VIABILITY OF EGGS OF *ANOPHELES ALBIMANUS* AND *ANOPHELES QUADRIMACULATUS* WHEN DRIED AND STORED AT VARIOUS TEMPERATURES

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ABSTRACT. A previous technique used to dry eggs of *An. albimanus* Wiedemann was adapted for storing dried eggs of *An. albimanus* and *An. quadrimaculatus* Say for future use. This system makes it possible to stockpile eggs for at least 5 days at 10–12°C with little loss of viability and for as much as 14 days with greater reduction in hatch. The procedure has application when continuous supplies of reared insects are required or when mass rearing is involved because excess eggs can be stored during periods of high production for use when production drops below normal.

In the past it has not been possible to store *Anopheles* mosquito eggs for future use since the females usually oviposit on the surface of water, and the eggs hatch in about 2 days when exposed to the proper temperature. Dame et al. (1978) described a new method for drying the eggs of *Anopheles albimanus* Wiedemann that facilitated the accurate stocking of rearing trays by volumetric measurement of dried eggs. This system was an improvement over that described by Ford and Green (1972), where trays were set up with estimated numbers of first instar larvae. The possibility therefore existed that dried anopheline eggs might be stored as are eggs of certain species of *Aedes* and *Psorophora*. The ability to stockpile *Anopheles* eggs would make it unnecessary to collect eggs daily in a small-scale operation, but also in a mass rearing program it would provide an emergency back-up capability in the event there was a sudden unexpected reduction in egg production in the adult colony. It was for these reasons that this research was conducted.

METHODS AND MATERIALS

The experiments were conducted in environments of controlled temperature as follow:

Temperatures (±1°C)	*Maintained by*
5	Household refrigerator
10	Environmental chamber
12	Environmental chamber
14	Environmental chamber
16	Environmental chamber
26	Laboratory room, thermostatically controlled

TEST OF CONTAINERS AND TEMPERATURES. All eggs from separate cages of *An. albimanus* and *An. quadrimaculatus* Say were collected over a 16-hr period and then washed and filtered through 20-mesh screen to remove dead mosquitoes and other debris. The collections from the 2 species were then each separated into 3 samples (approximately equal), aged for an additional 24 hr on the surface of water at 26°C, and treated as follows:

1. The first sample was divided into 160 sub-samples of about 500 eggs each. Each sub-sample was placed on the surface of water in a 3-oz plastic cup (wet method), and 32 of the cups were placed at each of 5 storage temperatures (5, 10, 12, 14, and 16°C). (The wet method was not tested at 26°C, since that temperature would have induced batch in all the cups where the eggs were on water.)

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2. The second sample was dried for 30 min by an air current drawn through it by an electric fan (Dame et al. 1978) and then measured volumetrically by sifting eggs into 192 small vials (vial method) made from disposable 1-ml glass pipettes (graduated into 0.01 ml units) at the rate of 0.01 ml eggs/vial (approximately 700–800 eggs). Thirty-two vials were placed in each of the 6 storage temperatures.
3. The third sample was dried for 30 min as in (2) above and then divided into 6 sub-samples that were placed in separate 150-ml amber glass bottles with screw caps (bulk method). One of those sub-samples was placed in each of the 6 temperatures for storage.

On the day the eggs were placed in storage, 2 control samples from each of the 3 treatments were set up to determine the initial percent hatch before storage. Hatch was determined by first placing a quantity of eggs in a 3-oz waxed paper cup with 75 ml well water and 1.5 ml of a 2% food solution containing liver powder and yeast (1:1); that had been filtered through muslin cloth to remove the larger food particles. The samples were then held in a temperature-controlled cabinet at 28°C. After 24 hr, a sample of eggs was taken from each hatch cup with a camel-hair brush, placed on filter paper, and then examined microscopically. The percent hatch was recorded based on 100 eggs examined.

Two samples of eggs from each experimental storage method were taken daily from each temperature for 2 weeks and prepared as previously described for determining hatch. Samples stored on the surface of water in cups were removed and placed in fresh cups, and 1.5 ml of food was added to each cup. Samples stored in vials were poured into hatch cups already prepared with food and water. From those samples stored in bulk in glass bottles two sub-samples (0.01 ml each) from each temperature were measured daily into vials and poured into separate hatch cups prepared as before. The glass bottles were then returned to their respective temperatures. All hatch cups were then placed in a temperature-controlled cabinet (28±0.5°C) for 24 hr, and the percent hatch was determined. The entire test was replicated 3 weeks later.

TEST OF PUPAL PRODUCTION. Another test was conducted to determine the pupal production we could expect from eggs stored at the minimum acceptable temperature. Eggs from both *An. albimanus* and *An. quadrimaculatus* adults were collected, dried, and stored at 10°C in glass screw-cap bottles. On days 0, 1, 3, 7, 10 and 14 after the eggs were dried, 2 samples from each species were measured volumetrically (0.085 ml for *An. albimanus* and 0.07 ml for *An. quadrimaculatus*) and placed in hatch cups as previously described.The following day the percent hatch was determined microscopically, and the contents of each hatch cup were placed in separate plastic rearing trays and reared according to techniques described by Dame et al. (1978). The water in the rearing trays was maintained at 29±0.5°C. On days 6, 7, and 8 after the initial set, pupae were removed and counted.

RESULTS AND DISCUSSION

Recently it was shown that *An. albimanus* eggs could survive a drying process (Breeland et al. 1970). The present study shows that *An. albimanus* and *An. quadrimaculatus* eggs not only survive drying but also can withstand considerable periods of storage. Table 1 summarizes the data for *An. albimanus* and *An. quadrimaculatus* eggs stored by 3 methods at 6 temperatures. For determination of the most effective storage method and temperature, the daily hatch was averaged for each consecutive 7-day period to determine the relative viability of the eggs.

The storage of dried eggs in bulk was obviously the most effective method for both species, since hatch was considerably better with this technique. The average

Table 1. Average daily hatch of *Anopheles albimanus* and *Anopheles quadrimaculatus* after storage at different temperatures for 14 days (mean of 2 replications).

Type of Storage	Storage period (days)	Daily hatch at indicated temperature (°C) 5	10	12	14	16	26
		An. albimanus					
Wet	1–7	71	75	82	78	76	
	8–14	7	20	36	4	4	
Vials	1–7	87	87	88	81	77	18
	8–14	9	10	12	3	1	0
Bulk	1–7	88	91	92	91	91	72
	8–14	44	72	68	43	49	16
		An. quadrimaculatus					
Wet	1–7	74	74	75	65	63	
	8–14	22	41	3	1	1	
Vials	1–7	66	57	58	51	50	21
	8–14	6	1	0	1	0	0
Bulk	1–7	77	84	85	82	79	63
	8–14	51	55	59	44	55	9

hatch for *An. albimanus* eggs during the first 7 days was above 90% at all temperatures except the lowest (5°C) and the highest (26°C). Also the average hatch from all temperatures for the last 7 days was much higher in bulk storage than with either of the other 2 methods. Wet storage was somewhat less effective during the first 7 days than storage in vials, although the average hatch after 14 days was similar from both these methods.

Although the average hatch of *An. quadrimaculatus* eggs after storage also showed that bulk storage was the best of the 3 methods tested, the hatch of that species, in general, was not as good after storage as was that of *An. albimanus*. However, the average daily hatch for bulk storage was above 80% after 7 days at 10, 12 and 14°C. With the other 2 methods, the average daily hatch was 75% or below during the 1st week and 41% or below during the 2nd week.

Table 2 shows the daily hatch of eggs of both species after all 3 methods of storage at 10°C for the entire 14-day period. The hatch was still 90% or higher for *An. albimanus* from all 3 methods after 3 days, but by the 14th day hatch was 4% from the wet storage, 1% from the vials, and 56% for bulk. With *An. quadrimaculatus* the results were different than with *An. albimanus*. The wet storage method was

Table 2. Effects of storage time at 10°C on the hatchability of eggs of *Anopheles albimanus* and *Anopheles quadrimaculatus* (mean of 2 replications).

Storage time (days)	Percent hatch of eggs stored: Wet *An. albimanus*	Wet *An. quadrimaculatus*	Dry in Vials *An. albimanus*	Vials *An. quadrimaculatus*	Bulk *An. albimanus*	Bulk *An. quadrimaculatus*
0	91	81	95	94	95	92
1	90	79	95	88	95	94
2	95	84	96	88	97	89
3	90	91	96	81	95	75
4	68	73	93	65	96	85
5	82	62	84	45	90	81
6	61	59	81	21	88	81
7	38	71	61	9	78	83
8	55	56	43	4	82	74
9	34	48	17	1	80	64
10	22	49	4	1	78	68
11	11	37	2	1	76	55
12	10	42	1	1	66	52
13	3	28	1	0	66	39
14	4	26	1	0	56	34

not as effective as the bulk method, but it did hold up almost as well. The hatch from the vial method dropped below 50% after only 4 days and was below 10% on day 7. The hatch with the bulk method was 83% after 7 days.

Table 3 shows the pupal production from *An. albimanus* and *An. quadrimaculatus* eggs stored dry in bulk at 10°C for various periods of time. Production of *An. albimanus* pupae from eggs stored for as much as 3 days was greater than production with no storage. Tests were not conducted with eggs stored for 4–6 days, but our subsequent use of stored *An. albimanus* eggs in colony production has shown little or no reduction in pupal production when storage time does not exceed 5 days. Thereafter, a decline in production usually occurs, as indicated in these data. However, some production (450 pupae per tray) was obtained even after 14 days of egg storage. For storage periods in excess of 5 days, an adjustment in the number of eggs per rearing tray could be made according to the percent hatch.

Table 3. Pupal production from dried eggs of *Anopheles albimanus* (0.085 ml) and *Anopheles quadrimaculatus* (0.07 ml) stored in bulk at 10°C for various periods (mean of 2 replications).

Number days stored	*An. albimanus*		*An. quadrimaculatus*	
	% Hatch	Avg. no. pupae per tray	% Hatch	Avg. no. pupae per tray
0	89	3210	87	2400
1	97	3240	91	2580
3	98	4530	92	2970
7	94	1680	80	1560
10	96	870	80	[a]
14	55	450	31	300

[a] Trays from this storage period suffered high larval mortality from overfeeding.

The pupal production from *An. quadrimaculatus* eggs stored in bulk at 10°C showed a similar trend to that of *An. albimanus*. After 3 days of egg storage the pupal production was equal to or better than that of eggs with no storage; however, it declined after 7 days or more storage time. Nevertheless, some pupae were produced after 14 days of storage.

This study shows that storage of 2 species of *Anopheles* eggs is feasible, and possibly would be for other anopheline species as well. The technique would be particularly useful in a sterile male release program or other programs of mass rearing since eggs could be stockpiled during a time of plenty and used a few days later if required during a time of unexpected poor egg production. Many times unexpected declines in egg production last only a few days, and a system of egg storage might often prevent the reduced production of insects that would occur if a stockpile of eggs was not available.

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