OPERATIONAL AND SCIENTIFIC NOTES

A NEW METHOD OF TESTING *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* (H-14) FORMULATIONS ON *AEDES TAENIORHYNCHUS* IN AN ABANDONED RICE IMPOUNDMENT IN SOUTH CAROLINA[1]

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Rearchers have recognized the need to develop means of testing larvicides under seminatural conditions to obtain results that are comparable to operational applications. Methods employed for testing *Bacillus thuringiensis* var. *israelensis* (H-14) formulations have included evaluation of effectiveness of applications to large rice fields (Lacey and Inman 1985, Sandoski et al. 1985, 1986), tests in smaller habitats such as natural standing pools and water holes (Purcell 1981, Weber and Lake 1986) and small rice plots (Hembree et al. 1980, Dame et al. 1981, Stark and Meisch 1983). The need for a technique of conducting inexpensive, replicated tests of *B. thuringiensis* (H-14) formulations in impounded marsh led us to develop another method of field testing.

The impoundments are former rice fields which now consist of areas of marsh surrounded by dikes with tidal floodgates which can be adjusted to drain, flood or maintain levels of water on the marsh. Low areas in the impoundments where water remains after drainage or rainfall accumulates were selected as test sites since sampling indicated that oviposition was heavy in the periphery of these areas. We observed that bottomless 30-gal (113-liter) galvanized steel trash cans sunk ca. 1 ft (30 cm) into the clay subsoil would maintain water levels independent of the water level in the marsh. We decided to make use of this feature in conducting a series of larvicide tests. The following is presented as an example of the type of studies conducted in these cans.

A study of the efficacy of Abbott granular *B. thuringiensis* (H-14) formulations 30969–104 (200 ITU/mg), 30969–105A (200 ITU/mg) and 30969–105B (200 ITU/mg) was began on August 13, 1987, at the International Center for Public Health Research near McClellanville, SC. In conducting these tests a small impoundment was partially flooded and the presence of mosquito larvae confirmed. Twelve cans (3 for each formulation tested and 3 for controls) were sunk into the soil. The water in the cans was dipped out along with any larvae for predators and replaced with screened water. The cans were then allowed to stand for 24 h before testing. Because all cans contained submergent vegetation, very little mud was stirred up when water was reintroduced. Therefore, mud was not a factor in influencing the efficacy of *B. thurigiensis* var. *israelensis* (H-14). Furthermore, it was found that due to lack of suspended mud, it was easy to remove any predators or indigenous mosquito larvae.

Based on a surface area of 1,923 cm^2, water in the cans was treated with 0.108 g of 200 ITU/mg granular *B. thuringiensis* var. *israelensis* (H-14) larvicide for an application rate equivalent to 5.6 kg/ha.

Prior to treatment, 370 third instar *Aedes taeniorhynchus* (Wiedemann) larvae were introduced into each can. This density was representative of densities recorded in marsh breeding sites (Wallace, unpublished data). Two means of measuring the effects of the larvicides were used: 1) larval and pupal densities were assessed daily by taking 3 standard dipper samples from each can (samples were returned to cans); and 2) after pupae had appeared, cans were covered with emergence trap lids, and adults were counted daily. A second introduction of 370 third instar larvae was made at 3 days posttreatment. These larvae were obtained by raising the water level in the impoundment and flooding additional marsh. This resulted in the hatch of more eggs and was repeated as necessary to obtain larvae for testing.

Results obtained by dipper sampling of the cans were inconsequential. Blind dips often recovered either no larvae or occasionally large numbers due to the tendency of larvae to aggregate. Since larval and pupal densities were difficult to assess, adult emergence was used as a measure of the effects of the larvicides. Results of the first introduction, days 1–6 (Fig. 1), indicated that formulation 105A decreased emer-

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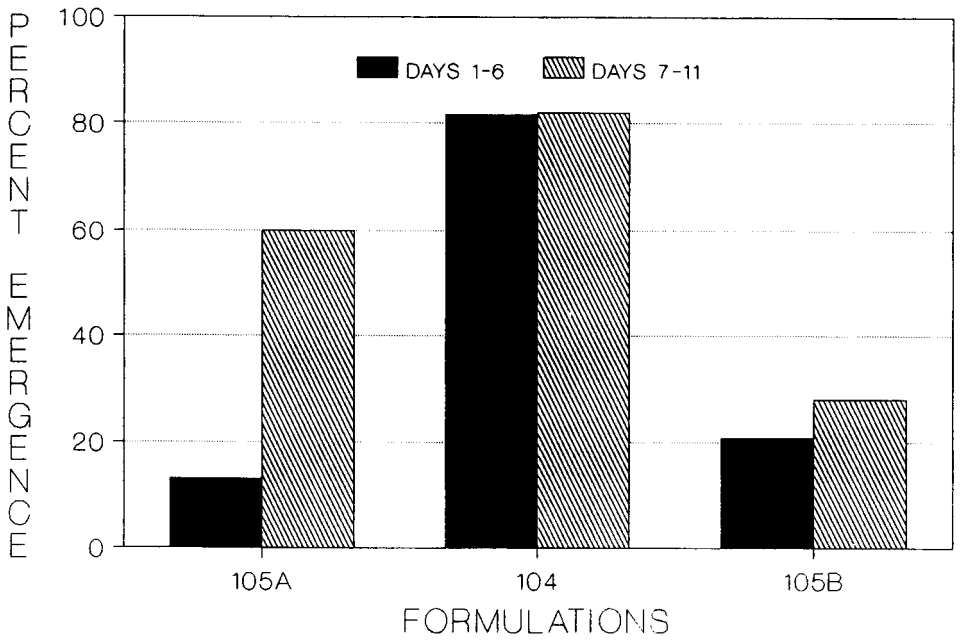

Fig. 1. Percent emergence of adult *Aedes taeniorhynchus* exposed to formulations of *Bacillus thuringiensis* var. *israelensis* (H-14).

gence 86.9%. Formulations 105B and 104 decreased emergence 79.2 and 18.4%, respectively, in comparison to the control. The second introduction (to test residual effects), days 7–11 (Fig. 1), indicated that the effectiveness of formulation 105A in reducing adult emergence had declined to 40%. Formulation 105B proved to have the most residual activity, reducing adult emergence 72%. Formulation 104 exhibited the least residual activity decreasing adult emergence 18%.

There are 3 main disadvantages to this method of testing larvicides against salt marsh mosquitoes. The first is that since the movement of water and larvae is restricted, the larvae may not be exposed to as much toxicant as they would be in an open system and, therefore, mortality may be reduced. Second, the small size of the containers requires that they be treated with very small amounts of larvicide which increases the possibility of significant variation in the actual dosage of insecticide applied. This disadvantage is currently being addressed by the development of larger test containers. Third, the emergence trap tops restrict the amount of sunlight reaching the surface of water in the cans and possibly affect the development of larvae and degradation of the larvicide.

Advantages of this method of conducting larvicide trials are the ease with which different larval instars can be obtained for testing over an extended period of time and the minimal amount of handling and transport that is required in counting the larvae and transferring them to the cans for treatment. Since this method makes use of mosquitoes reared in the natural habitat and the cans contain a small portion of this habitat, we believe that this method simulates most of the environmental conditions that may affect the performance of a larvicide applied against natural populations of marsh mosquitoes.

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