# EFFECT OF VARIOUS DIETARY FORMULATIONS ON THE DEVELOPMENT OF THE MOSQUITO *CULISETA INCIDENS* (THOMSON) (DIPTERA:CULICIDAE)[1]

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For rearing mosquito larvae in laboratories a number of substances and various combinations of them have been tested as sources of food. Among these are yeast, bread crumbs, commercial fish food, dog biscuits, bacteria and many kinds of infusions.

Species of *Anopheles, Aedes* and *Culex* have been the subjects of most of these studies. Wide variability in the results occurred, and it was evident that the requirements of each species have to be studied separately. It was reported that the larvae of *Culiseta inornata* grow well in a medium consisting of Difco Brain Heart Infusion, baker's yeast, and ground whole-wheat bread crumbs, added to Bates' "medium S," (McLintock, 1952). The studies reported here were an effort to determine the most suitable medium for rearing *Culiseta incidens.*

MATERIALS AND METHODS. For the present investigation, egg rafts of *Culiseta incidens* were collected in Alum Rock Park in San Jose, California. Individual egg rafts were floated in small containers of distilled water. A few of the first instar larvae that hatched in each container were examined and identified as *C. incidens,* then all of the young larvae (24 hours old) were pooled. Next, 100 larvae from this pool were placed in each of 33 finger bowls (7 inches in diameter and 2½ inches deep). Each bowl contained 500 milliliters of distilled water. For the purpose of observing the effects of different dietary formulations and establishing standardized media for rearing larvae of *C. incidens,* the following diets were fed to segregated groups of the larvae:

Group 1. 10 mg. of brewer's yeast.
Group 2. 20 mg. of brewer's yeast.
Group 3. 40 mg. of brewer's yeast.
Group 4. 100 mg. of whole-wheat bread crumbs (prepared by drying slices of the bread in an oven at 45° C and then grinding them as finely as possible in a mortar).
Group 5. 200 mg. of whole-wheat bread crumbs.
Group 6. 200 mg. of whole-wheat bread crumbs plus 20 mg. of brewer's yeast.
Group 7. 300 mg. of whole-wheat bread crumbs plus 30 mg. of brewer's yeast.
Group 8. 1000 mg. of protein mash food[3]

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[3] Ace Hi Upland Game Bird Starter Mash, California Milling Corporation, Los Angeles. Analysis by the Food and Drug Laboratory, California State Department of Public Health as follows: 27.1 percent crude protein, 4.0 percent fat, 4.8 percent crude fiber, 7.9 percent ash, 8.9 percent moisture, 45.1 percent starch, 0.7 percent reducing sugar, and 1.5 percent was not indicated in the analysis. (After Kardos, 1959.)

(obtained from Bakersfield Encephalitis Field Laboratory, California, where this material was used as food for rearing *Culex tarsalis*).

Group 9. 330 mg. of protein mash food.

Group 10. 330 mg. of brewer's yeast.

Group 11. 330 mg. of whole-wheat bread crumbs.

There were three replicates in each food group. Equal amounts of food were added to each bowl every 3 days until the larvae in the bowl pupated.

Each bowl was kept covered with a piece of glass, and a slow stream of air was bubbled continuously through the medium (except group 8). This air input was necessary to prevent the formation of a surface pellicle. For group 8, the water was changed every 3 days, but one-third of the old stock solution was retained so that the amount of alteration of the larval environment would be minimized. This method was adopted in rearing *C. tarsalis* at Bakersfield Encephalitis Field Laboratory.

Observations were made daily, and all visible changes in larval instars were recorded. Upon pupation the number of pupae from each container was recorded; then each batch was placed in a small container covered with a glass chimney, 7 inches tall and 3½ inches in diameter. The top of the chimney was covered with a piece of mosquito netting fastened by a rubber band. A hole about one-half inch in diameter was cut in the netting and was plugged with cotton, allowing for removal of adult mosquitoes with a mechanical aspirator. When they emerged, adult mosquitoes were sexed, recorded and each batch was transferred to a gallon jar covered with mosquito netting. On top of the netting a wet pad of cheesecloth was placed so that the relative humidity inside the jar would remain constantly high. The adult mosquitoes were kept in the jar without food, and daily observations revealed the number of deaths that occurred. All of the dead mosquitoes were transferred to a desiccator and dehydrated for exactly 10 days before being weighed on an analytical balance.

The pH of each medium was measured and recorded at the beginning of the experiment and again at the time when all mosquito larvae had pupated in any given container.

Larvae in groups 1 to 8 were reared at room temperatures, which ranged from 67° to 76° F, between October 10 and November 16, 1966. Larvae of groups 9, 10, and 11 were reared at room temperatures, which ranged from 65° to 75° F, between October 30 and December 1, 1966.

RESULTS. The results are presented in Table 1. In groups 1, 2 and 3 the length of time required for the first instar larvae to reach pupation decreased as the concentration of yeast increased. Analysis of variance showed that groups 1, 2, and 3 differ significantly from one another at the 5 percent confidence level, and groups 9, 10, and 11 also were significantly different in the developmental period. Groups 4 through 8 did not show any difference in the length of time required to reach pupation.

An analysis of variance indicated that differences in percent pupation among various groups were significant at the 5 percent probability level. The LSD was 8.4.

The data on the effect of various dietary formulations on sex ratio were analyzed by the chi-square method. It was found that the sex ratio was significantly different in groups 2, 3, and 9, but not in the remaining groups.

Analysis also showed that differences in percent adult emergence among various groups were significant. The LSD was 8.5.

An analysis of variance revealed that differences in dry weights of the adult mosquitoes among various groups were significant. (LSD was found to be 0.05.)

DISCUSSION. In groups 1, 2, and 3, the speed of larval development was shortened as the quantity of yeast was increased. This finding was in agreement with the experimental results obtained by earlier workers (Herms, 1928; Frost *et al.*, 1936). It should be noticed, however, that there

TABLE 1.—Effect of different formulations of diet on the development of *C. incidens*.

| Group | Food | Days from 1st instar to 1st pupation | Percent pupation | Sex ratio diff. | Percent of larvae becoming adults | Dry wt. per mosquito (mg.) | |
|---|---|---|---|---|---|---|---|
| | | | | | | males | females |
| 1 | 10 mg. yeast | 25.0 | 6.0 | no | 5.7 | .39 | .50 |
| 2 | 20 mg. yeast | 20.3 | 16.3 | yes | 11.3 | .28 | .60 |
| 3 | 40 mg. yeast | 15.0 | 45.0 | yes | 18.0 | .41 | .53 |
| 4 | 100 mg. bread crumbs | 9.0 | 93.3 | no | 82.0 | .57 | 1.08 |
| 5 | 200 mg. bread crumbs | 9.0 | 86.3 | no | 83.7 | .61 | 1.07 |
| 6 | 20 mg. yeast & 200 mg. bread | 9.0 | 88.3 | no | 81.3 | .67 | 1.12 |
| 7 | 30 mg. yeast & 300 mg. bread | 9.0 | 92.3 | no | 89.3 | .66 | 1.17 |
| 8 | 1000 mg. prot. mash food (water changed every 3 days) | 9.0 | 93.3 | no | 90.3 | .68 | 1.20 |
| 9 | 330 mg. protein mash food | 8.7 | 56.3 | yes | 55.3 | .65 | 1.13 |
| 10 | 330 mg. yeast | 10.0 | 82.7 | no | 76.3 | .71 | 1.24 |
| 11 | 330 mg. bread crumbs | 11.0 | 86.7 | no | 83.7 | .73 | 1.21 |

was no increase in speed of larval development beyond group 4 (i.e., 100 mg. of bread crumbs group). This suggests that 100 mg. every 3 days should be sufficient for the larvae to develop into pupae at the shortest possible length of time. The prolonged periods required in groups 1, 2, and 3 appeared to be attributable to an insufficient quantity of food. It should also be noticed, however, that there were significant differences in the speed of larval development among groups 9, 10 and 11, each of which received identical amounts of food. These three groups were reared at lower temperatures which might account for the differences in developmental time.

As can be observed in Table 1, the percent of pupation was significantly different among groups 1, 2, 3, 9 and 10, while it was not statistically different among groups 4, 5, 6, 7, 8, and 11. The percent of pupation in groups 1 (6 percent), 2 (16.3 percent) and 3 (45 percent) increased as the amount of yeast was increased. This obviously suggests that the lower percent of pupation was due to relatively inadequate quantities of food. For both groups 8 and 9 protein mash food was used, but in the former group three times as much food was supplied to the larvae. In group 8, 90.3 percent of pupae were obtained whereas only 56.3 percent

of pupae were obtained in group 9. That difference did not seem to relate to the quantity of food available, but rather to the fact that the renewal of water in group 8 every 3 days seemed to favor the survival of the larvae.

It was found, through the author's experience in the mass rearing of *C. incidens,* that often less than 330 mg. of protein-mash food per 100 larvae produced satisfactory pupation rate (and emergence rate), provided the water was renewed at appropriate intervals. It is suggested that the low pupation rate in group 9 was possibly due to the accumulation of toxic materials produced by microorganisms in the food which, after reaching a certain level, resulted in harmful effects on the mosquito larvae. Although the percent of pupation obtained in group 10 was not significantly different from groups 5, 6, 8 and 11, it was different from groups 4 and 7. It appeared that yeast alone was not as good a larval food as bread crumbs or protein-mash. A mixture of yeast and bread crumbs appeared to be satisfactory food for the mosquito larvae.

With regard to the effect of various dietary formulations on sex ratio, the table shows that in groups 2, 3, and 9 there was a significantly low productivity. It is interesting to note that smaller quantities of yeast usually resulted in more males whereas sufficient quantities of food supplies gave rise to equal sex ratio. This finding appears to be contrary to that of Herms (1928), who found that large quantities of yeast resulted in more males, whereas smaller quantities of food gave rise to more females. However, it is believed that females usually require more energy for the development of ovaries and consequently more food is needed. When the quantity of food was limited, it is reasonable to assume that the males would have a better chance to survive than the females. It was shown that no significant difference in sex ratio developed in group 1, even though the females slightly outnumbered the males. Better results might have been obtained (and consequently the statistical analysis of the data might have been more meaningful) if more replicates had been used.

With respect to the effect of various dietary formulations on the percent of adult emergence, the table shows that it followed more or less the same pattern as that of pupation. Although there was not a statistically significant difference between group 1 and group 2 or between group 2 and group 3, there was significantly faster adult emergence in group 3 than in group 1. However, it was observed that, as the quantity of yeast was increased, the percent of adult emergence increased. The factor responsible for the considerably lower percent of adult emergence in group 9 is the same as that for the low pupation rate, as already indicated in the discussion of the effect on pupation.

In general, males are lighter in weight than females in all groups. The adult mosquitoes (both males and females) from groups 1, 2 and 3 appeared to be relatively lighter in weight than the ones from the remaining groups. However, there were no significant differences among groups 1, 2 and 3. Although the females in group 2 appear to be heavier than those in group 1 or group 3, this may have been insignificant because of very small sample size. The adult mosquitoes in group 4 appeared to be significantly lighter in weight than those in groups 6, 7, 8, 9, 10 and 11. It seems evident, therefore, that the dry weights of adult mosquitoes are influenced directly by the quantity of food available to their larvae. Insufficient amounts of food produced markedly "light" mosquitoes (e.g., groups 1, 2, and 3). Although 100 mg. of bread crumbs (group 4) appeared to be sufficient to produce a high percent of pupation and adult emergence, the dry-weight of adults was slightly reduced. Since the mosquitoes from groups 5 to 11 did not show any significant variation in dry-weight, this seemed to suggest that any excess of larval food supplies beyond the optimum level required for normal development would not drastically increase the weight of adult mosquitoes.

SUMMARY. Of those foods tested, the

best dietary formulations for *C. incidens* were: (1) 1000 mg. of protein-mash food per 100 larvae every 3 days, provided the water is partly renewed each time the food is added; (2) a mixture of 30 mg. of yeast and 300 mg. of whole-wheat bread crumbs per 100 larvae every 3 days; or (3) 200 to 330 mg. of bread crumbs per 100 larvae every 3 days.

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