REVISION OF THE ARTICLE "CONSOLIDATION OF LARVAE AFTER SEPARATION OF PUPAE IN THE MASS PRODUCTION OF *ANOPHELES ALBIMANUS*"[1]

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ABSTRACT. Experiments showed that consolidation of larvae remaining in rearing trays after the 1st harvest of pupae was economically beneficial in mass rearing *Anopheles albimanus* Wiedemann. There was no significant difference (P=0.05) in pupal production or insect quality between the different consolidation schemes and the unconsolidated controls. However, there was a significant difference in insect quality between the 3rd pupal harvest and the first 2 harvests. The economics of using only 2 pupal harvests and a consolidation of pupae after the 1st harvest (4:1) is also shown.

INTRODUCTION

A major consideration in the mass rearing of insects is conservation of space, especially when several days are required from the time eggs are set in rearing containers until the desired life stage has been harvested. Also, with some insects, several days may be required for harvesting because of differential developmental time of the immature stages. When this is the case, after some of the insects have been removed from the rearing containers, consolidation of the remaining younger life stages into fewer containers reduces the space required for rearing, which also permits the use of additional rearing units. Ford and Green (1972) usually consolidated the larvae of *Anopheles albimanus* Wiedemann from 2 trays into 1 after the 1st harvest of pupae; after the 2nd harvest, the remaining larvae were discarded. However, their needs for maximum production were limited since they were only maintaining a small colony for laboratory use. Dame et al. (1974) consolidated *An. albimanus* larvae from 2 rearing trays into 1 after the 2nd harvest of pupae and discarded the remaining larvae after the 3rd harvest. From the ca. 2000 newly-hatched larvae they put into a rearing tray they estimated about 90% (1800) were recovered as pupae with 3 harvests. Also, the production needs of these researchers was less than 50,000 male pupae per day, which is a relatively small operation in insect mass rearing. Bailey et al. (1979) used the same type of rearing container as Dame et al. (1974) but used greater numbers and improved techniques for setting trays and for feeding the larvae. They harvested pupae on 3 consecutive days and did not consolidate trays, producing an average of 3822 pupae per tray and discarding an average of 1554 larvae per tray (22.9%) after the 3rd harvest. Bailey, et al. (1980) reported techniques for the mass production of more than 1 million sterile *An. albimanus* males per day. They harvested pupae on 3 consecutive days with no consolidation of pupae in trays. In a program of this scope, available space is a critical factor in maximizing production. If consolidation of tray contents were possible, much more space would be made available for additional rearing. The increased production could be significant, since the

[1] Editor's Note: Because of an inadvertence on the part of the editor and subsequent mysterious events the original, unrevised version of this article was sent to the printer, and the error was not discovered until the March number was actually "in press." The authors had revised the paper in response to reviewers' suggestions. The editor takes full responsibility for the fact that his mistake was discovered too late and apologizes to the authors. This paper is meant to replace the paper which appeared in Vol. 40 (1):15–18.

success of a sterile male program is directly dependent on the number of males that are available for release. The ratio of sterile males:normal males must exceed a critical threshold corresponding to a level of sterility that insures population reduction.

We therefore felt that a more thorough study of consolidation of *An. albimanus* larvae in their rearing containers might be beneficial. Experiments were conducted with the improved rearing system of Bailey, et al. (1979) to determine if consolidation of rearing trays after the 1st and/or 2nd harvest of pupae would result in increased production and then prove beneficial in mass rearing *An. albimanus*.

MATERIALS AND METHODS

The Santa Tecla strain of *An. albimanus* (an insecticide-susceptible strain that had been in colonization for ca. 4 years) was used in all the consolidation studies. The larvae were reared in ABS plastic rearing trays (56 x 43 x 7.5 cm high). Three liters of water were placed in each tray 24 hr prior to adding the newly hatched larvae to allow the water to reach the desired rearing temperature (29±0.5°C). The water temperature in the trays was maintained by electric heating tapes regulated by electronic proportional controllers (Dame et al. 1978). At the same time water was put in the trays, individual samples of 0.085 ml of dried eggs (ca. 6780 eggs) were measured and placed on the surface of water in hatch cups and held at 29±0.5°C for 24 hr. Just before the newly hatched larvae were poured into the trays (Bailey et al. 1979) 150 ml of an aqueous suspension containing 2.25 g of liver powder:yeast:hog supplement (1:1:1) was added to the water in each tray, and 72 hr later each tray received another 150 ml of the 1:1:1 mix. On each of the next 2 days, each tray received 150 ml (3 g dry ingredient) of a suspension of hog supplement in water.

The 1st pupae were produced on the 6th day after the newly hatched larvae were put in the trays. At the time the pupae were separated from the larvae using the cold water technique described by Bailey et al. (1979) (modified from Weathersby 1963). Each of the 4 replicates of the experiment consisted of 28 rearing trays, handled as follows:

	Group					
	*A**	*B*	*C*	*D*	*E*	*F*
No. trays started	2	2	4	8	4	8
No. trays after 1st harvest (day 6)	2	2	4	8	2	2
No. trays after 2nd harvest (day 7)	2	1	1	1	1	1

*Control

Consolidation of trays after pupal separation was accomplished by combining the larvae from 2 or more trays into 1 tray. The trays in the control group (A) were not consolidated, beginning with 2 trays per replicate and returning the contents of each tray to the original after each pupal separation. The larvae in the remaining trays went through various degrees of consolidation. Beginning with 2, 4, or 8 trays per group, the larvae in some groups of trays (B, C, and D) were not consolidated after the 1st pupae were removed, however, the larvae in all groups except the control were ultimately consolidated into 1 tray after the 2nd separation.

After each separation the total number of pupae in each group was measured volumetrically and then 1-ml samples were counted to determine the approximate number of pupae per original rearing tray. After the pupae had been separated 3 times, the larvae remaining in each group were discarded. Also, after each separation, samples of 300 pupae from each group were placed in small cages (15 x 25 x 20 cm high) and the number of emerging adults (males and females) was recorded. These adults were then held for 7 days with cotton pads soaked with 10% sugar water and the percentage surviving was recorded to determine the relative longevity of adults within each group.

Analysis of variance was used to determine whether consolidation of trays affected size of pupae, number of pupae per tray, emergence and survival of adults. The analysis also compared each of the 3 successive harvests of pupae to determine if 3 exposures of the mosquitoes to the cold water had any detrimental effects.

RESULTS AND DISCUSSION

The effects of the different degrees of consolidation on pupal size (as indicated by the number of pupae per ml), pupal production, percent adult emergence, adult survival, and the relative size of adults are reported in Table 1. The production of pupae per rearing tray ranged from 3404 to 4024, but an analysis of variance showed no significant differences between consolidation schemes. Also, there was no significant difference in data from any of the groups that would indicate that insect quality, i.e. pupal size, adult emergence, or adult survival, was affected by consolidation. However, this study did indicate that in all groups there was a significant (P=.95) decrease in pupal size and adult emergence and survival of the mosquitoes related to the number of consecutive pupal separations, which can be attributed in part to the additional handling and exposure to ice water. The pupae were significantly smaller with each succeeding separation, however adult emergence and adult survival was affected only in the 3rd separation, with no differences noted between the 1st and 2nd separations.

Even though the degree of consolidation in this experiment had no significant

Table 1. Results of various degrees of consolidation of larvae in rearing trays after beginning to harvest pupae when mass rearing *Anopheles albimanus* (mean of 4 replications).

	Consolidation scheme					
Harvest	2:2:2	2:2:1	4:4:1	8:8:1	4:2:1	8:2:1
			No. pupae/ml			
1	274	266	286	276	266	281
2	293	296	289	291	319	312
3	312	306	324	327	324	323
Avg.	293	289	300	298	303	305
			No. pupae/tray			
1	824	966	1309	1085	631	817
2	2232	2359	2334	2332	2313	2322
3	760	499	381	452	517	264
Total	3186	3824	4024	3869	3462	3404
			Percent adult emergence			
1	92.4	91.4	94.5	93.5	91.6	91.5
2	95.4	90.9	91.6	93.1	90.7	92.0
3	64.9	74.8	53.8	61.0	54.4	55.2
Avg.	84.2	85.7	79.8	82.5	78.9	79.6
			Percent adult survival (males)			
1	91.9	90.0	87.4	90.0	93.1	91.4
2	73.8	77.3	81.8	83.5	81.0	82.7
3	66.1	70.1	65.6	56.4	73.8	53.0
Avg.	77.3	79.1	78.3	76.6	82.6	75.7
			Percent adult survival (females)			
1	91.3	95.0	91.5	96.5	95.7	96.1
2	89.2	91.5	91.6	92.4	88.1	86.0
3	79.0	86.6	79.4	77.0	86.0	82.2
Avg.	86.5	91.0	87.5	88.6	89.9	88.1

effect on pupal production or insect quality, the inferior quality of the mosquitoes obtained from the 3rd separation points out an important practical aspect of consolidation in the mass production of *An. albimanus*. The reduced emergence and longevity of mosquitoes from the 3rd separation must be considered in the overall economics of production. Using the workforce outlined by Bailey, et al. (1980), calculations were done to show that consolidation reduces the overall workload and at the same time increases the available space for additional trays (Table 2). The economics of using a system of 2 pupal harvests with a 4:1 consolidation of larvae after the 1st harvest is apparent. With 3 pupal harvests and no consolidation (the system used by Bailey et al. 1980) ca. 12,688 pupae could be produced per man-hr. By reducing the harvests to 2, but with no consolidation, the maximum number of trays set per day could be increased, and the man-hr required per day decreased, but with no significant increase in efficiency (pupal production/man-hr). However, the quality of insect could be improved by this method, as those inferior individuals from the third harvest would be eliminated. But a system using 2 harvests and a 4:1 consolidation after the 1st harvest would increase the maximum number of trays set and decrease the required number of man-hr for a significant increase in efficiency (26.8%). Thus, we suggest that a system of mass rearing *An. albimanus* involving 2 pupal harvests and consolidation of tray contents after the 1st harvest (4:1) would increase both the efficiency of the operation and the quality of the mosquitoes produced.

Table 2. Production potential and rearing efficiency of *Anopheles albimanus* using 3 different harvesting schemes with 2400 tray spaces available.

	Harvesting scheme		
	3 Harvests no consolidation	2 harvests no consolidation	2 Harvests—4:1 consolidation after first harvest
Maximum no. trays set/day	266	300	328
No. trays harvested/day	798	600	410
Pupal production/day	3,816	3,056	3,140
Total daily pupal production	1,015,056	916,800	1,029,920
Man-hr required/day[a]	80	72	64
Pupal production/man-hr	12,688	12,733	16,093
Increased efficiency/man-hr (%)	—	0.4	26.8

[a] 0.12 man-hr/tray set and 0.06 man-hr/tray harvested.

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