Table 4.—Results of competitive mating test between male horn flies sterilized with tepa and normal flies.

| Number | | | | Percent | | | |
|---|---|---|---|---|---|---|---|
| Sterile ♂♂ | Normal ♂♂ | Normal ♀♀ | Eggs | Hatch | Sterility | Corrected[a] sterility | Expected sterility |
| | | | *Test no. 1* | | | | |
| 0 | 41 | 116 | 548 | 71 | 29 | | |
| 41 | 40 | 116 | 561 | 56 | 44 | 21 | 51 |
| 125 | 38 | 113 | 494 | 24 | 76 | 66 | 77 |
| | | | *Test no. 2* | | | | |
| 0 | 39 | 53 | 444 | 66 | 34 | | |
| 23 | 41 | 49 | 355 | 51 | 49 | 23 | 36 |

[a] Corrected for control sterility by Abbott's formula.

dinyl benzoquinone compound was effective at 1 μg/fly (Harris, unpublished data). Thus 2–4 times more chemical was required to sterilize the stable fly than the horn fly on a dose/weight basis.

The data in Table 4 suggest that sterile males released on caged steers were not so competitive as normal males since the percentage sterility of eggs collected was lower than expected. However, the competitiveness of sterile males did approach that of normal males in 1 test. Similar results were reported by Lewis and Eddy (1964) for flies sterilized by gamma radiation.

In the large-cage test, 2366 normal horn flies were reared in the test cage and 3676 sterile ♂ were released into the test cage. Since the sex ratio was 1:1 (672 of the 1376 flies examined were ♂ and 704 ♀), the number of normal males was about 1180, and the ratio of sterile males to normal males was about 3:1. This ratio would give an expected sterility of 76%; however, the actual sterility after correction for normal sterility by Abbott's formula over the test period was 60%. Thus these data indicate also that sterile males are less competitive than normal males.

## REFERENCES CITED

**Hamilton, E. W., and P. A. Dahm. 1960.** A versatile automatic microapplicator. J. Econ. Entomol. 53 (5): 853–6.

**Harris, R. L. 1962a.** Laboratory colonization of the horn fly, *Haematobia irritans* (L.). Nature (London) 196 (4850): 91–92.

**1962b.** Chemical induction of sterility in the stable fly. J. Econ. Entomol. 55 (6): 882–5.

**Harris, R. L., R. A. Hoffman, and E. D. Frazar. 1965.** Chilling vs. other methods of immobilizing flies. J. Econ. Entomol. 58 (2): 379–80.

**Knipling, E. F. 1960.** Use of insects for their own destruction. J. Econ. Entomol. 53 (3): 415–20.

**Lewis, S. F., and G. W. Eddy. 1964.** Some effects of gamma radiation on the horn fly. J. Econ. Entomol. 57 (2): 275–7.

**Lindquist, A. W. 1961.** Chemicals to sterilize insects. J. Washington Acad. Sci. 51 (7): 109–14.

# Ecological and Nutritional Studies on *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae). I. The Development of an Artificial Diet and a Laboratory Rearing Technique[1]

YOUSEF H. ATALLAH and L. D. NEWSOM

Department of Entomology, Louisiana State University, Baton Rouge

## ABSTRACT

Sixteen experimental diets were formulated and tested for rearing *Coleomegilla maculata*. Only one proved to be satisfactory for rearing this coccinellid continuously under laboratory conditions. The basic ingredients of this diet were casein, sucrose, wheat germ, soybean hydrolyzate, glycogen, butter fat, a liver fraction, corn oil, brewer's yeast, dextrose, cotton leaf extract (carotenoids and sterols), ascorbic acid, salt mixture, vitamins, antibiotics, agar, and water.

The value of mass culture and liberation of indigenous entomophagous insects for control of insect pests is debatable. A satisfactory synthetic diet would greatly facilitate studies of the type needed to establish the potential of this method of insect control. The objective of this study was to develop a synthetic diet for rearing *Coleomegilla maculata*. A knowledge of the biology and feeding habits of this species is basic for such a study.

Forbes (1883) found that the guts of several coccinellids contained spores of fungi, aphids, mites, and pollen. Fungus spores constituted the major part of the food in the species he studied. A comparative study on some aphid-feeding coccinellids was carried out by Clausen (1915). Discussing insect dietary, Brues (1946) stated that *C. maculata* was found to subsist in major part on spores and

[1] Portion of a dissertation presented by the senior author for the Doctor of Philosophy degree, Louisiana State University. Accepted for publication May 28, 1966.

pollen, but eggs and larvae of beetles were found also in its gut. He concluded that this mixed diet apparently represents a transition stage, similar to that which occurred at the time predatory insects first arose from phytophagous insects. Thompson (1951) stated that predaceous insects have a high degree of specificity in host relations; however, they may be less specific than parasites. Putman (1958) studied several coccinellids and stated that they fed primarily on aphids, except *Coleomegilla* (=*Ceratomegilla*), which was more or less phytophagous. Smirnoff (1958) was the first to rear coccinellids on a medium made from agar, sucrose, honey, water, royal jelly, alfalfa flour yeast, pulverized dry prey, and sometimes beef jelly. Smith (1960) stated that *C. maculata legni* Timberlake fed on various pollens developed from 1st-instar larvae to the adult stage, whereas other species failed. Szumkowski (1951a) reported that *C. maculata* showed no preference for aphids over moth eggs except when the latter had a protective covering of hair scales. His field observations indicated that *C. maculata* and *Cycloneda sanguinea* (L.) were effective egg predators of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and the cotton leafworm, *Alabama argillacea* (Hübner). Szumkowski (1951b) reared *Colemegilla maculata* larvae on meat, liver, Vitamin C, and "Multivitamin Roch." His best results (93% development) were with liver + Vitamin C. Requirement for vitamins other than those of the B group have never been clearly substantiated in nutritional studies (Lipke and Fraenkel 1956). Ascorbic acid proved to be indispensable for the growth and development of the boll weevil, *Anthonomus grandis* Boheman; the bollworm, *Heliothis zea* (Boddie); and the salt-marsh caterpillar, *Estigmene acrea* (Drury), when reared under aseptic conditions (Vanderzant et al. 1962); it was added to the bollworm diet developed by Berger (1963). Vitamin E (tocopherols) exerts an absolute effect on the last nymphal stadium of the male of the house cricket, *Acheta domesticus* (L.), in which spermatogenic activity is disrupted in the absence of the vitamin (Meikle and McFarlane 1965).

NATURAL DIETS.—*Materials and Methods.*—The biology and feeding habits of *C. maculata* were studied in the laboratory at 27–33°C and 65–85% RH under continuous fluorescent light provided by 400-w, Cool White lamps giving 70 ft-c. Adults were collected from cottonfields in Baton Rouge, La., and vicinity July 10, 1962. Cotton aphids, *Aphis gossypii* Glover; turnip aphids, *Hyadaphis pseudobrassicae* (Davis); and corn leaf aphids, *Rhopalosiphum maidis* (Fitch) were provided for food. The adult beetles were sexed, then pairs were placed in ½-pint or 1-pint ice cream cartons covered with a petri dish. The aphids were offered to the beetles on cotton leaves. The beetles usually oviposited on these leaves. The eggs were collected and held in 16×60-mm vials to hatch.

Immediately after hatching, 5 larvae were placed in each of a ½-pint or 1-pint ice cream carton with a petri dish cover and fed aphids until they pupated. When the adults emerged they were sexed and kept in pairs in the cartons.

*Results.*—Fresh milk was tried first as a diet for the larvae. Drops of milk on wax paper were placed in the rearing box. Different instars were tested. Neither the newly hatched larvae nor the larvae in later instars could develop on a fresh-milk diet. Most larvae survived for about 4–7 days, depending on the instar, then died without any development. Survivors made normal growth if transferred to an aphid diet.

Larvae held singly in ½-pint ice cream cartons and provided with stamens of cotton, which supplied an excess of pollen, did not survive although Smith (1961) found that *C. maculata legni* could develop on various pollens from 1st-instar larvae to the adult. The experiment was repeated 5 times with 5 larvae/replicate. All larvae died within 4 days.

Adult females provided with an excess number of cotton aphids consumed 5–11 aphids/hour with an average of 7. The number of aphids consumed per adult per day of 35 specimens studied was 168±117.

Data from 13 replicates with 5 individuals/replicate show that the average time in hours required for development was: egg stage 48±0; 1st larval instar 52±7; 2nd larval instar 46±11; 3rd larval instar 49±2; 4th larval instar 96±26; pupal stage 72±0; premating period 180±10; preoviposition period 252 ±24. Percent survival was 75–90.

Cannibalism was severe in all 4 larval instars. Although cannibalism among adults was observed in the laboratory, it is probably of relatively little, if any, significance under field conditions. Partial incompatibility was observed in some pairs, which resulted in a low percentage of egg viability. This condition was corrected by changing the male.

ARTIFICIAL DIETS.—*Materials and Methods.*—Sixteen experimental diets were formulated on the basis of the chemical analysis of aphids and calf liver, using the techniques that have been used successfully in developing artificial diets for the boll weevil (Earle et al. 1959) and for the bollworm (Berger 1963).

The technique for preparing the diets was:

1. Half the amount of water required was placed in a Waring Blendor® with all the ingredients except agar and mixed thoroughly for 3–4 min.
2. The other half was heated to boiling, then the agar was carefully dissolved in it. The agar solution was then added to the mixture in the blender, which was operated at high speed for 1½ min.
3. The diet was poured while still hot into the containers, where it solidified.

Table 1 shows the experimental diets formulated and tested for rearing *C. maculata*.

Specifications for the ingredients:

1. Casein—Vitamin free, Nutritional Biochemicals Corp., Cleveland, Ohio. "Devitaminized" by reprecipitation and exhaustive extractions with hot alcohol to remove both fat and water-soluble vitamins.
2. Wesson's salt—Nutritional Biochemicals Corp., Formulated by Wesson with modification of Osborne and Mendle salt mixture (Wesson 1932).
3. Sucrose—Mallinckrodt Chemical Works, St. Louis, Mo., analytical reagent.
4. Choline chloride—Mann Research Laboratory, New York 6, N. Y.
5. Vitamin suspension—Prepared in the following manner (Berger 1963):

| | |
|---|---|
| Distilled water | 100 ml |
| Niacinamide hydrochloride | 100 mg |
| Calcium pantothenate (dextrorotatory) | 100 mg |
| Riboflavin | 50 mg |
| Thiamine hydrochloride | 25 mg |
| Pyridoxine hydrochloride | 25 mg |
| Folic acid (crystalline) | 25 mg |
| Biotin, crystalline | 2.0 mg |
| Vitamin $B_{12}$ | 0.2 ml |

**Table 1.—Experimental diets formulated and tested for rearing *C. maculata*.**

| Ingredients | | Diet no. 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Water | ml | 22 | 22 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 80 | 80 | 60 | 60 | 100 | 100 | 100 |
| Casein | g | 15 | 5 | 5 | 10 | | 15 | 15 | 10 | 15 | 15 | 10 | | 7 | 6 | 6 | 6 |
| 4 Potassium hydroxide | ml | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | | | | | |
| Wessons salt | g | 1.0 | 1.0 | 1.4 | 1.4 | 1.4 | 1.4 | 1.4 | 1.4 | 1.4 | 1.4 | 1.4 | | 1.25 | 1.25 | 1.25 | 1.25 |
| Sucrose | g | 3.5 | 3.5 | 4 | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 3 |
| Wheat germ | g | | | | | | | | | | | | 5 | 4 | 4 | 4 | 4 |
| Choline chloride | g | .1 | .1 | .15 | .15 | .15 | .15 | .15 | .15 | .15 | .15 | .15 | | | | | |
| Vitamin solu. | ml | 1.0 | 1.0 | 1.6 | 1.6 | 1.5 | 1.5 | 1.5 | 1.6 | 1.6 | 1.5 | 1.5 | 3 | 3 | 2 | 2 | 3 |
| Ascorbic acid | g | .4 | .4 | .5 | .5 | .5 | .5 | .5 | .1 | .1 | .5 | .5 | | 0.4 | 0.4 | 0.4 | 0.5 |
| Tegosept | g | .15 | .15 | .10 | .10 | .10 | .10 | .10 | .1 | .1 | .1 | .1 | 0.1 | .1 | .2 | .2 | .2 |
| Aureomycin | g | .03 | .03 | .05 | .05 | .05 | .05 | .05 | .05 | .05 | .05 | .05 | .05 | .06 | .06 | .06 | .06 |
| Agar | g | 2.5 | 2.5 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.0 | 3.0 | 4 | 4 | 5 | 5 | 4 | 4 | 4 |
| Water | ml | 62 | 62 | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 90 | 90 | 100 | 100 | 100 |
| Casein, enzymatic hydrolyzate | g | | | | | 10 | | | | | | | | | | | |
| Fresh pork liver | g | | 200 | | | | | | | | | | | | | | |
| Soybean hydrolyzate | g | | | 15 | 10 | 10 | 5 | 5 | 10 | 5 | 5 | 5 | | 3 | 3 | 3 | 3 |
| Cholestrol | g | .1 | .1 | .1 | .1 | .1 | .1 | .1 | .1 | .1 | .1 | .1 | | | | | |
| Inositol | g | .1 | .1 | .1 | .1 | .1 | .1 | .1 | .1 | .1 | .1 | .1 | | | | | |
| Butter fat | g | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 3 | 2.5 | 2 | 2 | 2 |
| Chicken liver | g | | | | 25 | | | 25 | | | | | | | | | |
| Glycogen | g | | | | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 2.1 | 2 | 2 | 2 |
| Yeast extract | g | | | | | | 1.0 | 1.0 | 1 | 1 | 1 | 1 | | | | | |
| Sorbic acid | g | | | | | | | | .1 | .1 | .1 | .1 | .1 | .1 | .1 | .1 | .2 |
| Vitamin E | g | | | | | | | | | | | | | | | | |
| Liver fraction 2 | g | | | | | | | | | | | 5 | | | | | |
| Liver fraction 1–20 | g | | | | | | | | | | | | 15 | | 3 | | 3 |
| Liver fraction L | g | | | | | | | | | | | | | 3 | | 3 | 3 |
| Pollen | g | | | | | | | | | | | | 5 | 3 | | | |
| Homogenized fresh chicken eggs | g | | | | | | | | | | | | | | | 140 | |
| Cottonseed oil | ml | | | | | | | | | | | | 3 | | | | |
| Corn oil | ml | | | | | | | | | | | | | 2.5 | 2 | 2 | 2 |
| Brewer's yeast | g | | | | | | | | | | | | | | 1 | | 1 |
| Dextrose | g | | | | | | | | | | | | | | 1 | 1 | 1 |
| 50 cotton leaves extracted (carotenoids and sterols) | | | | | | | | | | | | | | | | | 1 |
| Total wt of diet (g) | | 108 | 300 | 172 | 200 | 176 | 177 | 202 | 177 | 177 | 198 | 198 | 196.2 | 191 | 234 | 373 | 237 |

6. Ascorbic acid (Vitamin C)—General Biochemicals, Chagrin Falls, Ohio.

7. Cholesterol—Fisher Scientific Co., Fair Lawn, N. J. (mp 148.2–149.2°C; $C_{27}H_{45}OH$, mol wt 386.665).

8. Inositol—Nutritional Biochemicals Corp.

9. Methyl *p*-hydroxybenzoate (Tegosept) – $HOC_6H_4COOCH_3$; mol wt 152.15; Eastman Organic Chemicals, Rochester 3, N. Y.

10. Aureomycin—chlortetracycline HCl. Lederle Laboratories Division, Pearl River, N..Y.

11. Agar—Difco Laboratories, Detroit 1, Mich.

12. Casein, enzymatic hydrolyzate—Nutritional Biochemicals Corp.

13. Soybean hydrolyzate—Nutritional Biochemicals Corp.

14. Glycogen—Eastman Organic Chemicals.

15. Yeast Extract—Difco Laboratories.

16. Sorbic acid—General Biochemicals.

17. Vitamin E—Nutritional Biochemicals Corp.

18. Liver fraction 2—Nutritional Biochemicals Corp.

19. Liver extract 1-20—Nutritional Biochemicals Corp.

20. Liver fraction L—Nutritional Biochemicals Corp.

21. Wheat germ—General Biochemicals.

22. Brewer's yeast—Nutritional Biochemicals Corp.

23. Dextrose—Nutritional Biochemicals Corp.

Vials 1.6×6.0 cm were convenient for rearing and handling the larvae. After preparing each diet, while still hot it was poured into the vials to a depth of 1–1.5 cm. These vials were kept in the refrigerator at a temperature of about 1°C until used. The rearing temperature was 27±2°C and 25% RH. A 50-w incandescent bulb provided a continuous light source. The adults were placed in a 1-pint carton with a vial of food and a vial of distilled water plugged with absorbent cotton. For purposes of holding the adults as many as 20 were kept in 1 box (Fig. 1). The females rarely oviposited under these conditions. For oviposition a pair or a mated female was placed in each box. The females laid their eggs in batches of

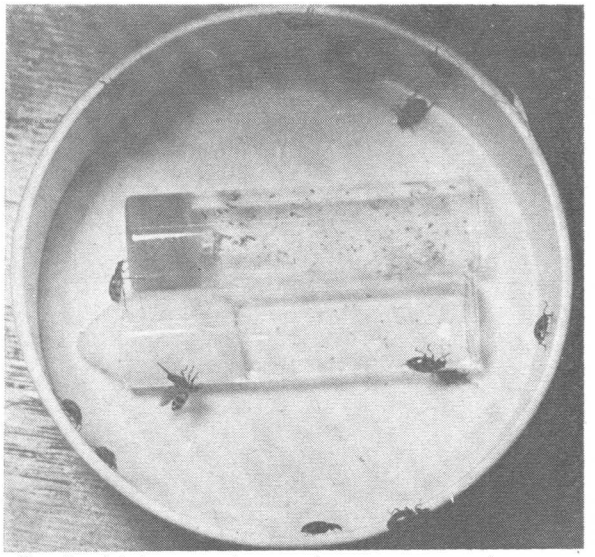

FIG. 1.—Adults held in a 1-pint carton with food and water.

3–29 mostly on the sides of the feeding tube (Fig. 2) or on the media. The eggs had to be removed promptly after deposition to prevent the adults from eating them. They were removed from the oviposition cages at 2-hr periods and held until they hatched. Newly emerged larvae were placed singly in feeding vials that were plugged with nonabsorbent cotton. The vials were held together with a rubber band and placed upside down to reduce the contamination, decay, and desiccation of the diet; also in this position the feces of the larvae would accumulate on the cotton plug, instead of the diet (Fig. 3). Sometimes it was necessary to change the feeding tube once or twice because the medium decayed or dried out. Trials were made to rear 2 or more larvae in 1 tube, but 1 always fed on the others. After the larvae completed their development they pupated in the vials, then emerged as adults. Adults of the same age were removed from the vials and kept together in boxes with food and water. To keep large numbers (more than 100) ½-gal cartons with a glass cover

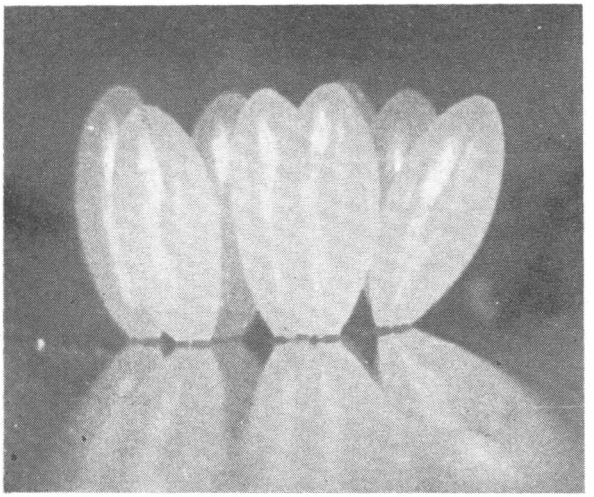

FIG. 2.—Eggs laid on the side of a feeding tube. Magnification ×25.

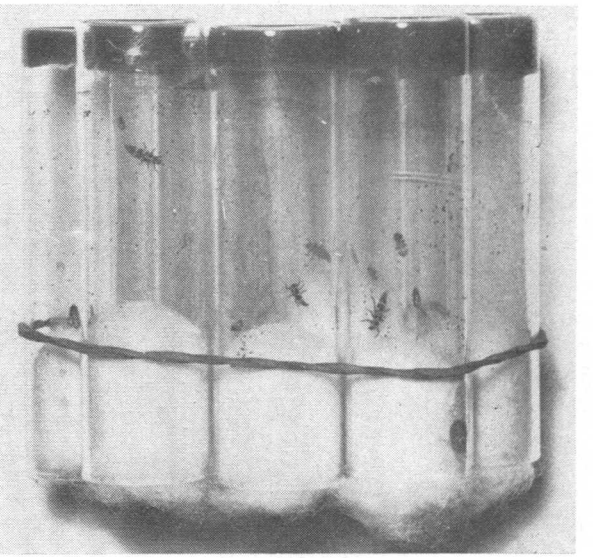

FIG. 3.—Larvae growing in the feeding tubes on Diet 16.

were used; a 5-cm-diam petri dish full of food and a vial of water were placed in the cage (Fig. 4). The food and water were changed every 4–7 days. Under these conditions the adults fed and mated but did not lay eggs. Fig. 5 shows a mating pair in 1 of the oviposition boxes feeding on Diet 16 plus Vitamin E, which appears to stimulate mating.

RESULTS.—The results of rearing individual larvae in single vials on diets 1–11 are summarized in Table 2.

Diet 1.—The individuals reaching the 3rd instar were undersized and less tanned than normal. The

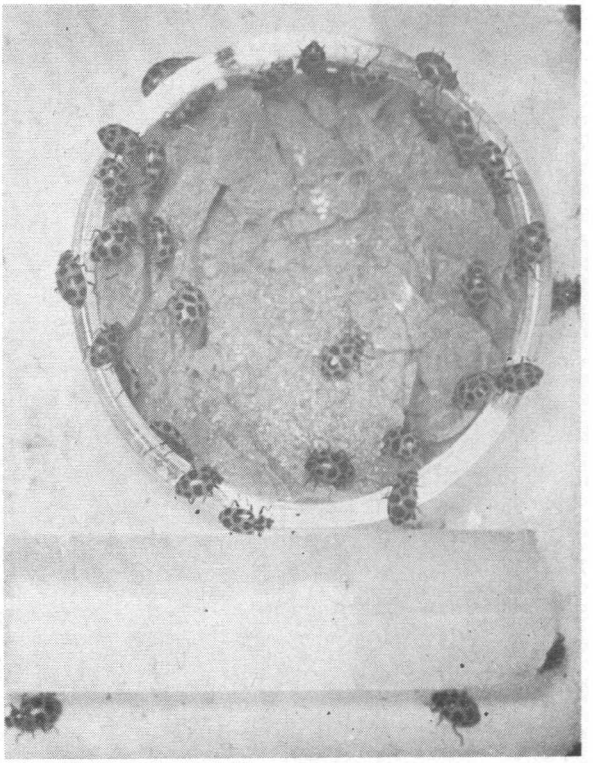

FIG. 4.—Adults feeding on Diet 16 in a ½-gal carton.

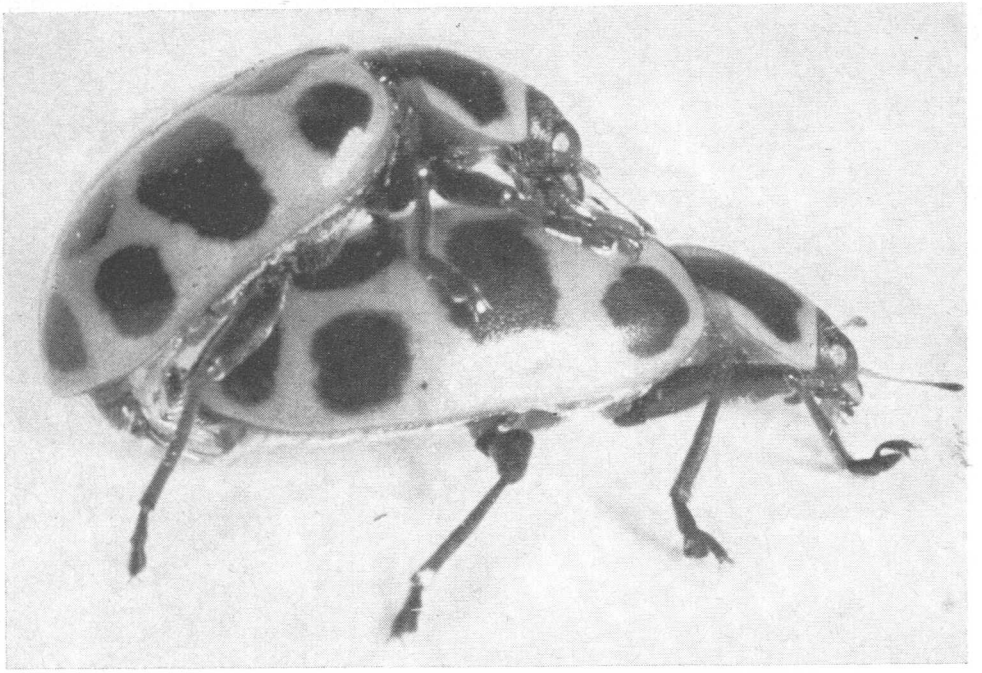

FIG. 5.—A mating pair held in one of the oviposition cages and fed on Diet 16 plus Vitamin E. Magnification ×14.

larvae were very slow in development. Most deaths occurred either immediately before or during molting.

Some larvae (not shown in Table 2) were reared in groups of 5 in 10-cm petri dishes. At the end of the 1st instar they became inactive, then after a day or 2 became cannibalistic. An average of 2 out of 5 survived to the 2nd instar. Usually 1 of the larvae fed on the others, then died in the 3rd or 4th instar. In 1 case a larva pupated and transformed to an adult with a deformed tarsus. It died after 1 day.

Adults obtained from larvae reared on liver and aphids then transferred immediately after emergence to Diet 1, fed and survived for 45 days without laying eggs. Mating seldom occurred. After 45 days the experiment was discontinued. When 4 adults were held together in 1-pint boxes cannibalism frequently occurred, especially when the adults were of different ages, e.g., 3 adults 2 days old fed on 1 adult 1 day old.

Diet 2.—Diet 2 was similar to Diet 1 except that 5 g of casein were used instead of 15, and 200 g of fresh pork liver were added. Larvae were placed in individual vials. The vials were changed every 4–5 days because of contamination with microorganisms. The larvae and adults were of normal size; larvae were slightly less tanned than normal. The adults were normally tanned. The tendency for cannibalism during the larval stages was less than in Diet 1. Decay of the diet appeared to be responsible for death of some larvae. The larvae completed development in 12 days on an average, compared with 10 days when reared on the cotton aphid. Adults reared on this diet mated rarely. In most cases the female rejected the male and sometimes tried to bite him. Even when the adults (regardless of sex) were feeding on the diet beside each other and 1 of them moved its leg, the other beetles would bite the moving leg. The females did not oviposit until their abdomens became

**Table 2.—Development of *C. maculata* on diets 1–11. Four to 15 specimens/replicate.**

| Diet no. | No. of replicates | No. of larvae | No. individuals and % of 1st-instar larvae reaching indicated stage of development | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2nd instar | | 3rd instar | | 4th instar | | Pupa | | Adult | |
| | | | No. | % | No. | % | No. | % | No. | % | No. | % |
| 1 | 10 | 99 | 69 | 70 | 7 | 7 | 1 | 1 | 0 | 0 | 0 | 0 |
| 2 | 5 | 50 | 48 | 96 | 45 | 90 | 41 | 82 | 41 | 82 | 41 | 82 |
| 3 | 6 | 60 | 25 | 42 | 21 | 35 | 17 | 28 | 7 | 12 | 2 | 3 |
| 4 | 7 | 70 | 61 | 87 | 61 | 87 | 60 | 86 | 60 | 86 | 60 | 86 |
| 5 | 6 | 50 | 17 | 34 | 13 | 26 | 9 | 18 | 7 | 14 | 3 | 6 |
| 6 | 6 | 48 | 21 | 44 | 17 | 35 | 17 | 35 | 17 | 35 | 16 | 33 |
| 7 | 6 | 60 | 58 | 97 | 53 | 97 | 57 | 95 | 56 | 93 | 55 | 92 |
| 8 | 5 | 52 | 47 | 90 | 3 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 6 | 54 | 25 | 46 | 21 | 39 | 19 | 35 | 19 | 35 | 17 | 31 |
| 10 | 5 | 41 | 27 | 66 | 21 | 51 | 7 | 17 | 4 | 10 | 2 | 5 |
| 11 | 5 | 39 | 29 | 74 | 22 | 56 | 22 | 56 | 21 | 54 | 19 | 49 |

distended with eggs, and it seemed that they were forced to oviposit. In contrast, the females fed on an aphid diet laid eggs before the abdomen was distended with eggs. The percentage of egg hatch varied from 50 to 100 and averaged 80.

Diet 3.—Diet 3 was the same as Diet 2 except that 15.0 g of soybean hydrolyzate was substituted for the pork liver, and ratios of the ingredients were altered slightly. High mortality during larval development was observed. Most of the 4th-instar larvae did not pupate. But when they did pupate high mortality occurred during pupal development.

The adults emerging were very weak and undersized. They died within 1 or 2 days after emergence. One adult had a deformed tarsus. No adults survived for more than 2 days regardless of diet. When 4th-instar larvae were fed on Diet 3, undersized adults were produced which survived.

Diet 4.—This diet had the same constituents as Diet 3 except that 10 g of casein were used instead of 5 g 10 g of soybean hydrolyzate were used instead of 15 g, and 25 g of chicken liver were added. Decay of the diet was a problem. Adults produced on it were normal in size and behavior. Copulation readily occurred. Egg laying was average compared with individuals fed on aphid diets.

Diet 5.—This diet differed from Diet 4 by substitution of 4 g of glycogen for the 25 g of fresh chicken liver, casein enzymatic hydrolyzate for casein, and reduction of sucrose from 4 g to 3. Although more satisfactory than Diet 3, it was inadequate.

Diet 6.—Diet 6 was similar to Diet 5 except that 15 g of casein were used instead of 10 g of casein enzymatic hydroylzate, 5 g of soybean hydrolyzate were used instead of 10 and 1 g of yeast extractive was added.

Except for high mortality during the 1st instar this diet appeared to be better than any previous diet without fresh liver. Improvement may have been due to adding the yeast extract and lowering the amount of soybean hydroylzate, which proved to have a toxic effect at high concentrations.

Diet 7.—This diet was similar to Diet 6 except for the addition of 25 g of fresh chicken liver. It was originally designed to increase the experimental population, since the culture was being lost at this point and was adequate for larvae and adults. Larvae fed and developed normally on it.

Adults had an average size, copulation occurred readily, and females laid eggs. The egg color was similar to that from females fed on aphids. The percentage of hatch averaged 83.

Diet 8.—Diet 8 was similar to Diet 5 except for the addition of 1 g yeast extract and 0.1 g sorbic acid for inhibiting growth of microorganisms.

Adults fed on this diet laid few eggs and, as in other diets containing soybean hydrolyzate in the concentration used, an apparent toxic effect on larvae was observed.

Diet 9.—This diet was similar to Diet 6 except that 0.1 g sorbic acid was added and the ascorbic acid was reduced from 0.5 to 0.1 g. It was similar to Diet 6 for larval development.

Adults fed on this diet, but copulation rarely occurred and females laid few eggs.

Diet 10.—This diet resembled Diet 6 except for an increase in the amount of distilled water and agar and the addition of 0.1 g sorbic acid. It was similar to Diet 9 except for the use of 160 ml of distilled water instead of 140 ml, 4 g of agar instead of 3, and 0.5 g of ascorbic acid instead of 0.1 g.

It proved to be quite suitable for adults. Feeding, mating, and egg-laying were better than on any other diet not containing liver. It did not support adequately larval development. Larvae developed on this diet until the 3rd instar when most of them died. About 5% of the larvae continued development to form undersized adults.

Diet 11.—Diet 11 resembled Diet 10 except for substitution of 5 g of "liver fraction 2" for 5 g of the casein.

It was adequate for larvae and adults but not so satisfactory as Diet 7, which contained fresh chicken liver.

Diet 12.—In this diet the following ingredients were eliminated: casein, potassium hydroxide, Wesson's salt, choline chloride, ascorbic acid, soybean hydrolyzate, cholesterol, inositol, yeast extract, and liver fraction 2. The following were added: wheat germ, liver extract 1:20, pollen, and cottonseed oil. The amounts of vitamin solution, butterfat and agar were increased. Liver extract used was a powdered 1:20 concentrate of liver containing appreciable quantities of the identified water soluble and B complex factors. Pollen was collected by honey bees. This diet allowed about 10% of the larvae to complete development, but adults did not feed well on it.

Diet 13.—Use of liver fraction L, a solubilized dry concentrate containing the alcohol insoluble fraction of liver, was the major change in this diet.

Starting with Diet 13 the data for development of larvae in different instars were discontinued since there was no considerable advantage in taking such data. Instead, data were taken for number of larvae, number of adults, and time required for development only.

Adults fed readily on it, copulated, and the females laid eggs. It was considered reasonably satisfactory (Table 3).

Diet 14.—The major difference between this and Diet 13 was substitution of liver extract for liver fraction L and elimination of pollen.

Adults fed readily on it, copulated, and laid eggs. However, the color of the eggs was abnormal in comparison to the color of the eggs laid by females fed on an aphid diet. The eggs were yellow instead of orange. Results are summarized in Table 3.

Diet 15.—This diet was similar to Diet 14 except for substitution of 140 g homogenized fresh chicken eggs for the 1 g of Brewer's yeast and Liver fraction "L" instead of liver extract concentrate. There was no significant difference between Diet 14 and Diet 15; however, microbial growth was more of a problem in the latter.

Diet 16.—The final and most satisfactory of the diets tested differed from all others mainly by the addition of an extract from 50 cotton leaves.

The cotton-leaf extract (carotenoids and sterols)

**Table 3.—Development of *C. maculata* on diets 13, 14, and 16. Two to 40 specimens/replicate.**

| Diet no. | No. replicates | No. larvae | No. adults | Period from egg to adult (days) |
|---|---|---|---|---|
| 13 | 11 | 209 | 94 | 22–50 |
| 14 | 9 | 82 | 31 | 23–51 |
| 16 | 7 | 39 | 35 | 21–37 |

was prepared according to the procedure described by Paech and Tracey (1955).

1. Leaves ground in a large blender and extracted 3 times with acetone.

2. Acetone extract diluted with 2 volumes of water.

3. Mixture extracted several times with ethyl ether. Ethyl ether extract reduced to dryness in a rotary evaporator under vacuum.

4. Sufficient ethanol (but not less than 10 ml) added to the dry residue to dissolve it completely and then 60% (w/v) aqueous potash (1 ml/10 ml ethanol) added with shaking. Mixture covered with nitrogen to prevent oxidation and left for 12–16 hr (preferably overnight) in the dark at room temperature. The solution then diluted with 3–4 times its volume of water and extracted with an equal volume of peroxide-free ethyl ether. Extractions continued until all the pigment was extracted. Combined ether extracts washed with about ½ their volume of warm water; repeated until all the soaps were washed from the ether layer.

5. Ether extract impregnated on casein, dried under vacuum until odor of the ether disappeared. Mixture kept frozen until used. Data obtained from use of this diet are summarized in Table 3.

Larval development was more uniform on Diet 16 than any of the others tested (Table 3). It was necessary to change the food once or twice during larval development. The adults reared on this diet fed readily, copulated, and the females oviposited. Copulation was increased by adding 0.2 ml Vitamin E. Eight generations of the beetles were reared on this diet in the laboratory without observed reduction in the viability of the culture after the 2nd generation. All the *C. maculata* used in toxicological and ecological studies conducted in the laboratory were reared on it. However, it failed to support *Coccinella novemnotata* Herbst, *Cycloneda* spp., or the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville. Adult *Olla abdominalis* (Say) fed on the diet, but neither copulation nor oviposition was observed.

ACKNOWLEDGMENT.—All photographs were made by J. H. Roberts.

REFERENCES CITED

**Berger, R. S. 1963.** Laboratory techniques for rearing *Heliothis* species on artificial medium. USDA-ARS 33–84. 4 p.

**Brues, G. T. 1946.** Insect Dietary. Harvard Univ. Press, Cambridge, Mass. 146 p.

**Clausen, C. P. 1915.** A comparative study of a series of aphid feeding Coccinellidae. J. Econ. Entomol. 8: 487–91.

**Earle, N. W., R. C. Gaines, and J. S. Roussel. 1959.** A larval diet for the boll weevil containing an acetone powder of cotton squares. J. Econ. Entomol. 52: 710–2.

**Forbes, S. A. 1883.** Notes on insectivorous Coleoptera. Bull. Ill. Nat. Hist. Surv. 1: 167–76.

**Lipke, H., and G. Fraenkel. 1956.** Insect nutrition. Annu. Rev. Entomol. 1: 17–44.

**Meikle, J. E. S., and J. E. McFarlane. 1965.** The role of lipid in the nutrition of the house cricket, *Acheta domesticus* L. (Orthoptera, Gryllidae). Can. J. Zool. 43: 87–98.

**Paech, K., and M. V. Tracey. 1955.** Modern Methods of Plant Analysis. Springer-Verlag, Berlin Gottingen. Heidelberg. 761 p.

**Putman, W. L. 1958.** Laboratory studies on the food of some coccinellids (Coleoptera) found in Ontario peach orchards. Can. Entomol. 89: 572–9.

**Smirnoff, W. A. 1958.** An artificial diet for rearing coccinellid beetles. Can. Entomol. 90: 563–5.

**Smith, B. C. 1960.** A technique for rearing coccinellid beetles on dry food and influence of various pollens on the development of *Coleomegilla maculata legni* Timb. (Col. Coccinellidae). Can. J. Zool. 38:1047–9.

**1961.** Results of rearing some coccinellid (Coleoptera: Coccinellidae) larvae on various pollens. Proc. Entomol. Soc. Ontario. 91: 270–1.

**Szumkowski, W. 1951a.** Observations on Coccinellidae. I. Coccinellids as predators of lepidopterous eggs and larvae in Venezuela. Proc. 9th Int. Congr. Entomol. 1: 778–81.

**1951b.** Observations on Coccinellidae. II. Experimental rearing of *Coleomegilla* on a non-insect diet. Proc. 9th Int. Congr. Entomol. 1: 281–5.

**1961.** Dietas sin insectos vivos para la cria de *Coleomegilla maculata* Deg. (Coccinellidae, Coleoptera). Agron. Trop. 10: 149–54.

**Thompson, W. R. 1951.** The specificity of host relations in predaceous insects. Can. Entomol. 83: 262–9.

**Vanderzant, E. S., M. C. Pool, and C. D. Richardson. 1962.** The role of ascorbic acid in the nutrition of three cotton insects. J. Insect Physiol. 8: 287–97.

**Wesson, L. G. 1932.** A modification of the Osborne-Mendle salt mixture containing only inorganic constituents. Science 75: 339–40.

# Ecological and Nutritional Studies on *Coleomegilla maculata* De Geer (Coleoptera:Coccinellidae). II. The Effects of Different Population Densities and Sex Ratios on Oviposition[1]

YOUSEF H. ATALLAH
Department of Entomology, Louisiana State University, Baton Rouge

ABSTRACT

Eight different population densities of adult lady beetles, *Coleomegilla maculata* De Geer, were tested in a standard space of 568 cc. The adults were provided with excess food and water. As the number of adults increased the number of eggs per female decreased. A greater degree of intraspecific competition occurred among females than among males and females. Oviposition was affected by the number of individuals and their sex. Maximum egg production was obtained by confining mated females singly in oviposition cages.

Much research has been done to study the effect of crowding on the fecundity of animals under laboratory conditions. Various techniques and designs have been used. Pearl (1932) found that as the popula-

[1] Accepted for publication May 31, 1966.