Assessment of Rainwater-Mediated Dispersion of Field-Sprayed *Bacillus thuringiensis* in the Soil

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(Received February 1, 1991; Accepted May 17, 1991)

The dispersion of field-sprayed *Bacillus thuringiensis* in the soil was assessed under naturally and artificially irrigated conditions. In tests on two field plots (mulberry plantations), there was no translocation of sprayed *B. thuringiensis* into the soil down to a depth of 10 cm, under a continuously rainwater-irrigated condition of the summer rainy season for about one month. When *B. thuringiensis*-sprayed soils were artificially irrigated with water equivalent to a 450-mm rainfall, *B. thuringiensis* was detected in the soil down to a depth of 3–6 cm. In irrigation tests using soil columns, *B. thuringiensis* was not capable of passing through the column of volcanic ash soil, but a few bacteria were detected in the flow-through water from a column of alluvium sand. The results suggest that the major factor causing the decrease in the level of *B. thuringiensis* is not a physical dilution due to the rainwater-mediated dispersion of bacteria into the soil.

Key words: *Bacillus thuringiensis*, dispersion, soil, effect of rainfall

INTRODUCTION

It is widely accepted that the field-applied *Bacillus thuringiensis* usually fails to grow in soils and gradually disappears from environments (Sekijima et al., 1977; Mortta and Kusuno, 1977; Pruett et al., 1980; Petras and Casida, 1985). Earlier investigators have shown that the decrease in the level of *B. thuringiensis* can be ascribed to one or more of the following biotic and abiotic factors: the presence of competitive soil microorganisms (Akiba et al., 1977; West et al., 1984 a, b), the acidity of the soil (Akiba et al., 1979, 1980; West et al., 1985), and the failure of *B. thuringiensis* spores to germinate in the soil (Akiba et al., 1979; Akiba, 1986; West et al., 1984 c). However, there are some difficulties in identifying the key factor causing the decrease in *B. thuringiensis* levels in a given soil environment. In the present study, experiments were conducted to determine whether the rainfall and artificial irrigation affect the level of *B. thuringiensis* in natural soils.

MATERIALS AND METHODS

*B. thuringiensis*. A commercial formulation of *B. thuringiensis*, NNI CELLSTART (Nihon Nohyaku and Kyowa Hakko Kogyo, Tokyo), was used in this study. The formulation was based on the strain AF101 belonging to *B. thuringiensis* serovar sotto (H antigen 4a; 4b).
**B. thuringiensis viable cell count.** Soils and soil filtrates were decimally diluted with sterile distilled water, and the dilutions were plated on pepton-polymyxin B (PP) medium, pH 8.5 (Saleh et al., 1969) consisting of peptone (10 g), agar (15 g), NaCl (5 g), polymyxin B (5 μg), and distilled water (1,000 ml). After incubation at 30°C for 48 hr, the number of colonies referable to the strain AF101 were counted.

Identification of the AF101 colonies was based on the morphological characteristics (Sekijima et al., 1977). In some tests, the colonies were examined with a phase-contrast microscope for the formation of crystals.

**Test plots.** The four field plots (1 × 2 m) were set in mulberry plantations, all located in Saitama Prefecture: (1) plot A, mulberry field, Saitama-ken Sericultural Experiment Station Kōnan Branch, Oosato-gun; (2) plot B, a private mulberry plantation on the riverbed of Arakawa R., the city of Kumagaya; (3) plot C, mulberry field, Saitama-ken Sericultural Experiment Station, the city of Kumagaya; (4) plot D, mulberry nursery garden, Saitama-ken Sericultural Experiment Station, the city of Kumagaya. The soil types were as follows: plot A, alluvium volcanic ash soil clay loam; plot B, alluvium sand; plot C, alluvium layer loam; plot D, sand.

**Spraying of B. thuringiensis and soil sampling.** A 1:1,000 dilution (10^8–10^9 spores/ml) of the formulation was prepared in distilled water. The soil surface (2 m²) was contaminated with bacteria by spraying 2,000 ml of the above dilution with a manual sprayer. In the bacteria-contaminated plot sampling pits were made to collect soils at depths of 0-1, 9-10, 19-20, and 29-30 cm for the plots A and B, and 0-0.5, 2.5-3, 5.5-6, and 8.5-9 cm for the plots C and D. The sampling was done weekly for 3-5 weeks and the sampling sites were shifted within the plot.

**Preparation of soil column and sampling of soil filtrates.** The soil column was prepared in a cylindrical glass funnel (17 cm in dia., 15 cm depth) with plastic net and cotton gauze at the bottom (Fig. 1). The soils used were collected from the plots A and B.

Fig. 1. Illustration of the soil column for assessment of the movement of Bacillus thuringiensis in the soil. A: the soil contaminated with B. thuringiensis, 6 cm thick. B: the soil free of B. thuringiensis, 6 cm thick. C: cotton gauze. D: plastic net. E: flow-through water.
B. thuringiensis in the Soil

The column consisted of two soil layers: the upper layer (1,400–1,500 g, 5–6 cm thick) was contaminated with B. thuringiensis and the lower layer (1,400–1,500 g, 5–6 cm thick) was free of B. thuringiensis. The soil columns received 4 to 5 irrigations of 500-ml distilled water for a total of 2,000–2,500 ml. The flow-through water was collected in 200 to 400 ml fractions every 10 min. The number of B. thuringiensis cells in sampled water was counted as described above. A 500 ml irrigation of distilled water corresponds to 22-mm of precipitation.

Moisture condition of the soils. The soil water content was determined with an infrared moisture meter (Kett Electric Laboratory, Tokyo). The basic intake rate was determined by the cylinder method (MIYOSHI and TANBARA, 1977).

RESULTS

Dispersion of B. thuringiensis into the soils

Table 1 shows the results of a study of vertical distribution of B. thuringiensis in the soils of plots A and B as a function of time. In both plots, B. thuringiensis was detected only in the surface soils (0–1 cm deep) but not in the soils 9–10, 19–20, 29–30 cm deep throughout the test periods. The number of B. thuringiensis cells detected in the surface soil immediately after spraying was 1.8–2.3 × 10⁶ cells/g of soil for the plot A and 2.2 × 10⁵–1.5 × 10⁶ cells/g of soil for the plot B. The time study revealed that the decrease in the number of B. thuringiensis cells was approximately 71% in the first week in the surface soil of the plot A. In the plot B, the number of cells was reduced by 99% within the first week. Figure 2 shows the frequency of rainfall during the test period (34 days) from June 22 to July 25, 1988. The rainfall occurred on 29 days; in particular, heavy rainfalls (11–34 mm/day) were recorded on 7 days.

The vertical distribution of B. thuringiensis in the soils of plots C and D was then investigated in detail (Table 2). In these experiments, the soils were artificially irrigated by 5-min showers on four days of the first week, followed by one 1.5-hr shower during the second week. The 5 min shower corresponds to a 25-mm rainfall, and the 1.5-hr shower is equivalent to a 450-mm rainfall. As shown in Table 2, in the plot C the number of B. thuringiensis cells in the surface soil (0–0.5 cm deep) was 1.0–1.4 × 10⁶ cells/g of soil immediately after the spray of B. thuringiensis. In contrast, no B. thurin-

| Soil depth (cm) |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|                 | 0 | 7 | 14| 20| 33| 0 | 6 | 19|   |   |   |   |   |   |   |   |   |   |   |   |
| 0–1             | 25.3±9.7 | 7.3±3.0 | 2.7±2.1 | 4.0±3.7 | 6.3±2.5 | 8.2±6.8 | 0.08±0* | 1.0±0.7* |   |   |   |   |   |   |   |   |   |   |
| 9–10            | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0   |   |   |   |   |   |   |   |   |   |   |
| 19–20           | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0   |   |   |   |   |   |   |   |   |   |   |

Table 1. Vertical distribution of Bacillus thuringiensis in field soils under naturally irrigated condition

a Average (±SE) of the two (*) or three experiments.
b Calciurn volcanic ash soil clay loam.
c Alluvium sand.
Fig. 2. Rainfall in the city of Kumagaya during the test period from June 22 to July 25, 1988. The data were based on the observation by the Kumagaya Local Meteorological Observatory. The arrow indicates the time of soil sampling (↓, plot A; ↓, plot B). The figure associated with the arrow indicates the day of postspray.

Table 2. Vertical distribution of Bacillus thuringiensis in field soils under artificially irrigated condition

<table>
<thead>
<tr>
<th>Soil depth (cm)</th>
<th>Number of viable B. thuringiensis cells (×10⁴)×g of soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plot Cb</td>
</tr>
<tr>
<td></td>
<td>Days postspray</td>
</tr>
<tr>
<td>0-0.5</td>
<td>1,200±100</td>
</tr>
<tr>
<td>0-0.5</td>
<td>430±0</td>
</tr>
<tr>
<td>0-0.5</td>
<td>270±10</td>
</tr>
<tr>
<td>2.5-3</td>
<td>0</td>
</tr>
<tr>
<td>2.5-3</td>
<td>0</td>
</tr>
<tr>
<td>5.5-6</td>
<td>0</td>
</tr>
<tr>
<td>8.5-9</td>
<td>0</td>
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<td>8.5-9</td>
<td>0</td>
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<td>8.5-9</td>
<td>0</td>
</tr>
</tbody>
</table>

a Average (±SE) of the two experiments.
b Alluvium layer loam.
c Sand.

giensis was found in soils 2.5-3, 5.5-6, and 8.5-9 cm deep. There was no significant change in this pattern of distribution for two weeks. However, a low level of B. thuringiensis (1.7×10⁴ cells/g) was detected in one of two soil samples collected from 2.5-3 cm depth.

In the plot D, the surface soil contained a high number of B. thuringiensis (6.5-7.1×10⁵ cells/g) just after the spray; however, no cells were isolated from soils 2.5-3, 5.5-6, and 8.5-9 cm deep. One week later, a small number of B. thuringiensis (3.3-5.0×10² cells/g) were found in the soil 3 cm deep. After two weeks, B. thuringiensis was present at a density of 3.3×10² cells/g in the soil 5.5-6 cm deep, but not in the soil 8.5-9 cm deep.

Assessment of B. thuringiensis movement in soil columns

The movement of B. thuringiensis was investigated in soil columns with artificial irrigation (Table 3). The upper layer soils contained B. thuringiensis at a density of
Table 3. Detection of Bacillus thuringiensis in in the flow-through water from soil columns

<table>
<thead>
<tr>
<th>Time post-irrigation (min)</th>
<th>Number of B. thuringiensis ($\times 10^3$)/ml of flow-through water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soil column</td>
</tr>
<tr>
<td>0-10</td>
<td></td>
</tr>
<tr>
<td>10-20</td>
<td></td>
</tr>
<tr>
<td>20-30</td>
<td></td>
</tr>
<tr>
<td>30-40</td>
<td>NT$^c$</td>
</tr>
<tr>
<td>40-50</td>
<td>NT$^c$</td>
</tr>
<tr>
<td>50-90</td>
<td>NT$^c$</td>
</tr>
</tbody>
</table>

$^a$ Ciluvium volcanic ash soil clay loam.
$^b$ Alluvium sand.
$^c$ Not tested.

The soil column consisted of two layers; the upper layer contained B. thuringiensis at a density of $2.4-4.5 \times 10^5$ cells/g and the lower layer was free of B. thuringiensis.

2.4–5.0 $\times 10^5$ cells/g of soil in both columns. When the plot A soil column was irrigated by distilled water, the flow-through water fractions, collected 0–10, 10–20, and 20–30 min after irrigation, did not contain any B. thuringiensis. In contrast, B. thuringiensis was detected at a concentration of 1.5–3.5 $\times 10^2$ cells/ml in the flow-through waters collected from the plot B soil column during the test period of 90 min.

Soil water contents and basic intake rates

Basic intake rate was determined to assess the permeability of the water into the soil. The results were as follows: plot A, 77.8 mm/hr; plot B, 63.6 mm/hr; plot C, 27.5 mm/hr; plot D, 120.4 mm/hr. The soil water content was as follows: plot A, 34.4%; plot B, 20.4%; plot C, 21.8%; plot D, 6.4%.

DISCUSSION

In the field plot tests, B. thuringiensis was detected in the soil at depths of 0–1 cm, but not in the soil below a depth of 10 cm, even under conditions of continuous irrigation of the rainy season. Other tests also gave similar results: the sprayed B. thuringiensis passed down into the soil only to a depth of 3–6 cm when bacteria-contaminated soils were artificially irrigated with water equivalent to a 450-mm rainfall. A reduction of approximately 1/1000 was observed in the density of B. thuringiensis at this depth. Since the basic intake rates were high (27.5–120.4 mm/hr) in the soils of the four plots used, it would seem that the rainwater can quickly permeate deep into these soils. However, it is clear from our results that the field-sprayed B. thuringiensis does not penetrate deeply into the soil.

In the irrigation tests using soil columns, B. thuringiensis was not capable of passing through the 6-cm column of volcanic ash soil, and only a few bacteria were detected in the flow-through water from the column of alluvium sand. This finding is in good agreement with the observation by Krieg (1983) who reported that B. thuringiensis spores could not pass through a 30-cm soil column.
These results strongly suggest that the decrease in the level of *B. thuringiensis* population in the soil is not attributable to the physical dilution of sprayed bacteria by the rainwater-mediated vertical dispersion. At present, there are two possible explanations for the disappearance of field-sprayed *B. thuringiensis* from the soil environment: (1) in natural soils *B. thuringiensis* spores fail to germinate due to the lack of germination factors (Akiba et al., 1979; Akiba, 1986; West et al., 1984 c), and are gradually inactivated by abiotic factors and (2) even if spore germination occurs, the soil microorganisms strongly suppress the growth of *B. thuringiensis* (Akiba et al., 1977; West et al., 1984 a, b).

It has been established that the soil components, such as aggregating clay minerals and silica of sand, absorb the organic materials and microbial cells (Brock, 1966). In particular, the volcanic ash soil, one of the predominant soil types in Japan, strongly absorbs various materials including agricultural chemicals (Nakamura, 1990). It is, therefore, very likely that the inability of *B. thuringiensis* to enter deep into the soil is due to the adsorption of bacterial cells to soil particles of the surface layer. More research is necessary on the physical interaction between *B. thuringiensis* cells and soil particles.

ACKNOWLEDGEMENTS

The author thanks Dr. M. Ohra, Kyushu University, for a critical reading of the manuscript.

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