



Pupal diapause in *Hypocosmia pyrochroma* (Lepidoptera: Pyralidae), a biological control agent for *Dolichandra unguis-cati* (Bignoniaceae)

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Received: 25 October 2021 / Accepted: 3 July 2024 / Published online: 22 July 2024
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Abstract

Cats claw creeper leaf-tying moth *Hypocosmia pyrochroma* (Lepidoptera: Pyralidae) enter pupal diapause in the soil from middle of autumn (April), in response to declining photoperiod. Proportion of larvae entering pupal diapause increased with decreasing Daily Solar Radiation (DSR), and all larvae completing development in winter under low DSR entered pupal diapause. Under natural photoperiod, adults emerged from pupal diapause, from late spring (October) to middle of summer (January), with peak adult emergence in late spring (November) and early summer (December). Pupae did not undergo diapause when the entire development (eggs and all larval instars) occurred under prolonged photoperiod (14 L:10D). However, it was not possible to terminate the pupal diapause either by prolonging photoperiod or by increasing the temperate regimes. Based on larval incidence in the field it is proposed that *H. pyrochroma* is a bivoltine species with overlapping generations.

Keywords Cat's claw creeper · Leaf-tying moth · Biological control · Diapause · Photoperiod

Introduction

The leaf-tying moth *Hypocosmia pyrochroma* Jones (Lepidoptera: Pyralidae), native to tropical South America, is a host specific and destructive biological control agent introduced against the weed cat's claw creeper, *Dolichandra unguis-cati* (L.) Lohmann (Bignoniaceae) in Australia (Dhileepan et al. 2007) and South Africa (King et al. 2011). Feeding and leaf tying by *H. pyrochroma* larvae severely damage foliage (Williams 2003) and result in reduced plant growth and subterranean tuber production (Snow et al. 2006). Adults are nocturnal and live for about two weeks under glasshouse conditions. Females lay about 150 eggs, singly, on the undersides of leaves and on the stems and the eggs hatch in 12 days. Emerging larvae feed destructively on cat's claw creeper by tying leaves together by silk webs, which create silken tunnels. Larvae go through six instars and take about 30 days to complete development.

Fully-grown larvae pupate in the soil, 2 to 3 cm below the soil surface. The pupal stage during summer lasts for about 30 days. Pupae undergo diapause from middle of autumn to late spring. The Day-Degrees (DD) required to complete a generation of *H. pyrochroma* was 877 (Dhileepan et al. 2013). In the glasshouse, the generation time (egg to adult) of *H. pyrochroma* without pupal diapause was 10 weeks. Based on DD requirements, potential number of generations *H. pyrochroma* can complete in a year in Australia has been suggested from two to three (Dhileepan et al. 2013).

Diapause, a period of suspended development at a specific developmental stage (egg, larval, pupal, or adult) of an insect, is triggered by environmental cues, like changes in daylight, temperature, or food availability (Denlinger 1986). Prolonged pupal diapause has been reported in several temperate lepidopteran species (Sims 1983). Yet, prolonged facultative diapause may occur in univoltine species, under extreme environmental conditions (Gill et al. 2017). In this study the role of photoperiod in the initiation of pupal diapause in *H. pyrochroma*, a tropical species is reported. Based on larval incidence in the field it is proposed that *H. pyrochroma* is a bivoltine species with overlapping generations, and not a multivoltine species as predicted by the DD model.

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Materials and methods

Hypocosmia pyrochroma

A colony of *H. pyrochroma* was established in a quarantine facility (22–27 °C; $65 \pm 5\%$ RH and natural photoperiod) at the Alan Fletcher Research Station, Sherwood, Queensland, Australia in 2005 for host specificity testing (Dhileepan et al. 2007). The insect was originally collected on cat's claw creeper from sites near Curitiba in Brazil and Posadas in Argentina by S. Naser and C.J. Cilliers in April 2002 (Williams 2003). The moth was approved for field release in Australia in 2007 (Dhileepan et al. 2007) and was field released from 2007 to 2010. Evidence of its field establishment was first observed in 2012, and since then the moth has dispersed more widely, but its establishment is restricted to riparian areas (Dhileepan et al. 2021).

Glasshouse studies

Biological studies were conducted in a quarantine glasshouse (20–27 °C; $65 \pm 5\%$ RH; natural photoperiod) from November 2005 to April 2008. Un-sexed 20–30 newly emerged moths were added to an insect-proof rearing cage (90 cm x 80 cm x 75 cm) filled with potted cat's claw creeper plants grown to at least 15 cm tall (28 plants in 125 mm pots). Moths were fed with cotton balls soaked with a solution of energy drink powder (Gatorade) and water. After two weeks, any live adult moths were removed, and the plants checked for egg hatching. Emerged larvae fed on the potted plants for 2–3 weeks and then the entire foliage with developing larvae were removed from the cage to avoid pupation in the potted plants. All larvae were transferred to transparent plastic boxes with fine holes in the bottom to drain excess water and filled with sterilised fine sand for pupation. Proportion of pupae undergoing diapause was recorded, along with pupal duration in relation to pupation month/season.

Studies in environmental growth cabinets

From April 2008 to December 2010, *H. pyrochroma* cultures were maintained in Environmental Growth Cabinets (27 ± 1 °C, $65 \pm 5\%$ RH and 14 L:10D). Newly emerged adults (10–20 pairs) were added to a 2 L transparent plastic container, and a corrugated paper towel was provided for oviposition. Adults were fed on diluted honey or Gatorade. After two weeks, unhatched eggs and newly emerged larvae were transferred into large plastic storage boxes (50 L) containing cut cat's claw creeper foliage, placed on the top of wire mesh (with the holes large enough for the larvae to drop down) with sterilized sand at the bottom of the box for

pupation. After 6 weeks, the sand in the bottom of the boxes was sieved to recover pupae. Pupae were stored in transparent plastic boxes (17 cm x 12 cm x 7 cm) for eclosion. Proportion of pupae undergoing diapause was recorded, along with pupal duration in relation to pupation month/season.

Field observations

Field monitoring of larval incidence and leaf-tying damage symptoms was conducted during 2018–2019 and 2020–21 seasons (observations in 2019–20 was suspended in March 2020, due to covid-19 related restrictions). Though the evidence of field establishment of *H. pyrochroma* was first noticed in 2012, consistent and more widespread field establishment was observed only from 2018 onwards (Dhileepan et al. 2021), and hence the field study was conducted from 2018 to 19 season. Larval activities of *H. pyrochroma* was monitored in three riparian (Boompa, Coominya, Moo-gerah) and one non-riparian (Oxley) sites at monthly intervals from October to May. In each site, a minimum of 10 trees was sampled for larval activities. If larvae were found, approximate larval instar was estimated visually. If there was no evidence of larval activity in 10 trees, the sampling was continued for more trees (> 20 trees) to make sure that there was no evidence of *H. pyrochroma* larvae at the site.

Data analysis

SigmaStat version 4 was used in all statistical analyses. Differences in pupal duration and proportion of adult emergence between pupae under diapause and pupae not in diapause, and differences in the number of adults emerging per day between months, were compared using One-way ANOVA, and the means compared using Tukey Test. Polynomial regression analysis was used to study the relationship between Daily Solar Radiation (DSR) (monthly mean) and proportion of developing larvae entering pupal diapause (data pooled from 2007 to 2008); and between the day (Julian) of pupal diapause initiation under natural photoperiod and the duration of pupal diapause. The DSR for the Brisbane region was sourced from the Bureau of Meteorology (<http://www.bom.gov.au>). All results in the text are presented as means \pm standard error (SEM).

Results

In the glasshouse, from late spring (November) to early autumn (March), under natural photoperiod and optimum temperature (22–27 °C) and humidity ($65 \pm 5\%$ RH), 82.7% of *H. pyrochroma* pupae emerged as adults in 32.9 ± 1.7 days. From late autumn (April–May). *H. pyrochroma*

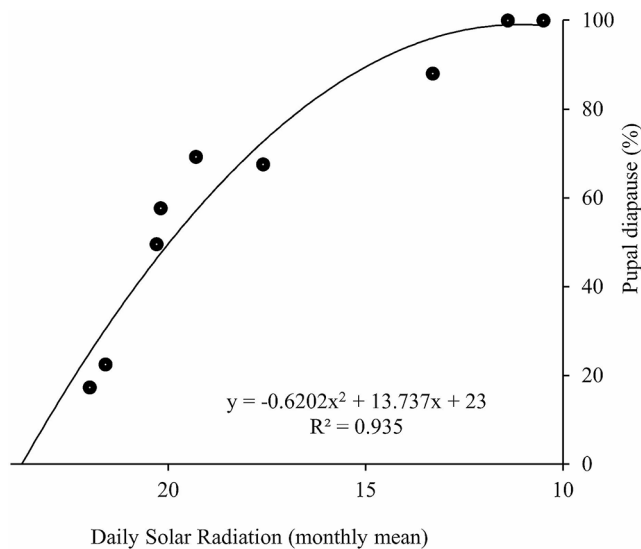


Fig. 1 Relationship between duration of daily solar radiation (monthly mean) and pupal diapause incidence (%) in *Hypocosmia pyrochroma* under natural photoperiod (data from 2007 and 2008 pooled)

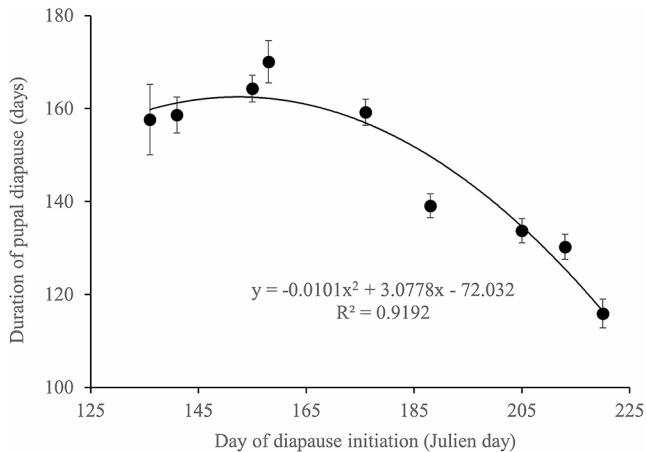


Fig. 2 Relationship between day of diapause initiation (Julien day) and the duration of pupal diapause (days, Mean \pm SE) for *Hypocosmia pyrochroma* under natural photoperiod in the glasshouse (data from 2007–2008)

entered pupal diapause in the soil, in response to declining photoperiod. The proportion of pupae entering diapause in autumn increased with decreasing Daily Solar Radiation (DSR) (Fig. 1). All larvae completing development in winter under low DSR (< 12) entered pupal diapause.

The duration of the pupal stage under diapause was significantly longer (147.6 ± 6.1 days) than in non-diapausing pupae (32.9 ± 1.7 days) ($F = 346.8$; $p < 0.001$). The duration of pupal diapause declined with the delay (increase in the Julien day) in the onset of pupal diapause (Fig. 2). Adults emerged from diapausing pupae from middle of spring (October) to middle of summer (January) (Fig. 3), with peak adult emergence (58%) in late spring (November) and

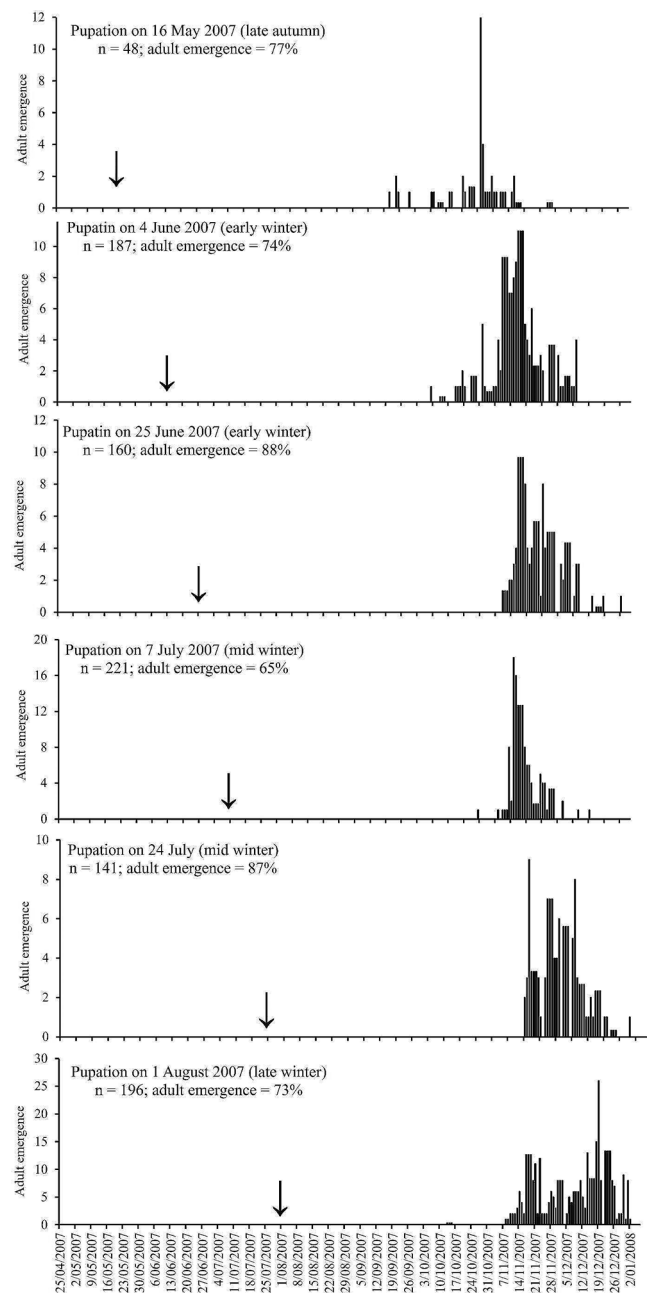


Fig. 3 Pattern of *Hypocosmia pyrochroma* adult emergence from pupal diapause in relation to time of pupation (from late autumn to early winter), under natural photoperiod (data for 2007–2008). Arrows indicate the week of onset of pupal diapause

(37%) early summer (December) (Fig. 4). There was no significant difference in proportion of adults emerged between non-diapausing ($82.7 \pm 1.4\%$) and diapausing ($76.9 \pm 3.7\%$) pupae ($F = 1.413$; $p = 0.260$).

In Environmental Growth Chambers, under optimum temperature (27 ± 1 °C), high humidity ($65 \pm 5\%$ RH) and longer photoperiods (14 L:10D), the larvae did not undergo pupal diapause. When the entire larval stage, from first instar to last, were exposed to prolonged photoperiod, it was

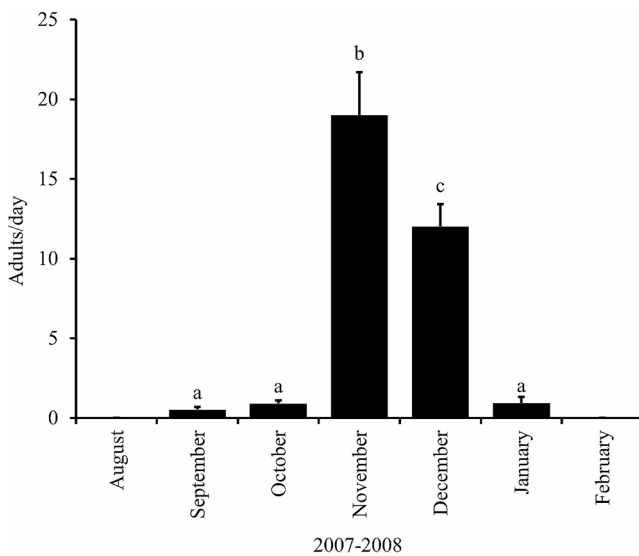


Fig. 4 Seasonal pattern of *Hypocsmia pyrochroma* adult emergence (Mean ± SEM) from pupal diapause under natural photoperiod (data for 2007–2008). One-way ANOVA. $F_{4,126} = 31.52$, $p < 0.001$. Means with same alphabets are not significantly different (Tukey test, $p < 0.05$)

possible to prevent the larvae from entering pupal diapause. However, exposure of diapausing pupae under prolonged photoperiod (14 L:10D) and high temperature ($30 \pm 1^\circ\text{C}$) did not result in the termination of the diapause.

In the field, evidence of larval activities was first noticed in late spring (late November) and continued till late autumn (May). Peak larval activities were seen from middle to late summer (January–February). From middle of autumn (April) onwards, most of the larvae found were in middle to late stages of development (instars 3 to 5). No larval activity was seen from June (early winter) till late November (late spring), as predicted from the glasshouse studies.

Discussion

Diapause in one of the basic means by which insects cope with unfavourable environmental conditions. Photoperiod is one of the main environmental cues followed by insects to predict the forthcoming seasonal changes and to adapt to these changes in their life-history traits (Hasan et al. 2020). This study provides evidence for the photoperiod induced pupal diapause in *H. pyrochroma*. Such prolonged diapause is more frequent in univoltine populations and least frequent in multivoltine populations (Sims 1983). Prolonged pupal diapause has been found in a number of temperate Lepidoptera (Sims 1983) but are uncommon in tropical Lepidoptera.

The pupal diapause in *H. pyrochroma* was not dependent on temperatures, as observed in temperature-controlled experiments where the developing larvae were exposed to a range of temperatures (12–40 °C) at 14 L:10D photoperiod

(Dhileepan et al. 2013). Larvae developing under natural photoperiod (with no supplementary light) in temperature-controlled glasshouse underwent pupal diapause from late autumn (April–May) until middle to late spring (October–November), in response to declining photoperiod. Photoperiodism plays a primary role in regulating the induction of diapause in insects (Denlinger 1986), and photoperiod-induced larval and pupal diapauses in family Pyralidae are well known (e.g., Hasan et al. 2020; Huang et al. 2009; Xiao et al. 2010; Xu et al. 2014).

Adult emergence after pupal diapause under natural photoperiod was erratic and inconsistent (Fig. 3), with adults continuing to emerge from late spring to late summer (Fig. 4), resulting in overlapping generations. This made the colony maintenance difficult, and restricted the field releases only to a few months, from late spring to early autumn, resulting in only limited number of field releases (Dhileepan et al. 2021). When the entire larval stage (from first instar) was exposed to prolonged photoperiod, it was possible to prevent the larvae from entering pupal diapause. This enabled mass rearing and field releases to continue throughout the year. As it was not easy to manipulate the photoperiod of large glasshouse facilities with artificial/ supplementary light sources, developing larvae were transferred to Environmental Growth Cabinets where the photoperiod was enhanced (14 L:10D), under optimum temperature ($27 \pm 1^\circ\text{C}$) and humidity ($65 \pm 5\%$ RH). This method prevented larvae entering pupal diapause during the winter months. However, once a pupa entered diapause, it was not possible to terminate the diapause either by prolonging photoperiod or by increasing the temperature regimes. Exposure of diapausing pupae to constant cold temperatures ($5 \pm 1^\circ\text{C}$ or $10 \pm 1^\circ\text{C}$) for one, two or three weeks in continuous darkness, and then transferring to a higher temperature ($30 \pm 1^\circ\text{C}$) under longer photoperiod (14 L:10D) also did not result in diapause termination (K. Dhileepan, unpublished data). Similar spontaneous termination of diapause without being induced by photoperiods (Jiang et al. 2010; Tobin et al. 2002) or temperatures, including chilling (e.g., Chen et al. 2014) has been reported in other lepidoptera as well. Further studies are needed to understand the factors needed to terminate pupal diapause in *H. pyrochroma*.

Incidence of fresh leaf-tying symptoms with newly emerged larvae in the field coincided with adult emergence from diapausing pupae in the glasshouse under natural photoperiod. Evidence of larval activities in late spring (November) suggest that the likely time of adult emergence in the field was in late spring (early to middle of November), two weeks (3 days of pre-oviposition period and 12 days of incubation period) before the neonate larvae were found in the field. Peak adult emergence (58%) in late spring (November) and (37%) early summer (December) in

the glasshouse coincided with peak leaf tying damage in the field in summer (December to February). In the field, adults emerging from pupal diapause from late spring (November) to early summer (December) laid eggs. Larval and pupal development completed in 10 weeks with the second-generation adults emerging from late summer (February) to early autumn (March). Larvae from the second-generation adults, from early autumn (March) underwent pupal diapause, due to shorter photoperiod. Based on larval incidence in the field we propose that *H. pyrochroma* is a bivoltine species with an overlapping generation, and not a multivoltine species as predicted from thermal and climate modelling studies. In the field *H. pyrochroma* completed a summer generation (from adults emerged from pupal diapause from the previous year) without pupal diapause, and an autumn generation with a prolonged pupal diapause (and the adults emerged in spring in the following year).

Acknowledgements The research was supported by the Queensland Department of Agriculture and Fisheries (QDAF). I thank Deanna Bayliss, Mariano Trevino, Jayd McCarthy, Dianne Taylor, Boyang Shi, Mohamad Mahbubur (QDAF) and Kevin Jackson (Goomboorian, Queensland) for technical support.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions

Declarations

Conflict of interest None.

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