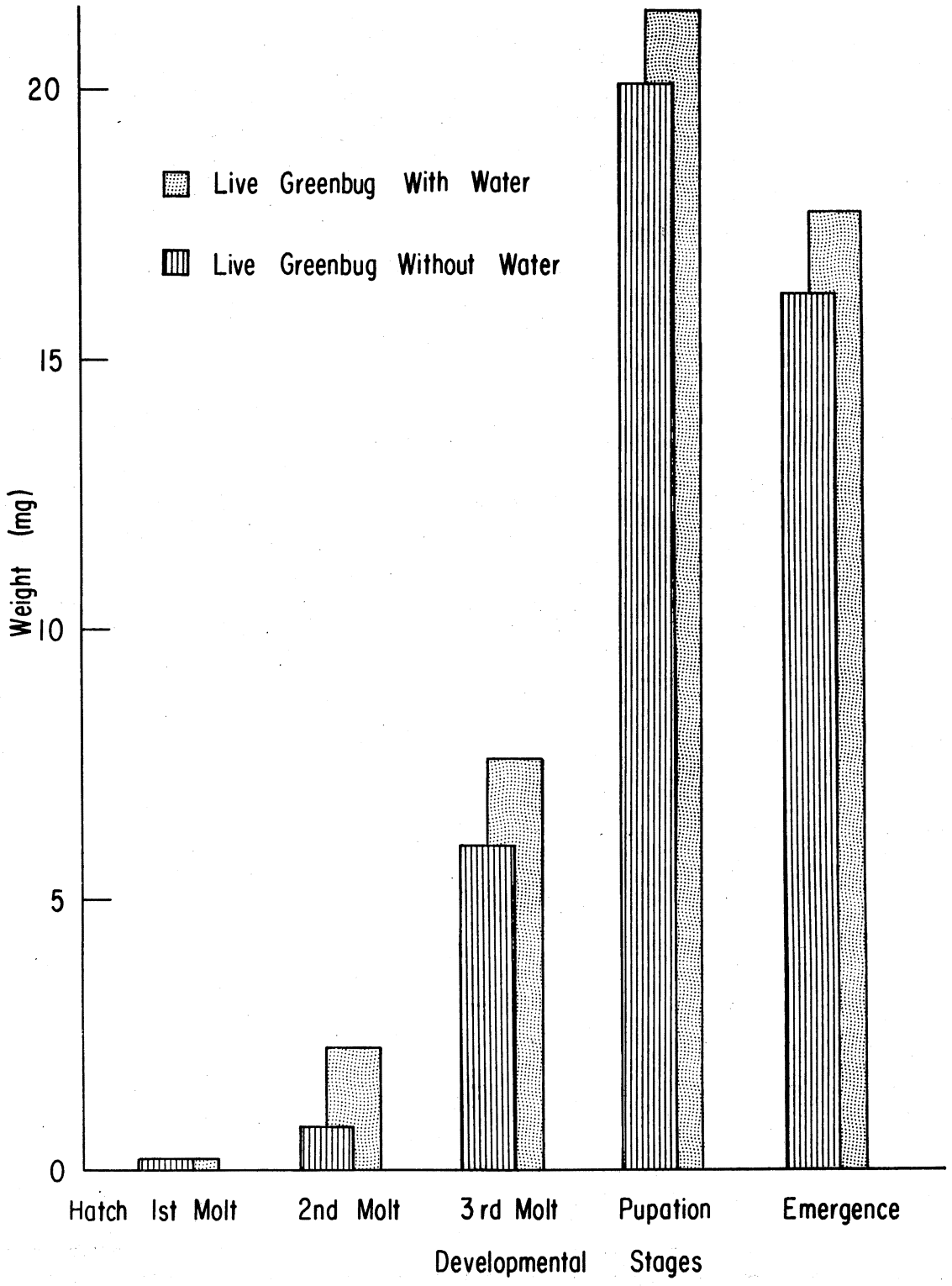

Figure 6. The effects of live greenbug with and without a supplementary source of water upon mean larval weights throughout various periods of development

Table 14. A comparison of the time (days) required for larval maturation as affected by feeding upon live greenbugs with and without a supplementary source of water.

Treatment	1st molt[1]	Inter[2]	2nd molt[1]	Inter[2]	3rd molt[1]	Inter[2]	Pupation[1]	Inter[2]	Emergence[3]
Live greenbug with water	2.00 a	2.50	4.50 a	3.05	7.55 a	3.19	10.74 a	3.26	14.00 a
Live greenbug without water	2.00 a	2.60	4.60 a	3.23	7.83 a	3.29	11.12 a	3.55	14.67 a
Standard deviation	0	----	0.601	----	0.837	----	0.768	----	0.891

[1]Means followed by the same letter, withon a column, are not significantly different at the 5 % level (Duncan).

[2]Interval time between developmental stages.

[3]Three days time added to compensate for the interval between hatch and the first molt.

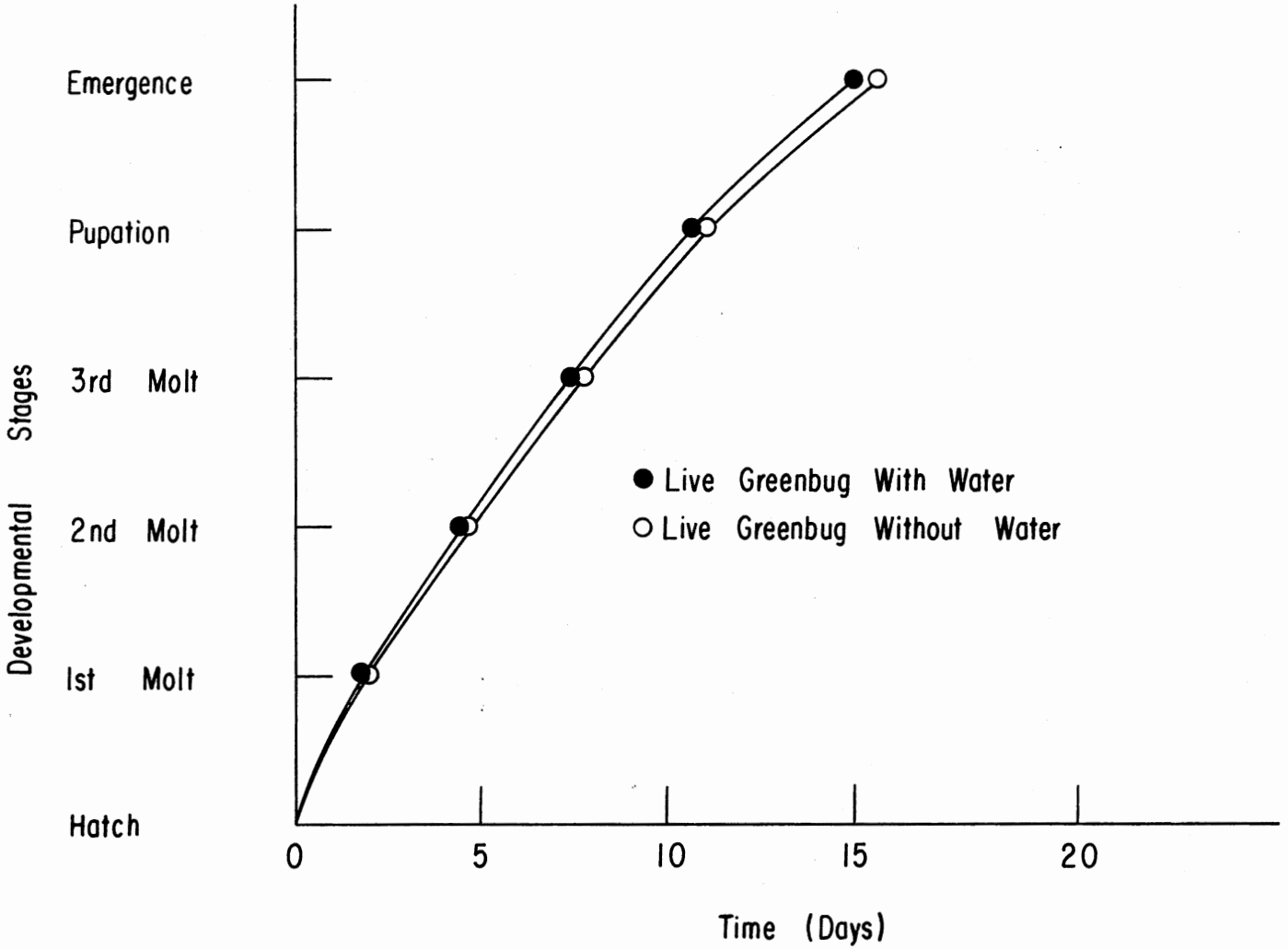

Figure 7. A comparison of the time (days) required for larval maturation as affected by feeding upon live greenbug with and without a supplementary source of water

bility for a requirement of supplemental water supplies for later instars or adults placed on the standard maintenance diet.

From this experiment, it is evident that survival of first instar larvae is dependent on parameters other than solely the presence of water. Those individuals developing on the live greenbug treatments appear to obtain sufficient moisture from their prey. The significant gain in mean weights observed at the pupal stage (Table 13) (Figure 6) are not explained but may be due to direct absorbtion of moisture from the air (Mellanby and French 1958). There may also be an increased requirement for water just before pupation.

Effects of Cannibalism on Larval Growth

When the larvae were allowed to cannibalize and feed on live greenbug 24 hours prior to their placement on standard maintenance diets 91.6% achieved one molt. No mortality was recorded. When larvae were hatched from isolated eggs and fed live greenbug 24 hours prior to their placement on a standard maintenance diet, 75.0% achieved one molt with no mortality. Larvae allowed to cannibalize for 24 hours prior to placement on a standard maintenance diet but provided no greenbug did not molt and had a 16.67% mortality. Larvae placed directly onto a standard maintenance diet with no prior 24 hour feeding period also did not molt and had a 16.67% mortality (Table 15).

A significantly greater mean weight was observed for the initial weight in, successive weigh dates, and maximum growth difference (Table 16) (Figure 8) of the following treatments: cannibalizing larvae and non cannibalizing larvae hatched in isolation, both provided with live greenbug, than was observed for treatments where the larvae cannibalized

Table 15. Frequency and percent molting, the number of mortalities, and percent mortality on a standard maintenance diet after a prior 24-hour feeding period on live greenbugs, standard diet, or cannibalization.

Treatment	No. reaching the 1st molt	Percent molting	No. of mortalities	Percent mortality
Cannibalize and live greenbug	11	91.6	0	0
Cannibalize	0	0	2	16.67
Isolated and standard diet	0	0	2	16.67
Isolated and live greenbug	9	75.0	0	0

Table 16. Mean weights and growth rate of larvae on a standard maintenance diet after a prior 24-hour feeding period on live greenbugs, a standard diet, or cannibalization.

Treatment	Initial weight (mg)[1]	After 4 days[1]	After 8 days[1]	After 15 days[1]	Maximum diff. Tot. growth period (mg)[1]
Cannibalize and live greenbug	0.497 a	0.653 a	0.813 a	0.725 a	0.332 a
Cannibalize	0.384 b	0.380 b	0.371 b	0.384 b	0.241 b
Isolated and standard diet	0.165 c	0.267 b	0.257 b	0.290 b	0.154 b
Isolated and live greenbug	0.510 a	0.671 a	0.666 a	0.805 a	0.293 a
Standard deviation	0.126	0.140	0.237	0.144	0.178

[1]Means followed by the same letter, within a column, are not significantly different at the 5 % level (Duncan).

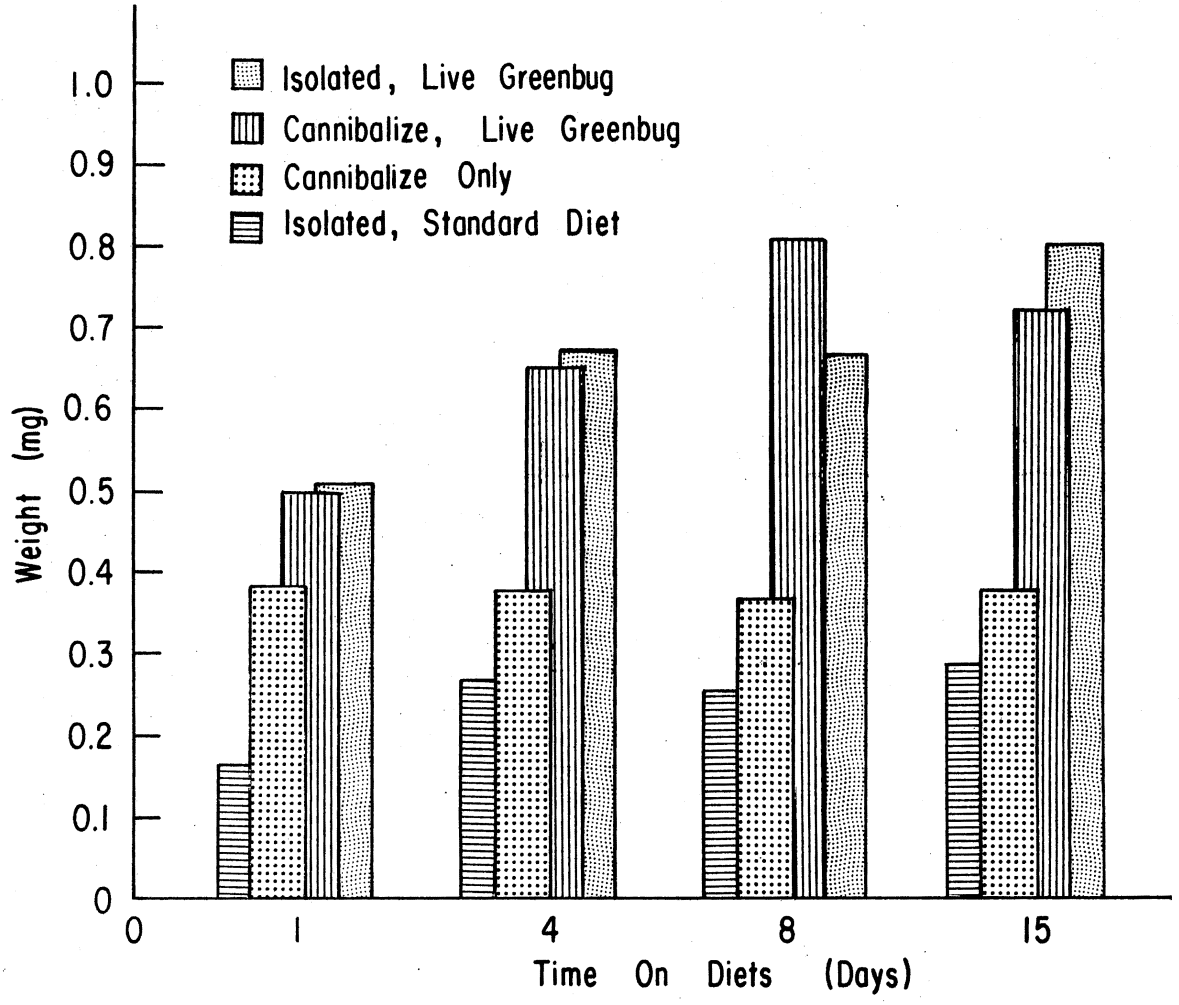

Figure 8. Mean weights of larvae on a standard maintenance diet after a prior 24-hour feeding period on live greenbug, a standard diet, or cannibalization

only, without live greenbug and where the larvae were hatched in isolation and placed upon a standard maintenance diet without a 24 hour feeding period. The growth rates of cannibalizing larvae and non cannibalizing larvae hatched in isolation, showed no significant difference on the standard maintenance diet.

The significantly higher mean weights per weigh date, greater growth rate and higher molting frequency observed for larvae within the treatments allowing a prior 24-hour feeding period are, evidently manifestations of the nutrient benefit received from the live greenbug. Cannibalism alone increased the initial weight, but did not maintain a weight significantly greater than isolated hatch larvae on a standard maintenance diet throughout the experiment.

Among predatory coccinellids cannibalism of unhatched sibling eggs apparently provides an important additional source of food for the first instar larvae, allowing them to complete the instar when prey density is low and thus gain additional strength and energy to pursue an extended searching pattern (Brown 1972; Hodek 1973; Dixon 1959; Dimetry 1974; Kaddou 1960; and Banks 1956 and 1957). The significance of the greater initial weight of cannibalizing larvae in these studies over that of isolated hatch larvae on standard diets coincides with the above authors findings in the field.

Effects of Various Liver Extract Diets on the Larvae

Effect of Liver Extract L Diet (L2)

In tests conducted to determine the effects of liver extracts in diets a significant difference was observed for the mean maximum weights

of larvae between each treatment in the liver L tests (Table 17). Those fed live greenbug achieved a greater weight than those individuals on the liver extract L (L2) diet (Table 6), the non-liver diet (NL1), (Table 6) and the standard maintenance diet attaining the least. Weights gained by larvae on the above diets were heaviest to lightest, respectively.

No significant difference in molting frequency by the larvae was observed on the live greenbug, L2, and NL1 diets. However, only four molts were achieved for those larvae on the standard maintenance diet (Table 18). Only a slight difference was observed in pupation between the first three diets, whereas, not one individual on the standard maintenance diet pupated. There was no difference in emergence between the live greenbug and L2 diets. However, there was a pronounced difference between the afore mentioned treatments and the non-liver (NL1) diet. Only 60% of the larvae emerged on the NL1 diet, considerably less than the above two treatments (Table 18).

The mean weights for those individuals feeding upon live greenbug were significantly higher than those individuals feeding upon the L2 diet (Table 19) (Figure 9). A significant difference was observed for the total time required for larval development between the live greenbug and L2 diets (Table 20) (Figure 10). Those larvae on the L2 diet required an additional 7.38 days to achieve adult development beyond the time required for those to mature that were fed live greenbug. A significant difference was also observed in the time required from the first to second molt, second to third molt, and third molt to pupation. However, the amount of time in the pupal stage was essentially equal for insects reared on either diet.

It is obvious that the L2 diet will not serve as an adequate substi-

Table 17. A comparison of mean maximum weights from larvae which have fed and developed on either live greenbugs or one of three artificial diets.

Treatment	Individuals/treatment[1]	$\bar{X}$ Maximum wt. (mg)[2]
Live greenbug	15	13.719 a
Liver L diet[3]	15	12.255 b
Non liver diet	15	10.167 c
Standard diet	15	2.140 d
Standard deviation		5.690

[1] $\bar{X}$ maximum weights were recorded on the second day of the fourth larval instar.

[2] Means followed by the same letter, within a column, are not significantly different at the 5 % level (LSD).

[3] Liver L was purchased from the United States Biochemical Corporation.

Table 18. Molting frequency, pupation frequency and percent, and emergence frequency and percent of insects as effected by development on live greenbugs vs. three artificial diets.

Treatment	Did not molt	Molted only once	Molted twice	Total[1] molts
Live greenbug	1	0	14	14
Liver L diet	2	0	13	13
Non liver diet	0	0	15	15
Standard diet	11	3	1	4

Treatment	Did not pupate	No. pupating	% pupation
Live greenbug	1	14	93
Liver L diet	2	13	87
Non liver diet	3	12	80
Standard diet	15	0	0

Treatment	Did not emerge	No. emerging	% emergence
Live greenbug	2	13	87
Liver L diet	2	13	87
Non liver diet	6	9	60
Standard diet	15	0	0

[1]Refers to the total number of individual larvae molting at least once per treatment.

Table 19. The effect of live greenbugs and an artificial diet containing a liver extract on mean larval weights throughout various periods of development.

Treatment	2nd molt (mg)[1]	3rd molt (mg)[1]	Pupation (mg)[1]	Emergence (mg)[1]
Live greenbug	3.00 a	5.80 a	12.62 a	11.53 a
Liver L diet	1.48 a	4.52 b	11.66 b	9.98 b
Standard deviation	2.32	2.89	5.76	5.69

[1]Means followed by the same letter, within a column, are not significantly different at the 5 % level (LSD).

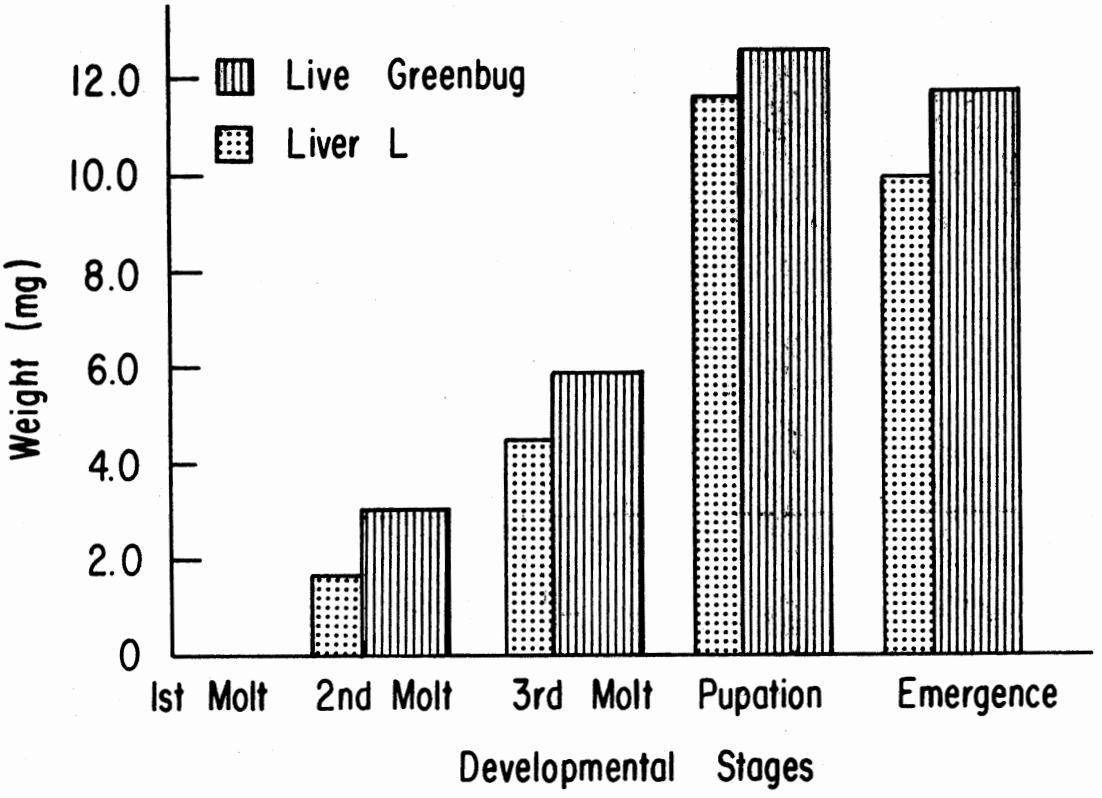

Figure 9. The effect of live greenbugs and an artificial diet containing a liver extract on mean larval weights throughout various periods of development

Table 20. A comparison of time required (days) for larval maturation as effected by feeding upon live greenbugs and an artificial diet containing a liver extract.

Treatment	2nd molt (days)	Inter.[2]	3rd molt	Inter.[2]	Pupation[1]	Inter.[2]	Emergence[1]	Hatch to Adult[3]
Live greenbug	2.21 a	2.22	2.21 a	5.21	9.64 a	4.21	13.85 a	18.85 a
Liver L diet	4.31 b	4.38	4.38 b	7.62	16.31 b	3.92	20.23 b	26.23 b
Interval diff.	----	2.16	----	2.41	-----	0.29	-----	-----
Standard deviation	1.99	----	2.99	----	4.62	----	4.75	-----

[1]Means followed by the same letter, within a column, are not significantly different at the 5 % level (LSD).

[2]Interval time between developmental stages.

[3]Three days time added compensating for the interval between hatch to the first molt.

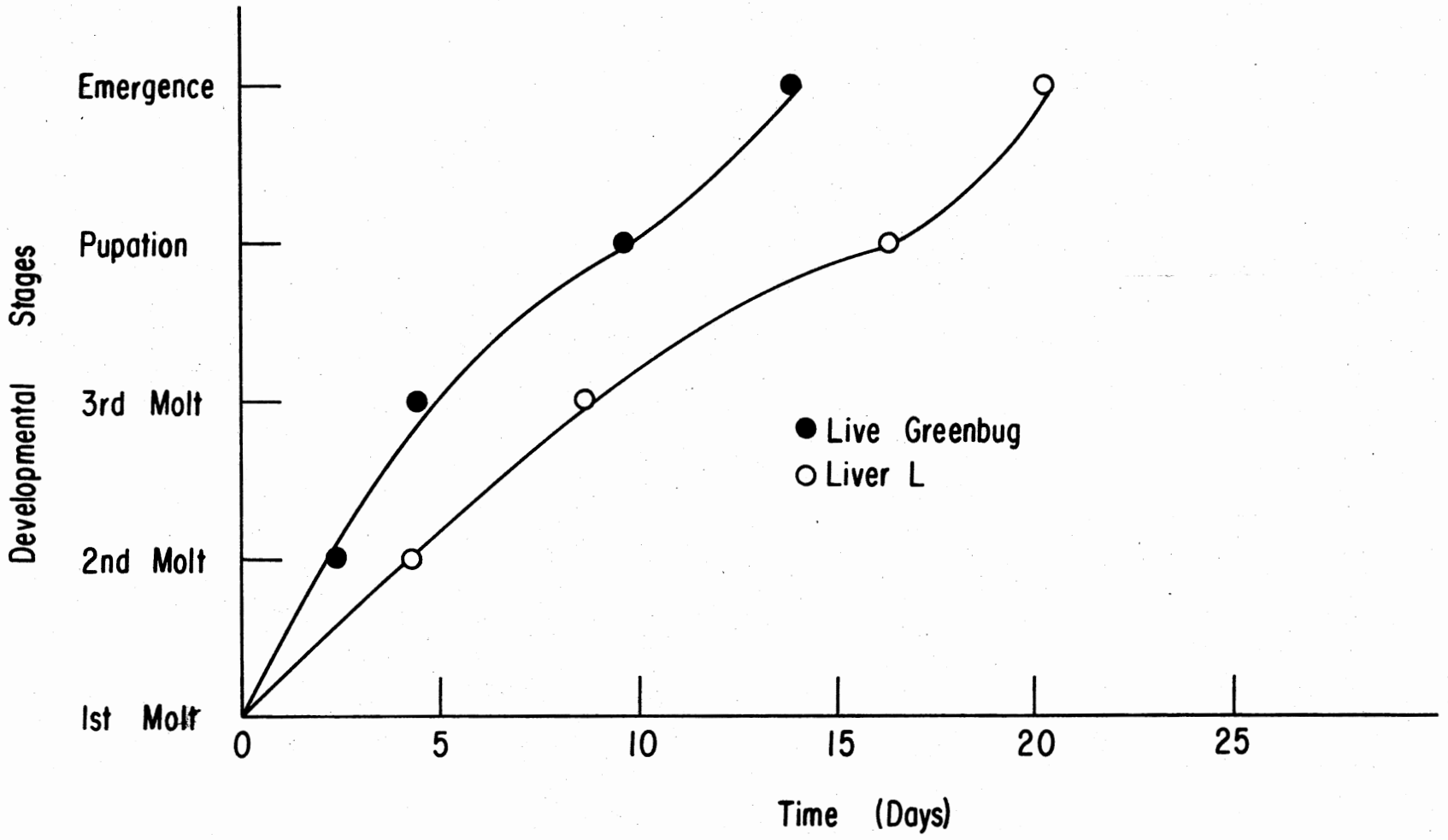

Figure 10. A comparison of time required for larval maturation as effected by feeding upon live greenbug and an artificial diet containing a liver extract

tute for the natural prey of the convergent lady beetle. However, the qualities inherent within the liver L extract, which is mainly the alcohol insoluble portion of the liver consisting of lipids and sterols, create a diet surpassing both the NL1 and standard maintenance diets in ability to stimulate growth and development of the larvae. These data may give a clue as to what portion of the artificial diets might be inadequate and require further investigation in the development of an acceptable diet.

Effect of Nutrient Deletions

Tryptophan is generally listed as one of the ten essential amino acids for insects and is significant in the formation of phenoxazine eye pigments, and ommochromes (Rockstein 1965). However, cystine (cysteine) is not listed as an essential amino acid but it has been found that lack of this amino acid effects moulting and growth in some insects (House 1972; Davis 1968c; and Freind 1958). Rockstein (1965) also mentions that omission of cystine on studies with Tribolium, Drosophila, Aedes, Lucilia, and Blattella has no effect on the number of larvae that reach the pupal stage nor the maturation time. However, it seems to be essential for normal pupal formation and metamorphosis. The following results of these experiments parallel Rockstein's statement.

In Table 21 it can be seen that no significant difference was observed for molting frequency nor for frequency of pupation between the diets L5, L6, and L7, however, the frequency of emergence did differ. On diet L6, considerably fewer emerged (54.5%) and, of those which emerged 66.7% did so without fully developed tibia or tarsi on one to all legs. This deformity also appeared in 10.0% of the emergents from the L7 diet.

Table 21. Molting frequency, pupation frequency and percent, and emergence frequency and percent of insects as effected by development on either live greenbug, an artificial liver diet, or two deletion diets.

Treatment	Did not molt	Molted only once	Molted twice	Total molts
Live greenbug	1	0	10	10
L5 (Liver conc. (1:20) plus liver 2	0	0	11	11
L7 (w/o CSM and powdered milk)	0	0	11	11
L6 (w/o Tryptophan and cystine)	2	0	9	9

Treatment	Did not pupate	No. pupating	% pupation
Live greenbug	1	10	90.9
L5	0	11	100.0
L7	1	10	90.9
L6	2	9	81.8

Treatment	Did not emerge	No. emerging	% emergence
Live greenbug	1	10	90.9
L5	1	10	90.9
L7	1	10	90.9
L6	5	6	54.5

[1]Refers to the total number of individual larvae molting at least once per treatment.

No deformities were encountered among emergents from the L5 diet or the live greenbug controls.

The mean weights for the larvae on the live greenbug treatment were significantly greater at all developmental stages than the remaining treatments. There was no significant difference observed for mean weights between the liver control (L5) and each of the two deletion diets (L6 and L7) (Table 22) (Figure 11) in spite of the deformities.

Larvae placed upon the live greenbug treatment required significantly less time to mature from hatch to adult, 16 days, than the remaining three diets which were 24.6, 26.0, and 26.83 days, respectively (Table 23) (Figure 12). No significant difference was observed for the total time required for metamorphosis or time within developmental stages between the liver control diet (L5) and each of the deletion treatments (L6 and L7).

From the above results it seems that either one or both of the amino acids tryptophan and cystine are necessary nutrient components of the liver control diet (L5). It may be assumed that either the insects do not possess the ability to synthesize these amino acids, or their deletion simply causes an imbalance within the nutrient ratio of the diet to an unfavorable degree. The resulting deformities produced by the omission of CSM and powdered milk could possibly be the result of deletions of unknown nutrient factors or simply an upset in the nutrient ratio of the diet. Since deformities were similar on both L6 and L7 diets, the necessary nutrients obtained from CSM and powdered milk could possibly be the two amino acids.

Table 22. The effect of live greenbugs, an artificial liver diet, and systematic deletion of nutrients from the artificial diets upon mean larval weights throughout various periods of development.

Treatment	2nd molt[1] (mg)	3rd molt[1] (mg)	Pupation[1] (mg)	Emergence[1] (mg)
Live greenbug	2.78 a	8.03 a	21.37 a	15.71 a
L5 (Liver conc. (1:20) plus liver 2)	1.69 b	4.20 b	10.68 b	8.61 b
L7 (w/o CSM and powdered milk)	1.50 b	4.53 b	10.73 b	7.89 b
L6 (w/o Tryptophan and cystine)	1.80 b	4.68 b	11.50 b	9.69 b
Standard deviation	0.39	1.25	2.31	1.95

[1]Means followed by the same letter, within a column, are not significantly different at the 5 % level (LSD).

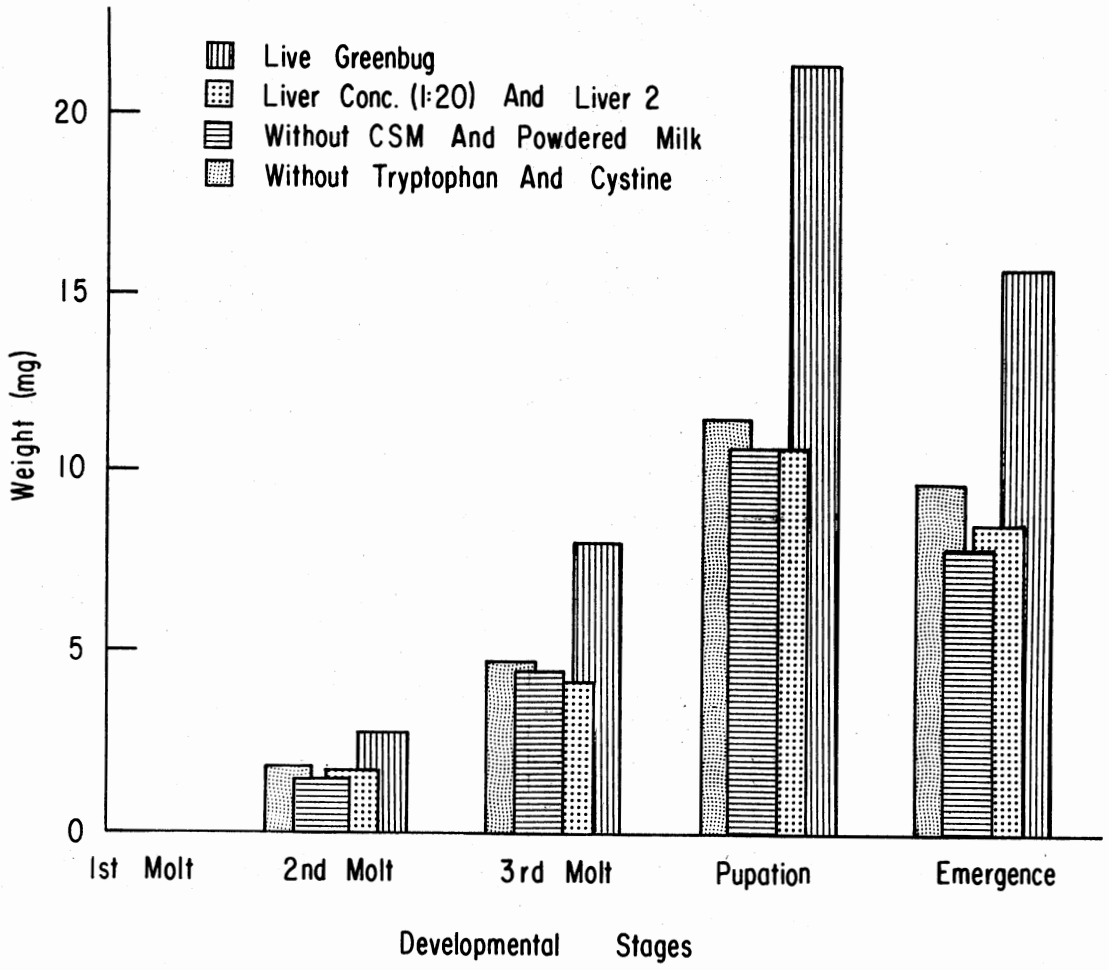

Figure 11. The effect of live greenbug, an artificial liver diet, and systematic deletion of nutrients from the artificial diets, upon mean larval weights throughout various periods of development

Table 23. A comparison of time required (days) for larval maturation as effected by feeding upon live greenbugs, an artificial liver diet, and an artificial diet with various nutritive components systematically deleted.

Treatment	Days 2nd molt[1]	Inter[2]	3rd molt[1]	Inter[2]	Pupation	Inter[2]	Emergence[1]	Hatch to adult[3]
Live greenbug	2.00 a	2.20	4.20 a	4.70	8.90 a	4.10	13.00 a	16.00 a
Liver conc. (1:20) plus liver 2	4.63 b	5.37	10.00 b	8.27	18.27 b	3.33	21.60 b	24.60 b
w/o CSM and powdered milk	5.09 b	6.18	11.27 b	7.03	18.30 b	4.70	23.00 b	26.00 b
w/o Tryptophan and cystine	4.11 b	5.44	9.55 b	8.01	17.56 b	6.27	23.83 b	26.83 b
Interval diff.[4]	----	3.46	-----	3.07	-----	2.23	-----	-----
Standard deviation	0.94	----	1.41	----	2.05	----	2.09	-----

[1]Means followed by the same letter, within a column, are not significantly different at the 5 % level (LSD).

[2]Interval time between developmental stages.

[3]Three days time added compensating for the interval between hatch to the first molt.

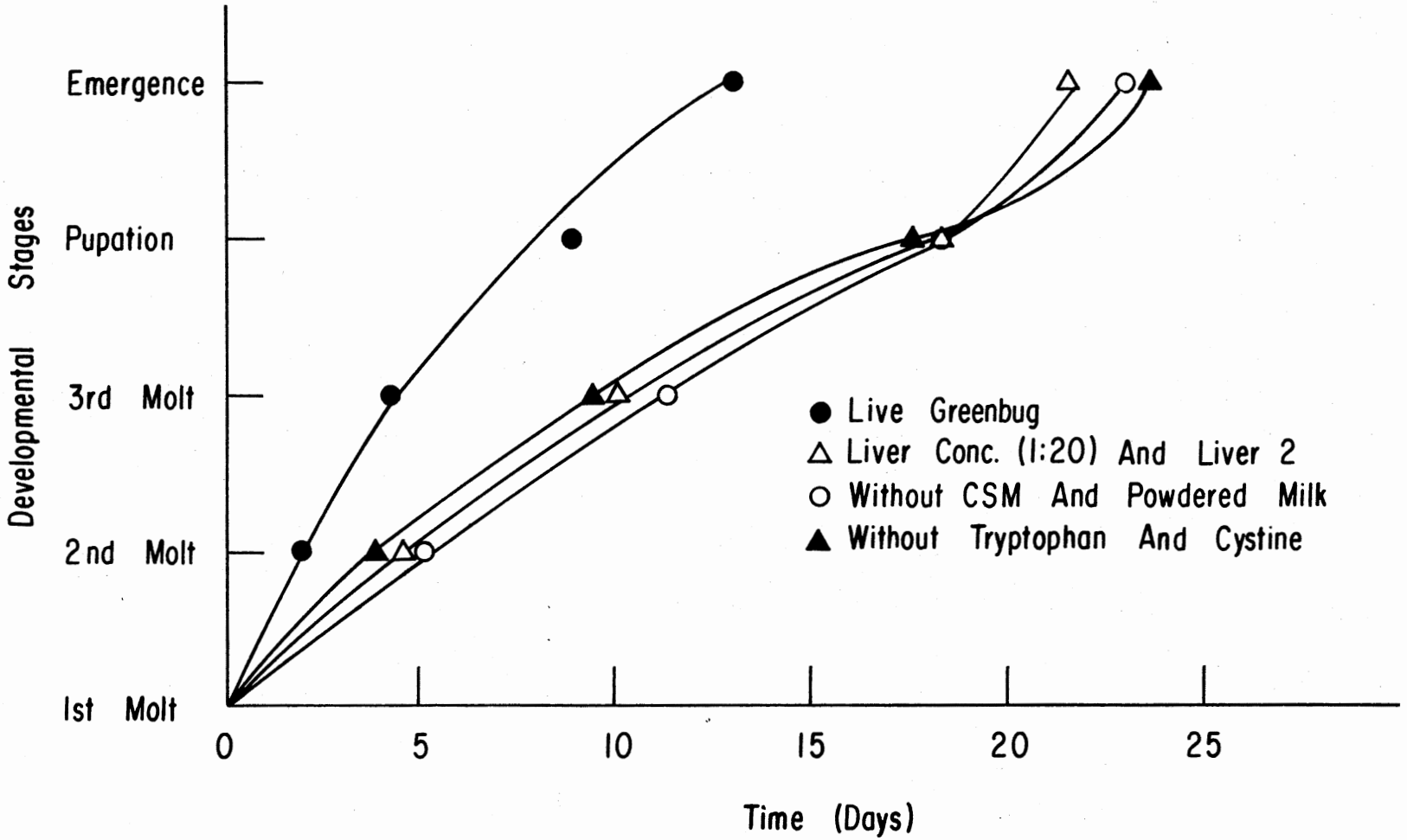

Figure 12. A comparison of time required for larval maturation as effected by feeding upon live greenbug, an artificial liver diet, and an artificial media systematically deleting various nutritive components

Effect of Liver Extract and Modified Vanderzant's Diet

The liver extract S diet (L5) (Table 6) was contrasted with two modified forms of Vanderzant's diet (V1) (Table 3). The modified V2 form was simply solidified and the modified V3 form was solidified with the addition of liver extract S (Table 3).

No significant difference was observed for the total molts between the live greenbug; the liver extract S (L4) diet (Table 6); and the modified Vanderzant's diets (L2 and L3) (Table 3). However, the frequency of molting for the V2 diet was definitely lower than the L4 or V3 diets, with only 4 larvae molting twice compared to 12 and 10 for the other two diets, respectively (Table 24). Pupation and emergence was identical for those reared on the live greenbug and L4 diets with higher pupation and emergence percentages than either the V2 or V3 diets, and the V3 diet (containing liver extract S) was higher than diet V2.

The mean weight of larvae at each developmental stage was significantly higher for those individuals on the live greenbug treatment than for other treatments. The mean weights of larvae for the L4, V2, and V3 diets show no significant difference for the second molt, third molt, and pupation. However, the mean weight of larvae upon emergence for the L4 diet was significantly greater than both the V2 and V3 diets. No significant difference was observed between either of the modified Vanderzant's diets (V2 and V3) (Table 25) (Figure 13).

Larvae placed upon the live greenbug treatment required significantly less time to mature from hatch to adult, 14.09 days, than either the L4, V2, or V3 diets, requiring 23.36, 35.00, and 29.57 days, respectively (Table 26) (Figure 14). The larvae placed upon the L4 diet required

Table 24. Molting frequency, pupation frequency and percent, and emergence frequency and percent of insects as effected by development on live greenbug, various forms of modified artificial diets, or a liver extract diet.

Treatment	Did not molt	Molted only once	Molted twice	Total molts
Live greenbug	1	0	11	11
L4 (Liver S)	0	0	12	12
V2 (Modified Vanderzant's)	2	6	4	10
V3 (Modified Vanderzant's plus Liver S)	0	2	10	12

Treatment	Did not pupate	No. pupating	% pupation
Live greenbug	1	11	91.7
L4	1	11	91.7
V2	10	2	16.7
V3	5	7	58.3

Treatment	Did not emerge	No. emerging	% emergence
Live greenbug	1	11	91.7
L4	1	11	91.7
V2	10	2	16.7
V3	5	7	58.3

[1]Refers to the total number of individual larvae molting at least once per treatment.

Table 25. The effects of live greenbug, various forms of modified artificial diets, and a liver extract diet upon mean larval weights throughout various periods of development.

Treatment	2nd Molt (mg)[1]	3rd Molt (mg)[1]	Pupation (mg)[1]	Emergence (mg)[1]
Live greenbug	4.143 a	13.567 a	20.493 a	17.205 a
L4 (Liver "S")	1.943 b	5.254 b	13.161 b	10.605 b
V2 (Vand. mod.)	1.664 b	4.075 b	11.805 b	8.651 c
V3 (Vand. mod.)	1.645 b	3.788 b	10.881 b	7.799 c
Standard deviation	0.499	1.939	3.071	2.471

[1]Means followed by the same letter, within a column, are not significantly different at the 5 % level (LSD).

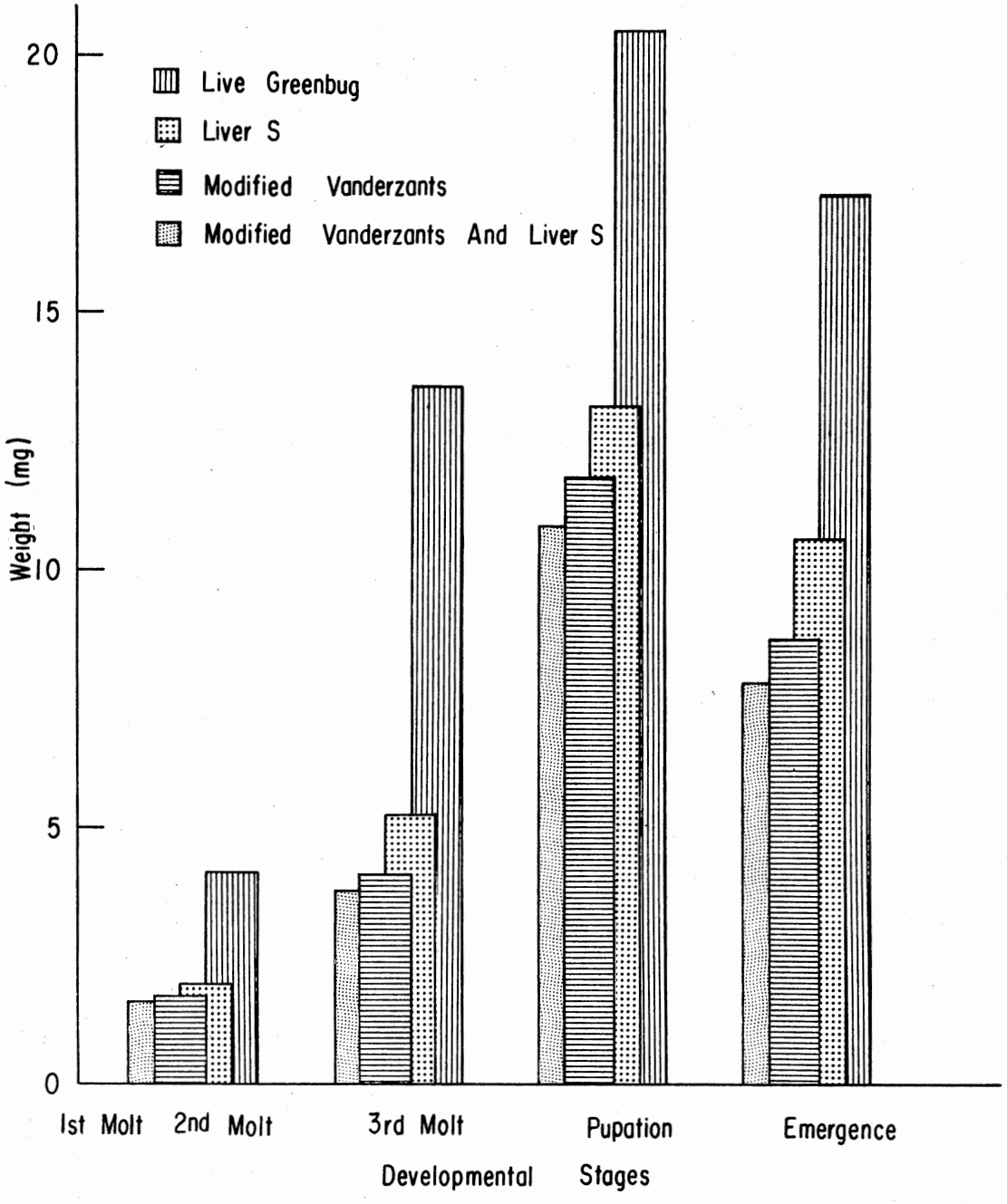

Figure 13. The effects of live greenbug, various forms of modified artificial diets, and a liver extract diet upon mean larval weights throughout various periods of development

Table 26. A comparison of the time required (days) for larval maturation as effected by feeding upon live greenbug, various forms of modified artificial diets, and a liver extract diet.

Treatment	2nd molt[1]	Inter[2]	3rd molt[1]	Inter[2]	Pupation[1]	Inter[2]	Emergence	Hatch to adult[3]
Live greenbug	2.00 a	1.82	3.82 a	3.91	7.73 a	3.36	11.09 a	14.09 a
L4 (Liver "S")	3.08 b	5.00	8.08 b	8.01	16.09 b	4.27	20.36 b	23.36 b
V2 (Modified Vanderzant's)	4.60 c	6.80	13.00 c	13.00	26.00 c	6.00	32.00 c	35.00 c
V3 (Modified Vanderzant's and Liver "S")	4.75 c	8.25	11.40 c	9.74	21.14 c	5.43	26.57 c	29.57 c
Standard deviation	0.67	----	1.34	-----	2.93	----	3.26	-----

[1]Means followed by the same letter, within a column, are not significantly different at the 5% level (Duncan).

[2]Interval time between developmental stages.

[3]3 days time added compensating for the interval between the hatch to the first molt.

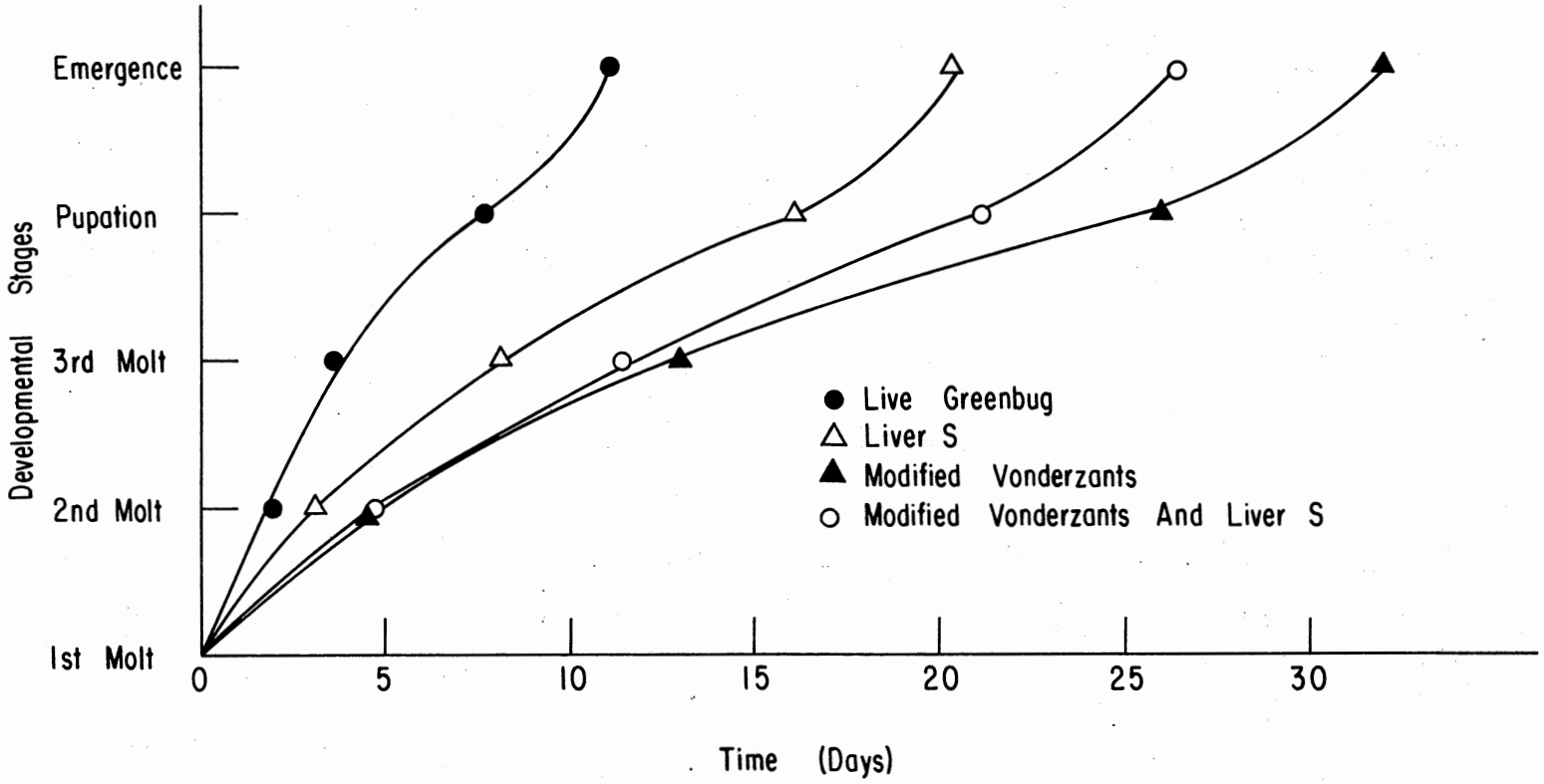

Figure 14. A comparison of time required (days) for larval maturation as effected by feeding upon live greenbug, various modified artificial diets and a liver extract diet

significantly less time to proceed from one developmental stage to the next, and less time for total maturation than either the V2 or V3 diets.

The results of this experiment indicate that the addition of liver extract S to the modified Vanderzant's diet, V3, increases the frequency of molting, pupation and emergence. This might provide another clue in the search for an adequate diet. The presence of liver extract S contributes little, however, toward increasing weight gains during development or lessening the total time required for maturation.

Comparative Effects of Liver Extracts L, S, and 2

A comparative study of three different commercial liver extracts was undertaken to ascertain if any differences existed in their ability to stimulate growth and development of the larvae. Each extract was incorporated into a different diet. The liver L extract was in diet L2, the liver 2 extract was in diet L3, and the liver S extract was in diet L4 (Table 6).

No significant difference was observed in molting frequency between the three liver extract diets (L2, L3, and L4), or the live greenbug treatment (Table 27). There was essentially no difference in frequency of pupation between the live greenbug and L2 diet. However, those individuals on L3 and L4 diets had a lower percentage of pupation. The frequency of emergence was significantly higher for larvae on the live greenbug treatment than for those upon the liver extract treatments. No significant difference in frequency or percent emergence was noted between the liver extract treatments.

Those individuals receiving the live greenbug treatment attained

Table 27. Molting frequency, pupation frequency and percent, and emergence frequency and percent of insects as effected by development on live greenbug or one of three natural diets, each containing a different liver extract.

Treatment	Did not molt	Molted only once	Molted twice	Total molts[1]
Live greenbug	1	0	11	11
L2 (Liver L)	1	0	11	11
L3 (Liver 2)	1	1	10	11
L4 (Liver S)	0	0	12	12

Treatment	Did not pupate	No. pupating	% pupation
Live greenbug	1	11	91.7
L2	2	10	83.3
L3	4	8	66.7
L4	4	8	66.7

Treatment	Did not emerge	No. emerging	% emergence
Live greenbug	1	11	91.7
L2	4	8	66.7
L3	5	7	58.3
L4	5	7	58.3

[1]Refers to the total number of individual larvae molting at least once per treatment.

significantly higher mean weights at each successive developmental stage than larvae receiving the various liver extract treatments (Table 28) (Figure 15). No significant difference was observed in mean weight either for developmental stages or emergence between the liver extract treatments.

Larvae receiving the live greenbug treatment required significantly less time to attain larval maturation than either of the three liver extract treatments (Table 29) (Figure 16). No significant difference in time requirements were observed between the three liver extract diets as the larvae proceeded from their first molt through to pupation. However, for the total time required for larval maturation (hatch to emergence), significantly less time was required for the L2 diet than for the L3 diet. This difference is due to a continual accumulation of slightly longer time requirements at each developmental stage for the L3 diet. Total time requirements for L4 diet are intermediate between the L2 and L3 diets.

For practical purposes of diet formulation it is doubtful that the use of one or another of the liver extract diets will have any profound effect upon larval development, regardless of slight differences in the nutrient composition due to the extracting process utilized. In such a oligidic diet, as is used, the small nutrient differences between liver extracts may not be revealed due to an excessive conglomeration of nutrients already present in the diet from chemically undefined sources. However, deficiencies and contributions obtained from these results could help direct further investigations in the search for adequate diets.

Table 28. The effect of live greenbugs, artificial diets, and the incorporation of a series of different liver extracts into the diets, Upon Mean Larval Weights Throughout Various Periods of Development.

Treatment	2nd Molt (mg)[1]	3rd Molt (mg)[1]	Pupation (mg)[1]	Emergence (mg)[1]
Live greenbug	4.677 a	12.257 a	23.071 a	18.575 a
L2 (Liver L)	1.754 b	5.022 b	11.260 b	10.124 b
L3 (Liver 2)	1.741 b	4.967 b	11.150 b	9.254 b
L4 (Liver S)	1.686 b	4.784 b	11.102 b	9.100 b
Standard deviation	0.567	1.652	1.987	1.997

[1]Means followed by the same letter, within a column, are not significantly different at the 5 % level (Duncan).

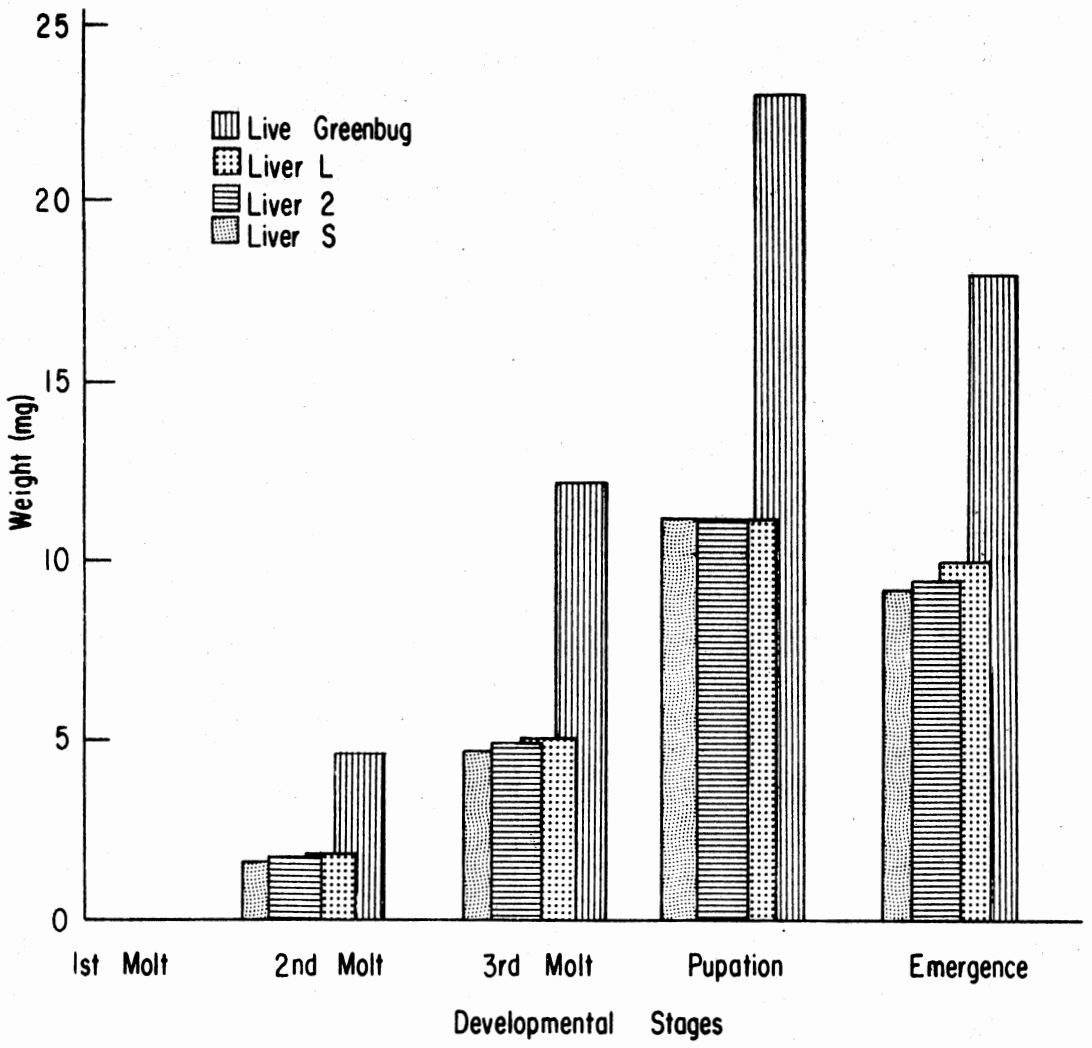

Figure 15. The effect of live greenbug and artificial diets that incorporate a series of different liver extracts upon mean larval weights throughout various periods of development

Table 29. A comparison of time required (days) for larval maturation as effected by feeding upon live greenbugs and artificial diets with different liver extracts.

Treatment	2nd Molt	Inter[2]	3rd Molt[1]	Inter[2]	Pupation	Inter[1]	Emergence[1]	Hatch to Adult[3]
Live greenbug	3.27 a	2.09	5.36 a	5.73	11.09 a	5.18	16.27 a	19.27 a
L2 (Liver L)	5.54 b	8.73	14.27 b	8.43	22.70 b	3.53	26.63 b	29.63 b
L3 (Liver 2)	6.08 b	8.82	14.90 b	9.85	24.75 b	5.82	30.57 c	33.57 c
L4 (Liver S)	7.09 b	7.33	14.42 b	10.33	24.75 b	3.82	28.57 b,c	31.57 b,c
Standard deviation	1.88	----	1.89	-----	2.79	----	2.99	-----

[1]Means followed by the same letter, within a column, are not significantly different at the 5 % level (Duncan).

[2]Interval time between developmental stages.

[3]Three days time added compensating for the interval between the hatch to the first molt.

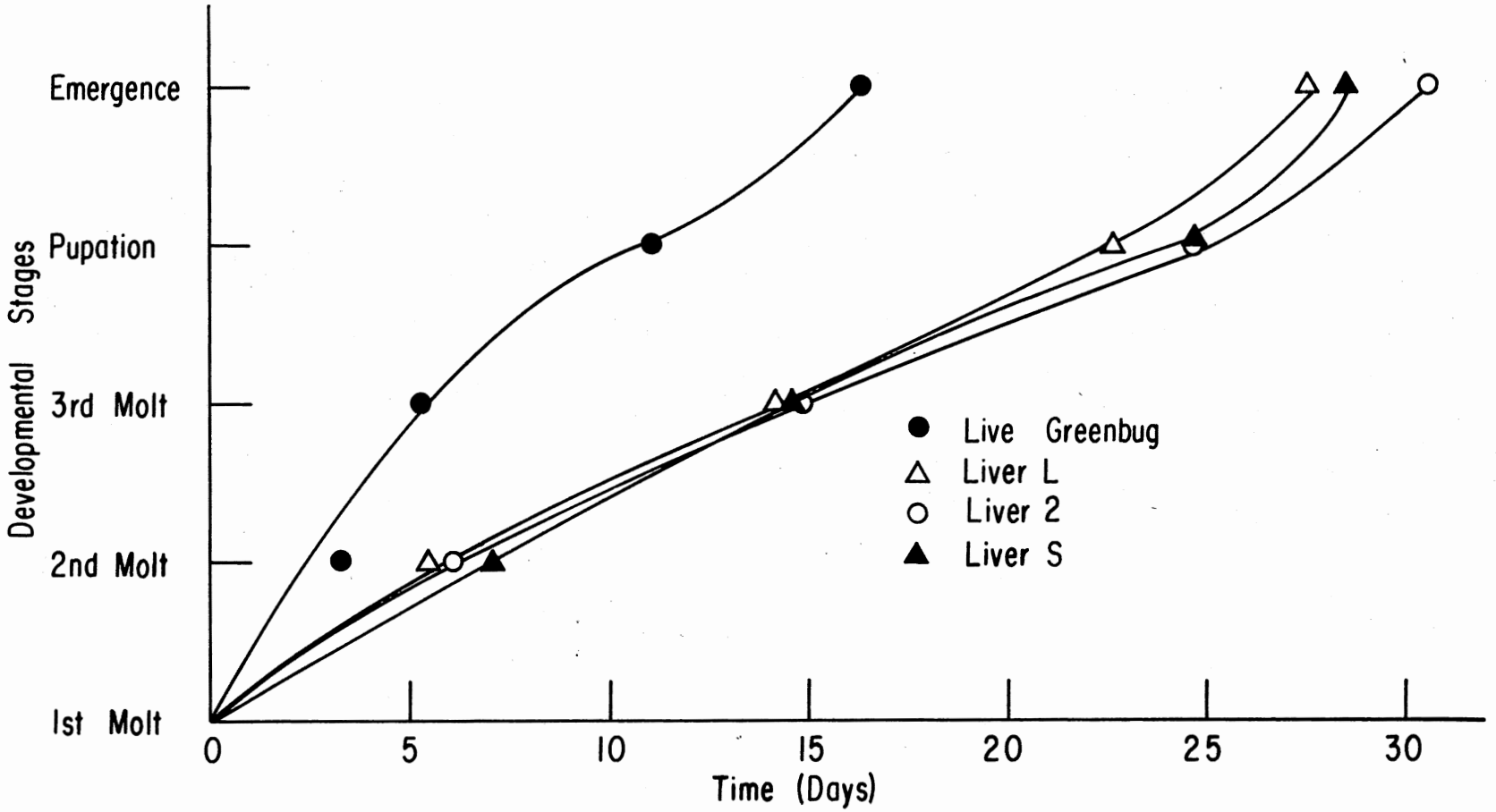

Figure 16. A comparison of time required (days) for larval maturation as effected by feeding upon live greenbug and artificial diets that incorporate a series of different liver extracts

Effects of Liver Extract L on First Instar Larvae

Due to previous success in rearing larvae from the first instar through to emergence on the liver extract L diet (L2) (Table 6) it was decided to attempt the same test only this time with newly hatched larvae. Live greenbug and the standard maintenance diet served as controls.

No significant difference was observed for frequency of molting, pupation or emergence between the L2 or live greenbug diets. Those few individuals, placed upon the standard maintenance diet, which molted, were unable to proceed through the remaining stages of larval development (Table 30).

The mean weight at each developmental stage was significantly higher for individuals on the live greenbug diet than those on L2 diets (Table 31) (Figure 17).

Larvae on the L2 diet required a significantly greater period of time at each developmental stage, and for complete development (35 days) than did those individuals on the live greenbug treatment (16 days) (Table 32) (Figure 18). A greater period of time was required for the development of newly hatched larvae than for the first instar larvae of previous experiments on the L2 diet. Of course, this is primarily due to the nutrients and greater weight acquired by the second instar larvae from their prior feeding period upon live greenbug.

This study, however, does provide conclusive evidence that newly hatched larvae will develope on a artificial diet without the necessity of a prior feeding period on live greenbug. Although, more time was required for larval development and their mean weight was significantly

Table 30. Molting frequency, pupation frequency and percent, and emergence frequency and percent of insects as effected by development on live greenbug, liver L extract, and standard maintenance artificial diets.

Treatment	Did not molt	Molted only once	Molted only twice	Molted three times	Total molts[1]
Live greenbug	0	0	0	12	12
L2 (Liver L)	0	1	0	11	12
Standard	9	3	0	0	3

Treatment	Did not pupate	No. pupating	% pupation
Live greenbug	2	10	83.3
L2	2	10	83.3
Standard	12	0	0

Treatment	Did not emerge	No. emerging	% emergence
Live greenbug	3	9	75.0
L2	3	9	75.0
Standard	12	0	0

[1]Refers to the total number of individual larvae molting at least once per treatment.

Table 31. The effects of live greenbugs, liver L extract, and standard maintenance artificial diets upon mean larval weights throughout various periods of development.

Treatment	1st Molt (mg)[1]	2nd Molt (mg)[1]	3rd Molt (mg)[1]	Pupation (mg)[1]	Emergence (mg)[1]
Live greenbug	1.423 a	4.289 a	10.029 a	22.631 a	19.490 a
L2 (Liver L)	0.602 b	2.012 b	4.434 b	11.306 b	9.896 b
Standard diet	0.485 b	-----	-----	------	------
Standard deviation	0.296	1.424	1.907	2.561	2.093

[1]Means followed by the same letter, within a column, are not significantly different at the 5 % level (Duncan).

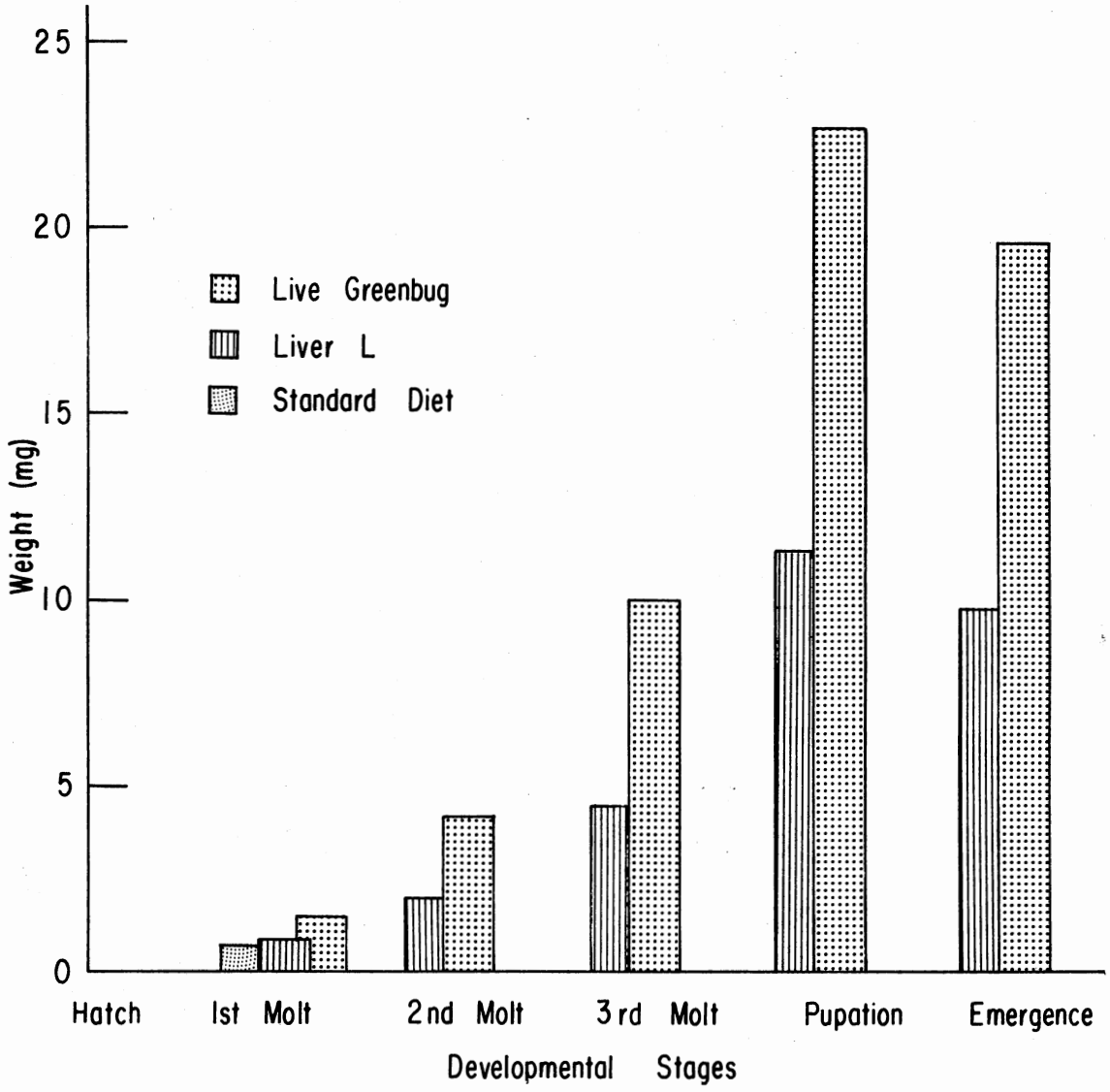

Figure 17. The effects of live greenbug, liver L extract and standard maintenance artificial diets upon mean larval weights throughout various periods of development (first instar larvae were utilized)

Table 32. A comparison of time required (days) for larval maturation as effected by feeding upon live greenbugs, liver L extract and standard maintenance artificial diets.

Treatment	1st molt[1]	Inter[2]	2nd molt[1]	Inter[2]	3rd molt[1]	Inter[2]	Pupation[1]	Inter[2]	Emergence[1]
Live greenbug	3.00 a	1.25	5.25 a	2.00	7.25 a	4.93	12.18 a	3.82	16.00 a
L2 (Liver L)	6.67 b	5.97	12.64 b	8.60	21.27 b	7.73	29.00 b	6.00	35.00 b
Standard diet	9.67 c	----	-----	----	-----	----	-----	----	-----
Internal diff.	----	4.72	-----	6.60	-----	2.80	-----	2.18	-----
Standard deviation	2.20	----	3.11	----	6.53	----	3.49	----	-----

[1]Means followed by the same letter, within a column, are not significantly different at the 5 % level (Duncan).

[2]Interval time between developmental stages.

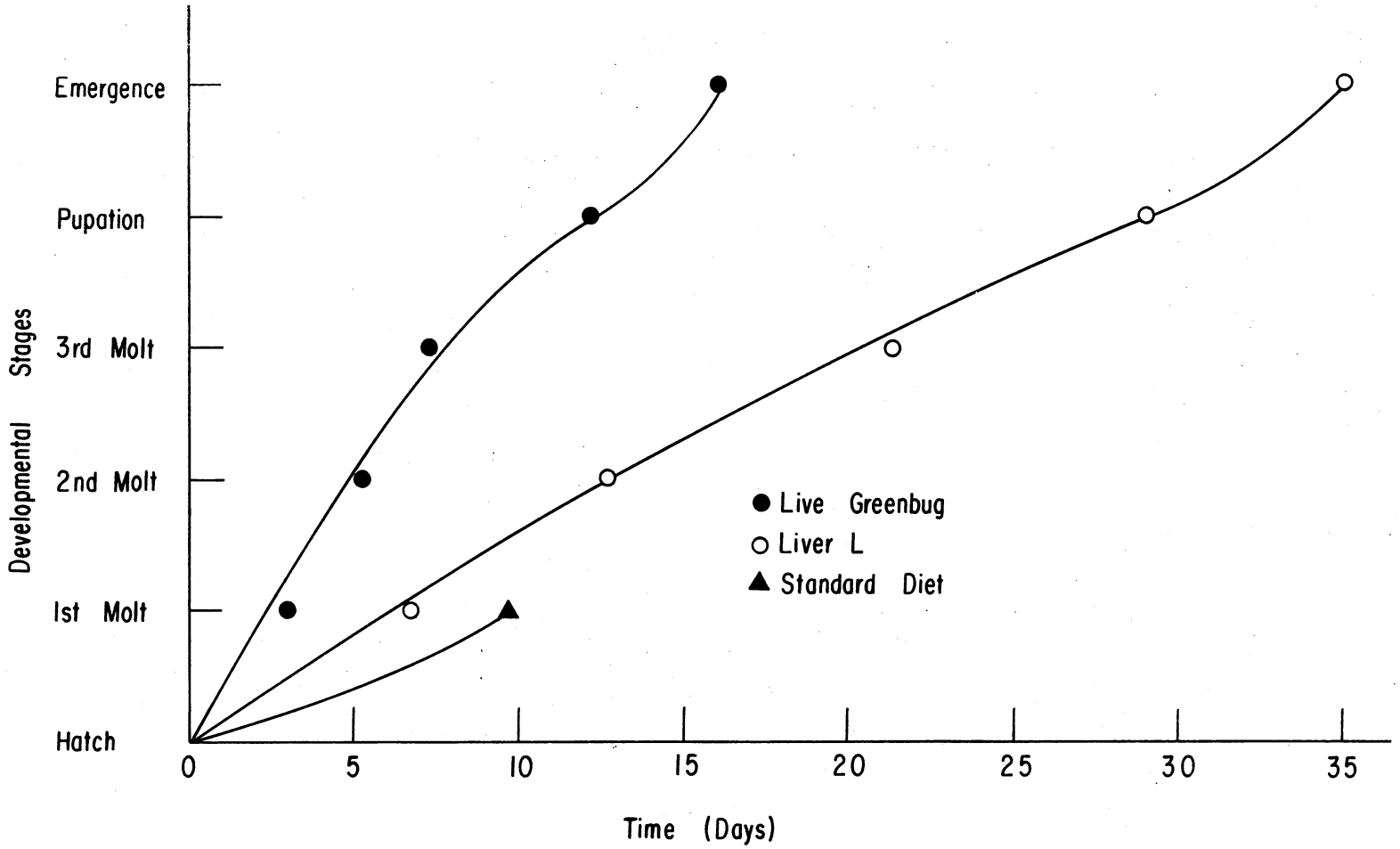

Figure 18. A comparison of time required (days) for larval maturation as effected by feeding upon live greenbug, liver L extract, and standard maintenance artificial diets (first instar larvae were utilized)

less than those individuals provided with live greenbug, their frequency of molting, pupation and emergence remained unaffected.

To date, this information has not appeared in the literature. Therefore, this diet contributes significant information toward the development of a balanced artificial diet and possible elucidation of important growth stimulants within the existing diet.

Effects of Supplemental Protein vs. Carbohydrate

In this study supplemental amounts of protein were added to one liver extract diet (L9) and supplemental amounts of carbohydrate to another diet (L10) (Table 6). The effects of the diets were compared according to their ability to stimulate growth and development in newly hatched larvae. A control group was set up on diet L4, containing liver extract S.

The frequency of molting between larvae on the L4 and L10 (high protein) diets proved only slightly different from one another. However, larvae receiving the carbohydrate diet L9 did not molt at all. Those individuals on the live greenbug treatment maintained a significantly higher molting frequency than was observed for the other treatments (Table 33).

Table 34 (Figure 19) shows that those larvae on the live greenbug treatment attained a significantly higher mean weight for weigh dates one and two, as well as a higher mean growth rate between the two weigh dates than those individuals upon the remaining treatments.

It may be determined from this experiment that supplemental supplies of protein or carbohydrate have neither a beneficial or negative influence upon the growth rate of first instar larvae. However, an excessive

Table 33. Molting frequency of larvae as effected by development on live greenbugs and a liver extract diet modified with supplemental protein and carbohydrate.

Diet	Did not molt	Molted only once	Molted twice	Total molts[1]
L10 (High protein)	8	3	0	3
L9 (High carbohydrate)	11	0	0	0
L4 (Liver S)	6	5	0	5
Live greenbug	0	0	11	11

[1]Refers to the total number of individaul larvae molting at least once per treatment.

Table 34. The effects of live greenbugs and a liver extract diet modified with supplemental protein and carbohydrate upon mean larval weights throughout a six day period.

Diet	$\bar{X}$ Weight one (mg)[1,2]	$\bar{X}$ Weight two (mg)[1,2]	$\bar{X}$ Growth $w_2 - w_1$ (mg)[1]
L10 (High protein)	0.168 a	0.463 b	0.295 a
L9 (High carbohydrate)	0.211 b	0.437 b	0.213 a
L4 (Liver S)	0.239 b	0.618 b	0.372 a
Live greenbug	1.571 b	7.254 c	5.684 b
Standard deviation	0.305	0.679	2.555

[1]Means followed by the same letter, within a column, are not significantly different at the 5 % level (Duncan).

[2]Weight one recorded after three days exposure to treatment. Weight two recorded on the sixth day of exposure.

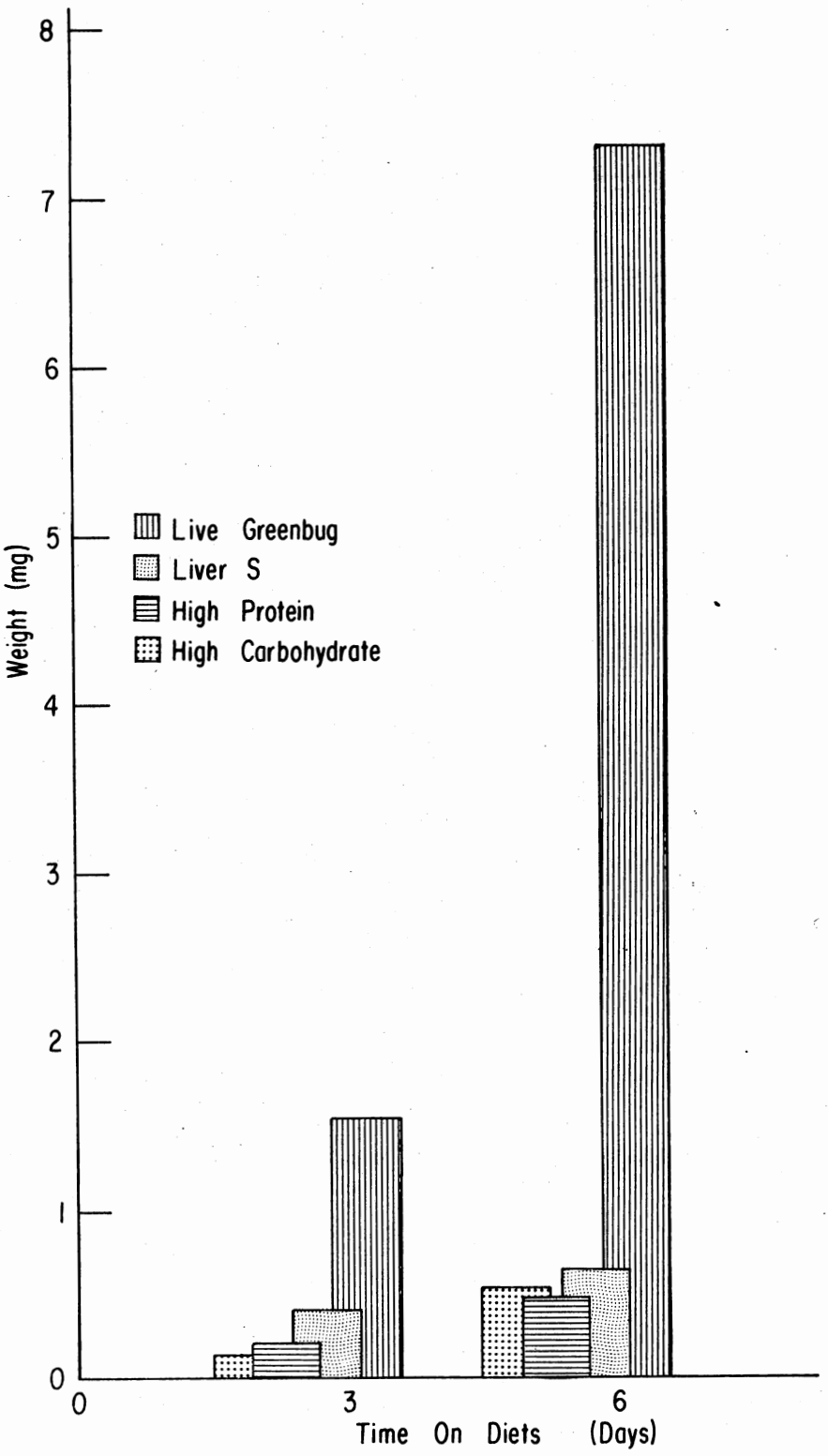

Figure 19. The effects of live greenbug and a liver extract diet modified with supplemental protein and carbohydrate upon mean larval weights throughout a six day period

quantity of carbohydrate impedes the normal process of larval development. This is in agreement with the comment by House (1962) that excessive sugar in the diet is detrimental to the growth of most larval insects. The additional carbohydrate probably upsets the existing ratio of nutrients within the diet to such a degree that the larvae would have to feed for an extended period of time before acquiring the quantity of other nutrients necessary for normal development.

Physiological and Behavioral Observations

As mentioned earlier, previous workers have tested whole liver and liver diets. Whole pork liver proved successful in the development of C. maculata, yet, the liver extract L was not as useful as liver extract 2 or the liver concentrate (1:20), which were incorporated into a diet later used in the successful laboratory rearing of C. maculata (Atallah and Newsom 1966).

In this study the liver extract L was successfully used in combination with nutrients found in diet L2 (Table 6) to promote the growth and development of H. convergens larvae. In preliminary tests, within the scope of this study, it was also shown that various liver diets will maintain the adults for an indefinite period of time. When paired and placed upon one of the several liver extract diets copulation was not an uncommon event. However, oviposition did not occur. Upon dissection of the females it was observed that production of eggs was not initiated. When ovipositing females were placed on the liver diets, after a previous week on a live greenbug diet, oviposition ceased within a day. When dissected, resorption bodies were found within the ovarioles.

Larvae reared from the second instar on the liver extract diets L2,

L3, and L4 were mated and placed on a live greenbug diet immediately after emergence. Within four to six days fertile eggs were oviposited by the females, although, not as many eggs were laid nor as often as individuals which developed on a diet of live greenbug. The process of rearing larvae on the L2 diet from the second instar and then mating and placing the emergents on a live greenbug diet was successfully carried through four generations before the females ceased laying fertile eggs.

The prominent features of adults reared upon the liver diets are their slightness in size, light pigmentation of elytra (Figure 20) and lethargic behavior compared to normal beetles. However, once they are placed upon a live greenbug diet the elytra become more deeply pigmented approaching normalcy and they become more active. It should be noted that only in exceptional cases will an adult fly after being reared for one generation in the laboratory regardless of the diet utilized, greenbug or otherwise.

Nearly 50% of the individuals emerging from the NL1, V3, L6, and L7 diets died within 24 hours after emergence. The majority possessed wringled elytra, extended wings, very light pigmentation of the elytra and exhibited extremely lethargic behavior, often refusing live greenbug and water.

Those individuals completing development, from the hatch, on a L2 diet were mated and placed on a live greenbug diet. Copulation was observed for nearly 60% of all pairs, but no oviposition occurred.

Figure 20. Slightness in size and lighter pigmentation of the elytra are prominent features of adult lady beetles reared upon artificial liver diets (left) vs. lady beetles reared upon live greenbug (right)

CHAPTER IV

SUMMARY AND CONCLUSIONS

Generally those coccinellids most responsive to synthetic or semi-synthetic diets may be classified as generalized feeders including Coleomegilla maculata and Hippodamia tridecimpunctata which are the more advanced species within the category of generalized feeders. Quite possibly the mixed diet of C. maculata represents a transitional stage from similar to intermitant periods when predatory insects were evolving from phytophagous insects (Brues 1946). This does not imply that the more specific feeders, H. convergens or Coccinella species, subsist solely on a single host, but that a physiological necessity for particular substances, nutritive or hormonal, from a specific host or group of hosts has evolved. This restricts the number of synthetic diets and the presentation techniques which could otherwise be successfully implemented.

Agar based diets containing a commercial liver source have proved most successful throughout this research in rearing and maintaining H. convergens in the laboratory. For the most successful results, larvae were normally fed live greenbug until reaching the second instar. However, diet L2 proved exceptional in its ability to stimulate growth and development of newly hatched larvae through to emergence without the necessity of a prior feeding period on live greenbug or other natural materials. This aspect of the study was emphasized because it provides information essential to the development of a balanced artificial diet

which does not appear in the literature.

Experiments where liver extracts were excluded from the diet obtained a lower mean weight and lower frequency of emergence than did diets provided with the liver extracts. Lipids, sterols, and unknown nutrient factors present within the extracts could possibly have been responsible for promoting the increased weight gains and facilitating successful emergence.

Generally, once the larvae emerge from the various liver extract diets utilized, they must be provided with live greenbug in order to achieve the vigor necessary for copulation and oviposition of fertile eggs. Unfortunately, the rate of oviposition and fecundity is extremely low and the few eggs laid by larvae reared from hatching on the L2 diet were infertile.

After rearing four such generations in the laboratory a phenomenal increase in larval mortality with concomittant decreases in oviposition and fecundity occur. This phenomena is also observed when rearing the coccinellids on live greenbug in the laboratory for numerous generations without periodic infusions from wild strains (Shull 1914).

Adults reared from second instar larvae on synthetic diets with a commercial source of liver extracts have lighter pigmentation of elytra, are quite lethargic, are much lighter in weight and smaller in size. Larvae also require in excess of 10 additional days to attain full development over those individuals reared on live greenbug. The above results are in close agreement with those of Ferran and Laforge (1975) who used a synthetic medium containing an unidentified liver powder.

Results show an apparent need for either or both amino acids, tryptophan and cystine, within the synthetic diet. Deletion of these

amino acids caused severe abnormalities in the emergents. Most prominent was the absence of tibia and tarsi on one or all legs for 66.7% of those emerging. However, deletion of CSM or powdered milk induced the same abnormalities in the emergents which indicates the milk solids may be providing these two nutrients. So, it is uncertain whether the deletion of tryptophan and cystine was simply a manifestation of the synthetic diet due to a nutrient imbalance or if there was an actual requirement for large amounts of the amino acids. Unfortunately, the method by which these components influence developmental processes has not been elucidated (Chen 1962).

The requirement for supplemental water during the first instar on a standard maintenance diet was found to be negligible. The effects of supplemental water on larvae which developed on live greenbug was also of little benefit until pupation. This may indicate an increased requirement for water just prior to pupation.

Numerous authors proved that sibling cannibalism was a very effective means of survival in the field utilized by newly hatched coccinellid larvae. The results of this study indicated that sibling cannibalism produced greater initial weight gains as expected, but this benefit did not affect the growth rate throughout the time period larvae spent on a standard maintenance diet.

The comprehensive inefficacy of synthetic diets for rearing aphidophagous coccinellids has prompted numerous researchers to return to supplemental additions of natural products (insect, plant, and animal parts), attempting adequate fortification of synthetic diets in use. However, it will be necessary, by systematic analysis of natural foods, deletion experiments, and assay of biochemicals to determine the particu-

lar substance(s) and possible ratios required to simulate the varied phases of growth, development, and oviposition of H. convergens, attained by feeding upon live greenbug.

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