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Climate change and pine processionary moth
Role of the host plant

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Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment has been made in the text.

January 31st, 2008   Edoardo Petrucco Toffolo

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Riassunto

La processionaria del pino (*Thaumetopoea pityocampa*), un'importante defogliatore dei pini in tutta l'area del Mediterraneo, ha mostrato un'espansione del suo areale nel corso degli ultimi decenni per effetto del cambiamento climatico. Nell'introduzione generale sono presentate alcune delle risposte che gli insetti stanno mostrando alla variazione delle condizioni ambientali; inoltre, sono delineate quali siano le principali caratteristiche che permettano ad una specie di rispondere più o meno positivamente al cambiamento climatico. Il lavoro sperimentale ha avuto come obiettivo l'aumento delle conoscenze sulla processionaria del pino al fine di svolgere previsioni attendibili sulle future risposte al cambiamento climatico. Lo scopo del primo lavoro è stato quello di conoscere il comportamento dell'insetto alle basse temperature, un importante fattore di mortalità per gli insetti. I risultati mostrano come la processionaria possa essere ritenuta moderatamente tollerante il congelamento, una strategia adatta per affrontare l'elevata variabilità presente ai limiti del suo areale e per permettere l'alimentazione e la sviluppo durante l'inverno. Gli esperimenti effettuati hanno permesso inoltre di formulare l'ipotesi che l'effetto cumulativo delle basse temperature possa essere un fattore di mortalità rilevante per questo insetto. Un elemento che deve essere considerato nel caso di un'espansione dell'areale è la presenza di piante-ospiti che permettano all'insetto di colonizzare con successo le nuove aree. La *performance* larvale mostra valori simili su *Pinus sylvestris* e *Pinus mugo*, le specie più diffuse nelle aree di espansione, rispetto a quelli mostrati su *Pinus nigra* (ospite primario) e questo sembra essere garanzia di successo nell'espansione. Tuttavia, la minor accettazione di *P. sylvestris* da parte delle femmine potrebbe determinare una minore entità degli attacchi di processionaria nelle formazioni pure di questa specie. Il terzo gruppo di esperimenti ha riguardato il comportamento delle processionaria su *P. mugo*. E' stata considerata la risposta all'aumento della concentrazione di CO_2 (effetto indiretto dovuto al cambiamento delle qualità dei tessuti della pianta ospite), e l'effetto della copertura nevosa, il più probabile fattore limitante la sopravvivenza su questa pianta-ospite, visto il suo portamento prostrato. La processionaria ha confermato la sua plasticità non mostrando differenze nella *performance* larvale su piante cresciute a differenti livelli di CO_2. La copertura prolungata dei nidi da parte della neve ha causato mortalità totale delle colonie; tuttavia, la diminuzione dell'altezza e del tempo di permanenza della neve, come

evidenziato da recenti studi e confermato dai nostri dati, potrebbe costituire un'ulteriore aspetto del cambiamento climatico, favorevole all'espansione e al mantenimento nella aree di nuova colonizzazione della processionaria del pino.

Summary

The pine processionary moth (*Thaumetopoea pityocampa*), an important defoliator of *Pinus* spp. in the Mediterranean area, is expanding its range in the last decades, in response to climate change. In the introduction I present the main evidences of the insect responses to the variations of climate. Moreover, I outline the main features that allow an insect species to respond to climate change. The aim of the experimental part is to increase the knowledge about the pine processionary moth, in order to improve the quality of predictions related to climate change. My thesis is based upon three main studies on the biology of the insect in the expansion area, i.e. cold tolerance of the larvae, role of the host-plant, and response to varying CO_2 level and snowpack.

In the first study I evaluate the processionary moth strategy to survive low temperature. Insects use different strategies to survive low temperatures, the knowledge of this strategies may have important consequences for predicting the effects of climate change. No exact data exist for *Thaumetopoea pityocampa* to allow conclusions on parameters determining survival of its larvae at low temperature, although there is some information about thermal demand of this species. In the study, the supercooling ability and long term survival under cold temperature were explored in different experiments. The supercooling point was analysed at various times during the winter; moreover, in order to estimate chill injury, the survival under accumulated negative temperature was tested in both laboratory and field. The trials of laboratory allowed also to study the starvation capability of larvae for extended period. Additionally, the larvae ability to tolerate freezing was tested for a short period in laboratory condition at very low temperature (-17°C), maintaining the colony in their nests. The larvae of *T. pityocampa* froze on average at –8°C, however, it is important to highlight that part of the tested larvae survived freezing. Moreover, the larvae groups inside their nest showed a high survival at -17°C, thereby demonstrating that they would survive such severe frost as it would occur in a cold winter night in the expansion area. The cumulative chill injury proved to be an important mortality factor in both laboratory and field experiment. The thermal sum required for 50% of mortality in the field are lower, and this is probably due to the presence of additional mortality factors or to effect of fluctuating temperature. Moreover, the experiments suggest

both the possibility to repair of chill injury and the ability to survive extended periods with temperatures that do not allow feeding. The absence of significant variation of SCP during winter season suggest that larvae do not undergo any special preparations for the cold period. The parameters obtained allow to classify the processionary as an organism moderately freeze tolerant. This appears to be a suitable adaptation to cope with highly variable temperatures that occur especially at the upper limits of its range and allows the larvae to resume feeding and development during winter periods with warm weather.

The second study evaluates the role of the host-plants in relation to range expansion, as novel hosts can be encountered in newly colonised areas. Because plant species often differ in their relative attractiveness and suitability for insects, insect preference for, and performance on, these hosts can determine recruitment potential in the current and future expansion areas. The experiments explore the relative roles of female preference and larval performance on three *Pinus* species at current range margin in the Italian Alps: *P. nigra* (primary host), *P. sylvestris* (secondary host), and *P. mugo* (novel host). Host use patterns in multiple insect populations were studied through choice and no-choice oviposition experiments in cages, field surveys of mixed stands, and laboratory and field monitoring of larval growth and mortality. It was predicted that a specific life-history trait – time limitation of short-lived females to lay a single batch of eggs – would act as a component of female performance, and lead to similar rates of host acceptance in no-choice settings. In the choice-experiment, *P. nigra* was accepted the most frequently while *P. sylvestris* was accepted the least frequently, confirming nest density patterns in the field. Contrary to prediction, females remained discriminating in no-choice settings in spite of time limitation. In contrast, relative growth rate and mortality of larvae did not differ significantly among the three hosts, highlighting a discrepancy between female preference and larval performance. Recruitment potential of *T. pityocampa* in future expansion into stands of *P. sylvestris* and *P. mugo* is evaluated by combining host quality, conservatism in oviposition behaviour, habitat suitability, and the opportunity for local adaptation.

The third study deals with the increase of atmospheric CO_2, that has been shown to have direct and indirect effects on insect species of various ecosystems, though the responses are often species-specific. I transplanted colonies of the pine processionary moth to a high elevation site well outside of the current range of the insect (Stillberg, Davos,

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Switzerland, m 2150), where trees of the mountain pine have been grown for five years in ambient and elevated CO_2 (568 ppm). The aim of the study was to evaluate the response of first instar larvae to extreme conditions of temperature and to an altered performance induced by the change of host metabolism under elevated CO_2. Contrary to expectations, larval mortality and relative growth rate did not differ between trees grown in elevated and ambient CO_2. In addition, colonies on native trees showed a higher mortality, likely because the needles were tougher than on planted trees. As extended snow cover may be an important mortality factor of larval colonies on the creeping trees of mountain pine, I tested the survival of colonies transplanted at two extreme sites of Eastern Alps. The snow cover extended over more than one month and proved to be an important mortality factor of larval colonies on mountain pine. I concluded that the first instar larvae of the pine processionary moth are not concerned by unusually low temperature and mediated changes in needle quality towards CO_2 increase whereas snow accumulation strongly affected larval mortality. The decrease of snow cover observed in the last decades, however, may reduce such a risk.

Chapter 1

Introduction

Observations on climate change

Numerous evidences show the important climate change involving the Earth in the last decades, although there are different interpretations of climate date trend and there is still an incomplete physical understanding of many components of the climate system and their role in climate change. Compared to the variations occurred in previous centuries due to natural drivers, the anthropogenic activity results to be a key in the recent climate change. The budget of global mean radiative forcing is amply positive and explained by the increasing concentration of various greenhouse gases in the atmosphere. The long-lived gases (carbon dioxide, methane, nitrous oxide) and ozone constitute the main forcing acting primarily to increase the atmospheric absorption of outgoing radiation. There are substances with opposite effect, as the aerosol, microscopic airborne particles, that reflect and adsorb incoming solar radiation, although the magnitude of these factors is smaller than that of anthropogenic gases (Solomon *et al.,* 2007).

These variations are at the base of the changes observed in other climate factors, some examples are the temperature increase (Hansen *et al.,* 2006), the snow depth and cover decrease (Hodgkins & Dudley, 2006; Lateneser & Schnebelli, 2007), ocean biochemistry change (pH and salinity) (Feely *et al.,* 2004) and sea level increase (Church & White, 2006). The availability of long records may permit a high confidence for these estimates. It is important to remember that I refer to average global values and there are large difference to spatial and temporal levels. In some factors the regional variability is very high as for the precipitation, that, whatever, has increased in many areas, due to increased of temperature that has favoured the evaporation. Finally there are some kind of factors for which the data-gathering and following analyse is very difficult, for example the drought intensity and duration, intense tropical cyclone, the heavy precipitation events, and the anomaly temperature, although, observational evidences have shown an increase in many regions (Solomon *et al.,* 2007).

Effects on ecosystems and insects

Physical and biological systems have already being affected by recent climate changes, in this case the main difficulty is the correct attribution of the effects to the anthropogenic climate change, distinguishing from the effects of natural climate variability and non-climate drivers, such as pollution, land use and urbanization (Rosenzweig *et al.,* 2007).

Insects

The main biological effects on ecosystem have concerned the range, the phenology, and the density in present range of animal and plant species (Parmesan, 2006). The evidence of the climate change role are always becoming stronger, as the number of studies that have the objective to know the effect on agricultural and natural ecosystem is increasing. Here I show the recent results regarding insect response and also the other ecosystems variations that could influence the insect responses.

Range shifts

Shifts in distribution range are commonly related to climate change. In 2000, the northern boundary of the green stink bug *Nezara viridula* (Pentatomidae), in central Japan, was 70 km northward compared with the northern edge recorded in early 1960s, the increase of winter temperature improved potential overwintering conditions for *Nezara viridula* (Musolin, 2007) .

The sachem shipper butterfly, *Atalopedes campestris*, has expanded its range from the northern California (1967) into eastern Washington in 1993, and is continuing to spread northward; in this case the winter temperature affect the survival of *A. campestris* at its northern range edge and the winter warming is a prerequisite for this butterfly's range expansion (Crozier, 2004).

Parmesan *et al.* (1999) carried out a study on 35 non-migratory European butterflies, the 63% species have ranges shifted to the north by 35-240 km during this century, and only 3% have shifted to the south. Most of the studies in species distribution concerns *Lepidoptera* (Konvicka *et al.,* 2003; Parmesan et Yohe, 2003), which may not be enough

representative of biodiversity as a whole; however, it is showed alike that range expansion is involving a wide range of taxonomic groups (Carroll *et al.,* 2004; Hickling *et al.,* 2005 and 2006). Some authors highlighted the weak evidence for range retraction at low-latitude and low-elevation boundaries (Hampe & Petit, 2005), that could be due to lack of thorough sampling and to the failure to attribute such a decline to climate change (Thomas *et al.,* 2006). In Britain, a study showed a consistent retraction of low/elevation boundaries of the range for four butterflies species over 19 year study period. The 70-100 km northwards (*Aricia artaxerxes*, *Erebia aethiops*) and 130-150 m uphill (*Erebia epiphron*) shifts were attributed to climate change, whereas the fourth species (*Coenonympha tullia*) was associated with habitat retraction (Franco *et al.,* 2006). The lower elevation limits for 16 butterflies species, out of 23 considered, have risen on average by 212 m in 31 years in Sierra de Guadarrama (central Spain) (Wilson *et al.,* 2005). These evidence of contractions and extinctions at low elevations and altitude boundaries of species range may represent a symptom of climate change-generated biodiversity loss (Wilson *et al.,* 2005); species with a limited climatic ranges, restricted habitat requirements, and small populations are typically the most vulnerable to extinction, such as endemic mountain species (Gitay *et al.,* 2002).

It is important to know also the response of plants, as for many insect herbivores the expansion possibility relies on the availability of their host plant. For the woody species the immobility and long generations times prevent a rapid response to changing environmental conditions, the possible response are in this case in situ evolutionary adaptation and environmental tolerance (Bierzychudek, 1982; Honay *et al.,* 2002); moreover, the availability of long time-series is limited. In a site of Swedish Scandes the range margin of tree and shrubs species showed a recent elevation (120-375 m) since the early 1950s (Kullman, 2002). Walther *et al.* (2005) highlighted the northward expansion of *Ilex aquifolium* range in different region of the range margins and related it to a coherent shift of local climatic data.

Phenology

Numerous observations of climate-change responses have involved alterations of species' phenologies. The first appearance of most British butterfly has advanced in the last two decades, moreover there is a concurrent advancement of peak appearance and longer

flight duration (Roy & Sparks, 2000); similarly there is a significant advance in the first appearance date for butterflies of the northwest Mediterranean Basin (Stefanescu *et al.,* 2003). As for the range shift, the most studied species belong to Lepidoptera, but there are some studies dealing with other groups. Gordo & Sanz (2005) have highlighted an advancement of the first appearance for *Leptinotarsa decemlineata*, *Bactrocera oleae* and *Apis mellifera* in Spain and Kiritani (2006) showed the same that for some crop pest species in southwestern Japan. The advances in phenology may affect different events of life-cycle, even if long time-series are hardly available. Through the development and validation of a descriptive model, winter moth egg hatching has advanced over the past 15 years in the Netherlands (Visser & Holleman, 2001). Regarding plant species, there is an evident signal of advancing leaf unfolding, flowering and fruiting in wild plant across Europe (Gordo & Sanz, 2005; Menzel *et al.,* 2006). As for many leaf-feeding herbivores synchrony in phenology with their host plants is crucial, a differential shift under novel environmental condition may cause synchrony loss. Currently, winter moth eggs tend to hatch before fresh oak leaves, with an elevated larval mortality (Visser & Holleman, 2001; van Asch & Visser, 2007).

Density

The species may respond with shifting density of individuals within their ranges. Future climatic condition will allow additional generations a year; the fall webworm, *Hyphantria cunea*, showed a shift of the life cycle in a recent colonization area of Japan (Fukui), where at least part of the population has tree generations per year at present, compared to two generations until the first half of the 1990s (Gomi *et al.,* 2007). Another important factor that further a greater population growth rates is the increase in fecundity, as it is shown in the marginal populations of *Hesperia comma* in Britain, where the females increase the egg-laying rate by higher temperature (Davies *et al.,* 2006). Moreover, the warmer conditions may allow an increased survival during overwintering, a critical stages of life-cycle (Crozier, 2004). Number of generations, survival, oviposition rate are factors that could determine variations at the level of population density, but the elevated number of interacting factors and the high local variations render this kind of studies very difficult. A long-term manipulative field experiment have shown an increase of *Auchenorrhyncha*

abundance in calcareous grassland ecosystems (Masters *et al.,* 1998); moreover, Roy *et al.* (2001) have elaborated a model that permits to predict the future changes in abundance of British butterfly species, and most species are predicted to increase under warmer climate. Regarding the population density, it is important to know the response of parasitoids and predators to climate change; the herbivorous insect could be favoured in expansion areas if they escape their natural enemies at higher elevations or latitudes by entering enemy-free space (Hodkinson, 1999).

Effects on forest ecosystems

Although forest ecosystems are in great measure object to human management, they show characteristics very different from other ecosystems. Their dynamic features are related to an ecosystem's ability to cope with disturbance/perturbations (Führer, 2000) even if in the commercial forestry, the stationary equilibrium is more or less artificially maintained. Changes in the environmental forcing functions caused by climate change may drastically reduce adaptation of local species and population (Larsen, 1995); moreover, the importance of forest society makes it important to influence the direction and timing of adaptation process measures (Kellomäki *et al*., 2005).

There are some papers that highlighted the importance to study the insect response, because the global change will have demonstrable changes in the frequency and intensity of pest outbreaks, particularly in the margins of host range (Ayres & Lombardero, 2000; Volney & Flemming, 2000). The insect may have a strong effect on production, biodiversity, structure, composition of the forest (Rosenzweig *et al.,* 2007). As the forest ecosystems show long cycles, there is the need to understand the direct effects of climatic variables on herbivores, and subsequently to predict regional patterns in abundance using historical climatic data and data scenarios of projected climates, that should be validated with geographical sampling programs (Ayres & Lombardero, 2000).

Recently, some models were suggested in relation to different aspects of the interactions between climate change, forest ecosystems and pest insects. The models showed the possible impact on boreal forests of Finland (Kellomäki *et al*., 2005), on different regeneration stages of the forest (Price *et al.,* 2001), on range and outbreak areas of the bark beetles *Dendroctonus frontalis* (Ungerer *et al.,*1999) and *Dendroctonus*

ponderosae (Williams & Liebhold, 2002); these last studies highlighted the range expansion with two different approaches, the first using a physiological model and the second a discriminant function model.

Some manipulative experiments were carried out to valuate the responses in possible future situations; these studies considered a limited number of climatic factors, due to the difficulties to recreate ambient conditions very different from the natural ones. At the Duke Forest free-air concentration (FACE) in North Carolina, Hamilton *et al.* (2004) have found a decrease in leaf damage by herbivorous arthropods in elevated CO_2 plots. Higher level of solar UV-B determine a lower leaf area consumption on *Nothophagus antartica* (Rousseaux *et al.,* 2004). The response at increasing of nutrients and temperature were assessed in a sub-artic dwarf shrub heath community. The various species of insect herbivore community showed a differed response to two treatments and treatments interaction; there was a trend to increase in abundance but the insect damage did not undergo variation at least for dominant dwarf shrub species (Richardson *et al.,* 2002).

There is some evidence that expected changes have already taken place; *D. ponderosae* has increased the number of infestation since 1970 in formerly climatically unsuitable habitats in British Columbia (Carroll *et al.,* 2004). In the early 1990s, *Argyresthia retinella*, a species not previously known for outbreaks, have significantly damaged the birch forest of north-western Norway, as a result of changed climatic condition (Tenow *et al.,* 1998).

The human activity has broken down many barriers to long-distance dispersal of plants and animals, therefore the response of the invasive alien species and the possibility of new introductions are two aspects that should be taken into account in relation to climate change. The climate change could further also these species with the increasing of survival and of generation number and with range expansion; moreover, the outbreak risk could be higher because of the low presence of natural enemies (Simberloff, 2000; Chornesky *et al.,* 2005).

Insect species features and climate change

The insects are commonly studied to valuate the effects of climate change because they have short generation time, which determines rapid responses, and because there are

numerous dataset available about monitoring of important pests and biodiversity indicators.

I have shown as the climatic change affects numerous taxonomic groups, however, the effects on insects surely differ among species, depending on their environment, life-histories, and ability to adapt. Some species may be adversely affected by climatic change, up to at extinction risk in worst situations, as for species that occupy narrow geographic ranges or narrow niches with highly constrained life styles in extreme environments (Bale *et al.,* 2002; Beaumont & Hughes, 2002; Parmesan, 2006).

On the other hand there are species with a greater ability to adapt to climate change, through phenotypic plasticity and rapid evolutionary response to strong selection, with some vanguard species benefiting from climate change (Bale *et al.,* 2002; Crozier, 2004). For example the range expansion is possible only for species with wide altitudinal and latitudinal distribution; moreover, the drivers of the species range must be climatic variables, as rich empirical evidence shows that low temperatures are the most constraining factor in the upper latitude and latitude (Gaston, 2003). Furthermore the availability of resources, such as the host plants, may be a limiting factor (Hill *et al.,* 1999). For example, the absence of host plant from high elevations prevents the possible expansion of *Aporia crataegi* in the mountains of Sierra de Guadarrama, (Merrill *et al.,* 2008). Similarly in northern Japan, the distribution of two ladybird species coincides with the geographic ranges of their host plant(s) (Koizumi *et al.,* 1999). Such species are monophagous, whereas there are plastic species that are able to occupy different habitats and to adapt to different hosts. The oligophagous and polyphagous species may find potential hosts even in areas where the original or preferred hosts are absent or much less abundant and they will accept those that are closely related to their host original host (Scriber & Ording, 2005).

The availability to accept new hosts can thus facilitate further expansion outside the geographic range of the original host (Gutiérrez & Thomas, 2000). A further important feature is the dispersal ability, as it is related to the possibility to colonize newly available areas; it is possible to hypothesise that in non-migratory species some individuals may always cross the species' boundary, and under climate warming some of these emigrants can be successful in founding new populations outside the former range (Parmesan, 2006). There is also the possibility of an evolutionary increase in dispersal ability in an expanding population in response to climate and habitat change (Hanski *et al.,* 2006; Hughes *et al.,*

2007). The females of *Pararge aegeria* have larger and broader thoraxes and smaller abdomens in the range margin (Hughes *et al.,* 2007), whereas the females of *Melitaea cinxia* have a higher [ATP]: [ADP] ratio in the new isolated populations (Hanski *et al.,* 2004). Both these morphological and metabolic features are related to greater adult flight ability. Hughes *et al.,* 2003 have individuated the decrease of reproduction effort as a possible cost of dispersal increase in *P. aegeria*, alike in wing dimorphic species ability; however, this trade-off between dispersal and fecundity is not found in dispersive females of *M. cinxia*, that exhibited no reduction in lifetime fecundity but only shorter life span than old population females (Hanski *et al.,* 2006).

Finally the colonizer population must be able to persist in new areas, that may be characterized by extreme conditions (Hill *et al.,* 1999), and species life history ought include appropriate strategies and plasticity to cope events that determine elevated mortality.

Insects and low temperature

I showed there are many studies that recognize the importance of low winter temperatures in shaping the northern distribution limits of insects (Ungerer *et al.,* 1999; Crozier 2004a; Musolin 2007). Many ectotherms, particularly those from temperate or cold regions, may cope with periods of potentially harmful, or even lethal, low temperatures during development or adult life (Zeilstra & Fisher 2005). There is a wide literature that study the different survival strategies at low temperature, the main classification concern the response at freezing of an insect's body fluid; depending on this, an organisms can be classified as freezing tolerant or intolerant (freeze avoidance) (Leather *et al.* 1993; Bale 1993, Duman *et al.* 1991). Another important aspect seems be the combined effect to temperature and exposure time, because there could be a cumulative chill injury (Turnock & Fields 2005). The understanding of cold hardiness strategies, especially in response to environmental variation, may have important consequences for predicting the regional effects of climate change (Addo-Bediako *et al.* 2000; Sinclair *et al.* 2003).
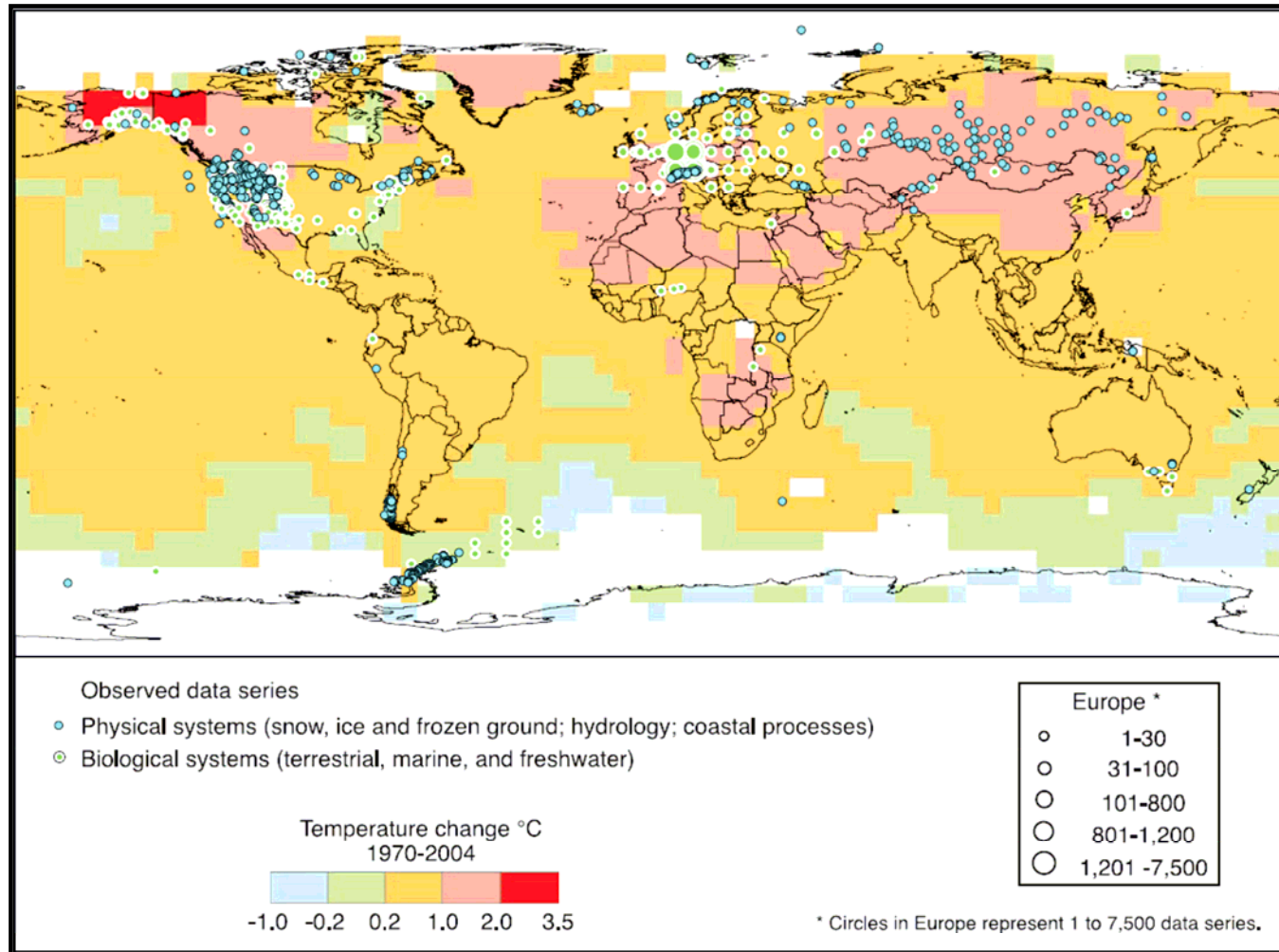
Observed data series
- Physical systems (snow, ice and frozen ground; hydrology; coastal processes)
- Biological systems (terrestrial, marine, and freshwater)

Temperature change °C
1970-2004

-1.0 -0.2 0.2 1.0 2.0 3.5

Europe *
- 1-30
- 31-100
- 101-800
- 801-1,200
- 1,201 -7,500

* Circles in Europe represent 1 to 7,500 data series.

Fig. 1.1 Locations of significant changes in observations of physical systems and biological systems, are shown together with surface air temperature changes over the period 1970 to 2004 (from Rosenzweig *et al.*, 2007).

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Pine processionary moth

The organism study is the pine processionary moth *Thaumetopoea pityocampa* ((Lepidoptera Notodontidae) (Denis & Schiffermüller, 1776)) (Plate 1), an important defoliator of *Pinus* and *Cedrus* species (Plate 1). The insect occupies the western part of Mediterranean basin, whereas in the eastern part the sibling species *Thaumetopoea wilkinsoni* occurs; the margin definition between the ranges of two species is still not totally clear (Salvato *et al., 2002*). The pine processionary moth range is characterized from a considerable size, it extent latitudinally from sub-Saharan region to southern to the north-central France (Paris Basin) and altitudinally from the sea level to 1200-1400 m in the Alps and to 1900-2001 in the Atlas (Schmidt *et al.,* 1997; Battisti *et al.*, 2005; Battisti *et al.,* 2006; Zamoum & Démolin, 2005). The pine processionary moth lives in very different environmental conditions, as it feeds on different plant-host such as *Pinus* spp. and *Cedrus* spp.. All over its range the insect is favoured by intense conifer cultivation, as *P. nigra*, *P. halepensis*, *P. pinaster*, but also exotic species (*Pseudotsuga menziesii*, *P. canariensis*, *P. radiata*) (Roques *et al.*, 2002; Mouna 2005). The winter temperature is considered be the range limiting factor in the northern and upper margins (Buffo *et al.,* 2007; Battisti *et al.*, 2005; Huchon & Démolin, 1970), given that the life-cycle of insect shows the particularity of larval development during the winter season. The larvae have gregarious behaviour in all stages of their development; they feed during the nocturnal hours, whereas during the day they remain inside silk nest, built on most sunny exposed parts of the tree.

This brief description highlighted the possibility of positive answers to climate change by the insect, as there are already a number of studies that have shown evidence about that. The pine processionary moth has been also taken as an example of the effects of climate change on organisms in the last report of IPCC (Rosenzweig *et al.*, 2007) (Fig. 1.1). Battisti *et al.* 2005 reported a recent latitudinal and altitudinal range expansion; in north-central France the range margin has shifted by 87 km northwards between 1972 and 2004, in Venosta/Vinschgau Valley (Alps) there was a altitudinal shift of 110-230 m upwards occurred between 1975 and 2004. Elaboration of a model and a translocation experiment have allowed to attribute the expansions to increased winter survival due to a warming trend over the past three decades. The rising temperatures are the cause of the uphill spread and attacks increase of pine processionary moth in two relict populations of Scots pine in

southern Spain. The insect defoliation reduced the growth and the reproductive ability of the pine, factors that could a threatened the conservation of a important ecosystem vulnerable to risk extinction (Hodar *et al.,* 2003). The study of processionary range has allowed to show the effect of the variation in extreme events intensity, such as during the summer of 2003. In that year an extraordinary range expansion to high elevation pine in Italian Alps occurred, with the upward shift attributed to increased nocturnal dispersal of females during the unusually warm night temperature in June-August 2003 (Battisti *et al.,* 2006). As an example of the response to the extreme summer of 2003 in two alpine valleys in north east Italy (Friuli Venezia Giulia district), I contributed a paper in the Proceedings of a IUFRO workshop on Methodology of forest insect and disease survey in Central Europe (Gmunden September 11-14, 2006) (see Annex 1). The occurrence of pine processionary moth was surveyed in the two valleys during three life cycles, recording the locations of extreme nests and evaluating the relationship between catch in pheromone traps and infestation level at same sites.

Plate 1

Ovipositing female on *P. nigra* (photo D. Zovi)

Winter nest on *P. nigra*

Third instar larvae feeding (photo D. Zovi)

Pupating larvae on soil

Aim of the research

In this thesis I introduce some new information useful to improve the knowledge about the   response of *Thaumetopoea pityocampa*to climate change. I evaluate the processionary moth strategies to survive low temperatures (Chapter 2). Subsequently I show some information about the ability of the processionary moth to colonize successfully the expansion areas in relation to different host-plants (Chapter 3), and the effects on larval performance and survival of others important factors involved in climate change, such as CO_2 and snow cover (Chapter 4).

The first contribution searches to set the cold hardiness of processionary moth, as low temperature is known to be an important mortality factor for active insect during winter. Until now, however, there are no data from this insect on the supercooling ability and long term survival under cold conditions. The experiments evaluate both supercooling point and survival of larvae inside the nest at temperature lower than the putative lethal threshold (-17°C); moreover, the possible effect of cumulative chill injury is estimated through field and laboratory experiments. The obtained results permit to delineate the strategy of the insect in respect to low temperature, considering the numerous classifications about this matter in the literature. Finally it is possible to evaluate the insect ability to cope with low temperature at its upper range margin.

The second contribution investigates the pattern of host use by the processionary moth in the Alps, where it is typically associated with Austrian black pine, *Pinus nigra*; in the course of the recent expansion, however, it has been increasingly encountering and colonising two other pine species: *P. sylvestris* (Scots pine), a secondary host utilised mostly at the margins of the past range of *T. pityocampa* , and a novel host *P. mugo* (mountain pine), which was only rarely colonised. To evaluate the relative role of host attractiveness and suitability in the patterns of host use by *T. pityocampa* in the Italian Alps, and to provide a basis for predictions on future expansion, I test the female preference patterns and larval performance on the three above mentioned hosts, in both laboratory and field experiments. If female oviposition patterns consistently showed little differentiation (hierarchy) in the controlled experiments, the natural colonisation pattern could be driven

largely by differential larval survival on the three hosts. However, if clear hierarchies in female preference were detected, larval performance could either counteract or amplify oviposition preference in the overall patterns of host use.

The main objective of third study is two-fold, the first being to assess survival and performance of young larvae of processionary moth in high-elevation FACE facility on mountain pine during the summer, under extreme environmental conditions for the insect as the site is located about 700 m above the present upper edge of the range. So I had the possibility to investigate if the change in food quality, due to the increase in carbon dioxide concentration, could be a critical factor for insect survival in a environment where the larvae already cope with extremely low temperature. The second aim is to evaluate the insect winter survival on mountain pine and black pine in stands near to the border of the range, where the expansion is occurring, with special attention to the role of the snowpack. The creeping habit of mountain pine furthers the cover of the nests for extended periods of time, and this could create a risk of colony mortality. I analyse the snow cover data of a possible expansion area, to know if the global trend of a decrease of snow cover is confirmed, because this factor could reduce the risk of winter mortality for the pine processionary moth.

The results of the papers and annexes offer the possibility to add useful information about the insect response to climate change particularly in the areas of new colonization. Through the analysis of negative and positive factors affecting insect colonization and persistence in the new range, I discuss the previsions about the future range expansion of the pine processionary moth and recognize aspects that need further studies.

Chapter 2

Cold tolerance of overwintering *Thaumetopoea pityocampa* larvae in an area of range expansion

Introduction

The pine processionary moth, *Thaumetopoea pityocampa* is a typical pest of coniferous forests with high socio-economic impact in Mediterranean countries. It mainly attacks *Pinus* spp. but it can also utilize *Cedrus* spp. and *Pseudotsuga menziesii* as hosts (Roques *et al.*, 2002). An expansion of the range of the pine processionary moth, both, latitudinally and altitudinally, in recent decades has been documented in detail for areas in France and Northern Italy and linked to warmer winter temperatures (Battisti *et al.*, 2005; Buffo *et al.*, 2007; Robinet *et al.*, 2007). In Northern Italy, e.g., the population in the Vinschgau/Venosta Valley has expanded its mean upper altitudinal limit on south exposed slopes from 1150 m to 1380 m in the last three decades (Battisti *et al.*, 2005). *T. pityocampa* overwinters in the larval stage without entering diapause or quiescence. Larvae are highly gregarious; they spin conspicuous silk nests on sun-exposed branches of the host tree as resting places for the colony during day time and periods of unfavourable weather conditions. Whenever abiotic conditions allow, larvae leave the nest during night hours and feed on the needles of the host tree (Huchon and Démolin, 1970).

Climatic conditions throughout the winter months are of critical importance for the occurrence of this species. Démolin (1969a) and Huchon and Démolin (1970) concluded from observational data that the range of pine processionary moth is defined by mean January minimum temperatures above -4°C and more than 1800 h of total annual sunshine. They further report that the lower lethal temperature is −6°C for an isolated larva and between −10°C and −12°C for a group of 200 larvae in the fourth instar. Moreover, these authors observed total mortality when the colony is exposed to −10°C for 10 hours. There is no mention on the methods used to determine the thresholds, nor to an indication of variation. These climatic limits sufficiently explained the stable range of *T. pityocampa* but were no longer consistent with the current range expansion of this insect (Robinet *et al.*, 2007). Therefore, a mechanistic understanding of the effects of winter temperature on *T. pityocampa* survival and consequently its range was sought. Temperature threshold data were derived from laboratory experiments and compared with experimental field data (Battisti *et al.*, 2005). The experiments revealed that feeding of *T. pityocampa* larvae occurs when night temperatures are >0°C but only if the temperature during the previous day was >6°C. The more conservative threshold of 9°C for this activation temperature

combined with the feeding threshold of 0°C was used in a model proposed by Battisti *et al.* (2005) to predict potential feeding time of *T. pityocampa* in areas of expanding range. Generally, survival of *T. pityocampa* observed in the field was reduced in areas with lower number of hours of predicted feeding; but the study also showed that stochastic temperature extremes can be important mortality factors (Buffo *et al.*, 2007).

Insects use different strategies to survive cold temperatures. Different schemes have been proposed to classify these strategies, classically focusing on whether or not an insect can survive freezing, i.e. whether it is freeze tolerant or freeze intolerant (e.g., Bale, 1993; Sinclair 1999). There is evidence that many insects suffer from injury well above freezing and that the effect of this chill injury can be cumulative (Turnock and Fields, 2005). To reflect the importance of the combined effect of time and temperature in determining survival of insects at low temperature, Nedvěd (2000) proposed a classification based upon freeze tolerance, susceptibility to cold shock, and cumulative chill injury. The temperature characterizing chill injury for a particular species has been termed the upper limit of cold injury zone (Turnock *et al.*, 1998; Nedvěd, 1998). No exact data exist from *T. pityocampa* to allow conclusions on parameters determining survival of its larvae at low winter temperatures. Démolin (1969a) and Huchon and Démolin (1970) based their assumptions of eco-physiological requirements mostly on observational data from the field. Therefore, a study was conducted to explore supercooling ability and long term survival under cold temperature to fill this gap in knowledge of *T. pityocampa* and to better understand the mechanisms underlying the ongoing expansion of the range of this Mediterranean insect. The supercooling point (SCP) of larvae collected in the field was analysed at various times during the winter. The SCP describes the temperature at which freezing of body water of the insect occurs. The ecological significance of SCP is discussed in Renault *et al.* (2002); they point out that chill injury above the SCP is important in many insects and that the SCP is no longer seen as adequate predictor of low temperature survival for many species (Turnock and Fields, 2005). But there are examples of some freeze intolerant species, in which SCP is thought to be appropriate to determine cold hardiness (Renault *et al.*, 2002). Since no knowledge exists for *T. pityocampa* in this respect, a SCP analysis was performed to provide these data for this insect. In order to estimate chill injury, survival under accumulated negative temperatures was tested in a

laboratory experiment. This allowed to study starvation capability of larvae for an extended period, an aspect that was found to be important when modelling feeding activity of the overwintering larvae (Battisti *et al.*, 2005). To see how the results from the laboratory relate to the field, the survival of overwintering larval colonies was measured on a study site in the expansion area of Vinschgau/Venosta and compared these data with accumulated negative temperatures. Additionally, the ability of larvae to tolerate freezing and survival of larvae inside their nests when cooled below the presumed lower lethal temperature for a short period – as would be the case in a typical cold winter night in the expansion area – was tested.

Materials and Methods

Analysis of super cooling point and hemolymph osmolality

Larvae were collected from infested *Pinus nigra* trees on south exposed slopes of the Vinschgau/Venosta valley, Province Bolzano, Northern Italy (for details on the study area see Battisti *et al.*, 2005). To study changes in the freezing behavior of larvae throughout the winter, samples were collected and their supercooling points (SCP) analyzed at various dates. Nests containing *T. pityocampa* colonies were cut from the trees and transported to the laboratory in Vienna on January 29 and November 26, 2003. Larvae were stored inside their nests at +3°C, 8L/16D photoperiod in a climate chamber until analysis of SCP in early February and early December 2003, respectively. For analysis of whole body SCP, a micro thermo element (copper-constantan) attached to a multi-channel recorder (Servogor 460, BBC Braun Boveri) was used. Larvae were positioned at the top of a thermo element in a 10-ml glass tube. The tube was submerged into a refrigerated diethylene glycol water bath; the temperature inside the tube decreased at an average rate of 1°C/min from +10°C to -25°C. The larvae were removed from the refrigerated bath immediately after recording the SCP and transferred to room temperature. After 2 h, larvae were checked and classified according to the degree of movement as response to strong disturbance with forceps; mortality was defined as absence of any movement. Hemolymph samples of additional fourth instar larvae from the January 2003 sample were collected. The osmolality of hemolymph samples pooled from three to five larvae was

analyzed in a micro osmometer (WESCOR 550 Vapor Pressure Osmometer). Calibration was based on pure water (0 mOsmol/kg) and a NaCl calibration solution (400 mOsmol/kg).

Additional *T. pityocampa* larvae were collected on the same site on February 12, 2004. In order to analyze changes in SCP at the end of the winter period, larvae were stored inside their nests under outdoor conditions in Vienna; *P. nigra* twigs were provided to allow feeding. A second group of larvae was stored inside the nests at +3°C/0°C, 8L/16D photoperiod in a climate chamber. Since larvae are not active at these temperatures, no food was provided. Analysis of SCP was performed as described above (but all runs were continued until minimum temperature was reached) at seven points in time from February to mid April.

Larval survival at low temperatures in the laboratory

Thaumetopoea colonies within their nests were collected from infested *P. nigra* trees on south exposed slopes of the Vinschgau/Venosta valley on November 5, 2004. The nests were stored under outdoor conditions in Vienna until setup of the experiment on November 17. Survival of 4th instar larvae was tested under different temperature regimes in climate chambers, relating to feeding thresholds of *T. pityocampa* (Battisti *et al.*, 2005): (1) temperatures that induce and allow feeding: +9°C/0°C; (2) day temperatures that induce feeding combined with night temperatures that do not allow feeding: +9°C/-5°C, +9°C/-10°C; and (3) temperatures that neither induce nor allow feeding: 0°C/-5°C, 0°C/-10°C. Photoperiod was set at 8L/16D. The coldest night temperature of -10°C was selected to test a minimum temperature just below the measured mean SCP of *T. pityocampa* larvae (see results of SCP analysis). Under each of the five temperature regimes, larvae were reared with or without food, respectively. Thirty larvae were put in ventilated 20 x 20 x 14 cm plastic boxes either with a twig of *P. nigra* with green needles or with a twig from which needles had been removed. Fresh food was provided every other week. Boxes were destructively sampled after 2, 4, 6, and 8 weeks of incubation; mortality was recorded and larvae were dissected in order to examine their midgut content. A sum of injurious cold temperatures in degree-hours was calculated by summing up the products of time (in h) and temperature (in °C) whenever temperature was below 0°C. In

lack of exact knowledge of the upper limit of the cold injury zone (Turnock *et al.*, 1998; Nedvěd, 1998) the temperature of 0°C was selected as basis for our thermal sum. This temperature has been shown to be the threshold for larval activity in *T. pityocampa* (Battisti *et al.*, 2005).

Colony survival during winter months in the field

The data used in this analysis come from the same experimental set up described by Battisti *et al.* (2005) and summarised here. Natural gradients to simulate temperature conditions experienced by the insect, as spatial analogues for climate change, were used. Two altitudinal gradients were established on the opposite slopes of Venosta/Vinschgau: 960-1450 m on the south slope, and 810-1190 m on the north slope. Each gradient involved three sites (south slope: 960, 1210, 1450 m; north slope: 810, 1010, 1190 m), representing the core, expansion, and external zones, respectively. Air temperature at each site was recorded with data loggers (Hobo, Tinytag). *T. pityocampa* colonies were collected at the egg stage on site and established at all sites along each gradient. A single egg mass producing a mean of 191.5 (SD 21.3) larvae was fixed to a branch of each of 20-30 trees of *P. sylvestris*, mostly from natural regeneration. Colonies were checked biweekly for survival until pupation. A sum of injurious cold temperatures was calculated as described above for the period between December 1 and February 28 of 2002-03. In 2003-04 only the two mid elevation sites on each slope were used and the data were collected as in 2002-03.

Survival of larvae inside nests at very low temperatures

Fifteen *T. pityocampa* colonies within nests were collected from *P. sylvestris* stands at six locations near Venosta/Vinschgau (Bondone, Caldonazzo, Fai, Rovereto, Storo, Tione) in Trentino, Northern Italy, in December 2005. Nests were individually stored outdoors at the Padova campus. Fresh *P. sylvestris* twigs were offered to the colonies weekly and feeding activity was estimated visually. In early February 2006, thirteen colonies that showed clear signs of activity were selected for the experiment. The experiment was performed in four consecutive trials, each lasting for five days. Size of the colonies used in the experiment did not differ among trials (mean 106.5±13.5 larvae;

ANOVA: $F_{4,18}$=0.61, P=0.66). Colonies were put into ventilated plastic boxes (50 x 35 x 40 cm) and transferred into rearing cabinets at 9L:15D photoperiod. After scotophase the light gradually increased through 2 h to the maximum intensity of 2500 lux. Light was kept constant for 5 h and decreased again through 2 h. Following the same scheme, temperature increased from +5°C night temperature to +15°C day temperature. Relative humidity was kept at 85% during scotophase while it decreased to minimally 60% during photophase. One group of colonies starved for 96 h in order to empty the guts whereas another group was continuously supplied with food. At the beginning of the fifth night, when the larvae were still inside the nests and temperature was +5°C, colonies were transferred into smaller boxes and put into a freezing cabinet capable of cooling to -17°C, i.e., 1°C below putative lethal temperature of *T. pityocampa* larvae. Temperature decreased gradually at 2.2°C/h for 10 h, reaching the minimum temperature of -17°C, and increased at 4.4°C/h for 5 h up to +5°C. Colonies were transferred back to the rearing cabinet at standard rearing conditions and provided with food. After two days, the nests were inspected, larvae counted and checked for mortality. A sample of dead larvae was dissected and the gut content analysed to determine time of death. Larvae (from the group that was constantly provided with food) that had green food particles in the gut were assumed to have died during the freezing trial. This was necessary because evaluation of mortality of larvae in the nest can only be done destructively and was therefore impossible before the trial.

Statistical analysis

All data were analysed with the software program SPSS 15.0.0 (SPSS Inc. 1989-2006). Data were tested for normal distribution using Kolmogorov-Smirnov test in SPSS' explore procedure. Normally distributed data were compared with independent samples t test or one-way ANOVA; data lacking normal distribution were compared using nonparametric tests. Relative frequencies were compared with χ^2 tests.

Results

Supercooling point and hemolymph osmolality of T. pityocampa larvae

The SCP of *T. pityocampa* larvae varied to a high degree averaging at ca. -8°C (Fig. 2.1). Analysis of SCP in larvae collected in January and November 2003 revealed a minimum SCP of -13.3.°C, a maximum of -1.1°C, and a mean of -6.4±0.2°C (n=189). The SCP was lower in third instars than in fourth instars in both trials; however, this difference was not statistically significant (t=-1.869, df=151, P=0.063 and t=-1.938, df=34, P=0.061, respectively).

The highest value of hemolymph osmolality in larvae collected in January 2003 was 508 mOsmol/kg, the mean was 441.8 ± 5.7 mOsmol/kg (n=32). Not all larvae died at the SCP and, generally, the SCP appeared to have an effect of survival. Larvae that were classified dead or moribund after freezing had lower SCPs (-7.6°C ± 0.7°C) than those classified as showing weak or strong movement (-5.2°C ± 0.5°C) (independent samples t test: t=-2.481, df=26, P=0.020). However, there was no clear threshold temperature for lethal freezing.

T. pityocampa larvae collected at the same locality in February 2004 showed a minimum SCP of -13.2°C, a maximum of -2.3°C, and a mean SCP of -7.7 ± 0.2°C (n=242) (Fig. 2.1). There were no significant differences between larvae stored at constant +3°C and larvae reared outdoors, except on the second day of analysis (March 10, 2003), when larvae from outdoors had significantly lower SCP (t=-3.108, df=45, P=0.003). SCP varied between the dates in outdoor-reared larvae. Larvae analysed on February 25 and April 1 had significantly higher SCP than those from March 10 (P<0.05, Tamhane's T2 tests following up significant ANOVA: F=5.444, df=5). This was mostly due to the variability of SCP in 4th instars. No significant variation occurred in larvae stored in the incubator at constant temperatures of +3°C (One-way ANOVA: F=0.410, df=4, P=0.801).

Larval survival at low temperatures in the laboratory

T. pityocampa larvae showed the capability of surviving long periods of starvation. Survival without food after 8 weeks was significantly lower than after 2 weeks at 9°/0°C

and 0°/-5°C (χ^2 tests, P<0.05) but not under 9°/-5°C. Ninety percent of the larvae from the latter treatment were alive after 8 weeks without food. Survival was low at 9°/-10°C and 0°/-10°C; in these cases survival was 3% and 10%, respectively after 2 weeks (Fig. 2.2). Whether or not food was provided had no significant effect on larval survival under our experimental conditions. Green needle particles indicating recent feeding occurred only in the midguts of larvae kept at 9°/0°C; this was the case in 0%, 27%, 11%, and 42% of the examined larvae after 2, 4, 6, and 8 weeks, respectively. Larvae from all other treatments had empty guts or brown, apparently predigested food particles. While the majority of larvae kept in treatments with daytime temperature of 9°C had either green particles or no particles in the gut, the majority of larvae kept at 0°C daytime temperature had brown particles.

Percent survival of larvae decreased with accumulating negative thermal sum (Fig. 2.3). A linear regression describes the decrease in survival with increasing sums of hour-degrees below 0°C (R^2=0.543; ANOVA: F= 45.12, P<0.01). At a negative thermal sum of ca. -3800 h°C, larval mortality reached 50%.

Colony survival during winter months in the field

Percent survival of *T. pityocampa* colonies in the field decreased with accumulating negative thermal sum (Fig. 2.4). A linear regression describes the decrease in survival with increasing sums hour-degrees below 0°C (R^2=0.719; ANOVA: F= 15.33, P<0.01).

Survival of larvae inside nests at very low temperatures

An average of 70.4% of *T. pityocampa* larvae survived exposure at -17°C for 1 h. Survival was higher in starved (74.0%) than in fed larvae (67.2.%) but the difference was not significant (t=-1.10, df=11, P=0.296). The larval activity (i.e., proportion of living larvae outside the nest) after freezing was higher in fed (69.4%) than in starved larvae (59.0%), but again the difference was not significant (t=-0.59, df=11, P=0.57). Gut content differed between treatments: 75% of dead larvae that were provided with food had green food particles in the midgut, while all larvae that starved before freezing had empty

midguts. There was no difference in survival among colonies from different locations ($F_{5,7}$=1.04, P=4.64).

Discussion

The larvae of *T. pityocampa* from the expanding population in Northern Italy apparently lack special adaptations to avoid freezing in order to be protected from cold winter temperatures. Larvae froze on average at -8°C. The supercooling point, however, does not adequately characterize cold hardiness in this insect as part of the tested larvae survived freezing. Higher SCPs were somewhat related to higher survival, however there was no clear-cut threshold temperature for such separation. Many larvae survived chilling down to -17°C (i.e., about 4°C below the lowest measured individual SCP) in groups inside their silk nest, thereby demonstrating that they would survive such severe frost as it would occur in a cold winter night in the expansion area. Thus, larvae could be classified as moderately freezing tolerant (Sinclair, 1999; Chown and Nicholson, 2004).

In this study it is shown that, despite being able to survive freezing, larvae suffered from cumulative chill injury. Larval survival decreased with increasing negative thermal sum (hour-degrees below 0°C) in both laboratory and field, but the thermal sum required for 50% mortality in the field was 3-4 times lower. Although the estimates of mortality differed in the two experiments (individual mortality in the laboratory and colony mortality in the field), they can be compared as previous work showed that they are strongly correlated (Buffo *et al.* 2007). Evidently additional mortality factors besides cold temperatures are working in the field, perhaps exacerbating the negative effects of low temperature. Another possible explanation of such a difference in the thermal sum may be given by the temperature regimes, as the larvae were subjected to constant temperature in the laboratory and naturally fluctuating temperature in the field. Given the same mean value, it is well known that fluctuating temperature is generally associated with different performance in insects (Dallwitz 1984, Hagstrum & Milliken 1991). Fluctuation around SCP values in the field can thus be hypothesised to be more harmful to the larvae than a long permanence at low but constant temperature.

Susceptibility towards accumulated chill injury has been accepted as very important determinant of cold hardiness of many insects (Renault *et al.*, 2002; Turnock and Fields, 2005). Nedvěd (1998) proposed the sum of injurious temperatures (SIT – the product of time and degrees of injurious temperature) at which 50% of the population would be killed as a parameter elegantly quantifying the level of susceptibility. However, it is necessary to have knowledge of what Turnock *et al.* (1998) called the upper limit of the cold injury zone. This parameter was not available for *T. pityocampa*. Thus, the temperature of 0°C was tentatively selected as base for our calculation of the negative thermal sum, which was shown to be the activity threshold for *T. pityocampa* larvae (Fitzgerald and Blas, 2003; Battisti *et al.*, 2005).

It is interesting to note the difference in survival at 9°/-5°C and 0°/-5°C at identical thermal sums – mortality was higher at the colder daytime temperature. This may be an indication that repair of chill injury is only possible at warmer day temperatures. Repair of cold injury is viewed as particularly important for insects overwintering in habitats with variable winter temperatures (Turnock and Fields, 2005), as is the case for *T. pityocampa* in its expansion area in the Italian Alps. Fluctuation around critical temperature may enhance cold injury and that can explain the lower thermal sum for 50% mortality in the field. At the sun-exposed slopes of the Vinschgau/Venosta valley that are inhabited by this insect, e.g., average temperatures in January 2003 varied between -4°C minimum and +4°C maximum air temperature and up to +16°C maximum temperature inside the nest (Battisti *et al.* 2005). Daytime temperature moreover determined the ability of the larvae to digest ingested food. Starved larvae at 9°C had empty midguts while the midguts of larvae at 0°C contained predigested food particles. This is interesting in the context of the temperature threshold for activation of night-time feeding. Battisti *et al.* (2005) showed that feeding at night occurred only when temperature on the preceding day was above 6°C. Our laboratory experiments also demonstrated a remarkable starvation capability of the larvae; given that temperature did not cause cumulative chill injury, a high proportion of larvae survived at least 8 weeks without feeding. Thus, the larvae possess the necessary endurance to survive extended periods with temperatures that do not allow feeding (i.e., below 0°C night and 9°C day temperature, Battisti *et al.*, 2005), a feature that will be of

particular importance to persist throughout severe winters in the areas of range expansion. It is likely, however, that accumulated cold injury may reduce the starvation potential.

The SCP of larvae averaged at -8°C. This is relatively high compared to many other insects – even tropical insects have often lower SCP – but it is in the range for freeze tolerant insects in temperate climate zones (Sinclair, 1999; Turnock and Fields, 2005).

Moreover, SCP showed no marked changes throughout winter season. Hemolymph osmolality in *T. pityocampa* larvae is comparable to summer values of freeze avoiding insects in temperate regions and, therefore, is markedly lower than in freeze avoiding insects in winter. Likewise, gas-chromatographic analysis of hemolymph showed no increased titres for sugars or sugar alcohols (Schopf, unpublished data). This indicates that *T. pityocampa* larvae do not undergo any special preparations for the cold period. Overall, adaptations for the cold period, also mechanical measures, such as emptying the gut, may not feasible for *T. pityocampa*, since the larvae are confronted with highly variable temperatures and have to be ready to feed throughout the winter whenever temperatures allow.

Field observations exist that winter survival of *T. pityocampa* depends on developmental stage of the larvae with younger larvae being more likely to suffer mortality (Démolin 1969a, Pérez-Contreras *et al.* 2003). However, SCP of young larvae did not differ from older instars; analysis of second instar *T. pityocampa* collected in Liguria (Italy) in October 2004 revealed a mean SCP of -9.1°C (unpublished data). On the other hand, the young larvae may still differ in their susceptibility to cumulative chill injury, and in any case they build smaller nests so that the heat trapping during daytime can be less effective.

The experiments with exposing larvae inside their nests to low temperatures showed that *T. pityocampa* can survive temperatures of -17°C for an hour when chilled at rates comparable to a typical cold winter night in the expansion area. Nevertheless, temperatures in the field may occasionally be even lower and can lead to 100% mortality of larvae as documented by Buffo *et al.* (2007); the likelihood of these conditions is higher at the upper altitudinal limit of the range. But the population is protected from eradication by such stochastic, catastrophic weather events by a flexible, extended diapause (Battisti *et al.*, 2005; Buffo *et al.*, 2007). In the study site, more than 90% of the larvae pupated in the

spring 1999 stayed in the pupal stage for up to 8 years in the soil where they are protected from extreme frost (Aimi *et al.* 2007).

Overall, this study gives first data on parameters characterizing survival of *T. pityocampa* larvae at cold temperatures. The high SCP together with the fact that part of the larvae can tolerate freezing indicate the this insect can be classified as "moderately freeze tolerant" (Sinclair, 1999; Chown and Nicholson, 2004). This appears to be a suitable adaptation to cope with highly variable temperatures that occur at the altitudinal limits of its range and allows the larvae to resume feeding and development during winter periods with warm weather. But besides its freeze tolerance it must be considered that *T. pityocampa* does suffer time/temperature related mortality due to cumulative chill injury as shown in our laboratory experiments as well as field data. According to Nedvěd's (2000) "Snow White and the seven dwarfs" classification, *T. pityocampa* may be listed in the class "Grumpy" for which freezing of body water and cold shock are not lethal but suffers from cumulative chill injury. It would be desirable to collect more experimental data for *T. pityocampa* larvae particularly in the temperature range between 0° and -7°C in order to determine the upper limit of the cold injury zone and investigate moderate positive temperatures for the repair of chill injury. However, the problems connected with handling and experimentally rearing the caterpillars with their air-borne urticating setae will further put constraints on intensive experimentation with this important pest species.
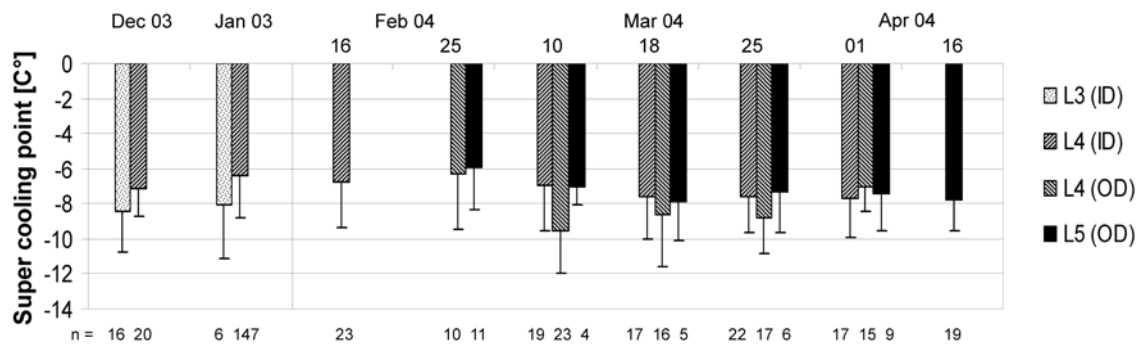
Fig. 2.1. Super cooling point (SCP) of *T. pityocampa* larvae of instars 3 to 5 (= L3 to L5) collected in Vinschgau/Venosta and analyzed at different points in time (means + SD). Larvae were either kept in incubators at +3°C (ID) or under outdoor winter conditions in Vienna (OD) between collection and analysis.
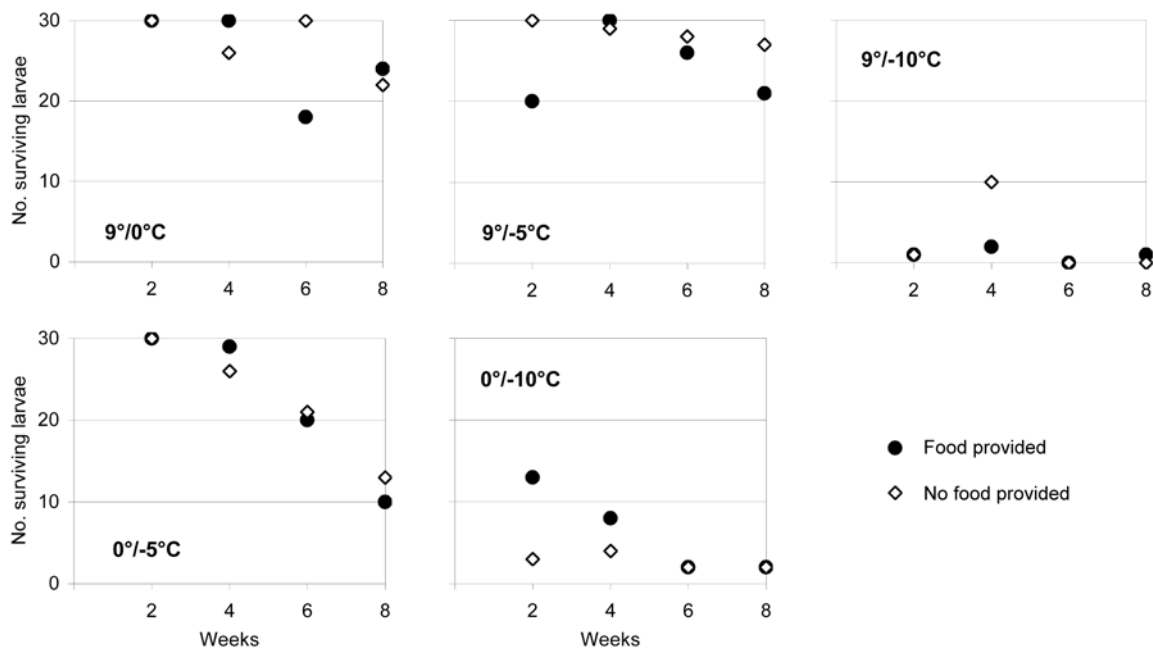
Fig. 2.2. Survival of *T. pityocampa* larvae after incubation for 2, 4, 6, and 8 weeks, respectively, at five different temperature regimes. Markers indicate whether larvae were reared on pine twigs with or without needles to provide food. According to analyses of larval gut contents, feeding occurred only in the 9°/0°C treatment.
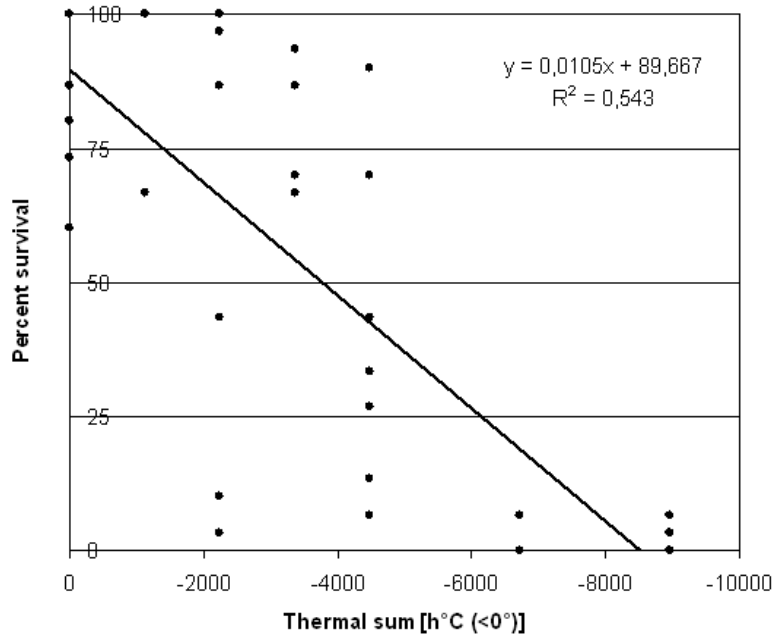
Fig. 2.3. Percent survival of *T. pityocampa* larvae in the laboratory depending on accumulating negative temperatures (sum of hour-degrees below 0°C). Treatments with or without food are shown together; feeding occurred in none of the treatments except 9°/0°C (thermal sum = 0 h°C).
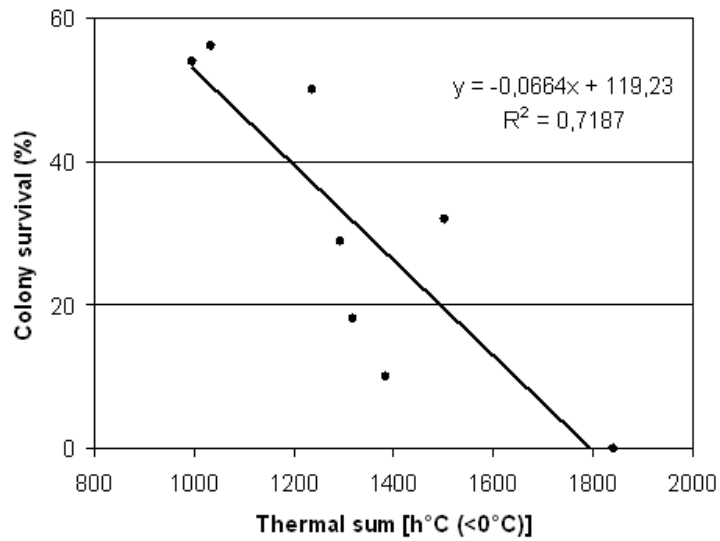
Fig. 2.4. Percent survival of colonies *T. pityocampa* translocated at different sites along the south and north slopes of Vinschgau/Venosta at the end February of 2003 and 2004, depending on accumulating negative temperatures (sum of hour-degrees below 0°C) in the period from December 1st to February 28th.

Chapter 3

Host plant use in the range expansion of the processionary moth *Thaumetopoea pityocampa*

Introduction

Many organisms depend on a single, or select few, species for food or other resources, with their distributions at least in part dictated by the availability of other species. For instance, specialised and oligophagous insect herbivores occur in regions that coincide with the geographic ranges of their host plant(s) (Koizumi *et al.*, 1999), and their local abundance often reflects the relative abundances of their preferred hosts (Strong *et al.*, 1984). Periodically, species may undergo expansions or shifts in their geographic ranges. Recent range expansions have been reported in a number of herbivorous insects, and attributed mainly to climate change (Thomas *et al.*, 2001; Root *et al.*, 2003; Battisti *et al.*, 2005) and human-caused habitat expansion (Gutierrez & Thomas, 2000). As insect herbivores colonise new areas, they may encounter novel host plants, and, except in the case of strict specialists, will often accept those that are closely related to their original host(s) (Scriber & Ording, 2005). Oligophagous species, in particular, may find potential hosts even in areas where their original or preferred hosts are absent or much less abundant. The availability of the new hosts can thus facilitate further expansion into regions beyond the geographic range of the original hosts (Gutierrez & Thomas, 2000).

At least in theory, ovipositing females are expected to prefer hosts that are the most suitable for larval development, and accept less suitable plant species only when the optimal (preferred) host is either absent or not found (Jaenike, 1978; Thompson, 1988; Awmack & Leather, 2002; but see Courtney & Kibota, 1989; Scheirs & De Bruyn, 2002). Host recognition and preferential oviposition should then lead to hierarchies of host use, and these hierarchies should be reflected in the frequency of host acceptance and measures of larval performance, such as larval mortality or relative growth rate (e.g. Jaenike, 1990; Thompson & Pellmyr, 1991). However, a novel host encountered during range expansion may fall anywhere within the preference-performance hierarchy because of the lack of common evolutionary history (Thompson, 1996).

As the range of an insect herbivore expands, habitats with multiple hosts may be replaced with single-host habitats in which the preferred host is missing and only the less preferred (or novel) host is present. Consequently, females may only encounter hosts perceived as less attractive in a multi-host setting. If discrimination among hosts is

behaviourally relatively fixed, I may expect that the preference hierarchy will still hold. Accordingly, the relative frequencies of females that accept each host for oviposition in a single-host (no-choice) setting should reflect the degree to which that host is accepted in a multi-host (choice) setting. On the other hand, a number of models of parasite-host systems predict that certain life history traits, such as egg load or longevity, may cause females to adaptively modify their host (or habitat) preference hierarchy (Levins & MacArthur, 1969; Ward, 1987; Mangel, 1989; Jaenike, 1990; Carriere, 1998), and to accept hosts more indiscriminately if not presented with a choice (e.g. Diaz-Fleischer & Aluja, 2003). For instance, species in which females are extremely short-lived as adults are under a strong time limitation to oviposit (Courtney, 1982; Leather & Burnand, 1987; Wiggins, 1997; Scheirs & De Bruyn, 2002; Jervis *et al.*, 2005). Persistent rejection of less attractive hosts may then severely compromise their realised fecundity, because strongly discriminating females may never encounter a more attractive host, or even another less attractive host, and may be exposed to other risks, such as predation and inclement weather. In such time-limited species, the original hierarchy of female preference in a multi-host setting may therefore break down in a single-host setting, and the differences in acceptance of each host may disappear.

In the context of this theoretical framework, I investigated the patterns of host use of the winter pine processionary moth (*Thaumetopoea pityocampa* Denis & Schiffermüller), an economically important defoliator of pines (*Pinus* spp.) in southern Europe. This insect has been expanding its latitudinal and altitudinal range in the Italian Alps over recent decades (Benigni & Battisti, 1999; Battisti *et al.*, 2005). In this region, *T. pityocampa* is typically associated with Austrian black pine, *Pinus nigra* Arnold, (Masutti & Battisti, 1990). However, in the course of the recent expansion, it has been increasingly encountering and colonising two other pine species: *P. sylvestris* L. (Scots pine), which historically represented a secondary host utilized less frequently and mostly at the margins of the past range of *T. pityocampa*, and a novel host *P. mugo* Turra (mountain pine), which was only rarely colonized in the past and probably did not support any viable populations. While population densities and outbreak frequencies vary among the host species (Masutti & Battisti, 1990), it is unclear whether these differences are driven by oviposition preference, larval performance, a combination of both, or by other factors. Importantly, females typically live only one night, and therefore are under strong time limitation to locate and accept a host on which to lay their single batch of eggs.

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To evaluate the relative role of host attractiveness and suitability in the patterns of host use by *T. pityocampa* in the Italian Alps, and to provide a basis for predictions on future expansion, I designed the following series of tests. First, I assessed the utilisation of the three hosts in mixed stands at two sites in the expansion range. Subsequently, I tested whether the hierarchy of host use at these sites was consistent with female *T. pityocampa* discrimination among the three hosts in controlled experiments. If the natural densities of the insect on the three hosts were driven primarily by differential female preference, the highest frequency of oviposition would be expected on the preferred host (*P. nigra*), relative to the secondary host (*P. sylvestris*) or the novel host (*P. mugo*) (Plate 2a). Second, I compared oviposition rates on each of the three hosts when females were not presented with a choice. Specifically, I tested the hypothesis that, due to limited female longevity, the original preference hierarchy in *T. pityocampa* would break down in a single-host (expansion) situation and frequency of oviposition would be similar on all three hosts. Finally, to check whether the observed preference patterns reflect the relative suitability of the hosts, I compared larval performance on the three hosts, both in laboratory and field experiments. If female oviposition patterns consistently showed little differentiation (hierarchy) in the controlled experiments, the natural colonisation pattern could be driven largely by differential larval survival on the three hosts. However, if clear hierarchies in female preference were detected, larval performance could either counteract or amplify oviposition preference in the overall patterns of host use. I discuss our findings in the context of *T. pityocampa*'s ecology, and present a synthetic perspective on its future expansion on the secondary and novel hosts in the Alps.

Materials and methods

Study system

Thaumetopoea pityocampa is a univoltine Lepidopteran oligophage on coniferous trees. Its main hosts in the study areas are *Pinus* spp., but it can also attack *Cedrus* spp. and the introduced *Pseudotsuga menziesii* (Roques *et al.*, 2002). Emergence of adults occurs after dusk, and females typically live only one night (Démolin, 1969b). After mating, females disperse by flight to search for a suitable host on which they lay their single batch

of 210-280 eggs (Masutti & Battisti, 1990). The larvae feed gregariously during autumn and winter, and construct a conspicuous silk nest, typically near the tip of a branch or treetop. *T. pityocampa* frequently reaches outbreak densities throughout the Mediterranean basin, and now extends its range well into the southern Alps and central France. Defoliation by the late larval instars can reduce tree growth (Laurent-Hervouet, 1986), and the larvae produce urticating hairs that can cause an allergic reaction (contact dermatitis) and pose a public health risk (Lamy, 1990). Because of its economic and medical importance, *T. pityocampa* populations have been monitored for many years, by means of winter nest censuses and pheromone trapping, as part of pest management programmes throughout the Mediterranean region.

Pinus nigra is considered the primary native host of *T. pityocampa* in the study area, and the planting of *P. nigra* outside its native range in the eastern Alps into many reforested regions of northern Italy has promoted population growth and outbreaks of *T. pityocampa* (Masutti & Battisti, 1990). Especially in mixed stands, it has also lead to increased colonisation and defoliation of the native *P. sylvestris*, which is at the southern edge of its distribution in Italy, and has historically been a secondary host for *T. pityocampa*, generally colonized at lower densities and in only certain parts of the insect's former range (Démolin, 1969b). In contrast, oviposition has been reported only infrequently on *P. mugo*. This pine is primarily a subalpine species with a restricted distribution largely outside the original range of *T. pityocampa*. Until recently, it was considered unsuitable for larval development due to climatic factors (especially snow cover) (Masutti & Battisti, 1990; A.B. pers. obs.).

Nest census

Colonisation of trees by *T. pityocampa* under mixed-stand conditions was surveyed in two censuses of winter nests (Table 3.1). Winter nests can be detected easily even at low population densities (Geri & Millier, 1985); in contrast, egg batches are inconspicuous and difficult to detect except on small trees. While a nest census likely underestimates the initial number of egg batches laid, due to early larval mortality, a previous study (Benigni & Battisti, 1999) did not detect any differences in overall larval mortality among the three pine species.

The first census was conducted in October 2004 at a site near Rovereto, Italy, at the edge of the historical range of the insect. Here, all three hosts grew intermixed at a low

density (mean distance between trees 4.7 ± 0.27 m), with *P. nigra* and *P. sylvestris* reaching 1.5 to 5 m in height, and the shrub-like *P. mugo* reaching 1-2 m. The number of nests present was assessed on all trees inside a randomly chosen area of about 0.4 ha, comprising 35 *P. mugo,* 58 *P. nigra,* and 55 *P. sylvestris* trees. The height of each tree was also measured to account for likely differences in apparency among the hosts; visual cues guide female host search before alighting (Démolin 1969b; Battisti *et al.,* unpubl. data).

The second census was carried out in November 2004 at a site in Venosta Valley, Italy, within the new range of the insect following a recent range expansion. Only *P. nigra* and *P. sylvestris* grew in this region. I chose a 3 ha site with natural regeneration, with isolated trees 3-8 m in height, growing in a transition between a meadow and a continuous pine stand. Nests on 58 *P. nigra* and 58 *P. sylvestris* trees selected at random within the site were counted.

Choice and no-choice experiments

To compare the results of nest surveys (natural colonisation patterns) with female oviposition preference, choice and no-choice experiments were designed in outdoor exclosures, in which I minimised confounding effects and standardised host parameters. The experiments were carried out on the campus of the University of Padova, Agripolis Legnaro, in four large, tunnel-shaped cages (Plate 2b). A 1 x 1 mm mesh net (Artes Politecnica, Santorso Vicenza, Italy) allowed near-ambient light and temperature conditions, and a black landscaping tarp (Artes Politecnica, Santorso Vicenza, Italy) covered the floor of the cages. Each cage was divided into two sections of 6 x 4 x 2.7 m, providing a total of eight rooms in which experiments could be carried out simultaneously.

Insect material

30 winter nests (about 5,000 larvae) were collected in December 2003 from each of eight populations from core (historical) and expansion (recent) (Plate 2b) parts of the insect's range (cf. Battisti *et al.*, 2005), chosen to include a wide geographic area and all the available hosts (Table 3.1). The larvae of each population were reared until pupation in the eight separate rooms, on pine branches from the host species from which they were collected. In late winter, the larvae were allowed to pupate in a 40 cm wide ditch running along the perimeter of each room, which was filled with sand (45%), peat (45%), and leaf

mould (10%) to a depth of 15 cm. To facilitate adult emergence and wing extension, wooden sticks (20 cm in length, 1.5 mm in diameter) were inserted in the ditch in vertical position. Emergence and oviposition were checked daily by counting and removing dead insects and egg batches.

Plant material

Pine trees used in the experiments were obtained from a forestry nursery situated in north-eastern Italy (Tolmezzo UD). To obtain individuals of the normally shrub-like *P. mugo* that would be of comparable size to those of *P. nigra* and *P. sylvestris*, I used the upright *P. mugo* var. *uncinata* Miller from a nursery in Aosta (north-western Italy). The plants were raised from local seed sources and were 5-6 years old, with an average height of about 120 cm.

To standardise tree appearance among hosts of varying architecture, I used an arbitrary measure of visual similarity to the insect, correlated with tree height (Battisti *et al*., unpublished data). The side-view total leaf area (A; hereafter, silhouette) was calculated using the formula

$A = L_{TB} * N * L_N * W$,

where L_{TB} = total length of branches bearing needles; N = mean number of needles per cm branch length; L_N = mean length of the needles; W = maximum width of the needle section, measured at the midpoint of needle length. In order to reduce variation among individual trees and reach comparable values of silhouette per host species, A was calculated for each plant and then plants were selected and grouped together such that collectively they would give a target silhouette of 9,000-12,000 cm^2. Characteristics of the trees used in the experiments are given in Table 3.2.

Experimental set-up

In the choice experiment, one pot each of the three pine species (*P. mugo*, *P. nigra*, *P. sylvestris*) was offered simultaneously in each room. Every morning, eggs and emerged females were counted, and then removed. After the collection of egg masses, the pots were rotated clockwise among room corners. In the no-choice experiment only one potted pine species per room was offered to the insects. The experiments started on 25 June 2004, at the beginning of the emergence period, which collectively lasted 36 days. For both types of

experiment, each insect population was tested for several nights in a row, depending on the availability of emerged insects and on the number of egg batches obtained. At least two choice and two no-choice experiments were running simultaneously on the same night with different populations. A complete randomisation of the experiments among the populations was not possible because of the unpredictability of adult emergence. While our cage experiments faced the usual challenges of obtaining mated females and egg batches under captivity, I achieved the highest rate of oviposition described in the literature for this species (up to 50%; Démolin, 1969b). In both experiments, the collected egg batches were kept in the laboratory at 24°C until they were used for larval performance experiments. Female realised fecundity was estimated by counting the total number of eggs per egg batch.

Larval performance experiments

To test for differences in host quality under a variety of conditions and for larvae of different ages, I conducted three separate experiments, measuring larval performance on the three host species.

In the first experiment, first-instar larvae from two core and three expansion populations were reared under laboratory conditions (Table 3.1). The larvae were obtained from egg batches laid during the preference experiments (see above). Larvae were fed one-year old needles of the three host species (*P. mugo*, *P. nigra*, *P. sylvestris*) that were collected either from potted trees or from naturally growing trees (hereafter, "needle origin"; potted or wild, respectively). In both cases, needles came from 10-15 trees and were randomised. In the bioassay, 15 full-sibling neonate larvae were placed inside each Petri dish (9 cm Ø), and provided with 750 mg of fresh needles of either potted or wild origin, replicated for each host species. One brood (egg batch) allowed to cover all six treatments, with the total number of replicates for each treatment corresponding to the number of broods used (Table 3.1). The needles were changed after three days (in all treatments before being consumed completely). After five days, all surviving larvae were weighed, and relative growth rate (RGR, mg mg^{-1} day^{-1}) was calculated using their initial and final weight (Bauce *et al.*, 2002). Percent mortality was also noted. Nitrogen content of the needles used in the experiments was assessed by the Kjeldahl method from samples taken before the bioassay started (Huhn & Schulz, 2002). Needle toughness was measured by

determining the force needed to penetrate the needles using a calibrated penetrometer. The leaf was fixed between two metal plates, and the penetrometer needle was inserted through the central part of the upper, rounded surface. Five needles were measured per tree, and three readings were taken per needle, avoiding the distal parts.

In the second experiment, larvae from two expansion populations were reared until the third instar on potted trees (Table 3.1) under outdoor conditions on the university campus. On each of 13 *P. mugo*, 13 *P. nigra*, and 13 *P. sylvestris* trees enclosed inside a 1 x 1 mm mesh net sleeve, I placed a single egg batch obtained from the preference experiments. As soon as the larvae moulted to the third instar, the total number of living larvae and weight of the survivors were determined. The original egg batches were collected to determine the number of hatched eggs (initial colony size).

In the third experiment, I compared larval performance from a core and an expansion population on each host species under field conditions across the 2004-2005 season. The experiment was conducted at a site near Cimolais (expansion area) (Plate 2b), where a sufficient number of trees of the three host species grew intermixed in an area of about 1 ha. Natural egg batches, collected at Venosta, northern Italy (expansion population) in mid July 2004, and at Rivoli Bianchi, north-eastern Italy (core population) in mid August 2004, were used. From each population, 20 egg batches were selected at random and assigned singly to 20 trees of each host species (a total of 120 batches). The egg batches were fixed to a branch and enclosed inside a 1 x 1 mm mesh net sleeve of 50 x 120 cm. After hatching, egg batches were collected to determine the number of hatched eggs (initial colony size). The sleeves were inspected periodically, and the colonies were transferred to another branch of the same tree when needles started to be in short supply. In mid November 2004, I collected half of the colonies from each population and host, and recorded larval survival, instar, and weight. The remaining colonies were left on the trees until early April 2005, when they were collected, analysed as above, and reared for another 4-5 weeks until pupation.

Statistical analyses

The distribution of nests among the pine species in the field (host colonisation pattern) was tested against the null hypothesis by the χ^2 test, and the number of nests per

tree by Kruskal-Wallis ANOVA by ranks (Sokal & Rohlf, 1995). To test for preference differences in the choice experiment, I used a log-linear analysis with three factors: host species, population type (core vs. expansion), and the original host (*P. nigra, P. sylvestris*), and included interaction terms between the three factors (Sokal & Rohlf, 1995). Log-linear analysis differs from the analysis of variance in that the latter tests for significance of main effects while log-linear analysis tests for significant interactions. Significance of an interaction term was assessed from the χ^2 value obtained by the difference of χ^2 values of models including or not including that interaction term.. Since the experiments involved a diffuse source of multiple emerging insects (rather than individual releases of single females), host trees could be treated as independent from one another. Before analyses, the numbers of egg batches laid on different hosts were first converted to relative frequency of egg batches (% egg batches), then corrected to account for the differences in the total number of females that had emerged in each population. The same correction, and the log-linear analysis, were also employed to test for oviposition frequency in the no-choice test. ANOVA was employed to compare the realised fecundity of females between core and expansion areas and among populations, again using the relative frequency of egg batches as the dependent variable. Tukey's test was used for pair-wise comparison of means. Differences in the performance of first-instar larvae among core and expansion populations, and host species, were tested using factorial ANOVA with relative growth rate and percent mortality of each population as variables. For the potted tree experiment and the field experiment with third instar larvae, the same variables were analysed using ANOVA and a log-likelihood G test (Sokal & Rohlf, 1995), respectively. In all cases in which ANOVA was exployed, the basic assumption of homogeneity of variance was met, and variables were tested without any transformation.

Results

Host selection patterns – nest census

In the mixed stand at Rovereto, nests were more abundant on *P. nigra* (65.5% of trees attacked) than on *P. mugo* (42.8%) and *P. sylvestris* (29.1%), with significant

differences on the whole ($\chi^2 = 8.17$, d.f. = 2, $p = 0.016$) and in any pair-wise comparison with the exception of *mugo-sylvestris*. The mean number of nests per tree differed significantly among hosts (Fig. 3.1; Kruskal-Wallis rank ANOVA $H_{2,149} = 14.8$, $p < 0.01$). However, *P. mugo* trees were significantly shorter than those of the other two species (ANOVA $F_{2,149} = 10.3$, $p < 0.01$), and hence less prominent in appearance. At Venosta, where *P. mugo* was absent, *P. nigra* tended to have more nests than *P. sylvestris* (34.5% and 17.2% of trees attacked, respectively; $\chi^2 = 3.3$, d.f. = 1, $p = 0.067$), and nests were more than twice as abundant per tree on *P. nigra* (0.58 ± 0.12, mean and SE, respectively) than on *P. sylvestris* (0.23 ± 0.07) (Kruskal-Wallis rank ANOVA $H_{1,111} = 4.7$, $p = 0.03$).

Female preference – cage oviposition experiments

In the choice experiment, *P. sylvestris* was clearly less preferred than either *P. nigra* or *P. mugo* ($\chi^2 = 90.1$, d.f. = 2, $p < 0.01$, log-linear), whereas I could not document any consistent preference for *P. nigra* over *P. mugo* ($\chi^2 = 1.26$, d.f. = 1, $p = 0.25$) (Fig 3.2). Overall, core and expansion populations did not show any significant differences in their oviposition preferences ($\chi^2 = 0.02$, d.f. = 1, $p = 0.90$ for *P. mugo* vs. *P. nigra*). However, there was a significant interaction between population type and host species ($\chi^2 = 10.6$, d.f. = 2, $p < 0.01$). For instance, *P. mugo* received more oviposition from the expansion than the core populations, whereas the trend was reversed for *P. nigra*. Also, the core populations largely preferred *P. nigra* over the other two hosts, while the expansion populations seemed to be less selective (Fig. 3.2). Other interaction terms were not significant.

In the no-choice experiment, a higher proportion of egg batches was laid on *P. mugo* and *P. nigra* than on *P. sylvestris* ($\chi^2 = 12.5$, d.f. = 2, $p < 0.01$) (Fig. 3.3), consistent with what was found in the choice experiment.

Oviposition was more frequent amongst females of some populations than of others ($\chi^2 = 31.9$, d.f. = 6, $p < 0.01$), but the populations did not differ in the mean number of eggs per egg batch ($F_{(6,262)} = 1.29$, $p = 0.27$) (Table 3.3). Realised fecundity (mean number of eggs per batch) was similar in both choice and no-choice experiments, and among the host species (Table 3.3; $F_{(2,230)} = 2.01$, $p = 0.14$).

Larval performance – laboratory and field experiments

In the Petri dish bioassay with neonate larvae, I found no significant differences in relative growth rate (RGR) between core and expansion populations, among host species, or between needle origin (potted versus wild trees). Neither were any of the interactions significant. Larval mortality, however, was slightly higher on wild plants ($F_{1,6} = 6.3$, $p = 0.04$). Nitrogen content differed significantly among hosts ($F_{2,6} = 16.6$, $p < 0.01$) but not between needle origin. Interestingly, *P. nigra* had a lower nitrogen content (0.81 ± 0.01 % dry weight) than *P. mugo* (0.97 ± 0.03 % DW) and *P. sylvestris* (1.2 ± 0.06 % DW). Needle toughness did not differ significantly among hosts ($F_{2,15} = 2.16$, $p = 0.15$), with a mean value of 72.5 ± 8.3 g for *P. mugo*, 93.2 ± 3.1 g for *P. nigra*, and 82.7 ± 8.4 g for *P. sylvestris*.

In the outdoor experiment on potted trees, both larval mortality and RGR were similar among host species ($F_{2,35} = 0.07$, $p = 0.93$ and $F_{2,35} = 2.56$, $p = 0.12$, respectively), with moderately low mean values of percent mortality (22.1 ± 7.3; 21.1 ± 8.6; 25.6 ± 10.8) and low mean values of RGR (0.058 ± 0.002 ; 0.053 ± 0.002; 0.061 ± 0.003 mg mg^{-1} day^{-1}) for *P. mugo, P. nigra,* and *P. sylvestris*, respectively.

In the field experiment, I found no differences in mortality and RGR among host species (Table 3.4), in spite of the fact that the few colonies alive at the end of the winter were only observed on *P. nigra* and *P. mugo*, and never on *P. sylvestris*. The effect of the host species was marginal in a log-likelihood test of percent mortality ($G = 5.52$, d.f. $= 2$, $p = 0.10$). Both the core and the expansion population contained colonies that survived until the end of the experiment; thus, the earlier hatching of the expansion population relative to the core population did not appear to enhance winter survival.

Discussion

Host utilisation is of central interest in range expansions of phytophagous insects, as novel hosts may provide means for colonisation of areas where the original or primary hosts do not occur. I explored the relative roles of female preference and performance, and larval performance to assess recruitment potential of *Thaumetopoea pityocampa* within and beyond its expansion areas in the Italian Alps. Below I discuss the observed discrepancy between female preference and larval performance, evaluate recruitment potential on the

secondary and novel hosts in the light of limited longevity of females, and provide a synthesis of host utilization and future range expansion of *T. pityocampa*.

Host acceptance: preference and performance of females

In the nest survey of the stand where all three pine species grew intermixed, *T. pityocampa* larval nests occurred more frequently on the primary host, *Pinus nigra,* than on the smaller-statured *P. mugo*, while *P. sylvestris* was the least attacked host even though its height was comparable to that of *P. nigra*. To test whether this pattern reflected a preference hierarchy in oviposition, I conducted a controlled experiment with the three hosts of comparable appearance (silhouette) presented to emerging females inside a cage. The basic hierarchy observed in the field was confirmed: the primary host (*P. nigra*) was preferred consistently, the secondary host (*P. sylvestris*) was accepted the least frequently, and the novel host (*P. mugo*) showed intermediate, or even equal, attractiveness relative to the primary host. Interestingly, in a study of *T. pityocampa* in a mixed stand in southern Spain, Hodar *et al.* (2002) found that field oviposition rates, measured as egg mass density, were similar between the indigenous subspecies of *P. nigra* and *P. sylvestris*, and lower on co-occurring *P. pinaster* (but see Calas, 1900). While Hodar *et al.* (2002) did not explicitly test female preference, the discrepancy between their and our field observations is not unexpected, as it could be attributed to region-specific characteristics of hosts and/or oviposition behaviour of the insect. In any case, the results presented here provide additional evidence that the distribution of larval colonies among the three hosts in multi-host stands is less likely to be generated by differential post-oviposition mortality (cf. Benigni & Battisti, 1999), and that female preference alone could drive the patterns.

Multiple-host stands with the primary and alternative hosts do not represent the typical situation in expansion areas of *T. pityocampa*, where the insect is increasingly faced with no-choice situations in single-host stands (pure *P. sylvestris*, or pure *P. mugo*). Under such conditions, the preference hierarchy may break down because of a conflict with female performance (Scheirs *et al.*, 2000; Scheirs & De Bruyn, 2002), since, after emergence, females only have one night to locate a suitable host for laying a single batch of eggs. Time limitation may then effectively make their oviposition behaviour less conservative by increasing acceptance,of normally less preferred hosts (Jaenike, 1978; Courtney, 1982; Mayhew, 1997; Rosenheim, 1999; Diaz-Fleischer & Aluja, 2003; but see

Stamp *et al.*, 2005). *T. pityocampa* females behaving conservatively may risk not ovipositing at all, because (1) the most attractive host may be rare or absent; (2) host-searching dispersal may lead them to areas with only non-hosts; and (3) the probability of pre-oviposition mortality due to predation, weather, or aging will increase over time. This system also lacks other components of female performance that could confound or obscure the effect of time limitation, such as feeding by adults (e.g. Scheirs *et al.*, 2000).

Contrary to expectations, I did not find support for the hypothesis that all hosts would be accepted equally in the absence of alternative choices. Rather, when each of the hosts was offered singly in the controlled no-choice cage experiment, the females showed near equal rates of host acceptance for *P. nigra* and *P. mugo*, but 50% lower oviposition rates on *P. sylvestris* than on either of the other hosts. Therefore, females behaved conservatively, following a similar pattern of host acceptance as in a multi-host setting. On the other hand, such conservative behaviour might be expected from an alternative perspective: because each female lays only a single batch of eggs, bet-hedging through repeated oviposition on a number of hosts is not possible in *T. pityocampa*, and continued search for another host may be a more adaptive strategy (but see Diaz-Fleischer & Aluja, 2003; also see next section). Finally, conclusions can only be made about the relative (not absolute) rates of differential host acceptance; oviposition would likely be higher under natural conditions than in the experimental setting. These results imply that the innate host recognition behaviour can override time limitation trade-offs with female performance, ultimately leading to a failure to reproduce in individuals that died before accepting a less attractive host.

Larval performance versus female host acceptance

The three hosts did not differ in larval growth and mortality: the larvae fared equally well on *P. nigra*, *P. sylvestris*, and *P. mugo*, and the three hosts appeared equally suitable even after considering a full range of larval stages, duration of feeding, and both controlled and field conditions. These results are in partial contrast with the findings of Hodar *et al.* (2002) in southern Spain, in which first-instar larvae performed much better on the local subspecies of *P. sylvestris* than on *P. nigra*, but agree with the observations by Devkota & Schmidt (1990) in Greece. I did not investigate possible differences in needle secondary chemistry (but none were found by Hodar *et al.*, 2002), but I did not find any link between

performance and the observed differences in nitrogen content, often invoked as a key determinant of insect performance (e.g., Underwood, 1994). Oligophagous insects can be expected to possess considerable physiological plasticity in dealing with hosts of different nutritional or chemical composition, and may maintain similar performance on related hosts (Roininen & Tahvanainen, 1989; Bernays & Chapman, 1994; Leyva *et al.*, 2000). Still, the lack of differences in suitability among the three hosts underlines a partial discrepancy between female preference and larval performance. *P. sylvestris* was accepted relatively infrequently for oviposition even though the larvae developed well on this host. Conversely, the offspring that developed on *P. nigra* or *P. mugo* did not derive any added benefit from the preferred choice of the mother.

It is not uncommon to find a mismatch between oviposition preferences and hierarchies of host suitability for larval performance, with examples from a variety of insect systems (Dethier, 1954; Wiklund, 1975; Jaenike, 1990; Mayhew, 1997; Leyva *et al.*, 2000). A substantial body of theory exists to account for these discrepancies, invoking either oviposition mistakes by females (e.g. Larsson & Ekbom, 1995), or additional factors, such as adult performance (e.g. Scheirs *et al.*, 2000), host plant abundance (e.g. Kuussaari *et al.*, 2000), habitat suitability (e.g. Rausher, 1979), sequestration of secondary metabolites (e.g. Björkman *et al.*, 1997), and enemy-free space (e.g. Denno *et al.*, 1990). The relatively high attractiveness of *P. mugo* in our study, especially in the no-choice situation, was somewhat unexpected. A variety of intrinsic factors influence host recognition in Lepidoptera, including needle chemistry (Schopf & Avtzis, 1987) and needle morphology (Renwick & Chew, 1994), and, possibly, females responded to phenotypic similarity between needle traits of the closely related *P. nigra* and the novel host. Equivocality in the patterns of preference and performance on novel hosts is the norm (Jaenike, 1990; Thompson, 1996; Barre *et al.*, 2002). In some cases, performance is lower on a novel host; for instance, *Panolis flammea* does not perform as well on *Pinus contorta* as on the native *P. sylvestris*, in spite of the occurrence of outbreaks on the novel host (Vanbergen *et al.*, 2003). In other cases, novel hosts appear perfectly suitable (e.g. *Papilio* butterflies; Scriber & Ording, 2005). The paradox of *T. pityocampa* females ovipositing infrequently on the otherwise suitable, historical, *P. sylvestris* presents a question to which ultimate rather than proximate answers are difficult to find without further study.

Implications on future range expansion

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The ability of an insect herbivore to complete its development on alternative hosts is one of the prerequisites for continuing range expansion beyond the extent of the distribution of its original hosts (Gutierrez & Thomas, 2000). On the one hand, larvae of *T. pityocampa* achieve comparable performance on all three hosts that occur at the range boundary in the southern Italian Alps; hence, the insect is not limited in its spread by the lack of hosts of sufficient quality. On the other hand, females show conservatism in host acceptance, frequently rejecting a suitable host despite compromising their own performance. Thus, female oviposition behaviour may limit recruitment potential, particularly in stands of pure *P. sylvestris*, compared with *P. nigra* stands. An intriguing scenario in areas dominated by *P. sylvestris* is the potential for future adaptation of populations in the expansion range (e.g., Thomas *et al.*, 2001, Vanbergen *et al.*, 2003), leading to a relaxation of the behavioural conservatism of *T. pityocampa*, particularly if gene flow is limited (e.g. Via, 1986). Although I found only suggestive evidence of this already occurring in the expansion populations used in our study (a significant population type x host interaction; Fig. 3.2), strong directional selection can be expected for increased acceptance of the less attractive host. The situation is likely to be different at sites dominated by *P. mugo*. While the results indicate that this novel host species is accepted as frequently as *P. nigra* in single-host settings, recruitment of *T. pityocampa* on *P. mugo* is likely to be limited by another, habitat-related, factor. Specifically, the smaller size and prostrate architecture of this predominantly subalpine shrub creates a risk of colony mortality when nests become buried under a heavy snowpack for extended periods of time (A.B., pers. obs.). Similarly, habitat suitability is the most important factor affecting host choice and survival in the related species, the acacia processionary moth *Ochrogaster lunifer* (Floater & Zalucki, 2000). Finally, both *P. sylvestris* and *P. mugo* occur extensively in areas beyond the current altitudinal and latitudinal range boundary of *T. pityocampa*, beyond which climatic conditions become suboptimal or even prohibitive for larval survival, mostly due to thermal requirements for larval feeding (Battisti *et al.*, 2005). The future expansion rates will therefore depend on the combination of host use patterns and climatic suitability of the habitat.

Table 3.1. Populations of *Thaumetopoea pityocampa* and sites used in the experiments, with an indication of which hosts were native and which were introduced in the sampling area. The numbers of nests, females, and egg batches used for each experiment are given for the nest census, preference experiments, and performance experiments, respectively.

Population	Type	Geogr. coord.	Altitude (m)	Host species: original (introduced)	Preference experiments			Performance experiments		
					Nest census	choice	no-choice	1st instar Petri dish	3rd instar potted tree	field test
Fumane	core	45°32′N - 10°52′E	630	*sylvestris (nigra)*		36		11		
Rivoli Bianchi	core	46°16′N - 13°06′E	240	*nigra*		74		17		60
Rovereto	core	45°50′N - 10°06′E	650	*sylvestris (mugo, nigra)*	74					
Cimolais	expansion	46°22′N - 12°21′E	760	*mugo, nigra, sylvestris*		174			20	
Friuli extreme*	expansion	46°22′N - 13°18′E	860-1200	*nigra*			407		20	
Moggio	expansion	46°28′N - 13°12′E	760	*mugo, nigra*		141	248	23		
Venosta N	expansion	46°37′N - 10°46′E	1090	*sylvestris*		132		20		
Venosta S	expansion	46°38′N - 10°46′E	1210	*sylvestris (nigra)*	44	68		12		60

* Friuli extreme nests were collected at the expansion edge in the south-eastern Alps within a radius of about 10 km from the location specified by the coordinates.

Table 3.2. Mean height and needle traits (± SE) of the trees used in the experiments. Different letters imply significant differences in pair-wise comparison of means (Tukey's test, $p < 0.05$).

Host	Tree height (cm)	Needle length (cm)	Needle perimeter (mm)	Leaf area (cm^2)
Pinus mugo	108 ± 10 b	3.9 ± 0.4 a	3.1 ± 0.2 b	10 179 ± 811 a
Pinus nigra	132 ± 17 a	6.6 ± 0.9 c	3.2 ± 0.2 b	9 010 ± 1148 a
Pinus sylvestris	132 ± 23 a	3.9 ± 0.6 a	3.5 ± 0.5 c	9 381 ± 890 a

Table 3.3. Percentage of female *Thaumetopoea pityocampa* that laid eggs in the choice and no-choice experiments (pooled), and mean number of eggs per egg batch (± SE) for the populations used in the study.

Population	Type	% ovipositing females	eggs / batch
Fumane	core	30.6	200 ± 8
Rivoli Bianchi	core	18.9	181 ± 11
Cimolais	expansion	19.0	189 ± 12
Friuli extreme	expansion	19.4	175 ± 13
Moggio	expansion	25.2	180 ± 12
Venosta N	expansion	18.2	202 ± 13
Venosta S	expansion	52.9	182 ± 10

Table 3.4. Summary of results from the field experiment on larval performance of *Thaumetopoea pityocampa*, carried out at Cimolais in 2004 - 2005, with the initial number of 20 egg batches for each host species of *Pinus*.

Population type	Host	Number of colonies alive in		Condition of colonies in November				
		November	May [1]	Total no. larvae alive ± SE	% larvae in 3rd instar	% larvae in 4th instar	3rd instar RGR [2]	4th instar RGR [2]
core	*P. mugo*	18	0	80.6 ± 18.5	51	49	0.058 ± 0.003	0.071 ± 0.001
	P. nigra	14	2 (43, 1)	96.1 ± 19.9	100	0	0.060 ± 0.003	-
	P. sylvestris	16	0	79.6 ± 7.8	89	11	0.061 ± 0.001	0.067 ± 0
expansion	*P. mugo*	12	1 (47)	74.4 ± 11.0	57	43	0.046 ± 0.001	0.052 ± 0.003
	P. nigra	13	1 (4)	69.1 ± 10.0	12	88	0.045 ± 0	0.054 ± 0.001
	P. sylvestris	10	0	58.1 ± 17.2	4	96	0.047 ± 0	0.055 ± 0.002

[1] Number of larvae found in each of the remaining colonies included in parentheses
[2] Mean ± SE of relative growth rate (mg mg[1] day[1])
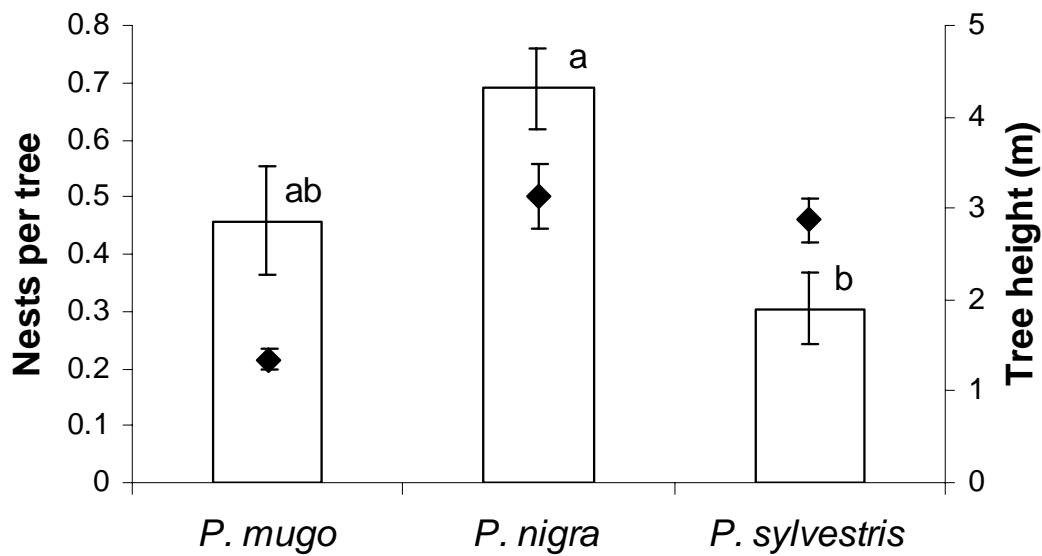
Fig. 3.1. Mean number (± SE) of winter nests of *Thaumetopoea pityocampa* per tree (columns) and mean tree height (± SE) (black diamonds) on three different *Pinus* species in a mixed stand at Rovereto, Italian Alps (n = 35, 58, 55, respectively). Different letters indicate significant differences in pair-wise comparisons of means of nests per tree (Kruskal-Wallis rank ANOVA, $p < 0.05$).
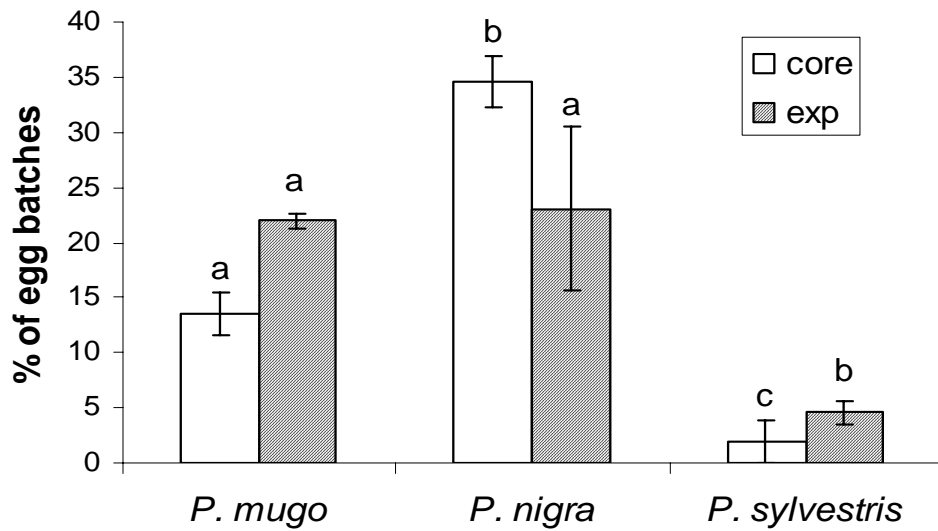
Fig. 3.2. Rate of host acceptance in the choice experiment: mean percentage (± SE) of egg batches laid on each host out of the total number of egg batches laid by females from two core populations (Fumane, Rivoli Bianchi) and four expansion populations (Cimolais, Moggio, Venosta N, Venosta S) of *Thaumetopoea pityocampa*. The total numbers of egg batches laid were 26 and 141, respectively. Different letters indicate significant differences in pair-wise comparisons of means within population types (χ^2 test, d.f. = 1, $p < 0.05$).
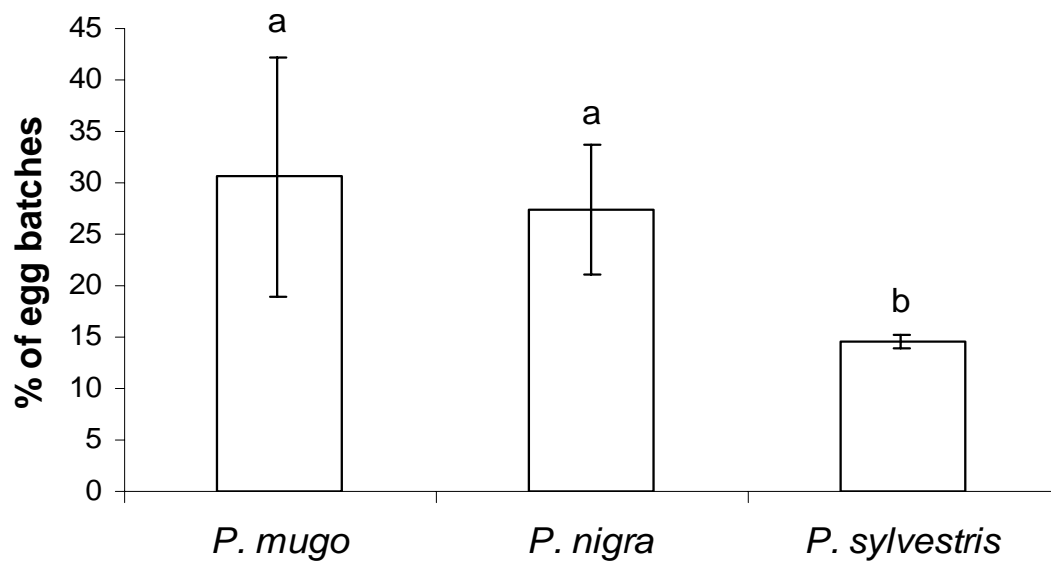
Fig. 3.3. Rate of host acceptance in the no-choice experiment: mean percentage (± SE) of egg batches laid on each host out of the total number of egg batches laid by females from two expansion populations (Moggio, Friuli) of the *Thaumetopoea pityocampa*. The total numbers of egg batches laid were 54 and 55, respectively. Different letters indicate significant differences in pair-wise comparisons of means within population types (χ^2 test, d.f. = 1, $p < 0.05$).

Plate 2a

Pinus nigra, primary host
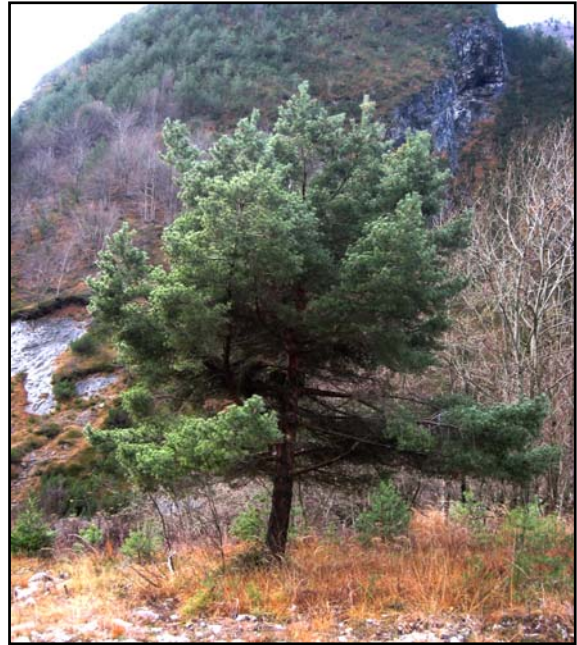
Pinus sylvestris, secondary host

Pinus mugo, novel host

Tunnel-shaped cages

Cimolais, site of field experiment on larval performance

Example of core area on *P. nigra* (below), and expansion area on *P. mugo* and *P. sylvestris* (right)

Chapter 4

Performance of *Thaumetopoea pityocampa* under elevated CO$_2$ and snow cover in the Alps

Introduction

Numerous studies have shown that it is possible to detect the effects of a changing climate on ecological systems (McCarty 2001; Parmesan 2006), taking into account all components of food webs (Samways 2007). However, the studies of climate change effects on ecosystems is complex because of many potentially interacting factors, e.g. species, genotype.

A principal driver of climate change is the increasing carbon dioxide concentration. It has increased by 31% since the pre-industrial time, from 280 ppm to more than 370 ppm (Karl & Trenberth 2003) and is expected to increase in the next 100 years to 500-900 ppm, depending on models of projection (Solomon *et al.* 2007). This change have a significant impact on herbivorous insect; the CO_2 enrichment, combined with greenhouse gases increasing, is the main cause of global warming, causing ecological changes in the distribution (Yukawa *et al.* 2007; Hickling *et al.* 2006) and in the phenology of insects (Gordo & Sanz 2005, Roy & Sparks 2000). These conditions may further the colonization of new potential hosts (Harrington *et al.* 2001; Agosta 2006) but few studies have explored that aspect. Moreover, the CO_2 increase may have indirect effect on the herbivorous insect due to changes occurring to their host plants; the main factors being the variation in food indicated by tissue C/N ratio, water content, leaf toughness and carbon-based defence compounds (Schädler *et al.* 2007). However, the responses of different plant species show enormous interspecific variation, as do herbivores feeding on them.

Recent studies have shown that herbivorous insects grown in elevated CO_2 may respond with a lower development and higher mortality (Coviella *et al.* 2002; Knepp *et al.* 2007); other studies showed no variation of performance but a higher fecundity in female and increasing of lipid concentration in males (Goverde *et al.* 2002), and again no effect on growth rate, larval instar duration and pupal weight (Karowe 2007). Others studies highlighted the effects of increased CO_2 even on insect population level (Stiling *et al.* 2003). In addition, responses of different host species to increased CO_2 may differentially affect the performance of oligophagous/polyphagous herbivores (Agrell *et al.* 2006; Schädler *et al.* 2007). A possible effect is the change of food preference with potential consequences for population dynamics of the plants (Goverde & Erhardt 2003; Agrell *et al.* 2005; Agrell *et al.* 2006) although there are studies where no preference shifts were

observed (Dìaz *et al.* 1998; Lederberger *et al.* 1998). The first experiments of CO_2 enrichment were carried out under controlled conditions on detached plant material or directly on plants grown in greenhouse, and the artificiality of these experiments may out processes of ecological relevance and lead to detection of effects not occurring in the field (Hunter 2001; Goverde *et al.* 2002; Knepp *et al.* 2005, Knepp *et al.* 2007). However, data are now available from FACE facilities set up in the field, even in the tree canopy (e.g. Hatteschwiler & Schafellner 2004) as well as for sites located at the alpine treeline (Asshoff & Hättenschwiler 2005).

The pine processionary moth (*Thaumetopoea pityocampa* (Denis & Schiffermüller, 1776)) is an insect pest of pines that is expanding its range as a consequence of climate change (Battisti *et al.* 2005), including novel hosts that may become readily exploited, as it was observed for the mountain pine in the Alps (Chapter 2). The mountain pine group includes two major forms known as upright (*Pinus uncinata* Ramond) and dwarf or creeping (*Pinus mugo* Turra), differing mainly in their growth habit (Vidakovic 1991). As the stands of mountain pine are extensively distributed beyond the historical range of *T. pityocampa*, it is likely that new problems will arise if the current trend of expansion will be maintained. However, the expansion could be limited by the accumulation of snow on the branches during the winter, when the larvae need to leave the nest for feeding (Buffo *et al.* 2007).

The main objective of the study is two-fold, first to assess survival and performance of young larvae of processionary moth in high-elevation FACE facility on mountain pine (*P. uncinata*) trees during the summer; the site location, about 700 m above the present upper edge, permits to test the larval development at extreme temperature conditions. So I had the possibility to investigate if the change in food quality, due to the increase in carbon dioxide concentration, could be a critical factor for insect survival in an environment where the larvae already cope with extremely low temperature. On the other hand, low temperature may be more limiting than low needle quality, that is tolerated by larvae. The second aim is to evaluate the insect winter survival on mountain pine (*P. mugo*) and *P. nigra* in stands near to the border of the range, where the expansion is occurring, with special attention to the role of the snow-pack. The creeping habit of mountain pine furthers the cover of the nests for extended periods of time (Table 3), and this could create a risk of

colony mortality. I have analysed the snow cover data of a possible expansion area, to know if the global trend of a decrease of snow cover is confirmed, because this factor could reduce the risk of winter mortality for the pine processionary moth.

Materials and methods

FACE experiment

The experiment was carried out at Stillberg (Davos) (Plate 3) in the Swiss Central Alps. The site is located on a slope oriented towards North-East. The long-term annual precipitation is 1050 mm, the average temperature is - 5.8°C in January and 9.4°C in July (Schönenberger & Frey 1988). The soil is classified as a Ranker (U.S. system: Lythic Haplumbrept) with a 10-cm-deep organic soil underlain by siliceous bedrock (Paregneis, Schönenberger & Frey 1988). The experimental site consists of a 5 ha afforestation area planted in 1975 with three tree species (*Larix decidua* L., *Pinus cembra* L., *Pinus uncinata* Ramond). In 2001, an area of approximately 2500 m^2 situated at 2180 m of elevation was selected to provide an experimental setup for a CO_2–enrichment study at tree line (Hättenschwiller *et al.* 2002; Handa *et al.* 2005). At the time of the experiment, there were 9 trees of mountain pine that were grown for 5 years under elevated CO_2 atmosphere (556 ppm) from June to September and 10 trees grown under ambient CO_2 (357 ppm) (see Hättenschwiller *et al.* 2002 and Handa *et al.* 2005 for details about CO_2-enrichment system and experimental set-up). The leaf chemistry of needles of the enrichment experiment was shown in previous studies (Hättenschwiller *et al.* 2002; Handa *et al.* 2005).

I used sets of insects for the experiment consisting of artificially created groups of 50 first instar larvae. At the end of July 2005, 30 egg batches were collected from several trees in Venosta/Vinschgau valley, Italian Alps and transferred to laboratory. After hatching, groups of 50 larvae were formed by picking individuals from different colonies. The larvae were provided with needles of mountain pine in Petri dishes. The food was renewed every 2-3 days and the dead larvae replaced with new ones. On August 8th I transferred and exposed the larvae groups on nine trees under elevated and ten trees under ambient CO_2 conditions, all belonging to the enrichment experiment; in addition, I selected ten native trees of mountain pine (*P. uncinata* form) under ambient condition, growing on the same slope at slightly lower elevation (2030 m). The trees were of a size similar to

those used in the enrichment experiment. On each tree, I selected one lateral branche that were protected with a 0.1 mm mesh net sleeve, about 40 cm long. On every branch, a group of 50 first instar larvae was gently transferred from the Petri dish to the needles inside the sleeve (Plate 3). About one month later (September 9th), the sleeves were opened and their content (faeces, dead larvae, fallen needles) transferred into a vial. Then the larvae were taken from the twig with a brush and put into a Petri dish. Later the amount of faecal pellets was measured by volume assessing in a micropipette and the larvae were counted and weighed, and the larval instar was assessed. After freezing at $-20°C$, the dry weight was obtained after 24 h of exposure at $60°C$. The relative growth rate (RGR mg^{-1} $mg^{-1}day$) was calculated for the groups of 50 larvae using both dry and fresh weight and using the initial weight from three groups of 15 larvae. At each site, temperature was recorded hourly by data loggers (Hobo, Onset Computer Corporation, Pocasset, MA). At the end of the experiment I took a sample of three needle pairs for each tree to measure needle toughness, by determining the force needed to penetrate the needles using a calibrated penetrometer constructed for this purpose. Each pine needle was fixed between two metal plates, and the probe (Bohemia insect pin ®, diameter 0.55 mm) of the penetrometer was inserted through the central part of the upper, rounded surface. Three readings were taken per needle, avoiding the distal parts.

Transplant experiment

The experiment was carried out in the two sites in South-Eastern Alps. The first (Resia) is located in area of recent colonization by the side of insect; it is placed on north slope and situated at altitude around 500 m. There is present a natural stand where the mountain pine (*P. mugo* form) is the main species associated with *P. nigra.* The second site (Tanamea) is situated at 850 m on northwest slope, in this place the main species present is mountain pine (*P. mugo* form) with isolated plants of *P. nigra.*

At the end of July 2002, 75 egg batches were collected in pine stands close to the experimental area. Before hatching, each egg batch was tied to the branch of a separated tree, and the larval development was checked every two weeks, noting the larval development stage (based on the size of faeces visible though the silk), nest size and recent feeding. Close to pupation the nests were collected and placed individually in a pot. The pot

was closed with polythene net to avoid the larvae escape. The larvae were regularly fed, on the bottom of the pot I positioned sand to consent the pupation of the larvae. During the adult emergence period the pots were checked every four days and the all present adults were collected and subsequently the sand winnowed to find diapausing cocoons, indicating the completion of life-cycle of an insect.

As snow resulted to be an important limiting factor to insect survival, I analysed daily data of the snow in addition to temperature in the period December - April. Four sites close to the experimental area were chosen in the elevation range of 540-900 m; I selected the medium height of snow and the number of days with snow cover higher than 20 cm as variables, which were analysed for the period 1972-2007.

Statistical analyses

The larval performance on three different trees (% survival and RGR) were analysed with a one-way ANOVA, likewise the data of needles toughness were tested. In all cases in which ANOVA was employed, the basic assumption of homogeneity of variance was met, and variables were tested without any transformation. Tukey's test was used for pairwise comparison of means. The larvae distribution in different stages was analysed by a χ^2 test in three treatments, comparing observed frequencies with those expected calculated on the base of medium value of three treatment. The number of faecal pellet was analysed by ANCOVA using the number of larvae as a covariate. The temperatures in different sites were analysed by a Student's t-test. The survival in the transplant experiment was analysed by a χ^2 test, comparing observed frequencies at different sites and host plant species with those expected calculated on the base of medium values.

Results

CO_2 experiment

The groups of 50 larvae did not show any difference in mortality between ambient and elevated CO_2 whereas those exposed on native trees under ambient conditions showed a higher mortality ($F_{2,25}$ = 4.895, P < 0.01) (Fig. 4.1). The larval stage was more advanced on native trees under ambient CO_2 (χ^2 = 10.69, d.f. = 4, p = 0.03) (Fig. 4.2). The RGR did not differ among treatments using either dry ($F_{2,25}$ = 0.678, P = 0.317) (mean = 0.036, SE =

0.0044) or fresh weight ($F_{2,25} = 0.706$, P = 0.502) (mean = 0.038, SE = 0.0045). During the experiment, the mean minimum daily temperature was 4.9°C, with several nights with temperature below or around 0°C, and the maximum daily temperature was 19°C at FACE site (2180 m), whereas at lower site (2030 m) the values were 0.61 °C and 2.63 °C higher, respectively (t-test, minimum temperature: t =5.27, d.f. = 62, p < 0.01; maximum temperature: t = 7.00, d.f. = 62, p < 0.01). The volume of faecal pellets did not differ among treatments ($F_{2,24} = 1.316$, P = 0.286). The needles of native trees under ambient CO_2 were significantly tougher than those of trees grown at higher elevation under both elevated and ambient CO_2 ($F_{2,25} = 24.41$, P < 0.01) (Fig. 4.3).

Transplant experiment

There was no difference in the colony survival on *P. mugo* and *P. nigra* located in Resia insect larvae before and after the cold period ($\chi^2 = 3.64$, d.f.=1, p=0.056; $\chi^2 = 1.02$, d.f.=1, p=0.311). In the comparison between two different sites on *P. mugo*, the survival was similar before the cold period ($\chi^2 = 1.763$, d.f.=1, p=0.184) but differed dramatically after ($\chi^2 = 6.373$, d.f.=1, p=0.0115), as total mortality was observed in Tanamea, where colonies were buried in snow for more than one month (Table 1). The medium height of snow cover and the number of days with snow cover higher than 20 cm showed a decreasing trend in the period 1972-2006 (Fig 4.4), although there was strong variability among years.

Discussion

The elevated CO_2 had no effect on the herbivorous insect, *Thaumetopoea pityocampa*, although there was significant variation at the plant level, consisting in an increase of non-structural carbohydrates and a reduction of surface leaf area (Handa *et al.* 2005). Both survival and performance of larvae did not differ between individuals fed on plants of mountain pine exposed to ambient and elevates CO_2. However, the larvae exposed on native mountain pines growing at slightly lower altitude showed higher mortality than those on plants at the upper site, but a significant difference was observed only when compared to elevated CO_2 trees. As expected the larval stage reached by the larvae at lower site was more advanced compared to the upper site due to temperature-dependent growth.

In spite of the generally low temperature for the development of first instar larvae, about 10°C lower than that observed at cold sites at the edge of the range (unpublished data) I observed a considerable survival also on a novel host, as *P. mugo,* that opens the question about the possibility of a successful development of the insect at range's edge. Final survival of transplanted colonies at two extreme sites on mountain pine showed that snow cover is preventing larval activity and establishment, whereas low temperature is not the major constraint factor. However, as snow cover is decreasing in the last decades, I may expected that range expansion will further occur in *T. pityocampa*.

Mountain pine responded to CO_2 enrichment with an increase of non-structural carbohydrates (Hättenschwiler *et al.* 2002), as it has been pointed for other conifers (Griffin *et al.* 1996; Runion *et al.* 1999). The increase was due to variation in starch fraction, whereas the concentration of soluble sugars was unchanged (Handa *et al.* 2005). The increase of starch may positively affect herbivore insects, improving the digestion efficiency and increase fat reserves (Goverde 1999; Asshoff & Hättenschwiler 2005). The accumulation of NSC in needles, however, may involve a lower N concentration per unit mass and a increase of C/N ratio; these are indicators of lower food quality for herbivorous insects (Mattson 1980). Moreover, the reduction of specific leaf area may cause needles to be heavier and tougher (Handa *et al.* 2005), although difference in needles toughness did not result significant in our study.

The studies carried out under similar situations have shown contrasting responses on larval performance. Several highlighted a negative response (Hättenschwiller & Schafellner 1999; Chen *et al.* 2005) whereas others showed no variations (Goverde *et al.* 2002; Willams *et al.* 2003) or a a positive effect (Goverde *et al.*1999). The response of the processionary could be due to opposite effect of two factors, as the dilution in N content may buffer the benefits of increased amounts of starch and sugars that may be phagostimulatory to herbivores (Stiling & Cornelissen 2007). Moreover, the same quantity of faeces produced in the two treatments does not support the hypothesis of compensatory feeding, that has been associated with increase of C/N ratio and lower tissue quality (Williams *et al.* 1994), although some recent studies have suggested that a differential post-ingestive adsorption may compensate for diminished food quality (Barbehenn 2004). In the study of Stastny *et al.* (2006) the performance of the processionary moth larvae did not

differ between three host-plant species with different nitrogen content, showing a high plasticity. This may display such a post-ingestive compensatory behaviour. Leaf toughness, however, can be a serious constrain to larval development especially in early instars (Tanton 1962; Zovi *et al.* in press). It is common that plant phenotype may show different resistance to herbivore attack (Preszler & Price 1988). In our experiment there were two phenotypes of mountain pine (native vs. introduced) which differed in needles toughness, a physical hurdle to larval feeding, that may explain the higher larval mortality on native mountain pine grown at lower altitude.

Although leaf toughness may be an important determinant of larval survival on some hosts, it rarely happens that may cause total mortality as the insect shows a high adaptive potential to tough needles (Zovi *et al.* in press). Extreme temperature and snow cover appear to be the most important abiotic factors for assessing the impact of climate change on alpine ecosystem (Inouye *et al.* 2000; Theurillat & Guisan 2001). The snow cover of the nest was a major constraint factor for survival during winter in our transplant experiment. The creeping habit of mountain pine, a species most widespread at the insect range edge, makes it possible that colonies become buried by snow when the snow is as high as 20 cm (unpublished data). The mean snow depth, the duration of continuous snow cover and the number of snowfall days in the Swiss Alps all have showed very similar trends in the period 1931-99: a gradual increase until the early 1980s followed by a statistically significant decrease towards the end of the century, mainly at low and mid altitudes (Beniston 1997; Lateneser & Schnebelli 2003). Our data from north-eastearn Alps showed a similar trend to the Swiss Alps. There is no universal agreement, however, on snow cover in the past century as Brown (2000) did not detect evidence of a significant long-term decrease of spring snow in the northern hemisphere.

There are few studies that consider the effect of the change in snow cover on ecosystems. In a study about growth of Norway spruce saplings the decrease in snow cover showed two opposite effects, a positive one due to decrease of attack by the black snow mold (*Herpotrichia nigra*) and to longer growing season, and a negative response of saplings to exposure to low temperatures (Cunningham *et al.* 2006). Vanbergen *et al.* (2003) suggested that the reduction of snow lie in the Scottish moorland, conceivable with climate change, had increased the probability of winter moth (*Operophthera brumata*)

outbreaks on *Calluna vulgaris*, through improvement of adult emergence and higher breeding success. Finally, *Atalopedes campestris* is a lepidopteran that cannot survive for a longer period under snow as larvae, and its future expansion could be favoured by the decrease of snow (Crozier 2004). If snow cover reduction may relax the conifer hosts from the attack of snow-associated pathogens like the black snow mold, it may contribute to worse the attack of organisms that are normally limited by snow, such as the winter moth and the pine processionary moth. In the latter case, it can be easily predicted that the expansion on mountain pine will continue as long as temperature increase will be associated with a decrease of snow cover and the insect will not be limited by higher CO_2. Interestingly, the temperature and the land-use change have been considered as predictors of range expansion of mountain pine in the alpine region (Dirnböck *et al.* 2003), likely leading to an altitudinal shift of plant and insect communities in mountain habitats.

Table 1. Survival of colonies transplanted on different host pines at range's edge in Friuli district, north-eastern Italy.

Site	Host	No. colonies	% survival November	% survival February
recent expansion	*P. nigra*	27	40.7	14.8
recent expansion	*P. mugo*	27	66.7	25.9
outside the range	*P. mugo*	21	46.6	0
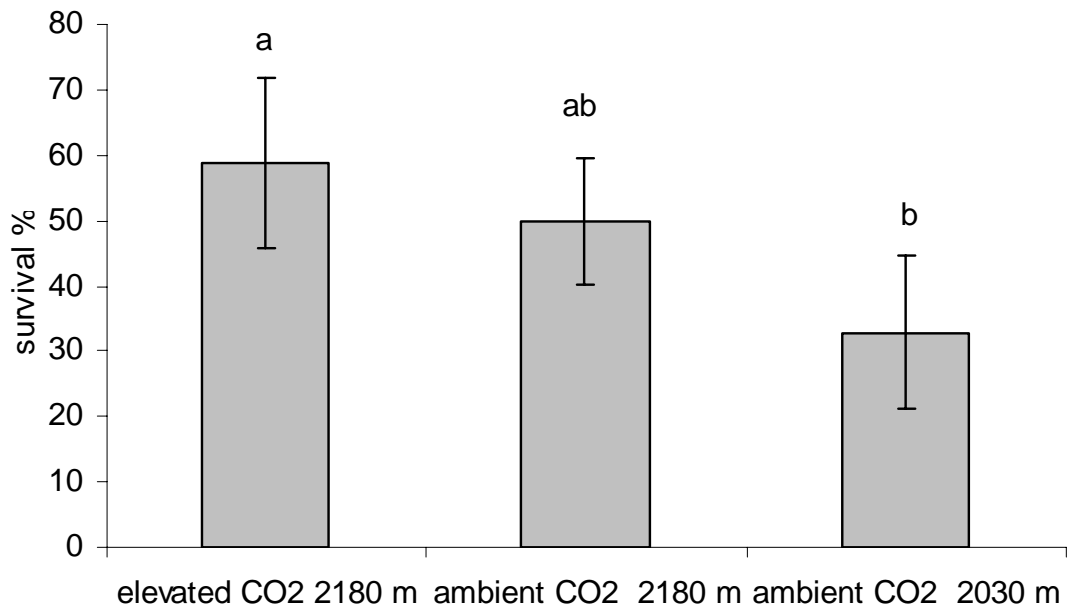
Fig. 4.1. Mean percentage (+/- SE) of survival of first instar larvae of Thaumetopoea pityocampa transplanted at different sites and CO_2 regimes at Stillberg, Switzerland, during the summer 2005. different letters indicated significant differences in pairwise comparison of means % survival (Tukey test, $p < 0.01$).
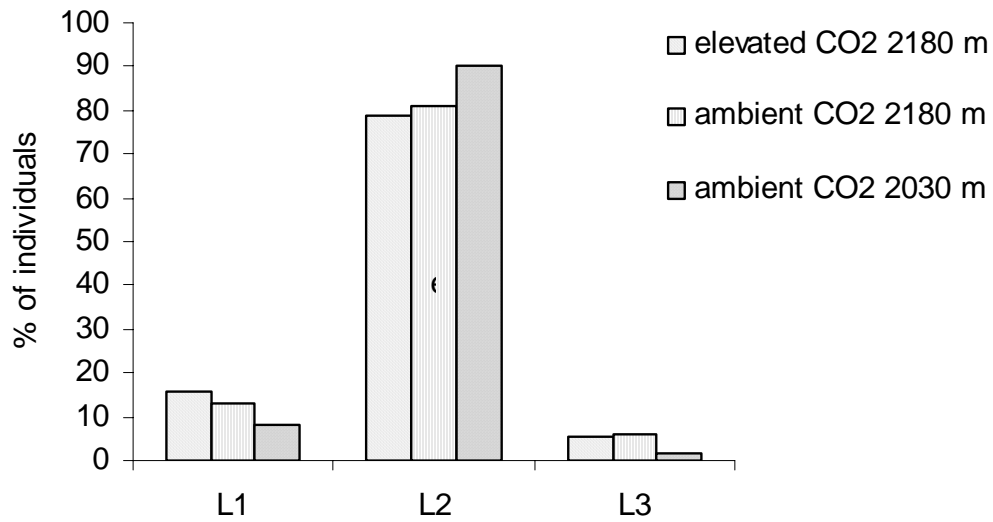
Fig. 4.2. Larval instar reached by the larvae at the end of the CO_2 experiment on different host plant types.
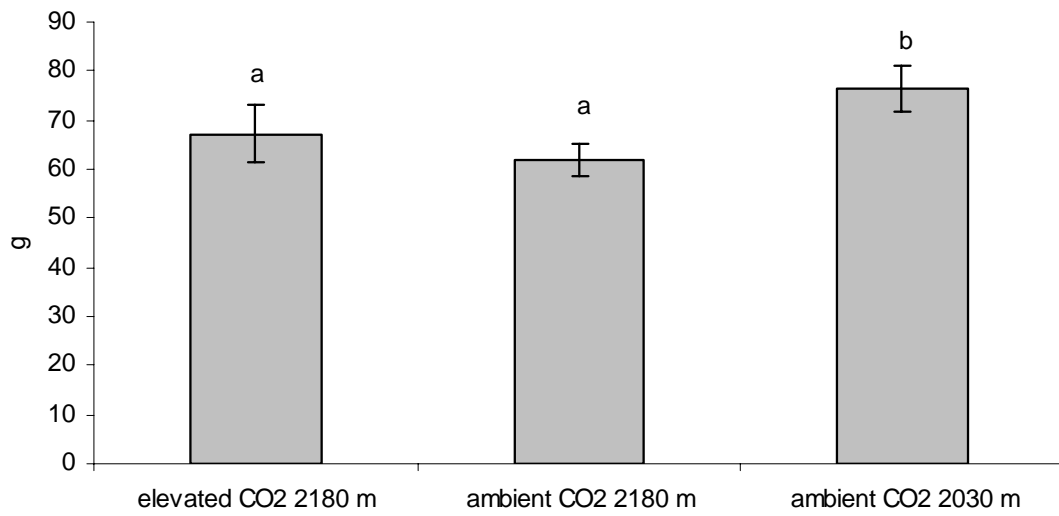
Fig. 4.3. Needle toughness of different host plant types measured at the sites of the CO_2 experiment. Error bars show one SE. Different letters indicate significant differences in pairwise comparisons of means (Tukey test, $p < 0.01$).
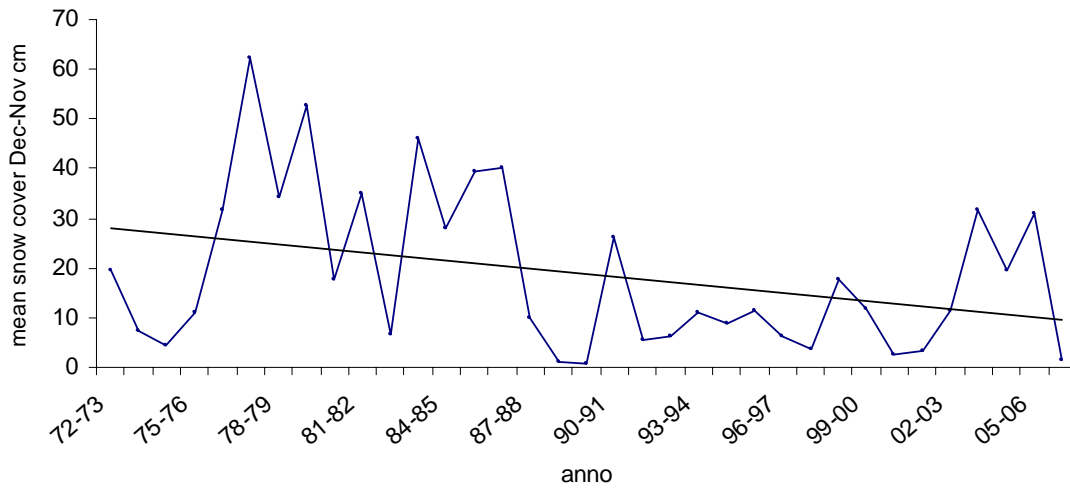
Fig. 4.4. Mean snow cover during winter period (December-April) from 1972-73 to 2006-07 at four weather stations between 540 and 900 m of elevation in the Alps of Friuli district, NE Italy. The line indicates the regression of mean snow cover over time (y = - 0.53x + 28.40, R^2 = 0.1145).

Plate 3

Site of FACE experiment at Stillberg (Davos)
(photo R. Asshoff)

A particular of the sleeve enclosing
the larval colony (photo R. Asshoff)

P. mugo under snow

Different habit of hosts: creeping *P. mugo* on the left and
upright *P. nigra* on the right

Conclusion

The pine processionary moth has shown a positive answer to temperature increase. In addition it has been shown to respond also to extreme events, as proved by the expansion occurred in two alpine valleys of north-eastern Italy after the hot 2003 summer (Annex 1).

The observations are in agreement with what is known from literature (Battisti *et al.* 2006) and add to the general response of the insect to the warming trend over the past decades (Battisti *et al.,* 2005). The response mechanism is based on a model based on larval feeding requirements, elaborated on populations of processionary moth in the Alps and in France (Battisti *et al.,* 2005; Robinet *et al.,* 2007). If the expected temperature increase and expansion rate of the insect are confirmed, the shift will be of 2.7 km per year poleward and a 7.0 m per year (southern slope) or 2.9 m per year (northern slope) upward.

Availability of suitable host plants is the first condition to allow the expected expansion. Latitudinally, for example in France, the wide use of *Pinus* spp. in forestry and as ornamental trees assures the possibility to colonize new areas, although the forests are very fragmented (Robinet *et al.,* 2007). I have shown that the larval performance on *P. sylvestris* and *P. mugo*, the host-plants more widespread at high latitude/altitude, is comparable with that on the primary host *P. nigra* in the core area. The lower acceptance of *P. sylvestris* by female moths may limit the recruitment potential, particularly in pure stands of this plant. The good female acceptance and larval performance make *P. mugo* a host favourable to expansion.

Even in case of mountain pine trees growing under elevated CO_2 concentration and having thus a lower food quality, the negative consequences known for other insects were not observed because of a high plasticity in larval feeding. Other experiments on mountain pine proved the hypothesis that colony mortality is total when the nests stay for a long period of time under snow cover, as it may be the case on this host because of high branch flexibility. The insect occurrence on *P. mugo*, however, may be favoured by the decreasing trend of snow cover, that is another consequence of climate change.

It has been mentioned that one possible factor limiting the expansion northward could be the insufficient insolation (Battisti *et al.,* 2005), as areas approximately two degrees farther north of the present range can not support winter feeding because the activation threshold of temperature is not achieved. According to the expected to expansion

rate (2.7 km/year), the processionary moth could reach its northern margin of the range in approximately 80 years. The low insolation could limit the potential expansion on the northern slope of mountains, whereas the main factor limiting the expansion on the southern slopes remains the night winter temperature. I have also contributed to a study (Annex 2), where an evolution of the model proposed by Battisti *et al.* (2005) is suggested. This model is based on thermal sum instead of temperature thresholds and helps to understand how feeding can be related also to an accumulation of degree-days even at very low temperature. The solar radiation remains a major factor affecting the biology of the processionary moth but thermal sum may also have an adaptive value, especially under extreme conditions such as higher latitude and northern slopes. Based on this, I can predict that the moth could expand beyond the predicted limits if thermal sums add on the low level of radiation and thus allow the larvae to feed and survive.

The concept of thermal sum has been shown to be useful also when the negative temperatures are considered, because of the risk of cumulative chill injury that is associated with the exposure to temperatures below 0°C. The analysis of cold hardiness in this species suggests an adaptation to cope with fluctuating temperature through winter , that may reach extreme values at upper range boundaries and in new colonization areas. The moderate freezing tolerance strategy assures survival also in case of severe frost, as it would occur in cold winter nights in the expansion area. Here I also showed that expanding populations may survive the putative lower lethal temperature known for the processionary moth. The larvae capability to tolerate extended periods of starvation is a further factor associated with the strong plasticity of the processionary moth.

Further studies should focus on the plasticity of life history traits, and related environmental factors, as this seems to be the key to understand the great potential of the pine processionary moth to exploit any host plant that may become accessible via new plantations or climate change.

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Acknowledgments

Annex 1

Climate change and expansion of the pine processionary moth, *Thaumetopoea pityocampa*, in northern Italy

I contributed to all experimental parts and to the paper writing.

Abstract

We studied the occurrence of *Thaumetopoea pityocampa* in two alpine valleys (Friuli Venezia Giulia district, north-eastern Italy), in order to know the response of the pine processionary moth to the hot summer of 2003. Extreme nests were located in the two valleys during three life-cycles. Moreover we studied the relationships between catch in pheromone traps and infestation level at the same sites. The numerous nights with high temperature in 2003 have facilitated the flight of the females and the colonization of new areas. This expansion has confirmed the successful colonization of the novel host *P. mugo*, previously recorded as occasional host of the pine processionary moth.

Key words: *Thaumetopoea pityocampa, Pinus sp,,*range, temperature, monitoring

1. Introduction

Global warming is predicted to cause distributional changes in organisms whose geographic ranges are controlled by temperature (Ungerer et al. 1999). A recent latitudinal and altitudinal expansion of the pine processionary moth, *Thaumetopoea pityocampa* (Denis et Schiffermüller) (Lep. Notodontidae) has been reported (Battisti et al. 2005). In north-central France (Paris Basin), its range boundary has shifted by 87 km northwards between 1972 and 2004; in northern Italy (Alps), an altitudinal shift of 110-230 m upwards occurred between 1975 and 2004. By experimentally linking winter temperature, feeding activity, and survival of *T. pityocampa* larvae, these authors attributed the expansions to increased winter survival due to a warming trend over the past three decades. Given that the present distribution of the oligophagous *T. pityocampa* is not constrained by the distribution of its present or potential hosts (Stastny et al. 2006), and that warmer winters will cause the number of hours of feeding to increase and the probability of the lower lethal temperature to decrease, a further latitudinal and altitudinal expansion is expected.

Climatic anomalies may produce, or accelerate, geographic range expansions of species limited by temperature or other climatic variables (Carroll et al. 2003). Battisti et al. (2006) reported a recent rapid shift of the range limit of *T. pityocampa* during the record hot summer of 2003 in southern Europe, that has the potential to be both permanent, and to have important implications on species range dynamics in general. The colonization of extreme, high-elevation sites was attributed to increased nocturnal dispersal of females during the unusually warm night temperatures in June–August 2003. Importantly, the colonies established at extreme sites survived the winter and produced offspring in 2004, although the range did not expand further due to low night temperatures that year.

Pheromone trapping has been considered for a long time as a mean to monitor populations of *T. pityocampa* and to explore gene flow at extreme sites (Salvato et al. 2005). The pheromone trapping method relies on a positive relationship between trap capture and local population density (Thorpe et al. 1993). Unfortunately, most of the field studies so far have failed to find a correlation between the number of males caught in pheromone traps and local infestation levels, traditionally expressed as the number of nests per tree. Jactel et al. (2006), however, detected a positive and significant correlation between the number of males and the level of infestation in the following generation. These findings suggest that pheromone-baited traps, operated according the framework proposed by Jactel et al. (2006), provide a suitable tool for monitoring of *T. pityocampa* populations.

In this paper, we describe the situation of a two alpine valleys in the expansion area

of *T. pityocampa* in north-eastern Italy, by comparing surveys carried out in the past with the present distribution. We used nest censuses and pheromone trap catches as indicators of population density.

2. Materials and methods

2.1 Study area and organism

The study area is located in the Friuli Venezia Giulia region, north-eastern Italy, and it includes the two alpine valleys Cimoliana and Settimana, in an altitudinal range between 600 and 1100 m (Fig. 1 and 2). Both natural pine forest and pine plantations are widespread in this area. In the lower part of the valleys the main species is *Pinus nigra*, occurring mainly on south-eastern slopes; this species is progressively replaced by *Pinus sylvestris* in the upper, colder parts of valleys. The creeping pine *Pinus mugo* is commonly associated with the other two pine species, and it forms pure stands beyond the tree line. Most of pine forests have high tree density and low height and diameter, because of low soil fertility and steep slope, making landslide frequent.

Larvae of the winter pine processionary moth *Thaumetopoea pityocampa* feed gregariously on pine foliage during the winter. Pupation occurs in spring, and adult emergence in summer is immediately followed by mating and oviposition. Females typically live only one night. The insect is distributed throughout the Mediterranean, and has long been known to produce spectacular outbreaks (Matthioli, 1568). Forest inventories generally report a scattered distribution of outbreaks, with usually no more than two years of strong defoliation in a row, after which most of the trees recover (Masutti & Battisti, 1990).

2.2 Insect survey

There are records of processionary moth occurrence in the study area in the last 20 years, made available by the Forest Service of Friuli Venezia Giulia district, indicating the presence of medium and intense defoliation events in the study area. In an attempt to describe the upper limit of moth's range, we surveyed the localization of the extreme colony tents ('nests') during 2002-2004. The winter nests were used to outline the boundary range as they are easy visible. Furthermore, a sample of these was collected and inspected in the laboratory to verify the survival of the larvae. For each of the two studied cycles (2002-03 and 2003-04), the area was divided in homogenous zones as far as nest density, indicated as the number of nests on forty pine trees. Density was compared between years by Student t-test.

We had access to data from a pheromone trap monitoring network working in the area in 1987-1991 and 1993. There were 5 traps in Cimoliana valley and 4 in Settimana valley. In the summer of 2003 a set of similar type of funnel traps (Supergreen), loaded with 0.2 mg of pheromone, was located in the same places. Two additional traps were used in each valley, located at the site where the extreme nest was pointed out in the winter and 300-400 m upstream. The traps were checked every week from July to September.

3. Results

3.1 Outbreak history

During the last 20 years, three attacks of pine processionary moth were recorded, precisely in 1988-89, 1992-93, and 1998-99. In the Cimoliana valley, the damaged area (11.2 ha with a defoliation higher than 25%) was located on the south-eastern slope of the lower part of the valley, mainly on *P. nigra* (Fig. 1). In the Settimana valley, the outbreak occurred in an area of 14 ha of pure plantation of *P. nigra*, at the beginning of the valley on the southern slope (Fig. 2). A second outbreak occurred only in the Cimoliana valley in 1998-99, on an area of 27.6 ha located in the same area of previous years, with a defoliation of 45% (Fig. 1). The catch of males in pheromone traps was not correlated with the outbreak of 1988-89 (Fig. 3). The captures were elevated in the first year of sampling, decreasing in the subsequent years around 20 males/trap, and increasing in 2003, when the mean catch was captured about 100 males.

The weekly catches offered the possibility to assess the seasonal phenology. Male emergence started at the end of June and peaked on 19 of July (Fig. 4). There was a second and smaller peak before mid August, and the catch was over at the beginning of September. The catch pattern was similar in all years of monitoring. Traps located at the site where the extreme nest was found in the previous winter, captured 14 males in Cimoliana and 9 males in Settimana valley. Traps located 300-400 m upstream the extreme nest, captured three males only in Cimoliana valley.

3.2 Range expansion

The nest density did not show any variation between the two years in both valleys. In the Cimoliana valley, there were 11.36 nests (SD=5.23, n=11) on forty pine trees in 2002-03 and 11.54 (SD=5.75; n = 11) in 2003-04. In the Settimana valley the mean density was higher than in the Cimoliana valley in particular 21.14 nests on forty plants (SD =9.02, n=7) in 2002-03, and 26.42 (SD=16.26, n=7) in 2003-04 . No significant differences were observed between the two years in both valleys. The zones showed variable density, the most attacked having more than one nest per tree, but defoliation was limited, excepted in few spots.

The range boundaries varied during the sampling period. In the 2002-03 several nests were recorded on the south-eastern slope of both valleys from the river bed, mainly on *P. mugo*, to the pines groups growing on outcrops of the valley's slopes up to an elevation of about 1000 m. In the valley bottom there were small nests up to an elevation of 850 m. During the following season (2003-04) the insect range expanded, and the extreme nests were found at a higher altitude in the valley bottom (900 m), at a distance of about 300 m. In 2004-05 the range edges did not show any variation with respect to the previous season.
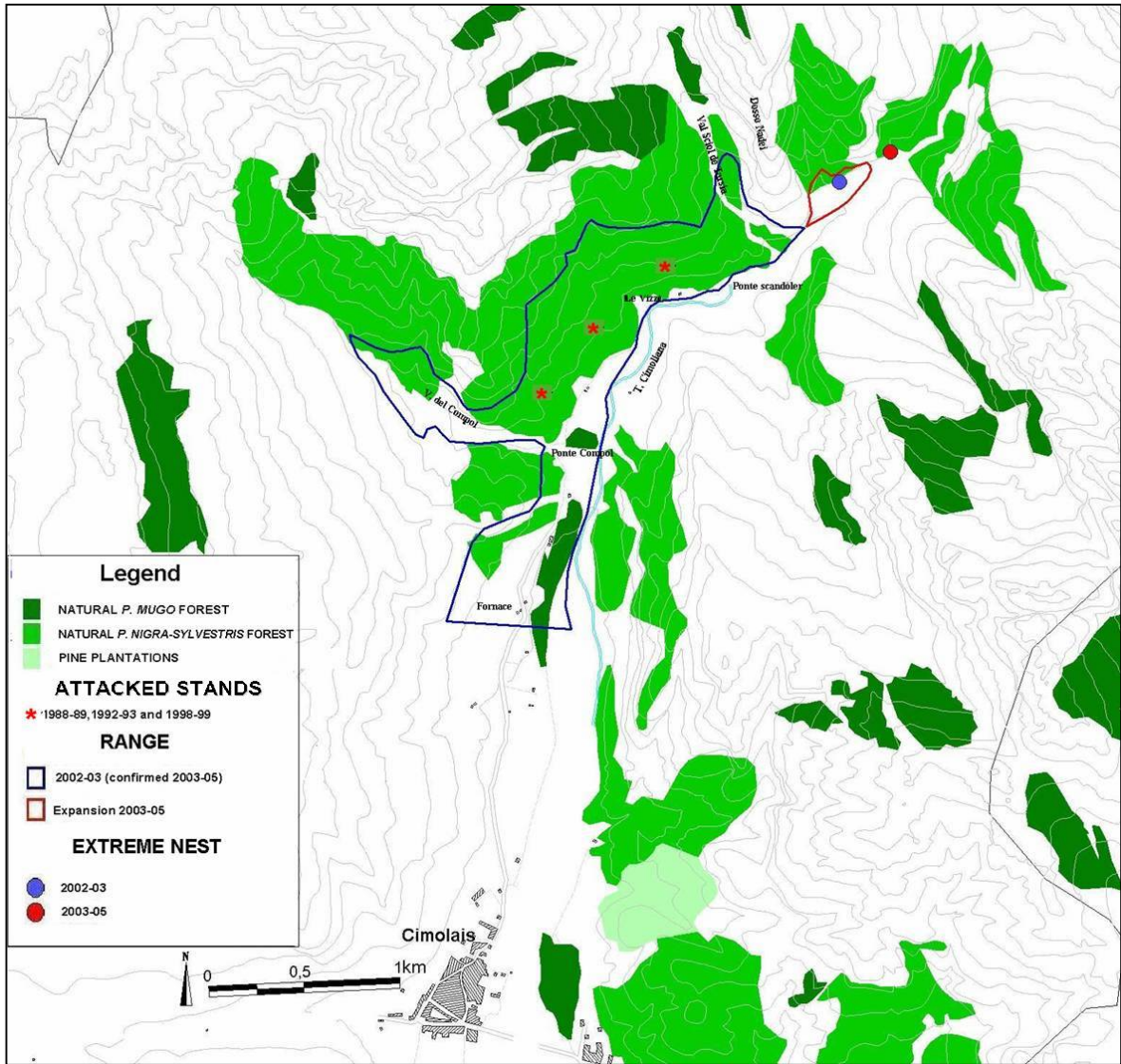
Figure 1. Map of the Cimoliana valley, north-eastern Italy, with the indication of the pine forests, the attacked stands, the range edge limited to the 2002-05 survey and the expansion range.

In the Settimana valley, the processionary was initially established on the most sun exposed slopes on both sides of the river, with the extreme nests around 900 m of elevation. In the 2003-04 the moth expanded further north and on shaded slopes, with extreme nests around 920 m and distant 880 m from those of previous cycle. In this case the most colonized host was *Pinus mugo*. As in the Cimoliana valley, there was no variation in the cycle 2004-05.
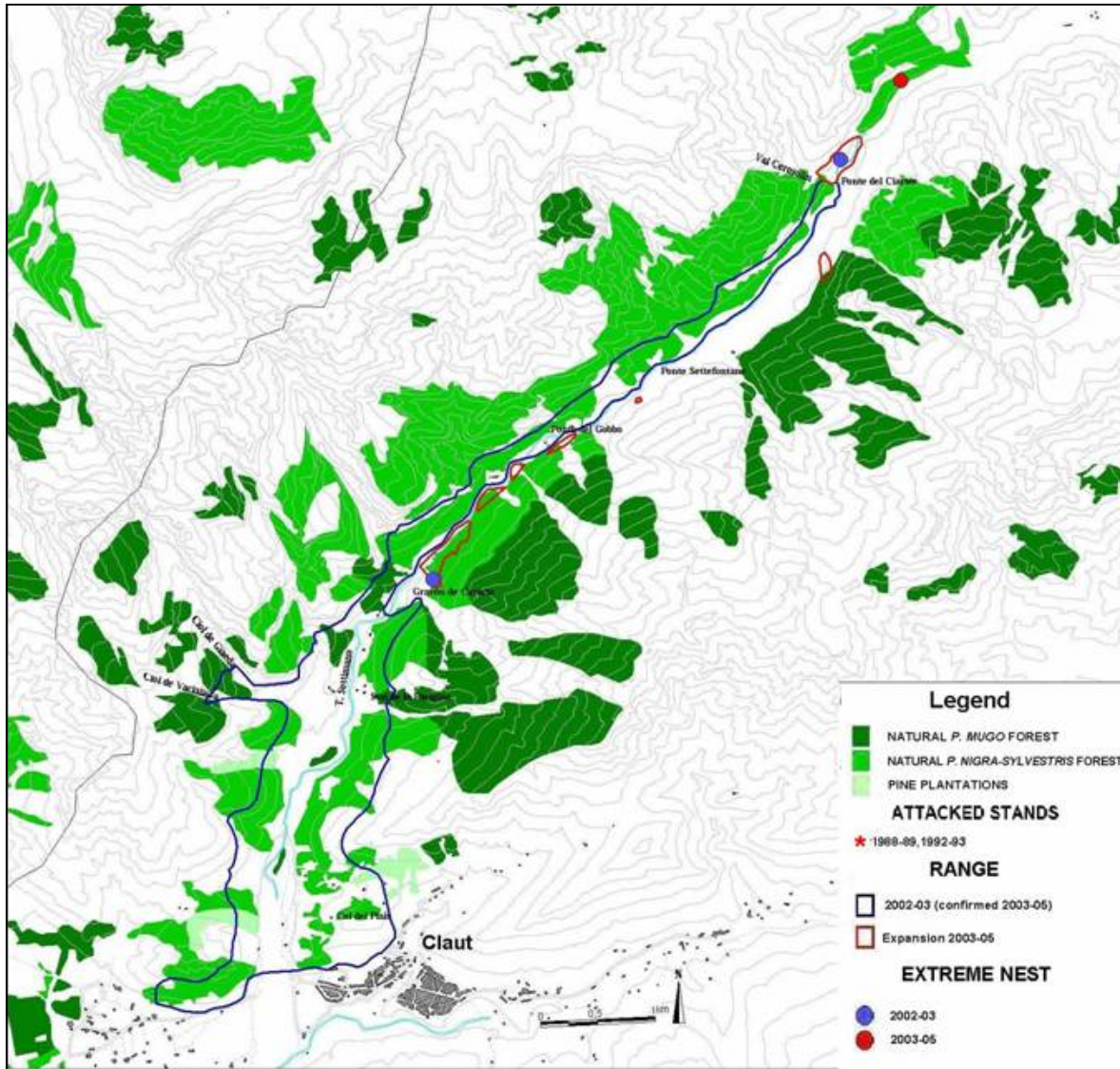
Figure 2. Map of the Settimana valley, north-eastern Italy, with the indication of the pine forests, the attacked stands, the range edge limited to the 2002-05 survey and the expansion range.
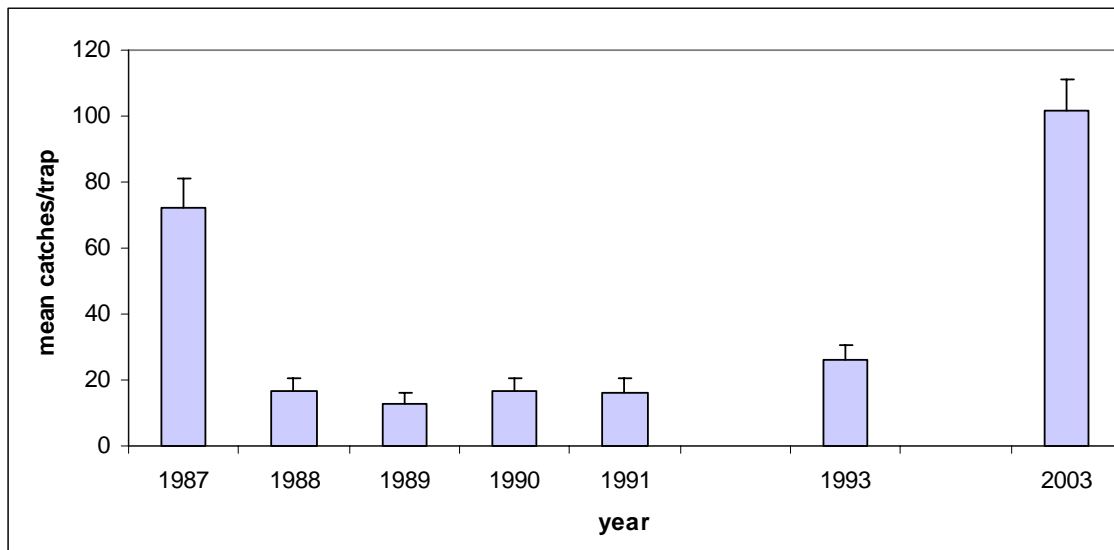
Figure 3. Catches of adult males (mean + 1 SE) in pheromone traps (n=5) during sampling years.
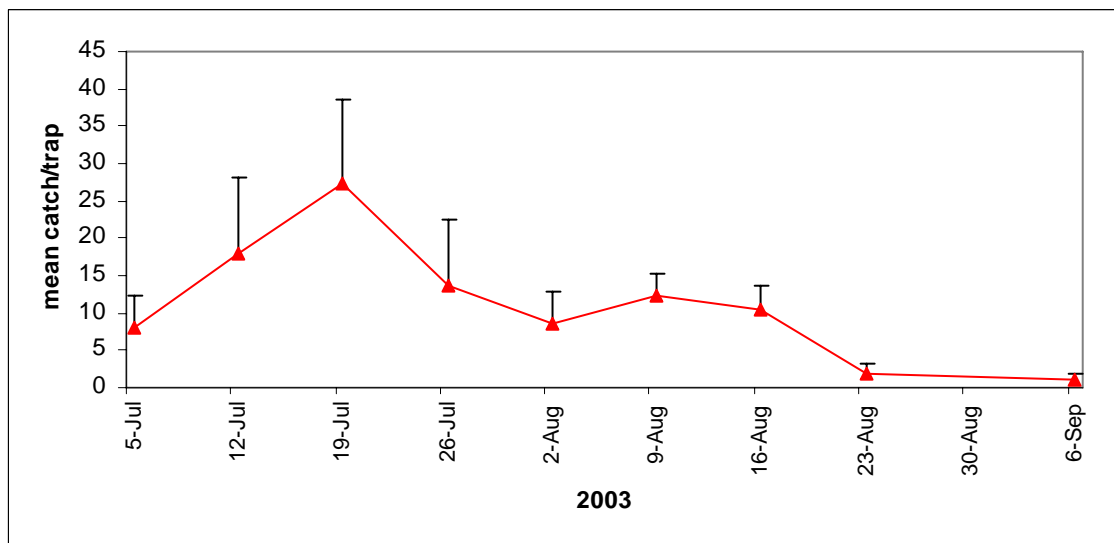

Figure 4. Catch of adult males (mean + 1 SD) in pheromone traps (n=5) in 2003

4. Discussion and conclusion

In the study period, intense defoliation events occurred every four to six years in Cimoliana valley and every four years in Settimana valley, showing thus some periodicity. Several authors described similar periodical outbreaks, every three-seven years in a Mediterranean pine plantation (Longo et al. 1989), six-seven years in an alpine area (Nicolini 1987), four-six years in Trieste Karst and in the pre-alpine areas of Friuli Venezia Giulia (Stergulc &

Frigimelica 1999), six years in France (Abgrall & Bouhot 1990). After a year of intense defoliation, the population density decreases quickly as a consequence of both change in food quality and activity of the natural enemies (Battisti 1988, Masutti & Battisti 1990, Hodar et al. 2004). The enemies usually do not contain the eruptive phase but may have an important function in the successive phases. However, outbreaks are not as regular as those described for the larch bud moth *Zeiraphera diniana* (Turchin et al. 2003), and in the last twenty years there was no indication of intense defoliations in the two valleys. Therefore population dynamics seem to depend also on other unpredictable factors, such as climatic conditions on a large scale, and the occurrence of extended diapause on a small scale.

The captures of pheromone trap did not allow a satisfactory prediction of the outbreaks, as in 1988 the catch value was low but in the following winter there was strong infestation with defoliation; on the contrary, in 1987 and 2003 numerous males were captured, but in following winter there was no indication of intense defoliation was observed. The difficulty to find a relation between catch and infestation was shown (Tiberi & Niccoli 1984, Roversi 1985, Devkota et al. 1992), however Jactel et al. (2006) recently showed a good correlation between male catch and nest density. The possible reasons of the different outcome rely on the monitoring design. In our study, as in many monitoring activities, the traps were placed on the border of the forest, often near roads, to facilitate the periodical inspections. The conditions in this location can be very different with respect to the forest, thereby the enhanced attractive power of the traps does not mirror the actual density of the insect. Moreover, the area of nest census is much wider than that of Jactel et al. (2006) trial.

Many studies analyzed and argued the climate change phenomenon occurring in the last decades (IPPC 2001), including the exceptionally hot summer of 2003 (Luterbacher et al. 2004; Beniston 2004). Climate change has been invoked to explain numerous variations in natural ecosystem such as range expansion (Hill et al. 1999, Wilson et al. 2005), phenological changes (Stefanescu et al. 2003), and acceptance of new hosts (Gutierrez & Thomas 2000). We can recognize the 2003 summer as one possible factor that has determined the range expansion of the insect in the two valleys. The numerous nights with high temperature may have facilitated the flight of the females and the colonization of new areas, as shown by Battisti et al. (2006). This expansion has confirmed the successful colonization of the novel host *P. mugo*, previously recorded as occasional host of the pine processionary moth (Benigni & Battisti 1999, Stergulc et al. 2000). Stastny et al. (2006) recently showed with laboratory and field trials that the relative growth rate and the mortality of larvae did not differ among three pine species growing in the expansion range of the moth, *P. nigra* (primary host), *P. sylvestris* (secondary host), and *P. mugo* (novel host). The reason of low occurrence and absence of outbreaks on *P. mugo* was previously explained by the separation of the ranges and by the low performance of the colonies (Devkota 1990). Since large stands of creeping pine occur in areas next to the insect range edge, we hypothesize that the pine processionary moth will colonize them, if climate change will maintain the current trend and strength. The main question is if the colonization, especially that due to the extreme 2003 event, will be followed by a successful establishment at the new locations. We hypothesize that some natural-history traits of the processionary moth may allow a long-term persistence of the population. According to Gaston (2003), the establishment depends on availability of critical resources, viable effective population size, and climatic factors. We have seen that the host plant is not a limiting factor (Stastny et al. 2006); as the female deposits all her eggs in a single batch,

in case of colony survival, there will be some tens of adults ready to persist in the same areas or establish in nearby zones. Moreover, the larvae can feed at low temperatures and this allows them to survive winters beyond the range edge (Battisti et al., 2005). A further element is the prolonged diapause at the pupal stage. The study of factors regulating the diapause is complex and numerous aspects remain unknown. Markalas (1989) identified as main factors the soil moisture and the fat reserves of insect at the time of pupation. The prolonged diapause, extended up to 5-7 years (Démolin 1969) or 9 years (Halperin 1990), is an insurance against local extinction: even if the lethal temperatures or starvation kill all the colonies in a unfavourable year, other members of the same cohort can survive as diapausing pupae in the soil, and some of them may emerge in a favourable year. This behaviour is known for many insects (Hanski 1988, Menu et al. 2000) and it is an example of bet-hedging strategy.

5. Acknowledgements

6. References

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Annex 2

Feeding strategy under harsh winter conditions in the pine processionary moth *Thaumetopoea pityocampa*

Abstract

Insects generally overcome the harsh winter conditions of the temperate region in a dormant phase, but a few developed the possibility to be active and feeding. The pine processionary moth *Thaumetopoea pityocampa* is an excellent example. It feeds on coniferous hosts, mainly *Pinus*, and may produce outbreaks in various areas of southern Europe. The larvae generally feed at night and spend daytime inside the nest built on the most sunny exposed parts of the tree. Based on a model of winter feeding recently developed, predicting that thresholds of day and night temperature drive the insect's activity, we hypothesized that the nest heat collection has an important role in determining the larval foraging schedule. We studied the response of the larvae to temperature in a laboratory experiment consisting in offering food to starved larval colonies, and measuring the production of fecal pellets over time at five constant temperatures in the range of 5-19 °C. In addition, we measured hourly temperature within and outside larval nests situated on southern and northern facing slopes in the Italian Alps. As expected, faeces production was positively correlated with temperature during the digestion period as well as with feeding time. In the field, the temperature was 11.7 °C higher in the nest than in the air on the southern slope, but only 0.9 °C on the northern slope. A new model based on thermal sum predicted about the same number of feeding events than the model based on temperature thresholds on the southern slope. However, many more feeding events were predicted for the northern slope by the thermal sum model, as they resulted from the accumulation of degree-hours that was not possible to estimate based on the threshold model. The new predictions fit better with the survival data of colonies on the northern slope. When the nest effect on temperature was excluded from the model, the predicted number of feeding events decreased by 20 %. This amount does not seem to fully justify the building of this costly structure, and we hypothesized that the nest can play other roles in the insect's life, such as protection from natural enemies.

Key words: *Pinus*, larval feeding, nest, temperature, Lepidoptera.

Introduction

Insect species that are active during winter are particularly suitable for studying the effect of temperature because their feeding and survival are often directly affected (Leather et al. 1993). For such species, the critical temperature for survival is often recorded in winter (Sinclair 1999), but the effects of temperature on survival may also be indirect, through mediating host plant availability or feeding activity (Jordano et al. 1991). However, insects evolved a remarkable diversity of adaptive behaviors and morphologies to overcome cold temperature (Heinrich 1996). Many of the behavioral responses to varying thermal environments involve microhabitat shifts or modifications. For example, in the Lepidoptera, behaviors such as basking (Forsman 2000), gregariousness (Stamp 1980; Bryant et al. 2000), and the construction of shelters (i.e., tents, nest or bolsa) (Casey et al. 1988; Joos et al. 1988; Fitzgerald and Underwood 2000) have been shown to effectively regulate body temperatures. In this way, many species are able to achieve and maintain specific body temperatures, which often coincide with the optimum of one or more physiologically important processes, such as feeding rate (Sherman & Watt 1973; Lactin & Johnson 1995), feeding efficiency (Porter 1982), growth rate (Knapp & Casey 1986), development rate (Lactin & Johnson 1996; Bryant et al. 2000) and metabolic efficiency (Kukal et al. 1988). However, insects capable of thermoregulating within certain

environmental constraints must compromise among these processes (Knapp and Casey 1986). In spite of the number of ways used by insects to overcome harsh winter conditions of the temperate region, very few developed the possibility to be active or feed during winter.

The larvae of pine processionary moth (*Thaumetopoea pityocampa* Denis & Schiffermüller, Lepidoptera, Notodontidae) are economically important pine defoliators active during winter. The species, that occurs in the Mediterranean area and in Southern Europe (Démolin 1969), in the last three decades, expanded its range latitudinally and altitudinally (Battisti et al. 2005). Adults emerge from pupae in the soil during mid-summer. The female lays eggs in clusters of 150 − 300 on conifer needles, mainly on *Pinus* spp. The larvae that hatch in late summer are nocturnal central foragers. They are strongly social and feed on pine needles throughout the winter. In late winter or early spring the larvae leave the tree to pupate in the soil. Late larval instars produce microscopic toxic hairs as a defense, presumably against vertebrate predators.

The details of the mechanisms behind the nocturnal feeding rhythm are not known but the day temperature in the nest seems to be a key component; e.g., a model based on minimum nest and night air temperatures required for larval feeding predicted the 82% of the colony survival in the Italian Alps (Battisti et al. 2005, Buffo et al. in press).

In this paper we hypothesize that the nest heat collection plays an important role in determining the larval foraging schedule. Specifically, we aimed at examining the thresholds used in the model by Battisti et al. (2005) and test whether or not the conditions for food processing in the nest can explain the observed relationships between larval feeding behavior and temperature. We studied this in a laboratory experiment using faeces production as a proxy of food processing. Moreover, we measured winter temperature within and outside larval nests in the field, in order to assess the role of the nest in the larval foraging schedule through a new model.

Material and methods

Experiment in environmental cabinets

Food processing was examined in environmental cabinets at five temperatures selected within the range usually experienced by the larvae in their nests. Larvae for the experiment were sampled from field populations. Fifty colonies, with their nests, were collected in December 2005 from *Pinus sylvestris* L. stands at six locations in the Trentino district, Northern Italy. Minimum distance between locations was 25 km. Branches with a nest (30 − 40 cm in length) were sampled, brought to the campus of Padua University, and kept outdoors in a large cage until the start of the experiment. Mixing between larval colonies was prevented by suspending each branch to the roof of the cage using oiled metal wire. At weekly intervals, fresh branches were offered to the colonies, and the feeding activity was visually estimated. In early January 2006, thirty colonies that showed clear signs of feeding were selected for the experiment. The colonies, with their nest and branch, were divided into five groups of six each (one from each site), and all needles were removed from the branches. Each group was designated to one of the five temperatures to be used in the experiment. The size of the colonies used in the experiment did not differ among treatments (mean = 106.5, SE = 13.5, min = 40, max = 356, ANOVA, $F_{(4,18)}$ = 0.61, P = 0.66). Only

two cabinets were available at a time; because each trial lasted for 15 days, the experiment went on from mid January until late February.

The larval colonies had to be conditioned to the experimental cabinets and prepared for the different temperature treatments before the main experiment could start. Thus the study was divided into a pre-treatment period and a digestion period (Fig. 1). The pre-treatment period was further divided into a conditioning, a starvation and a feeding phase. During the conditioning phase the colonies were free to feed "ad libitum" for 120 hours (5 nights). The larvae were then starved for 96 hours (4 nights). A pre-treatment period was used because the colonies, caged outdoors, fed irregularly and asynchronously due to low winter temperatures. Thus, we used the conditioning phase to obtain larval colonies that fed for four consecutive nights. The starving phase served the purpose to have all the larvae with an empty gut at the start of the feeding phase. This phase started at the beginning of the tenth night (N0) (Fig. 1). At that time, a fresh pine shoot, standardized to 6 cm in length, was offered as food to the colonies. After the larvae had left the nest to start feeding, the portion of the branch with the nest was removed, leaving the colony free to feed through the night. At the same time a faeces collector was placed beneath the larvae. The colonies were observed at hourly intervals in order to document when the feeding started.

Throughout the conditioning, starving, and feeding phases temperature, light, and humidity were set to resemble field average winter conditions for all the colonies. The photo-period was set at 9L:15D. After the dark period, light increased gradually through two hours, kept at a constant level (2500 lux) for five hours, and started to decrease reaching darkness two hours later. Following the same scheme, the temperature increased from the night level of +5 °C to the daily maximum of +15 °C through four hours. Four hours later, at dusk, the temperature again reached +5 °C through two hours and remained constant throughout the night. Relative humidity was constant (85%) during the dark period, but following the increase in temperature for four hours in early light period it became lower (60%). After four hours at constant level, almost at the end of the light period, it gradually decreased and returned to 85% at the beginning of the night.

The period of digestion – the process of main interest - started at the end of the night N0 (Fig. 1). From that time, the colonies were exposed at one of the treatment temperatures (+5.0, +8.5, +12.0, +15.5, +19.0 °C), which remained constant through the following 82 hours, i.e., until the end of the experiment. Humidity and light did not differ from the previous setting. During the trials, 28 colonies were in 3rd instar and two in 4th instar (assigned to the +12 and +19 °C trials). Needles not eaten during the feeding phase were removed at the end of the following light period (D1). At the same time, the percentage of food consumption was visually estimated. Within the cabinet, each of the six colonies was kept in a box in order to prevent mixing among larval colonies and to avoid contact with urticating hairs during inspections. The boxes (50 × 35 × 40 cm) were made of semi-transparent plastic with a piece of filter paper (10 × 6 cm) on the upper part to ensure gas exchange and control of humidity. Into each box a branch was suspended to the roof with oiled metal wire.

The faeces produced by each colony were collected every two hours. A hard-paper funnel, located beneath the colony directed the faeces into a sector of a rotating vessel divided into twelve equal sectors. The vessel was attached to an electric timer and completed one round in 24 hours, ensuring that each of the twelve sectors remained under the funnel for just two hours. At the end of the experiment, all the faeces were counted, dried at 105 °C for 24 hours, and weighed.

Nest temperature measurements
Temperatures outside and inside natural nests were recorded throughout the winter 2002 − 2003 in the Venosta/Vinschgau Valley, an Est-West oriented valley located in the South Tyrol district, north Italy (Battisti et al. 2005). Five nests were selected for the measurements, three on the northern slope (at 900, 990, 1040 m), and two on the southern slope (at 1200 and 1450 m). Temperatures were recorded from 3rd December to 28th February using data loggers (Hobo, Onset Computers, Pocasset, Massachusetts, USA). Nest temperatures were recorded by means of a probe inserted into the nests.

Modeling
Two mechanistic models were examined in order to explore the processes that underlie the feeding pattern of *T. pityocampa*. The number of feeding nights during winter was assumed to be critical for *T. pityocampa* (Battisti et al. 2005). This variable was investigated using models that took into account the nest and air temperature measured in the field, and different assumptions about the relationships between temperature and both feeding and food processing. Our field data, and the models that were developed, allowed us to test also the theoretical question about the importance of the nest thermal gain for the induction of feeding activity.

The first model, that we refer to as the "feeding threshold" model (FT) was developed by Battisti et al. (2005) and is based on two critical temperature thresholds. The model assumes that nocturnal feeding is induced by the following conditions: (1) the nocturnal air temperature is above 0 ◦C for at least one hour, and (2) the nest temperature during the previous day exceeds +9 °C during one hour or more.

In the second model, referred to as the "feeding thermal sum" model (FTΣ), the day temperature threshold of the FT model was replaced by the minimum thermal sum (T_Σ) required for food processing (min$T_{\Sigma(food)}$). Thus, the FTΣ model estimates the occurrence of larval nocturnal foraging under the following two conditions: (1) the nocturnal air temperature is above 0 °C for at least one hour and (2) the nest T_Σ(above 0 °C) after the end of the previous meal, exceeds min$T_{(food)}$.

Larval locomotion becomes quite slow at low temperature, and thus, one hour is probably not enough for colony feeding (Fitzgerald 1995). Therefore, a modified FTΣ model was developed (FT$\Sigma4$), stating that the first condition needs to be satisfied for four consecutive hours instead of one in order for the larvae to engage in nocturnal feeding. We used the same three models to explore the role of the nest. The number of winter feeding nights was recalculated using the air temperature in order to satisfy both models conditions and to exclude any nest effect. Thus, the differences in the number of feeding nights between the models calculated with and without the nest temperature estimated the relevance of the day thermal gain, obtained during larval resting in the nest, for the nocturnal feeding. The number of feeding nights was estimated in each of the five field sites using all the models described, in order to compare the FT with the new FT$\Sigma4$ models. Moreover, the FTΣ was included in the analysis in order to explore the effect of the introduction of the T_Σ in the FT model without any other additional effect.

The sensitivity of the models to their parameter values was explored for the FT$\Sigma4$ models in order to examine whether or not the differences observed among models were real, or simply related to the selected parameters. The sensitivity was tested by analyzing

the following alternatives in the formulation of the FTΣ_4 model: (1) increase in T$_{\Sigma(food)}$ from 36 to 48 °C·h, (2) reduction of the nocturnal temperature threshold from 0 to −1 °C, (3) increase of the shortest time with nocturnal temperature above the threshold (0 °C) from four to six hours.

Minimum thermal-sum used in the models (T$_{\Sigma(food)}$)

The minimum thermal sum for food processing (T$_{\Sigma(food)}$) was calculated based on data reported by Battisti et al. (2005). They observed that *T. pityocampa* larvae kept at constant temperature in an environmental cabinet feed during the night after they have experienced a T$_\Sigma$ equal to, or higher than, 48 °C·h during the previous day. They observed, however, that the larvae do not feed if the T$_\Sigma$ is 24 °C·h. Based on these results we assumed that minT$_{\Sigma(food)}$ should be between 24 °C·h and 48 °C·h. From our measurements, the T$_\Sigma$ during days with maximum nest temperature around +9 °C (i.e. the first condition of the FT model; see Modeling), resulted equal 36 ± 9.6 °C·h (n= 10). Because 36 is also the central value of the range 24 − 48 °C·h see above, we assumed minT$_\Sigma$ equal to 36 °C·h.

Statistical analysis

The effects of temperature on faeces production were tested with ANCOVA. Two periods were analyzed: the first was the feeding phase (N0), and the second was the following day (D1), corresponding in wild larvae to the inter-feeding period. Four variables were tested for a relationship with colony faeces production: total amount of food ingested, number and age of larvae, and feeding time. Feeding time was defined as the number of hours between the beginning of feeding activity and the end of the feeding phase (i.e. night N0). The variables that significantly correlated with faeces production were included in the ANCOVA as covariates. The Tukey's test was used to test for differences between paired treatments. The use of a linear model in order to explain the faeces production was considered appropriate because the residuals of the regressions were normally distributed. Due to the difficulties to have accurate weight of small samples of faeces, and the strong correlation found in our samples between weight and number ($R^2 = 0.89$), all the analyses were performed using the number of fecal pellets. All the variables were checked for normal distribution (Lilliefors, Shapiro-Wilk tests) and the number of fecal pellets was log-transformed in order to pass the test. A running mean over a period of six hours (n = 3) was used to smooth the temporal dynamics of faeces production.

The mean of the maximum and minimum daily temperatures, and their frequencies, were calculated for both the air outside and inside the nest, and differences were tested with T-test. Moreover, the average temperatures for entire study period were also calculated.

The number of feeding nights estimated by FTΣ_4, and the other three models used to test its sensitivity, were pooled among slopes. Differences between air and nest, and among slopes, were tested with paired T-tests.

Results

Experiment in environmental cabinets

Faeces production was positively correlated with temperature. There was a significant positive relationship between faeces production and temperature during the first ten hours of the digestion period (D1) ($\beta = 0.61$, $P = 0.001$) as well as feeding time ($\beta = 0.85$, $P < 0.001$) (MULT. LIN. REG. $R^2_{adj} = 0.56$, $F_{(2,20)} = 14.73$, $P < 0.001$), with significant differences among treatments (ANCOVA, $F_{(4,17)} = 3.35$, $P = 0.03$) (Fig. 2). During the feeding period (N0), faeces production was related to the number of larvae in the colony (β

= 0.40, P = 0.009), and the total amount of food ingested (β = 0.56, P = 0.001) (MULT. LIN. REG., R^2_{adj} = 0.69, $F_{(2,20)}$ = 25.71, P < 0.001). Feeding time during N0 differed among treatments (min = 4, max = 13, ANOVA, $F_{(4,18)}$ = 3.85, P = 0.02), but not the total amount of food consumed (mean = 38.6%, SE = 7.8, min = 2%, max = 100%). Larval age was not correlated with faeces production, thus this variable was excluded from the analysis.

Faeces production started during N0, shortly after the beginning of feeding activity, and two thirds (66%) of the total amount were produced during the treatment period. Faeces production was 44%, 26% and 30% during the first day (D1), the first night (N1), and the second day (D2) of the treatment period, respectively (Fig. 1).

Nest temperature in the field

There was a substantial difference in temperature between the nest and the air on the southern but not on the northern slope. On average, the nest on the southern slope showed a maximum daily temperature (Tn_{max}) of +16.4 ± 8.1 °C, which was 11.7 °C higher than the air ($T_{(174)}$ = 12.5, P = 0.001). Nest temperature on the northern slope, however, did not differ from the surrounding air temperature (2.8 ± 3.6, $T_{(174)}$ = 1.323, P = 0.188). On the northern slope, Tn_{max} was much lower than on the southern (3.7 ± 5.4, $T_{(174)}$ = 12.23, P = 0.001).

Two temperatures (+5 and +20 °C) illustrated the marked differences in the daily thermal gain in the nests on the two slopes. On the southern slope, Tn_{max} exceeded 20 °C for almost 2 hours (1.85 ± 0.86 h) approximately every other day (1.9 ± 1.86 d). Such high temperatures, however, were never reached in the nests on the northern slope and even the Tn_{max}> 5 °C did not reach such high frequency (2.87 ± 4.10 d) on the northern slope. Moreover, on the northern slope, Tn_{max} remained continuously below +5 °C for long periods (12 d), but not on the opposite slope (3 d), where it was exceeded almost every day (1.11 ± 0.39 d).

The mean minimum daily temperature on the northern slope was lower in the nests (−4.4 ± 3.5 °C) than in the air (−2.9 ± 3.1, $T_{(174)}$ = 12.23, P = 0.001). No such differences were recorded on the southern slope ($T_{(174)}$ = 12.23, P = 0.378).

Modeling

The estimates of feeding nights differed between the FTΣ and FT models. FTΣ estimated a larger number of feeding nights on the northern slope than FT; however, this was not the case on southern slope were the estimates were almost the same . The FTΣ4 gave about the same results than the FTΣ model (Fig. 3, Nest).

Larger estimates of feeding nights for FTΣ and FTΣ4 vs. FT were found when the effect of the nest thermal gain was not considered in the model. In this case the FTΣ model estimated on average many more feeding nights than FT, on both slopes (Fig. 3, Air).

The results of the test about the sensitivity of FTΣ4 model to its parameters are consistent with the pattern described above (Fig. 4). Thus, for FTΣ4 the number of potential feeding nights resulted larger on the southern than on the northern slope, independently on the nest thermal gain. On the southern slope the number of feeding nights resulted always larger, particularly when the nest effect was included in the model. Conversely, the nest did not affect feeding activity on the northern slope (Fig. 4).

Discussion

Our results show how intricate are the relationships between winter nocturnal feeding and nest day temperature in *Thaumetopoea pityocampa* larvae. The experiment in environmental cabinet showed a positive correlation between faeces production and temperature. Modeling based on nest and air field temperature confirmed the role of nest heat collection to increase the number of feeding nights throughout the winter. Moreover, feeding simulations based on thermal sums estimated more feeding occasions on the northern slope than predicted by the temperature threshold model.

In order to successfully estimate the digestion dynamics it was necessary to remove the nests in the cabinet experiment. The absence of nests during the digestion period resulted in colonies being exposed to light, a condition that they never experience in the field. However, all the colonies underwent the same conditions and it is reasonable to assume that the relative responses among treatments were not affected by the absence of the nest.

Food processing is a temperature-dependent process (Stamp and Casey 1993) that can be indirectly measured by faeces production. In our experiment, the faeces production increased proportionally with temperature (Q10 = 7) during the day following the feeding (D1). The linear increase among the tested temperatures suggests that the physiological optimum for gut emptying of *T. pityocampa* is above +19 °C (Fig. 2). After feeding, the larvae emptied the gut in a few days (Fig. 1); thus, due to the relation between temperature and food processing, low temperature during the inter-feeding periods may reduce, or even prevent, gut emptying. However, after each foraging bout the larvae spend all the day in the nest, and thus gut-emptying is strongly influenced by incident solar radiation.

Nests of *T. pityocampa* obtain large thermal gain through absorption of solar radiation (Breuer et al. 1989, Fitzgerald and Blas 2003). At the study sites, the average difference in maximum daily temperature between nest and air was 9.6 °C. High winter temperatures (> 20 °C) were frequently recorded inside the nests, but never in the air that only occasionally exceeded 10 °C. Thus, as a result of resting in a sun-exposed nest, the larval temperature can increase; this enhances digestion efficiency and extends the time during which minimum thermal conditions required for nutrient absorption, growth, and development are met.

Thaumetopoea pityocampa larvae show a unique and fixed nocturnal feeding schedule that seems to be induced by the previous day temperatures (Battisti et al. 2005). In an experiment in environmental cabinets, Battisti et al. (2005) showed that feeding bouts only start if the larvae experience a temperature above +9 °C during the previous day, even if the night temperature allows feeding activity (i.e. Tair > 0/ − 2 °C) (Fitzgerald and Blas 2003). Thus, low day nest temperatures can strongly limit the number of winter larval feeding bouts and affect colony survival (Buffo et al. in press). Therefore, it seems as if the thermal gain, obtained by the larvae resting in the nest, can increase the number of feeding occasions and plays a key role in larval growth and survival. It is not clear, however, what mechanism(s) explain the link between day temperature and night feeding. However, because food processing is related to day temperature, could be the day gut-emptying rate, or the nutrient uptake that induce the larval feeding and, through larval communication, the colony synchronization (Abisgold and Simpson 1987, Timmins and Reynolds 1992, Simpson and Raubenheimer 1993, Bernays and Singer 1998). Thus, even a small gut-emptying such as that observed at +5°C in our cabinet experiment may induce the subsequent feeding as described by Battisti et al. 2005.

Such as feedback could be the signal that induces the colony synchronization The strength of the communication, therefore, could be the last signal that induces the colony to leave the nest and search for food. The finding that the faeces production in D1 was proportional to temperature (Fig. 2), implies that gut emptying, and nutrient assimilation, depend on the total amount of thermal energy available. Therefore, it seems reasonable that a feedback induced by the level of food assimilation has to be related to the thermal sum above the minimum temperature required to activate food processing, and not only to a temperature threshold (e.g. +9 °C).

Based on this, we simulated the feeding behavior of *T. pityocampa* larvae in order to examine how different assumptions about mechanisms can affect the estimation of the number of winter feeding nights. The simulations showed that, on the northern slope, the models based on the $T\Sigma$ (FTΣ, FTΣ_4) estimate a larger number of feeding nights in comparison with the model based on the temperature threshold (FT). There were no differences, however, in model outputs for the southern slope (Fig. 3). These results show that the FTΣ model confirmed the prediction of the FT model on the southern slope. Moreover, the higher number of feeding nights estimated on the northern slope seems to better justify the small difference of colony survival observed by Battisti et al. (2005) between the northern (13.7%) and the southern slope (22.3%).

The simulations made without considering the nest thermal gain (Fig. 3) showed a surprising result, as thermal sum based on air temperature estimated a number of feeding nights not so different from that estimated by nest temperature. Both the FTΣ and FTΣ_4 models gave results that fall within the range estimated by the simulations that included the nest thermal effect. These results suggest that the nest, on average, does not induce more than 20% of the feeding nights (8 on 40) estimated on the southern slope (Fig. 3). The low sensitivity of the FTΣ_4 model to the selected parameters supported this conclusion.

From a strictly foraging point of view, it seems strange that *T. pityocampa* feeds during the night and does not also feed during the day because the night temperature seems to be the major limit to foraging. We think that the answer has to be searched in the relation between the larvae and both their hosts and enemies. In general, a major constraint on caterpillar foraging is risk of mortality from natural enemies (Stamp and Casey 1993). *Thaumetopoea pityocampa* larvae are not selective feeders, but feed on mature needles, a food source that does not change much in quality during winter. Thus, the larvae do not have to maximize growth rate in a race against seasonal decline of food quality, as is the case for many spring-feeding lepidopterans (Stamp and Casey 1993). Consequently, to forage only at night, and hiding themselves in a dense silk nest during the day, seemingly is an optimal strategy in order to minimize the encounters with parasitoids and day-active predators not sensitive to the toxic hairs. Moreover, the larvae can increase the nutrient uptake from digesting food at the higher temperature gained by mean of the nest. The higher silk spinning at colder sites (Démolin 1967) may support the thermoregulatory role of the nest, even if its effects on the colony (number of feeding nights, nutrient assimilation efficiency) have to be further clarified.

To conclude, two main selective forces seem to work in concert and to constrain the winter-active larvae of *T. pityocampa* to feed strictly during the night: risk of mortality from day-active predators, and minimum thermal requirement for physiological processes. In general, insect larvae are not able to run off from predators once they have been detected. Instead, blending with the surrounding environment and nocturnal foraging are the main alternative strategies to avoid these threats (Stamp and Casey 1993). Cryptic

larvae with an active feeding behavior would not be able to survive the winter because the low temperature would strongly limit feeding and food digestion. To bask in the sun, on the other hand, would expose larvae to natural enemies. By thermo-regulating and digesting sheltered in a sunny nest, protected against predators, *T. pityocampa* has evolved the only option presumably at hand to feed in harsh winter conditions, such as at high altitudes in the Alps.

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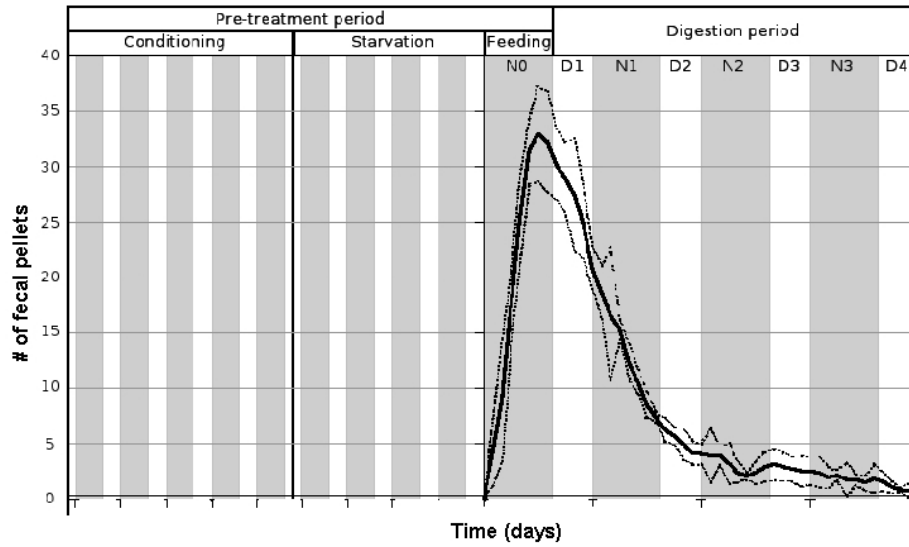
Fig. 1. Experimental conditions in the environmental cabinets. The grey and white background show the photo-period. The continuous line is a running-mean (n=3) of the temporal dynamics of faeces production ± SE (dashed line). The conditioning phase was used to obtain larval colonies that fed for four consecutive nights. The starving phase served the purpose to have all the larvae with an empty gut at the start of the feeding phase. This phase started at the beginning of the tenth night (N0). The food left was removed on D1 and the production of fecal pellets monitored through four days at different constant temperatures.
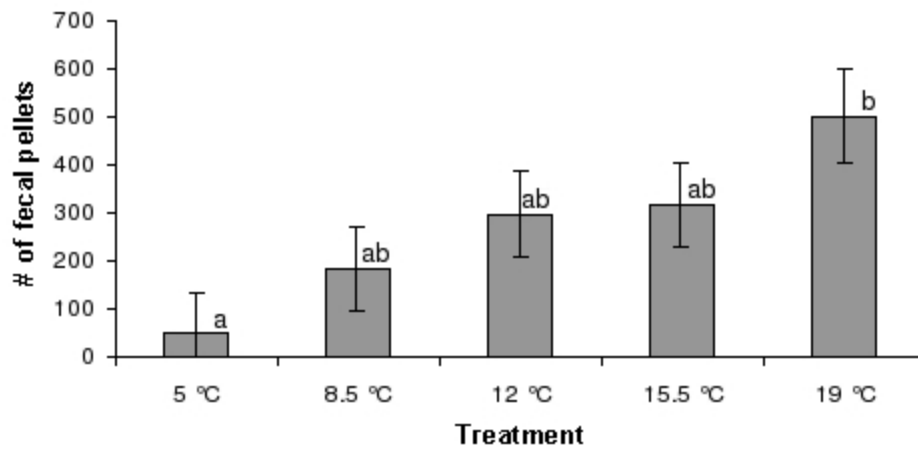
Fig. 2. Colony faeces production during the first ten hours (D1) of the experiment. The bars shows mean and standard deviation, corrected for feeding time (ANCOVA, $F_{(4,17)} = 3.35$, $P = 0.03$). Differences between paired treatments were tested by Tukey's test ($p < 0.05$).
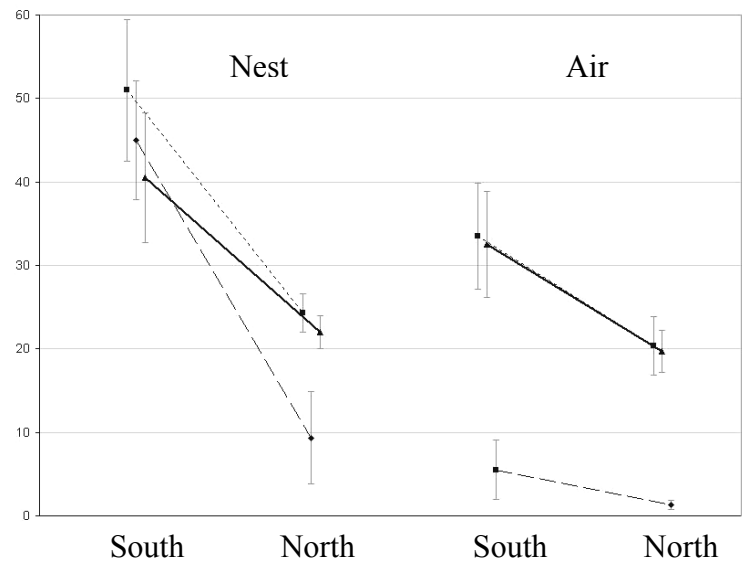
Fig. 3. Number of feeding nights (mean ± SD) estimated by models with (left) and without (right) the thermal effect of the nest, on the southern and northern slope of Venosta/Vinschgau Valley, Italian Alps. The three models are the feeding threshold FT (--) (Battisti et al. 2005), the feeding thermal sum $FT\Sigma$ (...), and the feeding thermal sum extended to 4 consecutive hours of night temperature suitable for feeding $FT\Sigma_4$ (—).
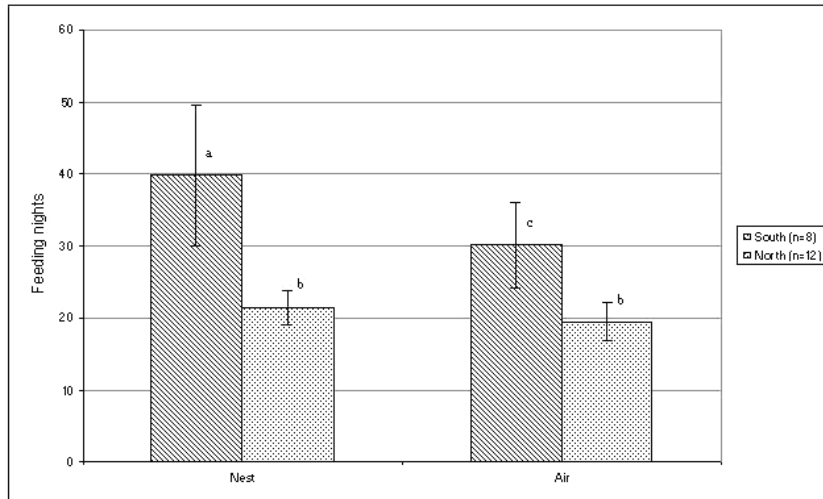
Fig. 4. Mean (± SD) of feeding nights estimated for each nest and slope of Venosta/Vinschgau Valley, Italian Alps, by the models based on the thermal sum $FT\Sigma_4$. The bars on the left ('nest') indicate the calculation based on daytime temperatures measured inside the nest while the bars on the left ('air') are based on daytime temperatures measured outside of the nest. The difference within each slope shows the contribution of the nest in increasing the number of feeding nights. Differences were tested with paired T-tests.