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Survival of *Bacillus thuringiensis* Spores in Soil†

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Bacillus thuringiensis **spores and parasporal crystals were incubated in natural soil, both in the laboratory and in nature. During the first 2 weeks, the spore count decreased by approximately 1 log. Thereafter, the number of spore CFU remained constant for at least 8 months. *B. thuringiensis* did not lose its ability to make the parasporal crystals during its residence in soil. Spore survival was similar for a commercial spore-crystal preparation (the insecticide) and for laboratory-grown spores. In contrast to these results, spores that were produced in situ in soil through multiplication of added vegetative cells survived for only a short time. For spore additions to soil, variations in soil pH had little effect on survival for those spores that survived the first 2 weeks of incubation. Also without effect were various pretreatments of the spores before incubation in soil or nutritional amendment or desiccation of the soil. Remoistening of a desiccated soil, however, caused a decrease in spore numbers. Spores incubated in soil in the field did not show this, but the degree of soil desiccation in nature probably never reached that for the laboratory samples. The good survival of *B. thuringiensis* spores after the first 2 weeks in soil seemed to be a result of their inability to germinate in soil. We found no evidence for the hypothesis that rapid germination ability for spores in soil conferred a survival advantage.**

Bacillus thuringiensis produces endospores during growth. Simultaneous with spore production, it forms a diamond-shaped, crystalline, refractile, parasporal inclusion body which, after partial degradation, is specifically insecticidal for lepidoptera larvae. For some strains of *B. thuringiensis*, however, the crystal shows activity against the larvae of Japanese beetles, blackflies, and mosquitoes (2). The crystals are produced commercially in large amounts for use as an insecticide against the gypsy moth. The commercial preparations, however, contain both the spores (the sporangium has lysed) and the parasporal crystals suspended (usually) in a menstruum for spraying. Thus, any ecological or other considerations of the commercial preparations should consider not only the crystals but also the spores and suspending menstruum. The application as a mixture is not just an economic consideration, because the spores themselves also have the insecticidal capability of the crystals. Thus, the glycoprotein protoxin making up the crystal is also a component of the coat of the spore, and both sources are active as relates to insecticidal powers (2).

The relative ability of *B. thuringiensis* spores to germinate has distinct ecological significance. The spores respond poorly, or not at all, to germinants which are considered optimal for *Bacillus subtilis* (2). Heat shocking does bring on germination, but the conditions must be right so that the heat shocking doesn't cause a belated loss of crystal-producing ability (2). Depending on the strain, *B. thuringiensis* may produce spores that germinate either slowly or rapidly (14). This is not affected by the presence or absence of the crystal. In the larval gut there is little competition from other organisms, so rapid spore germination is not required. The situation is different in soil, however. Stahly et al. (14) feel that rapid germination ability for the spores in soil may confer a survival advantage.

Ignoffo and Garcia (4) concluded that UV light plays a role in the death of *B. thuringiensis* vegetative cells and spores in nature, although their experiments were performed in a petri plate in the absence of soil. Also, they did not consider the crystal even though it apparently was present in their "wettable powder" formulation.

Saleh et al. (11, 12) studied the survival of *B. thuringiensis* in soil. Although they used commercial preparations, they did not consider crystal survival or any effects that the crystals might be having on survival of the spores. They heat shocked their soil preparations just before running spore plate counts but did not account for the vegetative cells of *B. thuringiensis*. They found (11) that, for field applications to cabbage and lettuce, *B. thuringiensis* could be recovered from three of the four soils tested for at least 14 to 40 days. However, there was no indication of percent survival. Laboratory experiments with natural soil, but no plants, showed a possible slight increase in spore numbers during 32 days of incubation in soil, but the total count (all organisms) and the total spore count also increased. They also carried out experiments of the latter type for neutral-pH soil amended with dried alfalfa (12). They concluded that, because the counts rose (although fluctuating markedly with time), the *B. thuringiensis* spores had germinated, multiplied, and resporulated. The results were not verified by counting the vegetative cells. When acidic soil was amended with alfalfa, the spores were stated to have germinated, but without survival of the resulting vegetative cells. However, sterile acidic soil was stated to prevent germination without affecting viability of the spores.

Kiseler (quoted in reference 10) found spores of *B. thuringiensis* still present in soil (percent reduction not stated) at 6 months after addition but not at 1 year. On the basis of laboratory trials with many soil isolates, he concluded that soil contains plenty of soil bacteria that are antagonistic to *B. thuringiensis*. However, the antagonism may have been only toward the vegetative cells. Pruett et al. (10) inoculated natural soil (in the laboratory) with a commercial *B. thuringiensis* preparation and incubated the soil for 135 days. Spores were plated without heat shocking, which may mean that vegetative cells were included in the count. During incubation, the CFU decreased to 24% of the original level.

During the spraying of trees for gypsy moths, large num-

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bers of spores and crystals are added to soil. It is not known whether these spores and crystals might accumulate in soil or what ecological role they might play. However, this accumulation would mean a decreased requirement for spraying new spores and crystals into the environment each year. From an opposing viewpoint, Uchida et al. (16), and others cited by these workers, have shown that the virulence of *Bacillus anthracis* is plasmid associated. It is also known that *B. anthracis* and *B. thuringiensis* are closely related organisms (3). Nonetheless, it is not known whether plasmids of these two bacteria can move between them in nature. This possibility probably would only have significance if *B. thuringiensis* should accumulate in nature. The object of the present study was to evaluate the survival of *B. thuringiensis* spores (crystals present) and vegetative cells in natural soil, both in the field and in the laboratory. Also of interest was survival of the ability to produce the crystals. Untreated spores, plus and minus the commercially used menstruum, and pretreated spores were examined for survival. The effects of soil pH, nutrient amendment of the soil, and drying of the soil during incubation were also tested.

MATERIALS AND METHODS

Media. Spore counts were performed on 0.1-strength heart infusion broth (HI)-Gelrite (Kelco, San Diego, Calif.) medium (7). This consisted of 0.35 g of $MgCl_2 \cdot 6H_2O$ and 1.25 g of HI in 500 ml of distilled water. After this had dissolved, 3.0 g of Gelrite was added. Sterilization was by autoclaving. The medium was tempered at 60°C before plates were poured. The oxgall-rose bengal agar (8) used to enumerate fungi from soil consisted of KH_2PO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.05 g; peptone, 5.0 g; glucose, 10.0 g; and Bacto-Agar (Difco Laboratories, Detroit, Mich.), 20 g. These components were autoclaved, and then 10 ml of a filter-sterilized streptomycin solution was added after tempering. The streptomycin solution contained 0.3 g of streptomycin sulfate (Sigma Chemical Co., St. Louis, Mo.) per 100 ml of distilled water. Glucose-nitrate agar (5) was used for the enumeration of actinomycetes. It consisted of KH_2PO_4, 0.1 g; $NaNO_3$, 0.1 g; KCl, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; glucose, 1.0 g; agar, 15 g; in 1 liter of distilled water (pH adjusted to 7.0). Total counts of soil bacteria used 0.1-strength HI agar. Unless otherwise noted, medium components were Difco products.

Spores and crystals. Dipel 4L is a commercial insecticide preparation (Abbott Laboratories, North Chicago, Ill.) which is used as a spray for the gypsy moth. It contained the spores and parasporal crystals of *B. thuringiensis* (H-type 3a3b-2) suspended in an oil emulsion. A pure culture of *B. thuringiensis* was isolated from this preparation. It was maintained on 0.1-strength HI agar. Incubation was at 27°C.

Vegetative cells of *B. thuringiensis* for use as an inoculum for spore production were grown in nutrient broth. Portions (50 ml) of nutrient broth in 250-ml baffle-bottom flasks (Bellco Glass, Inc., Vineland, N.J.) were inoculated by loop from a slant and shaken for 18 h at 27°C. This gave vegetative cells without spores. The cells from five flasks were washed and suspended in 5 ml of distilled water. For spore production, 0.1-ml portions of the washed cells were spread onto 0.1-strength HI agar plates supplemented with 0.01% $MnSO_4 \cdot H_2O$. After incubation for 1 week at 27°C, sterile distilled water was added to each plate to cover the spore lawns. The spores were suspended by scraping the plates with a glass spreading rod, and the suspensions were pooled. The spores plus parasporal crystals in the suspensions were washed three times with sterile distilled water. When the spore-crystal pellets were resuspended, care was taken to prevent formation of a hydrophobic foam layer at the surface. The number of spores in the final suspension was determined by plate count. Storage was at 5°C. The spores in these preparations and those observed in all studies were examined by phase-contrast microscopy to be sure that they were phase-bright.

***B. thuringiensis* counts.** Recovery of *B. thuringiensis* from soil was accomplished by blending the soil in a Waring blender. A 10-g portion of the soil (the amount in one bottle) was placed in a blender with 30 ml of sterile tap water and subjected to intermittent blending at low speed. The procedure consisted of 30 s of blending, a 30-s rest period, and finally, another 30 s of blending. The blended sample was then allowed to settle for 2 min before further dilutions were made in sterile tap water. Two dilution series were made for each soil sample. The initial dilution of one series was subjected to a 20-min, 80°C heat treatment; the other series was not heated. The heating eliminated vegetative cells from the count and, theoretically, activated the spores for germination. Activation, however, did not prove to be necessary.

Colony counts were performed by spreading 0.1-ml portions of the dilutions onto 0.1-strength HI-Gelrite plates. Inoculated plates were incubated at 27°C for 1 week. Initial *B. thuringiensis* colony counts were made after 15 h of incubation. *B. thuringiensis* colonies had a unique appearance on this medium solidified with Gelrite, and they appeared earlier than the colonies for other sporeforming bacteria. The young colonies were white and thin with a cottony appearance and irregular edge. Older colonies looked more like those of other sporeforming bacteria, except for a characteristic filamentous edge. All colonies of sporeforming bacteria spread out less on this medium than on media solidified with agar (7, 9). After the initial reading, the plates were reincubated for 6 days, and the *B. thuringiensis* colonies were checked for spore and crystal formation by phase-contrast microscopy.

Spores in nature. The fate of *B. thuringiensis* spores in nature was studied at various soil sites. Soil was collected from each site before spore addition, and the pH and natural *B. thuringiensis* counts were determined. Two areas at each site were cleared of debris and inoculated with spore-crystal suspension. The suspension was mixed with the soil to a depth of approximately 5 cm. An initial *B. thuringiensis* count of the soil, immediately after spore addition, was made to determine the zero-hour count. Soil was collected from each area at the sites at weekly intervals and processed as in soil bottle experiments. The soil was not sieved, but grass was removed. These soils, and most other soils used in this study, were Hagerstown silty clay loams.

Soil bottles. Soil bottles were prepared by adding 10.0 g of natural (nonsterilized) soil sifted through a 2-mm sieve to 1-oz (28-g) screw-cap bottles. The bottled soil was then brought to 60% of its moisture-holding capacity (MHC) by addition of the appropriate spore suspension. To maintain a constant soil moisture level, the bottles were weighed on day zero and at weekly intervals thereafter. Sterile distilled water was added to maintain the day zero weight. The caps were loose during incubation.

Soil bottles in which the soil was amended with various liquids were prepared as above except that a more concentrated spore suspension was added to obtain a final concentration of 10^7 spores per g of soil. This brought the soil to only approximately 40% of MHC. The bottles were then incubated at 27°C for 2 days before sterile liquid amendments were made to obtain a final moisture level of 60% of

MHC. The amendments were double-strength nutrient broth, double-strength HI, or solutions of 2.0% tryptone, 2.0% casein (washed from milk; Fisher Scientific Co., Fair Lawn, N.J.), 1.0% $MgCl_2 \cdot 6H_2O$, 1%; $CaCl_2 \cdot 2H_2O$, 1.0% $MnCl_2 \cdot 4H_2O$, or 0.1% L-alanine plus 5.0% glucose.

Soil bottles with sterile soil were prepared by placing 10.0 g of sifted soil in bottles with caps loosely fitted. The bottles were autoclaved at 121°C by the following time schedule. On day 1, the bottles were autoclaved for 2 h and then incubated at 27°C until day 2. On day 2, the bottles were autoclaved for 2 h and incubated until day 4. On day 4, the bottles received 17 h of sterilization. Sterility was checked by diluting the soil and plating on 0.1-strength HI agar plates.

Spore pretreatments. Heat-activated spore suspensions were prepared as follows. A spore suspension (crystals not removed) containing approximately 10^8 spores per ml was placed in an 80°C water bath for 20 min. Timing began when the suspension reached the 80°C temperature. The activated spores were cooled immediately.

Spores (plus crystals) were suspended in a solution of 0.01% L-alanine–0.5% glucose. After 24 h at room temperature, the spores (plus crystals) were suspended in distilled water to give 10^8 spores per ml. The nutrient broth pretreatment was for 2 min at room temperature, followed by immediate washing of the spores (plus crystals) in a refrigerated centrifuge and suspension in distilled water at 10^8 spores per ml. For pretreatment with sodium dodecyl sulfate (USP; Fisher), suspension was for 1 h in 0.1% sodium dodecyl sulfate. The spores (plus crystals) were then washed and suspended at 10^8/ml in distilled water.

Spore stain. A modified Schaeffer-Fulton endospore stain and staining procedure were used for staining endospores and crystals, both from in situ soil samples and for laboratory cultures (9).

Nematodes. Nematodes were collected and counted by the Baerman funnel technique. The collection apparatus consisted of a large funnel, a milk filter, a piece of rubber tubing with a clamp, and a collection vial. The tubing with clamp was attached to the end of the funnel, and the milk filter was placed inside the funnel. A 50-g portion of soil was placed on the milk filter. The clamp was tightened, and water was slowly added to the soil until the soil was just submerged.

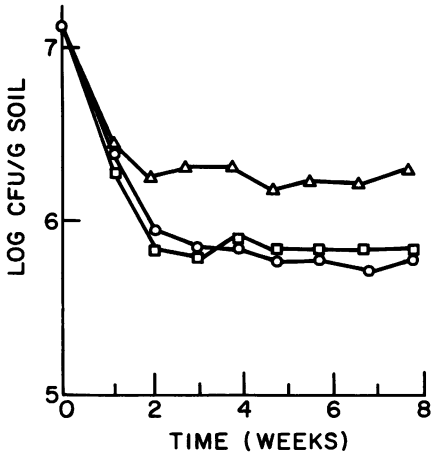

FIG. 1. Counts of viable heat-resistant *B. thuringiensis* spores during incubation in situ in soil in the field. Values are averages for adjacent sampling areas at each site. Soils: fallow field site (pH 6.5) (△); pine tree site (pH 4.9) (○); oak tree site (pH 4.2) (□).

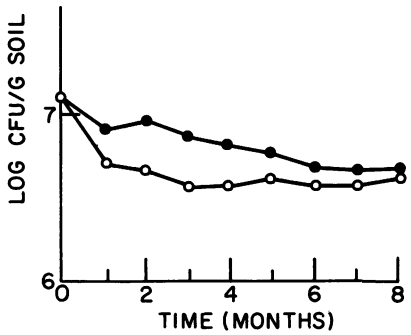

FIG. 2. Viable counts of heat-resistant *B. thuringiensis* spores during incubation at 27°C (○) and 4°C (●) for 8 months in natural soil A maintained at 65% of MHC.

The water-soaked soil was allowed to sit at room temperature for 48 h, and then the collecting vial was placed over the opening in the tube and the clamp was loosened. The collected water, now containing the nematodes, was placed on a watch glass, and the nematodes were counted with the aid of a dissecting microscope.

RESULTS

Spore survival. A mixture of *B. thuringiensis* spores and crystals was applied to three different soils in situ in nature. The applications were made at two adjacent sites for each soil. The sites were located at a pine tree stand, at an oak tree stand, and in a fallow field. The soil pH values were, respectively, 4.3, 4.9, and 6.5. At the time of application, these soils (and other soils used in this study) did not contain detectable crystal-producing, spore-forming rods. This conclusion was based on examination of colonies occurring on 10^{-3} dilution plates of the soils. During the first 2 weeks (Fig. 1), the *B. thuringiensis* spore count decreased by approximately 0.8 log for the pH 6.5 soil and 1.3 log for the pH 4.9 and 4.3 soils. After this, the counts held steady for the next 6 weeks. Microscopic examinations were made of slides that had been inserted in the soil at each site. Spores and crystals had been applied to each slide before insertion, and the slides were inserted vertically so that one end protruded just above the soil. Over the 8-week incubation period, there was no change in the staining characteristics of the spores or crystals. The crystals retained their size and unique shape, and there was no apparent change in their numbers (on the slides). During the 8-week incubation, there was 9.9 cm of rainfall. The average of the mean daily air temperatures was 17.3°C; the range was 6.7 to 26.1°C.

B. thuringiensis spore survival in soil was also determined for soils incubated in bottles in the laboratory. Spores plus crystals were added to soil A (pH 6.0) and incubated for 8 months at 4 and 27°C. There was relatively little death of the spores (Fig. 2). In similar experiments, but with an 8-week incubation period, our spore-crystal preparation was compared with Dipel, the commercial preparation that is sprayed in nature for control of the gypsy moth. At both incubation temperatures, the spore survivals of our preparation and of Dipel were identical. Therefore, the oily fluid used as a suspension agent in Dipel had no effect on spore survival. This experiment was repeated with the same soil, but the soil had been sterilized. Again, there was no difference between Dipel and our spore-crystal preparation. With the sterile soil, the spores neither germinated nor died: i.e., the spore count remained constant during incubation.

TABLE 1. Effect of *B. thuringiensis* spores and crystals on counts of indigenous organisms in soil A

Organisms	Counts per g of soil	
	Spores not added	Spores added[a]
Total bacteria	5×10^7	1×10^8
Actinomycetes	2×10^5	1×10^6
Fungi	3×10^3	5×10^3
Nematodes	15	50
Protozoa[b]	2	2

[a] Zero-hour spore count was 2×10^7/g of soil.
[b] By the method of Singh (13).

The *B. thuringiensis* spore survival responses to soil pH that were seen in the field (Fig. 1) were also seen for soils incubated for 8 weeks in soil bottles in the laboratory at 27°C. Our spore-crystal preparation was used. Three soils, other than those for Fig. 1, were used. Soil A was pH 6.0 and was used in other experiments. The other two came from pine tree stands and had pH values of 4.2 and 5.0. The first two were central Pennsylvania soils; the third was from Endwell, New York. The *B. thuringiensis* spore survival in these soils was identical to that shown in Fig. 1. The pH 6.0 and 5.0 curves resembled the upper curve of Fig. 1; the pH 4.2 curve resembled the lower curve. Thus, slightly greater initial spore death occurred at soil pH values below 5.0.

At least for the higher-pH soils (lower-pH soils were not tested), the addition of a spore-crystal suspension containing 1 log fewer spores did not affect the results. The initial death phase, followed by survival of the remaining spores, was still seen.

The pH 6.0 soil (soil A above) was incubated for 2 weeks at 27°C with our *B. thuringiensis* spore-crystal preparation. This time span allowed occurrence of the initial decrease in spore numbers (Fig. 1 and 2). After this time, the numbers of bacteria, actinomycetes, fungi, protozoa, and nematodes were determined for this soil and for similar soil incubated without spore-crystal addition. All of these, except protozoa, increased moderately in numbers in response to the spores (Table 1). We have noted that soil nematodes placed on lawns of *B. thuringiensis* spores and crystals cleaned out the spores, but not the crystals, in their track through the spores. In instances in which the spores were excreted, the spores were no longer phase bright. We could not judge the viability of the excreted spores. There was no apparent effect on the crystals.

Spores plus crystals were added to samples of soil A (pH 6.0), and the samples were adjusted with distilled H_2O to 40, 60 or 80% of MHC before incubation at 27°C. The moisture content of the samples was maintained during incubation, except for some of those at 60% of MHC, which were allowed to dry out over the first 5 weeks of incubation. At 5 weeks, water was added to the latter samples to bring them back to 60% of MHC. Excess water in the soil (80% of MHC) was slightly detrimental to initial spore survival but not thereafter (Fig. 3). Although drying of the soil had little effect on the spores, remoistening of the dried soil did cause a decrease in spore viability.

Pretreatment of spores. *B. thuringiensis* spores (plus crystals) were heated for 20 min at 80°C before addition to soil A for incubation at 4 and 27°C. The heat treatment had no effect on spore survival, i.e., the results were as in Fig. 1. When the preheated spores were added to sterile soil A and incubated as above, there was neither spore germination nor spore death. The numbers remained constant. Thus, the preliminary heat treatment did not induce germination. Similar spores, not contacted with soil but diluted and plated on laboratory media, germinated with ease and did not require the heat treatment.

Other germinants were tried for pretreatment of the spores before their incubation in natural and sterile soil. L-Alanine-glucose pretreatment had no effect on spore survival. A 2-min (room temperature) pretreatment with nutrient broth followed by refrigerated centrifugal washing of the spores induced the initial stages of spore germination before they were added to the soil. In soil, these spores exhibited an initial decrease in CFU of 1.2 log, but the numbers remained constant thereafter. A comparison of counts for pasteurized and nonpasteurized dilutions (at the time of plating) showed that the initial disappearance of the spores in the soil was due to death and not merely germination of the spores to give vegetative cells. In sterile soil, the pretreated spores increased in CFU by 0.7 log during week 1 and then maintained constant numbers.

Spores were pretreated with sodium dodecyl sulfate to reduce their hydrophobic nature and clumping before incubation in soil. This treatment had no effect on spore survival.

Soil amendment. Spores that had not been pretreated were added to soil, and the soil was then amended with nutrients or germinants. The soil was incubated for 2 days with the spores (plus crystals) before the amendments were made. There was no effect on the spores for soil amendments with L-alanine-glucose or solutions of $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, or $MnCl_2 \cdot 4H_2O$. For amendments with nutrient broth, HI, and tryptone broth, the spore count decreased, as for nonamended soil, over the first 10 days. During the next 20 days, however, the spores rose 0.8 log and then fell back 1.0 log. The counts did not change after this.

To determine what was happening during the above rise and fall in spore count, nutrient broth and the spores were added simultaneously to the soil, and the soil dilutions for platings were tested both with and without heating at 80°C for 20 min. During the first 4 days some of the spores

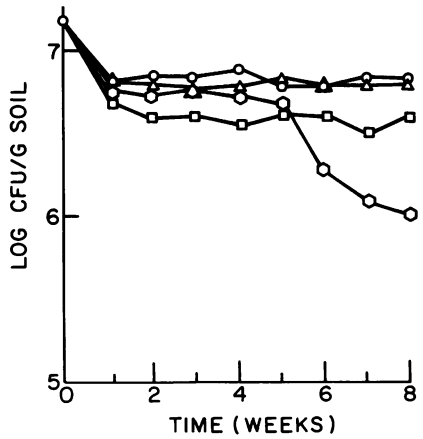

FIG. 3. Effect of soil moisture content on spore survival. Viable counts of heat-resistant *B. thuringiensis* spores incubated in natural soil A were maintained at different moisture contents. Symbols: △, 40% of MHC; ○, 60% of MCH; □, 80% of MHC; and ⬡, soil initially at 60% MHC but allowed to dry out. Distilled H_2O was added to the latter at 5 weeks to bring the moisture content back to 60%.

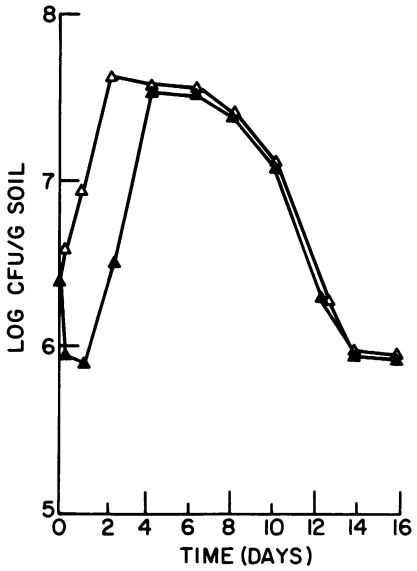

FIG. 4. Viable count of total *B. thuringiensis* cells (△) and heat-resistant *B. thuringiensis* spores (▲) during incubation of the spores in natural soil amended with double-strength nutrient broth. The soil was maintained at 65% of MHC.

germinated, multiplied, and then resporulated (Fig. 4). However, spores that were formed in situ in the soil died, and by 14 days the spores present were those that had not germinated originally.

DISCUSSION

Our results showed good agreement for survivals of *B. thuringiensis* spores incubated in soil in situ in nature and those incubated in soil bottles in the laboratory. Agreement was also good for spores studied as the commercial insecticide preparation and spores produced in the laboratory. The oil emulsion suspension fluid for the commercial preparation had no effect on spore survival in soil. The commercial preparation contained parasporal crystals in addition to the spores. Therefore, the crystals were not removed from the laboratory-produced spores before testing in soil. These crystals, however, seemed to survive intact in soil, but no attempt was made to determine whether their insecticidal properties had also survived. The ability of *B. thuringiensis* to produce the crystals was not lost during its residence in soil: i.e., all colonies of *B. thuringiensis* recovered from soil produced the crystals.

B. thuringiensis spores decreased in numbers by approximately 1 log during the initial 2 weeks of their incubation in soil. The numbers did not change thereafter. Thus, the spore preparations contained some spores that died easily in soil, but also contained other spores that did not. Spores that were produced in situ in soil, instead of in the laboratory, fell in the category of spores that died easily in soil.

All samples were plated both with and without heat treatment. Except for the special case presented in Fig. 4, the results were similar. These results, and the results from Fig. 4, indicated that the spores that survived in soil did not have the ability to germinate in soil, although they germinated with ease when they were recovered from soil and plated on laboratory media. Spore germination in soil was not increased or decreased by soil pH or various pretreatments of the spores before soil incubation. In addition, amendments of the soil and the presence of various soil moisture levels, including soil desiccation, had little effect. Thus, it was as if there was a sporostasis of some kind operating in the soil, such as the bacteriostasis reported by Brown (1), that protected the spores by keeping them from germinating. Stahley et al. (14) considered a different possibility; i.e., they felt that a rapid germination ability for spores in soil might confer a survival advantage. Our results do not show this.

Remoistening of desiccated soil in soil bottles, as opposed to desiccation per se, appeared to kill some of the spores. Experiments in the field, however, did not show this die-off. Although there was considerable fluctuation in soil moisture content for the soils in the field, the moisture content probably never dropped to the 2 to 3% of MHC values that are obtained for soil allowed to dry in soil bottles (6). Thus, die-off of *B. thuringiensis* spores in nature due to remoistening of thoroughly dried soil probably is a rare event if it occurs at all. For the soil bottles it is possible that the remoistening of the desiccated soil released either inorganic or organic nutrients (15) or toxic materials that directly affected the spores, e.g., caused germination of the spores. The released nutrients could also cause germination or growth of other soil organisms that could destroy the spores (9). It is also possible, however, that something about the remoistening of thoroughly dried soil prevented the physical release of the spores from the soil for plate count enumeration. This would lead to a false conclusion that the spores had died.

ACKNOWLEDGMENTS

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