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Not just the cones: *Dioryctria mendacella* (Lepidotera Pyralidae) also attacks grafted pine shoots

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Abstract

The pine cone moth, *Dioryctria mendacella* Staudinger, is one of the most important pests affecting cones of Mediterranean pines in Portugal and in southern Europe. The females lay eggs on pine cones where the larvae cause damage to tissues and seeds resulting in deformations, abnormal growth, cone abortion and seed loss. Attacks of *D. mendacella* are restricted to cones, but in the spring of 2021, an unusual infestation by Lepidoptera larvae in the grafted shoots of young *Pinus pinea* L. trees was observed in Coruche, Portugal. Pine shoots exhibited resin exudation, frass, wilting and drying of the scion and the upper stock. Shoots were collected and taken to the laboratory, and emerging adult moths were identified as *D. mendacella* by morphological (including genitalia observations) and molecular analysis. We recorded 49.5% of failed graftings and considering nearby plantations (in the absence of insect attacks) we estimated *D. mendacella* was responsible for around 17% of the grafting failure. Cones collected from pines within the plantation showed no insect attack. We discuss why this pine cone moth population may have adopted this new feeding habit, and its possible implications to modern stone pine silviculture where grafting is a frequent technique.

Key words: pine cone moth, *Pinus pinea*, cone pest, coneworm, feeding habits, graft.

Introduction

Stone pine (*Pinus pinea* L.) cones are one of the most important non-wood forest products in Mediterranean forests, being collected annually from standing trees to obtain the edible seeds (pine nuts), which provide substantial incomes for forest owners (Mutke *et al*., 2012; Calama *et al*., 2020). In Portugal, stone pines occur both in natural ecosystems and in pure and mixed planted forests, in an area exceeding 193,000 ha which represents 6% of the country´s forested area (ICNF, 2019). Stands are predominantly managed for nut production and, secondarily, for timber production (Freire *et al*., 2019), with grafting employed to accelerate entry into production and improve cone yield of plantations (Mutke *et al*., 2012; Loewe-Muñoz *et al*., 2022).

Along with the cone weevil, *Pissodes validirostris* Gyllenhall (Coleoptera Curculionidae) (Roques, 1983), the pine cone moth, *Dioryctria mendacella* Staudinger (Lepidotera Pyralidae) is one of the most important pests of immature cones of *P. pinea* (Romanyk and Cadahia, 1992; Mutke *et al*., 2013; Calama *et al*., 2017), with the larvae boring galleries into cones of all ages and causing a reduction on production and yield (Roques, 1983; Innocenti and Tiberi, 2002). Despite its pest status, *D. mendacella* has a little-understood and complex life cycle with a minimum of two annual generations (Romanyk and Cadahia, 1992; Calama *et al*., 2017), with a prolonged flight period resulting on the infestation of cones during several months and on different phases of maturation.

Attacks by *D. mendacella* are restricted to cones causing resin exudates, partial abortion of the cones and severe damage to pine nuts. Nevertheless, a noteworthy attack on pine shoots was recently observed in Southern Portugal, representing the first record of this feeding habit which had not previously been reported for this widespread and well-studied forest pest, and which is here documented.

Materials and methods

In June 2021, local forest managers detected severe infestations by an unknown insect in a young *P. pinea* plantation near Coruche, southern Portugal (38.9088005 - 8.5151394; 46 m a.s.l.; 30.14 ha). The pines were four years old and had been grafted in mid-April. Local vegetation included scattered trees of maritime pine (*Pinus pinaster* Aiton) and cork oak (*Quercus suber* L.), along with adult (≈30 years) stone pine trees adjacent to the plantation.

A sample of 87 attacked shoots were randomly collected and taken to the INIAV laboratories at Oeiras for detailed analysis. Nine shoots were dissected, and the remaining were kept at 20 °C and 70% humidity to obtain adults.

Emerging insects were identified to species level based on morphological characters following the keys of Dombroskie (2011) and Knölke (2007). To confirm identification, a genitalia preparation for two additional specimens was made, following Robinson (1976) with minor adaptations.

Two specimens were randomly selected for molecular analysis. Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, US). Mitochondrial DNA (mtDNA) from the cytochrome c oxidase I gene (COI) was amplified using a polymerase chain reaction (PCR) with the primers LCO1490 and HCO2198 (Folmer *et al*., 1994) in the thermocycler Biometra TAdvanced (Analytik Jena, Germany). All reactions used a 25 µL volume, containing 12.5 µL of Supreme NZYTaq II DNA polymerase Master Mix (NZYTech), 1 µL of

DNA template, 1 µL of each forward and reverse primer (10 µM) and 8.5 µL of molecular-grade water (Merck). The amplification profile consisted of an initial denaturation of 94 °C for 2 minutes followed by 40 cycles of 94 °C for 1 minute, 45 °C for 2 minutes, and 72°C for 3 minutes and a final extension of 72 °C for 5 minutes (Folmer *et al*., 1994). Amplified products were purified using ExoSAP-IT™ PCR Product Cleanup Reagent (ThermoFisher Scientific) following the manufacturer's protocols and submitted to the Sequencing facility at INIAV. The newly obtained sequences were edited, analyzed using BioEdit v7.2.0 (Hall, 2007) and compared to those of *D. mendacella* and other relevant sequences of *Dioryctria* spp. available in the GenBank database using the BLAST homology search. Sequences generated in this study were deposited in GenBank, accession numbers OK559625 and OK559626.

To calculate the rate of failed graftings, in October we examined 210 grafted trees in random groups of 30 from seven alternating rows, from the edge to the interior of the stand. We could not discriminate the cause of the failure (insect or not) because the dried grafts were removed and destroyed in June/July as a sanitary measure, but we used the grafting failure in 2020 and 2021 in nearby plantations free of the insect attack (provided by the TMF company) to compare with our observations.

To assess the population levels of *D. mendacella* in the field we made a detailed visual observation of insect damage on 37 cones, of all ages, from grafted and non-grafted pines in the plantation.

Results

Insect attacks were restricted to the grafted shoots, which exhibited abundant resin exudation, frass, wilting and drying of the scion and the upper stock. Externally, attacks caused masses of white pitch exuding from the feeding sites of larvae, with occasional small oval emergence holes visible (only one per graft) (figure 1). Dissection of the shoots revealed Lepidoptera larvae and pupae in galleries in the xylem of the stock shoot, where the graft was wrapped with a protective plastic ribbon.

Figure 1. External insect damages and oval emergence hole on the grafted shoots.

Brownish frass and excrements, of small size and irregular shape, were evident at feeding sites, often mixed with resin. Irregular galleries were totally or partially concealed inside the stock shoot xylem, with approximately 4-6 cm in length (figure 2).

Figure 2. Detail of the larval gallery on the stock after removing part of the resin and frass.

A total of 48 adult moths emerged from the pine shoots over a period of four weeks. Adults were morphologically identified as *D. mendacella*, through both external characters and genitalia observation (Knölke, 2007). The molecular analysis corroborated the morphological identification, with BLAST hits sequences of COI mtDNA (635 bp) 100% identical (e-value 0.0) to *D. mendacella* voucher BC ZSM Lep 51355 (accession number KX040713) from Spain.

No other insect species were recovered from the shoots.

Grafting failed on 49.5% of the 210 pines analysed, while in nearby locations without insect attack the failure rate was 32 ± 10% (mean; SD); accordingly, we estimated *D. mendacella* infestations accounted for 17% of the grafting failures.

None of the cones collected in the plantation were attacked by insects.

Discussion and conclusions

To our knowledge, this is the first report of *D. mendacella* feeding on pine tissues other than cones. In Portugal *D. mendacella* is a well-known pest, considered the most abundant and important insect damaging pine cones (Sousa *et al*., 2017). Feeding on pine shoots has not previously been recorded for this species, although in 2017 a similar attack was observed on a nearby plantation (4.5 Km distant), even though at that time the insects were not identified (Santos Silva, personal observation).

It is unclear why this shoot-feeding habit occurred. With the trees producing few cones due to their young age, very high populations of the pine cone moth could have compelled the females to lay eggs on a less-preferred breeding substrate; nevertheless, the cones in the plantation were all free of attacks, suggesting the local population levels were not unusually high. Although no molecular differences from other populations were found, further research on more variable molecular markers (microsatellites, SNPs) could provide additional information on a possible genetic differentiation at the population level.

An important aspect of this newly observed feeding habit is that the larvae were found only in grafted shoots. We could not determine if oviposition occurred on the grafts or on other pine tissues (cones, bud scales) and the larvae subsequently migrated to the grafts after overwintering, as it occurs with *Dioryctria amatella* (Hulst) on slash and longleaf pines in North America (Meeker, 2021). Irrespective of the breeding material, larval development occurred on the grafting wounds, similarly to *Dioryctria abietivorella* (Grote) which also feeds on graft unions (Ruth, 1980) and on terminal shoots previously wounded by other insects (Shu *et al*., 1997), and to *Dioryctria sylvestrella* (Ratzeburg) which feeds on stems of young trees that have suffered mechanical damage (Langstrom *et al*., 2004), including pruning (Jactel *et al*., 1994). This suggests that the wounding and mechanical damage on the grafted shoots may have promoted this new feeding habit, although it remains unclear why it occurred only now if the local populations of *D. mendacella* have cohabited with grafted pines for several years (Cordeiro and Lopes, 1992; Carneiro *et al*., 2007), without causing similar damage (except for the 2017 attack).

Although new for *D. mendacella*, a comparable behaviour of shifting feeding habit was previously reported for *Dioryctria mutatella* Fuchs (=*Dioryctria simplicella* Heinemann), damaging shoots of young plantations of *Pseudotsuga menziesii* (Mirb.) Franco, in alternative to pine cones (Vouland *et al*., 1990). Similar behaviour has also been reported for *Dioryctria abietella* (Denis et Schiffermuller) feeding primarily on cones but also on twigs, buds, and insect-induced galls (Roques, 1983). In North America, larvae of some *Dioryctria* spp. feed on diverse host tissues such as needles, buds, flowers, shoots, and galls, in addition to 1st and 2nd year cones (Roe *et al*., 2011; Whitehouse *et al*., 2011; Meeker, 2021). Overall, these observations suggest that larvae of several *Dioryctria* spp. can feed on multiple pine tissues other than cones, even in the case of primarily cone-feeding species.

The attacks in Coruche caused the drying of the scion and upper stock, affecting the growth of the main shoot and resulting in the need to repeat the grafting. We recommend a detailed investigation of the local populations of *D. mendacella* to assess the origin of these shoot-infesting larvae (i.e., identification of the oviposition sites), and whether if this phenomenon is exceptional or if it represents an established and expanding breeding and/or feeding behaviour. If disseminated and recurring, this behavioural shift may affect pine cultivation and impact the widespread use of grafting in modern *P. pinea* silviculture.

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References

CALAMA R., FORTIN M., PARDOS M., MANSO R., 2017.- Modelling spatiotemporal dynamics of *Pinus pinea* cone infestation by *Dioryctria mendacella*.- *Forest Ecology and Management*, 389: 136-148.

CALAMA R., GORDO J., MUTKE S., CONDE M., MADRIGAL G., GARRIGA E., ARIAS M. J., PIQUÉ M., GANDÍA R., MONTERO G., PARDOS M., 2020.- Decline in commercial pine nut and kernel yield in Mediterranean stone pine (*Pinus pinea* L.) in Spain.- *iForest*, 13: 251-260.

CARNEIRO A., HALL D'ALPUIM M., VACAS DE CARVALHO M. A., PESSOA J., CARRASQUINHO I., 2007.- *Manual ilustrado de enxertia do pinheiro manso*.- Estação Florestal Nacional, Oeiras, Portugal.

CORDEIRO N. R., LOPES L. M., 1992.- Enxertia do pinheiro manso.- *DGF Informação*, 3: 9-11.

DOMBROSKIE J. J., 2011.- A matrix key to families, subfamilies and tribes of Lepidoptera of Canada.- *Canadian Journal of Arthropod Identification*, 17: 1-129.

FOLMER O., BLACK M., HOEH W., LUTZ R., VRIJENHOEK R., 1994.- DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates.- *Molecular Marine Biology and Biotechnology*, 3: 294-299.

FREIRE J. A., RODRIGUES G. C., TOMÉ M., 2019.- Climate change impacts on *Pinus pinea* L. silvicultural system for cone production and ways to contour those impacts: a review complemented with data from permanent plots.- *Forests*, 10: 152-169.

HALL T., 2007.- *BioEdit. Biological Sequence Alignment Editor for Win95/98/NT/2K/XP*.- Ibis Biosciences, Carlsbad, CA, USA. [online] URL: http://www.mbio.ncsu.edu/BioEdit/bioedit.html

ICNF, 2019.- *6° inventário florestal nacional, 2015, relatório final*.- Instituto da Conservação da Natureza e das Florestas, Lisboa, Portugal. [online] URL: http://www2.icnf.pt/portal/florestas/ifn/ifn6

INNOCENTI M., TIBERI R., 2002.- The cone and seed pests of *Pinus pinea* L. in central Italy.- *Redia*, 85: 21-28.

JACTEL H., MENASSIEU P., RAISE G., 1994.- Infestation dynamics of *Dioryctria sylvestrella* (Ratz.) (Lepidoptera: Pyralidae) in pruned maritime pine (*Pinus pinaster* Ait.).- *Forest Ecology and Management*, 67: 11-22.

KNÖLKE S., 2007.- *A revision of the European representatives of the microlepidopteran genus Dioryctria Zeller, 1846 (Insecta: Lepidoptera: Pyralidae: Phycitinae)*.- Ludwig Maximilians-Universität München, München, Germany.

LANGSTROM B., HELIÖVAARA K., MORAAL L. G., TURČÁNI M., VIITASAARI M., YLIOJA T., 2004.- Non-coleopteran insects, pp. 501-538. In: *Bark and wood boring insects in living trees in Europe, a synthesis* (LIEUTIER F., DAY K. R., BATTISTI A., GREGOIRE J. C., EVANS H. F., Eds).- Springer, Dordrecht, The Netherlands.

LOEWE-MUÑOZ V., DEL RÍO R., DELARD C., BALZARINI M., 2022.- Enhancing *Pinus pinea* cone production by grafting in a non-native habitat.- *New Forests*, 53: 37-55.

MEEKER J. R., 2021.- *Southern pine coneworm, Dioryctria amatella (Hulst) (Insecta: Lepidoptera: Pyralidae)*.- Entomology and Nematology Department, UF/IFAS Extension, EENY325.

MUTKE S., CALAMA R., GONZALEZ-MARTÍNEZ S. C., MONTERO G., GORDO F. J., BONO M., GIL L., 2012.- Mediterranean stone pine: botany and horticulture, pp. 153-201. In: *Horticultural Reviews Volume* 39 (JANICK J., Ed.).- Wiley-Blackwell, Hoboken, NJ, USA.

MUTKE S., PIQUÉ M., CALAMA R., 2013.- AGROPINE 2011 meeting conclusions.- *Options Méditerranéennes Série A. Séminaires Méditerranéens*, 105: 111-112.

ROBINSON G. S., 1976.- The preparation of slides of Lepidoptera genitalia with special reference to the Microlepidoptera.- *Entomologist's Gazette*, 27: 127-132.

ROE A. D., WELLER D. R., WELLER S. J., 2011.- Complexity in *Dioryctria zimmermani* group: incongruence between species limits and molecular diversity.- *Annals of the Entomological Society of America*, 104: 1207-1220.

ROMANYK N., CADAHIA D., 1992.- *Plagas de insectos en las masas forestales Españolas*.- ICONA: Colección técnica, Spain.

ROQUES A., 1983.- *Les insectes ravageurs des cônes et graines de conifères en France*.- INRA, Versailles, France.

RUTH D. S., 1980.- *A guide to insect pests in Douglas-fir seed orchards*.- Pacific Forest Research Centre, Canadian Forestry Service, Victoria, British Columbia, Canada.

SHU S., GRANT G. G., LANGEVIN D., LOMBARDO D. A., MACDONALD L., 1997.- Oviposition and electroantennogram responses of *Dioryctria abietivorella* (Lepidoptera: Pyralidae) elicited by monoterpenes and enantiomers from eastern white pine.- *Journal of Chemical Ecology*, 23: 35-50.

SOUSA E., PIMPÃO M., VALDIVIESSO T., NAVES P., BRANCO M., 2017.- Cone pests of stone pine in the Mediterranean Basin.- *Options Méditerranéennes Série A. Séminaires Méditerranéens*, 122: 91-107.

VOULAND G., CORNIC J.F., DU MERLE P., 1990.- Un cas de dépérissement de jeunes Douglas lié à des attaques d'insectes (*Dioryctria mutatella*, Lepidoptere Pyralidae; *Pityogenes quadridens*, Coleoptere Scolytidae).- *Revue Forestière Française*, 42: 510-512.

WHITEHOUSE C., ROE A. D., STRONG W., EVENDEN M., SPERLING F. H., 2011.- The biology and management of North American cone-feeding *Dioryctria* species.- *The Canadian Entomologist*, 143: 1-34.

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