INTRASPECIFIC COMPETITION IN *AEDES AEGYPTI* (L.) LARVAE: I. EQUIPMENT, TECHNIQUES AND METHODOLOGY[1]

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The importance of competition as a regulatory mechanism of animal populations is, at present, a subject of wide controversy among ecologists. In fact, a precise definition of competition, agreeable to most authors, is lacking (see Birch 1955, Klomp 1961, and Milne 1961). Nonetheless, most investigators agree that competition does exist, and the present confusion and disagreement results from an inadequacy of detailed information.

Investigators have approached the study of competition from opposing ends and Park (1954) and Miller (1964) exemplify this dichotomy. Park's (1954) classical work with *Tribolium* beetles examined the end results of competition occurring among all life stages of the beetles. He compiled extensively detailed data on population sizes over time resulting from egg, larval, pupal and adult competition and the interaction of each life stage with the others. From these data Park (1962) drew conclusions as to the capacity and mechanisms of competition. In contrast, Miller (1964) examined one life stage of *Drosophila melanogaster* and *D. simulans*. He studied extensively those environmental factors of greatest importance to the larval development of each species. Subsequently, he was able to predict the outcome of competition between the two species under a given set of environmental conditions.

These two classical approaches to the investigation of competition demonstrate a growing dichotomy in experimentation among modern ecologists. Miller's emphasis and efforts are placed on the total and exact control of experimental factors. All parameters are investigated and observed for each of two similar species. Predictions, based on single species population performance, would be made as to the expected results of a two species population. Any change caused by the introduction of an unknown, such as the effect of two species living together in the same environment, is noted and attributed to this unknown. Conclusions are drawn, based on the type and degree of change from the expected. On the other hand, Park's approach involves the investigation of a field problem using a convenient laboratory organism. Environmental factors are quantitatively and qualitatively set and controlled for both species, individually and together. Conclusions are therefore not based on changes from the "normal" or expected for each species, but are based on differences between two populations, a population of one species and a population of two; with environmental factors in both cultures exactly duplicated quantitatively and qualitatively.

Rather than discussing the different meanings of competition, a working definition used in these studies is presented. Competition is an interaction among the individuals of one or more species that mutually depend on a limited resource. The interaction for the limited resource may adversely affect the involved species, either individually or collectively. In subsequent reports, as experimental results merit, a more precise definition will be formulated. At present, the accepted criterion for demonstrating the competi-

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tive process in action is to show that all competing organisms are detrimentally affected.

The work presented in this paper begins a series of studies on larval competition of *Aedes aegypti* (L.). As an initial step in this series of experiments an attempt was made to develop a standard rearing technique. With these data the requirements for optimal larval development would be available for *Ae. aegypti* larvae. In future experiments studying the competitive capacity of *Ae. aegypti,* any divergence from this norm would indicate the "interference" due to the competitive process.

MATERIALS AND METHODS

CULTURE REARING TECHNIQUES. The culture of *Aedes aegypti* was obtained from Rutgers University 2 years prior to experimentation. Rearing techniques were identical to those described previously (Chevone and Peters, 1969) with the following modifications: culture room R.H. maintained at 65 ± 5 percent, brewers' yeast as larval food, and larvae reared in a salt solution developed by Trager (1935).

EXPERIMENTAL REARING TECHNIQUES. Eggs, no more than 8 weeks old, were placed in Trager's salt solution and vacuum-hatched at 20 psi with a Gast vacuum pump, Model 0406-V2-154, for 15 minutes (Barr and Al-Azawi, 1958). This technique provided greater than 75 percent hatch and yielded larvae of uniform age. Forty larvae per dish were then counted into petri dishes 100 mm O.D. by 20 mm deep containing 75 ml of salt solution. A suspension, consisting of 140 mesh brewers' yeast, was pipetted volumetrically into each dish and Trager's salt solution was added to bring total volume per dish to 80 ml. Previous work in our laboratory had shown that 1 larva/2 ml did not detrimentally affect larval development or pupation. Vasquez (1966) found that densities of 1 larva/0.75 ml or less first produced adverse effects upon pupation of *A. aegypti,* and Morlan *et al.* (1963) found that in mass rearing, 1.4 larvae/ml and 1.4 larvae/cm^2 surface area yielded favorable pupation.

Experiments were conducted in a Percival E-57 environmental chamber. Environmental conditions were 12-12 LD period; 90 ± 5 percent R.H.; L-temperature (air) $84 \pm 1.0°$ F., D-temperature (air) $76 \pm 1.0°$ F., mean temperature $80 \pm 0.4°$ F. The water temperature is within the optimal range of 26–29° C. and the fluctuating temperature produces faster development than a constant temperature at the mean of the range as shown by Headlee (1941).

Media in petri dishes had varying temperatures depending upon position in the chamber. The primary source of this variation was the air stream in the chamber causing a 2–3° F. temperature difference among dishes. To reduce this variable, the petri dishes were randomly arranged daily within the chamber. The air stream also produced variation in evaporation of water from the petri dishes, causing a 5-15 ml loss per dish over a 24-hour period, regardless of the 90 percent R.H. This variable was reduced by covering the petri dishes. Grommets of polyethylene tubing prevented water from sealing the top and bottom dishes as condensation occurred at the daily temperature drop, and also allowed for a minimal circulation of air over the water's surface. Water evaporation was reduced to 0–2.0 ml per dish per 24 hours. Later experiments were conducted in petri dishes with three 1/16-inch beads of glass on the upper edge of the dish bottoms.

STANDARDIZATION OF CONDITIONS FOR LARVAL DEVELOPMENT TO PUPATION. Environmental conditions of light, water temperature, water evaporation, and R.H. were adequately controlled and highly replicable in the Percival chamber. Larvae of uniform age were produced by vacuum hatching eggs from a single oviposition cone for 15 minutes. In all experiments, media were changed daily to prevent possible pollution.

A specific daily amount of food was the only undetermined factor of primary importance influencing larval development. Previous work in our laboratory had shown that food/ml rather than food/

larva was the limiting factor if density did not exceed 1 larva/0.75 ml water. Fay (1964) gave the following amounts of dog chow in milligrams to *A. aegypti* larvae: 0.1, 0.2, 0.3, and 0.6 on days 1, 2, 3, and 4–6 respectively. We conducted 6 experiments to determine the optimal amounts of daily food required to produce fastest pupation, least pollution and least mortality under the above conditions. Five replicates of each treatment were run. The experiments were as follows:

Experiment I

Milligrams of yeast/ml of Trager's solution

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
I	0.10	0.15	0.20	0.25	0.30
II	0.20	0.25	0.30	0.35	0.40
III	0.30	0.35	0.40	0.45	0.50
IV	0.40	0.45	0.50	0.55	0.60
V	0.50	0.55	0.60	0.65	0.70

Experiment II

Milligrams of yeast/ml of Trager's solution

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
I	0.10	0.25	0.40	0.65	0.50
II	0.10	0.25	0.40	0.80	0.50
III	0.10	0.25	0.40	0.95	0.50
IV	0.10	0.25	0.40	1.10	0.50
V	0.10	0.25	0.40	1.25	0.50

Experiment III

Milligrams of yeast/ml of Trager's solution

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
I	0.10	0.25	0.40	0.80	0.30
II	0.10	0.25	0.40	0.80	0.40
III	0.10	0.25	0.40	0.80	0.50
IV	0.10	0.25	0.40	0.80	0.60
V	0.10	0.25	0.40	0.80	0.70

Experiment IV

Milligrams of yeast/ml of Trager's solution

Treatment	Day 1
I	0.10
II	0.08
III	0.06
IV	0.04
V	0.02

Experiments V and VI

Milligrams of yeast/ml of Trager's solution

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
I	0.10	0.25	0.40	0.80	0.50
II	0.10	0.25	0.40	0.80	0.50
III	0.10	0.25	0.40	0.80	0.50
IV	0.10	0.25	0.40	0.80	0.50
V	0.10	0.25	0.40	0.80	0.50

Daily observations were made on amount of food left in each treatment. That treatment which showed trace amounts of food remaining after 24 hours was chosen as the optimal and used as the daily food requirement in the next experiment.

Time of pupation was the criterion used to determine relative rates of larval development in the different treatments of experiments I, II, III, and V. Observations were recorded every 2 hours (Experiments I, II, and III), or 4 hours (Experiment V), for 24–26 hours after appearance of the first pupa in any replicate. In experiments I and V the pupae were killed by boiling and separated by sex. Each sex group was collectively dried at 100° C. for 1½ hours and weighed to the nearest 0.1 mg. on a Mettler single-pan balance. Individual pupal weights were computed and statistically analyzed.

Experiment IV was run to check the selection of 0.10 mg/ml as the proper level for day 1. The level had been previously selected as best in Experiment 1, but since it was the lowest level tried in that experiment the possibility remained that levels lower than 0.10 mg/ml might be optimal. Criteria for evaluating the results in Experiment IV were: a) rate of ecdysis to instar II as determined by counts of molted skins, and b) amounts of food remaining at the molt.

Experiment V was run to check the final rearing method for pupation rate and variation in weight. Termination of the experiment was at either 90 percent pupation or 24 hours after initial pupation; whichever occurred first.

Experiment VI was conducted to determine maximum percent survival with the rearing technique as used in Experiment V. The dishes were not changed after day 5.

Analysis of ecdysis or pupation rate was conducted as previously reported by Chevone and Peters (1969), modifying the data and using a one-way nested analysis design.

RESULTS AND DISCUSSION

EXPERIMENT I. The initial time of pupation in this experiment was not recorded, but observations showed that pupation occurred a maximum of 112 hours after hatch. Table 1 gives mean dry weights of male and female pupae and mean mortality in each treatment. Insufficient data prevented any comparison of rates of larval development to pupation.

Observations on daily amounts of unconsumed food gave the following results:

Day 1—Treatment I (0.1 mg/ml) had trace amounts of unconsumed yeast. All other treatments showed progressively increasing amounts of unconsumed food. No pollution had occurred.

Day 2—Treatment I (0.15 mg/ml) had no food remaining. Treatment II (0.25 mg/ml) had trace amounts of food remaining. All other treatments showed progressively increasing amounts of unconsumed food. Treatments IV and V showed slight pollution.

Day 3—Treatments I and II (0.20 and 0.30 mg/ml) had no yeast remaining. Treatment III (0.40 mg/ml) had trace amounts of unconsumed yeast. Treatments IV and V showed increasing amounts of unconsumed yeast. Slight pollution was present.

TABLE 1.—Mean larval mortality and pupal dry weights of male and female *A. aegypti* reared to pupation with different daily amounts of brewer's yeast in Experiment I.

Treatment	Mean dry weight (mg)		Mean % Mortality
	Males	Females	
I	.4145	.6727	2.01
II	.4712	.7539	1.51
III	.5272	.8611	4.58
IV	.5371	.9012	9.50
V	.5283	.9096	15.00

Day 4—All treatments had no yeast remaining.

Day 5—Treatment I (0.30 mg/ml) had no yeast remaining. Treatments II and III (0.40 and 0.50 mg/ml) had trace amounts of unconsumed yeast. Treatments IV and V had excess amounts of unconsumed yeast and moderate pollution.

The following daily food requirements were considered optimal from this experiment: day 1, 0.1 mg/ml; day 2, 0.25 mg/ml; day 3, 0.40 mg/ml. Insufficient total amounts of food available to the larvae in the first 3 treatments are reflected in the low mean weights of the male and female pupae (Table 1). A progressive increase in mean weights of male pupae occurs through treatment IV; and of female pupae through treatment V. The general trend of increasing mortality is probably attributable to pollution. In treatments IV and V, this pollution occurred on days 3 and 5. Mean percent mortality figures per replicate (5 reps./treatment) were as follows:

	Mean % Mortality/Replicate	
	Day 3	Day 5
Treatment IV	1.0	9.0
Treatment V	3.0	7.9

Mortality on these 2 days accounted for more than 80 percent of the total mortality in each treatment. Food requirements for the first 3 days of larval development were determined in this experiment.

EXPERIMENT II. Initial pupation in treatments I, II, IV and V (0.65, 0.95, 1.10 and 1.25 mg/ml) began 102 hours after hatch; in treatment III (0.80 mg/ml) pupation began 103 hours after hatch. Analysis of variance showed no significant difference, $p<.01$, between treatments. However a high variation, $p<.01$, occurred among replicates within treatments. Table 2 presents mean developmental rates of pupation and mean mortality rates to pupation in Experiment II.

No dry weights were taken for this experiment. Although treatment II (0.80 mg/ml) had the highest mean rate of development (1.37 hrs. longer that the lowest mean rate) and a high mortality rate for the experiment, this treatment was chosen as the optimal food requirement for day 4. No significant difference, $p<.01$, was found among mean pupation rates or mean mortality rates. Justification for this decision was based on the amounts of unconsumed food remaining at the end of day 4. They were as follows: Treatment I, no food; Treatment II, trace amounts; Treatment III, slight food, slight pollution; Treatment IV, excess food, moderate pollution; Treatment V, excess food, moderate to high pollution. From the recorded data and observations, 0.80 mg/ml appears to be slightly less than the optimal food requirement and 0.95 mg/ml slightly in excess. Amounts of 0.85–0.90 mg/ml may be optimal. Dry weights might have further clarified the optimal food requirement for day 4.

TABLE 2.—Mean pupation rates and mean mortality of *A. aegypti* larvae reared to pupation on different daily amounts of brewer's yeast in Experiment II.

Treatment (Day 4)	Mean rate of pupation (Hours after hatch)	Mean % Mortality
I (0.65 mg/ml)	111.96	4.52
II (0.80 mg/ml)	112.83	7.14
III (0.95 mg/ml)	112.37	5.94
IV (1.10 mg/ml)	111.47	7.58
V (1.25 mg/ml)	111.95	5.50

EXPERIMENT III. Initial pupation began 99 hours after hatch (in Treatments I and II, 0.30 and 0.40 mg/ml). Table 3 gives the mean rate of pupation and mean mortality to pupation.

Observations on the amount of unconsumed food remaining were as follows: Treatment I, no food remaining; Treatment II, very slight trace of food remaining; Treatment III, trace amounts of food remaining. The other treatments contained progressively increasing amounts of unconsumed food and became increasingly polluted.

Analysis of variance showed no significant difference, $p<.01$, between treatments. A significant difference, $p<.05$, occurred among replicates within treatments.

TABLE 3.—Mean rates of pupation and mortality of *A. aegypti* larvae reared to pupation on different daily amounts of brewer's yeast in Experiment III.

Treatment (Day 4)		Mean rate of pupation (Hours after hatch)	Mean % Mortality
I	(0.30 mg/ml)	110.96	5.02
II	(0.40 mg/ml)	110.88	3.06
III	(0.50 mg/ml)	111.03	2.01
IV	(0.60 mg/ml)	110.92	3.03
V	(0.70 mg/ml)	111.58	5.08

Optimal food requirement for day 5 was determined as 0.5 mg/ml based on amount of unconsumed food remaining at the end of the 24 hour period.

EXPERIMENT IV. Of the 5 feeding levels tested, 0.1 mg/ml proved best based on amounts of unconsumed food remaining after all larvae had molted to Instar II. Levels below 0.10 mg/ml had no trace of unconsumed food. Rate of ecdysis was fairly uniform throughout the treatments, with average rate of ecdysis for larvae in 0.10 mg/ml slightly better than in the other treatments (see Fig. 1). Therefore, 0.10 mg/ml was retained as the optimal food ration for day 1 under the rearing conditions described above.

EXPERIMENT V. This check of the rearing technique for variability in pupation rate and pupal weights yielded the following results. Average pupation rate over a 24-hour period from the time of initial pupation in any dish was 87.1 percent of the initial population. The average number of surviving larvae at the end of the 24 hour pupation period was 2.3+/dish or 5.8 percent of the initial population. Therefore survival to the end of the experiment was 92.9 percent. Average pupation within the 24 hour period was 93.8 percent of all surviving to the end of that period.

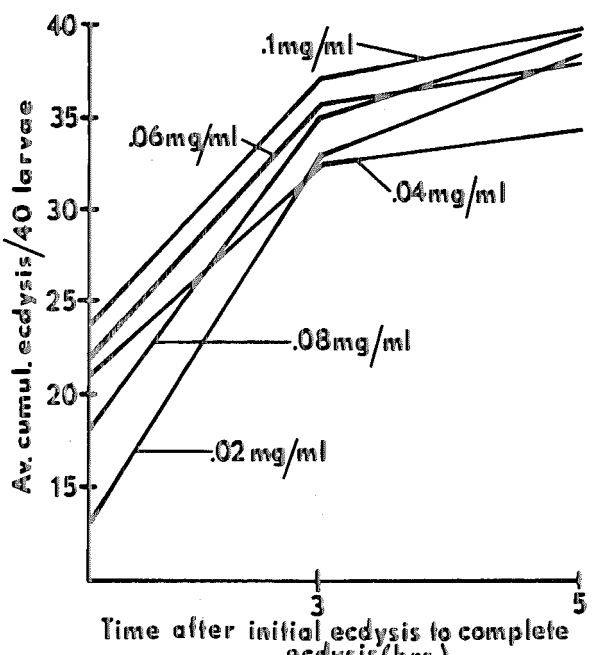

FIG. 1.—The influence of food ration on average rate of ecdysis by 1st instar *Aedes aegypti* (L).

The 25 replicates used in Experiment V were grouped in fives to duplicate handling and analysis of variance as used in Experiments, I, II, and III. Table 4 shows the analysis of variance for pupation rates in Experiment V.

There is no statistically significant difference between treatments (all treatments were the same) but there is a highly significant difference between replicates within treatments. Further analysis of the data reveals that more than half of this variability was due to one of the five groups. Each group was handled separately and it is possible that something happened to Group 5. There were 16 larvae left in Group 5 at the end of the experiment whereas the other groups had either 10 or 11 larvae. This means that an average of 3.4 percent fewer larvae had pupated in Group 5 than in the other groups.

TABLE 4.—Analysis of variance for pupation rates in Experiment V

Source	df	S.S.	M.S.	F.
Trt.	4	186.66	46.66	0.47
Rep.:Trt.	20	1969.75	98.48	2.77
Error	846	30006.60	35.47	
Total	870			

Average dry weights of pupae produced in Experiment V were calculated. Weights were as follows:

	Male	Female
Average	0.316 mg	0.642 mg
Standard deviation	0.042	0.059

EXPERIMENT VI. This was a duplication of Experiment V to determine total pupation under the rearing technique developed from the previous experiments. Conditions were identical to Experiment V except that the average temperature was 1° F. lower than in Experiment V. Mean pupation in Experiment VI was 91.8 percent, over a period of 5 days after initial pupation. Standard error of the mean for survival to pupation was $S_x = 0.487$.

CONCLUSIONS

The rearing technique developed in this series of experiments provides conditions suitable for predictably uniform growth of *Aedes aegypti* (L.) larvae. Our previous experience indicates that differences in rates of larval development are apparently controlled by slight differences in quantities of food. We feel that the feeding schedule developed in this study provides enough accuracy in regulating growth rates to warrant its use in bioassay of the effects of larval density in *Aedes aegypti* (L.) larvae. Experiments on crowding and starvation phenomena using the rearing technique herein described will be reported in the future.

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A FIELD STUDY OF AN AIRBORNE TOXIC EFFECT OF BAYGON[1] RESIDUAL SPRAY

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Few studies have been carried out on the "fumigant" effect of residual insecticides. Davidson (1952), in commenting on the mortality occurring among mosquitoes in cages hung in sprayed houses, but not in contact with sprayed surfaces, considered that this was due, in the case of DDT and dieldrin, to airborne particles and in the case of HCH to fumigant and particulate effects. The airborne effect has not been studied sufficiently to assess its role in the control of vector-borne diseases. Neither has the actual mechanism of this effect been thoroughly examined. Attention is now being given to this matter in connection with the WHO Program for the Evaluation and Testing of New Insecticides.

Baygon has been shown to be an effective insecticide for controlling anophelines (Gahan *et al.*, 1966; Pant and Self, 1966; Pant *et al.*[4]). Unpublished reports from WHO/PAHO Insecticide Testing Team in El Salvador showed that when mosquitoes were caged in the vicinity of sprayed surfaces they were killed. It was concluded by the above team that the lethal effect of this insecticide was due to microparticles floating in the air.

Pant and Self (1968) showed that mosquitoes were knocked down when exposed to glass plates treated with Baygon without actually coming in contact with the insecticide. An unpublished report of the *Anopheles* Control Research Unit, Kaduna[4] has also shown that the rate of loss of the active ingredient of Baygon from the insecticide-impregnated cheesecloth was very rapid and was in conformity with the half life of this material on glass fiber filters reported to be of the order of 13 days by Hadaway.[5] Baygon has the vapor pressure of 0.01 mm Hg at 120 ° C.[6]

Because biting and resting activities of malaria vectors frequently take place in the immediate vicinity of huts, it might be an advantage to have an insecticide which would not only kill those anophelines resting on a sprayed surface but would also kill those not in direct con-

[1] Ortho-isopropoxyphenyl methylcarbamate, also known as Unden, Bayer 39007, OMS 33, arprocarb, and propoxur.

[2, 3] Project Leader and Entomologist respectively of the *Anopheles* Control Research Unit, Kaduna, Nigeria.

[4] Unpublished report of the WHO *Anopheles* Research Unit, Kaduna, 1966, submitted by C. P. Pant, L. S. Self, M. Ramasamy and P. Renaud, WHO Document VBC/67.15.

[5] Unpublished communication by A. B. Hadaway to WHO, from the Tropical Pesticides Research Unit, Porton, U.K.

[6] Pesticides Chemicals Official Compendium, Association of American Pesticides Control Officials, Inc., 1966.