EXPERIMENTS ON THE POSSIBLE USE OF *BACILLUS THURINGIENSIS THURINGIENSIS* BERLINER IN THE CONTROL OF CROP PESTS

II. Susceptibility of Some Lepidopterous Pests to *B. thuringiensis*

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ABSTRACT

Two commercial preparations of *Bacillus thuringiensis thuringiensis* Berl. were used in laboratory tests on two noctuids: *Prodenia litura* (F.) and *Plusia orichalcea* (F.); two pyralids, *Leucinodesorbonalis* Guen. and *Chilo partellus* (Swinh.); and one papilionid, *Papilio demoleus* L.

On the basis of the results obtained it was evident that all the larval stages of *Papilio* were highly susceptible to both bacterial preparations, while *Prodenia* showed moderate susceptibility. The larvae of *Plusia*, *Leucinodes* and *Chilo* showed less susceptibility to one of the bacterial preparations tested. The results suggest that *Papilio demoleus*, a serious pest of young citrus in nurseries in India, may be efficiently controlled through spray applications of *B. thuringiensis*.

The larvae of *P. demoleus* exhibited a fairly rapid paralysis followed by an increase of blood alkalinity after ingestion of spores, whereas *P. litura* larvae did not show any sign of paralysis or appreciable change in the pH of the haemolymph.

THE formulations of microbial insecticides based on *Bacillus thuringiensis thuringiensis* are widely used in the control of several lepidopterous pests with some success. Basic research, particularly on the physiology and mode of action of the bacterium, has aided in its efficient use in the field. The literature on the diseases caused by spore-forming bacteria has been fully reviewed by Heimpel and Angus (1963).

Venkatraman *et al.* (1962) reported the results of preliminary tests on the pathogenicity of *B. thuringiensis thuringiensis* for some crop pests in India. The present paper deals with further laboratory tests on the tobacco worm, *Prodenia litura* (F.), the cabbage semilooper, *Plusia orichalcea* (F.), the egg plant borer, *Leucinodes orbonalis* Guen., the sorghum stalk borer, *Chilo partellus* (Swinh.), and the citrus butterfly, *Papilio demoleus* L.

MATERIALS AND METHODS

Bakthane L 69, manufactured by Rohm and Haas Co., and which is said to contain 75×10^6 viable spores/gram, and Thuricide W.P., a product of Bioferm Corporation, were used in the tests. The spore concentration of Thuricide was diluted approximately to that of Bakthane L 69, but the crystal concentrations were not taken into account. Skimmed milk powder was used as sticker in all tests. The hydrogen-ion concentration of the haemolymph and midgut contents of diseased larvae were measured by pH indicators.

Larval source and treatments

Prodenia litura (F.) (Noctuidae).—A common pest of tobacco, castor, cauliflower, cabbage. The larval stages were reared in the laboratory at a temperature of 24–29° C., and 50–70% R.H. Pairs of newly emerged moths were confined in oviposition cages with potted castor plants. Moths readily laid clusters of eggs on leaves. Larvae on hatching from the egg clusters were transferred to fresh leaves every day. Four replications of 25 1st instar larvae each, were used for pathogenicity tests. Castor leaves treated with Bakthane L 69 were exposed to larvae for 36 hours and then provided with fresh leaves for 7 days or until death. In the control, the leaves were treated with distilled water containing sticker. In the case of 2nd, 3rd, and 4th instars both the bacterial insecticides were tested. The later instars were starved for 6 hours piror to exposure. Six replications, each comprising of 10 larvae, were used in tests. The larvae were exposed to treated leaves for 24 hours and then fed on fresh leaves up to 10 days; the surviving caterpillars were taken as unaffected. A layer of soil was provided in rearing cages for pupation.

Papilio demoleus L. (Papilionidae).—A serious pest of citrus plants, particularly in the seedling stage. Eggs were collected in sufficient numbers from citrus nurseries and the larvae were reared in the laboratory on citrus leaves. Larvae were divided into three groups, *viz.*, 1st and 2nd instars, 3rd instar, 4th and 5th instars. Five larvae from each of these groups were

picked at random and exposed to treated citrus leaves for 36 hours, using four different spore concentrations.

Plusia orichalcea F. (Noctuidae).—A serious pest of cruciferous and leguminous crops. 2nd instar larvae collected in the field were exposed to leaves of *berseem* treated with Bakthane L 69 for 36 hours. These were then transferred to fresh *berseem* (alfalfa) leaves and kept under observation for about 6 days. Replicates in these tests consisted of 10 larvae each.

Leucinodes orbonalis Guen. (Pyralidae).—Commonly known as *brinjal* borer, boring the shoots and fruits of the egg plant *Solanum melongena.* Different stages were collected in the field and young shoots of egg plant treated with Bakthane L 69 were provided to the larvae. Six replications of 5 larvae each were used and the observations were continued up to 72 hours after ingestion of spores. Mortality counts were recorded after splitting open the shoots.

Chilo partellus (Swinh.) (Pyralidae).—A common stalk borer of maize and sorghum. Different stages were collected from sorghum fields and were used in these tests. Maize stems after splitting, were treated with Bakthane L 69 and the larvae exposed to treated stems for 36 hours. They were later transferred to fresh untreated pieces of stem and observations continued for 72 hours.

Results and Discussion

The first instar larvae of *P. litura* were most susceptible to Bakthane L 69. A high mortality occurred 2–3 days after ingestion of spores, while 98% accumulative mortality was obtained after 7 days (100 larvae, 50 in control). The results of tests designed to determine the susceptibility of 2nd, 4th and 5th instars of *P. litura* are summarised in Table I. Most of the 2nd instar larvae died on third and fourth days with no appreciable mortality after the sixth day. Bakthane L 69 showed slightly higher pathogenicity for the 2nd instars as compared to Thuricide, while at higher concentrations of both bacterial concentrations, the larvae were moderately susceptible. The emergence of moths from survivals showed that higher concentrations of Bakthane L 69 adversely affected moth emergence; the moths which emerged from Thuricide-treated larvae had deformed and stunted wings, and were short-lived.

All larval stages of *P. demoleus* were highly susceptible to the bacterial insecticides; a 100% mortality occurred in every case in less than 24 hours after ingestion of spores, at same concentrations as under test in Table I.

TABLE I

Susceptibility of larvae of P. litura *to* B. thuringiensis (60 *larvae per treatment*) Mortality after 10 days

Spores/ml.$\times 10^7$	Bakthane L 69		Thuricide	
	2nd instar	4th and 5th instars	2nd instar	4th and 5th instars
$18{\cdot}75\times 10^7$	51·66	36·66	20·00	23·33
$37{\cdot}50\times 10^7$	70·00	50·00	38·33	36·66
$56{\cdot}25\times 10^7$	90·00	63·33	51·66	40·00
$75{\cdot}00\times 10^7$	96·66	68·66	80·00	46·66
Control	1.66	6.66	..	..

Against *P. orichalcea*, Bakthane L 69 was found highly pathogenic to 2nd instar, most of the mortality occurring within 48 hours after ingestion of spores (concentration of $37{\cdot}5\times 10^7$; 50 larvae, 20 in control).

The early larval stages of *L. orbonalis* gave same results as the foregoing species, whereas the late instars had low pathogenicity. Most of the early larvae which had ingested the spores failed to bore in, while those which succeeded in burrowing died within the shoots.

The results of tests on *C. partellus* indicated that Bakthane L 69 was moderately pathogenic for the early larval instars (90% mortality after 72 hours; 50 larvae, 25 in control).

On the basis of results obtained it is evident that all larval stages of *Papilio demoleus* were highly susceptible to both the bacterial preparations, while *Prodenia litura* showed moderate susceptibility. *Plusia orichalcea, Leucinodes orbonalis* and *Chilo partellus* had shown low susceptibility to Bakthane L 69. These results suggest that *P. demoleus* may be controlled in the field by *B. thuringiensis* products.

The hydrogen-ion concentration of the haemolymph of *P. demoleus* larvae increased within 60 minutes after ingestion of spores (Fig. 1). There was cessation of feeding when pH rose to 6·7. In about 150 minutes the larva showed signs of general paralysis and it was moribund when the pH rose beyond 7·0. This is in agreement with the findings of other workers

on *Bombyx* which are summarised by Heimpel and Angus (1959), though the paralysis developed at a slower rate in *P. demoleus*. On comparing the symptoms of *P. demoleus* larva, it can be grouped under category I of Heimpel and Angus (1959), in that the larvae exhibited fairly rapid paralysis followed by an increase of blood alkalinity. Amlost all the larval stages stopped feeding within 90 minutes after ingestion of spores, followed by regurgitation and diarrhœa containing undigested plant tissues. There was a sign of gut paralysis, apparently masked by the general paralysis as observed by Heimpel and Angus (1959). On the other hand, *P. litura* larvae which had ingested bacterial spores did not show any apparent sign of paralysis or change in pH of the haemolymph. The infected larvae stopped feeding in about 24 hours and septicemia set in in about 72 hours, followed by death. *P. litura* may be placed under Group III of Heimpel and Angus.

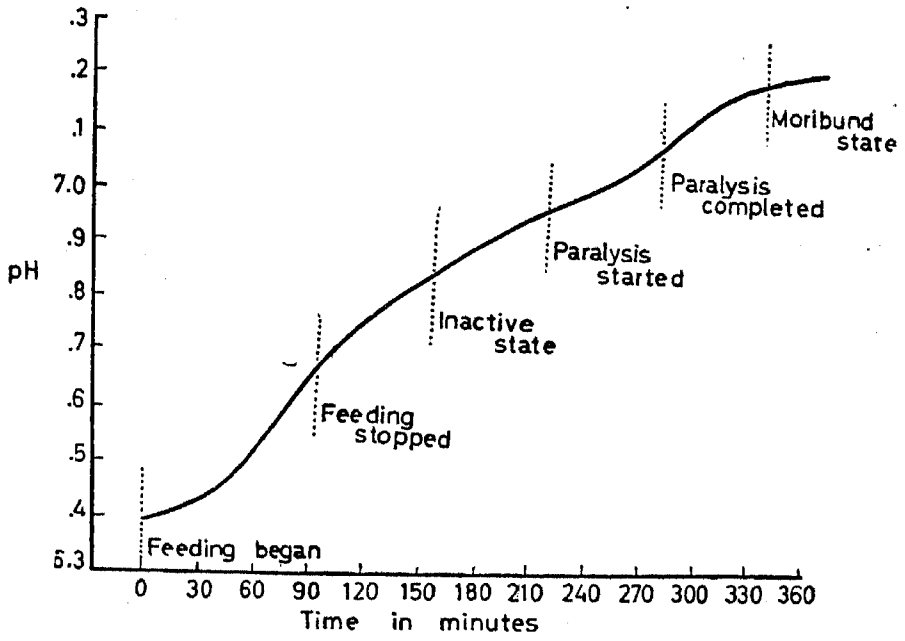

FIG. 1. Changes in hydrogen-ion concentration of the blood of *P. demoleus* after ingestion of *B. thuringiensis* spores.

A study of the hydrogen-ion concentration of the larval gut of *P. demoleus* may throw further light on the mode of action. Heimpel and Angus (1963) stated that the different results reported by various workers on *B. thuringiensis* are not strictly comparable in that they were derived by a variety of methods, and the response of a species is modified by various factors. There is need for fundamental studies of the toxic action of the crystals present in the different strains of *B. thuringiensis* in order to standardize the different commercial products. There is also a necessity to carry out more trials against a great

number of pest species over a wide area of the world as pointed by Steinhaus (1959).

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