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Nutritional Studies of an Aphidophagous Coccinellid *Harmonia axyridis*[1] II. Significance of Minerals for Larval Growth

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A diet consisting of drone honey bee brood provided an adequate nutrition for larvae of an aphidophagous coccinellid, *Harmonia axyridis* PALLAS. These beetles, however, could not grow on the diet lacking a fraction which is hot-water soluble, cationic and not adsorbed on active charcoal. A defined mixture of minerals could substitute for this fraction. The mineral requirements for the larval growth of the beetle were thus elucidated and shown to be substantial, especially for potassium.

INTRODUCTION

For rearing the aphidophagous coccinellid *Harmonia axyridis* PALLAS, freeze-dried powder of drone honey bee brood was shown to be better as a diet than several natural products such as yeast, liver, etc. (MATSUKA and OKADA, 1975). Several other aphidophagous, coccidophagous and chrysomelid-larva-feeding lady beetles and aphidophagous lacewings also can grow on the drone powder and water only (MATSUKA et al. 1972; OKADA et al., 1974). A previous investigation by fractionating this artificial diet showed that removal of the cationic fraction from a hot water extract of drone powder made it unsuitable for larval growth of *H. axyridis* (MATSUKA and OKADA, 1975). The possibility that the minerals contained in this fraction are required for the larval growth has been examined and confirmed in this report.

Mineral requirements for insect growth have so far been reported in several cases (see DADD, 1970). ATALLAH and NEWSOM (1966) added WESSON's salt mixture to an artificial diet for rearing the predaceous coccinellid *Coleomegilla maculata* and VANDERZANT (1969) prescribed another mixture for the predaceous lacewing *Chrysopa carnea*. These diets included, however, soy bean products and other natural substances which may be expected to meet certain of the mineral requirements of these predaceous insects. Investigations using holidic diets for rearing aphids, *Myzus persicae* (DADD and MITTLER, 1966) and *Acyrthosiphon pisum* (AUCLAIR and SRIVASTAVA, 1972) indicate the type of minerals which may be obtained from the prey of such predaceous insects.

MATERIALS AND METHODS

Old larvae and/or young pupae of drone honey bees, *Apis mellifera* L., were blended

[1] Coccinellidae : Coleoptera

and lyophilized. The drone powder was fractionated as follows. After extraction of lipids (21.8%) with ethyl ether using a SOXHLET apparatus, the defatted powder was twice treated for 20 min with water at 70°C to obtain water-soluble and residual fractions (latter, 44.8%). Amberlite IR-45 (OH^- form) anion exchange resin was added to the water-soluble fraction and its filtrate was treated with the cation exchange resin Dowex 50W-X2 (H^+ form). Substances not adsorbed on either resin constituted the neutral fraction (27.6%). The anion fraction (2.5%) was eluted from the Amberlite with 0.2 N HCl. The cations eluted from the Dowex resin with 4 N NH_4OH was treated with active charcoal, and subsequently eluted with 50% ethyl alcohol to obtain "cation-A" fraction (1.3%). The unadsorbed material constituted "cation-B" fraction (1.9%). All fractions except lipid were lyophilized and mixed in the ratios found in the original drone powder. The ratios obtained in one of the fractionations (given in brackets above) were used in the experiments described here.

To homogenize the constituents, all the fractions were dissolved or suspended and then lyophilized. The powdery diet was given to each larva in 5-cm petri dishes in a similar manner to that described previously (OKADA et al., 1972). Every feeding experiments were started with 20 replicates of single newly hatched larva.

RESULTS AND DISCUSSION

The larval growth of *H. axyridis* on the reconstituted diet, comprized of lipids, water-soluble and residual fractions was fairly good when compared to that obtained with original drone powder (MATSUKA and OKADA, 1975). If the water-soluble fraction was partitioned into 4 portions and then all the constituents recombined, larval and pupal period, pupal weight and the emergence of the adults were similar or inferior to those obtained on a diet lacking any one of the fractions except cation-B (Table 1). Possibly the procedure caused some change in each fraction and the modifications jointly resulted in a lower dietary value. The table shows that neutral materials, although they made up a large part (82.6%) of the water-soluble fraction (see Method), could be eliminated from the reconstituted diet. There was minor detrimental effect of the removal of the anionic fractions as MATSUKA and OKADA (1975) reported or of the cation-A which was adsorbed on charcoal, because several adults emerged on the diet lacking each of this fraction. The other cationic fraction (cation-B) which was not

Table 1. DEVELOPMENT OF *Harmonia axyridis* LARVAE ON THE RECONSTITUTED DRONE POWDER

Diet		% Development to			Pupal weight (mg) (mean±SD)	Average (L+P) period (days)
Base	Fraction deleted	4th-L	Pupa	Adult		
R+L+S[a]	None	90	85	85	33.8±4.5	21.9
R+L+CM[a]	None	45	35	30	19.6±1.5	35.0
〃	Neutral fraction	90	85	80	21.7±4.8	23.2
〃	Cation-A	60	60	50	18.5±3.7	30.7
〃	Cation-B	0	0	0	—	—
〃	Anions	30	30	25	20.0±3.0	29.8

[a] Hot water-soluble material (S) was mixed with lipids (L) and residual (R) fractions. All 4 sub-fractions from S (see Method) were used as a combined mixture (CM).

Table 2. Effect of Minerals on the Larval Development of *Harmonia axyridis*

Salts in diet	% Development to				Pupal weight (mg) (mean±SD)	Larval period (days)
	2nd-L	3rd-L	4th-L	Pupa		
None (cation-B deficient)	0	0	0	0	—	—
Wesson's salts (5%)	100	90	75	50	20.5±1.6	18.5
Myzus' salts[a] (4%)	95	85	75	55	19.5±4.0	19.9
Myzus' salts (6.9%)	100[b]	95	68	32	19.4±3.5	20.5
Ditto-4/5 K_3PO_4	90	20	0	0	—	—
Ditto-4/5 $MgCl_2$	85	75	50	25	19.7±1.9	20.6
Ditto-4/5 minor salts	100	95	70	35	16.8±4.1	21.7

[a] Salt mixture according to Dadd and Mittler (1966) but supplied as: K_3PO_4 500.0 mg, $MgCl_2$ 200.0 mg, $FePO_4 \cdot 4H_2O$ 1.0 mg, $ZnSO_4$ 0.3 mg, $CuSO_4 \cdot 5H_2O$ 0.3 mg, $MnSO_4$ 0.3 mg.

[b] It means 19 larvae.

adsorbed on charcoal were clearly essential: i.e., larvae could not develop to the 4th instar in the absence of this fraction. Dietary essentiality of several amino acids, sugars, cholesterol, vitamins and minerals has been established for many insects (House, 1974). Incomplete fractionation by our procedure may distribute these essential substances among several fractions. For example, the residual (R) fraction composed mainly of polymers of amino acids, sugars and nucleotides may contain trace levels of some of the other essential nutrients and may offset the effect of omitting a certain fraction.

In order to examine whether minerals could be used as a substitute for the required cation-B fraction, several inorganic salts were mixed with cation-B-deficient reconstituted diet. Since *H. axyridis* is predatory on aphids, we used a modification of the salt mixture developed for the aphid *Myzus persicae* (Dadd and Mittler, 1966). Wesson's and the aphid salt mixtures were found to supply enough inorganic matter for larval growth when added at a level of 4—6.9% to the reconstituted diet, indicating the validity of the inference (Table 2). Reduction in the level of $MgCl_2$ or of the other 4 minor components (Fe, Mn, Cu and Zn) of the aphid salt mixture to one fifth of the original content (total 6.9% salts) did not give a marked effect, but reduction of K_3PO_4 to one fifth caused severe mortality and only 43rd-instar and no 4th-instar larvae developed from 20 young larvae. Since anionic fraction was shown to be dispensable (Table 1 and Matsuka and Okada, 1975), this result would come from a deficiency of potassium and not of phosphorus. The one fifth level of K_3PO_4 means 5400 ppm of potassium in the modified diet. The necessity of an even higher level may be a specificity of the predaceous larva in comparison with the minimal requirement of 2000 ppm K for the flour beetle, *Tribolium confusum* (Medici and Taylor, 1966). The quantitative mineral requirements of *H. axyridis* larva, however, can not be clarified using these drone brood fractions, because they are so crude that scattering of a component among some fractions is inevitable. Development of chemically defined, holidic diet is necessary for further progress. In preliminary trials, holidic diets made by combining amino acids (in the ratios in which they occur in drone pupae according to Tomoda et al., 1975), sucrose, cholesterol, vitamins and the inorganic salts used in this report have failed to support larval growth of the coccinellid.

The insect's salt requirements were confirmed in another way. Although no adult *H. axyridis* developed on a mixture of dried yeast and sucrose, compared to the success of Hagen and Tassan (1970) in rearing aphidophagous lacewings, 4 adults

Table 3. LARVAL DEVELOPMENT ON THE YEAST+SUGAR DIET WITH OR WITHOUT *Myzus*' SALTS

Diet	% Development to			Average pupal wt. (mg)	Developmental period (days)
	4th-L	Pupa	Adult		
Dried yeast+sucrose (7 : 3)	70	15	0	14.2	—
Ditto+*Myzus*' salts[a] (6.5 : 3 : 0.5)	75	50	20	16.3[b]	28.8

[a] See legend of Table 2 for the composition.
[b] Four adults had an average pupal weight of 19.4 mg.

developed from 20 young larvae on the addition of the aphids' salts at 5% (Table 3). It is the other problem that the pupal weights of these and those in previous tables are only around 20 mg except when supplying drone powder as it is. HUKUSHIMA and TAKEDA (1975) succeeded to rear *H. axyridis* by adding yeast extract to a chemical diet but pupal weight was also at a level of 16.7—18.5 mg. These small individuals had a longer developmental period. Since drone powder by itself sustained pupal weight of above 30 mg (e.g. OKADA et al., 1972), it probably includes growth-promoting factor(s) other than inorganic salts. The effects on the larval growth of *H. axyridis* of adding each drone powder fraction to a minimal, chemically defined diet are now being examined.

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