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## MICROBIAL CONTROL

### 1. Laboratory Tests on the Susceptibility of the Lepidopterous Pests of Cabbage in Ceylon to commercially prepared *Bacillus Thuringiensis* Berliner

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#### INTRODUCTION

OVER emphasis on the control of insect pests by chemicals had retarded the development of other methods of control, particularly, that based on the use of biological agents. The fact that chemical control tends to magnify the problems it contends with, has convinced many entomologists of the need to use chemicals with more caution than has been done in the past. There is a growing body of evidence that could be adduced to support this line of thought and bolster up the view that pest control be based, as far as possible, on natural controls. With an awakening of interest in biological methods of control, chemicals could be employed in such a way as to supplement rather than replace these natural controls.

At present, more highly toxic insecticides are being used in Ceylon, for the control of Cabbage pests, than for those of any other economic crop. In general, unrestricted application of insecticides induce innumerable deleterious side effects such as—

1. Causing secondary outbreaks of arthropods, other than those, against which control was originally directed.
2. Producing a rapid resurgence of treated species, which necessitates repeated application of insecticides.
3. Producing strains that are resistant to insecticides.
4. Destroying beneficial insects, for example, parasites, predators and pollinators.

Apart from the above drastic side effects, excessive treatment aggravates the problem of residues, hazards to persons, livestock and wild life subjected to contamination by drift. Toxic insecticide

residues on food are injurious to human beings, but the precise nature of the harm done is yet unknown. In Ceylon, the problem of residues on crops is even more serious, in that at present there is no legislation in force to supervise and control the use of insecticides, and run on the lines adopted by the Food and Drugs Administration, Department of Health, Education and Welfare in the United States of America.

It seems worthwhile therefore, to investigate any new material or method of control that would not induce any of the adverse effects of chemicals and which offer the possibility of reduced chemical residues.

### BACTERIAL DISEASES USED IN INSECT CONTROL

The existence of bacterial diseases in insects had been known from very early times. Entomogenous bacteria pathogenic to insects have been isolated by many workers, Berliner (1911), d'Herelle (1911), Mattes (1927), Metalnikov and Chorine (1928, 1929), and Steinhaus (1941). Of these entomogenous bacteria, the spores of *Bacillus popilliae* isolated from the Japanese beetle, *Popillia japonica* (Newman) (Dutky, 1940) and *Bacillus thuringiensis* isolated from the Mediterranean flour moth, *Anagasta* (*Ephestia*) *Kuhniella* Zeller, are now being produced on a commercial basis.

### BACILLUS THURINGIENSIS BERLINER

*Bacillus thuringiensis* is a rod bacteria morphologically related to a common spore-forming soil bacterium *Bacillus cereus*. However, it differs from the latter by belonging to the group that forms crystalline parasporal bodies. The pathogenicity of *Bacillus thuringiensis* appears to be connected with the protein crystal or parasporal body found in the sporangium along with the endospore. The spindle shaped crystalline inclusion which is freed from the cell with the spore persists indefinitely and is extremely toxic to the gut of the larvae.

The mode of action of *Bacillus thuringiensis* is not completely known. Although it was at first thought that the bacillus caused a septicaemia which was followed by death, more recent work by Heimpel and Angus (1959) suggests the mode of action to be much more complex.

According to these workers, there are three modes of action each related to the type of insect ingesting the spores. In type I group of suspects, e.g., *Bombyx mori*, *Protoparce quinquemaculata* and *P.*

*sexta*, the insects become stricken by a general paralysis on ingesting the bacillus. The toxic parasporal bodies are believed to damage the gut wall thereby producing a leakage of the alkaline gut contents into the blood. This is followed by a rise in the blood pH which induces the paralysis. In type II, *Bacillus thuringiensis* induces a general gut paralysis which stops persistalsis and consequently ingestion of more food, causing death in 2 to 4 days. *Nymphalis antiopa* and *Malacosoma disstria* fall into this category. *Anagasta kuhniella* is the sole representative of type III. In this case, the invasion of the body cavity by the vegetative cells produces a true septicaemia.

*Bacillus thuringiensis* is very virulent towards a number of lepidopterous insect species. With continued research, however, its host range may widen to include suspects among the Diptera and Coleoptera. It has so far, demonstrated pathogenicity, for *Colias philodice eurytheme* Boisduval (Steinhaus, 1951); the imported cabbage worm, *Pieris rapae* Linnaeus and the diamond back moth, *Plutella maculipennis* Curt. (Tanada, 1956); the codling moth, *Carpocapsa pomonella* Linnaeus (Stephens, 1952) and the horn worm, *Protoparce quinque-maculata* Haworth and *P. sexta* Johanssen (Rabb, 1957). *Bacillus thuringiensis*, however, is reported to have shown limited effectiveness in the control of the cabbage webworm, *Hellula undalis* F. and the cabbage looper, *Trichoplusia ni* Hubner (Tanada, 1956).

### CABBAGE CATERPILLAR PESTS OF CEYLON

Eight species of leaf eating caterpillars have been recorded on cabbages in Ceylon, but *Plutella maculipennis* Curt. (Plates I and II) *Plusia eriosoma* Daubl, *Crocidolomia binotalis* Z. and *Hellula undalis* appear to be the four most predominant and widely spread species causing considerable damage to cabbages.

Field and Laboratory observations show that *Plutella maculipennis* is subjected to a high degree of parasitisation by the braconid wasp, *Apanteles plutellae* Kurdj. (Plate III). The latter has been recorded to possess some of the attributes that mark the efficient parasite. According to Lloyd (1940), *Apanteles plutellae* had shown considerable discrimination and frequently rejected hosts already parasitised. Another parasite, *Anilastus* species, belonging to the family Ichneumonidae was also recorded on *Plutella maculipennis*.

The cabbage looper, *Plusia eriosoma* was also observed to be effectively checked by a biological agent. In this case, the entomogenous fungus *Spicaria rileyi* (Farlow) Charles (Plate IV) belonging to the class *Deuteromycetes* was the controlling factor. Since, the fungus

was observed to be more prevalent in insect populations of high densities, it is construed, that certain of the effects of this micro-organism are dependent upon the density of the host population. Therefore, *Spicaria rileyi* acts as a density-dependent control factor.

*Spicaria rileyi* was also observed to be prevalent only in certain parts of the up country districts. In other words, the environmental limits of this widely distributed micro-organism was confined to narrow zones. This may be explained by the fact that the ecological niche of *Spicaria rileyi* may be so constituted that the occurrence of proper conditions of nutrition, temperature and humidity are limited. A thorough study, of the epizootiology of this disease causing fungus will however be necessary, before an evaluation of its efficiency, as a regulatory factor of insect populations, could be accurately assessed.

### METHODS AND MATERIALS

The materials used in these tests were samples of commercially prepared *Bacillus thuringiensis* Berliner received from Rohm and Haas Company, Philadelphia 5, Pennsylvania, U.S.A. Their product Bakhane L-69 contains as its active ingredient 75 billion viable spores of *Bacillus thuringiensis* per gram of inert powder. Determination of potency based upon the number of viable spores per gram of dry material was not checked by germination tests.

All the test insects were field collected, obtained from the Welimada-Nuwara Eliya districts. Treated and control insects were caged separately, in sterilised glass tubes three inches long with a diameter of one inch. The test insects were inoculated with the bacterium *per os*. They were provided with four circular pieces of cabbage leaf having an approximate area of 3.142 sq. inches each. The cabbage leaves were dipped in a suspension of the pathogen one and a half hours before they were used for feeding. Four concentrations of the pathogen, 1 gram; 0.75; 0.5 and 0.25 gram respectively, of the dry powder mixed with a standard quantity of wheat flour in 100 ml water was used. Larvae that had devoured the treated leaves provided at the commencement of the experiment, were supplied with fresh un-contaminated leaf thereafter. All the larvae that died during the period of observation after treatment, were examined microscopically, especially to determine if their death was caused by the pathogen. In cases of doubt, infection was confirmed by recovering the bacillus on plates of nutrient agar. Records were kept of the number of individuals that died during a period of 1-4 days and of the number of individuals parasitised.

SUSCEPTIBILITY OF THE LEPIDOPTEROUS PESTS OF CABBAGE IN CEYLON  
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## RESULTS

The present study was confined to an evaluation of susceptibility to infection of the four most predominant species. However, a few individuals, ranging from 5 to 10 in number, of other species of caterpillars, that included *Agrotis ypsilon* Roh., *Euxoa segetum* Schiff. and *Prodenia litura*, were tested at a concentration of 0.25 gram per 100 ml water and were found to be highly susceptible.

In the first laboratory test 120 first and second instar *Plutella* larvae were tested. Sixty of these were treated and 60 were maintained as checks. The results of the test are tabulated in Table I.

*Bacillus thuringiensis* was found to be very virulent on the larvae, killing them within a period of 24 hours. The high incidence of deaths recorded in the controls are presumed to be due to mechanical injuries, incurred during handling and transfer into tubes. No symptoms of bacillary infection was observed in them.

In the second laboratory test, 225 fifth instar larvae of each of the three pests, *Plutella*, *Hellula*, and *Crocitolomia* were tested. Groups of 45 larvae of the three species were tested at each level of treatment and 45 were maintained as a check. The results of these tests are tabulated in tables II, III and IV.

The results show that the bacillus is very virulent on *Plutella* and *Crocitolomia* at all four levels of treatment. *Hellula undalis* however, seemed to be less susceptible than the other caterpillars at the lowest dosage level. Death in all three cases took place in approximately 1-3 days.

Since there was some difficulty in obtaining healthy specimens of *Plusia* from the field owing to a high incidence of infection by the fungus *Spicaria rileyi*, only 75 individuals of this species were tested. The results of this test, tabulated in Table VI, shows that *Plusia* is quite susceptible to the bacillus. In this case too, death occurred in 1-3 days.

The laboratory tests seem to indicate that *Bacillus thuringiensis* is capable of bringing about a marked reduction in the larval populations of the four insect species that go to form the bulk of the "caterpillar complex" on cabbages. On the other hand, the present study has revealed that there is a certain amount of natural control of these insects by either the fungus *Spicaria* or the baconid parasite, *Apanteles plutellae*. Combination control by the two agents may be therefore possible, when *Bacillus thuringiensis* is used, in areas where the parasite or fungus is giving partial control.

In evaluating the results of control obtained with *Bacillus thuringiensis*, the nature of its action has to be considered somewhat differently from that of insecticides. Insects treated with the bacillus generally stop feeding within a few hours after ingesting the spores, but actual mortality takes place within 24 to 48 hours. The failure of the bacillus to destroy a pest species almost immediately as is the case with most insecticides, is compensated by the fact that feeding ceases within minutes of the application.

In order to demonstrate its effectiveness in slowing down feeding activity, the percentage of the foliage consumed, within a 24 hour period, by 15 individuals of *Plutella* and 15 individuals of *Plusia* from each of the four levels of treatment was compared with that of the controls. The results of the comparison are tabulated in Table VI.

### DISCUSSION

Although, the use of pathogenic micro-organisms is still in its infancy the fact that they do not as a rule directly effect other beneficial organisms such as parasites and predators, aside from giving good control, make them very valuable weapons that could be utilised in our fight against insect competitors for the food resources of the earth. Tanada (1956) for instance, has reported that pathogenic micro-organisms, applied to crucifers, for the control of four insect pests, had no marked effect on insect parasites and predators, especially those attacking hosts other than the four insects studied. For this reason, it is believed that *Bacillus thuringiensis*, if utilised, would play an important role in future control programs.

In recent years there have been numerous examples of the adverse effects of insecticides on insect parasites and predators resulting in high host density. While, on the one hand, the use of chemicals, ignoring its impact on both beneficial and harmful insects, is short-sighted, it is unrealistic to think that biological control is a panacea for all problems of insects. A method of control, that would combine the use of chemicals and biological agents was therefore forthcoming.

Unfortunately, for a long time, these two approaches had been looked upon as two alternatives in pest control. One of the reasons for the apparent incompatibility of biological and chemical control is the failure to recognise control of insects as a complex problem in ecology (Smith and Hagen, 1959). According to these workers, chemical control and biological control can be used in a complementary manner. The ecological approach that makes full use of biological

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controls, and chemicals where necessary and in a manner least distruptive to the former, has been termed Integrated control (Smith and Hagen, 1959).

The great expansion in interest in the utilisation of micro-organisms such as *Bacillus thuringiensis* opens new horizons for adopting the integrated control method which undoubtedly has enormous advantages and promise for the future. The scope for the use of micro-organisms in integrated control programs appear to have been widened in recent times with the work of Telenga (1958), in Russia who has reported that the combination sprays containing insecticides and micro-organisms gives greater control than either used alone.

### SUMMARY

*Plutella maculipennis* Curt., *Crocidolomia binotalis* Z., *Hellula undalis* and *Pulsia eriosoma* appear to be the four most predominant and widely spread species attacking cabbages in Ceylon.

2. *Hellula undalis*, *Crocidolomia binotalis* and particularly *Plutella maculipennis* were subjected to a high degree of parasitization by the braconid wasp, *Apanteles plutellae* not previously reported.

3. The Ichneumonid, parasite, *Anilastus* sp. was also reared from *Plutella maculipennis*.

4. *Plusia eriosoma* was found to be affectively checked by the entomogenous fungus *Spicaria rileyi*.

5. Laboratory tests show that *Bacillus thuringiensis* is very effective on the four most important species of caterpillars attacking cabbages.

6. Combination control by biological control agents and the bacillus may be possible when the micro-organism is used in areas where the parasite or fungus is giving partial control.

7. It is believed that the great expansion in interest in the utilisation of micro-organisms such as *Bacillus thuringiensis* would open new horizons for adopting the integrated method of control.

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TABLE I

Susceptibility of 1st and 2nd instar larvae of *Plutella* to infection by *B. thuringiensis*

| Dosage (grams of<br>Bakthane L-69/<br>100 ml. water) |    | Number<br>tested | Killed by bacillus |      | Parasitised |   | % alive at the end<br>of two weeks |
|------------------------------------------------------|----|------------------|--------------------|------|-------------|---|------------------------------------|
|                                                      |    |                  | No.                | %    | No.         | % |                                    |
| 1 gram                                               | .. | 15               | .. 15              | 100  | .. 0        | 0 | 0                                  |
| 0.75 gram                                            | .. | 15               | .. 15              | 100  | .. 0        | 0 | 0                                  |
| 0.50 gram                                            | .. | 15               | .. 14              | 93.3 | .. 0        | 0 | 0                                  |
| 0.25 gram                                            | .. | 15               | .. 14              | 93.3 | .. 0        | 0 | 0                                  |
| Control                                              | .. | 60               | .. 6*              | 0    | .. 0        | 0 | 90                                 |

TABLE II

Susceptibility of 4th—5th instar larvae of *Plutella* to infection by *B. thuringiensis*

| Dosage (grams of<br>Bakthane L-69/<br>100 ml. water) |    | Number<br>tested | Killed by bacillus |      | Parasitised |      | % alive at the<br>end of two weeks |
|------------------------------------------------------|----|------------------|--------------------|------|-------------|------|------------------------------------|
|                                                      |    |                  | No.                | %    | No.         | %    |                                    |
| 1 gram                                               | .. | 45               | .. 30              | 66.7 | .. 15       | 33.3 | 0                                  |
| 0.75 gram                                            | .. | 45               | .. 31              | 68.9 | .. 13       | 28.7 | 2.2                                |
| 0.50 gram                                            | .. | 45               | .. 32              | 71.1 | .. 7        | 15.6 | 13.3                               |
| 0.25 gram                                            | .. | 45               | .. 36              | 80.0 | .. 4        | 8.9  | 11.1                               |
| Control                                              | .. | 45               | .. 1*              | 0    | .. 20       | 44.4 | 53.3                               |

\*Death due to causes other than bacillary infection.

TABLE III

Susceptibility of 4th—5th instar larvae of *Helicula* to infection by *B. thuringiensis*

| Dosage (grams of<br>Bakthane L-69/<br>100 ml. water) |    | Number<br>tested | Killed by bacillus |     |    |      | Parasitised |   |    |      | % alive at the<br>end of two weeks |      |
|------------------------------------------------------|----|------------------|--------------------|-----|----|------|-------------|---|----|------|------------------------------------|------|
|                                                      |    |                  | No.                |     | %  |      | No.         |   | %  |      |                                    |      |
| 1 gram                                               | .. | 45               | ..                 | 38  | .. | 84.4 | ..          | 6 | .. | 13.3 | ..                                 | 2.2  |
| 0.75 gram                                            | .. | 45               | ..                 | 41  | .. | 91.1 | ..          | 2 | .. | 4.4  | ..                                 | 4.4  |
| 0.50 gram                                            | .. | 45               | ..                 | 38  | .. | 84.4 | ..          | 4 | .. | 8.9  | ..                                 | 6.7  |
| 0.25 gram                                            | .. | 45               | ..                 | 29  | .. | 64.4 | ..          | 6 | .. | 13.3 | ..                                 | 22.2 |
| Control                                              | .. | 45               | ..                 | 12* | .. | 0    | ..          | 2 | .. | 4.4  | ..                                 | 68.9 |

TABLE IV

Susceptibility of 4th—5th instar larvae of *Crocidolomia* to infection by *B. thuringiensis*

| Dosage (grams of<br>Bakthane L-69/<br>100 ml. water) |    | Number<br>tested | Killed by bacillus |     |    |      | Parasitised |   |    |     | % alive at the<br>end of two weeks |      |
|------------------------------------------------------|----|------------------|--------------------|-----|----|------|-------------|---|----|-----|------------------------------------|------|
|                                                      |    |                  | No.                |     | %  |      | No.         |   | %  |     |                                    |      |
| 1 gram                                               | .. | 45               | ..                 | 34  | .. | 75.6 | ..          | 4 | .. | 8.9 | ..                                 | 15.6 |
| 0.75 gram                                            | .. | 45               | ..                 | 44  | .. | 97.8 | ..          | 1 | .. | 2.2 | ..                                 | 0    |
| 0.50 gram                                            | .. | 45               | ..                 | 43  | .. | 95.6 | ..          | 2 | .. | 4.4 | ..                                 | 0    |
| 0.25 gram                                            | .. | 45               | ..                 | 40  | .. | 88.9 | ..          | 3 | .. | 6.7 | ..                                 | 4.4  |
| Control                                              | .. | 45               | ..                 | 20* | .. | 0    | ..          | 4 | .. | 8.9 | ..                                 | 46.7 |

\*Death due to causes other than bacillary infection.

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TABLE V

Susceptibility of 4th—5th instar larvae of *Plusia* to infection by *B. thuringiensis*

| <i>Dosage (gram of<br/>Bakthane L-69/<br/>100 ml. water)</i> |    | <i>Number<br/>tested</i> | <i>Killed by bacillus</i> |    |          |      | <i>Parasitised</i> |    |          |      | <i>% alive at the<br/>end of two weeks</i> |   |
|--------------------------------------------------------------|----|--------------------------|---------------------------|----|----------|------|--------------------|----|----------|------|--------------------------------------------|---|
|                                                              |    |                          | <i>No.</i>                |    | <i>%</i> |      | <i>No.</i>         |    | <i>%</i> |      |                                            |   |
| 1 gram                                                       | .. | 15                       | ..                        | 11 | ..       | 73.3 | ..                 | 4  | ..       | 26.7 | ..                                         | 0 |
| 0.75 gram                                                    | .. | 15                       | ..                        | 12 | ..       | 80   | ..                 | 3  | ..       | 20   | ..                                         | 0 |
| 0.50 gram                                                    | .. | 15                       | ..                        | 14 | ..       | 93.3 | ..                 | 1  | ..       | 6.7  | ..                                         | 0 |
| 0.25 gram                                                    | .. | 15                       | ..                        | 8  | ..       | 53.3 | ..                 | 7  | ..       | 46.7 | ..                                         | 0 |
| Control                                                      | .. | 15                       | ..                        | 4* | ..       | 0    | ..                 | 11 | ..       | 73.3 | ..                                         | 0 |

\*Death due to causes other than bacillary infection.

TABLE VI

Comparison of % foliage consumed during a 24-hour period by treated and untreated  
*Plutella* and *Plusia* larvae

| Insect          |    | Dosage<br>(Grams of <i>Bakthane</i><br><i>L-69</i> /100 ml. water) | Percentage foliage consumed |    |      |     |      |
|-----------------|----|--------------------------------------------------------------------|-----------------------------|----|------|-----|------|
|                 |    |                                                                    | Av.                         |    | Max. |     | Min. |
| <i>Plutella</i> | .. | 1                                                                  | ..                          | 13 | ..   | 25  | 0    |
|                 | .. | 0.75                                                               | ..                          | 13 | ..   | 50  | 0    |
|                 | .. | 0.50                                                               | ..                          | 15 | ..   | 70  | 0    |
|                 | .. | 0.25                                                               | ..                          | 30 | ..   | 50  | 0    |
|                 | .. | Control                                                            | ..                          | 30 | ..   | 50  | 0    |
| <i>Plusia</i>   | .. | 1                                                                  | ..                          | 13 | ..   | 50  | 0    |
|                 | .. | 0.75                                                               | ..                          | 10 | ..   | 25  | 0    |
|                 | .. | 0.50                                                               | ..                          | 15 | ..   | 60  | 0    |
|                 | .. | 0.25                                                               | ..                          | 15 | ..   | 50  | 0    |
|                 | .. | Control                                                            | ..                          | 70 | ..   | 100 | 50   |

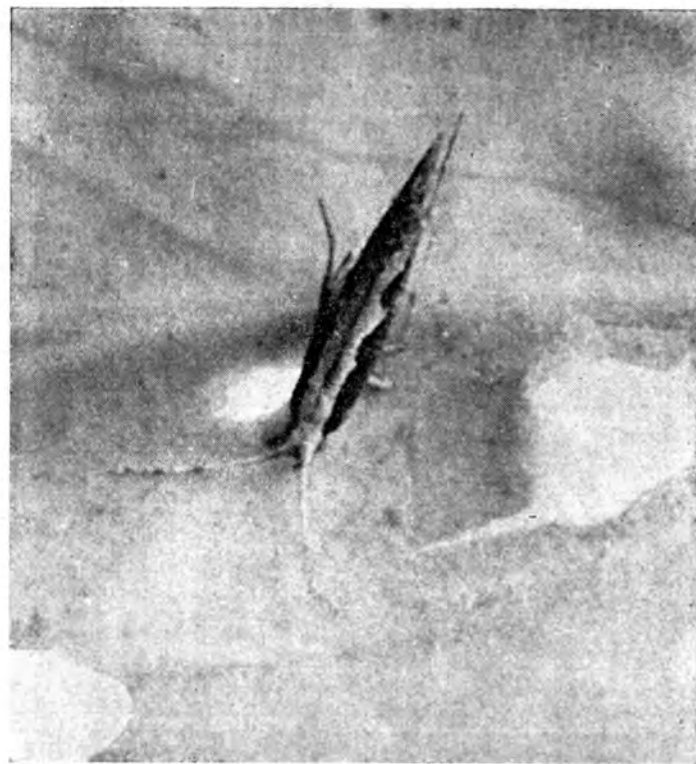


Plate I.—The adult Diamond Back Moth,  
*Plutella maculipennis* Curt.

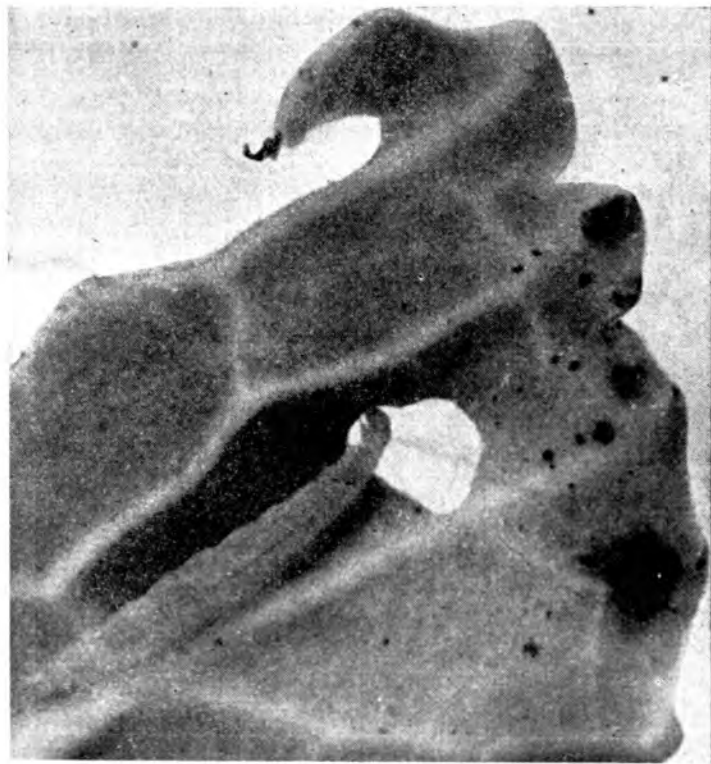


Plate II.—Shows the characteristic damage caused by  
the larva of *Plutella*.

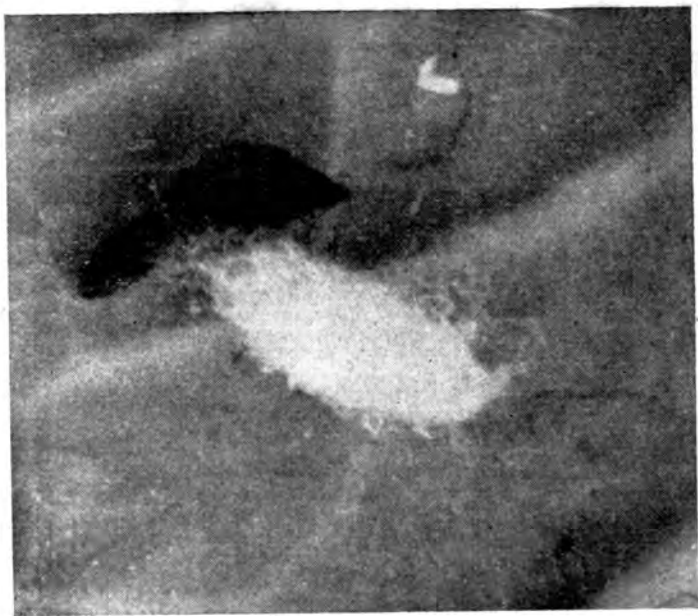


Plate III.—The larva of *Plutella* with the attached cocoon of the parasite *Apanteles plutellae* Kurdj., which emerged from its body (much enlarged).

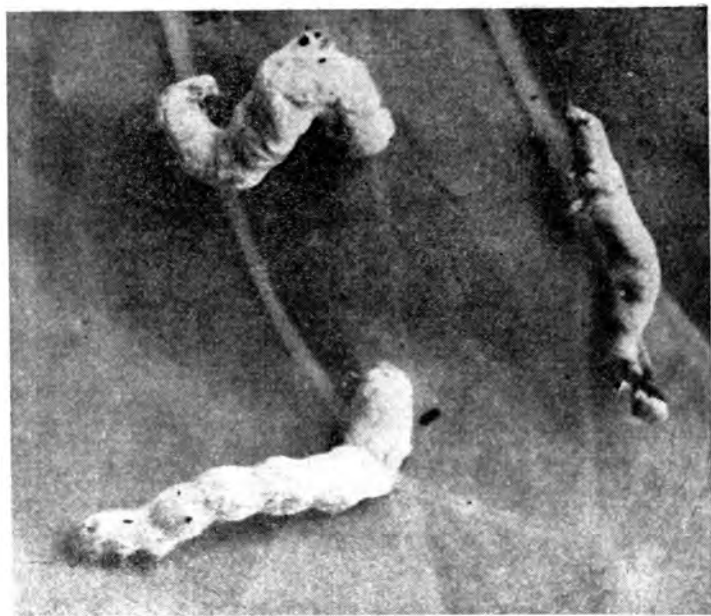


Plate IV.—The Cabbage looper, *Plusia eriosoma* Daubl. killed by the fungus, *Spicaria rileyi*.

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Larvae of the Diamond Back Moth *Plutella maculipennis* Curt. and the Cabbage Looper, *Plusia eriosoma* Daubl. dead of infection with *Bacillus thuringiensis*.