J Plant Dis Prot (2025) 132:64
https://doi.org/10.1007/s41348-025-01061-w

ORIGINAL ARTICLE

Methyl jasmonate spray for the protection of broad-leaf trees against oomycete and fungal pathogens

F. Javier Dorado[1] · **Iryna Matsiakh**[2,3] · **Álvaro Camisón**[1] · **Jaime Olaizola**[4] · **Carmen Romeralo**[5] · **Juan Antonio Martín**[6] · **Johanna Witzell**[7] · **Alejandro Solla**[1]

Received: 24 October 2024 / Accepted: 9 January 2025

Abstract

Methyl jasmonate (MeJA) is a volatile hormone produced by plants in response to stress. Exogenous application of MeJA enhances resistance to pathogens in conifers, but little is known if resistance is also enhanced in broad-leaf trees. This article reports on five independent experiments carried out to determine the preventive and curative effects of MeJA spray in broad-leaf trees in response to relevant pathogens. In *Castanea sativa* seedlings, preventive MeJA spray at 1 mM was the most effective dose against *Phytophthora cinnamomi*, and protection lasted one year. For *Quercus ilex* and *Q. suber* seedlings, double spray of MeJA at 0.2 mM, before and after inoculation, and single spray of MeJA at 1 mM before inoculation were the most effective treatments against *P. cinnamomi*, respectively. MeJA spray had no effect on the mycorrhization of plants. *Quercus robur* and *Fraxinus excelsior* plants were sprayed with 0, 0.2, 1, 5 and 10 mM MeJA, before and after *Phytophthora plurivora* infection, but no protection was observed. Finally, *Ulmus minor* trees were sprayed at 1 and 10 mM MeJA, and protection against *Ophiostoma novo-ulmi* was dose- and genotype-dependent. It is concluded that MeJA spray can induce resistance in broad-leaf trees against widespread and highly virulent pathogens, but the effect may vary depending on the tree species and pathogen combination. Protection of broad-leaf trees could be obtained only if the appropriate dose and timing is used.

Keywords MeJA · Induced resistance · Tree protection · Plant immunity · Invasive pathogen

Introduction

European chestnut (*Castanea sativa* Mill.), holm oak (*Quercus ilex* L.), cork oak (*Quercus suber* L.), pedunculate oak (*Quercus robur* L.), common ash (*Fraxinus excelsior* L.) and field elm (*Ulmus minor* Mill.) are native tree species in European forests. Alarmingly, the ecological functions and economic values linked to these species are increasingly threatened by several fungal and oomycete pathogens. For example, the invasive *Phytophthora cinnamomi* is widespread in southern Europe and threatens chestnut, holm oak and cork oak forests (Martín-García et al. 2015; Antonelli et al. 2023; Serrano et al. 2024). *Phytophthora plurivora* previously known as *P. citricola* is frequently found on a broad range of host species including pedunculate oak and ash (Jung et al. 2018). Dutch elm disease caused by the fungus *Ophiostoma novo-ulmi* decimated the populations of native elms since its introduction to Europe in early 1970s (Martín et al. 2023). To secure the future of native European tree species, sustainable, environmentally friendly methods are urgently needed.

✉ Alejandro Solla
asolla@unex.es

[1] Faculty of Forestry, Institute for Dehesa Research (INDEHESA), Universidad de Extremadura, Avenida Virgen del Puerto 2, 10600 Plasencia, Spain

[2] Southern Swedish Forest Research Centre, SLU Forest Damage Centre, Swedish University of Agricultural Sciences, Sundsvägen 3, 234 56 Alnarp, Sweden

[3] Institute of Forestry and Park Gardening, Ukrainian National Forestry University, Pryrodna Street 19, Lviv 79057, Ukraine

[4] IDForest, Biotecnología Forestal Aplicada, Calle Curtidores 17, 34004 Palencia, Spain

[5] Institute of Forest Sciences, (ICIFOR-INIA), CSIC, Carretera La Coruña Km 7.5, 28040 Madrid, Spain

[6] Escuela Técnica Superior de Ingeniería de Montes, Forestal y del Medio Natural, Centro para la Conservación de la Biodiversidad y el Desarrollo Sostenible (CBDS), Universidad Politécnica de Madrid, Calle José Antonio Novais 10, 28040 Madrid, Spain

[7] Department of Forestry and Wood Technology, Linnaeus University, 35195 Växjö, Sweden

Phytohormones are small signalling molecules, which regulate plant development and adaptation to stress, and are among the most studied compounds used to control biotic damage (Thakur and Sohal 2013; Camisón et al. 2019; Ali 2021). Jasmonates including jasmonic acid (JA) are a major class of oxylipin-derived phytohormones crucial to plant defence against herbivores and necrotrophic pathogens (Zas et al. 2014; Wang et al. 2020). Exogenous application of methyl JA (MeJA), a volatile derivative of JA, activates several defence-related metabolic pathways leading to the accumulation of phytoalexins, enhanced carotenoid biosynthesis and increased activity of enzymes involved in the scavenging of reactive oxygen species and the synthesis of phenolic compounds (Jiang and Yan 2018; Ghorbel et al. 2021).

In conifer trees, the application of MeJA induces resistance to harmful insects (Sampedro et al. 2011; Whitehill et al. 2014; Jiang and Yan 2018; Puentes et al. 2021) and pathogens (Krokene et al. 2008; Gould et al. 2009; Puentes et al. 2021; Wilkinson et al. 2022; Nunes da Silva et al. 2025) under controlled and field conditions (Berggren et al. 2023; Krokene et al. 2023; Zas et al. 2014). Side effects of MeJA-induced resistance in conifers have been documented, mostly in pine (*Pinus* spp.) and spruce (*Picea* spp.) (Huynh et al. 2024), and include transiently reduced tree growth and direct phytotoxicity (Gould et al. 2008, 2009; Sampedro et al. 2011; Zas et al. 2014; Fedderwitz et al. 2020). By contrast to conifer trees, the effectiveness of MeJA as a resistance inducer and the occurrence of side-effects in broad-leaved trees are much less known (but see Tianzi et al. 2018; Vivas et al. 2012; Whitehill et al. 2014).

There are significant gaps in our understanding regarding the durability and ecological consequences of MeJA treatment on trees (Huynh et al. 2024). For instance, the effects of MeJA treatments on the interactions between trees and beneficial soil microbes have not been studied. Most broad-leaved trees form symbioses with ectomycorrhizal fungi that have beneficial effects on plant nutrition and drought tolerance, and provide protection against root pathogens, such as *P. cinnamomi* (Cairney and Chambers 1997). Thus, impaired mycorrhization can lead to reduced resilience of forests to drought, more frequent infections by soil-borne pathogens, and reduced tree growth (Corcobado et al. 2015). It has been suggested that allocation costs arising from induced defence may have negative effects on the formation and functioning of mycorrhizae (Walters and Heil 2007), but as far as authors know these effects were not tested.

Here, we report results of five independent experiments in which the efficacy of exogenous application of MeJA to induce resistance in (i) *C. sativa*, *Q. ilex* and *Q. suber* seedlings against *P. cinnamomi*, (ii) *Q. robur* and *F. excelsior* seedlings against *P. plurivora* and (iii) *U. minor* trees against *O. novo-ulmi* was tested. We tested the hypothesis that MeJA spray protects broad-leaf trees against oomycete and fungal pathogens. The effect of MeJA spray on the growth of *Q. ilex*, *Q. suber*, *Q. robur* and *F. excelsior* was studied. Additionally, the impact of MeJA spray on the mycorrhization of chestnut, holm and cork oak seedlings was assessed.

Materials and methods

Plant material

Plant material from six deciduous tree species that are native in European forests, i.e. *C. sativa* (European chestnut), *Q. ilex* (holm oak), *Q. suber* (cork oak), *Q. robur* (European oak), *F. excelsior* (European ash) and *U. minor* (field elm), were used (Table 1). Since the induced response of trees varies depending on the species, progeny and age (Van Loon 1997; Puentes et al. 2021), we tested this variation by using plant material heterogeneous in age and origin. The six-month-old seedlings of *C. sativa*, *Q. robur* and *Q. suber* originated from natural forests in Extremadura, Spain, and were purchased from La Dehesa nursery (Valdeobispo, Spain). The two-year-old seedlings of *Q. robur* originated from Orrängen, Götaland, and were obtained from Ramlösa Plantskola AB nursery (Helsingborg, Sweden). The two-year-old *F. excelsior* plants, cloned through stem cuttings from six genotypes selected for their superior growth, were obtained from the clone collection of Forestry Research Institute of Sweden, Skogforsk (Ekebo, Sweden). The seven-year-old *U. minor* trees, replicated in vitro from Atinia (*U. minor* var. *vulgaris* = *U. procera*) (Gil et al. 2004), and CC-VG4.2 clones within the Spanish elm breeding program were selected because of their high susceptibility to Dutch elm disease. All trees were grown in pots in a controlled environment glasshouse at ~ 25 °C (65–70% relative humidity and natural daylight) and watered once a week, except the elms, which were planted in an experimental plot at 1 × 1 m spacing at Centro Nacional de Recursos Genéticos Forestales Puerta de Hierro, Madrid, Spain.

Pathogens and inoculum preparation

Three pathogens were used in the study (Table 1). First, an A2 strain of *Phytophthora cinnamomi*, since this oomycete is responsible for the ink disease in chestnut and decline of holm oak and cork oak and causes widespread mortality of trees in southern Europe (Jung et al. 2018). The strain, coded P90, was isolated from the rhizosphere of a *Q. ilex* tree in Puebla de Guzmán, Spain, and has been shown to be highly virulent in *Q. ilex* (San-Eufrasio et al. 2021). The inoculum was prepared following Jung et al. (1996) and incubated at 23 °C in darkness for four weeks.

A virulent strain of *Phytophthora plurivora* (AV1007), isolated in 2016 from a bleeding canker on a diseased

Table 1 Summary of tree species, pathogens and doses of MeJa tested and main results of five independent experiments (– = result not available; yes = at least one dose of MeJA protected/cured trees from infection)

	Tree species (development stage)	Pathogen	MeJA dose (mM)	Effect tested	Increased resistance	Effect on growth	Effect on mycorrhization (experiment 5)
Experiment 1	*Castanea sativa* (seedlings)	*Phytophthora cinnamomi*	0, 0.04, 0.2, 1, 5, 10	Preventive; preventive + curative	Yes	–	No
Experiment 2	*Quercus ilex* (seedlings)	*Phytophthora cinnamomi*	0, 0.2, 1	Preventive; curative; preventive + curative	Yes	Negative	No
	Quercus suber (seedlings)	*Phytophthora cinnamomi*	0, 0.2, 1	Preventive; curative; preventive + curative	No	Positive	No
Experiment 3	*Quercus robur* (seedlings)	*Phytophthora plurivora*	0, 0.2, 1, 5, 10	Preventive; curative; preventive + curative	No	No	–
	Fraxinus excelsior (seedlings)	*Phytophthora plurivora*	0, 0.2, 1, 5, 10	Preventive; curative; preventive + curative	No	No	–
Experiment 4	*Ulmus minor* (young ramets)	*Ophiostoma novo-ulmi*	0, 1, 10	Preventive	Yes	–	–

European beech (*Fagus sylvatica*) in Malmö, Sweden (Vetukuri et al. 2018), was used. The inoculum was obtained from mycelial cultures on potato dextrose agar (PDA) that had been incubated at room temperature in the dark for 2 weeks.

Ophiostoma novo-ulmi isolate (CC-CC1) was obtained in 2020 from an infected *U. minor* tree in Cáceres (Spain). Isolate mycelial plugs were grown on malt extract agar (MEA) two months prior to the experiment at 22 °C in the dark and were subcultured every 15 days. Four days before the experiment, mycelial plugs from fresh colonies were grown in Erlenmeyer flasks with liquid Tchernoff medium (Martín et al. 2023) under constant shaking at 22 °C to induce sporulation. Spores were collected by centrifugation and resuspended in sterile distilled water.

Inoculation methods

Inoculation of *Phytophthora cinnamomi* was carried out by using the soil infestation method (Jung et al. 1996). Briefly, the soil was inoculated by mixing 12 mL of inoculum with the first 3 cm of substrate in each individual cell, taking care not to damage the roots of the seedlings. After inoculation, to promote sporangia production and zoospore release, the seedlings were watered, left for one day and then flooded with non-chlorinated water for two days.

Phytophthora plurivora was inoculated using the stem inoculation method (Kurbetli et al. 2022). A wound was made in the bark in the middle of the stem using a sterile scalpel, and a mycelial plug from the edge of a growing *P. plurivora* colony was inserted into the wound and sealed with Parafilm® (American National Can Co., Neenah, USA).

Ophiostoma novo-ulmi was inoculated with a spore suspension at a concentration of 10^6 blastospores mL^{-1} (Martín et al. 2010). Two drops of inoculum were applied into the xylem of each tree through a transverse cut made with a sharp blade 5 cm above the level of the soil, allowing absorption of the inoculum. At the end of the study, *O. novo-ulmi* and each *Phytophthora* species were re-isolated from symptomatic tissues of inoculated plants.

Experimental layout

In five independent experiments, suspensions at different concentrations of MeJA (Sigma-Aldrich, #39270-7) in 0.5% ethanol (v/v) in deionised water were used. The solutions were shaken vigorously until a uniform milky emulsion was obtained. The aerial part of each tree was sprayed with ca. 2 mL of each solution separately up to run-off, and the aerial part of control plants was sprayed with 0.05% ethanol (v/v) in deionised water. Trees treated with different MeJA concentrations were kept in separate greenhouse cabinets for 48 h and then arranged in a fully randomized design. The growth conditions of plants were 25 °C, 60% relative humidity and natural daylight. Elm trees were MeJA-sprayed with ca. 1 L per tree by using a motorised wheelbarrow sprayer. In all experiments, the soil surface of trees was covered by filter paper to prevent the product from dripping onto the ground.

Experiment 1. MeJA to protect *Castanea sativa* against *Phytophthora cinnamomi*

In May 2021, six-month-old chestnut seedlings were sprayed with MeJA at 0, 0.04, 0.2, 1, 5 and 10 mM concentrations based on previous research (Vivas et al. 2012; Zas et al. 2014; López-Villamor et al. 2021). Six days later, the trees were challenged with *P. cinnamomi* soil infestation. To test a double application of MeJA, four days after being *P. cinnamomi*-infested, half of the inoculated trees were sprayed again, at the same concentration. This period was selected to coincide with pathogenesis of ink disease (Camisón et al. 2019), particularly when the pathogen switches into a necrotrophic phase (Fernandes et al. 2021).

The plants were ca. 25 cm high and were arranged in root trainers of 15 cells, each cell containing a single plant and 33 cL of *Sphagnum* peat (Kekkilä Professional®, Vantaa, Finland). One root trainer per MeJA concentration was used to assess the effect of the preventive treatment, and one root trainer per MeJA concentration was used to assess the effect of the preventive + curative treatment ($n = 15$ plants). An additional root trainer of non-treated plants was used as a control ($n = 15$). Thus, the experiment included 195 trees corresponding to 6 MeJA concentrations × 2 treatments × 15 plants, and 15 additional control trees. In April 2022, the vegetative budburst of all plants was assessed following Solla et al. (2014). In May 2022, all plants were inoculated with *P. cinnamomi* for a second time. Tree mortality was assessed monthly for 2 years, and tree growth was not assessed.

Experiment 2. MeJA to protect *Quercus ilex* and *Q. suber* against *P. cinnamomi*

Based on results from Experiment 1, in May 2022 one-year-old holm oak and cork oak trees were sprayed at 0, 0.2 and 1 mM MeJA concentrations. In order to evaluate the effect of MeJA as a preventive, curative or preventive + curative treatment against *P. cinnamomi* (*Pc*), the following eleven groups of plants were used: (i) treated at 0 mM and not inoculated (CC), (ii) treated at 0.2 mM and not inoculated (0.2), (iii) treated at 1 mM and not inoculated (1), (iv) treated twice at 1 mM (1 + 1), (v) treated at 0 mM and *Pc*-inoculated (*Pc*), (vi) treated at 0.2 mM and *Pc*-inoculated (0.2 + *Pc*), (vii) treated at 1 mM and *Pc*-inoculated (1 + *Pc*), (viii) *Pc*-inoculated and treated at 0.2 mM (*Pc* + 0.2), (ix) *Pc*-inoculated and treated at 1 mM (*Pc* + 1), (x) treated at 0.2 mM, *Pc*-inoculated and treated at 0.2 mM (0.2 + *Pc* + 0.2) and (xi) treated at 1 mM, *Pc*-inoculated and treated at 1 mM (1 + *Pc* + 1). *Pc* inoculation was performed one week after the MeJA treatments, and the second MeJA treatment was applied ten days after soil infestation. The timing of the second treatment was selected to coincide with pathogenesis of *Pc* in the two oak species (Luque et al. 2002), particularly when the pathogen switches into a necrotrophic phase (Redondo et al. 2015).

The *Q. ilex* and *Q. suber* plants were ca. 15 and 24 cm high, respectively, and were arranged in root trainers of 24 cells, each cell containing a single plant and 33 cL of *Sphagnum* peat (Kekkilä Professional®, Vantaa, Finland). One root trainer per treatment was used ($n = 15$ plants). The experiment comprised 528 trees corresponding to 2 species × 11 treatments × 24 plants. Tree mortality was assessed monthly for one year, and tree height was assessed before treatments and at the end of the vegetative period.

Experiment 3. MeJA to protect *Q. robur* and *Fraxinus excelsior* against *P. plurivora*

In July 2021, two-year-old European oak and European ash trees were sprayed at 0, 0.2, 1, 5 and 10 mM MeJA concentrations. Twelve days later, the trees were challenged with *P. plurivora*. To test a possible curative effect of MeJA, eight days after being *P. plurivora*-infected, a group of non-treated trees was sprayed at the same five MeJA concentrations. To test a possible effect of a combination of preventive and curative MeJA treatment, an additional group of treated and *P. plurivora*-infected trees were sprayed with same concentrations.

The *Q. robur* and *F. excelsior* plants were in average 63 and 44 cm high, respectively, and were planted in 3L pots containing *Sphagnum* peat (Kekkilä Professional®, Vantaa, Finland). The experiment comprised 180 trees corresponding to 2 species × 5 MeJA concentrations × 3 groups × 6 plants. Two months after inoculation, tree height was measured; then, plants were harvested and the outer bark/epidermis of the plants was removed, to measure the lesion length above and below the point of inoculation (Kurbetli et al. 2022). Tree height was assessed before treatments and at the end of the vegetative period.

Experiment 4. MeJA to protect *Ulmus minor* against *Ophiostoma novo-ulmi*

In May 2022, 7-year-old ramets of two field elm clones were sprayed with MeJA at 0, 1 and 10 mM concentrations based on previous research (Vivas et al. 2012). One week later, the trees were challenged with *Ophiostoma novo-ulmi*. At this time, trees were 2.6 to 3 m tall. The experiment comprised 30 trees corresponding to 2 clones × 3 MeJA concentrations × 5 ramets (i.e. tree replicates). Disease severity was evaluated 30, 60 and 120 days after inoculation by recording the percentage of leaf wilting in the crown (Martín et al. 2008). Tree growth was not assessed.

Experiment 5. Does MeJA spray affect ectomycorrhizal infection and symbiosis?

In spring 2022, single and double MeJA spray treatments were applied before and after inoculation of *Pisolithus tinctorius* on *C. sativa*, *Q. ilex* and *Q. suber* trees. MeJA at 0 and 1 mM and six-months-old seedlings were used. To test if MeJA affects mycorrhizal infection, the plants were sprayed once (45 or 15 days) or twice (45 and 15 days) before inoculation with *P. tinctorius*. To test if MeJA alters mycorrhizal symbiosis, the plants were sprayed once (45 or 15 days) or twice (45 and 15 days) after inoculation with *P. tinctorius*.

Inoculation was carried out in June 2022 by using a spore suspension in distilled water as inoculum. At this time, *C. sativa*, *Q. ilex* and *Q. suber* plants were ca. 12, 10 and 23 cm high, respectively, and were arranged in root trainers of 15 cells, each cell containing a single tree and 33 cL of *Sphagnum* peat (Kekkilä Professional®, Vantaa, Finland). Ten mL of inoculum including ~ 1.6×10^7 spores/mL was directly injected into the substrate of each tree by using a plastic syringe (González-Ochoa et al. 2003). The experiment comprised 180 inoculated trees corresponding to 3 species × 2 MeJA concentrations × 2 tests (before *vs* after inoculation) × 3 treatments × 5 seedlings, and five additional non-inoculated trees per species.

Four months after inoculation, the percentage of root tips colonized by *P. tinctorius* was assessed in five root samples taken randomly from each tree. Approximately 50 lateral root tips per tree were examined under a stereomicroscope (Olympus SZX10, Japan) and classified as mycorrhized (typically shorter, thicker and yellow) or non-mycorrhized (Sebastiana et al. 2013). Tree height was assessed before treatments and at the end of the vegetative period.

Statistical analyses

To analyse the time to death of trees inoculated with *P. cinnamomi*, survival time analysis based on Kaplan–Meier estimation was used (Solla et al. 2011). To evaluate the effect of MeJA treatments on tree height, leaf wilting, necrosis length or percentage of mycorrhization, one-way analysis of variance (ANOVA) was performed using the treatment as a single factor. To identify significant differences between means, Tukey's multiple comparison tests at $p < 0.05$ were used. Data were analysed with Statistica v10.0 (StatSoft Inc. 2011).

Results

MeJA at 1 mM protected chestnut against *P. cinnamomi*

In Experiment 1, at the end of the first vegetative period, mortality of *P. cinnamomi*-infected *C. sativa* trees subjected to 0 mM MeJA was 40% (Fig. 1A). Preventive

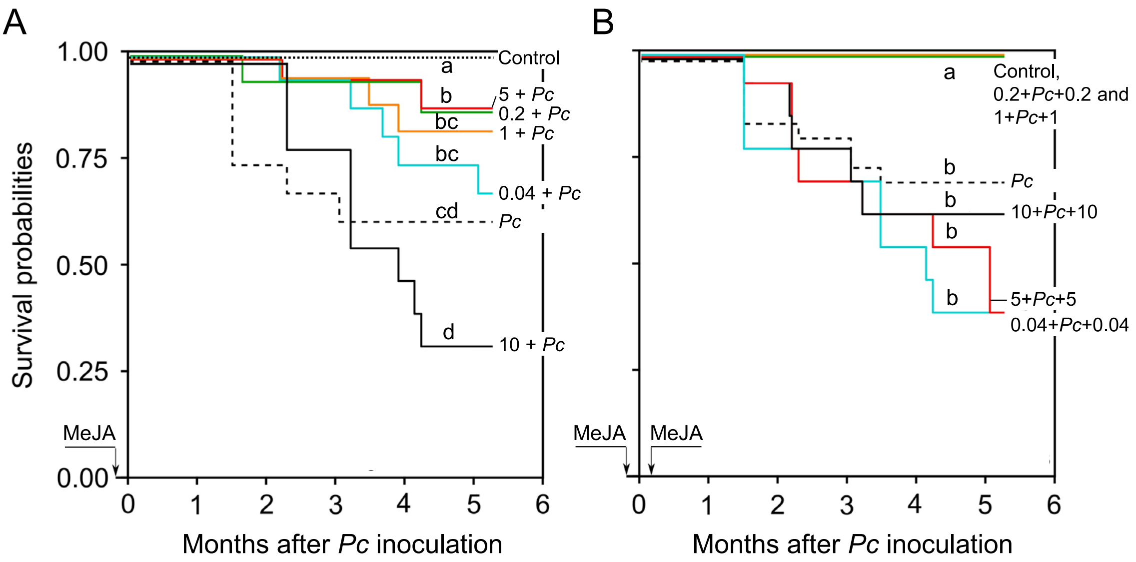


Fig. 1 Survival probabilities of *Castanea sativa* trees sprayed with methyl jasmonate (MeJA) and challenged with *Phytophthora cinnamomi* (*Pc*; preventive treatment) (A), or sprayed with MeJA, challenged with *Pc* and sprayed with MeJA again (preventive treatment + curative treatment) (B). Trees were neither sprayed nor inoculated (control, dotted line) or sprayed at 0 mM (black dashed line), 0.04 mM (blue line), 0.20 mM (green line), 1 mM (orange line), 5 mM (red line) and 10 mM (black line) MeJA concentrations and inoculated ($n = 15$). Different letters indicate significant differences between survival curves (log-rank test, $p < 0.05$)

treatments of MeJA at 0.2, 1 and 5 mM reduced tree mortality in relation to the 0 mM MeJA treatment by 53, 62 and 67%, respectively (Fig. 1A). On the contrary, the preventive MeJA treatment at 10 mM enhanced tree mortality in relation to the 0 mM MeJA treatment by 75% and caused phytotoxicity in young leaves ten days after application (Fig. 2A).

By spraying the trees again after inoculation, MeJA at 0.2 and 1 mM was able to impede mortality, fully protecting all the 15 *P. cinnamomi*-infected chestnuts during the whole vegetative period (Fig. 1B). The protection induced by MeJA at 1 mM after single (preventive) and double spray lasted two additional years, i.e. no additional tree mortality was observed in 2022 and 2023 although trees were re-inoculated in 2022. The protection induced by MeJA at 0.2 mM and other concentrations failed after the plants were re-inoculated in 2022 (Fig. S1). One year after treatments and *P. cinnamomi* infection, only in trees sprayed with 1 mM MeJA (Fig. S2), leaves started to flush ca. 15 days earlier than the leaves of control trees.

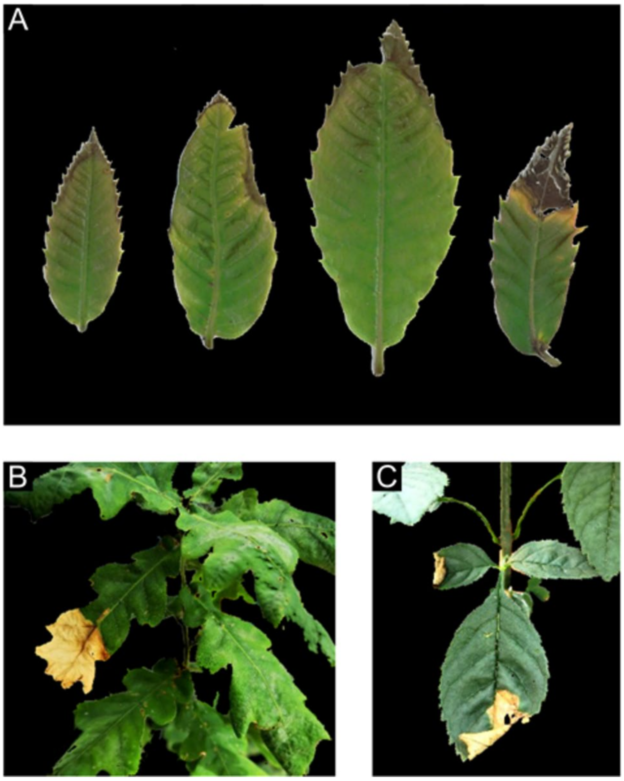


Fig. 2 Side effects caused by methyl jasmonate (MeJA) foliar spray at 10 mM in *Castanea sativa* **A**, *Quercus robur* **B** and *Fraxinus excelsior* **C**. Three weeks after spraying, yellowing and necrosis started in the leaf apex and expanded towards the petiole

MeJA at 0.2 mM protected holm oak against *P. cinnamomi*

In Experiment 2, one year after inoculation, *Q. ilex* and *Q. suber* trees sprayed with water (control treatment) and challenged with *P. cinnamomi* showed 79 and 12% mortality, respectively. In *Q. ilex*, MeJA spray increased the survival of trees, with the treatments 0.2 + *Pc* and 0.2 + *Pc* + 0.2 providing the best protection (Fig. 3). In *Q. suber*, MeJA spray did not increase the survival of trees, and some of the treatments (i.e. 0.2 + *Pc* + 0.2, 1 + *Pc* and *Pc* + 1) increased tree mortality (results not shown).

Tree height in *Q. ilex* was significantly impaired by *Pc* inoculation and/or MeJA treatments (Fig. 4A). The treatments that showed best protection in *Q. ilex* (i.e. 0.2 + *Pc*, 1 + *Pc* and 0.2 + *Pc* + 0.2) did not enhance tree growth (Fig. 4A). However, in *Q. suber* MeJA spray at 0.2 and 1 mM as preventive and at 0.2 mM as curative enhanced tree growth (Fig. 4B).

MeJA did not protect *Q. robur* and *F. excelsior* against *P. plurivora*

In Experiment 3, two months after inoculation with *P. plurivora*, lesions of 31.5 ± 11.2 and 22.2 ± 2.1 mm in length were observed on the stems of *Q. robur* and *F. excelsior* trees, respectively. In contrast, the length of lesions on mock-inoculated *Q. robur* and *F. excelsior* trees were 0.8 ± 0.7 and 1.0 ± 1.1 mm respectively. MeJA spray did not protect or cure any of the two species against *P. plurivora* (Fig. 5) and did not alter tree growth (results not shown). MeJA at 5 and 10 mM was slightly toxic for *Q. robur* and *F. excelsior*, as necrosis in the tips of some leaves was observed (Fig. 2B, C, respectively).

Protection of MeJA against *O. novo-ulmi* was dose- and genotype-dependent

In Experiment 4, one and two months after inoculation of the highly susceptible Atinia elm clone, leaf wilting was lowest when trees were preventively sprayed with MeJA at 1 mM (Fig. 6A). However, at the end of the experiment, MeJA spray was not able to decrease the leaf wilting caused by *O. novo-ulmi* in this clone (Fig. 6A). In the susceptible CC-VG4.2 clone, preventive treatment of MeJA at 10 mM reduced leaf wilting in relation to the 0 mM MeJA treatment by 70% (Fig. 6B). No phytotoxic effects of MeJA were observed.

MeJA did not affect ectomycorrhizal symbiosis

According to Experiment 5, MeJA spray at 1 mM did not influence mycorrhizal infection or symbiosis of *C. sativa*, *Q.*

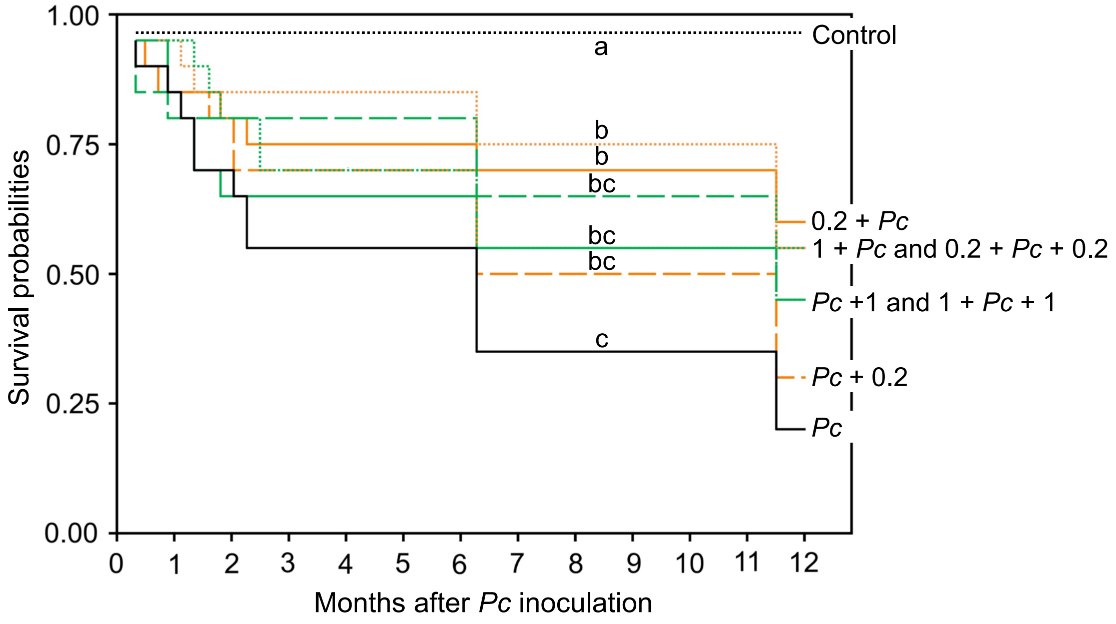


Fig. 3 Survival probabilities of *Quercus ilex* trees sprayed with methyl jasmonate (MeJA) at 0.2 mM (0.2 + *Pc*, orange line) and 1 mM (1 + *Pc*, green line) and challenged with *Phytophthora cinnamomi* (*Pc*) (preventive treatments), inoculated with *Pc* and sprayed with MeJA at 0.2 mM (*Pc* + 0.2, orange dashed line) and 1 mM (1 + *Pc*, green dashed line) (curative treatments), sprayed with MeJA at 0.2 mM (0.2 + *Pc* + 0.2, orange dotted line) and 1 mM (1 + *Pc* + 1, green dotted line) before and after being challenged with *Pc* (preventive + curative treatments), or neither sprayed nor inoculated (control, black dotted line) ($n = 15$). Different letters indicate significant differences between survival curves (log-rank test, $p < 0.05$)

ilex and *Q. suber* trees in relation to control plants, irrespective of single or double treatments. All trees showed vital ectomycorrhizal tips and percentages of mycorrhization with *P. tinctorius* per tree ranging from 19.6 ± 1.6 to 26.3 ± 4.0% (data not shown). At the end of the vegetative period, *P. tinctorius*-inoculated trees were ~ 15% taller than non-inoculated trees, irrespective of the species. MeJA spray at 1 mM did not significantly influence final height of trees (Table S1).

Discussion

Exogenous application of MeJA has been widely studied to help reducing the adverse effects of pest damage in forest trees (Sampedro et al. 2011; Semiz et al. 2012; Whitehill et al. 2014; Zas et al. 2014; Nunes da Silva et al. 2025). However, the potential of MeJA to protect deciduous trees from fungal and oomycete pathogens has remained poorly studied. In this study, the efficacy of MeJA foliar spray against pathogens was tested in six broad-leaved forest species widespread in Europe. In chestnut, this is the first study to evaluate the effect of MeJA in inducing resistance to *P. cinnamomi*. In a previous work, we observed that the presence of jasmonic acid (JA) and its conjugate JA-Ile in chestnut was related to constitutive and inducible resistance to *P. cinnamomi* (Camisón et al. 2019). Accumulation of JA and other oxylipins was observed in chestnut when inoculated with *P. cinnamomi* (Saiz-Fernández et al. 2020). In our work, we observed that MeJA spray at 0.2 mM protected chestnut seedlings from *P. cinnamomi*, even if treatments were applied after inoculation. This finding is in accordance with the previously described role of jasmonic acid in providing resistance to chestnut and other plant species (Cooper and Rieske 2008; Wang et al. 2011; Sulaiman and Bello 2024; Nunes da Silva et al. 2025) and emphasises the potential of MeJA in environmentally friendly control of ink disease.

Holm oak and cork oak trees are also suffering severe and widespread damage by *P. cinnamomi* (Encinas-Valero et al. 2022; Serrano et al. 2024). It has been shown that *Q. ilex* is more susceptible than *Q. suber* to *P. cinnamomi* (de la Mata et al. 2024). Indeed in the present study, cork oak seedlings were almost unaffected by the pathogen. In holm oak seedlings, MeJA spray at 0.3 mM increased the emission of green leaf volatile compounds (Semiz et al. 2012). These compounds are related to the expression of genes activating the synthesis of oxylipins that ultimately produce jasmonates in plants (Naeem et al. 2015). Jasmonate induction has been described as part of the defensive machinery of *Q. ilex* against *P. cinnamomi,* and exposure to MeJA of

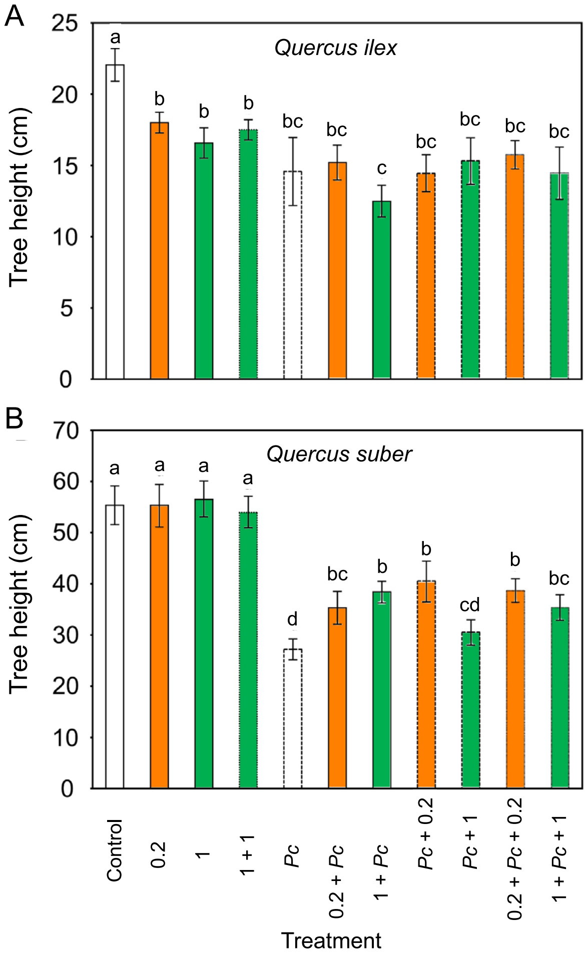


Fig. 4 Height of *Quercus ilex* **A** and *Q. suber* **B** trees sprayed with methyl jasmonate at 0 mM (white columns), 0.2 mM (orange columns) and 1 mM (green columns) within preventive, curative and preventive + curative treatments against *Phytophthora cinnamomi* (*Pc*) ($n = 15$). Vertical bars are standard errors and different letters indicate significant differences between values within each species and treatment (Tukey's HSD test, $p < 0.05$)

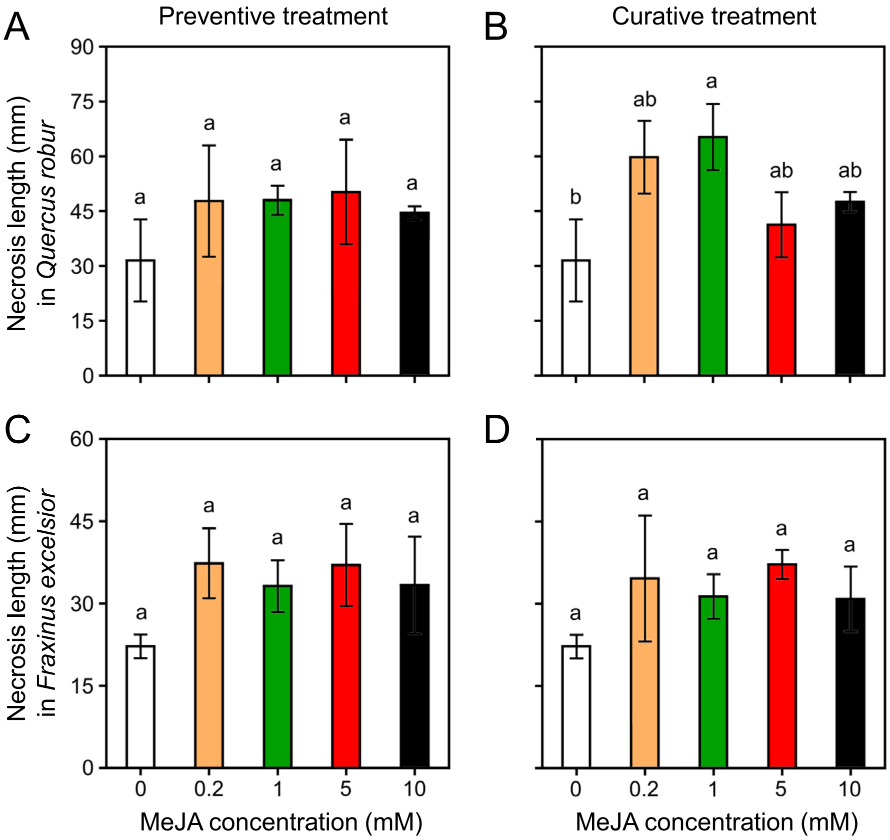
Q. ilex embryonic lines has resulted in an increase of abscisic acid, jasmonic acid and phenolic compounds (Morcillo et al. 2022). Our results agree with the previous studies and provide evidence that MeJA has long-lasting effects as a defence elicitor, probably leading to increased long-term resistance in *Q. ilex*, as it was observed in *P. pinaster* (Vázquez-González et al. 2022). Treatments with MeJA (0.2 mM) could thus help control *Phytophthora* in holm oak woodlands, where seedling establishment and tree regeneration often fail mainly due to *Phytophthora* soil infestation (Martín-García et al. 2015). In any case, the protection observed in the present work on seedlings should be tested in adults.

In the last 13 years, 56 chemical products have been tested to control *Phytophthora* of forest trees (López-García et al. 2024), and some studies have shown limiting or inconsistent results. Several factors could explain why MeJA treatments did not protect *F. excelsior* and *Q. robur* against *P. plurivora*. First, the dose and the timing may not have been optimal to induce the defences. Second, the development of stem necrosis, used as a proxy for disease development (Cleary et al. 2017) may not fully correspond to the trees' resistance against root infections, as indicated for *P. plurivora* (Macháčová et al. 2024). Moreover, the high virulence of the pathogen used may allow the resistance threshold of the plants to be surpassed (Vivas et al. 2012). Therefore, the failure of MeJA treatments observed here should be considered with caution and the results validated in experiments by using soil infestation.

The plant species may also have played a role in the lack of protection provided by MeJA in *F. excelsior* and *Q. robur*. In a previous work, variation of MeJA to induce resin duct formation in pine species was species-dependent (López-Villamor et al. 2021) and other studies have shown that genotypes, families and provenances within species can exhibit different responses to MeJA (e.g. Moreira et al. 2013; López-Goldar et al. 2018; Puentes et al. 2021). However, no overall differences in the induction of resistance by MeJA when comparing different *Pinus* and *Picea* species through a meta-analysis were found (Huynh et al. 2024).

The results showed that MeJA was able to mitigate the external symptoms of elms affected by Dutch elm disease, although leaf wilting reduction was dependent on the MeJA concentration and the elm genotype used. The best concentration was 1 mM MeJA for the highly susceptible Atinia clone, and 10 mM for the moderately susceptible CC-VG4.2 clone. The observed differences between the elm genotypes in the response to MeJA supports previous research in conifers also showing that MeJA effect is highly dependent on the genotype (Zeneli et al. 2006; López-Goldar et al. 2018; Moreira et al. 2013). The idea that the higher the host susceptibility, the higher the MeJA dose required, supported in our study when comparing the optimal dose against *P. cinnamomi* in the least susceptible *Q. ilex* (0.2 mM) *vs* the more susceptible *C. sativa* (1 mM), was not supported in the case of the elm genotypes tested. Thus, the relationship between susceptibility and the dose required does not seem straightforward and factors such as the type of disease (e.g. vascular *vs* soil-borne), the biological scale (e.g. tree species, families or genotypes), the timing of MeJA application and environmental factors can strongly affect the outcome of the induced resistance (Eyles et al. 2010). Previous research showed that MeJA at 50 and 100 mM did not protect *U. minor* against *O. novo-ulmi* (Vivas et al. 2012). Our results with lower doses were promising but again not enough to protect elm trees in the long term. More research by using lower MeJA concentrations and more elm genotypes is needed to determine if MeJA could be a useful compound to fight against *O. novo-ulmi*.

Fig. 5 Necrosis length in the stem of *Quercus robur* **A**, **B** and *Fraxinus excelsior* **C**, **D** trees inoculated with *Phytophthora plurivora*. Trees were treated with MeJA at different concentrations before (preventive treatment **A**, **C**) and after (curative treatment **B**, **D**) being inoculated ($n = 6$). Vertical bars are standard errors, and different letters indicate significant differences between values within each species and treatment (Tukey's HSD test, $p < 0.05$)



Some of the main problems encountered in the use of resistance inducers in plants are the effects on yield and growth, phytotoxicity and the negative effects on beneficial mutualistic interactions (Heil 2002; Walters and Heil 2007). JA-mediated signalling regulates the trade-off between plant growth and the expression of defence (Li et al. 2022), and temporarily reduced plant growth was observed following treatment of trees with MeJA (Gould et al. 2008; Krokene et al. 2008; Vivas et al. 2012; Huynh et al. 2024). Here, we found a negative effect of MeJA on the growth of *Q. ilex* but not on that of *Q. suber* (Fig. 4), indicating species-dependent sensitivity of tree growth to MeJA application within *Quercus* species. As a novelty, we report early leaf flushing one year after treatment with 1 mM MeJA in *C. sativa* (about 15 days earlier than controls; Fig. S2), and although further research is needed, this result may indicate that MeJA spray would allow increased exposure of chestnut to late frosts and herbivory.

Phytotoxicity due to repeated and/or high concentration of MeJA application has been reported in *Pinus* spp., *Fraxinus* spp., *Q. ilex* and *U. minor*, expressed as yellowing and necrosis of especially the younger leaves located in the upper part of the crown (Gould et al. 2009; Semiz et al. 2012; Vivas et al. 2012; Whitehill et al. 2014). We observed phytotoxicity in *C. sativa*, *Q. robur* and *F. excelsior* trees that were treated with MeJa 10 mM, which probably explains why treatments with this concentration were not effective. Treatments that induce resistance may also impact mutualistic interactions of treated plants with beneficial organisms. Particularly the inducers that enhance suberization and lignification of tissues (Martín et al. 2008, 2012), resulting in broad-spectrum resistance in plants, could alter plant–microbe mutualisms such as mycorrhization and nodulation (Heil 2002; Walters and Heil 2007). Our results indicate that MeJA spray in chestnut, holm oak and cork oak seedlings did not affect *P. tinctorius* establishment or alter the mycorrhization rates.

Besides the benefits of applying MeJA to combat forest diseases, additional potential side effects and ecological implications should be considered. MeJA treatments can alter the production of volatile organic compounds (VOCs) (Amo et al. 2022), also produced by natural plant defence signalling (Dicke and Baldwin 2010), and may affect non-target organisms such as pollinators, herbivores and beneficial insects (Thaler 1999). The application of MeJA to *Salix cinerea* in a field trial led to increased attractiveness of oak saplings for insectivorous predators, including invertebrates and birds (Mrazova et al. 2023). Furthermore,

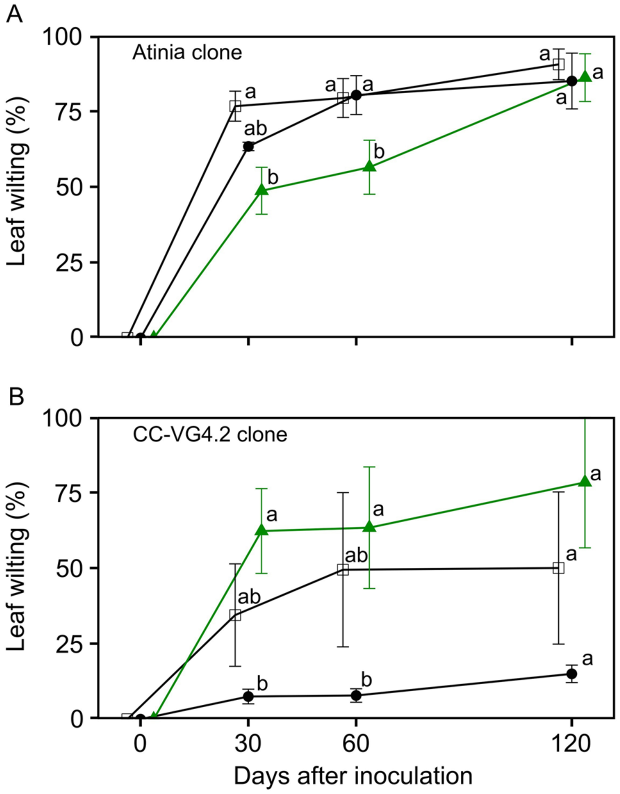


Fig. 6 Changes in leaf wilting percentages in Atinia **A** and CC-VG4.2 **B** *Ulmus minor* clones sprayed with MeJA at 0 mM (empty squares), 1 mM (green triangles) and 10 mM (black circles) and challenged with *Ophiostoma novo-ulmi* ($n = 5$). Vertical bars are standard errors, and different letters indicate significant differences between values within each day (Tukey's HSD test, $p < 0.05$)

MeJA treatments may induce changes in plant biochemical pathways and nutrient content by altering root exudates and bacterial rhizosphere communities (Doornbos et al. 2011; Carvalhais et al. 2013), which are crucial for ecosystem functioning and soil health (Pieterse et al. 2014). Lastly, continuous use of MeJA in agricultural or ecological settings may lead to the development of resistance in target organisms or unintended adaptations in non-target organisms, influencing the efficacy of future treatments and ecological balance (Heil and Baldwin 2002; Thaler et al. 2012). Thorough consideration of these implications is thus needed to guide the responsible use of MeJA in management strategies of forests.

Conclusion

For more than 20 years, methyl jasmonate (MeJA) has been used to study inducible defences in conifers and to increase their resistance to pests and pathogens. Despite the numerous studies on the subject, few attempts have been made to quantify if MeJA protects broad-leaf trees against oomycete and fungal pathogens. Here, we present evidence of protective effects of MeJA on chestnut, holm oak and field elm, three of the most widespread and ecologically important tree genera in temperate and Mediterranean forests. Our findings, summarized in Table 1, emphasize the potential of MeJA as a resistance inducer, but also point out several knowledge gaps and a general need of field studies to explore the usefulness of MeJA treatments in sustainable protection of the threatened broad-leaf forests in Europe.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s41348-025-01061-w.

Acknowledgements The authors are very grateful to Dr. Francisco Ruiz-Gómez (University of Córdoba) and Dr. Ramesh Vetukuri (Swedish University of Agricultural Sciences) for kindly providing us with the *P. cinnamomi* and *P. plurivora* isolates, respectively. Thanks to the encouragement provided by Rafael Coullaut and Ignacio Baños. We thank the Spanish elm breeding program (UPM-MITECO) for providing the elm material and the isolate and Jorge Domínguez for his technical assistance during MeJA application in elms.

Author contributions The concept and design of the experiments were prepared by AS, FJD and AC. JW and AS acquired the funding and were in charge of supervision and project administration. FJD, IM, AC and JAM performed the experiments, JO assessed mycorrhization, and CR helped with data sampling. FJD and JAM analysed the results, FJD and AS prepared the first draft of the manuscript, and all authors revised, edited, and approved the paper.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. The work was supported by Lithium Iberia S.L. (123/20 contract) and University of Extremadura (VI-23 Action). The experiment in Sweden was supported by a Carl Tryggers Stiftelse (CTS 18: 424) Grant, which financed a postdoc position for I.M. A.C. was supported by a postdoctoral 'Margarita Salas' contract from Universidad de Extremadura funded by the Spanish Ministry of Universities. Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature.

Data availability Data will be made available on request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Ali B (2021) Salicylic acid: an efficient elicitor of secondary metabolite production in plants. Biocatal Agric Biotechnol 31:101884. https://doi.org/10.1016/j.bcab.2020.101884

Amo L, Mrazova A, Saavedra I, Sam K (2022) Exogenous application of methyl jasmonate increases emissions of volatile organic compounds in Pyrenean oak. Biology 11:84. https://doi.org/10.3390/biology11010084

Antonelli C, Biscontri M, Tabet D, Vettraino AM (2023) The never-ending presence of *Phytophthora* species in Italian nurseries. Pathogens 12:15. https://doi.org/10.3390/pathogens12010015

Basso V, Kohler A, Miyauchi S, Singan V, Guinet F, Šimura J, Novák O, Barry KW, Amirebrahimi M, Block J, Daguerre Y, Na H, Grigoriev IV, Martin F, Veneault-Fourrey C (2020) An ectomycorrhizal fungus alters sensitivity to jasmonate, salicylate, gibberellin, and ethylene in host roots. Plant Cell Environ 43:1047–1068. https://doi.org/10.1111/pce.13702

Berggren K, Nordkvist M, Björkman C, Bylund H, Klapwijk MJ, Puentes A (2023) Synergistic effects of methyl jasmonate treatment and propagation method on Norway spruce resistance against a bark-feeding insect. Front Plant Sci 14:1165156. https://doi.org/10.3389/fpls.2023.1165156

Cairney JWG, Chambers SM (1997) Interactions between *Pisolithus tinctorius* and its hosts: a review of current knowledge. Mycorrhiza 7:117–131. https://doi.org/10.1007/s005720050172

Camisón A, Martín MÁ, Sánchez-Bel P, Flors V, Alcaide F, Morcuende D, Pinto G, Solla A (2019) Hormone and secondary metabolite profiling in chestnut during susceptible and resistant interactions with *Phytophthora cinnamomi*. J Plant Physiol 241:153030. https://doi.org/10.1016/j.jplph.2019.153030

Carvalhais LC, Dennis PG, Badri DV, Tyson GW, Vivanco JM, Schenk PM (2013) Activation of the jasmonic acid plant defence pathway alters the composition of rhizosphere bacterial communities. PLoS ONE 8:e56457. https://doi.org/10.1371/journal.pone.0056457

Cleary MR, Blomquist M, Vetukuri RR, Böhlenius H, Witzell J (2017) Susceptibility of common tree species in Sweden to *Phytophthora cactorum*, *P. cambivora* and *P. plurivora*. For Pathol 47:e12329. https://doi.org/10.1111/efp.12329

Cooper WR, Rieske LK (2008) Differential responses in American (*Castanea dentata* Marshall) and Chinese (*C. mollissima* Blume) chestnut (Falales: Fagaceae) to foliar application of jasmonic acid. Chemoecology 18:121–127. https://doi.org/10.1007/s00049-008-0399-y

Corcobado T, Moreno G, Azul AM, Solla A (2015) Seasonal variations of ectomycorrhizal communities in declining *Quercus ilex* forests: interactions with topography, tree health status and *Phytophthora cinnamomi* infections. Forestry 88:257–266. https://doi.org/10.1093/forestry/cpu056

de la Mata R, Cuenca B, Luquero L, Moreno G, Solla A (2024) Genetic variation in susceptibility of *Phytophthora cinnamomi*-infected holm oak in the absence or presence of severe drought. Forestry. https://doi.org/10.1093/forestry/cpae045

Dicke M, Baldwin IT (2010) The evolutionary context for herbivore-induced plant volatiles: beyond the “cry for help.” Trends Plant Sci 15:167–175. https://doi.org/10.1016/j.tplants.2009.12.002

Doornbos RF, Geraats BP, Kuramae EE, Van Loon LC, Bakker PA (2011) Effects of jasmonic acid, ethylene, and salicylic acid signaling on the rhizosphere bacterial community of *Arabidopsis thaliana*. Mol Plant-Microbe Interact 24:395–407. https://doi.org/10.1094/MPMI-05-10-0115

Encinas-Valero M, Esteban R, Hereş AM, Vivas M, Fakhet D, Aranjuelo I, Solla A, Moreno G, Curiel Yuste J (2022) Holm oak decline is determined by shifts in fine root phenotypic plasticity in response to belowground stress. New Phytol 235:2237–2251. https://doi.org/10.1111/nph.18182

Eyles A, Bonello P, Ganley R, Mohammed C (2010) Induced resistance to pests and pathogens in trees. New Phytol 185:893–908. https://doi.org/10.1111/j.1469-8137.2009.03127.x

Fedderwitz F, Björklund N, Anngren R, Lindström A, Nordlander G (2020) Can methyl jasmonate treatment of conifer seedlings be used as a tool to stop height growth in nursery forest trees? New for 51:379–394. https://doi.org/10.1007/s11056-019-09737-6

Fernandes P, MaChado H, de Céu Silva M, Costa RL (2021) A histopathological study reveals new insights into responses of chestnut (*Castanea* spp.) to root infection by *Phytophthora cinnamomi*. Phytopathology 111:345–355. https://doi.org/10.1094/PHYTO-04-20-0115-R

Ghorbel M, Brini F, Sharma A, Landi M (2021) Role of jasmonic acid in plants: the molecular point of view. Plant Cell Rep 40:1471–1494. https://doi.org/10.1007/s00299-021-02687-4

Gil L, Fuentes-Utrilla P, Soto Á, Cervera MT, Collada C (2004) English elm is a 2,000-year-old Roman clone. Nature 431:1053. https://doi.org/10.1038/4311053a

González-Ochoa AI, de Las HJ, Torres P, Sánchez-Gómez E (2003) Mycorrhization of *Pinus halepensis* Mill. and *Pinus pinaster* Aiton seedlings in two commercial nurseries. Ann for Sci 60:43–48. https://doi.org/10.1051/forest/2009125

Gould N, Reglinski T, Spiers M, Taylor JT (2008) Physiological trade-offs associated with methyl jasmonate - induced resistance in *Pinus radiata*. Can J Forest Res 38:677–684. https://doi.org/10.1139/X07-193

Gould N, Reglinski T, Northcott GL, Spiers M, Taylor JT (2009) Physiological and biochemical responses in *Pinus radiata* seedlings associated with methyl jasmonate-induced resistance to *Diplodia pinea*. Physiol Mol Plant Pathol 74:121–128. https://doi.org/10.1016/j.pmpp.2009.10.002

Heil M (2002) Ecological costs of induced resistance. Curr Opin Plant Biol 5:345–350. https://doi.org/10.1016/s1369-5266(02)00267-4

Heil M, Baldwin IT (2002) Fitness costs of induced resistance: emerging experimental support for a slippery concept. Trends Plant Sci 7:61–67. https://doi.org/10.1016/S1360-1385(01)02186-0

Huynh NB, Krokene P, Puentes A, Mageroy MH (2024) Over 20 years of treating conifers with methyl jasmonate:meta-analysis of effects on growth and resistance. For Ecol Manag 561:121893. https://doi.org/10.1016/j.foreco.2024.121893

Jiang D, Yan S (2018) MeJA is more effective than JA in inducing defense responses in *Larix olgensis*. Arthropod-Plant Interact 12:49–56. https://doi.org/10.1007/s11829-017-9551-3

Jung T, Blaschke H, Neumann P (1996) Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. Eur J for Pathol 26:253–272. https://doi.org/10.1111/j.1439-0329.1996.tb00846.x

Jung T, Pérez-Sierra A, Durán A, Jung MH, Balci Y, Scanu B (2018) Canker and decline diseases caused by soil-and airborne *Phytophthora* species in forests and woodlands. Persoonia 40:182–220. https://doi.org/10.3767/persoonia.2018.40.08

Krokene P, Nagy NE, Solheim H (2008) Methyl jasmonate and oxalic acid treatment of Norway spruce:anatomically based defense responses and increased resistance against fungal infection. Tree Physiol 28:29–35. https://doi.org/10.1093/treephys/28.1.29

Krokene P, Kohmann K, Huynh NB, Mageroy MH (2023) Methyl jasmonate, salicylic acid, and oxalic acid affects growth, inducible defenses, and pine weevil resistance in Norway spruce. Front Plant Sci 14:1155170. https://doi.org/10.3389/fpls.2023.1155170

Kurbetli İ, Woodward S, Aydoğdu M, Sülü G, Özben S (2022) *Phytophthora plurivora* and *Phytophthora pseudocryptogea* isolated from soils supporting declining oaks (*Quercus robur* L.) in İstanbul, Turkey. For Pathol 52(e12782):1. https://doi.org/10.1111/efp.12782

Li C, Xu M, Cai X, Han Z, Si J, Chen D (2022) Jasmonate signaling pathway modulates plant defense, growth, and their trade-offs. Int J Mol Sci 23:3945. https://doi.org/10.3390/ijms23073945

López-García N, Romeralo C, Rönnberg J, Witzell J (2024) Control and management of *Phytophthora* damage in forestry-a systematic mapping study. For Pathol 54:e12878. https://doi.org/10.1111/efp.12878

López-Goldar X, Villari C, Bonello P, Borg-Karlson AK, Grivet D, Zas R, Sampedro L (2018) Inducibility of plant secondary metabolites in the stem predicts genetic variation in resistance against a key insect herbivore in Maritime pine. Front Plant Sci 9:1651. https://doi.org/10.3389/fpls.2018.01651

López-Villamor A, Zas R, Pérez A, Cáceres Y, Nunes da Silva M, Vasconcelos M, Vázquez-González C, Sampedro L, Solla A (2021) Traumatic resin ducts induced by methyl jasmonate in *Pinus* spp. Trees 35:557–567. https://doi.org/10.1007/s00468-020-02057-9

Luque J, Parladé J, Pera J (2002) Seasonal changes in susceptibility of *Quercus suber* to *Botryosphaeria stevensii* and *Phytophthora cinnamomi*. Plant Pathol 51:338–345. https://doi.org/10.1046/j.1365-3059.2002.00713.x

Macháčová M, Tomášková I, Corcobado T, Nagy Z, Milanović S, Janoušek J, Pešková V, Čepl J, Gezan S, Nakládal O, Zumr V, Kalyniukova A, Milenković I, Jung T (2024) Response of *Alnus glutinosa* to *Phytophthora* bark infections at ambient and elevated CO_2 levels. Front for Glob Change 7:1379791. https://doi.org/10.3389/ffgc.2024.1379791

Martín JA, Solla A, Domingues MR, Coimbra MA, Gil L (2008) Exogenous phenol increase resistance of *Ulmus minor* to Dutch elm disease through formation of suberin-like compounds on xylem tissues. Environ Exp Bot 64:97–104. https://doi.org/10.1016/j.envexpbot.2008.05.004

Martín JA, Solla A, Witzell J, Gil L, García-Vallejo MC (2010) Antifungal effect and reduction of *Ulmus minor* symptoms to *Ophiostoma novo-ulmi* by carvacrol and salicylic acid. Eur J Plant Pathol 127:21–32. https://doi.org/10.1007/s10658-009-9567-3

Martín JA, Solla A, García-Vallejo MC, Gil L (2012) Chemical changes in *Ulmus minor* xylem tissue after salicylic acid or carvacrol treatments are associated with enhanced resistance to *Ophiostoma novo-ulmi*. Phytochemistry 83:104–109. https://doi.org/10.1016/j.phytochem.2012.07.017

Martín JA, Domínguez J, Solla A, Brasier CB, Webber JF, Santini A, Martínez-Arias C, Bernier L, Gil L (2023) Complexities underlying the breeding and deployment of Dutch elm disease resistant elms. New for 54:661–696. https://doi.org/10.1007/s11056-021-09865-y

Martín-García J, Solla A, Corcobado T, Siasou E, Woodward S (2015) Influence of temperature on germination of *Quercus ilex* in *Phytophthora cinnamomi*, *P. gonapodyides*, *P. quercina* and *P. psychrophila* infested soils. For Pathol 45:215–223. https://doi.org/10.1111/efp.12159

Morcillo M, Sales E, Corredoira E, Martínez MT, Segura J, Arrillaga I (2022) Effect of methyl jasmonate in gene expression, and in hormonal and phenolic profiles of Holm oak embryogenic lines before and after infection with *Phytophthora cinnamomi*. Front Plant Sci 13:824781. https://doi.org/10.3389/fpls.2022.824781

Moreira X, Zas R, Sampedro L (2013) Additive genetic variation in resistance traits of an exotic pine species: Little evidence for constraints on evolution of resistance against native herbivores. Heredity 110:449–456. https://doi.org/10.1038/hdy.2012.108

Mrazova A, Tahadlová MH, Řehová V, Sam K (2023) The specificity of induced chemical defence of two oak species affects differently arthropod herbivores and arthropod and bird predation. Arthropod-Plant Interact 17:141–155. https://doi.org/10.1007/s11829-023-09951-2

Naeem M, Zainal Z, Ismail I (2015) Green leaf volatiles:biosynthesis, biological functions and their applications in biotechnology. Plant Biotechnol J 13:727–739. https://doi.org/10.1111/pbi.12368

Nunes da Silva MN, Santos CS, Solla A, Gamir J, Flors V, Sampedro L, Zas R, Vasconcelos MW (2025) Resistance of *Pinus pinea* to *Bursaphelenchus xylophilus* explained by the dynamic response of phytohormones, antioxidant activity, and stress-related gene expression. Trees 39:21. https://doi.org/10.1007/s00468-024-02594-7

Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker PAHM (2014) Induced systemic resistance by beneficial microbes. Annu Rev Phytopathol 52:347–375. https://doi.org/10.1146/annurev-phyto-082712-102340

Puentes A, Zhao T, Lundborg L, Björklund N, Borg-Karlson AK (2021) Variation in methyl jasmonate-induced defense among Norway spruce clones and trade-offs in resistance against a fungal and an insect pest. Front Plant Sci 12:678959. https://doi.org/10.3389/fpls.2021.678959

Redondo MÁ, Pérez-Sierra A, Abad-Campos P, Torres L, Solla A, Reig-Armiñana J, García-Breijo F (2015) Histology of *Quercus ilex* roots during infection by *Phytophthora cinnamomi*. Trees 29:1943–1957. https://doi.org/10.1007/s00468-015-1275-3

Saiz-Fernández I, Milenković I, Berka M, Černý M, Tomšovský M, Brzobohatý B, Kerchev P (2020) Integrated proteomic and metabolomic profiling of *Phytophthora cinnamomi* attack on sweet chestnut (*Castanea sativa*) reveals distinct molecular reprogramming proximal to the infection site and away from it. Int J Mol Sci 21:8525. https://doi.org/10.3390/ijms21228525

Sampedro L, Moreira X, Zas R (2011) Resistance and response of *Pinus pinaster* seedlings to *Hylobius abietis* after induction with methyl jasmonate. Plant Ecol 212:397–401. https://doi.org/10.1007/s11258-010-9830-x

San-Eufrasio B, Castillejo MÁ, Labella-Ortega M, Ruiz-Gómez FJ, Navarro-Cerrillo RM, Tienda-Parrilla M, Jorrín-Novo JV, Rey MD (2021) Effect and response of Quercus ilex subsp. *ballota* [Desf.] Samp. seedlings from three contrasting Andalusian populations to individual and combined *Phytophthora cinnamomi* and drought stresses. Front Plant Sci 12:722802. https://doi.org/10.3389/fpls.2021.722802

Sebastiana M, Pereira VT, Alcântara A, Pais MS, Silva AB (2013) Ectomycorrhizal inoculation with *Pisolithus tinctorius* increases the performance of *Quercus suber* L. (cork oak) nursery and field seedlings. New for 44:937–949. https://doi.org/10.1007/s00572-018-0823-2

Semiz G, Blande JD, Heijari J, Isik K, Niinemets Ü, Holopainen JK (2012) Manipulation of VOC emissions with methyl jasmonate and carrageenan in the evergreen conifer *Pinus sylvestris* and evergreen broadleaf *Quercus ilex*. Plant Biol 14:57–65. https://doi.org/10.1111/j.1438-8677.2011.00485.x

Serrano MS, Villa-Sanabria E, Homet P, Gutiérrez E, Gómez-Aparicio L (2024) Impact of a drier climate on the exotic pathogen *Phytophthora cinnamomi* in Mediterranean forests differing in soil properties and species composition. For Ecol Manag 556:121721. https://doi.org/10.1016/j.foreco.2024.121721

Solla A, Aguín O, Cubera E, Sampedro L, Mansilla JP, Zas R (2011) Survival time analysis of *Pinus pinaster* inoculated with *Armillaria ostoyae*: genetic variation and relevance of seed and root traits. Eur J Plant Pathol 130:477–488. https://doi.org/10.1007/s10658-011-9767-5

Solla A, López-Almansa JC, Martín JA, Gil L (2014) Genetic variation and heritability estimates of *Ulmus minor* and *Ulmus pumila* hybrids for budburst, growth and tolerance to *Ophiostoma novo-ulmi*. iForest 8:422–430. https://doi.org/10.3832/ifor1227-00

StatSoft Inc (2011) STATISTICA. Data analysis software system. Tulsa: StatSoft. http://www.statsoft.com

Sulaiman MA, Bello SK (2024) Biological control of soil-borne pathogens in arid lands: a review. J Plant Dis Prot 131:293–313. https://doi.org/10.3390/horticulturae10040357

Thaler JS (1999) Jasmonate-inducible plant defences cause increased parasitism of herbivores. Nature 399:686–688. https://doi.org/10.1038/21420

Thaler JS, Humphrey PT, Whiteman NK (2012) Evolution of jasmonate and salicylate signal crosstalk. Trends Plant Sci 17:260–270. https://doi.org/10.1016/j.tplants.2012.02.010

Thakur M, Sohal BS (2013) Role of elicitors in inducing resistance in plants against pathogen infection: a review. ISRN Biochem 3:762412. https://doi.org/10.1155/2013/762412

Tianzi G, Congcong Z, Changyu C, Hui L, Kairu H, Shuo T, Xudong Z, Dejun H (2018) Effects of exogenous methyl jasmonate-induced resistance in *Populus* × *euramericana* 'Nanlin895' on the performance and metabolic enzyme activities of *Clostera anachoreta*. Arthropod-Plant Int 12:247–255. https://doi.org/10.1007/s11829-017-9564-y

Van Loon LC (1997) Induced resistance in plants and the role of pathogenesis-related proteins. Eur J Plant Pathol 103:753–765. https://doi.org/10.1023/A:1008638109140

Vázquez-González C, Sampedro L, López-Goldar X, Solla A, Vivas M, Rozas V, Lombardero MJ, Zas R (2022) Inducibility of chemical defences by exogenous application of methyl jasmonate is long-lasting and conserved among populations in mature *Pinus pinaster* trees. For Ecol Manag 518:120280. https://doi.org/10.1016/j.foreco.2022.120280

Vetukuri RR, Tripathy S, Malar CM, Panda A, Kushwaha SK, Chawade A, Andreasson E, Grenville-Briggs LJ, Whisson SC (2018) Draft genome sequence for the tree pathogen *Phytophthora plurivora*. Genome Biol Evol 10:2432–2442. https://doi.org/10.1093/gbe/evy162

Vivas M, Martín JA, Gil L, Solla A (2012) Evaluating methyl jasmonate for induction of resistance to *Fusarium oxysporum*, *F. circinatum* and *Ophiostoma novo-ulmi*. For Syst 21:289–299. https://doi.org/10.5424/fs/2012212-02172

Walters D, Heil M (2007) Costs and trade-offs associated with induced resistance. Physiol Mol Plant Pathol 71:3–17. https://doi.org/10.1016/j.pmpp.2007.09.008

Wang J, Song L, Gong X, Xu J, Li M (2020) Functions of jasmonic acid in plant regulation and response to abiotic stress. Int J Mol Sci 21:1446. https://doi.org/10.3390/ijms21041446

Wang Y, Liu GJ, Yan XF, Wei ZG, Xu ZR (2011) MeJA-inducible expression of the heterologous JAZ2 promoter from *Arabidopsis* in *Populus trichocarpa* protoplasts. J Plant Dis Prot 118:69–74. https://doi.org/10.1007/BF03356384

Whitehill JGA, Rigsby C, Cipollini D, Herms DA, Bonello P (2014) Decreased emergence of emerald ash borer from ash treated with methyl jasmonate is associated with induction of general defense traits and the toxic phenolic compound verbascoside. Oecologia 176:1047–1059. https://doi.org/10.1007/s00442-014-3082-8

Wilkinson SW, Dalen LS, Skrautvol TO, Ton J, Krokene P, Mageroy MH (2022) Transcriptomic changes during the establishment of long-term methyl jasmonate-induced resistance in Norway spruce. Plant Cell Environ 45:1891–1913. https://doi.org/10.1111/pce.14320

Zas R, Björklund N, Nordlander G, Cendán C, Hellqvist C, Sampedro L (2014) Exploiting jasmonate-induced responses for field protection of conifer seedlings against a major forest pest, *Hylobius abietis*. For Ecol Manag 313:212–223. https://doi.org/10.1016/j.foreco.2013.11.014

Zeneli G, Krokene P, Christiansen E, Krekling T, Gershenzon J (2006) Methyl jasmonate treatment of mature Norway spruce (*Picea abies*) trees increases the accumulation of terpenoid resin components and protects against infection by *Ceratocystis polonica*, a bark beetle-associated fungus. Tree Physiol 26:977–988. https://doi.org/10.1093/treephys/26.8.977