SCIENTIFIC NOTE

CHEMICALS USEFUL FOR SEPARATING EGG MASSES OF THE SCREWWORM[1]

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Screwworms, *Cochliomyia hominivorax* (Coquerel), are serious economic pests that cause myiasis of warm-blooded animals in the tropical-subtropical regions of the Western Hemisphere. A successful eradication program has eliminated screwworms from the United States, Mexico and most of Central America using the sterile insect technique (SIT) (Galvin and Wyss 1996). The success of SIT depends upon the production of sufficient quantities of flies. Because of the large number of flies produced, small changes resulting in more efficient rearing can lead to significant cost savings for the eradication program.

Currently at the mass rearing facility, female screwworms lay their eggs in sheets on an egging board; eggs are removed with a spatula, and they are then divided and weighed before they are placed in the larval rearing medium (Brown 1984). Efficiency would be increased if eggs could be volumetrically, mechanically added to the medium (using a 'dosifier') but this could be accomplished only if a method was available for separating the egg masses without loss of viability.

Previous reports (Fredeen 1959) found sodium hydroxide (NaOH) useful for separating the egg masses of Simuliidae. LaChance et al. (1964) reported on separating egg masses in 1% NaOH to determine the number and hatchability of eggs laid by individual females; Rawlins et al. (1983) separated screwworm egg masses for an insecticide study by soaking them in a 1% NaOH solution for 15 to 25 minutes. Although NaOH was reported useful for separating screwworm egg masses, no data were published demonstrating the optimum concentration, time of exposure, or effect on survival of the embryo. Our objectives were to determine the effects of NaOH exposure on screwworm embryos and compare the efficacy of NaOH, sodium carbonate (Na_2C0_3) and potassium hydroxide (KOH) for separating egg masses from three screwworm strains.

Costa Rica 92 (CR92), the strain previously used in the mass rearing facility, was used in the NaOH studies while the strains CR92, J2 (developed from collections in Jamaica) and Linc01 (resulting from crosses of six wild-type screwworm strains) were used in the studies comparing NaOH, Na_2C0_3 and KOH. Adults were kept at 25°C, 50% RH and 12:12 photoperiod. Eggs were collected for ~30 min from 7-10 days-old females.

First, about 100 mg of eggs were placed in either 1 or 2 % (weight/volume) NaOH, occasionally agitated for 30 sec and sampled after 10 min. Thereafter, samples were taken at ~ 15-min intervals with the final sample taken after 100 min. Two samples of 100 eggs

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were taken at each time interval, incubated and categorized as hatched (only the chorion remained), sclerotized (mouth-hooks and/or setae were visible through the chorion), or undeveloped (no structures were obvious). This was replicated four times. The time the eggs remained in the NaOH solutions had a significant effect on the embryo survival but the concentration of NaOH had no significant effect. Survival was ~71.9% for both concentrations at 10 min and progressively decreased to ~27% at 100 min. From these data, the optimum length of time egg masses should be left in NaOH is 10 min or less. Similar mortality occurred in a preliminary study where, for removal of the NaOH-treatment, eggs were transferred to and submerged (for 5 to 15 min.) in distilled water after soaking in NaOH for 10 min. This indicates that the mortality was due to lack of oxygen rather than to effects of the NaOH. To further support the importance of oxygen for the survival of screwworm embryos, if spacers are not used to keep a seal from forming between the top and the bottom of the petri dish, egg hatch does not occur (using spacers is standard practice in our laboratory).

About 100 mg of eggs were then placed in each of three scintillation vials containing 6 ml of 0.5%, 1% or 2% (w/v) solution of NaOH. The eggs were soaked in the solutions, with an occasional 30 sec agitation, for 10 min. Eggs were then pipetted onto moistened filter paper in a Buchner funnel and rinsed with distilled water. Two samples of 100 eggs from each treatment were placed onto moist filter paper in 100 mm petri dishes and incubated at 37° C. Two petri dishes (subsamples) for each concentration were set up each day (replicated 14 times). After about 18 hr, the eggs were examined and categorized as above. The mean percentages of egg hatch (±S.E.) for the three concentrations of NaOH (79.7±1.3 for 0.5%; 79.5±1.7 for 1%; 80.3±1.8 for 2%) were not significantly different. Egg mass separation was noticeably slower and less efficient in the 0.5% solution.

Egg masses were then divided into five portions; each portion was placed on moist filter paper in a petri dish and incubated for 0, 1, 2, 4, or 6 h at 37° C. After incubation, egg masses were divided in half and placed in either 1 or 2% NaOH with periodic agitation for 10 min before sampling, further incubated at 37° C, and then categorized as above. This was replicated five times. Neither age of the embryos at the time of placement into the NaOH nor concentration of NaOH had a significant effect on the percentage of eggs hatching. The mean percentage (±S.E.) of hatching ranged from 74.5±2.6 for eggs 0 h old in 2% NaOH to 81.2±1.2 for eggs 4 h old in 1% NaOH.

About 10 mg (~ 220 eggs) of freshly laid, untreated eggs were then placed in a 100 mm petri dish containing moist filter paper for comparison to NaOH treatments. A sample of ~100 mg of eggs was treated with 1% or 2% NaOH, with occasional agitation, for 10 min. Two samples of 100 eggs from each NaOH concentration were transferred to 100 mm petri dishes with moist filter paper. Egg hatch was categorized as before after incubation for 18-20 h at 37° C. This was replicated six times. There was no significant difference between the mean egg hatch of the treated and untreated eggs.

Finally, NaOH, Na_2C0_3 and KOH were mixed as 1%, 4%, and 2% solutions (w/v), respectively. A small mass of eggs was placed in each solution and mildly agitated for 5 min. Each solution was then poured though filter paper and the eggs were rinsed with distilled water. One hundred eggs were counted onto moist filter paper, incubated and the hatch was categorized as before. This was replicated three times with each of the three strains (CR92, J2 and Linc01). No significant differences were found between treatments of eggs from the J2 and Linc01 screwworm strains. KOH and NaOH were superior to Na_2CO_3 for CR92 (Table 1). There also was a trend, although not statistically significant, that KOH was generally less detrimental to the survival of the embryos.

We have shown that NaOH, KOH or Na_2CO_3 can be used to separate egg masses of the screwworm. NaOH is currently used in our laboratory to simplify our routine monitoring of egg hatch for the 15 strains currently maintained for research. Berkebile et al. (2000) used NaOH to separate egg masses and prepare them for permeabilization and further research with cryopreservation; the technique will be useful in genetic research requiring work with individual eggs.

TABLE 1. Effect of Sodium Hydroxide (NaOH), Sodium Carbonate (Na_2CO_3) and Potassium Hydroxide (KOH) on the Survival of Embryos from Three Strains of Screwworm

Strain	Treatment	Survival X±SE[a]
CR92	NaOH	73.55±1.35a
	Na_2CO_3	61.38±1.46b
	KOH	79.27±4.79a
J2	NaOH	64.52±4.72a
	Na_2CO_3	76.07±2.12a
	KOH	74.45±5.61a
Linc01	NaOH	76.89±5.93a
	Na_2CO_3	76.09±4.58a
	KOH	84.99±2.82a

[a] Values for the same strain followed by different letters are significantly different at $\alpha = 0.05$.

Separating egg masses could also be useful in the eradication program's mass rearing facility. This would allow eggs to be applied to the developmental medium by a volumetric 'dosifier'. The speed and accuracy at which the eggs could be applied to the media would reduce labor costs currently required to handle eggs and could enhance the efficiency of mass rearing.

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