rapid reproduction of the spotted alfalfa aphid; high humidity is detrimental. Also, the regions of the world in which *T. maculata* is a serious pest of alfalfa are semiarid, and temperatures are relatively high while the aphid is active. Since the average annual rainfall from April to September in the study areas ranges from about 20 to 25 in. and the RH averages about 75% at 8:00 AM and about 65% at noon during July (Anonymous 1941), the environmental conditions and the low temperatures during the winter months are probably highly unfavorable to the rapid reproduction of the species.

REFERENCES CITED

Anonymous 1941. Climate and man. *In* Yearb. Agr. USDA.

1966. Plant Pest Control Division, Agr. Res. Serv. USDA. Coop. Econ. Insect Rep. 16 (44): 1038.

Bishop, J. L., and D. Crockett. 1961. The spotted alfalfa aphid in Virginia. Va. Agr. Exp. Sta. Tech. Bull. 153.

Dickson, R. C., E. F. Laird, Jr., and G. R. Pesho. 1955. The spotted alfalfa aphid (yellow clover aphid on alfalfa). Hilgardia 24 (5): 93–118.

Graham, H. M. 1959. The effects of temperature and humidity on the biology of *Therioaphis maculata* (Buckton). Univ. Calif. Publ. Entomol. 16 (2): 47–80.

Howe, W. L., and G. R. Pesho. 1960. Spotted alfalfa aphid resistance in mature growth of alfalfa varieties. J. Econ. Entomol. 53 (2): 234–8.

Mackauer, M., and P. Stary. 1967. Note of section. *In* V. Delucchi, and G. Remaudiere [eds.] Index of World Aphiidae Index of Entomophagous Insects. Le Francois, Paris. 195 p.

Muesebeck, C. F. W. 1956. Two new parasites of the yellow clover aphid and the spotted alfalfa aphid. Brooklyn Entomol. Soc. Bull. 51: 25–28.

Van den Bosch, R. 1957. The spotted alfalfa aphid and its parasites in the Mediterranean region, Middle East, and east Africa. J. Econ. Entomol. 50 (3): 352–6.

Improved Laboratory Production of Eggs of *Coccinella septempunctata*[1,2,3]

W. A. SHANDS,[4] R. L. HOLMES,[5] and GEDDES W. SIMPSON[6]

Tests made at Presque Isle, Maine, from 1964 to 1966 showed that small plots (18×50 ft) were inadequate for the evaluation of the effectiveness of *Coccinella septempunctata* L. as a predator of aphids on potatoes. Larvae of this beetle did considerable moving between plots, even when the plots were separated by strips of soil 6–9 ft wide that were planted to oats or kept bare. Such movement was greatly reduced when the plots were separated by 18-cm aluminum flashing placed on edge and coated near the top on each side with a 2½-cm band of Fluoro Glide® (a duPont Fluorocarbon resin). However, larvae thus confined did not behave normally. Once outside the small plots, they attempted to escape the aluminum barriers rather than to reenter the plots to search the potato plants for aphids. It was apparent that much larger plots or fields would be required to determine the effectiveness of this insect as a predator of aphids on potatoes, thus necessitating many more beetle eggs than for the small-plot tests.

Studies were directed toward the mass production of predator eggs, the 1st step being to devise a technique for handling larger number of ovipositing beetles. The oviposition cage used to obtain eggs of *C. septempunctata* for the small-plot tests was a cylindrical ½-pint, pasteboard ice cream carton covered with the lid of a plastic petri dish. A paper liner 5 cm wide covered the wall, and a piece of paper (2½×2½ cm) was fastened flat against the inside of the lid to facilitate collection of the eggs. Also, a piece of paper about 2½×5 cm was folded to form a tent 2 cm high and placed on the floor of the cage to provide a shelter under which the beetles could hide. Each cage contained a single pair of beetles, and 1 technician could take care of 80 cages/day, including the daily collection of eggs. However, if such a procedure were used to produce the numbers of eggs needed for the large-plot tests, many workers would be necessary to handle the eggs for sustained weekly introductions in replicated fields of potatoes.

METHODS AND MATERIALS.—An oviposition cage was made from a clear plastic box about 15×30×9 cm. For an oviposition substrate it contained 2 pieces (8.5×8.5 cm) of single-phase, A-flute corrugated pasteboard coated with 33% Jello® (Fig. 1A). Once the Jello congealed and dried enough not to be tacky the 2 pieces of corrugated pasteboard were arranged so the flutes of one piece were at right angles to and touching those of the other, forming a multicelled sandwich. A similar 12×12-cm sandwich was also satisfactory, and an acceptable sandwich was made by placing 1 piece of either size of this pasteboard on white paper toweling similarly coated with strawberry Jello. After any of the 3 sandwiches was placed flat on the bottom of the cage (Fig. 1B), a 5-cm layer of fluffy excelsior was arranged over it (Fig. 1C). Balls of absorbent cotton or pieces of sponge wetted with liquid food supplement or water were placed in the corners on the cage floor, aphid-infested foliage dorsal side up was laid on top of the excelsior (Fig. 1D), the beetles were introduced, and the cage cover was put in place (Fig. 1E).

The cage was made of 32-mesh Saran® screen glued to the rim of the lid (center cut out) of the plastic box or of white cotton cloth stretched over the top of the cage and held in place with the rim of the lid. When 25 beetles of undetermined sex were placed in a cage, about ⅔ were females.

The infested foliage was changed daily at the same time the sandwiches were collected and replaced. Also, fresh water was supplied daily, and freshly mixed liquid food supplement was supplied twice a week. (The mixture was 4 g protein hydrolysate, 0.4 g air-dried bee's pollen, 5 ml honey, and 45 ml water.) The excelsior and the cage were changed about once a week or as often as needed. Also, for our production schedules, the eggs were refrigerated at 10°C for not more than 1 week.

The egg clusters were separated from the pasteboard sandwich by soaking the sandwich for a few minutes in lukewarm water that was being gently agitated to dissolve the coating of Jello between the eggs (adhesive-tipped) and the pasteboard. Also, most of the eggs were separated by this procedure. (Separated eggs were required for field application in a water spray.)

[1] Coleoptera: Coccinellidae.
[2] Received for publication May 16, 1969.
[3] Mention of a trade name does not constitute recommendation, guarantee, or warranty of the product by the USDA.
[4] Entomology Research Division, Agr. Res. Serv., USDA, Orono, Maine 04473.
[5] Resigned September 1967. Univ. Maine Agr. Exp. Sta., Orono, Maine 04473 (under Cooperative Agreement no. 12-14-100-2473 (33)), and Entomology Research Division, Agr. Res. Serv., USDA, Presque Isle, Maine 04769.
[6] Univ. Maine Agr. Exp. Sta., Orono, Maine 04473.

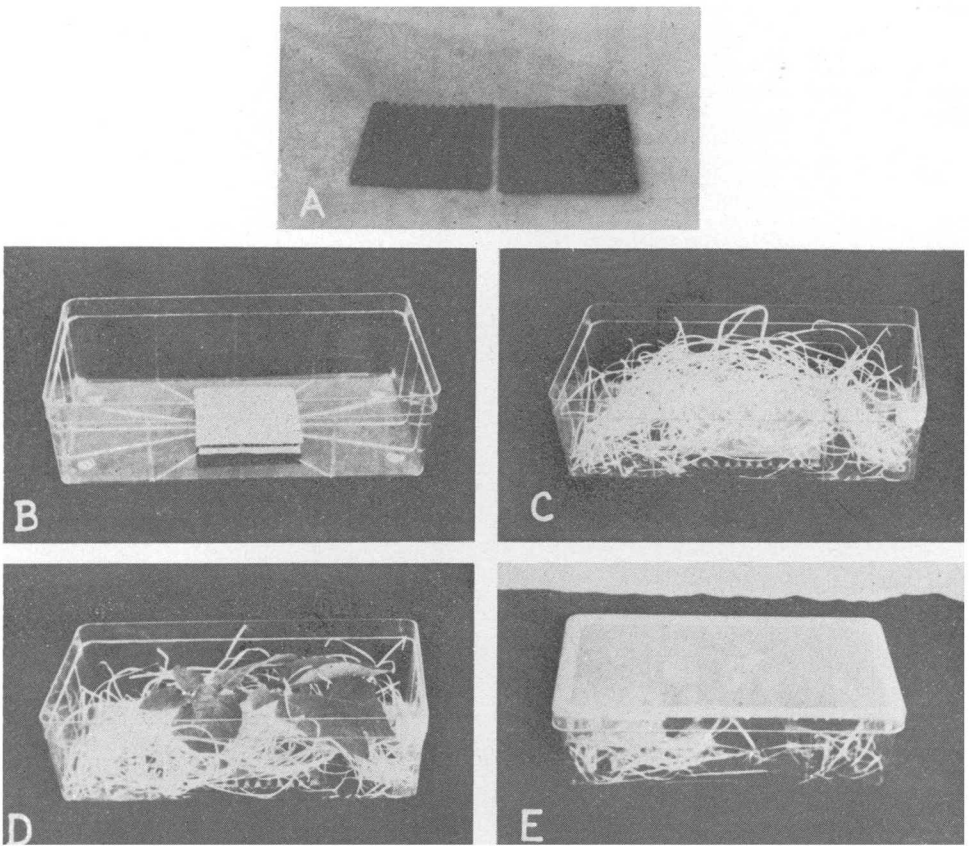

FIG. 1.—Two pieces of single-phase, A-flute corrugated pasteboard coated with 33% Jello solution (A) are put together to form a multicelled 8.5 × 8.5 = cm sandwich and placed in the middle on the cage floor (B). Fluffy excelsior is spread over the cage floor to a depth of about 5 cm (C), and aphid-infested foliage dorsal side up is laid on the excelsior (D). The cage top (32-mesh Saran screen glued to the cutout lid of the plastic shoe box) is installed after the beetles and water or liquid food supplement are introduced (E).

DISCUSSION.—The sandwich appeared to attract females ready to oviposit, and its multicelled interior provided varying degrees of shelter, isolation, darkness, or of other conditions favoring or inducing oviposition. An indefinite but substantial part of this attractiveness was the result of the coating of strawberry Jello: apparently olfactory, gustatory, and thigmotactic responses of the female beetles were involved. (Strawberry was superior to the other flavors of Jello that we tested.) Nearly all the oviposition occurred inside the cells of the sandwich when the infested leaves used as beetle food in the cage were radish, *Raphanus sativus* L.; or Chinese cabbage, *Brassica chinensis* L.; occasional clusters were laid on potato, *Solanum tuberosum* L., if foliage of this plant was used, and many were deposited on infested leaves of collards, *Brassica oleracea* L. Var. *acephala* De Candole, when that foliage was made available.

Cannibalism of the deposited eggs was less in the new oviposition cage that holds 25 beetles than in the old one that held only 1 or 2. In fact, little cannibalism of eggs occurred in the new cage unless a deficiency of food existed. Factors that may have influenced this decreased cannibalism were the increased traveling space provided by the excelsior for nonovipositing beetles, the lack of attractiveness of the sandwich to nonovipositing beetles, and the isolation of the egg clusters in the sandwich.

With the new cage, no evidence appeared of any stress that would influence the size of the egg clusters. Clusters deposited by females in the small cages contained an average of between 21.5 and 32.1 eggs, depending on the time of the year, the diet provided the beetles, and other factors; in the new cage, the average was 25.7 in a test involving 569–991 beetles/day during 35 days in midsummer.

The improved technique made possible the production of eggs on a scale adequate for sequential introductions in large-plot tests. With optimum conditions, a skilled technician could tend and collect the eggs daily from 112 cages, containing 2800 beetles. If each of the ca. 1800 ♀ in these cages deposited 1 cluster of 25 eggs each day, the 1 technician could collect 315,000 eggs/week.

The major limiting factor remaining in the production of enough eggs for large-plot testing is diapause in the adult female. Although the condition is not well understood, the external manifestations of diapause generally include failure to oviposit, variable but reduced activity, and erratic, reduced consumption of food. We have found no populations of the beetle entirely free of diapausing females, and the percentages for insectary-bred populations have varied from about 5 to 100% depending considerably upon rearing conditions during the larval stage. Also, diapause has endured from only a few days to more than 1 year. The types of diapause in this beetle are not well understood (Bonnemaison 1964, Hodek 1966); it may be obligatory, facultative, or a combination of the two. However, obligatory diapause, which is closely associated with uni- or multivoltinism and is genetic in nature (Hodek and Čerkasov (1961), was induced or largely prevented by varying conditions that affect facultative diapause, photoperiod, temperatures, and food. In our tests, we found that food in the larval stage and quality of light in the adult stage were

important factors influencing diapause in the adult females. Moreover, different populations of the beetle showed large variations in the percentage of diapausing females.

We have so far achieved only 64% of the possible level of production for 32 days or longer. However, with recent improvement in technique, the higher production may soon be attained or surpassed.

We are grateful to R. C. Riley, Rutgers—The State University of New Jersey, for suggesting the use of gelatin-coated paper as an oviposition substrate from which the eggs can be removed readily by solubilizing the gelatin.

REFERENCES CITED

Bonnemaison, L. 1964. Observations écologiques sur la Coccinelle à 7 points (*Coccinella septempunctata* L.) dans la region parisienne (Col.). Bull. Soc. Entomol. France 69: 64–83.

Hodek, I. 1966. Voltinism and diapause in aphidophogous insects, p. 97–102. *In* I. Hodek [ed.] Ecology of Aphidophogous Insects. Dr. W. Junk, The Hague.

Hodek, I., and J. Čerkasov. 1961. Prevention and artificial induction of imaginal diapause in *Coccinella septempunctata* L. (Col.: Coccinellidae) Entomol. Expt. Appl. 4: 179–90.

Rearing and Nutrition of the Olive Fruit Fly.[1] 1. Improved Larval Diet and Simple Containers[2,3]

M. E. TZANAKAKIS,[4] A. P. ECONOMOPOULOS, and J. A. TSITSIPIS

"Democritus" Nuclear Research Center, Greece

Ten generations of the olive fruit fly, *Dacus oleae* (Gmelin), were successfully reared in our laboratory prior to July 1966, on 2 artificial diets designated as M and P (Tzanakakis and Economopoulos 1967). Since then, more than 25 additional generations of the same insect stock have been completed satisfactorily by using interchangeably 1 of diets M, P, or N, or slight modifications of them. There were no indications of weakening of the colony, and the highest yield in flies and egg production reported to date have been obtained (Economopoulos and Tzanakakis 1967). The present paper gives the composition of diet N, which is the best of the diets we have tested long enough to recommend, some modifications of diet N, and a description of simple containers for easy collection of the pupae.

METHODS AND MATERIALS.—Diet N was a modification of diet F which was found promising in the early part of our work (Tzanakakis et al. 1966). It had the same composition in nutrients and preservatives as diets M and P. It differed only as to the inert ingredients, thickening being achieved with both agar and cellulose. The composition was as follows: tap water 55 ml, agar fine powder (Merck) 0.5 g, cellulose powder (no. 123 for column chromatography, Schleicher & Schüll) 20 g, brewer's yeast powder (Fix) 7.5 g, soy hydrolyzate enzymatic (Nutritional Biochemicals Corp.) 3 g, roasted peanuts (as sold on the market) 6 g, sucrose (granular) 2 g, olive oil (Minerva) 2 ml, Tween-80 0.75 ml, potassium sorbate (Merck) 0.05 g, Nipagin (methyl-*p*-hydroxybenzoate, Merck) 0.2 g, and 2N HCl 3 ml.

The preparation was similar to that for diet M (Tzanakakis and Economopoulos 1967). The cellulose was added last. After spreading the diet in trays on other containers, 10 min were allowed for some water to evaporate, grooves were made, eggs were spread over the surface, and the tray was covered. Grooves of some kind or scarification were needed to obtain a satisfactory yield of good pupae. We used parallel grooves, approximately 1 cm apart, running the whole length of the container and reaching the bottom, crossed at right angles by similar grooves. The larval food was thus cut in 1-cm squares.

The most convenient containers were shallow circular trays of hard plastic, 1–1.5 cm deep (Fig. 1). An empty tray served as lid for a full one. Each tray contained 500–800 g of larval food at a depth of 5–8 mm, and was seeded with 1000–4000 eggs. The trays were maintained at 25±1°C. When the larvae reached full growth, the cover tray was lifted 2 mm by inserting clips at 3 points of the periphery of the diet tray. The peripheral slit thus created allowed the larvae, which were searching for a suitable pupation site, to leave the tray. They fell to a larger collection tray below, which contained a little sawdust. The fully grown larvae or young pupae were thus collected with little labor as frequently as desired. A battery of frames allowed the stacking of several dozen trays and the collection of all the pupae in only 1 collection tray. This efficient and simple system was used by Nadel and Peleg (1968) for mass rearing larvae of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). A small percentage of the larvae pupated on or in the food. To minimize this percentage, the cover tray was lifted as little and late as possible, and the room was maintained above 60% RH.

RESULTS AND DISCUSSION.—When newly laid eggs were placed on diets N, M, or P, hatchability was low (Table 1). Following collection, a 24-hr incubation period on moist filter paper in a petri dish was allowed before eggs were placed on the diet. The filter paper was soaked with water, or preferably with 0.02% sodium benzoate solution (Lopez 1965).

By the time the larvae were fully grown, limited yeast growth and fermentation odor were detected in many trays. This condition has not adversely affected the consistency of the yield or the quality of the flies produced. It has not been determined whether the larvae do better with such live yeast on the diet.

The yield in pupae of diet N was usually 20% higher than of diets M and P. In 9-cm plastic cups, yields as high as 80% pupae from hatched eggs were recorded on several occasions. The percent yield dropped as the size of container and the number of eggs per gram of diet increased. In 32-cm round trays with 2 eggs/g diet the usual yield in pupae was 30–40% of the eggs. The average pupal weight[5] was usually 4.5–5.8 mg. In common with some other fruit fly species (Christenson and Foote 1960), the olive fruit fly has an endogenous periodicity as to the time of day the fully grown larvae leave their diet to pupate. Each day, under controlled photoperiod,

[1] Diptera: Tephritidae.

[2] Parts of the present text were presented, in an abbreviated form, at the 7th FAO Conference on the Control of Olive Pests and Disease, Palermo, Italy 2–10 May 1967.

[3] Received for publication Apr. 24, 1969.

[4] Present address: Faculty of Agriculture and Forestry, University of Thessaloniki, Greece.

[5] Pupae were weighed when at least 4 days old, i.e. after the period of abrupt weight loss.