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CHANGES IN METABOLIC RESERVES OF THREE SPECIES OF APHIDOPHAGOUS COCCINELLIDAE (COLEOPTERA) DURING METAMORPHOSIS

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Newly moulted fourth instar larvae of *Coccinella 7-punctata, Adalia 2-punctata* and *Propylea 14-punctata* contained only small amounts of reserve fat and glycogen. The larvae fed voraciously and accumulated reserves of fat and glycogen faster than water. During the pupal stage, these reserves were rapidly consumed so that the young adult contained relatively more water than the pupa. During metamorphosis, 53 to 75% of the fat and 71 to 87% of the glycogen was consumed, but only small quantities of water were lost. Newly emerged adult *C. 7-punctata* and *A. 2-punctata* reared as larvae on *Acyrthosiphon pisum* were not only heavier but contained more actual water, fat and glycogen than those reared on *Aphis fabae*. By contrast, adult *P. 14-punctata* reared on *A. pisum* were lighter and contained less water than those fed on *A. fabae*, but their fat and glycogen contents were nearly the same when fed on either aphid species. Newly emerged adult *A. 2-punctata* that had fed as larvae on *Microlophium evansii* on nettles in the field were heavier and contained more reserves than others that had fed as larvae on *A. fabae* on bean plants in the field. Adult *A. 2-punctata* that had fed as larvae on *A. fabae* in the field were also heavier and contained more reserves than those reared from larvae fed on laboratory reared *A. fabae*.

Holometabolous insects need a store of energy, usually fat, for their metamorphosis from larva to adult insect. Fat is usually most abundant just before metamorphosis (WIGGLESWORTH, 1953).

No quantitative work has apparently been done on the accumulation and use of reserves during the metamorphosis of Coccinellidae. This paper provides such information on reserve fat and glycogen in *Coccinella septempunctata* L., *Adalia bipunctata* (L.) and *Propylea quattuordecimpunctata* (L.) and describes the effect of diets of two different aphid species on the reserves of their larvae, pupae and young adults.

METHODS

Coccinellid larvae of the three species were reared from eggs in small Perspex cages similar to those described by KADDOU (1961). A hole of 25 mm diameter in a piece of Perspex (50 mm × 50 mm × 5 mm) was covered above by a leaf of broad bean (*Vicia faba*) on which aphids fed, and below by a piece of muslin to allow ventilation. Fresh leaves were provided every 2—3 days.

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Sufficient aphids (*Acyrthosiphon pisum* Harris or *Aphis fabae* Scop.) of various ages were provided twice or more each day to ensure that the coccinellid larvae always had an excess of food.

The cages were kept at 21°—23° and 60—70% R.H.

A sample of about ten newly moulted fourth instar larvae of each species of coccinellid was taken and the initial live and dry weight, water, fat and glycogen contents of each insect determined. Similar determinations were made when the remaining larvae were 2 and 4 days old and sometimes when 3 or 5 days old; new pupae and newly emerged adults were similarly treated.

Pupae of *A. bipunctata* were collected in the field from broad bean plants infested with *A. fabae* in 1963 and 1964, and also in 1964 from stinging nettles (*Urtica dioica* L.) infested with *Microlophium evansii* Theo. Newly emerged adults from these pupae were similarly analysed.

Insects of each sample were weighed alive individually on a torsion balance (sensitivity, 0.1 mg) and again after being dried to a constant weight at 70° to obtain their dry weight and water content.

The dried insects were next extracted with petroleum-ether (B.P. 40°—60°) for 24 hr in a Soxhlet apparatus to remove phospholipids, neutral fats, fatty acids and other ether-soluble substances. After extraction, the lean weights and fat contents were obtained by re-weighing each insect.

After fat extraction, the insects, singly, in pairs or in threes, were digested in 1—1.5 ml 30% KOH and heated in boiling water for 30 min. Glycogen was precipitated from the solution, dissolved in distilled water and estimated colorimetrically by the method of SEIFTER *et al.* (1950) except that the glycogen was estimated directly by comparing the samples with known concentrations of pure glycogen treated in the same way and not with glucose solutions.

Fat and glycogen are expressed as percentages of dry weight.

RESULTS

Table I summarises the results. The duration of the last larval and pupal instars, longest in *C. 7-punctata* (12 days), intermediate in *A. 2-punctata* (10—11 days) and shortest in *P. 14-punctata* (9 days), seems to be associated with the size of the insects, the smaller the species the shorter the duration of metamorphosis (the mean live weights were respectively, 35—39 mg, 9—14 mg and 7—8 mg).

Dry weight and water content

A last instar larva kills 60—75 per cent of all the aphids that a coccinellid larva kills during its whole life and most of the reserves are laid down during this stage. During the last instar all three species increased greatly in dry weight (Table I, Fig. 1).

Larvae of *C. 7-punctata* and *A. 2-punctata* fed on *A. pisum* were heavier in live and dry weight, increased faster in weight and contained more water than those fed on *A. fabae*, and the resulting pupae and young adults were also heavier; larvae of *P. 14-punctata* increased in live and dry weight faster when fed on *A. pisum*, but were finally lighter and contained less water than those fed on *A. fabae* (Table I).

TABLE I

Mean water, fat and glycogen contents (with standard errors) of samples of ten coccinellids during metamorphosis, after being reared as larvae on Aphis fabae *(A) and* Acyrthosiphon pisum *(B).* (L = larva; pp = pre-pupa; P = pupa; At = adult)

	Age	Live weight (mg)	Dry weight (mg)	Water (mg)	Fat (mg)	Glycogen (μg)
Coccinella 7-punctata						
A	0 days L	11.8 ± 0.4	2.4 ± 0.1	9.4 ± 0.3	0.1 ± 0.02	9.8 ± 0.9
B		11.2 ± 0.5	2.0 ± 0.1	9.2 ± 0.4	0.1 ± 0.01	18.2 ± 0.02
A	2 " L	36.5 ± 1.0	9.6 ± 0.4	26.9 ± 0.7	1.7 ± 0.2	177.9 ± 19.2
B		38.0 ± 1.1	9.9 ± 0.3	28.1 ± 0.8	1.9 ± 0.1	176.1 ± 9.1
A	4 " L	45.3 ± 1.6	12.1 ± 0.4	33.2 ± 1.2	2.4 ± 0.1	361.7 ± 20.8
B		47.5 ± 1.1	12.6 ± 0.3	34.9 ± 0.9	2.4 ± 0.1	399.0 ± 20.7
A	6 " P	40.9 ± 1.1	11.0 ± 0.3	29.9 ± 0.9	2.0 ± 0.09	211.2 ± 12.5
B		42.0 ± 1.5	11.0 ± 0.3	31.0 ± 1.3	2.0 ± 0.06	251.0 ± 15.7
A	12 " At	35.1 ± 1.3	8.3 ± 0.3	26.8 ± 1.0	0.7 ± 0.05	47.4 ± 2.1
B		39.0 ± 1.0	8.7 ± 0.2	30.3 ± 0.8	0.8 ± 0.06	57.3 ± 5.6
Adalia 2-punctata						
A	0 days L	4.9 ± 0.1	1.0 ± 0.04	3.9 ± 0.1	0.1 ± 0.02	3.4 ± 0.5
B		5.3 ± 0.3	1.2 ± 0.08	4.1 ± 0.3	0.1 ± 0.02	4.8 ± 0.6
A	2 " L	12.2 ± 0.8	2.9 ± 0.3	9.3 ± 0.5	0.5 ± 0.07	46.2 ± 4.6
B		15.1 ± 0.5	4.3 ± 0.2	10.9 ± 0.3	0.9 ± 0.07	95.4 ± 19.8
A	4 " L	12.3 ± 0.5	3.3 ± 0.2	9.0 ± 0.4	0.8 ± 0.04	53.8 ± 2.7
B	4 " pp	17.0 ± 0.5	5.6 ± 0.2	11.4 ± 0.4	1.5 ± 0.07	124.1 ± 10.6
A	5 " pp	10.5 ± 0.2	3.0 ± 0.08	7.5 ± 0.2	0.7 ± 0.03	66.9 ± 2.4

TABLE I (Ctd)

Age	Live weight (mg)	Dry weight (mg)	Water (mg)	Fat (mg)	Glycogen (μg)
A p	9.2 ± 0.2	2.7 ± 0.07	6.5 ± 0.2	0.6 ± 0.03	57.0 ± 3.6
6 days					
B	16.0 ± 0.8	5.0 ± 0.2	11.0 ± 0.6	1.3 ± 0.07	65.6 ± 1.0
(1 day old)					
p					
A 11 days	9.2 ± 0.4	2.3 ± 0.2	6.9 ± 0.3	0.2 ± 0.02	11.0 ± 1.7
At					
B 10 "	14.2 ± 0.7	3.9 ± 0.2	10.3 ± 0.5	0.7 ± 0.04	26.1 ± 2.2
At					
Propylea 14-punctata					
A	4.3 ± 0.2	1.1 ± 0.06	3.2 ± 0.1	0.1 ± 0.01	9.6 ± 0.8
0 days					
B	4.3 ± 0.2	0.8 ± 0.05	3.5 ± 0.2	0.1 ± 0.02	7.1 ± 2.1
L					
A	11.5 ± 0.3	3.1 ± 0.08	8.4 ± 0.2	0.5 ± 0.03	60.3 ± 5.2
2 "					
B	11.2 ± 0.5	2.8 ± 0.10	8.4 ± 0.3	0.5 ± 0.03	71.8 ± 3.8
L					
A pp	10.0 ± 0.3	2.6 ± 0.09	7.4 ± 0.2	0.5 ± 0.02	57.7 ± 2.5
3.5 "					
B pp	9.7 ± 0.4	2.6 ± 0.20	7.1 ± 0.3	0.5 ± 0.02	82.1 ± 6.7
2 "					
A P	9.8 ± 0.4	2.6 ± 0.11	7.2 ± 0.3	0.4 ± 0.02	61.2 ± 2.6
4.5 "					
B P	8.5 ± 0.2	2.2 ± 0.06	6.3 ± 0.2	0.4 ± 0.02	46.2 ± 2.2
A	8.2 ± 0.3	2.0 ± 0.07	6.2 ± 0.2	0.2 ± 0.01	12.1 ± 1.3
9 "					
B	7.6 ± 0.2	1.7 ± 0.04	5.9 ± 0.2	0.2 ± 0.01	11.3 ± 1.1
At					

During the last larval instar, dry weight and water content increased about 3—5 times in *C. 7-punctata*, by 2—4 times in *A. 2-punctata* and by 1.5—2.5 times in *P. 14-punctata*, but decreased during the pupal stage. All three species accumulated and used dry matter and water in the same way during metamorphosis (Fig. 1). The proportion of water was about 80 per cent at the start of the last larval instar and about 70% at its end, but the proportion increased to 76% in the newly emerged adult.

Fat and glycogen contents

The fat and glycogen contents also increased rapidly during the last larval instar and decreased rapidly during the pupal stage (Table I, Fig. 2). Fat and glycogen increased 23 and 36 times respectively in larvae of *C. 7-punctata*; increases of 7 to 14 times (fat) and 15 to 25 times (glycogen) occurred in the smaller species, *A. 2-punctata*, and of 4 times (fat) and 5 to 10 times (glycogen) in the smallest species, *P. 14-punctata*.

In all species, 53 to 75% of the fat and 71 to 87% of the glycogen accumulated by the larva was consumed during metamorphosis and, as a result, the proportion of water in the young adult increased. The ratio of water/fat decreased during the larval stage because fat accumulated faster than water. During the pupal stage fat was consumed rapidly, and water, produced by the oxidation of fats, increased relatively faster (Table II).

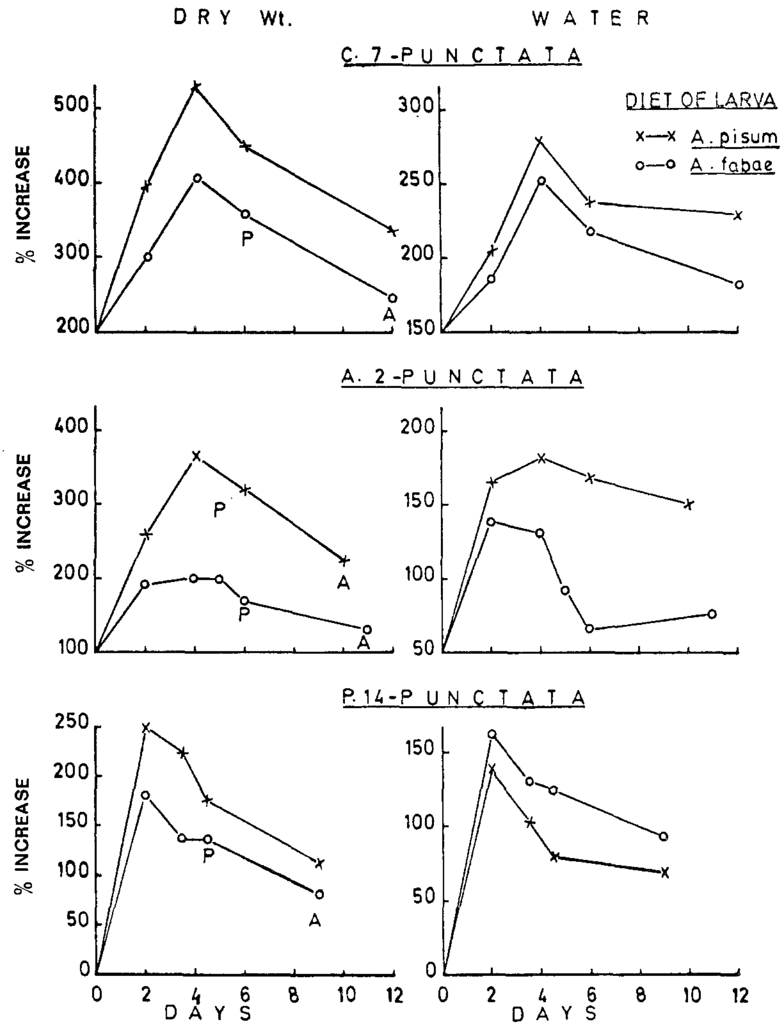

Fig.1. Rates of change of dry weight and water content of coccinellids during metamorphosis, as percentages of the values of newly moulted last instar larvae. P = pupa, A = adult.

TABLE II

Water/fat ratios during metamorphosis of coccinellids

C. 7-punctata							
Days	0	2	4	6	12		
A. fabae	94.0	15.8	13.8	14.8	38.1		
A. pisum	92.0	14.8	14.5	15.5	37.9		
A. 2-punctata							
Days	0	2	4	5	6	10	11
A. fabae	39.0	18.6	11.2	10.7	10.8	—	34.5
A. pisum	44.0	12.1	7.6	—	8.5	14.7	—
P. 14-punctata							
Days	0	2	3.5	4.5	9		
A. fabae	39.0	16.8	14.8	18.0	31.0		
A. pisum	35.0	16.0	14.2	15.7	29.5		

Newly emerged adult *C. 7-punctata*, and especially *A. 2-punctata*, reared during larval life on *A. pisum*, were not only heavier (see above) but contained more water, fat and glycogen than those reared on *A. fabae*. Adult *P. 14-punctata* reared on *A. pisum* were, however, lighter and contained less water than those fed on *A. fabae* but their fat and glycogen contents were nearly the same when reared on either aphid species (Table I).

Although females from the field were heavier than males and contained more fat and glycogen, the proportions of these reserves were about the same in both sexes. Insects of both sexes that had fed as larvae on *M. evansii* on nettles were heavier, had a smaller proportion of water, and contained more fat and glycogen (but in the same proportion) than those that had fed on *A. fabae* on beans (Table III).

The analyses of adult *A. 2-punctata*, collected as pupae from bean fields in 1964 and which as larvae had fed on *A. fabae*, were almost identical to those of similarly fed insects in 1963 (Table III).

TABLE III

Mean water, fat and glycogen contents (with standard errors) of newly emerged adult A. 2-punctata *collected as pupae from nettles and broad beans*

Date	larval food		n	Live weight (mg)	Dry weight (mg)	Water (mg)	Fat (mg)	Glycogen (μg)
Aug. 1963	*A. fabae* (Broad bean)	♂	(10)	10.2 ± 0.3	2.2 ± 0.1	8.0 ± 0.2	0.5 ± 0.04	13.7
		♀	(10)	12.9 ± 0.2	2.9 ± 0.1	10.0 ± 0.1	0.6 ± 0.03	16.3
Aug. 1964	*A. fabae* (Broad bean)	♂	(12)	10.5 ± 0.2	2.4 ± 0.1	8.1 ± 0.2	0.5 ± 0.03	12.1 ± 2.3
		♀	(12)	12.9 ± 0.3	3.0 ± 0.1	9.9 ± 0.3	0.6 ± 0.04	20.8 ± 1.6
July 1964	*M. evansii* (Nettle plants)	♂	(9)	11.1 ± 0.4	3.2 ± 0.1	7.9 ± 0.3	0.7 ± 0.04	16.6 ± 2.1
		♀	(11)	14.0 ± 0.3	3.9 ± 0.1	10.1 ± 0.2	0.8 ± 0.04	26.3 ± 1.5

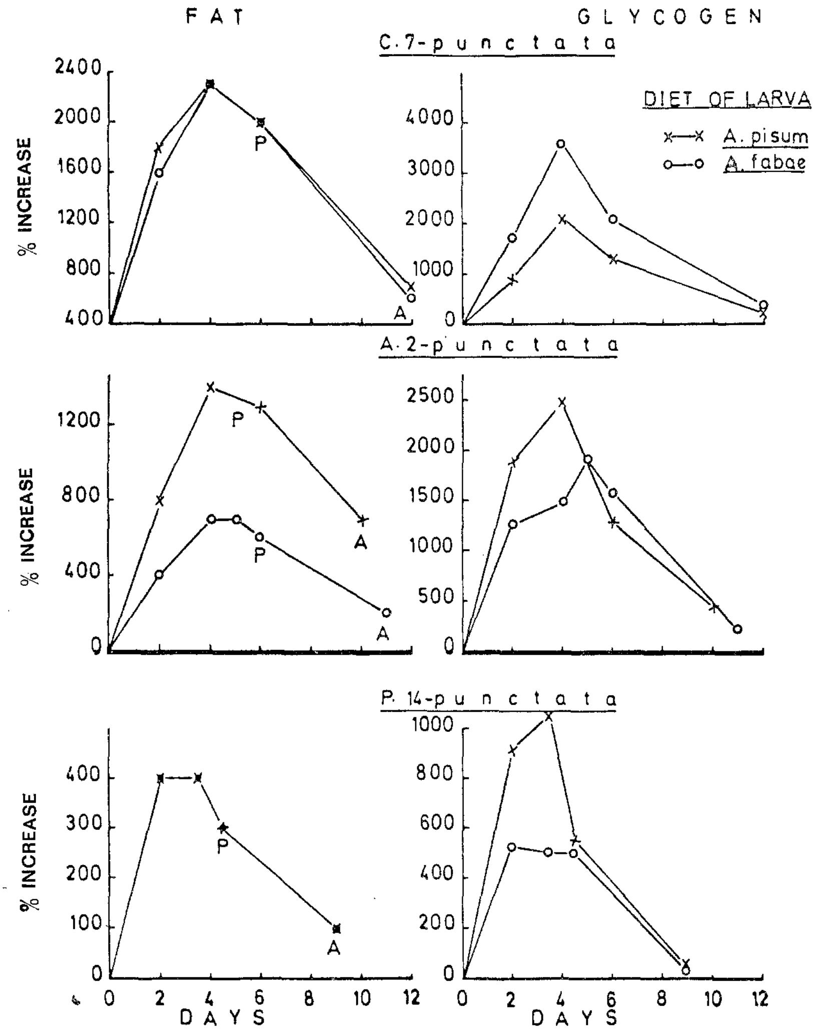

Fig. 2. Rates of change of fat and glycogen content of coccinellids during metamorphosis, as percentages of the amounts present in newly moulted last instar larvae. P = pupa. A = adult.

Young adult *A. 2-punctata* that had developed as larvae on *A. pisum* in the laboratory did not differ significantly in weight, water or fat content from those that had developed in the field on the similar *M. evansii* on nettles (cf Tables I and IV), which suggests that the insects reared in the laboratory on *A. pisum* were typical in these respects. But adult *A. 2-punctata* reared as larvae on *A. fabae* in the laboratory were lighter and contained less water, fat and glycogen than those that had developed on *A. fabae*-infested bean plants in the field (Table IV). The *A. fabae* used for laboratory rearing were apparently less nutritious than those occurring in the field. Cockbain (1961) showed that unflown alate *A. fabae* from a laboratory culture were smaller and contained less fat than alatae collected outdoors.

TABLE IV

Mean water, fat and glycogen contents (with standard errors) of newly emerged adult A. 2-punctata *reared as larvae in the laboratory and the field on various aphids*

Place	larval food	Live weight (mg)	Dry weight (mg)	Water (mg)	Fat (mg)	Glycogen (μg)
Field	*A. fabae*	11.8 ± 0.3	2.8 ± 0.1	9.0 ± 0.8	0.5 ± 0.03	16.4 ± 2.1
Laboratory	*A. fabae*	9.2 ± 0.4	2.3 ± 0.2	6.9 ± 0.3	0.2 ± 0.02	11.0 ± 1.7
Field	*M. evansii*	12.7 ± 0.4	3.6 ± 0.1	9.1 ± 0.3	0.8 ± 0.03	221. ± 2.3
Laboratory	*A. pisum*	14.2 ± 0.7	3.9 ± 0.2	10.3 ± 0.5	0.7 ± 0.04	26.1 ± 2.2

DISCUSSION

Coccinellid larvae in the early fourth instar contained much water and little fat and glycogen. The larvae fed voraciously and accumulated reserves relatively faster than they did water, which also increased in quantity. Only small amounts of water were lost during moulting to the pupal stage and during the emergence of the young adult from the pupa. Water lost by evaporation was, presumably, balanced by the water produced by the oxidation of metabolic reserves during the metamorphosis.

FAST (1964), whose lists do not include any references to Coccinellidae, reviewed the literature on fat in insects and pointed out that comparisons of analyses from various insects are almost impossible, because extraction techniques differ so much. The proportion of fat of the live weight of the Coccinellidae I studied increased to a maximum of 5 to 9% at the end of the fourth instar and decreased to 2 to 5% in the newly emerged adult. Other adult predacious insects (Carabidae, Dytiscidae and Notonectidae) listed by Fast had fat contents of 5 to 7% of the live weights, but nothing is known of the age of the insects and whether they had fed. The only information on the changes in fat reserves of Coccinellidae during metamorphosis are those of YERMOLENKO (1963) who studied the changes in fat content of the coccidophagous *Cryptolaemus montrouzieri* Muls. The newly emerged adult of this species contained 36 to 41% fat of the dry weight, whereas my estimates of fat in newly emerged adults of the three aphidophagous species were much less, ranging from 9 to 22% of the dry weight in laboratory and field reared insects.

The proportion of fat consumed by insects during metamorphosis varies from 38 to 92%, and of glycogen from 73 to 100% (NEEDHAM, 1942). My results with Coccinellidae show that 53 to 75% of the fat and 71 to 87% of the glycogen accumulated by the larvae is consumed during metamorphosis.

The larvae of the largest species, *C. 7-punctata*, contained more fat and glycogen than the smaller *A. 2-punctata*, which contained more of these reserves than the smallest species, *P. 14-punctata*. During metamorphosis, the largest species consumed a much greater quantity of fat and glycogen than the smaller species; the average proportions of fat used up were respectively 71%, 64% and 60% and the average proportions of glycogen consumed were 86%, 82% and 83%.

ATWAL & SETHI (1963) reported that larvae and pupae of *C. 7-punctata* were heavier and the pupae contained more dry matter when larvae were fed on *Lipaphis erysimi* than on *Macrosiphum granarium* or *Aphis gossypii*. The live and dry weights, and the water, fat and glycogen contents of larvae, pupae and adult *A. 2-punctata*, and to some extent *C. 7-punctata*, were much smaller when the larvae were reared on *A. fabae* instead of *A. pisum* and this effect appeared in the late fourth instars. I have already reported that the fecundity of *A. 2-punctata* is also greatly decreased when the adult beetles feed on *A. fabae* instead of *A. pisum* (HARIRI, 1966). The cause of these adverse effects of *A. fabae* on *A. 2-punctata* are still unexplained but *A. pisum* seems to be more nutritious for this species of coccinellid, for although all three species were given an excess of either *A. fabae* or *A. pisum* and presumably fed at a maximum rate, *A. 2-punctata* in particular was adversely affected by *A. fabae*.

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ZUSAMMENFASSUNG

VERÄNDERUNGEN IN DER STOFFWECHSELRESERVE VON DREI APHIDOPHAGEN COCCINELLIDEN (COLEOPTERA) WÄHREND DER METAMORPHOSE

Frischgeschlüpfte Larven des 4. Stadiums von *Coccinella septempunctata*, *Adalia bipunctata* und *Propylea quattuordecimpunctata* enthielten nur geringe Mengen Reservefett und Glykogen, aber viel Wasser. Die Larven frassen gierig und häuften Vorräte von Fett und Glykogen schneller an als Wasser. Während des Puppenstadiums wurden diese Reserven schnell verbraucht, so daß die jungen Imagines relativ mehr Wasser als die Puppen enthielten. Während der Metamorphose wurden 53—75% der Fettes und 71—87% der Glykogens verbraucht, aber nur kleine Mengen Wasser verloren. Frischgeschlüpfte Käfer von *C. septempunctata* und *A. bipunctata*, die als Larven mit *Acyrthosiphum pisum* gefüttert worden waren, waren nicht nur schwerer, sondern enthielten auch mehr freies Wasser, Fett und Glykogen als die mit *Aphis fabae* aufgezogenen. Im Gegensatz dazu waren erwachsene *P. quattuordecimpunctata*, die sich von *A. pisum* genährt hatten, leichter und enthielten weniger Wasser als solche, die mit *A. fabae* gefüttert worden waren, aber ihr Fett- und Glykogengehalt war fast der gleiche, wenn sie mit anderen Blattläusen ernährt worden waren. Frischgeschlüpfte Käfer von *A. bipunctata*, die als Larven im Freien auf Brennesseln *Microlophium evansii* gefressen hatten, waren schwerer und enthielten mehr Reservestoffe als andere, die als Larven im Freien auf Ackerbohnen von *A. fabae* gelebt hatten. Erwachsene *A. bipunctata*, die als Larven im Freien *A. fabae* gefressen hatten, waren ebenfalls schwerer und enthielten mehr Reservestoffe als diejenigen, die im Laboratorium mit *A. fabae* gefüttert worden waren.

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