6.0 The effect of propolis on the metabolic rate and metamorphosis of the greater wax moth *Galleria mellonella*

6.1 Abstract

Among the moth pests of the honeybee the greater wax moth *Galleria mellonella* (Lepidoptera: Pyralidae) causes the greatest damage, unless controlled at an early stage, because it feeds on wax, pollen, and cocoons of the bee larvae. This leads to the destruction of honeycombs and subsequent deterioration of the weakened colonies. For controlling this pest, natural products are second to none, not least because the use of synthetic substances carries with it the problem of residues which remain in the beehive to affect the bee products. This chapter reports results of calorimetric investigations on the effects of the bee natural insecticidal glue, propolis, on pupal metamorphosis and the metabolic rate of different larval instars.

Experiments were performed by batch calorimetry to record the heat flow rate of individual larvae/pupae before and after the treatment, which consisted of dipping L5, L6, and L7 instars in a graded series of different concentrations of ethanol-dissolved propolis for 30 s before blotting them. The heat production rates were then recorded for 7 h (short period experiment) or during the entire pupal metamorphosis (long period experiment).

The 5th larval instar (L5) showed higher sensitivity to propolis treatment than L6 and L7 whereby total mortality was obtained by 4% propolis for L5 and 8 to 10% for the latter. The treatment of the late L7 stage with non-lethal doses of propolis shortened the duration of pupal metamorphosis significantly. An untreated larva required 6.8 ± 0.8 d (mean ± SE, n=5) between larval-pupal and pupal-adult ecdysis, whereas this time was shortened to 5.4 ± 0.9, and 4.8 ± 0.5 d after treatment with 1% and 2% propolis, respectively. Though all treated larvae went through larval-pupal ecdysis, 40% and 100% of those treated with 2% and 4% propolis, respectively, displayed abortion of pupal metamorphosis and died. These results indicate that propolis is toxic at higher concentrations and acts as an insect growth regulator at lower ones.

6.2 Introduction

Among the wax moth pests of the honeybee the greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) causes the greatest damage, leading to material and financial losses. The larval stage of *G. mellonella* (with its 7 instars), the only feeding stage with the longest life span of all developmental stages, builds its silk-lined feeding tunnel in the honeycomb and feeds on wax, pollen, faeces and cocoon of the bee larvae. This voracious nature of the larva leads to

the destruction of the honeycomb and the subsequent death of weak colonies. Adults do not feed because they have atrophied mouth parts (Charrière and Imdorf 1997).

The greater wax moth can be controlled by biological, physical, and chemical methods, but most of these methods are either inefficient or expensive for the small-scale beekeeper. In addition, most chemical methods are associated with residue problems in honeybee products. The most commonly used biological control methods include: (i) the control with the wasp *Trichogramma spp*., that infests the eggs of the wax moth resulting in the emergence of a wasp rather than a moth from the egg of the latter (Bollhalder 1999); (ii) toxin-containing spores of the bacterium *Bacillus thuringiensis,* the toxins being released after spores are ingested by the moth larvae, subsequently damaging the intestinal wall and killing the latter; (iii) the use of male/female moth pheromone (Fraser 1997) to lure and trap the female/male moth; and (iv) using sterile male release technique, this method has been effective under laboratory conditions (Caron 1992). The use of pheromone traps is not as such effective since males find their partners not only by chemical means but also by the use of ultrasound (Caron 1992). Physical methods of wax moth control include the storage of combs at low temperature (<15 °C), frost treatment, and heat treatment (Charriére and Imdorf 1997). Each of these methods, however, has its own drawbacks and is too expensive to use by most small-scale beekeepers. Chemical control methods are also used to prevent or stop the destruction of honeycombs by wax moths. The most commonly used chemical fumigants include sulphur dioxide, acetic acid, formic acid, and paradichlorobenzole (PDCB). Even though these chemicals can be used in case of emergency they have their own limitations in that a number of them cause irritation and poisoning of bees and human beings. Some chemicals used for wax moth control, such as PDCB, contaminate honeybee products, mainly honey and wax (Wallner 1991). Storage of combs in modified atmospheres such as high partial pressure of CO_2 (Yakobson et al. 1997) can also be applied, but mainly by large scale beekeepers.

An alternative, and most likely the best solution, to solve the residue problems associated with chemical treatments, and the financial costs incurred by physical control methods, would be to use natural products that are at hand to the beekeeper and free of the aforementioned problems. One such honeybee product is propolis.

Though propolis is found inside the beehive, it does not play a significant role against the parasites, pests, and pathogens of the honeybee *in situ*. However, the *in vitro* experiments, presented in the previous chapters (3 to 5), demonstrated that propolis is varroacidal. The findings of a number of researchers confirmed that propolis is bactericidal and fungicidal against

pathogens of the honeybee that cause disease such as foulbrood, chalkbrood, and others (Lindenfelser 1967, König and Dustmann 1988).

The potential residue free use of propolis, compared to the commercially available expensive, hazardous and residue-associated insecticidal agents employed in the combat against *Galleria mellonella,* provide an incentive to investigate its insecticidal action against this wax moth. The aim of this chapter is to demonstrate the insecticidal and/or insectistatic (abort insect larval/pupal development) actions of propolis.

Calorimetric techniques were used for the various investigations. Calorimetry is a useful tool in the continuous monitoring of different developmental processes throughout the whole life cycle of individual insects without interference in their normal physiological activities. Several researchers have employed calorimetry in the investigation of insect growth and development; among others for the well studied *Galleria mellonella* by Löhr et al. (1978), Schmolz and Schulz (1995), Harak et al. (1996), Lamprecht (1997), Lamprecht (1999), Schmolz and Lamprecht (2000). Moreover, the insect growth regulator and toxic effects of plant secondary metabolites on insects have been investigated calorimetrically (Kuusik et al. 1995). Standard bioassay methods, such as the Petridish bioassay, demonstrate the results of extreme cases of biological activity, such as lethality of a certain concentration, or its impotence, demonstrated by the survival of the organisms after treatment. Biological activities of sublethal concentrations and their effects on development of organisms could, however, be monitored online by the use of the calorimetric method. This latter method was found to be highly sensitive in the investigations of effects of plant secondary metabolites on insect metamorphosis (Kuusik 1995) and on the Varroa weakening action of propolis demonstrated in the previous chapters (3 to 5). The calorimetric method is highly sensitive since it monitors the heat generation rate of an organism, which is directly determined by the metabolic rate. In addition to its high sensitivity, the calorimetric method enables the researcher to judge the mode of action of an insecticidal/insectistatic agent.

6.3 Materials and Methods

6.3.1 Animal and culture conditions

The greater wax moth *G. mellonella* was cultured in a plastic container (25x25x10 cm) at ambient temperature of 30 °C, relative humidity of ca. 70% and 24 h darkness. The culture medium (larval food) consisted of 22% maize flour, 11% wheat flour, 11% bruised wheat, 11% milk powder, 5.5% yeast, 17.5% beeswax, 11% honey, 11% glycerine. All the larval stages and eggs were kept together separated from the pupal and adult stages.

As the early larval stages are too small to handle and too delicate to be used for the purpose of the present investigations, only the 5th (L5), 6th (L6), and 7th (L7) larval instars were chosen. Identification of each larval instar was made by the width of the head capsule and its weight as parameters (for details, see Sehnal 1966). Only larval instars with weight and head capsule width values nearest to the mean of the corresponding range were considered.

6.3.2 Calorimetric experiments

The calorimetric experiments were performed using 3 isoperibolic heat conduction batch calorimeters with different vessel volumes. All investigations with L5 were conducted with a Biocalorimeter B.C.P.-600 (Thermanalyse, München, Germany) with a vessel volume of 12 cm^3, and a sensitivity of 44.73 μV mW^{-1}. For the corresponding experiments with L6 and L7 larvae, 2 Calvet calorimeters (SETARAM, Lyon, France) with vessel volumes of 15 cm^3 and 100 cm^3, respectively, were used. Each of these calorimeters has 2 measuring and 2 reference vessels. The sensitivities of the instruments amounted to 62.63 and 44.21 μV mW^{-1} for the 2 vessels with volume of 15 cm^3 and 51.15 and 53.67 μV mW^{-1} for the 2 vessels with volume of 100 cm^3.

In order to avoid starvation and behavioural change the larvae were provided with sufficient food for the entire experimental period. Air exchange between the vessel content and the surrounding took place through the openings in the lid of the Pyrex glass vessels. 2 types of calorimetric investigations were performed: short and long time experiments.

6.3.2.1 Short period experiments

The aim of these experiments was to investigate the effect of different sublethal concentrations of propolis on the heat production rate of the 3 larval instars mentioned (L5, L6, and L7), and to compare the change in the sensitivity to propolis, if any, with changing larval instar. Both the measuring and reference vessels were supplied with equal amounts of food to avoid asymmetry of non-experimental factors in the 2 vessels. The presence of the larval food in the vessels does not affect stability and level of the baseline. After establishment of the baseline, a pre-weighed larva was placed into the measuring vessel, and the heat production rate was recorded for ca. 4 h. Then the larva was removed from the vessel and treated with propolis, as described below. The treated larva was put back into the calorimeter and the heat production rate was again recorded for 7 h. Each experiment was done 6 times and results are presented as mean ± SE.

6.3.2.2 Long period experiments

These experiments were conducted only with L7. The aim was the evaluation of the effect of sublethal concentrations of propolis on metamorphosis and development of the pupal stage. This could answer the query whether sublethal concentrations of propolis, without remarkable effects on the larva, could cause abortion of pupal development, or either shorten or prolong the pupal development time. The heat production rate of the untreated, pre-weighed larva was recorded for a day in order to observe its activity before treatment. The larva was removed from the calorimeter, weighed again, treated with the desired propolis concentration and put back into the calorimeter. The heat production rate was recorded further until adult emergence, with weight measurements every 24 h. The mean weight between 2 consecutive weighings was used in the calculation of the specific heat production rate in this period (24 h). In cases where there was no adult emergence recording was continued for a total of 25 to 30 days and finally the calorimetric vessel was opened. The pupa was then removed and inspected for life under a binocular microscope by pricking it with a blunt needle. The maintenance of a constant weight during pupal development was also used as a preliminary clue for the death of the organism. Each treatment, including the controls, was done 5 times and the values are presented as mean ± SE.

6.3.3 Propolis preparation and larval treatment

Propolis samples obtained from the research beehives of the Institute of Zoology, Free University of Berlin, were extracted in 70% ethanol. From the extracted and dried propolis sample, a 10% w/v (g ml^{-1}) propolis stock solution was prepared in 55% v/v ethanol. The desired concentrations for treatment (0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 10.0 % w/v) were obtained by diluting the stock solution with 55% ethanol.

Treatments were made by dipping the larvae in 5 ml propolis solution in a 30 ml vial for 30 s. After the allocated treatment time the larvae were removed with a pair of tweezers taking care not to damage them, and placed on a pad of absorbent paper towels for 1 min, to blot fluid, which would otherwise disturb the calorimetric signal and prolong the experimental time undesirably. Double control experiments were carried out by dipping the larvae in 55% ethanol and in distilled water. After being properly blotted the larvae were put back into the calorimeter. Recording of the heat production rate started after a thermal equilibration time of 30 to 45 min, which is always needed after replacing the calorimetric vessel.

6.4 Results

Unless it is clearly stated, all values in this work are given as mean ± SE.

6.4.1 Metabolism and growth of untreated larvae

The wet weight of the larval stages increased drastically, from a mean value of 23.0 ± 2.5 mg at L5, achieving its maximum mean value of 236.8 ± 48.1 mg at the 7th larval instar (Fig. 6.1). These values are means of the larval instars used in the present investigation. Otherwise, the weight change during the entire larval developmental stage ranges from < 1 mg for L1, to nearly 400 mg at the 7th larval instar of some individuals. This drastic increase in weight was then followed by a nearly uniform drop during pupal metamorphosis, as it will be seen in the next sections.

The mean total heat production rates of untreated larvae increased with larval age from L5 (1.7 ± 0.2 mW) to L7 (6.5 ± 0.4 mW) and dropped drastically at the pupal stage (1.9 ± 0.3 mW). The specific heat production rates, however, followed a reverse pattern, except at the pupal stage, dropping considerably from L5 (78.9 ± 8.9 mW g^{-1}) to the pupal stage (8.2 ± 3.1 mW g^{-1}) (Fig. 6.2).

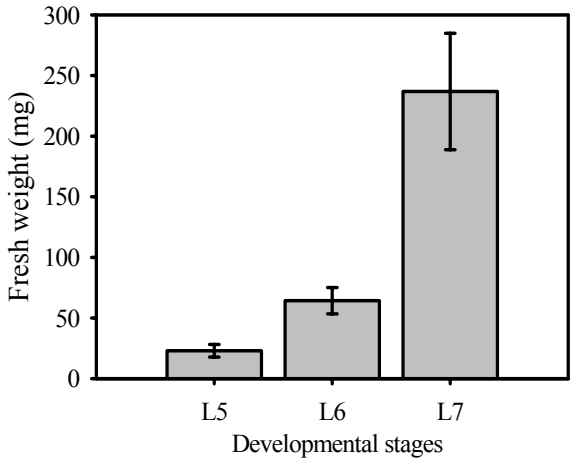


Fig. 6.1 Mean fresh weight (mg) ± SD of the 5th, 6th and 7th instar larvae of the greater wax moth *Galleria mellonella*. n = 54 for L5 and n = 36 for L6 and L7 each.

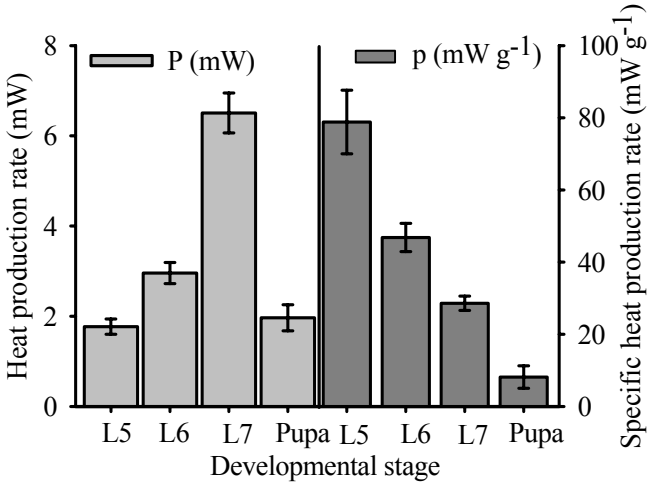
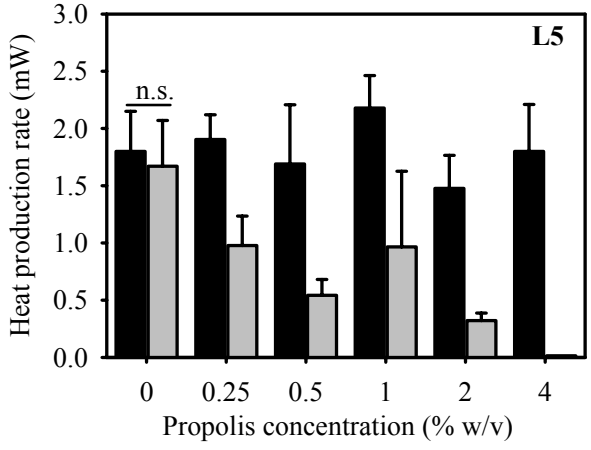
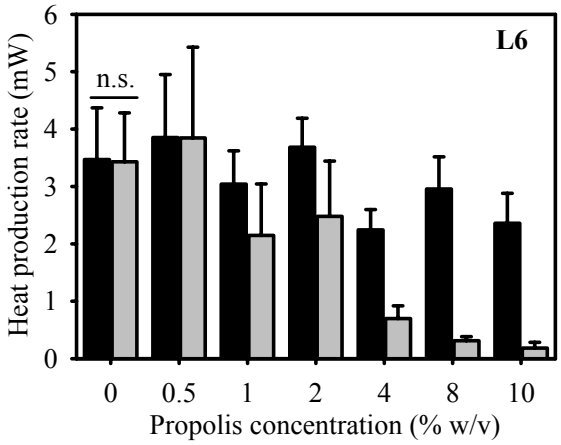


Fig. 6.2 Heat production rate (*P*, mW) per individual larva and specific heat production rate (*p*, mW g^{-1}) of L5, L6, L7 and pupa of the greater wax moth *Galleria mellonella*. L5, L6, and L7 represent the 5th, 6th, and 7th instar larvae, respectively. Mean ± SE, n = 54 for L5 and pupa and n = 36 for L6 and L7 each.





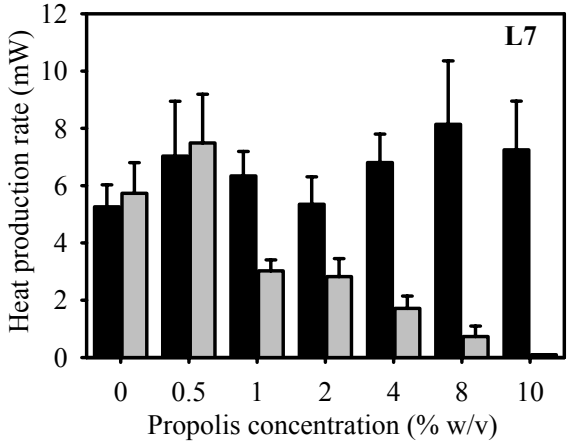


Fig. 6.3 Effect of treatment with different concentrations of propolis, in 55% ethanol, on the heat production rate (per animal) of the different instar larvae: L5, L6, and L7 of the greater wax moth *Galleria mellonella*. Mean ± SE, n = 9 (for L5) and n = 6 for L6 and L7. n.s. = no significant difference (paired sample t-test, p> 0.05).
■ Before treatment
□ After treatment

6.4.2 Short period experiments

The 5th larval instar was highly sensitive to propolis treatment compared to the 6th and 7th instars. Whereas the 2 latter instars did not display sensitivity even to 0.5% propolis, the heat production rate of the 5th larval instar was reduced by 48% due to treatment with 0.25% propolis (Fig. 6.3). Treatment with 4% propolis resulted in 100% mortality of L5 and reduced the heat production rate of L6 and L7 to 25% - 30% of the initial values (Fig. 6.3). Although the heat production rate of L5 dropped by 7.4%, this change was not statistically significant (paired sample t-test, p = 0.09); and the other 2 larval instars did not show any observable sensitivity to the control treatment (Fig. 6.3). The 6th and 7th larval instars were sensitive to treatments with propolis concentrations of ≥ 1%, with 10% propolis resulting in 100% mortality of L7 and a decrease of the power-time *(p-t)* curve to the baseline. The same concentration reduced the heat production rate of L6 by 95% (Fig. 6.3 b and c). In addition to the change in the total heat production rate, the change in the specific heat production rate showed a similar pattern (Fig. 6.4). The dose-response curves of residual specific heat production rate versus propolis concentration were the same for L6 and L7, but L5 displayed a different pattern (Fig. 6.5).

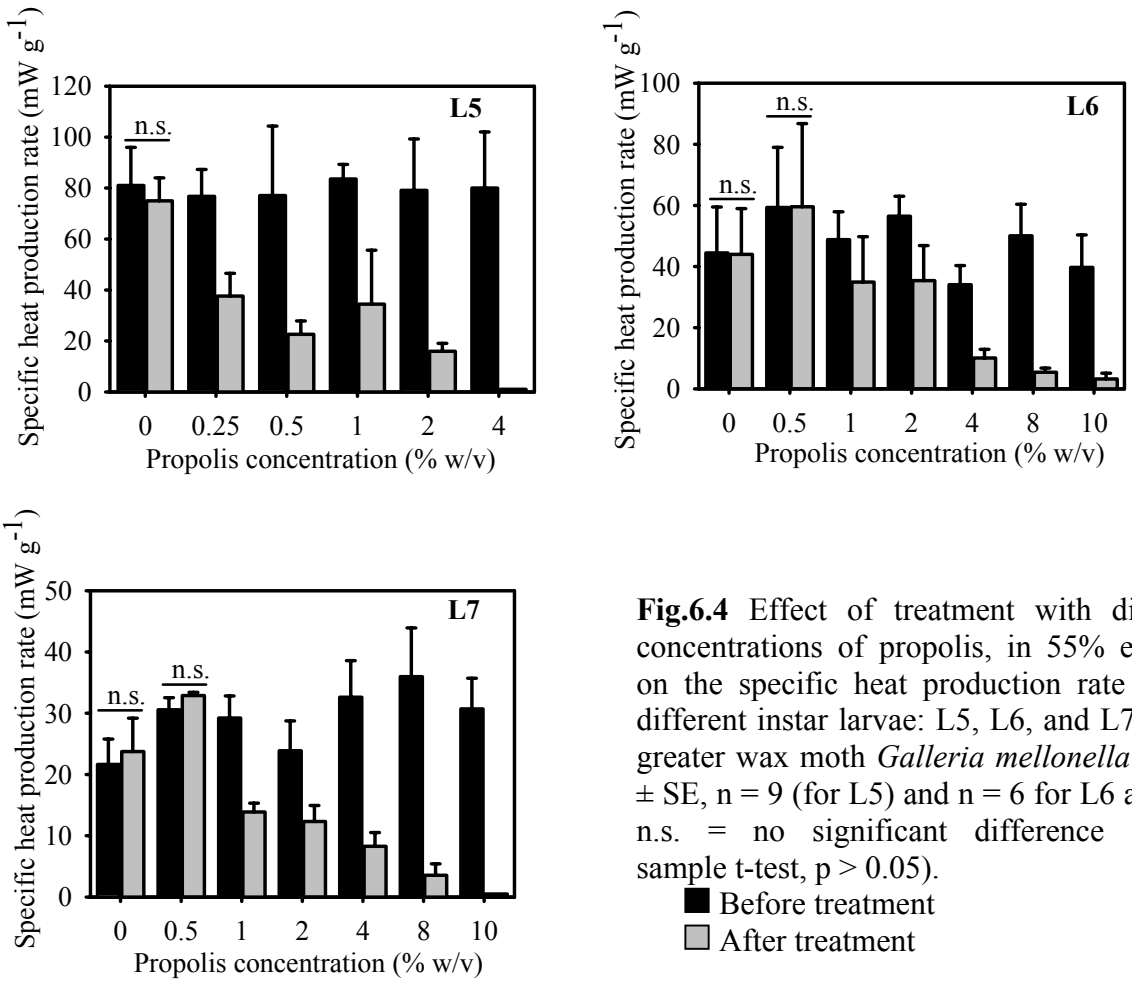


Fig.6.4 Effect of treatment with different concentrations of propolis, in 55% ethanol, on the specific heat production rate of the different instar larvae: L5, L6, and L7 of the greater wax moth *Galleria mellonella*. Mean ± SE, n = 9 (for L5) and n = 6 for L6 and L7. n.s. = no significant difference (paired sample t-test, $p > 0.05$).
■ Before treatment
□ After treatment

6.4.3. Long period experiments

The typical (control) long period *p-t*, curve of *G. mellonella* development in the last larval and the pupal stage showed a drastic drop in the heat production rate from the late L7 to the pupal stage through the prepupa (late L7 enclosed in silk cocoon) (Fig. 6.6 a).

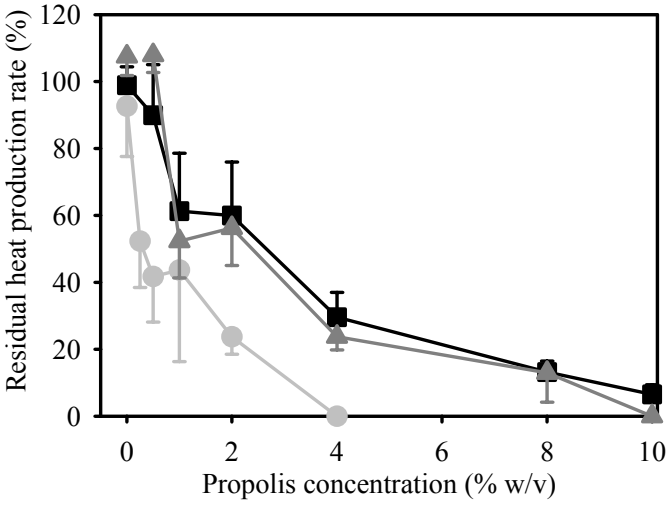
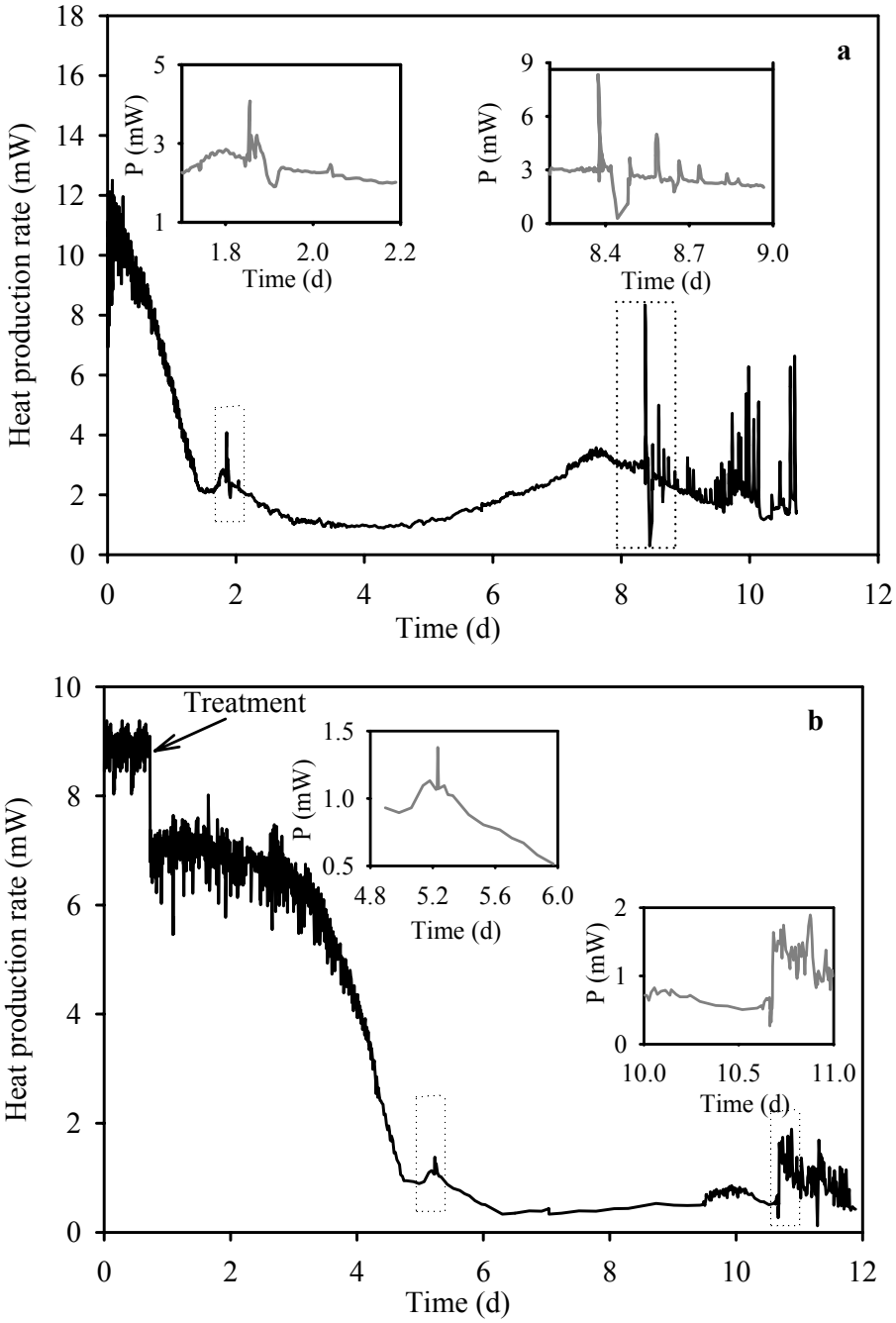


Fig. 6.5 Percentage residual specific heat production rates of the 5th (L5), 6th (L6) and 7th (L7) larval instars of the greater wax moth *Galleria mellonella* after treatment with various concentrations of propolis in 55% ethanol. Mean ± SE, n = 9 (L5) and n = 6 (L6 and L7).

● L5
■ L6
▲ L7

The transition from prepupa to the pupal phase around day 2 was accompanied by a sharp peak followed by a trough at ecdysis. The pupal heat production rate then dropped from a mean value of 2.2 ± 0.9 mW to 0.9 ± 0.3 mW in 1 day and remained at this level for the next 3 days. The heat production rate started to ascend between the 3rd and 4th days after pupation and achieved a maximum value of 3.6 ± 0.8 mW on the 6th day after pupation. The pupa-adult moulting took place 6.6 ± 0.7 days after the larva-pupa moulting. This last moulting was accomplished after a strong muscular contraction activity displayed by a sharp peak of 8.2 ± 0.45 mW followed by a trough of 0.2 ± 0.1 mW (Fig. 6.6 a).



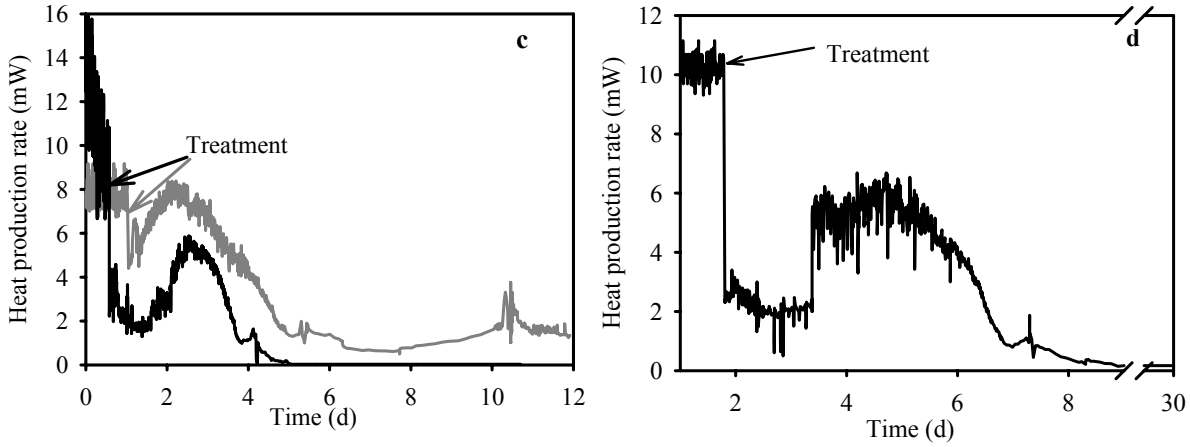


Fig. 6.6 Typical power time (*P-t*) curves of the development of *Galleria mellonella* from the late 7th larval instar to adult emergence. (a) 307 mg larva without treatment, (b) 173 mg larva treated with 1% propolis, (c) 209 mg (i) and 308 mg larvae (ii) treated with 2% propolis, and (d) 189 mg larva treated with 4% propolis. Note the differing vertical scales. Treatment period was 30 s. The insets are enlarged portions of the larval-pupal and pupal-adult ecdysis, marked by rectangles on the curve underneath the corresponding inset.

The 5 pupae treated with 1% propolis during the late L7 stage successfully completed their development to adult emergence. A representative example of pupal development after treatment with 1% propolis is displayed in Fig. 6.6 b. Though some of the larva-pupa and pupa-adult ecdyses were accompanied by peaks and troughs on the *p-t* curves, they were much weaker than the control peaks and near to the "resting" heat production rates. Some of the ecdyses displayed only exothermic peaks but no troughs (Fig. 6.6 b). The pupal-adult moulting of these treated organisms showed a unique feature in that there was no sharp exothermic peak followed by a trough, unlike the controls; rather the moulting was displayed by raising the level of the curve to a higher value. The pupal metamorphosis lasted 5.38 ± 0.9 d, which is shorter than that of the control.

Among the 5 larvae treated with 2% propolis, only 3 completed pupal development whereas 2 of them, even though they accomplished the larva-pupa ecdysis, were unable to complete their pupal development. The peaks and troughs during ecdyses were not as strong as in the case of the controls. In addition to that, the emerged adults, like the ones of 1% propolis treatment, did not show the typical *p-t* curves displayed by the controls; it was rather weak locomotory (flying) activity (cf. Fig. 6.6 a, b, and c). The pupal metamorphosis after treatment with 2% propolis lasted 4.8 ± 0.5 d.

Though the larvae treated with 4% propolis performed the larva-pupa ecdyses, the pupal development was aborted in all the 5 larvae investigated. These results indicate that, though the larvae survived the treatment and had a residual heat production rate of ca. 25% (Fig. 6.5), they were too weak and too unhealthy to go through pupation.

Apart from the difference in the heights of peaks (exothermic) and depths of troughs (endothermic) associated with moulting; the treatment with different concentrations of propolis introduced a significant difference in the length of the pupal metamorphosis (Table 6.1).

Table 6.1 Effect of propolis on length of pupal metamorphotic phase
After the late L7 stages of the greater wax moth *Galleria mellonella* were treated with sublethal concentrations of EEP, they were allowed to go through metamorphosis. The length of time (days) needed to complete this phase was recorded. A 1-way ANOVA and the Tukey's HSD post hoc test ($\alpha = 0.05$) were employed. Tukey`s test results with identical letters show no significant difference, but different letters do. Mean ± SE, n = 5.

Treatment	Length of pupal metamorphosis (d)	Tukey`s test
Control (no treatment)	6.6±0.7	a
Control (55% ethanol)	6.8±0.8	a
1% propolis	5.4±0.9	b
2% propolis	4.8±0.5	b

The specific heat production rate during pupal development showed a typical U-shaped curve for the controls. The curves for the treated pupae were, however, flatter with a smaller heat production rate in the late pupal and adult stages (Fig. 6.7).

The change of weight during the pupal development displayed similar patterns regardless of the treatment (Fig. 6.8). All pupae investigated showed a uniform loss of weight with developmental time until adult emergence.

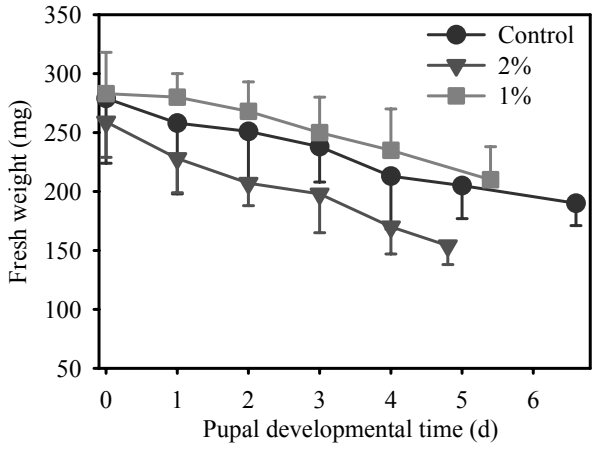


Fig. 6.7 Specific heat production (mW g^{-1}) of the greater wax moth *Galleria mellonella* during pupal development after treatment of the 7th instar larva with different concentrations of propolis. The pupal developmental time was counted starting from the larval-pupal moulting day as zero. Mean ± SE, n = 5.

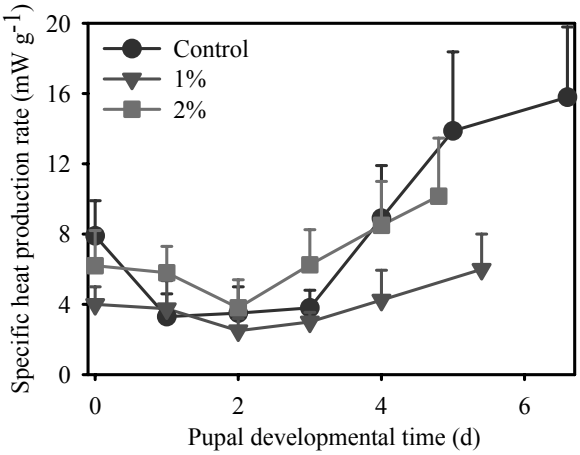


Fig. 6.8 Loss of weight of the greater wax moth *Galleria mellonella* during pupal development after treatment with different concentrations of propolis at the 7th larval instar. Only pupae that successfully completed pupal development were considered. The day of larval-pupal moulting was regarded as day zero. Mean ± SE, n = 5.

6.5 Discussion

The wet weight of larvae increased exponentially from the 5th to the 7th larval instar and dropped uniformly during pupal metamorphosis. This is because the larval stage is a "feeding machine", continuously consuming available food in order to accumulate enough reserve food for the entire phase of pupal metamorphosis and for the flying and reproductive activity of adults. As the pupal phase does not feed and hence completely depends on the reserve food accumulated during the feeding larval stage, its weight decreases continuously and at constant rate during metamorphosis. The uniform drop in the weight of the pupa during metamorphosis indicates that the rate of utilization of reserve food during this phase of *Galleria* development is constant. The tissue composition (proportion of fat, proteins and carbohydrates) remains almost constant at the various larval and pupal stages, changing only in the adults with increasing fat proportion (Schmolz et al. 1999).

The heat production rate increased from 1.77 ± 0.17 for L5 to 6.51 ± 0.44 for L7 mainly due to the increase in the wet mass. However, the specific heat production rate decreased from 78.8 ± 8.8 for L5 to 28.6 ± 2.0 for L7 and 8.2 ± 3.1 for the pupal stage. This decrease in the specific heat production rate is mainly because the bulk of the increased weight of L6, L7 and the pupal stages is reserve food and not metabolizing tissue. In the 5th larval stage, the main component of the larval weight is metabolizing tissue, leading to a very high specific heat production rate (Schmolz and Schulz 1995, Schmolz et al. 1999).

The higher sensitivity of the 5th larval instar to propolis treatment is due to at least 2 factors: the thin and relatively permeable cuticular layer, leading to a greater penetration of propolis; and the very high specific heat production rate, incorporating the toxin into the metabolic machinery at a faster rate.

Since the larval cuticle, or exoskeleton, stretches only to a limited extent it must be shed periodically to accommodate the rapidly growing body size of the larva, which could double daily during the first 10 days under ideal conditions (Morse 1978). Though the basic outer layers of the new cuticle are formed before shedding the old one, additional layers of endocuticle are added and sclerotization of outer layers increases with developmental days, throughout the duration of the instar (Semple et al. 1992). This indicates that the strength and thickness of the cuticle increases with age of the larval instar. The life spans of L5, L6, and L7 under ideal conditions are 2.2, 3.0, and 7.5 days, respectively (Sehnal 1966). Thus, L5 has the thinnest cuticle with high permeability and L7 has the thickest cuticular layer that impedes penetration of the lipophilic components of propolis. It is therefore highly plausible to state that, the thickness of the larval exoskeleton plays a role in the insecticidal action of propolis.

The higher specific heat production rate of the L5 plays a role in the faster penetration of propolis across the cuticle, and its accumulation at higher concentrations in the tissue. Higher metabolic rates are associated with increased transport of hydrocarbons and lipids through lipid pore canals across the cuticular layer (Renobales et al. 1991). The transport of hydrocarbons and lipids across the lipid pore canals to the cell surface may allow inward transport (penetration) of nonpolar pesticides, as displayed by direct correlation between active biosynthesis of hydrocarbons and transport to the surface and penetration by pesticides, especially the nonpolar ones (Theisen et al. 1991). As the majority of bioactive components of propolis are nonpolar, the analogy of penetration of nonpolar pesticides and propolis across the cuticular layer is reasonable. In addition to penetration via transport mechanisms, propolis may attack the cuticle aggressively and penetrate by destroying underlying structures.

When moulting, the cuticle begins to separate from the epidermis, the larva reduces feeding activity and becomes quiescent. Each active stage in the larval life is thus followed by a sluggish premoulting period (Snodgrass 1935). This quiescent stage in the transition from larva to pupa is accompanied by the declining heat production rate and a "U-shaped" *p-t* curve shortly before larval-pupal ecdysis. In this quiescent phase, a part of the old cuticle is degraded, resorbed and recycled by the epidermal cells for the formation of the new cuticular layer. Final break up of the old exoskeleton is achieved by peristaltic contraction of abdominal muscles, raising blood pressure in the thorax and splitting the former at the weakest point, usually along the mid dorsal line (Semple et al. 1992). This contraction of abdominal muscles is accompanied by a sharp peak on the *p-t* curve, and the break of the old cuticle and subsequent release of exuvial fluid is shown by the trough of evaporational heat loss. The height and area of the sharp peak and the depth of the trough indicate the amount of energy spent on contraction of the muscles and evaporation of exuvial fluid, respectively.

The treatment with propolis disturbs the above described typical moulting activity features of *Galleria mellonella*. After treatment with 1% propolis, all larvae were able to go through the metamorphotic phase and emerge as adult, but the exothermic and endothermic peaks were smaller than those of the controls. The adult emerged after the unusual moulting behaviour displayed a very weak flying activity demonstrated by the form of the *p-t* curve (cf. Fig. 6.6 a with b and c). The weak flying activity could be the result of malformed/underdeveloped flying muscles and or wing structures. This could be plausible as the metamorphotic phase/morphogenetic stage is significantly shortened due to treatment, ending up in the improper formation/deformation of body structures.

With the increase in the concentration of propolis, the length of the pupal phase was shortened significantly from 6.8 ± 0.8 d (ethanol control) to 4.8 ± 0.5 d (2% propolis) (Table 6.1). This suggests that propolis accelerates the development of the larval/pupal stage of *Galleria mellonella*. The unusually higher rate of metamorphosis may lead to malformed and immature individuals.

The biological activity of propolis displayed on *Galleria mellonella* is comparable to that of insect growth regulators (IGR) and toxicants, calorimetrically investigated by several researchers. Among others, Kuusik and colleagues (Kuusik et al. 1993, Kuusik et al. 1995) and Harak et al. (1999) elucidated that IGR and toxic compounds/mixtures interfere with the form of the *p-t* curve of insect development, even leading to abortion of metamorphosis. It was stated (Strong and Dickman 1973) that IGR could act to inhibit, retard or even accelerate insect developmental processes. The biological activity of propolis on *Galleria mellonella* obtained in the present investigation fits with those that accelerate insect development. It was also demonstrated by several researchers (Snodgrass 1935, Williams 1967, Strong and Dickman 1973, Theisen et al. 1991, among others) that the application of IGR at the larval stage resulted in the disruption of pupal development and early adult emergence. In addition, normal ecdysis was not achieved. Additionally, treated larvae may give rise to morphologically deformed adults that are unable to fly properly (Metwally and Sehnal 1973).

The use of moderate concentrations of propolis, such as 4%, in the control of *Galleria mellonella* is reasonable since it is toxic and kills the early larval stages immediately, facilitates larval-pupal ecdysis, and aborts pupal development. The practical significance of such concentrations of propolis is that they help to avoid the use of higher propolis concentrations that could probably affect the quality of honeybee wax and also avoid unnecessary wastage of propolis. Propolis can naturally occur in beeswax to a certain degree, but higher concentrations may be undesirable in some uses of wax, such as in the cosmetic industry where propolis may cause allergy in very few individuals.