was 10 to 14 times lower than that of the three strains of *C. brevilineata* (248–337 pmol/mg protein/60 min).

On the other hand, hydrolysis of fenitroxon was very weak, and similar, in the two species. This experimental evidence leads us to conclude that the high activity of the fenitroxon detoxication by binding protein in the soluble fraction should be one of the mechanisms of the insensitivity to fenitrothion in *C. brevilineata*.

The role of esterases as binding protein or "sequestration" to protect AChE from inhibition by oxygen analogs of OP insecticides have been reported with the green rice leafhopper, *Nephotettix cincticpes* (Hosokawa and Motoyama, 1981; Motoyama et al., 1984) and the peach-potato aphids, *Myzus persicae* (Devonshire and Moores, 1982). Konno and Shishido (1989) and Suzuki et al. (1993) also reported that the fenitroxon binding by some protein in the soluble fraction was the principal mechanism of fenitrothion resistance in the rice stem borer and the cotton aphid, respectively. However, the present study is the first evidence that an aquatic insect also can protect AChE from inhibition by oxygen analogs of OP insecticides by binding protein in the soluble fraction.

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Partial Rearing of a Phytophagous Lady Beetle, *Epilachna vigintioctopunctata* (Coleoptera: Coccinelidae)[1]

Maki Murata, Kikuo Iwabuchi and Jun Mitsuhashi

Laboratory of Applied Entomology, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan

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The phytophagous lady beetle, *Epilachna vigintioctopunctata*, is an oligophagous insect which feeds only on solanaceous plant leaves. Only a few reports have been published on the rearing of phytophagous beetles, including this species, on an artificial diet. Wardojo (1969) and Hsiao and Fraenkel (1968) reared the larvae of the Colorado potato beetle, *Leptinotarsa decemlineata*, on artificial diets. Kogan (1971) reared the larvae of the Mexican bean beetle, *Epilachna varivestis*, on artificial semiliquid diets. The diets used for the rearing of these species, however, could not support the entire larval growth.

Kono (1980) showed that *E. vigintioctopunctata* could be reared on sliced potato tubers as an alternative diet, but that the development of the fat body would be delayed.

The development of a suitable artificial diet for

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this species will facilitate physiological studies of it, and will also point the way for the studies of nutritional requirements and host plant selection. In the present study, a feeding method using liquid diets absorbed on sheets of toilet paper was found to be suitable for the rearing of *E. vigintioctopunctata*.

MATERIALS AND METHODS

Insects. Adults of *E. vigintioctopunctata* were collected in fields in Tokyo from potato (*Solanum tuberosum*) and horse-nettle (*Solanum carolinense*) plants. The adults and larvae were routinely reared on *S. carolinense* leaves in plastic cups (100 mm dia. × 55 mm ht.) with small vent holes in the lid and on the side at 25°C under a 16L–8D photoperiod.

Preparation of diets. Several types of diets were formulated. The basic solid diet consisted of 1 g agar, 1 g WESSON's salt mixture, 0.4 g choline chloride, 0.1 g cholesterol, 0.4 g L-ascorbic acid, 10 g *S. carolinense* dry leaf powder, and 100 ml distilled water. A modification of this diet was made by replacing the leaf powder with a cellulose powder with an absorbed ethanol extract of *S. carolinense*. Another solid diet tested, designated as diet D, was the diet A solidified by the addition of cellulose powder and agar (Table 1). In the liquid-diet category, the diets A, B, C and E in Table 1 were tested. Sheets of toilet paper were cut into disks to fit inside a plastic Petri dish (90 mm dia. × 20 mm ht.). Four such disks were stacked on the bottom of the Petri dish. One ml of component Group II in Table 1 was absorbed into the paper and was left at room temperature until its ethanol evaporated. Then, 2.5 ml of component Group I of Table 1 was absorbed into the paper.

Table 1. Compositions of artificial diets tested for rearing *E. vigintioctopunctata* larvae

Component	Diet (g)				
	A	B	C	D	E
Group I					
Casein	5	5	5	5	—
Casein hydrolysate	5	5	5	5	—
Amino acid mixture[a]	—	—	—	—	0.962
WESSON's salt mixture	0.1	0.1	0.1	0.1	0.1
L-Ascorbic acid	0.2	0.2	0.2	0.2	0.2
Vitamin B mixture[b]	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Inositol	0.1	0.1	0.1	0.1	0.1
Choline chloride	0.1	0.1	0.1	0.1	0.1
Sucrose	5	5	5	5	5
Cellulose powder	—	—	—	5	—
Agar	—	—	—	1.5	—
Distilled water	100 ml	100 ml	100 ml	100 ml	100 ml
Group II					
Cholesterol	1	1	1	1	1
β-Sitosterol	—	0.5	—	—	0.5
Stigmasterol	—	—	0.5	—	—
Linolenic acid	25 μl	25 μl	25 μl	25 μl	25 μl
Linseed oil	3 ml	3 ml	3 ml	3 ml	3 ml
Sorbic acid	0.2	0.2	0.2	0.2	0.2
Chloramphenicol	0.125	0.125	0.125	0.125	0.125
99.5% Ethanol	100 ml	100 ml	100 ml	—	100 ml

[a] See Table 2.

[b] 0.01 g folic acid, 0.01 g biotin, 0.05 g riboflavin, 0.1 g thiamine-HCl, 0.1 g Ca-pantothenate, 0.05 g pyridoxine-HCl, 0.1 g *p*-aminobenzoic acid, 0.1 g nicotinic acid and 0.05 g carnitine were dissolved in 500 ml of distilled water.

We used toilet paper (King®; Suzukami Paper Works, Shizuoka) because it was found to absorb the liquid diets better than filter paper.

Rearing of larvae. The larvae were placed individually on artificial diets or on host plant leaves in Petri dishes at the beginning of the fourth instar stage (the last instar). All Petri dishes were placed into a plastic box containing a sheet of wet paper on the bottom. They were then incubated at 25°C, under a 16L–8D photoperiod. The humidity was high enough to protect the diets from desiccation. Each larva was weighed every day. When the larvae reached the prepupal stage, they were transferred onto a sheet of filter paper and were kept under dry condition. After emergence the adults were reared on *S. carolinense* leaves and mated with adults from a stock colony. The ability of oviposition was recorded for each female.

Table 2. Composition of amino acid mixture of diet E

Component	mg per 100 ml D.W.
L-α-Alanine	25
L-β-Alanine	25
L-Arginine HCl	60
Na-L-Asparate	110
L-Cysteine	25
Na-L-Glutamate	130
Glycine	50
L-Histidine	25
L-Isoleucine	55
L-Leucine	70
L-Lysine HCl	60
L-Methionine	25
L-Phenylalanine	55
Hydroxy-L-Proline	10
L-Proline	40
L-Serine	35
L-Threonine	50
L-Tryptophan	40
L-Tyrosine	12
L-Valine	60

The diet was evaluated by an index on gravimetric records. The index numbers were calculated from the following formula: $Gr=(W_{max}-W_0)/W_0$, where W_{max} was maximum weight attained by the larvae during the experimental period and W_0 was the initial weight of the larvae. The mean W_0 of the tested larvae was 7.1 ± 0.5 mg (mean$\pm$SD). In cases when the larvae did not reach the pupal stage and their weight had stopped increasing, the rearing was terminated.

RESULTS AND DISCUSSION

At first, the basic solid diet and its modifications were tested. Neither the dry leaf powder diet nor the diet with ethanol extract of host plants could elicit feeding behavior. In the latter case, however, the diet did attract the larvae. Several modifications of diet form were made taking physical

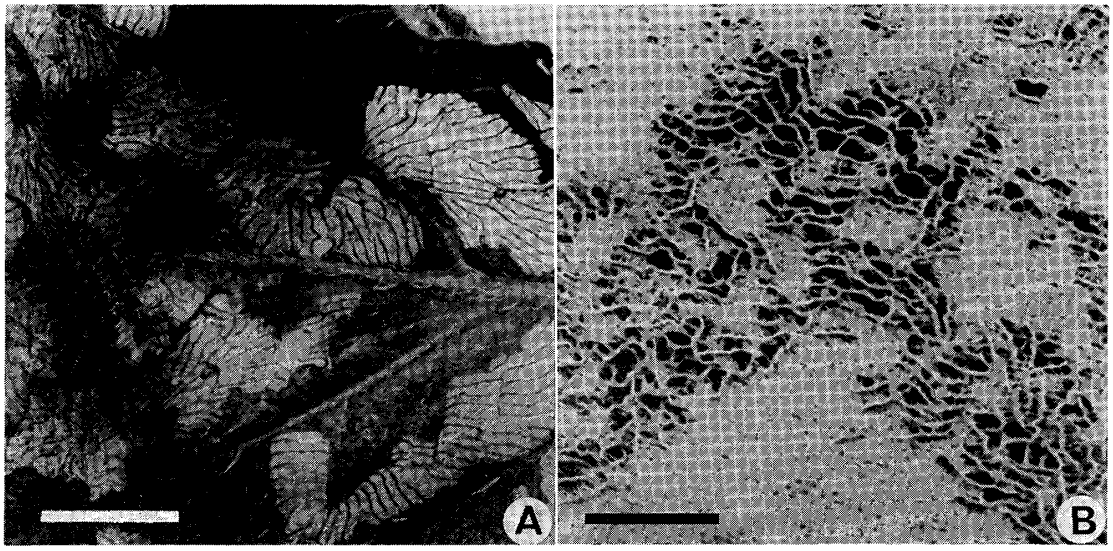

Fig. 1. Feeding marks of *E. vigintioctopunctata*. A: On host plant (*S. tuberosum*); B: On sheets of toilet paper. (Scale bars=5 mm)

Table 3. Development of *E. vigintioctopunctata* fourth instar larvae on artificial diets

Diet	*n*	Growth index[a]	Number of pupae obtained (rate,%)	Duration of the fourth instar stage (days)[a]	Number of adults obtained (rate,%)	Duration of pupal stage (days)[a]	Adult weight (*n*) (mg)[a]
Host plant	10	3.2±0.2	9 (90)	4.0±0.0	9 (90)	4.4±0.5	19.8±2.1 (9)
A	10	2.1±0.5	9 (90)	8.4±1.0	3 (30)	4.5±0.5	15.0 (2)
B	20	2.4±0.4	19 (95)	6.1±1.4	14 (70)	4.4±0.5	14.4±3.4 (13)
C	10	2.0±0.7	5 (50)	6.2±1.0	3 (30)	4.6±0.5	12.0±1.8 (3)
D	10	2.0±0.4	4 (40)	8.5±1.3	0 (0)	—	—
E	10	1.6±0.2	10 (100)	10.1±1.1	10 (100)	4.2±0.4	10.5±1.1 (10)

[a] Mean±SD.

properties of the diet into consideration. As a result, it was found that only liquid diets absorbed into sheets of toilet paper could elicit a stable feeding. Feeding marks similar to those found on the host plant were left on the tissue (Fig. 1). Larval developments on diets A–E and on host plants are shown in Table 3. The duration of the pupal stage was about 4 days irrespective of the diet used. The pupae which did not emerge were dissected 5 days after pupation to observe their internal condition. Solid diet D was not suitable because the duration of the fourth instar larvae was somewhat prolonged (8.5 days), the number of pupae obtained was the lowest and no adults emerged. Liquid diets (A, B, C and E) were preferred by the larvae. Little difference in preference was found among these diets. Comparing the rearing on diet A with that of diet B, β-sitosterol seemed to stimulate the development. However, stigmasterol showed no stimulatory effect (Table 3, A and C). For amino acids, the balance of each amino acid was referred to the amino acid compositions of an insect tissue culture medium (WYATT, 1956) and an artificial diet for *Bombyx mori* (ITO and ARAI, 1965). The diet containing amino acid mixture (diet E) depressed the growth index to its lowest level (1.6±0.2) and prolonged the duration of the fourth instar (10 days). However, all the larvae fed on diet E reached the adult stage. This result indicates that the diet E was qualitatively superior to diet B, but the amount of diet uptake by larvae was inferior to that of diet B probably because some amino acids are impalatable to the larvae. Adults of both sexes were obtained with about the same ratio in these rearings. When the female adults emerged from diets A–E and copulated with males reared on natural host plants, they laid fertilized eggs which proceeded to hatch, even though some adults exhibited malformed wings due to a failure to molt. The male adults obtained from artificial rearing could make the females, reared on natural host plants, lay fertilized eggs. The diets A–E were also tested for the third instar larvae. Some of the larvae reached the fourth instar but none of them reached the pupal stage.

The feeding process of *Epilachna* has been described by HOWARD (1941) as <scrape and suck>. KOGAN (1971) used a semiliquid rather than a solid diet in rearing *Epilachna varivestis*. In the present study, liquid diet absorbed on sheets of toilet paper was found suitable for the feeding of *E. vigintioctopunctata*. As regards the components of the diets, it became evident that β-sitosterol stimulated larval growth, and that plant-derived substances are not required for feeding. The use of this feeding method will improve the efficacy of the diets and will facilitate further feeding experiments.

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