

Evaluation of Pheromone Trap Devices for the Capture of *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae) in Southern Europe

Christos G. Athanassiou,¹ Nickolas G. Kavallieratos,^{2,3,4} David Pardo,⁵ José Sancho,⁵ Marco Colacci,⁶ Maria C. Boukouvala,^{2,3,7} Anastassia J. Nikolaidou,¹ Demetrius C. Kondodimas,³ Enrique Benavent-Fernández,⁸ Santiago Gálvez-Settier,⁸ and Pasquale Trematerra⁶

¹Laboratory of Entomology and Agricultural Zoology, Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Phytokou str., Nea Ionia, Magnissia 38446, Greece (athanassiou@agr.uth.gr; nstacey.789@gmail.com),

²Laboratory of Agricultural Zoology and Entomology, Department of Crop Science, Agricultural University of Athens, 75 Iera Odos str., Athens, Attica 11855, Greece (nick_kaval@hotmail.com; bouk@hotmail.gr), ³Laboratory of Agricultural Entomology, Department of Entomology and Agricultural Zoology, Benaki Phytopathological Institute, 8 Stefanou Delta str., Kifissia, Attica 14561, Greece (d.kontodimas@bpi.gr), ⁴Corresponding author, email: nick_kaval@hotmail.com, ⁵SANSAN PRODESING S.L., Pol. Industrial Los Vientos, C/Virazón 1 CD 1506, Náquera, Valencia 46119, Spain (tecnico@sansan.es; jose.sancho@sansan.es),

⁶Department of Agricultural Environmental and Food Sciences, University of Molise, Via De Sanctis, Campobasso 86100, Italy (marco.colacci@unimol.it; trema@unimol.it), ⁷Laboratory of Organic Chemistry, Department of Chemistry, University of Ioannina, Panepistimioupolis, Ioannina 45110, Greece, and ⁸AIMPLAS, Plastics Technology Centre, València Parc Tecnològic, Gustave Eiffel, 4, Paterna, Valencia 46980, Spain (ebenavent@aimplas.es; sgalvez@aimplas.es)

Subject Editor: John Trumble

Received 24 December 2016; Editorial decision 12 January 2017

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 and 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 with 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 device, monitoring, improved trapping, male adult catch

Thaumetopoea pityocampa (Denis and Schiffermüller) (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 (Jacktel et al. 2006). This is particularly important in the case of pine trees located in urban and suburban 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 respiratory 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 *Thaumetopoea pityocampa*-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 (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 (Jacktel et al. 2006). Furthermore, monitoring of larvae of *T. pityocampa* is not easy owing 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, Jacktel 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 nonadhesive ones. Similar results on the effectiveness of adhesive versus nonadhesive pheromone-baited traps have also been reported by Athanassiou et al. (2007) from one area of central Greece. Jacktel 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 (Jacktel 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).

Materials and Methods

Experimental Sites

The experiments were carried out in four different sites, two in Greece (Thessaly and 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 ~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, whereas 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 ~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, whereas 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 ~35 ha of pines, with 650 trees per ha, which in majority are *P. halepensis* with few *Pinus pinea* L. (Pinales: Pinaceae). The climate is warm and temperate. The average minimum temperature was 6.8°C, whereas 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 ~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, whereas 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* in the previous years.

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 consists of the dark brown plastic rectangular parallel-epiped body (24.1 cm in length, 10 cm in height, and 10 cm in width); one elastic band; one insect reservoir (plastic bag; 29.5 cm in length and 41.5 cm in height), of which the upper part (29.5 cm in length and 19.5 cm in height) is transparent and the lower part (29.5 cm in length and 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 and 4 cm in slant height), bearing peripherally eight rectangular parallelogram openings (0.4 cm in length and 2.4 cm in height), with a lid at the large base; and two nylon cords that the trap device is hanged from (Fig. 1a–c). 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 (Fig. 1a and c). 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 (Fig. 1a and b). 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 and 7 cm in slant height each), having the directions of their cut vertexes to the center of the body (Fig. 1d). The small base of each truncated pyramid is permanently attached to a cube (2 cm in edge) that is diametrically opened from both sides (Fig. 1d). The body is also opened at the bottom (Fig. 1e). A prominent dark brown plastic ellipsoid ring (22.3 cm in large diameter, 8.7 cm in small diameter and 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 and 2.5 cm in slant height; Fig. 1e). The bag is attached to the body by the elastic band that surrounds the ellipsoid ring (Fig. 1c). 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 consists of two half light brown plastic bodies (18 cm in length, 18.5 cm in maximum height and 14.5 cm in maximum width each), providing a spindle type construction; one insect reservoir (semitransparent plastic bag; 26.5 cm in length and 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 (Fig. 1f and g). Each half body bears at the maximum periphery two cylindrical projections (0.5 cm in diameter and 0.7 cm in high; Fig. 1f) and two small holes (0.8 cm in diameter), forming a conceivable rectangular parallelogram (11 cm in length and 9.5 cm in height). The two half bodies join each other by entering the projections inside holes and form the joint body (Fig. 1g). 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 (Fig. 1g). The pheromone container is fixed centrally on the top, inside a hole (2.4 cm in diameter) of the joint body (Fig. 1g). 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 and 9 cm in slant height each), having the directions of their cut vertexes to the center of the body (Fig. 1h and i). The small base of each truncated cone is permanently attached to a cylinder (2 cm in diameter and 1.8 cm in height) that is opened from both sides (Fig. 1i). A prominent rectangular parallelepiped (5.5 cm in height, 20 cm in length and 2.5 cm in width) is formed at the bottom of the joint body (Fig. 1j). The parallelepiped is opened at the top and bottom forming a new internal rectangular parallelepiped (2.5 cm in height, 24 cm in length and 2.5 cm in width; Fig. 1j). The bag is attached to the external parallelepiped of the joint body with the elastic band (Fig. 1g). 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 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 and 9.3 cm 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 and 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 and 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 and 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 (Fig. 1k and l). 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 (Fig. 1l). 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 (Fig. 1k). The pheromone container is fixed centrally on the top of the disk inside a hole (2.4 cm in diameter; Fig. 1k). 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 and 7 cm in slant height; Fig. 1k). The moths can enter the body through its opening and finally are trapped inside the container.

Prototype 4 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 (Fig. 1m). Thus, Prototype 4 holds two pheromone lures.

Prototype 5 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 liter capacity); one dark brown plastic body that is composed by two opened anterolateral rectangular parallelepipeds (2.7 cm in height, 17.5 cm in length and 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 (Fig. 1n–p). 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 (Fig. 1o). 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 and 2 cm in slant height) and a cylinder (4.5 cm in diameter and 2.2 cm in height) that is opened from both sides (Fig. 1p). The body, with the disc, is screwed upon the container (Fig. 1p). The moths can enter the body through its opening and finally are trapped inside the container.

Prototype 6 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 and 3.2 cm in slant height) and a cylinder (4.5 cm in diameter and 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 (Fig. 1q–s). The trap device is hanged as in the case of Prototype 3. The body bears three small holes (as in Prototype 3; Fig. 1r). The cover joins the body as in Prototype 3 (Fig. 1s). The joint body and cover are screwed upon the container (Fig. 1s). The moths can enter the body through its opening and finally are trapped inside the container.

All trap devices were baited with lures containing 1 mg of the sex pheromone component (*Z*)-13-hexadecen-11-ynyl acetate (Trécé



Fig. 1. Trap devices that were tested and their parts: (A) Prototype 1 in parts, (B) Pheromone container, (C) Prototype 1 fully composed, (D) Lateral aspect of Prototype 1, (E) Basal part of Prototype 1, (F) Prototype 2 in parts, (G) Basal part of Prototype 2, (H) Lateral aspect of the Prototype 2 half part body (external part), (I) Lateral aspect of the Prototype 2 half part body (internal part), (J) Prototype 2 fully composed, (K) Prototype 3 in parts, (L) Prototype 3 fully composed, (M) Internal part of body of Prototype 4; (N) Prototype 5 in parts; (O) Dorsal aspect of body and disc of Prototype 5, (P) Prototype 5 fully composed, (Q) Prototype 6 in parts, (R) Dorsal aspect of body of Prototype 6, (S) Prototype 6 fully composed.

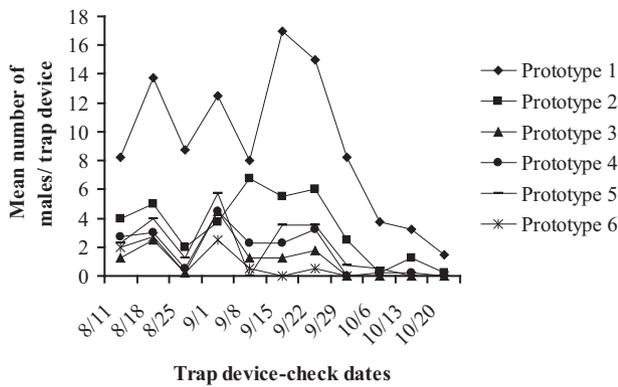


Fig. 2. Mean number of *T. pityocampa* male adults captured in each trap device in Thessaly during the experimental period.

Inc., Adair, OK). Prototype 4 contained two lures with 1 mg of the sex pheromone each.

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 ~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 d). 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 wk.

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 Inc. 2012). Before the analysis, counts were transformed to log (x + 1), to normalize variances and standardize means (Athanasios et al. 2002, 2004; Kavallieratos et al. 2005; Athanassiou et al. 2007, 2008). 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).

Results

Thessaly

In total, 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. 2). 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 1). Significantly more male adults were captured in Prototype 1 than in the other trap devices (Table 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 3). Within different dates, significant differences were noted among trap devices in 9 out of the 11 trap device-check dates (Table 4).

Attica

In total, 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. 3). 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 in November, captures were extremely low. Trap device and date, as well as their interaction, were significant (Table 1). Significantly more male adults were captured in Prototype 1 than in the other trap devices (Table 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 3). Within different dates, significant differences were noted among trap devices in 6 out of the 13 trap device-check dates (Table 4). No significant differences were recorded among the trap devices either early or late in the experimental period.

Molise

In total, 1,640 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). The highest number of male adults was recorded during early August. Trap device and date were significant (Table 1). Significantly more

Table 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		
	df	F	P	df	F	P	df	F	P	df	F	P
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 × 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 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 0.1a	0.5 \pm 0.1a	1.1 \pm 0.1a	0.2 \pm 0.1a
Prototype 2	0.5 \pm 0.1b	0.1 \pm 0.3b	0.6 \pm 0.1b	0.1 \pm 0.1c
Prototype 3	0.2 \pm 0.1c	0.3 \pm 0.1b	0.7 \pm 0.1b	0.1 \pm 0.1bc
Prototype 4	0.3 \pm 0.1bc	0.3 \pm 0.1b	0.7 \pm 0.1b	0.1 \pm 0.1bc
Prototype 5	0.3 \pm 0.1bc	0.1 \pm 0.1b	0.7 \pm 0.1b	0.2 \pm 0.1ab
Prototype 6	0.2 \pm 0.1c	0.2 \pm 0.1b	0.7 \pm 0.1b	0.2 \pm 0.1ab
F	22.2	7.4	3.7	6.1
P	<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.

male adults were captured in Prototype 1 than in the other trap devices (Table 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 3). Significant differences were noted among trap devices in 3 out of the 6 trap device-check dates (Table 4).

Valencia

In total, 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. 5). 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 1). Significantly more male adults were captured in the Prototype 1 than in Prototype 2, Prototype 3, and Prototype 4 (Table 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 3). Significant differences were noted among trap devices in 3 out of the 14 trap device-check dates (Table 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. Jacktel 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 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

(Jacktel 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 have a repulsive activity of the adults that were approaching the trap devices, as has been noted for other species (Manoukios 2016). In our experiments we used funnel-like trap devices, as 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 as the number of male adults that was recorded in Prototype 1 was 2–12 \times higher than those recorded in the other trap devices. The highest number of male adults captured in all experimental areas was owing 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, as 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 *Thaumetopoea wilkinsoni* Tams (Lepidoptera: Thaumetopoeidae) (Cyprus, Israel, Lebanon, and 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, Simonato et al. 2013, Kerdelhué et al. 2015). 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 as any identification based on

Table 3. Correlation coefficient values for captures between pairs of different trap devices during the monitoring period

Pair of trap devices/Area	Thessaly	P	Attica	P	Molise	P	Valencia	P
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 = 37, for Molise df = 22, for Valencia df = 62; two-tailed *t*-test at 0.05.

Table 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.

morphological characters is extremely difficult (Frérot and Démolin 1993, Kerdelhué et al. 2009, Simonato et al. 2013, Kerdelhué et al. 2015). 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

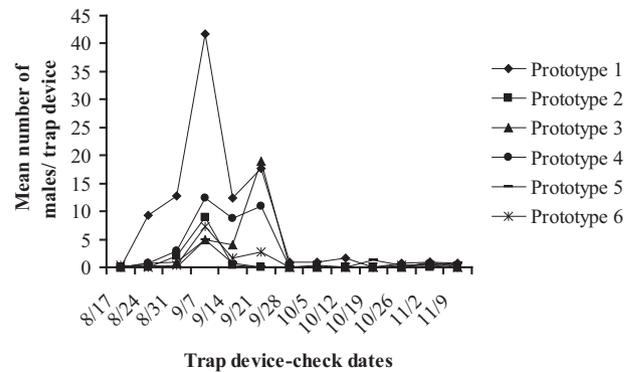


Fig. 3. Mean number of *T. pityocampa* male adults captured in each trap device in Attica during the experimental period.

given period of time, the captures should be representative of the actual changes of male adult 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 wk earlier when Funnel trap devices were compared with 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 *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.

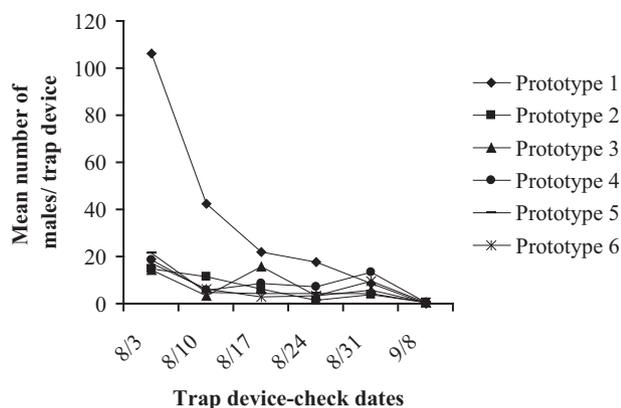


Fig. 4. Mean number of *T. pityocampa* male adults captured in each trap device in Molise during the experimental period.

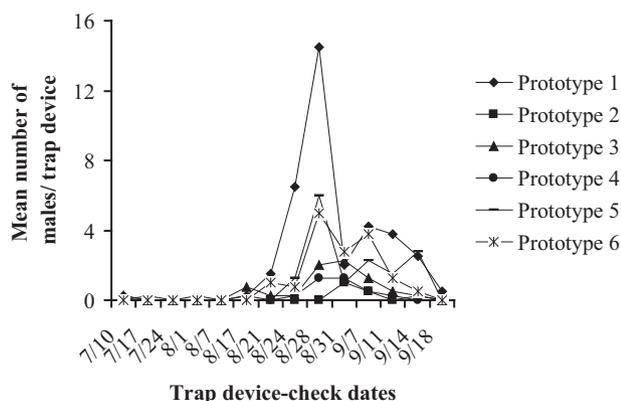


Fig. 5. Mean number of *T. pityocampa* male adults captured in each trap device in Valencia during the experimental period.

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 as 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 toward these directions.

Acknowledgments

We would like to thank G. Balotis and D. Markoyiannaki Printziou for their technical assistance. This study was funded by the EU grant "Innovative eco-friendly traps for the control of Pine Lepidoptera in urban and recreational places" (LIFE PISA: LIFE13 ENV/ES/000504).

References Cited

- Athanassiou, C. G., N. G. Kavallieratos, F. T. Gravanis, N. A. Koukounitsas, and D. E. Roussou. 2002. Influence of trap type, pheromone quantity and trapping location, on capture of the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). *Appl. Zool. Entomol.* 37: 385–391.
- Athanassiou, C. G., N. G. Kavallieratos, and B. E. Mazomenos. 2004. Effect of trap type, trap color, trapping location and pheromone dispenser on captures of male *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 97: 321–329.
- Athanassiou, C. G., N. G. Kavallieratos, S. F. Gakis, L. A. Kyrtza, B. E. Mazomenos, and F. T. Gravanis. 2007. Influence of trap type, trap colour, and trapping location on the capture of the pine moth, *Thaumetopoea pityocampa*. *Entomol. Exp. Appl.* 122: 117–123.
- Athanassiou, C. G., N. G. Kavallieratos, and B. E. Mazomenos. 2008. Almond seed wasp (Hymenoptera: Eurytomidae), sex pheromone: Effect of trap type, trap position, blend ratio and time of the day on male attraction. *Bull. Entomol. Res.* 98: 535–541.
- Avtzis, D. N., D. P. Papachristos, and A. Michaelakis. 2016. Pine processionary moths in Greece refined: introduction and population structure of *Thaumetopoea pityocampa* mtDNA ENA clade in Attica, Greece. *J. Pest Sci.* 89: 393–402.
- Battisti, A., G. Holm, G. Fagrell, and S. Larsson. 2011. Urticating hairs in arthropods: Their nature and medical significance. *Annu. Rev. Entomol.* 56: 2003–2220.
- Battisti, A., M. Avci, D. N. Avtzis, M. L. Ben Jamaa, L. Berardi, W. Berretima, M. Branco, G. Chakali, M. A. El Alaoui El Fels, B. Frérot, et al. 2015. Natural history of the processionary moths (*Thaumetopoea* spp.): New insights in relation to climate change. pp. 15–79. In A. Roques (ed.), *Processionary moths and climate change: An update*. Springer, The Netherlands.
- Berardi, L., M. Branco, M. R. Paiva, H. Santos, and A. Battisti. 2015. Development time plasticity to the pine processionary moth (*Thaumetopoea pityocampa*) populations under laboratory conditions. *Entomologia* 3: 19–24.
- Bonsignore, C. P., and F. Manti. 2013. Influence of habitat and climate on the capture of male pine processionary moths. *Bull. Insectol.* 66: 27–34.
- Branco, M., M. R. Paiva, H. M. Santos, C. Burban, and C. Kerdelhué. 2017. Experimental evidence for heritable reproductive time in two allochronic populations of pine processionary moth. *Insect Sci.* (in press).
- Devkota, B., and G. H. Schmidt. 1990. Larval development of *Thaumetopoea pityocampa* (Den. and Schiff.) (Lepidoptera: Thaumetopoeidae) from Greece as influenced by different host plants under laboratory conditions. *J. Appl. Entomol.* 109: 321–330.
- Devkota, B., M. Breuer, and G. H. Schmidt. 1992. Observations on the flight activity of the pine processionary moth *Thaumetopoea pityocampa* (Den. and Schiff.) in Greece, using synthetic sex-pheromone and light traps (Insecta: Lepidoptera: Thaumetopoeidae). *Boll. Zool. Agrar. Bachic.* 24: 147–157.
- Frérot, B., and G. Démolin. 1993. Sex pheromone of the processionary moths and biosystematic considerations within the genus *Thaumetopoea* (Thaumetopoeidae: Thaumetopoeinae). *Boll. Zool. Agr. Bachic.* 25: 33–40.
- Jacktel, H., P. Menassieu, F. Vétillard, B. Barthélémy, D. Piou, B. Frérot, J. Rousselet, F. Goussard, M. Branco, and A. Battisti. 2006. Population monitoring of the pine processionary moth (Lepidoptera: Thaumetopoeidae) with pheromone-baited traps. *For. Ecol. Manage.* 235: 96–106.
- Kavallieratos, N. G., C. G. Athanassiou, G. N. Balotis, G. Th. Tatsi, and B. E. Mazomenos. 2005. Factors affecting male *Prays oleae* (Lepidoptera: Yponomeutidae) captures in pheromone-baited traps in olive orchards. *J. Econ. Entomol.* 98: 1499–1505.
- Kerdelhué, C., L. Zane, M. Simonato, P. Salvato, J. Rousselet, A. Roques, and A. Battisti. 2009. Quaternary history and contemporary patterns in a currently expanding species. *BMC Evol. Biol.* 9: 220.
- Kerdelhué, C., A. Battisti, C. Burdan, M. Branco, A. Cassel Lundhagen, K. İpekdal, S. Larsson, C. Lopez Vaamonde, E. Magnoux, E. Mateus, et al. 2015. Genetic diversity and structure at different spatial scales in the

- processionary moths. pp. 227–263. In A. Roques (ed.), *Processionary moths and climate change: An update*. Springer, The Netherlands.
- Li, S., J. J. Daudin, D. Piou, C. Robinet, and H. Jactel. 2015. Periodicity and synchrony of pine processionary moth outbreaks in France. *For. Ecol. Manag.* 354: 309–317.
- Manoukis, N. C. 2016. To catch a fly: Landing and capture of *Ceratitis capitata* in a Jackson trap with and without an insecticide. *PLoS ONE* 11: e0149869.
- Martin, J. C. 2015. Development of environment-friendly strategies in the management of processionary moths. pp. 411–427. In A. Roques (ed.), *Processionary moths and climate change: An update*. Springer, The Netherlands.
- Martin, J. C., R. Mazet, M. Correard, E. Morel, and A. S. Brinquin. 2012. Nouvelles techniques de piégeage pour réguler la processionnaire du pin: piégeage phéromonal des adultes, piégeage comportemental des larves: Des expériences prometteuses de piégeage de masse. *Phytoma* 655: 17–22.
- Moneo, I., A. Battisti, B. Dufour, J. C. García Ortiz, M. González Muñoz, F. Moutou, P. Paolucci, E. P. Toffolo, J. Rivière, A. I. Rodríguez Mahillo, et al. 2015. Medical and veterinary impact of the urticating processionary larvae. pp. 359–409. In A. Roques (ed.), *Processionary moths and climate change: An update*. Springer, The Netherlands.
- Robinet, C., C. E. Imbert, J. Rousselet, D. Sauvard, J. Garcia, F. Goussard, and A. Roques. 2012. Human-mediated long-distance jumps of the pine processionary moth in Europe. *Biol. Invasions* 14: 1557–1569.
- Robredo, F. 1980. Tratamientos masivos con diflubenzurón contra la procesionaria del pino en Espana. *Bol. Serv. Plagas* 6: 141–154.
- Robredo, F., and E. Obama. 1987. Soybean oils as ULV carrier in forest spraying using *Bacillus thuringiensis*. *Meded. Fac. Landbouwwet. Rijksuniv. Gent.* 52: 757–762.
- SAS Institute Inc. 2012. Using JMP 10. SAS Institute Inc., Cary, NC.
- Simonato, M., A. Battisti, C. Kerdelhué, C. Burban, C. Lopez Vaamonde, I. Pivotto, P. Salvato, and E. Negrisol. 2013. Host and phenology shifts in the evolution of the social moth genus *Thaumetopoea*. *PLoS ONE* 8: e57192.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry*, 3rd ed. Freedman and Company, NY.
- Snedecor, G. W., and W. G. Cochran. 1980. *Statistical methods*. Iowa State University Press, Ames, IA.
- Trematerra, P. 2016. *Applied urban entomology*. Aracne editrice, Italy.
- Zhang, Q. H., and M. R. Paiva. 1998. Female calling behaviour and male response to the sex pheromone in *Thaumetopoea pityocampa* (Den. and Schiff.) (Lepidoptera: Thaumetopoeidae). *J. Appl. Entomol.* 122: 353–360.
- Zhang, Q. H., F. Schlyter, A. Battisti, G. Birgersson, and P. Anderson. 2003. Electrophysiological responses of *Thaumetopoea pityocampa* females to host volatiles: Implications for host selection of active and inactive terpenes. *J. Pest Sci.* 76: 103–107.