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EFFICACY OF GROUND ULTRA-LOW VOLUME LARVICIDAL TREATMENTS USING TEMEPHOS AND *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AGAINST *CULEX PIPIENS* LARVAE

ROGER ERITJA AND CARLES ARANDA

Consell Comarcal del Baix Llobregat, Parc Torreblanca, 08980 Sant Feliu de Llobregat, Spain

ABSTRACT. Spray drift determinations of truck-mounted ULV applications of temephos and *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) in simulated field trials were conducted in the Baix Llobregat area near Barcelona, Spain, using larval *Culex pipiens* as bioindicators. A 50:50 temephos : oil mixture applied at 350 ml/min, while traveling 9 kph, resulted in 100% control of larvae 10–50 m from the point of application (with the exception of 82% mortality at 30 m); control decreased rapidly to 50% at 55 m. Mortality due to *B.t.i.* application ranged from 92 to 100% within 12 m, and generally decreased therafter to about 11% at 30 m.

Applications of larvicides in flooded areas must be evaluated using cost and efficiency ratios, especially when the most effective and economic method of treatment, such as aircraft application, cannot be used. *Aedes caspius* (Pallas) breeds in salt marshes along the Llobregat River delta, near Barcelona, Spain. This species is highly pestiferous to nearby human settlements. These marshes also border the Barcelona Airport, a fact that makes scheduling of aerial application of insecticides extremely difficult because of the constant flow of air traffic. In the event of severe flooding, the area to be treated could reach more than 350 ha. Because such flooding is infrequent, an application crew cannot be routinely hired to deal with this situation. Therefore, when flooding occurs, treatment with knapsack sprayers may be incapable of larviciding the entire marsh area, and tank-mounted vehicles are restricted by their limited mobility. Dense marsh vegetation and adverse climatic conditions make applications of larvicides even more difficult.

In recent years, technology for ground application of ultra-low volume (ULV) adulticides has focused on development of operational avenues for applying larvicides, especially for *Aedes aegypti* (Linn.) control (Pant et al. 1973, Gratz 1991). This technology is economical and more convenient than conventional technology because it allows application of undiluted products or products with reduced dilution and eliminates the need for high-volume formulations with their increased payloads and larger tanks. However, ULV droplet behavior is complex and must be studied as it is influenced by wind direction and velocity and larval habitat characteristics (Pant et al. 1973) as well as by canopy and droplet size, among other factors (Sandoski et al. 1986). Thus, efficiency must be determined by assessing swath width and droplet size as they are determined by dilution and characteristics of the application device.

In view of the difficulties in controlling larval populations of *Ae. caspius* generated by extensive flooding, it was decided to test drift patterns and deposition of larvicidal products to determine whether a truck-mounted ULV aerosol machine could be used against this species' larvae in flooded areas.

Larvicides used in this area of Spain since 1983 have been temephos (Abate® 50E) and *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) (Bactimos®). Although *B.t.i.* is more environmentally desirable, its use has caused some operational troubles because of irregular dispersion when applied by knapsack sprayer. These problems with *B.t.i.* have led to greater use of temephos. The objectives of this study were to test spray drift pattern and maximum distance achieved by both temephos and *B.t.i.* using ULV application. These data will help in evaluating whether ULV ground application equipment could later be used on local *Ae. caspius* breeding sites.

The distance and spatial deposition pattern of both insecticides was determined using an autogenous insecticide-susceptible strain of *Culex pipiens* Linn. that had been maintained in our insectary since August 1993. This species was used instead of *Ae. caspius* because it was easier to handle and a homogeneous population was available. Abate 50E is a 50% wt/vol temephos liquid formulation, supplied by American Cyanamid, Bactimos, 1,200 ITU/mg was supplied by Novo Nordisk (Denmark). Temephos was mixed 50:50 with a common agricultural oil (Oleanol®, Schering); Bactimos was diluted in water 40:60. Two previous tests were carried out (data not presented) to initially evaluate the spray drift for each product as well as the best dilution rates. Other more concentrated Bactimos mixtures were found to clog the insecticide lines of the sprayer used in these tests.

Test plots were set up with 13 rows of 3 plastic cups (medical urine sampling pots, Eurotubo, Spain), containing 100 ml of distilled water, in an area which simulated an *Ae. caspius* breeding site. Although distilled water could be suspected of causing osmotic stress for the larvae, it was used in these tests, as it has been for years, as the standard larval medium in the insectary. Cups were 7.5 cm high and 5.7 cm diam at the opening, with a surface area of 25.5 cm^2. The 3 columns were located perpendicularly to the path of the truck, forming a grid consisting of 13 sets of 3 test points at 13 different distances from the point of discharge.

Information obtained in previous assays led us to change some distances in the actual tests for adaptation to different drift behaviors. The 3 tests presented were conducted in February 1994 with the temephos mixture applied at 300 ml/min and 350 ml/min and the *B.t.i.* at 400 ml/min. Rows of cups were spaced 4, 5, and 2 m apart. The ranges of distances from the application line were from 4 to 60 m, from 10 to 70 m, and from 6 to 30 m for each test. Insecticides were applied using a Curtis Maxi-Pro® ULV, mounted on a truck driven at 9 kph. In order to provide uniform coverage, the spraying system was turned on 10 m before the grid, and shut off at an equal distance the other side. The line pressures were 0.42 kg/cm^2 for the temephos mixture and 0.21 kg/cm^2 for the *B.t.i.* Following manufacturer's specifications, predicted droplet size (mass median diameter) was 30 μm for the *B.t.i.* formulation and 16 μm for temephos.

As measured by a portable anemometer (Anemo Deuta, Germany), tests were carried out at zero wind speed. After treatment, cups were tightly closed and brought to the laboratory, where approximatively 20 4th-instar *Cx. pipiens* larvae were added to each cup. Four additional cups per test containing distilled water were used in the laboratory as control replicates. Larvae were reared in the insectary at 26°C, and adequate food (triturated rat biscuit mixed with microencapsulated crustacean food, plus 1% choline chloride) was provided. The number of dead larvae was counted 24 h later, and pupae were discarded. Results expressed as the arithmetic mean of the 3 cups at each distance are shown in Fig. 1 together with the standard deviation for the mean.

The temephos mixture at a dose of 300 ml/min gave uneven results. Between 3 and 40 m, mortality ranged from 100 to 82.5% but fell off beyond that distance. This irregularity was apparently caused by a lower coverage of one of the rows. At a dose of 350 ml/min, the temephos mixture led to 100% mortality at nearly every distance up to 50 m. Beyond that, mortality dropped drastically. In one of 3 cups located at 30 m, mortality was much lower than in the other 2. The *B.t.i.* mixture gave good results from 6 to 12 m, with a minimum mean mortality of 91.6%; beyond this distance efficacy dropped to values ranging from 3 to 18% between 24 and 30 m.

Only one larva out of 240 died in the 3 control sets (0.4%). Therefore, data were not corrected for natural mortality. If the entire test area up to the furthest cups could be considered as the area treated, the temephos test resulted in a dose of 70 and 85 g AI/ha; the *B.t.i.* treatment delivered 1.07 liters/ha.

The *B.t.i.* formulation used in this study did not appear to be suitable for application with the Curtis Maxi-Pro because of the formation of coagulated masses in the insecticide lines. A minimum *B.t.i.* dose of 1.5 liters/ha is necessary to achieve acceptable control of *Ae. caspius* larvae (Eritja and Aranda, unpublished data). With the *B.t.i.* formulation used in this study, the output rate would have to be over 600 ml/min, a volume that cannot be produced by the Maxi-Pro generator. Another device, such as a vortical-type nozzle, could probably be used instead of the airshear nozzle to avoid these problems (World Health Organization 1990).

Tidwell et al. (1994) reported ULV-applied mixtures of permethrin and undiluted *B.t.i.* at *B.t.i.* dosages of 788, 1,577, and 2,586 ml/ha were successful in controlling both indoor and outdoor *Ae. aegypti* larvae in complex urban environments. These authors obtained a swath of more than 90 m with a Scorpion™ 20 ULV forced-air generator (Berry Corp., Lexington, KY). Output rates higher than 1,500 ml/min at a 4-kph speed would be required to achieve an actual dosage of 2,586 ml/ha. Thus, the Curtis Maxi-Pro, which is not a forced-air generator and relies on ambient air currents to achieve drift, has limitations in delivering the *B.t.i.* formulation used in the present study, as its output rate does not exceed 500 ml/min, and the maximum effective swath did not exceed 30 m.

In the circumstances encountered in our study, the selection of temephos as the product of choice is indicated. This product has many advantages over *B.t.i.*: a relatively low dosage rate of 150 to 200 ml/ha, physical homogeneity,

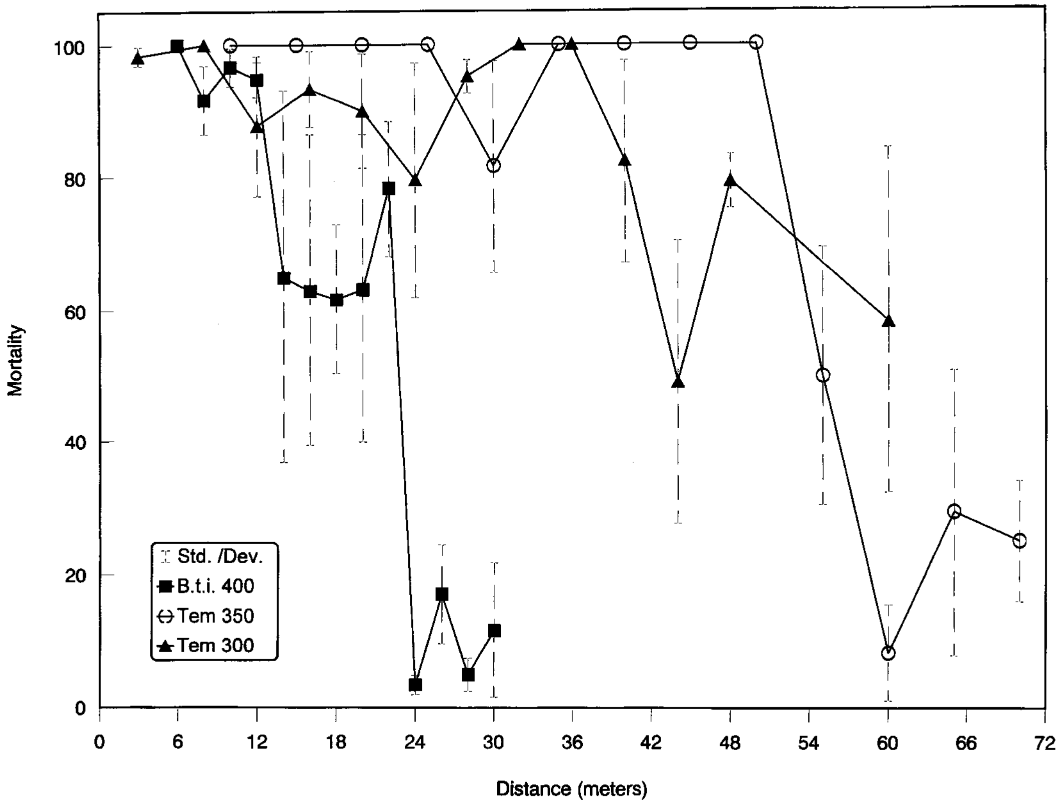

Fig. 1. Percent mortalities of the 3 tests. Each symbol represents the mean for the 3 cups at each distance. The SD of the mean is represented by a dashed line.

good mixing with oils, and a better dispersal in water once applied. However, it has a lower LD_{50} than *B.t.i.*, thus increasing toxicity risk to nontarget species.

Tests yielded evidence of effective deposition of temephos on water surfaces and drift up to 50 m, indicating the possibility of use for operational larviciding purposes. In the event the results of the 350 ml/min temephos test can be duplicated under actual field conditions, several economies would result. Ultra-low volume technology results in a lower product payload. In addition, the crew required is reduced by a ratio of 3:1, as one technician can apply larvicide in a 50-m swath instead of the 3-person team usually required to treat that area with knapsack sprayers. Finally, treatment time is reduced more than 90% and time spent for people and material setup before and after applications is greatly reduced, which enhances the mobility of the application operation. These combined benefits could confer a greater than 98% manpower/time savings for a ULV operation.

We conclude that the spray drift obtained in the test with temephos provided drift and deposition patterns that might be appropriate for use against *Ae. caspius*. However, the noted lack of uniform coverage, which is very likely to occur under operational conditions, remains to be studied. This phenomena will be tested by further experimentation under field conditions, targeting *Ae. caspius* in flooded areas with dense marsh vegetation.

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