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.

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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]

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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-

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Table 1.—Effect of different population levels on egg production of *C. maculata*.[a]

Level no.	Population level ♂	Population level ♀	Experiment 1 Batches	Experiment 1 Eggs	Experiment 2 Batches	Experiment 2 Eggs
1	0	1	4.8±3.6	22.6±13.7	5.0±2.0	29.1±14.7
2	0	2	3.8±0.9	17.5±6.9	3.7±1.2	18.0±8.6
3	1	1	2.8±1.5	14.8±8.4	3.1±1.3	17.2±5.3
4	2	2	1.5±1.0	5.8±3.9	1.7±0.9	6.3±4.1
5	3	3	0.0	0.0	0.2±.4	1.0±2.4
6	0	5	.0	.0	.0	0.0
7	5	5	.0	.0	.0	.0
8	0	4			.2±.4	.8±2.0

[a] Experiment 1 had 4 replicates and experiment 2 had 6 replicates.

tion density of *Drosphila melanogaster* (Meigen) increased in a limited or closed universe, the rate of egg production per female per day decreased. He stated also that the effect of population density upon egg laying was immediate and probably resulted from collision or the interfering action of the flies upon one another. Pearl concluded that crowding affects 3 major functions, food intake, energy output in muscular activity, and oviposition.

Later, several biologists arrived at the same conclusions on the basis of similar studies. Among them were Kluijver (1951), who worked with wild populations of the great tit, *Parus major* L. (after Odum 1962); Frank (1952) with laboratory cultures of the crustacean *Daphnia pulicaria* (Forbes); and Watt (1955) with the confused flour beetle, *Tribolium confusum* Jacquelin du Val. Nicholson (1957) stated that in cultures of the sheep blow fly, *Lucilia cuprina* Wiedemann, governed by larval competition only, a large number of flies laid so many eggs that larval overcrowding prevented any offspring from reaching maturity; whereas in those which were governed by adult competition alone, adult crowding prevented any eggs being produced. Nicholson observed also that the small flies laid fewer eggs. Flanders and Badgley (1963) found that with a constant food supply the periodicity of increase and decrease of the Mediterranean flour moth, *Anagasta kuehniella* (Zeller), appeared to be the result of variations in the intraspecific competition for requisites.

Atallah and Newsom (1966) developed an artificial diet and a satisfactory laboratory rearing technique for *Coleomegilla maculata* De Geer. Before undertaking a "mass" rearing program for this predator it was necessary to study the effects of intraspecific competition on oviposition.

MATERIALS AND METHODS.—Eight different population levels of adults were tested in a standard space of 568 cc provided by a 1-pint paper ice cream carton. The adults were provided with an excess of the synthetic diet formulated according to the method described by Atallah and Newsom (1966).

The population levels were:

1. One mated ♀ and 1 tube of diet
2. Two mated ♀ and 1 tube of diet
3. One ♂ and 1 ♀ and 1 tube of diet
4. Two ♂ and 2 ♀ and 2 tubes of diet
5. Three ♂ and 3 ♀ and 2 tubes of diet
6. Five mated ♀ and 2 tubes of diet
7. Five ♂ and 5 ♀ and 2 tubes of diet
8. Four mated ♀ and 2 tubes of diet

Water was supplied in 10-cc shell vials stoppered with absorbent cotton. Water was changed at 4-day intervals. The food was changed each 36 hr. The eggs were collected at 12-hr intervals— 9 AM and 9 PM. Two experiments were conducted during the summers of 1963 and 1964.

RESULTS.—Summarized data for egg production under the different population levels studied are shown in Table 1. The figures in experiment 1 were the average of 4 replicates. There were 6 replicates in experiment 2.

Obviously, intraspecific competition exerted an important regulating effect on egg production. Even 1 ♀ laid more eggs than 2 ♀ together. A female confined with a male laid more eggs than the average laid per female where 2 were caged together. Four individuals, 2 ♂ and 2 ♀ (level 4), laid more eggs than 4 ♀ (level 8). It seems that not only the number of individuals present in a space but also the sex of these individuals affects oviposition. The data indicate that at a certain population level there may be a greater degree of intraspecific competition among females than among males and females. The presence of another female inhibits oviposition more than the presence of a male; compare levels 2 and 3; 4 and 8; and 5, 6, and 8.

No eggs were deposited when more than 4 ♀ were held in 1 cage.

In a rearing program for *C. maculata*, maximum egg production should be obtained by confining mated females singly in oviposition cages. Satisfactory egg production was obtained by caging together 1 ♂ and 1 ♀. The latter method was used, in the rearing techniques reported previously (Atallah and Newsom 1966).

REFERENCES CITED

Atallah, Y. H., and L. D. Newsom. 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. Entomol. 59:

Flanders, S. E., and M. E. Badgley. 1963. Prey-predator interactions in self-balanced laboratory populations. Hilgardia. 35: 145–83.

Frank, P. 1952. A laboratory study of intraspecies and interspecies competition in *Daphnia pulicaria* and *Simocephalus vetulus*. Physiol. Zool. 25: 178–204.

Kluijver, H. N. 1951. The population ecology of the great tit, *Parus m. major* L. Ardea 39: 1–135.

Nicholson, A. J. 1957. The self-adjustment of populations to change. Cold Spring Harbor Symposia on Quant. Biol. 22: 153–73.

Odum, E. P. 1962. Fundamentals of Ecology. 2nd ed. W. B. Saunders Co. Philadelphia and London. 384 p.

Pearl, R. 1932. The influence of density of population upon egg production in *Drosophila melanogaster*. J. Exp. Zool. 63: 57–84.

Watt, K. E. F. 1955. Studies on population productivity. I. Three approaches to the optimum yield problem in population of *Tribolium confusum*. Ecol. Monogr. 25: 269–90.