



UNIVERSITY OF AGRICULTURE  
IN KRAKOW



Forest prevention and control strategy implemented by Portugal against the priority quarantine pest in Europe pine wood nematode (*Bursaphelenchus xylophilus* (Steiner et Bühner) Nickle et al.) since its detection in 1999

### the EPPO Jens-Georg Unger Plant Health Fellowship

#### The fellowship participant:

Magdalena Kacprzyk

University of Agriculture in Krakow  
Department of Forest Ecosystems Protection

#### The fellowship supervisor and host institution:

José Manuel Gomes Rodrigues  
Institute for Nature Conservation and Forests, IP  
Department of Forest Management and Enhancement

**The fellowship period: 26-06-2023 to 26-08-2023**



## Fellowship Background



### Portugal - Containment Stage

Presence of pine wood nematode since 1999

Well-established national system for forest pest management and risk governance

Implementation of coordinated control and containment programmes (monitoring, sanitary measures, movement restrictions)

Strong institutional cooperation (ICNF & DGAV) and structured reporting systems

Extensive practical experience in managing quarantine forest pests



### Poland - Prevention Stage

Absence of pine wood nematode, but high risk of introduction and spread

Dominance of Scots pine (*Pinus sylvestris*) → high ecosystem vulnerability

Increasing stress factors (drought, European mistletoe) → weakened stands and higher susceptibility

Additional threat: presence of PWN vector *Monochamus galloprovincialis*

**Need for proactive preparedness**

## Fellowship Objectives

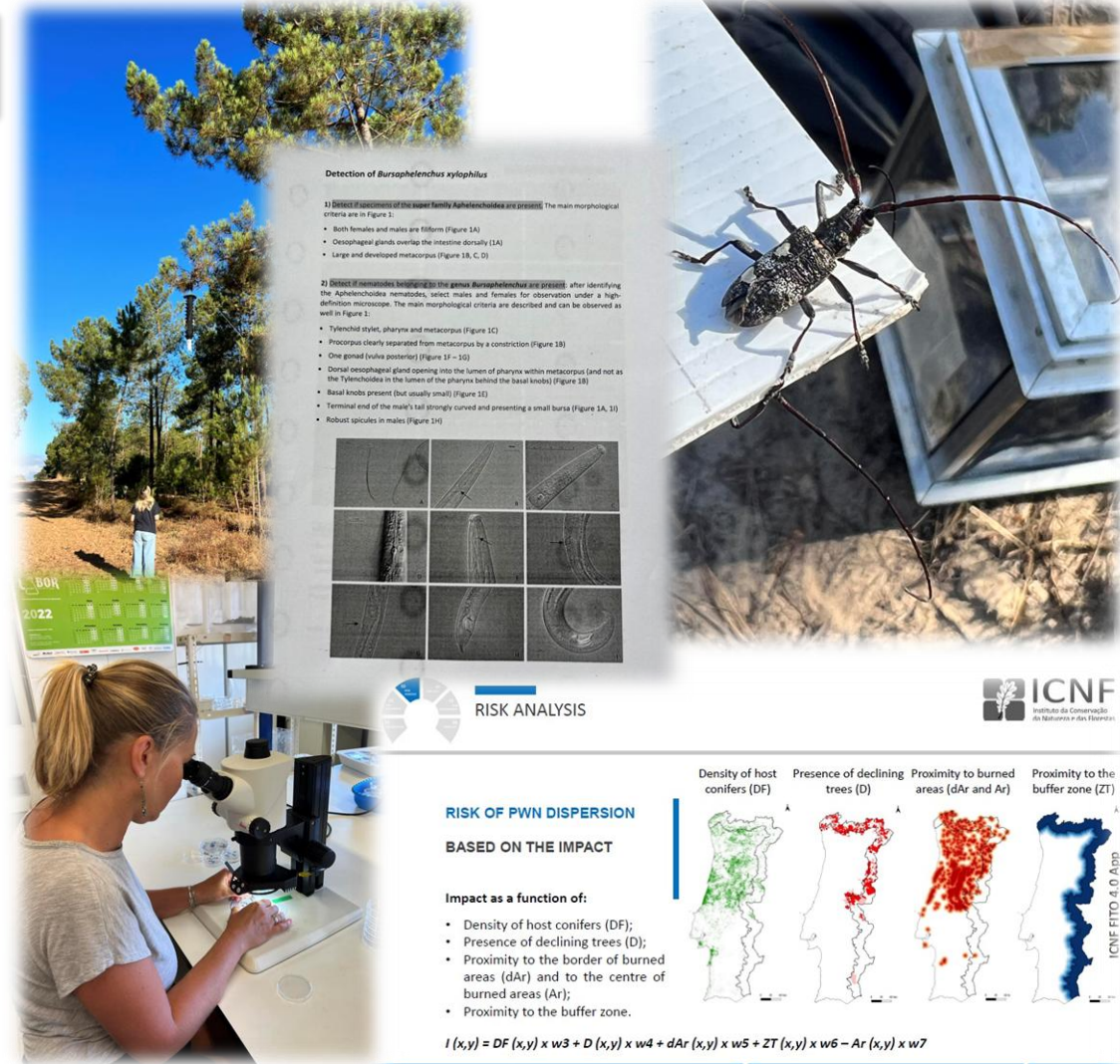
Analyse phytosanitary governance in the context of **PWN** management in Portugal

Understand risk-based management of the pine wood nematode

Explore advanced monitoring and reporting systems

Evaluate control and prevention strategies

Identify solutions applicable to Poland



**Detection of *Bursaphelenchus xylophilus***

1) **Detect** if specimens of the super family Aphelenchoidea are present. The main morphological criteria are in Figure 1:

- Both females and males are filiform (Figure 1A)
- Oesophageal glands overlap the intestine dorsally (1A)
- Large and developed metacarpus (Figure 1B, C, D)

2) **Detect** if nematodes belonging to the genus *Bursaphelenchus* are present after identifying the Aphelenchoidea nematodes, select males and females for observation under a high-definition microscope. The main morphological criteria are described and can be observed as well in Figure 2:

- Tylenchoid stylet, pharynx and metacarpus (Figure 1C)
- Procorpus clearly separated from metacarpus by a constriction (Figure 1B)
- One gonad (valva posterior) (Figure 1F – 1G)
- Dorsal oesophageal gland opening into the lumen of pharynx within metacarpus (and not as the Tylenchoidea in the lumen of the pharynx behind the basal knobs) (Figure 1B)
- Basal knobs present (but usually small) (Figure 1E)
- Terminal end of the male's tail strongly curved and presenting a small bursa (Figure 1A, 1I)
- Robust spicules in males (Figure 1H)

**RISK ANALYSIS**

**RISK OF PWN DISPERSION BASED ON THE IMPACT**

Impact as a function of:

- Density of host conifers (DF);
- Presence of declining trees (D);
- Proximity to the border of burned areas (dAr) and to the centre of burned areas (Ar);
- Proximity to the buffer zone.

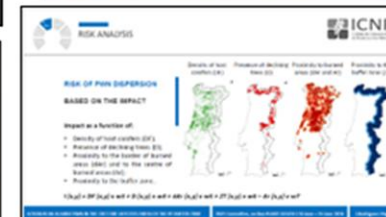
$I(x,y) = DF(x,y) \times w3 + D(x,y) \times w4 + dAr(x,y) \times w5 + ZT(x,y) \times w6 - Ar(x,y) \times w7$

ICNF FITO 4.0 App

## Lectures, seminars, and experts consultations with ICNF, INIAV, and DGAV specialists in Portugal

### Key topics

- ✓ Phytosanitary legislation, prevention strategies, and forest protection against biotic pests (ICNF: José Manuel Rodrigues).
- ✓ Evolution and management of Pine Wilt Disease: from eradication to containment strategies (INIAV: Luís Bonifácio Maria Lurdes Inácio, Maria Filomena de Sousa Nóbrega).
- ✓ Phytosanitary measures reducing the risk of quarantine pests in wood packaging materials used in international trade (DGAV, Phytosanitary Inspection and Vegetative Propagation Materials Division - DIFMPV: Maria Clara de Almeida Serra, João Nuno Barbosa)



## Laboratory diagnostics and reference lab (the National Institute for Agricultural and Veterinary Research, INIAV, I.P.) visits



Training on PWN identification following EPPO standards, including EU sampling and nematode extraction procedures (PM 7/119 (1)), and practical work in morphological and molecular identification techniques (PM 7/4 (3))

## Forest surveys



Training in pheromone trapping protocols for *Monochamus galloprovincialis*

Collection of beetles and analysis of trap catches (target and non-target species)



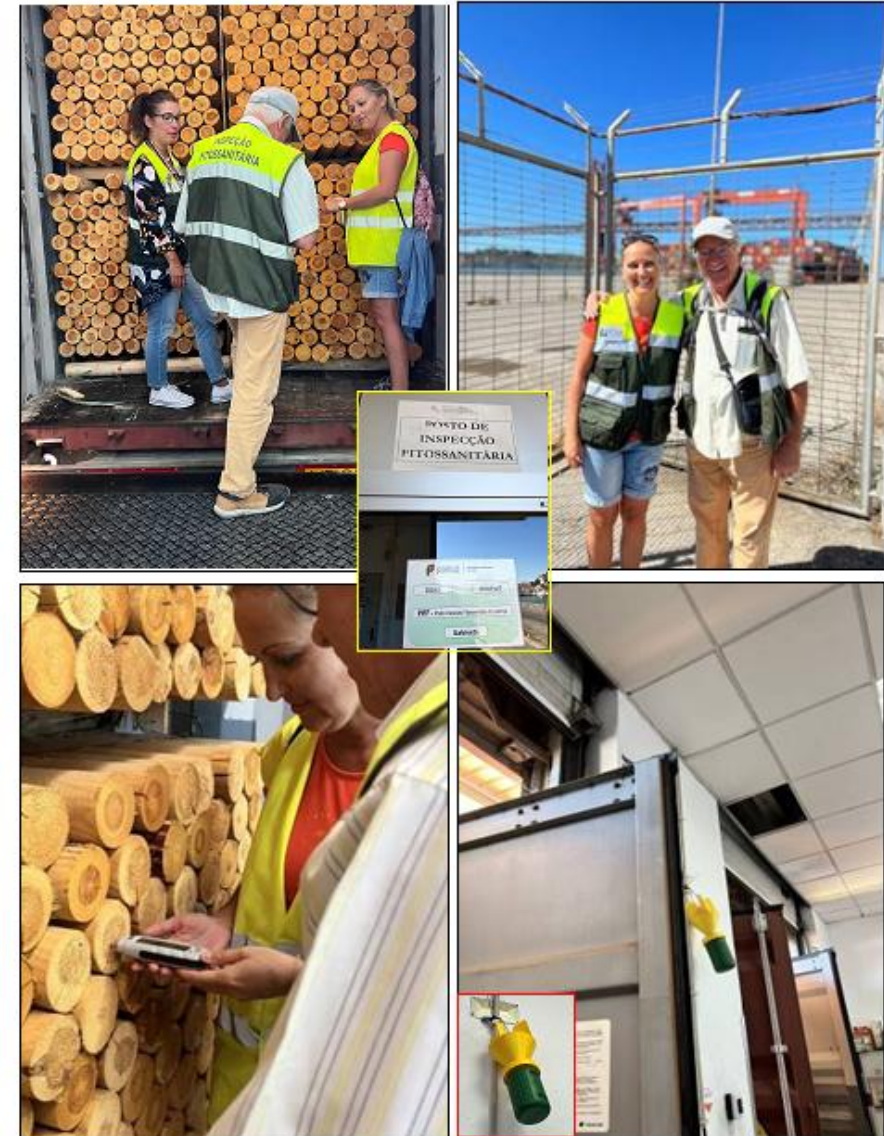
Training in diagnostic procedures for trees infected with PWN  
Research on oleoresin production and its impact on secondary pests



Participation in study on modelling *M. galloprovincialis* flight dispersal in heterogeneous landscapes

## Inspections of Wood and Phytosanitary Controls

- ❖ Detailed wood assessment: moisture measurement, bark presence, and insect exit holes
- ❖ Identification of infestation symptoms, including signs of PWN vector
- ❖ Familiarization with import documentation (phytosanitary certificates, inspection protocols)
- ❖ Observation of pheromone-based insect monitoring systems in ports
- ❖ Knowledge of early detection methods for invasive species at points of entry
- ❖ Practical use of the TRACES system for inspection reporting



Inspections of imported pine wood (*Pinus* spp.) with ICNF experts (Forest Phytosanitary Division)

## Plant Health Inspections and *Xylella fastidiosa* Surveillance

- ❖ Assessing phytosanitary status and control measures of *X. fastidiosa* in Portugal and the EU
- ❖ Implementing monitoring strategies in Demarcated Zones (100 × 100 m grid, buffer areas)
- ❖ Understanding epidemiology and economic impact since 2019 (forestry and ornamental hosts: *Quercus* spp.)
- ❖ Reporting field data using the DGAV Web-GIS platform



Participation in ICNF plant health inspections targeting early detection and eradication of *Xylella fastidiosa* in the Demarcated Zone of Massamá e Monte Abraão (Espirra nursery area), including collection of plant samples and sap-feeding insects as potential vectors in buffer zone field surveys (A). Training in molecular diagnostic techniques for detection of *X. fastidiosa* in plant material in the FitoLab (Instituto Pedro Nunes, Coimbra) (B)

## Application of Fellowship Knowledge in **Teaching**

- ✓ Transfer of knowledge and experience gained during the internship to teaching activities
- ✓ Conducting classes (lectures and exercises) for second-cycle (MSc) students
- ✓ Enriching course content with up-to-date practical and expert knowledge

### **Courses delivered:**

- ❖ *Strategy and tactics of forest protection against diseases and harmful insects*
- ❖ *Phytosanitary protection in trade of plants and forest products within the EU*



## One-month **scientific internship** at INIAV (Oeiras, Portugal, 2024), building on EPPO Fellowship cooperation (2023)

### Objectives

- ✓ Intensive training in diagnostics of nematodes associated with insects and wood using morphological and molecular techniques.
- ✓ Practical use of Polish materials (wood and insect samples) in analyses.
- ✓ Advanced training in PWN detection and research methods.
- ✓ Development of ideas for research projects and international cooperation (INIAV/ University of Lisbon/ University of Agriculture in Krakow)



#### CERTIFICATE OF COMPLETING AN INTERNSHIP

The host institution Instituto Nacional de Investigação Agrária e Veterinária, I.P.; INIAV, Av. da República, Quinta do Marquês, 2780-157 Oeiras, Portugal

confirms the completion of internship by **Magdalena Kacprzyk, Ph.D., Eng**

in the period: **01.07.2024 - 31.07.2024.**

During the internship, the Intern was supervised by: **Maria de Lurdes Inácio, Ph.D., Luis Filipe Bonifácio, Ph.D.**

Oeiras, 28th august 2024



## Enhancing **cooperation** with INIAV: Dr. Ana Rita Varela, Dr. Maria Lurdes Inacio, Dr. Luis Bonifácio

### 1. Advanced Monitoring Optimization

Development and optimization of pheromone-based trapping systems for longhorn beetles (Coleoptera: Cerambycidae), aimed at enhancing detection sensitivity, selectivity, and operational efficiency within integrated forest pest surveillance frameworks.



### 2. Biodiversity and Vector - Nematode Interactions

Assessment of the diversity of target and non-target beetle species and their associated nematodes in the monitoring of the pine sawyer beetle (*Monochamus galloprovincialis*) in Scots pine (*Pinus sylvestris*) and maritime pine (*Pinus pinaster*) stands

**Manuscript in preparation:** Varela A.R., Kacprzyk M., Bonifácio L., Inácio M.L. *New records of insect-associated Bursaphelenchus spp. in Portugal and Poland supported by multilocus phylogenetic analyses and a review of Bursaphelenchus species and their insect hosts.*

**Manuscript published**

Forest Pathology

WILEY

Forest Pathology

ORIGINAL ARTICLE

**Additional Records of *Potensaphelenchus stammeri* (Nematoda: Aphelenchoididae) in Association With *Spondylis buprestoides* (Coleoptera: Cerambycidae) Across Europe**

M. L. Inácio<sup>1,2</sup> | M. Kacprzyk<sup>3</sup> | A. R. Varela<sup>1,4</sup> | S. Costa<sup>5</sup> | L. Bonifácio<sup>1,2</sup>

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**Presentation of joint research results** (Dr. Luis Bonifacio) in IUFRO Working Party 7.03.10 Conference “Forests’ Future 2025: Bark Beetle Outbreak in Central Europe”, 22-26 September 2025, Průhonice, Czech Republic



Forests’ Future 2025

#### Bark Beetle Outbreak in Central Europe – Consequences and Future Challenges

Průhonice, September 22nd – 25th 2025

Book of Abstracts

Edited by Vít Šrámek, Miloš Knížek et al.



Conference is organized under the auspices of the Czech Minister of Agriculture Marek Věšný



Conference is organized as joint meeting with IUFRO WP 7.03.10: Methodology of forest insect and disease survey

Conference partners:



FORESTS’ FUTURE 2025

#### Diversity of target and non-target beetles and associated nematodes in monitoring of the pine sawyer beetle *Monochamus galloprovincialis* (Coleoptera, Cerambycidae) in Scots pine and Maritime pine stands - a case study from Poland and Portugal

Magdalena Kacprzyk<sup>1)</sup>, Luís Bonifácio<sup>2,3)</sup>, Ana Rita Varela<sup>2,4)</sup>, Maria Lurde Inácio<sup>2,3)</sup>, Maria José Silva<sup>4)</sup>, Wiktor Bębas<sup>1)</sup>, Magdalena Koziol<sup>1)</sup>

<sup>1)</sup> Department of Forest Ecosystems Protection, University of Agriculture in Krakow, Al. 29 Listopada 46, 31-425 Krakow, Poland

<sup>2)</sup> Instituto Nacional de Investigação Agrária e Veterinária (INIAV, IP), Quinta do Marquês, 2780-159 Oeiras, Portugal

<sup>3)</sup> GREEN-IT Bioresources for Sustainability, ITQB NOVA, Av. da República, 2780-157 Oeiras, Portugal

<sup>4)</sup> MED, Mediterranean Institute for Agriculture, Environment and Development and CHANGE Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Evora University, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Biotechnical methods that use chemical lures to attract harmful forest insects are an important component of prevention and management efforts of integrated forest protection (Sukovata and Skrzecz, 2022). Moreover, due to the ongoing climate change, globalization of trade of wood and wood commodities, the pressure from native and non-native pests is increasing on forests (Schafstall et al., 2024). In Europe, one of the challenges of today’s strategy is to protect pine stands against the quarantine pinewood nematode *Bursaphelenchus xylophilus* (Steiner et Bühner, 1934) Nickle, by monitoring its vector, the pine sawyer beetle *Monochamus galloprovincialis* (Oliv.) (Coleoptera, Cerambycidae), using different pheromone traps (Álvarez et al., 2015; Sousa et al., 2015; Jactel et al., 2019; Sukovata et al., 2022), baited with Galloprotect-2D lure (SEDQ, Spain). The lure includes the specific pheromone for *M. galloprovincialis* but also pine volatiles and bark beetles’ pheromones (Pajares et al., 2010). As a result, many other bark- and wood boring beetles (Coleoptera, Curculionidae, Scolytinae) and longhorn beetles (Coleoptera, Cerambycidae) are also caught in traps (Rodrigues et al., 2015; Sousa et al., 2015; Allison et al., 2024).

In the spring and summer of 2024 the occurrence of *M. galloprovincialis*, bark- and wood boring beetles in Scots pine (Poland: Włoszczowa, Knyszyn) and Maritime pine (Portugal: Samora Correia; Tróia) stands were

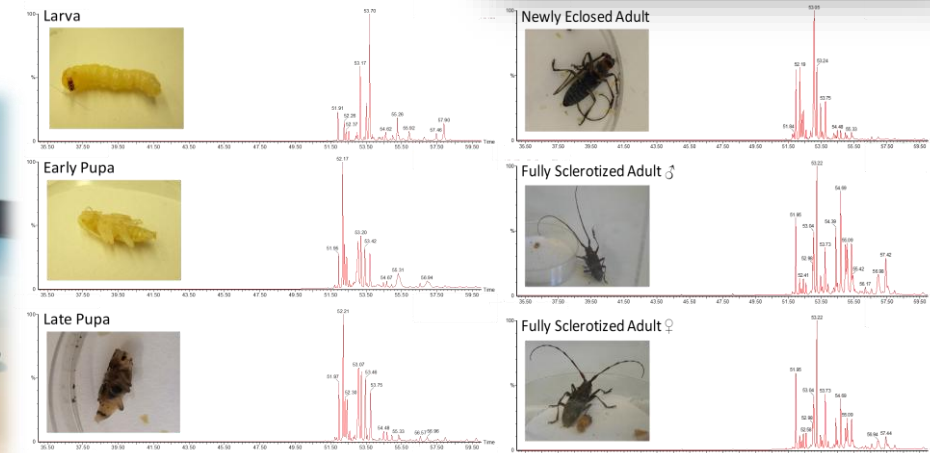
Enhancing **cooperation** with INIAV: Dr. Fernanada Simões, Dr. Luis Bonifácio and the University of Lisbon: Prof. Ana Cristina da Silva Figueiredo

## 3. Microbiome of ambrosia beetles

Investigation of the bacteria of ambrosia beetles colonizing deciduous and coniferous trees and their role in tree disease processes.

## 4. Chemical Ecology of Insects

Study of cuticular hydrocarbon compounds in *Monochamus* spp. and development of an innovative method to disrupt transmission of the pine wood nematode (**partner in a project submitted in Portugal**)



## Enhancing **cooperation** with the University of Lisbon: Prof. Ana Cristina da Silva Figueiredo and INIAV: Dr. Jorge M. S. Faria

### 5. Natural compounds from *Viscum album* spp. as potential biopesticides

Exploration of *Viscum album* as a source of bioactive compounds for sustainable nematode (PWN, *Globodera* spp.) management, integrating chemical profiling of essential oils and assessment of their nematocidal potential.

#### Manuscript under review

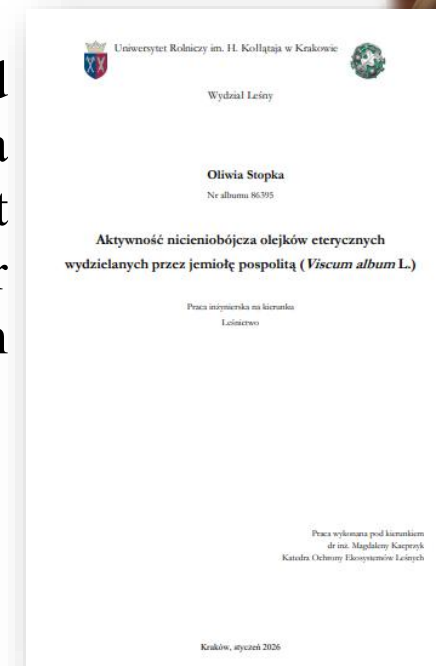
##### Industrial Crops & Products

*Viscum album* subsp. *album*, subsp. *abietis* and subsp. *austriacum* from Poland: essential oil variability assessment and comparison with a literature survey  
--Manuscript Draft--

Manuscript Number:	INDCRO-D-25-06400R1
Article Type:	Research Paper
Section/Category:	Non-food bioactive products
Keywords:	<i>Viscum album</i> , mistletoe, essential oil, volatiles, GC-FID, GC-MS

Magdalena Kacprzyk <sup>a</sup>, Alexandra M. Machado <sup>b</sup>, Luís Bonifácio <sup>c,d</sup>, Jorge M. S. Faria <sup>c,d</sup>, Ana Cristina Figueiredo <sup>b\*</sup>

**Bachelor's thesis** developed through collaboration during a two-month Erasmus+ student internship (27 July–27 September 2025) at the University of Lisbon and INIAV Institute.



## Enhancing **cooperation** with the University of Lisbon: Prof. Ana Cristina da Silva Figueiredo and INIAV: Dr. Jorge M. S. Faria

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Exploration of *Viscum album* as a source of bioactive compounds for sustainable nematode (PWN, *Globodera* spp.) management, integrating chemical profiling of essential oils and assessment of their nematicidal potential.

**Presentation of joint research results (Dr. Alexandra Machado)** in 55th International Symposium on Essential Oils (ISEO 2025), 7-10 September 2025, Sarajevo, Bosnia and Herzegovina

**Manuscript in preparation:** Kacprzyk M., Figueiredo A. C., Faria J. M. S., Machado A. M., Stopka O., Inácio M., Bonifácio L. *Nematicidal Activity of Essential Oils Isolated from European Mistletoe (*Viscum album* L.)*



ISEO 2025  
BOOK OF ABSTRACTS



PP - 39 ISEO2025, Sarajevo, Bosnia and Herzegovina - September 07-10, 2025

Essential oils variability of *Viscum album* subsp. *album*, *Viscum album* subsp. *abietis* and *Viscum album* subsp. *austriacum* from Poland

Kacprzyk, M.<sup>a</sup>, Machado, A.M.<sup>b,\*</sup>, Bonifácio, L.<sup>c,d</sup>, Faria, J.M.S.<sup>c,d</sup>, Figueiredo, A.C.<sup>b</sup>

<sup>a</sup>University of Agriculture in Krakow, Department of Forest Ecosystems Protection, Al. 29 Listopada 46, Krakow, Poland

<sup>b</sup>Centre for Ecology, Evolution and Environmental Changes (CE3C) & CHANGE - Global Change and Sustainability Institute, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, Lisboa, Portugal

<sup>c</sup>Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Quinta do Marquês, Oeiras, Portugal

<sup>d</sup>GREEN IT Bioresources for Sustainability, ITQB NOVA, Av. da República, Oeiras, Portugal

\*Corresponding author: [ampmachado@fc.ul.pt](mailto:ampmachado@fc.ul.pt)

**Keywords:** *Viscum*, mistletoe, essential oil, volatiles, GC-FID, GC-MS

**Objective:** *Viscum* L. species (mistletoes) are hemiparasitic epiphytes well-known as traditional medicinal plants in Africa, Asia, and Europe, which are also relevant for nutrient cycling, biodiversity maintenance, and the survival of species that depend on forests [1, 2]. Nevertheless, recent evidence shows that they also contribute to trees decline and mortality, particularly in massively infested host plants in regions with seasonal water deficit [2]. The objective of this work was to expand knowledge on the ecological relevance of volatiles from mistletoe by assessing the chemical variability of essential oils (EOs) isolated from the leaves (L) and wooden (W) parts of *Viscum album* subsp. *abietis* (Va\_abi), *Viscum album* subsp. *album* (Va\_alb), and *Viscum album* subsp. *austriacum* (Va\_aus), collected in Poland. Mistletoe volatile profiles were compared with those from the parasitized portions of two hosts, *Pinus sylvestris* and *Acer rubrum*. **Methods:** EOs were isolated by hydrodistillation and analysed by Gas Chromatography with Flame Ionisation Detection (GC-FID) for quantification and by Gas chromatography-Mass spectrometry (GC-MS) for component identification [3]. *Viscum* volatile profiles were used in the evaluation of the chemical correlation among the samples by cluster analysis. **Results:** The EOs yield was low (< 0.05%, v/d.w), but 186 compounds were identified in all *Viscum* and host EOs. Agglomerative cluster analysis based on the quantitative variation of the analyzed samples evidenced two main clusters poorly correlated, despite the qualitative similarity of the main components. Va\_abi and Va\_aus leaf EOs were dominated by *n*-nonanal (7-15%), *n*-hexanal (1-14%), and *trans*- $\beta$ -farnesene (1-13%), whereas 2-*trans*,4-*trans*-decadienal (7-13%), and palmitic acid (9-11%), were the main components of the EOs of the woody parts of the same species. Va\_alb leaf EOs were dominated by *trans*- $\beta$ -farnesene (13-23%),  $\alpha$ -pinene (traces-11%) and *n*-tricosane (2-11%). **Conclusions:** The results obtained highlighted the tendency of separation of leaf EOs from those of the woody parts, with most leaf EOs from Va\_abi and Va\_aus showing a higher correlation than each with Va\_alb leaf EOs. The comparison between the present data and an updated survey of the existing literature reinforces the need for further studies to confirm *n*-hexanal, *n*-nonanal, 2-*trans*,4-*trans*-decadienal, *trans*- $\beta$ -farnesene and palmitic acid as representative dominant components of *V. album* volatiles, independently of the subspecies, variety, or host.

#### ACKNOWLEDGMENTS

Magdalena Kacprzyk was supported by the MSHE (Poland) SUB/040013-D019 and Own Rector's Scholarship Fund for academic teachers at the University of Agriculture in Krakow. Partially funded by FCT/MCTES (Portugal) through CE3C UID/00329/2025, CHANGE, and GREENIT (DOI: 10.54499/UIDB/04551/2020 and DOI: 10.54499/UIDP/04551/2020).

#### REFERENCES

- [1] Song C., Wei X.-Y., Qiu Z.-D., Gong L., Chen Z.-Y., Ma Y., Shen Y., Zhao Y.-J., Wang W.-H., Lai C.-J.-S., Yang R., (2021). Exploring the resources of the genus *Viscum* for potential therapeutic applications. *Journal of Ethnopharmacology*, 277, 114233
- [2] Inácio, E. G., Vitoriano, C., Camarero, J., (2024). Long term effects of mistletoe removal on radial growth of semi-arid Aleppo pine forests. *Forests*, 15, 1113
- [3] Póvoa O., Faria N., Lopes V., Machado A. M., Figueiredo A. C. (2024). Coriander (*Coriandrum sativum* L.) from Alentejo (South Portugal) - Ethnobotany and Potential Industrial Use. *Food*, 13, 929

### Establishment of international scientific contacts and engagement with EPPO expert networks

- ✓ Participation as an observer in the 28th meeting of the EPPO Panel on Quarantine Pests for Forestry (October 2023) - representing the first-ever involvement of a Polish representative in the Panel's work
- ✓ Since 2024 - member of the EPPO Panel on Quarantine Pests for Forestry



29th Meeting of the Panel on Quarantine Pests for Forestry (York, GB, 2024-10-16/18) (EPPO archive)



28th Meeting of the Panel on Quarantine Pests for Forestry (Kildare, Ireland, 2023-10/31-11/02) (EPPO archive)



30th Meeting of the Panel on Quarantine Pests for Forestry (Paris, 2025-11-12/14) (EPPO archive)



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