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Over 20 years of treating conifers with methyl jasmonate: Meta-analysis of effects on growth and resistance

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ABSTRACT

- For more than 20 years, methyl jasmonate (MeJA) has been used to study inducible defenses in conifers and to increase tree resistance to pests and pathogens. Despite the numerous studies on the subject, no attempts have been made to summarize or quantify how MeJA affects resistance and growth in conifers. Here we present a quantitative meta-analysis of the effects of MeJA treatment on the conifer genera *Pinus* and *Picea,* two of the most economically and ecologically important tree genera in boreal, temperate, and alpine forests.

- A literature search yielded 120 relevant papers. We summarized the key experimental methods used in these papers and performed a meta-analysis of how MeJA affects tree growth and resistance to pests and pathogens.

- The results show that MeJA negatively affects tree growth, with an overall effect size of −0.63. The overall effect size of MeJA for tree resistance was −0.76, indicating that MeJA treatment significantly reduces tree damage caused by biotic stressors.

- Although our meta-analysis shows that MeJA is effective in enhancing conifer defenses, there are still gaps in our understanding of the durability and ecological consequences of MeJA treatment. We provide suggestions for how future research should be conducted to address these gaps.

1. Introduction

Methyl jasmonate (MeJA) has been used for more than 20 years to study inducible defenses in conifers. Despite numerous publications on the topic, there has been no previous systematic analysis of the effectiveness of MeJA to increase conifer resistance to pests and pathogens. Likewise, although negative effects of MeJA on tree growth often have been reported, this has not been quantified across studies. With more and more publications on MeJA treatment of conifers being published, there is now enough data to perform a quantitative analysis of how MeJA affects tree resistance and growth. We focus this meta-analysis on pine (*Pinus*) and spruce (*Picea*), the two conifer genera that have been most intensively studied and that include some of the most economically and ecologically important tree species in boreal, temperate, and alpine forests.

MeJA is a volatile, methyl ester derivative of the phytohormone jasmonic acid. According to plant defense paradigms, the jasmonic acid signaling pathway regulates herbivore and necrotrophic pathogen defense responses (Broekgaarden et al., 2015). Whether there is crosstalk between jasmonic acid and other defense hormone in conifers is not

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clear (Arnerup et al., 2013; Wilkinson et al., 2022). Wounded or attacked plants emit MeJA, which can sensitize defense responses in neighboring plants (Baldwin et al., 2006).

Like all plants, conifers defend themselves through a combination of constitutive defenses that are present all the time and inducible defenses that are activated in response to insect attack, pathogen infection, and other stresses (Fig. 1) (Krokene, 2015). Studies of long-term (lasting months to years) inducible defenses in conifers started about 25 years ago, when it was discovered that trees that had been wounded some weeks previously became much more resistant to fungal infection (Christiansen et al., 1999). Later, similar long-term effects on resistance were observed in trees that had been subjected to a sub-lethal fungal infection or treated with MeJA (Mageroy et al., 2020). Long-term inducible resistance, also called acquired resistance, can be the result of prolonged defense activation or defense priming (Wilkinson et al., 2019) (Fig. 1). Prolonged defense activation occurs when induced defense responses remain up-regulated for weeks or months and provide resistance against subsequent attacks. In the case of defense priming, exposure to a biotic or abiotic stimulus (a priming stimulus) enables the plants to launch a quicker or stronger induced defense response to a subsequent attack (a challenge) by a pest or pathogen (Wilkinson et al., 2019). Defense priming has been studied most intensively in short-lived model plants such as *Arabidopsis* but is also important in long-lived species such as conifers (Mageroy *et al.*, 2020; Wilkinson et al., 2019)

Primed or prolonged activation of inducible defenses in pine and spruce can be triggered by external application of chemical priming stimuli such as MeJA (Fig. 1). MeJA, the volatile ester-derivative of the defense hormone jasmonic acid (JA), is released from damaged plant tissue and acts as an internal or external long-range signaling molecule (Cheong and Choi, 2003; Heil and Ton, 2008). Upon entering the cell, MeJA is demethylated and conjugated with the amino acid isoleucine to trigger JA-regulated defenses (Wu et al., 2008). Unlike JA, it is thought that volatile MeJA can freely diffuse through plant membranes (Cheong and Choi, 2003).

In this study, we summarized all attainable publications on MeJA treatment of *Pinus* or *Picea* between 1999 and 2022. We then used meta-analysis to evaluate the effects of MeJA on *Pinus* and *Picea* growth and resistance. We used this analysis to suggest a protocol for MeJA usage in conifer research. We also identified research questions that still need to be explored before MeJA treatment can be applied in integrated pest management.

2. Materials and methods

2.1. Literature search

We identified publications on MeJA treatment of *Pinus* and *Picea* species by searching the Scopus literature database (Burnham, 2006) using the following search terms:

TITLE-ABS-KEY (("methyl jasmonate" OR "methyljasmonate") AND ((Pinus) OR (Picea) OR (pine) OR (spruce)))

This search yielded 134 records. To ensure the most complete literature survey possible, we also searched Web of Science (Birkle et al., 2020) using the following terms:

All fields ("methyl jasmonate" OR "methyljasmonate") AND (Pinus OR Picea OR pine OR spruce)

This search yielded 289 records. Both literature searches were performed on November 23, 2022. We manually checked all records by reading the abstract and/or methods. Articles were excluded if they were not primary research articles or did not include *Pinus* or *Picea* and/or MeJA treatment in the experimental methods. This initial screening gave a list of 113 articles from the Scopus search and 111 articles from the Web of Science search. Most articles (106) were in common between the searches, giving a total of 118 primary research articles on MeJA treatment of *Pinus* and/or *Picea*. These articles are listed in Supporting information dataset S1, together with two additional articles that did not show up in the database searches but were found otherwise (Supporting information file - Figure S1).

2.2. Systematic summary

For each of the 120 studies, we recorded several parameters including: year of publication, tree species, plant material, MeJA concentration, MeJA application method, any surfactant used, experiment type, challenge type, analyses, etc. These parameters are further described in the metadata provided with Supporting information dataset S1. Descriptive graphs were produced in R (version 4.3.0) using “ggplot2” (Wickham, 2016), “ggbreak” (Xu et al., 2021), and “patchwork” (Pedersen, 2022).

2.3. Meta-analysis

To investigate the overall effects of MeJA on *Pinus* and *Picea* growth and resistance, we conducted a meta-analysis. From the 120 studies originally found, we further selected studies that contained data that

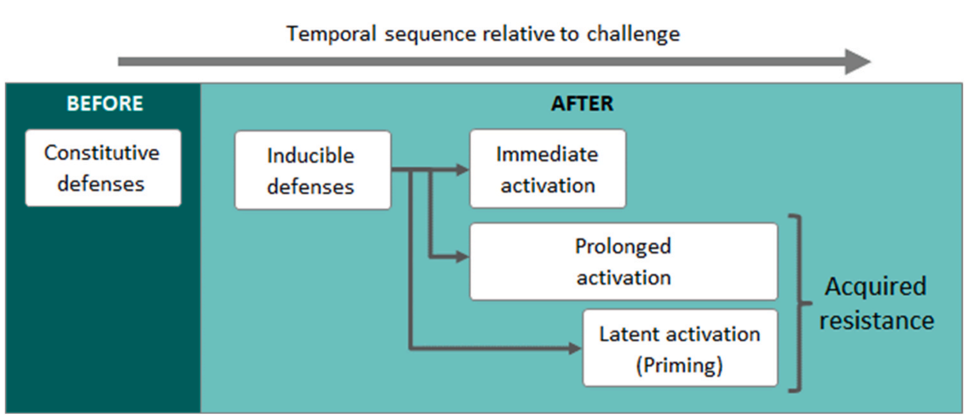

Fig. 1. Timeline of activation of conifer defenses. *Pinus* and *Picea* have both constitutive (preformed, innate) and inducible (acquired) defenses to biotic challenges. Inducible defenses can be activated by pest attack, pathogen infection, or methyl jasmonate application. Inducible defenses can be further divided into immediate, prolonged, and primed depending on when they are activated. Immediate defenses are activated directly after exposure to a stimulus and attenuate within a few weeks. Prolonged defenses are induced after exposure to a stimulus and maintained over a months to years. Primed defenses may be slightly induced after exposure to a stimulus, but quickly return to basal levels. However, upon a subsequent challenge they are rapidly and/or more strongly induced.

could be used to estimate effect sizes for growth or resistance parameters.

2.3.1. Effects of MeJA on growth

To quantify the effects of MeJA application on plant growth, each study was carefully examined and retained if:

1.The study included at least one assessment of a growth parameter between a non-treated (control) group and a MeJA-treated group.

2.Growth parameters included plant height, stem diameter, or biomass. Photosynthetic rate and other physiological measurements were not included as a growth parameter.

3.The results of the growth parameter assessment were presented either in the main article or in the supplementary files. Studies that mentioned the assessment of growth in the method section but did not present the results were disregarded.

2.3.2. Effects of MeJA on resistance

To quantify the effects of MeJA application on plant resistance, each study was carefully examined and retained if:

1.The study included at least one resistance assay experiment with a challenge applied to a non-treated group and a MeJA-treated group.

2.The challenge was a biotic stress, either in the form of a pest infestation with e.g. insects or nematodes, or a pathogen challenge with e.g. a fungal or bacterial infection.

3.Resistance effects were measured by quantifying pest/pathogen performance (larval development, number of eggs deposited, number of attacks, feeding rate or meal duration) or damage caused by the pest/pathogen (plant mortality, disease incidence, lesion length, debarked area). Studies that included a resistance bioassay but did not measure a resistance phenotype were excluded.

2.3.3. Data extraction and effect size calculation

Further inclusion criteria were applied for data extraction and effect size calculations. Studies were retained if:

1. The experimental design was adequately explained and comprehensible, with a sample size provided for each control and treated group.
2. Results were presented for both control and MeJA-treated plants, including statistical data for the response parameters (mean and standard deviation, standard error, or confidence interval; number of individuals or percentage of events; F-value and degrees of freedom in ANOVA analyses).

Studies were excluded if:

1. The experimental design was unclear and lacked information necessary for further data extraction.
2. The study did not report sample size.
3. Data were presented only in figures using a log scale.
4. The study presented p-values or F-values/Chi-squared values without degrees of freedom.

For data extraction and calculations of effect sizes and standard errors, we followed the *Cochrane Handbook for Systematic Reviews of Interventions* (Chapter 5 and 6) (Higgins et al., 2022) and *Doing Meta-Analysis with R: A Hands-On Guide* (Chapters 3, 4, and 17) (Harrer et al., 2021). Most of the studies we extracted data from included multiple experiments and/or measured several growth or resistance parameters. In these cases, each parameter and/or experiment was recorded as a single observation (data point). Data were extracted from texts, tables, and Figures presented in both the main article and the supplementary files. Data from Figures were extracted using the GUI_juicr function from the "juicr" package (v 0.1) (Lajeunesse, 2021) in Rstudio version 4.1.2. We extracted and calculated two types of effect size: Hedge's g and log risk ratio. Hedge's g effect sizes were calculated using the sample size, mean, and standard deviation of each group, extracted from t-test coefficients or one-way ANOVA F-values with degrees of freedom. Log risk ratios were calculated for data presented only as a percentage or number of incidences, without standard deviation, standard error, or confidence interval. Data that were not presented as a binary response (such as multiple ordinal responses on a scale from 0 to 5) were dichotomized by pooling all unhealthy/symptomatic categories. When no incidences of a response were recorded (e.g. "no eggs were found"), these values were set to 0.5, as zero values hinder the calculation of log risk ratio. Lastly, effect sizes with a standard error equal to 0 were omitted from the final dataset. The final dataset on effects of MeJA on growth comprised 21 studies with 262 observations (Supporting information dataset S2) and the dataset on effects on resistance comprised 33 studies with 317 observations (Supporting information dataset S3).

2.4. Statistical analyses

Because there were multiple effect sizes per study, we used a three-level meta-analysis model to calculate pooled effect sizes from all the extracted data. Pooled effect sized are presented without units, as they show the magnitude of an effect and if the effect is positive or negative. Rstudio version 4.1.2 (1.11.2021) was used for all the statistical analyses. The statistical model was fitted using the function "rma.mv" from the "metafor" package (Viechtbauer, 2010). The argument "random = ~ 1|Study_ID/es.id" was added to the function to indicate the three-level meta-analysis model, with "Study_ID" being the name we used for each individual study (e.g. "Kozlowski et al., (1999)", consisting of the first author, and the publication year) and "es.id" being the number ID of each observation within each study (as there were often multiple observations in each study).

Results were presented as forest plots that were generated using the "forest" function from the "meta" package (Balduzzi et al., 2019). Data were first aggregated to the individual study level based on the marginal variance-covariance matrix from the three-level model. This was done to reduce the number of observations shown in the plot. Each aggregated individual study is then presented in the forest plot as box plot. The box plot shows the estimated effect size of each study with the size of the box indicating the weight of the study and the error bars indicating the 95 % confidence interval (CI) of the estimated effect size. Weight of the study correlates with the variance of the study, or how concise their results are, with smaller variance meaning more weight. This also means that smaller studies with fewer observations usually have larger variances and thus contribute less weight to the pooled estimate effect size. The overall pooled effect size for growth or resistance effects was not reduced to the aggregated values from each study but was shown as the pooled effect from all observations.

Between- and within-study heterogeneity were analyzed following the three-level meta-analysis model. The "var.comp" function from the package "dmetar" (Harrer et al., 2019) was used to calculate and visualize Higgins & Thompson's I^2 (a measure of the percentage of variability in effect size caused by factors other than sampling error) for each level in the model.

To further investigate the source of high between-study heterogeneity we performed meta-regression. We separately tested effects of the variables "MeJA concentration", "challenge date after treatment" (for resistance analysis only), "challenge species" (for resistance analysis only), "sampling date after treatment" (for growth analysis only), "surfactant", "plant species", and "plant material". The argument "mod = ~ [name of variable]" was included in the rma.mv model to indicate variables for meta-regression. Pairwise comparisons were made for categorical variables using Tukey's post-hoc tests (applying the Holm methodology for p-value adjustment) with the function "glht" in the "multcomp" package (v1.4-16) (Hothorn et al., 2008).

Finally, we tested for publication bias by generating funnel plots (using R's "funnel.rma" function) and running Egger's regression tests for funnel plot asymmetry. Egger's regression tests were conducted by including the standard errors of the effect sizes as a moderator in the meta-regression model. We also calculated Rosenthal's fail-safe numbers using the function "fsn" from the "metafor" package (Viechtbauer, 2010). If the fail-safe number is larger than the critical value 5k+10, with k being the number of observations, publication bias is concluded to be minimal.

3. Results

3.1. Systematic summary of MeJA treatment studies

Studies describing the exogenous application of MeJA to *Picea* species first appeared in 1999 and involved treatment of young white spruce (*Picea glauca*) and Norway spruce (*Picea abies*) (Fig. 2). Studies on *Pinus* species have been published since 2003 (Fig. 2). Up to 2022, the three most studied species are *Picea abies*, radiata pine (*Pinus radiata*), and maritime pine (*Pinus pinaster*) (Fig. 2). Only for *Pinus* has MeJA been applied across all ontogenetic stages, from seeds to mature (44-year-old) trees (Fig. 3; note that seeds are grouped with 'other' plant materials in the figure). For *Picea*, seedlings to 100-year-old trees have been treated (Fig. 3). For both genera, also single cells and callus cultures have been treated with MeJA under *in vitro* conditions. For *Picea* species, most studies have been done on clonal plants propagated via cuttings or somatic embryogenesis. In contrast, for *Pinus*, families, provenances, and populations have been studied.

Almost 80 % of all studies have been conducted in controlled environments, such as labs, greenhouses or growth chambers (Fig. 3). Much fewer studies have evaluated the effects of MeJA in the field. MeJA treatment has usually been done during late spring and summer (Fig. 3). A few studies have applied MeJA in the fall or winter, but these experiments were usually conducted in controlled environments or in areas with mild climates (Supporting information dataset 1).

The doses of MeJA that have been investigated include different concentrations, application volumes, and application frequencies. In most studies, MeJA concentrations ranged from 0.001 mM and 0.1 mM for *Pinus* and *Picea*, respectively, to a maximum of 100 mM for both genera. However, a few studies applied very high concentrations and appear to be outliers (Fig. 4). For example, Richard and co-workers (Richard et al., 1999) reported using 459 mM and Graves and co-workers (Graves et al., 2008) used 4166 mM in *Picea* (Supporting information dataset S1). In most cases, these higher concentrations were applied to older plants and resulted in tree mortality (Fig. 4). The MeJA application volume applied spans from 0.05 to 800 mL for *Pinus* and 0.025–150 mL for *Picea* (with one study applying 2 L) (Graves et al., 2008)(Supporting information dataset S1). However, some studies only report concentration and not the amount applied to plants. In terms of application frequency, most studies only applied MeJA once, but a few studies treated plants two or three times. One study treated plants up to seven times (Reglinski et al., 2009) (Supporting information dataset S1). For studies that treated plants more than once, a two-week interval between treatments was the most common, but intervals ranged from one day up to 1 year (Supporting information dataset S1).

To treat plants with MeJA, one needs to prepare a suspension that includes a carrier solution and perhaps a surfactant. Our literature search showed that most studies used ethanol or Tween® to solubilize MeJA (Fig. 3). Tween® is a polysorbate that enables the dispersion of hydrophobic particles (such as MeJA) in aqueous solutions. The most commonly used concentrations were 0.1 % Tween-20® or 2.5 % ethanol (Supporting information dataset S1). A few studies used a product known as Du-Wett®, an organosilicon designed for the application of e.g., insecticides and plant growth regulators. Several studies used no surfactant at all or did not mention the use of a surfactant (Supporting information dataset S1).

MeJA can be applied to plants in different ways. Our literature search revealed that the most common way to treat *Pinus* and *Picea* species is by spraying the MeJA solution onto the plant material (Fig. 4). Less frequently used methods include absorption of MeJA from e.g. a cotton pad or filter paper, using a growth medium that contains MeJA, and painting the solution onto the plant material using a brush (Fig. 4). Other rarely used application methods are immersion of the plant material into the MeJA solution, exposing plant material to MeJA gas vapors, and soil drenching with MeJA solution. Finally, one study injected MeJA solution directly into the tree stem.

After application, effects of MeJA treatment on plant defense, growth, and resistance can be evaluated in different ways. The most

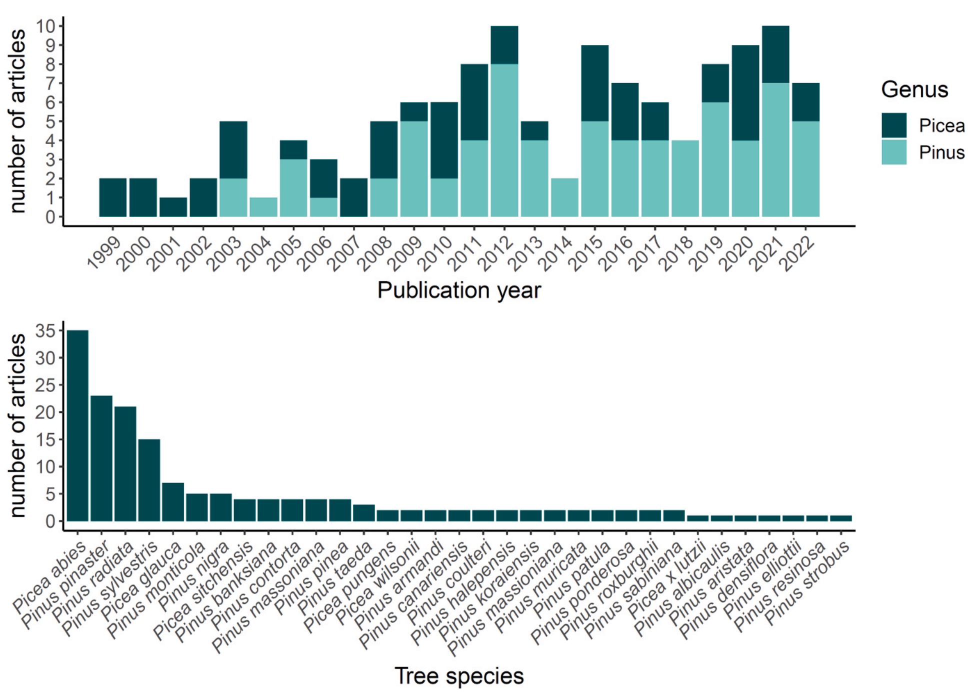

Fig. 2. Studies on methyl jasmonate treatment of *Pinus* and *Picea* species: the number of articles published by year (top panel) and by tree species (bottom panel).

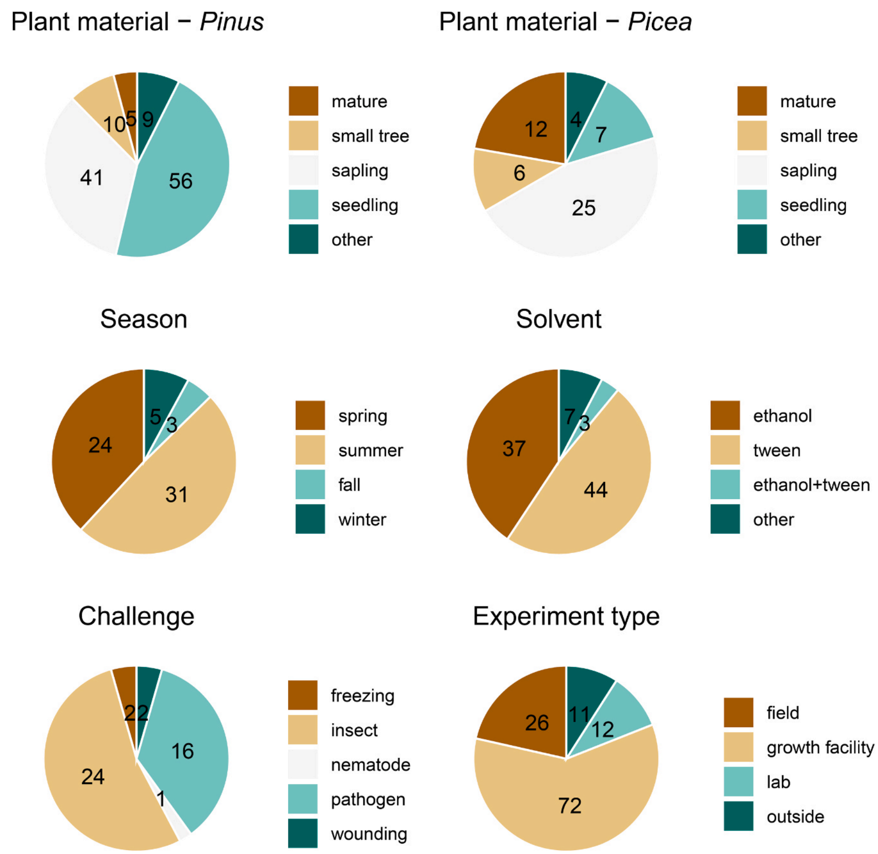

Fig. 3. Summary of published studies on methyl jasmonate (MeJA) treatment of *Pinus* and *Picea* species. Pie graphs show the number of studies broken down to plant material (for *Pinus* and *Picea* separately), season when MeJA was applied, solvent used to solubilize MeJA, the biotic or abiotic challenge applied to plant materials after MeJA treatment, and the type of experiment that was performed (field, lab, etc.). Plant material categories were defined as: seedling = < 1 yr.; sapling = 1–4 yrs.; small tree = 4–15 yrs.; mature = > 15 yrs. 'Other' includes seeds, pollen, calli, and cells.

common post-treatment analysis included evaluating changes in gene expression, protein expression, metabolites, physiology, and anatomy (Supporting information dataset S1). Many studies assessed plant resistance by challenging MeJA-treated and untreated plants with a biotic agent, simulated damage, or other stressors. The most common biotic stressors used were insects, followed by fungal pathogens (Fig. 3). Bark beetles (particularly in the genus *Ips*) and the large pine weevil (*Hylobius abietis*) were the most used insect stressors. The bark beetle-associated bluestain fungus *Endoconidiophora polonica* was the most used fungal pathogen, but many different fungal pathogens have been used, including diplodia tip blight (*Diplodia pinea*) and bluestain fungi in the genus *Grosmannia*. Plant responses to these challenges were monitored from 5 hours to 3 years after MeJA treatment.

MeJA treatment directly induces the expression of some defense genes and primes the expression of others (Mageroy et al., 2020). The most well-studied MeJA-responsive defense genes in *Pinus* and *Picea* are terpene biosynthesis-related genes [e.g. Kim et al., 2009; Zulak et al., 2009]. Other defense-related genes found to be induced by MeJA treatment include phenylpropanoid biosynthesis genes and pathogenesis-related (PR) genes [e.g. Šņepste et al., 2018; Yaqoob et al., 2012]. For example, phenylalanine ammonium lyase (PAL), the first enzyme in phenylpropanoid biosynthesis, is initially induced by MeJA treatment before returning to near-baseline expression (Wilkinson et al., 2022). Most of the PR genes that are differentially expressed in response to MeJA are initially up- or downregulated after treatment, followed by a return to constitutive expression levels (Wilkinson et al., 2022). Other PR genes show a MeJA-primed expression pattern, meaning that they are strongly induced in MeJA-treated plants in response to a subsequent triggering challenge, such as wounding (Mageroy et al., 2020).

Defense gene expression is regulated by the defense hormones JA, SA, and ethylene (ET). In a time-course transcriptome analysis of 2-year-old spruce saplings, genes in the biosynthesis pathways of all three hormones were found to be induced by MeJA (Wilkinson et al., 2022). This study also showed that many hormone-regulated transcription factors that in turn regulate defense genes, are upregulated after MeJA treatment (Wilkinson et al., 2022). MeJA treatment also changes the expression of miRNAs, which are involved in both transcriptional and post-transcriptional regulation of genes. Many of the miRNAs differentially expressed in response to MeJA are predicted to target receptors that detect pathogens (Wilkinson et al., 2021). These miRNAs may also have an important function in guiding RNA-directed DNA methylation, which contributes to long-term MeJA-induced resistance and defense priming (Mageroy et al., 2020; Wilkinson et al., 2022).

MeJA also induces anatomical changes in conifers. The most commonly observed anatomical response to MeJA treatment is the formation of terpene-filled traumatic resin ducts along the cambium-xylem border [e.g. Heijari et al., 2005; Franceschi et al., 2002; Martin et al., 2002]. In *Picea*, incipient traumatic resin ducts begin to appear in the cambial zone around 9 days after MeJA treatment and are fully formed by around 25 days after treatment (Martin et al., 2002). Additionally, swelling of polyphenolic cells in the secondary phloem is observed after MeJA application [e.g. Franceschi et al., 2002; Hudgins et al., 2003]. These cells play key roles in conifer defenses to pests and pathogens (Hudgins et al., 2003).

Corresponding to the anatomical changes in cells involved in tree resistance, MeJA treatment also induces chemical defense responses. As mentioned previously, the induction of traumatic resin ducts in MeJA-treated trees is accompanied by moderate increases (2–3-fold, depending on genotype) in terpene concentrations, which return to near constitutive levels by 4 weeks after treatment (Erbilgin et al., 2006;

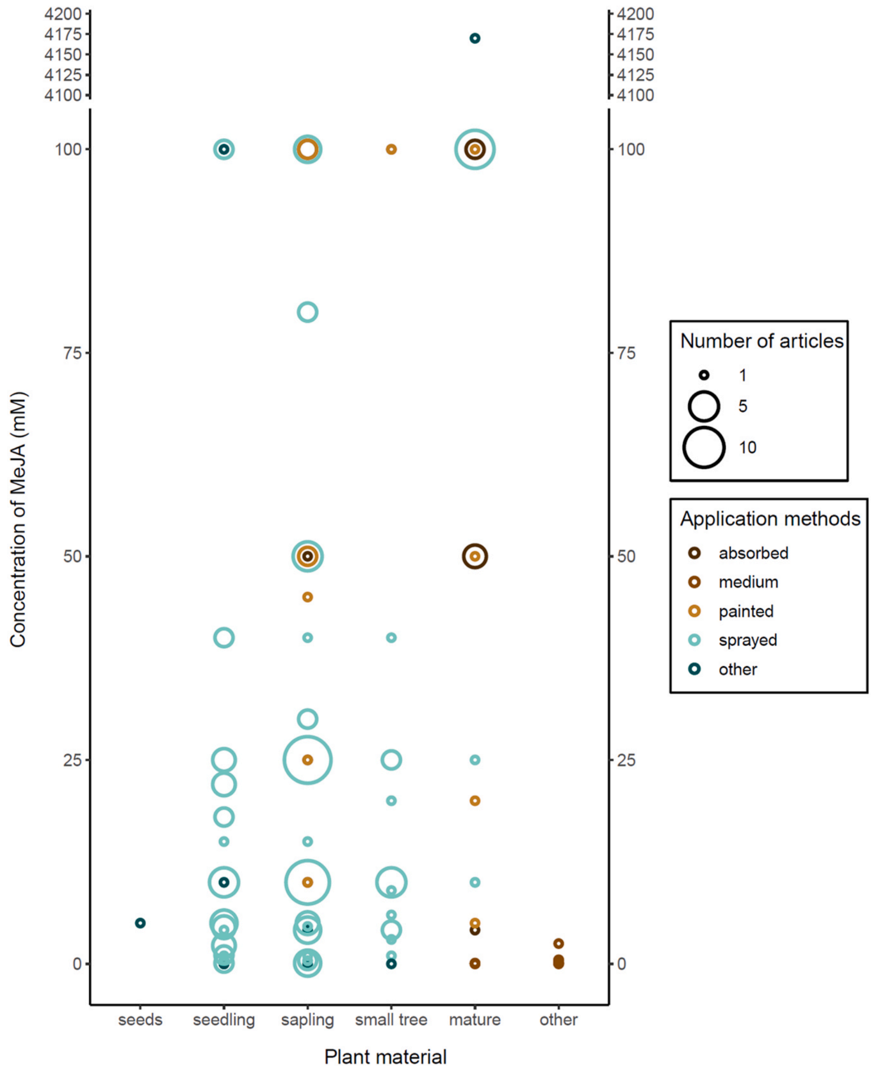

Fig. 4. Number of published studies on methyl jasmonate (MeJA) treatment of *Pinus* and *Picea* species grouped by plant material, MeJA concentration, and MeJA application method. Plant materials indicated by "other" include seeds, pollen, calli, and cells. Application methods indicated by "other" include immersion, root treatment, stem injection, and exposure to gas vapor. Plant material categories were defined as: seedling = < 1 yr.; sapling = 1–4 yrs.; small tree = 4–15 yrs.; mature = > 15 yrs.

Mageroy et al., 2020; Zulak et al., 2009). However, when these MeJA-treated trees are subsequently challenged, a much stronger terpene induction (6–60 fold) is observed (Mageroy, Wilkinson, *et al.*, 2020). Polyphenolic compounds have also been observed to increase after MeJA treatment (Liu et al., 2013; Moreira et al., (2012a)).

3.2. Meta-analysis on effects of MeJA treatment

Effects on plant growth and resistance to biotic stress are two commonly investigated responses to MeJA application across studies. We explored if the pooled effect size of MeJA on these important traits were significant. Analysis of funnel plots and Egger's regression test revealed an asymmetry in the published results, with an under-representation of studies reporting negative results. This suggests that there is a publication bias on effects of MeJA on both growth and resistance (Supporting information file - Figure S2). However, calculation of Rosenthal's fail-safe numbers indicated that a large amount of non-significant findings would be required to reject the overall conclusions of our meta-analyses (Supporting information file - Table S1).

3.2.1. Effects on growth

Many studies report negative effects of MeJA treatment on growth [e.g. Sampedro et al., 2011; Heijari et al., 2005]. Our meta-analysis showed a significant negative effect of MeJA treatment on growth in both *Pinus* and *Picea*, with a pooled effect size of −0.63 (CI = [-0.90; −0.37], Fig. 5). However, there is much between-study heterogeneity (I^2 = 57.54 %, Supporting information file - Figure S3). Meta-regression showed that this heterogeneity could be due to MeJA concentration, plant species, plant material, or sampling date after MeJA treatment, but not surfactant type. Predictably, higher MeJA concentrations had a greater effect on tree growth than lower concentrations (coefficient estimation: −0.0007, CI = [-0.0010; −0.0005]). Less growth reduction was observed with increasing time between MeJA-treatment and sampling (coefficient estimation: 0.0006, CI = [0.0001; 0.0010]). At the species level, growth reduction was significantly smaller in *Picea abies*, *Pinus pinaster*, and *Pinus resinosa* than in *Picea x lutzii*, *Pinus radiata,* and *Pinus sylvestris* (Supporting information file - Table S2 and S3). Additionally, negative effects of MeJA on growth were significantly milder in saplings and seedlings compared to mature trees (Supporting information file - Table S4 and S5).

3.2.2. Effects on resistance

Induction of defense responses can also affect conifer resistance to biotic challenges. From our meta-analysis of effects of MeJA on conifer

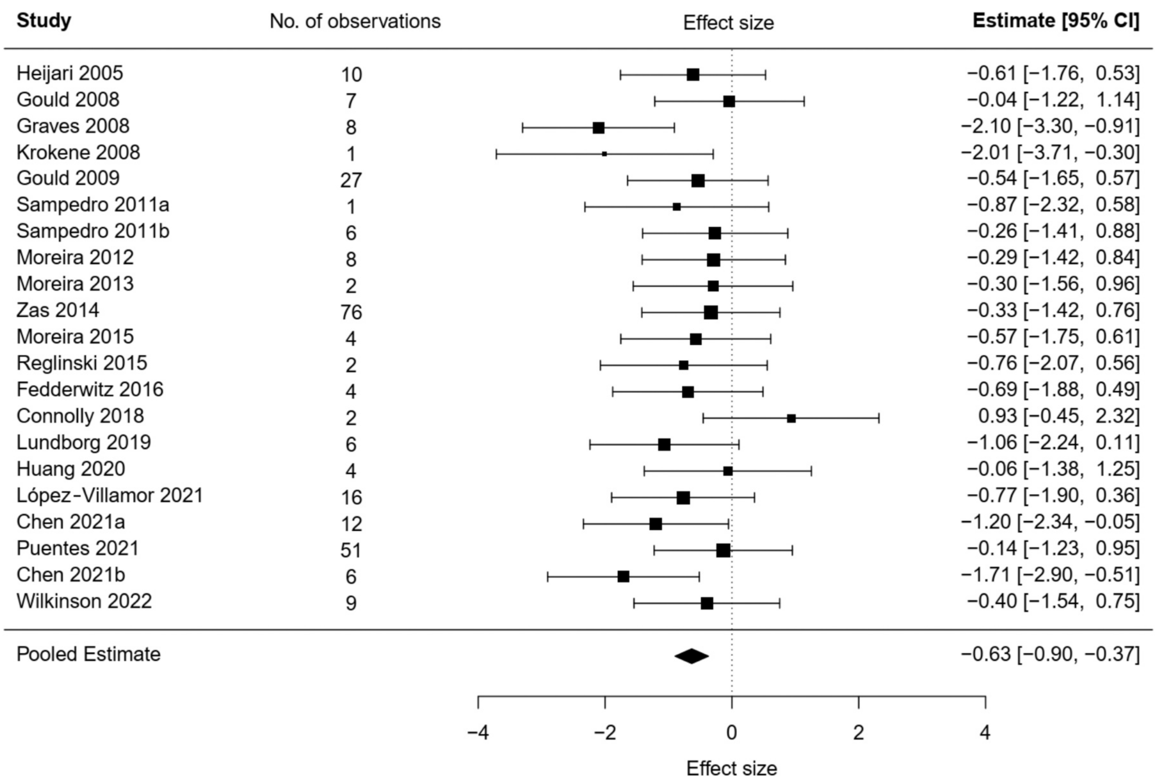

Fig. 5. Forest plot of effect sizes of methyl jasmonate (MeJA) treatment on growth in *Pinus* and *Picea* species. Studies included in the analysis are shown on the left. "No. of observations" is the number of individual observations (growth parameters or sub-experiments) extracted from each study. Box plots show the estimated effect size of each study with the size of the box indicating the weight of the study and the error bars indicating the 95 % confidence interval (CI) of the estimated effect size. The estimated effect size and 95 % CI for each study are also given in the column on the right. The pooled estimate at the bottom of the forest plot indicates the pooled estimated effect size of all observations from all 21 studies. A negative effect size indicates a negative effect of MeJA on growth.

resistance, we found the overall effect of MeJA treatment relative to controls to be −0.76 (CI = [-0.98; −0.55], $p < 0.001$) (Fig. 6). This indicates that MeJA application can decrease damage levels caused by pests/pathogens and/or decrease their performance, regardless of plant material, plant species, MeJA concentration, or type of biotic challenge used. There was substantial between-study heterogeneity (67.06 %) in the effect of MeJA on plant resistance (Supporting information file - Figure S3). Meta-regression analysis indicated that the heterogeneity is mostly due to variation in MeJA concentration and plant material. Higher MeJA concentrations tend to result in greater reduction in damage and/or performance of biotic agents. Pairwise comparison between plant materials showed that small trees are significantly less resistant than mature trees, saplings, and seedlings (Supporting information file - Table S6 and S7). However, effects of MeJA on resistance did not differ significantly between plant species, type of biotic challenge [pests (i.e., insects or nematodes) vs. pathogens (i.e., fungi or bacteria)], surfactant used, or the timing of the challenge relative to MeJA treatment.

4. Discussion

4.1. MeJA as a tool in conifer research

MeJA has been a very important tool in dissecting and deciphering conifer inducible defenses and their role in resistance. Initially, MeJA was thought to only directly induce defenses such as formation of traumatic resin ducts and swelling of polyphenolic cell (Franceschi et al., 2002). This early work resulted in the identification of many important genes in defense chemical biosynthesis (e.g. Fäldt et al., 2003; Ro et al., 2005; Schmidt and Gershenzon (2007). More recently, it was realized that MeJA induces complex defense responses that include direct induction of defenses, prolonged upregulation of defenses, and priming of defense (Mageroy et al., 2020b). MeJA is now being used to understand epigenetic regulation of conifer defense memory (Fossdal et al., 2024).

4.2. Practical use of MeJA

Although MeJA has been a very important tool in understanding conifer defenses, it is challenging to come up with a recommended MeJA treatment regime for protecting conifers from pests and pathogens. MeJA application methods, doses, plant species, plant age, and genetics have varied among published studies. The most used application method in the literature has been to spray aboveground plant tissues with a MeJA solution (Fig. 4). This method often induces traumatic resin duct formation in the sapwood and increases resistance to pests and pathogens in plants of different ages (Martin et al., 2002; Heijari et al., 2005). Our meta-regression analysis showed that MeJA-induced resistance can differ with plant age (Supporting information file - Table S5 and S6) and MeJA concentration used. Ontogenetic differences and the confounding effects of bark structure and permeability could cause this variation in the required MeJA concentration to induce resistance in plants of different developmental stages. For example, lower MeJA doses can be effective in seedlings with thin bark, while higher doses are needed in mature trees with thick cork bark (Fig. 7).

MeJA treatment has similar effects on tree resistance in all *Pinus* and *Picea* species, according to our meta-analysis. Although conifer species did not seem to be important in explaining variation in MeJA-induced resistance, previous studies have found that genotypes, families, and provenances can vary in their responses to MeJA (Zeneli et al., 2006; Heijari et al., 2008; Semiz et al., 2012; Moreira et al., 2013; López-Goldar et al., 2018; Puentes et al., 2021). For example, total

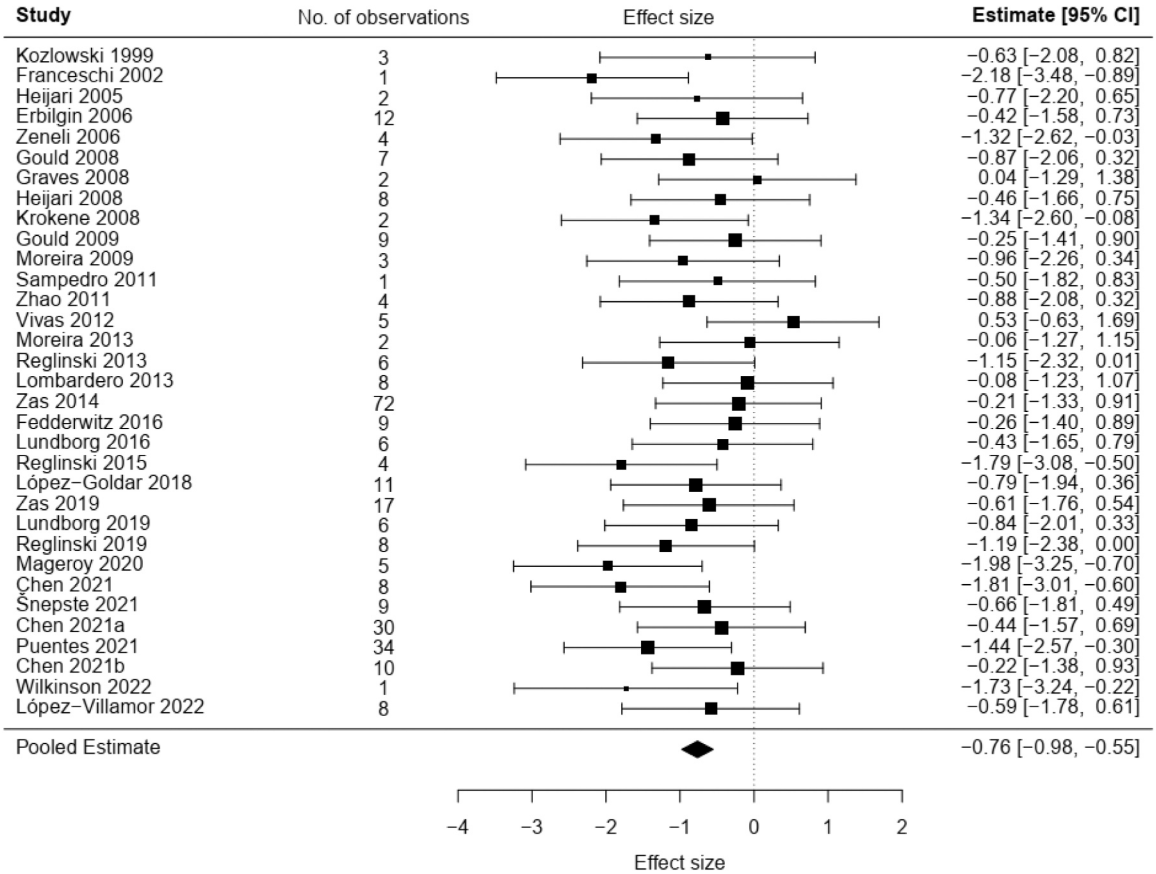

Fig. 6. Forest plot of effect sizes of methyl jasmonate (MeJA) treatment on resistance of *Pinus* and *Picea* species to pests and pathogens. Studies included in the analysis are shown on the left. "No. of observations" indicates the number of individual observations (resistance measurements or sub-experiments) extracted from each study. Box plots show the estimated effect size of each study with the size of the box indicating the weight of the study and the error bars indicating the 95 % confidence interval (CI) of the estimated effect size. The estimated effect size and 95 % CI for each study are also indicated in the column on the right. The pooled estimate at the bottom of the forest plot indicates the pooled estimated effect size of all observations from all 33 studies. A negative effect size indicates that MeJA reduces damage symptoms or performance of biotic stressors.

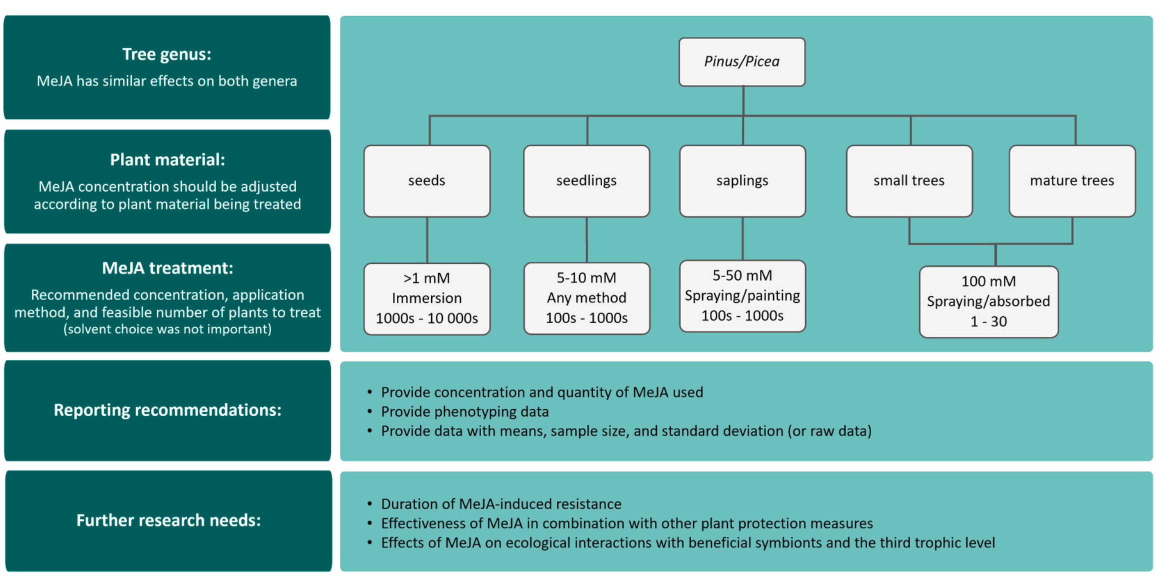

Fig. 7. Summary of effects of methyl jasmonate (MeJA) treatment on resistance and growth of *Pinus* and *Picea* species, recommendations for MeJA treatment and reporting, and further research needs. Plant material categories: seedlings = < 1 yr.; saplings = 1–4 yrs.; small trees = 4–15 yrs.; mature trees = > 15 yrs.

needle phenolics and volatile terpene emissions after MeJA treatment vary among half-sib families in *P. pinaster* and among *Pinus sylvestris* provenances in Turkey, respectively (Semiz et al., 2012; Moreira et al., 2013). Such intra-specific variability in responsiveness to MeJA does not hinder the use of MeJA as a practical tool since most plant materials examined thus far respond positively to treatment. Yet, intra-specific variability should be quantified and considered before MeJA is used for plant protection. Studying *P. abies* clones, Puentes and co-workers (Puentes et al., 2021) found that for some clones resistance to *H. abietis* and the bluestain fungus *E. polonica* did not increase significantly after MeJA treatment. For clones that were highly resistant to damage in a constitutive, non-treated state there was no benefit of MeJA treatment (Puentes et al., 2021). Similarly, Heijari and co-workers (Heijari et al., 2008) treated four different seed origins of *P. sylvestris* plants with MeJA, but plants from only one or two of these became more resistant to the pine sawflies *Neodiprion sertifer* and/or *Diprion pini*. For practical implementation of MeJA treatment, it is important to examine the variation in MeJA-inducibility in breeding populations and seed orchards used to produce forest regeneration material. This will help identify plant materials that will benefit most from treatment with MeJA.

When developing a MeJA treatment protocol for conifers, negative aspects of MeJA treatment must also be considered. For example, our meta-analysis showed that MeJA has a significant negative effect on plant growth across studies (Fig. 5) [e.g. Lajeunesse, 2021; Kraus *et al.*, 2019; Berglund et al., 2016]. However, the severity of this growth effect seems to be species-, dose-, and age-dependent (Heijari et al., 2005). Additionally, reduced growth seems to be temporary and result in a short period of stalled growth followed by a recouping period when treated plants catch up with the height of untreated control plants (Fedderwitz et al., 2020; Zas et al., 2014). Under field conditions, MeJA-treated plants may also grow better than untreated plants if herbivory pressures are high and treated plants suffer less herbivory. The relatively short-term negative effect of MeJA treatment on growth was also apparent in our meta-regression results, as growth reduction was less pronounced with increasing time since MeJA treatment. Thus, the negative effects of MeJA treatment on growth may be negligible when considered over a 3- to 5-year period, especially in environments with high pest or pathogen pressures.

In addition to any negative effects of MeJA treatment on plant growth, it is important to consider cost and practicality aspects when evaluating the potential of MeJA in practical plant protection. Although MeJA treatment has been shown to effectively reduce the colonization success of bark beetles and/or their associated bluestain fungi in mature *P. abies* trees [e.g. Mageroy et al., 2020a; Zeneli et al., 2006] the use of MeJA to protect mature trees at the forest scale is neither economically or logistically feasible. The most effective tree protection was obtained by spraying the stem of mature trees with 100 mM MeJA (21.7 mL/L) (Mageroy et al., 2020a). It is clearly not practical or economical to spray whole forest stands with large volumes of a chemical that costs 135€ for 25 mL ($139/25 mL) (www.sigmaaldrich.com [21 September 2022]). However, MeJA treatment could be a practical management option on smaller scales, such as protecting a few trees of great significance in private gardens, arboretums, camp sites, adventure parks, or in smaller stands used for resin tapping.

Contrary to mature trees, using MeJA to protect conifer seedlings or saplings appears to have practical potential. Many studies have shown that treating conifer seedlings and saplings with MeJA at lower doses (< 10 mM) induces resistance against pests and pathogens [e.g. Wilkinson et al., 2022; Chen et al., (2021a); Reglinski et al., 2019]. MeJA can even be applied before winter storage of saplings that are distributed and planted out in the spring (Chen et al., (2021b)). Spraying can be done with equipment that is already used in forest nurseries. The relatively low MeJA dose and total volume needed to protect younger plants will greatly reduce the cost of application.

Seed treatment with MeJA might be another effective way to protect germinating conifer seedlings and saplings. MeJA treatment of seeds has shown promise as a plant protection tool in angiosperms such as tomato (Król et al., 2015) and rice (Kraus and Stout, 2019), but data on the effectiveness of seed treatment in conifers is very limited (Berglund et al., 2016; Vivas et al., 2012). If seed treatment with MeJA is feasible in conifers it would provide a very cost-efficient way to protect many plants with very little effort and cost.

Although our meta-regression analysis indicated that the effect of MeJA on resistance varies between plant materials, there were no significant pairwise differences between the response of mature trees, seedlings, and saplings (Supporting information file – Table S5 and S6). Additionally, meta-regression analysis showed that negative effects of MeJA on growth were significantly milder in seedlings and saplings than in mature trees. This contrast was not very robust, due to the relatively low number of studies of growth effects of MeJA on mature trees. Still, the available data suggests that any negative effects of MeJA treatment of seedlings and saplings tend to be mild and most likely are compensated by the benefits of increased biotic resistance.

MeJA may also be combined with other management tools to achieve greater levels of plant protection. For example, MeJA treatment may be combined with physical stem-coating of conifer saplings which is used in several European countries to protect saplings from feeding by *H. abietis* (Galko et al., 2022). Additionally, saplings that have been treated with MeJA in nurseries can be planted at sites prepared by soil scarification to reduce *H. abietis* damage (Wallertz et al., 2018). Recently, MeJA has also been shown to increase the resistance of *P. abies* saplings to *H. abietis* attack when MeJA is applied to plants produced by somatic embryogenesis (SE) (Berggren et al., 2023). SE is a propagation method that produces new clonal individuals *in vitro* using somatic tissue from a mother plant. Producing somatic embryos by SE requires high levels of plant growth regulators and plant hormones, such as ABA and ethylene (Méndez-Hernández et al., 2019; von Aderkas et al., 2018). Among other things, these hormones mediate stress responses in plants (Müller and Munné-Bosch, 2021) and may cause strong upregulation of genes involved in various stress responses in somatic embryos (Winkelmann, 2016). Secondary metabolites also accumulate to a greater extent in somatic embryos compared to normal zygotic embryos that develop from seeds (Winkelmann, 2016). When SE-generated *P. abies* plants are treated with MeJA, they become very resistant to *H. abietis* attack (Berggren et al., 2023). In fact, combined SE and MeJA treatment acts synergistically and increases plant survival to a much greater extent than either factor does alone (Berggren et al., 2023). A possible mechanism responsible for this synergistic effect is defense priming. The high concentrations of plant hormones and growth regulators that SE plants are exposed to early in life constitutes a strong stress that may act as a priming stimulus, activating latent (primed) induced defenses. The subsequent MeJA treatment may act as a triggering stress that elicits a stronger and more rapid activation of defenses once plants are attacked by *H. abietis*.

Before SE or seed application methods can be recommended for protection of conifer saplings, we need more knowledge about the durability of MeJA-induced resistance. Most studies of how MeJA affects conifer resistance have been short-term. Only six studies have conducted experiments that tested for increased resistance more than one year after MeJA treatment (Erbilgin et al., 2006; Heijari et al., 2008; Zhao et al., 2010; Zas et al., 2014; Chen et al., (2021b); Vázquez-González et al., 2022). If the positive effects of MeJA treatment persist for several years, it might be most practical and cost-effective to apply MeJA treatment when plants are younger.

4.3. Ecological impacts of MeJA treatment

Most MeJA studies have been conducted under controlled conditions (Fig. 3). While these studies provide fundamental understand of conifer responses to MeJA treatment, results from controlled experiments may not be directly applicable to field conditions with various ecological interactions. Therefore, it is also important to explore the ecological impacts of MeJA treatment in conifers. Studies on MeJA have so far focused either on effects on the tree itself or on tree resistance to pests and pathogens. No studies have examined effects on other biotic interactions, such as those between conifers and root microbial communities. Root microbes, such as ectomycorrhiza (ECM) and plant-growth-promoting rhizobacteria (PGPR), play important roles in boreal coniferous forests (Uroz et al., 2016; Smith and Read, 2008). Increasing evidence shows that ECM and PGPR can promote conifer growth and resistance via different mechanisms (Velmala et al., 2018), such as direct induction of beneficial compounds, direct competition with or inhibition of pathogens, physical protection of roots, and perhaps priming of tree defenses (Smith and Read, 2008). Successful symbiosis between conifers and beneficial microbes depends on soil conditions, microbial diversity, host genotype, host metabolites, and other factors (DeVan et al., 2023; Velmala et al., 2013). MeJA has been shown to change host metabolites and could affect root exudates, which can thus change soil conditions and affect the microbial composition. Therefore, before MeJA is used for plant protection purposes it is important to assess if MeJA-induced resistance can interfere with the vital symbiosis between conifers and root microbial communities.

4.4. Publication bias

We found some evidence of publication bias in the literature on MeJA treatment of *Pinus* and *Picea* species. Even though the fail-safe number was large for both meta-analyses (Table S7), suggesting that many non-significant findings would be required to reject our overall conclusions, the funnel plots showed clear asymmetries that indicate publication bias. Non-significant results or incomplete data from small studies are less likely to be published than significant results. Such publication bias towards clear positive or negative results means there might be unpublished results that could have changed our estimated overall effect sizes. Another note for caution is that our meta-regression post-hoc tests should be interpreted carefully, as the number of observations is very limited for some explanatory variables (Table S2, S4 and S6). Differences in the number of observations between explanatory variables might be the result of researcher bias in study interests and choice of experimental design. For example, it might be more common to study younger plants because they are easier to handle when measuring resistance or growth parameters. Additionally, scientists tend to work with tree species that are important in their region and there are trends in what kind of research that is funded (Šimundić, 2013; Higgins et al., 2022). Thus, many factors may influence what kind of studies that get published.

MeJA has been a very useful tool to understand the mechanisms and complexity of conifer induced resistance. The use of MeJA also has practical potential as a forest management tool to boost the resistance of newly planted *Pinus* and *Picea* saplings. We should not expect that MeJA or other plant-derived chemicals can completely replace currently used pesticides, but rather that MeJA could be useful in combination with other tools in integrated pest management. By boosting tree resistance, MeJA might thus help reducing pesticide use and environmental pollution. Before MeJA can be used in practical pest management we need to understand more about how MeJA affects conifers and their interactions with other organisms (Fig. 7). Further research questions include: “How long does MeJA-induced protection last?”; ”How does MeJA affect different plant genotypes?” “How can MeJA be integrated with other management tools?”, and “How does MeJA treatment affect interactions between conifers and beneficial microbes that improve forest carbon sequestration and resilience?”.

CRediT authorship contribution statement

Paal Krokene: Writing – review & editing, Writing – original draft, Visualization, Methodology, Conceptualization. **Melissa Mageroy:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Adriana Puentes:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Conceptualization. **Ngan B Huynh:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data is provided in the supplementary files.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foreco.2024.121893.

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