

Rapid Communication

First report of damage in olive orchards in Slovenia caused by *Aculus olearius* Castagnoli (Prostigmata: Eriophyidae)

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Citation: Devetak M, Hobljaj S, Jančar M, Rot M, Tatti A, Malagnini V, Baldessari M, Pedrazzoli F, de Lillo E (2026) First report of damage in olive orchards in Slovenia caused by *Aculus olearius* Castagnoli (Prostigmata: Eriophyidae). *BioInvasions Records* 15(2): 339–348, <https://doi.org/10.3391/bir.2026.15.2.10>

Received: 18 February 2025

Accepted: 19 December 2025

Published: 16 March 2026

Handling editor: Laura Garzoli

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Abstract

During a field survey conducted in olive orchards (*Olea europaea* L.) in western Slovenia over two consecutive years (2022–2023), severe symptoms of bud browning, flower drying, leaf curling, and shoot deformations were observed. Symptomatic flower buds and leaves were collected in late May and early June for laboratory analysis. Stereomicroscopic examination confirmed a significant infestation of eriophyid mites. Morphological characterization identified the presence of *Aculus olearius* Castagnoli, marking the first record of this species in Slovenia. A molecular characterization was also performed and sequences were compared with other *Aculus* spp. High densities of mite populations feeding on flower buds and peduncles led to the premature dropping of flowers and young fruits, a phenomenon increasingly observed in recent years. This study documents the first occurrence of *A. olearius* in Slovenia and highlights its potential economic impact on olive production.

Key words: eriophyid mites, *Olea europaea*, molecular characterization, host-pest interactions, premature fruit drop

Introduction

To date, at least 30 different plant-feeding mite species are known to cause damage to olive trees (Castagnoli and Oldfield 1996). Among them, 12 species belonging to the Eriophyidae family are mostly present only on cultivated olive trees (*Olea europaea* L.) and some also on wild olives (*Olea europaea* L. subsp. *sylvestris*) (Tzanakakis 2003).

Aculus olearius Castagnoli is considered a pest of olive trees in Italy (Castagnoli and Oldfield 1996), Greece (Hatzinikolis and Kolovos 1985 even though it was previously reported by Papaioannou-Souliotis in 1982 into the annual report of the Benaki Phytopathological Institute), and Spain (González et al. 2000). This mite is recorded in Turkey as well,

causing significant damages to olive production in some regions (Çetin and Alaoğlu 2006; Ersin et al. 2020). Recently, *A. olearius* was also reported in the Kingdom of Saudi Arabia, where it affected olive plantations and caused losses in oil production (Ksibi et al. 2022). The phenology of plants can influence the biology of the species and the vulnerability of the plant organs. In Tuscany (central Italy), where this species was described and studied for the first time, young leaves, floral organs, and young fruits are the most susceptible to this mite, and the infestation of inflorescences was observed from bud burst until fruit set, from early May to the first half of June (Castagnoli 1977). The same behavior was observed in Turkey, where mite population peaks were found at the end of April on the buds and again at the end of May on the young fruits, with a slight population decline occurring at flowering (Çetin and Alaoğlu 2006). In late June, when the diameter of olive fruits reached 5 mm, a sudden and significant population decline was observed (Tzanakakis 2003).

During high population growth, the mite can also colonize the stems (González et al. 2000). Browning and withering of both flower buds and developing fruits, leading to large yield losses, were reported in association with the *A. olearius* outbreak on olives in Italy (Castagnoli 1977). Deformations of buds, leaves, and olive fruits were observed in the Kingdom of Saudi Arabia (Ksibi et al. 2022). In connection with feeding on leaves and buds, greenish-yellow spots and malformations on the young leaves, green and brown sunken spots on buds, split sections on fruits, and fruit deformations have also been described (Çetin and Alaoğlu 2006).

The spread of *A. olearius* to new regions may be facilitated by changing climatic conditions, including milder winters and warmer springs, which favour the overwintering and reproduction of eriophyid mites. This expansion could represent a significant threat to olive production, particularly in regions where olive cultivation is marginal and already challenged by abiotic stresses. Understanding the biology and ecology of *A. olearius* in these new environments, such as in Slovenia, is crucial for developing effective management strategies to mitigate its impact.

Several olive trees showed symptoms on flower buds and leaves in western Slovenia in spring 2022, in orchards where both organic and integrated management were applied. As this damage was similar to that associated with eriophyid mite attacks, we decided to investigate the species composition of mites developing on olive plantations, describing and documenting the evolution of this symptomatology. In addition, we decided to combine morphological and molecular analysis in order to determine the eriophyid mite species.

Materials and methods

Inspection of olive trees and record of the symptoms of eriophyid mite attacks were conducted in different Slovenian olive plantations and gardens. Symptomatic olive shoots were collected in five locations in the Goriška



Figure 1. Map of eriophyid mites (*Aculus* spp.) records in western Slovenia.

Table 1. Data on eriophyid mite occurrence in Slovenia in 2022 and 2023.

Location name	Sampling date	GPS Coordinates (DD)		Type of location	Olive varieties	Mite occurrence (year)
Kromberk	11-May-2023	45.9635916667	13.6887833333	olive orchard	Istrska belica - Bianchera	2023
Lokvica	24-May-2022	45.8619	13.6061305556	olive orchard	Istrska belica - Bianchera / Leccino	2022
Strunjan	19-May-2022 24-May-2023	45.5282694444	13.6158805556	olive orchard	Istrska belica - Bianchera	2022/2023
Solkan	26-May-2022 12-May-2023	45.9667222222	13.6455666667	garden	Istrska belica - Bianchera	2022/2023
Mohorini	9-May-2023	45.8759972222	13.6862916667	garden	Istrska belica - Bianchera	2023

region, Carst, and the Slovenian Istria in May and June 2022 and 2023 (Figure 1, Table 1). Field observations were referred to the trees' phenological stage as described in the Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale (Sanz-Cortés et al. 2002).

Five to seven symptomatic branches, 30–40 cm long, were sampled at each location and transferred to the laboratory to determine the mite presence. A Nikon SMZ-2B stereomicroscope (Nikon Corporation, Tokyo, Japan) with magnification up to 100× and a compound microscope Nikon Eclipse Ni-U (Nikon Corporation, Tokyo, Japan) were used for mite detection.

Eriophyid mites collected on May 2023 in Kromberk were cleared in Keifer's booster and mounted in Keifer's medium II (Keifer 1975). A phase contrast compound microscope (Olympus BX50, Tokyo, Japan) was used for the morphological characterization of the specimens, and their measurements were taken according to Amrine and Manson (1996), as modified by de Lillo et al. (2010). A Canon Mark III mounted on the Olympus BX50 was used to take pictures, which were later optimized with Adobe Photoshop 21.2.2. The genus was determined using the generic key provided

Table 2. Accession numbers of the eriophyid mites sequenced.

Sample ID	Species	Location name	Sampling date	N° individuals	Source	Accession number
F10(E09-C10)	<i>Aculus olearius</i>	Kromberk	11-May-2023	10	leaf	PP373065
F1(A09-G09)	<i>Aculus olearius</i>	Kromberk	11-May-2023	1	leaf	PP373066
M5(C09-A10)	<i>Aculus olearius</i>	Kromberk	11-May-2023	5	floral buds	PP373067
M5(F09-D10)	<i>Aculus olearius</i>	Kromberk	11-May-2023	5	floral buds	PP373068

by Amrine et al. (2003), through comparisons with the new genera described after that publication and with the species known on olive trees (Nuzzaci and Parenzan 1983) with particular regard to the description of *A. olearius* given by Castagnoli (1977).

DNA of four samples of different consistencies (two from leaves and two from flower buds) collected at the location of Kromberk in May 2023 was sequenced for molecular characterization (Table 2). DNA was extracted with Chelex® 100 (Bio-Rad) (Walsh et al. 1991), and the cytochrome oxidase subunit I (COI) gene was amplified using the universal primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al. 1994). A polymerase chain reaction (PCR) was performed using the protocol described in the EPPO PM 7/129 (2) (EPPO 2021) and the GoTaq® Green Master Mix (Promega), with the following conditions: 2 min at 95 °C, 5× (45 sec at 95 °C, 45 sec at 45 °C, 1 min at 72 °C), 35× (45 sec at 95 °C, 45 sec at 50 °C, 1 min at 72 °C), 5 min at 72 °C. PCR products, after purification with ExS-Pure™ (NimaGen), were sequenced with the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) on an Applied Biosystems 3130 xl Genetic Analyzer (Carlsbad, CA, USA) at the Sequencing and Genotyping Facility of Fondazione E. Mach.

The sequenced COI regions were visualized using Chromas software (Technelysium Pty Ltd) and then edited with BioEdit version 7.2.5 (Hall 1999). Forward and reverse reads were assembled, and the resulting consensus sequences were submitted to the NCBI GenBank database (accession numbers are provided in the Results section).

A multiple sequence alignment was conducted to analyze the phylogenetic relationships of samples within species belonging to the genus *Aculus*. Only nucleotide sequences that were reliably identified and assigned to specific species were included. One representative sequence per species was selected based on verified sequence homogeneity. Sequences were aligned using the ClustalW algorithm implemented in MEGA-X version 10.1.1 (Kumar et al. 2018) with default parameters. The initial alignment included 16 sequences of *Aculus* species, one sequence of *Aculops lycopersici* (Tryon), and one sequence of *Aceria tosichella* Keifer, which was used as the outgroup. *Aculops lycopersici* was included in the comparison based on the fact that the rounded broad frontal lobe of this species is typical and distinctive of the genus *Aculus* (Denise Navia, pers. comm. to EdL on June 9th, 2022). Accession numbers in GenBank of all compared sequences are

reported in the drawn phylogenetic tree. The total alignment length was initially of 692 positions. To enhance alignment quality, terminal regions were trimmed, resulting in a final alignment of 573 positions. Phylogenetic analysis was performed using MEGA-X version 10.1.1 (Kumar et al. 2018) employing the maximum likelihood (ML) method. Model selection identified the Jukes-Cantor model (Jukes and Cantor 1969) as the best fit for the data. Statistical support for the inferred phylogenies was assessed using bootstrap analysis with 1000 replicates.

Results and discussion

The morphological characterization was done on a single sample of population collected during late spring only in Kromberk. All qualitative and quantitative characters match completely with the original description given by Castagnoli (1977) (Figure 2). In particular, the body was spindle-shaped, the frontal lobe was broad based and typical of the *Aculus* genus, the prodorsal shield was subtriangular, its pattern was composed of seven cells in the median field (four close to the rear margin of the prodorsal shield plus three on the mid distance between the anterior and posterior margins of the prodorsal shield), a median line could be recognized on the first and last 1/3 of the shield, the tubercles of *sc* setae were cylindrical and set on the rear margin of the prodorsal shield, the *sc* setae were back oriented, the coxae were covered by fine granules, the coverflap was ornamented by longitudinal striae on the distal part and transverse granulated rows at its base. Only the short spines on the anterior border of the frontal lobe were not always clearly detectable. The quantitative characters largely match the original description, even though they can be influenced by ecological factors (plant variety, physiology of the plant, climatic conditions, physiology of the mite). It should be pointed out that a strict comparison could not be done with the original description (Castagnoli 1977), because that does not respect the current standard, does not give ranges of the morphometric values for each measured character, and details on the measurement detection were not specified. However, the size of the body, legs and their setae, and the number of dorsal and ventral semiannuli of the studied population are fully coherent with the original description.

Severe symptoms of mite attacks on flower buds and leaves were recorded at all five locations, indicating a high infestation level, despite the different growing systems of the olive trees (organic/integrated olive production; olive orchard/garden). By analyzing the collected material, we found that the mites were present at the beginning of flowering, in the phenological development stages 55 (flower cluster completely expanded and floral buds start to open) – 57 (the green-coloured corolla is longer than calyx) (Sanz-Cortés et al. 2002).

The most typical symptoms observed on olives were browning and desiccation of flower buds, leaf curling, heavy deformations of leaves and

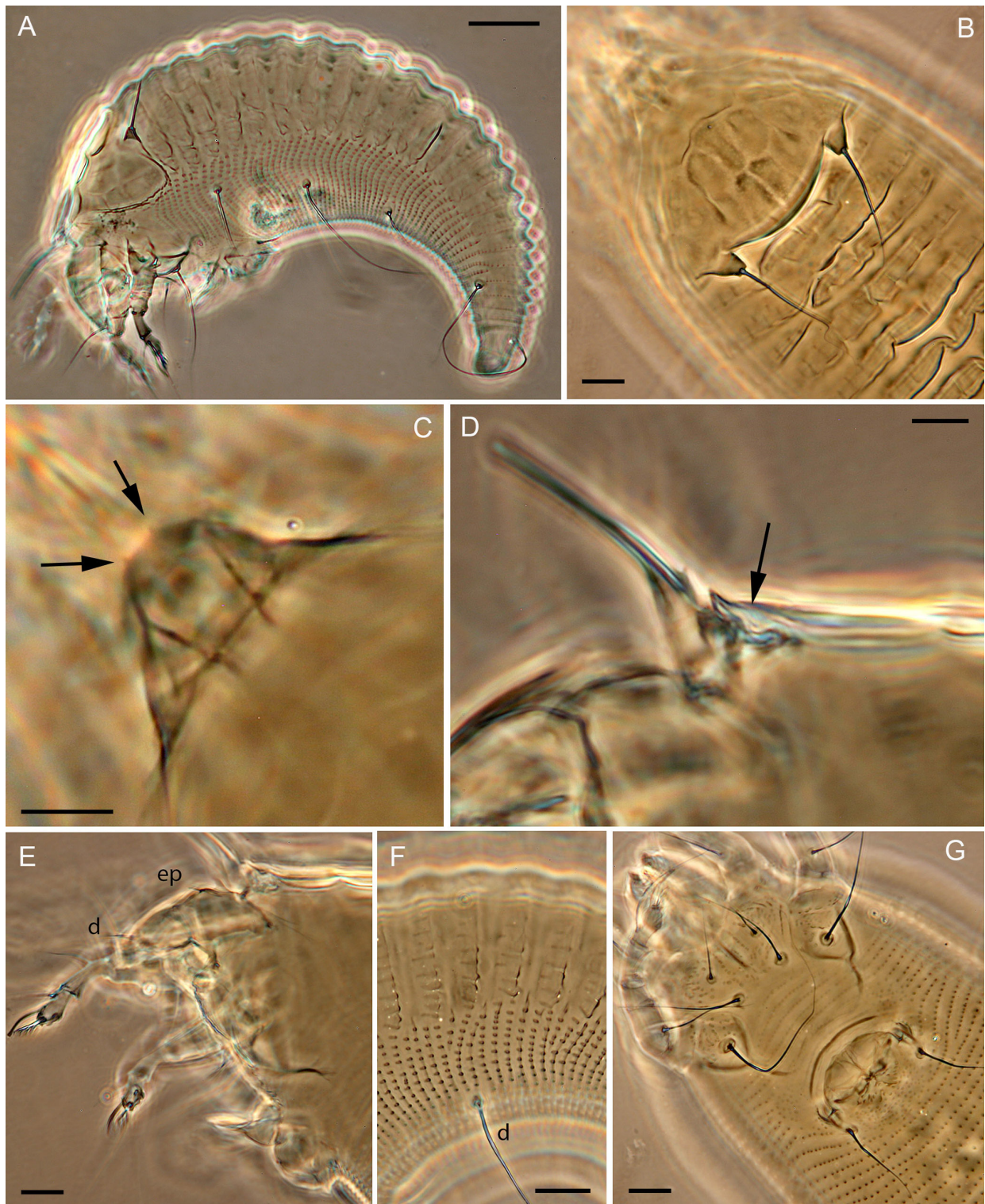


Figure 2. Light micrographs of *Aculus olearius*: A) lateral view of a female; B) detail of the prodorsal shield and first dorsal semiannuli, dorsally view; C) detail of the frontal lobe, dorsal viewed (arrows mark the short spines on the border); D) lateral view of the frontal lobe and chelicerae (arrow marks the frontal lobe); E) lateral view of the gnathosoma and prosoma of a female (d: palp genual seta; ep: palp coxal seta); F) details of the dorsal and ventral semiannuli at the level of the opisthosomal seta d, lateral view; G) ventral view of the coxal and genital area of a male. Scale bars: 20 μm for A; 10 μm for B, E, F, and G; 5 μm for C and D. Micrographs by Enrico de Lillo

leaf shoots (Figure 3). When sampled after the phenological stage BBCH71 (corresponding to a fruit size about 10% of final size), the mite populations were lower on the exposed plant surfaces and difficult to find.



Figure 3. Attacks of eriophyid mites on olive: A), C) olive floral bud desiccation; B) leaf deformation (Lokvica); D) symptoms of leaf curling on young shoots (olive orchard located in Kromberk, near Nova Gorica). Photo by Marko Devetak

The sequences obtained from the molecular characterization of the population sampled at a single location were subjected to BLAST searches against the database, but no exact matches with previously sequenced species were found. Evolutionary history was inferred by using the ML method and Jukes-Cantor model (Jukes and Cantor 1969). The tree with the highest log likelihood (-6036.55) is shown in Figure 4. All codon positions (1st, 2nd, 3rd) and noncoding regions were included. Positions containing gaps or missing data were excluded by applying the complete deletion option. A total of 547 positions were in the final dataset.

The ML phylogenetic tree indicates that the four sequences cluster closely with other species of the genus *Aculus* and exhibit minimal divergence among themselves (Figure 4). Notably, the sequence from the single mite sample identified as F1(A09-G09) aligns with those from pooled samples, suggesting homogeneity in the sequences obtained from grouped mites. Consequently, these four sequences have been deposited in NCBI GenBank under the name *Aculus olearius* (accession numbers provided in Table 2). Unfortunately, the phylogenetic analysis was limited by the scarcity of reliably identified *Aculus* sequences available in public databases. Furthermore, ongoing revisions in the taxonomy of eriophyid mites imply that genus

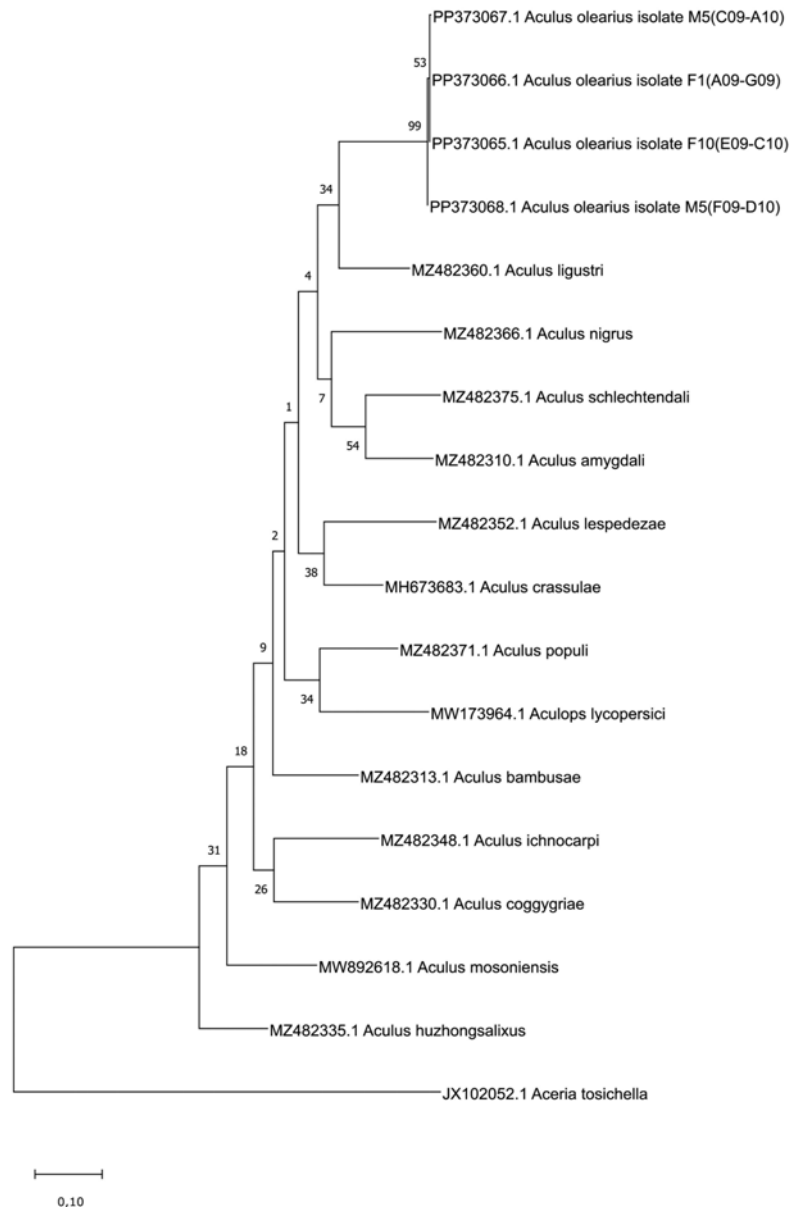


Figure 4. Maximum likelihood phylogenetic tree built with the cytochrome oxidase I (COI) sequences of different eriophyid mites. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying the Maximum Parsimony method. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

definitions are subject to change, which complicates species identification but will ultimately improve the usability and accuracy of molecular data. Secondly, the fact that the sequence of *Aculops lycopersici* is well nested into the *Aculus* tree supported the hypothesis of a new genus combination for this species.

The discovery of *A. olearius* in Slovenia represents a shift in the distribution of this pest, likely influenced by changing climatic conditions. The ability of *A. olearius* to cause economic damage in a region previously unaffected by eriophyid mites underscores the need for regular monitoring and the development of integrated pest management (IPM) strategies. Given the limited availability of acaricides registered for use in olive orchards in

Slovenia, alternative control methods, such as the use of more eco-sustainable products and/or the introduction of predatory mites, should be explored. Additionally, further research is needed to understand the population dynamics of *A. olearius* in different climatic conditions, olive varieties and its interaction with other olive pests.

Conclusions

In Slovenia, as in other northern countries where olive production is possible, we have not faced symptoms caused by eriophyid mites yet. In this country, they are still classified as marginal pests, and the abiotic conditions do not allow them to spread and cause economic losses. Because of climate change with temperature fluctuations, mild winters, and different precipitation regimes, more attention should be paid to these phytophagous mites in the future. Considering that the lists of registered plant protection products according to the environmental requirements are evolving and some broad-spectrum and non-selective pesticides such as pyrethrins, pyrethroids (deltamethrin), and neonicotinoids (acetamiprid) are already applied to control the olive fly (*Bactrocera oleae* Gmelin) and the brown marmorated stink bug (*Halyomorpha halys* Stål), the definition of pest control strategies is becoming more and more challenging. In this complex context, the effects of sulfur-based pesticides or other acaricides should be tested, and, in addition, the contribution of beneficial organisms, such as predatory mites, should be investigated as well.

Aculus olearius represents a new record for Slovenia. It was characterized for its COI sequence which was compared to other *Aculus* species pointing out their affinity. The first record of *A. olearius* in Slovenia highlights the growing threat posed by eriophyid mites to olive production in regions previously unaffected by these pests. The observed damage, including premature fruit drop and shoot deformations, underscores the need for proactive management strategies. Given the potential for climate change to facilitate the spread of *A. olearius*, continuous monitoring and research are essential. Future studies should focus on the efficacy of sulfur-based acaricides and the role of natural enemies in controlling mite populations. Additionally, the development of predictive models based on climatic data could help anticipate and mitigate future outbreaks.

Authors' contribution

MD and MR contributed to research conceptualisation, sampling design and methodology. All authors contributed to investigation and data collection. MD, AT and FP conducted data analysis. MD and FP wrote the original draft. All the other co-authors contributed to reviewing and editing the manuscript.

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Acknowledgements

The authors thank Prof. Enrico De Lillo for the precious help in the morphological determination of the samples used in this study. Three anonymous reviewers are thanked for providing comments on an earlier version of the manuscript.

Funding declaration

This research was funded by Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection (AFSVSPP), Ministry of Agriculture, Forestry and Food, Ljubljana, Slovenia. The funders have no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

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