

Susceptibility of Larvae of *Hemerocampa leucostigma* to Several Varieties of Crystalliferous Bacteria¹

H. W. ROSSMOORE, L. ELDER, AND E. A. HOFFMAN

Department of Biology, Wayne State University, Detroit, Michigan 48202

Received October 17, 1969

Twelve isolates belonging to six H-antigen serotypes were evaluated for their effectiveness against *Hemerocampa leucostigma* larvae. All except one, *Bacillus finitimus* var. *finitimus* produced high levels of mortality in 4 days. In addition, we found no difference in susceptibility to *Bacillus thuringiensis* var. *thuringiensis* among first-through fifth-instar larvae.

INTRODUCTION

Larvae of the white-marked tussock moth, *Hemerocampa leucostigma*, are primarily an urban nuisance. About 3 years ago, we discovered large numbers of this species voraciously consuming leaves on a variety of deciduous ornamental and shade trees on the Wayne State University campus. The use of chemical insecticides is restricted on the campus and the limited applications were unsuccessful. The extensive restrictions now placed on chemical insecticides even for the home gardener emphasize the need to find alternative methods of control for any potentially dangerous pest. We selected 12 isolates belonging to 6 serotypes (Bonnetoi and De Barjac, 1963) of *Bacillus thuringiensis* varieties that were previously used against *Lymantria dispar* (Ruperez and Rossmoore, 1965). For purposes of reference we have used the taxonomic scheme suggested by Heimpel (1967).

MATERIALS AND METHODS

Test Insect

First- through fifth-instar larvae of *H. leucostigma* were raised from field-col-

lected egg masses taken from an isolated maple tree on the Wayne State University campus. The female adult deposits the eggs on the pupal case after emergence, usually during the last 2 weeks in August. After collection, the eggs were stored at 10°C for a sufficient time to break diapause and incubated under diurnal conditions (14 hr of light at 26°C and 10 hr of dark at 18°C) at a relative humidity of 60% until hatching. The larvae were maintained on maple leaves that had been picked and stored at -70°C. We noticed no untoward effects on larval growth, development, or morbidity from this diet.

Bacteria

Twelve isolates belonging to six serotypes (Table 1) were grown from an active culture in half-strength veal heart infusion broth (Difco) supplemented with 100 mg CaCl₂ and 5 mg of MnCl₂ per liter. Incubation was at 30°C with shaking at 120 strokes/min. Sporulation and concomitant crystal formation occurred within 24 hr in all cultures at which time incubation was continued an additional 24 hr to permit sporangial lysis. Spore-crystal ratios of each culture was checked microscopically with Smirnov's (1962) staining procedure. The

¹ Contribution No. 259, Department of Biology.

TABLE 1
SOURCE AND DESIGNATION OF CRYSTALLIFEROUS *Bacillus* SP. USED

| Isolate no. | Varietal designation | Serotype | Source | Original designation |
|-------------|--|----------|---------------|----------------------------|
| 1 | <i>B. thuringiensis</i> var. <i>thuringiensis</i> | 1 | A. M. Heimpel | 996 (N. R. Smith) |
| 2 | <i>B. thuringiensis</i> var. <i>thuringiensis</i> | 1 | A. M. Heimpel | Galleriae Krieg & Franz |
| 3 | <i>B. thuringiensis</i> var. <i>amuscatotoxicus</i> | 1 | A. M. Heimpel | Va Weiser |
| 4 | <i>B. funitimus</i> var. <i>funitimus</i> | 2 | A. M. Heimpel | Finitimus Heimpel |
| 5 | <i>B. thuringiensis</i> var. <i>alesti</i> | 3 | A. M. Heimpel | Anduze Vago |
| 6 | <i>B. thuringiensis</i> var. <i>sotto</i> | 4 | A. M. Heimpel | Sotto Ono |
| 7 | <i>B. thuringiensis</i> var. <i>dendrolimus</i> | 4a | A. M. Heimpel | Dendrolimus Talalaev |
| 8 | <i>B. thuringiensis</i> var. <i>entomocidus</i> | 6 | A. M. Heimpel | 1328 Steinhaus |
| 9 | <i>B. thuringiensis</i> var. <i>subtoxicus</i> | 6 | A. M. Heimpel | 1124 Steinhaus |
| 10 | <i>B. thuringiensis</i> var. <i>aizawa</i> | 7 | A. Ruperez | HA-3 Aizawa |
| 11 | <i>B. thuringiensis</i> var. <i>aizawa</i> | 7 | A. Ruperez | IH-A Aizawa |
| 12 | <i>B. thuringiensis</i> var. <i>pacificus</i> | 7 | A. Ruperez | T63-L4 Aizawa |

spore-crystal suspensions were washed twice in sterile, distilled water at 10,000 rpm and finally suspended in 0.05 M Tris buffer (Tris (hydroxymethyl) aminomethane) at pH 7.2 with 0.25% Neutronyx 656 (Alkylphenol polyglycol ether, Onyx Chemical Co.) to facilitate wetting. At this point, viability was determined by standard plate count, and spore-crystal ratios were rechecked. Final suspensions were adjusted to approximately 10^9 spores/ml.

Infection of Larvae

First- through fifth-instar larvae were fed on foliage sprayed with various dilutions of each bacterial isolate. An aliquot of the highest concentration of isolate No. 1 was autoclaved for 20 min and served as a control. Tests were performed in 1-pt mason jars, covered with Saran (Dow Chemical

Company) and containing 10–15 larvae. Mortality results are based on a minimum of 100 larvae for each dosage and larval stage. Animals were offered sprayed leaves for 2 days at which time they were given fresh, uninfected food.

RESULTS AND DISCUSSIONS

Relative Resistance of First- to Fifth-Instar Larvae to Bacillus thuringiensis var. thuringiensis (996)

All instars ceased feeding within 10 min after ingesting the highest spore-crystal concentration. At the next lowest concentration, larvae continued to feed for at least 1 day but not more than 2; within 2 hr these animals were noticeably sluggish. The lowest level in no way affected voracity. Im-mobility in all instars was observed at the

highest dosage within 12 hr but not at any other level. There was no significant difference in mortality among the five age groups (Table 2). This contrasts with the report of Morrison and Perron (1963) who found that susceptibility of *Galleria mellonella* to *Bacillus thuringiensis thuringiensis* decreased from first to fifth instar. It would be difficult to draw any definitive conclusions from any one species since the actual amount ingested per larvae is not readily ascertained. Feeding habits vary among species, and it is not always possible to correlate directly food utilized with food consumption. We did, however, attempt to assay the repeatability of the application procedure by checking random centimeter square areas for viable spores before and after spraying. The counts averaged $2 \times$

$10^6/\text{cm}^2$ with separate areas varying no more than replicate plates from one area. Using this information, we calculated the average lethal dose of isolate No. 1 for fourth and fifth instars by the following formula:

$$\frac{\text{spore-crystals/cm}^2 \times \text{area eaten}}{\text{no. of larvae in container}}$$

The area eaten was determined by before-and-after measurements of the leaf with a Keufel and Esser planimeter. The dose was 6×10^5 spore-crystals/larvae.

Comparison of Crystalliferous Isolates on Fifth-Instar Larvae

Of the 12 isolates utilized, only 1 proved ineffective (Table 3). There was reduced feeding with sluggishness followed by almost complete lack of movement within 12 hr in all but the *finitimus* isolate. In general, the effective dose levels and time course of action followed the pattern already described for the first to fifth instar with isolate No. 1. In both studies we noticed a delayed immobilization that we are hesitant to call general paralysis. This occurs only at the highest dose level, some 12 hr after ingestion. Thus, *H. leucostigma* larvae do not respond either as rapidly or as dramatically as do larvae of *Bombyx mori*. However, the lack of movement noticed is greater than we have observed with *L. dispar*, which qualifies as a Group II species according to criteria of Heimpel and Angus (1959). The differential mortalities were mostly within the range of experimental error. However, in two cases isolates 1, 2, and 3 of serotype 1 and isolates 10, 11, and 12 of serotype 7, the most effective suspension seemed to be from the variety lacking the thermostable exotoxin, var. *amuscatotoxicus* and var. *pacificus*. This observation only serves to eliminate the exotoxin as a contributory factor in larval death. Except for the expected ineffective-

TABLE 2
RESPONSE OF DIFFERENT LARVAL STAGES OF
Hemerocampa leucostigma TO *Bacillus*
thuringiensis VAR. *thuringiensis*

| Larval stage | Dose (spores/cm ² of leaf surface) | Mortality % (4 days after feeding) |
|----------------|---|------------------------------------|
| L ₁ | 4.0×10^7 ^a | 95 |
| | 2.0×10^7 | 87 |
| | 2.0×10^6 | 58 |
| | 2.0×10^5 | 2 |
| L ₂ | 4.0×10^7 | 97 |
| | 2.0×10^7 | 81 |
| | 2.0×10^6 | 60 |
| | 2.0×10^5 | 4 |
| L ₃ | 4.0×10^7 | 93 |
| | 2.0×10^7 | 89 |
| | 2.0×10^6 | 61 |
| | 2.0×10^5 | 0 |
| L ₄ | 4.0×10^7 | 90 |
| | 2.0×10^7 | 83 |
| | 2.0×10^6 | 65 |
| | 2.0×10^5 | 0 |
| L ₅ | 4.0×10^7 | 95 |
| | 2.0×10^7 | 85 |
| | 2.0×10^6 | 50 |
| | 2.0×10^5 | 3 |

^a Both sides of leaves sprayed. In addition, an autoclaved suspension was sprayed on both sides and offered to all larvae. This produced no mortality.

TABLE 3
RELATIVE EFFECTIVENESS OF 12 CRYSTALLIFEROUS
ISOLATES AGAINST *Hermerocampa leucostigma*
LARVAE^a

| Isolate no. | Dose (spores/cm ² of leaf surface) | Mortality % (4 days after feeding) |
|-------------|--|--|
| 1 | Autoclaved suspension as control | 0 |
| 1 | 2.0 × 10 ⁷ | 85 |
| | 2.0 × 10 ⁶ | 50 |
| | 2.0 × 10 ⁵ | 3 |
| 2 | 1.6 × 10 ⁷ | 70 |
| | 1.6 × 10 ⁶ | 29 |
| | 1.6 × 10 ⁵ | 0 |
| 3 | 1.5 × 10 ⁷ | 95 |
| | 1.5 × 10 ⁶ | 35 |
| | 1.5 × 10 ⁵ | 3 |
| 4 | 1.8 × 10 ⁷ | 0 |
| | 1.8 × 10 ⁶ | 0 |
| | 1.8 × 10 ⁵ | 0 |
| 5 | 1.3 × 10 ⁷ | 64 |
| | 1.3 × 10 ⁶ | 33 |
| | 1.3 × 10 ⁵ | 0 |
| 6 | 1.3 × 10 ⁷ | 84 |
| | 1.3 × 10 ⁶ | 36 |
| | 1.3 × 10 ⁵ | 3 |
| 7 | 1.6 × 10 ⁷ | 84 |
| | 1.6 × 10 ⁶ | 34 |
| | 1.6 × 10 ⁵ | 0 |
| 8 | 1.5 × 10 ⁷ | 72 |
| | 1.5 × 10 ⁶ | 39 |
| | 1.5 × 10 ⁵ | 2 |
| 9 | 1.3 × 10 ⁷ | 53 |
| | 1.3 × 10 ⁶ | 30 |
| | 1.3 × 10 ⁵ | 0 |
| 10 | 1.9 × 10 ⁷ | 63 |
| | 1.9 × 10 ⁶ | 37 |
| | 1.9 × 10 ⁵ | 2 |
| 11 | 1.6 × 10 ⁷ | 67 |
| | 1.6 × 10 ⁶ | 34 |
| | 1.6 × 10 ⁵ | 0 |
| 12 | 1.9 × 10 ⁷ | 84 |
| | 1.9 × 10 ⁶ | 42 |
| | 1.9 × 10 ⁵ | 4 |

^a Fifth-instar larvae were used in all experiments.

ness of *Bacillus finitimus*, we found that all the other varieties were fairly active, the lowest being var. *subtoxicus*. However, the dose applied in that case is 50% lower than

for var. *thuringiensis*, isolate No. 1. If mortalities are theoretically adjusted based on dose differences, then the disparities among the various isolates all but disappear. We verified this by offering fifth-instar larvae a dose double of that offered previously (both sides of leaves sprayed). This finding suggests that *H. leucostigma* is fairly atypical in being susceptible to so many of the characterized crystalliferous varieties. Reports for other species including *L. dispar* (Grigороva, 1964; Vankova, 1964; Ruperez and Rossmoore, 1965), *Trichoplusia ni* (Broersma and Buxton, 1967), and *B. mori* (Angus and Norris, 1968) suggested that differential susceptibility to designated varieties is the more common phenomenon. Indeed, Heimpel (1967) uses resistance and susceptibility of *B. mori* and *Pieris brassicae* to give varietal status to *B. thuringiensis* var. *subtoxicus* and *B. thuringiensis* var. *entomocidus*. The susceptibility of *H. leucostigma* to such a broad spectrum of utilizable agents makes it a good candidate for biological control.

REFERENCES

- ANGUS, T. A., AND NORRIS, J. R. 1968. A comparison of the toxicity of some varieties of *Bacillus thuringiensis* Berliner for silkworm larvae. *J. Invertebr. Pathol.*, **11**, 289-295.
- BONNEFOI, H., AND DE BARJAC, H. 1963. Classification des souches du groupe *Bacillus thuringiensis* par la détermination de l'antigène flagellaire. *Entomophaga*, **8**, 223-229.
- BROERSMA, D. B., AND BUXTON, J. A. 1967. A comparative study of the action of six crystalliferous bacteria on the cabbage looper, *Trichoplusia ni*. *J. Invertebr. Pathol.*, **9**, 58-69.
- GRIGOROVA, R. 1964. Deux souches de *Bacillus thuringiensis* Berliner isolés des chenilles du *Bombyx dispar* et *Lymantria dispar*. *Entomophaga Mem.*, **2**, 179-191.
- HEIMPEL, A. M. 1967. A taxonomic key proposed for the species of the "crystalliferous bacteria." *J. Invertebr. Pathol.*, **9**, 364-375.
- HEIMPEL, A. M., AND ANGUS, T. A. 1959. The site of action of crystalliferous bacteria in

- Lepidoptera larvae. *J. Insect Pathol.*, **1**, 152-170.
- MORRISON, F. O., AND PERRON, J. M. 1963. Sensibilité des différents stades larvaires de *Galleria mellonella* L. à *Bacillus thuringiensis* Berliner. *Phytoprotection*, **44**, 106-115.
- RUPEREZ, A., AND ROSSMOORE, H. W. 1965. Consideraciones sobre bacterias del grupo *Bacillus cereus-thuringiensis* patógenas para la *Lymantria dispar* L., *Bol. Serv. Plagas. Forest.*, **8**, 51-59.
- SMIRNOFF, W. A. 1962. A staining method for differentiating spores, crystals, and cells of *Bacillus thuringiensis* (Berliner). *J. Insect Pathol.*, **4**, 384-386.
- VANKOVA, J. 1964. *Bacillus thuringiensis* ir praktischer anwendung. *Entomophaga Mem.* **2**, 271-291.