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XXX CICLO

Management of *Thaumetopoea pityocampa* (Den. and Schiff.)
in urban and semi-urban environments
using eco-friendly techniques

(S.S.D. AGR/11)

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SHORT ABSTRACT AND KEYWORDS

In urban and suburban areas larvae of the pine processionary moth, *Thaumetopoea pityocampa* (Denis and Shiffermüller, 1775), cause serious defoliation to *Cedrus*, *Pinus* and *Pseudotsuga* trees and their urticating hairs cause health problems for humans and domestic animals.

The research activities of my PhD thesis were aimed at the development of eco-friendly innovative technologies for Integrated Pest Management (IPM) of *T. pityocampa* in urban and semi-urban environments.

The following research activities were performed:

- a) Evaluation of the effectiveness of trunk barrier trap devices to capture the caterpillars that descend down the trees. In this study Ecopiège® barrier traps, adhesive barriers and a prototype barrier trap were tested. The result showed that the adhesive barrier was not able to stop the migrant caterpillar. In contrast, the Ecopiège® barrier trap and the prototype trap have a high capture capacity, and the prototype is easier and quicker to install than the Ecopiège® barrier trap.
- b) Management of *T. pityocampa* infestation using trunk barrier traps. Eight devices were placed on an equal number of trees in a public park located in Campobasso with 50 infested trees of *Pinus nigra*. After larvae trapping significantly fewer male adults were captured during the summer of 2016 in comparison with 2015. Similarly, significantly fewer nests were formed on the experimental trees in winter 2016 and 2017 compared with 2015. The results show the potential of the trunk barrier trap devices as a control method for the management of *T. pityocampa* infestations after long-term application.
- c) Pheromone trap comparison tests. Six different devices were tested during the 2015 flight period of *T. pityocampa* (between July and

September). Most of the adults were captured in August. From the trap devices tested, Prototype 1 was found to be superior to the other devices. In 2016 with the same protocol as 2015, 5 different devices were tested. This test selected an even more efficient prototype than the previous year's trap.

d) Management of *T. pityocampa* infestation using mating disruption technique. In a tourist recreational area, 2 plots composed of one hectare each were identified in 2015 and 2016. Mating disruption (MD) pheromones were applied in a plot. The other plot was used as a control. In both plots, about 600 infested trees of *Pinus halepensis* were present. MD demonstrated a high effectiveness. In both 2015 and 2016, the number of males monitored by pheromone traps and the number of nests present in the plot with MD were lower than males and nests recorded in the plot without pheromones. MD can be also applied in private gardens, public parks in urban and semi-urban areas.

Keywords: *Thaumetopoea pityocampa*, Integrated Pest Management, trunk barrier traps, pheromone traps, mating disruption.



EXTENDED ABSTRACT

INTRODUCTION

The pine processionary moth *Thaumetopoea pityocampa* (Denis and Schiffermüller, 1775) is by far the most important insect defoliator of pine forests in southern Europe and North Africa in terms of its temporal occurrence, geographic range and socioeconomic impact. The range expansion of this insect is acknowledged to be directly associated with the recent climate warming as larval feeding activity and survival are favoured from the warming trend in winter temperatures observed in Europe. Furthermore, the third instar larvae develop urticating setae that produce irritants that affect humans and warm-blooded animals.

The research activities of my PhD thesis were aimed at the development of eco-friendly innovative technologies for Integrated Pest Management (IPM) of the pine processionary moth in urban and semi-urban environments.

In particular, the following research activities were performed:

- a) evaluation of the effectiveness of trunk barrier trap devices (adhesive barrier, commercial trap and prototype trap) to capture the caterpillars that descend down the trees (Chapter 2);
- b) management of *T. pityocampa* infestation using trunk barrier traps; effective in trapping caterpillars for 2 consecutive years in relation to the associated male adult population and the number of winter nests on infested trees (Chapter 3);
- c) pheromone trap comparison tests; evaluation of the effectiveness of different pheromone traps in order to capture adults (Chapter 4);
- d) management of *T. pityocampa* infestations using the mating disruption technique (Chapter 5) .

MATERIALS AND METHODS

Evaluation of the effectiveness of trunk barrier traps

The experiments were carried out in a private garden in the city of Campobasso (Molise region, south-central Italy) that it is mainly composed of *Pinus nigra* trees.

In 2015, a study was carried out to assess the effectiveness of 2 caterpillar capturing techniques during their descent from infested trees. In this regard, the Ecopiège® barrier traps (La Mésange Verte, Bages, France) and the adhesive barrier were used. In the study area, 12 pines were selected with a range of 3-15 nests per tree. On 6 pines, the Ecopiège® barrier trap was positioned above the adhesive barrier (this arrangement was used to intercept the larvae that escaped the trap, allowing it to evaluate its effectiveness). In the remaining 6 pines, the positions of the 2 capture systems were reversed to calculate the interference by the adhesive barrier.

In 2016, a study was carried out to assess the effectiveness of the prototype trunk trap. In the study area, 4 pines were selected with a range of 2-8 nests per tree. On the 4 pines, the prototype trunk trap was positioned above an adhesive barrier.

Management of *T. pityocampa* infestation using trunk barrier traps

The experiment was carried out in an public park in the city of Campobasso with *Pinus nigra*; a nearby urban park of 2 hectares, with the same species of pines was used as control area.

For 2 consecutive seasons (2015-2016) in the experimental area, the Ecopiège® barrier traps were used in order to capture caterpillars that went down from the trees; in total, 8 traps were placed on 8 trees with a variable number of nests.

For the monitoring of the *T. pityocampa* male adult population in the experimental and control areas, 8 funnel traps (Nova-Trap, Novapher, Milano, Italy), baited with pheromone dispenser [(Z)-13-hexadecen-11ynyl acetate], were used; 4 traps were installed in the experimental area and the rest in the control area. Between 2015 and 2017, the winter nests built by *T. pityocampa* caterpillars on the tested pine trees of the experimental area were annually counted in January by visual inspection.

Pheromone trap comparison tests

The experiments of pheromone trap comparisons were carried out in a coastal touristic pinewood forest near Petacciato (Campobasso province) that is mainly composed of *Pinus halepensis*.

During the summer of 2015, 6 different pheromone traps were used. The traps, baited with a pheromone dispenser [(Z)-13-hexadecen-11ynyl acetate], were placed inside the canopy 4-5 m above the ground and around 100 m from each other. Checks were carried out once a week.

In 2016, with the same protocol described above, but with the checks that were carried out twice a week, 5 different pheromone traps were used.

Management of *T. pityocampa* infestation using mating disruption technique

The experiments were carried out in a coastal touristic pinewood forest near Petacciato that is mainly composed of *Pinus halepensis*.

During 2015 and 2016, 2 plots composed of 1 hectare each were identified in the study area. One plot was treated with the formulated mating disruption (MD) pheromone (Z)-13-hexadecen-11ynyl acetate in paste and was positioned at a height of about 4 m from the ground, reaching a concentration of 20 g/ha of the active ingredient. The other plot was not treated with MD and was used as a control. Within each of the plots, 2 pheromone traps (G-trap type, SEDQ,

Barcelona, Spain) were placed to monitor the progress of the *T. pityocampa* population.

In January 2016 and 2017, the winter nests realized by *T. pityocampa* wintering caterpillars on colonised pine trees of the experimental plots were visually counted. Counting of winter nests was performed on 12 trees positioned around each pheromone monitoring trap. A total of 48 trees were considered in the both the MD-treated plot and the non-MD-treated plot.

RESULTS AND DISCUSSION

Evaluation of the effectiveness of trunk barrier traps

In 2015, a total of 7837 caterpillars were captured; the first captures were recorded in the middle of March, when the average maximum temperature was 13-14°C, and the last captures were recorded at the end of April.

During 2016, a total of 487 caterpillars were trapped, the first caterpillars were captured in mid-March when the average maximum temperature was 10-11°C, and the last captures were in mid-April.

The results of the trials showed a high effectiveness for commercial Ecopiege® and the prototype trunk trap with rates of 95% and 94%, respectively. In contrast, the effectiveness of the adhesive barrier trap was around 55%. The results show that the adhesive barrier traps were not able to stop the migrant caterpillar given that only a small percentage of them were captured during the procession period. Conversely, the commercial and prototype traps had a high capture capacity, and of these, the prototype trap was easier and quicker to install than the Ecopiege® trap.

The trapping of the processionary caterpillars by barrier trunk traps is a powerful concept for use on individual pines in parks and gardens.

Management of *T. pityocampa* infestation using trunk barrier traps

Significantly more caterpillars were captured in trunk barrier trap devices in 2015 compared to 2016. Significantly fewer nests were formed on average on the evaluated pine trees in 2016 (0.8 ± 0.3) and 2017 (0.4 ± 0.2) in comparison with 2015 (2.0 ± 0.3).

In 2015, the first larvae were captured at the end of the third week of March and the last one at the end of April. The highest number of captures was observed between the last week of March and the first week of April.

Similarly, in 2016 the first larvae captures were recorded in mid-March and the last ones at the end of the first week of April, whereas most larvae were captured during the fourth week of March.

During the entire experimental period, a total of 532 (376 in 2015 and 156 in 2016) male adults were captured in the pheromone traps in the experimental area. Significantly more male adults were trapped in 2015 in comparison with 2016. In 2015, the first captures of *T. pityocampa* male adults were recorded during the first week of July, while male adults were continuously captured until the beginning of September, with the maximum at the beginning of August. In 2016, male adult captures were recorded between mid-July and mid-September.

During the entire experimental period, a total of 1340 (676 in 2015 and 664 in 2016) male adults were captured in the pheromone trap devices in the control area. No significant differences were noted in captures of male adults between the 2 years.

Significantly fewer male adults were captured in pheromone trap devices in the experimental area in comparison with the control area in 2016 but not in 2015. In the control area, male adults were trapped from mid-July for both years until the end of the first week of August in 2015 and the beginning of September in 2016. The highest number of captured male adults was recorded during the last week of July in 2015 and the first week of August in 2016.

This experimental approach clearly showed that the use of trunk barrier traps for the mass trapping of *T. pityocampa* caterpillars needed at least two years (or more) for effective management of the insect population.

Pheromone trap comparison tests

In total during 2015, 1640 male adults of the pine processionary moth were captured in the pheromone traps. The flight of *T. pityocampa* males started in early August and lasted until the first week of September. The highest number of male adults was recorded in early August. Significantly more male adults were captured in prototype 1 (with a total of 808 male adults) than in the other traps.

During 2016, 2817 male adults of the pine processionary moth were captured in the pheromone traps. The flight of *T. pityocampa* males started in the second half of July and lasted until the first week of September. The highest number of male adults was recorded in early August. Significantly more male adults were captured in prototype 7 (with a total of 1671 male adults) than in the other traps.

Management of *T. pityocampa* infestation using mating disruption technique

In the study area in both 2015 and 2016, pheromone traps produced the first catches of the *T. pityocampa* adults during last 2 weeks of July. The presence of males in the traps lasted until the beginning of September, with the maximum presence during second and third weeks of August.

During the trial in 2015, the pheromone funnel traps for monitoring of *T. pityocampa* captured 402 adult moths: 49 individuals were collected in the plot treated with MD, and 353 individuals were collected in the untreated control plot. In the MD plot, adults were found during the third week of August. On the contrary, in the control plot (non-MD-treated) adult males were trapped from the last week of July until the end of August with a maximum presence on the last day of July and early August.

During the trial realised in 2016, the G-traps for the monitoring of *T. pityocampa* males captured 435 adult moths: 35 specimens were collected in the plot treated with MD and 400 were collected in the control plot (non-MD-treated). In the MD plot, adults were collected from the first to the third week of August. On the contrary, in the control plot (non-MD-treated) adult males were trapped from the third week of July until the first week of September, with a maximum presence before the middle of August.

In 2015 and 2016, data comparison between the plot treated with MD and the untreated control plot was significant.

In 2016, the average number of nests per tree found in the plot with MD (0.12 nests/tree) was less than the same found in the plot without MD (0.87 nests/tree). Similar results were found in 2017 with 0.04 nests/tree in the plot with MD and 0.50 nests/tree in the plot without MD.

According to data collected during my research, MD is able to disturb the activity of *T. pityocampa* adults. This activity was evident both in the first and second experimental year (2015 and 2016) when considering the decreased number of adults recorded in the monitoring pheromone traps and the decreased number of nests counted on the experimental trees.

CONCLUSIONS

The pine processionary moth is not a problem limited to the Mediterranean and northern Africa given that it has naturally expanded into higher altitudes and latitudes. It has begun invading the northern regions of Europe, where it has not able to previously develop.

The allergens which are contained in the setae of the *T. pityocampa* caterpillars consist of a serious threat to public health in both southern and northern European countries. For these reasons, controlling *T. pityocampa* and/or

protecting humans and animals from its impact is an increasing necessity in urban areas due to the associated health problems.

In urban areas, the capture of larvae with the trunk barrier trap devices as demonstrated by my research could serve as a valuable, environmentally-friendly and cost-efficient alternative for management of *T. pityocampa*, as trap devices are installed once and provide protection for years. Additionally, MD is a particularly interesting control method in that it can be applied in public parks, recreational areas and other public areas. In small city parks and recreational areas, the efficiency of this technique must be evaluated and adapted if necessary.



CHAPTER 1

GENERAL INTRODUCTION AND STATE-OF-THE-ART



1.1 THE GENUS *THAUMETOPOEA* HÜBNER, 1820

The genus *Thaumetopoea* Hübner, 1820 is included in the subfamily Thaumetopoeinae within the Notodontidae family. This genus has great importance in forestry and urban greening because their larvae feed on broadleaved and coniferous trees; such defoliation makes plants more susceptible to attacks by other insects. Furthermore, the third instar larvae develop urticating setae as an effective defence strategy against vertebrate predators that act as irritants to humans and warm-blooded animals.

Following Agenjo (1941) and Kiriakoff (1970) the genus *Thaumetopoea*, *sensu lato* includes a dozen species in the Palearctic region and 1 in the Afrotropical region. De Freina and Witt (1982) split it in 2 genera, *Thaumetopoea* Hübner *sensu stricto* and *Traumatocampa* Wallengren, 1871, with a further separation of the new genus *Heliantocampa* de Freina and Witt, 1985 from *Traumatocampa* using morphological characteristics of the adults (mainly the chantus, the frontal process of males) (de Freina and Witt, 1987). Molecular phylogenies support a parallel evolution of the morphological traits used to separate *Thaumetopoea* into the 3 distinct genera; this result suggests that all species should be treated as members of a single genus *Thaumetopoea sensu lato* (Simonato *et al.*, 2013).

Roques and Battisti (2015) reported 15 species belonging to *Thaumetopoea* genus: *T. pityocampa* (Denis and Shiffermüller, 1755); *T. processionea* (Linnaeus, 1758); *T. pinivora* (Treitschke, 1834); *T. solitaria* (Freyer, 1838); *T. herculeana* (Rambur, 1840); *T. cheela* Moore, 1883; *T. jordana* (Staudinger, 1894); *T. apologetica* Strand, 1909; *T. wilkinsoni* Tams, 1925; *T. bonjeani* (Powell, 1922); *T. libanotica* Kiriakoff and Talhouk, 1975; *T. dhofarensis* Wiltshire, 1980; *T. ispartaensis* Doğanlar and Avcı, 2001; *T. sedirica* Doğanlar, 2005; *T. torosica* Doğanlar, 2005. In addition, 3 new species were recently described: *T. loxostigma* Hacker, 2016; *T. hellenica* Trematerra

and Scalercio, 2017, and *T. mediterranea* Trematerra and Scalercio, 2017 (Hacker, 2016; Trematerra *et al.*, 2017).

1.2 *THAUMETOPOEA PITYOCAMPA* (DENIS AND SHIFFERMÜLLER, 1755)

1.2.1 Distribution and host plants

The pine processionary moth *T. pityocampa* is by far the most important insect defoliator of pine forests in southern Europe and North Africa, both in terms of its temporal occurrence, geographic range and socioeconomic impact (Jactel *et al.*, 2015).

T. pityocampa is oligophagous on *Pinus* species; occasionally it can be found on other conifers such as *Cedrus*, *Pseudotsuga* and *Larix* species. In order of pest preference, the European and Mediterranean Plant Protection Organisation (2004) reported the following decreasing order of host species: *Pinus nigra* var. *austriaca*, *P. sylvestris*, *P. nigra* var. *laricio*, *P. pinea*, *P. halepensis*, *P. pinaster* and *P. canariensis*, followed by *Cedrus atlantica* and finally *Larix decidua*. However, different authors (Tiberi *et al.*, 1999; Petrakis *et al.*, 2005; Stastny *et al.*, 2006; Niccoli *et al.*, 2008; Paiva *et al.*, 2011; Carillo-Gavilán *et al.*, 2012) do not always agree on the order of host preference, which seems to greatly vary between geographical regions or types of experiments. The local adaptation and evolutionary processes probably explain these apparently contradictory results (Jactel *et al.*, 2015).

The pine processionary moth is present in regions in the Atlantic and Mediterranean climates in Europe (Albania, Bosnia and Herzegovina, Bulgaria, Croatia, France, Greece, Italy, Macedonia, Montenegro, Portugal, Serbia, Slovenia, Spain, and Switzerland), in part of European Turkey and in North Africa (Algeria, Morocco, Tunisia and Libya) (Roques *et al.*, 2015).

T. pityocampa is not a problem in that is limited to the Mediterranean and northern African countries given that it has naturally spread to higher altitudes and latitudes (Battisti *et al.*, 2005; Robinet *et al.*, 2010; Roques *et al.*, 2015). The range expansion of this insect is acknowledged to be directly associated with the recent climate warming due to the favouring of the larval feeding activity and survival due to the warming trend in winter temperatures observed in Europe (Robinet *et al.*, 2013). The temperature rise due to global climate warming favour the winter survival of the pest and enhances its feeding activity in regions where it could not develop before (Hóðar *et al.*, 2003; Battisti *et al.*, 2005; Buffo *et al.*, 2007). For this reason, *T. pityocampa* has been retained by the Intergovernmental Panel on Climate Change (IPCC) as one of the few model insects used as an indicator of global warming.

In the Paris basin (central France), more colonies become progressively established as a result of the accidental transportation of *T. pityocampa* individuals by humans; in the same area, its expansion into the north of France since 2000 is due to a temperature increase has been estimated at approximately 5.6 km/year (Robinet *et al.*, 2012).

1.2.2 Morphology and life cycle

The adult male has a wingspan of 30-40 mm (Fig. 1.1 A). The female is bigger with a wingspan of 35-50 mm (Fig. 1.1 B). Both sexes have a hairy thorax, a stout abdomen and a tuft of large scales covering the last segments. Forewings are whitish-grey with 3 dark transverse bands. Hindwings are white with a dark spot in the anal region.

The eggs are spherical and white and laid in groups cylindrically around conifer needles (Fig. 1.1 C, D). The egg mass is 25-40 mm wide and about 5 mm high and can contain 70-300 eggs. Abdominal scales of the female act as camouflage, protect and cover the egg mass.

The caterpillar develops through 5 instars. The body of the first 2 instars is green with a black head capsule. After the second moult, the integument appears dark, and the setae of the lateral and ventral part vary from white to dark yellow. The dorsal setae range from yellow to dull orange and are borne on red-brown verrucae. The third instar caterpillar develops urticating setae on integumental areas of the abdominal tergites called mirrors, owing to their reflective properties (Moneo *et al.*, 2015). These mirrors increase in number with larval moults until the last instar, where they occur on 8 abdominal segments. The full-grown caterpillar is 38-45 mm in length (Fig. 1.1 G).

The pupa is the obtecta type and is about 20 mm in length (Fig. 1.1 H). Initially, it has a pale brown-yellow colour that later change to dark red-brown. The cremaster is bluntly rounded, with 2 robust, curved spines.

The life cycle of the pine processionary moth is normally annual but may extend over 2 years at high altitude or in northern latitudes for the part or whole of the population (Fig. 1.2). In addition, experimental results in cages by Salman *et al.* (2016) indicate that *T. pityocampa* can extend its pupal diapause of 7 years; Démolin (1969) reported the diapause of pupae extended up to 6 years under controlled, semi-field condition.

At colder sites, adults emerge as early as June whereas emergence can be delayed until September at warmer sites (Battisti *et al.*, 2015). In general, females have a short life span (only 1 or 2 days), whereas males live longer (Zhang and Paiva, 1998). The day after emergence and mating, females oviposit on pines nearest to their pupation site, even if though they can fly for more than 10 km (Battisti *et al.*, 2015). Females lay about 70-300 eggs in batches on needles. The eggs hatch after 30-45 days (in July at colder sites and in October at warmer sites) and aggregate in colonies. The caterpillars feed on needles and spin silken nests (autumn nests) (Fig. 1.1 E) that are abandoned with each move until the fourth instar when the winter nest (Fig. 1.1 F) is built. The winter nest is a large silk bag up to 20 cm in length where the caterpillars spend the cold season

(Dajoz, 2000). Caterpillars leave trees in typical head-to-tail processions (Fig. 1.1 G) to search for suitable sites in soil for cocoon spinning between February and May and as early as December at warmer sites (Battisti *et al.*, 2015); a female is usually at the head of the procession (Dajoz, 2000). The procession occurs at temperatures of 10-22°C; at lower temperatures, the colonies regroup and at higher temperatures they bury themselves wherever suitable conditions exist. When a suitable site is found, such as open areas and forest edges (Démolin, 1971), caterpillars burrow 5-20 cm below the ground where they weave a cocoon and pupate. Cocoons remain in the soil for the next few months until the emergence of moths (Battisti *et al.*, 2015). As previously mentioned, the pupal stage may have a diapause of many years.

In 1997, a temporary shift in the population of *T. pityocampa* was recorded in Portugal. These new population of caterpillar development took place during the summer (summer population), while in the normal population it took place during the winter (winter population) (Pimentel *et al.*, 2006; Berardi *et al.*, 2015; Branco *et al.*, 2017). These two populations coexist in the same ecosystem. Santos *et al.* (2007) reported this phenological shift as a plausible case of allochronic differentiation between sympatric populations of the pine processionary moth. In the summer population, adults started emerging from the soil by the end of April. Eggs are laid between the end of April and early July; hatching starts about 1 month later. Larval development is much faster than the winter population, probably because it takes place during the warmer months. By the end of September, the caterpillars have reached the fifth instar and descend from the trees in procession for pupation. The pupae enter a winter diapause, which is just the opposite of the summer diapause of the winter population (Battisti *et al.*, 2015).

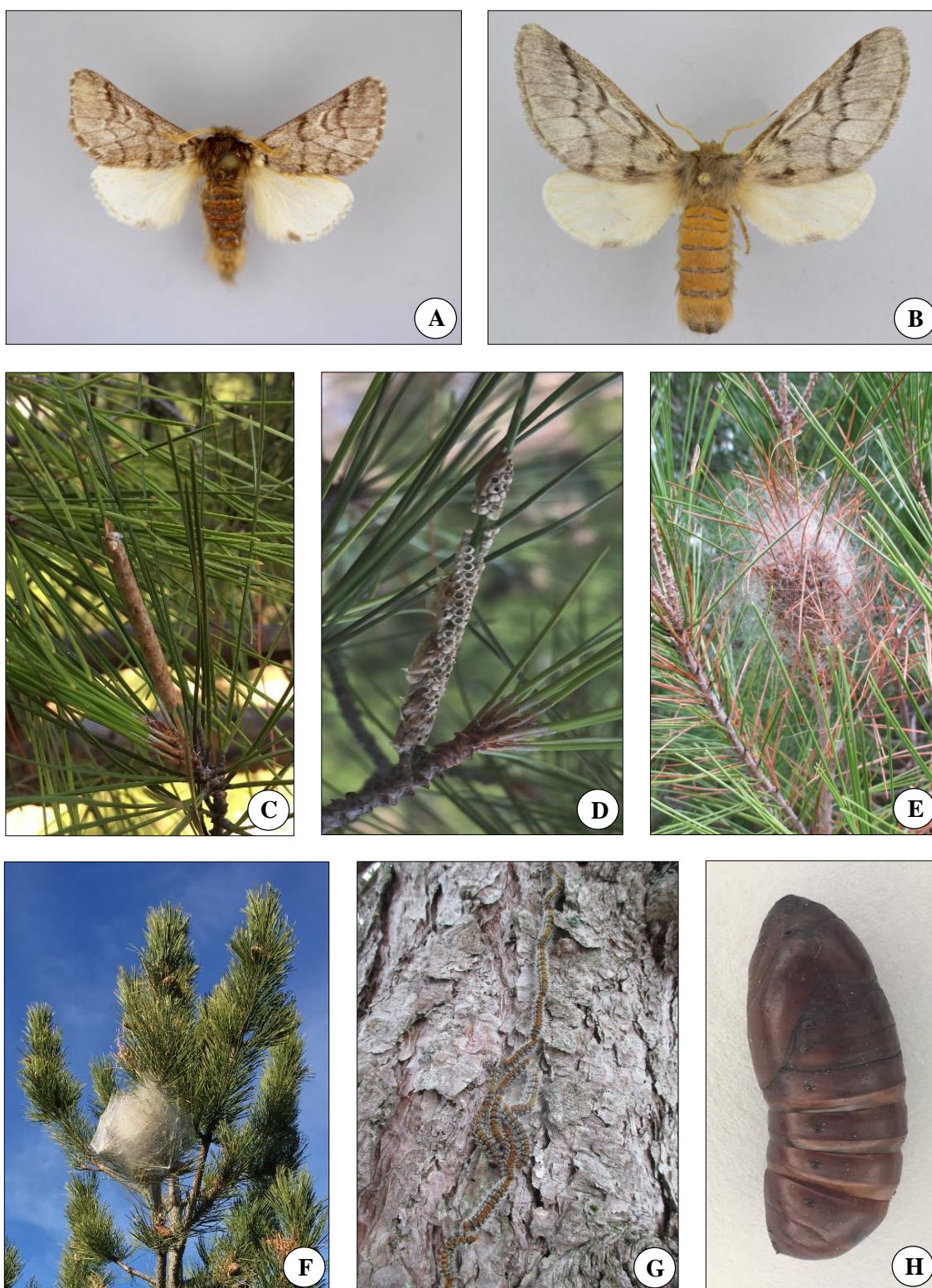


Figure 1.1 *T. pityocampa*: adult male (A) and female (B), egg batches (C and D), autumn nest (E), winter nest (F), procession (G), pupa (H).

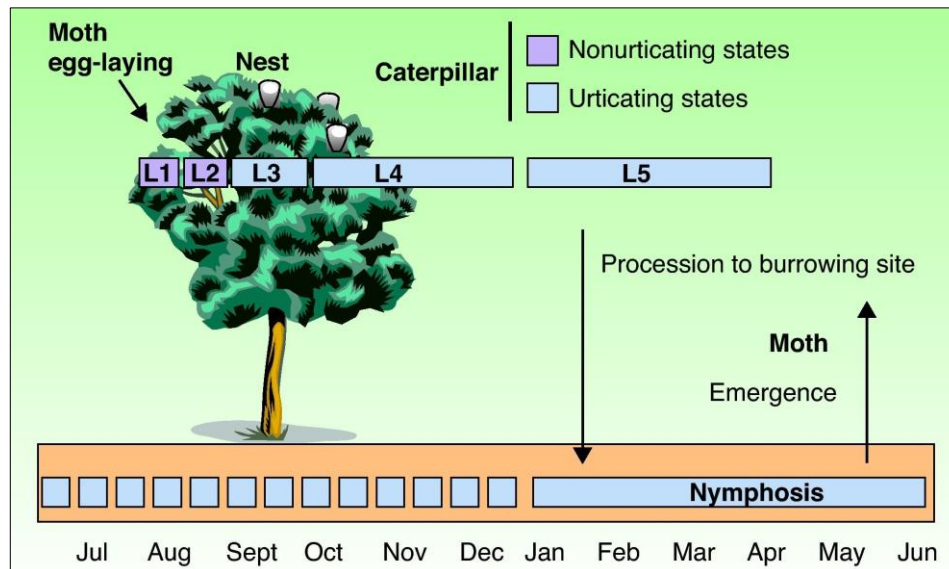


Figure 1.2 Annual biological cycle of *T. pityocampa* (from Vega et al., 2011).



Figure 1.3 Aspects of the use of the environments near pine-woods: grazing animals (A), people (B and C), pets (D) (pictures A, C and D from web).

1.2.3 Tree defoliation and urticating setae

The caterpillars of *T. pityocampa* feed on the foliage of host trees during the cooler months of the year causing significant defoliation (Fig. 1.4 A and B). Initially caterpillars feed on current year needles near the oviposition site, but soon after they switch to old needles for the rest of the development (Battisti *et al.*, 2015).

Defoliation damage is extremely serious in young reforested areas and young plantations where it may lead to the death of trees. Adult trees are rarely killed by this species, but even in cases where they are able to survive the infestation, tree growth is seriously affected (Jactel *et al.*, 2006).

Jacquet *et al.* (2012) conducted a meta-analysis based on 45 study cases, to estimate the effect of processionary moth defoliation on tree growth. They reported a reduction in pine growth by about 20% when the defoliation was between 5% and 24%, whereas severe defoliation (>50%) induced growth losses of almost 50%. In addition to reduced growth, the infested trees became stressed which makes them more susceptible to other agents, including attack by secondary pest species, such as wood and bark boring insects or fungi.

Apart from the defoliation, the third instar caterpillars develop urticating setae. This characteristic likely evolved against predators (Battisti *et al.*, 2011) but can accidentally affect farm animals, pets and humans, who eventually disturb the larvae or live near infested trees. For these reasons the infestations of *T. pityocampa* in urban, suburban, recreational and touristic areas produce several health problems in humans or animals (pets and domestic livestock) (Fig. 1.4 C-G). Because of this problem, in Italy exist a mandatory control measure under ministerial decree October 30, 2007.

The last instar caterpillar has approximately 1,000,000 urticating setae with a density of 60,000 setae/mm² (Moneo *et al.*, 2015). Setae have a length ranging from 46 to 681 µm. The distribution can be considered bimodal with a first peak in the class of 50-100 µm and a second in the class of 200-250 µm

(Petrucchio Toffolo *et al.*, 2014). The release of setae by the caterpillar was explored by Démolin (1963), who showed that the caterpillar may actively open the integumental mirrors when disturbed.

Inflammatory reactions occur in most individuals after exposure to setae of the caterpillar of *T. pityocampa*. Setae are considered a rich source of allergens; Rodriguez-Mahillo *et al.* (2012) identified 7 allergens that are delivered to humans or animals by intradermal injection, including the main allergen that could match the taumetopoein protein (a previously described protein with mast cell-degranulating properties) (Lamy *et al.*, 1986).

These allergens enter the skin at the site of setae penetration, either through direct contact with a live or dead caterpillar or as a result of wind dispersal (aero-mediated contamination), which can occur over considerable distances and enhance an immune response in infected people (Rodriguez-Mahillo *et al.*, 2012). Thus, the exposed tissue suddenly shows local symptoms such as vesicles (due to fluid accumulation), itching and secondary flare and reddening, which results from the inflammatory reaction. The reaction can be delayed up to 24 hours and is generally localised to the area of contact. Sometimes the patient needs hospitalisation to recover (Moneo *et al.*, 2015).

In addition to cutaneous reactions, other rare symptoms that are attributed to the pine processionary caterpillar have been also reported in humans: conjunctivitis, keratitis, uveitis, ocular itchiness and respiratory issues (Vega *et al.*, 2003, 2011; Artola-Bordás *et al.*, 2008; Bonamonte *et al.*, 2013; Julienne *et al.*, 2015; Battisti *et al.*, 2017). These reactions also affect farm animals (cattle, goats, horses and sheep) that have come into contact with urticating setae while grazing grass and pets (dogs and cats). The dog is the species most affected by urtication; this can be explained by their curiosity during forest walks and even gardens. Oral damage following ingestion of caterpillars or contact with them is often predominant (Kaszak *et al.*, 2015; Pouzot-Nevoret *et al.*, 2017). Some fatal cases have been reported after angioedema and asphyxia (Moneo *et al.*, 2015).



Figure 1.4 *Defoliation caused by T. pityocampa caterpillars on pine tree (A and B), urtications on humans (C and D) and animals (E, F, and G) caused by contact with the urticating setae of the T. pityocampa caterpillars (pictures D, E, F and G from web).*

1.2.4 Management of infestation

Management of *T. pityocampa* quickly developed in Europe beginning at the end of the nineteenth century because of the risks related to the urticating caterpillars and the defoliating threatening pine forests and plantation (Martin, 2015).

Preventive methods can be applied in new conifer plantations. Martin (2015) suggested surrounding these plantations with a belt of broad leaf trees at the edge in order to slow down pest dispersal, which is generally more abundant at the edge and will simultaneously support natural enemies' activity. Other strategies are choosing plants that are less susceptible to infestation and the design of mixed forests. Jactel and Brockerhoff (2007) showed there that pine trees growing in monospecific stands have a higher risk of being attacked by insect pest than those growing in mixed stands.

Common environmentally friendly strategies in the management of pine processionary moth are: removal of egg masses, removal of nests, trapping of the caterpillar, spraying microbial insecticides, adult trapping, mating disruption and biocontrol.

Removal of egg masses. This strategy must be applied before the egg hatches into the first instar caterpillar and has the disadvantage of not being exhaustive because of the difficulty of visualising the egg masses in the foliage. It is also highly time-consuming and involves a lot of manpower (Martin, 2015).

Removal of nests. Removal of autumn nests offers the possibility of manipulating larval colonies when they are not yet urticating but spotting all colonies may also be difficult. Removal of winter nests is generally reserved for the areas at high risk to the population because of the urticating properties of the caterpillars at the present time; it requires individual protective equipment for the manipulators. In addition, the winter nest are often difficult to access when located on tree tops (Martin, 2015). Winter nest counting is also a method to monitor the population, though it is not easy when the trees are tall or grow in

high density stands. This strategy may lead to unreliable estimations at the beginning of outbreaks when the population levels are still low (Jactel *et al.*, 2006).

Trapping of caterpillar. Special barrier traps can be applied for the capture of the caterpillars on their way down from the infested plants. Martin *et al.* (2012) reported the high efficacy of the trap device Ecopiège®, which is installed around the tree trunk as a ring before the start of the procession activity. This technique is a powerful concept for use on individual pines in parks and gardens.

Spraying microbial insecticides. One of these methods is the spraying of tree foliage using *Bacillus thuringiensis* var. *kurstaki* (Btk). This technique has been in use since 1980 (Zamoun and Démolin, 2004). Such formulations are efficient on the first 4 larval stages of practically all lepidopteran species, but have virtually no incidence on natural enemies (Martin, 2015). The effectiveness of Btk was tested in several experiments (Battisti *et al.*, 1998; Shevelev *et al.*, 2001; Cebeci *et al.*, 2010; Zamoum *et al.*, 2016). However, the Btk formulations are influenced by the climatic conditions of the area during and after applications; Martin (2015) reported the degradation of Btk formulations by ultraviolet light occurs quickly after spreading. The action only persists for 8-12 days. One other group of insecticides that can be used against *T. pityocampa* is Insect Growth Regulators (IGRs). In an early study, Halperin (1980) noted that the application of the IGR diflubenzuron was effective for young larval control of this species. Hence, one of the most promising active ingredients for this purpose is the bacterium-based insecticide spinosad, which has proven very effective for the control of *T. pityocampa* larvae, at relatively low dose rates (Semiz *et al.*, 2006). However, even in this case, the use of IGRs and other novel insecticides is not easy, due to the height of the trees, the concerns about the use of sprayings in the urban and suburban areas and the fact that most IGR based formulations are not registered for use in the urban environment.

Adult trapping. In 1981, the chemical structure of the female sex pheromone of *T. pityocampa* was identified. Since its identification, research has mainly been focused on the synthesis of the sex pheromone in sufficient quantities for the monitoring of pine processionary moth populations and the development of mass trapping methods for direct control (Zhang and Paiva, 1998). The use of large capacity traps baited with synthetic sex pheromone constitutes a control technique aimed to capturing massive numbers of male moths in order to significantly decrease mating probability. All of the traps and marketed pheromones do not have the same efficiency in capturing male moths (Martin, 2015) and can be influenced by several factors, including trap placement, type of trapping, pheromone source and, other visual stimuli such as the colour of the trapping surface (Jactel *et al.*, 2006; Athanassiou *et al.*, 2007; Brockerhoff *et al.*, 2013). Martin (2015) reported that a minimum of 4 traps is necessary to result in any effect even for a small surface. Six traps per hectare are needed for larger areas.

Mating disruption. Mating disruption (MD) technology uses synthetic sex pheromones, during the male flight period in order to confuse males and limit their ability to locate calling females, thus reducing the proportion of mated females and subsequent egg laying. There are many examples of the successful use of MD against agricultural and crop insect pests, but few studies exist regarding the use of this system for the management of pine processionary moth. Preliminary experiments of MD carrier against *T. pityocampa* with promising results were reported by Baldassari *et al.* (1994) and Halperin (1985). During 2004 and 2005, interesting data were also cited by Martin and Frérot (2006).

Biocontrol. There are a number of predators, parasitoids and diseases that play a role in the biological control of *T. pityocampa*. Eggs are attacked by wasp parasitoids, ants and orthopteran predators (Way *et al.*, 1999; Battisti *et al.*, 2015; Mirchev *et al.*, 2015). *T. pityocampa* larvae and pupae are attacked by dipteran and hymenoptera parasitoids, dipteran predators and birds (Bergström and

Bystrowski, 2011; Battisti *et al.*, 2015; Tarasco *et al.*, 2015). Some species of insectivorous birds (tits and hoopoes) and bats have been cited as regular predators of the adult pine processionary moth (Barbaro and Battisti, 2011; Charbonnier *et al.*, 2014). Fungi and bacteria can also play a role in the biological control of the pine processionary moth. The installation of tit nesting boxes can facilitate the establishment of this bird and can contribute to the ecological control of the moth population. Studies are looking at the possibility of applying a massive release of egg parasitoids but they lack cost-effectiveness (Martin, 2015).

Chemical insecticides are still used in areas where a higher efficacy is requested but their use is constantly decreasing.

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CHAPTER 2

EVALUATION OF THE EFFECTIVENESS OF TRUNK BARRIER TRAPS



2.1 INTRODUCTION

General information on the pine procession moth is reported in Chapter 1. In this chapter, tests on trunk barrier traps are reported.

In 2015, a study was carried out to assess the effectiveness of 2 caterpillar capturing techniques during their descent from infested trees; the Ecopière® barrier traps and the adhesive barrier were used.

In 2016, a study was carried out to assess the effectiveness of a prototype trunk trap.

2.2 MATERIALS AND METHODS

2.2.1 Experimental area

The experiments were carried out in a private garden in the city of Campobasso (Molise region, South-Central Italy, 740 m/a.s.l.) with 40 trees of *Pinus nigra* J. F. Arnold (Pinales: Pinaceae) (Figs. 2.1-2.2). The trials were conducted in 2015 and 2016.

2.2.2 Placement and inspection of trunk barrier and adhesive barrier trap devices

In 2015 in the study area, 12 pines were selected with a range of 3-15 nests per tree. On 6 pines, the Ecopière® barrier trap was positioned above the adhesive barrier (this arrangement was used to intercept the larvae that escaped the trap, allowing it to evaluate its effectiveness) (Fig. 2.3 A). In the remaining 6 pines, the positions of the 2 capture systems were reversed to calculate the interference by the adhesive barrier (Fig. 2.3 B). The barrier traps were installed on March 2 and were checked every 3-4 days.



Figure 2.1 *Aerial view of the experimental area (from Google Earth).*



Figure 2.2 *Vegetational aspects of the experimental area.*

The Ecopière® barrier trap (La Mésange Verte, Bages, France) was made of a flexible flange that surrounded the trunk. A bag was connected to the flange by a cylindrical pipe (2.5 cm diameter, 40 cm height) that left an opening of 4.9 cm².

The adhesive barrier (Zapi Garden, Conselve, PD, Italy) was made of 10 cm wide transparent plastic tape with the 2 sides sprinkled with glue.

In 2016 in the study area, 4 pines were selected with a range of 2-8 nests per tree. On the 4 pines, the prototype trunk trap was positioned above an adhesive barrier. The barrier traps were installed on 22 February and were checked twice a week.

The prototype trunk trap (Fig. 2.3 C) consisted of a black 15.5 cm wide plastic deflector, a strip of double density foam, a strap, 3 clamps, a slightly curved rectangular parallel pipe downspout (2.8 cm x 6.0 cm x 17.7 cm) that left an opening of 16.8 cm².



Figure 2.3 *Trunk trap devices that were tested: Ecopière® (upper) and adhesive barrier (lower) (A), adhesive barrier (upper) and Ecopière® (lower) (B), prototype trap used in 2016 (C).*

2.2.3 Data analysis

For each trial, the effectiveness of the Ecopière® traps, the adhesive barrier, and the prototype trunk trap was expressed in percentage (%) as follows: the number of caterpillars in Ecopière® traps / (the number of caterpillars in Ecopière® traps + the number of caterpillars in adhesive barriers) x 100, or the number of caterpillars in adhesive barriers / (the number of caterpillars in Ecopière® traps + the number of caterpillars in adhesive barriers) x 100, or the number of caterpillars in prototype trunk traps / (the number of caterpillars in prototype trunk traps + the number of caterpillars in adhesive barriers) x 100, respectively.

2.3 RESULTS

In 2015, a total of 7837 caterpillars were captured; the first captures were recorded in the middle of March, when the average maximum temperature was 13-14°C, and the last captures were recorded at the end of April.

When the Ecopière® traps were installed upon the adhesive barriers they captured a total of 5322 caterpillars, while the adhesive barrier captured only 260 caterpillars (Fig. 2.4). With the 6 traps in this arrangement, the effectiveness of Ecopière® traps was between 87% and 100%, with an average of 95%.

When the adhesive barriers were installed upon the Ecopière® traps they captured in total 1255 caterpillars, while the Ecopière® traps captured 1030 caterpillars (Fig. 2.5). With the 6 traps in this arrangement, the effectiveness of adhesive barriers was between 10% and 71%, with an average of 55%.

During 2016, a total of 487 caterpillars were trapped. The first caterpillars were captured in the middle of March when the average maximum temperature was 10-11°C, and the last were captured in the middle of April.

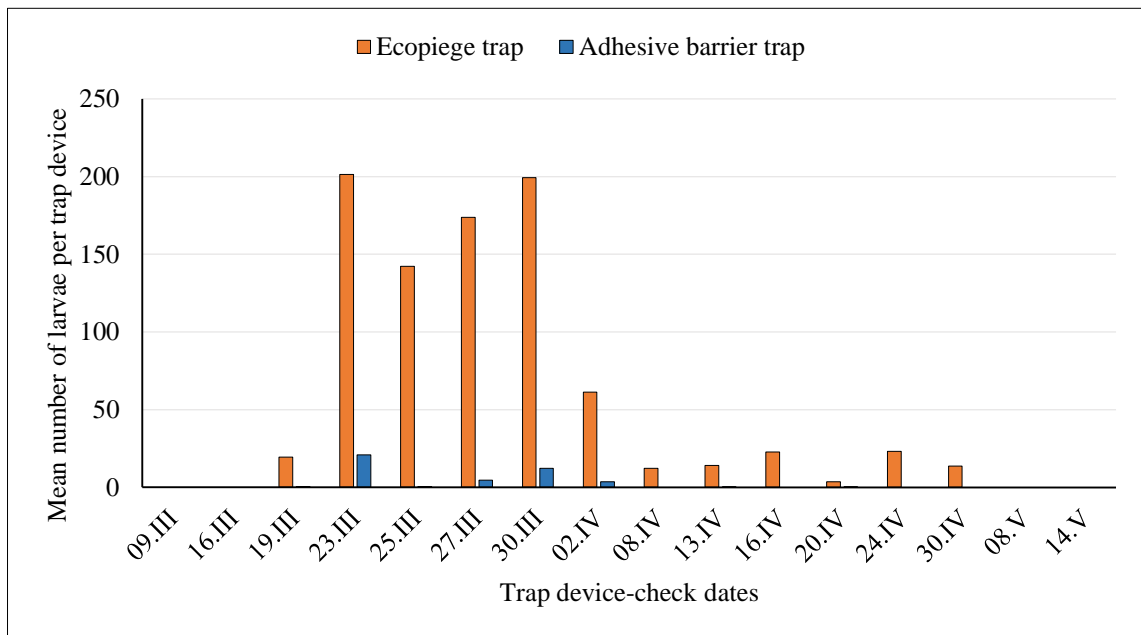


Figure 2.4 Mean number of *T. pityocampa* larvae captured when the Ecopiege® traps were installed upon the adhesive barriers in 2015.

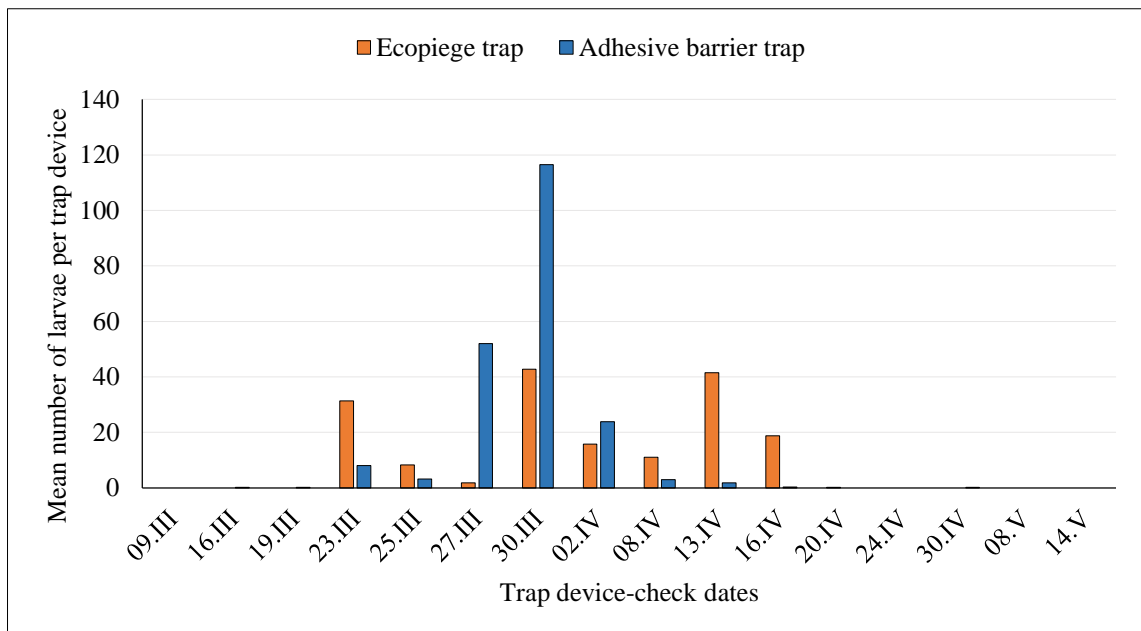


Figure 2.5 Mean number of *T. pityocampa* larvae captured when the adhesive barriers were installed upon the Ecopiege® traps in 2015.

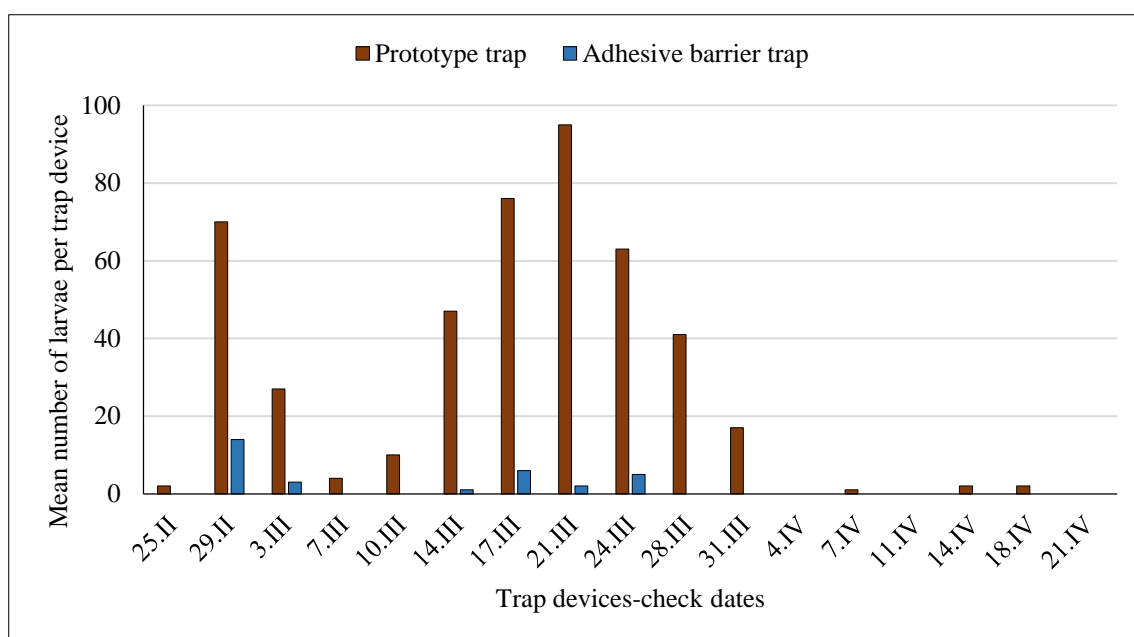


Figure 2.6 Mean number of *T. pityocampa* captured when the prototype traps were installed upon the adhesive barriers in 2016.

The prototype trunk trap captured 457 caterpillars in total, while the adhesive barrier only captured 30 caterpillars (Fig. 2.6). The effectiveness of the 4 prototype had a range between 89% and 97%, with an average of 94%.

The results of the trials show a high effectiveness for commercial Ecopière® and the prototype trunk trap with 95% and 94% respectively. In contrast, the effectiveness of the adhesive barrier trap was approximately 55%.

2.4 DISCUSSION

The results show that the adhesive barrier traps were not able to stop the migrant caterpillar given that only a small percentage of them were captured during the procession period. This is because the larvae have the ability to cross the adhesive barrier by passing over the other glued larvae. Furthermore, when it rains the wet glue loses its adhesive properties. Martin (2015) reported a similar

result in National Institute of Agricultural Research (INRA, France) tests that revealed the completely inefficiency of the adhesive barrier because the caterpillars have the ability to cross the glue band without remaining stuck.

In addition to poor performance, adhesive strips interfered with non-target species such as birds and lizards.

Instead, the commercial trap had a high capture capacity with an effectiveness of 95%. A similar result using the Ecopiege® trap was also confirmed by Martin *et al.* (2012) after a single year of application in southern France.

This research also introduced a novel trunk trap device (prototype trap) that is cheaper and can be installed more quickly than the commercial traps and has a similar high-capture capacity.

The trapping of the processionary caterpillars by barrier trunk traps is a powerful concept for use on individual pines in parks and gardens (see Chapter 3).

2.5 REFERENCES

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CHAPTER 3

MANAGEMENT OF *T. PITYOCAMPA* INFESTATION USING TRUNK BARRIER TRAPS

The general results of an international collaboration “LIFE-PISA project (LIFE13 ENV/ES/000504)” are included in the following publication:

Colacci M, Kavallieratos NG, Athanassiou CG, Boukouvala MC, Rumbos CI, Kontodimas DC, Pardo D, Sancho J, Benavent-Fernández E, Gálvez-Settier S, Sciarretta A, Trematerra P (2017) Management of the pine processionary moth, *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae), in urban and suburban areas: trials with trunk barrier and adhesive barrier trap devices. *Journal of Economic Entomology*: doi: 10.1093/jee/tox270

Parts related to my PhD research are reported using font 13, parts related to other groups are reported in font 10.

MANAGEMENT OF THE PINE PROCESSIONARY MOTH, *THAUMETOPOEA PITYOCAMPA* (LEPIDOPTERA: THAUMETOPOEIDAE), IN URBAN AND SUBURBAN AREAS: TRIALS WITH TRUNK BARRIER AND ADHESIVE BARRIER TRAP DEVICES

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Abstract

In urban and suburban areas larvae of the pine processionary moth, *Thaumetopoea pityocampa* (Denis and Schifferrmüller), cause serious defoliation to *Cedrus*, *Pinus*, and *Pseudotsuga* trees and health problems to humans and domestic or farm animals by their urticating setae. In this study, we present the results of biennial trials (2015-2016) on the management of *T. pityocampa* infestations using commercial or LIFE-PISA prototype trunk barrier and adhesive trap devices in Greece (Attica and Volos), Spain (Valencia), and Italy

(Molise). In Attica, for both 2015 and 2016, the commercial trunk barrier trap devices captured significantly more *T. pityocampa* wintering migrant larvae compared to the adhesive barrier trap devices, indicating also their high capture capacity. The total performance of the trunk barriers trap devices was 99.8% in 2015 and 99.6% in 2016. In Volos and Valencia, no significant differences were recorded between captures in commercial and LIFE-PISA prototype trunk barrier trap devices. In the tests that were conducted in Molise, the commercial trunk barrier trap devices exhibited high effectiveness in capturing the wintering migrant larvae during their procession, before they reach the ground for pupation. Moreover, significantly fewer male adults were captured by pheromone trap devices during summer 2016 in comparison with 2015 in the experimental area. Similarly, significantly fewer nests were formed on the experimental area trees in winter 2016 and 2017 compared with 2015. Our results show the potential of the trunk barrier trap devices in the management of *T. pityocampa* numbers after long-term application in urban and suburban areas.

Keywords: *Thaumetopoea pityocampa*, novel trunk trap device, monitoring, male adults, winter nests, wintering larvae.

3.1 INTRODUCTION

The pine processionary moth, *Thaumetopoea pityocampa* (Denis and Schiffermüller), is a pest of *Cedrus*, *Pinus* and *Pseudotsuga* trees in the Mediterranean area (Hódar *et al.*, 2003; Cielsa, 2011; Battisti *et al.*, 2015). The larvae cause severe defoliation to trees, which become much more susceptible to insect attacks or to water and thermal stresses (Carus, 2004; Kanat *et al.*, 2005; Lombardero *et al.*, 2016), and health problems to humans (Battisti *et al.*, 2011; Rodriguez-Mahillo *et al.*, 2012; Moneo *et al.*, 2015) or animals (pets and

domestic) (Kaszak *et al.*, 2015) in urban, suburban, recreational and touristic areas (Athanassiou *et al.*, 2017). The larvae have urticating hairs (setae) from the third instar onwards, which are considered as a rich source of allergens. These allergens enter the skin, either through direct contact with alive or dead larvae or as a result of wind dispersal (aeromediated contamination), which can occur over considerable distances (Rodriguez-Mahillo *et al.*, 2012; Battisti *et al.*, 2017). As a result dermatological, respiratory, ophthalmic, and other hypersensitivity reactions are commonly addressed due to *T. pityocampa* infestations (Vega *et al.*, 2003, 2009, 2011; Artola Bordás *et al.*, 2008). Sometimes the patients need hospitalisation to recover (Ziprkowski and Roland, 1966; Battisti *et al.*, 2011; Moneo *et al.*, 2015). Dermatological problems are observed in occupational settings (lumberjacks, woodcutters, other forestry personnel, residential gardeners, nurserymen, stockbreeders, resin collectors, and entomologists) and even more in extra occupational situations, such as tourers and campers (Hosler, 2010; Battisti *et al.*, 2011). Individuals of every age can be affected (Hosler, 2010) but children seem to suffer more often from general allergy symptoms, because they are more curious and their exposure to larvae is more common (Vega *et al.*, 2003; Hosler, 2010).

Adults of *T. pityocampa* fly in the evening during summer and autumn. A few hours after emergence and mating, the adult females oviposit on the nearest conifers. Seventy to 300 eggs are laid together forming a sleeve around a pair of needles. After a period of 30-45 days larvae hatch and build communal silken nests amongst branches. They leave nests at dusk to feed on needles and return at dawn. Their development includes 5 instars. They overwinter in the nests. When temperatures increase between 10 and 22 °C, during late winter and early spring, larvae of fifth instar leave the tree in a litany procession during daytime larvae. The larva at the head of the procession is commonly a female, leading the colony to open places or forest margins inside soil to pupate at a depth of 5-20 cm. The cocoons remain in the soil till next summer when the adults start emerging.

However, the cocoons may fall to diapause under unfavourable environmental conditions and stay in the soil for several years before adult emergence (Devkota *et al.*, 1992; Dajoz, 2000; Battisti *et al.*, 2006, 2015; Athanassiou *et al.*, 2017).

Conifers constitute a major part of “natural” trees in recreational areas in the urban and the suburban environment in southern Europe. Hence, the presence of conifer trees is common in schools, parks and other public areas, which means that susceptible individuals (i.e., children) are more likely to be affected by *T. pityocampa* larvae (Vega *et al.*, 2003). Allergic reactions can occur not only when the larvae are present, but also during the following season because of the persistence of allergenic setae in the remains of the winter nests (Lamy, 1990; Vega *et al.*, 2011; Bonamonte *et al.*, 2013; Battisti *et al.*, 2017).

Given that aerial insecticidal treatments against *T. pityocampa* for the suppression of outbreaks may not be effective (Cayuela *et al.*, 2011), the most effective strategy for the control of this moth species involves a combination of preventive methods, such as the conservation and enhancement of plant biodiversity (Jactel and Brockerhoff, 2007; Jactel *et al.*, 2015), and curative methods, namely the removal of eggs, larvae mass trapping, application of sex pheromones aiming to mass trapping and mating disruption of male adults, mechanical removal and destruction of nests, incorporation of repellents against egg laying, installation of nest boxes for insect-eating birds, biological control using parasitoids or the design of risk maps that may direct the application of control measures (Halperin, 1985; Artola Bordás *et al.*, 2008; Barbaro and Battisti, 2011; Cayuela *et al.*, 2011; Auger-Rozenberg *et al.*, 2015; Jactel *et al.*, 2015; Martin, 2015; Rossi *et al.*, 2016; Athanassiou *et al.*, 2017).

Control treatments against *T. pityocampa* larvae become urgent when the infested trees are close to inhabited places, where severe health problems may be associated with the presence of the larvae. Thus, an effective control system should aim to the considerable reduction of the number of larvae, based always on a reliable monitoring chain (Rossi *et al.*, 2016). From the aforementioned

alternative control methods against *T. pityocampa*, very little information is available on the mass trapping of the processing larvae. For example, Martin (2015) reported the high efficacy of a trap device, installed around the tree trunk as a ring before the start of the procession activity, for the capture of *T. pityocampa* larvae in southern France. However, to the best of our knowledge, there are no data on the impact of this type of trap device to the *T. pityocampa* infestation levels and its efficiency in comparison with other trap device under long-term use.

Therefore, the objectives of the present study were to evaluate the efficacy of trunk barrier trap device (experiments in Attica, Greece), the comparison of a prototype with a commercial trunk barrier trap device (experiments in Volos, Greece and Valencia, Spain) and the effect of trapping the wintering migrant processionary larvae with trunk barrier trap devices for two consecutive years on the associated male adult population and the number of winter nests on infested trees in Molise (Italy).

3.2 MATERIALS AND METHODS

3.2.1 Experimental areas

All trials were conducted between 2015 and 2017. The experiments were carried out in four different areas, two in Greece (Attica and Volos), one in Spain (Valencia), and one in Italy (Molise). In Greece, the first experimental area was a suburban forest in Amarousion, Attica, southern Greece, at 270 m above sea level (a.s.l.), with approx. 65 ha of *Pinus halepensis* Miller (Pinales: Pinaceae). This forest belongs to the Institute of Agricultural Sciences that is a public entity of the Ministry of Rural Development and Food of the Hellenic Republic (Greece). Apart from this experimental area, trials in Greece were conducted also at a low elevation (188 m/a.s.l.) pine forest on Goritsa hill at the edge of the city of Volos (Magnesia, Thessaly). This hill covers a total area of 120 ha of pine trees, mainly *Pinus brutia* Tenore (Pinales: Pinaceae) and to a lesser extent *P. halepensis*. In Spain, the trials were conducted in Porta Coeli, an area inside the Serra Calderona Natural Park, Valencia (200 m/a.s.l.) with 600 ha of *P. halepensis*. The chosen experimental area in Italy was a public park located in the town of Campobasso (740 m/a.s.l.), Molise region, central-

south Italy with 50 trees of *Pinus nigra* J. F. Arnold (Pinales: Pinaceae). A nearby urban park of 2 ha with about 1000 *P. nigra* trees was used as control area, where no trunk trap devices were installed (Figs. 3.1-3.2). The selection of the areas was based on the fact that they were heavily infested by *T. pityocampa* the previous years.

3.2.2 Placement and inspection of trunk barrier and adhesive barrier trap devices

Greece

Attica

The PROCEREX trunk barrier trap (AGH Protecta, Vaucluse, France) and the handmade adhesive barrier trap device were evaluated for the capture of *T. pityocampa* wintering migrant processionary larvae. Each trunk barrier trap device consisted of the following parts: a transparent 10 cm wide plastic deflector, a holding spring, two strips of double density foam with one beveled edge, a small bag containing a steel spring, a strap, three clamps, a slightly curved rectangular parallelepiped downspout (2.7 cm x 5.6 cm x 16.5 cm) that left an opening area of 15.1 cm², and a collecting bag for the larvae (Fig. 3.3 A and B). The adhesive barrier trap device is made of a 25 cm brownish bag material (in order to reduce the crossing risk), coated by a brush with entomological glue (Tem-O-Cid, Kollant S.p.a., Vigonovo, Italy), that was positioned around the trunk (Fig. 3.3 A and B). Based on preliminary observations, the trunk trap devices were installed at the beginning of February in 2015 and after mid of February in 2016. The trap devices were disassembled after the end of the procession period, approximately at the beginning of April in 2015 and at the end of March in 2016. Five trunk barrier traps were installed at equal number of trees with five nests on each tree, at a height of approximately 2 m above the ground. An adhesive barrier trap device was positioned 0.5 m below each trunk barrier trap device to verify its effectiveness by checking the number of migrant larvae that stuck on the adhesive surface in relation with the number of larvae found in each respective trunk barrier trap device. At five other pine trees with five nests each, equal number of trunk barrier trap devices and respective adhesive barrier trap devices were installed, with two trap devices on each tree, but their positions were reversed, in order to study the effectiveness of the adhesive barrier trap device. Different trees were selected for experimentation in 2015 and 2016. Trap devices were inspected every two days. The captured larvae were carefully removed from devices, sorted in pre-labeled plastic bags and transferred to the laboratory for counting. As commencement of trunk or adhesive barrier trap device-check date, we considered the date on which the first captures were recorded.

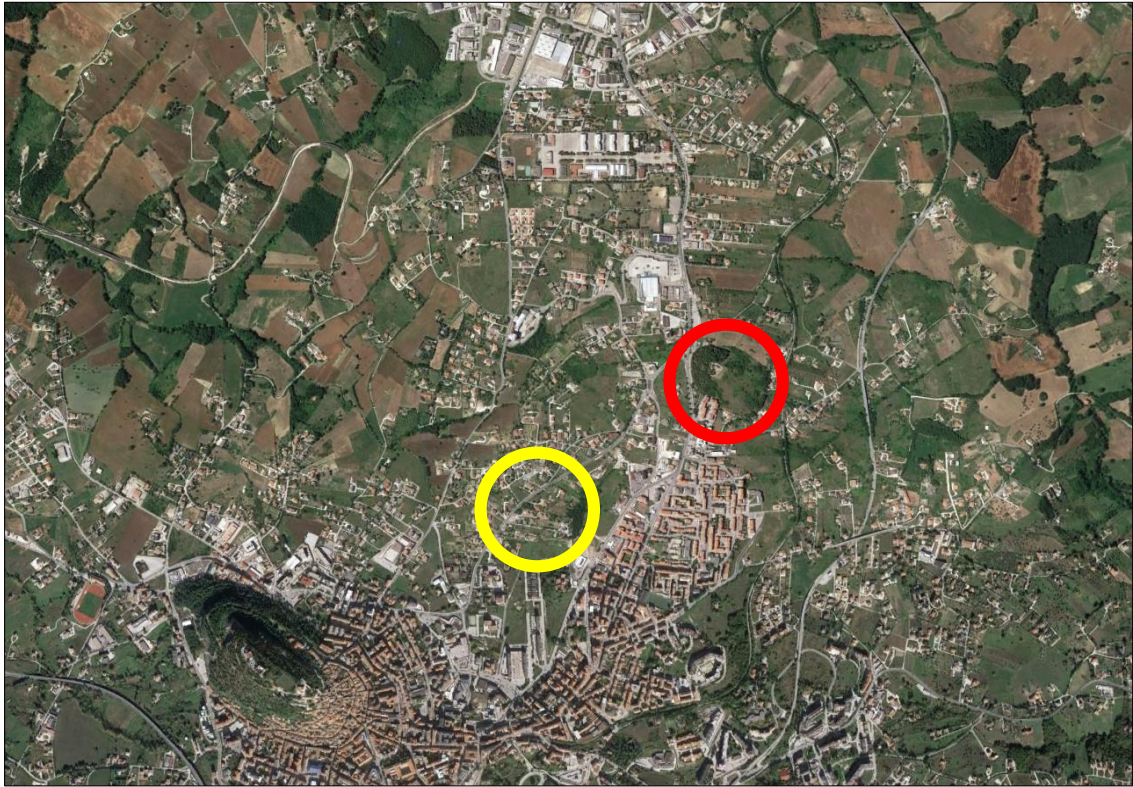


Figure 3.1 Aerial view of the areas in Italy: experimental area (circled in yellow), control area (circled in red) (from Google Earth).



Figure 3.2 Vegetational aspects of the areas in Italy: experimental area (A), control area (B).

The performance of the trunk barrier trap device and the adhesive barrier trap device was expressed in percentage (%) as follows: $\text{number of larvae in the trunk barrier trap device} / (\text{number of larvae in the trunk barrier trap device} + \text{number of larvae in the adhesive barrier trap device}) \times 100$, or $\text{number of larvae in the adhesive barrier trap device} / (\text{number of larvae in the adhesive barrier trap device} + \text{number of larvae in the trunk barrier trap device}) \times 100$, respectively.

Volos

The trials in this experimental area were conducted in 2016. In this trials, the PROCEREX trunk barrier trap device was compared with a LIFE-PISA prototype trunk barrier trap device, constructed by AIMPLAS (Plastic Technology Centre, Valencia, Spain) and SANSAN PRODESING S.L. (Valencia, Spain). Each LIFE-PISA prototype trunk barrier trap device was consisted of the following parts: a black 15.5 cm wide plastic deflector, a strip of double density foam, a strap, three clamps, a slightly curved rectangular parallelepiped downspout (2.8 cm x 6.0 cm x 17.7 cm) that left an opening area of 16.8 cm², and a collecting bag for the larvae (Fig. 3.3 C). Based on preliminary observations, the trap devices were installed after mid of February and disassembled after the end of the wintering larvae procession period. Four commercial and four LIFE-PISA prototype trunk barrier trap devices were installed at equal number of trees with four *T. pityocampa* nests on each tree, at a height of approximately 2 m above the ground. Moreover, handmade depositories were constructed by using the multi-layer material ISO-ROLL PUR (Imper Italia s.r.l., Borgaro, Italy) formed as a cylinder (15 cm in height) positioned around the trunks (Fig. 3.3 C). The periphery of each depository abtained three times the periphery of the plastic deflector. The entire internal part of the depositories was coated by a brush with entomological glue (Tem-O-Cid, Kollant S.p.a., Vigonovo, Italy). The depositories were installed 0.5 m below each trunk barrier trap device to verify its effectiveness by checking the number of migrant larvae that were trapped in the depositories in relation with the number of larvae found in each respective commercial or LIFE-PISA prototype trunk barrier trap device. Trap devices were inspected every three days and the captured larvae were treated as described above. The performances of the commercial and the LIFE-PISA prototype trunk traps were evaluated as above.

Spain

The PROCEREX trunk barrier trap device and the LIFE-PISA prototype trunk barrier trap device were used for the capture of *T. pityocampa* larvae as described above. Based on preliminary observations, the trap devices were installed in the experimental area after late January 2016, and disassembled after the end of the larval procession period, at mid of April 2016. Depositories were made using the same raw materials as in the LIFE-PISA prototype trap without funnel and bag and installed as described above. Trap devices were inspected every seven or fifteen days and captured larvae were treated as described above. The performances of the commercial and the LIFE-PISA prototype trunk traps were evaluated as above.

Italy

The Ecopiège trunk barrier trap device (La Mésange Verte, Bages, France) was used for the capture of *T. pityocampa* wintering migrant processionary larvae. Based on preliminary observations, the trap devices were installed on the same trees in the experimental area after mid of February in 2015 and 2016 and disassembled after the end of the procession period, approximately at the beginning of May in both 2015 and 2016. As commencement of trunk barrier trap device-check date, we considered the date on which the first captures were recorded. The trunk barrier trap device was made of a flexible flange that surrounded the trunk and a bag, connected to the flange by a cylindrical pipe (2.5 cm diameter, 40 cm height) that left an opening area of 4.9 cm² (Fig. 3.3 D). In total, eight trunk barrier trap devices were installed on equal number of trees at approximately 1.2 m height above the ground, to prevent children and/or animals to come in contact with the captured larvae. Each tree had variable number of nests. The trunk barrier trap devices were inspected every 3-4 days, whereas the captured larvae were carefully removed from the trunk barrier trap devices, sorted in pre-labeled plastic bags and transferred to the laboratory for counting.

3.2.3 Placement and inspection of pheromone trap devices

Italy

For the monitoring of *T. pityocampa* male adults population, eight funnel trap devices (Novapher, Milano, Italy) were used in both experimental and control areas. Four traps were installed in the experimental area while the remaining in the control area. All trap devices were baited with dispensers, containing the sex pheromone component (Z)-13-hexadecen-11-ynyl acetate (Pest Control Products, Chieti, Italy) (Athanasios *et al.*, 2007, 2017; Frérot and Démolin, 1993). Based on earlier observations in this region, the pheromone trap devices were installed on the canopy of trees at a height of 2-3 m above the ground in early July and disassembled after the end of the flight period

(Athanassiou *et al.*, 2017). The distance among pheromone-baited traps was about 100 m. Devices were checked every 3-4 days and rotated clockwise to minimize the influence of the individual trapping location. As first pheromone trap-check date, we considered the date on which the first captures were recorded. During each inspection, the captured male adults were noted and removed from the devices.

3.2.4 Winter nests

Italy

From 2015 to 2017, all *T. pityocampa* winter nests on the tested pine trees were annually counted by visual inspection in the experimental area (Jactel *et al.*, 2006). Visual inspection was carried out in January, when the formed on the trees nests were more easily visible (Trematerra, 2016).

3.2.5 Data analysis

Greece

Attica

The two-tailed *t* test at $n - 2$ df and 0.05 probability (Snedecor and Cochran, 1980) was used to compare the larvae captures in the trunk barrier trap vs. adhesive barrier trap devices in 2015 and 2016.

Volos

The two-tailed *t* test at $n - 2$ df and 0.05 probability (Snedecor and Cochran, 1980) was used to compare the larvae captures in the commercial vs. LIFE-PISA prototype trunk barrier trap devices in 2016.

Spain

The two-tailed *t* test at $n - 2$ df and 0.05 probability (Snedecor and Cochran, 1980) was used to compare the larvae captures in the commercial vs. LIFE-PISA prototype trunk barrier trap devices in 2016.

Italy

The two-tailed t test at $n - 2$ df and 0.05 probability (Snedecor and Cochran, 1980) was used to compare:

- a) the larvae and the average number of larvae per nest in 2015 vs. 2016;
- b) the male adults captures in 2015 vs. 2016. In this case, we tested the hypothesis that the adult population trend between years was different: a decrease in *T. pityocampa* population was expected in the experimental area, due to the application of trunk barrier trap devices, but not in the control area;
- c) the number of trapped adults in experimental vs. control area within each year (2015, 2016). Data on the numbers of the recorded nests were analysed by using a one-way ANOVA, with year as main effect. In all cases, means were separated by the Tukey-Kramer (HSD) test at 0.05 probability (Sokal and Rohlf, 1995).

Before the analysis, counts were transformed to $\log(x + 1)$, to normalize variances and standardize means (Athanasios *et al.*, 2002, 2004, 2007, 2008, 2017; Kavallieratos *et al.*, 2005).

A linear regression analysis was carried out to assess the relationships between number of nests vs. number of larvae, sampled on the same tree in the experimental area, by merging the 2015 and 2016 samplings.

All analyses were conducted using the SPSS software (SPSS Inc., 2009).



Figure 3.3 *Trunk trap devices that were tested: (A) PROCEREX (upper) and an adhesive (lower) used in the trials in Attica, Greece, (B) Adhesive (upper) and a PROCEREX (lower) used in the trials in Attica, Greece, (C) LIFE-PISA prototype (upper) and a depository (lower) used in the trials in Volos, Greece, (D) Ecopiège used in the trials in Italy (pictures A and B by N. G. Kavallieratos; picture C by C. G. Athanassiou).*

3.3 RESULTS

3.3.1 Greece

Attica

Significantly more wintering migrant larvae were captured in trunk barrier trap devices in comparison with the adhesive barrier trap devices installed below the trunk traps in 2015 ($t = -3.4$, $df = 168$, $P < 0.01$) and 2016 ($t = -5.3$, $df = 168$, $P < 0.01$). A total of 4399 larvae (2414 in 2015 and 1985 in 2016) were captured in the trunk barrier trap devices, whereas only few larvae (5 in 2015 and 8 in 2016) were captured in the adhesive barrier trap devices installed below the trunk traps, indicating the high trapping efficacy of the trunk trap devices for both years. In 2015, the first captures were recorded during the first week of March and the latest at the beginning of April (Fig. 3.4). During 2016, the first larvae were captured during the third week of February and the last ones at the third week of March (Fig. 3.5). When the adhesive barrier trap devices were installed upon the trunk trap devices, significantly fewer wintering larvae were captured in the adhesive barrier trap devices in comparison with the trunk barrier trap devices both in 2015 ($t = -2.9$, $df = 168$, $P < 0.01$) and 2016 ($t = -5.0$, $df = 168$, $P < 0.01$). A total of 741 larvae were captured (i.e., 300 in 2015 and 441 in 2016) in the adhesive barrier trap device and 2629 larvae (i.e., 1257 in 2015 and 1372 in 2016) were captured in the trunk barrier trap devices. In this case, the trapping efficacy of the trunk trap devices was also high since it ranged between 66.5% and 92.1% in 2015 and 69.7% and 87.9% in 2016 (Fig. 3.6). In contrast, the overall performance of the adhesive barrier trap devices was poor for both years since they never captured $> 34\%$ of the migrant larvae (Fig. 3.7).

Volos

The mean numbers of larvae captured in the commercial or LIFE-PISA prototype trunk barrier trap devices and in their depositories in the trial in Volos are presented in Table 3.1. The first wintering migrant larvae were captured in the mid of February and the last at the beginning of March. In all inspection dates, no significant differences were recorded between the mean numbers of captures in the commercial and the LIFE-PISA prototype trunk barrier trap devices. The performances of the commercial and the LIFE PISA prototype trunk barrier trap devices were high and ranged from 92.3% to 100% and from 90.9% to 100%, respectively.

3.3.2 Spain

The first larvae were captured at the beginning of February while the last at the end of March (Table 3.2). No significant differences were recorded between the mean numbers of captured larvae in commercial and LIFE-PISA prototype trunk barrier trap devices at any inspection date. As in the case of Volos, the performances of both types of barrier trap devices were also high and ranged from 96.7% to 98.9% (commercial) and from 90.0% to 98.8% (LIFE PISA prototype).

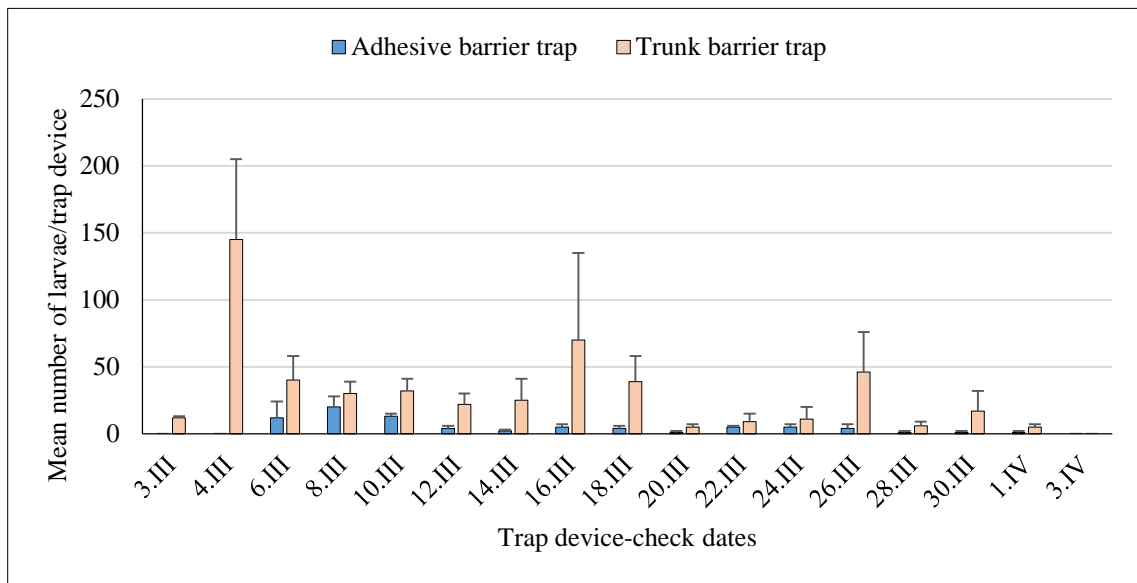


Figure 3.4 Mean number (+ SE) of *T. pityocampa* larvae captured in trunk and adhesive barrier trap devices in Attica, Greece in 2015 ($n = 5$).

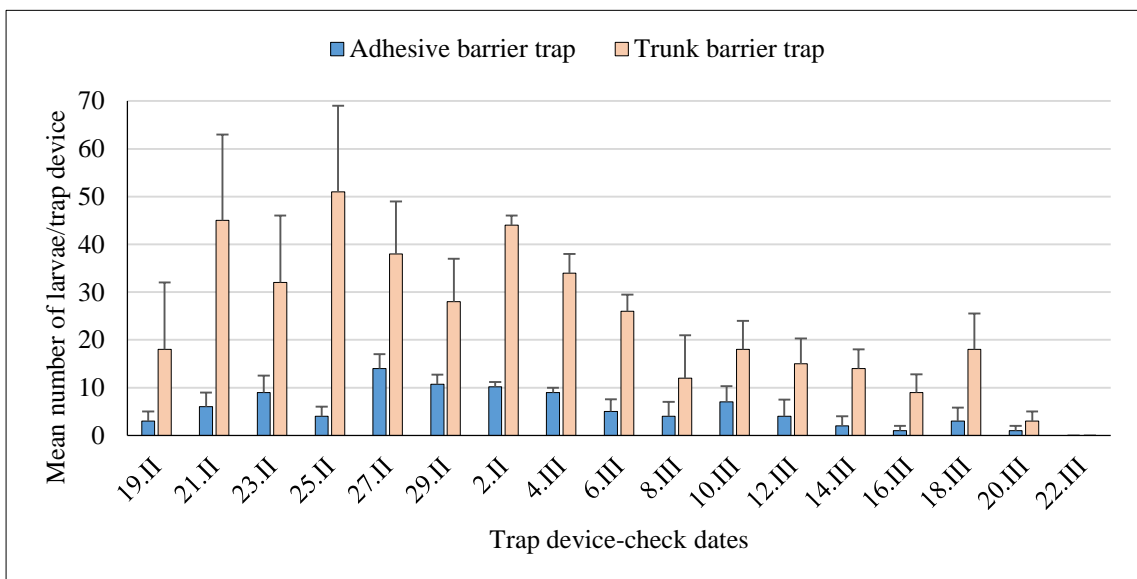


Figure 3.5 Mean number (+ SE) of *T. pityocampa* larvae captured in trunk and adhesive barrier trap devices in Attica, Greece in 2016 ($n = 5$).

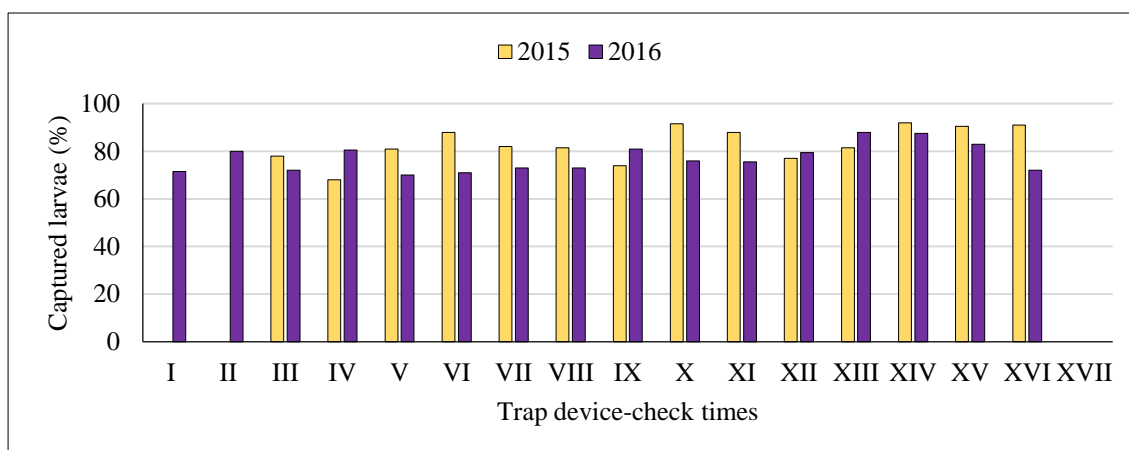


Figure 3.6 Percentage (%) of *T. pityocampa* larvae captured in trunk barrier trap devices installed below the adhesive barrier trap devices in Attica, Greece in 2015 and 2016 ($n = 5$). On axis X, I corresponds to 03 March 2015 and 19 February 2016, II to 04 March 2015 and 21 February 2016, III to 06 March 2015 and 23 February 2016, IV to 08 March 2015 and 25 February 2016, V to 10 March 2015 and 27 February 2016, VI to 12 March 2015 and 29 February 2016, VII to 14 March 2015 and 02 March 2016, VIII to 16 March 2015 and 04 March 2016, IX to 18 March 2015 and 06 March 2016, X to 20 March 2015 and 08 March 2016, XI to 22 March 2015 and 10 March 2016, XII to 24 March 2015 and 12 March 2016, XIII to 26 March 2015 and 14 March 2016, XIV to 28 March 2015 and 16 March 2016, XV to 30 March 2015 and 18 March 2016, XVI to 01 April 2015 and 20 March 2016, and XVII to 03 April 2015 and 22 March 2016.

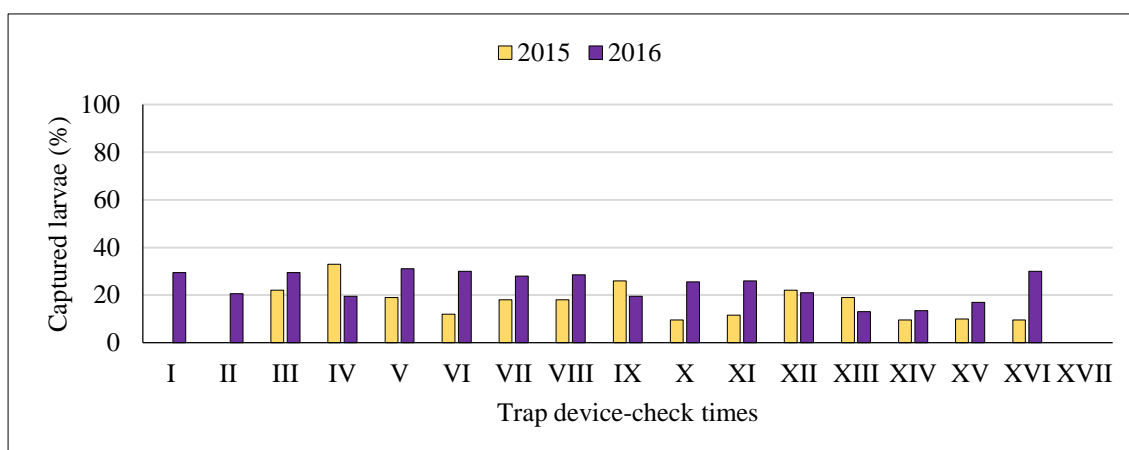


Figure 3.7 Percentage (%) of *T. pityocampa* larvae captured in adhesive barrier trap devices installed above the trunk barrier trap devices in Attica, Greece in 2015 and 2016 ($n = 5$). On axis X, I corresponds to 03 March 2015 and 19 February 2016, II to 04 March 2015 and 21 February 2016, III to 06 March 2015 and 23 February 2016, IV to 08 March 2015 and 25 February 2016, V to 10 March 2015 and 27 February 2016, VI to 12 March 2015 and 29 February 2016, VII to 14 March 2015 and 02 March 2016, VIII to 16 March 2015 and 04 March 2016, IX to 18 March 2015 and 06 March 2016, X to 20 March 2015 and 08 March 2016, XI to 22 March 2015 and 10 March 2016, XII to 24 March 2015 and 12 March 2016, XIII to 26 March 2015 and 14 March 2016, XIV to 28 March 2015 and 16 March 2016, XV to 30 March 2015 and 18 March 2016, XVI to 01 April 2015 and 20 March 2016, and XVII to 03 April 2015 and 22 March 2016.

Table 3.1 Mean number (\pm SE) of captured larvae in commercial and LIFE PISA prototype trunk barrier trap devices and their performances in Volos in 2016 ($n = 4$).

| Commercial trunk barrier trap devices | | LIFE PISA prototype trunk barrier trap devices | | Performance of commercial trunk barrier trap devices | | Performance of LIFE PISA prototype trunk barrier trap devices |
|---------------------------------------|---------------|--|-------|--|--------|---|
| Date | | | t | P | | |
| 2/19 | 0.5 ± 0.3 | 1.1 ± 0.4 | 1.1 | 0.31 | 100.0% | 100.0% |
| 2/22 | 3.3 ± 0.2 | 3.0 ± 0.4 | -0.82 | 0.45 | 97.9% | 98.3% |
| 2/25 | 3.6 ± 0.1 | 2.7 ± 0.6 | -1.5 | 0.19 | 95.3% | 100.0% |
| 2/28 | 2.5 ± 0.7 | 2.1 ± 0.2 | -0.5 | 0.62 | 92.3% | 90.9% |
| 3/2 | 3.1 ± 0.6 | 3.4 ± 0.2 | 0.4 | 0.74 | 95.0% | 100.0% |
| 3/5 | 2.8 ± 0.6 | 1.1 ± 0.8 | -1.7 | 0.14 | 96.9% | 91.0% |
| 3/8 | 1.5 ± 0.2 | 0.7 ± 0.4 | -1.8 | 0.11 | 100.0% | 100.0% |

Table 3.2 Mean number (\pm SE) of captured larvae in commercial and LIFE PISA prototype trunk barrier trap devices and their performances in Valencia in 2016 ($n = 4$).

| Commercial trunk barrier trap devices | | LIFE PISA prototype trunk barrier trap devices | | Performance of commercial trunk barrier trap devices | | Performance of LIFE PISA prototype trunk barrier trap devices |
|---------------------------------------|---------------|--|------|--|-------|---|
| Date | | | t | P | | |
| 2/4 | 0.5 ± 0.5 | 0.6 ± 0.4 | 0.1 | 0.92 | 98.9% | 91.2% |
| 2/18 | 1.9 ± 0.4 | 2.4 ± 0.3 | 1.0 | 0.39 | 97.8% | 98.8% |
| 2/25 | 1.7 ± 0.4 | 1.6 ± 0.3 | -0.1 | 0.91 | 95.2% | 98.6% |
| 3/3 | 1.6 ± 0.2 | 1.2 ± 0.5 | -0.8 | 0.43 | 98.6% | 97.6% |
| 3/16 | 1.2 ± 0.2 | 1.4 ± 0.2 | 0.5 | 0.65 | 98.7% | 96.4% |
| 3/31 | 0.4 ± 0.2 | 0.5 ± 0.2 | -0.5 | 0.62 | 96.7% | 90.0% |

3.3.3 Italy

The numbers of *T. pityocampa* migrant larvae captured in the trunk barrier trap devices and the number of nests for each tree between 2015 and 2017 are reported in Table 3.3.

Significantly more larvae were captured in trunk barrier trap devices in 2015 in comparison with 2016 ($t = 5.8$, $df = 206$, $P < 0.01$). During 2015, the highest captures occurred in the 1 and 5 trunk barrier trap devices while the lowest captures observed in 6 and 8 (Table 3.3). During 2016 the highest captures occurred in 1 and 8 trunk barrier trap devices while the 2, 3 and 6 did not capture larvae.

Significantly fewer nests were formed on average on the evaluated pine trees in 2016 (0.8 ± 0.3) and 2017 (0.4 ± 0.2) in comparison with 2015 (2.0 ± 0.3), i.e., before the installation of the trunk barrier trap devices ($F = 11.4$, $df = 2$, 23 , $P < 0.01$). In 2015, the maximum number of winter nests, i.e., 15, was observed on tree 5 while the minimum number was present on trees 3, 6, 7 and 8 with 3 nests. In 2016, the maximum numbers of nests were observed on trees 1 and 8, i.e., 3 nests, while on trees 2, 3 and 6 no nests were formed. In 2017, the maximum number of nests was observed on tree 1, with 3 nests, while on trees 3-7 no nests were formed. The total average number of larvae per nest significantly decreased from 644.3 in 2015 to 78.3 in 2016 ($t = -5.6$, $df = 14$, $P < 0.01$).

In 2015, the first larvae were captured at the end of the third week of March and the last at the end of April (Fig. 3.8). The highest number of captures was observed between the last week of March and the first week of April. Similarly, in 2016 the first larvae captures were recorded in the mid of March and the last ones at the end of the first week of April, whereas most larvae were captured during the fourth week of March (Fig. 3.9).

During the entire experimental period, a total of 532 (i.e., 376 in 2015 and 156 in 2016) male adults were captured in the pheromone trap devices in the experimental area. Significantly more male adults were trapped in 2015 in

comparison with 2016 in the experimental area ($t = 2.3$, $df = 150$, $P = 0.02$). In 2015, the first captures of *T. pityocampa* male adults were recorded at the first week of July. Male adults were captured until the beginning of September, with a maximum at the beginning of August (Fig. 3.10). In 2016, male adult captures were recorded between the mid of July until mid of September.

During the entire experimental periods, a total of 1340 (i.e., 676 in 2015 and 664 in 2016) male adults were captured in the pheromone trap devices in the control area. No significant differences were noted in captures of male adults between the two years in the control area ($t = -1.4$, $df = 118$, $P = 0.18$). Significantly fewer male adults were captured in pheromone trap devices in the experimental area in comparison with the control area in 2016 ($t = -4.7$, $df = 142$, $P < 0.01$) but not in 2015 ($t = -0.825$, $df = 142$, $P = 0.41$). In the control area, male adults were trapped from mid of July for both years till the end of third week of August in 2015 and beginning of September in 2016 (Fig. 3.11). The highest numbers of male adult captures were recorded during the last week of July in 2015 and the first week of August in 2016.

Regression analysis was significant ($F = 111.6$, $df = 15$, $P < 0.01$, $R^2 = 0.89$) and highlighted the strong relationship between the number of nests and the number of larvae on the same tree (Fig. 3.12).

Table 3.3 *Number of nests and captured larvae of T. pityocampa between 2015 and 2017 in Molise, Italy.*

| Tested tree | 2015 | | 2016 | | 2017 |
|-------------|-----------------|--------------------------|-----------------|--------------------------|-----------------|
| | Number of nests | Number of trapped larvae | Number of nests | Number of trapped larvae | Number of nests |
| 1 | 8 | 1129 | 3 | 81 | 3 |
| 2 | 11 | 705 | 0 | 0 | 1 |
| 3 | 3 | 261 | 0 | 0 | 0 |
| 4 | 8 | 704 | 1 | 5 | 0 |
| 5 | 15 | 1591 | 1 | 12 | 0 |
| 6 | 3 | 157 | 0 | 0 | 0 |
| 7 | 3 | 207 | 1 | 14 | 0 |
| 8 | 3 | 110 | 3 | 61 | 2 |
| Total | 54 | 4864 | 9 | 173 | 6 |

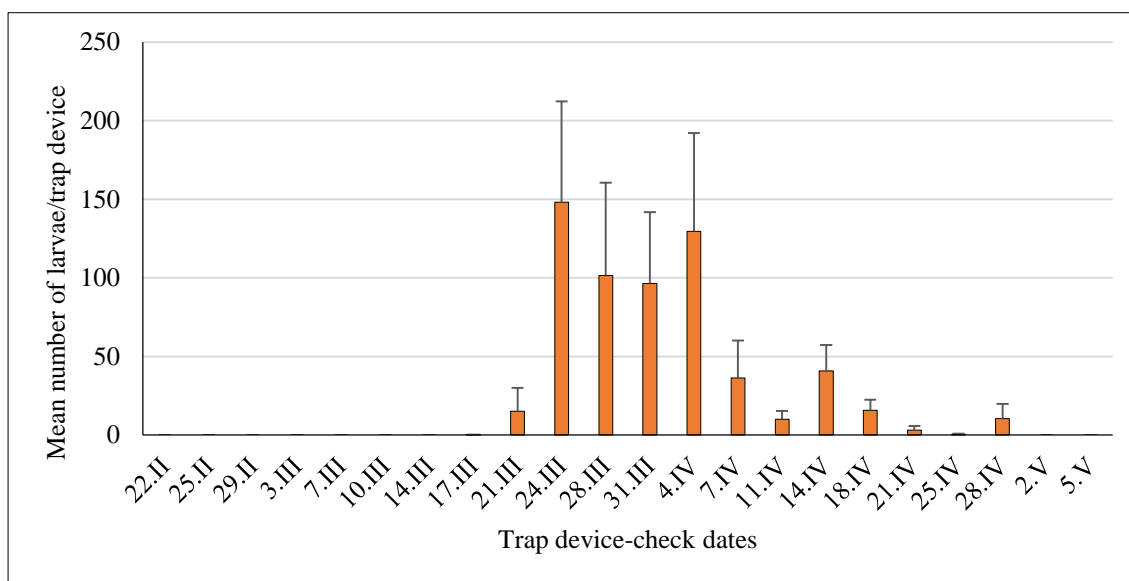


Figure 3.8 Mean number (+ SE) of *T. pityocampa* larvae captured in trunk barrier trap devices in the experimental area in Molise, Italy in 2015 ($n = 8$).

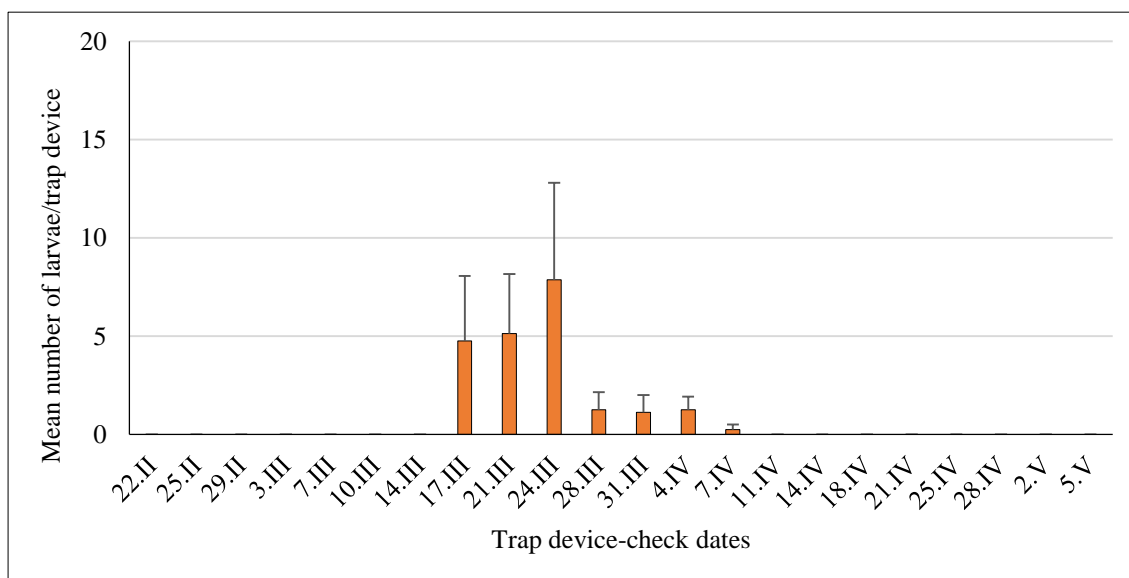


Figure 3.9 Mean number (+ SE) of *T. pityocampa* larvae captured in trunk barrier trap devices in the experimental area in Molise, Italy in 2016 ($n = 8$).

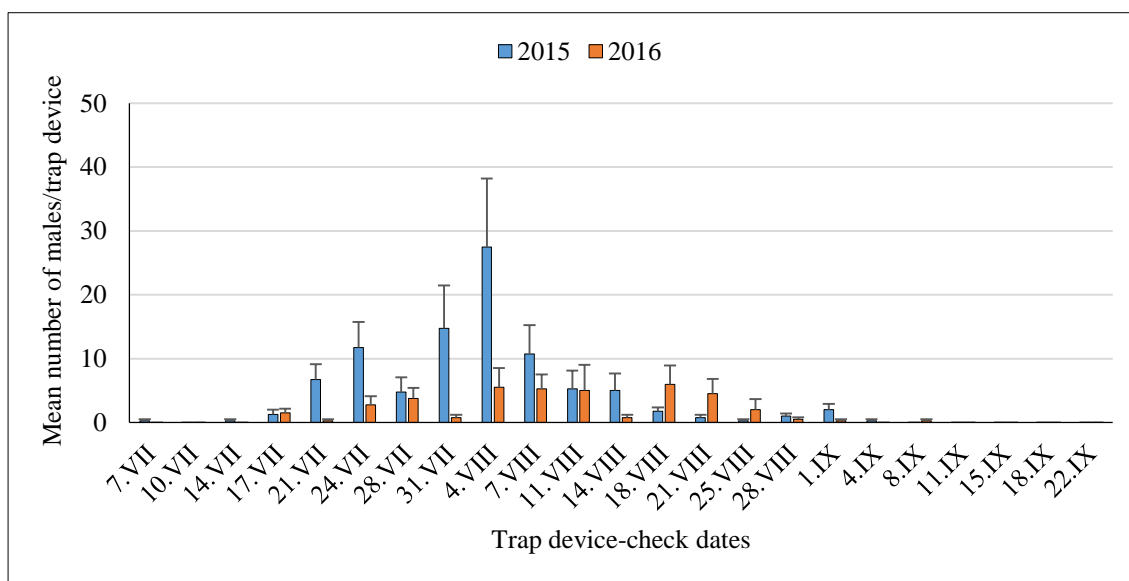


Figure 3.10 Mean number (+ SE) of *T. pityocampa* male adults captured in pheromone-baited funnel trap devices in the experimental area in Molise, Italy in 2015 and 2016 ($n = 8$).

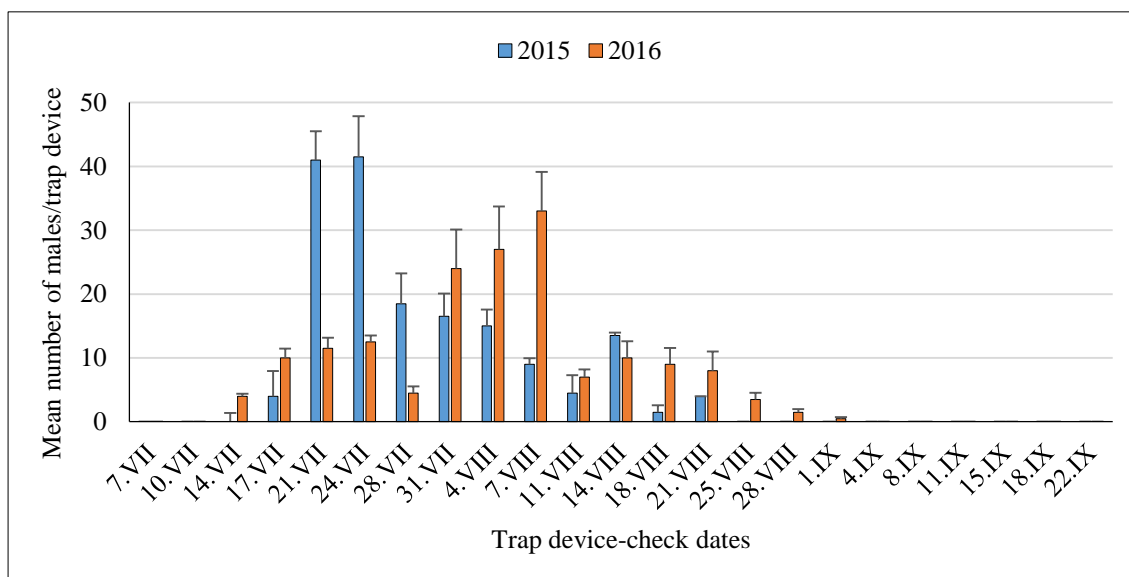


Figure 3.11 Mean number (+ SE) of *T. pityocampa* male adults captured in pheromone-baited funnel trap devices in the control area in Molise, Italy in 2015 and 2016 ($n = 8$).

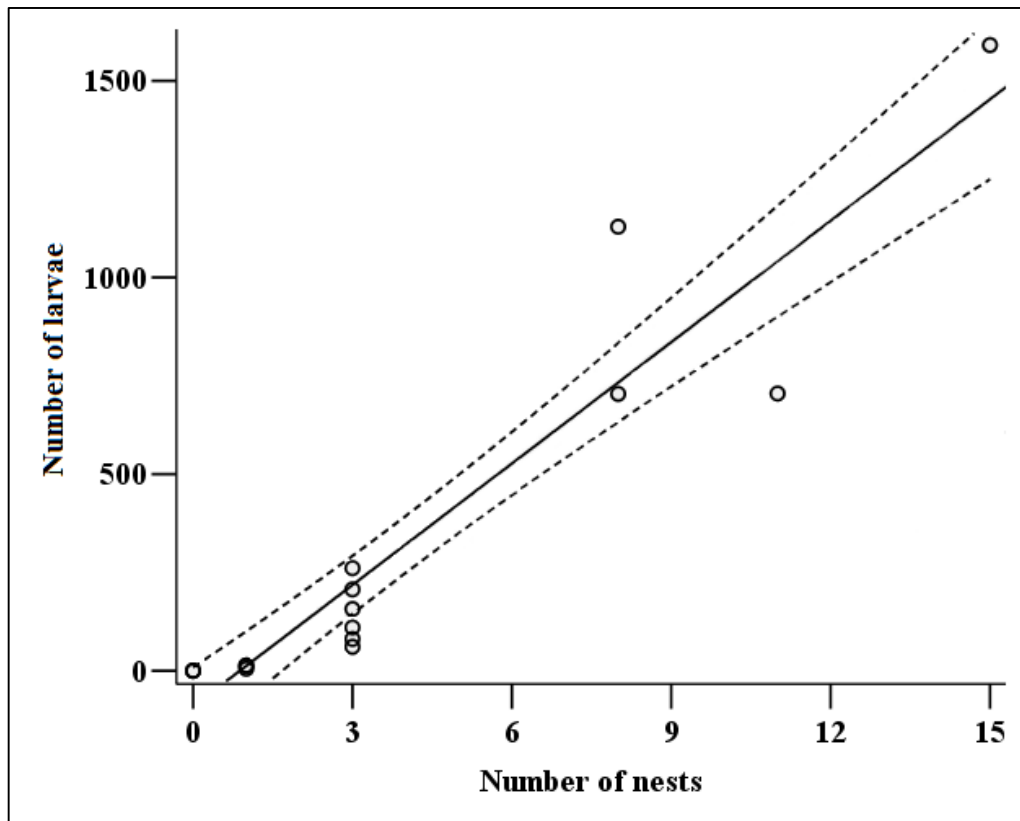


Figure 3.12 Linear regression analysis between number of nests vs. number of larvae, sampled on the same tree in the experimental area, by merging the 2015 and 2016 samplings. Dashed lines indicate the confidence interval at $P = 0.05$.

3.4 DISCUSSION

Thaumetopoea pityocampa is not a problem that is limited to the Mediterranean and northern African countries, given that it has naturally spread to higher altitudes (as in northern Italy) and latitudes (as in central and eastern France) (Battisti *et al.*, 2005; Robinet *et al.*, 2010; Li *et al.*, 2015). The temperature rise due to global climate warming favors the winter survival of the pest and enhances its feeding activity in regions where it could not develop before (Hódar *et al.*, 2003; Battisti *et al.*, 2005; Buffo *et al.*, 2007). It should be noted that *T. pityocampa* larvae develop continuously throughout the winter and

they are easily detectable due to their visible winter nests (Battisti *et al.*, 2005, 2011). In Paris basin (central France) progressively more colonies become established as a result of the accidental transportation of *T. pityocampa* individuals by humans (Robinet *et al.*, 2012). In the same area, its expansion to the north since 2000 due to temperature increase has been estimated at approximately 5.6 km/year (Robinet *et al.*, 2012). It becomes evident that the allergens which are contained in the setae of the *T. pityocampa* larvae consist a serious threat for public health in both southern and northern Europe (Rodriguez-Mahillo *et al.*, 2012). Therefore, an effective control of *T. pityocampa* larvae could help to the better manipulation of medical incidences at least during their procession period.

According to our trials in Italy, the trunk barrier trap devices installed in the experimental area were able to intercept the majority of *T. pityocampa* migrant larvae that were coming down from the winter nests. As a result of this interception, a reduction on the number of the captured larvae, male adults and winter nests was observed during the second year of the trial. Considering that in the control area no adult population variation was observed during these two years, the difference observed in the experimental area could have been, at least, partially determined by the trapping effect on larval population. Apart from the so-far used method of counting the winter nests on trees (Jactel *et al.*, 2006), the trunk barrier trap devices can serve as an additional tool for the monitoring of *T. pityocampa* populations due to the strong relationship between the number of nests and the number of larvae.

The high capture capacity of the trunk barrier trap devices was also confirmed during the Greek trials. Martin (2015) reported that the Ecopiège trunk barrier trap devices exhibited 96.5% efficiency after a single year of application in southern France. Our tests revealed that the commercial trunk barrier trap (e.g. PROCEREX) performed even better (>99.5%) for both years. This could be attributed to the structural differences of these two types of trunk trap devices. For instance, the opening area of the downspout in PROCEREX trap device is 2.5 times bigger than in Ecopiège, allowing more migrant larvae to pass and fall into the bag. Although no significant differences were noted regarding captures of

larvae in Volos and Valencia between LIFE-PISA prototype and PROCEREX trunk trap devices, we noticed that in the former more larvae were captured in total than the latter, i.e., 95.6 and 98.0% vs. 97.2 and 98.4% performance, respectively. As aforementioned, the opening area of the downspout of LIFE-PISA prototype is 1.1 times bigger than the corresponding of PROCEREX. These findings indicate that the larger the opening area of the downspout is, the more larvae are captured in the bags. However, further experimentation is needed to clarify this issue by testing variable opening areas and / or shapes of downspouts. Last but not least, although no quantitative measurements were taken, we observed that the whole construction of the LIFE-PISA prototype enabled us to install it easier and faster than commercial trunk barrier trap device.

Our experimental approach clearly showed that use of trunk barrier trap devices for the mass trapping of *T. pityocampa* larvae need at least two years for the effective management of the *T. pityocampa* population. Given that re-infestations of the pine trees by *T. pityocampa* cannot totally be avoided (Martin, 2015), the use of trunk barrier trap devices should be repeated every year to ensure a long-term effect. The number of captures of male adults in pheromone-baited trap devices and a simple counting of the formed winter nests were useful indicators of the efficacy of the trunk trapping method. Similarly, Jactel *et al.* (2006) reported that the population levels of *T. pityocampa* in France, Italy and Portugal, as monitored by pheromone-baited trap devices during summer, were significantly and positively correlated with the density of winter nests.

As far as it concerns the adhesive barrier trap devices, they were not able to stop the wintering migrant larvae given that only a small percentage of them were captured during the procession period. Similar observations have also been reported from France (Martin, 2015). In our trials, we noticed that when several larvae stuck on the adhesive surface of the barrier trap device, other larvae could step upon them and continue their movement downwards. Furthermore, as *T. pityocampa* larvae are hairy (Monsel *et al.*, 2016), they could avoid the direct contact with the glue of the band and finally cross the trap.

Any management measures against *T. pityocampa* should be implemented in such a way so as to achieve the eradication of the larvae from crowded urban areas, (i.e., pedestrian and protected areas, private gardens, schools, hospitals,

hotels), and keep numbers of larvae in suburban areas or forests below hazardous levels (Trematerra, 2016). In urban areas, the capture of larvae with the trunk barrier trap devices, as it is demonstrated in our study, could serve as a valuable, environmentally-friendly and cost-efficient alternative method for the *T. pityocampa* management, as trap devices are installed once and provide protection for several years. In heavily infested suburban areas or forests, biocidal treatments with entomopathogenic bacteria, i.e. *Bacillus thuringiensis* Berliner var. *kurstaki* (Bacillales: Bacillaceae) or insect growth regulators (IGRs) can be applied against II-III instars larvae in late September or October, and against wintering migrant larvae from February to April, depending on the local conditions (Martin and Bonneaux, 2006; Trematerra, 2016). Chemical insecticides are still used in areas where a higher efficacy is required but their use is constantly decreasing. Further studies are considered necessary for the successful implementation of novel control systems that will effectively suppress the population of *T. pityocampa*, alone or in combination with the management tools that are currently used against this species.

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CHAPTER 4

PHEROMONE TRAP COMPARISON TESTS

The general results of an international collaboration “LIFE-PISA project (LIFE13 ENV/ES/000504)” are included in the following publication:

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Parts related to my PhD research are reported using font 13, parts related to other groups are reported in font 10.

EVALUATION OF PHEROMONE TRAP DEVICES FOR THE CAPTURE OF *THAUMETOPOEA PITYOCAMPA* (LEPIDOPTERA: THAUMETOPOEIDAE) IN SOUTHERN EUROPE

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Abstract

The development of reliable monitoring techniques can offer valuable sources of knowledge on the control of *Thaumetopoea pityocampa* (Denis and Schiffermüller) (Lepidoptera: Thaumetopoeidae). Nevertheless, there is a knowledge gap on the simultaneous large scale monitoring of *T. pityocampa* male adult population by using novel trap devices. Thus, the influence of type of trap device on the capture of male adults of *T. pityocampa* was evaluated in four areas with pine trees in southern Europe; two in Greece (Thessaly, Attica), one in

Italy (Molise) and one in Spain (Valencia). Six different novel trap devices, i.e., Prototype 1, Prototype 2, Prototype 3, Prototype 4, Prototype 5 and Prototype 6, were tested during 2015 between July and November. In general, the male adult catches lasted longer in the two sites of Greece compared to Molise and Valencia. Hence, in Thessaly, captures started in early August and remained at high levels until late September. In Attica, captures started in mid August and lasted until early November. In contrast, for both Molise and Valencia most of the male adults were captured in August, while male adult catches were recorded until September. From the trap devices tested, Prototype 1 was found superior than the other devices, regardless of the area, with the exception of Valencia, where there were no differences in the overall captures among Prototype 1, Prototype 5 and Prototype 6. In most of the combinations tested, there was a positive and significant correlation among captures of *T. pityocampa* in pairs of different trap devices, indicating that most of them gave similar population fluctuations. Our results suggest that Prototype 1 should be selected for the monitoring of *T. pityocampa* male adult population.

Key words: *Thaumetopoea pityocampa*, trap devices, monitoring, improved trapping, male adult catches.

4.1 INTRODUCTION

The pine processionary moth *Thaumetopoea pityocampa* (Denis and Schifferrmüller) (Lepidoptera: Thaumetopoeidae) is probably the most commonly known species that infests pine trees in the Mediterranean area (Devkota and Schmidt, 1990; Devkota *et al.*, 1992; Zhang *et al.* 2003; Athanassiou *et al.*, 2007; Kerdelhué *et al.* 2009). It can cause serious defoliations, which eventually weaken the trees or even lead to death, especially in the cases of infestations by

secondary colonizers, such as wood and bark boring insects or fungi (Athanassiou *et al.*, 2007). Nevertheless, even in cases that the trees are able to survive the infestation, tree growth is seriously affected (Jactel *et al.*, 2006). This is particularly important in the case of pine trees located in urban and sub-urban areas, such as parks, recreational and residential areas, especially in the coastal zones of the Mediterranean basin, where pines constitute an essential floristic element. Apart from the infestation per se, larvae of this species release urticating hairs that are able to cause serious skin and eye irritation problems, strong allergic reactions and respiration disorders to humans or animals (Moneo *et al.*, 2015).

Despite the fact that, until recently, *T. pityocampa* was considered a species restricted only in the Mediterranean basin, recent reports revealed that it has already expanded in areas of northern Europe that were regarded as processionary pine moth-free (Robinet *et al.*, 2012). For example, Li *et al.* (2015) showed that *T. pityocampa* has moved far northern than initially considered, and it is now a common pest at the areas of Bretagne and central France, often with extreme population outbreaks. In fact, some of these “long jumps” to central and northern Europe are considered as “human mediated”, but they are also associated with climate change (Robinet *et al.*, 2012; Li *et al.*, 2015). Currently, *T. pityocampa* is regarded as an invasive species for many areas of Northern-Central Europe and its further expansion is likely to carry on in next years (Robinet *et al.*, 2012; Battisti *et al.*, 2015). Moreover, in Portugal, it has been recently revealed that there are two allochronic populations of *T. pityocampa*, with shifted phenologies, which coexist in the same ecosystems (Berardi *et al.*, 2015; Branco *et al.*, 2016).

From a phytosanitary point of view, as from the perception of the economic damage in forests, urban or suburban areas and public health issues, it is essential to draw an action plan not only for controlling its further spatial distribution, but also for drastically reducing larval populations in already heavily

infested areas. Chemical control of *T. pityocampa* has relied on a relatively narrow range of active ingredients, such as those that are based on the bacterial insecticide *Bacillus thuringiensis* Berliner var. *kurstaki* (= Btk) (Bacillales: Bacillaceae) and those that are based on insect growth regulators (IGRs) (Robredo, 1980; Robredo and Obama, 1987; Trematerra, 2016). The development of reliable monitoring techniques can offer valuable sources of knowledge on the control of *T. pityocampa* (Martin, 2015). Nevertheless, monitoring strategies face several drawbacks in the case of *T. pityocampa*. For instance, counting of winter nests is not easy when the trees are tall or grow at high density stands and may lead to unreliable estimations in the beginning of outbreaks when the population levels are still low (Jactel *et al.*, 2006). Furthermore, monitoring of larvae of *T. pityocampa* is not easy due to the health risks that are associated with the possible contact with the larvae during sampling (Battisti *et al.*, 2011). Hence, monitoring of male adults is one of the solutions suggested, as there are no allergenic risks, while at the same time, trapping can be less laborious than direct (absolute) sampling. In an earlier study, Jactel *et al.* (2006) compared different types of pheromone-baited trap devices for the capture of *T. pityocampa* male adults in one area in France and one area in Portugal on the basis of the mean number of captured male adults per trap device per day, and reported that those with adhesive surfaces were able to capture more male adults than non-adhesive ones. Similar results on the effectiveness of adhesive vs. non-adhesive pheromone-baited traps have also been reported by Athanassiou *et al.* (2007) from one area of central Greece. Jactel *et al.* (2006) showed that the increase of pheromone dose significantly increased the captures of male adults on sticky trap devices. Furthermore, sticky trap devices caught significantly more male adults at the top of the tree crown in comparison with lower (i.e., mid and breast) heights. The authors also reported that the mean numbers of male adults captured on sticky pheromone traps baited with 0.2 mg of the commercial *T. pityocampa* sex pheromone (pityolure) per day were significantly positively

correlated with the density of winter nests per hectare. Athanassiou *et al.* (2007) indicated that the pine density significantly affected trap device performance and that trap device color should be considered as one of the factors that can affect captures. Given that different trap devices exhibit variable capture efficacy (Jactel *et al.*, 2006; Athanassiou *et al.*, 2007; Martin, 2015), the evaluation of novel trap devices, in terms of their capture sensitivity and capacity, could optimize pheromone based monitoring protocols of the male adult population of *T. pityocampa*. However, any experimentation should be conducted at the same time frame, in a large scale in order to obtain results that will be widely applicable. Therefore, the objective of the current study was to simultaneously evaluate six different novel trap devices on the capture of *T. pityocampa* male adults, during 2015 in four areas that are located in three countries of southern Europe (Greece, Italy, and Spain).

4.2 MATERIALS AND METHODS

4.2.1 Experimental sites

The experiments were carried out in four different sites, two in Greece (Thessaly, Attica), one in Italy (Molise), and one in Spain (Valencia). The first site in Greece was in the hill of Goritsa (180 m a.s.l., Magnessia, Thessaly, central Greece). This area is covered by approx. 120 ha of pines with 200 trees per ha which in majority are *Pinus brutia* Tenore (Pinales: Pinaceae) and secondarily *Pinus halepensis* Miller (Pinales: Pinaceae). The climate of this area is warm and temperate. The average minimum temperature was 7.2°C while the average maximum temperature was 27.2 °C. The annual rainfall was 491 mm. The second one was in Amarousion (270 m a.s.l., Attica, southern Greece). This area includes an approx. 65 ha of *P. halepensis* forest with 180 trees per ha. The climate is warm and temperate. The average minimum temperature was 8.7 °C while the average maximum temperature was 26.7 °C. The annual rainfall was 456 mm. The third area in Italy was in Petacciato (10 m a.s.l., Campobasso, Molise, central Italy). This area is covered by approx. 35 ha of pines with 650 trees per ha, which in majority are *P. halepensis* with few *Pinus pinea* L. (Pinales: Pinaceae) (Figs. 4.1-4.2). The climate is warm and

temperate. The average minimum temperature was 6.8 °C while the average maximum temperature was 23.5 °C. The annual rainfall was 686 mm. The area of Spain was located in Porta Coeli (179 m a.s.l., Serra, Valencia, eastern Spain), which is covered by approx. 600 ha of *P. halepensis* with 600 trees per ha. The climate is warm, temperate and subtropical. The average minimum temperature was 8.6 °C while the average maximum temperature was 23.7 °C. The annual rainfall was 469 mm. The selection of the areas was based on the fact that they were heavily infested by *T. pityocampa* the previous years.

4.2.2 Trap devices

Six trap devices were used for the experimentation: Prototype 1, Prototype 2, Prototype 3, Prototype 4, Prototype 5 and Prototype 6.

Prototype 1 (Fig. 4.3 A) consists of the dark brown plastic rectangular parallelepiped body (24.1 cm in length, 10 cm in height, 10 cm in width); one elastic band; one insect reservoir (plastic bag) (29.5 cm in length, 41.5 cm in height) of which the upper part (29.5 cm in length, 19.5 cm in height) is transparent and the lower part (29.5 cm in length, 22 cm in height) is black; one green pheromone plastic container that has the shape of a truncated cone (2.8 cm in large diameter, 2 cm in small diameter, 3.8 cm in height, 4 cm in slant height), bearing peripherally 8 rectangular parallelogram openings (0.4 cm in length, 2.4 cm in height), with a lid at the large base; and two nylon cords that the trap device is hanged from. At the four angles of the top of the body there are four triangular constructions with a small hole (0.7 cm in diameter) at the top each that help to hang the trap device. The pheromone container is fixed centrally on the top, inside a hole (2.4 cm in diameter) of the body as an inverted truncated cone. The body is opened on the right and left sides shaping two internal square truncated pyramids (10 cm in edge at large base, 2 cm in edge at small base, 5.5 cm in height, 7 cm in slant height each) having the directions of their cut vertexes to the center of the body.

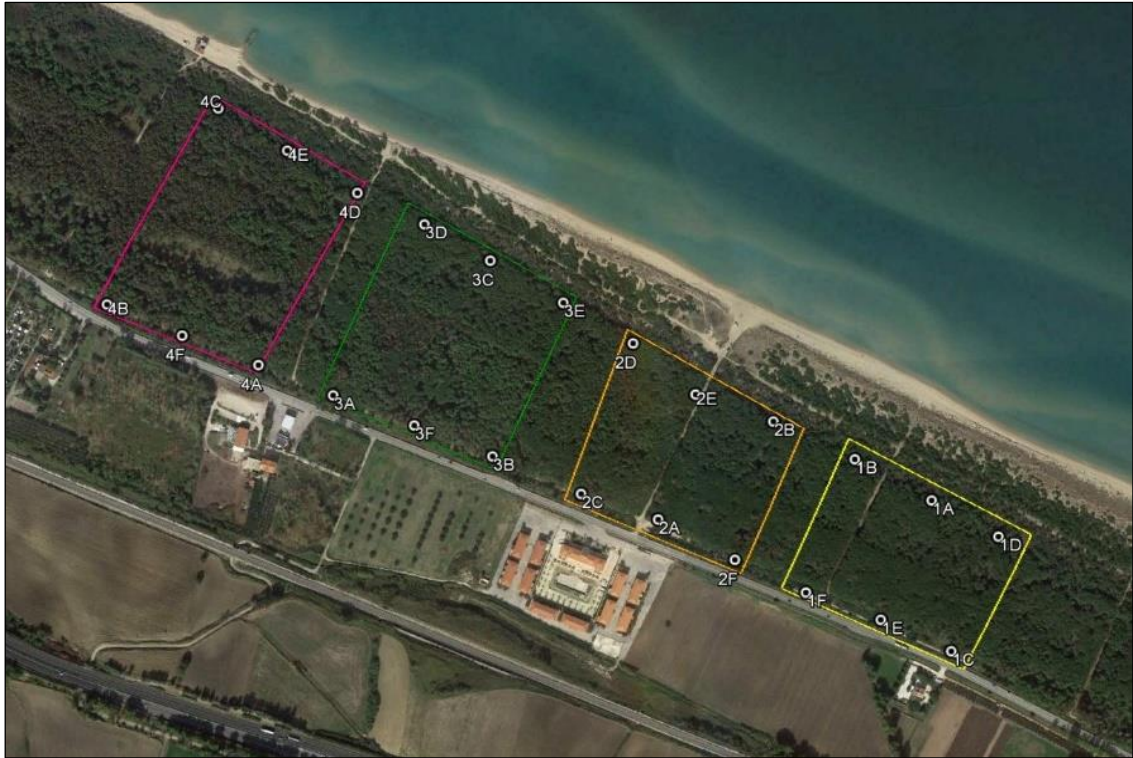


Figure 4.1 Aerial view of the experimental site in Italy with highlight the location of the blocks and traps (from Google Earth).



Figure 4.2 Vegetational aspects of the experimental area.

The small base of each truncated pyramid is permanently attached to a cube (2 cm in edge) that is diametrically opened from both sides. The body is also opened at the bottom. A prominent dark brown plastic ellipsoid ring (22.3 cm in large diameter, 8.7 cm in small diameter, 2.2 cm in height) surrounds this opening that has the shape of an inverted isosceles pyramid (16.5 cm in edge at large base, 5 cm in edge at large base, 13.5 cm in edge at small base, 1.5 cm in length of edge at small base, 2.5 cm in slant height). The bag is attached to the body by the elastic band that surrounds the ellipsoid ring. The moths can enter the body from the right and left openings, pass through the opening at the bottom and finally are trapped inside the bag.

Prototype 2 (Fig. 4.3 B) consists of two half light brown plastic bodies (18 cm in length, 18.5 cm in maximum height, 14.5 cm in maximum width each), providing a spindle type construction; one insect reservoir (semitransparent plastic bag) (26.5 cm in length, 30.5 cm in height); one green pheromone plastic container with lid (as in Prototype 1); one elastic band; and one nylon cord that the trap device is hanged from. Each half body bears at the maximum periphery two cylindrical projections (0.5 cm in diameter, 0.7 cm in high) and two small holes (0.8 cm in diameter) forming a conceivable rectangular parallelogram (11 cm in length, 9.5 cm in height). The two half bodies join each other by entering the projections inside holes and form the joint body. At a distance of 2.5 cm from the vertex of each body there are two discoid constructions (diametrically opposed) with a small hole (0.4 cm in diameter) each at the center that helps to hang the trap device. The pheromone container is fixed centrally on the top, inside a hole (2.4 cm in diameter) of the joint body. The body is opened on the right and left sides shaping two internal truncated cones (2 cm in small diameter, 11 cm in large diameter, 8 cm in height, 9 cm in slant height each) having the directions of their cut vertexes to the center of the body. The small base of each truncated cone is permanently attached to a cylinder (2 cm in diameter, 1.8 cm in height) that is opened from both sides. A prominent rectangular parallelepiped

(5.5 cm in height, 20 cm in length, 2.5 cm in width) is formed at the bottom of the joint body. The parallelepiped is opened at the top and bottom forming a new internal rectangular parallelepiped (2.5 cm in height, 24 cm in length, 2.5 cm in width). The bag is attached to the external parallelepiped of the joint body with the elastic band. The moths can enter the joint body from the right and left openings, pass through the opening at the bottom and finally are trapped inside the bag.

Prototype 3 (Fig. 4.3 C) consists of a large inverted truncated conical plastic transparent insect container (12 cm in diameter at the top, 10.8 cm in diameter at the bottom, 9 cm in height, 9.3 in slant height); a truncated conical green plastic body (13 cm in large diameter, 10.7 cm in small diameter, 6.5 cm in height, 7.6 cm in slant height) bearing three small holes on its top (1.9 cm in diameter each) forming a conceivable equilateral triangular (8.3 cm in height, 9.3 cm in base); a green plastic cover that is composed by a disk (12 cm in diameter) and three cylindrical projections (1.8 cm in diameter, 3.2 cm in height each) triangularly and permanently attached to its lower part; one green pheromone plastic container (as in Prototype 1); and one nylon cord that the trap device is hanged from. The top disc and the body join each other by entering the projections inside holes. The joint top disc and body is semiscrewed upon the insect container. At a distance of 0.5 cm from the periphery of the disc there are two holes (0.4 cm in diameter) (diametrically opposed) that help to hang the trap device. The pheromone container is fixed centrally on the top of the disk inside a hole (2.4 cm in diameter). The body is opened in the center shaping an internal inverted truncated cone (7.5 cm in large diameter, 3.5 cm in small diameter, 6.5 cm in height, 7 cm in slant height). The moths can enter the body through its opening and finally are trapped inside the container.

Prototype 4 (Fig. 4.3 D) is a Prototype 3 with the following modification: there is one more green pheromone plastic container (as in Prototype 1) that is

placed in the internal part of the body as truncated cone. Thus, Prototype 4 holds two pheromone lures.

Prototype 5 (Fig. 4.3 E) consists of one transparent insect container (bottle like) (31 cm in height, 3.5 cm in diameter at the top, 8 cm in diameter at the bottom, 1.5 l capacity); one dark brown plastic body that is composed by two opened anterolaterally rectangular parallelepipeds (2.7 cm in height, 17.5 cm in length, 5 cm in width each) forming an isosceles cross; one dark brown plastic disc (10 cm in diameter) that partially and centrally covers the upper part of the body; one green pheromone plastic container (as in Prototype 1) that is fixed centrally at the top of the disc inside a hole (2.4 cm in diameter); and two nylon cords that the trap device is hanged from. On the periphery of the disc there are four discoid constructions with a small hole (0.3 cm in diameter) each at the center, forming a conceivable square that help to hang the trap device. The body is opened centrally at the bottom to be permanently attached to an inverted truncated cone (7.2 cm in large diameter, 4.5 cm in small diameter, 1.7 cm in height, 2 cm in slant height) and a cylinder (4.5 cm in diameter, 2.2 cm in height) that is opened from both sides. The body, with the disc, is screwed upon the container. The moths can enter the body through its opening and finally are trapped inside the container.

Prototype 6 (Fig. 4.3 F) consists of one transparent insect container (as in Prototype 5); one dark brown plastic body that is a disc (20 cm in diameter) with a central circular opening (8 cm in diameter), which is permanently attached below with an inverted truncated cone (8 cm in large diameter, 4.5 cm in small diameter, 2.8 cm in height, 3.2 cm in slant height) and a cylinder (4.5 cm in diameter, 2.2 cm in height), opened from both sides; one green plastic cover (as in Prototype 3), one green pheromone plastic container (as in Prototype 1) that is fixed centrally at the top of the disc, inside a hole (2.4 cm in diameter); and one nylon cord that the trap device is hanged from. The trap device is hanged as in the case of Prototype 3. The body bears three small holes (as in Prototype 3). The

cover joins the body as in Prototype 3. The joint body and cover is screwed upon the container. The moths can enter the body through its opening and finally are trapped inside the container.

All trap devices were baited with lures containing with 1 mg of the sex pheromone component (Z)-13-hexadecen-11-ynyl acetate (Trécé Inc., Adair, OK, USA). Prototype 4 contained two lures with 1 mg of the sex pheromone each.

4.2.3 Placement and inspection of trap devices

In all areas, there were four blocks, with the exception of Attica, where there were three blocks. Each block contained one trap device from each type, hence, there were 24 trap devices for each site (18 in Attica). The distance among trap devices in the same block was approx. 100 m and among blocks 100 m or more. The trap devices were suspended in the test sites on early July within 2015 to be able to detect the male adult flight initiation, based on previous trappings from earlier years. As first trap device-check date for each experimental site was considered the one in which the first captures were recorded. The trap devices were inspected for captured male adults at weekly intervals, until the end of the male adult catches, with the exception of Valencia, where trap devices were checked at shorter intervals, from early September until the end of the flight period (3 - 4 days). During each inspection, the male adults that had been captured were recorded and removed from the trap devices. After the termination of this procedure, the trap devices were rotated clockwise within each block to minimize the influence of the individual trapping location. Each trap device was placed with its lower part at a height of 2 - 3 m from the ground. The lure was replaced every 4 weeks.



Figure 4.3 Funnel trap devices that were tested: Prototype 1 (A), Prototype 2 (B), Prototype 3 (C), Prototype 4 (D), Prototype 5 (E), Prototype 6 (F).

4.2.4 Data analysis

The data were analysed by using a two-way ANOVA, separately for each site, with trap device and date as main effects. All analyses were conducted using the JMP 11 software (SAS Institute, 2013). Before the analysis, counts were transformed to $\log(x + 1)$, to normalize variances and standardize means (Athanasidou *et al.*, 2002, 2004, 2007, 2008; Kavallieratos *et al.*, 2005). Means were separated by the Tukey-Kramer (HSD) test at 0.05 probability (Sokal and

Rohlf, 1995). Moreover, the correlation coefficient values between pairs of trap devices was also calculated, in order to estimate the synchronization between pairs of catches among different trap devices throughout the monitoring period, separately for each site. These values were tested for departure from zero by using the two-tailed t test at $n - 2$ df and 0.05 probability (Snedecor and Cochran, 1980).

4.3 RESULTS

4.3.1 Thessally

A total of 796 male adults were captured in the trap devices, during the entire experimental period. The flight of *T. pityocampa* male adults initiated in early August and lasted until end of October (Fig. 4.4). The highest number of male adults was recorded during mid September, but captures were relatively high since late August. Trap device and date were significant (Table 4.1). Significantly more male adults were captured in Prototype 1 than in the other trap devices (Table 4.2). Moreover, significantly more male adults were captured in Prototype 2 than in Prototype 3 and Prototype 6. The correlation coefficient values for the 15 pairs of trap devices were positive and significant, with the exception of two pairs (Prototype 1 - Prototype 3 and Prototype 1 - Prototype 6) (Table 4.3). Within different dates, significant differences were noted among trap devices in nine out of the eleven trap device-check dates (Table 4.4).

4.3.2 Attica

A total of 604 male adults were captured in the trap devices. In Attica, the flight period of *T. pityocampa* males was initiated in mid August and lasted until early November (Fig. 4.5). The highest number of male adults was recorded during early September, while the overall captures were high also from late August, but captures were relatively high from late August and the following weeks till mid September. In contrast, during October and until the end of the monitoring period on November, captures were extremely low. Trap device and date, as well as their interaction, were significant (Table 4.1). Significantly more male adults were captured in Prototype 1 than in the other trap devices (Table 4.2). The correlation coefficient values for the pairs of trap devices were positive and significant, with the exception of two pairs (Prototype 2 - Prototype 3 and Prototype 3 - Prototype 5) (Table 4.3). Within different dates, significant differences were noted among trap devices in six out of the thirteen trap device-check dates (Table 4.4). No significant differences were recorded among the trap devices either early or late in the experimental period.

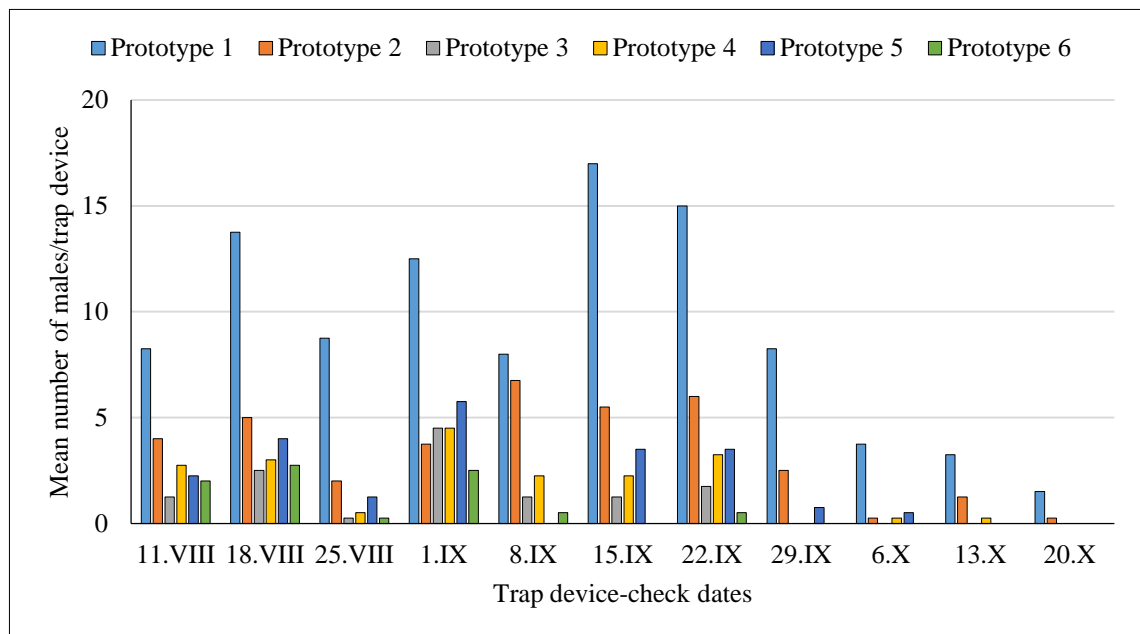


Figure 4.4 Mean number of *T. pityocampa* male adults captured in each trap device in Thessaly during the experimental period.

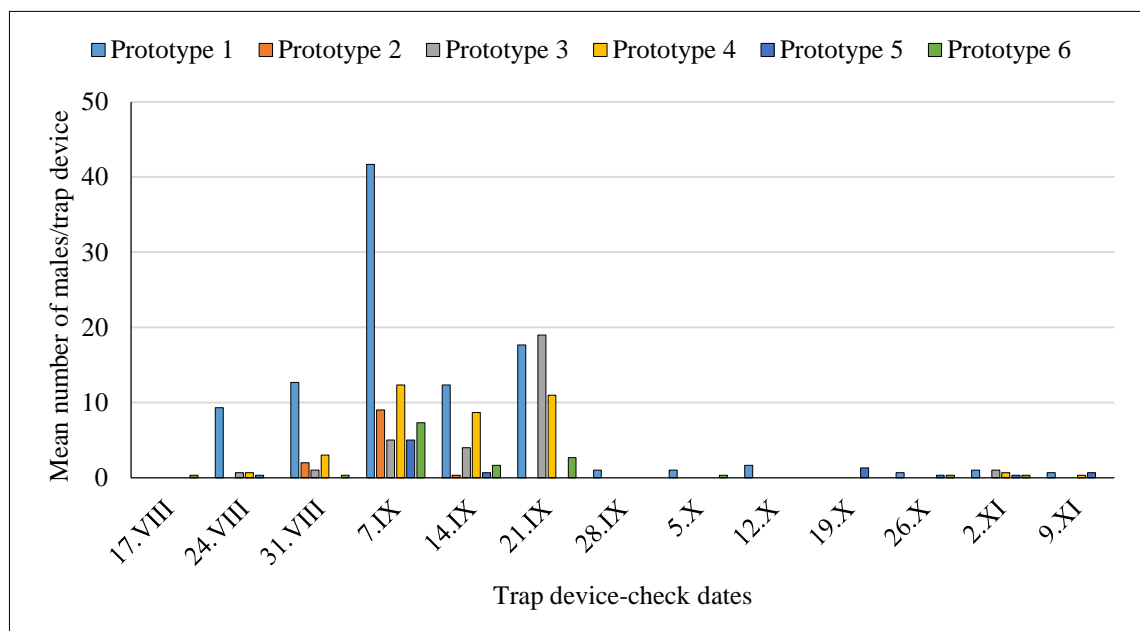


Figure 4.5 Mean number of *T. pityocampa* male adults captured in each trap device in Attica during the experimental period.

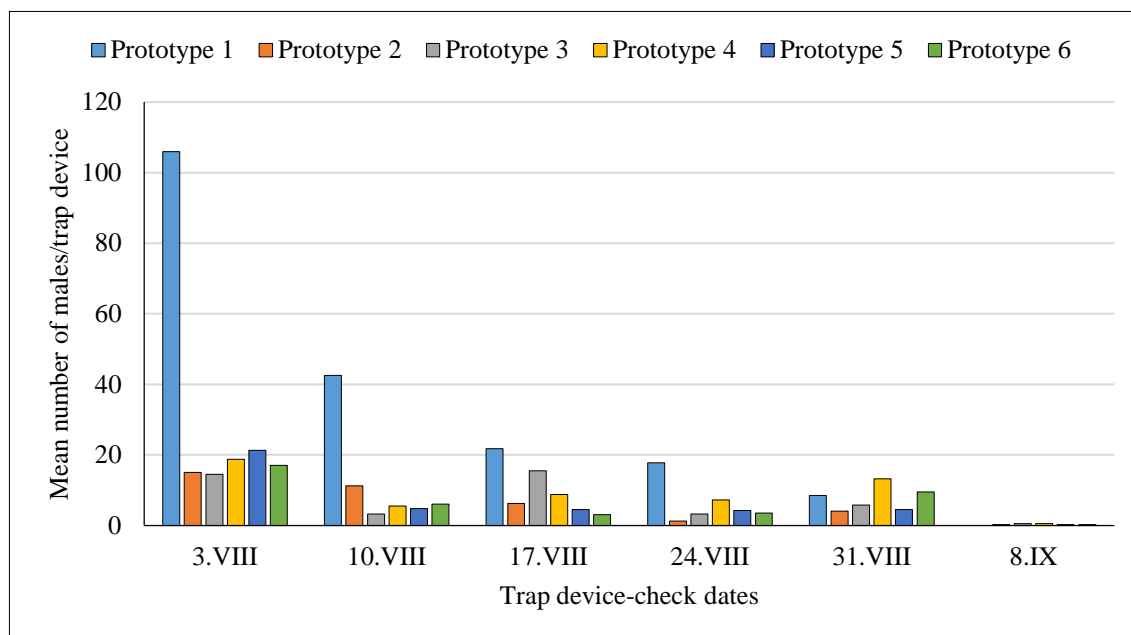


Figure 4.6 Mean number of *T. pityocampa* male adults captured in each trap device in Molise during the experimental period.

4.3.3 Molise

A total of 1640 male adults were captured in the trap devices. In Molise, the flight of *T. pityocampa* male adults started in early August and lasted until the first week of September (Fig. 4.6). The highest number of male adults was recorded during early August. Trap device and date were significant (Table 4.1). Significantly more male adults were captured in Prototype 1 than in the other trap devices (Table 4.2). The correlation coefficient values for the pairs of trap devices were positive and significant with the exception of two pairs (Prototype 2 - Prototype 3 and Prototype 3 - Prototype 6) (Table 4.3). Significant differences were noted among trap devices in three out of the six trap device-check dates (Table 4.4).

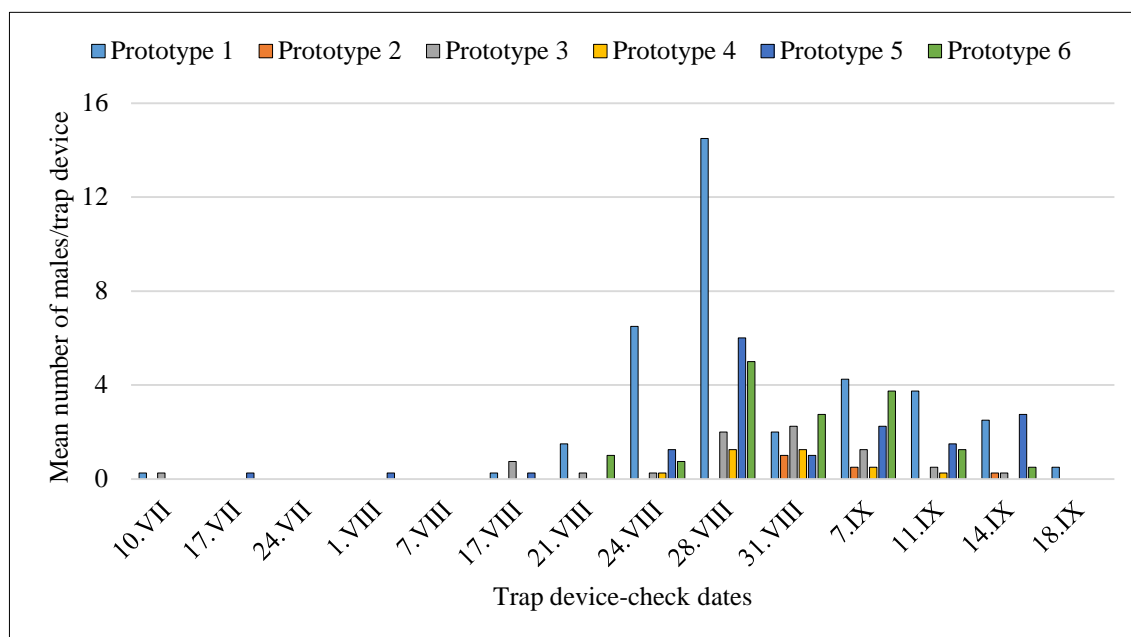


Figure 4.7 Mean number of *T. pityocampa* male adults captured in each trap device in Valencia during the experimental period.

4.3.4 Valencia

A total of 322 male adults were captured in the trap devices during the entire monitoring period. In Valencia, the flight of *T. pityocampa* male adults started in early July and lasted until mid September, but the vast majority of male adults were captured between late August and early September (Fig. 4.7). The highest number of male adults was recorded during late August, while captures in the previous weeks were negligible. Trap device and date were significant (Table 4.1). Significantly more male adults were captured in the Prototype 1 than in Prototype 2, Prototype 3 and Prototype 4 (Table 4.2). The correlation coefficient values for the pairs of trap devices were positive and significant for twelve pairs but not for the Prototype 1 - Prototype 4, Prototype 2 - Prototype 4, Prototype 2 - Prototype 5) (Table 4.3). Significant differences were noted among trap devices in three out of the fourteen trap device-check dates (Table 4.4).

Table 4.1 ANOVA parameters for main effects and their interaction of catches of *T. pityocampa* male adults in trap devices for the experimental areas during the monitoring period.

| Area | Thessaly | | | Attica | | | Molise | | | Valencia | | |
|--------------------|----------|----------|----------|--------|----------|----------|--------|----------|----------|----------|----------|----------|
| Source | df | <i>F</i> | <i>P</i> | df | <i>F</i> | <i>P</i> | df | <i>F</i> | <i>P</i> | df | <i>F</i> | <i>P</i> |
| Trap device | 5 | 35.1 | <0.01 | 5 | 30.2 | <0.01 | 5 | 7.5 | <0.01 | 5 | 8.3 | <0.01 |
| Date | 10 | 15.4 | <0.01 | 12 | 45.3 | <0.01 | 5 | 27.4 | <0.01 | 13 | 9.9 | <0.01 |
| Trap device x date | 50 | 1.1 | 0.27 | 60 | 3.8 | <0.01 | 25 | 1.5 | 0.08 | 65 | 1.1 | 0.23 |

For Thessaly total df = 263, for Attica total df = 233, for Molise total df = 143, for Valencia, total df = 335; HSD test at 0.05.

Table 4.2 Mean number (\pm SE) of *T. pityocampa* male adults captured in each trap device in the four experimental areas during the monitoring period.

| Trap device/ area | Thessaly | Attica | Molise | Valencia |
|-------------------|------------------|-----------------|-----------------|------------------|
| Prototype 1 | 0.8 \pm 0.1 a | 0.5 \pm 0.1 a | 1.1 \pm 0.1 a | 0.2 \pm 0.1 a |
| Prototype 2 | 0.5 \pm 0.1 b | 0.1 \pm 0.3 b | 0.6 \pm 0.1 b | 0.1 \pm 0.1 c |
| Prototype 3 | 0.2 \pm 0.1 c | 0.3 \pm 0.1 b | 0.7 \pm 0.1 b | 0.1 \pm 0.1 bc |
| Prototype 4 | 0.3 \pm 0.1 bc | 0.3 \pm 0.1 b | 0.7 \pm 0.1 b | 0.1 \pm 0.1 bc |
| Prototype 5 | 0.3 \pm 0.1 bc | 0.1 \pm 0.1 b | 0.7 \pm 0.1 b | 0.2 \pm 0.1 ab |
| Prototype 6 | 0.2 \pm 0.1 c | 0.2 \pm 0.1 b | 0.7 \pm 0.1 b | 0.2 \pm 0.1 ab |
| <i>F</i> | 22.2 | 7.4 | 3.7 | 6.1 |
| <i>P</i> | <0.01 | <0.01 | 0.01 | <0.01 |

Within each column, means followed by the same letter are not significantly different, for Thessaly df = 5, 263, for Attica df = 5, 233, for Molise df = 5, 143, for Valencia df = 5, 383; HSD test at 0.05.

Table 4.3 *Correlation coefficient values for captures between pairs of different trap devices during the monitoring period.*

| Pair of trap devices/ area | Thessaly | <i>P</i> | Attica | <i>P</i> | Molise | <i>P</i> | Valencia | <i>P</i> |
|----------------------------|----------|----------|--------|----------|--------|----------|----------|----------|
| Prototype 1 - Prototype 2 | 0.41* | 0.01 | 0.60* | <0.01 | 0.68* | 0.01 | 0.26* | 0.04 |
| Prototype 1 - Prototype 3 | 0.21 | 0.17 | 0.66* | <0.01 | 0.49* | 0.02 | 0.34* | 0.01 |
| Prototype 1 - Prototype 4 | 0.48* | 0.01 | 0.77* | <0.01 | 0.58* | 0.01 | - 0.01 | 0.93 |
| Prototype 1 - Prototype 5 | 0.48* | 0.01 | 0.43* | 0.01 | 0.75* | <0.01 | 0.34* | 0.01 |
| Prototype 1 - Prototype 6 | 0.27 | 0.08 | 0.64* | <0.01 | 0.63* | 0.01 | 0.41* | 0.01 |
| Prototype 2 - Prototype 3 | 0.34* | 0.02 | 0.24 | 0.14 | 0.33 | 0.12 | 0.47* | <0.01 |
| Prototype 2 - Prototype 4 | 0.49* | 0.01 | 0.60* | <0.01 | 0.67* | 0.01 | 0.06 | 0.65 |
| Prototype 2 - Prototype 5 | 0.50* | 0.01 | 0.63* | <0.01 | 0.57* | 0.01 | 0.08 | 0.55 |
| Prototype 2 - Prototype 6 | 0.39* | 0.01 | 0.48* | 0.01 | 0.46* | 0.01 | 0.43* | 0.01 |
| Prototype 3 - Prototype 4 | 0.66* | <0.01 | 0.76* | <0.01 | 0.40* | 0.05 | 0.44* | 0.01 |
| Prototype 3 - Prototype 5 | 0.58* | <0.01 | 0.28 | 0.09 | 0.52* | 0.01 | 0.36* | 0.01 |
| Prototype 3 - Prototype 6 | 0.50* | 0.01 | 0.77* | <0.01 | 0.35 | 0.10 | 0.55* | <0.01 |
| Prototype 4 - Prototype 5 | 0.67* | <0.01 | 0.41* | 0.01 | 0.55* | 0.01 | 0.33* | 0.01 |
| Prototype 4 - Prototype 6 | 0.63* | <0.01 | 0.70* | <0.01 | 0.47* | 0.02 | 0.36* | 0.01 |
| Prototype 5 - Prototype 6 | 0.46* | 0.01 | 0.54* | 0.01 | 0.76* | <0.01 | 0.42* | 0.01 |

An asterisk declares that value is significantly different from 0, for Thessaly df = 42, for Attica df Attica = 37, for Molise df = 22, for Valencia df = 62; two-tailed *t*-test at 0.05.

Table 4.4 ANOVA parameters for different dates of catches of *T. pityocampa* male adults in the trap devices for the experimental areas.

| Thessaly | | | Attica | | | Molise | | | Valencia | | |
|----------|-------|-------|--------|------|-------|--------|-----|------|----------|-----|------|
| Date | F | P | Date | F | P | Date | F | P | Date | F | P |
| 8/11 | 0.3 | 0.90 | 8/17 | 1.0 | 0.46 | 8/3 | 3.1 | 0.03 | 7/10 | 0.8 | 0.56 |
| 8/18 | 5.2 | 0.01 | 8/28 | 17.8 | <0.01 | 8/10 | 3.6 | 0.02 | 7/17 | 1.0 | 0.45 |
| 8/25 | 2.9 | 0.04 | 8/31 | 4.0 | 0.02 | 8/17 | 2.5 | 0.07 | 7/24 | - | - |
| 9/1 | 1.0 | 0.47 | 9/7 | 5.4 | 0.01 | 8/24 | 4.9 | 0.01 | 8/1 | 1.0 | 0.45 |
| 9/8 | 3.1 | 0.04 | 9/14 | 4.5 | 0.02 | 8/31 | 0.4 | 0.84 | 8/7 | - | - |
| 9/15 | 7.4 | 0.01 | 9/21 | 22.4 | <0.01 | 9/7 | 0.6 | 0.70 | 8/17 | 1.4 | 0.27 |
| 9/22 | 17.2 | <0.01 | 9/28 | 3.5 | 0.04 | | | | 8/21 | 6.8 | 0.01 |
| 9/29 | 123.8 | <0.01 | 10/5 | 2.3 | 0.11 | | | | 8/24 | 1.7 | 0.20 |
| 10/6 | 10.3 | <0.01 | 12/10 | 2.7 | 0.07 | | | | 8/28 | 1.8 | 0.17 |
| 10/13 | 5.2 | 0.01 | 10/19 | 1.0 | 0.46 | | | | 8/31 | 0.4 | 0.86 |
| 10/20 | 5.0 | 0.01 | 10/26 | 1.3 | 0.32 | | | | 9/7 | 0.7 | 0.63 |
| | | | 11/2 | 0.7 | 0.61 | | | | 9/11 | 2.8 | 0.05 |
| | | | 11/9 | 1.1 | 0.43 | | | | 9/14 | 4.1 | 0.01 |
| | | | | | | | | | 9/18 | 1.0 | 0.45 |

For Thessaly, Molise and Valencia df = 5, 23, for Attica df = 5, 17; HSD test at 0.05.

4.4 DISCUSSION

Despite the fact that there are some data available referring to the trapping of *T. pityocampa* male adults, there are very few studies regarding the influence of trap device on the capture of this species, which reflects the perception that its phenology is still poorly understood. Jactel *et al.* (2006) reported that Funnel trap devices were much less effective than plate sticky trap devices on the capture of *T. pityocampa* male adult individuals in 1999 in France and 2003 in France and Portugal. In an earlier work from Greece, which was actually carried out in the same area with the current study (Goritsa), Athanassiou *et al.* (2007), noted that the adhesive trap devices Delta and Pherocon II performed better than Funnel on the capture of *T. pityocampa* male adults, during experiments that were carried out in summer and fall of 2002 and 2003. In this regard, adhesive trap devices may have some advantages over the use of trap devices that have no adhesive surface, such as Funnel, in terms of detection sensitivity. However, in the case of *T. pityocampa*, there may be some certain drawbacks in the use of sticky Delta over the use of Funnel trap devices. For instance, male adults of this species have large bodies, fact that means that the sticky surface is quickly saturated during catches (Jactel *et al.*, 2006). As a result, additional male adults that approach these trap devices are not eventually captured. On the other hand, Funnel traps are considered “high capacity” trap devices and can serve for this purpose (Martin *et al.*, 2012). Athanassiou *et al.* (2007) used the organophosphorus insecticide dichlorvos (as a solid formulation) inside the funnels as a killing agent, which might had a repulsive activity of the adults that were approaching the trap devices, as has been noted for other species (Manoukis, 2016). In our experiments we used funnel-like trap devices, since the insects are captured in the trap devices with a similar mechanism to Funnel, i.e., by entering from openings at the upper part of the devices and moving downwards. Based on the results from all areas, Prototype 1 was proved superior to the other five trap

devices used. This was evident since the number of male adults that was recorded in Prototype 1 was 2 to 12 times higher than those recorded in the other trap devices. The highest number of male adults captured in all experimental areas was due to captures in this trap device, which reflected the more clear difference in capture capacity between Prototype 1 and the other trap devices. Hence, in Valencia (Spain), where captures were relatively low, considering the overall data, there were no significant differences between the captures of Prototype 1 and Prototype 5 or Prototype 6 trap devices. Conversely, in Molise, where the highest numbers of male adults were recorded, Prototype 1 was clearly more effective on the capture of *T. pityocampa* male adults than all the other trap devices.

Apparently, by considering a number of previous studies, it becomes evident that *T. pityocampa* has different patterns of male adult catches among different areas (Devkota *et al.*, 1992; Zhang and Paiva, 1998; Berardi *et al.*, 2015), a fact that it is also evident from the findings of the present study. In Thessaly and Attica, the male adult catches lasted considerably longer than the other areas tested. This is important since the accurate assessment of the seasonal abundance of the male adults is informative in order to time control measures that are friendly to the environment, i.e., mass trapping and mating disruption for reducing mating process (Martin, 2015). Furthermore, an accurate monitoring system of *T. pityocampa* male adult population will also provide valuable information for the exact application of the rapid degradable Btk based microbial insecticides against larvae (Martin, 2015). In Thessaly, the highest number of captures was recorded during September, and male adult catches continued with relatively high captures until October. Similar results have also been reported by Athanassiou *et al.* (2007) for the same area. In contrast, in Molise (Italy) the period of male adult catches was extremely short, while most of the male adults were captured considerably earlier, i.e., during August. Previous preliminary observations of the authors for these areas also confirm these male adult catches

patterns. The different patterns may indicate the influence of local environments and habitats (Bonsignore and Manti, 2013) but also the influence of other factors. In Mediterranean, *T. pityocampa* [Iberian Peninsula, France, Balkan Peninsula without the island Crete (Greece), a part of Turkey, Morocco and part of Algeria] occurs with the eastern pine processionary moth, *Thaumetopoea wilkinsoni* Tams (Lepidoptera: Thaumetopoeidae) (Cyprus, Israel, Lebanon, part of Turkey) and the clade provisionally named *Thaumetopoea pityocampa* ENA (part of Algeria, Tunisia, Libya), forming a species complex, with distinct geographical distribution (Kerdelhué *et al.*, 2009, 2015; Simonato *et al.*, 2013). However, in a recent study, Avtzis *et al.* (2016) found that the ENA clade is present in Attica (Greece) probably introduced by Libya. At the same time, *T. pityocampa* is expanding to the north of Europe (Robinet *et al.*, 2012). Members of the complex can be separated with molecular methods since any identification based on morphological characters is extremely difficult (Frérot and Démolin, 1993; Kerdelhué *et al.*, 2009, 2015; Simonato *et al.*, 2013). Still, the adult male flight period of *T. wilkinsoni* is poorly understood given that both *T. pityocampa* and *T. wilkinsoni* respond to the same pheromone (Frérot and Démolin, 1993). Hence, the long period of male adult catches for Thessaly and Attica, along with the peak records which were recorded later than the other areas, may not be attributed to the presence of *T. pityocampa* *s.str.* necessarily but to a mixed population (Avtzis *et al.*, 2016). In the case of Attica, Avtzis *et al.* (2016) assumed that the port of Piraeus, which is the largest passenger and mercantile port of Greece, could play a role for the introduction of ENA clade haplotypes in this area. Similarly, the proximate to Goritsa large passenger and mercantile port of Volos could be a similar nodal insect source.

Capture capacity consists one of the most important elements of a given trap device. However, apart from the capture capacity *per se*, which is expressed as the number of captured individuals in a given period of time, the captures should be representative of the actual changes of population densities in the test

area. For example, Athanassiou *et al.* (2007) also found that the male adult catches of *T. pityocampa* ended 5 - 6 weeks earlier when Funnel trap devices were compared to Delta or Pherocon II trap devices, suggesting that some devices may not be good indicators on reflecting seasonal fluctuation and may underestimate the presence of male adults. Based on our results, the correlation of the captures between pairs of trap devices was fair in most of the cases examined, but in some cases there were asynchronies arising mostly by the low number of male adults that were captured in one or in both trap devices of a given pair. If the estimation of the peak period is one of the most important questions that need to be answered by the use of a trapping protocol, then any asynchronies may lead to wrong conclusions and, concomitantly, to ineffective measures. For the cotton pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), Athanassiou *et al.* (2002) noted that the peak of the male adult catches, based on the Funnel trap devices, was much later than the one that was given by two adhesive trap devices, a fact that was very likely to affect trap-oriented chemical control. In this regard, trapping should be combined with additional sampling of the other life stages of *T. pityocampa*, in order to have a holistic and accurate view of its phenology.

To our knowledge, this is the first study that examined in parallel the influence of numerous novel trap devices on the capture of male adults of *T. pityocampa* in the extensive area of southern Europe. Our findings suggest that Prototype 1 was found to be the most effective trap device for the capture of *T. pityocampa* male adults, and therefore it should be selected for the monitoring of this species. However, it is not significantly more sensitive to low population densities than Prototypes 5 and 6. The development of novel pheromone trap devices that have high capture capacity is important since they can be used for the mass trapping of male adult individuals of *T. pityocampa* which is a potential method for the control of this noxious species (Martin, 2015). At the same time, our study revealed a different pattern and male adult flight duration of *T.*

pityocampa, which may indicate the simultaneous presence of different members of the *Thaumetopoea* species complex. Additional experimental work is needed towards these directions.

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ADDENDUM

EVALUATION OF PHEROMONE TRAP DEVICES IN 2016

The experiment of pheromone trap comparison, during the summer 2016, was carried out in a coastal touristic pinewood near Petacciato (Province of Campobasso) that is mainly composed of *Pinus halepensis*.

Five different prototype pheromone traps [Prototype 1 (the most effective trap of the 2015 experiment), Prototype 7, Prototype 8, Prototype 9, Prototype 10] were used (Fig. 4.6). The traps, baited with a pheromone dispenser [(Z)-13-hexadecen-11ynyl acetate], were placed inside the canopy 4-5 m above the ground and around 100 m from each other. Checks were carried out twice a week.



Figure 4.6 Funnel trap devices tested in 2016: Prototype 1 (A), Prototype 7 (B), Prototype 8 (C), Prototype 9 (D), Prototype 10 (E).

For statistical purposes, 4 repetitions were performed and for each control, the traps were rotated clockwise, so as to reduce the influence of the position. The traps were installed on July 7 and were retired on 22 September, when there was the end of pine processionary moths flight period.

The data was analysed by using a two-way ANOVA, with trap and date as main effects. Means were separated by the Tukey-Kramer (HSD) test at $p < 0.05$. Before the analysis, counts were transformed to natural log ($x + 1$), to normalize variances and standardize means.

In total, 2817 male adults of pine processionary moth were captured in the traps. The flight of *T. pityocampa* males started in the second half of July and lasted until the first week of September (Fig. 4.7). The highest number of male adults was recorded in early August. Significantly more male adults were captured in Prototype 7 (with a total of 1671 male adults) than in the other traps ($F = 84.9$, $df = 4$, $P < 0.01$).

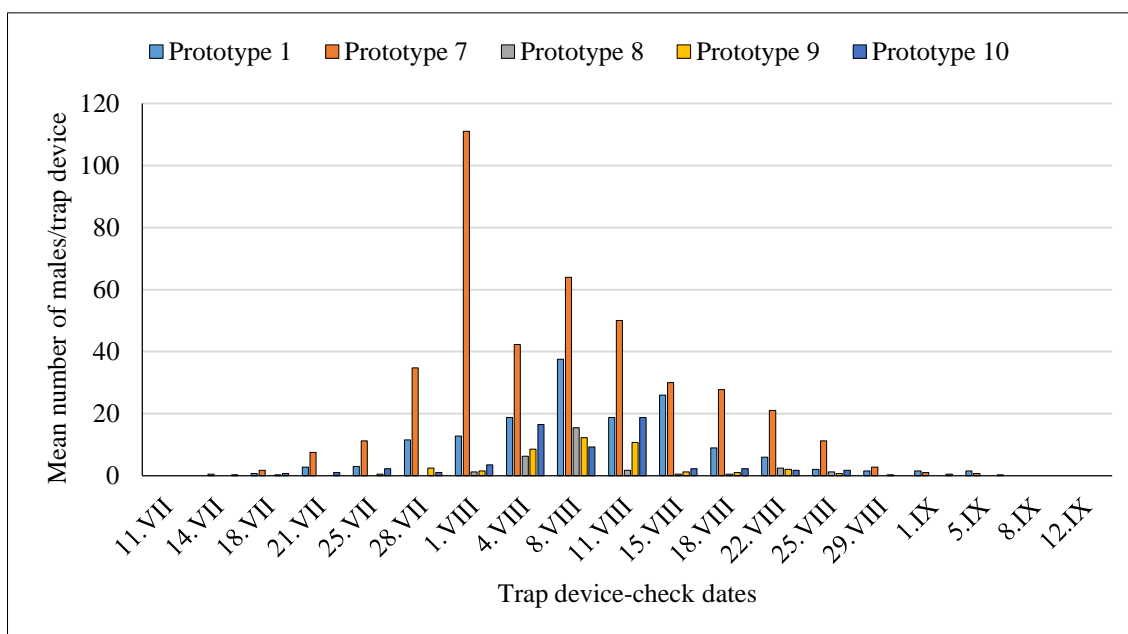


Figure 4.7 Mean number of *T. pityocampa* male adults captured in each trap device in Molise during the 2016 experimental period.

CHAPTER 5

MANAGEMENT OF *T. PITYOCAMPA* INFESTATION USING MATING DISRUPTION TECHNIQUE



5.1 INTRODUCTION

General information on the pine procession moth is reported in Chapter 1. For the management of *T. pityocampa* infestation, the present chapter reports the results obtained by the use of pheromones as mating disruption (MD) technique in a recreational area in central Italy. Activity of MD was verified for 3 years (2015, 2016, 2017), controlling variation of *T. pityocampa* male adults in the area and number of winter nests on infested trees around traps.

5.2 MATERIALS AND METHODS

5.2.1 Experimental sites

Pest management of *T. pityocampa* was carried out in a recreational area located in the area of Marinelle, Petacciato, province of Campobasso (Molise region, south-central Italy) (Fig. 5.1). During 2015 and 2016 in the study area 2 plots composed of 1 hectare each were identified. In 1 plot, pheromones were applied for MD of *T. pityocampa* males. No MD pheromone was applied to the other plot as a control.

Approximately 600 trees of *Pinus halepensis* infested by the pine processionary moth were present in the MD experimental plot. Another plot covered by approximately 600 trees of *P. halepensis* close to the experimental MD area was used as a control area.

5.2.2 MD application

One plot was treated with the formulated pheromone (Z)-13-hexadecen-11-ynyl acetate in paste (commercially available by ThauPi-polymix, NovAgrica, Greece) that it was positioned in small amounts (drops) on the trunk or on branches at a height of about 4-5 metres from ground, reaching a concentration

of 20 g/ha of the active ingredient (Fig. 5.2). The other plot was not treated with MD and was used as control.

In 2015, the pheromone in paste was applied on 27 July at the first flights of the *T. pityocampa* adults; in 2016 the pheromone was applied on 21 July.

5.2.3 Pheromone monitoring traps

For the monitoring of *T. pityocampa* adult populations, 4 pheromone traps of the G-trap model (SEDQ, Barcelona, Spain) were used. Two traps were positioned in the MD experimental plot and 2 traps were positioned in the control plot without MD.

The traps were baited with pheromone dispensers impregnated with (Z)-13-hexadecen-11-ynyl acetate (Kenogard, Barcelona, Spain) and placed on the canopy of trees at about 5-6 meters from the ground. The pheromone dispensers remained on the trees for 3 months.



Figure 5.1 Aerial view of the experimental site with highlight the plot with MD (in red), the plot without MD (in blue), the monitoring traps (from Google Earth)

In 2015, the monitoring pheromone traps were suspended in the test plot on 11 June and retired on 22 September; in 2016, the G-traps were installed on 7 July and removed on 22 September at the end of the *T. pityocampa* flight period.

In 2015 and 2016 the pheromone traps were checked every 3-4 days. During each inspection, the male adults that had been captured were recorded and removed from the trap devices.

5.2.4 Counting of winter nests

In the years 2016-2017, the winter nests realised by *T. pityocampa* wintering larvae on colonised pine trees of the experimental plots were visually counted. Counting of winter nests was performed on 12 trees positioned around each pheromone monitoring trap. In the MD-treated and untreated plots, a total of 48 trees were considered. Over the 2 years of the study (2016-2017), these activities were performed in January.

5.2.5 Data analysis

The statistical analysis on adult populations was performed using an independent samples t-test, where the independent variable was treatment (untreated area and area treated with MD).

Data on the number of recorded nests were analysed by using two-way analysis of variance (ANOVA) with treatment and year as main effects. Means were separated by the Tukey-Kramer (HSD) test at $p < 0.05$ (Sokal and Rohlf, 1995).

Before all analyses, counts were transformed to natural log ($x + 1$) to normalise variance and standardise means. All analyses were conducted using the SPSS software (SPSS Inc. 2009).



Figure 5.2 Application of pheromone paste on *Pinus* trees (A, B, and C), small amount (drops) of pheromone paste on branch (D).

5.3 RESULTS

In the territory of Marinelle in both 2015 and 2016, pheromone traps realised the first catches of the *T. pityocampa* adults during last the 2 weeks of July. The presence of males in traps lasted until the beginning of September, with a maximum presence during the second and third weeks of August (Figs 5.3-5.6).

During the trial performed in 2015, the pheromone traps for monitoring of *T. pityocampa* captured 402 adult moths: 49 individuals were collected in the plot treated with MD and 353 individuals were collected in the control untreated control plot. In the MD-treated plot, adults were collected during the third week of August. On the contrary, in the untrated control plot adult males were trapped from the last week of July till the end of August with a maximum presence on the last day of July and late August (Fig. 5.4).

During the trial performed in 2016, the G-traps for monitoring of *T. pityocampa* males captured 435 adult moths: 35 specimens were collected in the plot treated with MD and 400 were collected in the untreated control plot. In the MD-treated plot, adults were collected from the first to third week of August. Conversely, in the untrated control plot, adult males were trapped from the third week of July till the first week of September, with a maximum presence before middle of August (Fig. 5.6).

In 2015, data comparison between MD-treated plot and untreated control plot was significant. Statistical analysis was performed on 22 samples per plot with $t = -5.664$, $df = 42$ and for two-tailed t-test: $P < 0.01$. In 2016, data comparison between MD-treated plot and untreated control plot was significant. Statistical analysis was performed on 28 samples per plot with $t = -4.662$, $df = 54$ and a $P < 0.01$ for two-tailed t-test.

In 2016, the average number of nests per tree found in the MD-treated plot (0.12 nests/tree) was less than the same found in the untreated control plot (0.87 nests/tree). Similar results were found in 2017, with 0.04 nests/tree in the MD-

treated plot and 0.50 nests/tree in the untreated control plot. Statistical analysis of the number of winter nests per year is not significant ($F = 3.8$, $df = 1$, $P = 0.06$), but it is significant for the treatment ($F = 26.9$, $df = 1$, $P < 0.05$).

5.4 DISCUSSION

The *T. pityocampa* moth will be not a problem limited to the Mediterranean and northern African countries given that it has naturally expanded into higher altitudes and latitudes and has begun invading northern regions of Europe where it has not previously had the ability to develop (Battisti *et al.*, 2005; Buffo *et al.*, 2007; Robinet *et al.*, 2007, 2010, 2014). Battisti *et al.* (2011) reported that larvae of *T. pityocampa* developed continuously throughout the winter. Thus, the allergens that are contained in the setae of the *T. pityocampa* larvae consist of a serious danger to public health for both southern and northern European countries (Battisti *et al.*, 2011; Rodriguez-Mahillo *et al.*, 2012).

Insect control through the use of synthetic semiochemicals to disrupt normal mating behaviour is now operational for a number of species of Lepidoptera. Even though success has been demonstrated, failures also exist. Forest pests have been considered suitable targets for control by MD since the host range is invariably narrow and large forest plantations make area wide application possible. A major problem, with a few notable exceptions, has been the absence of suitable methods to assess the significance of the damage caused by the pests in anything other than outbreak situations. Migration of adults may be a problem in some species but little is known about the majority.

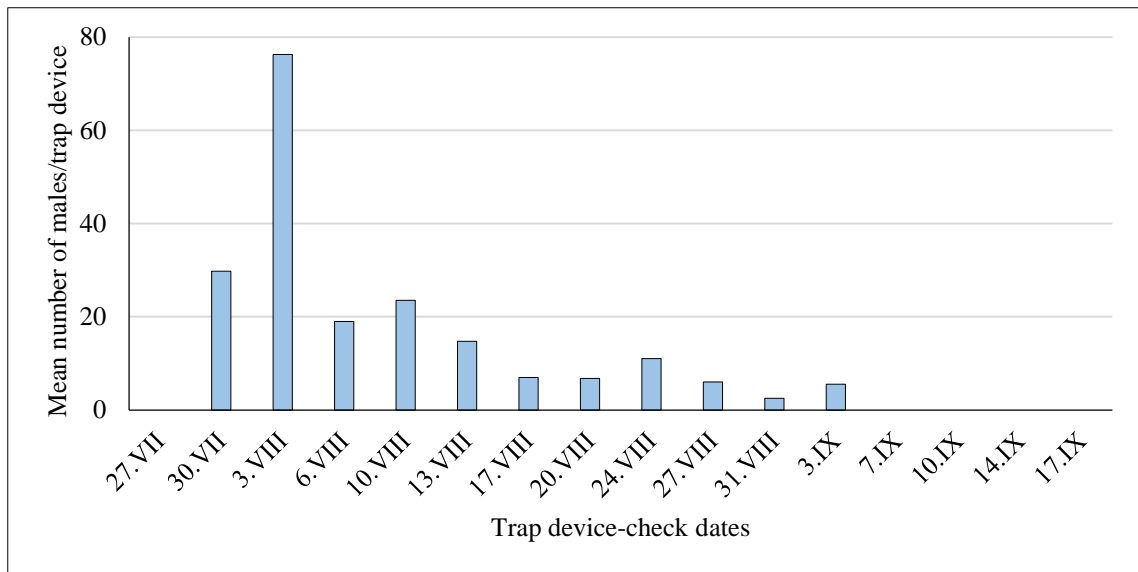


Figure 5.3 Mean number of *T. pityocampa* male adults captured in the Marinelle recreational area during 2015.

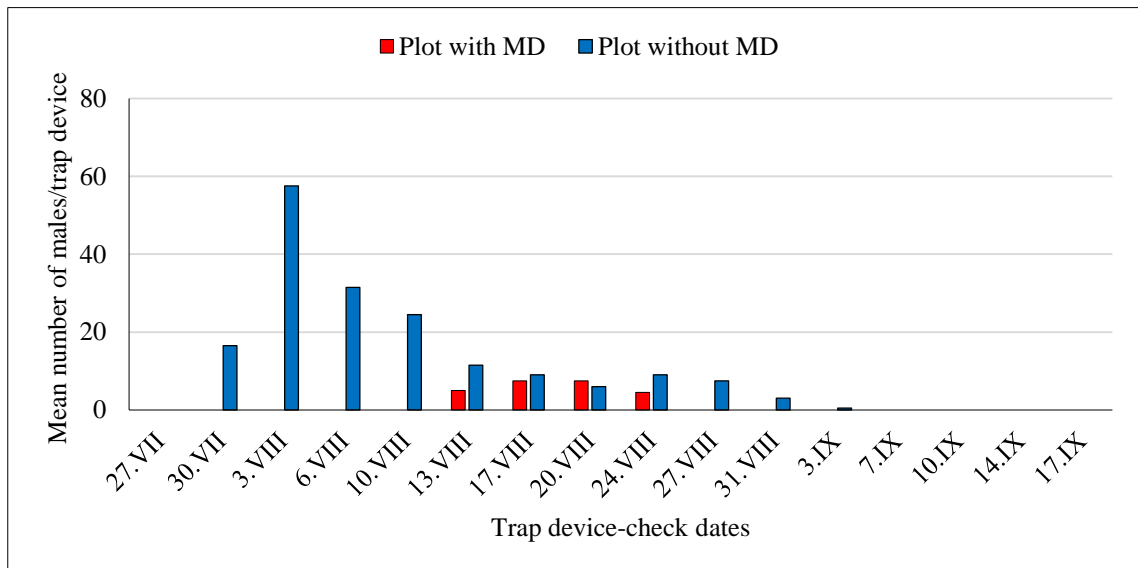


Figure 5.4 Mean number of *T. pityocampa* male adults captured in the experimental plots with MD or without MD during 2015.

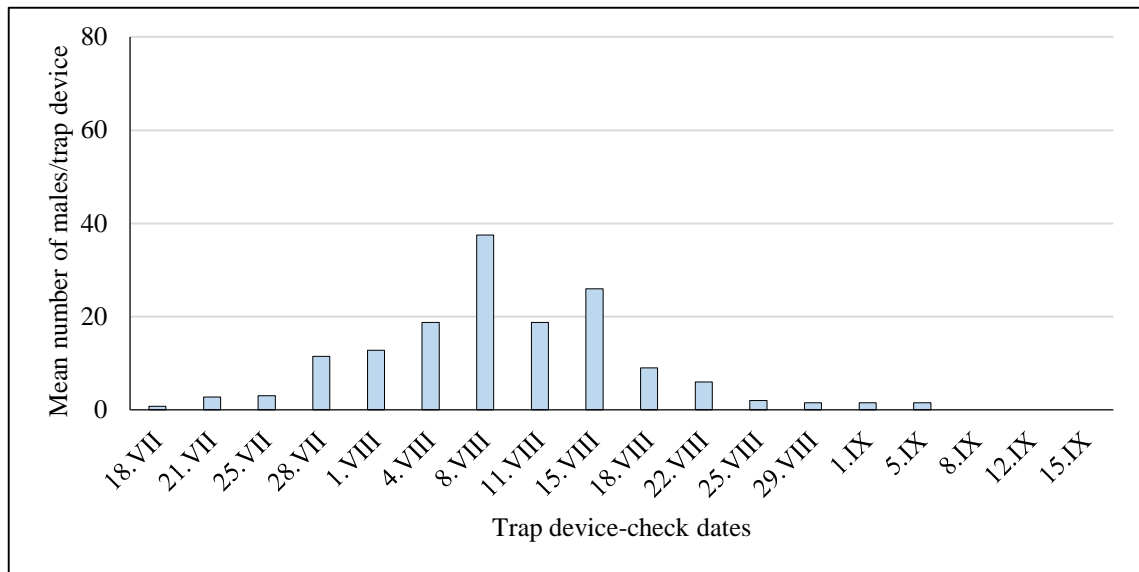


Figure 5.5 Mean number of *T. pityocampa* male adults captured in the Marinelle recreational area during 2016.

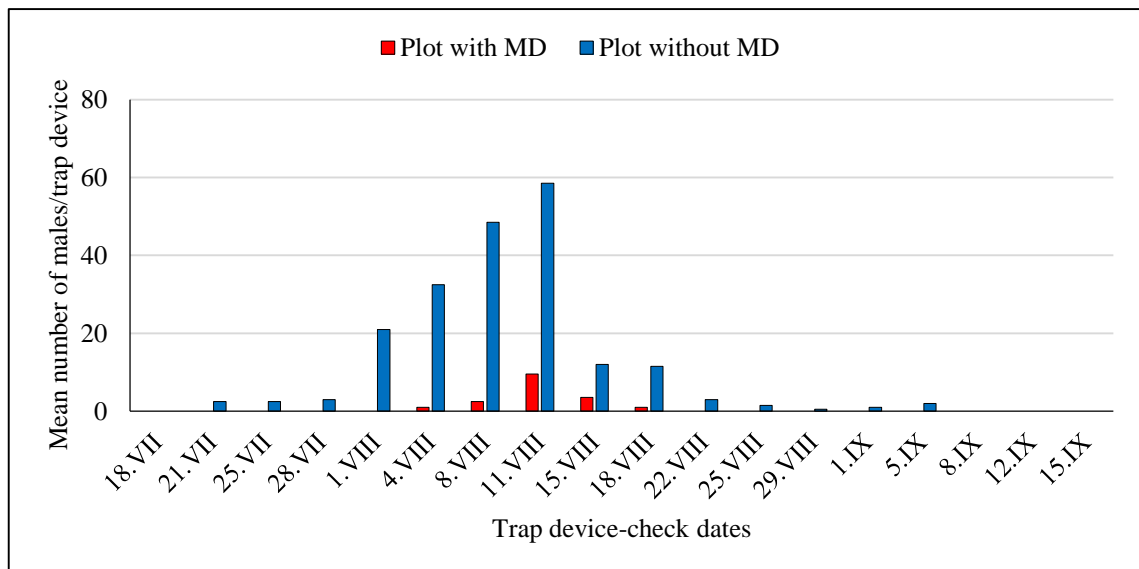


Figure 5.6 Mean number of *T. pityocampa* male adults captured in the experimental plots with MD or without MD during 2016.

Preliminary experiments on pheromone application and MD carried out against the pine processionary moth with promising results were reported by Halperin *et al.* (1985) and Baldassari *et al.* (1994). During 2004 and 2005, interesting data were also cited by Jactel *et al.* (2006) and Martin and Frérot (2006).

According to this experiment, MD is able to disturb the activity of a large quantity of *T. pityocampa* adults. This activity was evident both in the first and second experimental year (2015 and 2016) as evidenced by the decreased number of adults recorded in the monitoring pheromone traps and the decreased number of nests counted on the experimental trees.

In 2015, when comparing the adult population between the experimental plot with MD treatment and the untreated control plot, it was possible to note that the population revealed in the MD treated plot was lower than the population present in the untreated control plot (with 49 and 353 adults trapped, respectively). In 2015, the experiment utilising the MD technique, in the study area of Marinelle, was found to be as effective as the 2 traps that were placed in the plot treated with MD that captured only 12.19% of the total of adults during the monitoring time.

In 2016, the population density revealed in the MD treated experimental plot was lower than the population present in the untreated control plot (with 35 and 400 adults trapped respectively). In 2016, the experimentation of the MD technique was also found to be effective as the 2 traps that were placed in the plot treated with MD captured 8.05% of the total of adults during the monitoring time.

In the MD treated experimental plot comparing adult populations present between 2015 and 2016, it was possible to note a decrease in the presence of *T. pityocampa* adults during the second year of MD application (49 and 35 adults respectively). On the contrary, in the untreated control plot the population of *T. pityocampa* increased from 353 adults to 400 adults.

The effectiveness of the MD technique is also supported by the count of winter nests. In fact, in 2016, after 1 year of MD treatment, the number of nests in the plot treated with MD (0.12 nests/tree) was found to be less than the number of nests present in the plot not treated with MD (0.87 nests/tree). Similar results were also found in 2017 after 2 years of treatment.

MD is a particularly interesting control method that can be applied in public parks, recreational areas and public areas. Nevertheless, MD requires treating surfaces large enough to support air saturation of the sex pheromone. In the small city parks and recreational areas, the efficiency of this technique must be evaluated and adapted if necessary. It is useful to suggest that any curative methods used in this context should be repeated every year to ensure a long-term effect.

Users of the MD application should be aware that the method does not limit the possibility of re infestation in the following year in case there are female moths flying in from the surrounding areas.

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CONCLUSION AND FUTURE PERSPECTIVES

The pine processionary moth, *T. pityocampa* is considered one of the most destructive conifer pests of both natural and artificial pine forest of southern Europe and the Mediterranean basin (Zhang *et al.*, 2003). In addition to pine trees (*Pinus*) it can occasionally be found on other conifers as *Cedrus*, *Pseudotsuga* and *Larix*.

The pine processionary moth will not be a problem limited to the Mediterranean and northern African countries given that it has naturally expanded into higher altitudes and latitudes and is now invading the northern regions of Europe where it was not able to previously develop (Battisti *et al.*, 2005; Robinet *et al.*, 2010; Roques *et al.*, 2015)

The larvae ruin the needles and cause stress to the trees, which results in reduced photosynthesis and tree growth (Jacquet *et al.*, 2012). Furthermore, the third instar larvae develop urticating setae that cause irritation to humans and warm-blooded animals. Erythema is due to the mechanical tear of the skin caused by the penetration of the bristles and contact with substances of the affected organism; Rodriguez-Manillo *et al.* (2012) isolated 7 allergens that are injected with the puncture, including the primary allergen that could be associated with the taumetopoeine protein.

Setae can be transported by the wind at great distances. The risk for people and animals is very high. In rural infested areas of the Mediterranean region, about 12% of the population has reactions due to contact with the setae of pine processionary moth (Rodriguez-Manillo *et al.*, 2012). Also for this reason, in Italy exist a mandatory control measure under ministerial decree October 30, 2007, in order to prevent sanitary and health hazards for human and animal health.

The management of the pine processionary moth is especially needed in urban and semi-urban environments because of the risks related to the urticating caterpillars. The tolerance level is zero nests per tree when children are present, such as in the yards of kindergardens, schools and public parks. Similarly, it is very close to zero in the semi urban parks with high public frequentation (Martin, 2015).

Given that *T. pityocampa* is a univoltine species, monitoring of the male adult activity is essential, in order to time any control measures. Results of my studies suggest that Prototype 7 trap devices should be selected for this purpose.

It was known that trunk trap devices help in the collection of larvae (Martin *et al.*, 2012), but their impact on the overall population of the insect was unknown. My research showed that in urban areas, the capture of larvae with the trunk barrier trap devices could serve as a valuable, environmentally-friendly alternative method for *T. pityocampa* management, as trap devices are installed once and provide protection for years. In addition, a novel trunk trap device that is cheaper than commercial available traps and can be installed more quickly was introduced.

Additionally, MD as a control method is particularly interesting in that it can be applied in public parks, recreational areas and public areas. In small city parks and recreational areas, the efficiency of this technique must to evaluated and adapted if necessary.

In the future, a lot of attention will be given regarding the disclosure of control techniques to the stakeholders and end users of the area subjected to control. The expansion of the distribution area of the *T. pityocampa* will mean that areas with no present infestations will be affected and this will increase the number of people who may be affected by urtications. A continuous improvement of the monitoring and control techniques of the populations of *T. pityocampa* must be carried out and such techniques will have to be adapted to the environmental conditions of the new colonisation sites.

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LIST OF PAPERS (Scientific articles and Congress abstracts)

Scientific articles

- Trematerra P, **Colacci M** (2015) I Tortricidi del Friuli Venezia Giulia e della Slovenia nelle collezioni del Museo Friulano di Storia Naturale (Lepidoptera Tortricidae). *GORTANIA - Botanica, Zoologia*, **36** (2014): 69-86.
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