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Field efficacy of Bacillus thuringiensis var. israelensis[1]

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Field experiments with *Bacillus thuringiensis* var. *israelensis* were carried out in the Swiss canton of Wallis to control mosquito larvae belonging to the species *Aedes vexans* MEIGEN, *A. cataphylla* DYAR and *A. communis* DEGEER. The main experiment, conducted in a wildlife reservation, resulted in a total mortality of the mosquito larvae within 1,5 day. *B.t.* var. *israelensis* was highly active against all three mosquito species; younger larval stages were more susceptible. No adverse effect was observed on other water-inhabiting invertebrates. Since the experiments were successful under extreme conditions such as low water temperature and heavy vegetation, it is expected that application of *B.t.* var. *israelensis* will also be promising in more moderate and tropical climates.

In some parts of the canton of Wallis, situated in the middle of the Swiss Alps, a major mosquito problem exists. Relatively large mosquito populations develop as a result of the annual spring thaw. Snowmelt in alpine regions causes a rise in the level of ground water, which leads to the formation of temporary ponds and swamps during May an June along the Rhone valley. Mosquitoes developing in these flooded areas are numerous enough to present a significant annoyance to the inhabitants of the region. The prevalent species in the plain of the Rhone is *Aedes vexans* MEIGEN, whereas in the mountainous regions *A. cataphylla* DYAR and *A. communis* DEGEER predominate. Because of the particular hydrodynamic situation, only one generation develops per year. Adults live for up to three months.

Large breeding sites of *A. vexans* are located in swampy grounds in and around the wildlife reservation of Pouta Fontana situated about 10 km east of Sion, producing an extremely high mosquito population in Pramagnon, a village adjacent to the reservation.

Mosquito populations of the reservation were controlled in 1978 by the use of Dimilin®. In 1979 enough *Bacillus thuringiensis* var. *israelensis* became available to carry out field tests. This new microorganism was isolated by GOLDBERG & MARGALIT (1977) and classified as a new serotype 14 by DE BARJAC (1978a). Several laboratory studies have shown that this organism is active against larvae of mosquito species belonging to the genera *Aedes, Anopheles* and *Culex* (DE BARJAC, 1978b, 1978c; DE BARJAC & COZ, 1979) as well as against blackflies (WEISER & VANKOVA, 1978). The present study was undertaken to evaluate field efficacy of *B. thuringiensis* var. *israelensis*. The experiments were carried out in a region were the mosquito populations have been carefully studied over the last few years by RABOUD (1980). Field assay methods were used without modifications from his studies.

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MATERIALS AND METHODS

Microorganism

B.t. var. *israelensis* (serotype 14). This strain is designated in the collection of the Department of Microbiology of the Swiss Federal Institute of Technology, CH-8092 Zürich, as LBG B 4439.

Cultivation

Media used for the cultivation of other varieties of *B. thuringiensis* were also suitable for growth, sporulation and crystal formation of the variety *israelensis*. Material for the field experiments was cultured in the following medium (g/l): soya bean meal (fatty) = 35; corn starch = 12.15; malt extract = 2.0; $K_2HPO_4.2H_2O$ = 1.3; $MgSO_4.7H_2O$ = 0.2; $CaCl_2.2H_2O$ = 0.08; $MnSO_4.7H_2O$ = 0.08; the pH was adjusted to 7.2.

For the preliminary field experiments (ponds A, B and C) the organism was grown in 500 ml Erlenmeyer flasks with 100 ml medium on a rotary shaker. Incubation temperature was held at 30 °C. For the experiment in the wildlife reservation a 300 l fermenter was used to prepare the material.

Processing and application of the sporulated material

The first batch prepared in Erlenmeyer flasks was centrifuged and freeze-dried. The freeze-dried powder was resuspended in H_2O before the field application. A slight ultrasonic treatment assured a homogenous distribution of the material. The freeze-dried preparation was only used in the first preliminary field trial; for the other field trials the whole fermentation broth was applied without further processing. If storage was necessary prior to use, the broth was frozen at -20 °C.

Determination of activity

Larvae of *Aedes aegypti* L. were used to determine the activity of the *B.t.* var. *israelensis* preparations in the laboratory prior to the field experiments. Bioassays were conducted with 2nd instar larvae in 250 ml serum bottles containing 200 ml of tap water at a temperature of 23 °C. Fifty larvae were transferred to each bottle. As a standard value of activity the active material was compared with the minimal lethal dose (MLD) obtained within 6 h (i.e. 100% mortality in 6 h). The MLD averaged 7000 spores per ml water. The biological activity of the fermentation broths varied by a factor of 8 according to the preparation method.

Description of the field plots

The first preliminary trial was conducted in two small shallow ponds (A and B) near Nax, a village situated near Sion at an altitude of 1200 m. Pond A

had a surface of 32 m^2 with an average depth of 0.1 m. The ground consisted of silty and rocky material. Pond B had a surface of 20 m^2 and an average depth of 0.3 m. The bottom was covered by a deep layer of organic litter. The population of the mosquito larvae was calculated at 100–150 per liter in each pond, and was composed of *A. cataphylla, A. communis* and *A. pullatus* COQUILLETT, with the latter only being present as a small percentage of the total. Larvae were in their 1st to 4th stage of development. Water temperature during the 3 day period of the experiment was 5–8 °C in the morning, reaching a maximum of 18–24 °C in the afternoon.

The second preliminary trial was carried out in the region of Mayen de la Dzour, near Sion, at an altitude of 1500 m. The pond (C) had a surface of 1060 m^2 and an average depth of 0.3 m, and was situated in a coniferous forest. The pond bottom consisted of peat. Water temperature was 10 °C at the time of treatment, and fluctuated between 8 °C and 15 °C during the 2-day period of the experiment. The mixed population of *A. cataphylla* and *A. communis* amounted to more than 200 larvae per liter in the 1st to 4th instar.

The main field experiment in the wildlife reservation of Pouta Fontana (D) near the village of Pramagnon covered an area of 7500 m^2. The average depth was estimated at 0.3 m, varying between 0.1 m and 0.6 m. The larval population of *A. vexans* averaged 50–100 per liter. Water temperature was fairly constant at about 15 °C. The treatment was impeded by underbrush which covered the whole area and which was difficult to penetrate.

The check consisted of samples of 10 liters of water taken from the infested ponds or from the reservation just before treatment. As no significant mortality of *Aedes* occurred (fig. 3) it was assumed that the larval and pupal mortality in the treated ponds was entirely caused by *B.t.* var. *israelensis.*

Method of application

Aliquots of the resuspended freeze-dried material or the whole fermented broth were diluted with water to give a predetermined final concentration for the plots to be treated. The suspensions were spread on the surface of the water with portable motorized sprayers. With one tank load (12 l), 500 m^2 of water surface could be treated.

Time of application and doses

The first preliminary trials (ponds A and B) were carried out on May 9, 1979 using the freeze-dried preparation. The MLD determined in the laboratory was adjusted for the volume of the pond. It was then increased by a factor of 4 because the experiment was conducted under field conditions, against different target species, and against 3rd and 4th larval instars instead of 2nd. Therefore, pond B received 3 times as much active ingredient as pond A when the dose was related to surface area.

The second preliminary trial (pond C) was carried out on May 22, 1979 using the fermentation broth. Because of the very promising results obtained in the first trials, the MLD was increased only by a factor of 2, so that the pond received half the dose of *B.t.* var. *israelensis* applied to pond B.

The wildlife reservation of Pouta Fontana (D) was treated on June 6, 1979 using the fermentation broth. Because of the dense vegetation in the areas infested by *A. vexans* and of the great variation of water depth, it was decided to increase the MLD by a factor of 2.5 as compared with that applied to pond B.

Determination of the mortality in the field

The field efficacy of *B.t.* var. *israelensis* was determined by the larval mortality measured by two different methods.

The first one consisted of sampling living larvae in the treated plots at the time of treatment and at intervals of 6, 12, 24, 36 and 48 h post treatment. Larvae were placed in ethanol (70%) and brought to the laboratory for determination of species and instar. The second method consisted of collecting randomly 50 4th stage larvae 2 h after treatment and placing them in floating units (RABOUD, 1980) in treated areas as shown in fig. 1. The same amount of 4th stage larvae was collected immediately before treatment and confined to the same floating units placed in untreated water. Mortality in the floating units was also measured at the time intervals mentioned above.

RESULTS AND DISCUSSION

In pond A (Nax) maximum mortality reached 70% after 3 days, whereas in pond B (Nax) 100% mortality was observed 2 days after treatment (fig. 2, A and B).

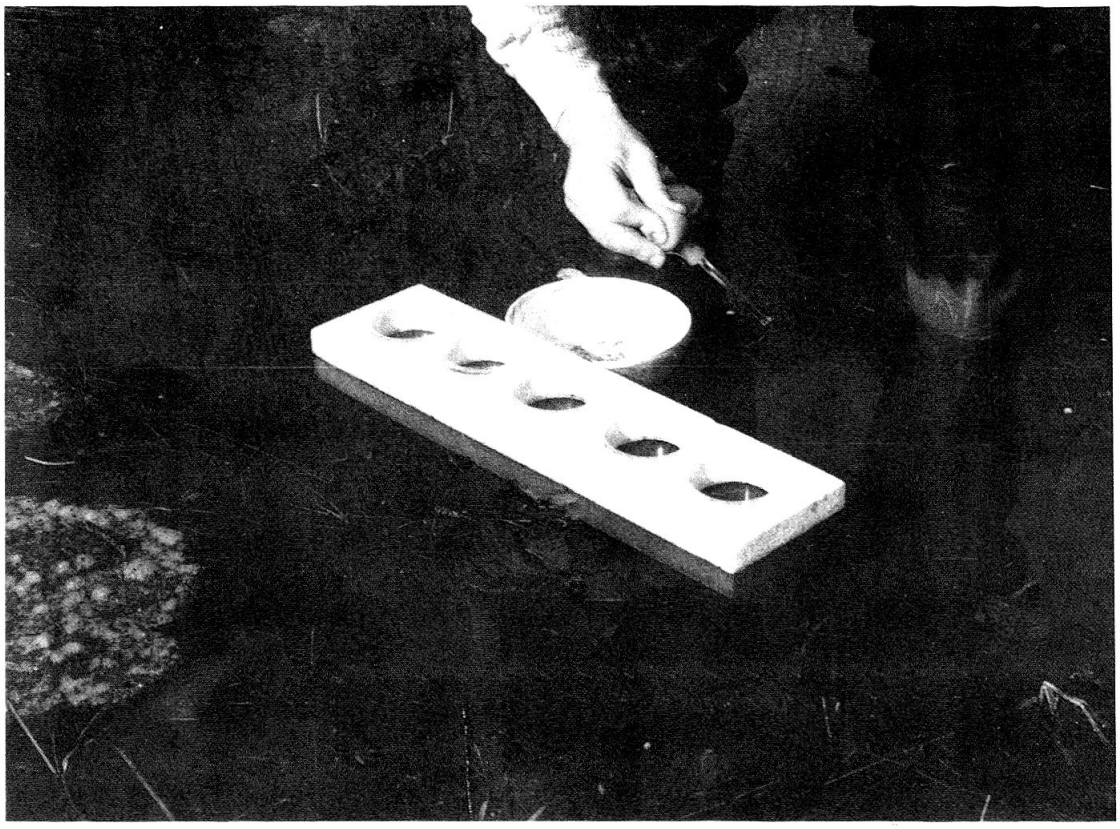

Fig. 1: View of the arrangement of the floating unit supporting five plastic cups containing 25 larvae each. Larval escape through the bottom is prevented by a gauze (200 mesh/cm^2).

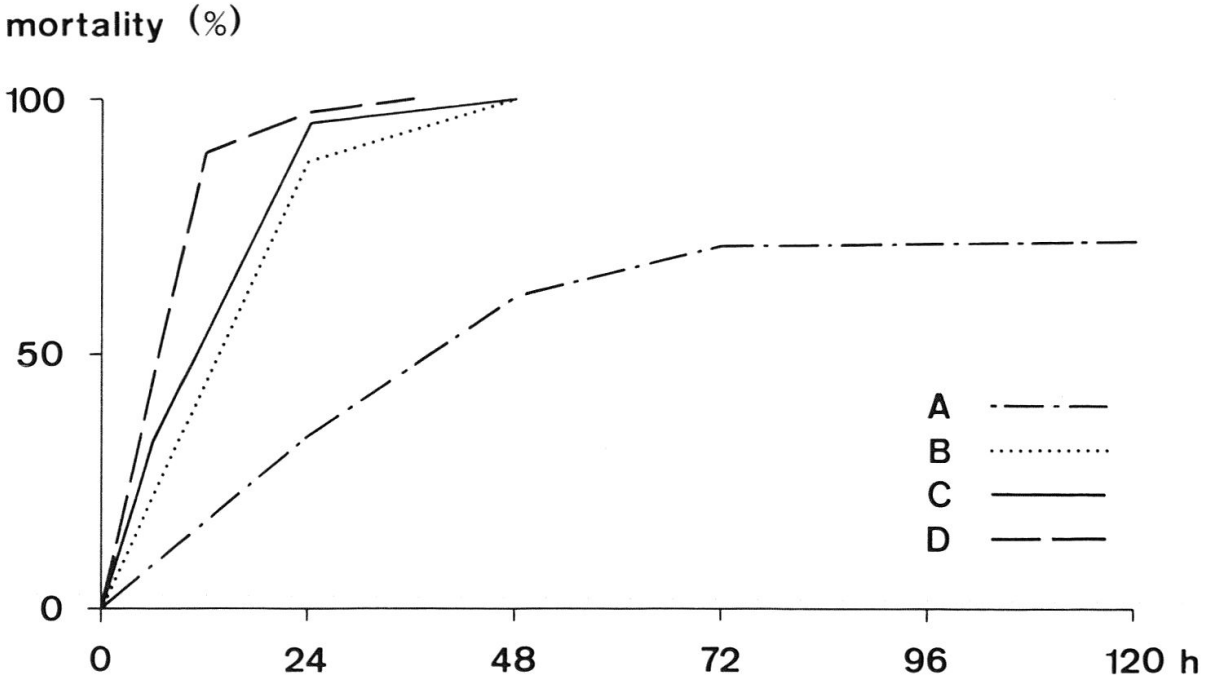

Fig. 2: Mortality of *Aedes* sp. after treatment with *Bacillus thuringiensis* var. *israelensis*. Ponds A and B with 1st to 4th instar larvae of *A. pullatus, A. communis* and *A. cataphylla;* pond C with 4th instar larvae of *A. communis;* wildlife reservation D with 4th instar larvae of *A. vexans*.

Excellent results were also obtained in pond C (Mayen de la Dzour), where mortality of *Aedes* amounted to 100% 2 days after treatment (fig. 2, C) in spite of the reduced dose of *B.t.* var. *israelensis*. In the wildlife reservation D (Pouta Fontana) larval mortality reached 100% 36 hours after treatment (fig. 2, D), but the dose of *B.t.* var. *israelensis* used was 2.5 times higher than in pond B. The 4 species of *Aedes* occurring in the treated areas showed a similar susceptibility to *B.t.* var. *israelensis*.

The results obtained in pond B show, as compared with those in pond A, that the volume of the water has little or no effect on the *Aedes* mortality and that the surface area alone should be considered for the determination of the dose. In fact, the dose used in the treatment of pond B was the same as in pond A if related to the volume of the water, but 3 times higher when related to surface area. The corresponding *Aedes* mortality is clearly different as shown in fig. 2. In ponds B and C as well as in the wildlife reservation (D) the progression of *Aedes* mortality was very similar; these 3 areas had the same average depth of the water.

The results obtained in the wildlife reservation (D), where the dose was 10 times the MLD for *A. aegypti* or 2.5 times the dose used in pond B, indicate that little additional effect is obtained through the increase of the dose of *B.t.* var. *israelensis*. In fact, with the assumption that the *Aedes* species in the treated areas have a similar susceptibility to *B.t.* var. *israelensis,* 100% mortality occurred 48 h after treatment in C, only 12 h later than in D. The more rapid development of mortality in D may have been influenced by the higher temperature of the water rather than by the higher dose of *B.t.* var. *israelensis*.

The different larval stages of *Aedes* have a different level of susceptibility to *B.t.* var. *israelensis,* younger stages being more susceptible than older (fig. 3), as shown by comparing age distribution in check and treated samples at intervals

following treatment. Mortality of the 2nd and 3rd instar larvae of *A. communis* and of *A. vexans* was 100% within 24 hours after treatment. The larvae of *A. cataphylla* seem to be slightly more tolerant to *B.t.* var. *israelensis* than those of *A. communis* (fig. 3, mortality 24 h after treatment).

The pathogen used is very effective for the control of *Aedes* larvae and pupae under field conditions. Although the experiments were conducted under unfavorable conditions (low water temperature, dense vegetation), the results obtained were satisfactory. It may be speculated that the pathogen can be more efficiently applied in zones with warmer climate.

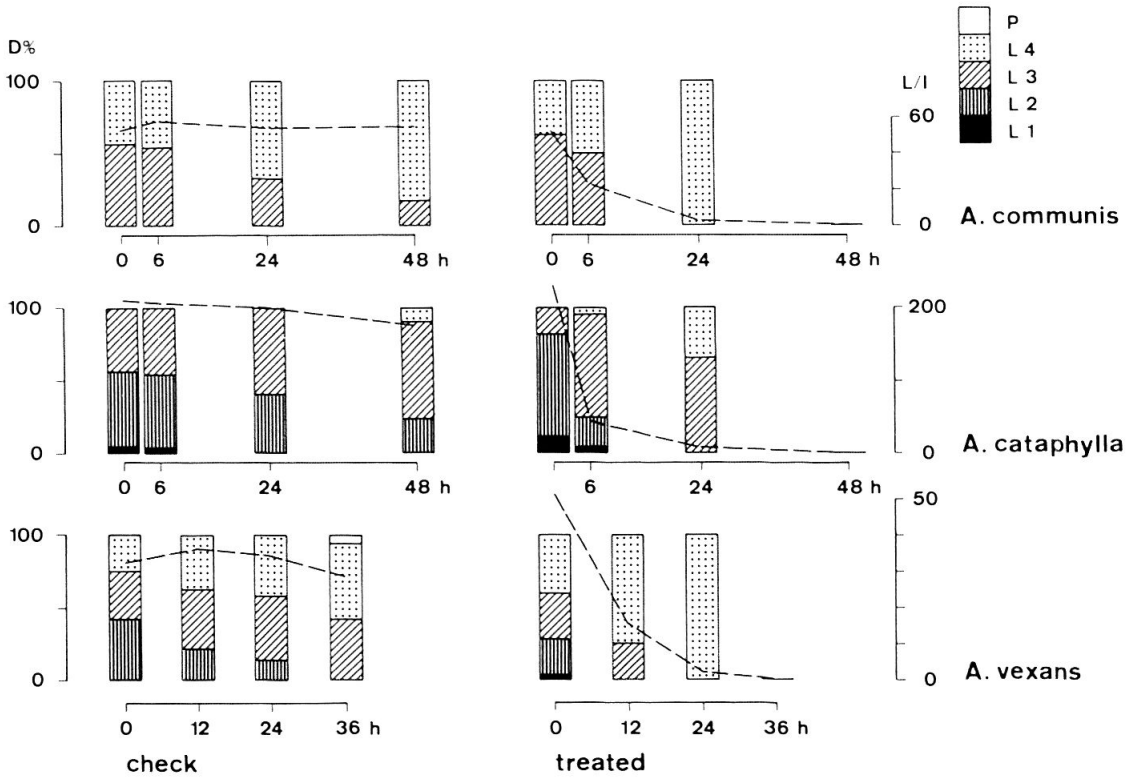

Fig. 3: Susceptibility of larvae and pupae of *Aedes communis* and *A. cataphylla* to *Bacillus thuringiensis* var. *israelensis* observed in the treated pond at Mayen de la Dzour and of *A. vexans* to *B.t.* var. *israelensis* in the wildlife reservation of Pouta Fontana (D%, distribution of the different stages in %; L/l, number of larvae per liter, broken line; L_1 to L_4, 1st to 4th larval stages; P, pupae).

During summer 1979 mosquitoes were practically absent from the region of Mayen de la Dzour, which is a recreation area. In the wildlife reservation of Pouta Fontana the treatment with *B.t.* var. *israelensis* did not include the entire reservation area and could not eliminate the mosquito plague; however, a considerable reduction of the adult population was obtained in the area. The dose of *B.t.* var. *israelensis* applied in the reservation was higher than necessary and could be reduced to the same dose applied in pond B. Thus, efficient control of *Aedes* larvae should be feasible with 10 to 20 liters of fermented broth per ha and the number of spores in the reservation water shortly after treatment would range between 5000 and 10 000/ml.

Direct application of the fermented broth is of course not the ideal method. It was used to assure that no loss of the small inclusion bodies occurred through centrifugation or other processing of the sporulated culture medium. An ideal

formulation would be a granulate which is dissolved as soon as it comes in contact with the water surface.

No undesirable side effects were noticed on other organisms. For example, the development of ostracods, stoneflies, dytiscides (larvae and adults), dragonflies and tadpoles continued normally.

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