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# Annual flight activity of *Dioryctria mendacella* (Lepidoptera: Pyralidae) in southern Portugal

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Abstract The pine cone moth, Dioryctria mendacella, is one of the most important pests affecting cones of Mediterranean pines in southern Europe. The larvae cause damages to cone tissues and seeds, but despite its pest status, the biology of D. mendacella is largely unknown, specifically the seasonal flight activity of the adults. To overcome this, we studied the annual flight activity of D. mendacella in southern Portugal with traps baited with the sex pheromone. Fifteen white delta sticky traps were placed on the lower branches of grafted Pinus pinea trees, and pyralid moths were collected weekly for one year. We captured a total of 3.555 individuals, corresponding to a flight period from February to early December. Initial captures were relatively low but subsequently increased during the summer and early autumn months, and peaking in September. However, no captures occurred from early December to late February. Multiple regression analysis detected a positive and significant correlation between moth captures and temperatures at dusk, suggesting a

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P. Naves · E. de Sousa GREEN-IT Bioresources for Sustainability, ITQB NOVA, Av. da República, Quinta Do Marquês, 2780-157 Oeiras, Portugal dominant twilight/nocturnal flight activity for *D. mendacella*. Overall, we conclude that Delta traps baited with the sexual pheromone are effective in capturing adult moths of *D. mendacella* throughout the year, and our results suggest the occurrence of two (or more) annual generations for this pest, supporting similar inferences from other studies. The use of traps baited with sex-pheromones is a new tool for the development of integrated pest management strategies against *D. mendacella*, offering also the possibility of studying in detail the biology and population dynamics of this pest.

**Keywords** Pine cone moth · Flight phenology · Pheromone-baited traps · *Pinus pinea* · Portugal

## Introduction

The pine cone moth, *Dioryctria mendacella* Staudinger (Lepidotera: Pyralidae) is one of the most important pests affecting pine cones in Europe and Northern Africa, with larvae feeding on cones of all ages and causing significant damage (Romanyk and Cadahia, 1992; El Alaoui El Fels and Roques, 2006; Mutke et al., 2013; Calama et al., 2017). Attacks are particularly important in Stone pine (*Pinus pinea* L.) production, affecting up to 20–30% of the nut production and being more severe in years of low harvest, when the damages may impact up to 70% of the pine-cones (Calama et al., 2017). Stone pine cones are harvested



for edible seeds (pine nuts) and are one of the most important non-wood forest products in Mediterranean forests, particularly in Spain and Portugal (Calama et al., 2020; Mutke et al., 2012).

The biology of *D. mendacella* has proven difficult to study, with all life stages present in spring and autumn and probable multiple generations which may overlap throughout the year. In the Iberian Peninsula, two annual generations can occur (Garre et al., 2022), although more generations are possible under favourable conditions (Romanyk & Cadahia, 2003). The lack of detailed knowledge on the life cycle of *D. mendacella* is surprising considering its importance as a forest pest, and additional research on its biology is needed to develop efficient control strategies (Calama et al., 2017; Romanyk & Cadahia, 2003).

The identification of the sex pheromone of *D. mendacella* offers new possibilities for using baited traps to study the seasonal flight patterns of the pest (Hall et al., 2017). Studies show that pine cone moth females produce (Z,E)-9,11-tetradecadienyl acetate (ZE9,11–14:Ac) and (Z,Z,Z,Z,Z)-3,6,9,12,15-pentacosapentaene (ZZZZZ3,6,9,12,15–25:H), with the former eliciting a strong EAG response from males while no response could be recorded for the latter. In field trapping tests, both compounds were individually unattractive to males, but blends of the two were highly attractive (Hall et al., 2017).

Considering these recent developments and appraising the importance of *D. mendacella* as a pest of stone pines (Naves et al., 2022; Sousa et al., 2017), in this paper we describe the flight activity of the pine cone moth in a location in southern Portugal for one year, studied with pheromone-baited traps in one of the most important cone-production regions of the country, and providing new and important data for the characterisation of the life cycle of this poorly-studied forest pest.

### **Materials and Methods**

White Delta sticky traps (Kenogard), totalling fifteen, were placed on the mid-lower branches (3 to 4 m above ground) of grafted *P. pinea* trees (20 years old) near Canha, southern Portugal (38°45′00′N; 8°31′55′′W; altitude 72 m). The region has a climate classification of 'Csa' according to the Köppen-Geiger system, corresponding to a typical Mediterranean climate with hot and dry-summers and mild and wet winters (Peel et al., 2007).

The pine forest where the study was carried out was subjected to grazing by cows and vegetation included herbaceous plants in the understory, Ulex shrubs and occasional cork oak (Quercus suber L.) trees. Traps were placed in pines distant  $\approx 20$  m apart, and baited with a red rubber septum impregnated with the pheromone attractant "Dioryctria mendacella" product number 50398 (Pherobank), containing (Z,E)-9,11-tetradecadienyl acetate and (Z,Z,Z,Z,Z)-3,6,9,12,15-pentacosapentaene at 0.55 mg/lure (0.79 g/kg). Pheromones were changed every four weeks, before the five-week replacement recommended by the manufacturer. Traps were placed from 18 March 2021 to 24 March 2022, collecting weekly (every 6-7 days) the insects captured and replacing the white sticky cards.

The cardboards with insects were taken to the INIAV laboratories at Oeiras for identification and counting. Adult moths were identified by the senior author (PN) to species level based on morphological characters described by Knölke (2007), considering the wing colouration and venation, unipectinate antennae, and body/wing dimensions.

When morphological identification was uncertain, the damaged or dubious specimens which could not be clearly assigned as D. mendacella were subjected to DNA sequence-based identification. Genomic DNA was extracted from individual insects using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, US) according to the manufacturer's instructions. A fragment of the mitochondrial gene cytochrome c oxidase I gene (COI) was amplified using the universal primer pair LCO1490 and HCO2198 (Folmer et al., 1994). Polymerase chain reaction (PCR) was conducted in a 25 µL final reaction volume, containing 12.5 µL of Supreme NZYTaq II DNA polymerase Master Mix (NZYTech, Lisbon, Portugal), 1 µL of DNA template, 1 µL of each forward and reverse primer (10 µM) and 8.5 µL of molecular-grade water (Sigma-Aldrich, St. Louis, MI, USA). Amplification reactions were performed in the thermocycler Biometra TAdvanced (Analytik Jena, Germany) and the temperature program for amplification consisted of an initial denaturation of 94 °C for 2 min followed by 40 cycles of 94 °C for 1 min, 45 °C for 2 min, and 72 °C for 3 min and a final extension of 72 °C for 5 min (Folmer et al., 1994). Amplified products were visualized under UV light on a 1.5% agarose gel to confirm successful amplification. All the PCR products of the expected size were purified using ExoSAP-IT<sup>™</sup> PCR Product Cleanup Reagent (ThermoFisher Scientific, Pittsburg, PA, USA) following the manufacturer's instructions and then bidirectionally sequenced using the Sequencing facility at INIAV (Oeiras, Portugal). Nucleotide sequences were edited and analyzed using BioEdit v7.2.0 (Hall, 2007). The resulting consensus sequences were multi-aligned using ClustalW and the alignment revealed no differences. Molecular identification was made by comparing our sequences with those of *D. mendacella* and other relevant sequences of Dioryctria spp. available in the GenBank database of the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) homology search.

#### Statistical analyses

A parametric analysis of variance test (ANOVA) was used to compare mean annual captures between traps and between meteorological seasons, which in the Northern Hemisphere comprises March, April, and May for spring, June, July, and August for summer, September, October, and November for autumn, and December, January, and February for winter. The Fisher Least Significant Difference test (LSD) was used to compare means within each significant factor in the ANOVA.

To evaluate whether environmental factors were associated with weekly capture rates, we used multiple regression to model weekly captures of D. mendacella using local climatic variables provided by a conventional weather station installed 150 m from the field plot. The following variables were used in model construction: mean, maximum, and minimum daily temperatures, daily temperatures at sunrise and sunset, daily temperatures at the beginning (end of astronomical twilight) and after (beginning of astronomical twilight) "dark" night, number of days with a maximum temperature equal or above 20°C, 25°C, 30°C or 35°C, and number of days with a minimum temperature equal or below 15°C, 10°C or 5°C, daily mean values (or counts of days) over the previous 7-days were backward calculated. Each week×trap combination was treated as a unit of replication for model construction. Tests were made on the STATIS-TICA software, version 12 (StatSoft), and incorporate at Type I error rate of  $\alpha = 0.05$  for assigning statistical significance.

## Results

Over a period of one year, we captured a total of 3.555 male *D. mendacella*, with a flight period extending for more than eight months.

In 2021 insects were captured in the first week of sampling, from 25 March (Julian day 84) onwards, with initial captures relatively low during the spring months but increasing throughout the summer/ autumn months. There was a peak in September, with the highest catches on September 23<sup>rd</sup> (Julian day 266) with 332 males in one week, and captures continued until December 9<sup>th</sup> (Julian day 343), this being the last capture of the civil calendar year.

The absence of captures occurred during 11 weeks of the year, from early December to late February, with the first moths appearing on February 24<sup>th</sup> (Julian day 55) 2022 (Figs. 1 and 2).

Overall, each trap captured a mean of  $237 \pm 78$  (mean  $\pm$  SD) moths during the year, with significant differences between traps (F=3.1435; df=14; p<0.001). The highest weekly captures by a single trap were 34 moths, occurring during the week ending on September 23<sup>rd</sup>.

There were significant differences in captures between meteorological seasons (F=14.5747; df=3; p < 0.001), with a contrast of lower captures in winter and spring and higher numbers in summer and autumn (Fig. 3).

The multiple regression model was a relatively strong fit for predicting weekly trap captures ( $R^2=0.798$ ; F=10.767; df=3; p<0.001), and only detected a significant (and positive) correlation between captures and temperatures at the beginning of the "dark" night (end of astronomical twilight) (Table 1), while for the other variable's the correlations were not statistically significant.

Most of the insects were alive and in good conditions when collected and could be clearly identified as *D. mendaccella* by morphological characters. In order to assert the identity of 21 dubious specimens, a molecular identification was performed by sequencing a fragment of the COI gene, resulting in a PCR product of about 675 bp in agreement with the expected size established for this region. Fig. 1 Weekly annual captures of *Dioryctria mendacella* (mean $\pm$ SE) on pheromone traps from March 2021 to March 2022, Canha, Southern Portugal







BLAST hits showed that the partial sequences of COI gene were 100% identical (e-value 0.0) to *D. mendacella* isolates PP17 and PP16 from Portugal (accession numbers OK559625 and OK559626) (Naves et al., 2022), and to voucher BC ZSM Lep 51,355 (accession number KX040713) from Spain.

Novel sequences from this study were deposited in GenBank, accession numbers OP269725 to OP269745.

Very few other insect species were caught in the traps, and by-catch captures consisted mainly of Muscidae flies (Diptera).





Table 1 Estimated
regression parameters for
climatic variables after
a Multiple Regression.
Statistically, significant
correlations are marked in
bold

Climatic variables	В	SE	t-statistics	<i>p</i> -value
Mean daily temp	-0.890	0.967	-0.921	0.363
Maximum daily temp	0.012	0.746	0.015	0.988
Minimum daily temp	0.059	0.639	0.405	0.688
# days temp.≥20°C	0.146	0.297	0.489	0.627
# days temp.≥25°C	0.587	0.296	1.985	0.054
# days temp.≥30°C	-0.434	0.225	-1.925	0.062
# days temp.≥35°C	0.083	0.124	0.672	0.506
# days temp.≤15°C	-0.049	0.119	-0.408	0.686
# days temp.≤10°C	-0.013	0.196	-0.065	0.949
# days temp.≤5°C	-0.265	0.263	-1.008	0.320
Temperature at sunrise	0.627	0.839	0.747	0.460
Temperature at sunset	0.299	0.359	0.835	0.409
Femp. beginning ''dark'' night	0.971	0.429	2.262	0.029
Temp. end "dark" night	-1.117	0.840	-1.330	0.191

### Discussion

The Delta trap baited with the "Dioryctria mendacella" 50,398 pheromone was effective in capturing adult male moths of *D. mendacella* throughout the year and was very specific with very few by-catch captures of other insects.

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We report evidence for a prolonged period of flight activity extending from February to early December, with a peak in September. The seasonal flight activity of *D. mendacella* had not been studied in detail until now: isolated captures were reported from central Portugal from May to June and from August to November (Pires & Corley, 2007), while in the Algarve (southern Portugal) specimens are sporadically caught from February to November each year (Banza, 2022), resulting in an overall pattern of seasonal activity compatible with the one we report. In Spain, the seasonal flight activity is reported on April-June and October–November, suggesting a bivoltine life cycle of two annual generations (Gómez de Aizpirúa, 1991), although more generations are possible under favourable conditions (Romanyk & Cadahia, 2003). Our continuous captures throughout the year do not allow us to discriminate different generations; nevertheless, the increasing captures from June onwards suggest a first annual generation completed in early summer, with a subsequent second, or even third generation completed in early autumn and/ or with overlapping/sister generations also possible. The overlap of life stages among generations is common to several *Dioryctria* species worldwide (Merkel & Fatzinger, 1971; Whitehouse et al., 2011).

Other species of the Genus *Dioryctria* also exhibit extended seasonal flight activity (Grant et al., 1993; Merkel & Fatzinger, 1971; Roe et al., 2006; Whitehouse et al., 2011), although not as prolonged as we report for *D. mendacella*. Flight peaks of other *Dioryctria* species usually occur in June-July (Menassieu et al., 1989; Whitehouse et al., 2011) or August (Roe et al., 2006), and not in September as reported here for *D. mendacella*.

Regression analysis detected a positive and significant correlation only between moth captures and temperatures at the beginning of the "dark" night (end of astronomical twilight), which in the longest summer days of June/July initiates at 22:03 (10:03 PM), local time. Our results suggest that D. mendacella flight activity may take place mainly during the astronomical twilight/early night hours, which agrees with reports for other species of the genus that are active soon after dark (Fatzinger & Asher, 1971; Trudel et al., 1995; Menassieu et al., 1989). For Dioryctria disclusa Heinrich the flight of males in response to sex pheromone occurs between 3 to 5 h after sunset (DeBarr & Berisford, 1981), while for D. abietivorella it begins 2 h after sunset, peaks 4 h after sunset, and ends 1 h after dawn (Whitehouse et al., 2011). Furthermore, Triggiani (1986) mentions that Dioryctria pineae has crepuscular habits in Italy, while Menassieu et al. (1989) report nocturnal reproductive flight activity for Dioryctria sylvestrella in south-west France.

Although the traps were placed relatively close to each other ( $\approx 20$  m apart), there were significant differences in the number of captured moths. The discrepancies could result from differences in population density between trees with traps, considering that *D. mendacella* has a low dispersal propensity at

the local scale (Calama et al., 2017), although additional studies are needed to clarify this.

The pine cone moth D. mendacella is one of the most important pests affecting cone production in the Mediterranean region, and, furthermore, has recently caused damage to P. pinea grafted shoots in southern Portugal (Naves et al., 2022). Considering the experience of other species of the Dioryctria genus, the use of traps baited with sex-pheromones allows for to study biology and population dynamics of D. mendacella and offers a convenient, inexpensive, and specific survey technique to monitor the population levels between years and locations (DeBarr & Berisford, 1981; DeBarr et al., 2000; Grant et al., 1993; Hanula et al., 1984a, 1984b; Löfstedt et al., 2012; Roe et al., 2006; Strong et al., 2008; Whitehouse et al., 2011). According to Whitehouse et al. (2011), there is great potential for the use of synthetic sex pheromones to control pestiferous Dioryctria species, with trapping used on mating-disruption approaches within integrated pest management strategies incorporating silvicultural, mechanical, and biological techniques (Hall et al., 2017). Pheromonebased control tactics require information derived from reproductive-behaviour studies, which for D. mendacella should focus on characterising the phenology and seasonal flight activity in regions with contrasting climatic conditions, on assessing the most efficient location of traps on the tree canopy and trap density within the stands, and on quantifying the production/economic benefits in using traps as monitoring and control tools against this pest.

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Authors' contributions Pedro Naves and Edmundo de Sousa prepared the field trials, Pedro Naves conducted the field trials

and identified the insects, Filomena Nóbrega made the molecular analyses, Pedro Naves, Filomena Nóbrega and Edmundo Sousa wrote the manuscript text, Pedro Naves prepared the figures. All authors reviewed the manuscript.

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**Data availability** The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

#### Declarations

Ethics approval and consent to participate Not applicable.

**Consent for publication** All authors agreed with the content and give explicit consent to submit.

Competing interests Not applicable.

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