THE EFFICACY OF BACILLUS THURINGIENSIS VAR. ISRAELIENSIS AGAINST LARVAE OF THE BLACKFLY ODAGMIA ORNATA (MEIG.) (SIMULIIDAE) AT LOW TEMPERATURES

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Abstract. The effect of the suspension of Bacillus thuringiensis var. israelensis spores on larvae of the blackfly Odagmia ornata was studied in the laboratory and under field conditions of a natural biotope in southern Bohemia. The preparation Moskvar was used and its effect was tested in laboratory at temperatures 0.1—2.9 °C and 17—19 °C. Although O. ornata larvae were able to filter feed on a lethal dose of the preparation even at a lower temperature than 2.9 °C, no marked manifestation of mortality was observed at low temperatures in comparison with a control sample.

Bacillus thuringiensis serovar H-14, also designated as var. israelensis is one of the most promising biological agents in the control of mosquitoes and blackflies today. Its wide application is due to the efforts of the research workers of the Special programme for research and training in tropical diseases UNDP (World Bank) WHO. Commercially produced formulations and the experience with them are mostly associated with the tropics where B. thuringiensis H-14 is sometimes the only control agent against which the treated larvae have no resistance. This applies primarily to larvae of Simulium damnosum complex in Africa, against which bacterial suspensions are used to a great extent (more than 600,000 litres of preparation annually). S. damnosum is a vector of onchocerciasis in Africa is quite an exceptional case. Elsewhere larger groups of different blackfly species occur and their effect as pests annoying man or animals predominates over the accidental transmission of filarial worm diseases. In Europe noted are annual losses in pastured cattle in the environs of Golubac where blackflies emerge in great abundance. Their larvae live attached to the rocky bed of the Danube river at the Iron Gate. Likewise noted are the outbreaks of blackflies in boreal regions of Europe and Asia where especially abundant broods emerge in the spring and interfere with every human activity in the landscape. A typical example is the Angara river basin in Siberia, where aerial control measures against enormous masses of blackflies attacking workers at the building site of a dam on the Angara river had to be carried out several times daily. The outbreaks in the temperate climatic zone are unusual in that the blackfly populations are relatively not migrating, but continuously building up, their larvae sometimes covering the entire stream bed and their eggs in clusters weighing sometimes over 1 kg.

Unlike the warm and swiftly flowing rivers in Central Africa with great variability of water level during the rainy and dry seasons, the blackfly occurrence in boreal regions depends on a lesser fluctuation of stream levels, a lesser water flow and mainly on lower water temperatures. The larval populations here mostly survive under a thick layer of ice in the winter at water temperatures near to 0 °C. Nevertheless, control measures against these populations should still be taken, at best before their pupation in April or at the beginning of May. The subject of the present paper was the response of the blackfly larvae to B. thuringiensis H-14 under such conditions.
The effect of *B. thuringiensis* H-14 on the blackfly larvae was first reported by Undeen and Nagel (1978), and Weiser and Vaňkóvá (1978), while shortly before them Lacey and Mulla (1977) tested the efficacy of 13 strains hitherto known to be effective against caterpillars and achieved 64–88% blackfly mortality in laboratory tests after 24-hr bacterial exposure at a concentration of 10 ppm (10 mg/l). Later studies (Guillet and Escaaffre 1979, Lacey and Federici 1979, Molloy and Jannack 1981 or Undeen et al. 1981) were carried out by means of different laboratory testing facilities with water temperature of 10°C, the tests in the tropics at temperatures above 20°C. Because the said tests mostly solved the problems of local application, no attention was paid to the relationship of the water temperature and the bacterium's effect on the blackfly larvae. In mosquitoes this relationship was already studied. Wraith et al. (1981) compared the effect of *B. thuringiensis* H-14 on mosquito larvae at 13°C and 21°C and ascertained the necessity of higher LD₅₀ at a lower temperature in older larvae of *Aedes stimulans*.

**MATERIALS AND METHODS**

A sample of the Moskitor preparation (produced by the agricultural cooperative Stoklovice) containing 1.500 million biological units in 1 ml was used in the tests. The efficacy test was conducted according to the WHO method (Rashikesh and Quelemie 1981) using the standard IPS 80. The field tests were conducted on a small stream near the village Čerňo Dub (district of České Budějovice, southern Bohemia, CSSR). The stream is cannibalized, with a flat, stone-covered bed. It is flanked by a dense vegetation hanging down and partly submerged in the water. The width of the bed is 1.5 m in diameter. The water depth is 20 cm on the average, speed of water flow 65 cm/sec., the average flow 200 l/sec.

<table>
<thead>
<tr>
<th>Site</th>
<th>No. of larvae/dm² before B. t. t. application 7. iv. 1983</th>
<th>No. of larvae/dm² 48 hours after B. t. t. application 9. iv. 1983</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control — 10 m</td>
<td>142</td>
<td>108</td>
</tr>
<tr>
<td>0 m</td>
<td>260</td>
<td>103</td>
</tr>
<tr>
<td>50 m</td>
<td>166</td>
<td>185</td>
</tr>
<tr>
<td>100 m</td>
<td>144</td>
<td>128</td>
</tr>
<tr>
<td>200 m</td>
<td>280</td>
<td>134</td>
</tr>
<tr>
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</tr>
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<td>400 m</td>
<td>185</td>
<td>201</td>
</tr>
<tr>
<td>Air temperature/Water temperature</td>
<td>3.6/1.1°C</td>
<td>0.2/0.2°C</td>
</tr>
</tbody>
</table>

The following sites were selected for controls: — 10 m (upstream from the site of application), 0 m (site of application), 50 m, 100 m, 200 m, 300 m and 400 m downstream from the site of application. Doses of 2 × 10⁹ spores ml⁻¹ were used for treatment.

The water temperature in the stream varied between +0.1°C and +0.2°C at air temperatures from 3.6°C to —0.2°C. At the given sites grass samples with attached blackflies were collected, corresponding to 5–10 dm² of substrate area from each site and brought to the laboratory. Here the blackfly larvae were counted, the substrate area was measured and converted to number of larvae per 1 dm² (Table 1). The samples were collected 30 min. before application and 48 hrs after the application of Moskitor. On the third day after application the stream became icebound throughout its length. At the control site and at the sites 0, 50, 200 and 400 m samples of larvae were successively collected and brought to the laboratory where they were kept in groups per 100 specimens each in 200 cm³ of stream water at the temperature of 17–19°C (Table 3). The siring and water movement were provided by aquarium diaphragm pump. During the trial the larvae were not fed.

### Table 2. Mortality of larvae in laboratory test

<table>
<thead>
<tr>
<th>Date</th>
<th>16/3</th>
<th>17/3</th>
<th>18/3</th>
<th>19/3</th>
<th>20/3</th>
<th>21/3</th>
<th>22/3</th>
<th>23/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period after (in hours) B. t. t. application</td>
<td>24</td>
<td>48</td>
<td>72</td>
<td>96</td>
<td>120</td>
<td>144</td>
<td>168</td>
<td>192</td>
</tr>
</tbody>
</table>

### Table 1. Number of larvae found in the field one hour before and 48 hours after application of B. t. var. g. larvae per 1 dm² of substrate

<table>
<thead>
<tr>
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<th>No. of larvae/dm² before B. t. t. application 7. iv. 1983</th>
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</table>

Each sample tested contained 100 larvae, the number of dead specimens express simultaneously the percentage of mortality. The first line indicates the mortality of larvae and the number of pupae (P) found on different days. The second line shows total mortality of larvae and total numbers of pupae (P). The full line marks the boundary between temperatures.
The laboratory tests were conducted about one month later, using larvae of the same population from the same locality, but taken from the sites which had not been treated in the first trial. The larvae were kept in air-dried vessels of 200 cm³ volume of stream water at temperatures of 0.1 to 2.9°C or at 17–19°C (Fig. 1). Before the beginning of the test the larvae were kept in the vessel for 24 hrs and the larvae damaged during transport were discarded. Table 2 records the results of the test. Fifteen minutes after application of the B. thuringiensis suspension the water in all vessels was changed three times with the exception of two vessels (B1 and B2), in which the larvae were kept in the B. thuringiensis suspension. All blackfly larvae, both in the field and laboratory tests, belonged to the species Odonata ornata Mg. The smallest larvae in the laboratory tests were longer than 3 mm, about 25% accounting for larvae of the last instar.

Table 3 Mortality of larvae transferred from field to laboratory after application of B. thuringiensis var. israelensis. Each sample contained 100 larvae of O. ornata.

<table>
<thead>
<tr>
<th>Group</th>
<th>Larvae collected at different sites 7 h, 1/2 hour after B. thuringiensis application</th>
<th>Larvae collected at different sites 9 h, 48 hours after B. thuringiensis application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sites</td>
<td>9.8. 10³</td>
</tr>
<tr>
<td></td>
<td>0 m</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>50 m</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>200 m</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>400 m</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>−10°C</td>
<td>1</td>
</tr>
</tbody>
</table>

**Fig. 1. Diagram of the arrangement of laboratory test. B1-B6—samples treated by B. thuringiensis var. israelensis, C1-C4—control samples. The full line marks the boundary between temperatures. Each sample contained 100 larvae.**

**RESULTS**

The mortality of larvae transferred to the laboratory from the field 30 min. and 48 hrs after application of Moskitor is shown in Table 3. The control of the treatment outcome in the field (Table 1) shows that nature marked changes took place in the disintegration of the population which could be due to the effect of the bacterium. Because of the frozen stream it was impossible to observe continually the changes in the fauna. About three weeks after the bacterial application a fishpond was being filled above the test site and the results of later comparisons did not reveal whether the changes in the number of larvae in the locality, after the ice had thawed, resulted from the hydrotechnical changes (distribution and changes of water flow, volume of water) or were due to the effect of B. thuringiensis var. israelensis.

Table 2 presents the outcome of larval mortality in the laboratory test (see diagram in Fig. 1). In all cases the larval mortality in samples treated with Moskitor was distinctly higher than that in the control samples, provided that the larvae were kept at the temperature of 18°C. Conversely, when the larvae were kept at a low temperature, the larval mortality in samples was insignificant. At the average temperature of 18°C a 100% mortality of larvae was manifest within 72 hrs at the latest, while at low temperatures the larvae survived as long as 12 days and their mortality in the treated sample differed from that in the control samples only insignificantly. In the samples where constant presence of B. thuringiensis was preserved, the effect of the preparation in warm water became manifest twice as fast as in cold water (Table 2, conf. samples B1 and B2).

**DISCUSSION**

The literature contains data recording that the effect of B. thuringiensis H-14 is reduced at lower temperatures of environment, often quite distinctly (Lacey et al. 1978, Lacey and Federici 1979, Molloy et al. 1981), but the temperatures observed by these authors were relatively higher—above 10°C. As Mansingh and Steele (1973) ascertained, the physiological processes in blackfly larvae somewhat change at temperatures lower than 4°C. Our investigations showed considerable differences in the larval mortality during various combinations of basic conditions. Marked differences were noted between the mortality of larvae in nature and that in samples transferred to the laboratory. Therefore, we conducted additional laboratory tests with two values of temperature to which the larvae were exposed. The majority of published studies were carried out by means of complex laboratory facilities and were directed at conditions in the tropics, essentially different from the conditions under which the blackflies occur in boreal regions. In the streams of the temperate zone the effects of B. thuringiensis were tested by Molloy and Jamnback (1981) and by Undeen and Colbo (1980). The latter authors conducted one test in the field at the water temperature of 3°C, but they did not report the mortality of larvae in the field. The low mortality of larvae collected 48 hrs after treatment in the gradually frozen stream is conspicuous. The data on the population in the stream were obtained with difficulty due to the fact that artificial substrates used for standard counting of larvae, such as PVC strips floating in the stream, were not suitable when the water level gradually froze over, because under such conditions the larvae tend to migrate towards the bottom. A considerable mortality of larvae collected in the treated sites, when on top and at the beginning of the cold samples brought to the laboratory, shows that a large percentage of larvae is able to filter feed on lethal dose of the preparation even at a low temperature, and even in
competition with saproplex which was released in the stream when the fishesponds situated upstream was being emptied. It seems likely that the stirred up saproplex and loam particles forced the larvae to stop feeding temporarily, as is also reported elsewhere (Gaugler and Mollov 1980).

The laboratory experiment was directed at a detailed analysis of the effect of Bt. var. tarentolae against blackfly larvae at low temperatures. Denaturation could not be involved, as the Bt. var. tarentolae suspension retains its activity about half a year, provided that it is stored at the temperature of 2°C. This means that the reduced susceptibility of blackfly larvae is not caused by inactivity of bacteria. There are many other factors which play a part in the manifestation of larval intoxication and their mortality. It became evident that at a higher concentration of the preparation the manifestation of intoxication and mortality can be achieved in 100% of larvae even at a low temperature. When the doses were lower, the bacterial effect vanished very soon. A marked difference was always observed in the manifestation of intoxication and mortality of larvae, when the temperature of water containing larvae was increased. This factor indicates that the larvae at low temperature can not ingest the food from water to such an extent that enough active bacteria and crystals accumulate to kill them. The accumulated bacterial dose remained in the gut of larvae tested longer than 144 hrs. Due to the conditions of the experiment and to the high stress caused by the transfer of larvae from nature to the laboratory microaquarium and their longer maintenance in it, the manifestations of larval intoxication and mortality after 144 hrs both in the test and in the controls are strongly influenced by the stress and the results cannot be compared with certain confidence. No larva lived after 168 hrs one larva pupated and after 216 hrs another two larvae did so, so that larvae not damaged in the test were able to complete their development irrespective of the conditions of the test. In the remaining samples pupation took place only in the controls, after the larvae had been transferred to a warmer water. In natural locality the larvae pupated as early as the collecting day and pupation progressed relatively fast because the water temperature rose to 8°C. In contrast, the pupation of O. ornata is unusual at temperatures below 3°C and the occurrence of pupae in the tests may be connected with the delayed effect of toxin.

The stress conditions in the tests with non-colonized blackflies pose considerable problems in the evaluation of the test results. Nevertheless, the tests clearly show that the ingestion of a lethal dose of Bt. var. tarentolae does not represent the only prerequisite for intoxication and death of the host. In addition, also higher temperature is necessary, as it apparently triggers the host's digestive and metabolic activity and thereby creates conditions for the manifestation of intoxication. The relationship should be borne in mind, when the treatment of streams against blackflies is contemplated. It is still not clear, however, to what extent the response of blackfly larvae to thermal gradients is used by blackflies to avoid critical temperatures. It should be appreciated that in nature the life processes in the genus Prosimulium usually take place not only under cold conditions and that metabolism of the larva must be adapted to low temperatures, while Odagmia has no optimum of development in a warmer season and according to temperature it can accelerate its development from four months to less than one. The effect of the bacterium in a cold season is inhibited both by a lesser food intake as well as by lower intensity of digestion and higher temperature means of sedimentation, the interception of the bacterium by filtering rheobions and dilution in the water flow.

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**References**


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