

THE EFFECTS OF VARIOUS OLIGIDIC SYNTHETIC DIETS ON THE GROWTH OF *HIPPODAMIA CONVERGENS*¹

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An experimental oligidic diet (our formulation) and an established diet (Vanderzant, 1969) were used to evaluate the ability of three commercial liver extracts (L, 2, and S) to promote growth and development of *Hippodamia convergens* Guérin-Ménéville. The mean weight of adults (10.6 mg) and the time required to complete development (20 days) were significantly better on our experimental diet than on the Vanderzant's diet with or without liver extract (8.0 mg and 30 days). Growth of controls fed the aphid, *Schizaphis graminum* (Rondani), was significantly better, with heavier pupae (17.2 mg) and a shorter developmental time (11 days). The adult weights and development times on the three liver extracts were similar but none was as good as the live aphid diet. Deletion studies showed that the omission of tryptophan (2 g) and cystine (2 g) resulted in adult morphological deformities (absence of fully developed tibia and tarsi). Deformities from the omission of CSM and powdered milk were not as pronounced. *H. convergens* larvae were successfully reared on the liver extract diet through four generations when 1st-instars and adults were supplemented with live aphids. A synthetic diet for maintaining adults in storage is also reported.

Development of a suitable artificial diet for the aphidophagous coccinellid, *Hippodamia convergens* Guérin-Ménéville, is requisite to development of mass-rearing programs intent on promoting bionomic or laboratory studies. To date, the majority of artificial diets developed for carnivorous coccinellids have failed to stimulate adequate development and/or oviposition (Hodek, 1973).

Diets incorporating mammalian liver or liver extracts have given varying degrees of success. Szymkowski (1952) attained successful growth and development of *Coleomegilla maculata* De Geer larvae with raw meat supplemented with fresh liver and Warren & Tadić (1967) induced oviposition by adding a vitamin solution (Deca-Vi-Sol) to the liver. However, these diets were not suitable for *H. convergens* (Szymkowski, 1961).

Smith (1965) elicited oviposition in *H. convergens* fed minced pork liver and Atallah & Newsom (1966) developed a successful diet for

C. maculata by replacing raw liver with commercial liver extract 2 or liver concentrate (1:20).

This study examined the ability of three commercial liver extracts to support growth and development of *H. convergens* larvae. Consideration also was given to the morphological and behavioral effects induced by deleting the supplemental tryptophan (2 g) and cystine (2 g) or CSM (10 g) and powdered milk (10 g) from the experimental oligidic diet.

MATERIALS AND METHODS

Rearing procedures. The culture techniques of Starks & Burton (1977) were used to rear the aphid, *Schizaphis graminum* (Rondani). A stock colony of *H. convergens* was established with individuals field-collected from alfalfa and housed either in a rearing room or growth chamber under the following parameters: 16:8 LD, 23.3—25.6°, and 30—40% RH. During the winter, an increase in RH to 65—80% seemed to facilitate oviposition. Plastic cups (30 ml) with paperboard insert lids were used as oviposition chambers for mated adults and as isolation chambers for larvae to prevent cannibalism. Adults and larvae were fed live aphids from colonies reared on sorghum plants in the greenhouse. Mated pairs were provided with an excess of 100 aphids daily, a drop of honey-water, and a cotton ball saturated with

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water. Eggs were laid on the walls or lid of the plastic cup. After eggs were deposited, adults were transferred to fresh cups, so eggs could be collected. After egg hatch, larvae were placed in individual cups and 1st-instars were fed 20, 2nd-instars 30 and 3rd- and 4th-instars 75—100 live aphids/24 hr. When pupation occurred, the cups were set aside until adult emergence. Newly emerged adults were fed 75 aphids for 24 hr before they were paired for mating or storage. Stored adults were provided with a maintenance diet listed in Table I, moist cotton and excelsior.

Nutrient components and tests. An oligidic media was developed for maintenance and experimental diets. Oligidic pertains to a medium in which natural products supply most dietary requirements in contrast to holidic, a medium of chemically defined constituents, and meridic, a medium with a holidic base to which is added minor amounts of unknown substances (Dougherty, 1959).

Soy and casein enzymatic hydrolysates provided the chief sources for protein in all but the maintenance diet while torula yeast, CSM (corn-soy-milk blend) (Burton 1970), gelatin, and powdered milk supplied protein and inorganic salts. Diets were supplemented with 2 g each of tryptophan and cystine because of their reported nutritive and attractive qualities (Chumakova, 1962, Saad Ben & Bishop, 1976, Hagen *et al.*, 1976). Various commercial liver extracts, S, L, and 2 (United States Biochemical Corp.) provided protein, glycogen, vitamins, and unidentified active components.

Sucrose and honey were the major sources

of carbohydrates and wheat germ oil was the primary source of lipid and sterol. The vitamin-B solution was that developed by Vanderzant (1969). Ascorbic acid, inositol, and choline chloride, essential for many insects, were added to all diets, Sorbic acid (2, 4-hexadienoic acid) and methylparaben (p-hydroxybenzoic acid methyl ester) were added in minute quantities to inhibit growth of microbial contaminants. The nutrient components were blended with agar at 45°, and poured into individual 30 ml plastic cups to a depth of 0.5—1.0 cm. Vanderzant's diet (1969) was modified by solidification in an agar base. The ingredients for the basic experimental diet are listed in Table I. The following diets were tested: 1) The experimental diet containing singularly the liver extract S, L, or 2 (liver-S, L, or 2), 2) The modified Vanderzant's diet with or without liver extract S, Vand-S and Vand-O, respectively, and 3) in the deletion studies the experimental diet containing liver extract 2 was compared to the same diet from which the supplemental tryptophan and cystine or the CSM and powdered milk were omitted. Experiments contrasting liver extracts S, L, and 2 were conducted 2—4° below normal experimental conditions (23.3—25.6°), resulting in prolonged development. All experiments used 2nd-instar larvae that had been fed live aphids during the 1st-instar. Fifteen larvae were used for each treatment. All experiments were accompanied by controls fed live aphids and all larvae were block randomized by weight before each experiment. Mean maximum weight, developmental time, mortality, and pupation

TABLE I

Nutrient components of standard adult maintenance diet and experimental oligidic diet for H. convergens (in g unless indicated differently)

Nutrients	Stand. maint.	Exp. diet	Nutrients	Stand. maint.	Exp. diet
Agar	5.2	7.0	Soy enzymatic hydrolysate	—	10.0
Distilled water	200.0 ml	250.0 ml	L-cystine	—	2.0
Sucrose	32.0	12.0	DL-tryptophan	—	2.0
Honey	12.0	20.0	Liver extracts (L, 2, or S)	—	10.0
Torula yeast	10.0	10.0	Wheat germ oil	—	3.0
CSM (Corn, soy, milk)	10.0	10.0	Vitamin B-solution (Vanderzant, 1969)	—	5.0 ml
Gelatin	10.0	5.0	Choline	—	1.6
Ascorbic acid	1.0	1.0	Inositol	—	0.6
Powdered milk	—	10.0	Sorbic acid	0.1	0.12
Casein enzymatic hydrolysate	—	10.0	Methylparaben	0.1	0.12

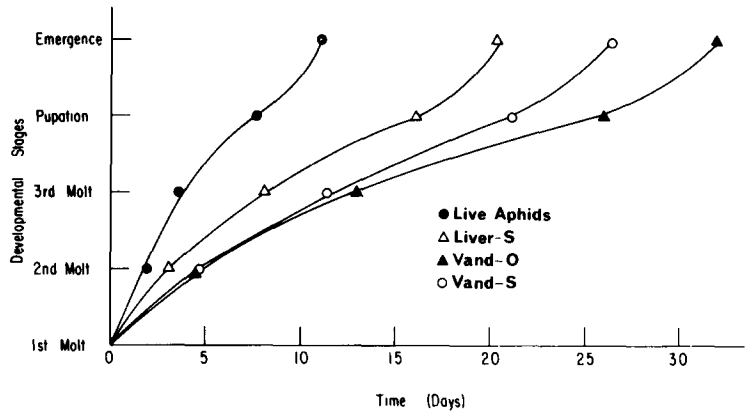


Fig. 1. Time required (days) for larval development of *H. convergens* when fed live aphids, a liver-S diet, and Vanderzant's modified diets, with (Vand-S) and without liver-S (Vand-O).

were recorded. Upon emergence, adults were followed for 48 hr.

RESULTS AND DISCUSSION

Liver extract studies. The liver, banana, and dry mix diet experiments, first performed by Smith (1965) were repeated in our laboratory. Although adults oviposited, both fecundity and fertility were low and larvae did not complete development. We developed an oligidic diet using a complex variety of natural and chemically defined substances with the intention of simplifying the diet at some future date.

In preliminary experiments on the effects of liver extracts in artificial diets, those larvae fed diets supplemented with liver-S were significantly heavier than larvae fed on a diet modified with Vanderzant's (1969) and supplemented with liver-S (Vand-S) or without the extract (Vand-O). However, larvae reared on live aphids were significantly heavier than those on liver-S.

Both liver-S and aphid controls attained 91.7% pupation. The Vand-S and Vand-O averaged 58.3% pupation. Adult emergence was 100% of pupae from all four treatments.

Larvae grown on liver-S required significantly less time for the individual developmental stages and less time for total development than either the Vand-S or Vand-O diets (Fig. 1). Those fed live aphids required significantly less time (11 days) from 1st-larval molt to adult than either the liver-S, Vand-S and Vand-O diets which required 20, 26, and 32 days, respectively. When comparing only the Vand-S and the Vand-O there is evidence that the addition of liver extract S decreased total development time (Fig. 1) and increased the frequency of pupation and emergence, but did not facilitate

weight gains (Table II). Thus, liver extract S may contain certain nutrients (i.e., lipids and sterols) that enhance development.

We compared liver-S with two other types, liver-L and 2, and found few differences. The development from the 1st-larval molt to adult, required 26 days on liver-L; 28 days on liver-S; and 30 days on liver-2. Insects fed live aphids required only 16 days. A pupation weight of 11.2 mg and 66.7% adult emergence were obtained with the extract diets compared to 23.0 mg and 91.7% for the live aphid control.

Deletion studies. The omission of supplemental tryptophan and cystine from the experimental diet had a profound effect on adult emergence. Only 81.8% pupation and 54.5% emergence were observed compared to 90–100% for the other treatments (Table III). Furthermore, 66.7% of the adults lacked fully developed tibia and tarsi. Effects from omission of CSM and powdered milk were less pronounced, with deformities observed in 10% of adults. No deformities occurred in either the aphid control or the liver-2 experimental diet. There was no significant difference in percentage of pupation or emergence between the liver-2 experimental diet, the CSM and powdered milk deletion diet, or the live aphid control. Interestingly, there was no difference between development time and mean maximum weights between the liver-2 experimental diet and the omission diets. The time and weight for both were approximately 23 days and 8 mg compared to 13 days and 21.4 mg for the aphid control.

Although tryptophan and cystine are present in torula yeast, CSM, casein and soy hydrolysates, additional amounts are important to de-

TABLE II
Effects of several diets upon the mean weight (mg) of H. convergens

Diet	2nd instar	3rd instar	Pupae	Adults
Liver S extract	1.943 b	5.254 b	13.131 b	10.605 b
Vanderzant's Modified with liver S (Vand-S)	1.664 b	4.075 b	11.805 b	8.651 c
Vanderzant's Modified without extract (Vand-O)	1.645 b	3.788 b	10.881 b	7.799 c
Live aphid control	4.143 a	13.567 a	20.493 a	17.205 a
S.D.	0.499	1.939	3.071	2.471

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

velopment, pupation and emergence. House (1972) stated that tryptophan was an essential amino acid for insects and that cystine has been found to be essential for normal growth and ecdysis. Atallah & Killebrew (1967) investigated the amino acid requirements of *C. maculata* by using ¹⁴C-labeled acetate, but tryptophan and cystine were not reported because of destruction during acid hydrolysis and/or inability of the assay to separate them. Our data agrees with Chefurka (1965) that cystine is essential for normal pupal formation and metamorphosis.

Other dietary effects. Our studies show that liver extract S can be successfully used in combination with nutrients found in the experimental oligidic or Vanderzant's diets (Table II) to promote the growth and development of *H. convergens* larvae. Our preliminary tests also show that liver extract diets maintained adults for an indefinite period of time and copulation was commonly observed between

paired adults. However, oviposition did not occur. Dissection of the females revealed that egg production was not initiated. When ovipositing females were placed on the liver diets, after a previous week on a live aphid diet, oviposition ceased within a day. Resorption bodies were found within the ovarioles.

When adults reared from the 2nd-instar on the liver extract diets were mated and placed on a live aphid diet immediately after emergence, fertile eggs were oviposited within 4–6 days, although not as many eggs were laid nor as often as from individuals which developed on a diet of live aphids. The process of rearing larvae on the liver-S diet from the 2nd-instar and then mating and placing the adults on a live aphid diet was carried through four generations before the females ceased laying fertile eggs.

The most distinct features of adults reared upon the liver extract diets were their small size, light pigmentation of elytra, and lethargic behavior compared to normal beetles. How-

TABLE III

Effects of several diets on percent pupation, adult emergence, and tibia-tarsi deformities of H. convergens

Diet	% pupation	% adult emergence	% adults deformed
Liver 2 extract	100.0	90.9	0
(10 g) CSM and (10 g) powdered milk deleted	90.9	90.9	10.0
(2 g) Tryptophan and (2 g) Cystine deleted	81.8	54.5	66.7
Live aphid control	90.9	90.9	0

ever, once placed upon a live aphid diet, the elytra became more deeply pigmented and adult activity increased. Nearly 50% of the adults reared on oligidic diets omitting supplemental tryptophan-cystine or CSM-powdered milk or diets without liver extracts died within 24 hr. The majority exhibited wrinkled and lightly pigmented elytra, extended wings, lethargic behavior and often refused live aphids and water.

The authors thank Drs. John R. Sauer, Ronald W. McNew and Douglas L. Dahlman for technical and statistical advice, and Ms. Tina Whittle for technical assistance.

RÉSUMÉ

Influence de différents régimes synthétiques oligidiques sur la croissance d'Hippodamia convergens

Deux régimes expérimentaux, celui établi par Vanderzant (1969) et un régime expérimental oligidique (notre formule) ont été utilisés pour examiner la valeur de trois extraits commerciaux de foie (L, 2 et S) pour assurer le développement et la croissance de *Hippodamia convergens* Guérin-Ménéville. Le poids moyen des adultes (10,6 mg) et la durée de développement (20 jours) sont significativement meilleurs avec notre régime expérimental qu'avec celui de Vanderzant avec ou sans extrait de foie (8,0 mg et 30 jours).

La croissance des témoins sur le puceron, *Schizaphis graminum* Rondani est significativement meilleure, donnant des nymphes plus lourdes (17,2 mg) et une durée de développement plus courte (11 jours). Les poids des adultes et les durées de développement sur les trois extraits de foie sont voisins, mais aucun n'égale ceux obtenus avec alimentation sur puceron.

La suppression du tryptophane (2 g) et de la cystine (2 g) provoque des malformations chez les adultes: absence de développement total des tibias et des tarse. Les malformations dues à la suppression de CMS et de poudre de lait sont moins prononcées.

Les larves de *H. convergens* ont été élevées avec succès pendant quatre générations sur régime avec extrait de foie quand les larves de 1er stade et les adultes ont reçu une alimentation complémentaire en pucerons vivants. Il est aussi possible de maintenir une souche d'adultes.

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