Hellenic Plant Protection Journal **13:** 42-53, 2020
DOI 10.2478/hppj-2020-0005

Efficacy of communication disruption of *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae) with low pheromone formulation

A. Michaelakis[1], E. Anastasaki[1], P.G. Milonas[1], D.P. Papachristos[1], D. Kontodimas[1], C.M. Pontikakos[2], D.G. Raptopoulos[3], N.A. Babilis[3] and M.A. Konstantopoulou[4*]

Summary Mating disruption (MD) has been a successful approach for pest control of several lepidoptera. Field trials to evaluate the efficacy of communication disruption of low pheromone load formulation on *Thaumetopoea pityocampa* were carried out in 2010 and 2011 in an urban park. The efficacy of MD was assessed by comparing male *T. pityocampa* catches in pheromone traps, between MD and Control areas. In the 1st year of the application the percentage of male inhibition ranged from 85 to 100% during the 1st month of the flight period and 95-100% during the whole flight period in the 2nd year. The pheromone remained in the polymeric matrix was almost 30% after 7 weeks under laboratory aging conditions. Combining the pheromone release results with the male disorientation results we can assume that after 7 weeks the remaining pheromone concentration was still sufficient to achieve MD. This study indicates that air permeation with the major sex pheromone component (*Z*)-13-hexadecen-11-ynyl acetate, at a rate of 20 g/ha for one application per season, can affect the orientation of *T. pityocampa* males. Since mating disruption is an environmentally safe method for pest control, it could be a valuable tool to control *T. pityocampa* in urban areas and parks.

Additional Keywords: mating disruption, pheromone formulation, pine processionary moth, release rate, wax matrix.

Introduction

The pine processionary moth (PPM), *Thaumetopoea pityocampa* (Denis and Schiffermüller) (Lepidoptera: Thaumetopoeidae), is an endemic insect of pine and cedar trees, existing both in rural and urban areas causing economic damage throughout temperate regions of the Mediterranean, including southwestern Europe, the Balkan Peninsula and North Africa (Huchon and Démolin, 1971; Battisti 1988; Battisti *et al.*, 2005; Simonato *et al.*, 2007; Yilmaz *et al.*, 2013; Kerdelhué *et al.*, 2014; Battisti *et al.*, 2015; Castagneyrol *et al.*, 2016). This lepidopteran pest causes significant economic losses. It impairs the vitality of pine forests due to defoliation, and growth retardation of pine trees and also augments their susceptibility to secondary pests in temperate regions (Devkota and Schmidt, 1990; Masutti and Battisti, 1990; Hódar *et al.*, 2002; Carus 2004; Erkan 2011; Jacquet *et al.*, 2012).

PPM larvae defoliate pine trees by feeding gregariously, from autumn to spring (Semiz *et al.*, 2006; Yilmaz *et al.*, 2013). They complete their life cycle annually, but in unfavorable conditions they may remain in pupal stage in the soil for several years (Salman *et al.*, 2019). With their urticating hairs PPM larvae induce a variety of allergic reactions, such as skin irritation, conjunctivitis, respiratory congestions and asthma in humans and animals (Battisti *et al.*, 2011; Vega *et al.*, 2011). Outbreaks of the species in areas previously unaffected by the insect can be favored by the presence of *Pinus* hosts (Stastny *et al.*,

[1] Department of Entomology and Agricultural Zoology, Benaki Phytopathological Institute, GR-145 61 Kifissia, Attiki, Greece.
[2] Region of Attica, Attiki, Greece.
[3] Novagrica Hellas S.A., Athens, Greece.
[4] Chemical Ecology and Natural Products Laboratory, Institute of Biosciences and Applications, NCSR "Demokritos", GR-153 41 Ag. Paraskevi, Attiki, Greece.
* Corresponding author: mkonstan@bio.demokritos.gr

2006) or as a result of climate change (Battisti *et al*., 2005, 2006, 2017).

In recent years there is an increasing interest in the prospect of exploiting environmentally safe strategies to control pine pests. This also applies for the PPM since the use of chemical pesticides for the control of this pest is not permitted in urban and suburban areas and parks. Although research has provided solutions for many agricultural problems, control of forest pest insects via integrated pest management tactics has developed at a slower pace (Corley and Jervis, 2012). Biological pest management practices have been used as alternatives to insecticides against *Thaumetopoea* sp. to reduce their damage on forestlands (Rausell *et al*., 1999; Kanat and Ozbolat, 2006; Semiz *et al*., 2006; Barbaro and Battisti, 2011; Yilmaz *et al*., 2013). Various methods such as destruction of winter nests and treatment with *Bacillus thuringiensis* have been tested in many studies for reducing the population of PPM larvae (Cebeci *et al*., 2010; Yilmaz *et al*., 2013).

Mating disruption (MD) has been a successful approach for pest control over the past few decades, and is now an accepted control option for a number of lepidopteran pests of fruits and vegetables (Cardé and Minks, 1995; Byers, 2007; Witzgall *et al*., 2010; Miller and Gut, 2015). To make MD successful as an insect management technique, synthetic pheromone needs to be present in the air in sufficient quantities over the course of the insects' mating period. As a general guide, application rates ranging between 10 g to 100 g per ha per season are required to achieve communication disruption, equivalent to, at least, 1 ng/m^3 aerial concentration (Bengtsson *et al*., 1994; Cork *et al*., 2008). Furhermore, the matrix that holds and releases the pheromones plays a significant role in the success of the mating disruption systems (Wilkins *et al*., 1984; Zdarek *et al*., 1988; Chamberlain *et al*., 2000; Zada *et al*., 2009).

There are several successful commercial formulations made of polyethylene tubes, cotton rolls, ropes, or bags baited with pheromones (Brown *et al*., 1992; Suckling 2000; Johansson *et al*., 2001; Hegazi *et al*., 2007; 2009). However, problems may arise when the matrix does not preserve the pheromone and allows either too high a release that exhausts the dispenser too soon, or too low a release that is less than optimal, primarily depending on the volatility of the components. Waxy blobs and similar materials are increasingly being used to dispense pheromone from many sources in mating disruption (Atterholt *et al*., 1998; Stelinski *et al*., 2005, 2007a,b; deLame *et al*., 2007).

The major sex pheromone component (*Z*)-13-hexadecen-11-ynyl acetate produced by the female *T. pityocampa* was first identified by Guerrero *et al*. (1981). Since its identification, research has mainly focused on the synthesis of the sex pheromone in sufficient quantities for the monitoring of PPM populations and the development of a mass-trapping method for direct control (Cuevas *et al*., 1983; Halperin, 1984, 1985, 1986; Tiberi and Niccoli, 1984; Roversi, 1985; Nicolini ,1987; Fabrias *et al*., 1989; Arsequell *et al*., 1990; Camps *et al*., 1990a,b; Devkota *et al*., 1992; Zhang and Paiva, 1998; Jactel *et al*., 2006; Battisti *et al*., 2015; Athanassiou *et al*., 2007, 2017).

Effective pheromone mating disruption formulations based on synthetic polymers have been developed for many species (*Grapholita molesta, Lymantria dispar, Paralobesia viteana* etc) and are commercially available. However to the best of our knowledge no such formulation with (*Z*)-13-hexadecen-11-ynyl acetate as active ingredient is commercially readily available.

Our study reports results of using a polymer formulation with the PPM sex pheromone as active ingredient applied consecutively for 2 years in "Attiko Alsos", a major hill-park in the Attica district. This park is a focal point within the city network with outdoors caffes, promenade walkways, sports courts etc. The treated area suffered severe PPM infestation raising concerns to visitors and the authorities. An effective treatment with minimal ecological footprint which would respect the non-target entomofauna could serve as a model for many parks in the Mediterranean basin facing similar problems.

Materials and methods

Pheromone formulation

The sex pheromone formulations used for monitoring and MD of *T. pityocampa* were provided by Novagrica Hellas SA (Athens, Greece). The MD formulation was a wax matrix plus pheromone [(Z)13-hexadecen-11-ynyl acetate, 95% pure by GC], similar to that reported by Atterholt et al. (1999). It contained 2 % (w/w) of the PPM pheromone.

Experimental fields

MD trials were conducted for two consecutive seasons from 2010 to 2011 in "Attiko Alsos" (23 hectares with trees, mostly pines). It is an urban, green and recreational area of significant ecological value for Athens (E23°45'37.37"; N38° 0'16.08"). The pines in that area are mainly *Pinus brutia* Ten. and to a smaller extent *Pinus halepensis* Mill., representing a low elevation Mediterranean forest.

Three experimental plots (0.7 ha each) were selected, one plot for control (CO) and two plots for MD trials. The CO plot was separated by 250m from the MD plots and the distance between the MD plots were approx. 30m. Baker *et al.* (2009) report that males of the sibling species *Thaumatopoea processionea* L. fly long distances whereas gravid females fly much shorter ones. Gravid PPM females are also expected to be weaker fliers than males. In addition, time limitation of short-lived females to lay a single batch of eggs is a factor suggesting limited dispersion of females in an area where their natural host is abundant (Stansthy *et al.*, 2006). Plots were more or less elongate and on a declivity of the park to avoid trap disturbance by passing-byers. MD plots were clearly demarcated (secondary traffic road and barren land strips). Trees of the experimental plots were of medium size (reaching 4m in height). Tree density was approximately 345 trees/ha.

A data-logging unit (EBI 20-TH, ebro Electronic GmbH & Co. KG, Ingolstadt Germany) was placed in area of study to measure temperature and air relative humidity during the period of MD implementation. Data logger was interfaced with a computer to record air temperature and air humidity at 1 hour intervals.

Pheromone application

In 2010 on the first week of August and in 2011 on the fourth week of August MD treatments took place in the experimental plots to control the PPM. Waxy polymer formulation containing 2 % (w/w) of the PPM pheromone was applied manually on the trunks of the pines at head height using caulking guns. On each application point a small blob, approx. 2 g was placed. The point sources were placed at the base of branches for protection against the sun. In our effort to have as much as possible homogenous spread of the polymeric matrix effort was made to apply at least one blob of the waxy formulation on every tree depending on tree density and canopy volume. The amount of pheromone applied was 20 g/ha.

Assessment of MD efficacy

The efficacy of the MD was assessed by comparing: i) male *T. pityocampa* catches in pheromone-baited traps and ii) egg density and hatchability in control and pheromone-treated plots and iii) monitoring the pheromone release rate at different temperatures under laboratory conditions.

Trap catches

In the MD and CO plots four Delta traps per plot (12 traps in total) (Novagrica Hellas S.A, Athens, Greece) were hung on the external south part of tree canopy at head height and not in the immediate proximity of any of the pheromone releasing waxy blobs.

Each trap was baited with gray septa (Novagrica Hellas S.A, Athens, Greece) loaded with 1 mg of (*Z*)-13-hexadecen-11-ynyl-acetate. The traps were serviced once per week. Moth inhibition due to pheromone treatment was assessed weekly by comparing the number of *T. pityocampa* captured in the Delta traps in MD and CO plots and was used to calculate the percentage of male inhibition according to the following formula: {[CO – MD)/CO]x100} representing the average male catches in untreated and treated plots respectively.

Egg density

The effect of mating disruption on oviposition of *T. pityocampa* was assessed by recording egg densities on pine needles. Egg masses were collected randomly in MD and CO plots five times during the experimental period (approx. every two weeks). Each time five sampling points were randomly selected in the MD or CO plots, but not at the border of the plot. On each sampling point, two neighboring trees were sampled and from these pines, 2 shoots (ca. 30 cm long) bearing egg masses were removed. In the laboratory, the number of unhatched eggs/sample was recorded. Collected eggs were kept in a climatic chamber (25 ±1 °C; 70% RH; 16:8 h L:D) until they either hatched, or were considered non-fertilized.

Pheromone release rate

Due to practical difficulties of collecting blobs samples (waxy polymer formulation) from the trees trunks, the determination of the release rate of the major sex component (*Z*)-13-hexadecen-11-ynyl acetate was carried out under laboratory conditions in two temperatures. Two (2) g of the wax polymer were weighed in small glass petri dishes and placed in constant temperature rooms at 20 and 28 °C. Three samples thereof were analyzed per sampling date and temperature as follows: prior to incubation, at 1, 2, 3, 4, and 7 days following incubation and then weekly for a total of 7 weeks. Samples were placed in a 20-mL vial (Machery-Nagel, Düren, Germany). Five mL of an internal standard solution of 1 mg/mL of methyl hexanoate in acetone (99.9% purity; Acros Organics, Geel, Belgium) was added to each vial. The samples were placed at -18 °C until analyses. Prior to analysis, samples were placed in a water bath at 65-68 °C for 3 min. Then they were vortexed for 1 min and placed again in the water bath for 2 more min. Samples were filtered through a polytetrafluoroethylene filter (PTFE) with a 0.45 μm pore size (Machery-Nagel, Düren, Germany).

A Thermo Scientific TRACE 1300 Series GC chromatograph (Milan, Italy) equipped with a flame ionization detector (FID) and a TG-1 ms capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) with helium as carrier gas at 1 mL /min was used for the analysis of samples. Column temperature was initially kept for 2 min at 50 °C, then gradually increased to 240 °C with a rate of 2 °C/min, and held for 10 min. The injector and detector temperatures were set to 220 and 250 °C, respectively. One microlitre of each sample was injected manually in splitless mode. For quantification of pheromone content, the internal standard method was used and was normalized for the original weight of each sample.

Data analysis

The field data were subjected to analysis of variance (ANOVA) (SAS Institute, 2000). Data are presented as means of male catches per trap and mean number of eggs, and egg hatchability. Means were normalized using the log(x+ 0.5) transformation. The data for eggs density and status were tested by using the tailed t-test at n-2 and P=0.01 (Snedecor and Cochran, 1989).

Results

Trap catches

During the 1st year of the study (2010), the average air temperature of the area was 29.6, 23.5 and 17.6°C and the average rainfall was 0.0, 37.0, and 116.6 mm in August, September and October respectively. In 2011 the average air temperature of the area was 27.6, 25.4 and 16.4°C and the average rainfall was 0.0, 0.0, and 37.2 mm in August, September and October respectively.

Mean weekly catches per pheromone trap for treated (MD) and untreated (CO) plots are shown in Fig. 1A for the 2010 trials. Capture data from the untreated plot indicated that the flight peak occurred on 17 September (40.25±5.8 males/trap/week). Of total male catches (20 August to 25 October), 65.6% were captured during September. In 2010, MD was applied on August 6th. Total trapped males were significantly lower (F=7.361, df=11, P=0.000) in MD plots (114.6±29.3 males/trap) than in CO (325 ± 49.7 males/trap) during the MD period. The percentage of male inhibition ranged from 85% to100% during the 1st month of the flight period (Fig. 1B).

In 2011 (Fig 2A), catches of males in the untreated plot indicated that flight peak occurred on 23 September (56±0.5 males/trap). Capture of moths in the MD plots was significantly low compared to CO traps (F=47.053, df=11, P=0.000). Trap catches on the CO plot started on 29 August. Of the total trapped males (29 August to 9 November), 77% were captured in September. Pheromone application was done on August 22nd. Trap captures in MD plots were significantly lower than in CO plot. Mean capture rates in MD and CO plots differed significantly each year. In 2011, the population density of *T. pityocampa* moths was generally, higher than in the previous year. The percentage of male inhibition ranged from 95-100% during the whole flight period (Fig. 2B).

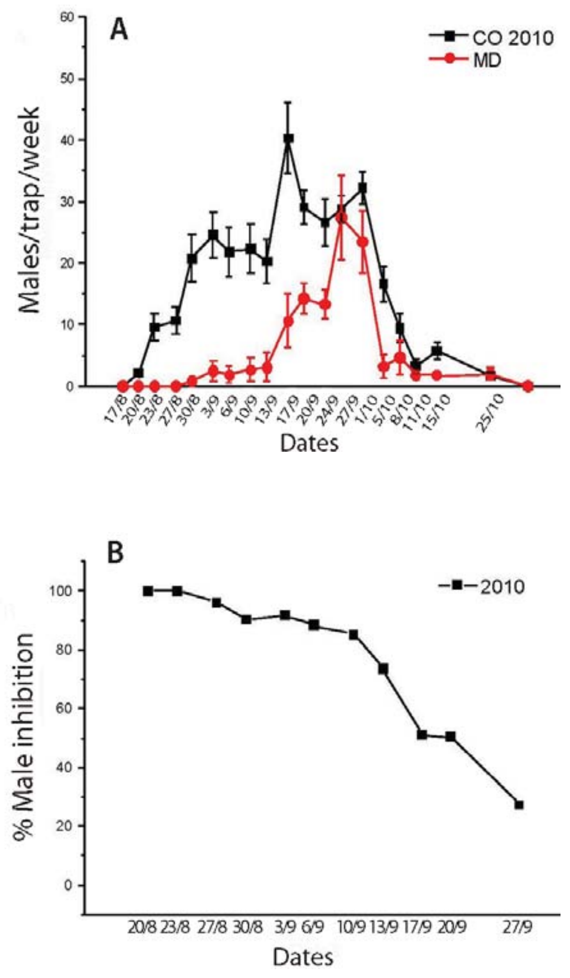

Figure 1. Captures of males (A) and male inhibition (B) of *Thaumetopoea pityocampa* in pheromone traps installed in plots treated with pheromone (MD) *vs* untreated-control (CO) plots at "Attiko Alsos", Attiki, Greece in 2010.

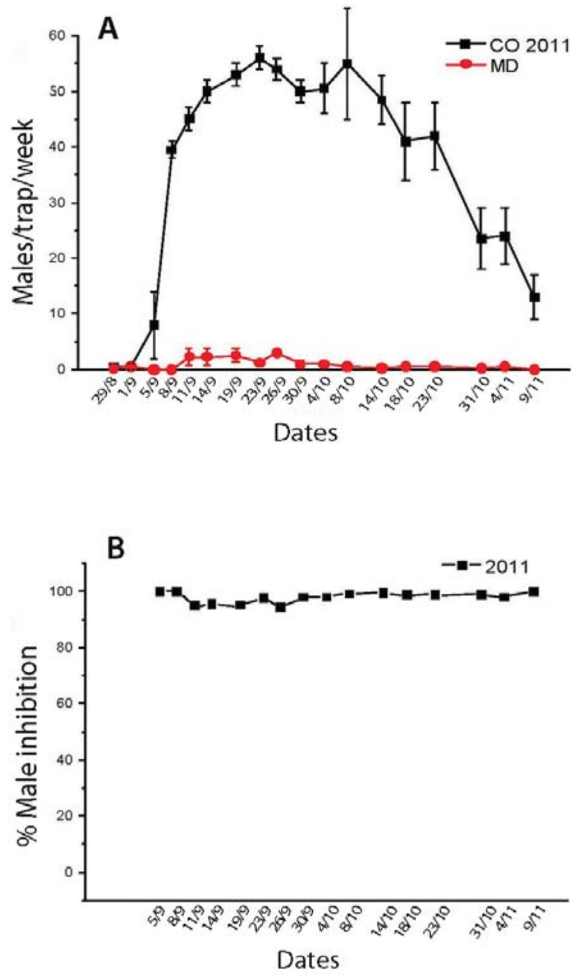

Figure 2. Captures of males (A) and male inhibition (B) of *Thaumetopoea pityocampa* males in pheromone traps installed in plots treated with pheromone (MD) *vs* untreated-control (CO) plots at "Attiko Alsos", Attiki, Greece, in 2011.

Egg density

In 2010 (Table 1) the number of *T. pityocampa* eggs/egg mass was significantly lower (t=14.370; df=49; P=0.000) in MD (91.24 ± 4.59 eggs/egg mass) compared to CO (172.24 ± 5.67 eggs). In 2011 (Table 1) number of *T. pityocampa* eggs was significantly lower (t = 12.610; df=49; P=0.000) in MD (76.44 ± 3.0 eggs) compared to CO (154.56 ± 3.62 eggs).

In 2010 hatchability of *T. pityocampa* eggs (Table 1, Fig. 3) was significantly lower (t= 9.023; df=49; P=0.000) in MD (35.28 ± 3.58 % eggs/egg mass) compared with CO (75.8 ± 1.49 %). In 2011 hatchability of *T. pityocampa* eggs (Table 1, Fig. 4) was also significantly lower (t = 16.851; df=49; P=0.000) in MD (20.34 ± 2.71 %) compared with CO (81.64 ± 1.56 %).

Pheromone release rate

The release rates of the sex pheromone component (*Z*)-13-hexadecen-11-ynyl acetate at the two examined temperatures are shown in Fig. 5 and fit a logarithmic decay curve. After 7 weeks of incubation almost 30% of the pheromone remained in the polymeric matrix (Fig. 5A). During the first week, the release rate was 1 mg per day at both temperatures. In the next week, the rate was slightly decreased at 0.63 and 0.7mg per day at 20 and 28ºC, respectively. Over the next weeks the average rate was 95μg per day (Fig.5B). As indicated by male disorientation results, after 7 weeks the remaining pheromone concentration was still sufficient to achieve mating disruption.

Discussion

The results indicated that air permeation with the major sex pheromone component (*Z*)-13-hexadecen-11-ynyl acetate, with 20 g/ha for one application per season, can affect

Table 1. *Thaumetopoea pityocampa* egg density (No ± SE) and hatchability from untreated (CO) and treated plots (MD) at "Attiko Alsos", Attiki, Greece, in 2010 and 2011.

Year	No of eggs/egg mass		% of hatched eggs/egg mass	
	CO	MD	CO	MD
2010	172.24 ±5.67	91.24 ±4.59	75.8 ±1.49	35.28 ±3.58
2011	154.56± 3.62	76.44 ±3.0	81.64 ±1.56	20.34 ±2.71

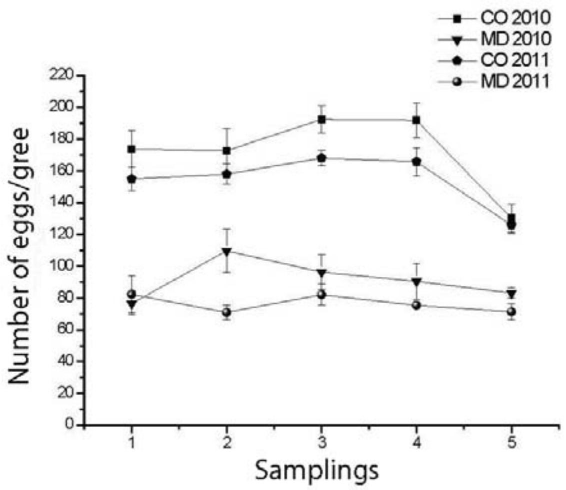

Figure 3. Egg density of *Thaumetopoea pityocampa* from samplings in untreated (CO) and treated plots (MD) at "Attiko Alsos", Attiki, Greece, in 2010 and 2011.

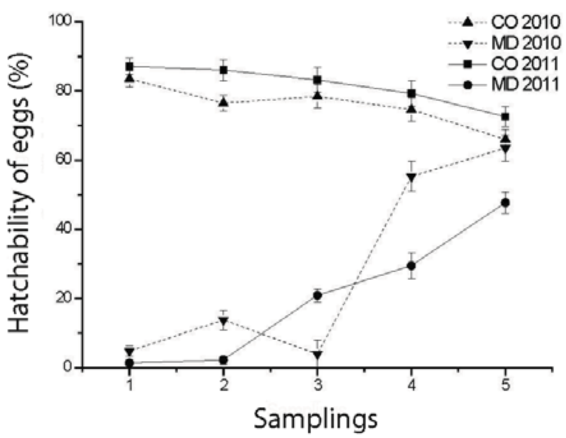

Figure 4. Hatchability of *Thaumetopoea pityocampa* from samplings in untreated (CO) and treated plots (MD) at "Attiko Alsos", Attiki, Greece, in 2010 and 2011.

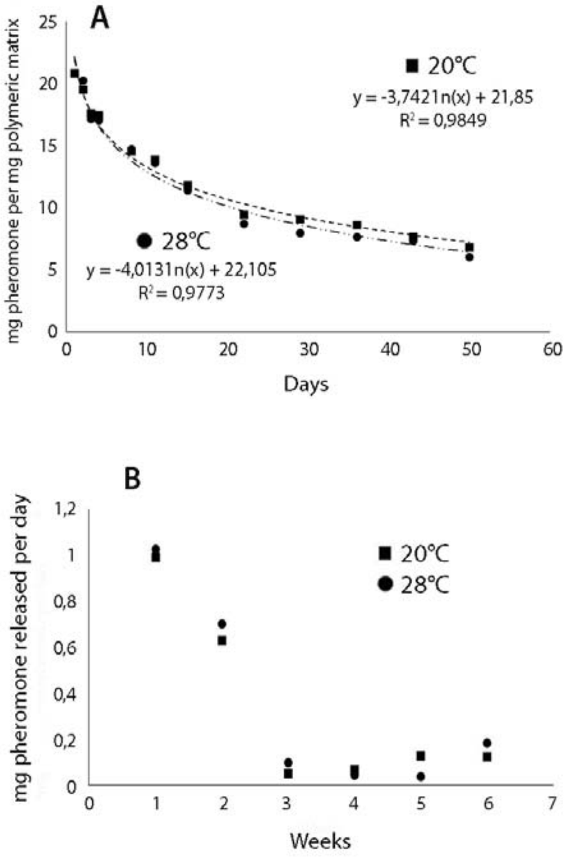

Figure 5. Release pattern of the major sex pheromone component (*Z*)-13-hexadecen-11-ynyl acetate at 20 and 28 °C (A) and the corresponding weekly loss (B).

the orientation of *T. pityocampa* males to female pheromone sources.

Successful mating disruption by a single pheromone component has also been reported for several Lepidoptera such as *Platyptilia carduidactyla* (Klun *et al.,* 1981), *Prays oleae* (Mazomenos *et al.*, 1999; Hegazi *et al.*, 2009) and *Phyllocnistis citrella* (Lapointe and Stelinski, 2011).

Captures of males in pheromone-baited traps were significantly lower in all pheromone-treated (MD) plots in comparison with the control (CO) during the study years. In 2010, polymer formulation of the pheromone was applied much earlier than the beginning of the flight. Thus, male disorientation was partially achieved ranging 85-100% during the first 4 flight weeks and then was reduced. For this reason, during the next trials in 2011 the MD was applied only a few days before the beginning of the flight, as has been verified by monitoring traps already set in place. Thus male disorientation was successfully achieved ranging 95-100% during the whole flight period. In 2011, the inhibition of successful orientation was almost complete even at the peak of the flight period of the moth. This observation is consistent with other studies on mating disruption, where the technique is most effective when applied in the correct time (Moffitt and Westigard, 1984; Borchert and Walgenbach, 2000; Hegazi *et al.*, 2007; 2009; Witzgall *et al.*, 2010).

Although during the second year of the application the population size of *T. pityocampa* was higher than in the first one, the number of *T. pityocampa* eggs collected from treated plots was significantly lower than those found in the control plot. The results are in agreement with the findings of Mazomenos *et al.* (1999), who reported that, in mating disruption trials, the reduction of moth populations is a gradual process and requires 2–3 years of continuous pheromone application to achieve control measures close to economically acceptable levels.

The shutdown of the trap captures was noticeable even from the first year and resulted to a further reduction in the oviposition patterns. During the two years, oviposition by female moths was reduced on the treated trees in comparison to untreated ones, but still indicated the presence of mated females. The fertile eggs in the treated plots may have been laid by females fertilized despite MD treatment or by gravid females immigrating from adjacent plots into the MD plots. Battisti et al. (2015) refer that *T. pityocampa* males may disperse over distances of 50–100 km. In general, immigration of gravid females into pheromone-treated areas from the surroundings is a common obstacle when using the MD technique (Knight, 1996; Mazomenos *et al.*, 1999), but can be avoided by extending pheromone application to a larger spatial scale. Besides the risk of invasion by mated females from neighbouring areas, the existence of "hot spots" *i.e.* areas with high moth densities, within the treated area, may allow significant mating. More knowledge on mating behaviour, dispersal and oviposition behaviour of this pest is still needed and studies in this respect should be encouraged.

The used pheromone formulation in our study with a load of 20 g/ha for one application per season, allowed the presence of sufficient pheromone concentration in the field for more than ten weeks of field exposure. This is a low pheromone load formulation compared to the pheromone concentration used in other species to achieve insect disorientation and communication disruption i.e. between 10 g and 100 g per ha (Bengtsson *et al.*, 1994; Cork *et al.*, 2008). In the case of *Prays oleae* the pheromone concentration used was 40 g a.i./ha per season (Hegazi et al. 2009), for *Palpita unionalis* was 80 g a.i./ha per season (Hegazi *et al.*, 2007), for *Spodoptera littoralis* was 40-60 g of a.i/ha (De Souza *et al.*, 1992), for *Grapholita molesta* was 44 g of a.i/ha (Arioli *et al.*, 2014), for *Phylocnistis citrella* was 75 g of a.i/ha (Lapointe and Stelinski, 2011), for *Cydia pomonella* was up to 100 g of a.i/ha (Witzgall *et al.,* 2010).

As both the formulation and the matrix to deliver pheromone are of paramount importance in determining the mode of action and the success of MD programs (Cardé and Minks 1995, Leonardt *et al.*, 1990, Weatherston 1990), the tested formulation-matrix combination seems promising for the effective MD of *T. pityocampa,* especially because it was attained with 20 g a.i./ha is. Since the cost of pheromone is still very high, achieving adequate levels of male sexual disorientation with less amounts of pheromone could make the use of this method more cost-effective (Gordon *et al.*, 2005). Last but not least, MD is an environmentally safe method for pest control in urban and suburban areas and parks and a valuable tool in Integrated Pest Management programs.

This research has been co-financed by the Region of Attica, Greece (former Prefecture of Athens – Piraeus) through the project "Protection of woods in urban areas from Thaumetopoea pityocampa: Control of the moth by pheromone mating disruption method combined with Bacillus thuringiensis".The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Literature Cited

Arioli, C., Pastori, P., Botton, M., Garcia, M., Borges, R. and Mafra-Neto, A. 2014. Assessment of SPLAT formulations to control *Grapholita molesta* (Lepidoptera: Tortricidae) in a Brazilian apple orchard. *Chilean Journal of Agricultural Research,* 74: 184-190.

Arsequell, G., Fabrias, G. and Camps, F. 1990. Sex pheromone biosynthesis in the processionary moth *Thaumetopoea pityocampa* (Denis and Schiffermüller) (Lepidoptera: Thaumatopoeidae) by delta-13 desaturation. *Archives of Insect Biochemistry and Physiology,* 14: 47–56.

Athanassiou, Ch., Kavallieratos, N., Gakis, S., Kyrtsa, L., Mazomenos, B. and Gravanis, F. 2007. Influence of trap type, trap colour, and trapping location on the capture of the pine moth, *Thaumetopoea pityocampa. Entomologia Experimentalis et Applicata,* 122: 117–123.

Athanassiou, C.G., Kavallieratos, N.G., Pardo, D., Sancho, J., Colacci, M., Boukouvala, M.C., Nikolaidou A.J., Kondodimas, D.C., Benavent-Fernández, E., Gálvez-Settier, S. and Trematerra, P., 2017. Evaluation of pheromone trap devices for the capture of Thaumetopoea pityocampa (Lepidoptera: Thaumetopoeidae) in southern Europe. *Journal of Economic Entomology,* 110: 1087-1095.

Atterholt, C.A., Delwiche, M.J., Rice, R.E. and Krochta, J.M., 1999. Controlled release of insect sex pheromones from paraffin wax and emulsions. *Journal of Controlled Release,* 57: 233-247.

Atterholt, C.A., Delwiche, M.J., Rice, R.E. and Krochta, J.M. 1998. Study of biopolymers and paraffin as potential controlled-release carriers for insect pheromones. *Journal of Agricultural and Food Chemistry,* 46: 4429–4434.

Baker, R., Caffier, D., Choiseul, J.W., De Clercq, P., Dormannsné-Simon, E., Gerowitt, B., Evtimova Karadjova, O., Lövei, G., Lansink, A.O., Makowski, D., Manceau, C., Manici, L., Perdikis, D., Porta Puglia, A., Schans, J., Schrader, G., Steffek, R., Strömberg, A., Tiilikkala, K., Coert van Lenteren, J. and Vloutoglou, I. 2009. Evaluation of a pest risk analysis on Thaumetopoea processionea L., the oak processionary moth, prepared by the UK and extension of its scope to the EU territory. EFSA Scientific Opinion of the Panel on Plant Health. The EFSA Journal (2009), 1195, 1-64

Barbaro. L. and Battisti, A. 2011. Birds as predators of pine processionary moth (Lepidoptera: Notodontidae). *Biological Control,* 56: 107-114.

Battisti, A. 1988. Host–plant relationships and population dynamics of the pine processionary caterpillar *Thaumetopoea pityocampa* (Denis and Schiffermuller). *Journal of Applied Entomology,* 105: 393–402.

Battisti, A., Avci, M., Avtzis, D., Ben Jamaa, M., Berardi, L., Berretima, W., Branco, M., Chakali, G., Alaoui El Fels, M., Frérot, B., Hódar, J., Ionescu-Mălăncus, I., Ipekdal, K., Larsson, S., Manole, T., Mendel, Z., Meurisse, N., Mirchev, P., Nemer, N., Paiva, M., Pino, J., Protasov, A., Rahim, N., Rousselet, J., Santos, H., Sauvard, D., Schopf, A., Simonato, M., Yart, A. and Zamoum, M. 2015. Processionary moths and climate change: An update Chapter 2 Natural History of the Processionary Moths (*Thaumetopoea* spp.): New Insights in Relation to Climate Change. Roques A. (Ed.) 2015, XVII, 427 p.

Battisti, A., Holm, G., Fagrell, B. and Larsson, S. 2011. Urticating hairs in Arthropods: Their nature and medical significance. *Annual Review of Entomology,* 56: 203-220.

Battisti, A., Larsson, S. and Roques, A. 2017. Processionary Moths and Associated Urtication Risk: Global Change Driven Effects. *Annual Review of Entomology,* 62: 323-342.

Battisti A, Stastny M, Buffo E., Larsson S., 2006. A rapid altitudinal range expansion in the processionary moth produced by the 2003 climatic anomaly. *Global Change Biology* 12: 662-671.

Battisti, A., Stastny, M., Netherer, S., Robinet, C., Schopf, A., Roques, A. and Larsson, S. 2005. Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. *Ecological Applications,* 15: 2084–2096.

Bengtsson, M., Karg, G., Kirsch, P.A., Lőfqvist, J., Sauer, A. and Witzgall, P. 1994. Mating disruption of pea moth *Cydia nigricana* F. (Lepidoptera: Tortricidae) by a repellent blend of sex pheromone and attraction inhibitors. *Journal of Chemical Ecology,* 20: 871–887.

Borchert, D.M. and Walgenbach, J.F. 2000. Comparison of pheromone-mediated mating disruption and conventional insecticides for management of tufted apple bud moth (Lepidoptera: Tortricidae). *Journal of Economic Entomology,* 93: 769–776.

Brown, D.F, Knight, A.L., Howell, J.F., Sell, C.R., Krysan, J.L. and Weiss, M. 1992. Emission characteristics of a polyethylene pheromone dispenser for mating disruption of codling moth (Lepidoptera: Tortricidae). *Journal of Economic Entomology,* 85: 910–917.

Byers, J.A. 2007. Simulation of mating disruption and mass trapping with competitive attraction and camouflage. *Environmental Entomology,* 36: 1328–1338.

Camps, F., Gasol, V. and Guerrero, A. 1990a. Inhibitory pheromonal activity promoted by analogs of sex pheromone of the processionary moth *Thaumetopoea pityocampa* (Denis and Schiffermüller) (Lepidoptera: Thaumetopoeidae). *Journal of Chemical Ecology,* 16: 1155–1172.

Camps, F., Gasol, V., Hernandez, Z. and Montoya, R. 1990b. Inhibition of the processionary moth sex pheromone by some haloacetate analogues. *Pesticide Science*, 29, 123–134.

Cardé, R.T. and Minks, A.K. 1995. Control of moth pests by mating disruption: successes and constraints. *Annual Review of Entomology,* 40: 559-585.

Carus, S. 2004. Impact of defolation by pine processionary moth (*Thaumetopoea pityocampa*) on radial, height and volume growth of Calabrian pine (*Pinus brutia*) trees in Turkey. *Phytoparasitica,* 32: 459-469.

Castagneyrol, B., Jactel, H., Brockerhoff, E., Perrette, N., Larter, M., Delzon, S. and Piou, D. 2016. Host range expansion is density dependent. *Oecologia,* 182: 779–788.

Cebeci, H.H., Oymen, R.T. and Acer, S. 2010. Control of pine processionary moth. *Journal of Environmental Biology,* 31: 357-361.

Chamberlain, D.J., Brown, N.J., Jones, O.T. and Casagrande, E. 2000. Field evaluation of a slow release pheromone formulation to control the American bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. *Bulletin of Entomological Research,* 90: 183–190.

Cork, A., De Souza, K., Hall, D.R., Jones, O.T., Casagrande, E., Krishnaiah, K. and Syed, Z. 2008. Development of PVC resin-controlled release formulation for pheromones and use in mating disruption of yellow rice stem borer, *Scirpophaga incertulas*. *Crop Protection,* 27: 248–255.

Corley, J.C. and Jervis, M.A. 2012. Forest pest management: a global challenge. *International Journal of Pest Management,* 58: 193-194.

Cuevas, P., Montoya, R., Belles, X., Camps, F., Coll, J., Guerrero, A. and Riba, M. 1983. Initial field trials with the synthetic sex pheromone of processionary moth *Thaumetopoea pityocampa* (Denis and Schiffermüller) (Lepidoptera: Thaumetopoeidae). *Journal of Chemical Ecology,* 9: 85–93.

deLame, F.M., Miller, J.R., Atterholt, C.A. and Gut, L.J. 2007. Development and evaluation of an emulsified paraffin wax dispenser for season-long mating disruption of *Grapholita molesta* in commercial peach orchards. *Journal of Economic Entomology*, 100, 1316–1327.

de Souza, K.R., McVeigh, L.J. and Wright, D.J. 1992. Selection of insecticides for lure and kill studies against *Spodoptera littoralis* (Lepidoptera, Noctuidae). *Journal of Economic Entomology,* 85: 2100–2106.

Devkota, B. and Schmidt, G.H. 1990. Larval development of *Thaumetopoea pityocampa* (Den. and

Schiff.) (Lepidoptera: Thaumetopoeidae) from Greece as influenced by different host plants under laboratory conditions. *Journal of Applied Entomology,* 109: 321–330.

Devkota, B., Breuer, M. and Schmidt. G.H. 1992. Observations on the flight activity of the Pine processionary moth *Thaumetopoea pityocampa* (Den. and Schiff.) in Greece, using synthetic sexpheromone and light traps (Insecta: Lepidoptera: Thaumetopoeidae). *Bolletino di Zoologia Agraria e di Bachicocultura,* 24: 147–157.

Erkan, N. 2011. Impact of pine processionary moth (*Thaumetopoea wilkinsoni* Tams) on growth of Turkish red pine (*Pinus brutia* Ten.) *African Journal of Agricultural Research,* 6: 4983–4988.

Fabrias, G., Arsequell, G. and Camps, F. 1989. Sex pheromone precursors in the processionary moth *Thaumetopoea pityocampa* (Denis and Schiffermüller) (Lepidoptera: Thaumetopoeidae). *Insect Biochemistry,* 19: 177–181.

Gordon, D., Zahavi, T., Anshelevich, L., Harel, M., Ovadia, S., Dunkelblum, E. and Harari, A. 2005. Mating Disruption of *Lobesia botrana* (Lepidoptera: Tortricidae): Effect of Pheromone Formulations and Concentrations. *Journal of Economic Entomology,* 98: 135-142.

Guerrero, A., Camps, F., Cole, J., Riba, M., Einhorn, J., Descoins, C.H. and Lallemand, J.Y. 1981. Identification of a potential sex pheromone of the processionary moth *Thaumetopoea pityocampa* (Lepidoptera, Notodontidae). *Tetrahedron Letters,* 22: 2013-2016.

Halperin, J. 1984. Pityolure traps as tools in macromapping of the distribution of the pine processionary caterpillar. *La Yaaran,* 34: 25–29 (in Hebrew).

Halperin, J. 1985. Mating disruption of the pine processionary caterpillar by pityolure. *Phytoparasitica,* 133: 221–224.

Halperin, J. 1986. Application of pityolure for detection and control of *Thaumetopoea pityocampa* (Denis and Schiffermüller) (Lepidoptera: Thaumetopoeidae) in Israel. *Bulletin OEPP/EPPO,* 16: 627–632.

Hegazi, E.M., Konstantopoulou, M.A., Herz, A., Mazomenos, B.E., Khafagi, W., Agamy, E., Zaitun, A., Abd El-Aziz, G., Showiel, S. and Abdel-Rahman, S.M. 2009. Is mating disruption effective in controlling the olive moth, *Prays oleae*? *Crop Protection,* 28: 181-189.

Hegazi, E.M., Konstantopoulou, M.A., Milonas, P., Herz, A., Mazomenos, B.E., Khafagi, W.E., Zaitun, A., Abdel-Rahman, S.M., Helal, I. and El-Kemny S. 2007. Mating disruption of the jasmine moth *Palpita unionalis* (Lepidoptera: Pyralidae) using a two pheromone component blend: a case study over three consecutive olive growing seasons in Egypt. *Crop Protection,* 26: 837–844.

Hódar, J.A., Zamora, R. and Castro, J. 2002. Host utilisation by moth and larval survival of pine processionary caterpillar *Thaumetopoea pityocampa* in relation to food quality in three *Pinus* species. *Ecological Entomology,* 27: 292–301.

Huchon, H. and Demolin, D. 1971. La bioécologie de la procesionnaire du pin. Dispersion potentielle—dispersion actuelle. *Phytoma,* 225: 11–20.

Jacquet, J.S., Orazio, C. and Jactel, H. 2012. Defoliation by processionary moth significantly reduces tree growth: a quantitative review. *Annals of Forest Science,* 69: 857-866.

Jactel, H., Menassieu, P., Vétillard, F., Barthélémy, B., Piou, D., Frérot, B., Rousselet, J., Goussard, F., Branco, M. and Battisti, A. 2006. Population monitoring of the pine processionary moth (Lepidoptera : Thaumetopoeidae) with pheromone-baited traps. *Forest Ecology Management,* 235: 96–106.

Johansson, B.G., Anderbrant, O., Simandl, J., Avtzis, N.D., Salvadori, C., Hedenstrom, E., Edlund, H. and Hogberg, H.E. 2001. Release rates for pine sawfly pheromones from two types of dispensers and phenology of *Neodiprion sertifer. Journal of Chemical Ecology,* 27: 733–745.

Kanat, M. and Ozbolat, M. 2006. Mass production and release of *Calosoma sycophanta* L. (Coleoptera: Carabidae) used against the pine processionally moth, *Thaumetopoea pityocampa* (Schiff.) (Lepidoptera : Thaumetopoeidae), in biological control. *Turkish Journal of Zoology,* 30(2): 181-185.

Kerdelhué, C., Battisti, A., Burban, C., Branco, M., Cassel-Lundhagen, A., Ipekdal, K., Larsson, S., Lopez-Vaamonde, C., Magnoux, E., Mateus, E., Mendel, Z., Negrisolo, E., Paiva, M.R., Pivotto, I., Rocha, S., Ronnas, C., Roques, A., Rossi, J.P., Rousselet, J., Salvato, P., Santos, H., Simonato, M. and Zane, L. 2014. Genetic diversity and structure at different spatial scales in the processionary moths. In A. Roques (Ed.), Processionary moths and climate change: An update. Dordrecht: Springer.

Knight, A.L. 1996. Why so many mated female codling moths in disrupted orchards. *Proceedings of the Washington State Horticultural Association,* 92: 213–214.

Klun, J.A., Haynes, K.F., Bierl-Leonhardt, B.A., Birch, M.C. and Plimmer, J.R. 1981. Sex pheromone of female artichoke plume moth, *Platyptilia carduidactyla. Environmental Entomology,* 10: 763–765.

Lapointe, S. and Stelinski, L. 2011. An applicator for high viscosity semiochemical products and intentional treatment gaps for mating disruption of *Phyllocnistis citrella. Entomologia Experimentalis and Applicata,* 141: 145-153.

Leonardt, B.A., Cunningham, R.T., Dickerson, W.A., Mastro, V.C., Ridgway, R.L. and Schwalbe, C.P. 1990. Dispenser design and performance criteria for insect attractants. pp. 113-130. In R.L. Ridgway, R.M. Silverstein and M.N. Inscoe (Eds.), Behavior-modifying chemicals for insect management. Applications of pheromones and other attractants. Marcel Decker, New York, 761 pp.

Masutti, L. and Battisti, A. 1990. *Thaumetopoea pityocampa* (Den. and Schiff.) in Italy Bionomics and perspectives of integrated control. *Journal of Applied Entomology,* 110: 229-234.

Mazomenos, B.E., Ortiz, A., Mazomenos-Pantazi, A., Stefanou, D., Stavrakis, N., Karapati, C. and Fountoulakis, M. 1999. Mating disruption for the control of the olive moth, *Prays oleae* (Bern) (Lep.: Yponomeutidae) with the major sex pheromone component. *Journal of Applied Entomology,* 123: 247–254.

Miller, J.R. and Gut, L.J. 2015. Mating disruption for the 21st century: Matching technology with mechanism. *Environmental Entomology,* 44: 427-453.

Moffitt, H.R. and Westigard, P.H. 1984. Suppression of the codling moth populations on peach in southern Oregon through mating disruption with sex pheromone. *Journal of Economic Entomology,* 77: 1513–1519.

Nicolini, G. 1987. Biologia della procesionaria del pino in Trentino. Lotta tradizionale e con attattiviti sessuali di sintesi. *Dendronatura,* 8: 8–49.

Rausell, C., Martinez-Ramirez, A.C., Garcia-Robles, I. and Real, M.D. 1999. The toxicity and physiological effects of *Bacillus thuringiensis* toxins and formulations on *Thaumetopoea pityocampa*, the pine processionary caterpillar. *Pesticide Biochemistry Physiology,* 65: 44–54.

Roversi, P.F. 1985. Ossrvazioni sull' impiego di trappole a feromone sessuale di *Thaumetopoea pityocampa* (Denis and Schiffermüller) sul promontorio del Gargano (Lepidoptera: Thaumetopoeidae). *Redia,* 68: 1–17.

Salman, M.H., Giomi, F., Laparie, M., Lehmann, P., Pitacco, A., Battisti, A. 2019. Termination of pupal diapause in the pine processionary moth *Thaumetopoea pityocamp*a *Physiological Entomology,* 44 (1) 53-59.

Semiz, G. Cetin, H. Isik, K. and Yanikoglu, A. 2006. Effectiveness of a naturally derived insecticide, spinosad, against the pine processionary moth, *Thaumetopoea wilkinsoni* Tams. (Lepidoptera: Thaumetopoeidae) under laboratory conditions. *Pest Management Science,* 62: 452–455.

Simonato, M., Mendel, Z., Kerdelhué, C., Rousselet, J., Magnoux, E., Salvato, P., Roques, A., Battisti, A. and Zane, L. 2007. Phylogeography of the pine processionary moth *Thaumetopoea wilkinsoni* in the Near East. *Molecular Ecology,* 16: 2273–2283.

Snedecor, G.W. and Cochran, W.G. 1989. Statistical Methods, 8th edn. Iowa State University Press, Ames, IA, USA.

Stastny, M., Battisti, A., Petrucco Toffolo, E., Schlyter, F. and Larsson, S. 2006. Host-plant use in the range expansion of the pine processionary moth, *Thaumetopoea pityocampa*. *Ecological Entomology,* 31:, 481-490.

Stelinski, L.L., Gut, L.J., Mallinger, R.E., Epstein, D. Reed, T.P. and Miller, J.R. 2005. Small plot trials documenting effective mating disruption of significant populations of oriental fruit moth, *Grapholita molesta* (Busck), using high densities of wax-drop pheromone dispensers. *Journal of Economic Entomology,* 98: 1267–1274.

Stelinski, L.L., Gut, L.J., Haas, M., McGhee, P. and Epstein, D. 2007A. Evaluation of aerosol devices for simultaneous disruption of sex pheromone communication in *Cydia pomonella* and *Grapholita molesta* (Lepidoptera: Tortricidae). *Journal of Pest Science,* 80: 225–233.

Stelinski, L.L., Miller, J.R., Ledebuhr, R., Siegert, P. and Gut, L.J., 2007B. Season-long mating disruption of *Grapholita molesta* (Lepidoptera: Tortricidae) by one machine application of pheromone in wax drops (SPLAT-OFM). *Journal of Pest Science,* 80: 109–117.

Suckling, D.M. 2000. Issues affecting the use of pheromones and other semiochemicals in orchards. *Crop Protection,* 19: 677–683.

Tiberi, R. and Niccoli, A. 1984. Osservazioni pluriennali sull' impiego di trappole con il feromone sessuale di *Thaumetopoea pityocampa* (Den. and Schiff.) (Lepidoptera: Thaumetopoeidae). *Redia,* 67: 129–144.

Vega, J.M., Moneo, I., Ortiz, J.C.G., Palla, P.S., Sanchis, M.E., Vega, J., Gonzalez-Muñoz, M. Battisti, A. and Roques, A. 2011. Prevalence of cutaneous reactions to the pine processionarymoth (*Thaumetopoea pityocampa*) in an adult population. *Contact Dermatitis,* 64: 220–228.

Weatherston, I. 1990. Principles of design of controlled-release formulations, pp. 93-112. In R.L. Ridgway, R.M. Silverstein and M.N. Inscoe lEds.), Behavior·modifying chemicals for insect management. Applications of pheromones and other attractants. Marcel Decker, New York, 761 pp.

Wilkins, R.M., McGuffog, D.R., Anerson, T.P., Plimmer, J.R., Pickett, J.A., Dawson, G.W., Griffith, D.C., Liu, X., MaCauly, E.D.M., Woodcock, C.M., Lie, R. and Meghir, S. 1984. Pesticides group symposium–recent developments in controlled release formulations for pest control. *Pest Science,* 15: 258–267.

Witzgall, P., Kirsch, P. and Cork, A. 2010. Sex Pheromones and their impact on pest management. *Journal of Chemical Ecology,* 36: 80–100.

Yilmaz, S., Karabőrklű, S. and Azizoğlu, U. 2013. Toxicity of native *Bacillus thuringiensis* isolates on the larval stages of pine processionary moth

Thaumetopoea wilkinsoni at different temperatures. *Turkish Journal of Agriculture and Forestry,* 37: 163-172.

Zada, A., Falach, L. and Byers, J.A. 2009. Development of sol–gel formulations for slow release of pheromones. *Chemoecology,* 19: 37–45.

Zdarek, J., Vrkoc, J., Hochmut, R. and Kolk, A, 1988. Male confusion on the nun moth with dispalure at high and low population densities. *Journal of Chemical Ecology,* 14: 537–547.

Zhang, Q.H. and Paiva, MR, 1998. Female calling behaviour and male response to the sex pheromone in Thaumetopoea pityocampa (Den. and Schiff.) (Lepidoptera: Thaumetopoeidae). *Journal of Applied Entomology,* 122: 353–360.

Received: 9 April 2019; Accepted: 16 November 2019

Αποτελεσματικότητα της παρεμπόδισης επικοινωνίας της πιτυοκάμπης, *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae), με χαμηλής συγκέντρωσης φερομόνη φύλου

Α. Μιχαηλάκης, Ε. Αναστασάκη, Π.Γ. Μυλωνάς, Δ.Π. Παπαχρήστος, Δ. Κοντοδήμας, Κ.Μ. Ποντικάκος, Δ.Γ. Ραπτόπουλος, Ν.Α. Μπαμπίλης και Μ.Α. Κωνσταντοπούλου

Περίληψη Η μέθοδος παρεμπόδισης των συζεύξεων (ΠΣ) έχει εφαρμοστεί με επιτυχία για τον έλεγχο πληθυσμών πολλών επιβλαβών λεπιδοπτέρων εντόμων. Τα έτη 2010 και 2011 πραγματοποιήθηκαν σε ένα αστικό πάρκο, πειράματα πεδίου για την αξιολόγηση της αποτελεσματικότητας της παρεμπόδισης της χημικής επικοινωνίας της πιτυοκάμπης, *Thaumetopoea pityocampa,* χρησιμοποιώντας μορφοτυποποιημένη φερομόνη φύλου σε χαμηλή συγκέντρωση. Η αποτελεσματικότητα της ΠΣ αξιολογήθηκε συγκρίνοντας τις συλλήψεις των αρσενικών *T. pityocampa* σε παγίδες φερομόνης, σε περιοχές που εφαρμόστηκε η ΠΣ και σε περιοχές που δεν έγινε καμία εφαρμογή (μάρτυρας). Τον 1^{ο} χρόνο της εφαρμογής της μεθόδου το ποσοστό του αποπροσανατολισμού των αρσενικών κυμάνθηκε από 85 έως 100% κατά τη διάρκεια του 1^{ου} μήνα της πτητικής περιόδου και 95-100% σε όλη τη διάρκεια ολόκληρης της πτήσης κατά το 2^{ο} έτος. Το ποσοστό φερομόνης που παρέμεινε στη μήτρα του πολυμερούς μετά από 7 εβδομάδες σε εργαστηριακές συνθήκες γήρανσης ήταν σχεδόν 30%. Συνδυάζοντας τα αποτελέσματα απελευθέρωσης της φερομόνης με τα αποτελέσματα του αποπροσανατολισμού των αρσενικών μπορούμε να συνάγουμε ότι μετά από 7 εβδομάδες η συγκέντρωση της φερομόνης που απομένει εξακολουθεί να είναι επαρκής για την επίτευξη της ΠΣ. Η μελέτη αυτή υποδεικνύει ότι η διάχυση του κύριου συστατικού της φερομόνης φύλου (Z) -13-δεκαεξανο-11-υνυλ οξικού εστέρα σε συγκέντρωση 20 g/ha στον αέρα σε μία εφαρμογή ανά πτητική περίοδο είναι επαρκής ώστε να επηρεάσει τον προσανατολισμό των αρσενικών του *T. pityocampa*. Δεδομένου ότι η παρεμπόδιση συζεύξεων είναι μια περιβαλλοντικά ασφαλής μέθοδος για τον έλεγχο των επιβλαβών εντόμων, θα μπορούσε να αποτελέσει ένα πολύτιμο εργαλείο για τον έλεγχο του *T. pityocampa* σε αστικές περιοχές και σε πάρκα.

Hellenic Plant Protection Journal **13:** 42-53, 2020