HODEK (1983) has attempted to make general explanations for the process of diapause termination by postulating two independently proceeding processes, tachytelic and horotelic processes. The former was postulated to proceed at a standard rate while the latter proceeds at a more accelerated rate. Since we have recently published data on summer diapause regulation in a similarly estivating chrysomelid beetle, *Pyrrhalta humeralis* (NAKAI and TAKEDA, 1995), comparisons of these species in such a scheme may be appropriate for characterization of this species. We assume that the horotelic process in *P. humeralis* depends on temperature and that the tachytelic process depends on photoperiods in such a way that the former proceeds at a higher rate at lower temperatures and the latter under short days (Fig. 2, left).

Similar analysis favors the notion that the horotelic process in *E. westwoodii* is accelerated at higher temperatures (Fig. 2, right). The tachytelic process is accelerated by short photoperiods, since the emergence was greatly accelerated by a photoperiodic shift from LD 18 : 6 to LD 13 : 11.

In conclusion, the rate of termination of diapause in *E. westwoodii* depends more on photoperiodic conditions than temperature but the latter strongly affects the rate of survival. High temperature accelerated the termination of diapause but induced high mortality. The mode of dependence on temperature and photoperiod is just the opposite of what was observed in *P. humeralis*, where low temperature accelerated the termination of diapause but photoperiod affected the survival rate significantly.

ACKNOWLEDGEMENTS

We thank Prof. S. MOMOI for his encouragement.

REFERENCES

- GOMI, T. and M. TAKEDA (1992) *J. Insect Physiol.* **38**: 665–670.
 HODEK, I. (1983) In *Diapause and Life Cycle Strategies in Insects* (V.K. BROWN and I. HODEK, eds.). Dr W. Junk Publ., The Hague, pp. 9–33.
 NAKAI, T. and M. TAKEDA (1995) *Appl. Entomol. Zool.* **30**: 295–301.

Cuticular Proteins Specific to the Last Larval Stadium in the Sweet Potato Hornworm, *Agrius convolvuli* (Lepidoptera: Sphingidae)¹

Ken TATEISHI and Jun MITSUHASHI

Laboratory of Applied Entomology, Faculty of Agriculture,
 Tokyo University of Agriculture and Technology,
 Fuchu, Tokyo 183, Japan

(Received 18 May 1995)

(Accepted 2 August 1995)

Key words: cuticle protein, electrophoresis, growth, ecdysis, *Agrius convolvuli*

In holometabolous insects, the larval, pupal and adult cuticles are markedly different, and each has stage specific proteins (CHIHARA et al., 1982; SRIDHARA, 1983; COX and WILLIS, 1985). Genes coding for

larval cuticular proteins of *Manduca sexta* are expressed during the feeding and growth phases and are suppressed at the initiation of moulting. Moreover, these genes are permanently suppressed by the small rise of ecdysteroid titre in the absence of juvenile hormone at the end of the larval stage (RIDDIFORD et al., 1986; REBERS and RIDDIFORD, 1988). In the cuticle of *Drosophila*, which forms a puparium from the last larval cuticle, cuticular proteins of the last instar are different from those of previous stadia (CHIHARA et al., 1982). In this study, we describe the presence of proteins specific to the last or immature larval cuticle in the sweet potato hornworm, *Agrius convolvuli*.

MATERIALS AND METHODS

Animals. *A. convolvuli* used in this study was obtained from the National Institute of Sericultural and Entomological Science. The larvae were kept under long photoperiod (16L : 8D) at 25 ± 1°C according to the methods described by KIGUCHI and SHIMODA (1994). They were reared on an SPLP-25 diet

¹ *Appl. Entomol. Zool.* **30** (4): 596–599 (1995)

This study was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science.

Fig. 1. Developmental changes of cuticular proteins shown by native polyacrylamide gel electrophoresis. Cuticular proteins were extracted from the dorsal abdominal integument of the each stage by a buffer containing 8 M urea. III–V, third to fifth stadia; P, pupa; A and B, last larval specific cuticular proteins; C, immature larval specific protein.

(KIGUCHI and SHIMODA, 1994) until Day 1 of the 5th stadium, and thereafter were given an SPLP-5 diet.

Preparation of cuticle. Cuticles were taken from blue colored larvae at the following stages: Day 2 of the 3rd stadium, Day 1, Day 2 and Day 3 (the day of head capsule slippage) of the 4th stadium and Day 0, Day 1, Day 2, Day 3, Day 4 (wandering stage) and Day 6 (pre-pupal stage) of the 5th stadium, and Day 5 of the pupal stage. Cuticles were collected from 10–20 4th stadium of each of the stages, or 3–10 5th stadia each of the stages and pupae. Dorsal integument from the 1st to the 6th abdominal segment was cut off and adherent tissues were scraped from the integument with a piece of rubber attached to a glass rod. The integument was rinsed with 20 mM Tris-HCl, 0.15 M NaCl, pH 7.5 (Tris-buffered saline: TBS), wiped with a filter paper, frozen on dry ice and stored at -100°C .

Extraction of cuticular proteins. Cuticular proteins were extracted as described by NAKATO et al. (1990). Cuticles were homogenized with a mortar and pestle, suspended in 10 vol (w/v) of TBS containing 1 mM phenylmethyl-sulfonyl fluoride (PMSF) and 10 mM 2-mercaptoethanol. The mixture was centrifuged at $1,000 \times g$ for 5 min and the supernatant was discarded. The pellet was washed twice with the same buffer and three times with a high concentration salt buffer containing 20 mM Tris-HCl, 0.6 M NaCl, 1 mM PMSF and 10 mM 2-mercaptoethanol, pH 7.5. Cuticular proteins were extracted from the pellet with 5 vol of 8 M urea containing 20 mM Tris-HCl,

pH 7.5, by occasional stirring for 15 min at 25°C (FRISTROM et al., 1978). After centrifugation, the supernatant was stored at -45°C .

Gel electrophoresis. Native slab gels were made using a 7.5% polyacrylamide resolving gel and 3% polyacrylamide stacking gel. Denaturing slab gels (12% polyacrylamide resolving gel; 3% polyacrylamide stacking gel) were made according to LAEMMLI (1970). Protein standards (Sigma) were run for determination of approximate molecular weights of proteins. More than 5 samples were analyzed at each stage.

All gels were stained by the silver stain kit, Silver Stain KANTO II (Kanto Chemical Co., Inc.).

RESULTS AND DISCUSSION

Cuticular proteins from third stadium larvae to pupae were extracted with 8 M urea and analyzed by electrophoresis on a 7.5% polyacrylamide native gel. The results were highly reproducible. A typical electrophoretogram is shown in Fig. 1. Several major larval cuticular proteins were resolved. One of slow migrating proteins (Fig. 1, C) existed only in the cuticles of immature larvae, the 3rd and the 4th stadium, and disappeared after Day 3 of the 4th stadium. Small amounts of two other slow migrating proteins (Fig. 1, A and B) existed in the 4th stadium and these increased in quantity at the 5th stadium. The slower migrating protein (Fig. 1, A) increased from Day 1 but decreased after Day 3 and then disappeared at the pupal stage. Another protein (Fig. 1,

Fig. 2. Developmental changes of cuticular proteins shown by SDS-polyacrylamide gel electrophoresis. Cuticular proteins were extracted from the dorsal abdominal integument of the each stage by a buffer containing 8 M urea. III–V and P as in Fig. 1; A–D, proteins of 60, 35, 32, 20 kDa; M, molecular weight markers.

B) increased in quantity from Day 0 and existed throughout the last larval and pupal stages. In the pupal stage most larval cuticular proteins disappeared, and two proteins specific to pupae appeared.

Cuticular proteins were also analyzed by electrophoresis on a 12% SDS-polyacrylamide gel (Fig. 2). Some proteins appeared only at the last larval stadium. A protein of 32 kDa (Fig. 2, C) appeared at Day 0 of the 5th stadium, increased in quantity toward Day 3 and disappeared at the pupal stage. The other two proteins (Fig. 2, A and B) also existed in slight quantities at the 4th stadium but increased in quantity from Day 0 to Day 3 of the 5th stadium. On the other hand, a protein of 20 kDa (Fig. 2, D) existed before the 5th stadium, and disappeared at Day 0 of the 5th stadium. The cuticular proteins of the pupal stage were completely different from those of the larval stages.

Most larval cuticular proteins of *A. convolvuli* were only seen during the growth phase. Genes of these proteins were apparently expressed during the growth phase and suppressed at the initiation of moulting. A similar phenomenon has been reported in the low molecular weight cuticular proteins of *M. sexta* (REBERS and RIDDIFORD, 1988). In *A. convolvuli* there were proteins that appeared specifically during the growth phase of the 5th stadium or before the 4th moulting (Figs. 1 and 2). The difference in protein synthesis between the 3rd and the 4th moults seems to be due to differences in the internal endocrine con-

ditions. In *M. sexta* the suppression of the m-RNA expressing larval specific proteins was reportedly caused by ecdysteroid in the absence of juvenile hormone (REBERS and RIDDIFORD, 1988). Therefore, the expression of the specific proteins for the last and immature stadia may be controlled by the titre of ecdysteroid and juvenile hormone.

The shape of the cuticle surface of last stadium larvae differed from those of previous stadia in *A. convolvuli*. There were many processes on the entire surface of the cuticle of immature stadium larvae, which were absent from last stadium larvae (data not shown). The relationship between the existence of the processes and the stadium specific proteins was not clarified, but those last stadium specific phenomena suggest the existence of an endocrinological change-over between the penultimate and the last stadium of *A. convolvuli*. The finding of cuticular proteins specific to the last or immature stadia of *A. convolvuli* may shed light on the mechanism of final larval stadium determination.

ACKNOWLEDGEMENTS

We thank Dr. K. KIGUCHI of Shinshu University for presenting us with the insects.

REFERENCES

- CHIHARA, C.J., D.J. SILVERT and J.W. FRISTROM (1982) *Devel. Biol.* **89**: 373–388.
- COX, D.L. and J.H. WILLIS (1985) *Insect Biochem.* **15**:

- 349–362.
- FRISTROM, J.W., R.J. HILL and F. WATT (1978) *Biochemistry* **17**: 3917–3924.
- KIGUCHI, K. and M. SHIMODA (1994) *Zool. Sci.* **11**: 143–147.
- LAEMMLI, U.K. (1970) *Nature* **227**: 680–685.
- NAKATO, H., M. TORIYAMA, S. IZUMI and S. TOMINO (1990) *Insect Biochem.* **20**: 667–678.
- REBERS, J.E. and L.M. RIDDIFORD (1988) *J. Mol. Biol.* **203**: 411–423.
- RIDDIFORD, L.M., A. BAECKMANN, R.H. HICE and J. REBERS (1986) *Devel. Biol.* **118**: 82–94.
- SRIDHARA, S. (1983) *Insect Biochem.* **13**: 665–675.

Conocephalus longipennis (DE HAAN)
(Orthoptera: Tettigonidae): A Suspected
Egg-Predator of the Rice Bug in the Muda
Area, West Malaysia¹

Kiyomitsu ITO, Ho Nai Kin²
and CHANG Poon Min³

Hokkaido National Agricultural Experiment Station,
Hitsujigaoka, Sapporo, Hokkaido 062, Japan

² Muda Agricultural Development Authority (MADA),
Ampang Jajar, 05990 Alor Setar, Kedah, Malaysia

³ Rice Research Centre, Malaysian Agricultural Research and
Development Institute (MARDI), Kepala Batas, 13200
Seberang Perai, Malaysia

(Received 22 May 1995)

(Accepted 4 July 1995)

Key words: *Conocephalus longipennis*, predator,
Leptocoris oratorius, egg, Malaysia

The rice bug, *Leptocoris oratorius*, is a serious pest of rice panicles in South and Southeast Asia. In the Muda irrigation area, known as “the rice bowl of Malaysia,” damage from this bug has been visible since the early 1980s; when farmers introduced direct-seeding rice cultivation (NIK MOHD NOOR and HIRAO, 1987; HIRAO and HO, 1987; ITO et al., 1992). To elucidate the impact of natural enemies against the population fluctuation of this bug, we attempted to clarify the fauna of the egg parasitoids. In the course of the study, we found that a longhorned grasshopper, *Conocephalus longipennis*, frequently fed on eggs of this bug. The present paper demonstrates that this grasshopper is an egg-predator of *L. oratorius* in the Muda area.

MATERIALS AND METHODS

Field-collected *L. oratorius* adults were kept in a transparent plastic rearing cage (30 × 30 × 30 cm). Rice panicles at the milk-ripe stage were provided as food and leaves of the rice plant as the oviposition materials. Since most females were gravid, they quickly laid eggs.

Pieces of leaves with *L. oratorius* eggs (0–1 d-old after oviposition) were fastened on cardboard strips (5 × 12 cm) using a stapler, and these were attached to rice plants in 3 different fields, Alor Mengkudu (Locality C-III), Lencong Barat (B-IV) and Simpang Sala (C-IV), and exposed for 3–5 d. After this period, the cardboard strips were retrieved and the eggs examined. No eggs were considered to have hatched during the exposure period, because most of the nymphs appeared from the eggs 3–4 d after being retrieved. No precipitation was recorded during the experimental period. Rice plants in the fields were from the milk-ripe to the dough-ripe stage. The experiment was conducted from February 5 through 18, 1995.

Several species of insects commonly found in the field were caught on February 7, 1995 and kept in small plastic cages (9 cm in diameter, 4 cm in height) with *L. oratorius* eggs and moistened filter paper for 4 d under ambient conditions (23–28°C) to verify whether or not they were predators of *L. oratorius* eggs.

Field-collected adults, 4th instar nymphs and 2nd instar nymphs of *C. longipennis* were confined individually in small plastic cages with *L. oratorius* eggs and moistened filter paper, and kept under ambient conditions to investigate the daily egg consumption.

Population densities of *L. oratorius* and *C. longipennis* were examined in the 3 fields by net-sweepings on February 13, 1995.

¹ Appl. Entomol. Zool. **30** (4): 599–601 (1995)