— 1946. Verzeichnis der in den Jahren 1941—1945 für die Fauna Finnlands neuhinzugekommenen Insektenarten. — Notulae Ent. 26, 122—142.
— 1953. Zur Kenntnis der Ichneumonidenfauna (Hym.) Finnlands VIII. — Notulae Ent. 33, 1—13.
NYLANDER, W. 1859. Hymenoptera et Diptera från Österbotten. — Not. Sällsk. Fauna Flora Fenn. Förhandl. 4, 245—249.
OEHLKE, J. 1966. Die westpalaearktischen Arten der Tribus Poemeniini (Hymenoptera: Ichneumonidae). — Beitr. Ent. 15, 881—892.
SCHMIEDEKNECHT, O. 1907. — Pimplinae. — In: Opuscula Ichneumonologica 3 (16—17), 1201—1360. Blankenburg in Thüringen.
— 1925. Tryphoninae. — In: Opuscula Ichneumonologica 5 (40), 3123—3202. Blankenburg in Thüringen.
TOWNES, H. 1970. Genera of Ichneumonidae 2. Gelinae. — Mem. Amer. Ent. Inst. 12, 1—537.

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Attempts at rearing Adalia bipunctata L. (Col., Coccinellidae) on different artificial diets

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About 60 variations of artificial diets were formulated and tested for rearing *Adalia bipunctata* L. The basic ingredients were: wheat germ, brewer's yeast, sucrose, honey, eggs, casein, liver, a salt mixture, vitamins, antibiotics, agar and water. A series of seven diets is presented showing increasingly promising results. On the best diets larval development was delayed by about 20—30 %, as compared with naturally fed larvae, and adult weight was about 30 % lower than in animals fed on *Myzus persicae* Sulz. On the best diets the percentage of adults emerging varied between 60 and 80 %, while the corresponding figure in naturally fed insects was 65—95 %. On these test diets matings were frequently observed, but no eggs were laid, although some adults survived for six months.

Many attempts have been made to rear coccinellid beetles on artificial diets. ATALLAH & NEWSOM (1966) have succeeded in rearing *Coleomegilla maculata* De Geer for generations on an artificial diet. SMIRNOFF (1958) has reared 19 coccinellid species on his artificial diet, but *Adalia bipunctata* was not among them. OKADA et al. (1972) reared *Harmonia axyridis* Pall. on lyophilized drone honeybee brood for 16 generations. Using the same diet, MATSUKA et al. (1972) succeeded in rearing *Menochilus sexmaculatus* Fabr. for three generations, but

Propylea japonica Thun., *Aiolocaria mirabilis* Motsch. and *Chilocorus kuwanae* Silv. for only one generation. Many adult coccinellids could survive on the drone powder up to one year. SMITH (1965) has reared *A. bipunctata* among other coccinellid species on his artificial diets.

The diet of ATALLAH & NEWSOM (1966) was chosen as a basis for reformulation because the first-instar larvae of *A. bipunctata* survived slightly longer on that diet in preliminary tests than on the diets of SMIRNOFF (1958) and SMITH (1965).

The purpose of the present investigation was to formulate and test an artificial diet on which *A. bipunctata* could be reared for generations. We are interested in *A. bipunctata* as a means of biological control of aphids in greenhouses.

Material and methods

Experimental animals and rearing methods

Adults of *Adalia bipunctata* were collected for egg-laying from the field at Tikkurila, Vantaa, South Finland, in the spring of 1973, and maintained in a greenhouse at the Agricultural Research Centre, Tikkurila. In the greenhouse the temperature varied between +20 and 35°C and the R.H. between 25 and 80 %. The photoperiod was about 18 L : 6 D. The adults were kept in glass jars (diameter 95 mm, height 55 mm) 5 to 10 per jar and fed daily on *Myzus persicae* Sulz., which is a suitable prey for *A. bipunctata* (BLACKMAN 1957). The jars were provided with wrinkled and wetted filter paper for retention of humidity and for egg-laying, walking and hiding. The jars were covered with thin gauze. The eggs were removed daily, counted and transferred to the insectarium at the Department of Physiological Zoology, University of Helsinki, where the rearing of larvae took place. In the insectarium the temperature was + 23 ± 0.5°C and the R.H. varied between 65 and 75 %. The rearing table was illuminated by three 40-W fluorescent tubes at a height of 150 cm. A photoperiod of 18 L : 6 D was used. Under these conditions the eggs hatched in three to four days. After the emerging larvae had consumed the egg remnants, they were reared individually in glass tubes (diameter 20 mm, height 55 mm) containing the diet to be tested. The tubes were plugged with cotton, and checked daily. If the feed was considered dry or contaminated, the animals were transferred on to fresh feed.

A. bipunctata has four larval instars, but a fifth instar sometimes occurred. An insect attached to its support, but still inside the last larval cuticle, was considered a prepupa. Emerged adults were reared in glass jars (4—15 per jar) containing the larval diet and a tube of water plugged with cotton.

Preparing the artificial diets

All the ingredients (Table 1), except aureomycin, the vitamins, agar and half of the water, were placed in a 500-ml glass container. After the ingredients had been ground to a fine suspension with an Ultra-Turrax-homogenizer (Janke & Kunkel KG.), the mixture was heated to +65°C under continuous stirring. After about 5 to 10 minutes a clear, hot solution of agar was added, and finally after two minutes the vitamins and the aureomycin were mixed in the diet. When the final mixture was evenly coloured, indicating good mixing, it was pipetted into the rearing tubes while still hot (1 to 1.5 ml/tube). The tubes were placed in small plastic bags and stored in a refrigerator at +1°C.

Results

Diet 1. — The first diet to produce adults differed considerably from that of ATALLAH & NEWSOM (1966). The development from hatching to adult emergence was very slow and took on an average 30.0 ± 1.2 days, while larvae fed on *M. persicae* completed their development in about 18 days. The rearing started with 193 first-instar larvae and 6 (3 %) reached the adult stage. The mean weight of these adults was only 4.4 ± 0.2 mg, compared with 10.2 ± 0.3 mg for adults (N = 49) fed during larval life on *M. persicae*. For development and mortality see Figs. 1, 2 and 3.

Diet 2. — This diet was similar to Diet 1 except for the addition of egg yolk. The rearing started with 76 first-instar larvae and 8 (about 10 %) reached the adult stage. The animals were still small (mean weight 4.4 ± 0.3 mg) and the developmental period long (30.1 ± 0.7 days). The adults were lighter in colour than normal; their behaviour seemed normal; they fed on the diet and some of them survived up to six weeks, but they did not mate or lay eggs. Figs. 1, 2 and 3.

Table 1. The formulae of the seven diets tested. The diet used by ATALLAH & NEWSOM (1966) is also included for comparison.

Ingredients		Number of the diet							(ATALLAH &
		1	2	3	4	5	6	7	NEWSOM 1966)
Water, distilled	ml	200.0	200.0	200.0	220.0	220.0	220.0	220.0	200.0
Wheat germ	g	2.0	2.0	2.0	2.0	5.0	5.0	5.0	4.0
Brewer's yeast	g	2.0	2.0	2.0	2.0	5.0	10.0	7.0	1.0
Casein, soluble BDH	g	1.5	1.5	1.5	1.5	—	—	—	6.0
Carrot-lipid-casein[1)]	g	3.0	3.0	3.0	3.0	3.0	3.0	3.0	—
Cotton-leaf-extract		—	—	—	—	—	—	—	1.0
Egg, yolk	g	—	20.0	20.0	—	—	—	—	—
Egg, whole	g	—	—	—	50.0	50.0	50.0	50.0	—
Sucrose	g	7.0	7.0	7.0	7.0	7.0	7.0	7.0	3.0
Liver fractions	g	—	—	—	—	—	—	—	6.0
Beef liver, fresh	g	20.0	20.0	50.0	50.0	50.0	50.0	50.0	—
Honey, natural	g	10.0	10.0	10.0	10.0	10.0	10.0	10.0	—
Glycogen	g	—	—	—	—	—	—	—	2.0
Soybean hydrolysate	g	—	—	—	—	—	—	—	3.0
Butter fat	g	—	—	—	—	—	—	—	2.0
Corn oil	ml	—	—	—	—	—	—	—	2.0
Dextrose	g	—	—	—	—	—	—	—	1.0
Amino acid solution[2)]	ml	—	—	—	—	—	20.0	—	—
Salt mixture[3)]	g	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.25
Ascorbic acid, Merck	g	0.5	0.5	0.5	0.5	1.0	1.0	1.0	0.5
Vitamin stock[4)]	ml	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0*)
Choline chloride[5)]	ml	1.5	1.5	1.5	1.5	1.5	1.5	1.5	—
DL-α-Tocopherol, Merck	ml	0.1	0.1	0.1	0.1	0.1	0.1	0.1	(0.2)
Nipagin[6)]	ml	1.5	1.5	1.5	1.5	1.5	1.5	1.5	0.2 g
Aureomycin[7)]	g	0.8	0.8	0.8	0.3	0.3	0.3	0.3	0.06
Sorbic acid, Fluka	g	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Agar, Bacto-agar, Difco	g	4.0	4.0	4.0	4.0	3.7	3.7	3.7	4.0
Total	g	258	278	308	358	362	387	364	237

1) Carrot lipids, extracted from 1 kg of washed and macerated carrots with chloroform-methanol (1:2). The extract still containing the solvent was added to 100 g of casein and evaporated to dryness under vacuum. The carrot-lipid-casein mixture was prepared monthly and stored at —18°C.
2) Amino acid mixture (Levamin Infundibile, Leiras, N-cont. 0.88 g/100 ml).
3) Salt mixture (BECKMAN et al. 1953).
4) Vitamin stock (IGNOFFO 1963) prepared monthly and stored at +1°C.
5) Choline chloride, 15 % solution in water.
6) Nipagin, 15 % solution in 95 % EtOH.
7) Aureomycin, veterinary grade, Cyanamide, chlortetracyclinechloride 55 g/kg.
*) The concentration of the vitamin stock was one-sixth of that of IGNOFFO (1963).

D i e t 3. — This diet differed from Diet 2 only by containing more than twice the amount of fresh liver. Larval development was more synchronous than on the previous diets. The rearing was started with 214 larvae and 58 (27 %) became adults. The period of development was shorter (about 25.0 ± 0.3 days), but the weight of the adults was still rather low (4.8 ± 0.2 mg). Figs. 1, 2 and 3.

D i e t 4. — This diet was similar to Diet 3,

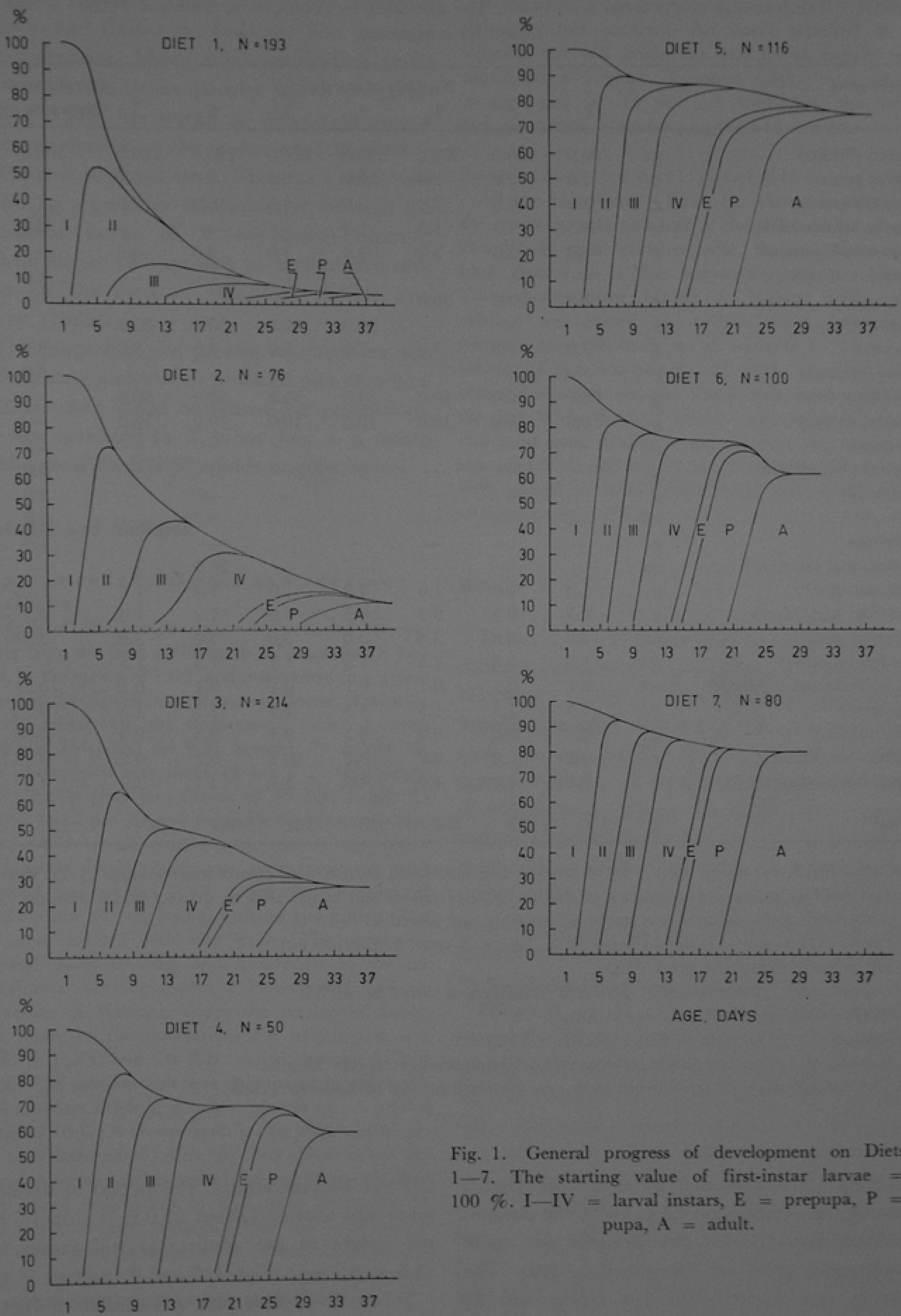

Fig. 1. General progress of development on Diets 1—7. The starting value of first-instar larvae = 100 %. I—IV = larval instars, E = prepupa, P = pupa, A = adult.

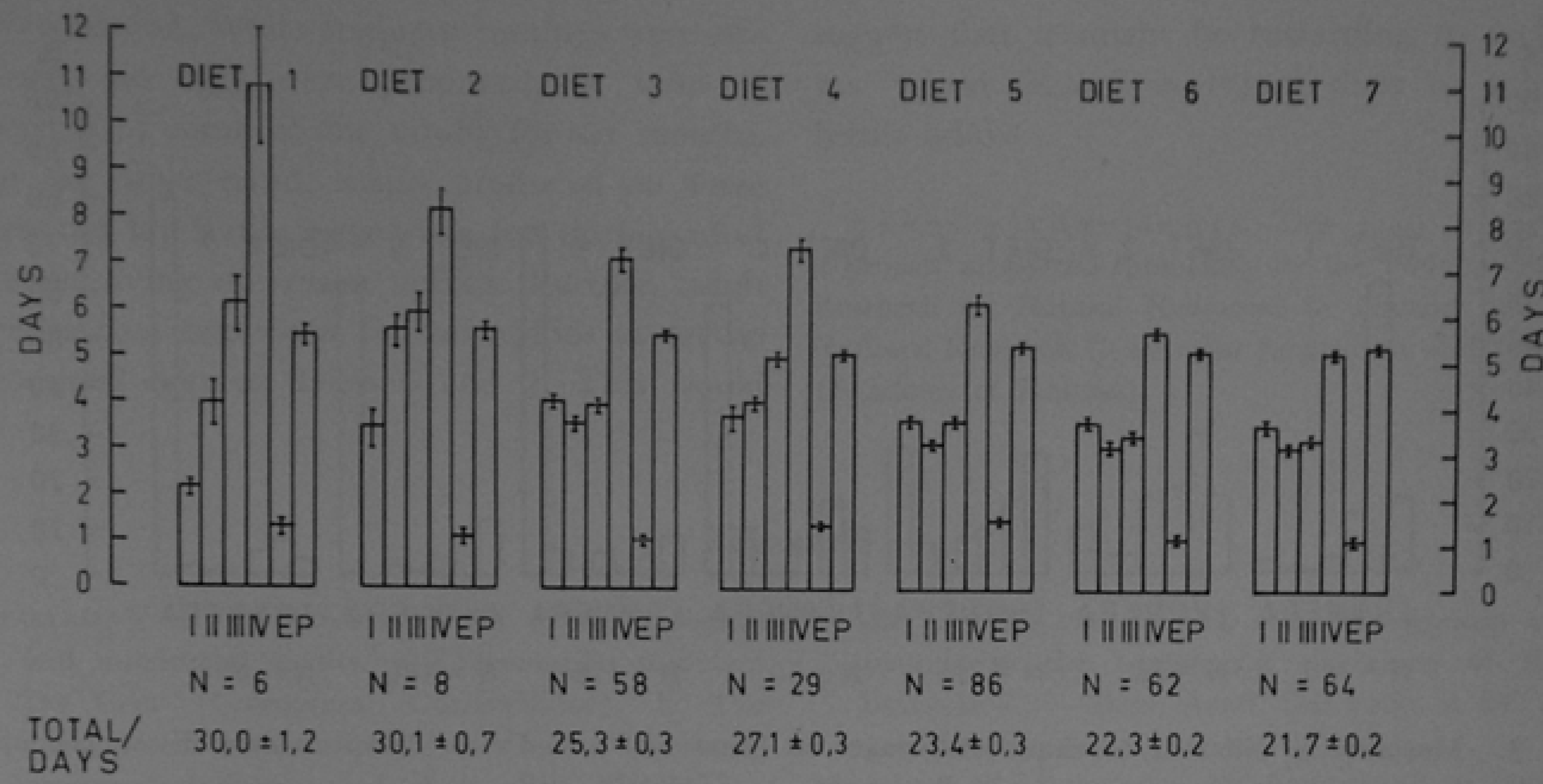

Fig. 2. Duration of the different developmental stages on Diets 1—7. Only emerged adults are considered. I—IV = larval instars, E = prepupa, P = pupa.

with the exception that whole egg was added instead of only yolk, and the amount of aureomycin was reduced. The yield from 50 first-instar larvae was 29 adults (58 %), with a mean weight of 5.0 ± 0.2 mg and a developmental period of 27.1 ± 0.3 days. Figs. 1, 2 and 3.

Diet 5. — This diet was similar to Diet 4, except for an increase in wheat germ and brewer's yeast. From 116 first-instar larvae 86 adults (74 %) emerged. Their mean weight was 5.5 ± 0.1 mg and the developmental period 23.4 ± 0.3 days. These adults were grown in groups of 4 to 15 and fed on the larval diet, on which they survived from one to eight weeks, without laying eggs. Figs. 1, 2 and 3.

Diet 6. — This diet differed from Diet 5 only by a further increase in brewer's yeast and the addition of a solution of amino acids. From 100 larvae 62 adults emerged. The mean weight was higher (7.1 ± 0.1 mg) and the development was completed in 22.3 ± 0.2 days. The coloration of the adults was normal. About 30 adults were reared together on the larval diet and many matings were observed, although no eggs were produced. Figs. 1, 2 and 3.

Diet 7. — This diet was similar to Diet 6, except for the omission of the amino acid solution and a reduction in the amount of brewer's yeast. From 80 first-instar larvae 64 adults (80 %) emerged. Their mean weight was 7.0 ± 0.1 mg and they completed development in 21.7 ± 0.1 days. These normally coloured adults mated frequently from about two weeks after their emergence, but no eggs were produced, although some of the animals survived up to six months on the larval diet. Figs. 1, 2 and 3.

Discussion

In the present study the formulation of an artificial diet for *Adalia bipunctata* was based solely on the method of trial and error. About 60 different compositions were tested and the seven diets described here illustrate the progress made. Length of the developmental period, adult weight, and the percentage of adults produced were chosen as criteria of success.

It is well known that insect development tends to be longer on artificial diets than on natural diets. For example, on the semi-synthetic diets

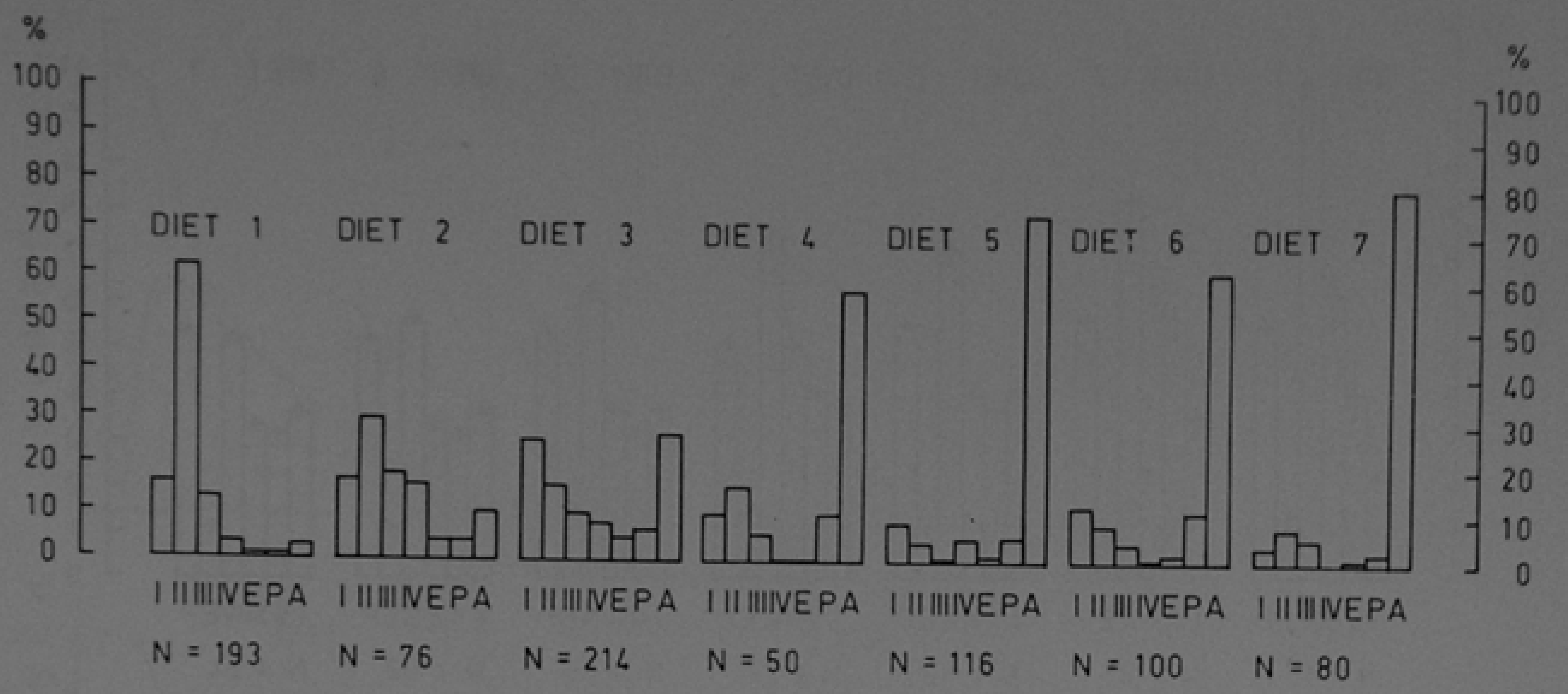

Fig. 3. Mortality in different developmental stages on Diets 1—7. I—IV = larval instars, E = prepupa, P = pupa, A = adult.

used for rearing *Pieris brassicae* L. the larval stage is two days longer than in naturally fed larvae (DAVID & GARDINER 1966, van der GEEST 1968, JUNNIKKALA 1969). The same tendency is evident in coccinellid beetles. In *C. kuwanae* the development is lengthened by 15 % and in *M. sexmaculatus* by 23—56 % (MATSUKA et al. 1972). According to MONTES OGAS (1970), the larval stage in *A. bipunctata* takes 12—14 days and the pupal stage 9—11 days. The total time from hatching to adult emergence at +23°C is thus 21—25 days. In the present study the larval stage was longer, taking 15—17 days on Diets 5, 6 and 7, but this was compensated by a pupal stage (pupa + prepupa) of only 6—7 days. On these three diets (5, 6 and 7) *A. bipunctata* completes development at +23°C in 23.4, 22.3 and 21.3 days, respectively. This represents a delay of about 20—30 % as compared with rearings on natural diets.

The weight of adult *A. bipunctata* reared on Diets 6 and 7 was not more than about 70 % of that of the animals reared on *Myzus persicae*. According to MATSUKA et al. (1972), the corresponding value varied from 60 to 83 % in *M. sexmaculatus,* was about 67 % in *C. kuwanae* and about 63 % in *P. japonica.* These percentages compare very well with those for *A. bipunctata.*

The percentage of adults emerging from a given number of first-instar larvae provides a rather good basis for the evaluation of some qualities of an artificial diet. Studies dealing with such diets for coccinellids show great variation in these values. In 16 generations of *H. axyridis* reared on honeybee brood, they varied between 38.6 and 60.6 % (OKADA et al. 1972). When ATALLAH & NEWSOM (1966) reared *C. maculata* on an artificial diet, they obtained an adult yield of about 90 %. The corresponding value was 3—19 % for *M. sexmaculatus,* 12.8 % for *P. japonica,* 8.6 % for *C. kuwanae* and only 1.2 % for *A. mirabilis,* on honeybee brood (MATSUKA et al. 1972). On the best diets of this study the yield of adults varied between 60 and 80 %. When the larvae were fed on *Acyrthosiphon pisum* Harris, the corresponding value was 70—95 % and on *M. persicae* 65—90 %.

The results of the present experiments can be considered encouraging. The main aim, however, that of producing a diet on which *A. bipunctata* could be reared for generations, was not achieved. While frequent matings were observed, no eggs were produced, in spite of survival of some of the adults for six months. On the other hand, adults produced on these diets did lay fertile eggs when fed during adult life on living or frozen aphids. Further, adult beetles that had been fed on aphids as larvae produced eggs on Diets 6 and 7. This would suggest that it might be rewarding to include an "aphid factor" in future diets to achieve fertile adults.

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References

Atallah, Y. H. & Newsom, L. D. 1966. Ecological and nutritional studies on Coleomegilla maculata De Geer (Coleoptera: Coccinellidae). I. The development of an artificial diet and a laboratory rearing technique. — J. Econ. Ent. 59, 1173—1179.

Beckman, H. F., Bruckart, S. M. & Reiser, R. 1953. Laboratory culture of the pink bollworm on chemically defined media. — J. Econ. Ent. 46, 627—630.

Blackman, R. L. 1967. The effect of different foods on Adalia bipunctata L. and Coccinella 7-punctata L. — Ann. Appl. Biol. 59, 207—219.

David, W. A. L. & Gardiner, B. O. C. 1966. Rearing Pieris brassicae (L.) on semi-synthetic diets with and without cabbage. — Bull. Ent. Res. 56, 581—593.

Geest, L. P. S. van der 1968. Effect of diets on the hemolymph proteins of larvae of Pieris brassicae. — J. Insect Physiol. 14, 537—542.

Ignoffo, C. M. 1963. A succesful technique for mass-rearing cabbage loopers on a semisynthetic diet. — Ann. Ent. Soc. Amer. 56, 178—182.

Junnikkala, E. E. 1969. Effect of a semi-synthetic diet on the level of the main nitrogenous compounds in the hemolymph of larvae of Pieris brassicae L. — Ann. Acad. Sci. Fenn. A IV 155, 1—10.

Matsuka, M., Shimotori, D., Senzaki, T. & Okada, I. 1972. Rearing some coccinellids on pulverized drone honeybee brood. — Bull. Fac. Agric., Tamagava Univ. 12, 28—38.

Montes Ogas, F. 1970. Biologia y morfologia de Eriopis connexa Germar 1824 y de Adalia bipunctata Linnaeus 1758 (Coleoptera). — Publicaciones del Centro de Estudios Entomológicos, Universidad de Chile 10, 43—56.

Okada, I., Hoshiba, H. & Maehara, T. 1972. [In Japanese]. Summary: Successive artificial rearing of a coccinellid beetle, Harmonia axyridis Pallas on lyophilized drone honeybee brood. — Bull. Fac. Agric., Tamagava Univ. 12, 39—47.

Smirnoff, W. A. 1958. An artificial diet for rearing coccinellid beetles. — Can. Ent. 90, 563—565.

Smith, B. C. 1965. Effect of food on the longevity, fecundity and development of adult coccinellids (Coleoptera, Coccinellidae). — Can. Ent. 97, 910—919.

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