A GRANULOSIS VIRUS ATTACKING THE LARVAE OF *PERSECTANIA EWINGII* WESTW. (LEPIDOPTERA : AGROTIDAE) IN SOUTH AUSTRALIA

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Summary

An epizootic of a virus disease in a larval population of *Persectania ewingii* Westw. (Lepidoptera : Agrotidae) in South Australia is described, and possible methods of its transmission in the population are suggested. The symptomatology of the disease during its progress is outlined. A description of the virus and its structure follows. The aetiology of the disease is described, and the paper concludes with a brief history of 5000 diseased larvae which were collected in the field.

I. INTRODUCTION

In the course of investigations into the biology of *Persectania ewingii*, the author came upon a severe epizootic of virus disease in a dense population of the larvae inhabiting a little valley near the township of Cherry Gardens, in the Mt. Lofty Ranges, some 20 miles south of Adelaide. The population was an isolated one, no other larvae of this species being found within 20 miles of the locality.

The area is typical tussock grassland (*Stipa pubescens* R.Br. the dominant), with a few scattered gum trees, and the larvae had eaten practically all the current season's growth of grass. There remained chiefly the tall, dry stalks which, in the previous year, had borne inflorescences, and each stalk carried one or more dead larvae.

II. METHOD OF SAMPLING, AND COMPOSITION OF POPULATION

Numerous living larvae, principally in their two final instars (fifth and sixth) were found in masses beneath frass and debris surrounding the bases of the grass plants, or under bark on the ground. To determine the degree of infection, 5168 larvae were collected, some from every type of minor habitat within the area.

These comprised 5141 recognizably diseased fifth and sixth instar larvae, and 27 fourth instar larvae which, although showing no symptoms of the disease at the time, developed it later. This uniformity of age composition of the population is characteristic of first generation larvae of this species. In "outbreak years," the adults emerge from their pupae in May, and the females oviposit and die within a fortnight. This results in the majority of larvae, in any one area, being within an instar of each other during development. The small number of younger larvae probably resulted from delayed emergence of a few

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adults. The larvae were brought back to the laboratory where 168 moribund specimens were used as a source of virus for infecting healthy larvae. The remaining 5000 were placed in cages for observation.

III. Transmission of the Virus

It was found that if larvae in any instar, from known healthy laboratory colonies, were placed in a cage with diseased larvae, or in one from which diseased larvae had recently been removed, all contracted the disease. Under these conditions, the possible modes of transmission appeared to be the eating of food contaminated with (1) body fluid from dead larvae, (2) saliva left on food by diseased larvae, (3) faeces of diseased larvae. In the field there are two additional possibilities: (4) the cannibalistic habits of starving larvae, and (5) through the agency of the ovipositors of parasitic Hymenoptera. These possibilities, except the fourth, were separately investigated. Transmission through cannibalism could not be tested since the disease is so infectious that proven agents of transmission could not, at the same time, be excluded. From the tests carried out, however, it appears certain that infection would follow the feeding of a healthy larva on a diseased one.

Attempts to inoculate healthy larvae, by piercing them with a needle previously thrust into diseased ones, met with varying success. The fact that results were sometimes positive, however, indicates that the ovipositor of hymenopterous parasites may be a significant agent in transmitting the virus from one population to another.

Method 1

Diseased larvae, after being washed several times with distilled water, were ground in a mortar with a little distilled water. The mixture was filtered to get rid of gross particles. The slightly opalescent, yellowish brown filtrate was sprayed on fresh grass in a sterilized cage, and 50 healthy larvae in various instars were allowed to feed on it for 24 hr. They were then divided into five equal batches, placed in clean cages, and given the same treatment as those in the laboratory colonies from which they came. Every larva was infected, and all except two died in the fifth instar. The two which survived were in the fifth instar when infected. These ultimately produced moths.

Method 2

Grass on which diseased larvae had fed was washed with a little distilled water, the liquid filtered, and the filtrate sprayed on clean grass. The procedure of the first method was then followed. Twenty-four larvae were infected (3 — 6 — 1 — 7 — 7) and later died; the remaining 26 showed no disease symptoms, and subsequently pupated. One pupa failed to develop; no cause for this was found. Twenty-five apparently normal moths emerged from the remainder. It is necessary to point out here that, as larvae which are recognizably diseased do not feed, larvae which had been infected by the first method were used. As these all died later from the virus, it was assumed that they were infected at the time that the test was applied.

Method 3

Frass, from recognizably diseased larvae, was ground with distilled water, filtered, and the filtrate used as in the first method. The results were inconsistent; in the five batches the infected specimens were 0 — 0 — 0 — 7 — 1.

The above results suggest that, in nature, infection through the ingestion of food contaminated with either virus-containing saliva, or frass, is important in the transmission of virus among the individuals of a population. While the entry of body fluid, by any means, into the body of a larva, appears to cause certain infection, it is not normally present until some larvae have died. By this time, the part of the population which will contract the disease appears to be already infected.

IV. Symptomatology of the Disease

Young diseased larvae, and all healthy ones, have a bold colour pattern, consisting of a series of narrow, longitudinal stripes of yellow, white, and orange, on a velvety, brownish black background. No symptoms are observable until infected larvae reach their fifth or final instars. When symptoms appear in fifth instar larvae, these invariably die. When symptoms are delayed until the sixth instar, most larvae die but a few complete their development. Time of infection appears to be decisive here.

(*a*) *First Stage*

The initiation of the virulent phase of the disease is marked by cessation of feeding, and restlessness. The larva climbs a grass stalk or similar object, and then descends. It may do this many times until a suitable place is found. It then takes up an inverted position which it maintains by hooking the crochets of the terminal larvapodia in small crevices in the stalk. The final site is apparently determined by the object's having the necessary roughness to enable the crochets to take a firm hold. Pieces of narrow glass rod, straws thinly coated with hard paraffin, or thin sticks smoothed with emery cloth and painted with shellac are repeatedly climbed but never selected. A favoured position is where a leaf or a pedicel grows out from the stem. In cages, the metal gauze at the ends is most frequently chosen. Once in position, the larva contracts so that it is shorter and stouter than a healthy resting larva of the same size. Soon the whole of the body weight is supported by the terminal larvapodia, and in consequence the posterior segments become fully extended (Fig. 1A). The larva shown in Plate 1, Figure 1, is typical and was the model from which the series of text figures was copied. If disturbed, the larva will crawl down the stem and ascend another, but normally it remains where it has attached itself.

(*b*) *Second Stage*

Other changes now follow in quick succession; within 12 hr, the colour pattern has faded. The cuticle assumes a dull lead-grey coloration and becomes wax-like and translucent. Liquefaction of the body contents begins at the anal end so that the insect becomes suspended by the cuticle of the terminal segments.

The anterior part of the body becomes distinctly swollen (Fig. 1*B*). Even in this stage, locomotion is still possible. If sufficiently stimulated, the insect will slowly crawl down to the soil surface, but is unable to re-ascend. It merely climbs any object sufficiently high for its head to be above the soil when suspended, or dies on the soil surface. In the field, the majority of the larvae were in this condition, probably having been dislodged by high winds which had blown a day or so before the area was examined.

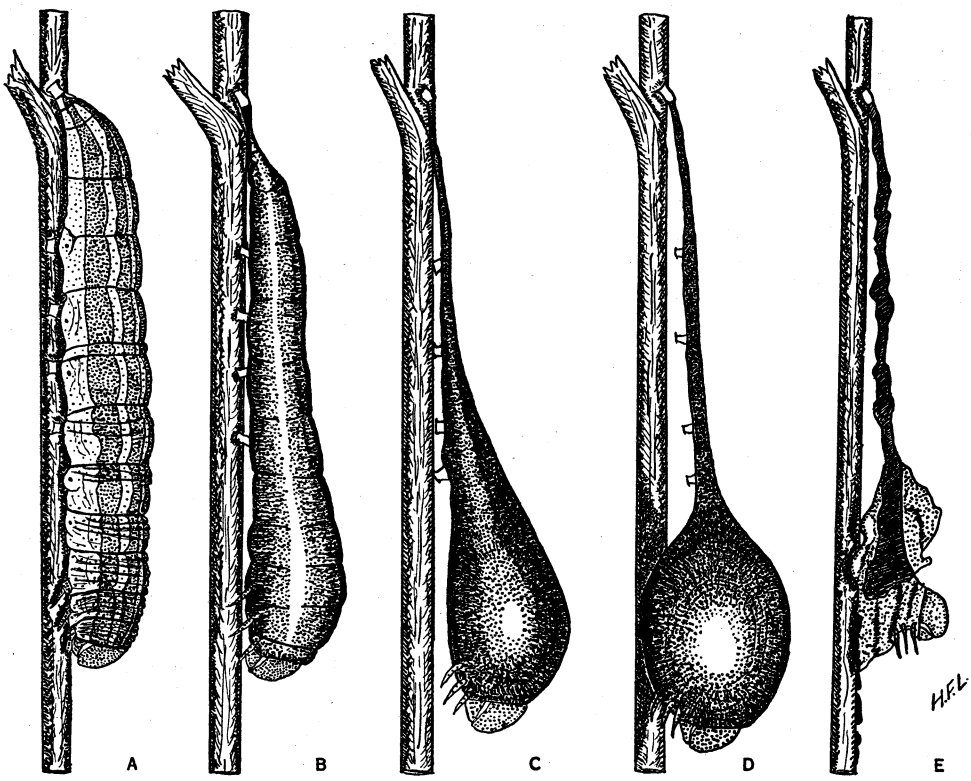
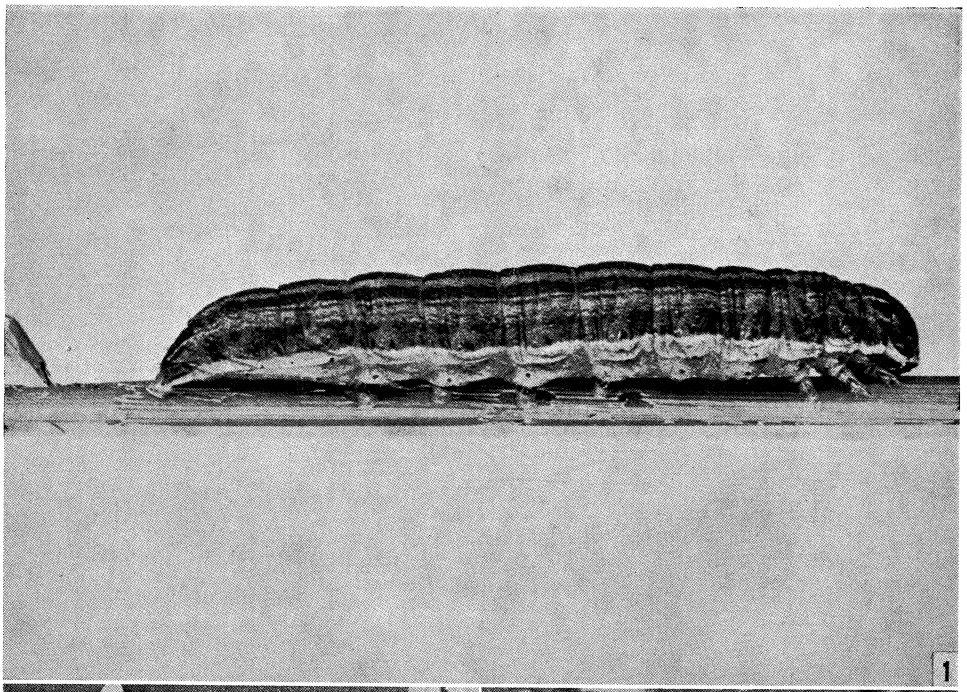


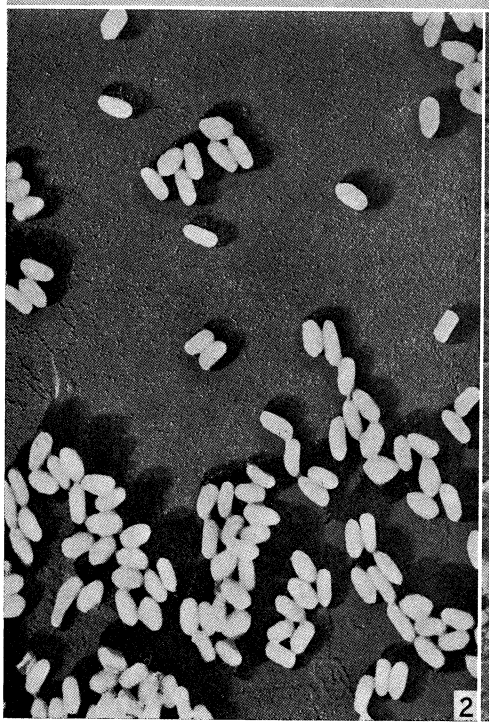
Fig. 1.—*A*, larva in position of final attachment. First stage of virulent phase. *B*, larva in second stage of virulent phase. Note colour changes and beginning of liquefaction at anal end. *C* and *D*, larva in third stage of virulent phase. In *D*, liquefaction is complete, *E*, rupture of cuticular sac and escape of dark brown body fluid.

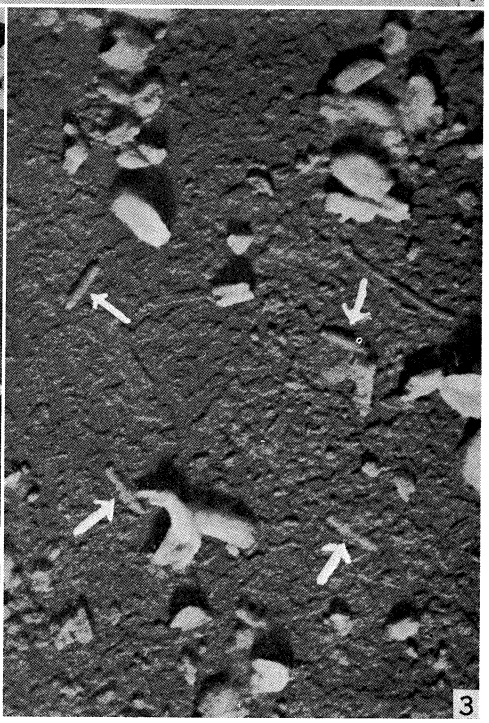
(*c*) *Third Stage*

Death occurs within 24 hr, with a progressive onset from posterior to anterior. Specimens are not uncommon which can still move the head and thoracic legs, but are incapable of moving any part of the body posterior to these. Liquefaction proceeds rapidly. The cuticle becomes uniformly blackish brown, and the anterior of the insect is distended to form a fluid-containing sac (Fig. 1*C*). Within a further 24 hr, liquefaction is complete, the swollen anterior being supported by the shrunken, collapsed cuticle which becomes

GRANULOSIS VIRUS DISEASE OF *PERSECTANIA*







delicate, and easily fractures (Fig. 1*D*). Soon, the sac ruptures, permitting the escape of a dark brown, odourless fluid. The dried, blackened cuticle remains attached until blown away by the wind or washed down by rain (Fig. 1*E*).

V. Internal Histological Changes and Description of the Virus

On dissection of a larva in the first stage of the virulent phase, the fat-body is seen to be greatly enlarged. The fatty material found in the fat-body of a healthy larva has largely disappeared and a dirty, whitish, opaque suspension fills the distended cells. Microscopic examination, under oil immersion, shows that the original contents of the cells have degenerated. The liquid which replaces them consists of numerous minute granules showing Brownian movement, suspended in an apparently colourless, clear fluid. No structural details of the particles can be observed.

(*a*) *The Granules*

Electron micrographs show the granules to be subovoid, elongate bodies with more or less parallel sides. They often appear to be united end to end in chains, but this is possibly an artifact (Plate 1, Fig. 2).

If treated with alkali, the granules swell and then rupture, and each is found to consist of a proteinaceous envelope enclosing a single rod of virus (Plate 1, Fig. 3).

This encapsulated virus body is typical of the granulosis group of insect viruses for which Steinhaus (1949) erected the genus *Bergoldia.* From the morphology of the granules, the species would appear to be closely allied to an as yet unnamed one found by Hughes and Thompson (1951) in the omnivorous looper (*Sabulodes caberata* Gn.).

The type material of the virus is retained in the collection of the University of California, Laboratory of Insect Pathology, Albany, California, U.S.A., where it will be available for eventual taxonomic study.

VI. Aetiology of the Disease

At present, there is no evidence to show how initial infection of the population occurred. Once this had taken place, however, certain predisposing factors may account for its rapid spread and for the high incidence of mortality in the area.

(1) The density of larval population was very high. At night no larva could wander more than a few inches without coming in contact with a neighbour, or crossing many times over the tracks of diseased larvae. The larval habit of congregating by day in dense masses round the bases of grass plants, down the stems of which body fluid from dead larvae was constantly being washed by rain and dew, placed them in a position ideal for being infected. Such contaminated grass, being the only available food, tended further to attract the larvae. The importance of crowding may be gauged from the fact that though

this species is subject to attack over wide areas, only an occasional larva contracts the disease when the population density is low.

(2) All larvae showed evidence of starvation; specimens which fed voraciously when supplied with food in the laboratory had very little food in the gut when collected. This condition would probably tend to lower their resistance to infection.

(3) Rainfall in the area is high (30 + in. average), and falls principally during the period of development of the species. While the epizootic was in progress both rainfall and number of cloudy days were much above average so that the larvae were exposed almost constantly to a mild, very humid atmosphere. In another locality where population density was at least as high, but where the rainfall for the corresponding period was only 11 + in., and bright sunny days were of frequent occurrence, not a single infected larva was found. There is, of course, the possibility that, in this case, the virus had not been introduced into the population.

VII. History of the Collected Larvae

Of the 5000 larvae collected, 27 mid-instar larvae showed no symptoms of the disease at the time of collection, but all developed it later; 4803 larvae died, and 195 attempted to pupate on the soil surface below litter. No cell-building in the soil, a characteristic of the normal larva when preparing to pupate, occurred. Two larvae could not be accounted for. The 195 which attempted pupation produced 51 adult moths and 144 parasites. The moths laid several thousand eggs but the larvae which hatched from these died in their third and fourth instars from a disease produced by a species of the *Bacillus cereus* group (identified by Dr. C. G. Thompson) before the presence or otherwise of virus could be determined.

Two interesting facts emerge from these results. The first is that the virus is not necessarily fatal to every infected larva. Dr. Thompson informs me that he has found that if a virus disease is contracted by the larvae of some species towards the end of their larval period, some may complete their development. This applies also to *P. ewingii*.

The second significant fact is that some parasites at least are immune to the virus. Of the 144 adult parasites, 11 were Hymenoptera (Braconidae) and 133 were Diptera (four species of Tachinidae). While the marked preponderance of Diptera may indicate that the larvae of *P. ewingii* are not preferred hosts of the Hymenoptera, there is also the fact that this order tends to parasitize first and second instar larvae, whereas the Tachinidae prefer later instars. Hymenopterous parasites would thus be exposed for a longer period to conditions which might not favour their development than would the dipterous. Much more information is required, however, before any definite verdict on these matters can be given.

VIII. Acknowledgments

I desire to acknowledge my indebtedness to Dr. Clarence G. Thompson, Insect Pathologist, University of California, for all the information concerning the structure and classification of the virus. He has kindly authorized my use of this information and of the electron micrographs, which are the work of Mr. K. M. Hughes of the same institution, to whom also I express my thanks.

IX. References

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Explanation of Plate 1

Fig. 1.—Final instar larva of *Persectania ewingii* in position of attachment (on vertical grass stem). The terminal larvapodia are hooked on the stem, the posterior segments are extended, and the anterior segments contracted.

Fig. 2.—Granules. × 14,600. (Photo by courtesy of C. G. Thompson.)

Fig. 3.—Granules after treatment with alkali. The granules have ruptured. Arrows indicate the liberated virus bodies. × 20,800. (Photo by courtesy of C. G. Thompson.)