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# Evaluation of Mating Disruption For the Control of *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae) in Suburban Recreational Areas in Italy and Greece

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## Abstract

*Thaumetopoea pityocampa* (Denis and Schiffermüller) is a severe defoliator of various species of *Pinus* and *Cedrus*, while the urticanting hairs produced by its larvae cause public health problems for humans and pets. In the present study, we report results of trials (from summer 2015 until winter 2017) of mating disruption for management of *T. pityocampa* in different areas of Italy and Greece. Overall, the total number of male moths captured in mating disruption-treated plots over each season (70) was significantly lower than the respective number in untreated plots (780). The total number of winter nests was likewise significantly less in the mating disruption plots (13) compared with control plots (147). Our results indicate that mating disruption can be an important tool for judicious, insecticide-free control of *T. pityocampa* in urban, suburban, and recreational areas, where many alternative control measures are not available.

**Key words:** Mating disruption, pheromones, *Thaumetopoea pityocampa*, pines, recreational areas

The pine processionary moth, *Thaumetopoea pityocampa* (Denis and Schiffermüller), is the main pest for *Pinus* (Pinales: Pinaceae) and *Cedrus* (Pinales: Pinaceae) in all countries around the Mediterranean. Its larvae can cause serious defoliation, especially in young trees, whereas larval setae may pose a serious threat for human and animal health, as they can cause respiratory and dermal problems (Ziprkowski and Rolant 1966, Rodriguez-Mahillo et al. 2012, Battisti et al. 2015, Moneo et al. 2015). These effects occur not only when the larvae are present, but also during the following season because of the persistence of allergenic hairs in the remains of winter nests (Lamy 1990, Vega et al. 2011, Bonamonte et al. 2013). This is particularly important in the case of urban, suburban, and recreational areas across the Mediterranean basin, given that pines are among the main tree species that occur in inhabited zones.

Currently, the most effective strategy for the control of this moth involves a combination of preventive methods (e.g., planting policies and methods for early detection) and curative methods (e.g., trapping of adults, elimination of caterpillar nests, trapping of caterpillars, and application of insecticides). Nevertheless, the methods that are used for this purpose may provide insufficient levels of control and/or endanger human health. For example, removal of the nests is not always possible in all trees, especially in high-density pine stands or when pine trees are tall. Moreover, insecticide applications in urban and suburban environments may be ineffective because some parts of the trees remain untreated. Chemical spraying in inhabited areas often triggers complaints by residents. Biological control is an option but has to be stringently applied to achieve a satisfactory level of control (Barbaro and Battisti 2011, Martin et al. 2012, Auger Rozenberg et al. 2015, Martin 2015).

Mating disruption has long been regarded as a viable option for noninsecticide control, especially in the case of Lepidoptera (Miller and Gut 2015). For mating disruption, artificial female pheromone is released in a treated area in order to compete with pheromone produced by calling females, and this can drastically reduce mate finding by males and thus mating (Cardé and Minks 1995). The most well-known example of successful mating disruption use in crop protection is its application against the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), in apple orchards (Angeli et al. 2007, Stelinski et al. 2007, Trona et al. 2009, Walker et al. 2013, McGhee et al. 2016). For instance, the application of mating disruption in commercial apple orchards in New Zealand considerably reduced the seasonal pheromone trap catches and the number of necessary insecticide sprays against *C. pomonella* (Walker et al. 2013). In storage environments, Athanassiou et al. (2016) found that a mating disruption formulation that contained *Z,E,*-9,12-tetradecadienyl acetate (ZETA), the male attractant of stored-product moths (Pyralidae), was able to reduce moth activity and progeny production of the Mediterranean flour moth, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae), in a commercial storage facility. In general, mating disruption has been successfully evaluated and commercialized for a very wide diversity of moths of economic importance (Gordon et al. 2005, Stelinski et al. 2007, Hoshi et al. 2016). Nevertheless, there are disproportionately few data for moth species that are important pests of forest trees, apparently due to the considerable cost of a mating disruption-based strategy applied on a large scale against these species, in relation to the actual benefits for the forest resource in financial terms. Still, mating disruption has demonstrated efficacy against important pests of forest trees, including gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae) (Schwalbe and Mastro 1988, Leonhardt et al. 1996, Thorpe et al. 1999, Onufrieva et al. 2008, 2019) and the Douglas fit tussock moth, *Orgyia pseudotsugata* (McDunnough) (Lepidoptera: Lymantriidae) (Cook et al. 2005).

*Thaumetopoea pityocampa* is a univoltine species (Athanassiou et al. 2007, 2017). Females lay eggs in summer around the pine needles, and larvae hatch after a period of 1–1.5 mo and feed at night on needles during summer and autumn. They construct communal silky nests among branches for protection during the day, and they overwinter in them until late winter or early spring. Larvae have five instars. Fifth instars leave the tree in a procession to pupate in the ground. Adult moths start emerging in the beginning of summer and fly at night until late autumn, depending on the prevailing temperatures (Battisti et al. 2015, Colacci et al. 2018). In a recent work, Athanassiou et al. (2017) found that the flight activity of this species in Greece is considerably longer than in Spain and Italy, which likely leads to long oviposition periods that require repeated insecticide applications, as eggs and newly hatched larvae may exist for several months. Univoltine species may be ideal for mating disruption, as a single annual application may lead rapidly to population suppression, without the need to reapply the method; however, timing of the application is critical. The potential of mating disruption for control of univoltine pests of forest trees has already attracted scientific interest (Halperin 1985, Schwalbe and Mastro 1988, Leonhardt et al. 1996, Thorpe et al. 1999, Anderbrant 2003). In one of the several studies regarding mating disruption application against *L. dispar*, Leonhardt et al. (1996) showed that low-density populations of this species were effectively controlled for at least 3 yr by annual aerial pheromone applications. However, the evaluation of the success of mating disruption systems has certain difficulties and requires replicates in space and time (Trematerra et al. 2011). Studies on mating disruption carried out against *T. pityocampa* have been limited mostly to pilot studies carried out several decades ago in small areas (Halperin 1986, Baldassari et al. 1994). At that time, the high cost of mating disruption application, as well as the repeated pheromone applications that were necessary to cover the entire moth flight period, were the main constraints for mating disruption implementation on a large scale (Halperin 1986). Currently, pheromone cost has been considerably reduced; moreover, the slow-release of pheromone can be easily achieved with novel types of dispensers. In this framework, the objective of the present study was to evaluate mating disruption for the control of *T. pityocampa* on a large scale, in different areas of Italy and Greece, for two consecutive years.

## Materials and Methods

### Experimental Sites

Trials were conducted from summer 2015 to winter 2017 in one site in Italy (Marinelle) and two sites in Greece (Goritsa Hill and the Institute of Agricultural Sciences [IAS]). An overview of the main characteristics of each site is provided in Table 1. Two 1-ha plots (two 2-ha plots at Goritsa Hill) at least 1 km distant from each other were randomly assigned to mating disruption or untreated control. In the second year of experimentation, the same plots were used as mating disruption and control plots in all sites. Apart from easy access, all three areas were selected because, in preliminary on-site inspections in January 2015, they had been found infested by *T. pityocampa*. In all areas in Italy and Greece, there were no insecticide applications during the entire experimental period.

### Application of Mating Disruption

In both years, traps baited with lures containing the sex pheromone component (13*Z*)-13-hexadecen-11-yn-1-yl acetate (commercially

**Table 1.** Main characteristics of the three experimental sites

| Site | Marinelle | Goritsa Hill | IAS |
|---|---|---|---|
| Location | Petacciato, Campobasso, Molise, central–south Italy | Volos, Magnissia, Thessaly, central Greece | Amaroussion, Attica, southern Greece |
| Coordinates | 42°02′27″N, 14°50′14″E | 39°21′10″N, 22°58′16″E | 38°03′45.1″N, 23°49′05″E |
| Total size | 35 ha | 120 ha | 65 ha |
| Main pine vegetation | *Pinus halepensis* Miller | *Pinus brutia* Tenore | *Pinus halepensis* Miller |
| Pine tree age | 50 yr | 70 yr | 70 yr |
| Pine tree density | 650 trees/ha | 200 trees/ha | 180 trees/ha |
| Use | Recreational park | Pine suburban forest | Pine suburban forest |

known as pityolure; Athanassiou et al. 2007) were suspended on pine branches to monitor adult male *T. pityocampa* flight activity before and after mating disruption application and to determine the beginning of the flight period (Table 2). In Marinelle, traps were distributed diagonally within the square plots. In Goritsa and IAS, each plot was divided into four subplots within which three pheromone traps were evenly distributed with a minimum 40-m separation. The mating disruption plots were treated with pityolure formulated in paste (ThauPi-polymix, 2% active ingredient, NovAgrica). A 250-g syringe operated with a standard caulk gun was used to apply pheromone paste in small drops of approximately 2.5 g, with one drop applied per tree. In Marinelle, a ladder was used for the application of the pheromone paste on pine branches at 4–5 m height. In Marinelle, approximately 50% of the trees present in each plot were treated with pheromone paste, whereas as in Goritsa and IAS the majority of the trees (>95%) received an application. Occasionally, landscape features of the plot did not permit application on certain trees in Goritsa and IAS. The total concentration of pityolure applied in each plot was 20 ± 0.5 g active ingredient/ha. For monitoring of *T. pityocampa* adult male flight activity before and after mating disruption application, pheromone traps were checked at regular intervals (twice weekly in Italy and weekly in Greece) to count and remove captured adults. The specific features of the mating disruption application process followed in each site are summarized in Table 2. Additionally, in winter 2016 and winter 2017, the winter nests that had been created by *T. pityocampa* larvae on colonized pine trees of the experimental plots (24, 240, and 160 pine trees per plot in Marinelle, Goritsa Hill, and IAS, respectively) were counted visually. In Marinelle, nest counts were performed on 12 trees positioned around each trap (Trematerra et al. 2019), whereas in Goritsa and IAS trees were selected randomly. Current-year nests were easily distinguished from older nests, as they were white and full of larvae, in contrast to old nests which were grayish-brown and empty of larvae.

### Data Analysis

The unit of replication was each site ($n$ = 3). Since male captures in traps were nonnormally distributed according to Shapiro–Wilk's test (Zar 1999), data were analyzed using the nonparametric Mann–Whitney $U$ test for independent samples (Helbig et al. 2016). Our response variable was the total number of adult *T. pityocampa* male captures in mating disruption-treated and untreated plots (after mating disruption application), and data were pooled for both years of experimentation at each site. The same procedure was also followed for the mean number of nest counts per pine tree. For all tests, statistical significance was set at $P$ = 0.05, whereas all analyses were conducted using the SPSS software (SPSS Corp. 2017).

## Results

The mean numbers of male *T. pityocampa* adults captured in the control and mating disruption plots for each trap-inspection date at each site in 2015 and 2016 are presented in Fig. 1A–F, whereas the total number of male adults captured in pheromone-baited traps after mating disruption application, the total number of nests, as well as the mean number of nests per pine tree in the mating disruption and the control plots in each site for each year of experimentation are listed in Table 3. The total number of male moths captured in mating disruption-treated plots after mating disruption application (139.0 ± 95.6; pooled for both years) was significantly lower than that in untreated plots during the same interval (1402.3 ± 782.9; df = 2, $z$ = −1.964, $P$ = 0.05). It is noteworthy that in Goritsa Hill in 2016 (Fig. 1D), the number of males captured in the mating disruption plots dropped considerably postapplication, whereas catches in the control site did not change during the same period. The mean number of winter nests per pine tree pooled for both seasons for each site was also significantly lower in the mating disruption plots (0.10 ± 0.02) compared with control plots (0.92 ± 0.38; df = 2, $z$ = −1.993, $P$ = 0.04).

## Discussion

According to the data reported here, mating disruption is able to disrupt the flight, mating behavior, and reproductive success of a large proportion of the population of *T. pityocampa*, which clearly suggests that this method can be used for the control of this species. Trap shut-down is not a reliable indicator for success of this method (Cardé and Minks 1995), and this is why we evaluated the number of nests in the treated and untreated areas, which further confirmed the efficacy of mating disruption.

One of the key elements in the successful implementation of mating disruption is the size of the area treated. When mating disruption is limited to a small area, it may fail due to immigration of

**Table 2.** Summary of the MD application process followed in the three experimental sites

| Site | Marinelle | Goritsa Hill | IAS |
|---|---|---|---|
| Plot size | 1 ha | 2 ha | 1 ha |
| Traps | G-traps[a] | Funnel traps[b] | Funnel traps[b] |
| Pheromone lure in trap | Kenogard[c] | ThauPi[d] | ThauPi[d] |
| Trap lure release rate | 10 µg/d | 10 µg/d | 10 µg/d |
| Pheromone application height | 4–5 m | 1.5 m | 1.5 m |
| Trap installation height | 5–6 m | 2 m | 2 m |
| Pheromone application 2015 | 27 July | 29 Aug. | 13 Aug. |
| Monitoring period 2015 | 11 June–22 Sept. | 8 Aug.–2 Oct. | 14 July–16 Nov. |
| Pheromone application 2016 | 21 July | 24 Aug. | 10 Aug. |
| Monitoring period 2016 | 7 July–22 Sept. | 10 Aug.–23 Nov. | 15 July–3 Nov. |
| Pheromone release rate from paste | 550 µg/d | 550 µg/d | 550 µg/d |
| Traps per plot | 2 | 12 | 12 |

[a]SEDQ, Barcelona, Spain.
[b]Uni-trap, Agrisence BCS, Pontypridd, United Kingdom.
[c]Barcelona, Spain.
[d]NovAgrica, Athens, Greece.

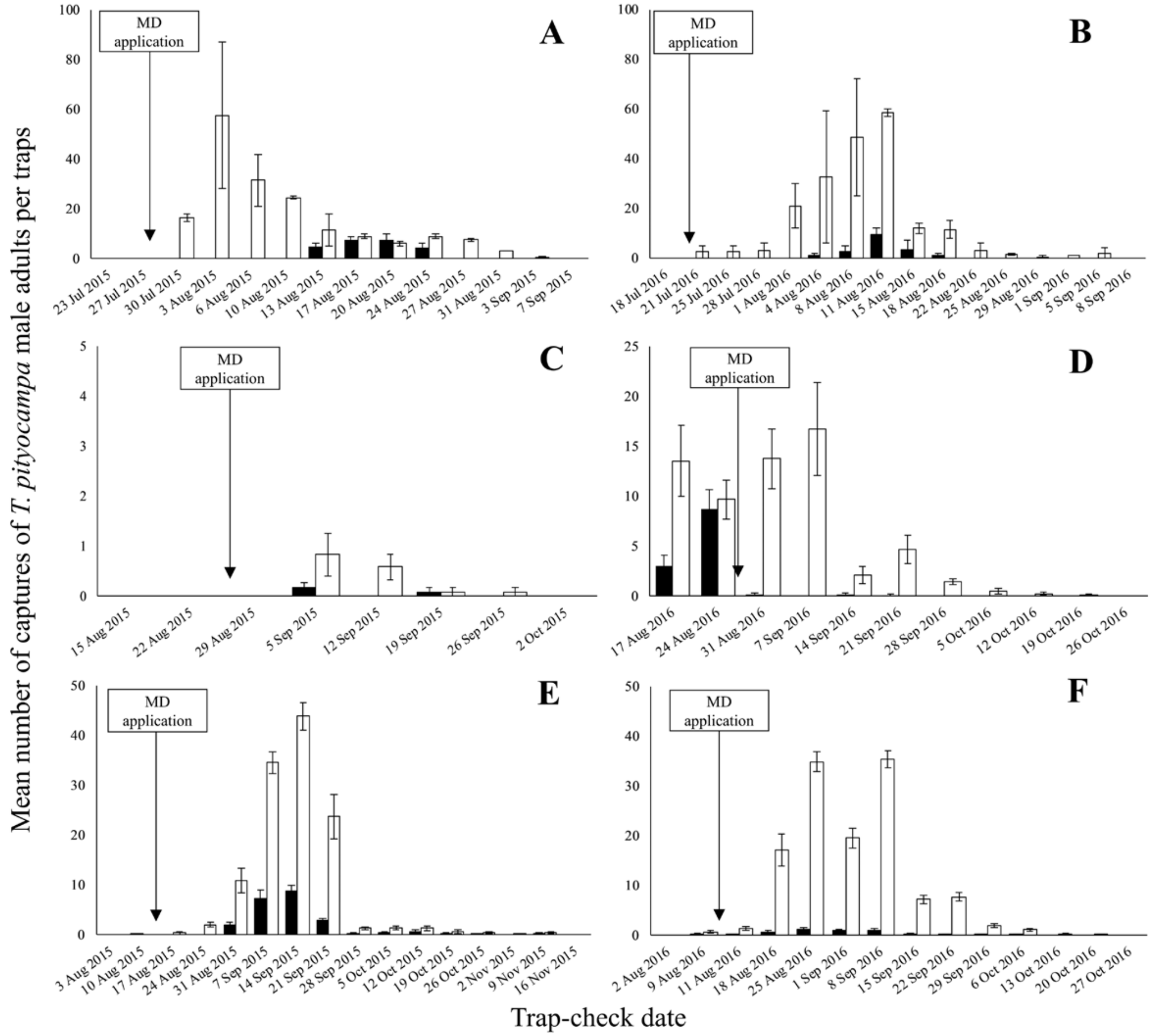


**Fig. 1.** Mean number (±SEM) of male adults of *T. pityocampa* captured in pheromone-baited traps in the control and mating disruption plots in Marinelle, Italy during 2015 (A) and 2016 (B), in Goritsa Hill, Greece during 2015 (C) and 2016 (D) and in IAS, Greece during 2015 (E) and 2016 (F).

mated females from surrounding, untreated areas, whereas large areas are overall less influenced by female immigration during application of mating disruption (Schwalbe and Mastro 1988, Sanders 1989, Il'ichev et al. 1998, Albajes et al. 2002, Ambrogi et al. 2006). For example, Il'ichev et al. (1998) reported that mating disruption application did not prevent damage at the edge of peach orchards, due to mated females of the oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), migration from adjacent peach orchards where mating disruption was not applied. It is often suggested that a barrier should be created at the peripheral zones in the mating disruption-treated area, through spraying conventional insecticides, in order to mitigate the influence of immigrating mated females from outside of the mating disruption-treated zone (Howell et al. 1992, Knight et al. 1995). Our experiments were carried out in relatively small plots surrounded by large areas of pine trees that were infested and untreated. Despite this, the method worked successfully, which is encouraging. This outcome may be attributed to the behavior of *T. pityocampa* adult females, which, in contrast with adult males, have very limited flight activity (Battisti et al. 2015). Hence, immigration of mated *T. pityocampa* females into mating disruption-treated areas would be expected to be rather limited, and this factor should increase the likelihood of success of the method. We assume that most of the nests found inside the mating disruption-treated areas originated from females able to mate within these areas, and not from females that migrated from untreated zones. However, further experimental work is needed to test this hypothesis.

Apart from the size of the treated area, the height on which the mating disruption paste and the pheromone traps are placed may affect the efficacy of the method and validity of its evaluation. For the related oak processionary moth, *Thaumetopoea processionea* L. (Lepidoptera: Thaumetopoeidae), Williams et al. (2013) showed that pheromone traps caught significantly higher numbers

**Table 3.** Total number of male adults of *T. pityocampa* captured in pheromone-baited traps, total number of nests, and mean number (±SEM) of nests per pine tree in the MD and the control plots in Marinelle, Italy, Goritsa Hill, Greece and IAS, Greece

| | | 2015–2016 | | | 2016–2017 | | |
|---|---|---|---|---|---|---|---|
| | | Marinelle | Goritsa Hill | IAS | Marinelle | Goritsa Hill | IAS |
| Total number of captures | Control | 353 | 19 | 1446 | 400 | 474 | 1515 |
| | MD | 49 | 3 | 272 | 35 | 5 | 53 |
| Total number of nests | Control | 21 | 235 | 71 | 12 | 564 | 59 |
| | MD | 3 | 50 | 18 | 1 | 14 | 8 |
| Number of nests/tree | Control | 0.87 ± 0.12 | 0.98 ± 0.11 | 0.44 ± 0.06 | 0.50 ± 0.12 | 2.35 ± 0.24 | 0.37 ± 0.06 |
| | MD | 0.12 ± 0.07 | 0.21 ± 0.05 | 0.11 ± 0.03 | 0.04 ± 0.04 | 0.06 ± 0.02 | 0.05 ± 0.02 |

of adults when placed in the upper part of the tree canopy of oak trees (10–15 m) compared with the lower (3–5 m) and middle (5–10 m) sections. However, trap height did not affect the number of males of the citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae; Lapointe et al. 2015), or the navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) (Girling et al. 2013), caught in pheromone traps, indicating that males of these species are active throughout the canopy. Our data suggest that the height at which the pheromone paste and the traps were placed may not play a critical role for mating disruption of *T. pityocampa*. Reduction in trap catches and nests was similar in Italy and Greece, irrespectively of the height at which the paste and the traps had been suspended.

Given that pine is a forest tree which is particularly abundant in the Mediterranean, and forests of Northern Europe, an area-wide application of mating disruption for the control of this species may not be viable, for two main reasons. First, due to the often large size of forest areas, treatment of each tree may not be practical in terms of human resources and access in all target areas. Second, mating disruption is more commonly used for agricultural pests for which increased yield over single years may compensate the cost of the application. In contrast, the benefits of such an application cannot be calculated easily for forests, and costs may not be recovered for many years. Nevertheless, the application of mating disruption against *T. pityocampa* could be justifiable for urban, suburban, and recreational areas, in terms of economic viability and ease of application. This is particularly important considering the risks for public health in urban and suburban areas by the presence of *T. pityocampa* larvae.

*Thaumetopoea pityocampa* is apparently becoming a more widespread problem. Numerous studies in recent years have documented that this species has invaded pine forests in Northern Europe, including forests with greater elevations and latitudes than were previously considered suitable for its development and population growth (Battisti et al. 2005, Buffo et al. 2007, Robinet et al. 2007, 2010, 2014). In this regard, mating disruption might be applied in invaded areas as a mean to mitigate further range expansion, similar to its use in the *L. dispar* 'slow-the-spread' program in the United States (Sharov et al. 2002). In this regard, trapping is essential in order to identify low density population zones that are ideal for mating disruption.

To conclude, our findings indicated that mating disruption of *T. pityocampa* by hand application of pheromone paste to individual pine trees was effective for the control of *T. pityocampa* in spite of large areas of untreated pines in adjacent areas. This method was evidently efficacious within suburban areas, as our study areas included such sites. Based on the above, mating disruption may provide a practical alternative to insecticide sprays and tree pruning for suppressing populations of *T. pityocampa*.

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