Abnormal Behavior of the Common Armyworm *Pseudaelia separata* (WALKER) (Lepidoptera: Noctuidae) Larvae Infected with an Entomogenous Fungus, *Entomophaga aulicae*, and a Nuclear Polyhedrosis Virus

Takashi OHBAYASHI and Kikuo IWABUCHI

Faculty of Agriculture, Tokyo University of Agriculture and Technology, Saiwai-cho, Fuchu, Tokyo 183, Japan

(Received May 29, 1991; Accepted July 15, 1991)

Daily migrational patterns of *Pseudaelia separata* (WALKER) larvae infected with *Entomophaga aulicae* and the nuclear polyhedrosis virus (PsNPV) were examined under laboratory conditions and compared to that of healthy larvae. The healthy larvae exhibited a daily rhythmic pattern of movement, i.e., feeding and movement on feeding plants at night and hiding them under the soil during the day through the 5th and 6th larval instars. When infected with either *E. aulicae* or PsNPV, the pattern of the movement was disturbed: the larvae crawled out from the soil even during the day and died near the top of the feeding plant. The symptoms first appeared two to three days and two days before the death in *E. aulicae*- and PsNPV-infected larvae, respectively. In the case of *E. aulicae*-infected larvae, a frequent vertical migration was observed during this period.

Key words: abnormal behavior, *Pseudaelia separata*, nuclear polyhedrosis virus, *Entomophaga aulicae*, pathogens

INTRODUCTION

Pathophysiological changes in insect behavior have been revealed by many investigators (e.g., reviewed by BENZ, 1963; MARTIGNONI, 1964; ENTWISTLE and EVANS, 1985; EVANS, 1989). A classical example is that of the larvae of the nun moth, *Lymantria monacha* (LINNAEUS), which gather at the top of trees in groups when infected with nuclear polyhedrosis virus (KOMAREK and BREINDL, 1924). This type of abnormal behavior has been also found in other insect species infected with fungi and viruses (SKAIFE, 1925; MACLEOD, 1963; SMIRNOFF, 1965; AOKI, 1981). Since the infection of insects with different pathogens often produces similar behavior, it has been considered that this type of reaction may be caused by a relatively common mechanism (BENZ, 1963). Some attention has been paid to the physiological mechanisms which lead to the abnormal behavior. However, most of the behavioral symptoms have not been noted in detail.

The common armyworm, *Pseudaelia separata* (WALKER), is a nocturnal feeder in the second half of its larval stage and exhibits a daily rhythmic activity: larvae come up to feed on plants at night and return into the soil during the day (SAITO et al., 1983). In another noctuid species *Mamestra brassicae* L., it has been reported that most larvae
infected with *Entomophthora* sp. climb up to the upper portion of plants before the death even in day time (Aoki, 1981).

In the present study, abnormal behavior of the common armyworm larvae infected with *Entomophaga aulicae* and the *P. separata* nuclear polyhedrosis virus (PsNPV) was investigated.

**MATERIALS AND METHODS**

*Insects.* The common armyworms used for the present study were obtained from stock culture. The insects were reared at 25°C on an artificial diet developed by Hattori and Atsuzawa (1980) with a minor modification under a photoperiod of 16 hr light and 8 hr darkness.

*Pathogens.* Protoplasts of *Entomophaga aulicae* were cultured at 25°C in Mitsushashi and Marahorosch’s serum free (MM-SFA) medium for insect cell cultures. The protoplasts were suspended in 5 μl of MM-SFA medium (1.1 × 10⁶ cells/ml) were injected into a proleg of the 5th instar, day 2, larvae with a syringe attached to a microdispenser. With PsNPV, 2 ml suspension of polyhedral inclusion body (PIB) (1.5 × 10¹⁰ PIB/ml) was mixed with 10 g of the artificial diet. The diets containing PIB were served to newly eclosed fifth instar larvae for 33 hr. *E. aulicae* F462 was obtained from Forestry and Forest Products Research Institute, Ministry of Agriculture, Forestry and Fisheries. The virus was the gift from Dr. Y. Kunimi of Tokyo University of Agriculture and Technology. The origin of the virus has been described elsewhere (Kunimi and Yamada, 1990).

*Experiments.* The experiments were designed according to the method described by Sato et al. (1983). Healthy or infected larvae were released individually on corn seedlings (15 cm in height) separately planted in plastic pots (10.5 cm in diameter). The soil surface was covered with rice husks to facilitate the larvae digging into the soil. The corn seedlings were kept at 25°C under a photoperiod of 16 hr light and 8 hr darkness. The illumination of about 600 lux was provided by an overhead fluorescent lamp. Observations were made hourly from day 2 of the 5th instar and continued until most of the infected larvae died. Behavioral characteristics and the position of each individual were recorded. When the infected larvae died, the cause of death was determined as follows. For *E. aulicae*-infected larvae, the dead larvae were kept separately in petri dishes (9 cm in diameter) containing wet filter papers. After about 12 hr, the discharge of conidia from the cadavers was examined. In the case of PsNPV-infected larvae, the dead larvae were dissected and examined for the presence of polyhedra under a microscope at a magnification of ×400. The data were collected from 18 healthy larvae, and 14 and 20 larvae infected with *E. aulicae* and PsNPV, respectively.

**RESULTS**

1. **Daily migrational pattern**

Daily migrational patterns of larvae during five successive days before their death (except healthy larvae) are shown in Fig. 1. Healthy larvae exhibited the daily rhythmic pattern in migration during the 5th and 6th instars. They burrowed into the soil towards the beginning of the photophase and crawled out soon after the onset
Abnormal Behavior of *P. separata* Larvae

Fig. 1. Daily migrational patterns of the fifth and sixth instar larvae; (A) healthy larvae, (B) *E. aulicae*-infected larvae, (C) PsNPV-infected larvae. In (B) and (C), zero indicates the day of the death. L and D indicate photophase and scotophase, respectively.

Fig. 2. Frequency (times) (mean±S.E.) of appearance of larvae from soil during photophase (A) and the duration (hr) (mean±S.E.) of the larvae's stay on feeding plants or above the soil level (B). Healthy larvae (○), *E. aulicae*-infected larvae (●) and PsNPV-infected larvae (▲). For broken lines see text.
of scotophase. During scotophase, most of the larvae were found on various parts of the feeding plant.

The daily rhythm in behavior was severely disturbed when the larvae were infected with pathogens. In the case of *E. aulicae*-infected larvae, they died 4.50±0.73 days after injection of the protoplasts, and the daily rhythmic pattern gradually disappeared from four days before their death (Figs. 1B, 2B). The amount of time the infected larvae stayed on the feeding plant, or above the soil level, during the photophase of a day was greater than that of healthy larvae. On the other hand, the frequency of vertical migration, during the photophase of a day reached a high level over a period of three to two days before the death (Fig. 2A). The time spent above the soil increased up to 14.50±0.63 hr during the photophase of the day before the death (Fig. 2B), but the frequency of vertical migration declined (Fig. 2A). The frequency of upward migration declined and the time of staying above ground diminished on the last day in Fig. 2 (broken lines), since most of the infected larvae died on the way during the photophase. Thus, the larvae infected with *E. aulicae* tended to actively move vertically three to two days before the death, and then remain on the feeding plant until the death.

In the case of PsNPV-infected larvae, they died 6.45±1.07 days after being served the NPV mixed diet. The first sign of abnormal behavior appeared two days before the death (Fig. 1C). During this period, the number of larvae in the soil diminished regularly with the advance of time even at photophase. The time spent above the soil by PsNPV-infected larvae was 3.70±1.09 hr and 11.80±1.29 hr during the photophase of days two and one before the death, respectively, and was longer than that of healthy larvae (Fig. 2B). The length of time spent above ground by the PsNPV-infected larvae one day before the death was similar to that of *E. aulicae*-infected ones. However, no great increase in the frequency of vertical migration occurred in the PsNPV-infected larvae on days three through one before the death (Fig. 2A).

2. Behavior of dying larvae

The larvae infected with *E. aulicae* often crawled out from the soil even during photophase, became restless and wandered on the soil or plants. Wandering behavior was observed on at least eight (57%) of 14 dying larvae. The appearance from the soil prior to the death and the wandering behavior started 33.4±15.5 hr and 6.8±1.4 hr before the death, respectively. Later, the dying larvae climbed the feeding plant and stayed at the upper parts of it. They also exhibited other abnormal behavior, such as feeding on withered parts of corn leaves. Shortly after the death, the larvae lost tension: the water contents of the body seemed to decrease, the larva became flaccid and laterally compressed. The discharge of conidia from all the dead larvae began during the next scotophase.

On the other hand, the dying NPV-infected larvae were less active and did not exhibit wandering behavior. They only moved upward and stayed on upper parts of the plant until the death. The appearance of larvae from the soil occurred 28.7±13.0 hr prior to the death. Finally, the larvae died hanging from the plants, as was the case with NPV-infected larvae of other insects. Microscopic observations revealed the presence of a large number of polyhedra in the body cavity of all the dead larvae infected with PsNPV.

The times when healthy and infected larvae crawled out from the soil are shown
Abnormal Behavior of *P. separata* Larvae

**Fig. 3.** Hourly rate of appearance of larvae from soil. A, healthy larvae; B, *E. aulicae*-infected larvae; C, PsNPV-infected larvae.

**Fig. 4.** Time of the death of larvae infected with *E. aulicae* (A) and PsNPV (B).

### Table 1. The site where *E. aulicae*- and PsNPV-infected larvae died

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>No. of larvae</th>
<th>Parts of feeding plants</th>
<th>Soil surface</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. aulicae</em></td>
<td>14</td>
<td>Upper 4 Middle 3 Lower 2 Folding leaf 0</td>
<td>5</td>
</tr>
<tr>
<td>PsNPV</td>
<td>20</td>
<td>Upper 9 Middle 9 Lower 0 Folding leaf 0</td>
<td>2</td>
</tr>
</tbody>
</table>

in Fig. 3. The bar graphs indicate that there were no specific times associated with the appearance of both the *E. aulicae* and the NPV-infected larvae. However, the death of the *E. aulicae*-infected larvae occurred exclusively during the late photophase, while that of the NPV-infected larvac did not occur at any specific time (Fig. 4).

### 3. Site of death

Sites on which the infected larvae died are summarized in Table 1. All the larvae, both the *E. aulicae*- and the NPV-infected ones, died on the feeding plants or above the soil level. In the *E. aulicae*-infected larvae, 64% died on leaf surfaces. On the other hand, 90% of the NPV-infected larvac died on leaf surfaces and all of them on the upper and middle parts of the leaves.
DISCUSSION

Daily migrational pattern of the fifth or sixth instar larvae of the common armyworm was disturbed by infection with either *E. aulicae* or PsNPV. The infected larvae appeared from the soil or remained on feeding plants even during photophase, while healthy larvae stayed in the soil. This agrees with the observation on upward migration of infected larvae in related species (Aoki, 1981).

The abnormal behavior may be due to a severely altered taxis induced by disturbed physiological conditions in the heavily diseased organism (Benz, 1963). Abnormalities in behavior may result from a taxis which is normally absent in the species concerned or which functions in other larval instars. The present study showed a positive phototaxis or a negative geotaxis which is usually absent in the common armyworm during the photophase. Manifestation of a positive phototaxis has been reported in the common armyworm reared under crowded condition (Iwao, 1967). Thus, the physiological mechanism in this phenomenon is similar to that of abnormal behavior observed in the present study.

In the case of the NPV-infected larvae, most of the dying larvae climbed up to near the top of corn seedlings, whereas less than 50% of the *E. aulicae*-infected larvae did so. On the other hand, restless and wandering behavior was exhibited more strikingly in the larvae infected with *E. aulicae*. Thus, it is likely that there are differences in pathophysiological events between larvae infected with *E. aulicae* and NPV.

The death at the upper parts of plants is not a general phenomenon of the common armyworm infected with any pathogen, because most of the larvae infected with *P. unipuncta* granulosis virus do not climb up to the feeding plants before dying. Thus, the upward movement before the death of the common armyworm may be one of the characteristics of the infection with *E. aulicae* and PsNPV.

ACKNOWLEDGEMENTS

The authors would like to thank Prof. J. Mituhashi and Dr. K. Satô of Tokyo University of Agriculture and Technology for their valuable advice and critical reading of this manuscript.

REFERENCES


Abnormal Behavior of *P. separata* Larvae

KOMÁREK, J. and V. BREINDL (1924)  
Die Wipfelkrankheit der Nonne und der Erreger derselben.  

KUNIMI, Y. and E. YAMADA (1990)  
Relationship of larval phase and susceptibility of the armyworm,  
*Pseudaletia separata* WALKER (Lepidoptera: Noctuidae) to a nuclear polyhedrosis virus and a granulosis virus.  

MACLEOD, D. M. (1963)  
Entomophthorales infections.  
In *Insect Pathology*, Vol. 2 (E. A. STEINHAUS, ed.).  

MARTIGNONI, M. E. (1964)  
Pathophysiology in the insect.  

SATO, Y., T. TANAKA, M. IMAFUKU and T. HIDAKA (1983)  
How does diurnal *Apanteles kariyai* parasitize and egress from a nocturnal host larva?  
*Kogyô* 51: 128–139.

SKAIFE, S. H. (1925)  
The locust fungus, *Emusa gyllii*, and its effects on its host.  

SMIRNOFF, W. A. (1965)  
Observations on the effect of virus infection on insect behavior.  