Acta Biochimica Polonica

Vol. 47 No. 1/2000

215–221

QUARTERLY

Review

Temperature-dependent regulatory mechanism of larval development of the wax moth (*Galleria mellonella*)✪

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Received: 27 October, 1999; accepted: 31 January, 2000

Key words: physiology, wax moth, *Galleria mellonella,* larval diapause, ecdysteroids, brain proteins, storage proteins

The mechanisms underlying larval diapause in the wax moth (*Galleria mellonella*) is one of the most throughly studied aspects. At the low temperature of 18°C, the last instar larvae did not pupate but transferred to 30°C they initiated development and pupation in a circadian manner. Different types of surgical manipulations including head-ligation, nerve cord-severance, implantation of the brain, prothoracic glands, accompanied with ecdysteroid titre measurements indicated that diapausing arrest of larval development at 18°C might be due to the nervous inhibition of their prothoracic glands.

Despite of decrease in the rate of total brain protein synthesis, sub-optimal temperature induced an increase in the content of two major brain proteins: 112 and 84 kDa 21–28 days after transferring to the lower temperature. In the haemolymph of diapausing *Galleria* larvae there was also an accumulation of some proteins. Three of proteins: 74, 76 and about 80 kDa were identified as a group of storage proteins (larval haemolymph proteins – LHP). The synthesis of 74 kDa and 76 kDa proteins started 24 h, and that of about 80 kDa – 96 h after transferring larvae from 30°C to 18°C. 20-Hydroxyecdysone inhibited synthesis of the 74 and 76 kDa proteins in larvae exposed to lower temperature. It seems, therefore, that larval diapause of *Galleria mellonella* is associated with the synthesis and accumulation of both the brain (PTTH?) as well as haemolymph storage proteins.

✪This work was supported in part by the State Committee for Scientific Research (KBN, Poland) grant No. 6PO4CO1916.

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Abbreviations: JH, juvenile hormone; LHP, larval haemolymph proteins; PG, prothoracic gland; PTTH, prothovacicotropic hormone.

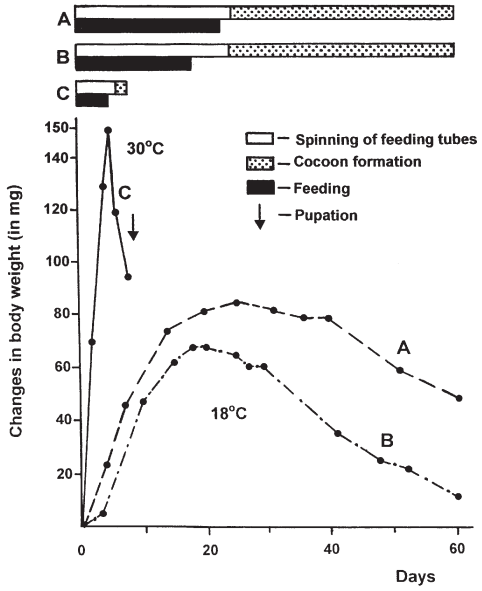


Figure 1. Effect of temperature on changes in body weight and behaviour of *Galleria mellonella* larvae which on the 1st day of the last instar were transferred to 18°C (their mean body weight at that moment was assigned a value of zero) and kept at this temperature singly (A — broken line), or in groups (B — broken-doted line).

The control, last instar larvae (C) were kept continuously at 30°C (solid line). Duration of behavioural processes is illustrated by the horizontal bars at the top of the figure. Determinations were performed on 30–35 individuals (Mikołajczyk & Cymborowski, 1993).

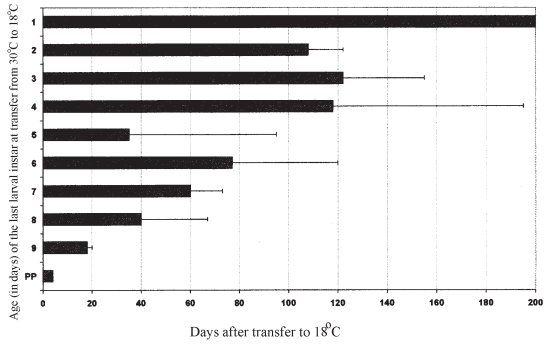


Figure 2. Effects of lower temperature on development and timing of pupation of *Galleria mellonella* larvae depending on age of the last instar at transfer from 30°C to 18°C.

Note that the larvae which were transferred to18°C on the first day of the last instar had never pupated. PP — prepupa. Each determination was performed on 25–30 individuals (±S.D.) (Mikołajczyk & Cymborowski, 1993).

Ecdysteroids are also involved in these processes.

The effect of temperature on insect growth and development is one of widely investigated aspects of insect physiology. Several studies have demonstrated that the wax moth *Galleria mellonella* (Lepidoptera, Pyralidae) is a very convenient model for studying temperature-mediated changes in insect physiology and endocrinology because of its unusual habitat in beehives characterized by constant temperature. Furthermore, this species reared at a stable and optimal temperature of 30°C during many years in laboratory, became very sensitive to any temperature changes. The exposure of *G. mellonella* last instar larvae to low temperature (0°C) and to sub-optimal temperature (18°C) cause different effects (Cymborowski, 1991). More detailed studies have indicated that two separate endocrine mechanisms affecting development are induced by low temperature stress (chilling stress) and by sub-optimal temperature. Exposure of the early last instar larvae to 0°C causes supernumerary moultings (Cymborowski & Boguś, 1976), induced by rapid and persistent increase in the juvenile hormone (JH) titre (Boguś & Cymborowski,

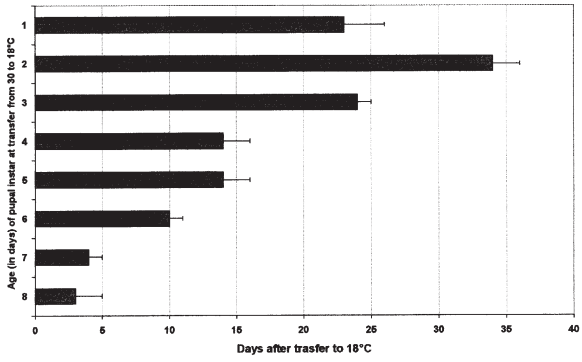


Figure 3. Effects of lower temperature on development and timing of imaginal moults of *Galleria mellonella* pupae depending on their age after transferring from 30°C to 18°C.

Further explanations as for Fig. 2 (Mikołajczyk & Cymborowski, 1993).

1981). This rapid increase in the JH titre is due to high allatotropic activity exhibited by the brain of chilled larvae (Boguś & Cymborowski, 1984). While chilling stress can cause supernumerary moultings, sub-optimal temperature causes prolongation of the last instar depending on the age at which the larvae were placed at 18°C (Mikołajczyk & Cymborowski, 1993). The most strong and profound impact of sub-optimal temperature has been observed when one-day-old last instar larvae were transferred from optimal conditions to 18°C. When *Galleria mellonella* larvae are reared at 30°C the last instar lasts about eight days and the pupation occurs on the ninth day, but at 18°C the last instar can persist for more than one year. The transfer of one-day-old last instar larvae to 18°C results in developmental arrest at the stage of the spinning larva and cause a marked delay in pupation. This developmental arrest, described as facultative larval diapause (Mikołajczyk & Cymborowski, 1993), is the consequence of low ecdysteroid titre and high titre of JH in the larval haemolymph due to changes in the neuroendocrine system (Muszyńska-Pytel *et al.,* 1993). The transfer of these larvae from 18°C back to the optimal temperature of 30°C results in the resumption of development and synchronous pupation within 4–7 days (Śmietanko *et al.,* 1989).

The wax moth is not the only known species in which development can be affected by sub-optimal temperature. The prolongation of instar length caused by temperature changes has been previously described in other insects species: *Dermestes lardarius* (Jacob & Fleming, 1980), *Manduca sexta* (Reynolds & Nottingham, 1985). In these cases the observed delay in development could be explained by the influence of lower temperature on the rate of growth and metabolic processes.

The development of *Galleria mellonella* is arrested by lowered temperature of 18°C at the stage of the last instar post-spinning larva. Further development was markedly prolonged and desynchronised (Fig. 1) when the insects of different stages (from 6th instar head capsule slippage larvae up to 8-day-old pupae) were transferred from optimal temperature of 30°C to 18°C. One-day-old last instar larvae transferred to 18°C arrested their development completely (did not pupate) but went through all behavioural phases characteristic of 30°C-reared larvae including formation of cocoons in which they remained as dauer larvae (Mikołajczyk & Cymborowski, 1993).

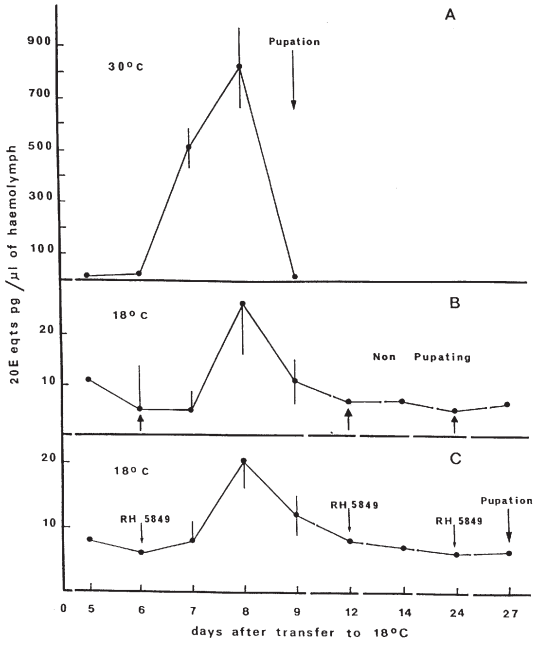


Figure 4. Effects of lower temperature on ecdysteroid titres in the haemolymph of *Galleria mellonella* larvae transferred from 30°C to 18°C on the 1st day of the last instar.

A – control, last instar larvae reared at 30°C; B – 1-day-old last instar larvae transferred to 18°C and treated (arrows) with 1 μl of acetone (solvent for RH-5849); C – 1-day-old last instar larvae transferred to 18°C and treated (arrows) with 10 μg of RH-5849 in 1 μl of acetone solution. Samples of haemolymph were assayed by enzyme immunoassay (EIA). Each point represents mean (±S.D.) of 3–6 separate determinations. Data are presented as picograms of 20-hydroxyecdysone (20E) equivalents per μl of haemolymph (Mikołajczyk & Cymborowski, 1993).

The transfer of penultimate (6th) instar larvae, showing head capsule slippage up to

8-day-old pupae, to 18°C caused prolongation of development regardless of the stage and the incidence and timing of subsequent moults was observed. However, when the older last (7th) instar larvae were placed in lower temperature, the length of their subsequent development was decreased and they pupated in shorter time as compared to controls kept at 30°C (Figs. 2 and 3). The transfer of 1–8-day-old pupae to 18°C resulted in an approximately 3–4 times bigger delay of imaginal moult as compared with the time required for an imaginal moult to occur in control insects (see Fig. 3).

Different types of surgical manipulations including head-ligation, nerve cord-severance, implantation of the brain, prothoracic glands (PG) or a fat body accompanied with ecdysteroid titre measurements indicated that diapausing arrest of larval development at 18°C might be due to the nervous inhibition of their PG (Muszyńska-Pytel *et al.*, 1993). Control larvae reared continuously at 30°C showed a large increase in haemolymph ecdysteroid titre (Fig. 4A). Whereas in larvae which were transferred to 18°C on the first day of the last instar and never pupated, ecdysteroid levels remained very low, except for one small increase that occurred on day 8 after a transfer to 18°C (Fig. 4B). Diapausing larvae pupated at 18°C after implantation of active PG or applying a non-steroidal ecdysone agonist RH-5849 (Fig. 4C). The inhibitory input probably comes from the brain and is transmitted down to the PG *via* the sub-oesophageal ganglion and paired cervical nerves. Severance of the nerve tracts at any level caused rapid activation of PGs in diapausing insects. Nervous inhibition of the PGs ceases about 6–9 h after a transfer of diapausing larvae from the lower temperature to diapause-terminating conditions at 30°C. As long as the PGs are under nervous inhibitory control they are refractory to humoral stimulation. The brains of diapausing larvae exhibit *in vitro* high prothoracicotropic activity (Muszyńska-Pytel *et al.,* 1993) which suggests that PTTH is accumulated in the brain

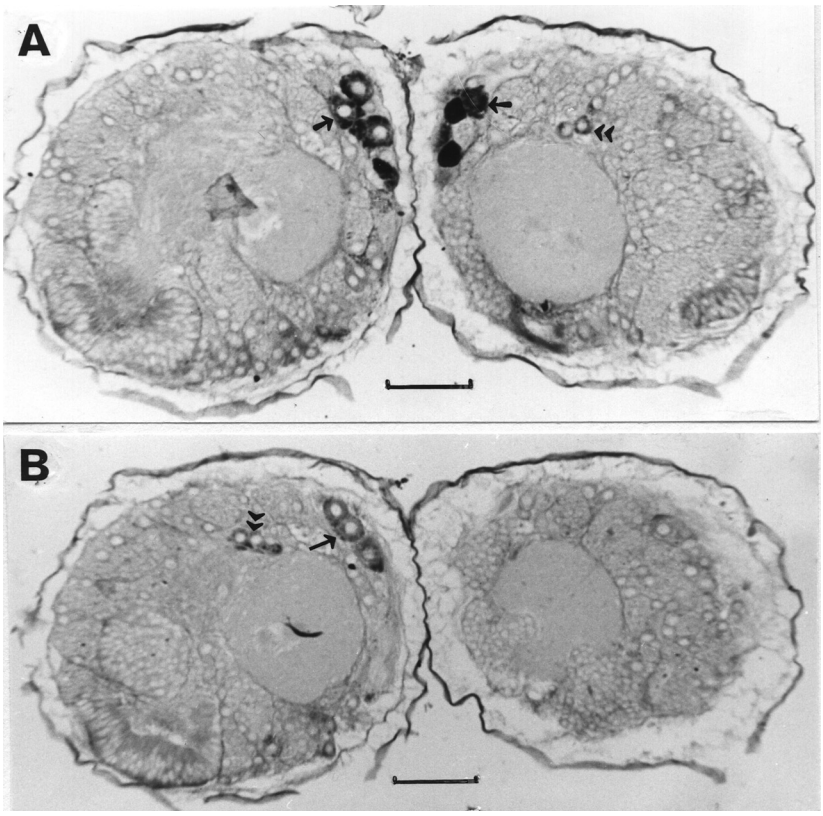


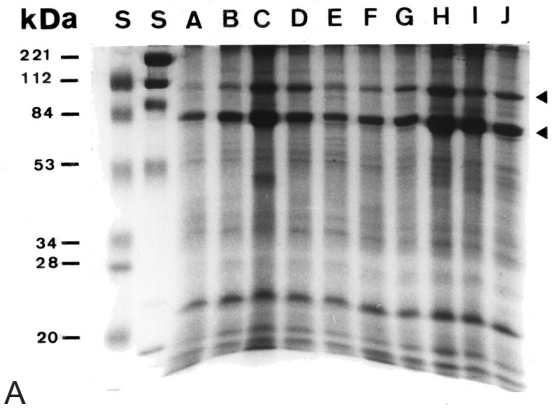
Figure 5. Neurosecretory cells of *Galleria mellonella* brain stained with paraldehyde-fuchsin.

A – Neurosecretory cells of protocerebrum of diapausing larvae which spent three months at 18°C. High degree of accumulation of neurosecretion both in the medial (arrows) and lateral (arrow heads) are observed. **B** – Neurosecretory cells of non-diapausing (control) larvae. Low degree of accumulation of neurosecretion in the same cells are observed. Scale bars equal 50 mμ (Cassier & Cymborowski, 1993).

during diapause. The largest group of peptiderdic neurosecretory cells in the brain of *Galleria mellonella* is located in its *pars intercerebralis* and lateral cells, located in *pars lateralis.* After three months spent at 18°C there was a high degree of accumulation of neurosecretion both in medial and lateral groups of *procerebrum* cells (Dogra & Tandam, 1964). In the brain of non-diapausing larvae taken on day 8 of the last instar the degree of accumulation of neurosecretion in these cells was very low (Cassier & Cymborowski, 1993; Fig. 5).

Diapausing larvae transferred to 30°C initiated development and pupated in a circadian manner. They exhibited also circadian rhythmicity of ecdysteroid titres in their haemolymph, with a period of approximately 24 h (Cymborowski *et al.,* 1989; 1991; Cymborowski, 1994). These results suggest that ecdysteroid synthesis by prothoracic glands exhibit significant daily increases and decreases, being most active at the time when the increase of ecdysteroids observed in the haemolymph of the control temperature-synchronised larvae. Therefore, it appears that the clock driving these rhythms is located within the prothoracic glands of the larvae of *Galleria*. It seems that ecdysteroids may be considered as a primary pacemaker for imposing rhythmicity over wide variety of developmental processes, and act as to maintain temporal order during development. In this sense, ecdysteroids may act as time-keeping compounds in insect body. Daily releases could act as internal Zeitgeber (timegiver), preparing the morphological, as well as physiological backgrounds for future developmental events, such as those of the last larval instar of *Galleria,* cumulating in pupal ecdysis (Cymborowski *et al.,* 1989).

The first of the tissue in the insect body responding to different environmental signals is the brain. Moreover the brain is the centre of



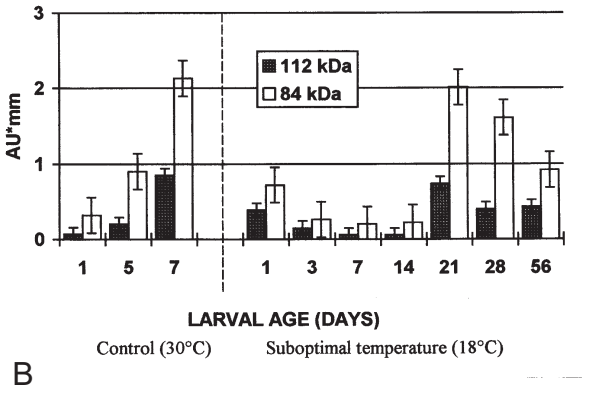


Figure 6A. SDS/PAGE of brain proteins of *Galleria mellonella* last instar larvae reared at 30°C (controls) and 18°C (sub-optimal temperature).

Lanes A–C, last instar larvae reared at 30°C: A: 1-day-old larvae. B: 5-day-old larvae. C: 7-day-old larvae. Lanes D–J, last instar larvae reared at 18°C. D: 1 day; E: 3 days; F: 7 days; G: 14 days; H: 21 days; I: 28 days; J: 56 days. Lane S, standard proteins. Arrowheads, two major brain proteins: 112 kDa and 84 kDa. The homogenate of 20 brains was used for each line.

Figure 6B. Changes in the level of 112-kDa and 84-kDa proteins in the brain of *Galleria mellonella* last instar larvae reared at 30°C (controls) and 18°C (sub-optimal temperature).

Content of proteins determined by scanning the protein bands (proteins were separated by SDS/PAGE) by Pharmacia-LKB densitometer, and GelScan XL computer analysis system was used for quantitation of the intensity of stained bands. AU*mm GelScan XL unit denotes the intensity of staining, i.e., proteins content (±S.D.). (After Chechłacz *et al.* 1998).

the neuroendocrine regulatory system and thereby is the key to understanding the biochemical basis for regulation of the developmental arrest by sub-optimal (18°C) temperature. The rate of protein synthesis in the brain of the last instar larvae kept at 18°C was only about 40% of the value characteristic for this tissue during normal development at 30°C. In spite of decrease in the rate of total brain protein synthesis, sub-optimal temperature induced an increase in the level of two major brain proteins: 112 and 84 kDa (Chechłacz *et al.*, 1998). These two proteins appear 21–28 days after transfer to the lower temperature (Fig. 6A and 6B). In the haemolymph of

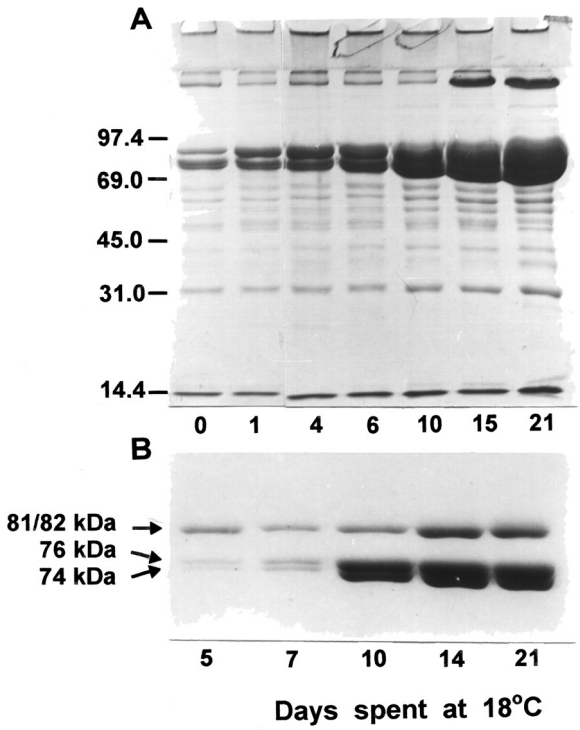


Figure 7. Changes in the electrophoretic pattern of haemolymph proteins of larvae reared at 18°C.

One-day-old last instar larvae were transferred from optimal temperature of 30°C to 18°°C, and then reared at this temperature for at least 4 weeks. Proteins were analysed in 10% polyacrylamide gel (A) and in 7.5% polyacrylamide gel (B). Proteins in 0.5 μl of haemolymph were loaded into each slot of the gel (Kłudkiewicz *et al.* 1996).

diapausing *Galleria* larvae there was also an accumulation of some proteins. Three of these proteins of 74, 76 and about 80 kDa were identified as a group of storage proteins (larval haemolymph proteins – LHP). The synthesis of 74 kDa and 76 kDa proteins started 24 h, and that of about 80 kDa – 96 h following a transfer of larvae from 30°C to 18°C, respectively (Fig. 7A and B). 20-Hydroxyecdysone inhibited synthesis of the 74 and 76 kDa proteins in larvae exposed to lower temperature (Kłudkiewicz *et al.*, 1996). It appears that larval diapause of *Galleria mellonella* is associated with the synthesis and accumulation of both the brain proteins (PTTH?) as well as haemolymph storage proteins. Whether these proteins are specific for induction of larval diapause of *Galleria mellonella* remains to be further investigated.

I am grateful to Dr D. L. Denlinger for reading the manuscript.

REFERENCES

Boguś, M.I. & Cymborowski, B. (1981) Chilled *Galleria mellonella* larva: Mechanism of supernumerary moulting. *Physiol Entomol.* **6**, 343–348.

Boguś, M.I. & Cymborowski, B. (1984) Induction of supernumerary molts in *Galleria mellonella*: Evidence for an allatotropic function of the brain. *J. Insect Physiol.* **30**, 557–561.

Cassier, P. & Cymborowski, B. (1993) Physiology of larval diapause in the wax moth, *Galleria mellonella*: An ultrastructural analysis. *Comp. Biochem. Physiol.* **105A**, 679–687.

Chechłacz, M., Michalik, J. & Cymborowski, B. (1998) Suboptimal temperature-dependent changes in the brain development and activity of *Galleria mellonella* larvae. *Arch. Insect Biochem. Physiol.* **38,** 66–73.

Cymborowski, B. & Boguś, M.I. (1976) Juvenilizing effect of cooling on *Galleria mellonella*. *J. Insect Physiol.* **22**, 667–672.

Cymborowski, B., Śmietanko, A. & Delbecque, J.P. (1989) Circadian modulation of ecdysteroid ti-

tre in *Galleria mellonella* larvae. *Comp. Biochem. Physiol.* **94A**, 431–438.

Cymborowski, B. (1991) Effects of cold stress on endocrine system of *Galleria mellonella* larvae; in *Hormones and Metabolism in Insects* (Ivanović, J., Janković-Hladni, M., eds.) pp. 99–114, CRS Press, Boca Raton, Ann Arbor.

Cymborowski, B., Muszyńska-Pytel, M., Porcheron, P. & Cassier, P. (1991) Haemolymph ecdysteroid titres controlled by a circadian clock mechanism in larvae of the wax moth, *Galleria mellonella*. *J. Insect Physiol.* **37**, 35–40.

Cymborowski, B. (1994) Circadian clock and ecdysteroid titres in the wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae) larvae. *Eur. J. Entomol.* **91**, 277–283.

Dogra, G.S. & Tandam, B.K. (1964) Adaptation of certain histological techniques for *in situ* demonstration of the neuro-endocrine system in insects and other animals. *Q. J. Microsc. Sci.* **105**, 455–466.

Jacob, T.A. & Fleming, D.A. (1980) Some observation on the effect of temperature and relative humidity upon pupation, fecundity and the longevity of *Dermestes landarius* L. (Coleoptera, Dermestidae). *J. Stored Product Res.* **16**, 79–80.

Mikołajczyk, P. & Cymborowski, B. (1993) Lower temperature influences developmental rhythms of the wax moth *Galleria mellonella*: Putative role of ecdysteroids. *Comp. Biochem. Physiol.* **105A**, 57–66.

Muszyńska-Pytel, M., Trzcińska, R., Aubry, M., Pszczółkowski, M.A. & Cymborowski, B. (1993) Regulation of prothoracic gland activity in diapausing larvae of the wax moth, *Galleria mellonella* L. (Lepidoptera). *Insect Biochem. Molec. Biol.* **23**, 33–41.

Kłudkiewicz, B., Godlewski, J., Grzelak, K., Cymborowski, B. & Lassota, Z. (1996) Influence of low temperature on the synthesis of some *Galleria mellonella* proteins. *Acta Biochim. Polon.* **43,** 639–644.

Reynolds, S.E. & Nottingham, S.F. (1985) Effect of temperature on growth and efficiency of food utilization of fifth-instar caterpillars of the tobacco hornworm, *Manduca sexta*. *J. Insect Physiol.* **31**, 371–378.

Śmietanko, A., Wiśniewski, J.R. & Cymborowski, B. (1989) Effect of low rearing temperature on development of *Galleria mellonella* larvae and their sensitivity to juvenilizing treatment. *Comp. Biochem. Physiol.* **92A**, 163–169.