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Pine Processionary Moth (*Thaumetopoea pityocampa*) and Great Tit (*Parus major*) in Portugal: Population Dynamics and Interactions

Lisboa

2004

Depósito legal nº 1
ISBN: 972-8893-02-7

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Dissertação apresentada para obtenção do Grau de Doutor em Ciências do Ambiente, pela Universidade Nova de Lisboa, Faculdade de Ciências e Tecnologia

Orientadores: Doutora Maria Rosa Paiva e
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Lisboa
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Acknowledgements

I would like to thank Prof. Maria Rosa Paiva, the supervisor of this thesis, for the giving me the opportunity to do this work and for providing all the means for its execution.

I sincerely thank Prof. Jan-Åke Nilsson, who acted as a co-supervisor of this thesis, for his helpful suggestions on statistics and for introducing me to the fascinating world of nest-boxes and cavity-nesters. I also thank him for receiving me in Lund and providing access to the library service.

I am grateful to Verónica Amparo, Marta Neves, Márcia Santos and Cláudia Ferreira, who were the team that made this work possible. I thank them for the discipline, enthusiasm and team spirit they showed even in the hardest tasks.

I also would like to thank Teresa Calvão and Rita Barrocas for collaborating in Chapter 4 and Ana Paula Martinho for collaborating in Chapter 5. Thanks are also due to Eveline Moura for collaborating in the fieldwork during the season 1999/2000.

I would like to express my gratitude to the Administrations of Apostiça and National Pine Forest of Leiria who provided access to the field sites, and to the meteorological services of Aerial Base of Monte Real and AFLOPS for providing the meteorological data used in the present work.

I also whish to thank my uncle Júlio Botas, and my sister Ana Pimentel for all the unconditional support and encouragement during the last stages of the present work.

This work was funded by Fundação para a Ciência e Tecnologia, Ministério da Ciência e Ensino Superior, with a PhD grant refª PRAXXIS XXI /BD/18 119/98.

Sumário

No presente trabalho estudou-se a dinâmica populacional de uma praga importante nas florestas de pinheiros em Portugal – *Thaumetopoea pityocampa* (Den. & Schiff.) – e de um dos seus predadores potenciais – *Parus major* (L.). As fases de ovo e larvares do lepidóptero foram estudadas em vários tipos de plantações de *Pinus pinaster* (Aiton) localizadas em três regiões do País: Península de Setúbal, Abrantes e Mata Nacional de Leiria. A localização geográfica dos pinhais e a estrutura dos povoamentos foram os factores mais importante para determinar as densidades populacionais de *T. pityocampa*, sendo as zonas de Setúbal e Abrantes preferencialmente atacadas em relação a Leiria e pinhais jovens e homogéneos mais susceptíveis ao ataque do que povoamentos mais maduros e heterogéneos. No entanto, uma população anómala de *T. pityocampa*, cujas larvas se desenvolvem durante o Verão ao invés do Inverno, como a população normal, atingiu elevadas densidades populacionais em Leiria, ao contrário da população normal. O impacte dos diversos factores de mortalidade nos estados imaturos do insecto (ovos e larvas) e das condições climáticas, são analizados e discutidos para todas as populações, assim como as possíveis implicações evolutivas do aparecimento da população anómala de *T. pityocampa* numa área de pinhal gerida para produção de madeira há centenas de anos. Uma vez que é a quebra da diapausa das pupas e a emergência dos adultos que define o ciclo de vida do insecto, foi a emergência precoce dos adultos na população dessincronizada de Leiria o factor que determinou a alteração do ciclo de vida do insecto. Os diferentes factores que influenciam as datas de emergência das populações normais e dessincronizada, ao longo dos anos, são também discutidos. Para o estudo das populações de *P. major* foram colocadas caixas-ninho na Península de Setúbal e Mata Nacional de Leiria, as quais foram monitorizadas ao longo de três anos. As caixas ninho aumentaram a densidade populacional de aves invernantes e nidificantes nas plantações estudadas, indicando ser de facto a ausência de cavidades nas árvores um factor limitante. Registou-se o mais precoce início de nidificação de *P. major*, o que indica que nos pinhais costeiros nacionais, a abundância de presas, ocorre mais cedo do que em outras zonas da Europa e Norte de África. Isto determina de facto uma sobreposição entre o final da fase larvar de *T. pityocampa* e o início da nidificação de *P. major*.

Palavras chave: *Thaumetopoea pityocampa*, *Parus major*, *Pinus pinaster*, dinâmica populacional, Portugal.

Abstract

In the present work, we studied a common outbreaking Lepidoptera species in Portuguese pine stands – *Thaumetopoea pityocampa* (Den. & Schiff.) - and one of its potential predators – *Parus major* (L.). The population dynamics of the immature stages of the Lepidoptera was studied in several types of *Pinus pinaster* (Aiton) plantations in three different areas: Setúbal Peninsula, Abrantes and National Pine Forest of Leiria. Location and plantation structure was the most important factors determining population density of *T. pityocampa.* Setubal and Abrantes was highly susceptible to attacks by the Lepidoptera, whereas Leiria had lower densities. Young and homogeneous pine stands was more susceptible to attacks than older and more heterogeneous pines stands. However, a desynchronized population of *T. pityocampa*, in which the larvae develops during summer instead of during winter, reached high densities also in Leiria. The impact of several mortality factors and climatic conditions on the immature stages of the insect (eggs and larvae), in normal and desynchronized populations are discussed, as well as possible evolutionary implications of the sudden appearance of the new version of *T. pityocampa*. The break of the pupa diapause and adult emergence times the annual life cycle of this insect. Adults from the desynchronized population emerged earlier than adults from the normal population, which in turn determined the change in the larvae development period. Different factors, potentially affecting the timing of adult emergence in both normal and abnormal populations are also discussed. To study *P. Major*, nest-boxes were placed in the areas of Setúbal and Leiria and they were monitored during three seasons. The nest-boxes increased the density of breeding and wintering birds in the studied pine plantations, indicating that a lack of natural holes are in fact a limiting factor for this populations. The earliest breeding start for this species was recorded in my study area, indicating that Portuguese coastal pines provide good breeding conditions earlier than in other areas of Europe and North Africa. This leads to an overlap between the end of the larvae stage of *T. pityocampa* and the beginning of the breeding season of *P. major*.

Key-words: *Thaumetopoea pityocampa*, *Parus major*, *Pinus pinaster*, population dynamics, Portugal.

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1. General Introduction

1.1. The forest ecosystem

The combined action of the geochemical and biochemical cycles in undisturbed forest ecosystems results in an active accumulation and retention of nutrients from the geochemical cycle. The organic forest floor that develops from decomposing litter promotes the chemical retention of nutrients, and the combined action of mycorrhizal roots and fungi provides an efficient biological mechanism for nutrient uptake and retention. Plants growing under conditions of low nutrient availability have evolved mechanisms of nutrient conservation, such as long foliage retention, leaching-resistant cuticles, chemical defences against losses to herbivores, infrequent reproduction, and efficient internal recycling. The biogeochemical efficiency of forests is one of the main reasons why forests are able to grow on soils of extremely low fertility. It is frequently observed that mature forests of similar composition and productivity can develop on areas varying in the inherent fertility of the soil. Part of this apparent independence of a mature forest from the nutritional status of the underlying mineral substrate arises from the tendency for forests to build up a forest floor, accumulate a capital of nutrients, and then operate largely from the nutrients in the biogeochemical cycle (Mattson 1980, Kimmins 1997).

Mediterranean type ecosystems are characterized by hot, dry summers and warm, wet winters. This climate type characterizes only 5% of the earth, but it is present in all inhabited continents – Mediterranean Basin, California, Central Chile, South Africa, and Southwest Australia – these areas have clear physiognomic similarities despite genetic diversity (Fouseki and Margaris 1981). Coniferous and sclerophyllous forests and shrub lands are widely distributed in this area and the evergreen leaves of Mediterranean vegetation are typically poor in nutrients and rich in recalcitrant compounds. In fact Mediterranean-climate ecosystems are characterized as low nutrient ecosystems and the efficient withdrawal of nutrients before leaf fall reduces the quality of litter, nutrients being conserved within aerial biomass thus reducing the dependence of individual plants on the soil system (Fouseki and Margaris 1981, Rapp *et al*. 1999).

The pines, that are a prominent component of the Mediterranean Basin flora covering 25% of the forested area, invaded the area 3.5 million years ago. They present a remarkable ecological plasticity and often occur under harsh environmental conditions or at high altitudes. This high plasticity probably explains the dynamic behaviour of most of these pines and the important roles that they play in vegetation dynamics around the Mediterranean Sea wherever human-induced disturbances are not too severe (Barbéro *et al*. 1998).

An idealized “pine prototype” would conform with the following profile: “a light-demanding, fast-growing, sclerophyllous tree that regenerates as even-aged cohorts following landscape-scale disturbance and retains its position in the landscape by exploiting aspects of its regeneration biology”. Among the factors that have contributed to the rapid range extension and population increase of pines are; abundant output of seeds from an early age; ability to recruit dense daughter stands on exposed sites soon after disturbance; effective mechanisms for long distance seed dispersal; a mating system that permits inbreeding and selfing in isolated trees; various life-history traits that confer resilience at the population level under a wide range of disturbance regimes; and the ability to colonize nutrient-poor sites (Richardson and Rundel 1998).

These characteristics helped to expand the range of the pine in South Western Europe (France and Spain) in recent decades. Pines are often invaders occupying the abandoned fields induced by the “set-aside” policy of the European Union and burned areas. On the contrary, in many areas of the southern part of the Mediterranean Basin, the shortage of fuel and timber is severe (e.g. in Algeria and Morocco) and the area covered with pine decrease drastically (Barbéro *et al*. 1998).

In Portugal the forested area covers about 40 % of the territory - about 3.4 million ha. About 30 % of this area is monocultures of maritime pine *Pinus pinaster* (Aiton), which constitutes 98 % of the stands - the other 2 % are *Pinus pinea* (L.) stands (DGF 1999, DGF 2001). Fossil, charcoal and palynological records indicate that *P. pinaster* has been present in Portugal for 55 000 years and was probably able to survive the last glaciation in sheltered areas at low altitudes close to the Atlantic Ocean (Figueiral 1995). In the 1870`s the area occupied by pines was just 210 000 ha, but in the 1960`s this area had increased to 1.3 million ha. Most of this increase was due to massive forestation campaigns at the end of the 14th century (DGF 1999). Ribeiro *et al.* (2001) did not find any discernible geographic pattern for the Portuguese populations of *P. pinaster* and they explain these results by the recent expansion of the species range and extensive gene flow among populations associated with the expansion. Areas covered by maritime pine has decreased in the last two decades due to replacement by the fast growing *Eucalyptus globulus* (Labill.) but the pine woods are still of important economical value for production of high quality timber and resin (Silveira da Costa 1984, DGF 1999, DGF 2001).

P. pinaster is considered to be drought resistant species that grows well in acid infertile sandy soils. It prefers high air moisture content and its distribution reaches 1000 m a.s.l., it is intolerant to frost and cold weather, thus being well adapted to the north coastal areas of Portugal (Silveira da Costa 1084). *P. pinaster* has a long needle life span – about six years - and an annual foliage mass turnover of just 18% (Warren and Adams 2000). Mass and nutrient of litter fall and its decomposition rate is lower in *P. pinaster* stands than in other evergreen Mediterranean forest ecosystems, indicating that this species is extremely efficient in recovering most of its nutrients, especially Nitrogen, before needles fall (Moro and Domingo 2000, Kavvadias *et al*. 2001, Santa Regina 2001).

1.2. Herbivory in forests

The extent to which plants are consumed by herbivores varies greatly between different types of ecosystems. Phytoplankton communities in aquatic ecosystems experience exploitation by herbivores of 60-99%, grassland plants loose between 28-60% of their net primary production to herbivores, while in forests only about 5-10% of the foliage is consumed. Under endemic insect densities (less than 10% foliage reduced) this average level represents only 1.5-3% of the net primary production. In grasslands, a very high percentage of aboveground plant biomass and of the net primary production is palatable, digestible and within reach of herbivores. In forests, on the other hand, the foliage corresponds to a much smaller proportion of the net primary production, the rest being woody tissues that are little used by herbivores, with the exception of thin roots and young twigs. Forest foliage tends to be less palatable and digestible than grassland foliage because of its physical and chemical characteristics, and much of it is physically out of reach of many herbivores (Kimmins 1997, Hunter 2001).

These budgets change drastically during periods of high insect herbivore abundance. Forest insects periodically increase to epidemic proportions, at which they can consume all of a deciduous tree’s foliage or several years of evergreen foliage production in a single outbreak. This can drastically reduce the biomass of primary producers in the ecosystem, resulting in the reduction of the net primary production for several years. Such episodes can also cause changes in nutrient cycles and nutrient availability in the soil through insect frass, dead insects, nutrient leaching from damaged leaves, changes in litter quality and changes in the structure of plant canopies and the cover that they provide, with concomitant changes in light availability, soil temperature and moisture. Such pulses of energy flow from the producer to the primary consumer level may be reflected in temporary expansions of the magnitude of energy flow and biomass all the way up the trophic chain (Kimmins 1997, Hunter 2001).

While outbreaking species of forest insects attract most research attention, the majority of forest insects have low, stable population sizes and never cause noticeable defoliation – for example only less than 2 % of macrolepidoptera tree feeding species undergo outbreaks (Nothnagle and Schultz 1987). Outbreaking and stable species of folivorous macrolepidoptera coexist in essentially the same habitat, on resources that are relatively predictable in time and space (Nothnagle and Schultz 1987, Hunter 1991). Differences in habitat stability and selective regimes cannot by themselves explain the patterns (Hunter 1991) although community structure can be very important (Faeth 1987, Redfearn and Pimm 1987).

According to Hunter (1991), forest outbreaking macrolepidoptera species have specific life cycle characteristics in response to weather, predation and changes in hosts. They typically have higher fecundity and host breadth, which allows them to rapidly exploit large sections of the environment when conditions are favourable. Furthermore, a disproportional number of outbreaking species are gregarious (12% of the total number of species analysed were gregarious, but 51% of them caused outbreaks whereas only 7% of the solitary species caused outbreaks), gregariousness was also found to be connected with egg-clustering and poor flying ability among females (Hunter 1991). In fact Tammaru and Haukioja (1996) connected Lepidoptera eruptivity with a capital breeding life-cycle – species in which adults depend mostly on resources derived from the larval period – and these characteristics are connected with low mobility in females, egg clustering and larval gregariousness.

1.3. Gregarious caterpillars

The relation between larval gregariousness and the capacity to attain very high population densities is not obvious. Insects are both small and slow in relation to many potential predators, which should have the capacity to consume an entire group once discovered. Besides, groups of caterpillars can suffer great losses from parasitoids (Stamp 1981, Knapp and Casey 1986), have higher risk of being infected by pathogens (Hochberg 1991) and may rapidly overexploit their larval resources because of competition for food within a colony which may result in the inability to finish development, and sometimes to death of all offspring of a female (Floater and Zalucki 1999).

In fact gregariousness remains rare during the larval stage in Lepidoptera: of the about 160 thousand described species of butterflies and moths only about 300 species are known, so far to exhibit gregarious or social life-styles for at least part of their larval development. However, it is a widespread phenomenon, occurring in some twenty or more butterfly and moth families, and is likely to have multiple origins, with different species coming to sociality by different paths (Costa and Pierce 1997).

Two pre-requisites are generally considered essential for this life-style to evolve in Lepidoptera: egg clustering (Stamp 1980) and aposematism – repellent defences and warning coloration (Sillen-Tullberg 1988, Sillen-Tullberg and Leimar 1988, Tullberg and Hunter 1996). Egg clustering evolved due to energetic constrains to decrease oviposition searching time (Courtney 1984, Wiklund *et al*. 1987). This is especially important in species where adults do not feed and have short life span. An increase in fecundity is connected with an increase in the size of the abdomen and, consequently, an increase in the energetic cost of flying for females (Wickman and Karlsson 1989, Tammaru and Haukioja 1996). Egg clustering may be enhanced if it reduces desiccation of the eggs, as has been demonstrated for the nymphalid *Chlosyne lacinia* (Geyer) (Clark and Faeth 1998). Repellent scent glands, long hairs or spines, coordinated “head-flickering” movements, regurgitation of host plant toxins and irritant substances are defensive tactics that work out much better in large groups performing synchronized group behaviour (Gamberale and Tullberg 1996, 1998, Hunter 2000, Ruf 2002). Taking into account that natural enemies are the main cause of mortality in immature herbivorous insects (Cornell and Hawkins 1995, Cornell *et al.* 1998), this may lead to higher larval survival in gregarious than in solitary species (Hunter 2000). Thus, these defences make it possible to overcome the disadvantage of belonging to a slow and highly visible group.

Once this life-style has evolved other advantages can be explored such as possibility to build large silk webs or tents which are multifunctional structures giving shelter from desiccation and protection from invertebrate predators and parasitoids (Damman 1987). Furthermore, the tent may transfer vibrations, which could enhance synchronized group behaviour (Ruf 2002) and serve as effective material for thermoregulation, keeping the metabolic heat production

(Breurer and Devkota 1990, Ruf and Fiedler 2000) or increasing temperature when the group bask in the sun (Joos *et al.* 1988, Breuer *et al.* 1989, Ruf and Fiedler 2002a).

Social behaviour can be advantageous for feeding facilitation in ways which include communal overcoming of plant defence structures (Clark and Faeth 1997, Fordyce and Agrawal 2001), optimal exploitation of resources (Tsubaki and Shiotsu 1982, Le Masurier 1994) or the development of a pheromone based communication system, which can be more or less sophisticated according to the caterpillars food demands (Peterson and Fitzgerald 1991, Fitzgerald and Underwood 1998a,b, Fitzgerald and Pescador-Rubio 2002, Fitzgerald 2003).

Solitary caterpillars are able to elevate their body temperature by exposing their preferably dark coloured bodies to the radiation of the sun, which enhances consumption and digestion rates and consequently the development rate (Casey 1976, Bryant *et al*. 2000). Their small size increases the rates of heating, but also of cooling, giving them a limited capacity of increasing their body temperature above the air temperature (Stevenson 1985a,b). In aggregation, caterpillars multiply their effective body mass, which results in much higher temperatures than in solitary larvae (Knapp and Casey 1986, Stevenson 1985a,b, Bryant *et al*. 2000). Aggregation can also be important for water balance in warmer climates when there is no need for an increased body temperature (Klok and Chown 1999). Furthermore, if caterpillars build tents, these structures may enhance temperature gains even further (Joos *et al*. 1988, Breuer *et al.* 1989, Fitzgerald and Underwood 2000, Ruf and Fiedler 2002a).

It is noticeable that sociality in caterpillars combined with tent building predominantly occurs in species that develop in early spring in temperate climatic areas, feeding on deciduous trees, like the eastern tent caterpillar *Malacosoma americanum* (F.) (Joos *et al*. 1988) and the small aggar moth *Eriogaster lanestris* (L.) (Ruf and Fiedler 2000, 2002a); or throughout the winter in warmer climates, feeding on evergreen species, like the madrone caterpillar *Eucheria socialis* (Westwood) (Kevan and Bye 1991), *Ochrogaster lunifer* (Herrich-Schaffer) (Floater 1997) and *Thaumetopoea pityocampa* (Den. & Schiff). Both types explore thermal conditions that are very restrictive for ectothermic insects.

The widely accepted classification of foraging behaviour in gregarious Lepidoptera includes patch-restricted, nomadic or central-place foragers (Costa and Pierce 1997). Patch-restricted foragers represent the simplest form of sociality, in which species typically construct shelters and feed on leaves incorporated within the structure. Nomadic foragers move in groups from one food patch to the next, and depleted sites are regularly abandoned. They do not construct three-dimensional silken shelters but often build silken pads for resting or moulting. Central-place foragers represent the most complex system of sociality in caterpillars. These species usually construct shelters but unlike patch-restricted foragers they feed outside these constructions in the surrounding vegetation. These species evolved a sophisticated communication system, which includes group cohesion, group defence, spatial orientation, and exchange of information about feeding sites (Costa and Pierce 1997.).

In central place foragers that move in processions, a chemical trail marker secreted from the tip of the abdomen is primarily responsible for eliciting trail following (Peterson and Fitzgerald 1991, Fitzgerald and Underwood 1998a,b, Fitzgerald and Pescador-Rubio 2002, Ruff *et al*. 2001, Fitzgerald 2003). This communication system can be more or less sophisticated according to the caterpillar's food demand. The eastern tent caterpillar is an example of a fine tuned trail-marking system. The species feed on the youngest leaves in the tree, which allow them to have a fast growth rate. To meet the food demands they need to find new sources of food and leave exhausted areas efficiently, dealing with a patchily distributed food supply. These larvae are able to quickly abandon exhausted food paths and recruiting their nest mates to new food sources (Peterson and Fitzgerald 1991). Other species, with slower growth rates, do not discriminate among patches of leaves of different nutritional quality and have a less efficient trail marking system (Fitzgerald and Underwood 1998a,b). According to Fitzgerald (2003), *T. pityocampa* seems to be the less demanding of all central place foragers studied. The caterpillars have the ability to distinguish new from weaker trails which enables them to move to communal feeding sites independently, and to find their way back to the nest after feeding, but colonies are relatively inefficient in abandoning exhausted feeding sites in favour of new sites and they do not recruit others to food.

1.4. Thaumetopoea moths

Moths belonging to the Thaumetopoeidae family are widespread throughout Eurasia, Africa and Australia. The processionary moths of the Mediterranean area belong to the genus *Thaumetopoea*. All *Thaumetopoea* species oviposit in clusters and live in aggregations. Some of the species living in warmer areas have lost the ability to build silky nests, but larvae of all species move on silky trails during their processions. In all species the larval develop in five instars and pupate in the soil, except *Thaumetopoea processionea* (L.), which develops during six instars and pupate in the nest. Nocturnal behaviour in late instars and adults, as well as urticating late instar larvae, are also common features. The known *Thaumetopoea* species can be grouped in species having egg diapause, the so called “summer processionary moths” which are *Thaumetopoea pinivora* (Treitschke), *T. processionea*, *Thaumetopoea bonjeani* (Powell), *Thaumetopoea solitaria* (Freyer) and *Thaumetopoea herculeana* (Rambur); and a pupal diapause the so-called “winter processionary moths”. *T. pityocampa*, *Thaumetopoea wilkinsoni* (Tams) and *Thaumetopoea jordana* (Staudinger) (Douma-Petridou 1989, Schmidt 1989, Halperin 1990) (see Table 1.1).

Chemistry of the pheromone components corroborates the split between the two groups of processionary moths except for *T. solitaria*, which differs from both groups (Frerot and Demolin 1993). All these species, except *T. herculeana*, which feeds on Geraniaceae, are reported as causing outbreaks in deciduous and coniferous trees.

Another well-known Thaumetopoeidae is the *O. lunifer* species complex, widespread in Australia, feeding on *Eucalyptus spp.* and *Acacia spp.*, which has a univoltine life cycle very similar to the *Thaumetopoea* species with pupal diapause (Floater 1996 a,b,c).

1.5. Birds as insect predators in forest ecosystems

For immature stages of herbivorous insect, natural enemies are known to be the major cause of mortality (Cornell and Hawkins 1995, Cornell *et al.* 1998). Predatory birds are the third trophic level in a forest ecosystem, and evidence suggests a “top-down” effect, having a potential impact on the community composition, plant productivity and individual plant fitness (Marquis and Whelan 1994). In fact, insectivorous birds are known to respond numerically to forest Lepidoptera outbreaks (Holmes *et al.* 1986, Haney 1999), effectively reducing the density of insect larvae in tree canopies (Holmes *et al*. 1979, Gunnarsson 1996) and improving growth of the trees (Marquis and Whelan 1994, Murakami and Nakamo 2000). These predators can cause heavy damage on early instar tent building caterpillars; effectively reducing the density of the population (Costa 1993; Ruf 2002) and several birds are known to be predators of *T. pityocampa* larvae and pupae (Gonzalez-Cano 1981, Battisti *et al*. 2000).

Table 1.1: Life-history of nine Thaumetopoeidae species: the eight species belonging to European *Taumetopoea* and the Australian specie of the genus *Ochrogaster*.

Specie	Cycle	Diapause	Flight period	Larval period	Nests	Host	Distribution	Reference
Thaumetopoea pinivora Treitschke, 1834	Bianual Univoltine	Egg+Pupa Pupa	July-August April-June	April-July June-August	Yes	*Pinus spp.*	Central and South Europe	Schmidt 1989
Thaumetopoea bonjeani Powell, 1922	Univoltine	Egg	August-September	March-July	Yes	*Cedrus atlantica* (Endl.)	North of Africa	Yousfi 1989
Thaumetopoea processionea Linnaeus, 1758	Univoltine	Egg	August-September	April-July	Yes	*Quercus spp.*	Central and South Europe	Pascual 1988a,b
Thaumetopoea solitaria Freyer, 1838	Univoltine	Egg	August-November	February-March	No	*Pistacea spp.* and *Cypressus spp.*	East Mediterranean	Schmidt 1989
Thaumetopoea herculeana Rambur, 1840	Univoltine	Egg	August-November	Spring	Yes	*Cistus spp.*, *Helianthemum spp.* and *Erodium spp.*	Iberian Peninsula, North Africa and Palestine	Schmidt 1989
Thaumetopoea pityocampa Denis & Schiffermüller, 1775	Univoltine	Pupa	July-September	September-March	Yes	*Pinus spp.* and *Cedrus spp.*	Mediterranean Basin	Schmidt 1989
Thaumetopoea wilkinsoni Tams, 1924	Univoltine	Pupa	August-November	October-March	Yes	*Pinus spp.*	Cyprus, Israel and Jordan	Schmidt 1989
Thaumetopoea jordana Staudinger, 1894	Univoltine	Pupa	September-December	November-March	No	*Rhus tripartita* (Ucria)	Israel, Jordan and Saudi Arabia	Halperin 1990
Ochrogaster lunifer Herrich-Schäffer, 1855	Univoltine	Pupa	October-November	November-May	Yes	*Acacia spp.* and *Eucalyptus spp.*	Australia	Floater 1996 a,b,c

Vegetation characteristics are the dominant factors in determining a forest bird community composition, distribution, and abundance (Holmes *et al.* 1986). A resource essential to the community of cavity-nesters, an essential part of the insectivore's guild in the forest, is the availability of trees containing cavities (Showalter and Whitmore 2001). Especially large trees with dead wood or with lightning scars are important for providing this resource (Ganey 1999). Due to modern forestry practices, managed forests consist of homogenous, even-aged stands of trees, where the systematic removal of such trees reduces the overall abundance, species diversity and richness of breeding and roosting birds (Lohr *et al*. 2002).

Great tit *Parus major* (L.) is a common cavity-nester all over Europe. It readily breeds in nest boxes and, upon provisioning such boxes, most of the great tits in a study area will breed in them, resulting in the control of virtually the whole population (Lack 1964, Perrins 1965). This fact has made the species extremely suitable as a model organism for solving ecological and evolutionary questions, and it has been intensively studied during the last fifty years (e.g. Lack 1964, Perrins 1965, Sanz 1998, Pettifor *et a*l. 2001, Tinbergen and Verhulst 2000). By now, long-term studies on the breeding biology of the species are available (Boyce and Perrins 1987, McCleery and Perrins 1998) and its feeding ecology is well known. Great tits are largely insectivorous and are a Lepidoptera larvae specialist during the breeding season (Betts 1955, Gibb and Betts 1963, Royama 1970, Cowie and Hinsley 1988). The relationship between oak, winter moth, and great tits has been studied for many years in Central West Europe, and this three-step food chain is a classical example in forest ecology (van Noordwijk *et al*. 1995, Buse *et al.* 1999).

Studies have connected great tit abundance and breeding performance to the abundance of its favourite prey (Seki and Takamo 1998, Naef-Daenzer and Keller 1999). Furthermore, great tits have been shown to be an effective predator on larvae, causing a reduction in larvae density and the damage to the primary producers (Murakami and Nakamo 2000, Mols and Visser 2002, Mols 2003). All these characteristics make the great tit potentially suitable as a pest control agent in young forest plantations where tree age homogeneity normally precludes breeding due to lack of natural breeding cavities.

1.6.Thesis outline

Due to the large extension of pine plantations in Portugal, the pine processionary moth *T. pityocampa* is of major economical importance as a defoliator, leading to important biomass losses (Laurent-Hervouët 1986, Markalas 1998). The distribution of maritime pine monocultures are mainly restricted to the north of the Tejo River and two major spots can be identified: the Central Pines, located exactly in the central area of the country and the Atlantic Coastal Pines located at the North West coast of Portugal. A smaller *P. pinaster* forest is found in the Setúbal Peninsula and thus, isolated from the other two areas. These three areas are known to have different climatic and geological characteristics, representing the main pine forests in the country and were therefore selected for this study of the population dynamics of *T. pityocampa* during three seasons (1999-2002). The overall aim with the study was to assess potential factors leading to outbreaks. This subject is developed in Chapter 2, with focus on the different mortality factors that are potentially important for the control of the population. This was done by monitoring the larval growth and development and assessing variation in the susceptibility of *T. pityocampa* attacks to different pine plantations, differing in location, structure and climatic variables.

In 1997 a desynchronized population of *T. pityocampa* had an outbreak in the oldest National Forest of the country, the National Pine Forest of Leiria, located in the area of the Atlantic coastal pines. Larvae of this abnormal population develop through the summer, being named the summer population (SP), while the normal version of the species develops through the winter, thus named the winter population (WP). The existence of this desynchronized population offers a unique opportunity to study the ecological and evolutionary factors behind its appearance and apparent success. This new version of *T. pityocampa* was also studied and compared to the normal population coexisting in the National Pine Forest of Leiria (see Chapter 3).

Abiotic factors, mainly climatic variables are known to induce outbreaks of forest insects (Martinat 1987) and affect their dispersion patterns (Pedgley *et al*. 1989, Showers *et al*. 1989, Pedgley and Yathos 1993). Factors connected with pine stand structure are also known to have great importance (Floater and Zalucki 1999), and Leiria is a national pine forest in which there is a good record of management operations during the last 30 years. The fact that the outbreak date of the Leiria desynchronized population of *T. pityocampa* is well known, and that it occurred in a managed pine wood with good records allowed to explore this subjects, which was made in Chapter 4.

The life cycle of this strictly univoltine species is timed by the break of the pupal diapause and adult flight period. Chapter 5 presents data collected during several years in the field and laboratory concerning the pupal period and adult phenology of both the winter populations and the abnormal summer population, allowing a comparison between them. The objective of this chapter is to understand biological and environmental factors that govern adult emergence timing in the normal WP and compare this with the desynchronized SP.

Great tit *P. major*, being a common cavity-nester and one of the most thoroughly studied bird species, was the most logical choice for studying the third trophic level in the ecosystem dealt with in the present study. Furthermore, great tits are known to be a predator on *T. pityocampa* (Gonzalez-Cano 1981). Thus, nest-boxes for *P. major* were placed in two of the areas - Setúbal and Leiria – covering SP and WP distribution areas in order to study the population dynamic of this bird species and to assess the mutual influence on the *T. pityocampa* population dynamic (see Chapter 6).

2. Dynamics of pine processionary moth: comparison of different maritime pine plantations in Central-South Portugal.

2.1. Introduction

The pine processionary moth *T. pityocampa* is an economically important pine pest in the Mediterranean region (e.g. Schmidt *et al.* 1990). Severe pine defoliation caused by the caterpillars frequently occurs, resulting into biomass loss and economic damage (Laurent-Hervouët 1986, Markalas 1998). Additionally, this moth also affects humans, since late instar larvae possess urticating hairs, which cause irritation to the skin and mucous membranes, often leading to severe allergic reactions (Lamy 1990). *T. pityocampa* is considered part of the native fauna and is normally present in pine stands across the country and is one of the most important phytophagous insect known to cause visible damages to this forest.

The larvae are gregarious and develop through five instars during the winter months (e.g. Schmidt *et al.* 1990). The caterpillars weave a silky nest, very important for their thermoregulation during this cold and wet season (Breuer *et al.* 1989). At the end of the 5th instar the larvae start a procession and pupate in the soil; a diapause occurs during the spring (Schmidt *et al.* 1990). Adult emergence extends throughout the summer but the timing is strongly dependent upon several factors, mainly altitude and latitude: at higher altitudes and latitudes an earlier period of emergence occurs (Masutti & Battisti 1990, Devkota *et al.* 1992).

Pine forest had gain importance in the last decades in the Mediterranean Basin due to the fact that pines are often invaders occupying the abandoned fields induced by the “set-aside” policy of the European Union and burned areas (Barbéro *et al.* 1998). In Portugal maritime pine *P. pinaster* covers about one million ha of the territory, which constitutes 98 % of the pine stands, and most of this area originated from massive plantations occurred in the 19th century (DGF 1999). Pure stands of this tree correspond to 65 % of all its area (DGF 2001). The Pine Processionary Moth gained this way importance. However, information about Mediterranean pine forest outbreaking species, its possible inducing and controlling factors and the relationship with forest structure is very fragmented and rudimentary.

The appearance of outbreaks in forest Lepidoptera have been connected with climatic extremes (Martinat 1987, Kamata 2000), variations in host quality (Haukioja 1980, Haukioja and Neuvonen 1987); pathogen induced cycling (Myers 2000), parasitoids (Berryman 1996), forest structure (Bragança *et al*. 1998b, Floater and Zalucki 2000) and site quality (Kamata 2000, Alfaro *et al*. 2001). In the present work, a study was made on the population dynamics and different factors with the potentiality of leading and/or controlling *T. pityocampa* outbreaks in Central-South Portugal.

2.2. Material and Methods

2.2.1. Areas of study

P. pinaster stands located in three areas of Central and South Portugal were selected for this study (Fig. 2.1): Península de Setúbal, Abrantes and Leiria National Pine Forest. Preliminary surveys indicated that *T. pityocampa* outbreaks are detected mainly in very young plots with trees below 10 years, so stands of this age class were preferably selected for our study. In Leiria, *T. pityocampa* nests are rarely detected in stands below this age, so plantations between 10-15 years old were selected.

National Pine Forest of Leiria is located 200 km north of Lisbon (Latitude: 39º50´N, Longitude: 8º57´W, Altitude: 30-50 m a.s.l.). It has an area of 11 023 ha, of which 10 828 ha are covered by pine stands of *P. pinaster*. Part of the area, 8 702 ha, is managed to produce high quality timber. The area close to the Atlantic Ocean evolves mainly by natural regeneration, resulting in low tree density and low growth rates. In the XIII century King Dinis included Leiria in the crown possessions and established the first management rules, mainly because of the protective function of the pinewood for stabilising the dunes and protect agricultural fields. However its origin probably predates this event (Ferreira and Gomes 2000).

The management scheme presently in operation dates from the end of the XVIII century, with some minor changes. The pinewood is divided into 334 rectangular plots of 30 to 40 ha each (except on the border areas), which are the management units. The trees within a plot are even-aged and the initial density is 2000–2500 trees.ha^{-1}. Adjacent plots are often planted at the same time, leading to large continuous areas with trees of the same age. Thinning is practised according to management targets so that, by the time trees reach their ideal size at about 80 years, tree density will be only 10 % of the initial one and a clear cut is made. Seven plots, aged 10-15 years, located in along the forest were selected for this study.

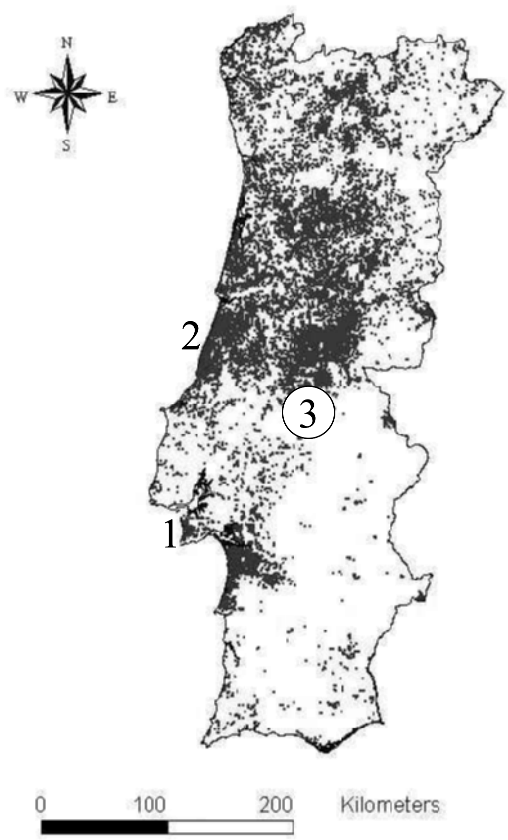


Figure 2.1: Field site locations 1) Apostiça; 2) Leiria; 3) Abrantes. The black spots are the Pine forests in Portugal.

Apostiça, is a private forest property with total area of about 4 000 ha located in the Setúbal Península, also close to the Atlantic Ocean but located south of Lisbon (38º30´N, 9º11´W, 35 m). The area has few *P. pinea* and *Quercus suber* (L.) spots, but is mostly a *P. pinaster* forest. This forest is the result of major *P. pinaster* plantations made during the 30's and 40's. Logging in part of the original stand, fires and natural regeneration has resulted in a low density and a more diverse forest. For the present study areas with a large amount of young trees were selected. Two spots 2000 m apart were selected: 1 - an area with high density of young trees (around six years) resulting from a clear cut and natural regeneration; 2 - a stand originating after a fire that occurred 20 years ago with a diverse age composition.

Two pine stands, separated by 60 km, were selected in the Abrantes region, Central Portugal. Barrada in the Tejo River flats (39º26´N, 8º04´W, 162 m) is an eight ha *P. pinaster* stand aged seven years. This small stand was surrounded by older pine stands, an olive tree stand and a *E. globulus* stand. Pucariça (39º32´N, 8º15´N, 230 m), is located north of the Tejo River, in the border of an area called the Central Pine, the largest continuous *P. pinaster* forest in Portugal (and in the World) that was the result of massive plantations made during the IXX cent. For this study an eight years old stand was selected.

Overall the Abrantes area has more a continental climatic type. Typically temperatures reach the lowest levels in the winter and the highest in the summer, with more number of days of frost per year. Setúbal Peninsula is more Mediterranean, with lower precipitation, and higher Global radiation and number of hours of sun per year. Leiria represents the coastal Atlantic area at north of Lisbon. It has a soft weather, with lower temperature fluctuations along the year, higher precipitation and higher air moisture content (Table 2.1).

Table 2.1: Climatic characterization of the three areas included in this study (Instituto do Ambiente 2003).

Parameters	**Setúbal Peninsula**	**Abrantes**	**Leiria National Pine Forest**
Number of days in the winter with minimum temperatures below 0ºC	< 2 days	10 - 30 days	2 - 15 days
Average minimum temperature of the coldest month in the winter	> 6 ºC	2 – 4 ºC	4 – 6 ºC
Number of days in the summer with maximum temperatures above 25ºC	20 – 100 days	100 – 120 days	< 20 days
Average maximum temperature of the hottest month in the summer	23 – 29 ºC	29 – 32 ºC	< 23 ºC
Average annual temperature	15 – 16 ºC	16 - 17,5 ºC	12,5 - 15,0 ºC
Number of hours of annual solar radiation	2 800 - 2 900 h	2 700 - 2 800 h	2 400 - 2 500 h
Total global radiation	160 – 165 Kcal/cm^2	145 – 150 Kcal/cm^2	140 – 145 Kcal/cm^2
Total precipitation	500 – 600 mm	600 –700 mm	700 – 800 mm
Average annual air moisture content at 9:00 a.m.	75 – 80 %	75 – 80 %	80 – 85 %
Number of days with frost per year	5 – 10 days	30 – 40 days	10 – 20 days

2.2.2. Precipitation data

Total precipitation was obtained from stations located near the studied sites: Leiria -Latitude: 39º50´N, Longitude: 8º53´W, Altitude: 52 m a.s.l.; Pucariça - Latitude: 39º32´N, Longitude: 8º20´W, Altitude: 127 m a.s.l.; Barrada - Latitude: 39º27´N, Longitude: 8º12´W, Altitude: 190 m a.s.l.; Apostiça - Latitude: 38º28´N, Longitude: 9º5´W, Altitude: 120 m a.s.l.. Total precipitation is the amount of precipitation, which reaches the ground in a specific period of time and is measured in millimetres with rain gauges.

2.2.3. Field surveys

The present study was based on survivorship and densities during the larval development in the field. Other similar studies on tent building caterpillars were based on colony survivorship, thus taking colony as the demographic unit (Costa 1993, Floater and Zalucki

1999, Ruf 2002). The assumption behind this measure is that, in highly social caterpillars, individual survivorship is closely tied to that of the group. However, preliminary surveys indicated striking differences in tree defoliation in the different areas studied. Thus, knowing how many individuals were causing the defoliation in each tree seems to be an important population dynamic parameter. To count and weigh caterpillars, nests were collected in the field and handled in the laboratory. Thus, this study is not based on the survivorship of a certain number of colonies in the field, but on the average variation in numbers of caterpillars in the different larval stages.

The study was made during three seasons between 1999 and 2002. During the larval period, samples of *T. pityocampa* nests and egg-batches were collected monthly from *P. pinaster* trees. The samples were always taken at the same location in each stand: in Leiria, in four plots located in the central and northern area of the wood; in Apostiça, at two locations of young trees in area 1 and 2, although in area 1 samples were only taken during the two last seasons; in Barrada and Pucariça, at one location for each stand.

Sampling occurred during five months, between October and February, covering the whole larval period. During the season 1999/00, samples of nests and egg-batches were taken randomly from the trees: 3 to 12 nests in each sampling location and all the egg-batches we could find, thus the sampling unit was nest/ egg-batch not tree.

In the last two seasons (2000/01 and 2001/02) samples were collected per tree. For each sampling date and sampling location, 3-12 attacked trees either with nests or showing signs of larval grazing, were randomly selected and all nests and egg batches were collected. The orientation of egg-batches and larval nests was recorded as heading towards NE, SE, SW, NW or along the axis of the tree. The nests and egg-batches which were along the tree axis or towards SE and SW were considered to be in the sunny part of the tree and the ones located at NE and NW were considered to be in the shade part of the tree.

After the larval period was completed (starting in March), an estimate of the attack level by *T. pityocampa* was made, expressed as the proportion of trees attacked and the number of nests.ha^{-1}. The diameter at breast height (DBH) of all trees within a specified area (see below) was measured and the number of nests present on each tree and its orientation recorded (following the same procedure as for the nest and egg-batch collection). These measurements always covered an area different from the one where the nests had been collected. This data is not available for the first season (1999/00) in Apostiça.

At the three sampling locations in Leiria, two transects with 15 m width were sampled, which followed two perpendicular axes inside the plot (between 1.35 and 1.99 ha in each plot summing to a total area of 12.77 ha). In Apostiça the measurements were made inside two squares of 50x50 m in Area 1 (a total of 0.5 ha), and three transects, 15 m wide and 760-960 m long, in Area 2 (a total of 3.78 ha). In Pucariça, we sampled inside four squares with an area between 0.10 and 0.18 ha (a total of 0.52 ha) and in Barrada inside two squares of 40x40 m (a total of 0.32 ha).

2.2.4. Laboratory

In the laboratory the structural characteristics of the egg-batches were recorded. The scale cover was removed from each egg-batch and the number of lines and number of eggs in a sample of lines were recorded. From this, the number of eggs per batch could be estimated. Furthermore, those eggs that did not hatch were counted and the number of eggs that were parasitized was annotated (black or with an emergence hole). Emerged parasitoids in the laboratory were identified.

For each population and season, 10 egg batches were randomly selected to measure egg volume. Egg length and width were measured at 70-fold magnification and egg size is reported as the volume of the egg (mm^3) assuming the egg to be a prolate spheroid and using the formula $V=1/6\pi w^2L$, where w is the egg width and L is the egg length (Berrigan 1991, García-Barros 2000).

The pine needles around which the females wrap their egg-batches constitute the first source of food for the newly hatched caterpillars. Since these needles eventually will dry and have a tendency to fall from the tree, the calculation of the number of eggs per tree, mortality of all batches and survival of the early larval instars were restricted to trees sampled in October and November. These calculations were only made during the last two seasons (2000/01 and 2001/02) because only then were the samples made on a per tree basis.

Each collected nest was opened and the larvae separated according to instar and counted. Each batch of larvae of the same instar, from each nest, was then weighted and the larvae batches were dried at 60º - 70º C to be able to calculate the average larvae dry weight by gravimetry for each instar.

The early instars of *T. pityocampa* build temporary nests that are quickly degraded by the harsh conditions during winter. Permanent nests are structures that allow the colonies to get rid of excrements, dead caterpillars and parasitoids pupae (e.g. *Phryxe caudata* Rond.) through its bottom side, material that is typically found on the ground bellow the attacked tree. This made it hard to get quantitative measures of the cause of larval mortality. However, when we found parasitoid pupae, we kept them in petri dishes until the imago emerged and could be identified. Potential invertebrate predators were also identified whenever found.

2.2.5. Life table

To estimate the mortality of *T. pityocampa* immature stages in the seasons 2000/01 and 2001/02 (when the samples were made on a per tree basis) we used the ratio of the number dying in a stage (d_x) to an initial number of eggs (l_0): i.e. d_x/l_0 (Bellows *et al.* 1992). Mortality in the egg stage was derived from direct observations of dead eggs in the egg-batches. The mortality of whole batches was calculated as the proportion of egg-batches in trees where all batches of larvae disappeared minus the egg mortality affecting these batches. The mortality of early instars (1st-3rd instar when larvae have their temporary nests and are still moving around the tree) was estimated as the larval mortality in trees where at least some larvae survived (number of larvae minus number of eggs in each tree). Both the mortality of whole batches and early larval instars mortality were estimated in trees sampled in October-November. Total mortality of larvae in the 4th-5th instar (when larvae have built their permanent nests) was estimated taking into account the final number of larvae per tree minus the calculated number of larvae that pass the 3rd instar. The number of deaths caused by *P. caudata* was counted directly from the number killed in nests where *T. pityocampa* larvae were found moulting to the 5th instar. Since *P. caudata* larval development is strictly connected with this stage, the procedure avoid that the mortality caused by the parasitoid will be underestimated due to losses of dead caterpillars and parasitoids pupae through the nests bottom.

2.2.6. Statistical analyses

The statistical analysis was performed using SYSTAT 10 and the probabilities shown refer to two-tailed tests. Chi-square tests are presented after the application of Yates' correction. Differences between means of two samples with normal distributions were tested with *t*-test. One-Way ANOVA was used to assess differences among groups of more than two samples. Post hoc multi-comparison tests (Tukey for unequal sample sizes) were used in order to detect which groups were significantly different from other groups. In the case of two sources of variation we used Two-Way ANOVA. These tests were performed if the assumptions of independence, homogeneity of variance, normality and additivity were verified. Normal distribution of each sample was tested with the Kolmogorov-Smirnov test for goodness of fit. If the evidence indicates that the assumptions for an analysis of variance or *t*-test cannot be maintained, variables were transformed by logarithmic transformation. Percentages and proportions were always transformed by arcsine transformation, even if the test assumptions were verified. If none of the transformations manage to make our data meet the assumptions, we used the analogous non-parametric methods. In the case of two samples we used the Mann-Whitney U-test and in the case of several samples the Kruskal-Wallis test.

Linear regression models were calculated to explain the variation of the dependent variable in terms of a linear function of an independent variable. Regression was also used as a method for accounting the variation of the dependent variable y in terms of the independent variable x. Multiple regressions were used to account the variation of the dependent variable y in terms of variation of several independent variable x. To determine whether two variables are independent or co-vary, we determined the correlation coefficients and its significance (Pearson correlation). These tests were performed in continuous variables. Significance was accepted at 5 % level. (Sokal and Rohlf 1995).

2.3. Results

2.3.1. Pine stands characterization

To characterize de different stands, we made a comparison of tree density, average DBH and Basal Area, obtained in the year 2001, once we have a measure on all the stands on that year. The pine stands all differed significantly about the DBH (One-way ANOVA: F=1145.686, df=4, p<0.001; Tukey<0.005) with the larger value found in Apostiça 2, the stand with the oldest pines, which also presented a more diverse tree size, indicated by the highest standard deviation (Fig. 2.2). A higher density of trees was found in Pucariça and a lower in Apostiça 2 (One-way ANOVA: F=25.095, df=4, p<0.001; Tukey<0.05). Apostiça 1 and Barrada presented a lower Basal Area than the other three areas (One-way ANOVA: F=30.947, df=4, p<0.001; Tukey<0.005) (Fig. 2.2).

A high proportion of small trees (less than 1.60 m high) were only found in both areas in Apostiça and Barrada and, as expected, this proportion decreased as the stands grew older (Table 2.2). These trees were not included in the calculations of DBH. In all the other plots this proportion was close to zero.

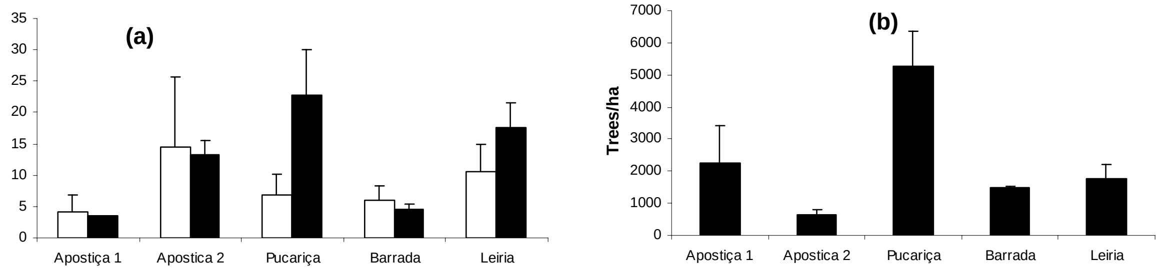


Figure 2.2: (a) Average tree diameter at breast height (DBH in cm; open bars), basal area ($m^2.ha^{-1}$; filled bars) and (b) tree density ($tree.ha^{-1}$). The bars represent the average (+ sd) for the year 2001 in each of the studied locations.

Table 2.2: Proportion (%) of trees less than 1.60 m high in the different seasons in Apostiça and Barrada.

Stand	1999/00	2000/01	2001/02
Apostica 1	-	16.0±1.7; 2	8.0±3.8; 2
Apostiça 2	-	17.3±12.4; 3	12.3±8.7; 3
Barrada	22.5±14.4; 2	2.5±2.3; 2	0.4±0.6; 2

2.3.2. Host selection and orientation

As would be assumed, the average DBH increases with age (years in Table 2.3). Except for Apostiça 2, the DBH of the attacked trees was larger than the DBH of non attacked trees (attack in Table 2.3). The different host selection behaviour in Apostiça 2 was due to the fact that in the season 2000/01 the common pattern was inverted since the attacked trees had a smaller DBH than non attacked trees (Fig. 2.3 and interaction in Table 2.3). However, in general female *T. pityocampa* selected the dominant trees in each stand, especially in young and homogeneous pine stands and, except in Apostiça, they never selected trees smaller than 1.60 m.

Among the attacked trees, a multiple regression indicates that the number of egg-batches per attacked tree depended both on the tree DBH (t=2.169, p=0.031) and on the pine stand (t=2.170, p=0.031) but not on the season (t=1.308, p=0.193). In general, we found no relation between egg-batch size and tree DBH except in the season 2001/02 in Apostiça 1 (r= 0.4; p=0.047), where females of low fertility selected smaller trees.

Table 2.3: Two-way ANOVA testing differences in DBH between years, of attacked and non attacked trees and their interaction in the different stands of the study. * significant test.

	Years			Attack			Interaction		
Stand	F-ratio	df	p	F-ratio	df	p	F-ratio	df	p
Apostiça 1	31.6	1	0.000*	133	1	0.000*	2.97	1	0.085
Apostiça 2	46.8	1	0.000*	9.6	1	0.002*	51.9	1	0.000*
Pucariça	3.07	2	0.047*	55.9	1	0.000*	0.15	2	0.86
Barrada	31.8	2	0.000*	110	1	0.000*	0.55	2	0.58
Leiria	11.0	2	0.000*	116	1	0.000*	0.44	2	0.64

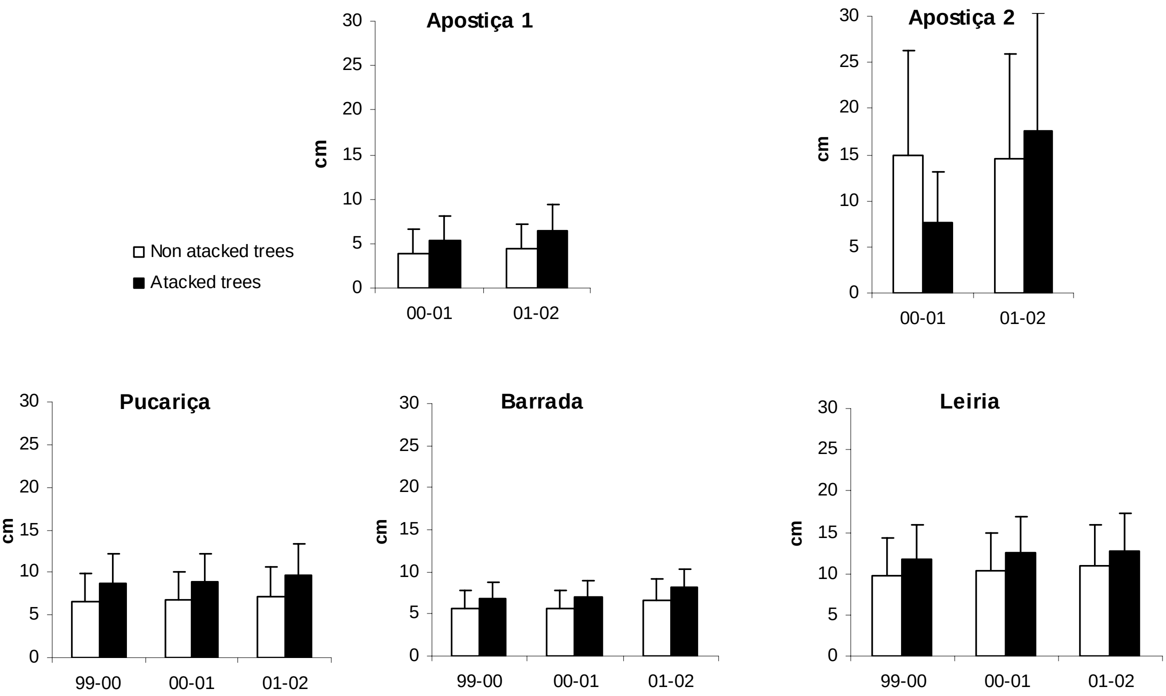


Figure 2.3: Mean DBH (+sd) of attacked (filled bars) and non attacked (open bars) trees during the different seasons in the five stands of the study.

Within each tree, females select the sunny part for egg laying (χ^2=29.06; df=1; p<0.001). During the first three instars the larvae are nomadic, spinning and abandoning a series of shelters constructed by enveloping a few needles in silk. When they reach the 4th instar they build a permanent cohesive structure and larvae become central place foragers. Larvae hatching in the shade either move their nests to the sunny part of the tree during their nomadic moves or die since the proportion of nests in the sunny part of the tree increases with age of larvae (χ^2=357.6; df=3; p<0.001) (Fig. 2.4).

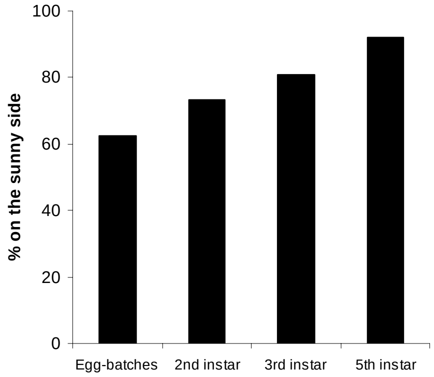


Figure 2.4: The proportion of egg-batches and nests of three age categories of developing *T. pityocampa* larvae situated in the sunny part of the tree. Data from all areas during two years.

2.3.3. Egg-batches analysis

In Apostiça, we found no significant difference in the number of eggs per egg-batch between stand 1 and 2 in the two seasons 2000/01 and 2001/02. However an interaction between stand and season was due to an increase in Apostiça 2 in the season 2001/02, the opposite of what was observed in Apostiça 1 (Table 2.4 and Fig. 2.5). Egg volume was higher in Apostiça 2 (0.69±0.09 mm^3, n=18) than in Apostiça 1 (0.62±0.06 mm^3, n=23). Egg mortality was higher in Apostiça 2 (Apostiça 1: 20.8±18.2, n=70; Apostiça 2: 30.0±21.9, n=88) (Table 2.4). Apostiça 1 was only sampled during two seasons and is not included in the full analysis.

Table 2.4: Two-way ANOVA testing for differences in the number of eggs per egg-batch, egg mortality and egg volume between the stands Apostiça 1 and 2 during two years of study. Egg mortality was arcsine transformed before statistical analyses. * significant test.

	Areas			Years			Interaction		
Parameters	F-ratio	df	p	F-ratio	df	p	F-ratio	df	p
Eggs/egg batches	0.30	1	0.59	0.22	1	0.64	6.74	1	0.010*
Mortality	11.4	1	0.001*	3.58	1	0.060	3.64	1	0.058
Egg volume	7.59	1	0.009*	0.37	1	0.549	0.93	1	0.34

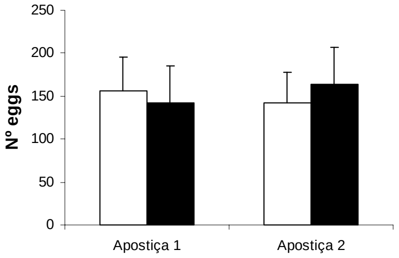


Figure 2.5: Mean (+sd) number of eggs per egg-batch, in the two stand of Apostiça in the seasons 2000/01 and 2001/02. Open bars represent 2000/01 and filled bars 2001/02.

The following analyses are based on samples from Apostiça 2, Pucariça, Barrada and Leiria from the three seasons 1999/00 – 2001/02. Overall, the number of eggs per egg-batch differed between the stands (Table 2.5; One-way ANOVA: F=18.708, df=3, $p<0.001$). This was due to Apostiça 2 having fewer eggs per batch when compared to Barrada and Leiria (Tukey: $p<0.01$) and Barrada had a significantly higher number of eggs per batch than Pucariça (Tukey: p=0.001). Thus, the number of eggs per batch varied in the pine stand sequence: Apostiça 2 < Pucariça < Leiria < Barrada (Fig. 2.6a). The differences between the years (Table 2.4; One-way ANOVA: F=6.64, df=2, $p<0.01$) were due to significantly fewer eggs per batch during the last season (2001/02) compared to the years 1999/00 and 2000/01 (Tukey: $p<0.01$). The relation between number of eggs per batch and season varied, however to some degree between areas as evidenced by a strong interaction term (Table 2.5). No significant differences were found between pine stands and seasons for the egg volume (0.68 ± 0.08 mm^3, n=111; Table 2.5) and no significant correlation was found between the egg volume and number of eggs (r=-0.13, Bonferroni $\chi^2=1.96$ P=0.16).

Table 2.5: Two-way ANOVA testing for differences in number of eggs per egg-batch, egg mortality and egg volume between the four areas and three years of study. Egg mortality was arcsine transformed before statistical analyses. * significant test.

	Stands			Years			Interaction		
Parameters	F-ratio	df	p	F-ratio	df	p	F-ratio	df	p
Eggs/egg batches	11.420	3	0.000*	3.510	2	0.031*	6.724	6	0.000*
Mortality	9.185	3	0.000*	21.812	2	0.000*	4.088	6	0.001*
Egg volume	1.72	3	0.169	1.182	2	0.311	1.321	6	0.26

The difference in egg mortality between the pine stands (Table 2.5; One-way ANOVA: F=1.18, df=3, $p<0.001$) was due to the fact that in Apostiça 2 and Barrada the mortality rate of the eggs was significantly higher than in Pucariça and Leiria (Tukey: $p<0.01$), especially so during the last two seasons (Fig. 2.6b). The difference between years (Table 2.5; One-way ANOVA: F=33.8, df=2, $p<0.001$) was due to a higher mortality rate during the last season compared to the two other seasons (Tukey: $p<0.02$). This increase was specially pronounced in Apostiça 2 and Barrada as evidenced by the significant interaction between pine stands and years (Table 2.5). Pucariça differed somewhat from the general pattern by having a higher mortality rate during 1999/00 than during the two following seasons (Fig. 2.6b).

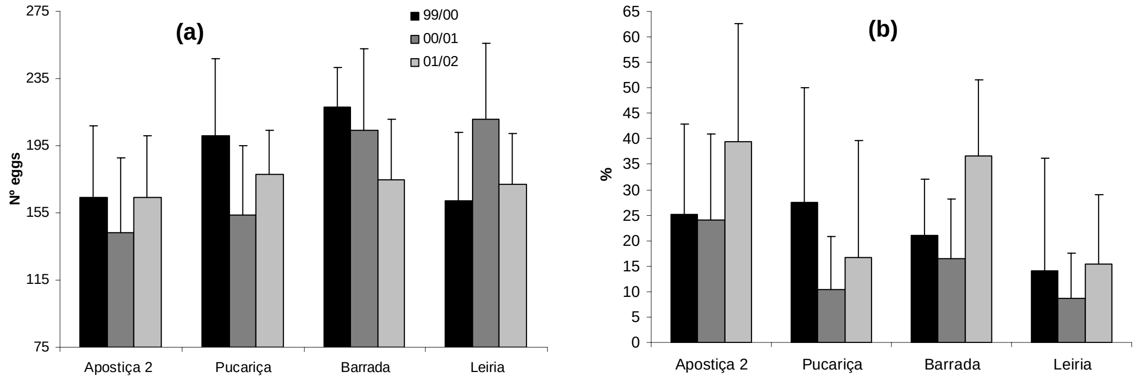


Figure 2.6: Mean (+sd) number of eggs per egg-batch (a) and mean (+sd) egg mortality (%) in the different pine stands and years under study.

2.3.4. Eggs per tree and initial larval survival

The proportion of trees with egg-batches, but without living larvae, can be used as an indicator of the mortality of whole batches of newly hatched larvae, since mobility of 1st and 2nd instars is very limited and movements among trees are hardly possible (own observations). This mortality represents the loss of the entire reproductive output of these females. This proportion of trees with total larval failure was significantly different in the five sampling sites during the two seasons of 2000/01 and 2001/02 ($\chi^2=10.59$; df=4; $p<0.05$) and was lower in Barrada (Fig. 2.7a). The highest whole batch mortality was found in the season 2000/01 in Pucariça with 73.3 % of the trees sampled in October and November with egg-batches, having no larvae.

There was a significant difference in the number of eggs per tree between the different areas (Table 2.6). This difference (One-way ANOVA: F=0.14, df=4, $p<0.001$) was due to more eggs being deposited per tree in Barrada (Tukey: $p<0.001$) than in the other pine stands (Fig. 2.7b).

In trees with egg batches where we also found larvae, it was possible to calculate survival of the hatched larvae in the early instar stage (1st-3rd instars). This gives us an idea of the larval mortality before they construct the permanent nests. However, we did not find any significant differences between the areas in this measure of survival (Table 2.6), which varied between

40 and 60 %. A negative correlation between the number of hatched larvae per tree and its survival was found in all areas, but it was only significant in Barrada (Table 2.7).

Table 2.6: Two-way ANOVA testing the differences in total number of eggs per tree and early larval survival in trees where both egg-batches and larvae were found between the five areas of study during the season's 00/01 and 01/02. Survival was arcsine transformed before analysis. * significant test.

	Stand			Years			Interaction		
Parameters	F-ratio	df	p	F-ratio	df	p	F-ratio	df	p
Eggs/tree	13.0	4	0.000*	0.83	1	0.37	1.15	4	0.34
Survival	2.12	4	0.084	3.82	1	0.053	1.60	4	0.18

Table 2.7: Correlation between the number of hatched eggs in a tree and the larval survival. *Significant correlation according to the Bonferroni probability test.

Stand	2000/2001, 2001/2002
Apostiça 1	r=-0.374; χ^2=3.389 p=0.066
Apostiça 2	r=-0.239; χ^2=1.030 p=0.310
Pucariça	r=-0.281; χ^2=1.031 p=0.310
Barrada	r=-0.414; χ^2=5.346 p=0.021*
Leiria	r= -0.101; χ^2=0.241 p=0.624

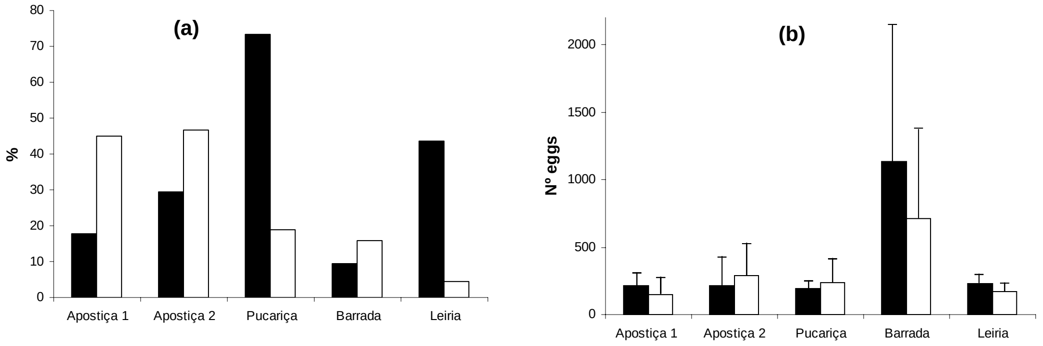


Figure 2.7: (a) Proportion (%) of trees with egg-batches but no living larvae and (b) the average (+sd) total number of eggs per tree in the seasons 2000/01 (filled bars) and 2001/02 (open bars).

2.3.5. Temporal changes in the number of larvae per nest and tree.

The number of larvae per nest in the 1st sample represents the number of larvae, mostly from the 2nd instar, which survived from one egg-batch. At this time larvae have a low capacity to move and individuals from different batches of the same tree do not get together, unless the egg-batches are deposited very close. This number indicates the early instar survival in each batch, in which at least some larvae survived and the optimum number of larvae needed per nest for initial survival. The number of larvae per nests in December the 3rd sample, represents the number of larvae in each batch that start to build a permanent nest. At this time, larvae originating from different egg batches in the same tree may have merged to build one nest. In December most larvae entered the 4th instar, the nests became permanent and the larvae central place foragers. The number in the 5th sample represents the final number from each nest that went to pupate.

The number of larvae per nest in the 1st, 3rd and 5th sample showed no difference between areas and years (seasons 2000/01 and 2001/02) in the two pine stands of Apostiça (Table 2.8). When analysing the four areas for which we also have this parameter in the season 1999/00, we found the 1st sample to differ between the years (Table 2.9) due to a smaller value in the season 2001/02 (One-way ANOVA: F=5.17, df=2, p=0.007; Tukey: p=0.006), but no difference between the stands. Thus, it seems that the high and variable mortality in the early instars, end up to level out the number of larvae in the first nests that hatched from egg-bathes of different sizes. In the 3rd sample there was no difference between pine stands and seasons (Table 2.9).

Table 2.8: Two-way ANOVA testing the differences in the number of larvae per nest in the 1st, 3rd and 5th sample in the two pine stands of Apostiça during the seasons 2000/01 and 2001/02. * significant test.

	Stands			Years			Interaction		
Sample	F-ratio	df	p	F-ratio	df	p	F-ratio	df	p
1st sample	0.545	1	0.465	2.690	1	0.110	0.662	1	0.421
3rd sample	2.913	1	0.098	0.896	1	0.351	0.136	1	0.715
5th sample	0.033	1	0.857	2.273	1	0.142	5.389	1	0.027*

Table 2.9: Two-way ANOVA testing the differences in the number of larvae per nest in the 1st, 3rd and 5th sample in the four areas of study during the seasons 1999/00, 2000/01 and 2001/02. * significant test. To explain the variation in the 5th sample, the number of larvae in the 1st sample was introduced as a covariate. This covariate was not significant (p = 0.32) related to the number of larvae in the 5th sample and were removed from the model.

	stands			Years			Interaction		
Sample	F-ratio	df	p	F-ratio	df	p	F-ratio	df	p
1st sample	1.56	3	0.203	4.48	2	0.013*	1.209	6	0.305
3rd sample	2.57	3	0.056	2.108	2	0.125	1.906	6	0.084
5th sample	3.50	3	0.018*	22.930	2	0.000*	3.364	6	0.004*

For the 5th sample, the difference between stands (Table 2.9) was due to a non-significantly smaller number of larvae in Leiria (One-way ANOVA: F=2.70, df=3, p=0.049; Tukey: p=0.09). The difference between the years (Table 2.9) was due to a higher survival of larvae in the season 1999/00 (One-way ANOVA: F=15.98, df=2, p=0.000; Tukey: p<0.001).

The minimum number of live larvae in one nest in the 1st sample was six and was recorded in Barrada in the season 2001/02 and the maximum number was 523 and was recorded in Leiria in 2000/01 and an average of 121.2±74.3 n=151 was found for all seasons and stands. In the

3rd sample the minimum number of larvae in one nest was 10 in Leiria and the maximum was 811 in Barrada, both in the seasons 2000/01 (average 103.5±95.7, n= 175). In the 5th sample a minimum of five larvae were recorded in Leiria 2001/02 and a maximum of 273 was recorded in Pucariça in 1999/00 with an average of 66.4±58.3 n=136.

The number of larvae per nest oscillates between months until December, after which numbers have a tendency to decrease (One-way ANOVA: F=15.98, df=2, p=0.000; October and December > January and February Tukey: $p<0.01$, November > February Tukey: $p<0.05$, Fig. 2.8). However a multiple regression indicates that the number of larvae per nest is predicted by the pine stand (t=7.390, p=0.000), and by the year (t=3.513, p=0.000), but not by the sampling month (t=0.676, p=0.500). The maximum number of larvae ever recorded in one nest was 1334 in Barrada in November 2000, consisting of larvae of 2nd-3rd instar, and in this area several nests with more than 500 larvae were recorded in November and December during the three seasons. Obviously larvae from different egg-batches in the same tree will get together. However, in January, the maximum number of larvae per nest recorded in Barrada decreased markedly to 346. After the larvae reach the end of the 3rd instar beginning of the 4th and start building the permanent nests, no colony was found empty, until the larvae start to pupate in February. Thus, colony extinction after this stage must be rare.

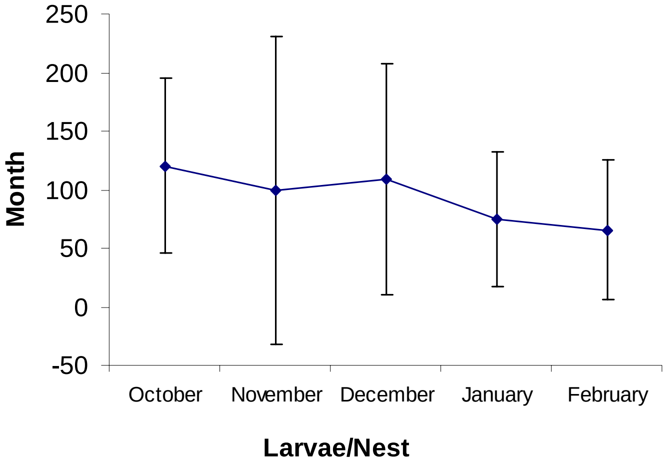


Figure 2.8: Temporal change in the number of larvae per nest along five sampling months. Average of all pine stands and the three seasons studied.

We found a significant difference in the number of nests per tree in both the 1st and 5th sample between the different areas (Table 2.10). This was entirely due to more nests per tree in

Barrada (Fig. 2.9). In the other areas, most trees just contained one nest. Thus, in Barrada, all larvae in the same tree did not aggregated in the same nest.

Table 2.10: Non-parametric tests of the differences between the number of nests per attacked tree in the five areas of the study and during the seasons 2000/01 and 2001/02. Data from 1st and 5th sample. * significant test.

Sample	Stands: Kruskal-wallis test			Years: Mann-Whitney U test		
	χ^2	df	p	χ^2	df	p
1st sample	23.486	4	0.000*	0.037	1	0.847
5th sample	17.768	4	0.001*	0.000	1	1.000

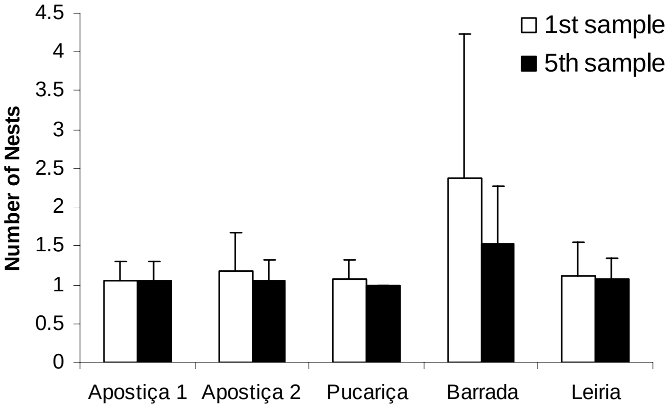


Figure 2.9: Average (+ sd) number of nests per tree in the first and fifth sample in the five stands during the seasons 2000/01 and 2001/02.

The number of larvae per tree in the 1st sample was significantly different between the areas (Table 2.11) due to a higher density in Barrada (One-way ANOVA: F=14.178, df=4, p<0.001; Tukey: p<0.001) (Fig. 2.10). In the 5th sample, the same difference between areas remained, although less pronounced (One-way ANOVA: F=5.159, df=4, p<0.001; Tukey: p<0.05) (Fig. 2.10). There was a tendency for a decrease in the number of larvae per tree in the season 2001/02, except in Leiria and Pucariça (Fig. 2.10). The value in the 5th sample didn't depend on the value in the 1st sample (Table 2.10), which means that mortality was different in trees with different initial larvae numbers. A multiple regression indicates that the number of larvae per tree is dependent on the pine stand (t=7.388, p=0.000), year (t=4.111, p=0.000) and also on the sampling month (t=-3.791, p=0.000), on the contrary to what was observed with the number of larvae per nest.

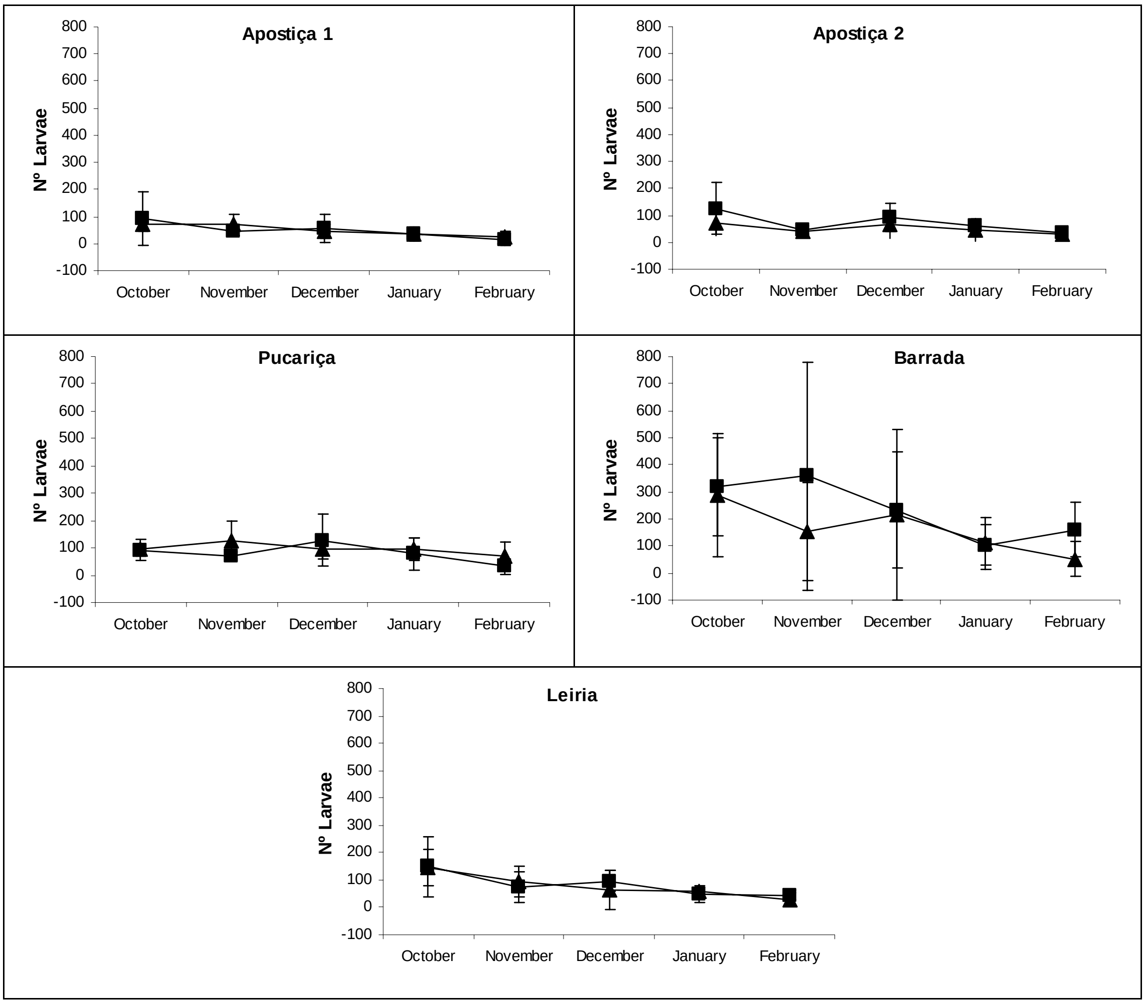


Figure 2.10: Temporal variation in the number of larvae per tree in the pine stands under study during two seasons. ■ - 2000/01, ▲- 2002/01. Average (± sd) of each month.

During the larval stage, the only mortality factor that was possible to quantify, was the one caused by *P. caudata*. The two areas of Apostiça had different death rates (Table 2.12), larvae in Apostiça 2 suffering from higher mortality than larvae in Apostiça 1 (t-test: $t_{44}=3.083$; $p=0.004$). The variation between the four areas: Apostiça 2, Pucariça, Barrada and Leiria during the three seasons of study (Table 2.11), depended on a higher mortality due to *P. caudata* in Barrada (One-way ANOVA: F=10.864, df=3, $p<0.001$; Tukey: $p<0.01$; Fig. 2.11). Differences between seasons (Table 2.11) were not confirmed by the One-Way ANOVA (One-way ANOVA: F=2.339, df=2, p=0.1), but *P. caudata* related mortality seems to be higher during the season 2000/01 (Fig. 2.11).

Table 2.11: Two-way ANOVA testing the differences in the number of larvae per attacked tree in the 1st and 5th sample in the five areas of study during the seasons, 2000/01 and 2001/02. The larvae density in the 1st sample had no significant (p = 0.43) effect on the density in the 5th sample. * significant test.

	Stands			Years			Interaction		
Sample	F-ratio	df	p	F-ratio	df	p	F-ratio	df	p
1st sample	13.408	4	0.000*	0.925	1	0.339	0.213	4	0.931
5th sample	9.091	4	0.000*	5.396	1	0.022*	8.386	4	0.000*

Table 2.12: Two-way ANOVA testing the differences in mortality (%) caused by *P. caudata* in nests with larvae at the end of the 4th instar in the two pine stands of Apostiça during the seasons 2000/01, 2001/02 and in Apostiça 2, Pucariça, Barrada and Leiria during three seasons. Mortality was arcsine transformed before used in statistical analyses. * significant test.

	Stands			Years			Interaction		
Stands compared	F-ratio	df	p	F-ratio	df	p	F-ratio	df	p
Apostiça	11.248	1	0.002*	6.016	1	0.018*	2.886	1	0.097
All pine stands	14.009	3	0.000*	4.252	2	0.016*	2.863	6	0.011*

2.3.6. Mortality during different stages of development

T. pityocampa survival from egg to the end of the larval period varied from 7 to 26% (Table 2.13). The mortality in the egg stage was lower in Leiria than in the other locations. Parasitoids play an important role in egg mortality and we found the Hymenoptera *Oencyrtus pityocampae* (Marcet.) and *Baryscapus servadeii* (Dom.) in all areas. In Leiria the Hymenoptera *Trichogramma embryophagum* (Htg.) was also found. Mortality due to parasitoids accounts for 15 to 77 % of the total egg mortality, the rest was due to embryonic mortality or mortality caused by parasitoids that failed to develop.

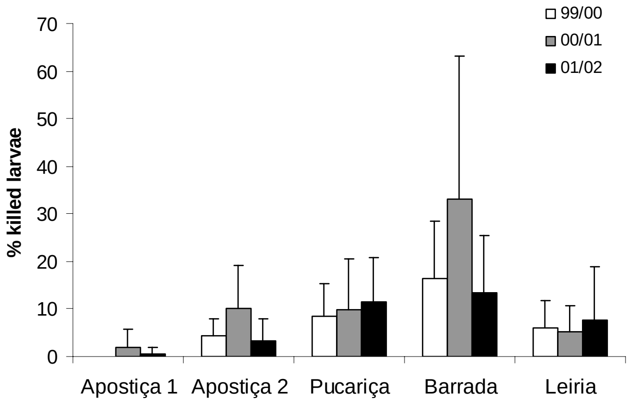


Figure 2.11: Mortality (%) caused by *P. caudata* in nests with larvae at the end of the 4th instar in all pine stands during three seasons of study.

Mortality of whole batches, probably shortly after hatching is very important and fluctuates widely in different seasons. This kind of mortality was very low in Barrada, but in this area a large number of egg batches per tree were recorded which might mask the true value. The loss of all larvae from one egg-batch in a tree with several other egg-batches would not be noticed and the correspondent mortality would be registered as mortality of 1st-3rd instars. The mortality in the 1st-3rd instar was high in all areas and seasons. Both early and 1st-3rd instar mortality were due to factors other than parasitism but were not possible to quantify with the present methodology.

Individuals of the family Tettigoniidae (Orthoptera) were found on the surface of temporary nests with 1st-3rd instar caterpillar in all areas studied until December. They were probably predating on the larvae (Martinho 2003), but all those nests had live larvae and the damages could not be quantified. Damages caused by birds are also hard to evaluate from visible cues on the temporary nests. An abundant arthropod predatory fauna was found inside the nests containing larvae between 3rd and 5th instar in all areas, specially the permanent nests. Individuals belonging to the Classes Diplopoda and Quilopoda were found, and also to the Families of insects Forficulidae (Dermaptera), and Carabidae (Coleoptera). Syrphidae (Diptera) larvae and pupae were found inside nests containing 5th instar larvae. Three species of Formicidae were found: *Formica fusca* (L), *Crematogaster scutellaris* (Olivier) and *Lasius emarginatus* (Olivier), but only represented by few individuals and never as a massive attack on a nest.

Table 2.13: Mortality (%) of eggs and different larval stages and final survival (%) in the different populations during the seasons 2000/01 and 2001/02.

Population	Season	Eggs		Loss of whole batches	1st-3rd instar	4th-5th instar		Final survival
		Parasitoids	Non-hatched			*P. caudata*	Other factors	
Apostiça 1	00/01	10.92	10.17	11.71	40.44	0.26	12.76	13.73
	01/02	12.08	8.42	25.36	19.51	0.12	23.98	10.58
Apostiça 2	00/01	13.13	10.88	19.09	30.63	2.57	3.25	20.44
	01/02	30.11	9.79	23.48	23.66	0.23	6.16	7.07
Pucariça	00/01	3.66	6.63	37.82	22.59	0.85	18.82	9.62
	01/02	9.77	6.93	10.67	28.89	4.51	13.75	25.47
Barrada	00/01	6.70	9.80	1.25	43.91	3.52	20.93	13.84
	01/02	21.66	15.05	2.56	24.50	2.19	27.00	7.05
Leiria	00/01	4.64	4.05	28.20	28.11	0.95	24.49	9.55
	01/02	1.33	7.36	5.44	27.47	2.37	31.15	24.89
Total		10.40±8.76	8.81±2.98	16.56±12.16	28.97±7.74	1.76±1.51	18.23±9.09	14.22±6.98

2.3.7. Larval development

A linear regression of the moisture content in the 1st, 2nd, 3rd, 4th and 5th instars showed a significant increase in moisture content with larval development (t=74.506 $p<0.001$). The larval moisture content decreased, however, significantly between the 5th instar and the pre-pupal stage (t-test: $t_{132} = 20.34$; $P < 0.001$; Fig. 2.12).

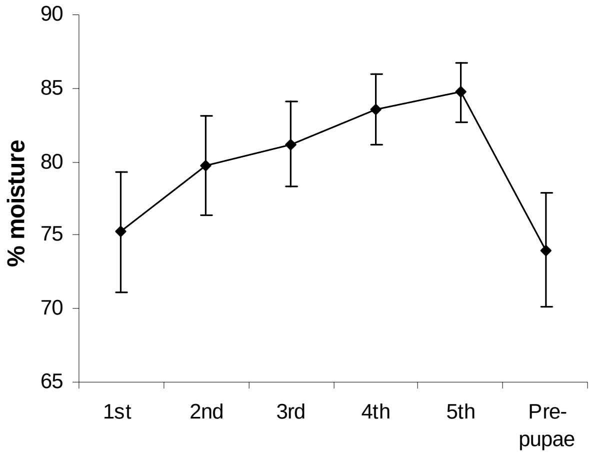


Figure 2.12: The moisture content of *T. pityocampa* larvae during the different stages of development (Average + sd).

In new-hatched larvae, hatched in the laboratory from egg batches obtained from Pucariça in 2000, we found the mass of a larva to be on average 0.59±0.03 mg wet weight (n=29 larval batches). With a moisture content of 75 % for 1st instar larvae (Fig. 2.12), the dry mass would be about 0.14 mg and since the egg size was similar in all populations, except Apostiça 1 in the season 2001/02, this can be considered to be the starting mass for almost all the populations. Once the pre-pupae final mass goes around 203 mg, *T. pityocampa* will go through a dry mass increase of about 1500 times during its larval development.

The drop of the pre-pupae moisture content makes them assume a torpedo like shape. This makes it possible to identify the 5th instar larvae that undergo the pre-pupation process, at a time when they have attained their final mass. Most of the larvae found at the pre-pupal stage, were kept in the laboratory where they pupated. For this reason, we only measured the final dry mass in a few samples (n=33; 203.0 mg ± 45.7) and we found no significant differences between the different areas or years ($p > 0.1$ in both cases).

A larger number of samples were obtained for the final wet weight (n=134; 743.7 mg ± 147.2) and significant differences were found between stands and years (Table 2.14). A One-way

ANOVA did not however, confirm the difference between the stands (One-way ANOVA: F=1.56, df=3, p=0.202). The differences between the years (One-way ANOVA: F=23.65, df=2, p<0.001) were due to a low final wet mass in the season 2000/01, a higher mass in 2001/02 and highest during the season 1999/00 (Tukey: p<0.002 in all comparisons; Fig. 2.13).

Table 2.14: Two-way ANOVA testing the differences in the pre-pupae final wet mass in the four stands in the seasons 1999/00, 2000/01 and 2001/02. * significant test.

Stands			Years			Interaction		
F-ratio	df	p	F-ratio	df	p	F-ratio	df	p
3.630	3	0.015*	27.024	2	0.000*	1.835	6	0.098

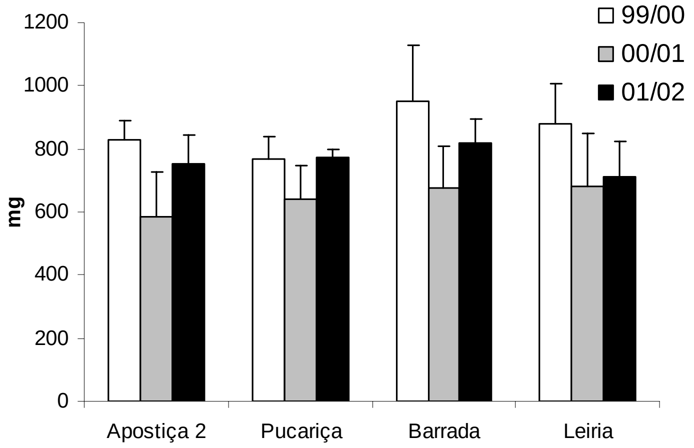


Figure 2.13: Final wet mass of pre-pupae in different pine stands and seasons (Average + sd).

The rate of development of the larvae was higher in Barrada and lower in Apostiça 1. Lower values were found in de season 2000/01 (Table 2.15). The winter 2000/01 corresponded to a climatically anomalous winter, with values of precipitation 2-3 times higher than the other two seasons studied, which can be considered as average seasons. A multiple regression, using precipitation, stand and year to explain the variation in growth rate, showed that only precipitation was significantly related to growth rate although area tended to explain some of the variation (precipitation: t = -2.53; p = 0.030; area: t = 2.00; p = 0.074; year: t = -0.81; p = 0.44).

A positive correlation was found between the larval speed of development and the final wet mass of pre-pupae on that season (r=0.782, p=0.003). A positive correlation, but with a marginal significance was also found between pre-pupae final wet mass and egg batch size on the following season (r=0.699, p=0.053).

Table 2.15: Linear regression between the dry mass of the caterpillars and the sampling dates. Total precipitation is calculated for the period September to February.

		Linear Regression	Precipitation (mm)
Apostiça 1	00/01	y=0.338x-6.652	810.4
	01/02	y=0.764x-23.351	309.4
Apostiça 2	99/00	y=1.351x-26.579	315.7
	00/01	y=0.729x-18.689	810.4
	01/02	y=1.097x-35.468	309.4
Pucariça	99/00	y=1.028x-23.275	288.1
	00/01	y=0.862x-2.356	1036.9
	01/02	y=1.136x-25.276	278.9
Barrada	99/00	y=1.863x-33.145	343.2
	00/01	y=1.308x-34.762	677.8
	01/02	y=1.526x-23.188	286.4
Leiria	99/00	y=1.076x-12.988	349.2
	00/01	y=0.828x-17.048	982.3
	01/02	y=1.131x-34.238	303.4

2.3.8. Larval distribution and densities

A major population crash was recorded in Pucariça, between the season's 1999/00 and 2000/01 (Fig. 2.14). This population crash was an isolated event, that wasn't repeated in any other stand, which precluded an evaluation of the evolution of *T. pityocampa* population dynamics between the seasons. This way we tested the differences between pine stands in the season with the higher attack rate recorded for each stand, so that we could evaluate the potential of a stand to be infested by *T. pityocampa*. The five areas differed in the proportion of attacked trees, with Barrada presenting a value higher that any other stand (One-way ANOVA: F=13.091, df=4, $p<0.001$; Tukey < 0.05). About the number of nests per ha, Barrada and Pucariça are different from Leiria and Apostiça 2 (One-way ANOVA: F=16.312, df=4, $p<0.001$; Tukey < 0.005), presenting Apostiça 1 a medium value. (Fig. 2.14). A multiple regression indicates that the final number of nests per ha is very dependent on the tree density of the pine stand (t=4.084, p=0.001), not on the average tree DBH (t=-0.280, p=0.783), nor the area of the pine stand location (t=-0.134, p=0.895).

A multiple regression indicates that egg-mortality is dependent on the proportion of attacked trees (t= 2.315 p=0.043) and it change considerably along the different seasons (t= 3088 p=0.011), but not with the different pine stands (t= 0.218 p=0.832). However we should notice that the higher rate of this type of mortality was found in Apostiça 2 and Barrada, two areas with very different attack rates by *T. pityocampa*. About mortality caused by *P. caudata* the pine stand explain part of the variation (pine stand: t= 2133 p=0.059; season: t= -0.233 p=0.820, proportion of attack trees: t = 1.436 p=0.182) although a significant relation was not found. Barrada, a pine stand with a high rate of *T. pityocampa* attack, presented the higher rate of mortality by this parasitoid. This indicates some density dependent mortality effect of parasitoids.

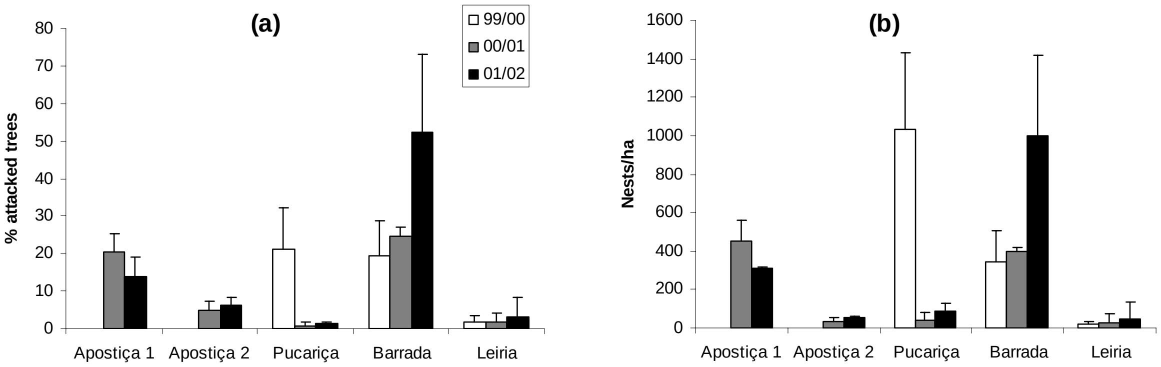


Figure 2.14: Level of attack of *T. pityocampa* in the different stands studied along the three seasons. a) % of attacked trees; b) Nests per ha (Average + sd).

2.4. Discussion

2.4.1. Host selection, egg-batches and nest orientation

From our results it is clear that dominant trees in young plots are preferably selected for oviposition. In all plots studied, aged 6-15 years old, *T. pityocampa* females selected the dominant trees except in Apostiça 2, the most heterogeneous plot with a large gap in tree age. Larger trees tended to receive more egg batches, especially in pine stands with high population densities like Barrada. *T. pityocampa* avoid very small trees (below 1.6 m), and oviposition on such trees was only observed in Apostiça.

Host size and insect size were correlated in highly host-specific species (Thompson 1983, Kirk 1991). *T. pityocampa* seems to require a minimum host size for egg laying, clumping their eggs in the same hosts even when other smaller hosts are available, as happened in Barrada. Selection of hosts according to its larger size is commonly described in Lepidoptera (Myers *et al.* 1981, Moore *et al.* 1988, Floater and Zalucki, 2000) and egg clumping on the same host even if these means lower larval survival (Myers *et al.* 1981).

Butterflies, moths and other insect herbivores often discriminate between host plants, depositing eggs on “high quality” plants that enhance offspring survival and performance (e.g.

Howlett *et al.* 2001). In contrast, other studies have found no evidence for higher egg densities on high-quality hosts. In these studies, host-plant “appearance”, influencing the likelihood of females locating a potential host (Courtney 1982, Karban and Courtney 1987) and the ability of females to balance the time/energy budget for finding potential oviposition sites (Underwood 1994), are suggesting to affect egg-laying patterns in a habitat. In this case, there is a complex relation between the patterns of use and the suitability of resources, leaving a part of suitable resources untouched (Soberon 1986).

According to Floater and Zalucki (2000) in a study made with a Thaumetopoea caterpillar with a similar life cycle as *T. pityocampa*, the proportion of high quality host-trees that receive eggs depends on the frequency of trees that are both of high quality and highly apparent. In young habitats most trees are small, and therefore relatively poor for larval performance, but because most trees are conspicuous females can exercise a high degree of host-plant discrimination and so deposit eggs on the best host-plants for offspring performance. In older habitats, however the closed structure of the habitat reduces the number of conspicuous host-trees amongst which females can choose, consequently females are forced to lay eggs on low quality hosts. So, the interaction between spatial variation on host quality and host appearance in a habitat would be expected to have important effects on the dynamics of these forest herbivores, including the frequency of pest outbreaks. All pine stands studied, except Apostiça 2, were even-aged young pine plantations with trees planted at a high density. Apostiça 2 is a more mature plot with lower tree density and a diverse age structure.

Females of *T. pityocampa* emerge with full complement of mature or nearly mature eggs and mating occurs a few hours after emergence (Zhang and Paiva 1998). Oviposition takes place late in the night of emergence or in the early hours in the morning (own observations). All eggs are laid as one clutch so that only one oviposition site needs to be found and the fact that adults can not feed are suggestive that females have been selected for fast oviposition. According to our observations, females of *T. pityocampa* can fly but they are poor flyers. They alternate periods of short flights with climbing vertically on a substrate with the wings stretched behind them. When they reach the top, they fly again downwind until they reach another substrate. This behaviour always takes place during the night. During the day they are inactive, so olfactory and tactile cues must be the most important for selection of an oviposition site. Young even-aged plots, with a high tree density, offer a large concentration of possible hosts in a limited space that can be searched in a short period of time, increasing the possibilities of finding a suitable host.

According to the model by Jones (1977) for oviposition in a gregarious caterpillar, the more potential hosts in a plot, the fewer eggs are laid on each plant, although more eggs can be produced in the plot as a whole. Thus, the more diverse tree size and age structure as well as the low density in Apostiça 2, reduces the number of potential hosts that can be searched in a certain time. As a result, lower quality hosts, in this case smaller trees, has to be accepted also increasing egg clumping in suitable hosts, even at a low density of moths. In Barrada, a higher population density of moths also makes egg clumping common. In this area, the number of eggs per attacked tree decreased in the last season, although the number of attacked trees and total number of egg-batches deposited increased. This might be due to the fact that the number of trees below 1.6 m decreased, as they grew, increasing the number of potential hosts.

Peres-Conteras and Tierno de Figueroa (1997) found a very significant positive correlation between the number of eggs per egg-batch and the host tree size in *T. pityocampa*. We could not, however, find such a relationship. It seems that *T. pityocampa* females select a host above a certain size, which might explain the common observation that pine plantations start to be attacked by the insect when the trees attain a certain size. The exception is Apostiça, which can be explained by *T. pityocampa* females having lower host choice possibilities due to the heterogeneity of the area. Another hypothesis is that lower fecundity females may accept small hosts when few hosts are available and in this area a lower fecundity was found. Thus, if food is more likely to run out on small hosts than on large ones, females would avoid food shortage by producing small clutch sizes on small hosts (Thompson 1983). In this way, females with a low egg load might be able to avoid competition.

Our study indicates that neither egg-batches nor caterpillar nests are randomly distributed on host tree. Sunny facing sections of the tree receive more eggs at all study sites. Oviposition in the sunny parts of a host has previously been found in *T. pityocampa* (Geri 1983, Geri and Miller 1985, Breuer *et al.* 1989) as well as in other tent social caterpillars (Moore *et al.* 1987). In some cases, the choice of warmer sections of the host has been connected to a faster rate of egg development (Williams 1981). It is also possible that some aspect of female behaviour is influenced by the exposure of the tree to the sun and thereby not being related to egg or larval survival (Moore *et al.* 1987). Several studies have suggested that egg distribution reflects areas of greatest female activity or areas with optimal conditions for mating and oviposition (Jones 1977, Rausher 1979, Courtney 1982).

Adult *T. pityocampa* are active only during the night. Mating occurs during the night when temperatures drop (Zhang and Paiva 1998). Not much is known about the timing and behaviour of oviposition, but females were often observed ovipositing in the early hours in the morning (own observations) following the night of emergence. Temperature or infrared gradients could influence the oviposition behaviour. Alternatively the female could respond to volatile emission from parts of the tree exposed to the sun during the day, since *T. pityocampa* female antennae are known to respond to its host volatiles emissions (Zhang *et al*. 2003).

Females will oviposit mostly during August, but the larvae will hatch one month later, mostly during late September to early October when Global Radiation is lower and rainfall can occur. The 1st instar larvae have a green colour, not dark or black like many social caterpillars, which would allow them to absorb the solar radiation (Bryant *et al*. 2000, Ruf and Fiedler 2002a). They also lack caterpillar setae, which are important for selective insulation, reducing the rates of convective heat exchange (Casey and Hegel 1981). Their small size increases the rates of heating but also of cooling, giving them a limited capacity of increasing their body temperature above the air temperature. The best thermoregulatory strategy for these animals is to control their body temperature by choosing micro-sites of the appropriate air temperature, in this case sunny locations (Stevenson 1985a, b). However, these larvae have a limited capacity to move to another location in the tree since 1st instar larvae are always found near their egg-batch (own observations). Thus, the location of the egg batch can be a very important factor for growth and survival of the early instar.

Moore *et al*. 1987 found that females from the western tent caterpillar also had a tendency to oviposit in the sunny area of the tree. However, this did not affect larval survival and the caterpillar's tents showed a tendency to be more homogeneously distributed in the tree than the egg-batches. In the present study, the nest distribution tends to become even more heterogeneously distributed in the tree as the larvae developed. This indicates either a better fitness of larvae in the sunny area of the tree or a tendency of larvae colonies to move to the sunny parts of the tree during their movements in the first three instars.

The nests of gregarious caterpillars have been connected to their thermoregulatory capacity either by keeping the metabolic heat production (Breurer and Devkota 1990, Ruf and Fiedler 2000) or increasing temperature by basking in the sun (Joos *et al*. 1988, Breuer *et al*. 1989, Ruf and Fiedler 2002a). In fact, location in the habitat (either at or away from the tent), orientation to the solar radiation and aggregation are behavioural mechanisms contributing to a higher body temperature of caterpillars in relation to the environment (Stevenson 1985a,b, Joos *et al.* 1988, Ruf and Fiedler 2002a). Thus, by locating their nests in sunny areas of the tree, *T. pityocampa* larvae receive some thermal advantages during their cold developmental season (Breuer *et al.* 1989, Breurer and Devkota 1990).

In *P. pinaster*, a higher photosynthetic rate at the upper part of the canopy results in needles having higher Nitrogen content than in the lower part of the canopy (Warren and Adams 2001). Young *P. pinaster* trees, growing in high-density stands, have a cone shape and permanent nests of *T. pityocampa* will preferably be located in the top of the tree. Thus, larvae will not only be in a more favourable thermal environment, but also close to more nutritious food.

2.4.2. Egg size, fecundity and larval batch size

The mean number of eggs per batch was lowest in Apostiça 2 in 2000/01 and Apostiça 1 in 2001/02 (142) and was highest in Barrada in 1999/00 – (218). The number of eggs per batch in Apostiça, was the lowest found for this species, only comparable to a study in Algeria with a mean of 154 eggs per egg-batch (Tsankov *et al.* 1995). The maximum batch size in this study was closer to the average found in the literature: 203-253 for different locations and years in Bulgaria (Tsankov *et al.* 1998), 193 and 208 in Greece (Bellin *et al.* 1990; Schmidt 1990) and 158-254 in four mountainous areas on the Iberian Peninsula (Schmidt *et al.* 1999). It was also clear that egg-batch size was not a population characteristic, but varied among years, indicating that it depends on environmental conditions.

It is generally acknowledged that there is a strong trade-off between egg size and number in insects (Berrigan 1991, Fox and Czesak 2000, Garcia-barros 2000) and this is due to that ovary volume scales to body size so that functional levels of locomotory performance can be maintained (Berrigan 1991). At eclosion the reproductive reserves available to the adult are restricted to the abdomen and their amount vary inversely with adults capacity to obtain nutrients (Boggs 1981). An increase in the size of the abdomen will lead to increased energetic costs of flying (Wickman and Karlsson 1989; Berrigan 1991). This is particularly important in species that do not feed as adults, like *T. pityocampa*, where all the resources used for reproduction are obtained during the larval stage, so-called Capital Breeders

(Tammaru and Haukioja 1996). In fact in *T. pityocampa* females, the abdomen size is strongly correlated with their number of eggs (Peres-Conteras and Tierno de Figueroa 1997).

In the present study egg size seem to be constant and not related to change in the number of eggs. Apostiça 1 appeared somewhat different presenting a low fecundity and the lowest egg size (0.61 mm^3 when the average for all the populations was 0.68 mm^3). Egg number seems to be larger in pine stands of high population densities like in Barrada (218 in the season 1999/00) and decrease sharply with a population crash as in Pucariça (from 201 in 1999/00 to 150 in 2000/01). A decrease in fecundity seems also to follow a season with lower pre-pupae mass as happened between the seasons 2000/01 and 2001/02. We found no trade off between egg size and number due to egg size being rather constant across areas and years.

Selection acts to maximize the reproductive success of the female parent such that eggs should be produced at an optimal size independently of female body size. If egg size should increase, there would have to be a positive correlation between egg size and offspring fitness (Smith and Fretwell 1974). According to Fox and Czesak (2000), the assumption of Smith and Fretwell (1974) that progeny fitness increase with progeny size and that there is a trade-off between progeny size and number, is true assuming that total reproductive effort is constant. If there is a change in the quantity of resources allocated to reproduction, egg size and number may change positively. Optimal allocation between egg size and number may depend critically on the female phenotype and should alter in relation to the conditions she encounters during the season (Parker and Begon 1986). Thus, the better the larval conditions, the higher fertility the female will have keeping an optimal egg size. If larval resources are drastically reduced, a minimum egg number will be kept and egg size decreases. This hypothesis is a good candidate to explain the results explained in Apostiça 1, where lower fertility was associated with small egg size. These might be due to the fact that survival in gregarious larvae depends on a minimum batch size (Stamp 1981, Fordyce and Agrawall 2001). In the present work it seems that the average egg-batch size for any *T. pityocampa* population never reduces to below 140.

Large egg-size in insects has been connected to fast development and hatchability of eggs, large hatchling mass (Ayres and Scriber 1994, Azevedo *et al.* 1997, Fischer and Fiedler 2001, Fisher *et al.* 2002), high larval feeding rate and larval and pre-adult development rates (Ayres and Scriber 1994, Azevedo *et al.* 1997). In fact Apostiça 1 presented the lowest larval speed of development for each season. However the population apparently did not suffer from a lower survival, although a decrease in the population number was observed in the season 2001/02.

If better larval development condition results in higher female fecundity, that will mean lower dispersion capacity from the adult eclosion area due to an increase in adult body size and decreasing flight capacity (Wickman and Karlsson 1989). Thus, if a plot has good conditions for larval development, the population density will have a tendency to increase even more in the next season, and the opposite will mean that the population will disperse more.

First instar larvae, emerging from one egg batch and feeding on the needles near by, usually have little opportunity to interact with individuals from other colonies. As a result, the primary colony structure is probably composed of siblings from one egg batch – unless several egg batches are laid very close on the same branch. Once the caterpillars have the tendency to move within the tree, older caterpillars experience a greater probability to interact with caterpillars from other colonies. This has also been found in other tent-building caterpillars (Costa and Ross 1993, Floater and Zalucki 1999).

The number of larvae per batch found in October, consisting mostly of larvae in the 2nd instar, was about 120 that is below the minimum number of eggs found in a batch which was 140. Several studies have reported on the need of a minimum group size for establishment in gregarious caterpillars (Stamp 1981, Fordyce and Agrawal 2001) and an optimal group size that maximises survival (Stamp 1981, Clark and Faeth 1997, Ruf 2002). It might even be so that egg batch size is above the optimal group size to compensate for egg mortality and early larval mortality (Stamp 1981). In *T. pityocampa,* the present work indicates a group size for early instars between 120 and 140 larvae, considerably lower than the average size of egg-batches.

Larval batch size is important for larval survival along all its development. Floater and Zalucki (1999) reported the extinction of colonies of the Australian processionary caterpillar whenever the number of larvae went below 10. *T. pityocampa* larvae move their colonies within the tree several times, building temporary loose nests each time, until it settles at the end of the 3rd instar (which usually happens in December) and a permanent nest structure is built. The production of silk is an energetically costly investment for any insect (Craig *et al.* 1999, Stevens *et al.* 1999) and an increase in the larval group size decrease the individual investment for the communal nest. The construction of the nest or tent in tent building caterpillars is considered to be important for survival (Ruff 2002) and in *T. pityocampa* this has to be done several times before the final establishment. It is probably an important survival factor to keep at least a minimum number of caterpillars per batch until final settlement. In the present work, about 100 individuals per batch were found at the time when most of the larvae were building their permanent nests, and the minimum number found was 10. The thermal ecology of gregarious caterpillars is also very dependent on group size by effectively increase their body mass (Stevenson 1985a) and the temporary nests have a limited capacity of increasing the temperature above the air temperature (Breuer *et al.* 1989).

2.4.3. Mortality factors and survival

Egg mortality found for *T. pityocampa* in the present study varied between 9% in Leiria in the season 2000/01 and about 40% in Apostiça 2 and Barrada in the season 2001/02. For this species, a larval hatching rate of 60-90%, can be considered as high when compared with data from other Mediterranean regions: 20-65% in Bulgaria (Tsankov *et al.* 1998); 56% in Algeria, (Tsankov *et al.* 1995); 68% in Greece (Schmidt 1990) and 47-80% in Spain (Schmidt *et al.* 1999).

According to the data obtained in the present work, the egg mortality, in which parasitoids play an important role, have a major impact on population density. Egg mortality seems to some degree to be dependent on density, as Barrada had the highest mortality rate and Leiria the lowest. Also the decrease in the population density in Pucariça was accompanied by a decrease in egg mortality. However, egg mortality does not seem to be strictly density dependent, since density cannot explain high rates of egg parasitism during in Apostiça 2. This area happens to be more heterogeneous and high levels of Hymenoptera parasitoids were found in heterogeneous forest when compared with even-aged monocultures (Bragança *et al.* 1998b).

Loss of whole batches in *T. pityocampa*, which represents the loss of the entire reproductive output of one female, can also be a very important mortality factor, but it varied considerably within areas and seasons. In demographic analysis of herbivorous insects, plant factors can cause important mortality in new-hatched larvae. Inability of neonate larvae to find leaves suitable for establishment can lead to high mortality, even if the same leaves are suitable for older instars (Zalucki *et al.* 2002). Weather factors also play an important role in this initial stage (Cornell and Hawkins 1995, Cornell *et al.* 1998).

Loss of entire colonies in the early instars has also been considered an important mortality factor in other tent building caterpillars and has been explained as failure to initiate feeding (Moore *et al.* 1988, Floater and Zalucki 1999), failure to build an initial tent (Ruf 2002) and predation (Costa 1993). Total loss of colonies in *T. pityocampa* has been explained by low host plant quality (Hodar *et al.* 2002). The highest proportion of this kind of mortality occurred in Pucariça during the season 2000/01, which coincided with a major population crash. This is in accordance with the hypothesis that hosts induce a chemical defence as a response to severe herbivory in the previous years (Haukioja 1980, Haukioja and Neuvonen 1987). The lowest proportion of loss of entire colonies was found in Barrada where the population was increasing. The loss of whole batches can also be due to Tettigoniidae (bush-crickets) predation – the most common predator during this initial development stage (own observation). These predators may also have an important impact on loss of whole batches before caterpillars hatch (Martinho 2003), which was not accounted for with the methodology used in the present work.

In batches that manage to establish in the host and in which at least some larvae survived, mortality was always high and proportional to the number of hatched caterpillars in a tree, especially in high density situations, like in Barrada. The present work shows that, although *T. pityocampa* larvae can attain very high concentrations of individuals because of merging of batches from several females, mortality is probably higher on these large aggregations. Numbers tend to level out before the final larval stages, when there is a dramatic increase in caterpillar biomass and consumption rate. Thus, this mortality does not seem to be due to depletion of resources neither to parasitoids, which did not appear until the 4th instar.

Other authors found increased mortality above an optimal group size in gregarious Lepidoptera (Stamp 1981, Tsubaki and Schiotsu 1982, Ruf 2002). Large larval aggregations have to move more to acquire food (Tsubaki and Shiotsu 1982), increasing the energetic cost of foraging and exposure to enemies. Predators and parasitoids often exploit clumped resources more efficiently than dispersed resources by concentrating their efforts in the area where the encounter rate is high (Royama 1970) and a tree infested with thousands of caterpillars is highly conspicuous. In fact, several females can lay their batches in the same tree, and there is a strong possibility of the resulting batches of larvae to merge. A higher mortality on larger aggregations makes the number of larvae per nest to fluctuate along the larval development. If we look at the number of larvae per tree, the numbers clearly decrease along the larval development, as expected.

Tettigoniidae demonstrated to be efficient predators of *T. pityocampa* egg-batches and larvae until the 3rd instar in laboratory tests (Martinho 2003). They were also found in the nests of larvae until the 3rd instar in the present study, indicating that they can be an important mortality factor in these stages. Several bird species are known to predate on *T. pityocampa* larvae. Great tits for example, predate on larvae of all instars, and this predation can be very important at high larval densities (Gonzalez-Cano 1981). Thus, birds do not seem to be hindered by the urticating substances in late instars (Gonzalez-Cano 1981), which has also been observed for bird predation on other tent-building caterpillars (Knapp and Casey 1986, Costa 1993, Ruf 2002). Holes apparently made by birds were occasionally found on permanent nests, but in none of them it led to colony extinction, and it was impossible to know how many caterpillars had been removed. It is difficult to estimate how many colonies are affected by bird predation, because caterpillars have the capacity to repair damaged nests (own observations).

After the 3rd instar, larvae start to build their permanent nests, become urticating and increase their rate of food consumption. Thus, this is the stage when competition would be most serious. However, on the contrary to what Hunter (2000) postulated for gregarious caterpillars, most of the immature mortality will occur before the final larval stage. This agrees with the suggestion that natural enemies will play such an important role in herbivore insect's demography that the effect of competition will be negligible (Cornell *et al.* 1998).

Although *P. caudata* is referred to as a major factor in the mortality of *T. pityocampa* (Biliotti, E. 1956, Geri 1983), the present work indicates that it does not seem to be a very important cause of mortality in the larval stage. This bivoltine Diptera laid an egg in 1st-3rd instar larva of *T. pityocampa* and the parasitoid larva will develop when the Lepidoptera starts moulting to the 5th instar, producing one parasitoid per parasitized larva. This 1st generation of parasitoids will lay their eggs in 5th instar larvae, where they will develop after *T. pityocampa* pupation, with 2-3 parasitoids emerging from each pupa (Biliotti 1956, Pimentel *et al.* 1999, Neves 2000). Thus, heavy mortality during the 1st-3rd instar will decrease the impact of these parasitoids on later larval stages. It should be noted that Barrada, the area with highest rate of attack and highest density of larvae, was the area with the highest proportion of parasitism and the mortality caused by this parasitoid seem to be dependent on *T. pityocampa* larval density. It has also previously been found that larger groups of larvae in a gregarious caterpillars suffers higher rate of parasitism (Stamp 1981). We should take into account that this parasitoid will also cause mortality during the pupal stage, so the mortality caused in the entire *T. pityocampa* life cycle may be much higher.

Predation by other arthropods also seems to play a very important role in reducing the population in all the *T. pityocampa* larval development stages. Floater and Zalucki (1999) indicate predation by arthropods to be the major cause of mortality in the Australian processionary caterpillar and Costa (1993) found a widespread low-level predation by these predators in eastern tent caterpillar colonies.

According to our data, mortality in the larval stages of *T. pityocampa* is divided into many factors and we cannot point out one as the most important. Furthermore, the importance of each factor changes between areas.

2.4.4. Larval development

A low pre-pupae mass was found in the season 2000/01, corresponding to a low speed of development and an exceptionally rainy season probably with consequent low levels of insulation. Has a probable consequence the fecundity in the following season decreased. The population in Barrada showed the highest rate of development, the highest larval final mass and the highest fecundity.

A relation between final mass and fecundity has been shown for Lepidoptera (Tammaru *et al.* 1996). The fact that fecundity is higher during the increase and peak phases of an outbreaking population and decreases when the population crashes is also known and such fluctuations in fecundity have been associated with fluctuations in population density (Ruohomäki and Haukioja 1992, Myers 2000).

Most of the mass increase in these caterpillars occurs during the coldest winter months, when average monthly air temperature stays around 10ºC. However, temperatures inside the nests were found to be as high as 35ºC under the incidence of solar radiation (Breuer *et al.* 1989). In fact Ruff and Fiedler (2002b) found that the number of foraging trips per day, in another tent building caterpillar, was mostly dependent on solar radiation and temperature, while precipitation only was of modulating significance. Caterpillars, being ectotherms, seem to benefit from favourable weather conditions by maximizing their rate of food intake up to a physiological threshold, when no further increase of digestion is possible. Temperatures also have an important effect on the time spent moulting, and this can also be an important factor for speeding up the rate of development (Ayres and Schriber 1994). Thus, the thermal environment experienced by *T. pityocampa* larvae, is dependent not only on the air temperature, which is not lower in rainy years, but on the number of hours of solar insulation. Solar insulation dramatically changes the temperature accumulation of the caterpillars. Since solar insulation is lower in rainy seasons, it may be part of the reason for why precipitation explains most of the variation in growth rate.

However, several studies indicate that different temperatures do not influence the final mass of the larvae, only the relative growth rate (Stamp and Bowers 1994, Lindroth *et al.* 1997, Frid and Myers 2002) that did not influence final mass (Knap and Casey 1986). Dry matter digestibility and nitrogen use efficiency varied across different hosts but not across temperatures, instead only the consumption rate varied with temperature (Ayres and Schriber 1994). Final pupal mass seems to be mostly dependent on host quality, especially on its Nitrogen content (Lindroth *et al.* 1997). However, the decrease in final mass with a long development period might be a consequence of increasing risks of predation and a halt in larval development might increase fitness in spite of decreasing female fertility (Ayres and Schriber 1994). In fact growth rates seem to be directly connected with fertility, as Barrada presented higher growth rates and fertility and Apostiça 1 presented lower values of this parameter.

2.4.5. Susceptibility of different areas

Forest structure plays an important role in determining high *T. pityocampa* population densities, presenting pine stands with high tree density a higher probability of an outbreak by *T. pityocampa*. Maritime pine stands with high densities (≥ 1 500 trees per ha) are mostly young even aged pure monocultures (DGF 2001) that did not pass through thinning operations. The differences found in both stands in Apostiça, illustrate this fact. Apostiça 1, the young even-aged homogeneous stand with a much higher tree density presented higher *T. pityocampa* population density than Apostiça 2, a more mature plot, with diverse tree age and low density.

As was discussed in point 2.4.1., an homogeneous, even-aged plot with high tree density potentially allowed the low mobility females of *T. pityocampa* to exercise a high degree of host selection, leading to better larval survival. This was the reason pointed by Floater and Zalucki (2000), to explain the higher susceptibility of young plantations to outbreaks of an Australian Thaumetopoea caterpillar with a similar life cycle as that of *T. pityocampa*, when compared to more mature stands. This is in agreement with the "resource concentration hypothesis" (Root 1973, Redfearn and Pimm 1987, Bragança *et al*. 1998a): Insect outbreaks tend to occur in dense homogeneous monocultures because herbivores can reach their host easily and successfully, allowing prompt population expansion. Theses systems also present a decrease in the natural enemy's abundance due to a more simple community structure (Bragança *et al*. 1998b).

In the present work we also found evidence that the area of the pine stand location is important in determine high population densities of *T. pityocampa.* The higher population densities were found in both stands in Abrantes. In Barrada the higher fertility and numbers of larvae per tree was found, parameters connected with population outbreak peaks. In Apostiça 1, although a high proportion of attacked trees was recorded (about 20 %), the population presented a very low fertility, which probably indicates worst development conditions, and compromise the capacity of the population to increase even more its numbers. In Leiria, only two plots out of seven studied, presented attack values above 1 % of infested trees. It's also curious the fact that the attack by *T. pityocampa* is visible in this area in stands older than 10 years, contrary of what is observed in the other two areas of the country. The infestation of a young plot by *T. pityocampa* probably depends on two factors: 1) colonization from surrounding areas, 2) good conditions for a high rate of increase inside the young plot. This way, in Leiria, areas with a previous low level of *T. pityocampa* population densities possibly surround the young plots, and somewhat the species have bad conditions to increase its numbers in those young plots.

P. pinaster is considered to be a drought resistant species that grows well in acid, infertile, sandy soils. It prefers high air moisture content and is intolerant to frost and cold weather, being well adapted to the north coastal areas of Portugal (Silveira da Costa 1984). Thus, the coastal dune system of Leiria is considered to be an optimal area for growth. On the contrary, the continental area of Abrantes, presents large fluctuations of temperature along the year and frequent days with frost. This might impose same stress in the host tree. Site quality was linked to forest insect's outbreaks (Kamata 2000, Alfaro *et al*. 2001, De Somviele *et al.*

2004). The common explanation proposed is that differences in soil characteristics change the host nutritional value and defences against herbivorous, affecting in a indirect way the insect development (Kamata 2000, Alfaro *et al*. 2001). Additionally climatic differences can have a direct effect in the larval development and adult fertility, as was proved in the present work (see discussion in point 2.4.4.). Leiria is in fact the area with lower levels of insulation, which might impose constrains in the larval development during the winter.

The present work was done only along three years, so we could not assess if *T. pityocampa* outbreaks present the long term cycles observed in other forest Lepidoptera outbreaking species (Myers 2000, Kamata 2000, Alfaro *et al*. 2001). We observed that *T. pityocampa* could attain very high densities in young plots. In Pucariça the outbreak reached its peak and collapse, in a pine stand with eight years old. This was an event only connected with this pine stand, once in the near by Barrada the insect population was increasing. This indicates that a smaller scale dynamic, connected with the landscape structure might play a very important role in *T. pityocampa* outbreaks. In Portugal, half of the *P. pinaster* stands are even aged monocultures, which makes half million ha. Clear cuts are made mostly in stands between 35 and 60 years (Silveira da Costa 1984). Leiria appears once again has an exception with clear cuts after 80 years (Ferreira and Gomes 2000). In fact, less than 20% of the even aged monocultures are above 60 years, and more than 40% are below 20 years (DGF 2001). Several studies indicate the preference of forest outbreaking insects for stands of the average and older age classes (Alfaro *et al*. 2001; De Somviele *et al*. 2004). However several factors can explain the *T. pityocampa* outbreaks observed in stands of younger age classes: 1) as was previously mentioned, the high resource concentration, once younger stands are the ones with higher densities; 2) a possible induced resistance and decreased nutritional value caused by herbivory (Haukioja 1980, Haukioja and Neuvonen 1987) in older stands which might have suffer from a previous *T. pityocampa* outbreaks, leading to a dispersion of the insect population to younger stands without a previous exposure to herbivory; 3) larger extensions of this younger pine stands, leading to an adaptation of the insect populations to the landscape dynamic.

2.5. Conclusions

In the present study, forest structure and its location appear to be important factors leading to *T. pityocampa* outbreaks. Young and even aged pine stands with high densities appeared as highly susceptible if located in South-Central area of the country. The same type of plantation located in the North West coast has a lower susceptibility. Future studies focusing the influence of soil and climatic variables on the nutritional quality of the host, would be useful to explain this patterns. On the other way more mature and diverse stands end up with a low susceptibility, even when located in high risk areas. In young and homogeneous plantations, the dominant trees are preferably attacked. Growth losses will therefore concentrate in this stratum. This imposes a problem for forest management once these are the trees that benefit most from thinning operations. Although weather interferes with larval development, and female's fertility, we could not find a clear impact on population densities, although these factors might lead to a reduction in the population rate of increase. Mortality factors affecting the early stages – eggs and early instar larvae – have a larger impact in reducing the population density. This way natural enemies affecting the early development stages are the best candidates for this pest biological control.

3. Dynamics of the pine processionary moth in the National Pine Forest of Leiria (Portugal): comparison of two desynchronized populations in the same pine stand.

3.1. Introduction

Recent theories connected Lepidoptera eruptivity with species-specific life cycle characteristics (Hunter 1991, Tammaru and Haukioja 1996), having some groups a large proportion of outbreaking species – like Noctuoidea and Bombycoidea – and others very few of this species or no species at all – like Sphingidae and Papilionoidea (Tammaru and Haukioja 1996). Although environmental factors and natural enemies can be important in determine population density fluctuations (Haukioja 1980, Haukioja and Neuvonen 1987, Martinat 1987, Berryman 1996, Myers 2000), specific characteristics as adults feeding and flying capacity (Tammaru and Haukioja 1996), larval gregariousness and development season (Hunter 1991) make outbreaking species different from the majority of forest insects which have low, stable population sizes and never cause noticeable defoliation (Nothnagle and Schultz 1987, Hunter 1991).

The processionary moths –Thaumetopoeidae- of the Mediterranean area belong to the genus *Thaumetopoea*. Of all its eight known species, only one - *T. herculeana* - which feeds on Geraniaceae, do not cause outbreaks, the other are reported as causing serious outbreaks in deciduous and conifers forests all over Europe and Mediterranean Basin (Douma-Petridou 1989, Schmidt 1989, Halperin 1990). The winter pine processionary moth *T. pityocampa* is maybe the most known of all processionary caterpillars, and is considered an economically important pine pest in the Mediterranean region, mainly due to the pine area expansion in the last century (e.g. Schmidt *et al*. 1990). According to Cabral (1979), *T. pityocampa* is considered part of the native fauna and is normally present in pine stands across Portugal.

Maritime pine has been present in Portugal since 55 000 years ago and was probably able to survive the latest glaciation's in sheltered areas at low altitudes close to the Atlantic Ocean (Figueiral 1995). Nowadays, most of the pine forests, which cover 1.2 million ha of the continental territory, are monocultures of this tree, and these figures were achieved mainly during the XIX century by massive forestation campaigns (DGF 1999). In fact Ribeiro *et al.* (2001) using polymorphic chloroplast microsatellite, didn't found any discernible geographic pattern for the Portuguese populations of *P. pinaster* and he explain the results by the recent specie range expansion and extensive gene flow among populations. National Pine Forest of Leiria, a managed pine wood located in the western coast of central Portugal, which extends over 11 000 ha, appeared somehow different, with the highest genetic variation, spanning most of the distribution range of the specie in Portugal. This proves the old origin of this pine and this area might have been the source of much of the transferred genetic material to the fast expansion of the specie on the country. In fact it was already in the XIII century that King Dinis included Leiria in the crown possessions and established the first management measures, mainly because of the protective function of the pinewood for stabilize dunes and protect agricultural fields. However its origin probably predates this event. Nowadays this National Forest provides an average annual yield off one million cubic meters of wood, 15% of each is constituted by trees with an individual volume of more than a cubic meter (Ferreira and Gomes 2000).

It was in this particular pinewood that in August 1997 an unusual outbreak of *T. pityocampa* was observed for the first time. On the September 1997 a very high density of larvae in a restricted area located at the south of the forest was reported. The larvae were already mostly in their last instar (M.R. Paiva personal observation), indicating that larval development had occurred during the summer instead of winter as it happens with the normal version of the specie. This desynchronized population of *T. pityocampa* has, since it was first recorded, been observed every year in the same area, attaining high densities. Here we present the results of a study of the population dynamics of both this unique population (summer population: SP) and of the normal one (winter population: WP), conducted between October 1999 and March 2003.

3.2. Materials and Methods

3.2.1. Area

National Pine Forest of Leiria is located 200 km north of Lisbon (Latitude: 39º50´N, Longitude: 8º57´W, Altitude: 30-50 m a.s.l.). For a description and location of the area see Chapter 2, point 2.2.1..

3.2.2. Meteorological data

Total insulation, total precipitation and average monthly temperature were obtained from the meteorological station of the Aerial Base of Monte Real (Latitude: 39º50´N, Longitude: 8º53´W, Altitude: 52 m a.s.l.), in the northern border of National Pine Forest of Leiria. Total insulation is the total number of hours of sunshine in a stated period of time. Total precipitation is the amount of precipitation, which reaches the ground in a stated period of time and is measured in millimetres with rain gauges. The average monthly temperature (ºC) is the average of the daily temperature during all days of the month. The daily temperature is calculated as the average of the recorded temperature in each of the 24 h of the day.

Global radiation corresponds to the total energy emitted by the Sun reaching the Earth. It includes the direct solar radiation and the diffuse sky radiation and is measured in Kcal/cm^2. The global radiation for our area and period of time was obtained by extrapolating the average monthly values observed at the 1938-1970 period in the area of Portugal between the Douro and Tejo Rivers - Central Portugal (Peixoto, 1981) with the insulation values for each year.

3.2.3. WP and SP- distribution and attack level

The present study was based on individual survivorship and densities along the larval development in the field and its final larval densities (see Chapter 2, point 2.2.3).

The study was conducted during three seasons for each population, between 1999 and 2002, in plots with trees aged 10-15 years. Between October and March, trees attacked by the WP were sampled monthly for *T. pityocampa* nests and egg batches in plots 24, 63 and 166 (Fig. 3.1). During the last two seasons (2000/01 and 2001/02) samples were also taken from plot 254. Between July and October of 2000, 2001 and 2002, SP samples were taken every two or three weeks in plots 282 and 298 (Fig. 3.1), located at the centre of the SP attack. For details see Chapter 2, point 2.2.3..

In the first season of WP sampling (1999/00), we randomly took 3-12 nests in each sampling location and all the egg-batches we could find, thus the sampling unit was nest not tree. In the first season of SP sampling (2000), samples were taken per tree, but the orientation of the egg-batches and larvae nests was not recorded.

After the WP larval periods were completed (starting in March), an estimate of the attack level by *T. pityocampa* was made, expressed as the proportion of trees attacked and the number of nests ha^{-1} (see Chapter 2, point 2.2.3 referring to Leiria). After the larval period of the first WP season (1999/00), surveys were made in plots 24, 63 and 166 (1.35-1.99 ha per plot, a total area of 5.14 ha). In the seasons 2000/01 and 2001/02 for the WP and 2000 and 2001 for the SP, surveys were made in seven plots, 24, 63, 166, 254, 283, 299 and 314 (1.35-1.99 ha per plot, a total area of 12.77 ha) covering all Leiria and the distribution area of both populations (Fig. 3.1). After the last season of the SP, the surveys were made in plots 283, 299 and 314 (1.95-1.99 ha, a total area of 5.90 ha) in March 2003. Nests from the two populations are easily distinguishable since SP larvae weaves very loose nests, that at the time of the year when the survey was conducted looked older than the ones from the WP, which appeared whiter, thicker and more glittering.

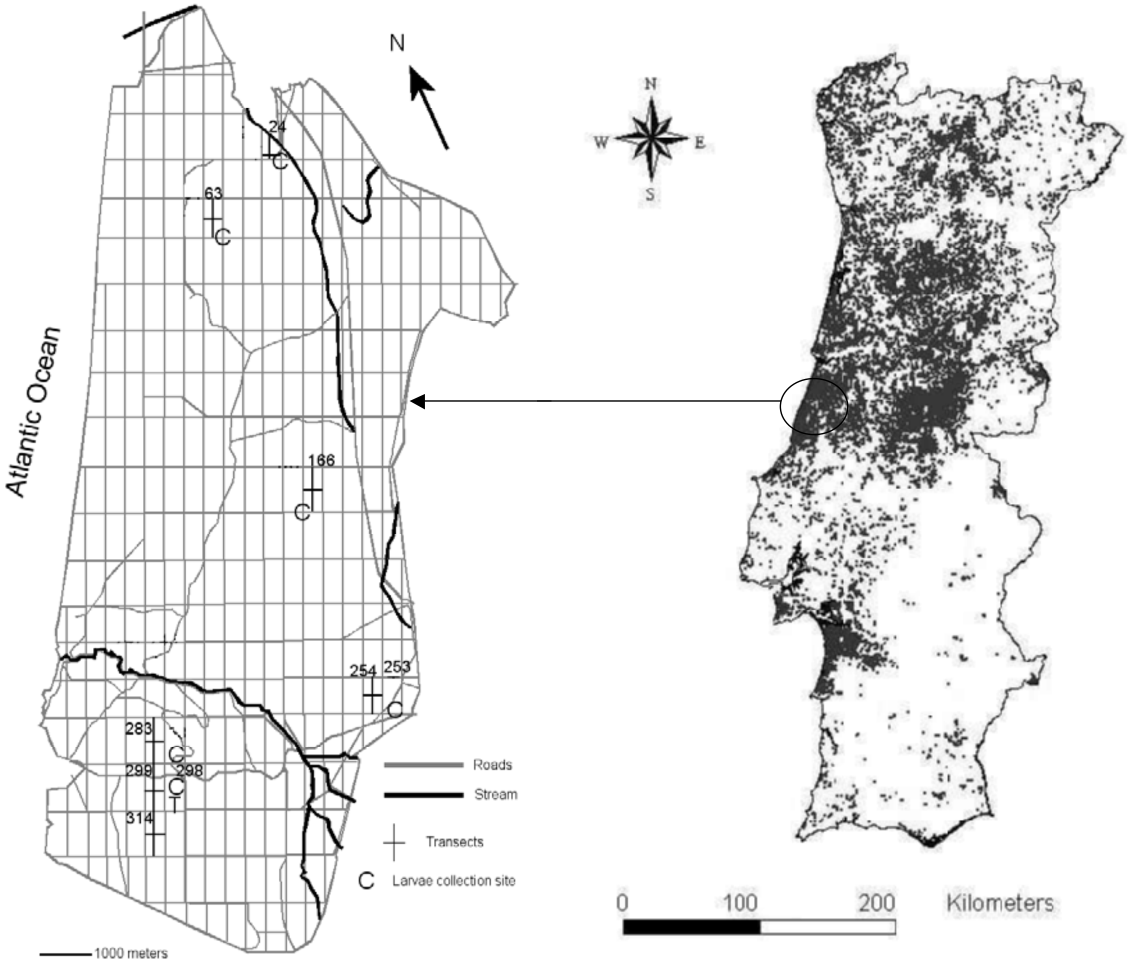


Figure 3.1: Location of the field surveys and larvae collection sites in National Pine Forest of Leiria, Portugal during 1999-2003.

3.2.4. Larvae and egg-batches

In the laboratory the structural characteristics of the egg-batches and mortality were recorded. The calculation of the number of eggs per tree and of the survival of the early larval instars was restricted to trees sampled in July and August for the SP, and in October and November for the WP. It wasn't possible to perform this type of calculation during WP first season because the samples weren't made per tree. Nests were opened and the larvae separated weighted and the average larval dry mass calculated by gravimetry (see Chapter 2, point 2.2.4, for details on the procedures).

3.2.5. Life table

To estimate the mortality of the *T. pityocampa* immature stages in the seasons 2000/01 and 2001/02, we used the ratio of the number dying in a stage (d_x) to an initial number of eggs (l_0) i.e. d_x/l_0 – see Chapter 2, point 2.2.5 - (Bellows *et al.* 1992)

3.2.6. Statistical analyses

The statistical analysis was performed using SYSTAT 10 and the probabilities shown refer to two-tailed tests. Chi-square tests are presented after the application of Yates' correction. Statistical analyses followed the procedures described in Chapter 2, point 2.2.6.

3.3. Results

3.3.1. Plot characterization

To characterize the different stands, we made a comparison of the variables obtained in the year 2001. All Plots included in this study had about 2000 trees ha^{-1} except Plots 63 and 299, which contained just over 1000 trees ha^{-1} (Fig. 3.2). The DBH in all Plots varying between 9.5 and 10.9 cm, with the diameter of trees in Plot 299 being a little larger (12.4 cm) (One way ANOVA: F=145.743, df=6, p=0; 299>24=254>166=283>63=314 Tukey<0.01). Also the Basal Area was very similar in all Plots (between 16.5 and 22.7 m^2 ha^{-1}) except in Plot 63 with a basal area of only 10.8 m^2 ha^{-1} (Fig. 3.2).

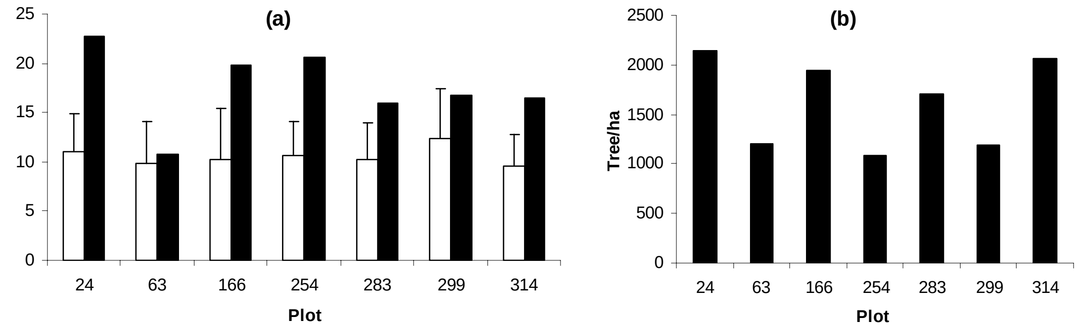


Figure 3.2: (a) Average (+s.d.) tree diameter at breast height (DBH in cm; open bars), Basal Area ($m^2.ha^{-1}$; filled bars) and (b) tree density ($tree.ha^{-1}$). The bars represent the values obtained for the year 2001 in each Plot.

3.3.2: Host selection and orientation

In the WP Plots (24, 63, 166), the average DBH increased with time (years) and the DBH of the trees attacked by the WP was larger than the DBH of non-attack trees. This was not the case in the SP Plots (283, 299, 314) where trees seem to be attacked irrespective of their DBH (Table 3.1 and Fig. 3.3).

Table 3.1: Two Way ANOVA testing differences between the average DBH of attacked and non-attacked trees and between the years in the two populations. The tests were performed on the three Plots in each population with a three year data-set. * significant test.

	Years			Attack			Interaction		
Population	F-ratio	df	p	F-ratio	df	p	F-ratio	df	p
WP	11.0	2	0.000*	116	1	0.000*	0.44	2	0.64
SP	141	2	0.000*	103.9	1	0.000*	71.9	2	0.000*

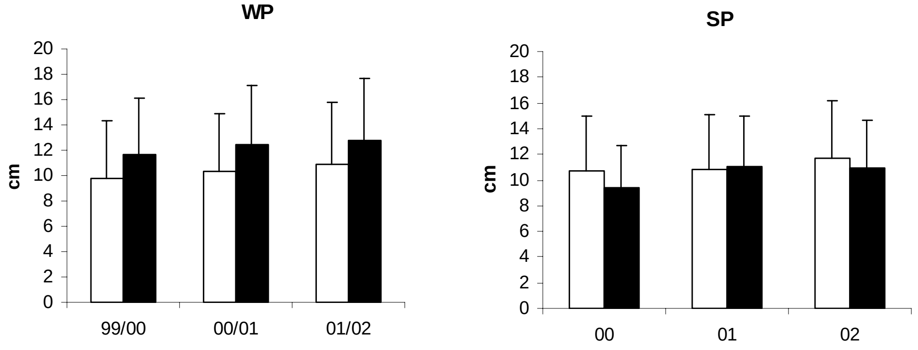


Figure 3.3: Mean (+s.d.) DBH of attacked (filled bars) and non-attacked (open bars) trees during three seasons in the WP and SP. Data from Plots 24, 63 and 166 for the WP and from 283, 299 and 314 for the SP.

In the WP, the females select the sunny part of the tree for oviposition (χ^2=5.53; df=1; p<0.05). During the first three instars the larvae are nomadic, spinning and abandoning a series of loose shelters constructed by covering a few needles in silk, but nests with larvae of the 4th instar looks like the permanent, cohesive structure and larvae become central place foragers. Since the proportion of nests in the sunny part of the tree increases with larval development (χ^2=30.6; df=3; p<0.001), larvae hatching in the shade move their nests to the sunny part of the tree during their nomadic moves or larvae in shaded nests have a higher mortality risk (Fig. 3.4).

In the SP, females select a spot for oviposition irrespective of being in the sun or in the shade (χ^2=1.11; df=1; p>0.2) but the larvae either move to the sunny part of the tree or shaded ones die (χ^2=198.91; df=3; p>0.001). In the SP there is a larger proportion of temporary nests in the shade than in the WP, but almost 100% of the permanent nests will be located in the sunny part of the tree, as in the WP (Fig. 3.4).

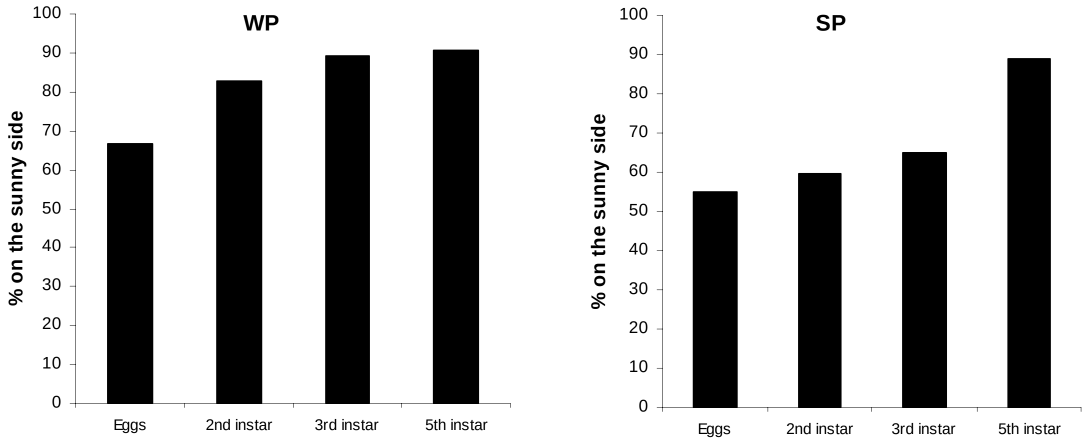


Figure 3.4: The proportion of egg-batches and larval nests situated in the sunny part of the tree in the WP and SP during two years of study.

3.3.3. Egg-batch analyses.

The number of eggs per egg-batch and the egg mortality was higher in the WP (Table 3.2 and Fig. 3.5). In the SP, the average number of eggs per egg-batch was similar in all years (One-way ANOVA: F=0.56, df=2, p=0.57) but in the WP, females produced more eggs per egg-batch in the season 2000/01 than in the other two seasons (One-way ANOVA: F=7.32, df=2, p=0.002; Tukey=0.001). Mean egg volume was generally higher in the SP (WP=0.70±0.09 mm^3 n=22; SP=0.77±0.08 mm^3 n=30; Table 3.2) but this difference was mostly due to large eggs being produced during the season 1999/00 (Fig. 3.5c), explaining the observed interaction in the two way ANOVA (Table 3.2). In spite of a tendency for egg volume to increase with egg numbers in the WP (Fig. 3.5), this was not significant (One-way ANOVA: F=2.10, df=2, p=0.15).

Table 3.2: Two Way ANOVA testing differences in the number of eggs per egg-batch, egg mortality (%), egg volume and ovary volume between populations and years. Egg mortality was arcsine transformed before statistical analyses. * significant test.

	Populations			Years			Interaction		
Parameters	F-ratio	df	p	F-ratio	df	p	F-ratio	df	p
Eggs/egg batches	0.16	1	0.00*	7.66	2	0.00*	3.66	2	0.03*
% mortality	14.45	1	0.00*	2.13	2	0.12	1.29	2	0.28
Egg volume	11.20	1	0.00*	0.86	2	0.43	6.42	2	0.00*

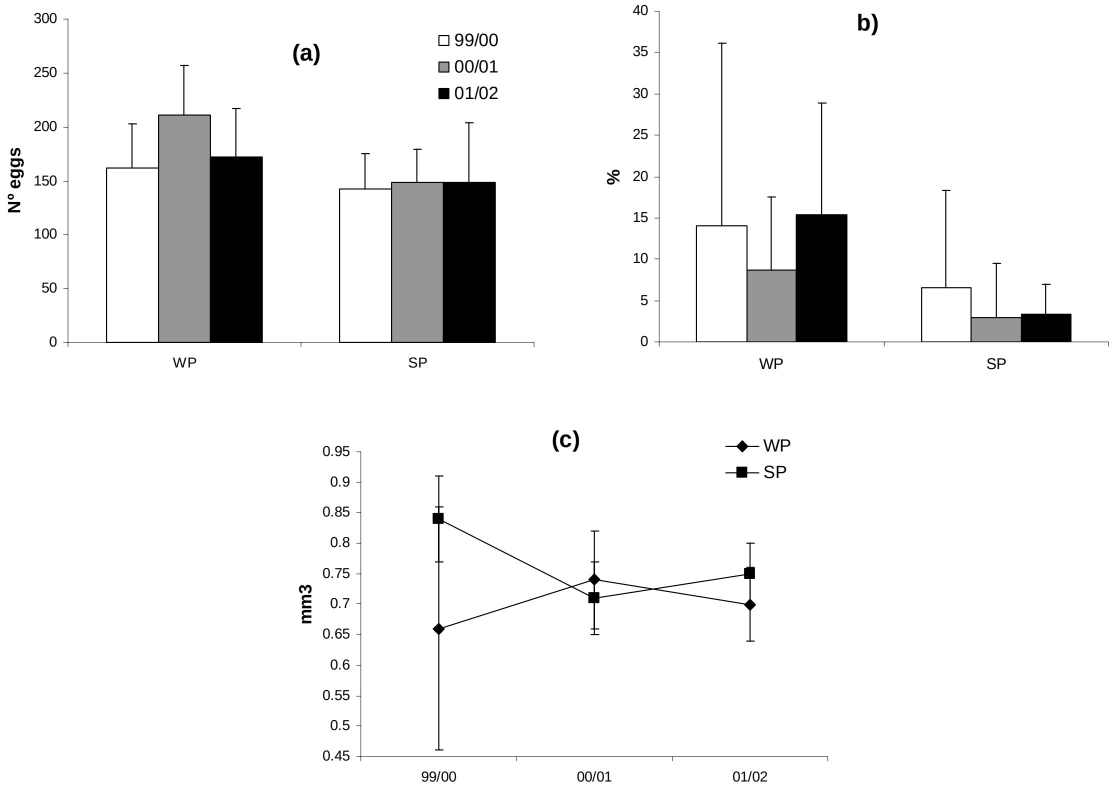


Figure 3.5: (a) Mean (+s.d.) number of eggs per egg-batch, (b) mean (+ s.d.) egg mortality (%), (c) mean (± s.d.) egg volume and in both populations during the three seasons.

3.3.4. Eggs per tree and initial larval survival

The proportion of trees with egg-batches, but without living larvae, varied significantly between the two populations and between the seasons (χ^2=5.63; df=1; p<0.02), because of a very high mortality of whole batches during the season 2000/01 in the WP (Fig. 3.6a). We found a higher number of eggs per tree in SP than in WP (Fig. 3.6b) and a decrease in both populations between the season 2000/01 and 2001/02 (Table 3.3). The survival of the hatched larvae in the early instar stages (1^{st}-3^{rd} instars) was similar between the populations and varied between 33 and 45 % (Table 3.3). Furthermore, we found no significant relation between the number of hatched larvae per tree and its survival in any of the populations (P > 0.6).

Table 3.3: Two Way ANOVA testing the differences in total number of eggs per tree and early larval survival in trees where both egg-batches and larvae were found in the two populations, WP and SP, during the seasons 2000/01 and 2001/02. Survival was arcsine transformed before analysis. *significant test.

	Populations			Years			Interaction		
Parameters	F-ratio	df	p	F-ratio	df	p	F-ratio	df	p
Eggs/tree	4.21	1	0.05*	6.43	1	0.01*	0.27	1	0.61
Survival	1.80	1	0.19	0.76	1	0.39	0.02	1	0.90

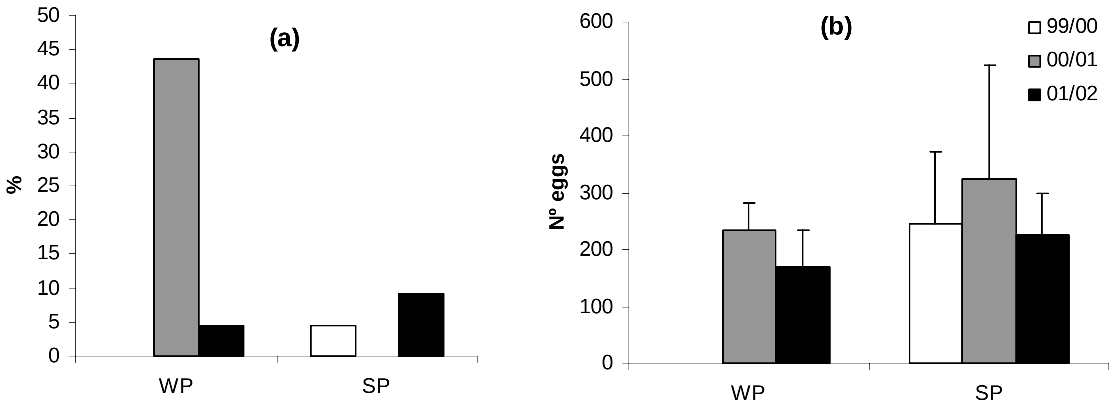


Figure 3.6: (a) Proportion (%) of trees with egg-batches but no living larvae and (b) the average (+s.d.) total number of eggs per tree in the different seasons.

3.3.5. Changes in the number of larvae per nest and tree

The number of larvae per nest in the 1^{st} sample represents the number of larvae, mostly from the 2^{nd} instar that survived from one egg-batch, since larvae from different batches of the same tree have little opportunity to get together. This sample is from September/October in the WP and in July in the SP. The number of larvae per nest in the 3^{rd} sample represents the number of larvae in each batch that start to build a permanent nest, which occurs when most larvae enter the 4^{th} instar. This occur in December for the WP, and at the end of August early September for the SP. The number of larvae in the 5^{th} sample represents the final number from each nest that pupate, which occur in February for the WP and October for the SP.

In the 1^{st} sample, the number of individuals per nest was similar in the two populations and in the different seasons (between 11 and 523; average: 124.6±68.8; n=83). In the 3^{rd} sample, we found a difference between the two populations (WP: n=45, 79.9±49.4; SP: n=55, 113.8±64.9; Table 3.4). In the 5th sample, the number of larvae per nest also differed between populations and this time also between seasons (Table 3.4). SP nests contained more larvae (65.34±42.83; n=53) than WP nests (42.22±28.94; n=46) and the number of larvae in the first season was higher than in the other two (One-way ANOVA: F=7.08, df=2, p=0.001; Tukey<0.05) (Fig. 3.7). The number of larvae in the 5th sample did not depend on the number found in the 1st sample as this number could not explain any of the variation in the 5th sample (p=0.3). The number of larvae per nest in was in fact more dependent on the population (t=19.187 p=0) than on the month of sample (t=-2.573 p=0.010), presenting higher values in the SP (Fig. 3.7).

The maximum number of larvae ever recorded in one nest in the WP was 523 in October 2000 with larvae of 2nd-3rd instar, and in the SP it was 338 in September 2000 with larvae of 3rd-4th instar. In neither WP nor SP, did we find any empty nests, i.e. colony extinction, after the building of the permanent nests.

Table 3.4: Two-way ANOVA testing the differences in the number of larvae per nest in the 1st, 2nd and 5th sample in the two populations and during the three seasons. * significant test.

	Population			Years			Interaction		
Sample	F-ratio	df	p	F-ratio	df	p	F-ratio	df	p
1st sample	0.276	1	0.601	0.798	2	0.454	0.333	2	0.717
3rd sample	7.512	1	0.007*	1.004	2	0.370	0.386	2	0.681
5th sample	5.537	1	0.021*	6.852	2	0.002*	0.069	2	0.934

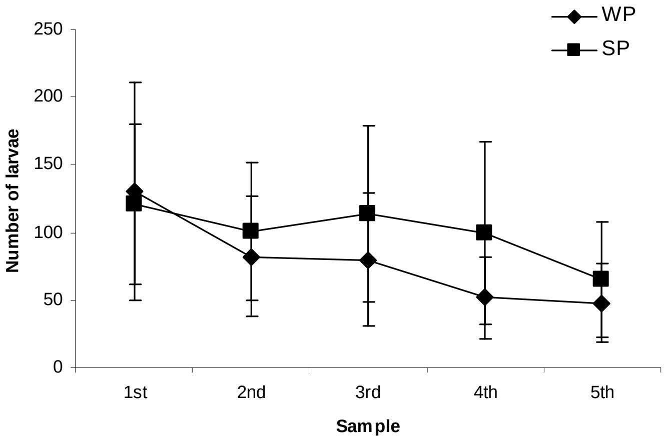


Figure 3.7: Temporal variation in the number of larvae per nest in the WP and SP. Average (±s.d.) of the three seasons for each sample/population.

In the season 2000/01 and 2001/02 the number of nests per tree in the 1st sample was higher in the SP than in the WP, a difference that disappeared in the 5th sample (Table 3.5 and Fig. 3.8). The number of larvae per tree was also higher in the SP, both in the 1st and 5th sample (Table

3.6 and Fig. 3.9). The number of larvae in the 5th sample was depended on the number found in the 1st sample (Table 3.7). The number of larvae per tree was dependent on the population (t=17.821 p=0) and month of sample (t=-5.978 p=0), presenting SP clearly higher values (Fig. 3.9).

Table 3.5: Tests for the differences between the number of nests per attack tree in the 1st and 5th sample and between the two populations during the season's 2000/01 and 2001/02. * significant test.

	Population: Mann-Whitney U test			Years: Mann-Whitney U test		
Sample	χ^2	df	p	χ^2	df	p
1st sample	11.536	1	0.001*	0.480	1	0.488
5th sample	2.109	1	0.146	1.833	1	0.176

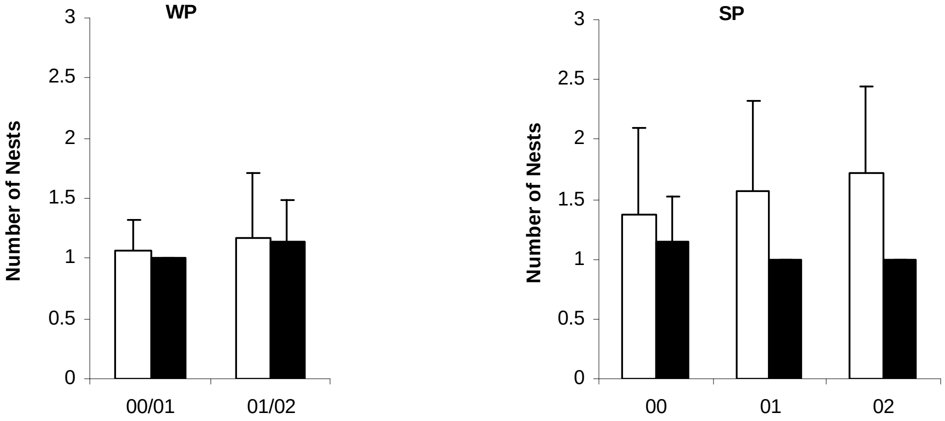


Figure 3.8: Average (+s.d.) number of nests per tree in the first (open bars) and fifth (filled bars) sample in the two populations during the seasons 2000/01 and 2001/02 for the WP and 2000, 2001, 2002 for the SP.

Table 3.6: Two-way ANOVA testing the differences in the number of larvae per attacked tree in the 1st and 5th sample between the two populations of study during the seasons 2000/01 and 2001/02. * significant test.

	Population			Years			Interaction		
Parameters	F-ratio	df	p	F-ratio	df	p	F-ratio	df	p
1st sample	6.185	1	0.016*	0.646	1	0.425	0.097	1	0.756
5th sample	8.315	1	0.005*	6.275	1	0.015*	0.073	1	0.787

Table 3.7: Two-way ANOVA testing the differences in the number of larvae per attack tree in the 5th sample between the two populations during the season's 2000/01 and 2001/02 and the co variation according to the 1st sample. *significant test.

Population			Years			Interaction			Covariate		
F-ratio	df	p	F-ratio	df	p	F-ratio	df	p	F-ratio	df	p
2.634	1	0.111	5.937	1	0.018*	0.194	1	0.662	6.349	1	0.015*

During the larval stage, the only mortality factor that was possible to quantify, was the one caused by *P. caudata*. We found no difference between populations or between seasons for this mortality factor (p>0.3). The average season/population mortality due to this factor varied between 4.9 % (SP 2000/01) and 8.9 % (SP 1999/00) of 4th instar larvae killed per nest (total average: 6.8±7.7, n=96).

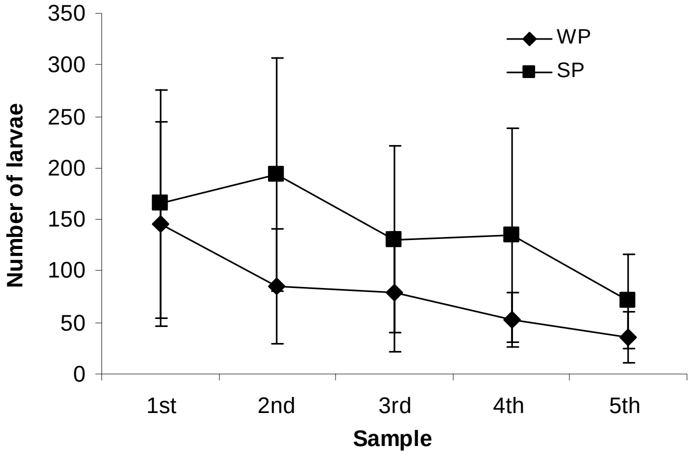


Figure 3.9: Temporal variation in the number of larvae per tree in the WP and SP. Average (±s.d.) of the different seasons for each sample/population.

3.3.6. The distribution of mortality factors during the egg and larval periods

T. pityocampa survival from egg to the end of the larval period varied from 10 to 25% in the WP and 15 to 35 % in the case of the SP (Table 3.8). The mortality in the egg stage was lower in the SP than in the WP, but had a low impact on the number of larvae pupating in both populations. Parasitoids play an important role in egg mortality and in the WP the Hymenoptera *O. pityocampae*, *B. servadeii* and *T. embryophagum* was found. In the SP, most of the parasitoids emerged before the egg-batches were collected. Mortality due to parasitoids accounts for 15.3% to 53.4% of the total egg mortality in the WP, and 9.2% to 51.25% in the SP. The rest of the egg mortality was due to embryonic mortality or mortality caused by parasitoids that failed to develop.

Mortality due to the loss of whole egg batches was very important in the WP during the season 2000/01. This had a large impact on the population during that season, which did not happen in the rest of the populations/seasons. The mortality in the 1st-3rd instar was high in both populations and seasons. Both types of mortality were due to factors other than the parasitism and were not possible to quantify with the present methodology.

Individuals of the family Tettigoniidae were found on the surface of the temporary nests with 1st-3rd instar caterpillar in both populations. They were probably predating on the larvae, but all those nests had live larvae and the damages were not possible to quantify. An abundant arthropod predatory fauna was found inside the nests, especially in the permanent nests. Individuals belonging to the Class Diplopoda, and to the Family of insects Forficulidae were found inside nests with larvae between 3rd and 5th instar in both populations. Additionally in the WP, Carabidae adults were fond in nest of the 3rd-5th instar and Syrphidae larvae and pupae were found inside nests with 5th instar larvae. Three species of Formicidae were found; *F. fusca*, *C. scutellaris* and *L. emarginatus*, but only in few numbers.

T. pityocampa permanent nest are structures that allow the colonies to get rid of excrements, dead caterpillars and parasitoid pupae, through its bottom side, which is typically found on the ground bellow the attacked tree. Only in the SP in the season 2001, we found a large number of dead larvae apparently killed by virus (liquefy tissues). The high mortality due to disease was the probable reason for the high mortality in the 4th-5th instar, observed in SP during this season, resulting in a low final survival (Table 3.8).

Although *P. caudata* is an important mortality factor when larvae are passing to the 5th instar, it does not have a large impact on the overall mortality of the immature stages. Holes apparently made by birds were occasionally found on the permanent nests, but it did not lead to colony extinction and it was impossible to know how many caterpillars had been removed. It is not possible to quantify the number of colonies affected by bird predation, because caterpillars have the capacity to repair damaged nests (own observations). A lot of caterpillars were found impaled in thorny vegetation in the SP distribution area, which also indicates bird predation.

Table 3.8: Mortality (%) of eggs and different larval stages and proportion (%) of eggs that produced larvae that pupated in the two populations (final survival) in the season's 2000/01 and 2001/02 for the WP and 2000, 2001 and 2002 for the SP.

Population/ Season		Eggs		Loss of whole Batch	1st-3rd instar	4th-5th instar		Final survival
		Parasitoids	Non-hatched			*P. caudata*	Other factors	
WP	00/01	4.64	4.05	28.20	28.11	0.95	24.49	9.55
	01/02	1.33	7.36	5.44	27.47	2.37	31.15	24.89
Total		2.99±2.34	5.71±3.90	16.82±16.10	27.79±0.46	1.66±1.00	29.48±5.71	17.22±10.85
SP	00	3.33	6.50	2.88	31.94	6.01	23.16	35.53
	01	0.27	2.64	0.00	33.76	1.79	46.94	14.57
	02	0.84	2.57	7.67	24.25	4.69	32.66	27.37
Total		1.48±1.63	3.90±2.25	3.52±3.88	29.98±5.05	4.16±2.16	36.41±12.81	25.82±10.56

3.3.7. Larval development

The final pre-pupae wet mass (general mean: 742.4 mg ± 135.5; n=90) did not differ between the populations (F=0.18; P=0.7), however the seasons differed significantly (F=8.20; P=0.001). The differences between the seasons were due to a higher final wet mass in the first season (Tukey: p<0.05, Fig. 3.10).

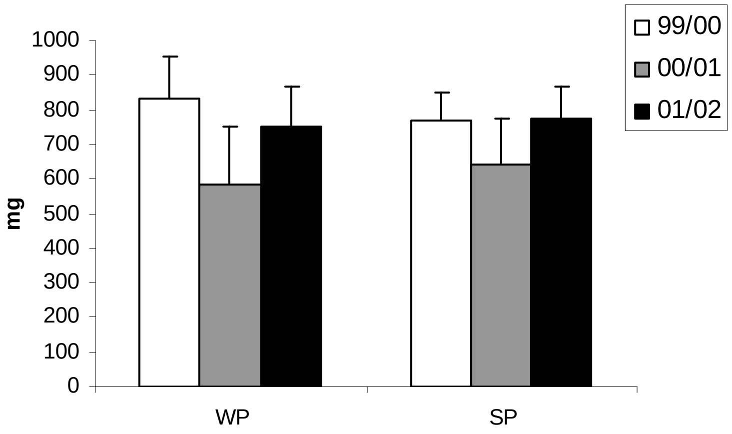


Figure 3.10: Average (+s.d.) final wet mass of pre-pupae in the two populations during three seasons.

During the three seasons of this study, the first larval nests of *T. pityocampa* SP were observed in mid to end July and the last processions occurred at the end of October. For the WP, the first nests were observed in early September, while the last processions occurred at the end of February to early March. Larvae were thus observed in the field during approximately 3.5 months for the SP and 6.5 months for the WP.

The growth rate of the larvae was calculated as a linear regression between the dry mass of the caterpillars and the sampling dates. The first sampling date is denoted as day 0 and was at the end of September to early October for the WP and at the end of July for the SP. The last sampling date, which was close to pupation, was at the end of February to early March for the WP (1999/00: day 103; 2000/01: day 136; 2001/02: day 134) and early to mid October for the SP (2000: day 76; 2001: day 85; 2002: day 84). The biomass of SP larvae increased almost twice as fast compared to that of WP larvae (Tables 3.9).

The number of hours of Insulation during the development time of WP larvae was higher than in the SP, but as the solar radiation is stronger during the summer, and the Global Radiation during the larval period was similar in both populations. The average temperature was higher during the SP larval period and the total precipitation was higher during the WP larval period

(Table 3.9). Larval development was more dependent on temperature and precipitation than on the other parameters (Table 3.10). It should be noted that the winter 2000/01 was exceptionally rainy with low insulation, resulting in the lowest rate of larval development during that season.

Table 3.9: Linear regression between the dry mass of the caterpillars and the sampling dates; total number of hours of insulation, total global radiation, total precipitation and average temperature during the months of September to February for the WP and mid July to mid October for the SP.

Parameters	WP			SP		
	99/00	00/01	01/02	00	01	02
Linear regression	y=1.08x - 12.99	y=0.83x - 17.05	y=1.13x-34.24	y=1.84x-20.72	y=1.94x-44.45	y=1.88x-24.45
Insulation (h)	1054	886	1048	827	698	681
Global Radiation (Kcal/cm^2)	42.7	41.3	46.2	51.1	43.12	42.5
Precipitation (mm)	349.2	982.3	303.4	105.0	118.8	107.6
Temperature (ºC)	13.1	13.9	13.5	18.7	19.5	19.3

Table 3.10: Multiple regression between the slope of the linear regression between dry mass of the caterpillars and sampling dates and climatic parameters for each population/season combination (see Table 3.10). * significant test.

	Linear regression: β
Insolation	t=-1.443; p=0.286
Global radiation	t=0.522; p=0.654
Precipitation	t=-15.212; p=0.004*
Temperature	t=15.661; p=0.004*

3.3.8. Distribution and density of larvae from the SP and WP

During the field surveys, the WP was recorded in all of the plots in all seasons, except in plot 253 in the season 2001/02. In the season 2000 the SP was recorded in three plots only, located in the southern part of Leiria (283, 299 and 314), but in 2001 this population was also detected in plots 166 and 63 (see Fig. 3.1). Although the SP reached plot 63 and 166 in 2002, less than 0.5 % of the trees were attacked. The hot spot of the SP were the plots in the south - 283, 299 and 314. In the first season of study, the most northerly of the plots –283- had the highest level of attack, but the moth seems to have a tendency to move south (Fig. 3.11 and 3.12). Due to these differences, we followed the same procedure as in Chapter 2, for comparison among normal populations (see point 2.3.8.). This way we compared the different Plots for each population, in the season when the attack rate was higher, so that we could evaluate the potential of each Plot to be infested, by each population.

The proportion of the trees attacked by the WP was below 1 % in all plots except the two in the north – 24 and 63 – which had an attack rate 15-400 times higher than in the other plots. The final number of nests per ha was 16-330 times higher. These two heavily attacked plots showed a clear increase in the attack rate over the years (Fig. 3.11 and 3.12).

The SP attacked a 2-4 times higher proportion of pines on its main area of distribution – plots 283, 299 and 314-, than the WP did in the two most attacked plots –24 and 63. The final number of larval nests ha^{-1} was about 2-5 times higher in the SP than in the WP.

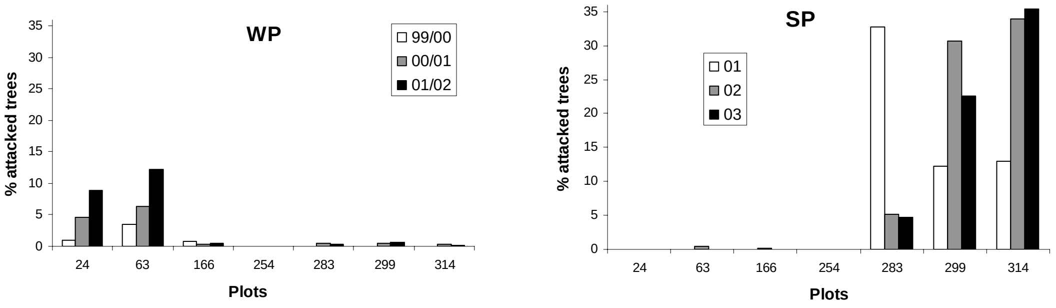


Figure 3.11: Proportion (%) of trees being attacked by the two populations in the different plots studied during the three seasons.

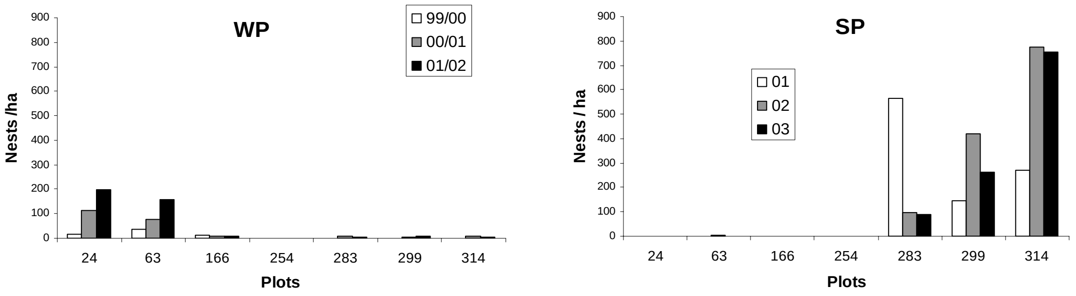


Figure 3.12: Number of larval nests per ha of the two populations in the different plots studied during the three seasons.

A multiple regression between the plot characteristics and localization in the Leiria Pine Forest, and the number of nests per ha, indicates that the attack rate is dependent on the plot location, not on its characteristics (Table 3.11).

Table 3.11: Multiple regression between the characteristics of each plot and its location and the number of nests per ha in each population. * significant test.

	WP	SP
Trees per ha	t= 0.661; p=0.545	t= 1.470; p=0.216
DBH	t= 1.809; p=0.145	t=-2.400; p=0.074
Location	t=-3.221; p=0.032*	t= 4.153; p=0.014*

3.4. Discussion

3.4.1. Host selection and orientation

Females of the normal WP select the dominant trees in the homogeneous young plots that we studied, just like what was found and discussed for normal winter populations in other areas of the country (Chapter 2, point 2.4.1). In the SP, on the other hand, we found no clear pattern of tree size selection.

The tree measurements were made in March for both populations, that means as soon as the WP larval period finished and before there was a significant increase in the tree size with. But the SP had finished the larval period in October and the major defoliation occurred in September-October, that means during the autumn leaf flush. Defoliations caused by *T. pityocampa* cause a loss in the maritime pine annual radial growth (Laurent-Hervouët 1986, Markalas 1998). The heavy defoliations observed in the SP attacked trees, that end up with a larger final number of larvae per tree than the trees attacked by the WP, might have caused a decrease in these tree radial growth, which might explain the differences found in both populations on the selection patterns. In the present work, we found that females from the normal population had the tendency to oviposit in the sunny areas of the tree, just like what was found and discussed for all normal populations of *T. pityocampa* in Portugal (Chapter 2, point 2.4.1) something that did not occur in the SP.

Most of the females from the WP oviposit during July-August, but the larvae hatch one month later, mostly during September, when the Global Radiation is lower and rainfall can occur. The SP larvae will hatch during the driest and sunniest month of the year (July), so egg-batch location in the tree might not be such an important survival factor. The exposition to stronger solar radiation might even have a negative effect on newly hatched SP larvae.

Adults of the SP emerge at a colder and wetter season (May-June: average temperature and total precipitation, 16.5ºC and 87.6 mm) than adults of the WP (July-September: 19.2ºC and 43.8 mm) and these are also the conditions that the eggs will face during the incubation period. We do not know how these different conditions will influence the female's oviposition behaviour. Since the warmer sections of the hosts are connected with a faster rate of egg development (Williams 1981), it would be advantageous from the egg point of view, to be placed in warmer sections of the tree, on the contrary of what was observed in the SP. In fact the scales covering the egg batches were much darker in the SP (own observations), indicating an increased need of capturing the lower solar radiation during the SP egg incubation period. Thus, the different oviposition patterns in the two populations might be due to a change in adult activity due to different conditions or to lower survival of newly hatched larvae in areas strongly exposed to solar radiation.

The nests for both populations, showed a tendency to have a more clumped distribution in the tree as larval developed. This indicates a preference of the larvae for the sunny area of the tree and that they have a tendency to move to the sunniest parts of the tree during the nomad movements in the firsts three instars. However, the location of temporary nests of 2nd and 3rd instars still differed between the populations with WP nests in sunnier locations than SP nests.

Orientation to the solar radiation and aggregation behaviour are behavioural mechanisms contributing to the body temperature excess of caterpillars in relation to the environment (Stevenson 1985a,b, Joos *et al.* 1988, Ruf and Fiedler 2002a). These facts explain the preferable location of the WP nests in sunny areas of the tree, as also reported by other authors (Breuer *et al.* 1989, Breurer and Devkota 1990), giving them some thermal advantages during their cold development season. In the SP the movement to more sunny locations is slower, but at the end the permanent nests will also end up mostly in sunny locations. This fact does not present an obvious advantage during its warmer developmental season. Young *P. pinaster* trees, growing in high-density stands have a cone shape, and permanent nests will preferably be located in the top of the tree. The higher photosynthetic rate at the upper part off the canopy of the host tree results in higher Nitrogen content (Warren and Adams 2001). This fact may help to explain the distribution of permanent nests in the SP.

Silk is an energetically costly investment for any insect (Craig *et al.* 1999, Stevens *et al.* 1999) and from our observations, SP caterpillars invest much less of their resources in their nest. In fact nests from the two populations are easily distinguishable since SP larvae weave very loose nests that usually look brownish and opaque and they are easy to handle in the laboratory since we only need to stretch the fibbers a bit to reach the larvae. On the contrary, nests from the WP are whiter, thicker and glittering and the fibbers have to be cut with a sharp pair of scissors. This indicates a lower need to invest in thermoregulation, once the thickness of the nests is connected with the larval group capacity to increase its temperature (Breuer *et al.* 1989). Also tent-building and behavioural thermoregulation in caterpillar not only allow them to increase their body temperature, but also to avoid overheating (Knapp and Casey

1986, Joos *et al*. 1988, Fitzgerald and Underwood 2000, Ruf and Fiedler 2002a) and water loss (Klok and Chown 1999), and this fact might helped the SP larvae to adapt to its new seasonal developing time.

3.4.2. Egg size, fecundity and larval batch size

The mean number of eggs per egg-batch for the SP (143-149) was the lowest found for this species, only comparable to a study in Algeria with a mean of 154 eggs per egg-batch (Tsankov *et al*. 1995) and with values found for Apostiça in the present study (142) (Chapter 2 - 2.4.2.). The mean number of eggs per egg-batch for the WP (162-211) was close to the average figures found in the literature: 203-253 for different locations and years in Bulgaria (Tsankov *et al*. 1998); 193 and 208 in Greece (Bellin *et al*. 1990; Schmidt 1990); 158-254 in four mountainous areas on the Iberian Peninsula (Schmidt *et al*. 1999).

A strong trade-off between egg-size and number was observed in the two populations in Leiria, with WP producing more eggs and SP producing larger eggs. This is a pattern generally found among insect species (Berrigan 1991, Fox and Czesak 2000, Garcia-barros 2000). However this trade-off wasn't observed among the different normal populations studied in the present work (see discussion in Chapter 2, point 2.4.2.)

Larger egg-size in insects has been connected to faster rate of egg development and hatchability, larger hatchling mass (Ayres and Schriber 1994, Azevedo *et al*. 1997, Fischer and Fiedler 2001, Fisher *et al*. 2002), higher larval feeding rate and faster larval and pre-adult development rates (Ayres and Schriber 1994; Azevedo *et al*. 1997). In fact newly hatched SP larvae from egg batches hatched in the laboratory, were larger than larvae from another WP – Pucariça - with similar egg size to the Leiria WP (Pucariça 2000: 0.59±0.03 mg Wet mass n=29 larvae batches; Leiria SP 2001: 0.71±0.05 mg Wet mass n=42 larvae batches).

The needles of *P. pinaster* have a long life span, about six years, and an annual foliage mass turnover of just 18 %. This species also has a low leaf specific area, which means tougher needles, with lower nitrogen content when compared with *Pinus radiata* (D. Don), a pine species with more flexible needles and a higher needle turnover rate (Warren and Adams 2000). In fact *T. pityocampa* larvae showed lower growth rate in *P. pinaster* when compared with the exotic *P. radiata* (Pimentel *et al*. 2000) and the maritime pine is considered a host species where the caterpillars show low survival (Hodar *et al.* 2000). In spite of this fact, in

Portugal, where most of the pine forests are plantations of *P. pinaster*, outbreaks of *T. pityocampa* are very frequent, but so far not in the National Pine Forest of Leiria (Chapter 2). The success of the SP might be due to a larger size of newly hatched caterpillars, which can be connected with a larger head capsule (Fischer *et al.* 2002) helping them to overcome the mechanic characteristics of the pine needles in that area.

However not always has a positive correlation been found between egg size and hatchlings fitness (Wiklund and Karlsson 1984) and few studies have demonstrated a relationship between progeny size and fitness in different environmental conditions (Fox and Czesak 2000). We should also keep in mind that in gregarious larvae, survival is also dependent on batch size (Lawrence 1990, Clark and Faeth 1997).

Temperature is an environmental factor known to strongly influence egg size in insects, although its influence on progeny fitness remains uncertain. Several studies have found an increase in egg size with altitude and latitude (Wiklund *et al.* 1987, Azevedo *et al.* 1996a, Fischer and Fiedler 2001). Selection for larger size was also found in laboratory populations of *Drosophila melanogaster* (Meigen) reared at lower temperatures (Azevedo *et al.* 1996a). In egg production two stages can be discerned; a differentiation stage with follicle production and a growth stage with yolk accumulation (Nation 2002). According to Ernsting and Isaaks (2000) egg size increase with temperature if follicle production is more temperature sensitive than yolk production. These authors also postulate that temperature influences the rate of egg production but the rate of egg production by it self is connected with egg size, lower rate of egg production resulting in larger eggs.

During the WP pupal period, occurring between March and August, the average temperature is 16.9 ºC and a precipitation of 239.5 mm, while in the SP pupal period, between October and May, the average temperature is 12.9 ºC and the precipitation 696.8 mm. So the SP pupae will develop at a lower temperature and higher moisture content in the soil and eggs will also develop at a lower temperature and insolation. In fact eggs from the SP have darker and larger scales, making egg-batches from the two populations easy to distinguish (own observations). The length of the pupal period is longer in the SP, which might lead to a slower rate of metamorphosis and egg production.

In SP, it seems, as the number of eggs per batch is kept constant at the lowest level found for the species. Thus, the number of eggs seems to be constrained by the need to produce large eggs as was found for populations of satyrids adapted to low temperatures (Wiklund *et al.* 1987). This might be due to a physiological adjustment to pupal development at lower temperatures or to the increase in the length of the metamorphosis. The trade-off between egg size and number might be a consequence of different female condition during oogenesis for the two populations. Larger eggs might also survive better during cold and less sunny whether during incubation and/or larger hatchlings might have a better survival during the first instars. The decrease in the number of eggs per batch is compensated by higher egg hatchability, although this seems to be the consequence of lower rates of parasitism.

The number of larvae per batch found in September/October for the WP and July for the SP, consisting mostly of larvae in the 2nd instar, was about 125. This is very similar to the number found in other WP`s in the country (Chapter 2, point 2.4.2.). It's known the need for a minimum group size for establishment in gregarious caterpillars (Stamp 1981, Fordyce and Agrawal 2001) and an optimum group size where survival is maximized (Stamp 1981, Clark and Faeth 1997, Ruf 2002). In *T. pityocampa* there is no information available for these group sizes, but the present work (Chapter 2) gave an indication that these sizes falls between 120 and 140. It has been suggested that egg batch size is above the optimum group size to compensate for egg mortality and early larval mortality (Stamp 1981). Once the average egg-batch size in the WP is larger than these minimum/ optimum sizes, this would help to explain why the egg batch size of SP does not seem to have a negative effect on its population dynamics.

WP *T. pityocampa* larval colonies move the location of the nest in the tree several times, building temporary loose nests each time, until it settles at the end of the 3rd instar, mostly in December, and a permanent nest structure is built. In the case of the SP this happens at the end of August - early September, in a warmer part of the year. We found on average 80 larvae per batch in the WP, at the time when most of the larvae were building their permanent nests. In the SP, we found an average of 110. The larger number of larvae in the SP, was probably due to a higher initial number of egg batches per tree. When larvae from these batches eventually ended up getting together, the initially higher number of batches per tree in the SP, was reduced to approximately one permanent nest per tree as in the WP.

As was discussed in Chapter 2 (point 2.4.2), batch size is important along *T. pityocampa* larval development. The most pointed reason in the fact that thermoregulation depends on batch size in gregarious caterpillars, with larger aggregations having more capacity to keep a constant temperature in relation to environmental temperature (Stevenson 1985a). Additionally, not only SP larvae have a lower need to invest in silk, as proved by its more loose permanent nests, but the larger batch size at the time of building their permanent nests potentially lead to an even lower individual investment in communal nests.

3.4.3. Mortality factors, survival and development

Egg mortality for both the SP and WP of *T. pityocampa* remained below 20 %. For this species a larval hatching rate of 80 %, can be considered as a high value, when compared with data for other Mediterranean regions: 20-65 % in Bulgaria (Tsankov *et al.* 1998); 56 % in Algeria, (Tsankov *et al.* 1995); 68 % in Greece (Schmidt 1990) and 47-80 % in Spain (Schmidt *et al.* 1999). Comparable results were only found for the Baleares Islands, where Alemany *et al.* (1994) reported a hatching success of 82-86 %.

The egg mortality of the WP in Leiria was also lower than in other WP in other areas of the country. Once most of the mortality of *T. pityocampa* before pupation is concentrated on egg and early larval stages (Chapter 2, point 2.4.3.), egg mortality, in which parasitoids play an important role, can have a major impact in reducing the population. The SP benefit from a low parasitoid population in the area of its outbreak. Thus, egg mortality ends up having a low impact in reducing the population in the outbreaking SP.

Loss of whole batches in *T. pityocampa*, which represents the loss of the entire reproductive output of one female, was an important mortality factor only in the WP in the season 2000/01. In the SP, this kind of mortality was low in all three seasons. In herbivorous insects, plant factors (Zalucki *et al.* 2002), weather factors (Cornell and Hawkins 1995, Cornell *et al.* 1998) seem to be the most important mortality factors in newly hatched larvae. Loss of entire colonies in the early instars was also considered an important mortality factor in other tent building caterpillars and this was explained as failure to initiate feeding (Moore *et al.* 1988, Floater and Zalucki 1999), failure in building an initial tent (Ruf 2002), predation (Costa 1993), and low host plant quality (Hodar *et al.* 2002) Thus, the SP seems to have benefited from high host quality, mild weather conditions and low predation level, reducing the loss of entire colonies to a minimum, which did not always happen in the WP or on other normal populations found in other areas of the country (Chapter 2, point 2.4.3.).

In batches that manage to get establish in the host, mortality was invariably high in both populations. We found no relation between the number of hatched larvae in a tree and survival in the SP. On the contrary, in normal WP, particularly at high population's densities (Chapter 2), we found a negative relation between the number of hatched larvae per tree and survival.

Increasing mortality in gregarious Lepidoptera above an optimal group size was found by other authors (Stamp 1981, Tsubaki and Schiotsu 1982, Ruf 2002). Predators and parasitoids often exploit clumped resources more efficiently than dispersed resources by concentrating their efforts in the area where the encounter rate is high (Royama 1970). However, in the present study the larger aggregations of caterpillars in the SP did not lead to an increase in mortality.

On the contrary of what was found for the normal populations on other areas of Portugal, where most of mortality occurs before 4th instar (Chapter 2, point 2.4.3.), in the SP mortality was higher after this stage. In fact a disease outbreak was apparent in this population on the season 2000/01, in 4th-5th instar larval nests, reducing the overall larval survival.

Like what was found in Chapter 2 for normal populations, *P. caudata* don't seem to be a very important cause of mortality in the larval stage. But in the SP it reaches 4-6 % mortality, which can be considered high. The high mortality caused by virus in the season 2001 in the SP, coincided with a decreased impact of the parasitoid, indicating that it's vulnerable to its host mortality.

Mortality in the immature stages of *T. pityocampa* is divided into many factors and we cannot point out one as the most important, just like what was generally found for normal populations (Chapter 2). The same mortality factors were found in WP an SP. However SP benefits from a low mortality in eggs and earlier instar larvae, and this stages mortality have a major impact in normal populations (Chapter 2). Except for the season 2001, survival in the SP was higher than in the WP of Leiria or other normal populations in other areas of the country.

3.4.4. Larval development

Larvae from the SP start their development at a higher mass, however both populations end up at a similar mass. Larger egg-size in insects and consequently larger hatchling mass has been connected to faster larval feeding rate and larval and pre-adult development rates (Ayres and Schriber 1994; Azevedo *et al.* 1997). This gives advantage in colder environments, once caterpillar growth is nearly exponential, and the proportional advantage is retained throughout larval development – even with equal growth rates the same pupal mass will be attained in less time as result of differences in hatching mass (Ayres and Schriber 1994). The paradox is that SP will develop under warmer and drier whether conditions.

In the Leiria WP and other normal populations, most of the mass increase of caterpillars occurs during the coldest winter months, when average monthly air temperature stays around 10ºC. Gregariousness and tent building allows caterpillars to explore geographical and temporal distributions colder than is optimal for development due to their capacity to create their own environmental temperature (Breuer *et al.* 1989; Bryant *et al.* 1997). Thus, it is possible that *T. pityocampa* has a higher optimum ambient temperature than found in its winter environment and the temperature during the summer gets closer to the optimum for development.

Tent-building caterpillars are any way dependent on climatic conditions. Just like what was found and discussed in Chapter 2, they are particularly dependent on the number of sunny days and solar radiation during its development time. In the WP, a lower pre-pupae mass was found in the season 2000/01, corresponding to a low speed of development recorded during that season and an exceptionally rainy season with low levels of insulation, decreasing the fecundity during the following season - 2001/02. These points even more towards the importance of weather for the population dynamics.

SP larvae develop during the season of maximum solar radiation and the highest number of hours of Sun per day, which certainly contributes to its high speed of development. As was referred previously, tent-building and behaviour thermoregulation allowed caterpillars not only to increase its temperature but also to avoid overheating through a thermal gradient inside the tent and caterpillars movements between shade and sun which allowed them to keep a constant body temperature (Knapp and Casey 1986, Fitzgerald and Underwood 2000, Ruf and Fiedler 2002a). These characteristics might help SP caterpillar avoid overheating in the summer, benefiting at the same time of favourable whether conditions.

Once SP larval development occurs during a more stable season it was not possible to establish a relation between different weather conditions along the season and larval speed of development, final mass and female fecundity on the next season, like in the normal populations (Chapter 2). But a clear decrease in the SP egg size was found in the season 20001. This exceptionally rainy season affected the SP pupal period between the seasons 2000 and 2001, and probably increased the environmental moisture experienced during diapause and metamorphosis, but it also correspond to a warmer winter, and in fact an earlier male flight period was observed in the field (Chapter 5). We do not know to which level this accounts for the clear decrease in the SP egg size in the season 2001. Soil moisture content is known to increase pupal mortality in *T. pityocampa*, but not fecundity (Markalas 1989). Topp and Kirsten (1991) found, however, that the temperature to which pupae of *O. brumata* were exposed had a major impact on their fertility.

Natural variation in concentration of plant nitrogen is of great importance to the fitness of Lepidoptera (Zalucki *et al* 2002). In central-place foragers feeding in deciduous hosts, synchronization with bud burst in early spring is very important (Joos *et al.* 1988, Ruff and Fiedler 2002b). In central-place foragers feeding on evergreen hosts, synchronization of newly hatched caterpillars with the production of new needles year leaf flush is also important (Yela and Herrera 1993; Floater 1997, Fitzgerald and Underwood 2000).

The larvae of the normal WP emerges in September by the time the first rains start and there are new needles available. SP newly hatched caterpillars of SP appear in mid to end July, in the dry season, but also with new needles available from the springtime flush. According to Medlyn *et al.* (2002), the nitrogen content of the new needles in *P. pinaster* from the area of Bordeaux in France, increased from the time when the needles emerged, at the end of September, over the winter and then declined from March onwards. However, this might be dependent on the provenience since in Morocco the decline in needle nitrogen content did not begin until August. Nitrogen is stored in needles over winter and during spring and summer it is reallocated to build new needles. This temporal pattern of total nitrogen content of needles was also found in other Mediterranean evergreen species (Fernádez-Escobar *et al.* 1999).

It seems, then that larvae from the WP will start their larval development with low nutrient levels but by the time they reach the 4th-5th instar, when consumption is higher, – they will have the highest levels of nitrogen in the needles. The larval development of the SP will occur during a period with low nitrogen content in its host compared with the normal population. We should take into account that SP larvae will grow under lower air moisture content and probably lower moisture food content, which might impose metabolic costs (Jindra and Sehnal 1989, 1990).

Several studies indicate that temperature does not influence the final mass of the larvae but only the relative growth rate (Stamp and Bowers 1994, Lindroth *et al.* 1997, Frid and Myers 2002). Final pupal mass seems to be dependent mostly on host quality, especially its nitrogen content (Lindroth *et al.* 1997). However, low levels of nitrogen in host species can lead to higher resorption of nitrogen and the cessation of spinning during the feeding period, which may be mechanisms for increasing nitrogen utilization for body growth (Jindra and Sehnal 1989). This way, SP larvae theoretically grow with lower host Nitrogen content, but compensatory metabolic mechanisms and probably its low need to invest in silk will lead to the same final mass as the normal WP.

3.4.5. Attack level of the two populations

SP of *T. pityocampa* remained at high densities in the same area of Leiria where was first discovered in 1997. The SP density on this area located at the south of Leiria National Pine Forest, measured as the proportion of attack tree and nests per ha, was higher than WP density in any point of the Forest. Additionally SP larvae attain larger numbers per attacked trees leading to visibly heavier levels of defoliation. In fact the WP has never been observed, at least in the past 50 years, to reach population levels similar to those of the SP (personal communication, administrative officers of National Pine Forest of Leiria). However variations occur throughout the pinewood, with plots located north having higher densities of WP. As was discussed in Chapter 2, the coastal dune system of Leiria is considered as an optimal area for the growth of *P. pinaster* and although it's a monoculture with even aged trees, *T. pityocampa* of the normal WP never reaches the densities that are observed in other areas of Portugal. Differences in density in both populations cannot be explained by the different structure of the studied plots and the summer and winter populations overlap geographically.

The field surveys indicated a movement of the SP in direction of the south, but in the more inland area of the pine (Plot 254), at the same latitude as the hot spot of attack this population wasn't detected (for more detailed discussion see Chapter 4). SP attack also starts to be detected in plots located at the north of the Pine Forest, but at a very low level.

3.4.6. Why is the change in the life cycle advantageous?

Taylor (1981) explored the importance of physiological time for insect life-history strategies and noted that while some insects may maximise fitness by developing as rapidly as possible, others may need to constrain growth to time their development relative to some environmental factor that was critical for their fitness. In the present work, two populations of the same species end up with a different physiological time, exploring different seasons in the same ecosystem with a dramatic consequence on its population dynamics.

The first question is why the normal version of *T. pityocampa* has larval development during winter? This species belongs to the family Thaumetopoeidae, where all the known species exhibit egg clustering, aposematism – repellent defences and warning coloration and gregarious caterpillars, all co-evolved traits (Stamp 1980, Sillen-Tullberg 1988, Sillen-Tullberg and Leimar 1988, Tullberg and Hunter 1996). This enables them to build tents and nests, which is also a common trait in the family, and serve as effective devices for thermoregulation (Joos *et al*. 1988, Breuer and Devkota 1990, Ruf and Fiedler 2002a). These traits and the existence of egg or pupal diapause enabled the different species of the family to explore less favourable thermal environments, being the species with egg diapause early spring feeders and the ones with pupal diapause, winter feeders.

The advantage of early spring feeders have been well studied in other gregarious caterpillars, feeding on deciduous trees in the Central-North Europe and America, like the eastern tent caterpillar *M. americanum* (Joos *et al*. 1988) and the small aggar moth *E. lanestris* (Ruf and Fiedler 2000 and 2002a). This species feed on the early spring leaves, which enable them to achieve a fast development rate and escape predation pressure that occurs later in the spring or during summer. The thermoregulatory capacity enables them to develop in a time of the year with low temperature but sunny whether.

T. pityocampa WP has a similar life cycle as two other *Thaumetopoea* species, *T. Wilkinson*, *T. jordana* (Halperin 1990) and to the Australian Thaumetopoeidae *O. lunifer* (Floater

1996a,b,c). These species are adapted to warmer climates, with mild winters or no winter at all. They start larval development in their evergreen host species with the early rains after a dry season, which also coincide with the existence of new leaves (Halperin 1990, Floater 1997). This synchrony with leaf flush and rain was found to be an important survival factor in *O. lunifer* (Floater 1997). Nothing is known about the importance of the synchrony of *T. pitiocampa* life cycle with its host phenology. Although this species seem to be a less demanding grazer in its late instars (Fitzgerald 2003), Lepidoptera first instar larvae are generally taken to be very sensitive to nutritional and climatic factors (Zalucki *et al.* 2002).

By developing during the winter, larvae also escape an important predation pressure. Larval development finishes in February-March and during this time birds of the genus *Parus* start to breed in coastal areas of Portugal (Chapter 6). These birds can be important predators of *T. pityocampa* larvae, especially great tits, which predates the larvae until the last instar (Gonzalez-Cano 1981). During the nestling and fledgling periods of insectivorous birds, the predation intensity on larvae increases dramatically due to the high density of birds during and after breeding (Holmes *et al.* 1979). Lepidoptera larvae are a major food for most passerine birds, especially when young are being fed (Betts 1955, Mols and Visser 2002) and a significant reduction of caterpillar numbers in the trees occurs at this time (Holmes *et al.* 1979).

Another important group of predators of *T. pityocampa* larvae are ants. Unlike other natural enemies, many predaceous ants possess the unique attributes of being able to survive even when prey is scarce and to respond quickly to increases in prey density (Way and Khoo 1992). Rodrigues (2003) found a halt in the activity of the native ant *Pheidole pallidula* (Nylander) between early November and April. Wasps are also known to be important Lepidoptera predators, with the capacity to restructuring their prey's communities (Beggs and Rees 1999). In temperate forests these predators' colonies grow fast during July-August, a time when food requirements are maximal (Steward *et al.* 1988). Thus, by developing during the winter, the *T. pityocampa* larvae escape the period of high activity of several predators.

A second question is why SP reaches outbreaking levels, something not observed in the WP of Leiria. The newly hatched larvae will have new needles available, but the early instars will develop during the dry season, which probably is connected with low nitrogen content in the needles. The temporal variation in the chemical composition of the needles was unfortunately not assessed in the present work. However larvae might take the advantage of a lower need to invest in silk, and other metabolic mechanisms such as a more efficient absorption of Nitrogen may help to overcome the theoretical disadvantage of growing with lower Nitrogen content. At the end, pre-pupae of both populations end up with the same final weight.

The newly hatched caterpillars of the SP originated from larger eggs and had a larger mass than newly hatched caterpillars from normal populations. The advantage of this in overcoming needle toughness of maritime pine is unknown.

The SP has its flight period at the time of forest birds breeding season and larval development starts during the bird fledging period. However, due to the forestry practice in the National Pine Forest of Leiria, the SP attack area consists of homogenous, even-aged stands, lacking dead trees and with a very low supply of natural cavities for bird breeding. This will probably strongly reduce the density of cavity nesting birds, e.g. tits, thereby reducing the risk of predation for the larvae considerable.

The SP larval development also takes place during the active period of both the native ant *P. pallidula* and the exotic *Linepithema humile* (Mayr), which have been demonstrated to predate on *T. pityocampa* larvae (Rodrigues 2003). In fact Way *et al.* (1999) did not find attacked trees by *T. pityocampa* in areas where the Argentine ant *L. humile* was present. However, in the present work, we never observed signs of attack by ants on *T. pityocampa* SP larvae. SP larval development also takes place during the period of larger need of food for the wasps. Community structure of this simple ecosystem might be responsible for the low predation pressure from these invertebrate predators, once monocultures are connected with low biodiversity and lower ecosystem stability (Bengtsson *et al.* 2000) and lower abundance of natural enemies (Bragança *et al.* 1998b).

The higher hatchling mass of SP larvae and the warmer weather conditions, result in a development rate twice the one of the normal population. Taking into account that natural enemies are the main cause of mortality in immature herbivorous insects (Cornell and Hawkins 1995, Cornell *et al.* 1998), speeding these stages will avoid much of the predation risk - the slow growth high mortality hypothesis (Benrey and Denno 1997), and in fact the SP larvae presented lower mortality at the earlier instars (1st-3rd) than the other populations.

This desynchronized population has originated in an area covered by an old managed forest that has been subject to human intervention for several hundred years. The normal version of the *T. pityocampa* population, the WP, has never been reported to reach outbreaking levels in this stand as reported in other pines in the country. Thus, the sudden appearance of the SP is of high importance not only from an ecological point of view but also for questioning forestry management practices.

4. Climate influence and spatial characteristics of a desynchronized population of *Thaumetopoea pityocampa* outbreak in the National Pine Forest of Leiria (Portugal): comparison with the normal population in the same area.

4.1.Introduction

A general theory relating outbreaks of forest insect herbivores to environmental conditions, mostly climatic anomalies, was first suggested by Graham (1939). The theory postulates that the periodic eruptive outbreaks of many forest insect herbivores are under long-term control, and a favorable climatic anomaly leads to "temporal lowering of environmental resistance" which allows a forest insect to realize its high reproductive ability. On this long-term control, forest structure and natural enemies also play an important role. The concept of population "release" originated with Solomon (1949), who believed that under certain circumstances natural control may become "disorganized". This would allow a population to temporarily escape from its important controlling agents, or "key factors", leading to an increase in number. Nowadays accumulated information indicates that forest outbreaking insects exhibit typical regular, and often predictable cycles (Zhang *et al.* 1999, Kamata 2000, Burleigh *et al.* 2002, Nealis *et al*. 2004). This cycles generally follow four distinct phases: an endemic and innocuous phase during which numbers are low and stable; a release phase, during which favourable conditions allow population increase; an outbreak phase, in which damaging defoliation occurs; and a population collapse phase (Martinat 1987).

Severe outbreaks play a key role in modifying forest structure, composition and renewal by selective mortality, reducing competition among trees and accelerating the growth rates of survivors and understory tree species (Zhang *et al.* 1999, Alfaro *et al.* 2001, Burleigh *et al.* 2002). Historical records and dendroecological studies indicate that severe outbreaks occurred in the past in natural non-managed forests (Zhang *et al.* 1999, Burleigh *et al.* 2002) and damaging outbreaks are likely to re-occur at regular intervals regardless of changes in forest structure and composition (Nealis *et al*. 2004). However modern forestry practices, with the implementation of large monocultures with even aged trees, were directly related to the occurrence of outbreaks (Bragança *et al.* 1998a, Floater and Zalucki 2000) and with the seriousness of its effects (Kamata 2000, Nealis *et al*. 2004)

In 1997 a desynchronized population of *T. pityocampa* was record for the first time, due to a very visible outbreak. This occurred in a restricted area in the oldest National Forest of the country, the National Pine Forest of Leiria, located in the area of the Atlantic coastal pines. Leiria has been submitted to active management for centuries. The abnormal population attained a much higher population density in its outbreaking area, than the normal version of the same species in any point of the Forest, coexisting geographically both populations (Chapter 3). Outbreaking forest insects are known to adapt to man-induced changes in forest structure and composition, changing its preferences of host age (Nealis *et al*. 2004) and adapting to non-native tree species (Ozaki *et al.* 2004). The outbreak of Leiria is unique because it was caused by a different population of a native insect species, on a native host tree species.

Climatic anomalies are known to occur in the years previous to forest insect's outbreaks (White 1969, Ozaki *et al.* 2004). Thus we began this study by examining temperatures and precipitation data, the most common variables connected with insect population fluctuations. There is a good record of management operations, stand structure, and age evolution in the last 30 years in the National Pine Forest of Leiria. This allowed us to explore the influence of the spatial-temporal dynamics of forest structure on the occurrence of this outbreak. The dispersion pattern of this population from the area where it was originated, between 1997 and 2000, was also studied. A comparison was made with the normal version of the species in the same Forest.

4.2. Methods

4.2.1. Area

The present study was performed along National Pine Forest of Leiria, located 200 km north of Lisbon (Latitude: 39º50´N, Longitude: 8º57´W, Altitude: 30-50 m a.s.l.). For a description and location of the area see Chapter 2. The plot characteristics (tree density, tree DBH (cm), tree height (m) and Basal Area (m^2/ha)) in each plot are regularly recorded by Administration of the National Pine Forest of Leiria, through several sampling points after the plots reach 20 years old. As a measurement of plot quality the Forest Administration uses the average height of the dominant trees at the age of 50 years (HD50), an attribute that was also used in the present work. In plots with age below 20 years data are rare and below 10 are inexistent. There is one sampling point per ha, and they are equidistant. The 2000 ha located near the

Atlantic are an unproductive protection area. This area evolves mainly by natural regeneration and the only human intervention is the cutting of dead trees. This way no information is available for this area, which is characterize by low tree density, diverse tree age, with an abundant understory. A map supplied by Administration of the National Pine Forest of Leiria (from 1980) was digitized using the program ArcView 3.2, and information concerning the plots attributes was associated.

4.2.2. Meteorological data

Total precipitation and average monthly temperature were obtained from the meteorological station of the Aerial Base of Monte Real (Latitude: 39º50´N, Longitude: 8º53´W, Altitude: 52 m a.s.l.), in the northern border of National Pine Forest of Leiria for the years 1981-2002. The average temperature and total precipitation for each SP season were calculated. It was considered that the annual SP season started at the beginning of the pupal period in November, when all the larvae had pupated in the field. The wind frequency is the average number of times in the month in which was registered each of the eight directions of the wind (N, NE, E, SE, S, SW, W, NW) expressed in percentage and the average speed value for each of the directions (Km/h). Average of a 15 years series (1960-74) was obtained for six months, between April and September, covering the flight period of the WP and SP.

4.2.3. Field surveys

Basic surveys registering the existence or absence of both SP and WP were conducted across National Pine Forest of Leiria in the winter 2000-01 and 2001-02. These surveys were extended north and south of the Forest. Additionally an estimate of the attack level by both *T. pityocampa* populations was made directly in the pinewood during three seasons (1999-2003), expressed as the proportion of trees attacked and the number of nests per ha. Surveys corresponded to seven plots (24, 63, 166, 254, 283, 299 and 314; 1.35-199 ha per plot, a total area of 12.77 ha), covering all Leiria Pine Forest, were made following the procedure described in Chapter 2 (point 2.2.3. corresponding to Leiria). Following the procedures used in Chapter 2 and 3, we used the higher attack rate recorded in the three seasons for each population in each plot, neglecting differences among the years. This way we evaluated the potential of each plot to be infested by *T. pityocampa* of both populations.

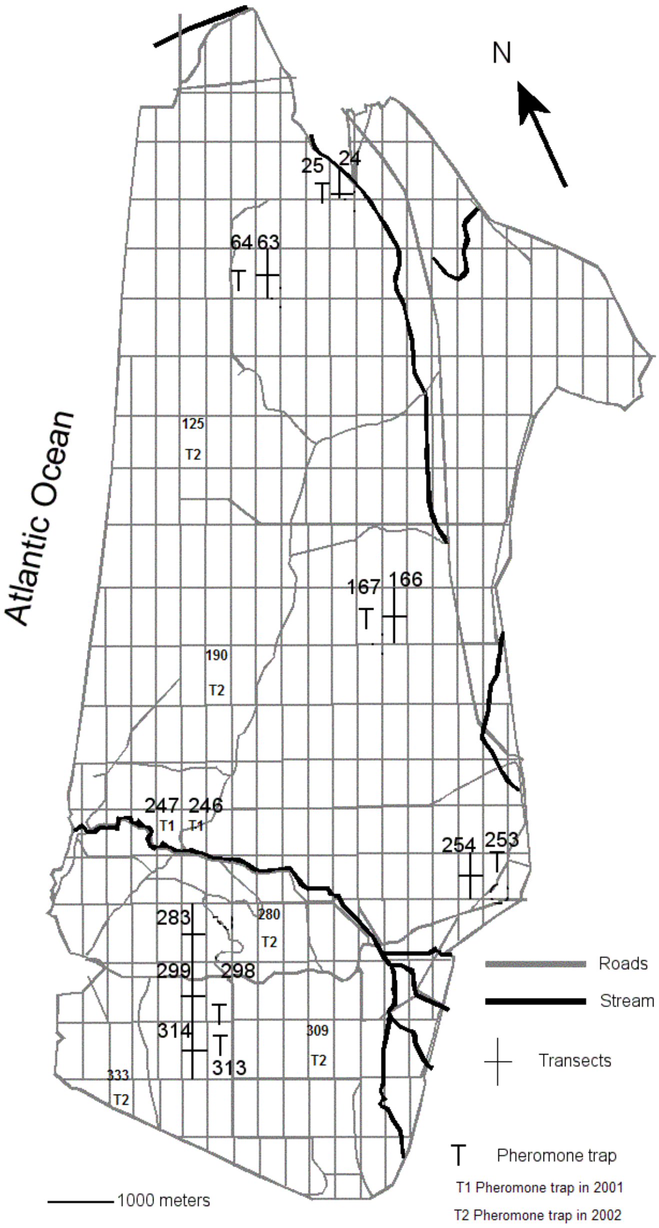


Figure 4.1: Location of the field surveys (transects) and pheromone traps in National Pine Forest of Leiria, Portugal during 1999-2003.

4.2.4. Seasonal flight activity

The dispersion of adults along the Pine Forest was estimated monitoring the male flight activity during the years 2001 and 2002, using traps baited with the female sex pheromone ((Z)-13-hexadecen-11-ynyl acetate identified by Guerrero *et al.* (1981). Funnel traps (AgriSense$^{\text{TM}}$), baited with one synthetic pheromone dispenser (AgriSense-BCS Ltd; TP058A140; BFL072) and a killing agent (DDVP strip) were used to capture males. All traps were placed at a height of 2.5 – 3.0 m, in mid April and checked weekly until October of each year, chemicals being replaced after 30 days. In the year 2001, eight traps were set up, distributed in plots 25, 64, 167, 253, 298 and 313, near the field surveys location, and in plots 246 and 247 (Fig. 4.1), the possible limit of the distribution of the SP according to surveys made in 2000. In the year 2002, the first six traps remained, but traps located in plots 246 and 247 were replaced by five new traps, at the new possible limit of the distribution of SP according to observations made in 2001 (plots 125, 190, 280, 309 and 333; Fig. 4.1).

4.2.5. Statistical analyses

The statistical analysis was performed using SYSTAT 10. Regression analyses were used to explore possible predictive relationships between the severity of attack by each population and plot attributes. Severity of attack was measured as the maximum proportion of attacked trees, number of nests per ha and trapped males recorded in each plot. Plots attributes included the location of the plot inside the Forest and the attributes of the surrounding plots, once the studied plots characteristics were similar and did not have any effect in the population level of both SP and WP as was discussed in Chapter 3. This attributes were the total continuous area of young plots (6-20 years) in which the studied plot was included; distance from the northern border of the Forest; average age and density of the surrounding plots in a circumference with 1 km radius, as a measure of the area heterogeneity. In the non-managed area, the tree age was considered 100 years – the longevity of the maritime pine, and tree density has 130 trees per ha – the minimum recorded inside the Forest. In managed plots below 20 years of age, tree density was taken as 2000 per ha, the maximum value recorded inside the Forest. As a measurement of plot quality, it was used the average height of the dominant trees in a plot at the age of 50 years (DH50). An evaluation of the average plot quality in a circumference with 1 km radius around each studied plot was performed through a One-Way ANOVA.

4.3.Results

4.3.1. Climatic variables

To assess the seasons with an anomalous weather, we calculate the Average and Standard Deviation of the 21 years series for temperature and precipitation, to detect the values that fall above or below the S.D. Regarding precipitation, the first season of the series with an anomalous weather was 94-95, the driest season, followed by a very wet season. These were exactly the two seasons previous to the SP outbreak in 1996-97, which can be considered as an average season regarding precipitation (771.3 mm), but had a high mean temperature. Temperatures in both 94-95 and 95-96, were also above the average, and 94-95 was once again an anomalous season (Fig. 4.2 and 4.3).

From April to October the wind blew predominantly from the directions N and NW but occasional spells can be expected from the directions NW and W, and less frequently from other directions. Directions from N to SW had similar speed (Fig. 4.4).

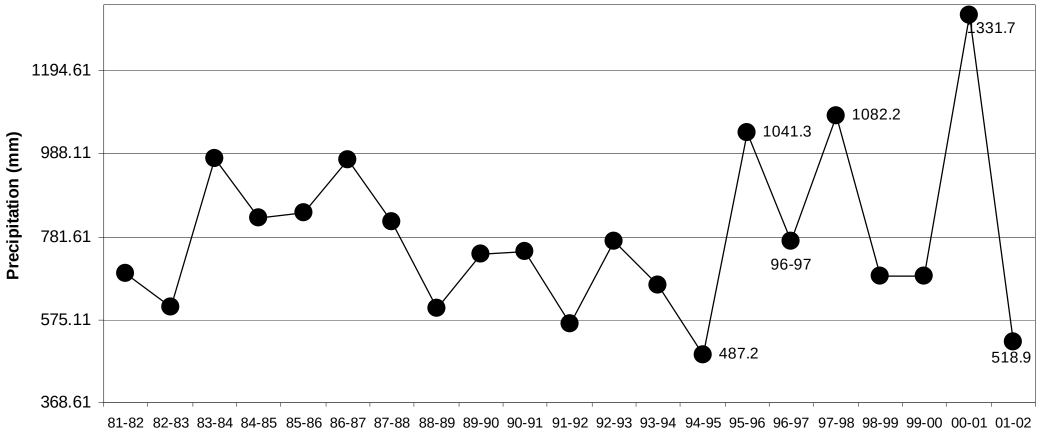


Figure 4.2: Total precipitation in a series of 21 years (1981-2001) (Latitude: 39º50´N, Longitude: 8º53´W). Average value for the series - 781.6 ± 206.4 mm. Extreme values, and the season of the SP outbreak (96-97) are indicated.

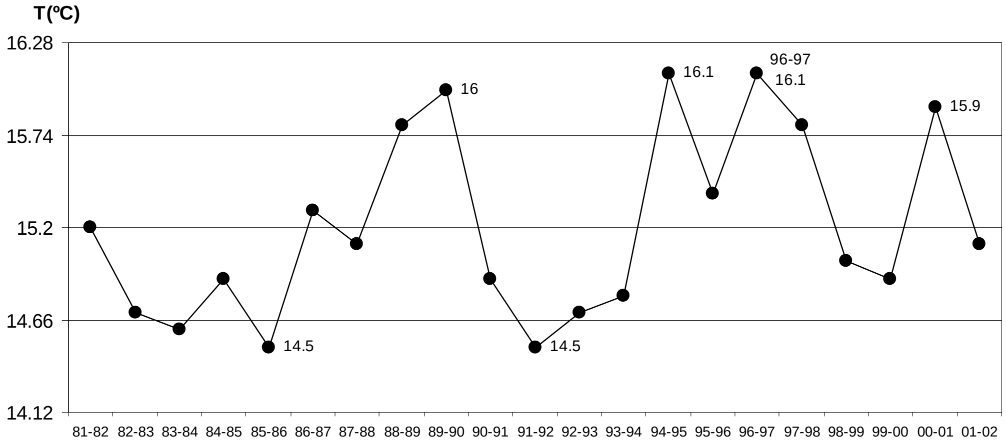


Figure 4.3: Average temperature in a series of 21 years (1981-2001) (Latitude: 39º50´N, Longitude: 8º53´W). Average value for the series - 15.20 ± 0.54 ºC. Extreme values and the season of the SP outbreak (96-97) are indicated.

4.3.2. Spatial variables in National Pine Forest of Leiria

In 1985, the largest area of plots with clear cuts and trees bellow six years was recorded - 1923 ha. Of this more than 900 ha were a continuous area in the south of the pine stand (Fig. 4.5). This large area appeared due to a large fire that occurred in 1981 and consequent clear-cut of plots with several ages. In 1997 trees in this area were more than 10 years old, and this was the area where the SP outbreak occurred.

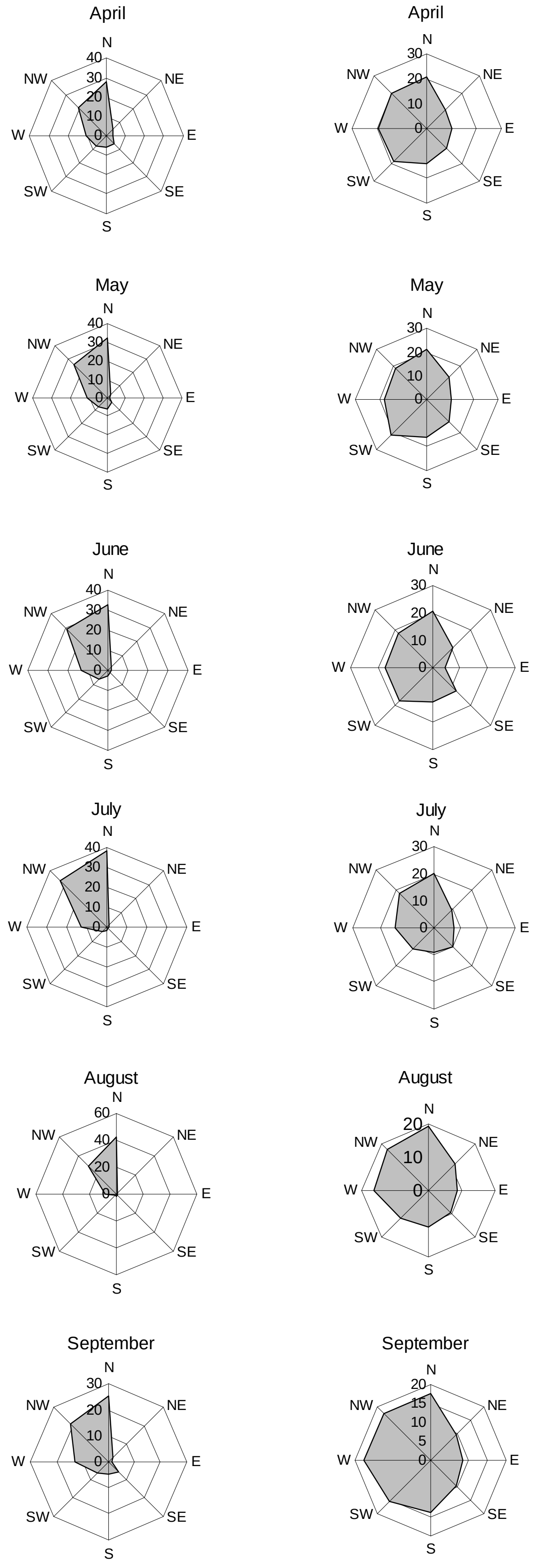
Due to the management scheme adopted by the administration, tree density decreases regularly with age due to thinning operations, while a constant increase in the DBH, height and Basal Area can be observed (Table 4.1 and 4.2). However the increase in the Basal Area with age is lower than that recorded for the other parameters, due to thinning operations performed over the time.

Table 4.1: Linear regression between plot age and different plot attributes. Original data supplied by Administration of National Pine Forest of Leiria, Marinha Grande, and obtained from the 1995 inventory.

Density	- 16.424x + 1256.761; F=360.570 p=0
DBH	0.482x + 5.559; F=1449.124 p=0
Height	0.231x + 5.287; F=848.589 p=0
Basal area	0.193x + 14.543; F=190.172 p=0

Table 4.2: Variation of tree parameters for the tree age classes established. Original data supplied by Administration of National Pine Forest of Leiria, Marinha Grande, and obtained from the 1995 inventory.

Plot age	Tree density per ha	DBH (cm)	Height (m)	Basal Area (m^2/ha)
10-20	700-2000	9-15.5	6-11.3	7.8-23.2
21-40	160-1700	12.2-36.3	7.5-21.7	11.6-31.3
>40	130-580	23.1-47.0	9.8-25.2	14.5-39.4



Wind Frequency: % Wind Speed: Km/h

Figure 4.4: Wind frequency and wind speed from each orientation during the time when the adults of T. *pityocampa* SP (April-June) and WP (July-September) are on the wing. Data obtained for a 15 years series: 1960-74 (Latitude: 39º50´N, Longitude: 8º53´W).

Figure 4.5: Evolution of tree age classes in the plots of National Pine Forest of Leiria from 1980 to 1997. Original data supplied by Administration of National Pine Forest of Leiria, Marinha Grande and maps obtained through the ArcView 3.2.

4.3.3. SP and WP in National Pine Forest of Leiria

During the surveys made in the winters of 2000-01 and 2001-02, SP was registered inside the National Pine Forest of Leiria in a strip near the coast, until 14 km counting from its southern limit. The population was visible in the non-managed area at a low level, but it was absent from the more inland areas (Fig. 4.7). The SP wasn't recorded at the north of National Pine Forest of Leiria, but high densities were recorded until the city of Nazaré, about 30 Km south of the initial outbreak area, and the limit of distribution of the Atlantic coastal pines (Fig. 4.6). This way a potential area of about 90 000 ha was already infested by the SP. The WP was present in all plots above 10 years old, being hard to detect in plots older than 40 years. It was detected in all the area of the surveys.

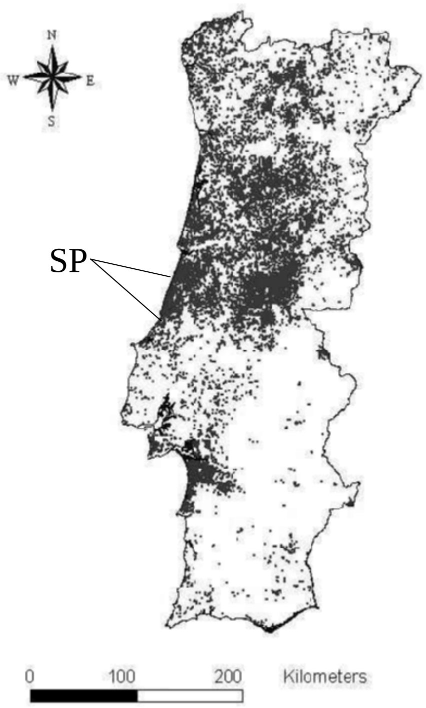


Figure 4.6: Location of *T. pityocampa* SP distribution area, delimited to the south by the city of Nazaré and to the north by the borders of National Pine Forest of Leiria. The density of pine stands across the country is indicated by black spots.

We can define a hot spot area of SP attack in the south of the Pine stand with high population levels (Fig. 4.7), the area where the SP outbreak was first recorded in 1997. This was confirmed by the results obtained in the surveys made on plots 283, 299 and 314 (30.8-35.4 % of attack trees and 419.8–752.8 nests per ha) and by the high number of males captured in plots 298, 309, 313 and 333 (total of 412 in 2001, and 486 in 2002, see Fig. 4.1). Few males were captured in 2002 by traps placed on Plots 125 and 190 in the unmanaged area (total of

11), confirming observations of the presence of the SP in this area. At the south limit in this area, in plots 246 and 247, 22 males were captured in 2001. Data indicates that the population is expanding towards the central and north part of the managed area of the pine, as shown by the presence of nests in plots 166 and 63, found for the first time in 2002 (0.1 and 0.4 % of attack trees, 2.1 and 4.4 nests per ha). In plot 166 two males of the SP were trapped in 2001, increasing to 10 in 2001. Concomitantly, eight males were also captured in plot 64 in 2002.

The SP was neither present in plots 24-25, nor in plots 253-254, both areas being separated from the SP distribution area by plots of older trees. In fact in plots with young trees in the southern part of the pine, close the high population area, but surrounded SW by older plots, the SP wasn't recorded (Fig. 4.7, see also Fig. 4.1). So it seems that the older plots act as an effective barrier to the dispersion of the population.

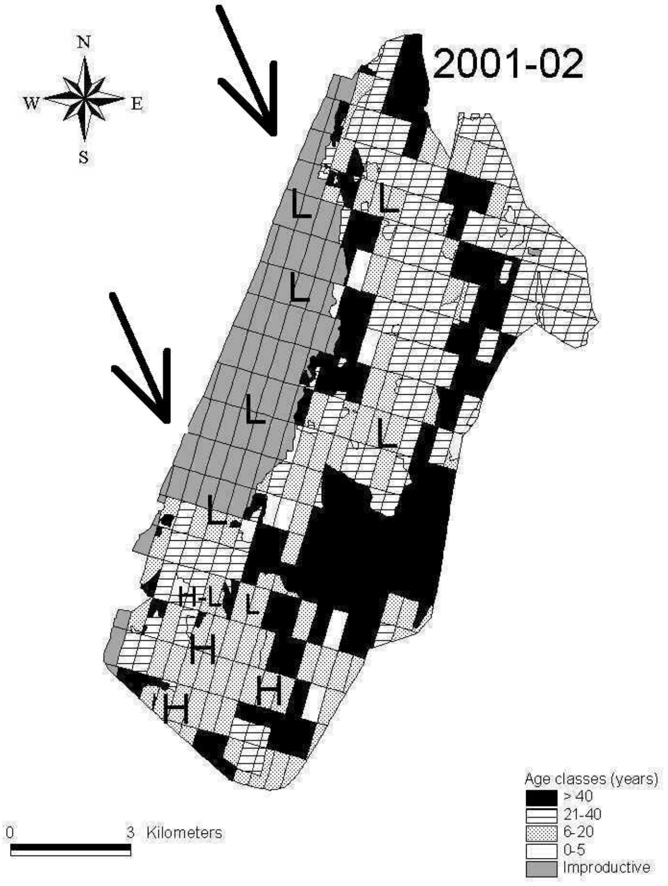


Figure 4.7: Management units and tree plots age of National Pine Forest of Leiria in 2001-2002, original data supplied by Administration of National Pine Forest of Leiria, Marinha Grande and the map was obtained through the ArcView 3.2. Arrows: direction of the wind during SP adults flight period; L: SP low-density attack; H: SP high-density attack.

The WP never reached the levels observed in the SP, but it was heterogeneously distributed along the Leiria Forest, with the two most northern plots - 24 and 63 - presenting the highest levels (8.9 and 12.2 % of attack trees, 198.5 and 158.3 nests per ha). In the other five studied plots, infestation level was always below 1 % of attacked trees and 13.5 nests per ha. Due to this low level of infestation, very few males were trapped during the WP emergence period: three males were captured in plots 25 and 63 in each of the studied year, other two in Plot 246 in 2001 and two in 2002 in plot 167 in the centre of the Forest, and one in plot 313 in the south of the Forest.

The total number of males captured in the traps during the SP flying period in the two years 2001 and 2002 was 78 times higher than the ones captured during the WP flying period. This indicates not only a higher population density, but also that there are more adult males from the SP dispersing across the Forest.

This way it seems that the SP originated in the coastal area of the National Pine Forest of Leiria, had the conditions to reach outbreak levels on the south of this pine, and it dispersed mainly in the direction SE following the direction of the dominant winds, that blow from NW, during the time of adult flight between April and June. Older pines plots seem to act as a barrier to its expansion.

The non-managed area near the Atlantic is considered a poor quality site due to the stress to which the trees are submitted in the proximity of the Ocean. No differences were found about site quality in the seven points where the field surveys were made (One-Way ANOVA: F=0.795, df=6, p=0.575), being the average HD50 17.9±3.4 m (n=125 plots).

The regression equation with the best statistical significance, to explain the level of attack by the SP is the one who relates the proportion of attacked trees in an infested plot with the total continuous area with trees aged 6-20 years in which the plot is included. This plot attribute also had a strong influence in the number of nests per ha and the number of trapped males. A significant equation was also found between the proportion of attacked trees in a plot and the average tree density of the surrounding plots (Table 4.3). However, this was probably due to the fact that young plots have higher tree density. These equations translate the fact that the occurrence of the SP outbreak was very localized in the larger continuous are of plots with high density of young trees inside the Forest. Although the population was found in other areas of the Forest, the density was always low. The WP attack level is better explained by the distance of the infested plot to the northern limit of the Forest (Table 4.3). A significant equation was found between the proportion of attacked trees by the SP and the distance from the north limit of the Forest, with the opposite slop of the WP. However this can be related to the fact that the large area with young plots is located in the south. In the case of the WP the distance by itself explains the infestation pattern.

Table 4.3: significant regression equations found between severity of attack by each population and plot attributes. * significant regression.

Regression	WP	SP
y= % attack trees; x= continuous area with plots aged 6-20 years	y = -0.008 x + 6.477; R^2= 0.324 $F_{1,5}$= 2.393 p=0.183	y = 0.049 x – 5.075; R^2= 0.975 $F_{1,5}$= 195.062 p=0.000*
y= Nests/ha; x= continuous area with plots aged 6-20 years	y = -0.140 x + 111.104; R^2= 0.348 $F_{1,5}$= 2.673 p=0.163	y = 0.854 x – 89.735; R^2= 0.894 $F_{1,5}$= 42.011 p=0.001*
y= % attack trees; x= average density of the surroundings	y = -0.003 x + 7.031; R^2= 0.044 $F_{1,5}$= 0.233 p=0.650	y = 0.039 x – 38.510; R^2= 0.727 $F_{1,5}$= 13.319 p=0.015*
y= trapped males; x= continuous area with plots aged 6-20 years	-	y = 0.205 x – 7.262; R^2= 0.642 $F_{1,5}$= 17.914 p=0.002*
y= % attack trees; x= distance from the northern limit of the forest	y = -0.841 x + 9.913; R^2= 0.770 $F_{1,5}$= 16.710 p=0.009*	y = 2.531 x – 5.739; R^2= 0.575 $F_{1,5}$= 6.775 p=0.048*
y= Nests/ha; x= distance from the northern limit of the forest	y = -14.734 x + 171.729; R^2= 0.843 $F_{1,5}$= 26.753 p=0.004*	y = 45.256 x – 107.509; R^2= 0.546 $F_{1,5}$= 6.011 p=0.058

Thus for the SP infestation to exceed 1 %, an area of more than 100 ha of continuous forest with 6-20 years is need. For each 100 ha added and increase of 5 % in the proportion of attack trees should be expected, until a maximum of 35 % recorded at the south of the Forest. A maximum of 10 % of attack trees by the WP should be expected in young plots near the north limit of the Leiria Forest. Ten km away from this limit the attack rate will decrease below 1%.

4.4. Discussion

Nowadays it is generally believed that atypical or anomalous weather is directly responsible for widespread changes in the abundance of many forest insects, although the mechanisms are rarely understood in detail (Martinat 1987, Zhang *et al.* 1999). Weather has both direct and indirect effects on phytophagous forest insect populations. Direct effects can be considered the ones, which act upon the insect behavior and physiology (e.g. Ozaki *et al.* 2004). However, indirect effects that act upon insect populations, through effects on host plants and natural enemies, may be more important than direct effects. Weather may influence the level of stress in the host plant, which in turn may alter its nutritional quality, chemical defenses or digestibility (for a review see Mattson 1980, Brodbeck and Strong 1987, Mattson and Haack 1987). This theory was first postulated by White (1969), who connected climatic extremes with psyllid outbreaks in Australia, and attributed the causes to changes in the nutritional quality, mainly to variations in the amino acid concentrations, on the host plants.

In the present work an outbreak of a desynchronized population of *T. pityocampa* was recorded after two years of extreme weather conditions, first a very dry year which coincided with a high seasonal temperature, followed by a very wet year. Additionally, in the season of the outbreak a high temperature value was also recorded. The differences in rainfall occurred mostly in wintertime and affected directly the population dynamic through its pupal period. Soil moisture content is known to increase the mortality of the pupae in *T. pityocampa* (Markalas 1989), and there is some evidence that it may decrease egg size (Chapter 3). This way the species population dynamic is supposed to be directly influenced by dry seasons. The larval period will occur during the summer, with a warmer and stable environment, but larval development may benefit from the stress caused in the trees by extreme weather, and both extreme precipitation and temperature values can act as stress factors on trees (Mattson 1980, Martinat 1987).

Taylor (1974) proved the existence of a “boundary layer” which describes a layer of air near the ground within which the insects are able to control their movements relative to the ground because their flight speed exceeds wind speed. Outside the boundary layer they, perforce, move downwind. The depth of this boundary layer depends on the wind speed, size of the insect and on its behaviour. An increase in the wind speed decreases the depth of the boundary layer and larger insects with higher flight capacity can control better its height of flight. A species whose economy is adjusted to a stable local environment and is therefore non-migratory, could not afford to enter the free-air zone. In contrast, the species with a fluid system of population dynamics would acquire flight behaviour patterns designed to break free of the boundary layer in order to effectively scan environment for suitable breeding sites. For example migrant aphids tend to take off upwards, attracted by light, but at some level the light stimulus will no longer be unilateral as it is at take off. Light may, then, distribute aphids relatively uniformly from take-off up to this level, but not above it (Taylor 1960).

During the past few decades, evidence has been accumulating that many species of moths, some of them major crop pests, are able to migrate hundreds of kilometres, due to meteorological driven long-range dispersal (Pedgley *et al*. 1989, Showers *et al*. 1989, Pedgley and Yathos 1993). This can be very important in dispersing imagoes for long distances from crops where pests had reach high population densities (Wolf *et al.* 1990).

According with our own observations females of *T. pityocampa* can fly, on the contrary of what was observed by other authors (Devkota *et al*. 1992). Shortly after the sunset, imagoes have a period of a few hours during which they are extremely active. They intercalate periods of short flight with climbing vertically in a substrate with the wings stretched behind (e.g. tree stem). When they reach the top they fly again downwind until they reach another substrate. This behaviour is observed only in females emerged on that day not in females with one or two days (own observations). This indicates a dispersion pattern in which females try to escape the boundary layer and enter the free air zone, taking advantage of the wind blow and occurs during few hours after emergence. These dispersion stops when temperature drops and females start their calling behaviour (Zhang and Paiva 1998). Evidence from this work indicates a clear dispersion pattern following the wind direction, which made SP to disperse south from the initial outbreak area, occurring at visible high densities in a distance of several km at the south of Leiria National Pine Forest. In Chapter 3, evidence was already found of a dispersion of SP imagoes from plots with a higher attack rate in the direction of plots located at the south.

However, *T. pityocampa* doesn’t appear as a long distance migratory insect and dispersion, dependent on suitable continuous pine stands, is stopped by older plots and by the end of the pine distribution, and this characteristic is probably very important for its population dynamic, allowing just a fast colonisation of young plantations from neighbouring high population areas. *T. pityocampa* is included in the so called *Capital Breeders* described by Tammaru and Haukioja (1996), which connected Lepidoptera eruptivity with female low mobility, and so a limited capacity to disperse from an area where resources are being completely depleted. In fact genetic analysis of *T. pityocampa* indicates that the species has a strong geographical structure, and gene flow is low. Also males appear to disperse more than females and long distance colonization by adult females seems to be a rare event (Salvato *et al*. 2002).

This unidirectional pattern of dispersion dependent on the direction of the wind, might be the probable cause of the higher population densities found for the WP in the two most northern plots of the Forest. The term “edge effect” was used to describe the commonest observation of a higher concentration of Lepidoptera eggs in the edge of plots than on its centre (Jones 1977). This edge effect depends on the species mobility and patterns of dispersion, but it is explained by the fact that edge areas accumulate more visits due to individuals that accidentally leave the plot and make an effort to return. The areas located behind the north limit of Leiria Forest are very heterogeneous, with a small village and agricultural fields. Since *T. pityocampa* dispersion pattern is very unidirectional, the north of the Forest is its only edge, from the insect point of view. The WP attack rate in northern plots will be at least in part be dependent on individuals who move out from other forest spots, ending up to get in Leiria after crossing an area without potential hosts.

Inside the Leiria National Pine Forest, the WP seems to lack the capacity to reach high levels, on the contrary to what was observed with the SP. The SP outbreak was clearly connected with a large continuous area with high densities of young trees. Since imagoes disperse manly from the N-NW directions, the origin of this abnormal population was either in the outbreaking area or in the non-managed area located at NW, where the SP was found at low levels. The outbreak seems to be connected exclusively with the forest structure. Although the non-managed area is considered as a poor quality site, it also presents a diverse and heterogeneous forest structure, contrary to the SP outbreaking area.

This way it seems that the SP of Leiria was able to take advantage of forest spatial characteristics that led to *T. pityocampa* outbreaks in other areas of the country (Chapter 2). Not only an outbreak of this unusual population was observed in a large continuous area of young plantations, but also older plantations seem to protect younger plots behind *T. pityocampa* wind path. Older plots have lower tree density, larger tree DBH and tree height and there is an increase in the Basal Area – a measurement of the total area occupied by trees. The Basal Area doesn’t increase as sharply with age as tree size, due to a strong decrease in density. The relative distribution of nutrients varies according to the age of the plants (Mattson 1980). The distribution of nitrogen among foliage, branches, and stems in a pine stand changes along the stand age. Most of the nitrogen in the very young stand is accumulated in the foliage, but the proportion in the stem increases with stand age. This reflects the continued accumulation of stem and branch biomass through the years (Kimmins 1996). This way the increase in Basal Area doesn’t reflect an increase in foliage density, which is the proportion consumed by *T. pityocampa*.

This is once again in agreement with the “resource concentration hypothesis” (Root 1973): Not only insect outbreaks tend to occur in dense homogeneous monocultures because herbivores can reach their host easily and successfully, but also young pine plantations present higher concentration of available nutrients for the development of the population, allowing prompt population expansion. The case of the SP outbreak is exceptional, since *T. pityocampa* was able to make use of the resources made available by human intervention in this Forest, only after a drastic change on its life cycle.

5. Adult flight of the pine processionary moth in Portugal: comparison of different populations.

5.1. Introduction

For short-lived organisms in seasonal environments, the timing of occurrence of different developmental stages may become a central target of life-history evolution (e.g. Taylor 1981; Hairston and Walton 1986). Among insects, diapause is the primary means of achieving seasonal synchronization (e.g. Smith 1988, Bradford and Roff 1997). The capacity for diapause is in fact wide spread among insects and has contributed to the success of the Class by allowing them to exploit seasonal resources and avoid harsh winters, dry seasons, or other seasonally adverse conditions. The arrest in development, accompanied by reduced metabolism, has been observed in diverse embryonic stages, different larval instars, pupae, pharate adults, and adults, but for any given species, the potential for diapause is most commonly restricted to a single stage. In a few cases diapause occurs at a specific stage in each generation, regardless of the prevailing environmental conditions (obligatory diapause), but more commonly environmental cues such a day length are utilized for the programming of diapause (facultative diapause) (Delinger 2002).

Extensive research on diapause during the past half century provided a comprehensive view of the environmental regulators of diapause, the hormonal system that direct its onset and termination, and the theoretical properties of the clock mechanisms involved in insect periodism (for a review see Takeda and Skopik 1997, Delinger 2002 and Nation 2002). However molecular information on diapause is available currently for only a few species, and in all cases the results are rather fragmentary (Dunlap 1999, Delinger 2002). Actual knowledge about molecules involved in insect circadian timekeeping comes mostly from the genetic research on the fruit fly *D. melanogaster* (for a review see Dunlap 1999, Scully and Kay 2000). But this species has only a weak diapause in the adult stage, and other insects would be much better models for examining diapause regulation (Delinger 2002).

In Lepidoptera, studies on environmental cues behind the onset and termination of diapause - basically thermoperiod and photoperiod - focus mainly in multivoltine species in the temperate regions. Commonly, the short daylenghts of the late summer signal the advent of winter (e.g. Adkisson 1966, Fantinou and Kagkou 2000, Miller *et al.* 2000, Roditakis and Karandinos 2001). Winter is thus anticipated long before the onset of low temperatures, allowing the insect to store additional energy reserves and seek a protected site for over-wintering (Delinger 2002). The increase in day lengths signals the advent of spring leading to the advert of diapause (Adkisson 1966).

Among Lepidoptera, there are also various taxonomic unrelated groups that have a common *fixed point* in their phenologies (Tammaru *et al.* 2001). Young larvae can develop successfully only on immature foliage of deciduous host trees in temperate areas and egg hatching in these early spring-feeders must therefore be synchronised with bud burst (Feeny 1970). In other climatic areas synchronisation with leaf flush in evergreen hosts and with the beginning of the wet season seem to be important (Floater 1997, Fitzgerald and Underwood 2000). This *fixed point* imposes a constrain that enforces strictly univoltine phenologies, even if the length of the season would allow more generations to develop. As a consequence, these Lepidoptera have extra waiting time to be divided between the different life stages, which can be spent on diapause (Tammaru *et al.* 2001).

According to Hunter (1991) outbreaking macrolepidoptera in northern hardwood trees are preferably univoltines, exhibiting egg diapause and being early spring feeders. Tammaru *et al.* (2001) used the term *winter-moths syndrome* (without reference to any particular taxonomic group) to characterize temperate species that exhibit adult flight and, hence, oviposition late in autumn and late flight is achieved by prolonged pupal periods. Afterwards these species also exhibit egg diapause. The most well known examples are the winter moth *O. brumata* (Topp and Kirsten 1991, Peterson and Nilssen 1998) and the autumnal moth *Epirrita autumnata* (Borkhausen) (Tammaru *et al.* 1999, Tammaru *et al.* 2001). An example of an univoltine species with a fixed point that do not have a diapause on its life cycle is the Mexican madrone butterfly *E. socialis*, inhabiting mountains areas in Mexico. However this species goes through a voluntary hypothermia during larval development, which occurs in winter, so that it will be prolonged (Fitzgerald and Underwood 2000). In the selection of the timing and duration of the different life stages its important the synchronization with its *fixed point*, mortality factors and metabolic costs (Mitter *et al.* 1979, Petterson and Nilssen 1998, Tammaru *et al.* 2001). Not much is known about the environmental cues that lead to avert of the diapause in this type of insects but its known to have a genetic base and strong adaptative value (Mitter *et al.* 1979, Tammaru *et al.* 1999).

The *Thaumetopoea* species of the family Thaumetopoeidae are all univoltine and can be grouped in species having egg diapause, the so-called “summer processionary moths” and a pupal diapause the “winter processionary moths” (Douma-Petridou 1989, Schmidt 1989, Halperin 1990).

In the “summer processionary moths” (*T. pinivora*, *T. processionea*, *T. bonjeani*, *T. solitaria* and *T. herculeana*), the eggs hatch after a winter diapause in earlier spring, followed by a fast larval development of about three months. Two of this species, *T. pinivora* and *T. processionea*, are early spring feeders in the colder areas of Central Europe, the first feeding on *Pinus spp.* and the second on *Quercus spp.* (Douma-Petridou 1989, Schmidt 1989, Halperin 1990).

The “winter processionary moths” (*T. pityocampa*, *T. Wilkinsoni* and *T. jordana*) all developed in warmer Mediterranean and semi-arid climates. The eggs are laid in summer or fall, and hatch without a previous diapause, occurring a long larval development through wintertime, followed by a pupal diapause in spring (Douma-Petridou 1989, Schmidt 1989, Halperin 1990). In these moths it’s the break of the pupal diapause and adult emergence during the summer that times the annual life cycle. Another well-known Thaumetopoeidae is the *O. lunifer* species complex, widespread in Australia, feeding in *Eucalyptus spp.* and *Acacia spp.*. These species, or species complex, has a very similar univoltine life cycle with the *Thaumetopoea* species with pupal diapause (Floater 1996 a,b,c).

The pine processionary moth *T. pityocampa* is one of the “winter processionary moths”, with its basic life-cycle features, inhabiting the Mediterranean area. Not much is known about the factors timing the annual life cycle of this species or the other “winter processionary moths”. The sudden appearance of a desynchronized population of *T. pityocampa* in National Pine Forest of Leiria (*T. pityocampa* SP), which was discussed in Chapter 3 and 4, points to a different timing of the adult emergence, which had a dramatic consequence on the population life-cycle timings and density.

In the present work we present data collected along several years for the adult phenology of both *T. pityocampa* populations (SP- summer population; WP- winter population) in Leiria and for other normal populations in Portugal. Laboratory and field data were recorded and analysed so that environmental cues that trigger adult emergence could be assessed.

5.2. Methods and Materials

5.2.1. Areas of study.

The areas of this study were: a) National Pine Forest of Leiria (Latitude: 39º50´N, Longitude: 8º57´W, Altitude: 30-50 m a.s.l.), where both the normal *T. pityocampa* WP and desynchronized SP were collected; b) Apostiça, located in the Setúbal Península, (38º30´N, 9º11´W, 35 m); c) Abrantes, where two pine stands, separated 60 km were selected: Barrada (39º26´N, 8º04´W, 162 m) and Pucariça (39º32´N, 8º15´N, 230 m). For a more detailed description of the areas see Chapter 2, point 2.2.1.

5.2.2. Laboratory studies

Late 5th instar larvae, which had entered the pre-pupal stage, were collected in the field from all populations studied, along six years - between 1998 and 2003. It was possible to collect per-pupae from the SP during all six years study. However the different WP`s were represented only in few years: Leiria WP – 2000, 2001 and 2002; Apostiça – 1999, 2000 and 2002; Barrada - 1998 and 2001; Pucariça - 2000 and 2002. This was due to field sampling miss pupation timing, or to very high pupal mortality due to unknown causes. In Apostiça in the year 2000 pre-pupae were collected in five sampling dates: 30th January, 9th February, 14th February, 16th February and 22nd February. In all the other populations/years pre-pupae were collected in one date only, when empty larval nests started to be found in the field, indicating the beginning of pupation.

Pre-pupae were placed inside plastic boxes (40x30x25 cm), containing a layer of forest soil about 10 cm deep, where pupation took place. The boxes were kept at room conditions, close to a window, and thus under natural daylight. Adult's emergences were recorded daily, every hour, and the adults sexed and placed individually in plastic goblets (8 cm diameter and 10 cm height) covert with a net. The pattern of activity and life span of each adult was also recorded.

Egg-batches were obtained from the laboratory populations of Leiria SP in 1999, and Pucariça 2000. Males and females were placed in an outdoor insectarium (4x4x2 m) shortly after eclosion. Fresh twigs of *P: pinaster* was supplied as substratum for oviposition. Egg-batches were collected every day and kept individually in petri dishes on the laboratory under semi-natural conditions. They were checked every day for determine eclosion time.

5.2.3. Field studies

The phenology of male flight activity was monitored using traps baited with the female sex pheromone ((Z)-13-hexadecen-11-ynyl acetate identified by Guerrero *et al.* (1981). Funnel traps (AgriSense^{TM}), baited with one synthetic pheromone dispenser (AgriSense-BCS Ltd; TP058A140; BFL072) and a killing agent (DDVP strip) were hung at a height of 2.5 – 3.0 m, in the second week of April and checked weekly until October of each year, in the National Pine Forest of Leiria and between early July-October for all other areas. Chemicals were replaced after 30 days.

Male flight activity was monitored along National Pine Forest of Leiria, during three seasons between 2001 and 2003 (eight traps in 2001, eleven in 2002 and three in 2003), according to the scheme described in Chapter 4 (point 4.2.4, see Fig. 4.1). In 2003 male flight was monitored only until the end of SP flight period in July. In Apostiça male flight was monitored during five years between 1998 and 2002, using three traps between 1998-2000, and two traps in 2001-2002. In Pucariça male flight was monitored during two seasons, 1999 and 2000 with five traps. Traps were located at least 200 m apart from each other.

5.2.4. Meteorological data

According to Cabral (1979), the pupal period *T. pityocampa* encompasses three stages: 1) pre-pupa, lasting a few days and characterized by an intense cell division; 2) an obligatory diapause, lasting several months, determining the length of pupal stage; 3) metamorphosis, which starts about one month before adult emergence. Due to this obligatory diapause climatic variables are more likely to have impact in the final months of the pupal period (Wiklund and Solbreack 1982). In this way, to evaluate the impact of climatic parameters upon the timing of adult emergence in the field, climatic data was analyzed starting three months before the beginning of the adult emergence for each population: between February-May for the SP, and May-August for the WP. To evaluate the impact of climatic factors during the flight period, weekly values were correlated to corresponded male captures in the field, until male flight reaches its peak.

Total precipitation, average, maximum and minimum monthly temperatures were obtained in the meteorological station of the Aerial Base of Monte Real for Leiria (Latitude: 39º50´N, Longitude: 8º53´W, Altitude: 52 m a.s.l), in Tapada da Ajuda – Lisbon, for Apostiça (Latitude: 38º42´N, Longitude: 9º11´W, Altitude: 60 m a.s.l.) and Alvega for Pucariça (Latitude: 39º28´N, Longitude: 8º03´W, Altitude: 51 m a.s.l).

5.2.5. Statistical analysis

The statistical analysis was performed using SYSTAT 10. Adult's emergence dates in the laboratory and field were transformed taking 15th April as day one– the first date in which emergences of SP adults were ever recorded. Differences between samples means were tested with One-Way ANOVA, t-tests, Kruskal-Wallis test, and Mann-Whitney U-test tests according to procedures described in Chapter 2 (point 2.2.6). Kendall`s coefficient rank correlation was used to establish the relation between climatic variables in the late pupal period and male flight median time in the field, due to the fact that the analysed date do no conform to a bivariate normal distribution. Regression analyses were used to explore possible predictive relationships between climatic variables and increase in male captures in the field. Significance was accepted at 5 % level (Sokal and Rohlf 1995).

5.3. Results

5.3.1. *Thaumetopoea pityocampa* laboratory populations

5.3.1.1. Seasonal pattern of emergence

Adults of the Leiria SP emerged in the laboratory over six seasons, beginning in 1998, which is since the outbreak of this population was first recorded (larvae pupated in September 1997). Larvae always pupated in September-October and adults emerged between April and June, being the emergence period clearly separated from all WP`s (Fig. 5.1). In the years when adult of both Leiria populations were obtained in the laboratory (2000, 2001 and 2002), the time gap between the last SP emergences and the first WP emergences felled between 33 days (2002) and 48 days (2001).

The beginning of the different WP's adult's emergence, occurred in July-August with a maximum of 20 days gap between the different years for each population. We obtained pupae simultaneously from Leiria WP, Apostiça and Pucariça in 2000 and 2002. In 2000 significant differences in emergences timings (One-way ANOVA: F=9.943, df=2, p=0.000) were due to an earlier emergence of Pucariça population compared with Apostiça (Tukey=0), but this time only 21 adults of Leiria WP emerged in the laboratory. In 2002, Leiria WP clearly presented an earlier emergence followed by Pucariça and then by Apostiça (One-way ANOVA: F=354.534, df=2, p=0.000; Tukey=0 for all comparisons). In 2001 Leiria WP also presented an earlier emergence than Barrada (t-test: t_{50}=-11.434; p=0.000) (see Fig. 5.1). Unfortunately it was not possible to obtain pupae from both Abrantes populations in the same year, but taking the data from all years together, Pucariça population presented a tendency for an earlier emergence than Barrada population (t-test: t_{754}=29.544; p=0.000, Pucariça: 26th July [13th July-15th August] n=635; Barrada: 9th August [1st –21st August] n=124).

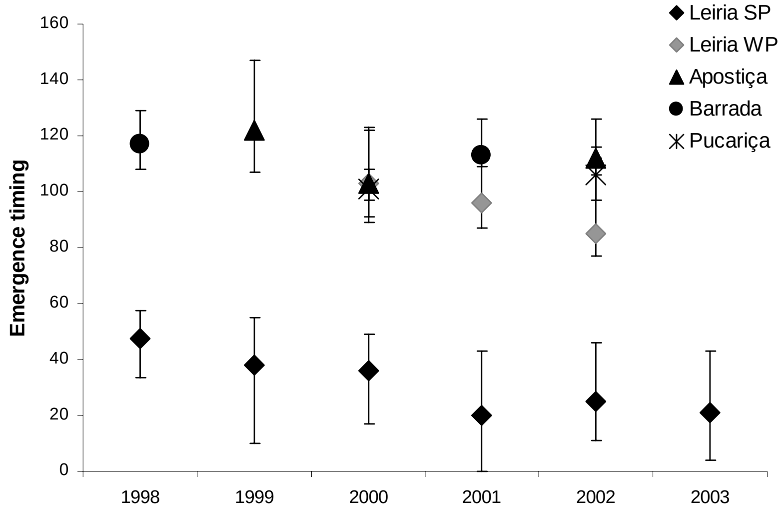


Figure 5.1: Median (dots) and range (bars) of adult emergence dates (counting 15th April as day 1) for *T. pityocampa* laboratory populations, in the different years.

Overall emergence timing was different among the different populations (One-way ANOVA: F=14229.810, df=4, p=0.000) in the order along the year: Leiria SP (10th May) < Leiria WP (13th July) < Pucariça (26th July) < Apostiça (10th August) = Barrada (9th August) (Tukey=0). All the WP`s pupate between the end of January and beginning of March, but pupal period length was also significantly different among the five laboratory populations studied (One-way ANOVA: F=4127.712, df=4, p=0.000): Leiria SP (217 days) > Apostiça (180 days) > Barrada (164 days) > Leiria WP (156 days) > Pucariça (144 days) (Tukey=0). This way the later emergence onset in Apostiça and Barrada, compared with Leiria WP and Pucariça was at least partly due to a shorter pupal period.

Adults of the Leiria SP showed the tendency to an earlier eclosion along the six studied years (One-way ANOVA: F=4127.712, df=4, p=0.000): 1998 (1st June) > 1999 (23rd May) =2000

(21st May) > 2002 (10th May) =2003 (6th May) > 2001 (5th May) (Tukey < 0.02) (Fig. 5.1). In 1997-98, the larvae were ready to pupate in early September, but the following years pre-pupae were collected at the end of September- early October (seasons 1999 and 2000) and at the two last seasons occurred by middle October. There was a significant difference in the length of the pupal period on the different seasons (One-way ANOVA: F=1505.354, df=5, p=0.000), which by decreasing order was 1998 (273 days) > 2000 (236 days) > 1999 (233 days) >2002 (210 days) = 2003 (208 days) > 2001 (204 days) (Tukey p=0), clearly the more recent years (2001-2003) having shorter pupal periods than the ones following the beginning of the outbreak (1998-2000).

In Apostiça, season 2000, the emergence timing of laboratory adults, increased positively with their pupation timing ($y = 0.372\ x + 92.240$; $R^2= 0.160$; $F_{1,198}= 37.578$ p=0), being both traits somewhat correlated. In each pupation date, the range of eclosion dates was anyway very large (Fig. 5.2). However a stronger negative relation was found between the pupation timing and pupal period length ($y = -\ 0.628\ x + 183.240$; $R^2= 0.350$; $F_{1,198}= 106.79$ p=0), indicating that a later pupation lead to a shortening in the pupal period, so that emergence timing wont be to much delayed.

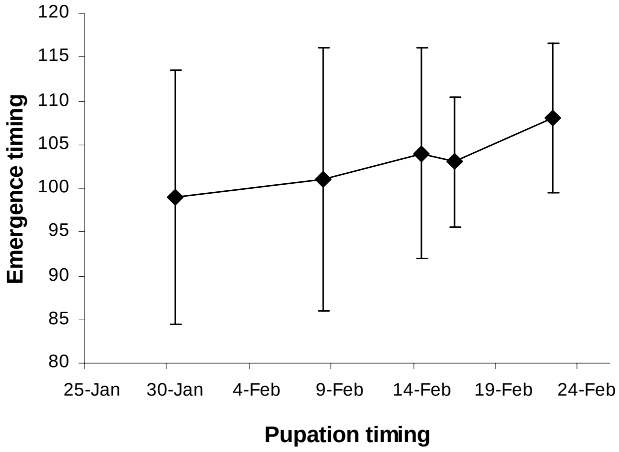


Figure 5.2: Median (dots) and range (bars) of adult emergence dates (counting 15th April as day 1) according to its pupation timing. Data from Apostiça *T. pityocampa* laboratory population, collected in the year 2000.

There was a significant difference between the incubation times of egg-batches obtained from the laboratory populations of Leiria WP in 1999 (40.0±2.2 days n=30) and Pucariça in 2000 (31.9±0.9days n=47) (t-test: t_{75}=22.866; p=0.000). Egg-batches of the Leiria WP ecloded between 8th and 16th of June 1999, and Pucariça egg-batches ecloded between 24th and 26th of August 2000.

For Leiria SP, there was a clear tendency for protogyny in adult's emergence: females emerge before males. In the WP emergences timings were similar for both populations. For all other WP's, there been a clear tendency for protandry: males emerged before the females. The variability of emergence timings, measured by the Standard Deviation, was larger for males in both Leiria populations, larger for females in Apostiça and Barrada, and similar for both sexes in Pucariça (Table 5.1). These results were achieved by a longer male pupal period in Leiria SP, a similar pupal period for both sexes in the Leiria WP, and a shorter male pupal period in all other WP`s (Table 5.2).

Table 5.1: Males and females variability in eclosion timings measured by the Standard Deviation; number of average days in which male eclosion precede female eclosion; t test testing the differences between males and females eclosion timing for each population. Data refers to the average of all years for each population. * significant test.

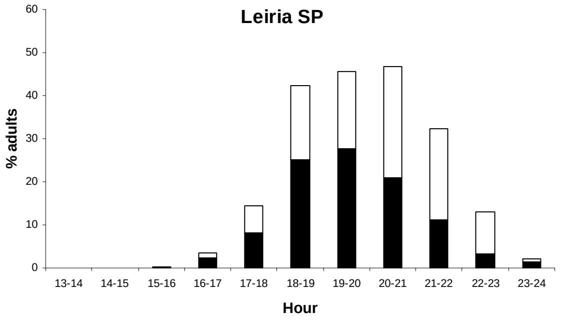
Population	Males s.d.	Females s.d.	Males precede females	t test
Leiria SP	10.8 days	10.2 days	-2.3 days	t_{1454}=4.113; p=0.000*
Leiria WP	9.4 days	8.9 days	-1.6 days	t_{164}=1.130; p=0.260
Apostiça	9.9 days	11.4 days	+6.3 days	t_{703}=-7.834; p=0.000*
Barrada	4.6 days	4.8 days	+4.0 days	t_{122}=-4.560; p=0.000*
Pucariça	5.2 days	5.2 days	+2.4 days	t_{633}=-5.912; p=0.000*

Table 5.2: Males and females variability in pupal length measured by the Standard Deviation; number of average days in which males pupal length exceed female's pupal length. t test testing the differences between length of males and females pupal period for each population and season. Data refers to the average of all years for each population * significant test.

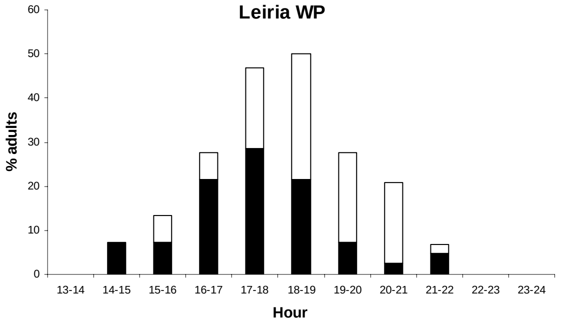
Population	Males s.d.	Females s.d.	Males exceed females	t test
Leiria SP	17.0 days	16.2 days	+2.2 days	t_{1454}=2.457; p=0.014*
Leiria WP	9.7 days	9.9 days	+0.9 days	t_{164}=1.130; p=0.548
Apostiça	8.2 days	10.2 days	-5.1 days	t_{703}=-7.311; p=0.000*
Barrada	4.8 days	4.0 days	-4.0 days	t_{122}=-4.659; p=0.000*
Pucariça	5.0 days	5.1 days	-2.5 days	t_{633}=-6.293; p=0.000*

5.3.1.2 Daily pattern of eclosion

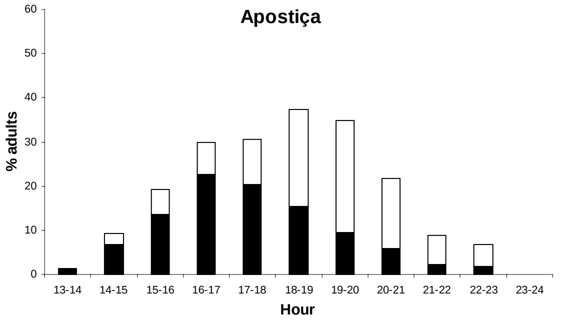
In all populations males showed a tendency to emerge earlier in the day than the females. Once again Leiria SP diverged from the common pattern, emerging the adults later in the day (Fig. 5.3). Eclosion in the SP started after 16 h, most males emerging on average between 18-21 h and most females between 18-22 h. By contrast, the normal WP starts emerging as early as 13 h, females mostly between 14-20 h, and males mostly between 15-21 h (Fig. 5.3). During May, when most of the SP eclosions were recorded, sunset occurred between 19:27 h and 19:52 h, concomitantly between mid July – mid August, when most of the WP`s eclosions were recorded, it occurred between 19:58 h and 19:29 h. Thus after 19-20 h, adult eclosion occurred under a very low light intensity, as it happen with a large proportion of SP adults.



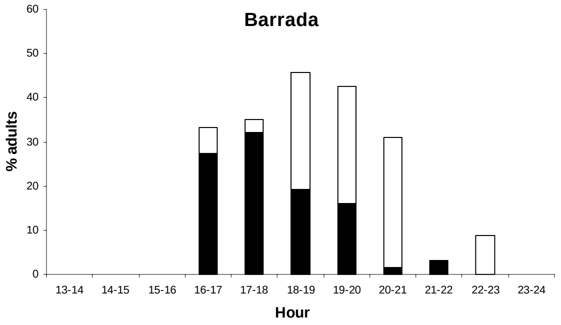
Data from seasons 1999, 2000, 2001, 2002; ♂=533, ♀=418.



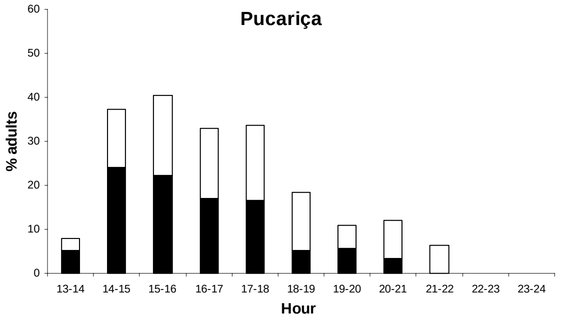
Data from seasons 2000, 2001, 2002; ♂=42, ♀=49.



Data from seasons 1999, 2000, 2002; ♂=220, ♀=197.



Data from seasons 1998, 2001; ♂=62, ♀=34.



Data from seasons 2000, 2002; ♂=228, ♀=189.

Figure 5.3: Daily pattern of male and female eclosion in laboratory populations of *T. pityocampa* originated from pre-pupae from different populations, Monte de Caparica, Portugal. Males - filled bars; females – open bars.

Males lived between one and eight days and females between one and six days, and males presented a longer mean longevity than females, both in the normal WP (t-test: t_{319}=10.296; p=0.000; ♂=4.13±1.13 days n=156; ♀=2.95±0.92 days n=165) as well as in the Leiria SP (t-test: t_{556}=4.322; p=0.000; ♂=3.71±1.10 days n=318; ♀=3.35±0.84 days n=240).

5.3.2. Male seasonal flight activity in the field

In the field, the Leiria SP started emerging over a time gap of 10 days, between 7th May in 2001 and 16th May in 2003, and males were trapped in the field during two months (Fig. 5.4). Male trap timings were clearly different in the three years (Kruskal-Wallis= 232.664, df=2, p=0), occurring flight peak earlier in 2001 - around May 21st - while in the other two years it occurred already in June (Fig. 5.4). In 2001 and 2002 more than 400 males of this population were trapped, more than 90 % of which at the outbreaking area. In 2003 only 187 males were captured in three traps placed in the outbreaking area (see Chapter 4).

High negative correlations were found between average monthly temperature in March, average minimum temperature and precipitation in March and February and the median date of male flight activity in the Leiria SP (Table 5.3). This means that high values of this climatic parameters leaded to an earlier male flight. In fact the year 2001, which presented an earlier male flight peak, also presented higher minimum temperatures and total precipitation in February and March (7.3-11.2 ºC, 125.2-167.6 mm, while in the other years varied between 5.8-8.9 ºC, 30.4-98.8 mm) and higher average temperature in March (14.4 ºC, while in the other years varied between 13.3-13.8 ºC).

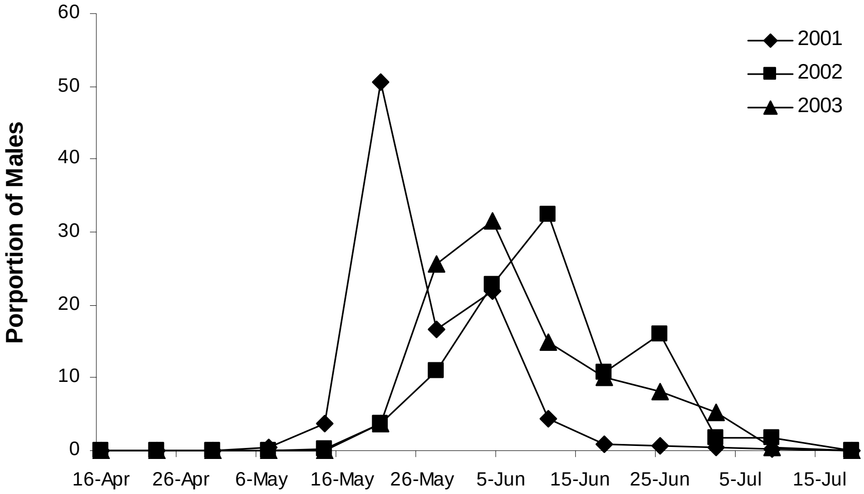


Figure 5.4: Seasonal flight activity of *T. pityocampa* males monitored by pheromone traps in the field, April- July 2001 - 2003. Values correspond to the proportion of trapped males along the flight period in each year. National Pine Forest of Leiria, Portugal.

Table 5.3: Kendall`s coefficient rank correlation between average, maximum and minimum monthly temperatures, total monthly precipitation in February-May and the median male flight date in the Leiria SP.

Month	Average Tem.	Maximum Temp.	Minimum Temp.	Precipitation
February	-0.333	-0.333	-1.000	-1.000
March	-1.000	0.333	-1.000	-1.000
April	-0.333	0.000	-0.333	0.333
May	-0.333	-0.333	-0.816	-0.333

Between mid July and mid September 2001 and 2002 five and six males were detected in traps placed along National Pine Forest of Leiria. These males were trapped regularly along two months, and according to its flight timing, belong to the WP. In traps placed in plots 64, 167, 246 and 313 (see Fig. 4.1) males from both SP and WP were trapped, confirming the fact that both populations coexist geographically.

The beginning of the Apostiça population flight period in the field occurred over a time gap of 12 days in five seasons, and 93 to 527 males were trapped during 1.5-2 months. Male trap timings were different in the five years studied (Kruskal-Wallis= 296.177, df=4, p=0), presenting 2000 and 2001 earlier flight peaks and 1998 and 1999 later flight peaks. Overall a large flight peak occurred in August, and typically a second smaller flight peak was recorded at earlier-mid September (Fig. 5.5). The beginning of the Pucariça population flight period in the field occurred over a time gap of six days on the two seasons studied, and 79-231 males were trapped in the field during about one month, occurring the flight peak in August (Fig. 5.6). Male trap timings were different in the two years studied (Mann-Whitney: X^2= 139.836, df=1, p=0), occurring the flight period later in 1999 (Fig. 5.6)

For both years studied male flight always started later in Pucariça than in Apostiça (1999: 11 days; 2000: 6 days), which coincided also with later flight profiles (1999: Mann-Whitney X^2= 42.083, df=1, p=0; 2000: Mann-Whitney: X^2= 17.710, df=1, p=0). This is the opposite of what was observed in the laboratory populations. In general laboratory populations always started emerging earlier than field populations, although in 1999 in Apostiça the timing was almost coincidental. This differences showed the tendency to be shorter in Apostiça than in Leiria SP and Pucariça (Table 5.4).

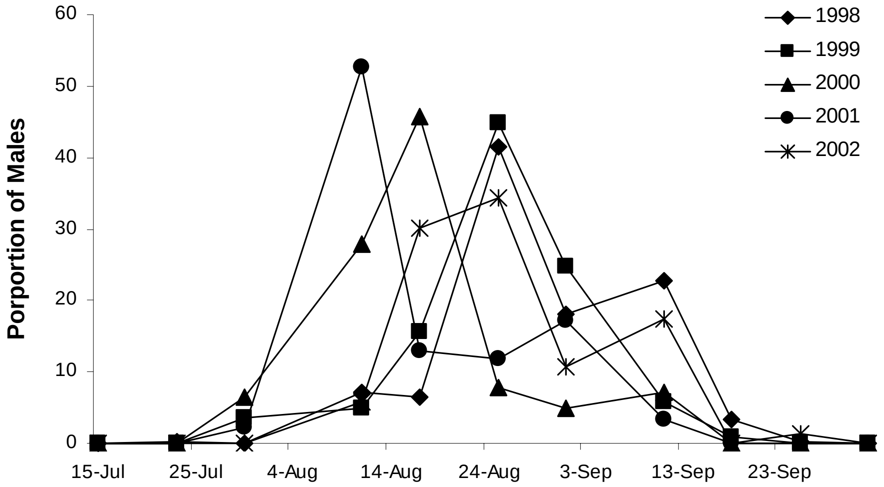


Figure 5.5: Seasonal flight activity of *T. pityocampa* males monitored by pheromone traps in the field, July-October 1998 – 2003. Values correspond to the proportion of trapped males along the flight period in each year. Apostiça, Portugal.

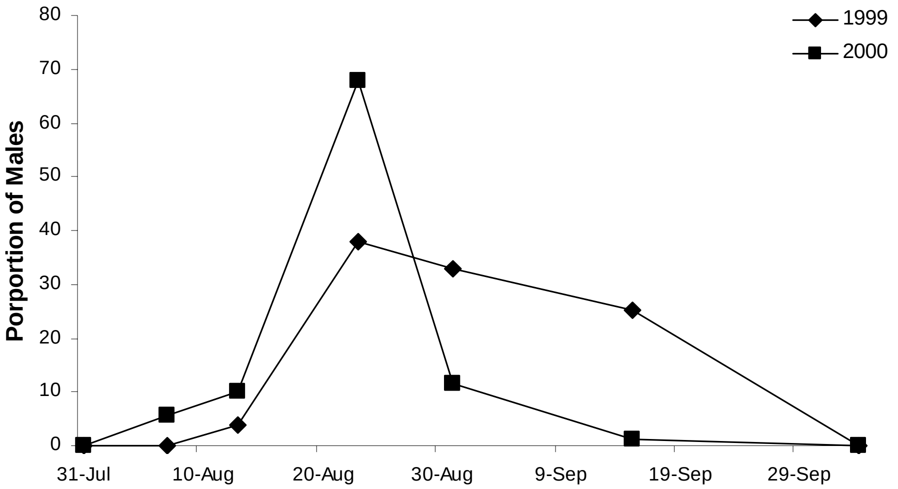


Figure 5.6: Seasonal flight activity of *T. pityocampa* males monitored by pheromone traps in the field, July- September 1999 and 2000. Values correspond to the proportion of trapped males along the flight period in each year. Pucariça, Portugal.

Contrary of what happen with the Leiria SP, no high correlations were found between climatic conditions on the months corresponding to the late pupal period and median date for male flight activity in both WP`s (Fig. 5.5). Late pupal period in the SP occurred any way under a much lower temperature than in the two normal WP (e.g. average monthly temperature, SP (February and March,): 11-14ºC, WP (May and June): 16-22ºC).

Table 5.4: Number of days in which eclosion of laboratory populations preceded the correspondent field population emergence (populations/seasons in which both laboratory and field adults were obtained).

Year	Leiria SP	Apostiça	Pucariça
1999	-	1	-
2000	-	16	25
2001	22	-	-
2002	16	7	-
2003	28	-	-

Table 5.5: Kendall`s coefficient rank correlation between average, maximum and minimum monthly temperatures, total monthly precipitation May-August and the median male flight date in the Apostiça and Pucariça populations.

Month	Average Tem.	Maximum Temp.	Minimum Temp.	Precipitation
May	0.300	0.250	-0.050	0.488
June	-0.050	0.250	-0.350	-0.450
July	0.683	0.586	-0.098	0.488
August	0.053	0.098	-0.150	0.450

Significant positive regressions were found between monthly temperatures and the increase of SP male captures in the field. In the two WP`s it was not possible to establish a pattern between the increase of male traps in the field and climatic variables (Table 5.6). Once again, the temperatures were lower during this period in the Leiria SP (aver. temp.:11.9-21.6ºC; max. temp.:15.4-28.7ºC; min. temp.:6-14.5ºC), than in the other normal WP`s (aver. temp.:19.5-26.4ºC; max. temp.:23-35.9ºC; min. temp.:10.7-20ºC).

Table 5.6: Linear regression between climatic variables and the increase in the number of male captures in the field. * significant regression.

Param.	Leiria SP	Apostiça	Pucariça
Av. Temp.	$y = 0.099 x + 14.409$ $R^2=0.349$; $F_{1,25}=13.376$ $p=0.001$*	$y = 0.021 x + 22.093$ $R^2=0.039$; $F_{1,43}=1.737$ $p=0.194$	$y = -0.042 x + 23.366$ $R^2=0.280$; $F_{1,16}=6.221$ $p=0.024$*
Max. Temp.	$y = 0.100 x + 19.177$ $R^2=0.199$; $F_{1,25}=6.207$ $p=0.020$*	$y = 0.049 x + 27.376$ $R^2=0.091$; $F_{1,43}=4.290$ $p=0.044$*	$y = - 0.040 x + 32.798$ $R^2=0.089$; $F_{1,16}=1.561$ $p=0.229$
Min. Temp.	$y = 0.100 x + 9.603$ $R^2=0.456$; $F_{1,25}=20.932$ $p=0$*	$y = -0.001 x + 16.750$ $R^2=0$; $F_{1,43}=0.005$ $p=0.946$	$y = 0.038 x + 13.658$ $R^2=0.194$; $F_{1,16}=3.856$ $p=0.067$
Precip.	$y = -0.102 x + 10.471$ $R^2=0.013$; $F_{1,25}=0.334$ $p=0.568$	$y = -0.051 x + 1.957$ $R^2=0.024$; $F_{1,43}=1.059$ $p=0.309$	$y = -0.035 x + 1.599$ $R^2=0.032$; $F_{1,16}=0.525$ $p=0.479$

5.4. Discussion

In the present study we present data from a population of *T. pityocampa* that became reproductively isolated from the normal populations through a break of the diapause occurring at a different time of the year, which leaded to a higher population density on its area of distribution (Chapter 3 and 4) thus indicating an evolutionary advantage.

Clear differences were found in adults' eclosion timings of different laboratory populations, which pupae were kept under the same semi-natural conditions. This points to eclosion timings being a population characteristic, with a different response to the same environmental cues. According to Giebultowicz (1999 and 2000) many cells in insect peripheral organs seem to have the molecular machinery necessary to "run" the circadian clock, suggesting that integration of life functions in the time domain may be achieved by a set of independently working clocks rather then by a central master clock. Experimental data demonstrate that the peripheral clocks are photoreceptive, so it's the sun that acts as a master oscillator coordinating all the clocks in the insect body. Photoperiod is a highly predictable environmental cue, which might be the best candidate to explain differences of emergence timings in *T. pityocampa* populations.

Onset and break of diapause in arthropods is in fact known to be a genetic characteristic at population level and considered to have a high adaptive value (Mitter *et al.* 1979, Smith

1988), with a rapid evolution in response to altered environmental conditions (Hairston and Walton 1986) or a trait open to constrain-free adaptive evolution (Tammaru *et al.* 1999). One example of adaptive value of diapause timing is the photoperiodic response curves among insect populations of varying geographic origin, which have shown an increase of critical photoperiod with increasing latitudes. At each new latitude invaded, natural selection assures that the diapause response mechanism of certain species will exploit the same temperature range at which the rest of the insect physiology has already adapted at previous latitudes. Once temperatures decrease with increasing latitudes, the break of the diapause will occur earlier (Pittendrigh and Takamura 1987; Bradshaw and Holzapfel 2001).

The geographical gradient on *T. pityocampa* emergence patterns proves its high adaptive value. Overall the emergence period occurs between June and October, being earlier at higher altitudes and latitudes (Masutti & Battisti, 1990; Devkota *et al.*, 1992). The harsher the winter, the earlier the adult's emerge. In the Greek mountains differences in emergence timings between 800 and 1400 m high are as large as one month (Devkota *et al.* 1992). Emergences of a laboratory population originating from a mountainous area of Northeast Portugal occurs in June-July (own unpublished data), while in Central and South Portugal (Abrantes and Apostiça) — adult emergence occurs mainly during August (Zhang and Paiva 1998, present work).

The WP`s of Pucariça and Leiria, in which the larvae presented a lower speed of development (Chapter 2), showed a shorter pupal period, and a tendency for an earlier onset of adult emergence under the same environmental conditions. These populations are from an area with a higher precipitation level during the winter, which means a lower insulation, and once that lead to a decrease in the speed of larval development (Ayres and Schriber 1994, Ruff and Fiedler 2002b, present work Chapter 2), there is an advantage for those populations to start larval period earlier, building their permanent nests earlier, and so adult emergence – that times the season in which larvae develop - will start earlier. In fact the first larval nests of Leiria normal population are systematically found in the field at the beginning of September, while on the other populations – Apostiça and Barrada – they are found at the beginning of October (own observations).

The differences in the median emergence dates in the Apostiça population (2000), following the pattern of the pupation dates indicates that both traits are no totally decoupled. However differences in the median adults eclosion time (9 days) was much lower than the differences in pupation dates (23 days). The coupling of both traits might just be due to genetic factors and not to the pupation timing *per si*, once earlier pupating caterpillars are probably the progeny of earlier flight adults. In the other populations/seasons it wasn't possible to recorded different pupation timings. However our observations suggest that pupation in the field occurs during a long period. In Apostiça processions were systematically observed since the end of January until the beginning of March, and in Leiria SP since the end of September until November. Emergence timings in the laboratory were any way very diverse, occurring during more than one month even for batches that pupate at the same time. Additionally the emergence periods overlap to a large extent between populations. The emergence period for Apostiça and Pucariça field populations was quite long (1-2 months), and it was recorded the existence of more than one emergence peak, and a variation in those peaks timings in the different years, without much connection with whether factors.

Our data indicates a rather constant incubation time in the laboratory semi-natural conditions for both Leiria SP and Pucariça egg-batches, although significant differences were found between both populations. In the more variable field conditions more diverse incubation timings might occur, once insect egg development is dependent on environmental temperature (Williams 1981). However the lack of egg diapause probably lead to the fact that egg eclosion in the field follow the pattern of adult flight, occurring during a long period of time. In fact the 1st and 2nd instar caterpillar were found in the field during all month of October and part of November, in Apostiça, Barrada and Pucariça (own observations). In insect species with a fine-tuned synchronization with its host, eggs or adult's emerge in just few days (e.g. Brockerhoff *et al.* 1990, Tikkanen and Julkunen-Titto 2003). However the variance in onset and break of diapause can also be largely neutral and may be maintained in a population simply because it is not selected against, and keeping a high level of variance in one population can be highly adaptive (Bradford and Roff, 1997; Tammaru *et al.* 1999), and this seems to be the case of *T. pityocampa*.

Unfortunately it was not possible to detect male flight peaks in the National Pine Forest of Leiria, corresponding to the WP, probably due to its low population level. According to the male flight profiles in the field, we have a very long flight period on this area – 4-5 months – since the beginning of May to mid September, longer than in any other study area. On this long flight period there seem to have occurred a directional selection favoring individuals emerging in one extreme of the range (mid-May to mid-July), leading to the earliest adult eclosion timing known to the species. However during larval development we clearly identified two populations in the field, which present separated emergences timings in the laboratory: the SP which 1st instar larvae occurs in mid July-early August and the WP which 1st instar larvae occurs during September. Only one month separates eclosion timings of larvae of both populations, but that month happens to be August, the warmest of the year, and that makes all the difference (see Chapter 3). Furthermore, there was a tendency for an earlier occurrence of emergence in the laboratory SP and a shortening in the pupal period along the six years studied, which might indicates a selection for an even earlier emergence of adults, stabilizing the larval development during the summer and separating even more this population from the normal WP.

The appearance of the SP in Leiria might give us a chance to study a phenomenon of allochronic isolation drove by ecology. The key question will be: which ecological factors drove the temporal shifting? This subject was approached in Chapter 3 and 4. According to Schluter (2001) the main difficulty of the ecological hypothesis for speciation has been the scarcity of examples from nature, and Leiria National Pine Forest might have given us one. Once the appearance of the SP occurred in an area with no geographical barrier to normal WP`s, this temporal isolation developed in sympatry. This way the SP can also be an addition to the once controversial, and nowadays increasingly accepted theories of sympatric speciation (Via 2001).

Herbivorous insects, due to the fact that they tend to be ecologically specialized in their use of particular plant *taxa*, are the best study systems for investigations of speciation ecology (Funk *et al.* 2002). Host races formation in plant-feeding insects provided the stronger evidence of sympatric speciation via natural selection (Drés and Mallet 2001). The appearance of the SP is somewhat different, since what occurred was a seasonal shift, not a host shift. In fact the different *Thaumetopoea* species differ no only in the use of different hosts, but also on the use of different seasons on the same host (see Table 1.1). This way *T. pinivora* larvae develops in *Pinus spp*. and *T. bonjeani* larvae develops in *Cedrus atlantica* (Endl.) during spring, while *T. pityocampa* develops on the same host species in the same geographical area during winter (Shmidt 1989, Yousfi 1989). The fact that all *Thaumetopoea* present gregarious larvae, which means larger capacity for thermoregulation (Joos *et al.* 1988, Breuer and Devkota 1990, Ruf and Fiedler 2002a), allowed them to explore not only different hosts in their evolutionary path, but also different seasons of the same host.

Once diapause is obligatory, the timing of adult emergence is dependent on two events: the diapause termination and the pupal rate of development afterwards. In Lepidoptera, as ectotherms, pupal rate of development is dependent on temperature (Topp and Kirsten 1991; Peterson and Nilsson 1998) and that is expected in *T. pityocampa*. If pupal development occurs one month before adult emergence, that means, February-March in Leiria SP and May-June in the normal WP, the desynchronized population will experience a much lower temperature during the development than the normal populations. In Leiria SP, warmer temperatures in February and March in the season 2001, seem to have resulted in an earlier adult emergence. On the normal WP`s, there wasn't any clear relationship of emergence time and weather at the end of pupal period, but on this case weather was far more warmer. The fluctuations of the SP adult's emergence timing on the different years due to weather conditions during pupal development might lead that both WP and SP still can interbreed in the field, although in the laboratory both this populations have separated emergence timings.

Temperatures influence not only the rate of development, but can also influence the response to the critical photoperiod (Pittendrigh and Takamura 1987, Roditakis and Karandinos 2001). Under natural over wintering conditions insects are usually capable of initiating development long before development is actually initiated. For example diapausing pupae of the flesh fly *Sarcophaga bullata* (Parker) from 40ºN are in a fixed period of latency during autumn and early winter and fail to break diapause in response to high temperatures at the time. However, by early January they are fully capable of responding to high temperatures, but the low temperatures that prevail at that time of the year prevent this from happening. Only when the soil temperatures rise in the spring is development observed in the field. So is possible to distinguish between diapause –the period of fixed latency – and post-diapause – the stage that is fully capable of initiating development when favourable conditions are present (Delinger 2001). For SP a larger influence of temperature during pupal development might be due to the fact that pupal development occurs with a lower degree-day level, making them more sensitive to a lowering in the temperature. It also might be that lower temperatures, which can occur during the month of February, decrease their sensitivity to the photoperiod, delaying the break of the diapause. Milder temperature helps to explain the earlier onset of adult emergence in laboratory populations of *T. pityocampa* comparing with the ones in the field.

Adult emergence in the normal population was synchronized with the hottest and driest days of the year. SP adult emergence occurred under different climatic conditions, in a timing of increasing temperature. A sharp drop in both light intensity and air temperatures at dusk is essential for eliciting the onset of male flight in *T. pityocampa*. A low light intensity and a drop in air temperatures are also essential to the onset of female calling (Zhang and Paiva 1998). But these observations were made during the emergence peak of the normal populations in August, when nocturnal temperatures were between 19-25ºC. Such temperatures are not expected at night during SP emergence period, and its unknown how limited low temperatures are to the adult's normal activities. However this might explain why the increase of SP male captures in the field followed the pattern of the increasing temperatures in spring, something that was not observed in the normal WP`s.

The originally observed rhythmic output in *Drosophila pseudoobscura* (Frolova & Astaurov) and the one that drove most of the early research on insect rhythms was pupal eclosion, which takes place in a defined window of time near dawn (Dunlap 1999). *T. pityocampa* adults also show a daily rhythmic output during the emergence period, different for males and females. Once the moth is strictly nocturne, its logical an emergence close to the sunset, so that adults can initiate activity as soon as light intensity decrease. In the desynchronized SP, a larger proportion of adults emerge already at night than in the normal WP. The photoperiod is not very different during the SP and WP emergence time (SP: 13:48-14:37 h; WP: 14:33- 13:38 h) so other environmental cues must be used for regulates the daily pattern of emergence. An increase of temperature to a certain level seem to be a good candidate, once SP, which emerge at a colder time, emerge later during the day.

Protandry is defined as the earlier emergence of males, being a common feature in life histories and could be the result of sexual selection on males to maximize mating, or alternatively an incidental by-product of other selection pressures on the sexes (Wiklund and Solbreck 1982). The first hypothesis is the adaptive one, and is connected with butterfly and moth species of seasonal environments, with non-overlapping generations, where male and female populations emerge according to given time curves of eclosion which are under genetic control, in which females mate only once and preferably on the day of the eclosion, male mortality is constant and age-independent, and the only prerequisite for mating to occur is that a male encounters the female (Fagerström and Wiklund 1982; Wiklund and Solbreck 1982; Nylin *et al.* 1993; Zonneveld 1996). Females are selected to minimize the time lag between eclosion and mating, minimizing the risk of pre-reproductive death and this is achieved when there is a maximum of living males in the population (Fagerström and Wiklund 1982). All this life-history traits occur in *T. pityocampa* population, and according to our data males live longer than females, another reason for the existence of protandry, which was observed in most of the WP. In Leiria SP, protogyny was found instead, and there is no adaptive reason for such phenomenon.

The differences in eclosions times may be a by-product of selection for other life history traits. In many butterflies males are smaller than females (which might be adaptive per se) and thus should have a shorter development time as an incidental by-product of size differences between sexes – the incidental explanation (Wiklund and Solbreck 1982). *T. pityocampa* adults have in fact sexual dimorphism, being males much smaller than females, but several works found that the evolution of both this traits – sexual size difference and protandry – are decoupled (Wiklund and Solbreck 1982; Nylin *et al.* 1993). In Lepidoptera protandry was in fact, found to be due to differences in pupal development time (Wiklund and Solbreck 1982; Topp and Kirsten 1991; Nylin *et al.* 1993). Pupal rate of development in both sexes was also found to respond differently to environmental factors, defining the degree of protandry (Wiklund and Solbreck 1982). According to Wiklund and Solbreck (1982), male pupal development time was relatively constant and female development was more dependent on environmental conditions. This hypothesis might explain the tendency towards protogyny under milder environmental conditions in the laboratory, once SP emerges earlier in the laboratory than the other normal WP`s. SP emergence timing seem to be very sensitive to climatic differences in the field, and the earlier emergence period observed in the laboratory might be stronger in the females. The fact that protogyny is observed in the laboratory, it doesn't mean it occur in the field.

Another explanation it would be a desynchronization of this new population to other environmental cues that might regulate protandry. Females in Apostiça and Barrada populations vary more than males in emergence timings than males (shown as standard deviation in Table 5.2). This was also found in other Lepidoptera species presenting protandry, and explained by a lower selection pressure on females for early eclosion than on males (Wiklund and Solbreck 1982, Peterson and Nilssen 1998). It's intriguing that in both populations in Leiria the variation in emergence timings is larger on males than on females, indicating. It might be that natural selection did not fine-tune yet the emergence timings on these populations.

6. Population dynamics of great tit in coastal pines in South-Central Portugal.

6.1. Introduction

Great tit *P. major* is a common forest cavity-nester bird all over Europe and is one of the most thoroughly studied bird species due mainly to the facility to keep track of a large proportion of the population in the field through the erection of nest-boxes (Lack 1964, Perrins 1965). The species is extremely suitable as a model organism and as been used for solving ecological and evolutionary questions during in the last fifty years (e.g. Lack 1964, Pettifor *et a*l. 2001, Sanz *et al*. 2000, Tinbergen and Verhulst 2000, van Noordwijk *et al*. 1995, Buse *et al.* 1999). These studies have been performed mostly in central (e.g. Perrins 1965, Tinbergen and Verhulst 2000) and northern Europe (e.g. Rytkönen and Orell 2001), although some recent studies have also been performed in the Mediterranean area (Barba and Gil-Delgado 1990, Barba *et al*. 1993 and 1995, Maicas and Fernandez Haeger 1996). Information on geographic variation, including the Mediterranean area, in breeding biology is available (Belda *et al.* 1998, Sanz 1998, Sanz *et al*. 2000, Encabo *et al.* 2002), however studies at lower latitudes are still scarce. In Portugal, to my knowledge only one study of *Parus spp*. populations has been conducted so far, a one-year study of the tit guild in São Jacinto – Aveiro (Fidalgo, 1990).

Due to modern forestry practices, many forests consist of homogenous, even-aged stands, lacking dead trees and with a very low supply of natural cavities for breeding. The fact that great tits are largely insectivorous (Betts 1955, Mols and Visser 2002) and easily occupy nest-boxes, make them potentially suitable as pest control agents in young forest plantations where tree age homogeneity normally precludes breeding due to lack of natural cavities. Additionally its breeding biology is tightly dependent on Lepidoptera larval abundance in the forest (Nager and van Noordwijk 1995).

In the present work a three-year study of two great tit populations was conducted in *P. pinaster* coastal pine plantations in Portugal, separated by about 200 Km. The purpose of the study was: 1) to assess how much nest-boxes can increase the population density of great tits in pine plantations; 2) to study the species population dynamics.

6.2. Methods

6.2.1. Areas of study

National Pine Forest of Leiria is located 150 km north of Lisbon (Latitude: 39º50´N, Longitude: 8º57´W, Altitude: 30-50 m a.s.l.). Three plots with trees aged 10-15 years were selected for placing the nest-boxes. Two of them, plots 24 (21 ha) and 63 (32 ha), are located at the north of the Forest, separated by about 1 km. These boxes were erected in the winter 2000/2001. At the south of the Forest, plot 314 (36 ha) was also selected for the study and nest boxes were put up in the winter 2001/2002. This plot is separated from the other two by about 10 Km (see Fig. 3.1). Plot 24 is located at the border of the Forest, close to *E. globulus* stands and agricultural fields. The other two plots are completely surrounded by pine stands. The nest boxes were placed at a density of one per ha at equal distance from each other.

Apostiça, is a private forest property with a total area of about 4 000 ha located in the Setúbal Península, also close to the Atlantic Ocean but located 40 Km south of Lisbon (38º30´N, 9º11´W, 35 m). A square with an area of 200 ha was selected and 52 nest-boxes were placed there in the season 2000/2001, at equal distances from each other. In the season 2001/2002, the number was increased to 68 and in 2002/2003 to 72. This area corresponds to Apostiça 2, described in Chapter 2, and was very open area with large tree age diversity and many large trees (DBH > 20 cm) mainly *P. pinaster*, with few *Q. suber* and *P. pinea*. For a more detailed description and characterization of these pine forests see Chapter 2, 3 and 4.

6.2.2. Climatic data

Maximum daily temperatures were shown to be the best environmental temperature measurements to be correlated with tits breeding phenology (McCleery and Perrins 1998, Buse *et al.* 1999). This way, total precipitation as well as maximum daily temperature were used to make the climatic characterization of the two studied areas during the breeding season. Data was obtained from the meteorological stations of the Aerial Base of Monte Real (Latitude: 39º50´N, Longitude: 8º53´W, Altitude: 52 m a.s.l.), at the northern border of National Pine Forest of Leiria and from Apostiça (Latitude: 38º30´N, Longitude: 9º11´W, Altitude: 35 m a.s.l).

6.2.3. Population dynamics on great tit

The breeding season was monitored during three years, between February and July 2001-2003 in Apostiça and in plots 24 and 63 in Leiria, and during two years (2002-2003) in plot 314. The nest boxes were checked at least once a week to determine the number of breeding pairs, laying date, clutch size, hatching date, nestling survival, proportion of second clutches and total number of fledged young produced per couple. Laying date was calculated assuming one egg was laid per day. Second clutches were assumed to be produced by females, who had completed a first clutch of eggs in the same or a nearby box. In the winters of 2001/2002 and 2002/2003 a survey was made to determine the number of nest-boxes occupied by roosting birds.

6.2.4. Statistical analyses

The statistical analysis was performed using SYSTAT 10. Great tit breeding dates were transformed taking 1st April as day one– the most commonly used procedure for analyse these species breeding timings (e.g. Sanz 1998). Differences between samples means were tested with One-Way ANOVA, and t-tests according to procedures described in Chapter 2 (point 2.2.6). To determine whether two variables are independent or co-vary, we determined the correlation coefficients and its significance (Pearson correlation). These tests were performed in continuous variables. Significance was accepted at 5 % level (Sokal and Rohlf 1995).

6.3. Results

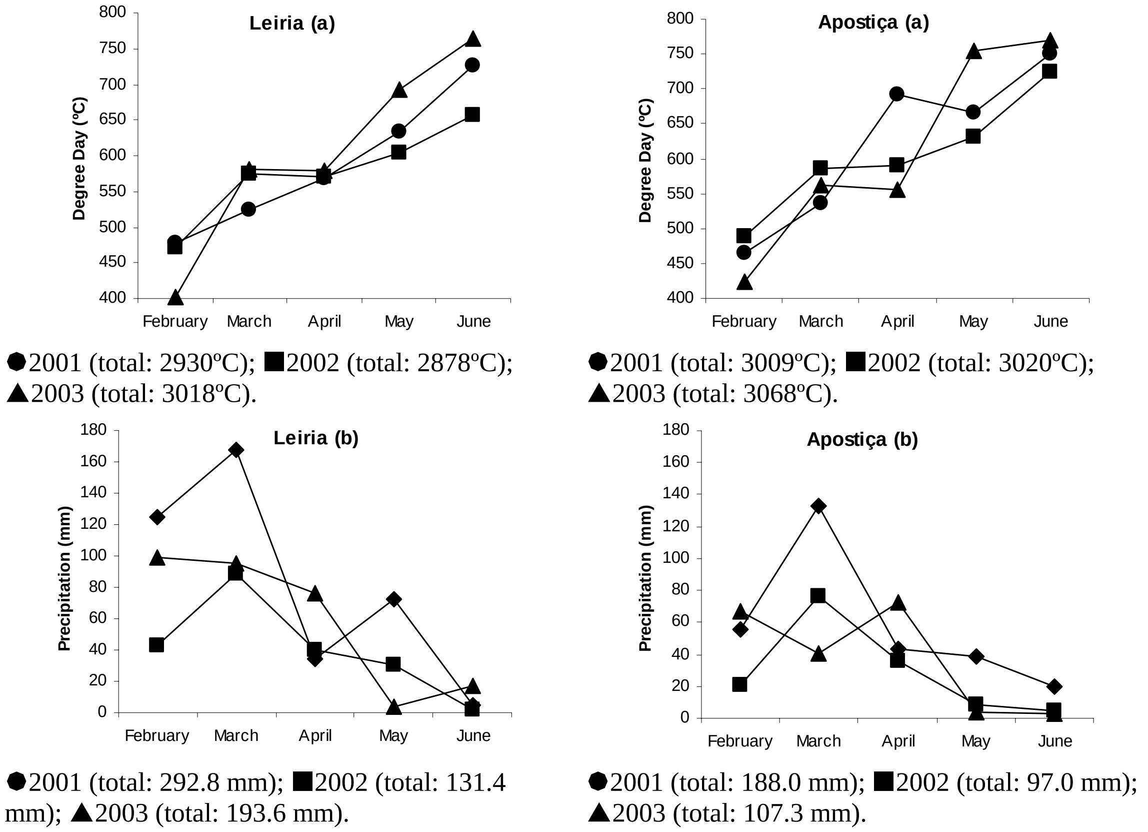
6.3.1. Climatic characterization of the two areas during the breeding season

The breeding seasons were characterized by an increase in the temperature levels and a decrease in precipitation in both areas (Fig. 6.1). In the first three months of the season, the areas did not differ significantly in the average maximum temperature, only the years (February: 16.2±2.3 ºC; March: 18.1±3.3 ºC; April: 19.2±3.3 ºC; Table 6.1). In February, 2001 and 2002 presented higher values (One-way ANOVA: F=25.64, df=2, p<0.001; 2001 and 2002 > 2003 Tukey<0.001), and in March, 2001 presented lower values (One-way ANOVA: F=3.62, df=2, p=0.028; Tukey=0.05). In the months of May and June, maximum temperature also differed between areas (Table 6.1), being Apostiça warmer (May: 22.1±4.3 ºC to 20.8±4.4 ºC; June: 24.9±4.1 ºC to 23.4±5.4 ºC). This time 2003 was the year with the higher values (May: One-way ANOVA: F=10.817, df=2, p<0.001; 2001 and 2002 < 2003 Tukey<0.01; June: One-way ANOVA: F=7.845, df=2, p<0.005; 2001 and 2003 > 2002 Tukey<0.02).

Table 6.1: Two-way ANOVA testing differences in maximum daily temperature, between the two areas and the three different seasons during the months of the breeding season.

	Years			Area			Interaction		
Month	F-ratio	df	p	F-ratio	df	p	F-ratio	df	p
February	24.177	2	0.000*	1.234	1	0.268	1.565	2	0.212
March	4.231	2	0.016*	0.013	1	0.910	0.410	2	0.664
April	0.330	2	0.719	0.199	1	0.656	0.935	2	0.394
May	10.940	2	0.000*	4.473	1	0.036*	0.307	2	0.736
June	7.992	2	0.000*	5.158	1	0.024*	2.361	2	0.097

The degree-day accumulation during the breeding season (sum of all daily maximum temperatures during a specified period) was higher in Apostiça during all breeding seasons studied (Fig 6.1a). Precipitation was always lower in Apostiça than in Leiria for each month/year. Overall the amount of precipitation in March was higher than in the other months (Fig. 6.1b), and in 2001 was exceptionally high: 167.6 mm in Leiria and 132.6 mm in Apostiça, being the average 85.2 and 97.8 mm, respectively.



●2001 (total: 2930ºC); ■2002 (total: 2878ºC); ▲2003 (total: 3018ºC).

●2001 (total: 3009ºC); ■2002 (total: 3020ºC); ▲2003 (total: 3068ºC).

●2001 (total: 292.8 mm); ■2002 (total: 131.4 mm); ▲2003 (total: 193.6 mm).

●2001 (total: 188.0 mm); ■2002 (total: 97.0 mm); ▲2003 (total: 107.3 mm).

Figure 6.1: (a) Degree day accumulation of the maximum monthly temperature; (b) Total monthly precipitation; along *P. major* breeding seasons.

Overall, 2001 stands out as being wetter in the beginning of the season, and 2003 as being colder at the beginning but warmer in the last two months of the breeding season than normal. Apostiça is a warmer and drier area than Leiria, especially at the end of tits breeding season.

6.3.2. Breeding timing

The median date for breeding onset in great tits occurred in March in both areas. The production of 1st clutches continued well into April in both areas and started as early as February in Apostiça during 2003 (Table 6.2). When testing for differences between years and areas in the mean start of egg laying, we found no differences between areas (F = 1.49; df = 1; P = 0.22). However, we found significant differences between years (F = 3.41; df = 2; P = 0.035) and for the interaction between year and area (F = 5.64; df = 2; P = 0.004). In Apostiça the difference between the seasons (One-way ANOVA: F=4.335, df=2, p=0.015) was due to an earlier laying in 2003 than in 2002 (Tukey: P=0.013), and in Leiria (One-way ANOVA: F=5.605, df=2, p=0.006) was due to an earlier laying in 2001 than in the other two years (Tukey<0.02).

Table 6.2: Median date and range of dates when the 1st and 2nd clutch was started. Sample size in parentheses.

	1st clutch start	2nd clutch start
Apostiça, 2001	16th March; 2nd March- 26th April (26)	8th May; 31st March – 27th May (17)
Apostiça, 2002	20th March; 2nd March – 25th April (38)	15th May; 12th March – 20th June (23)
Apostiça, 2003	11th March; 20th February-20th April (56)	6th May; 1st April-21st May (36)
Leiria 24, 2001	13th March; 1st -24th March (7)	10th May; 4th April– 21st May (7)
Leiria 24, 2002	21st March; 12th March –26th April (11)	14th May; 30th April – 30th May (6)
Leiria 24, 2003	26th March; 13th March-22nd April (14)	8th May; 19th April –22nd April (6)
Leiria 63, 2001	21st March; 18th -25th March (2)	17th May; 13th –21st May (2)
Leiria 63, 2002	21st March; 18th –27th March (10)	15th May; 2nd-23rd May (6)
Leiria 63, 2003	23rd March; 16th March – 5th April (12)	11th May; 28th April- 11th June (7)
Leiria 314, 2002	23rd March; 16th-27th March (4)	15th May;12th-17th May (4)
Leiria 314, 2003	21st March; 12th March-26th April (17)	12th May; 3rd-30th May (12)

6.3.3. Density of breeding pairs

Predation was unimportant in the present study. Although the entrance hole of nest-boxes in Apostiça was sometimes enlarged by woodpeckers *Dendrocopos major* (L), it only stopped tits from using those nest-boxes. Predation on clutches or adults were never observed in any of the areas.

Between 2-4 couples of crested tit *Parus cristatus* (L.) breed in Apostiça in each season studied. Onset of egg laying occurred between 1st March and 22nd April, producing a clutch of 5-6 eggs and 0-5 fledglings. No second clutch was ever recorded. In Leiria, one pair of crested tits bred in the same area in plot 63 during all the three seasons producing two clutches in 2001 and 2003, and one clutch in 2002. Onset of egg laying occurred between 1st March and 20th April in the first clutch, producing a clutch of 5-6 eggs and five fledglings, and between 24th April and 1st May, on the second clutch, producing a clutch of 4-5 eggs and 3-4 fledglings. Three breeding pairs of coal tit *Parus ater* (L.) were recorded in Leiria. One in Plot 24 in the season 2001, starting on 23rd of March, producing four eggs, however the nestlings died when they were 20 days old. In 2002, two pairs bred in Plot 314, starting on 5th and 9th April, both producing six eggs. In one of the attempts all nestlings died when one week old and in the other attempt all fledged.

The nest-boxes were however, mostly occupied by *P. major*. The area in Apostiça ended up with a lower density of nest-boxes per ha (0.26-0.36 ha^{-1}) than any plot in Leiria (1 ha^{-1}), although we increased the density of boxes with time. A higher number of breeding pairs per nest-box was found in Apostiça during the season 2003 (0.78 pairs/nest-box), indicating that the low density of nest-boxes during previous years might have restricted great tit density contrary to what happen in Leiria (maximum value found was 0.67 pairs/nest-box).

Breeding pairs density increased after the initial year in all the areas, indicating that it takes some time before density reaches a new equilibrium after the erection of nest-boxes (Fig. 6.2a). Plot 314 of Leiria in the second season (2003) had the highest number of nestlings produced per ha (4.39) recorded in this study (Fig. 6.2c). Only great tits were found roosting in the nest-boxes in winter. The density of breeding adults during springtime (Fig. 6.2a) was much higher than the density of roosting birds in the previous winter (Fig. 6.2b).

6.3.4. Evolution of the breeding parameters

In all the three plots in Leiria, we found a lower proportion of second clutches, during the second and third breeding seasons compared with the first season when the nest-boxes were erected and breeding density was low. During the first breeding season all first clutches were succeeded by a 2nd clutch in this area. In Apostiça, the frequency of 2nd clutches was constant over the years – 60-65 % (Fig. 6.3).

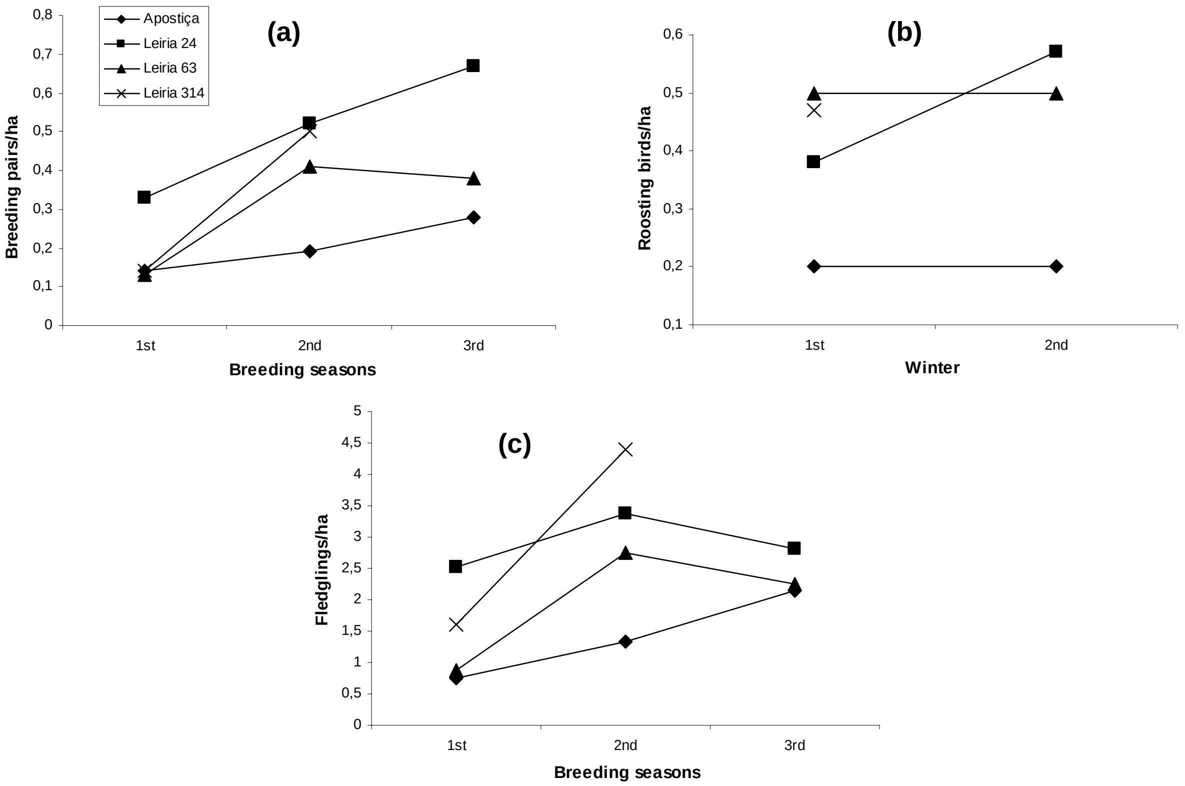


Figure 6.2: (a) Number of breeding couples per ha; (b) number of roosting birds per ha during winter; and (c) number of fledglings produced per ha. (b) and (c): in Leiria 314, 1st and 2nd breeding seasons corresponds to 2002 and 2003, and on all the other areas corresponds to 2001 and 2002; (b) in Leiria 314, 1st winter corresponds to 2002/2003, and on all the other areas corresponds to 2001/2002

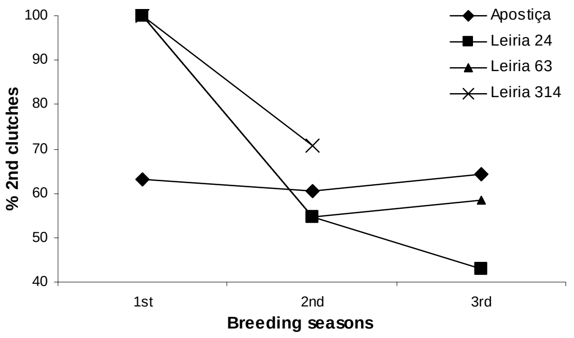


Figure 6.3: Evolution of the proportion of 2nd clutches along the breeding seasons. In Leiria 314, 1st and 2nd breeding seasons corresponds to 2002 and 2003, and on all the other areas corresponds to 2001 and 2002.

Average clutch size in the first clutch was larger than in the second clutch in both populations (Apostiça: t-test: t_{164}=12.206, p=0; Leiria: t-test: t_{149}=7.146, p=0). The number of fledglings produced per pair and nestling survival was also significantly higher in the first clutch in Apostiça (fledglings/pair: t-test: t_{155}=9.107, p=0; survival: t-test: t_{155}=5.473, p=0). However in Leiria the number of fledglings/pair was similar in both clutches (t-test: t_{148}=-1.632, p=0.105), and nestling survival was higher in the second clutch (t-test: t_{148}=-3.084, p=0.002) (see Table 6.3).

Clutch size in the first clutch in Apostiça was lower in the second season (One-way ANOVA: F = 3.969; df = 2; P = 0.022, 2002<2003, Tukey<0.02). However the number of fledglings produced per pair (One-way ANOVA: F = 6.871; df = 2; P = 0.002) and nestling survival (One-way ANOVA: F = 6.746; df = 2; P = 0.002) was lower in the first season (Tukey<0.02 for all comparisons). In Leiria, plots 24 and 63 (when we have a three year data set), although there was a tendency for clutch size in the first clutch to decrease with the years in each plot (see Table 6.3), a significant difference among the years was not found (One-way ANOVA: F = 2.338; df = 2; P = 0.107). Neither were significant the differences in the number of fledglings produced per pair (One-way ANOVA: F = 0.770; df = 2; P = 0.468), neither in the nestling survival (One-way ANOVA: F = 0.834; df = 2; P = 0.440).

Table 6.3: Clutch size (completed clutches), number of fledglings produced per clutch; proportion (%) of hatched nestlings that fledged and total number of eggs and fledglings produced per pair during the breeding seasons.

Area	Egg clutch size		Nº young per pair		Proportion fledging		Total per pair	
	1st	2nd	1st	2nd	1st	2nd	Eggs	Fledglings
Apostiça, 2001	8.4±1.2; 26	6.3±1.3; 16	4.2±2.7; 26	2.2±2.0; 16	53.9±34.5; 26	36.0±33.3; 16	12.5±3.4; 26	5.6±2.7; 26
Apostiça, 2002	8.1±1.1; 37	6.8±0.8; 21	6.0±2.1; 34	3.2±2.3; 20	79.1±27.2; 34	48.8±34.6; 20	11.7±3.8; 37	7.0±3.0; 38
Apostiça, 2003	8.7±1.0; 56	6.7±1.1; 35	6.2±2.3; 52	3.1±2.1; 35	75.2±26.4; 52	47.9±31.2; 35	13.0±3.7; 56	8.0±3.6; 54
Leiria 24, 2001	8.0±0.8; 7	6.4±0.8; 7	3.1±3.4; 7	4.4±2.2; 7	40.4±43.8; 7	77.6±36.8; 7	14.4±1.3; 7	7.6±2.5; 7
Leiria 24, 2002	7.1±1.6; 11	5.0±1.6; 6	4.2±2.6; 11	4.2±1.7; 6	63.3±38.6; 11	96.7±8.2; 6	9.8±3.7; 11	6.5±3.5; 11
Leiria 24, 2003	6.8±1.0; 11	5.7±0.5; 6	4.0±2.2; 11	2.5±1.4; 6	64.9±37.9; 11	57.5±33.1; 6	8.6±3.6; 11	4.5±2.6; 13
Leiria 63, 2001	8.0±0; 2	5.5±0.7; 2	5.5±0.7; 2	5.0±1.4; 2	68.8±8.8; 2	100±0; 2	13.5±0.7; 2	10.5±2.1; 2
Leiria 63, 2002	7.7±1.1; 10	6.6±1.1; 6	5.1±1.7; 10	5.0±1.7; 6	69.2±26.1; 10	89.0±17.3; 6	11.6±3.9; 10	8.1±3.4; 10
Leiria 63, 2003	7.3±1.1; 12	5.6±0.8; 7	3.7±2.5; 12	4.7±0.8; 6	54.4±36.0; 12	85.0±13.0; 6	10.3±3.4; 12	6.0±3.6; 12
Leiria 314, 2002	8.0±0.8; 4	7.0±0.8; 4	6.5±2.1; 4	7.0±0.8; 4	90.6±12.0; 4	100±0; 4	15.0±0.8; 4	13.5±1.7; 4
Leiria 314, 2003	7.6±1.1; 16	6.4±0.9; 12	5.4±2.7; 16	5.9±0.8; 12	73.1±33.8; 16	96.2±7.0; 12	11.9±3.3; 16	9.3±3.9; 16

In Apostiça the total number of eggs produced per pair was similar in all the three seasons (One-way ANOVA: F = 1.243; df = 2; P = 0.292), but in the first season the total number of fledglings was lower than in the other two (One-way ANOVA: F = 3.575; df = 2; P = 0.031; 2001<2003 Tukey<0.05). In plots 24 and 63 in Leiria, we found a decrease in the total number of eggs produced per pair (One-way ANOVA: F = 6.489; df = 2; P = 0.003; 2001 > 2002 and 2003 Tukey<0.05), probably due to the decrease in the proportion of second clutches. However this was not translated in a significant decrease in the total number of fledglings produced per pair with the seasons (One-way ANOVA: F = 2.953; df = 2; P = 0.061).

The clutch size in the first and second clutches was similar in all the three plots in Leiria (1st clutch: 7.4±1.1, n=73; One-way ANOVA: F=0.99, df=2, p=0.38; 2nd clutch: 6.1±1.1, n=50; One-way ANOVA: F=3.03, df=2, p=0.058). However, the number of fledglings produced varied between plots in both clutches (1st clutch: One-way ANOVA: F=3.17, df=2, p=0.048; 2nd clutch: One-way ANOVA: F=12.3, df=2, p<0.001) being larger in Plot 314 (1st clutch: Plot 24 vs. Plot 314, Tukey: p=0.039, Plot 24: 3.9±2.6 n=29; Plot 63: 4.4±2.2 n=24; Plot 314: 5.7±2.6 n=20; 2nd clutch: Plot 24 and Plot 63 vs. Plot 314, Tukey: p<0.05, Plot 24: 3.7±1.9 n=19; Plot 63: 4.9±1.2 n=14; Plot 314; 6.2±0.9 n=16). Nestling survival in first clutches, did not differ between plots (One-way ANOVA: F=1.74, df=2, p=0.18; 59.4±34.3 n=73), but in the second clutches survival was higher in Plot 314 (One-way ANOVA: F=3.69, df=2, p=0.033; Plot 24 vs. Plot 314, Tukey: p=0.026; Plot 24: 77.3±32.1 n=19; Plot 63: 88.9±14.3 n=14; Plot 314:97.1±6.2 n=16).

The total number of eggs produced per pair was similar in the three plots in Leiria (One-way ANOVA: F=2.03, df=2, p=0.14; 11.2±3.7 n=73). However, the total number of fledglings was much higher in Plot 314 (One-way ANOVA: F=8.97, df=2, p<0.001; Tukey: p<0.02; Plot 24: 6.1±3.1 n=29; Plot 63: 7.3±3.6 n=24; Plot 314: 10.4±3.8 n=20).

Overall clutch size in Apostiça was larger than in Leiria both in the first (t-test: t_{190}=-6.20197; p<0.001; Apostiça: 8.4±1.1, n=119; Leiria: 7.4±1.1, n=73) and second clutches (t-test: t_{190}=2.71; p=0.008; Apostiça: 6.6±1.1, n=75; Leiria: 6.1±1.1, n=50). The first clutch in Apostiça varied between 6-11 eggs, the most common clutch size being 8-9 eggs. The second clutch size varied between 4-9 eggs, with the most common egg clutch being 6-7 eggs. In Leiria the clutches were smaller than in Apostiça, with clutch size in the first clutch varying between 3-9 eggs, the most common being 7-8 eggs, and in the second clutch between 3-8, the most common being six. The number of fledglings produced per pair in the first clutch was higher in Apostiça (t-test: t_{183}=-3.03; p=0.003; Apostiça: 5.7±2.5, n=112; Leiria: 4.5±2.5, n=73), but in the second clutch the number of fledglings produced was higher in Leiria (t-test: t_{120}=-2.74; p=0.007; Apostiça: 3.8±2.3, n=73; Leiria: 4.9±1.8, n=49). We found no differences in first brood nestling survival between the two areas (t-test: t_{183}=1.44; p=0.15; 68.7±32.2), but nestlings in the second brood had higher survival in Leiria than in Apostiça (t-test: t_{120}=-4.96; p<0.001; Apostiça: 59.4±34.3, n=73; Leiria: 87.1±23.0, n=49).

The total number of eggs produced per pair was once again larger in Apostiça than in Leiria (t-test: t_{190}=2.27; p=0.024; Apostiça: 12.5±3.7, n=119; Leiria: 11.2±3.7, n=73), however the total number of fledglings was similar in the two areas (t-test: t_{190}=-1.21; p=0.23; 7.3±3.6, n=192).

In Apostiça and Leiria, we found a strong negative relation between the onset of egg laying in the first clutch and the number of eggs produced, the existence of a second clutch and so the total number of eggs produced per breeding couple. In Apostiça there was also a strong negative seasonal relation in the total number of fledglings produced, which was not found in Leiria. The start of egg laying of the first clutch was not related to the number of nestlings or nestling survival of that clutch (Table 6.4). In Apostiça, the timing of the second clutch was strongly related to the number of eggs and fledglings produced in this clutch, as well as to nestling survival, which was not the case in Leiria (Table 6.5). The total number of eggs produced per pair was positively related to the total number of fledglings produced in both areas (Apostiça: r=0.403, p=0; Leiria: r=0.626, p=0).

Table 6.4: Pearson correlation between the onset of egg laying in first clutches for each breeding pair (counting day 1 as 1st April) and the number of eggs and fledglings produced in the first clutch, nestling survival in the first clutch, production of a second clutch, and the total number of eggs and fledglings produced in the two areas studied. Bonferroni probability test, *significant test.

Parameter	Apostiça	Leiria
Nº eggs	r=-0.467, p=0.000*	r=-0.577, p=0.000*
Nº fledglings	r=-0.195, p=0.203	r=-0.067, p=1.000
Survival	r=-0.039, p=1.000	r=0.176, p=0.819
2nd clutches	r=-0.490, p=0.000*	r=-0.432, p=0.001*
Total eggs	r=-0.568, p=0.000*	r=-0.559, p=0.000*
Total fledglings	r=-0.434, p=0.000*	r=-0.288, p=0.080

Table 6.5: Pearson correlation between the start of egg laying in second clutches and the start of egg laying in the first clutch (counting day 1 as 1st April), the number of eggs and fledglings produced in that clutch, and nestling survival in the second clutch, in the two areas studied, in the total data set and in each separate season. Bonferroni probability test, *significant test.

Parameter	Apostiça	Leiria
Day of the 1st clutch	r=0.232, p=0.220	r=0.568, p=0.001*
Nº eggs	r=-0.362, p=0.009*	r=-0.159, p=1.000
Nº fledglings	r=-0.384, p=0.004*	r=0.057, p=1.000
Survival	r=-0.335, p=0.020*	r=0.185, p=1.000

There was no relation between the existence of a second clutch and the number of eggs and nestlings produced in the first clutch in any of the areas or years. Neither any correlation was found between the number of eggs and fledglings produced in the first clutch and the number of eggs in the second clutch (Table 6.6).

Table 6.6: Pearson correlation between the number of eggs and fledglings produced in the first clutch and the existence of a second clutch and the number of eggs produced in that second clutch (in the case of pairs that produced a second clutch). Bonferroni probability test, *significant test.

Area	Correlation		Total
Apostiça	Existence of a 2nd clutch	Eggs 1st clutch	r=0.198, p=0.062
		Fledglings 1st clutch	r=-0.035, p=1.000
	Nº eggs in the 2nd clutch	Eggs 1st clutch	r=0.256, p=0.057
		Fledglings 1st clutch	r=0.010, p=1.000
Leiria	Existence of a 2nd clutch	Eggs 1st clutch	r=0.066, p=1.000
		Fledglings 1st clutch	r=0.040, p=1.000
	Nº eggs in the 2nd clutch	Eggs 1st clutch	r=-0.004, p=1.000
		Fledglings 1st clutch	r=-0.065, p=1.000

6.4. Discussion

6.4.1.Breeding timings and success.

According to a study by Sanz (1998), laying date in great tits showed a significant quadratic relationship with latitude. The mean laying date in south-western and central Europe was found to be in April, in northern Europe in May, and in the northern Africa in late April or early May. The results obtained in the present work, with great tits starting to breed on average in the 2nd-3rd week of March, are the earliest records of breeding in the species. The beginning of breeding in our population was a few weeks earlier than in another coastal pine forest located 100 km north of the Leiria Forest (Fidalgo 1990), or in Mediterranean Spain at 39-41ºN (Belda *et al*. 1998) in which breeding started in mid April - early May.

The onset of breeding in different geographical areas is explained mostly by food availability, ambient temperature, and photoperiod (Sanz 1998). Other factors like the age of the breeding female and her establishment in the breeding area in the previous winter (Nager and van Noordwijk 1995) and heritability (Price *et al.* 1988, van der Jeugd and McCleery 2002) can also be important factors.

Photoperiod opens and closes a window during which reproduction in birds is possible, but within this window, the actual onset of egg laying is determined by additional fine-tuning and supplementary mechanisms that optimise the timing of breeding (Silverin *et al*. 1989, Silverin *et al*. 1993). The onset of gonadal development is regulated by an increase in the secretion of gonadotrophin that is regulated by the number of hours of daylight – the photoperiodic threshold (Silverin *et al*. 1989). Variation in photoperiodic threshold values may account for latitudinal differences in the timing of breeding among birds, being higher at higher latitudes. For example the day length threshold is 12 hr at 70ºN and 11 hr at 45ºN. Once photoperiod is a highly predictable cue, the onset of breeding of a population cannot be much earlier than the very first observation of an egg in a nest in that population (Silverin *et al*. 1993). In Leiria the very first observation of an egg in a nest was on 1st March and in Apostiça it was on 20th February. Once the vernal gonadal growth phase has started, it takes about six weeks for the birds to be able to breed (Silverin *et al*. 1993). Thus in our study populations, gonadal growth should start early/mid January, corresponding to a day length of 9-10 hr in latitudes of 38º30´N-39º50. This threshold is probably much lower than at Eastern Spain – 39 to 41ºN- where breeding starts much later (Belda *et al.* 1998), indicating different thresholds at similar latitudes.

The amount of food available for egg-laying females has also been predicted to be an important factor (Perrins 1970, Seki and Takano 1998). When experimentally provided with extra food, tits (*Parus sp.*) advance the timing of the egg laying by two or eight days (Nilsson and Svensson 1993, Nilsson 1994). The biosynthetic cost of egg formation, together with the cost of ovary-oviduct recrudescence, can be important in the timing of reproduction (Nilsson and Råberg 2001).

Great tits rely mostly on Lepidoptera larvae during the breeding season in central Europe (Betts 1955, Gibb and Betts 1963, Royama 1970), but during egg laying such food items are scarce. Tits have to feed on something else, for example spiders, for the start of breeding (Betts 1955, Eeva *et al.* 2000). In the pine ecosystem studied in the present work, Lepidoptera larvae of *T. pityocampa* reaches its peak biomass in February and can be very abundant in outbreaking situations. Since *P. major* is a predator of this caterpillar (Gonzalez-Cano 1981), they probably account for the timing of breeding of great tits. In the nest-boxes area of the Apostiça pine stand (Apostiça 2 – see Chapter 2), in the winters 2000/01 and 2001/02, *T. pityocampa* larval density in the month of February varied between 10.6-14.4 larvae/m^2, and in an area of young trees, located 1 000 m away, 79- 145.5 larvae/m^2 were found (Apostiça 1 – see Chapter 2). In Leiria, Plot 24 and 63 this figures were 27-83 larvae/m^2. These larvae are available for birds just before breeding season onset, and its density can be considered high, once the Lepidoptera larval abundance in central Europe coniferous forests at its peak in July is 10-15 larvae/m^2, when tits are still feeding its nestlings (Gibb and Betts 1963).

Additionally, in a marginal area of Plot 24 near a water stream, outbreaks of marsh fritillary larvae *Euphydrias aurinia* (Rottenburg) were observed in the understory vegetation in all the three seasons studied, during the months of February/March (own observations). This outbreak of *E. aurinia* is possibly related with the earlier onset of breeding in Plot 24 observed in 2001.

Studies have reported on a significant relation between laying date and early spring temperatures (Nager and van Noordwijk 1995), being earlier in years with high than with low temperature sums (McCleery and Perrins 1998). This relationship could arise directly because low temperature either increases the female's cost for maintenance at the expense of egg formation, or indirectly affecting the food abundance, resulting in an energy supply early in the season that is too low to allow both body maintenance and egg formation (Perrins 1970). However, experimental manipulation of overnight temperature in the pre-laying and laying period of *P. major*, did not effect the laying date, although cooling decreased the size of the eggs (Nager and van Noordwijk 1992). In the present study, it wasn't possible to establish a relationship between the timing of egg laying and environmental temperatures, although we only have a three years study. In Apostiça, in fact, birds started to breed earlier in the season of 2003, in which the coldest February month was recorded. This earlier onset of breeding might have been due to an older and more established female population, three years after the nest-boxes set up (Nager and van Noordwijk 1995). The earlier onset of breeding in Leiria in 2001, was mainly due to results obtained in Plot 24. It occurred under warm and wet climatic conditions, which might have enhanced the marsh fritillary larval outbreak.

Ambient temperature decreases with latitude and elevation and has been suggested to influence laying date along an elevational and latitudinal cline (Sanz 1998). The coastal pines where this study was performed have a very mild climate with soft winters. The average monthly temperatures in Apostiça in the months of January-February was 11-12 ºC and in Leiria 9-10ºC and the average minimum and maximum monthly temperatures remains between 6-17ºC. These temperatures are above the temperatures found when great tits start breeding in April-May in central Europe (8-11ºC; Nager and van Noordwijk 1995) and north Europe (4ºC; Eeva *et al*. 2000).

The synchronization between the peak of caterpillar abundance and the time when nestlings are about one week old and require maximum energy, is essential for nestling fitness being this fact crucial for the evolution of breeding timing (Perrins 1965, Naef-Daenzer and Keller 1999). In deciduous woods in Central Europe the timing of the start of breeding in tits is closely correlated with the time of caterpillar emergence, which in turn is correlated with tree bud burst, so that the timing of nestlings maximum food demanding will be synchronized with maximum food biomass, when caterpillars are about to pupate. Temperatures are crucial on this system once affects differently the development of caterpillars but the hatching and development of nestlings is unaffected (van Noordwijk *et al.* 1995, Buse *et al.* 1999). The timing of the larval stage has evolved to be as early as possible because of the marked seasonal decline in foliar quality with an increase in intensity of host-plant defences and a reduction in the available nitrogen and water (Feeny 1970). Tits breeding biology tends to follow the pattern of the primary producers (Buse *et al.* 1999).

In coniferous forests in central Europe, things are somehow different with tits breeding early having a lower success than later or second clutches. This has been explained with a later peak of food abundance in coniferous as opposed to deciduous forests (Gibb and Betts 1963, Perrins 1965). Habitat does not always affect the timing of breeding (Sanz 1998). This has been explained by gene flow from birds primarily adapted to deciduous forests, the original forest in central Europe, to small woods in a fragmented landscape, that do not allow adaptations to coniferous forests (Perrins 1965).

Not much is known about seasonal insect abundance in coniferous forests in the south of Europe. According to Maicas and Fernandez Haeger (1996) great tit present a later breeding onset in evergreen oak Mediterranean Forests than in coniferous forests. The Portuguese coastal area to the north of Lisbon, is characterized by large continuous forested areas of *P. pinaster*. The 11 000 ha of the National Pine Forest of Leiria have been a managed forest for the last 700 years. So, a well-adapted population of great tits should be expected. In Apostiça, there is a continuous area of 4 000 ha, but the pine has a much more recent origin, and the Setúbal Peninsula is characterized by a more heterogeneous landscape, with areas of cork oak. The tendency for a less synchronized breeding start in Apostiça compared to Leiria, might be due to gene flow from surrounding ecosystems in Apostiça, since breeding start is known to have a heritable component (Price *et al.* 1988, van der Jeugd and McCleery 2002).

The total number of fledglings produced per pair in the two areas was similar, although this was achieved by a much higher nestling survival of second clutches in Leiria compared to Apostiça. Furthermore, an early timing of the first clutch was important for the existence of a second clutch, in accordance of what was found by Verboven and Verhulst (1996). The mean proportion of second clutches is negatively correlated with latitude, and this likely is due to the short breeding season in the northern part of the species distribution (Sanz 1998). In Apostiça, an early onset of breeding of the second clutch was also important to the survival of nestlings in that clutch and to the total production of nestlings per pair. This also indicates a shortage of food late in the season in Apostiça, which is probably opposite to the situation in Leiria.

Higher temperature sums characterized Apostiça as compared to Leiria. This might speed up Lepidoptera development, reducing food availability for later second clutches. Apostiça is also a dryer area, particularly later in the season (May/June), and this also might constrain insect abundance (Yela and Herrera 1993). The higher nestling survival of second clutches in Plot 314 of Leiria is interesting. This plot hosts a very high abundance of adults of the desynchronized population of *T. pityocampa* during the end of May and June. Adult moths may be a major component of the nestling diet when they are abundant and there is a lack of Lepidoptera larvae (Barba and Gil-Delgado 1990).

The average number of fledglings produced in the first brood per pair was 5.7 in Apostiça and 4.5 in Leiria, below the average of six found for coniferous forests all over Europe (Sanz 1998). The survival of nestlings and the number of fledglings produced in the first brood was not dependent on the onset of breeding, contrary to the relation in deciduous woods in central Europe (Verhulst and Tinbergen 1991, Naek-Daezer and Keller 1999). However, the possibility of laying a second clutch did have an impact on the total number of fledglings produced per pair. The final number of nestlings per pair amounted to 7.3 in both areas. However, we do not know anything about the survival of nestlings after leaving the nest or their probabilties of recruitment. Early fledglings are known to have an advantage in escaping predation in central Europe (Naef-Daenzer *et al.* 2001). However, in the Mediterranean region the relationship between hatching date and the probability of recruitment was found to be non-linear and different between years (Monrós *et al*. 2002).

6.4.2. Clutch size

Clutch size is larger in the Apostiça population than in the Leiria population in both clutches. Great tits are considered to lay a clutch size that they can successfully rear given the local environment, thus an individually optimised clutch size (Pettifor *et al.* 2001). Clutch size is known to have a high heritability, although considerable variation in mean clutch size from year to year has been observed. This indicates that the species do not inherit the tendency to lay a clutch of a particular size, but rather to lay a clutch of a certain size in relation to the mean clutch size in the population at that time (Perrins and Jones 1974). Clutch size in great tits are affected by two major factors: 1) resource abundance for the laying female resulting in physiological constraints on her ability to lay a large clutch early enough in the season to ensure that resources will still be adequate for rearing the brood; 2) environmental variability, which may have severe consequences for the young in larger clutches in bad years, favouring pairs that produce an average clutch size (Perrins and Moss 1975, Boyce and Perrins 1987).

In Apostiça, a larger number of nestlings are produced in the first clutch than in Leiria. This is due to differences in the number of eggs laid since nestling survival in the first clutch is similar in the two areas. If food availability is higher in Apostiça at the beginning of the season, decreasing later, there is an advantage in the evolution of a larger first clutch size in that population. Also the tendency for higher temperatures in the area of Apostiça might be an important factor, since low temperatures limit clutch sizes (Visser and Lessels 2001). The clutch size also decreased with the season, being larger for the early pairs that is a common observation in this species (Perrins 1965).

Clutch size in both the first and second clutch, show a quadratic relationship with latitude, being higher at 55-60ºN (Sanz 1998, Encabo *et al.* 2002). The clutch size of 8.4 eggs found in the present work in Apostiça and 7.4 eggs in Leiria was somewhat lower than the average of 8.7 in other areas of coniferous forests (Sanz, 1998). Our clutch sizes are, however, higher than the ones found in different habitats in Eastern Mediterranean deciduous, evergreen oak and coniferous forests - between 5.9 and 7.3 (Maicas and Fernandez Haeger 1996, Belda *et al.* 1998)

The number of eggs and nestlings produced per pair in the first clutch did not seem to limit the proportion of second clutches or their size, especially not in Leiria, to the contrary of what happens in central and northern Europe (Smith *et al.* 1988, Tinbergen and Verhulst 2000).

6.4.3. Evolution of density.

The nest-boxes in the young plots of Leiria had a large impact in increasing the density of great tit breeding density in an area where the most abundant tit is the coal tit (Pina 1982). In fact Fidalgo (1992) reported that the most abundant tit in a coastal pine located 100 km north of Leiria was the coal tit, with a better breeding performance than great tits in nest-boxes. Fidalgo (1992) also found that the average beginning of breeding of coal tit was 16th March, close to the average start for great tits in the present work. In Apostiça, we found no coal tits, confirming that the limit of distribution of this bird is the Tejo River. According to our observations, the most abundant tit at the time of nest-boxes placement was the crested tit. In none of the areas there seem to be any differences in the start of breeding of the different tit species.

The increase in breeding density in Leiria affected the breeding performance, particularly decreasing the proportion of second clutches. The reproductive decisions in great tits are known to be affected by density (Both 1998a), and has been explained by the occupation of low quality territories (Dhondt *et al*. 1992) or by individual adjustments to increasing densities (Both 1998b, Both *et al*. 2000).

6.5. Conclusions

In the present work the earliest breeding onset timing of great tit was record, leading to a long breeding season in which the possibility of laying a second clutch plays an important role on the total number of fledglings produced per breeding couple. In Apostiça, the warmest and drier area, breeding is constrained at the end of the season, on the contrary of what happens in Leiria, leading to a development of a larger first clutch size. Overall data obtained indicates earlier peaks of insect abundance in Portuguese coastal pines than in other pine ecosystems in Europe, and *T. pityocampa* might play an important role.

Nest-boxes lead to an important increase in the breeding population of great tits in the pine plantations studied, and in fact lack of breeding holes on even aged homogeneous pine plantations, are an important limiting factors on the population of this insectivorous bird.

7. Final Considerations

In the present study, forest structure and its location appeared to be the most important factors determining population density of *T. pityocampa*. Especially young pine stands, with a high tree density, located in South-Central Portugal appeared to be most susceptible to attack. Pine stands located in the Central area of the country – the Abrantes region – had the highest proportion of attacked trees, number of larvae per tree and female fecundity of all the regions studied. Even if a high proportion of trees were attacked also in some areas of the South area – Apostiça –, the Lepidoptera there had low fecundity, which indicates worse conditions for development. On the other hand, the same type of plantations at the West Coast, an area considered to be optimal for the maritime pine, appeared to be less susceptible to attack. Old and heterogeneous pine plantations, even located in high susceptibility areas, had low populations densities.

Weather affects larvae development and female fecundity. During rainy years larval development rate decreased as well as larval final mass and female fecundity during the next season, although we could not find a clear impact on population densities. However, these factors might lead to a reduction in the rate of increase of the population.

Mortality in the immature stages – eggs and larvae – was due to several factors. Although larvae were predated until the very end of their development, the factors affecting the earlier stages – eggs and 1st-3rd instar larvae – had a larger impact in reducing population density.

In the National Pine Forest of Leiria (Central West Coast of Portugal) two populations of *T. pityocampa* coexist geographically. A normal population with the life cycle commonly described for the species, in which larvae develop during the autumn/winter, and a desynchronized population, in which larvae develop during the summer. Thus, two populations of the same species end up with a different physiological time, exploring different seasons in the same ecosystem with a dramatic consequence on the population dynamics, since the desynchronized population reaches densities never recorded for the normal population in this wood.

The newly hatched caterpillars of the desynchronized population originated from larger eggs and were heavier than newly hatched caterpillars from normal populations. The advantage of this to overcome host toughness and defences, in spite of a reduced initial batch size, was unfortunately not assessed in the present work, but it can potentially be of great importance in explaining the density differences between the two populations.

Mortality of egg and early instars was very low in the desynchronized population, but otherwise the same mortality factors were found in both populations. Additionally the desynchronized population has its flight period during the breeding season of the bird guild and larvae development starts during the fledgling period. Larval development also coincides with the peak of activity of other predators like ants and wasps. The normal population, due to its development during wintertime, avoids these potential predation risks. However, the forestry practices in the National Pine Forest of Leiria, create an homogenous, even-aged forest, lacking dead trees and with a very low supply of natural cavities for hole breeding birds, which probably decreased the predation risk to which this new population was naturally exposed. Community structure of this simple ecosystem might also have some importance in decreasing the other predation pressures.

The larva of the desynchronized population develops twice as fast as the normal one, due to the fact that it starts with a larger mass and its development occurs during a warmer season. This will certainly decrease the period of time when they are vulnerable to predation, thus decreasing larvae mortality. A decrease in the length of the larval period implies an increase in the length of the pupa period. However, since the predation risk probably is lower during the pupa period this is advantageous for the desynchronized population.

The outbreak of the desynchronized population in Leiria – recorded for the first time in 1997 – coincided with extreme climatic conditions in the previous years. In fact, 1995 was a drought year with precipitation values of 487.2 mm, followed in 1996 by a rainy year with precipitation of 1041.3 mm. It also happened in a large area of young homogeneous pines, with high tree densities, a factor that was related to outbreaks of the normal populations of *T. pityocampa* in other areas of the country. The dominant winds during adult flight, expanded the distribution of this desynchronized population towards south, ending at the limit of the Atlantic Coastal Pines.

In the pine processionary moth, the break in the pupa diapause times the life cycle of this strictly univoltine species, since there is no egg diapause. It was the earlier adult emergence in the desynchronized population of Leiria that determined the change in the life cycle. The adult emergence of laboratory populations of the desynchronized population was clearly separated from all the other *T. pityocampa* normal populations, showing the same time gap of at least 30 days as found in the normal population in Leiria. However, we could not prove reproductive isolation in the field. The desynchronized population proved to be much more sensitive to climatic fluctuations during its pupa period, potentially with a large impact on the timing of emergence of adults, opening the possibility that the two populations still can interbreed in certain years.

Populations of the normal version of *T. pityocampa*, in which larvae developed in rainy areas with low insulation –like Leiria–, showed a tendency for a shorter pupa period and an earlier adult emergence. In spite of this fact, there is a high level of within-population variance in the timing of emergence, indicating that this trait is not under strong selection pressure. The desynchronized population might have evolved due to the fact that adults with earlier emergence timing in the National Pine Forest of Leiria, had a better fitness, leading to directional selection.

The placement of nest-boxes in young pine plantations did increase the density of *P. major* breeding pairs and wintering birds, especially in Leiria, indicating that the lack of natural cavities are in fact a limiting factor in this type of habitats.

In the present work, I obtained the earliest record of breeding start in great tits –one of the most well studied bird species in the world. This indicates, that the Portuguese coastal pines, offer breeding conditions earlier than in other areas of Europe and North Africa. In Apostiça, the warmest and driest area of those studied, breeding is constrained at the end of the season, on the contrary to what happens in Leiria, leading to selection for larger first clutch sizes.

The beginning of the breeding period can in fact be related to food abundance in the breeding territories of the tits – in this case Lepidoptera larvae – and with the abundance of *T. pityocampa*. Thus, young homogeneous pine plantations, not only lead to outbreaks of *T. pityocampa*, but also the lack of older and dead trees within this type of ecosystem precludes breeding of one of its predators, the great tit.

The actual silvicultural practices not only offer conditions for high population levels of pest insects, but also may create a new scenario for outbreaking species that can potentially lead to ecological evolution. The appearance of the summer population in the Leiria National Pine Forest is a very interesting example. The overall results of this study may constitute important guidelines to forest managers so that silvicultural practices adopted make pine forests less susceptible to infestation by *T. pityocampa*, preventing high densities of the insect.

8. References

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