

Effect of habitat type and soil moisture on pupal stage of a Mediterranean forest pest (*Thaumetopoea pityocampa*)

Lucía Torres-Muros, José A. Hódar and Regino Zamora

Facultad de Ciencias, Department of Ecology, University of Granada. Avda. Fuentenueva s/n, E-18071, Granada, Spain

- Abstract**
- 1 Habitat selection is especially important for pupae of holometabolous insects because this stage is usually immobile and to a certain extent unable to react to environmental changes. In the present study, we analyze how habitat and soil moisture determine the fate of pupae of the pine processionary moth *Thaumetopoea pityocampa*, a defoliator pest in pine woodlands of Europe and the Circunmediterranean region. The pine processionary moth pupates buried in the soil and can spend up to 9 years in an extra-long diapause.
 - 2 We considered the impact that different habitats, as well as the manipulation of soil water conditions in the preferred habitat (i.e. in bare ground with null or sparse herbaceous vegetation), can exert on pupation.
 - 3 Less than half of the buried larvae successfully pupated, even in the most favourable habitats and soil water conditions, whereas, on average, 2.5% underwent extra-long pupae diapause (2 years after pupation).
 - 4 Although habitat influenced mainly pupation success and pupal survival, changing moisture conditions in the preferred habitat affected primarily the phenology of emergence, whereas pupal survival remained unaffected.
 - 5 The results of the present study reveal the importance of both habitat and soil moisture on the pupal stage, providing valuable information with respect to adequately forecasting the effects of changes in climate or land use on the population dynamic of this important forest pest.

Keywords Habitat, moth phenology, Pine processionary moth, pupal diapause, soil moisture.

Introduction

The pupal stage of holometabolous insects is a critical step of their life history. The lack of mobility makes pupae susceptible to high rates of predation, parasitism and/or diseases, and also limits their reaction capacity against extreme abiotic conditions (Cornell & Hawkins, 1995; Hawkins *et al.*, 1997). Thus, larvae often select a protected site before pupation, seeking shelter, camouflage and frequently adding a silk cocoon as protection (Sims & Shapiro, 1983; Ruszczuk, 1996; Stefanescu, 2004; Zheng *et al.*, 2011). A common solution is burrowing into the soil, where temperature changes are tempered and many predators and pathogens are excluded (Hogdson *et al.*, 1998), although there is also a notable risk of flooding, as well as rotting by the action of fungi and bacteria, and specialist predators (Kaya & Haya, 1981; Murray & Zalucki, 1990; Hogdson *et al.*,

1998; Zheng *et al.*, 2011). The pupal period not only imposes spatial immobility, but also allows temporal flexibility to suit the emergence time as an adult to favourable ambient conditions aboveground (Tauber & Tauber, 1976; Hayes, 1982; Tauber, 1986; Denlinger, 2002; Kingsolver *et al.*, 2011).

The extension of the pupation period, beyond the time strictly needed for metamorphosis, can be a result of mere quiescence, a simple arrest of the development lasting until ambient conditions change (e.g. until temperature increases) or diapause, a break of development somewhat independent of ambient conditions, as well as centrally or physiologically controlled (Danks, 1987; for diapause-related terminology, see also Kostál, 2006). In the case of the diapause, the entry in pupal diapause can be promoted by food during the larval period (Liu *et al.*, 2010; Metspalu *et al.*, 2013), as well as photoperiod and/or temperature (Kay, 1982; Sims & Shapiro, 1983; Xiao *et al.*, 2013), and the same applies to the exit (Shimoda & Kiuchi, 1997; Chen *et al.*, 2013). Thus, the beginning and the end of that time period can be partially modulated by environmental conditions, even those with strict

Correspondence: Lucía Torres-Muros; Tel.: +34 958 241000 ext. 20079; fax: +34 958 246166; e-mail: torresmuros@ugr.es

genetic control (Denlinger, 2002; but see Menu *et al.*, 2000). For this reason, the habitat selection for pupation is important not only for predation risk or shelter against adverse environmental conditions, but also for conditions of abiotic stimuli perceived by pupae.

In the current context of climate change, many of these abiotic stimuli (e.g. humidity or temperature) are expected to vary substantially. Taking into account the above consideration, we followed a field-experimental approach to analyze how different habitat and climate conditions affect the survival and phenology of a moth species that undergoes pupation in the soil. As a model species, we chose the pine processionary moth (PPM) *Thaumetopoea pityocampa*. This species represents an excellent case study because it combines univoltine life cycles with prolonged pupal diapause, and because of its ecological and economic importance as a forest pest (Battisti *et al.*, 2015). Unlike most temperate and Mediterranean Lepidoptera, the PPM has a peculiar 1-year development cycle because the larvae feed over the winter. Pupae spend several months (in spring) undergoing metamorphosis in the soil and moths emerge in early summer to autumn depending on elevation/latitude and temperature. Additionally, prolonged pupal diapause makes the population dynamics rather complex. The information available on the biology of the PPM adult and larva is quickly increasing (Battisti *et al.*, 2005; Stastny *et al.*, 2006; Buffo *et al.*, 2007; Hoch *et al.*, 2009; Dulaurent *et al.*, 2012), whereas knowledge of the pupa remains scarce (Markalas, 1989; Aimi *et al.*, 2006; Dulaurent *et al.*, 2011).

The present study aimed to analyze the effect of habitat on the survival and phenology PPM. Accordingly, we chose a mosaic landscape composed of patches of native forest and degraded habitats (hereafter, Habitat experiment). Selected habitats comprised open areas, shrubland and forest, with the main ones in the Mediterranean mountains, representing the trend towards replacement of cropland and pastures with shrubland and woodland. Moreover, because the present study aimed to determine the effects of climatic variability on the survival and phenology of PPM (hereafter referred to as the Water experiment), we experimentally varied the soil water content that could appear during the coming decades in the Mediterranean area (i.e. drier and wetter summer conditions). We simulated: (i) sporadic wetter conditions during the growing season, based on maximum historical records from the study area (Matías *et al.*, 2011), and (ii) drier climatic conditions during the growing season, in accordance with the A-2 scenario from the Intergovernmental Panel on Climate Change (IPCC).

Thus, the present study aimed to test the effect of different habitats (Habitat experiment) and soil moisture (Water experiment) conditions on: (i) burial success, as indicated by larvae becoming buried without dying in surface soil; (ii) pupation success and pupal survival, as indicated by larvae pupating after being successfully buried and pupae surviving after the first summer, respectively; (iii) extra-long pupal diapause, as indicated by pupae remaining buried after 2 years of the experiment; and (iv) moth emergence and phenology, as indicated by the number of emerged moths and the date by when this is achieved. These two experiments will provide valuable information on how the soil stages of life cycle of PPM will be affected by two important factors related to global change.

Materials and methods

Study area

The study was performed in La Cortijuela Botanical Garden, within Sierra Nevada National Park (1650 m.a.s.l.; 37°05'N, 3°28'W, Granada, SE Spain). This mountain area has a continental Mediterranean climate, with cold winters, and hot and dry summers. Rainfall is 870 ± 70 mm/year, mostly during spring and autumn, and the mean temperatures of coldest and hottest months (January and July, respectively) are 3.0 ± 0.3 and 21.6 ± 0.4 °C, respectively (mean \pm SE 1990–2012). The vegetation in the area is composed of patches under different management, namely pine plantations (*Pinus sylvestris* and *Pinus nigra*) with intermingled oaks (*Quercus ilex*), shrublands, open areas and patches of native forest (*P. sylvestris* var. *nevadensis*, *Acer opalus* ssp. *granatense*, *Taxus baccata*). The study lasted 3 years, starting in 2010, when there was a peak in the PPM population cycle, with severe defoliation in the area, whereas 2011 and 2012 had much lower infestations.

Experimental design

To analyze how the habitat affects the survival and phenology of PPM, we chose a mosaic landscape composed of patches of native forest and degraded habitats. The Habitat experiment had three levels: Open, in bare ground with null or sparse herbaceous vegetation; Shrubland, in areas covered by the dominant shrubs at the site (e.g. *Crataegus monogyna*, *Prunus ramburii* and *Genista speciosa*); and Woodland, formed by mixed forests of *P. sylvestris*, *P. nigra*, and *Q. ilex*. Each level had three replicates, consisting of plots of 2×2 m, in which the controlled burials of larvae were set. Accordingly, each plot had 11 breathable buried bags: 10 for larval pupation and one for the microclimatic stations. Pine processionary caterpillars in pre-pupation processions were collected to burial into the bags. A total of 50 larvae by bag were buried. Thus, the initial setting was 3 levels × 3 replicates × 10 bags × 50 larvae for habitat experiment, for a total of 4500 larvae (see Supporting information, Fig. S1).

The water experiment also had three levels: Drought, Control and Watering. All levels were set in the same bare-ground area, the preferred burial habitat (Robredo, 1963; Dulaurent *et al.*, 2011), and soil-water input was manipulated. The differentiation in these climate scenarios was based on the study by Matías *et al.* (2011). The Drought treatment was arranged by means of rain-exclusion shelters (Yahdjian & Sala, 2002) formed by a 2×2 m metal frame supporting V-shaped clear methacrylate bands without ultraviolet filters (Barlocast; Faberplast S.L., Spain), covering 35% of the surface, and intercepting the same percentage of natural water supply (rain). The level for rain exclusion was based on the SRES A-2 model by the IPCC (2007), where a reduction in summer rainfall of 30% is predicted from 1980–1999 to 2080–2099 for Mediterranean areas. Rainout shelters were placed from April to September, simulating drier and longer summers. The control treatment involved squares exposed to natural rainy conditions over the experimental period. In the Watering treatment, the sporadic summer storms characteristic of Mediterranean mountains were simulated. Accordingly, a

water-addition system comprising four sprinklers at the corners of the squares was set. Each week from the beginning of May until the end of September, a quantity of 12 mm of water was added, simulating a summer storm. If a natural storm occurred during a week, the irrigation pulse was not added. The total water added during the summer was 180 mm, which is equivalent to the mean summer rainfall of the five wettest summers of the 1902–2006 series in the study area. Each water level had three replicates, also consisting of plots of 2×2 m, with the same setting as the habitat experiment: 10 bags for larval pupation and one for the microclimatic stations. Thus, the initial setting also was 3 levels × 3 replicates × 10 bags × 50 larvae for Water experiment, for a total of 4500 larvae.

Plots for both experiments were selected in September 2009. Holes (diameter 20 cm, depth 40 cm) were opened with a power auger, and then bags were filled with the same soil extracted and placed into holes, ensuring the absence of previous processionary pupae. All bags (length 80 cm, diameter 20 cm) comprised fine wire netting (mesh size 1 mm × 1 mm) and, once set in soil, the upper part of the bag, which was not buried in soil, could be sealed to retain the emerging moths. Bags were left then untouched during 6 months to simulate as much as possible the level of soil compaction of the natural habitat.

Insects used in the experiments

In March 2010, pine processionary caterpillars in pre-pupation processions were collected in a forest gap 200 m far from the burial site. All larvae collected were pooled to ensure a mixture of genetic origins. Each bag within each plot, in both experiments, received 50 larvae and was sealed to avoid escapes, allowing the caterpillars to bury themselves. All burials were performed on the same day. Two days after making burials, all bags were checked to count the larvae that remained in the top soil because they failed to bury. The larvae were not replaced, although the number of larvae failing to bury was recorded, to obtain an exact number of the larvae buried in each of the experimental bags.

The initial scheme in each experiment included one bag extraction, randomly selected, per plot and year, before the period of moth emergence (May) to enable pupal survival to be checked. However, several bags in the Open (habitat) and Control (climate) treatments were accidentally damaged after the set-up of the experiment, which reduced the initial number of bags. To maintain a balanced number of bags for moth emergence in all squares, they were not replaced and, instead, the extraction bags for these treatments were merged. Consequently, the estimation of pupae survival was made on the basis of the same three extraction bags per year for the Open treatment of the habitat experiment and the Control treatment of the Water experiment (Open/Control hereafter).

Microclimatic conditions

Soil temperature (°C) was monitored on the soil surface (T_e) and 10 cm deep (T_i) using TMC6-HD air/water/soil temperature sensors (Onset Comp. Corp., Cape Cod, Massachusetts). Soil relative humidity (% Volumetric Water Content; VWC)

was measured with ECH₂O Dielectric Aquameter EC-20 sensors (Decagon Devices Inc., Pullman, Washington), integrating the values of the top 20 cm of soil. Total sun radiation (W/m²) was monitored with Silicon Pyranometer smart sensors (S-LIB-M003; Onset Comp. Corp.). All sensors were connected to HOBO U-12 data loggers (Onset Comp. Corp.). The whole set of microclimatic measurements was recorded in all plots, except radiation, which was only measured in the habitat experiment. All variables were recorded every 30 min and, on a daily basis, for the analysis, we calculated the average, maximum and minimum soil temperatures, average soil moisture, and average total irradiation from 1000 to 1400 solar time. The recording system was set for the whole study period; however, we restrict the analysis of environmental variables to the first experimental season. For temperatures and irradiance, the period spanned April to September 2010. For soil VWC, the period was restricted to mid-May to September 2010 because the addition of water was delayed until the end of the spring rains (Fig. 1). Winter conditions were analyzed for the period December to February.

Insect development assessment

Pupal survival per season was estimated by digging up one bag per plot and year (except for Open and Control, see Experimental design; total of 15 bags per year). For this, the bag was gently extracted, the mesh opened, and the soil cylinder carefully teased out, recording the number of larvae or pupae alive, emerged or dead, as well as the depth (in 2-cm intervals) at which pupae were found. Because all larvae were collected in the same place and adequately mixed before being buried, the presence of larval endoparasites was assumed to be homogeneous among treatments.

From the beginning of June until the end of September, and for the years 2010 and 2011, the bags were checked every other day to collect emerged moths from burials. These periodic samplings were programmed well before and after the usual emergence period aiming to prevent information loss. The rate at which moths emerged was estimated as the proportion of moths emerged per day in relation to the larvae successfully buried for a given bag.

The initial aim was to continue the experiment for as long as possible to gather information on extra-long pupae diapause. However, because of the extremely low moth emergence the second season of study, as well as the low percentage of pupae in extra-long diapause at the beginning of the third season, the experiment was ended in May 2012. At that time, all the bags were unearthed, carefully checked, and the number of pupae still alive was recorded.

Statistical analysis

Data were recorded and analyzed on a plot basis for climatic data and on a bag basis for burial success, burial depth, pupation survival, moth emergence and moth phenology.

Differences between treatments for each variable were analyzed with Tukey's honestly significant difference (HSD) all-pairwise comparisons. Habitat and Water experiments

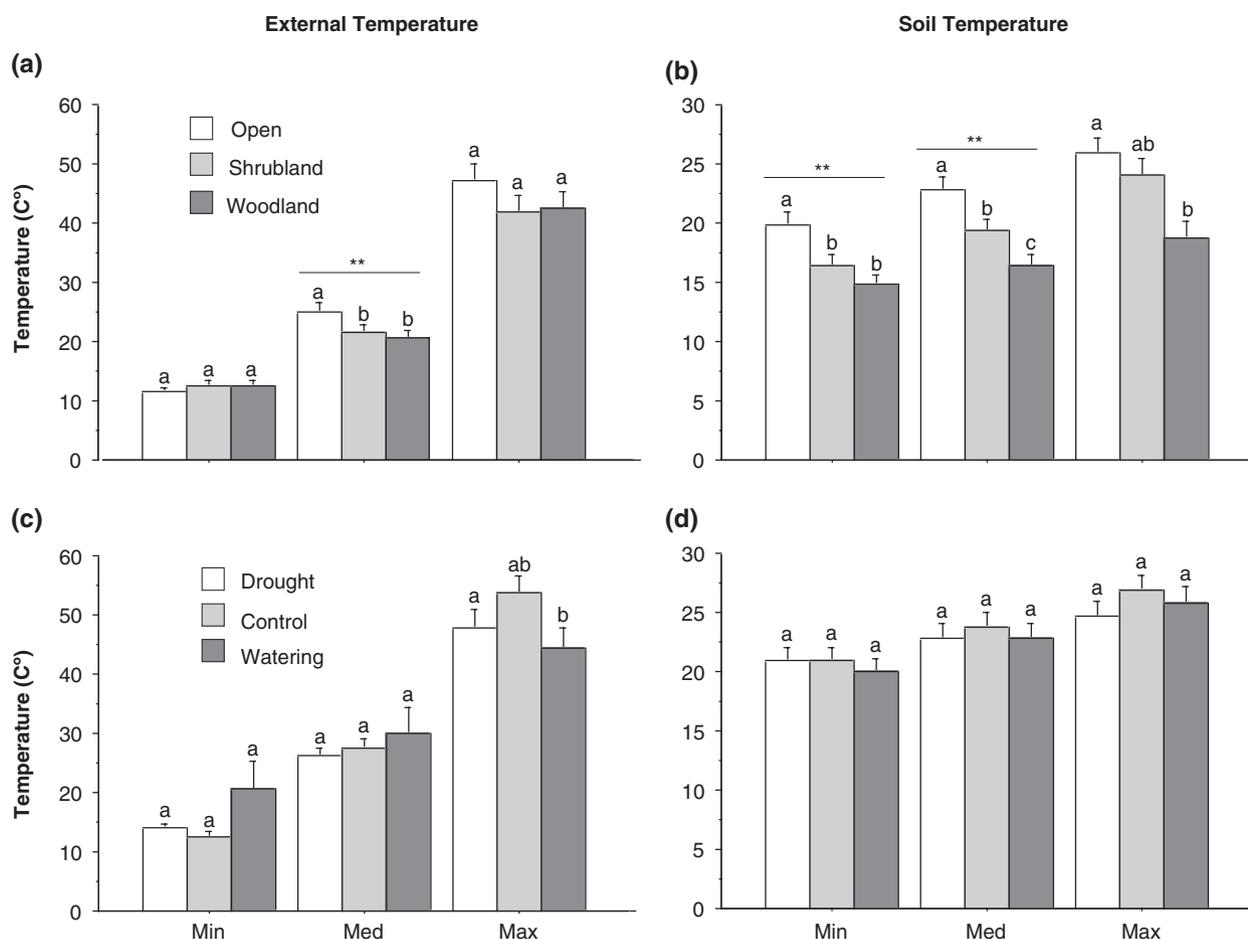


Figure 1 Soil temperatures in the Habitat (a, b) and Water (c, d) experiments for the period June to August 2010. Bars represent the mean \pm SE of minimum, mean and maximum temperatures at the soil surface (a, c) and at a depth of 10 cm (b, d). Letters on bars show the differences between treatments (multiple comparisons by Tukey means of contrasts) and symbols (*) show the effect of treatment at each temperature.

were analyzed independently, except for pupae survival (see Experimental design).

Climatic data were analyzed with linear mixed models, with a different model being used for each variable. In all models, treatment was considered the fixed factor and the random factor was plot.

Larval burial success and pupal survival were analyzed using a generalized linear mixed model (GLMM) with a binomial response. Pupation depth was analyzed using a linear mixed model and with respect to the number of emerged moths. Differences in the phenology of moth emergence were analyzed with a GLMM with Poisson and Gamma distribution, respectively. In all of these models, the fixed factor was treatment, with the exception of pupal survival data, where the fixed factors were treatment and year. The random factors were bag nested within a plot.

Mixed models were generated using the 'lme4' R package (Bates, 2011) and the 'glmmADMB' package (Fournier *et al.*, 2012), and *a posteriori* multiple comparisons were carried out using the 'multcomp' R package (Hothorn *et al.*, 2008). All statistical analyses were performed into the open source statistical language R, version 3.0.1 (R Core Team, 2013).

Results

Microclimatic conditions

From April to September, mean surface and soil temperatures, as well as minimum soil temperatures, were significantly different between treatments in the Habitat experiment (Fig. 1a,b), being higher in Open and lower in Woodland. This was also the pattern for irradiance, with the lowest value for Woodland, although this was similar to Shrubland (see Supporting information, Table S1), and with Open having the highest record, as well as being significantly different from the other two habitats (Tukey's HSD: both $P < 0.0001$, see Supporting information, Table S1). With respect to soil WVC, there were significant differences between habitats (see Supporting information, Table S1). Open and Woodland showed similar values, whereas the lowest values were in Shrubland (Tukey's HSD: Shrubland-Woodland, $P = 0.01$; Open-Shrubland, $P < 0.02$) (Fig. 2a). Regarding treatments in the Water experiment, no differences were found, either for surface or for soil temperatures (in all cases, $P > 0.16$) (Fig. 1c,d; see also Supporting information, Table S1), whereas differences were found for soil WVC, with this being lowest in Drought and highest in Watering (Tukey's HSD: Drought-Control, $P = 0.384$;

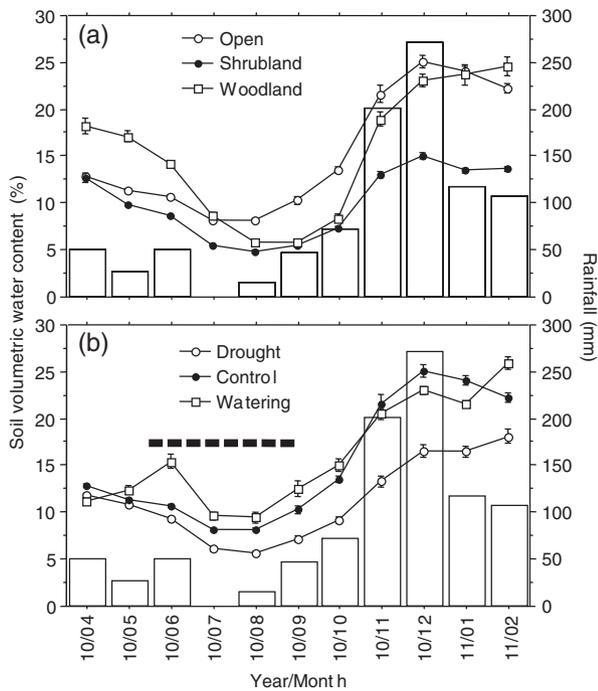


Figure 2 Soil volumetric water content (%; mean \pm SE) over time in the Habitat (a) and Water (b) experiments. Bars represent the amount of natural rainfall. The dotted line in (b) indicates the period of water addition in the Watering treatment and also that of rain exclusion in the Drought treatment.

Drought-Watering, $P = 0.008$; Control-Watering, $P = 0.323$) (Fig. 2b). Therefore, these results indicate an effect of applied treatments on the climatic variables.

Burial success

Approximately 94.6% of the larvae (8512 out of 9000) buried successfully. There were differences between habitats in burial success ($F_{2,5} = 12.113$, $P < 0.001$). For the Habitat experiment, 100% of larvae buried successfully in Woodland and Shrubland, whereas $96.7 \pm 0.1\%$ did so in Open, which significantly differed from the other treatments (Tukey's HSD: Open-Woodland and Open-Shrubland, $P < 0.001$; Woodland-Shrubland, $P = 0.999$). For the Water experiment, the lowest success was in Drought ($84.8 \pm 0.1\%$) and the highest was in Watering ($94.5 \pm 0.1\%$), with Control being intermediate ($90.7 \pm 0.1\%$). In this case, there were no statistically significant differences between treatments ($F_{2,5} = 1.952$, $P = 0.221$). The depth at which larvae buried to pupate was found to be quite similar between treatments, regardless of the Habitat ($F_{2,6} = 2.018$, $P = 0.1309$) or Water experiments ($F_{2,6} = 0.390$, $P = 0.672$). Almost all pupae (97.4%) were found at a depth between 4 and 10 cm, with more than half (56.2%) at a depth in the range 6–8 cm.

Pupation success and pupae survival

For Habitat experiment, the initial proportion of larvae that successfully pupated greatly varied between different treatments

($F_{2,7} = 19.226$, $P < 0.0001$) (2010 data in Fig. 3). Pupation rate was much higher in Open/Control ($39.4 \pm 6.5\%$; see Experimental design) than in Shrubland and Woodland ($2.7 \pm 0.7\%$ and $0.7 \pm 0.7\%$, respectively). Overall, significant differences were found between the Open and the other two habitats (Tukey's HSD: $P < 0.001$) but not between Shrubland and Woodland (Tukey's HSD: $P = 0.999$) (Fig. 3a).

Conversely, in the Water experiment, pupation success did not vary much between treatments. It was higher in Open/Control ($39.4 \pm 6.5\%$) and Watering treatments ($34.0 \pm 2.3\%$) and somewhat lower in Drought ($30.0 \pm 6.4\%$). Climate treatments did not significantly differ ($F_{2,7} = 0.119$, $P = 0.091$).

In both experiments, from the initial proportion of larvae that successfully pupated, the number of pupae remaining alive declined over time as a result of moth emergence and also because some of the pupae died during the extended diapause (Fig. 3). At the beginning of the third season, the number of pupae remaining alive in extended diapause varied between 5.3% in the Control/Open treatment and 0.7% in Woodland (Fig. 3b).

Moth emergence and phenology

A total of 771 moths emerged during the 3 years of study; of these, 764 emerged the first summer (2010) and thus we restricted our analysis to these data.

In the Habitat experiment, most of the moths were recorded in Open, registering the highest emergence rate ($12.3 \pm 1.9\%$) in this treatment. Interestingly, this proportion was found to decrease with an increasing vegetation habitat. Thus, just a few emerged in Shrubland ($4.8 \pm 1.1\%$ of successfully buried larvae) and no moths emerged in the Woodland treatment. The differences were significant between treatments ($F_{2,5} = 7.426$, $P = 0.0183$). Furthermore, emergence in the Shrubland treatment was much later in the season, with the difference in emergence pattern being significant (Tukey's HSD: Open-Shrubland, $P < 0.001$) (Fig. 4a).

In the Water experiment, there were no differences between treatments in the number of emerged moths ($F_{2,5} = 0.033$; $P = 0.967$). Moth emergence was slightly higher in the Drought treatment ($16.7 \pm 2.2\%$), although differences with respect to other treatments were negligible (Control: $16.4 \pm 1.4\%$, Watering: $15.3 \pm 1.5\%$; P values in the range 0.996–0.966). This was not the case for the emergence pattern ($P < 0.001$) (Fig. 4b), being earliest in the Drought treatment, intermediate in Control and latest in Watering (Tukey's HSD: Watering-Control and Watering-Dry, both $P < 0.001$; Control-Drought, $P = 0.4$).

Discussion

The results of the present study provide strong evidence indicating that the different soil moisture scenarios and the type of habitat significantly affect the emergence phenology and pupal survival, respectively. Furthermore, an important effect was also found regarding the phenology of moth emergence. These results offer valuable insights into how the life cycle of this forest pest can be conditioned by climate, providing relevant information about the differences in survival of pupae and moth emergence among the main habitats. In a context of global

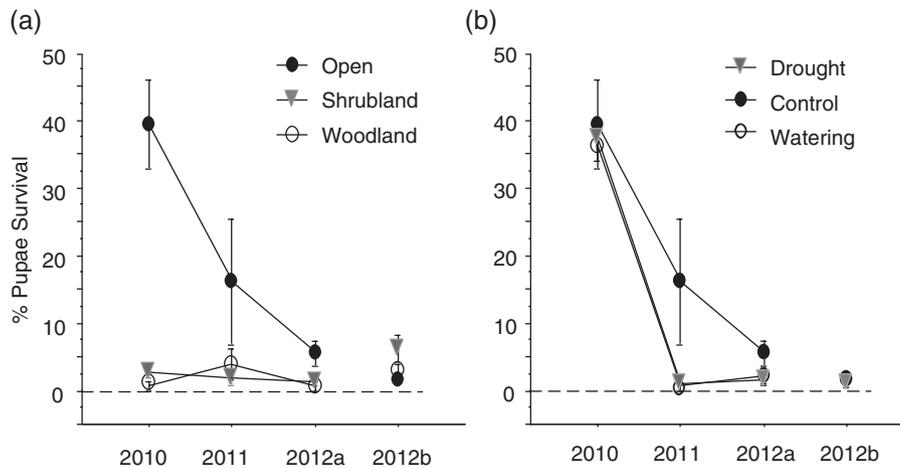


Figure 3 Mean \pm SE percentage of pupal survival per bag over the years in the Habitat (a) and Water (b) experiments. For the year 2012, the results of the programmed (2012a) and the total lift (2012b) are also shown.

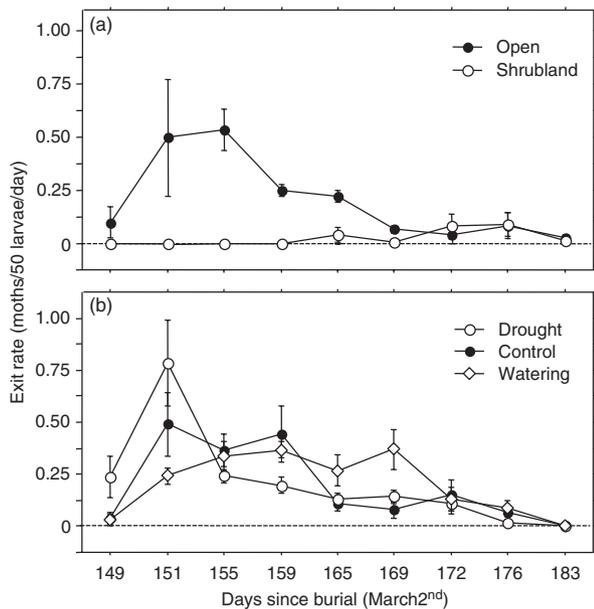


Figure 4 Timing of moth emergence for the Habitat (a) and Water (b) experiments. Results are expressed as mean \pm SE moth emergence rate by bag and day. There was no moth emergence in Woodland.

change in the Mediterranean region, with land use and climatic change as major drivers (Doblas-Miranda *et al.*, 2015), the study of the effect of these environmental conditions has even more importance.

Burial and pupation success

Burial depth was quite constant in our field experiments, irrespective of treatment. Dulaurent *et al.* (2011) also failed to find significant differences between habitats, although they did not report the usual depth for burial, as was also unreported by Markalas (1989). Montoya and Hernández (1998) stated that burial depth depends on soil structure (for *Spodoptera exigua*, see Zheng *et al.*, 2011), whereas Battisti *et al.* (2015) place this

variation at between 5 and 20 cm. A deeper burial provides greater protection against predators and soil abiotic conditions (e.g. see differences between T_i and T_e in Supporting information, Table S1). Nevertheless, a deeper burial also represents a major handicap for moth emergence (Capinera, 2008) such that, in hard clay soils, as is the case in the present study, this could be disadvantageous.

After burial, survival was much higher in Open, and conspicuously low in Shrubland and, particularly, Woodland. Larvae buried themselves easily in these two habitats but, once buried, they were unable to pupate, as indicated by the number of pupae still alive being extremely low 2 months after burial (Fig. 3a). Presumably this pattern is directly linked to soil temperature (Fig. 1a,b), with low temperatures limiting pupation success, as well as delaying moth emergence (for the effect of temperature on moth emergence, see Branco *et al.*, 2016). Bare soil has been previously described (Robredo, 1963; Démolin, 1969; Huchon & Demolin, 1970; Dulaurent *et al.*, 2011) as the most optimal habitat for pupation, and the percentage of survival on it (approximately 40%) is within the range of that reported in similar studies (37–62.7%: Dulaurent *et al.*, 2011; 44–90%: López-Sebastián, 2014). By contrast, the easy burial on Woodland and Shrubland contrasts with the high mortality on these habitats, which were nevertheless systematically avoided by natural processions (Robredo, 1963), suggesting that some strong source of pupal mortality (presumably fungus such as *Beauveria bassiana*; López-Sebastián, 2014; Battisti *et al.*, 2015) operates on them more intensely than in bare soil. Within the Water experiment, there was a lower burial success in Drought treatment and a higher in success in Watering, although burial success differences between the two extreme treatments were less than 10%. Moreover, these differences are not significant, which indicates that bare soil is the best habitat for burial of the pine processionary larvae, regardless of soil moisture.

Prolonged pupal diapause

The proportion of pupae entering prolonged diapause was low. Approximately 15% of initial larvae in Control/Open were alive

as diapausing pupae at the beginning of the second season and, by the third season, the live pupae comprised approximately 1–5%, depending on the treatment (Fig. 3). These values are not so different from reported by Abgrall (2001) ($6.7 \pm 1.6\%$) and Dulaurent *et al.* (2011) ($5.1 \pm 0.7\%$) in France and López-Sebastián (2014) (3.6% and 19.7% in two different locations) in Eastern Spain, although they contrast sharply with the findings of the study by Aimi *et al.* (2006) (50–100%) in the Italian Alps. The entry into pupal diapause has been attributed not only to the time spent by larvae in nonfeeding and silk-weaving activities (Démolin, 1969; Masutti & Battisti, 1990), but also to low temperatures during the pupal stage (12–14 °C: Démolin, 1974; Géri, 1980). Moreover, it is unlikely that soil moisture determines extra-long diapause, although it could affect the survival of extra-long-diapausing pupae (Turnock *et al.*, 1983; Leather, 1984; Markalas, 1989) and the exit of diapause, as previously induced by those other variables (Beck, 1980; Saunders, 1982; Tauber, 1986; Danks, 1987; Kostál, 2006).

Independently of the exact factor determining the engagement in extra-long diapause, the low proportion of pupae showing this strategy suggests that our population is basically univoltine, and that diapausing pupae might represent a reserve for the periods of very low population. It should be noted that the proportion of diapausing pupae is low but the entry into diapause happens during a population peak, such that the absolute number of pupae engaging in extra-long diapause is not low at all. The number of pupae remaining in extra-long diapause in the soil appears to be sufficiently high to allow the local populations to persist during the low infestation phases, which recur in this area every 5–6 years (Hódar *et al.*, 2012). Aimi *et al.* (2006) found prolonged diapause as the usual strategy in two zones of the Southern Alps, acting as an adaptive mechanism to escape adverse conditions. Also in the Alps, Tamburini *et al.* (2013) failed to show any negative effect of temperature on the PPM population dynamics and, conversely, found a negative density-dependent feedback with a 1-year lag as the main factor driving the population dynamics in both regions. This relationship was attributed to a deterioration of host quality and increased mortality caused by pathogens. In our case, the low level of extra-long diapause would explain the rather direct response that the population of PPM shows to climatic effectors (Hódar & Zamora, 2004; Hódar *et al.*, 2012). With a high proportion of pupae in extra-long diapause, producing high stocks of moths year after year, the responses to these climatic events should be necessarily smoothed or hidden (Tamburini *et al.*, 2013). However, when this response is based on the survival and reproductive success of the previous generation, as a result of the predominating univoltine strategy, the effect on the population is clear and direct (Hódar & Zamora, 2004; Hódar *et al.*, 2012).

Moth phenology

We found contrasting results for the phenology of nondiapausing moth emergence. With respect to habitat, moth emergence was strongly determined by the pupal survival, although habitat also changed the phenology of emergence, with this being delayed in Shrubland by approximately 3 weeks with respect to Open (Fig. 4a). Regarding the different watering scenarios, the proportion of emerging moths was similar between treatments,

although phenology varied from early in Drought to late in Watering (Fig. 4b). These results show that soil-water content and temperature can operate as a cue for moth emergence.

Previous studies concerning the effect of habitat and/or soil moisture on moth emergence (Markalas, 1989; Dulaurent *et al.*, 2011) have analyzed only the number of moths that emerged and not moth phenology, which prevents comparisons. These issues are pivotal because the time of moth emergence determines the time available for early-instar larvae in autumn before winter arrives (Arnaldo *et al.*, 2011) and, consequently, the fate of these young larvae. Our field experiments suggest that the drier the soil, the sooner the moth emerges (Fig. 4b). Thus, taking into account the aridification trends for the Mediterranean basin, we would expect an early oviposition and more time under optimal climatic conditions for the development of young larvae. This would be an advantageous scenario for the pest to survive during winter.

Conclusions

Our experimental results reveal the critical implications that the pupal stage of the insect's life cycle may have with respect to determining future population dynamics, and thus pupation in the PPM. An open and dry pupation site promotes an earlier emergence of moths, whereas vegetation cover or higher soil moisture delays emergence. According to the predictions of the future climate-change scenarios, a good part of the Mediterranean basin will undergo higher temperatures and lower rainfall, and woodlands will also be replaced by shrublands (Gibelin & Déqué, 2003; Christensen *et al.*, 2007; Matías *et al.*, 2012), which would be advantageous for pupae in open soils, at least when the low moisture does not prevent the moth emergence. Thus, a lower soil moisture, and the landscape changes resulting from this moisture change, could *a priori* favor this pest. Conversely, changes in land use caused by land abandonment may foster the recovery of the native vegetation cover (Peña *et al.*, 2007), potentially hampering the development of this pest. Therefore, two possible scenarios arise: (i) where aridification exerts a greater influence than land-use changes, the processionary moth will have more places to bury itself successfully, finding more open areas for an advanced moth (and larval) phenology, and (ii) where changes in land use are more important than climate, thus increasing the soil cover by shrubs and trees, the processionary will be less successful at this life stage. The PPM has displayed great demographic plasticity in contrasting situations, from quasi-univoltine strategy to preferential prolonged diapause, also being influenced by the local characteristics of the habitat (open, shrub, forest). All of these options represent a spatiotemporal complex scenario for the PPM interactions in the future (Arnaldo *et al.*, 2011), highlighting the need to consider all phases within the life cycle of an organism to adequately forecast the effects of global change.

Acknowledgements

The Andalusian Environmental Council, Junta de Andalucía, and the Direction of the Sierra Nevada National Park provided permission to work in La Cortijuela. Karim Senhadji

and Ramón Ruiz-Puche helped us during the setting up of the experiments. David Nesbitt checked the English version of the manuscript submitted for publication. The study was supported by projects PROPINOL (OAPN 022/2008) and CLAVINOVA CGL2011-29910 from the Spanish Ministry of Science and Innovation (MICINN), as well as FPU predoctoral grant from MCYT to LTM. We also thank the anonymous referees for their invaluable comments.

Supporting information

Additional Supporting information may be found in the online version of this article under the DOI reference: 10.1111/afe.12188

Table S1. Climatic description of the different treatments: mean \pm SE values of soil surface temperature T_e , soil (depth 10 cm) temperature T_1 , radiation (Rad) and % volumetric water content (% VWC) in the summer and winter for each experiment (Habitat and Water experiments). Units: °C, temperature; W/m², radiation. Summer comprises the period April to September (except %VWC, May to September); winter comprises the period December to February. *F*-(analysis of variance) and *P*-values are also shown. Furthermore, different lowercase letters indicate statistically significant differences between treatments (Tukey's test).

Fig. S1. Schematic showing the experimental design. Upper: the three treatments for the habitat experiment (Open, Shrubland and Woodland). Lower: the three treatments for the watering experiment (Control, Drought and Watering); all them were in open habitat. All treatments had three replicates in both experiments.

References

- Abgrall, J.F. (2001) *Le réseau surveillance processionnaire du pin en France 1969–1989*. Conception- Historique- Résultats, France.
- Aimi, A., Zocca, A., Minerbi, S., Hellrigl, K. & Battisti, A. (2006) The outbreak of the pine processionary moth in Venosta/Vinschgau: ecological and economic aspects. *Forest Observer*, **2**, 69–80.
- Arnaldo, P.S., Oliveira, I., Santos, J. & Leite, S. (2011) Climate change and forest plagues: the case of the pine processionary moth in Northeastern Portugal. *Forest Systems*, **20**, 508–515.
- Bates, D. (2011) *Linear Mixed Model Implementation in lme4*. University of Wisconsin, Madison, Wisconsin.
- Battisti, A., Stastny, M., Netherer, S. *et al.* (2005) Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. *Ecological Applications*, **15**, 2084–2096.
- Battisti, A., Avci, M., Avtzis, D. *et al.* (2015) Natural history of the processionary moths (*Thaumetopoea* spp.): new insights in relation to climate change. *Processionary Moths and Climate Change: An Update* (ed. A. Roques), pp. 15–79. Springer, The Netherlands.
- Beck, S.D. (1980) *Insect Photoperiodism*, 2nd edn. Academy Press, New York, New York.
- Branco, M., Paiva, M.R., Santos, H.M., Burban, C. & Kerdelhué, C. (2016) Experimental evidence for heritable reproductive time in 2 allochronic populations of pine processionary moth. *Insect Science*. DOI: 10.1111/1744-7917.12287.
- Buffo, E., Battisti, A., Stastny, M. & Larsson, S. (2007) Temperature as a predictor of survival of the pine processionary moth in the Italian Alps. *Agricultural and Forest Entomology*, **9**, 65–72.
- Capinera, J.L. (2008) *Encyclopedia of Entomology*, 2nd edn. Springer, Gainesville, Florida.
- Chen, C., Xia, Q.W., Fu, S., Wu, X.F. & Xue, F.S. (2013) Effect of photoperiod and temperature on the intensity of pupal diapause in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Bulletin of Entomological Research*, **104**, 12–18.
- Christensen, J.H., Hewitson, B., Busuioc, A. *et al.* (2007) Regional climate projections. *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, Chapter 11, ISBN: 78-0-521-88009-1. Cambridge University Press, U.K.
- Cornell, H. & Hawkins, B. (1995) Survival patterns and mortality sources of herbivorous insects: some demographic trends. *American Naturalist*, **145**, 563–393.
- Danks, H.V. (1987) *Insect Dormancy: An Ecological Perspective*. Biological Survey of Canada (Terrestrial Arthropods), Canada.
- Démolin, G. (1969) Comportement des adultes de *Thaumetopoea pityocampa* Schiff. Dispersion spatiale, importance Écologique. *Annales des Sciences Forestières*, **26**, 81–102.
- Démolin, G. (1974) *Réflexions générales sur la diapause et les diapauses renforcées chez la processionnaire du pin, Thaumetopoea pityocampa Denis et Schiff., Lepidoptera Thaumetopoeidae*. Compte-rendu de la Direction Générale de la Recherche Scientifique et Technique.
- Denlinger, D. (2002) Regulation of diapause. *Annual Review of Entomology*, **47**, 93–122.
- Doblas-Miranda, E., Martínez-Vilalta, J., Lloret, F. *et al.* (2015) Reassessing global change research priorities in mediterranean terrestrial ecosystems: how far have we come and where do we go from here? *Global Ecology and Biogeography*, **24**, 25–43.
- Dulaurent, A., Porté, A.J., van Halder, I., Vétillard, F., Menassieu, P. & Jactel, H. (2011) A case of habitat complementation in forest pests: pine processionary moth pupae survive better in open areas. *Forest Ecology and Management*, **261**, 1069–1076.
- Dulaurent, A., Porté, A., van Halder, I., Vétillard, F., Menassieu, P. & Jactel, H. (2012) Hide and seek in forests: colonization by the pine processionary moth is impeded by the presence of non host trees. *Agricultural and Forest Entomology*, **14**, 19–27.
- Fournier, D.A., Skaug, H.J., Ancheta, J. *et al.* (2012) AD model builder: using automatic differentiation for statistical inference of highly parameterized complex non linear models. *Optimization Methods and Software*, **27**, 233–249.
- Géri, C. (1980) Application des méthodes d'études démécologiques aux insectes défoliateurs forestiers. Cas de *Diprion pini* (Hyménoptère Diprionidae). Dynamique des populations de la processionnaire du pin *Thaumetopoea pityocampa* Schiff. (Lépidoptère Thaumetopoeidae) dans l'île de Corse. PhD Thesis. University of Paris-Sud, France.
- Gibelin, A. & Déqué, M. (2003) Anthropogenic climate change over the Mediterranean region simulated by a global variable resolution model. *Climate Dynamics*, **20**, 327–339.
- Hawkins, B., Cornell, H. & Hochberg, M. (1997) Predators, parasitoids and pathogens as mortality agents in phytophagous insect populations. *Ecology*, **78**, 2145–2152.
- Hayes, J. (1982) A study of the relationships of diapause phenomena and other life history characters in temperate butterflies. *American Naturalist*, **120**, 160–170.
- Hoch, G., Toffolo, E., Netherer, S., Battisti, A. & Schopf, A. (2009) Survival at low temperature of larvae of the pine processionary moth *Thaumetopoea pityocampa* from an area of range expansion. *Agricultural and Forest Entomology*, **11**, 313–320.
- Hódar, J.A. & Zamora, R. (2004) Herbivory and climatic warming: a Mediterranean outbreaking caterpillar attacks a relict, boreal pine. *Biodiversity & Conservation*, **13**, 493–500.
- Hódar, J.A., Zamora, R. & Cayuela, L. (2012) Climate change and the incidence of a forest pest in Mediterranean ecosystems: can the North

- Atlantic Oscillation be used as a predictor? *Climate Change*, **113**, 699–711.
- Hogsdon, P., Sivinsky, J., Quintero, G., Aluja, M. & Álvarez, S. (1998) Depth of pupation and survival of fruit fly (*Anastrepha* spp.: Tephritidae) pupae in a range of agricultural habitats. *Environmental Entomology*, **27**, 1310–1314.
- Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.
- Huchon, H. & Demolin, G. (1970) La bioécologie de la processionnaire du pin. Revue Forestière Française fascicule thématique 'La lutte biologique en forêt', France.
- IPCC (2007) *Climate Change 2007: The Physical Science Basis: Working Group I Contribution to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, U.K.
- Kay, I.R. (1982) The incidence and duration of pupal diapause in *Heliothis armiger* (Hübner) (Lepidoptera: Noctuidae) in Southeast Queensland. *Australian Journal of Entomology*, **21**, 263–266.
- Kaya, H.K. & Haya, A.H. (1981) Susceptibility of various species of lepidopteran pupae to the entomogenous nematode *Neoaplectana carpocapsae*. *Journal of Nematology*, **13**, 291–294.
- Kingsolver, J., Woods, A.H., Buckley, L., Potter, K., MacLean, H. & Higgins, J. (2011) Complex life cycles and the responses of insects to climate change. *Integrative and Comparative Biology*, **51**, 1–14.
- Kostál, V. (2006) Eco-physiological phases of insect diapause. *Journal of Insect Physiology*, **52**, 113–127.
- Leather, S.R. (1984) Factors affecting pupal survival and eclosion in the pine beauty moth, *Panolis flammea*. *Oecologia*, **63**, 75–79.
- Liu, Z., Gong, P., Li, D. & Wei, W. (2010) Pupal diapause of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) mediated by larval host plants: pupal weight is important. *Journal of Insect Physiology*, **56**, 1863–1870.
- López-Sebastián, E. (2014) *Estudio de la dinámica poblacional del lepidóptero Thaumetopoea pityocampa (Denis & Schiffermüller, 1775) (Notodontidae) en la provincia de Valencia (España)*. PhD Dissertation, Universitat de Valencia, Spain.
- Markalas, S. (1989) Influence of soil moisture on the mortality, fecundity and diapause of the pine processionary moth (*Thaumetopoea pityocampa* Schiff). *Journal of Applied Entomology*, **107**, 211–215.
- Masutti, L. & Battisti, A. (1990) *Thaumetopoea pityocampa* (Den. & Schiff) in Italy. Bionomics and perspectives of integrated control. *Journal of Applied Entomology*, **110**, 229–234.
- Matías, L., Zamora, R. & Castro, J. (2011) Repercussions of simulated climate change on the diversity of woody-recruit bank in a mediterranean-type ecosystem. *Ecosystems*, **14**, 672–682.
- Matías, L., Castro, J. & Zamora, R. (2012) Effect of simulated climate change on soil respiration in a mediterranean-type ecosystem: rainfall and habitat type are more important than temperature or the soil carbon pool. *Ecosystems*, **15**, 299–310.
- Menu, F., Roebuck, J.P. & Viala, M. (2000) Bet-hedging diapause strategies in stochastic environments. *American Naturalist*, **155**, 724–734.
- Metspalu, L., Kruus, E., Jõgar, K. *et al.* (2013) Larval food plants can regulate the cabbage moth, *Mamestra brassicae* population. *Bulletin of Insectology*, **66**, 93–101.
- Montoya, R. & Hernández, R. (1998) La procesionaria del pino. *Plagas de insectos en las masas forestales españolas*, pp. 67–84, Ministerio de Medio Ambiente, Spain.
- Murray, D. & Zalucki, M. (1990) Effect of soil moisture and simulated rainfall on pupal survival and moth emergence of *Hecoverpa punctigera* and *H. armigera* (Lepidoptera: Noctuidae). *Australian Journal of Entomology*, **29**, 193–197.
- Peña, J., Bonet, A., Bellot, J. *et al.* (2007) Driving forces of land-use change in a cultural landscape of Spain. A preliminary assessment of the human-mediated influences. *Modelling Land-Use Change, Progress and Applications* (ed. by E. Koomen, J. Stillwell, A. Bakema, H. J. Scholten), pp. 97–115. Springer, Netherlands.
- R Core Team (2013) *R: A Language and Environment for Statistical Computing*. R Foundation, Austria.
- Robredo, F. (1963) Las procesiones de crisalidación de *Thaumetopoea pityocampa*. *Boletín del Servicio de Plagas Forestales IV*, **12**, 122–130.
- Ruszczyk, A. (1996) Spatial patterns in pupal mortality in urban palm caterpillars. *Oecologia*, **107**, 356–363.
- Saunders, D.S. (1982) *Insect Clocks*, 3rd edn. Elsevier Science, U.K.
- Shimoda, M. & Kiuchi, M. (1997) Effect of chilling of diapause pupa on adult emergence in the sweet potato hornworm, *Agrius convolvuli* (Linné) (Lepidoptera, Sphingidae). *Applied Entomology and Zoology*, **32**, 617–624.
- Sims, S.R. & Shapiro, A.M. (1983) Pupal color dimorphism in California *Battus philenor* (L.) (Papilionidae): mortality factors and selective advantage. *Journal of the Lepidopterists' Society*, **37**, 236–243.
- Stastny, M., Battisti, A., Petrucco-Toffolo, E., Schlyter, F. & Larsson, S. (2006) Host-plant use in the range expansion of the pine processionary moth, *Thaumetopoea pityocampa*. *Ecological Entomology*, **31**, 481–490.
- Stefanescu, C. (2004) Seasonal change in pupation behaviour and pupal mortality in a swallowtail butterfly. *Animal Biodiversity and Conservation*, **27**, 25–36.
- Tamburini, G., Marini, L., Hellrigl, K., Salvadori, C. & Battisti, A. (2013) Effects of climate and density-dependent factors on population dynamics of the pine processionary moth in the Southern Alps. *Climatic Change*, **121**, 701–712.
- Tauber, M.J. (1986) *Seasonal Adaptations of Insects*. Oxford University Press, New York, New York.
- Tauber, M.J. & Tauber, C.A. (1976) Insect seasonality: diapause maintenance, termination and postdiapause development. *Annual Review of Entomology*, **21**, 81–107.
- Turnock, W., Lamb, R. & Bodnaryk, R. (1983) Effects of cold stress during pupal diapause on the survival and development of *Mamestra configurata* (Lepidoptera: Noctuidae). *Oecologia*, **56**, 185–192.
- Xiao, H., Wu, S., Chen, C. & Xue, F. (2013) Optimal low temperature and chilling period for both summer and winter diapause development in *Pieris melete*: based on a similar mechanism. *PLoS One*, **8**, 1–9.
- Yahdjian, L. & Sala, O. (2002) A rainout shelter desing for intercepting different amount of rainfall. *Oecologia*, **133**, 95–101.
- Zheng, X.L., Cong, X.P., Wang, X.P. & Lei, C.L. (2011) Pupation behaviour, depth, and site of *Spodoptera exigua*. *Bulletin of Insectology*, **64**, 209–214.

Accepted 26 August 2016