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RESEARCH

Effects of propagation method and methyl jasmonate treatment on stem bark wound healing in Norway spruce seedlings

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Abstract

Healing of stem bark wounds is important for minimizing pathogen infection risk, restoring nutrient transport and structural support in trees. Here, we explore how propagation through somatic embryogenesis (SE) and methyl jasmonate (MeJA) treatment affect wound healing ability in Norway spruce (*Picea abies*) plants. We inflicted a mechanical wound on the lower stem of MeJA- and non-treated plants produced via SE (emblings) or from seeds (seedlings). Visible signs of healing around the wound edges (onset of healing) were recorded 2 weeks post-wounding; wound size (exposed xylem) was measured every other week (June–September) in year 1, and May and September in year 2. Plant height and diameter were also measured. MeJA positively affected healing onset, with 48% more MeJA- than non-treated plants exhibiting early healing. This resulted in a sharp decrease in wound size for MeJA-treated plants 2–4 weeks post-wounding. However, these benefits only occurred early on, as MeJA reduced the overall healing rate (tissue growth/day) by 9%. For SE, fewer emblings (70%) showed early healing signs compared to seedlings (91%). Yet, non-treated emblings showed the highest healing rate during year 1; in year 2, these effects persisted with all emblings having a 61% faster healing rate and 68% more had completely closed their wounds relative to seedlings. Wounding did not affect growth, MeJA negatively affected diameter but not height, and overall emblings grew less than seedlings. We conclude that MeJA may stimulate stem wound healing initiation in Norway spruce, but slow down healing rate and vice versa for SE plants.

Keywords Emblings · *Picea abies* · Mechanical wounding · Plant tolerance · Seedlings · Somatic embryogenesis · Wound healing rate

Introduction

Plants can receive multiple types of damage to their reproductive and vegetative tissues. In trees, damage to the stem bark can be caused by herbivorous insects or mammals, as well as physical factors such as fires, storms, and avalanches, or logging machines. These biotic and abiotic factors can create stem wounds which can physically weaken the tree, allow entry of pathogens to the phloem and xylem, and eventually lead to stem decay (Neely 1988a; Vasiliauskas 2001; Vasaitis 2012) or even death in seedlings or young trees (Stephens and Westoby 2015). Stem wounds are healed and sealed by generating new parenchyma tissue (callus) from the edges around the wound, which subsequently differentiates and matures to become newly-formed bark (Neely 1979). For wounds that become infected, fungal development and stem decay decreases once wounds become smaller or are completely sealed (Biggs 1986;

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Vasiliauskas 2001; Vasaitis 2012; Romero 2014). Since stem wounds interrupt sap flow and nutrient translocation (Neely 1988a, b), faster repair allows the plant to return to a state in which resources are prioritized to growth and reproduction. Thus, to protect themselves, trees must heal their bark wounds as soon as possible.

Various attempts have been made to exogenously affect trees' healing abilities. For example, Biggs (1990) succeeded in accelerating bark wound responses in peach trees (*Prunus persica*) by using fungal cell wall extract and the sugar cellobiose. These compounds increased lignification (i.e., hardening) of the parenchyma cells that line the edge of the wound (Biggs 1990), which is one of the first responses following bark damage (Neely 1979). This is a desirable effect since lignification creates a barrier to pathogen entry and reduces water loss. The application of calcium directly to wounds, or increasing its availability in the soil, has also been shown to promote wound closure and reduce infection in sugar maple (*Acer saccharum*) and peach trees, respectively (Biggs 1990; Huggett et al. 2007). In a separate study, Bai et al. (2005) inflicted stem wounds and concurrently applied the plant growth regulator paclobutrazol to nine tree species, but observed negative effects on wound closure in five species and no effect in the remaining four. Similarly, fertilization did not affect the rate of wound closure in oak (*Quercus virginiana*) and grand fir (*Abies grandis*) trees (Filip et al. 1992; Ali and Luley 2006).

Overall, a wide range of compounds and interventions particularly within the field of arboriculture, have been tested to promote stem wound repair and limit infection (Clark and Matheny 2010). Despite extensive research, the influence of resistance-inducing methods on wound healing remains poorly understood. Plant resistance refers to their ability to reduce or prevent damage caused by biotic stressors (Nuñez-Farfán et al. 2007; Mitchell et al. 2016). Resistance can be induced either through natural damage or the application of specific treatments, such as phytohormones. Hormones like jasmonic acid (JA), salicylic acid (SA), ethylene (ET), and abscisic acid (ABA) play key roles not only in activating induced resistance, but also in regulating both local (at the wound site) and systemic wound responses (Savatin et al. 2014). Consequently, it is plausible that enhanced resistance mechanisms may also contribute to improved wound healing capacity. Supporting this notion, Mullick (1977; as cited in Hudler 1984) observed that bark feeding by the balsam woolly adelgid (*Adelges piceae*) was obstructed in resistant conifers due to the formation of an impermeable layer of tissue at feeding sites—an effect not observed in susceptible trees, where aphid saliva inhibited this response. Notably, the relationship between resistance and wound healing remains particularly understudied in young trees. While most existing studies focus on trees older than ten years, stem damage can be significantly more detrimental to juvenile individuals, for whom successful recovery is critical for survival during forest regeneration (Stephens and Westoby 2015).

In conifers, two resistance-inducing methods have recently been shown to decrease damage inflicted by a bark-feeding insect to Norway spruce seedlings. Berggren et al. (2023) examined resistance to a major conifer forest regeneration pest, the pine weevil (*Hylobius abietis*), which inflicts large stem wounds and can completely debark and eventually kill seedlings (Långström and Day 2004). The authors found that plants produced through somatic embryogenesis (SE) and subsequently treated with the plant hormone methyl jasmonate (MeJA), received 86% less stem damage than untreated seedlings produced through seeds (Berggren et al. 2023). Both of these resistance-enhancing methods have the potential to affect wound healing abilities of plants. Firstly, SE is a vegetative propagation process which uses plant growth regulators to first induce embryos from a donor tissue in vitro; these embryos are then stimulated to mature and grow to eventually become small plantlets that can be cultivated in nurseries. Many of the growth regulators used in SE are also those that trigger stress responses in plants, such as ABA (Méndez-Hernández et al. 2019; Müller 2021). Secondly, MeJA is naturally produced when plants are attacked or wounded, and activates resistance mechanisms which reduce damage by insects and pathogens (e.g. Chen et al. 2018, 2020; Senthil-Nathan 2019; Mageroy et al. 2020a; Mouden et al. 2021; Erazo-García et al. 2021; Puentes et al. 2021). Moreover, MeJA alone has been shown to negatively affect the rate of stem wound healing when applied to Norway spruce seedlings after wounding has occurred (Chen et al. 2023). However, the effects on healing of MeJA application before wounding, and together with the SE process, are not known.

Investigating the effects of SE and MeJA together on stem wound healing is important for several reasons. From a forest regeneration perspective, it is necessary to evaluate if enhanced plant resistance to insect damage through SE and MeJA can entail a cost. For example, if wound healing is slowed down by these factors and there is a greater risk of infection, seedling vigor and survival may be negatively affected. This could result in low seedling establishment, which may compromise the successful formation of a new forest stand. On the other hand, positive effects may also occur, as we observed that a larger proportion of Norway spruce SE-plants showed signs of wound healing relative to regular seedlings in the field, late in the growing season (Berggren et al. 2023 study, unpublished data). Evaluating these effects is also timely, as the use of SE and MeJA may become more widespread. Production of forest regeneration material through SE is expected to increase in European boreal forests (Rosvall et al. 2019; Fugeray-Scarbel et al. 2023; Thiffault et al. 2023), and MeJA is being proposed as a viable nursery treatment prior to forest planting (Chen et al. 2020; Nybakken et al. 2021). Moreover, examining the relationship between resistance-inducing methods and a recovery trait, such as stem wound closure, can provide ecological insight into trade-offs among plant defense strategies. In the present study, we conducted a mechanical stem damage experiment to investigate how the SE process alone and in combination with MeJA, can affect stem wound healing and plant growth in Norway spruce seedlings across two growing seasons. We aimed to answer the following questions:

1. Do SE-plants (emblings) and regular seedlings that have been first treated with MeJA (or water) and then stem-wounded, differ in (a) onset of wound healing and (b) wound closure over time?
2. Do SE-plants (emblings) and regular seedlings that have been first treated with MeJA (or water) and then stem wounded, differ in plant growth (height and diameter)?

After infliction of a mechanical stem wound, we examined plants for early signs of healing around the edges of the wound 2 weeks post-wounding (referred to as onset of healing). Wound size (exposed xylem) was measured every other week for 15 weeks (June-September) in year 1, and in May and September in year 2. We estimated wound healing rate (production of new tissue over time) and noted complete wound closure across the two years. Throughout the text, Norway spruce plants produced through SE are referred to as *emblings* and non-SE seedlings that were produce from seeds are referred to just as *seedlings*.

Materials and methods

Plant material

Plant material consisted of Norway spruce (*Picea abies* (L.) H. Karst) somatic plants (emblings) and seedlings obtained from the Natural Resources Institute Finland (Luke) (a total of 76 emblings and 80 seedlings). The 76 emblings used in the experiment originated from four genotypes (three full-sib families, from progeny-tested ‘plus’ trees from southern Finland belonging to the breeding program), and the 80 nursery seedlings were of more Northern origin. The cell lines were initiated and cryopreserved in 2014 following the methodology developed by Varis et al. (2017). For three of the genotypes (known as 14Pa607, 14Pa645, and 14Pa1324), proliferation and maturation were carried out in temporary immersion system (TIS) bioreactors according to the protocols of Välimäki et al. (2020). In vitro germination was carried out on Petri plates as described by Välimäki et al. (2020). The germinated embryos were transplanted to Plantek 81f containers (81 separate ventilated compartments, 85 cm^3 in size), filled with pre-fertilized semi-coarse sphagnum peat and grown in greenhouse and nursery as described by Tikkinen et al. (2018). For one of the genotypes (known as 14Pa4043), proliferation and maturation were carried out on Petri plates according to the protocol in Businge et al. (2012). Desiccation and in vitro germination were carried out as described in Dobrowolska et al. (2017), but with the following modifications: germination plates were first placed for one week in dark at 20°C, followed by one week in red light and then gradually increasing the light intensity to approximately 100 µmolm−2 s−1 at 20°C using fluorescent tubes. The germinated embryos were transplanted, acclimatized and fertilized as described in Le et al. (2021). Once the emblings had outgrown HIKO-V13 trays, they were re-potted into larger containers and grown over the summer (year 2021) outside at the Swedish University of Agricultural Sciences (SLU), Umeå campus. The 80 zygotic seedlings (seedlings) used in the experiment were obtained from Luke Suonenjoki research nursery, derived from seeds collected at an open pollinated seed plantation (commercial seed lot tracking code: EY/FIN M29-14-0023). Seedlings were grown in Plantek 81f containers and treated with Proline® 250 EC, Prothioconazole 250 g/l against plant diseases in autumn 2020.

Both emblings and seedlings had commenced their growing season when they were delivered from Finland to SLU, Uppsala campus Sweden, in June 2021. Emblings from Umeå (genotype 14Pa4043) arrived in September 2021. All plants were immediately repotted in 1 L (ø 13 cm) plastic pots and kept in two adjacent greenhouses, separating emblings and seedlings, for one year (light/temp. conditions summertime: 16 h/8 h light/dark and ~18/15°C day/night, and wintertime (natural light and temperature): minimum 9 h/15 h light/dark and ~6/3°C day/night). In April 2022, about 5 weeks prior to the experimental start, plants were repotted in 2 L (ø 16.5 cm) plastic pots. Plants were between 2 and 3 years old at the start of the experiment.

Experimental set-up

Methyl jasmonate treatment

On June 1, 2022, three weeks prior to the start of the experiment, MeJA treatment was applied to the plants. A total of 39 emblings and 40 seedlings (ca. half of the 76 and 80 available emblings and seedlings, respectively) received exogenous treatment with 10 mM methyl jasmonate (MeJA), following the methodology in Berggren et al. (2023). In summary, MeJA (95%, Sigma-Aldrich, ref. 392707) was dissolved in ethanol and poured into a plastic hand-sprayer bottle (Free-Syringe PC 1.5 L, Jape Products AB, Hässleholm, Sweden). Deionized water was added to achieve the final ethanol concentration of 2.5% (v: v), and the solution was vigorously shaken. Plants were arranged in rows outdoors, and the solution was sprayed on the aboveground parts of each plant for about one second but avoiding the newly-formed top shoots. Spraying distance was approximately 30 cm from the nozzle to the plant. After spraying, the MeJA-treated plants were placed in a separate greenhouse. Non-treated plants were sprayed similarly with deionized water to differentiate between MeJA induction and the spraying process.

Mechanical wounding

A total of 37 non-treated and 39 MeJA-treated emblings, and 40 non-treated and 40 MeJA-treated seedlings were used in the experiment. On the day of wounding, a rectangular wound (height: 40 mm, width: 5 mm) was incised on the lower part of the stem (the lower end of the wounds was situated ~ 1–2 cm from the soil surface) using a scalpel, avoiding side branches in the wound area (see Fig. 1). If numerous branches surrounded the lower stem, the wound was inflicted slightly higher or lower on the stem to prevent cutting the branches (as in Chen et al. 2023). Within the rectangular area, bark phloem was removed until the xylem was completely exposed, and tools were disinfected with 95% ethanol between each plant. To measure the actual size of the wound that was inflicted, a transparent plastic film was placed over the stem wound and tightly held to trace the outline of the wound with a black permanent marker. The outline drawings were taped to a sheet of white paper, and filled in with the marker so they became black rectangles. A piece of millimeter paper, 40 × 5 mm (to use as scale), was taped to each white sheet and sheets were subsequently scanned. QuPath image analysis software (version 0.4.3) (Bankhead et al. 2017) was used to measure the area and perimeter of each wound drawing and these are referred in the results as the initial wound sizes. Wounding occurred over two consecutive days, June 21–22, 2022, with half of

Fig. 1 Example of wound healing/size over time in one of the MeJA-treated seedlings (that healed fast). The first picture to the left (June 27, 2022) shows the size and shape of the incised wound four days after wounding. The following pictures (from left to right) were taken: 32 days (July 25, 2022), 55 days (August 17, 2022), and 75 days (September 6, 2022) after wounding

the plants wounded each day. Plants in each treatment were randomly wounded over the two days, and after wounding the plants were arranged in a semi-randomized block design (see Supplementary Text in the Supplementary material) in the greenhouse on two benches.

Wound healing and growth measurements

To evaluate the effects of treatment on the onset of healing, we visually examined plants for signs of healing two weeks post-wounding (July 5–6, 2022). A plant had initiated healing (recorded as yes or no) if we could observe light green cell masses along the edge of the wound (see Fig. 1); this mass should be the cambium and callus growing together, which will proliferate and eventually seal the wound (Chano et al. 2015). To estimate wound healing rate during the first year, the size of each wound (i.e., the exposed xylem) was measured using a millimeter paper every second week from July 5–6 until September 13–14, 2022 (a total of 12 weeks post-wounding). Height and width of the exposed xylem was measured at three points along the wound, at 25%, 50%, and 75% of the wound length, respectively, and the area of the exposed xylem was estimated in squared millimeters (mm^2). Wound size was measured in the same way during the second year, but only at two time points (May 19 and September 19, 2023). Complete wound closure (recorded as yes or no; yes = no xylem was visible, no = xylem remained visible and could be measured) was recorded once (September 19, 2023). After completing each measurement, plants were randomized to a new position following the same block design as before (see Supplementary Text). Wound healing rate (mm^2 growth of new tissue per day) was calculated for each plant as:

$$\frac{\text{First wound size measurement } (\text{mm})^2 - \text{final wound size measurement } (\text{mm})^2}{\text{Number of days between measurements}}$$

For year 1, the first wound size measurement occurred two weeks post-wounding (July 5–6, 2022) and the final wound size measurement occurred 12 weeks post-wounding (Sept. 13–14). Thus the number of days between measurements was 70 days. For year 2, the first and final wound measurements were taken on May 19 and September 19, 2023 respectively (i.e., 123 days between measurements). For years 1 and 2 together (cumulative rate), the first and final wound measurements were taken on July 5 or 6, 2022 and September 19, 2023 respectively (i.e., 454 days between measurements).

Plant stem height and basal diameter were measured on all plants the days prior to MeJA treatment (May 30–31, 2022), the days prior to wounding (June 20–21, 2022), and then approximately once a month after wounding (July 27–28, August 29–30, and September 13–14, 2022). Before MeJA treatment, the average plant height ± standard error of the mean (SEM) (and ranges) for emblings was 34.1 ± 1.1 cm (19.0 to 50.4 cm), and for seedlings 37.6 ± 0.6 cm (22.0 to 46.6 cm). The average plant diameter ± SEM (and ranges) for emblings was 4.61 ± 0.08 mm (3.25 to 6.51 mm), and for seedlings 4.79 ± 0.09 mm (2.72 to 6.39 mm). To estimate effects of treatments on growth, unwounded control plants were also included in the growth measurements. However, due to human error, sample sizes were very uneven between treatments (non-treated seedlings $n = 8$, MeJA-treated seedlings $n = 5$, non-treated emblings $n = 12$, and MeJA-treated emblings $n = 5$). For this group of plants, the average plant height ± SEM (and ranges) before MeJA treatment was for emblings 36.3 ± 1.9 cm (22.0 to 48.0 cm), and for seedlings: 36.0 ± 1.3 cm (26.6 to 43.2 cm). The average plant diameter ± SEM (and ranges) for emblings was: 4.85 ± 0.18 mm (3.41 to 6.57 mm), and for seedlings: 4.59 ± 0.17 mm (2.98 to 5.30 mm).

Statistical analyses

All analyses were conducted in R version 4.3.1 (R Core Team 2023). To evaluate differences between non-treated and MeJA-treated seedlings and emblings in stem wound healing abilities, various models were fitted. In all models, plant type (seedling or embling), MeJA treatment (10 mM or deionized water) and their interaction, as well as wounding day (wound inflicted on June 21 or 22, 2022, referred to as block or greenhouse bench 1 or 2), were used as fixed effects. The size of the actual inflicted wound (day 0) was included as a covariate (except in the plant growth analyses). Plant id was included as a random effect in the model examining effects on wound size over time, as repeated measures were done on the same plant.

Onset of healing and complete wound closure (0 = no, 1 = yes) two weeks after wounding and at the end of year 2, respectively, were analysed using generalized linear models with a binomial distribution (*glm*-function in the base stats R package; R Core Team 2023). Wound size over time (exposed xylem, mm^2) from the first to the last measurement (2 weeks to 12 weeks post-wounding, respectively), was analysed using a linear mixed model with time as a fixed squared term (*lmer*-function in the *lme4* package; Bates et al. 2015). Wound healing rate (mm^2 growth of new tissue per day, from the first to the last measurement) was analysed using linear models (*lm*-function in the base stats R package; R Core Team 2023) for year 1, year 2 alone, and cumulatively years 1 and 2. In the wound healing rate analyses of year 2 and years 1–2, plants that had completely closed their wounds by the end of the second growing season are included. We do not know exactly at which date their wounds closed completely, hence, their healing rate was calculated in the same way as for other plants (i.e., divided by the same number of days). The healing rate of these plants may actually be faster than we calculated, but this should not bias the results towards finding significance among treatments since estimates are just more conservative.

Effects on plant height and diameter were analysed using the difference (i.e., increment) between the last and the first measurement for each year (year 1: May 30–31 and Sept. 13–14, 2022; year 2: May 19 and Sept. 19, 2023). Height (cm) and diameter (mm) increment were analysed using a linear model including the *varIdent*-function (*gls*-function in the *nlme* package; Pinheiro et al. 2023) in year 1, and a generalized linear model in year 2 (*glm*-function in the base stats R package; R Core Team 2023). The *varIdent*-function allows different groups to have different variances instead of assuming homoscedasticity (constant variance), which is a standard assumption in regression models. In year 1, variances differed among plant types (variation among seedlings in plant growth was either higher or lower than among emblings) and the function *varIdent* allowed for this to be taken into account in the model.

Model validations were performed through inspection of residuals vs. predicted values, through simulation and plotting of scaled residuals and outliers using the *DHARMa* package (Hartig 2022), and by examining if assumptions of equal variances across treatments were met using the *LeveneTest*-function in the *car* package (Fox and Weisberg 2019). To meet the model assumptions of linearity, normality, and/or homoscedasticity (depending on the model), healing rate (in year 2 and cumulative years 1–2), height increment (in years 1 and 2), and diameter increment (in year 1) were square root-transformed. Additionally, diameter increment (in year 2) was log-transformed. Analysis of deviance (ANODEV) or Analysis of variance (depending on the model) (ANOVA) was used to test significance of main effects and interactions through the *Anova*-function in the *car* package (Fox and Weisberg 2019). ANODEV is similar to ANOVA but is designed for models that do not assume normally distributed errors. It uses deviance (likelihood-based measure) instead of sum of squares, and significance is assessed using a Chi-square test. In this study, the models where ANODEV was used were those for onset of healing, wound closure and plant height and diameter in year 1. Estimated means for each treatment were obtained through the *emmeans*-function in the *emmeans* package (Lenth 2023) for graph plotting. The *emmeans*-function calculates estimated marginal means (EMMs), also known as least-squares means. It adjusts for covariates in the models (e.g., initial wound size), and are thus more informative than raw means. We used these means to estimate the magnitude of effect sizes, which allowed us to evaluate how much the treatment affected the outcome as a proportion of the control condition. These were calculated as follows and are presented in the results section:

$$Effect\ size\ (proportion) = \frac{M1 - Mcontrol}{Mcontrol}$$

M_1 is the mean of the treatment group and $M_{control}$ is the mean of the control group (reference or untreated group, depending on the experimental design).

Results

Onset of healing

Two weeks after wounding, we noted that 76% of all the experimental Norway spruce plants were showing signs of healing, but the proportion of plants showing these signs differed significantly between treatments (Table 1). MeJA treatment promoted the onset of wound healing for both emblings and seedlings (Table 1, significant main effect of MeJA), with 48% more plants (effect size calculation can be found in the statistical section) showing healing initiation relative to non-treated plants (Fig. 2A). Irrespective of MeJA treatment, fewer emblings had shown signs of healing two weeks post-wounding (significant main effect of plant type, Table 1). In total, we noted that 91% of all seedlings and 70% of all emblings were showing signs of healing. Four weeks after wounding, all plants had initiated healing.

Table 1 Summary of results from models examining the effects of plant type and MeJA treatment on onset of healing and complete wound closure

Fixed effects	Onset of healing			Complete wound closure		
	χ^2	df	*P*	χ^2	df	*P*
Plant type	6.31	1	**0.01**	10.2	1	**0.001**
MeJA	16.99	1	**< 0.0001**	0.28	1	0.60
Initial wound size	0.10	1	0.75	2.03	1	0.15
Block	0.65	1	0.42	0.89	1	0.34
Plant type × MeJA	1.65	1	0.20	1.12	1	0.29

χ^2: Chi-square value; *df*: degrees of freedom; *P*: *p*-value; plant type (seedlings and emblings of Norway spruce); MeJA (methyl jasmonate) treatment (10 mM or water). Onset of healing (0 = no, 1 = yes) was measured in year 1 two weeks after wounding. Wound closure (0 = no, 1 = yes) was measured in year 2 at the end of the experiment (September 19, 2023). Initial wound size (at the day of wounding) and positional blocks in the greenhouse (plants were placed on two benches) were included as covariates. Significant effects ($P < 0.05$) are in bold

Complete wound closure

During year 2 of the experiment, we noted that 50% of all Norway spruce plants had completely closed their wounds, but the proportion differed significantly between plant types (significant main effect of plant type, Table 1). A total of 68% more emblings had closed their wounds relative to seedlings (Fig. 2B). We did not detect a MeJA × plant type interaction (Table 1), showing that the positive effects of somatic embryogenesis on wound closure were consistent for treated and untreated plants. Nonetheless, of all treatments, MeJA-treated emblings displayed the highest proportion of plants with completely closed wounds; 22% more than non-treated emblings and 74% more than non-treated seedlings (Fig. 2B).

Healing rate

We found that the stem wound healing rate of Norway spruce plants was influenced by MeJA treatment in year 1. MeJA had a negative effect on healing rate (significant main effect, Table 2); with treated plants experiencing a 9% slower rate compared to non-treated plants (Fig. 3A). However, these effects were somewhat more detrimental for emblings than seedlings, and this was supported by the close-to significant (at the $P<0.05$ level) interaction between MeJA and plant type ($P=0.06$, Table 2). Treated emblings suffered a 16% reduction in healing rate relative to non-treated emblings (Fig. 3A), while treated seedlings suffered a 1% decrease compared to non-treated seedlings. On the other hand, non-treated emblings displayed the fastest healing rate compared to all other treatments (11% greater than non-treated seedlings, Fig. 3A). These results are in contrast to those found for the onset of healing, where MeJA positively affected the early signs of healing and non-treated emblings were slowest to get started (Fig. 2A).

In the year following wounding, the rate of wound healing was significantly different between the two plant types (main effect of plant type, Table 2). Overall, emblings exhibited a 61% faster healing rate than seedlings (Fig. 3B). MeJA-treated emblings experienced the highest healing rate of all treatments (58% greater than non-treated seedlings, Fig. 3B) in year 2, which is in contrast to non-treated emblings having the highest healing rate in year 1. The lowest healing rate in year 2 was observed for MeJA-treated seedlings, which suffered a 32% decrease compared to non-treated seedlings (Fig. 3B). Cumulatively across both years, emblings exhibited the fastest healing rate, 20% faster than seedlings, with MeJA treatment slowing down the healing rate for both plant types by 6% (but this effect was not statistically significant) (Table 2; Fig. 3C).

Wound size over time

Changes in wound size over time during the first year were affected by whether plants were treated with MeJA or not (significant MeJA × time interaction, Table 3). Over the entire measurement period, MeJA positively affected changes in wound size, with treated plants having wounds that were on average 10% smaller compared to non-treated plants (Fig. 4). Even though we did not detect a significant plant type × MeJA × time interaction at the $P<0.05$ level (but it was close to it, $P=0.08$; Table 3), some slight differences among plant types could be observed through time. Non-treated emblings had slightly greater wound sizes throughout the experimental period, especially in the beginning. This is in line with our results on the onset of healing, as fewer non-treated emblings were showing early signs of healing (Fig. 2A). However, the healing rate of non-treated emblings was superior to all others (Fig. 3A), which resulted in a steep decrease in wound size after day 14 up until day 56 (Fig. 4). On the other hand, for treated emblings, the positive effects of MeJA on onset of healing resulted in a steep decrease in wound size early on, which remained despite MeJA slowing down their healing rate (Fig. 4). For seedlings, little difference was observed among treated and untreated plants in healing rate (Fig. 3A); but due to a slower start of untreated plants (Fig. 2A), they had slightly larger wound sizes by the end of the experiment relative to treated seedlings. By the end of the experiment, for all plants, the inflicted wounds had healed substantially and were reduced to 30–40% of their original sizes.

Plant growth

Plant height and diameter were differentially affected by plant type, but not by MeJA treatment (Table 4) in year 1. Emblings grew significantly less than seedlings both in height and diameter (Fig. 5A and C, Fig. S1). Height and diameter increment was 55% and 16% less for emblings than for seedlings, respectively. Changes in average height growth for all plants mainly occurred during the first 3 weeks, while diameter growth mostly happened towards the end of the experimental period (Fig. S1). Wounding on its own did not seem to affect the height or diameter of plants (compare wounded and unwounded plants, Fig. S1). Note that the unwounded plants were few, hence, analyses were not performed to distinguish significant differences between wounded and unwounded plants. Nonetheless, it is clear from the figure and mean comparisons that wounding did not negatively affect growth.

In year 2, plant height and diameter were still differentially affected by plant type (Table 4) and emblings continued to grow less than seedlings (Fig. 5B and D).

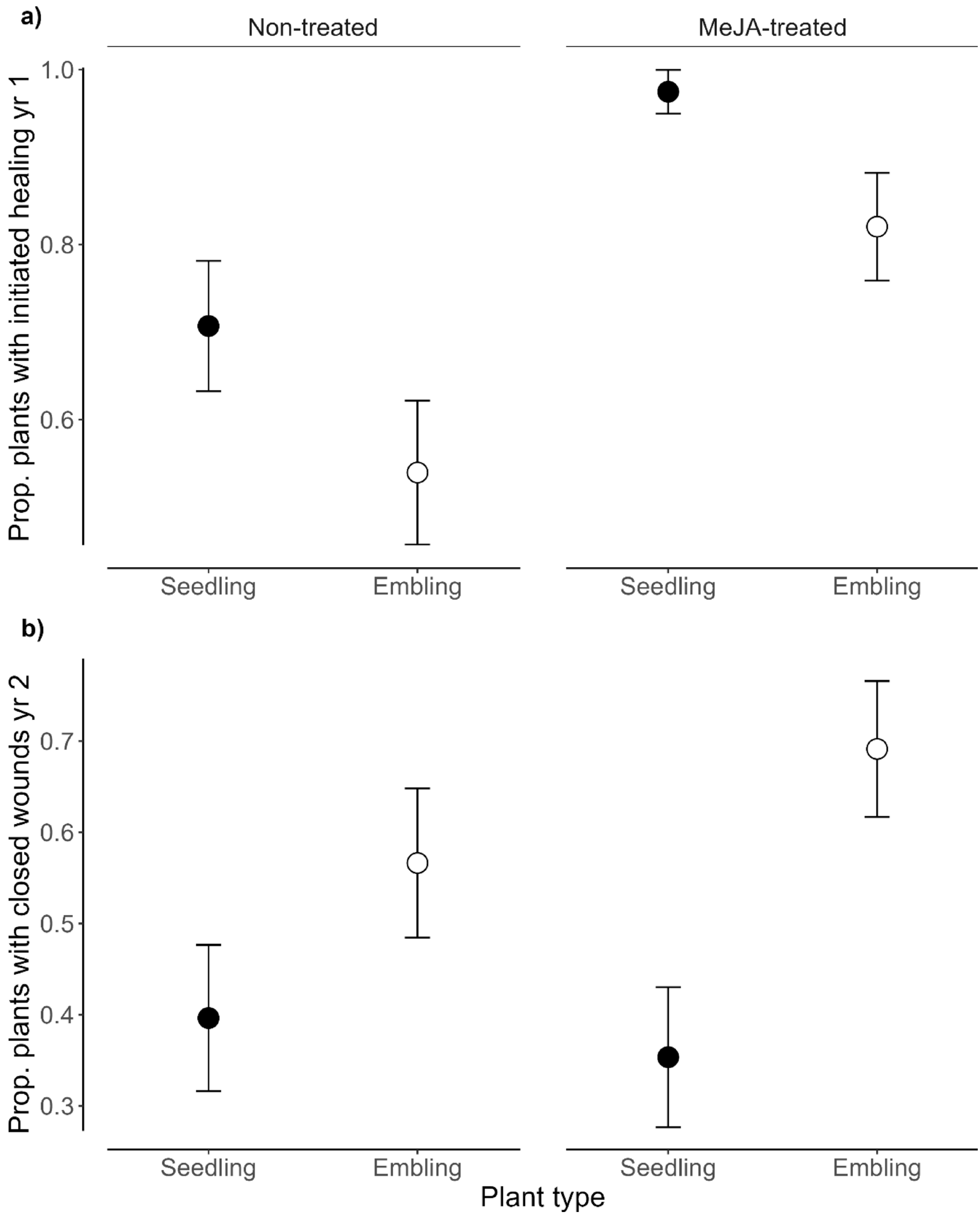
a)
Non-treated
MeJA-treated
Prop. plants with initiated healing yr 1
1.0
0.8
0.6
Seedling
Embling
Seedling
Embling
b)
Prop. plants with closed wounds yr 2
0.7
0.6
0.5
0.4
0.3
Seedling
Embling
Seedling
Embling
Plant type

Fig. 2 Estimated means (±standard error) of the proportion of Norway spruce (*Picea abies*) that had **a** initiated healing (signs of new tissue growth around the wound perimeter) two weeks post-wounding in year 1 (July 2022), and **b** closed their wounds (xylem completely covered with new tissue) in September year 2 (2023). Plant type consisted of seedlings (grown from seeds) or emblings (produced via somatic embryogenesis) treated or not with the plant hormone methyl jasmonate (MeJA, 10 mM or water applied once in June, 2022). Sample sizes were from left to right: non-treated seedlings $n=40$, non-treated emblings $n=37$, MeJA-treated seedlings $n=40$, MeJA-treated emblings $n=39$. Note that the y-axes do not start at zero

MeJA-treatment had a negative effect on both height and diameter increment, but it was only statistically significant for diameter (Table 4).

Discussion

Our study showed that the propagation method somatic embryogenesis (SE) and methyl jasmonate (MeJA) treatment can affect the stem wound healing abilities of Norway spruce plants, in terms of how fast new tissue is produced (healing rate) and complete closure of the wound. MeJA treatment prior to wounding positively affected the onset of healing for both emblings and seedlings, with an average of 48% more of the MeJA-treated than the untreated plants exhibiting early signs of healing along the wound edges two weeks post-wounding. However, MeJA negatively affected the overall wound healing rate for all plants. The opposite pattern was found for the effects of SE. Early signs of healing two weeks post-wounding were observed to a lesser extent in emblings than seedlings (70% vs. 91% of emblings and seedlings, respectively). Across both years though, emblings experienced faster healing rates and a greater frequency of wound closure than seedlings (68% more emblings had completely sealed their wounds relative to seedlings by year 2). In terms of plant growth, we found that emblings grew significantly less than seedlings. MeJA treatment reduced diameter but not height growth across years for all plants, and wounding did not affect either variable. We conclude that MeJA treatment may stimulate the initiation of bark wound healing processes in Norway spruce plants, but slow down the overall rate of tissue production (i.e., healing rate) and vice versa for the effects of the SE process. That is, emblings appear to be slower at initiating healing but once healing processes have begun, they exhibit superior healing and wound closure abilities.

Effects of MeJA

A positive effect of MeJA treatment on initiation of stem wound healing can be advantageous for plants. One of the most important benefits is that it could minimize the risk of pathogens entering through the exposed tissue, as it has earlier been reported that healing does not only prevent infection, but it can also stop it. Biggs (1986) showed that peach tree (*Prunus persica* (L.) wounds became resistant to *Cytospora leucostoma* canker fungus when a new periderm, at least three phellem cells thick, was produced around the wound perimeter. Periderm, the outer layer of tree bark, serves as a protective covering and is composed of phellem (suberized cells), phellogen (cork cambium), and phelloderm (parenchyma-like cells derived from the phellogen tissues). Biggs (1986) also found that even infection severity and occurrence decreased as healing progressed. In almond trees, bark wounds became resistant to the fungus *Ceratocystis fimbriata* 10–14 days after wounding (Bostock and Middleton 1987). For Norway spruce seedlings in forest regeneration sites, substantial debarking occurs due to pine weevil feeding and often results in girdled stems. To withstand such damage, seedlings need to grow fast in diameter (e.g., Thorsen et al. 2001) and quickly restore phloem connection and nutrient transport. Thus, for plants in our study, early onset of healing may not only be beneficial for minimizing risk of infection but also risk of plant death by girdling.

Given that MeJA signals plants to activate stress-related and defense functions, treatment with this hormone may accelerate onset of healing through direct or indirect effects. In response to wounding or pathogen invasion, one of the most important initial responses of conifers is lignification and suberization of cells around the wound/entry site (Franceschi et al. 2005). Hudgins and Franceschi (2004) found that MeJA accelerated lignification in Douglas fir (*Pseudotsuga menziesii*) saplings in the form of secondary phloem sclereids (stone cells), which were produced earlier than expected in treated vs. non-treated plants. In another study, it was found that wounding, pathogen infection, and MeJA treatment elicited similar expression patterns of nine defense genes in the bark and wood of 2-year old Norway spruce (Yaqoob et al. 2012). The authors found that MeJA strongly elicited expression of lignin-related genes (e.g., peroxidases) in the bark, and expression levels were higher than those for fungal inoculation and wounding alone (Yaqoob et al. 2012). However, for some of these genes examined, expression returned to basal levels 13 days after MeJA treatment. In our study, wounding occurred 21 days after MeJA treatment. Even though expression levels could have decreased with time, it is possible that some of these early response genes remained slightly upregulated or that activation was faster after wounding in MeJA-treated relative to non-treated plants. To further examine this, a study examining expression of wound repair genes specifically, and under a longer period after MeJA treatment would be needed.

Table 2 Summary of results from models examining the effects of plant type and MeJA treatment on wound healing ability year 2, and cumulatively year 1 to 2

Fixed effects	Wound healing rate yr 1			Wound healing rate yr 2			Wound healing rate yr 1–2		
	F	df	*P*	F	df	*P*	F	df	*P*
Plant type	0.61	1	0.44	4.17	1	**0.04**	11.60	1	**0.001**
MeJA	4.43	1	**0.04**	0.01	1	0.94	1.18	1	0.28
Initial wound size	8.85	1	**0.003**	0.00	1	0.98	3.33	1	0.07
Block	1.08	1	0.30	4.10	1	0.05	0.50	1	0.48
Plant type × MeJA	3.52	1	0.06	2.28	1	0.13	0.00	1	0.96

F: F-value; *df*: degrees of freedom; *P*: *p*-value; plant type (seedlings and emblings of Norway spruce); MeJA (methyl jasmonate) treatment (10 mM or water). Wound healing rate (mm^2 growth of new tissue per day) was analysed in year 1 from two weeks after wounding until September 14, 2022; 10 weeks (12 weeks post-wounding), in year 2 from May 19, 2023 to September 19, 2023 (17.5 weeks), and cumulatively for years 1 to 2 (July 6, 2022 to September 19, 2023). Initial wound size (at the day of wounding) and positional blocks in the greenhouse (plants were placed on two benches) were included as covariates. Significant effects ($P<0.05$) are in bold

Interestingly, other studies have found that MeJA does not influence the actual morphological changes that are expected to occur after wounding. For instance, Franceschi et al. (2002) found that MeJA induced the same set of anatomical responses that are elicited by fungal inoculation, but not those involving lignification or wound periderm formation in mature Norway spruce trees (30+years). Similarly, Kozlowski et al. (1999) found that lignin deposition did not differ between 7 days old MeJA- and non-treated Norway spruce seedlings. Discrepancies among studies can be due to different tree ages, but also MeJA concentration and application methods. Franceschi et al. (2002) state that they might have a used a concentration of MeJA that was too low, while Kozlowski et al. (1999) exposed seedlings in chambers to volatile MeJA added to cotton wool. Even if results on the potential direct effects of MeJA on wound repair mechanisms have varied, it is also possible for indirect effects to be occurring. It is well established that MeJA treatment results in the reprogramming of the cambial zone for production of traumatic resin ducts (Franceschi et al. 2002; Hudgins and Franceschi 2004). Moreover, it increases expression of genes involved in biosynthesis of JA, SA and ET (Wilkinson et al. 2022), which are major defence regulatory hormones and known to be mediating post-wounding responses (Savatin et al. 2014). Our study does not allow us to discern direct from indirect effects, but given ours and previous results there seem to be several avenues for MeJA to positively affect the onset of wound repair processes.

Despite its initial positive effects, MeJA had an overall negative effect on wound healing rate of Norway spruce plants during the first year. After the steep reduction in wound size promoted by MeJA treatment, wound healing was slowed down by 9% relative to non-treated plants. These effects could be due to the resource-costly morphological and chemical changes that occur a few weeks following MeJA treatment. In Norway spruce, traumatic resin duct formation occurs three to four weeks after treatment, and the phenolic content as well as the number of polyphenolic parenchyma (PP) cells have also been shown to increase after this time (Nagy et al. 2000; Franceschi et al. 2002; Krokene et al. 2008). Moreover, MeJA treatment results in substantial terpene production in Norway spruce, especially after wounding/damage (Erbilgin et al. 2006; Krokene et al. 2008; Zulak et al. 2009; Lundborg et al. 2016; Mageroy et al. 2020b). In addition, MeJA may temporally decrease photosynthetic capacity in various conifers (Heijari et al. 2005; Gould et al. 2008; Wilkinson et al. 2022).

Overall, it is possible that MeJA is beneficial for initial activation of wound repair processes, as many defense hormones are up-regulated following treatment. However, resources are soon after prioritized to anatomical and chemical responses, and as resource competition is exacerbated by reduced photosynthesis, it is likely that much of it is diverted away from wound healing. In line with this idea and our results, Chen et al. (2023) found that MeJA-treated Norway spruce seedlings exhibited much slower wound healing rates (ca. 15% slower) relative to non-treated plants. The authors applied MeJA one week after plants were mechanically wounded in the stem, thus, it is probable that repair mechanisms were already active when treatment occurred. MeJA treatment likely increased resource allocation to the expected defense responses, and negatively affected the ongoing healing processes. We were able to observe its initial positive effects since we applied MeJA before wounding. From a plant protection perspective, timing of treatment relative to stem damage/wounding may be key to taking advantage of MeJA's beneficial effects.

Effects of SE

In contrast to the effects of MeJA, the SE process appears to have slowed down the onset of healing but positively affected the overall wound healing rate of Norway spruce plants. We cannot pinpoint the exact mechanisms behind the effects on onset of healing, but we observed a similar phenomenon in one of our earlier experiments. In Berggren et al. (2023), we examined the amount of pine weevil damage received by MeJA-treated and non-treated Norway

Fig. 3 Estimated means (±standard error) of Norway spruce (*Picea abies*) wound healing rate (mm^2 growth of new tissue per day) during **a** year 1 (two weeks post-wounding until September 14, 2022; 70 days), during **b** year 2 (May 19, 2023 to September 19, 2023; 123 days) and **c** years 1–2 (July 6, 2022, to September 19, 2023; 454 days). Plant type consisted of seedlings (grown from seeds) or emblings (produced via somatic embryogenesis) treated or not with the plant hormone methyl jasmonate (MeJA, 10 mM or water applied once in June, 2022). Sample sizes were from left to right: non-treated seedlings $n=40$, non-treated emblings $n=37$, MeJA-treated seedlings $n=40$, MeJA-treated emblings $n=39$. Note that the y-axis does not start at zero

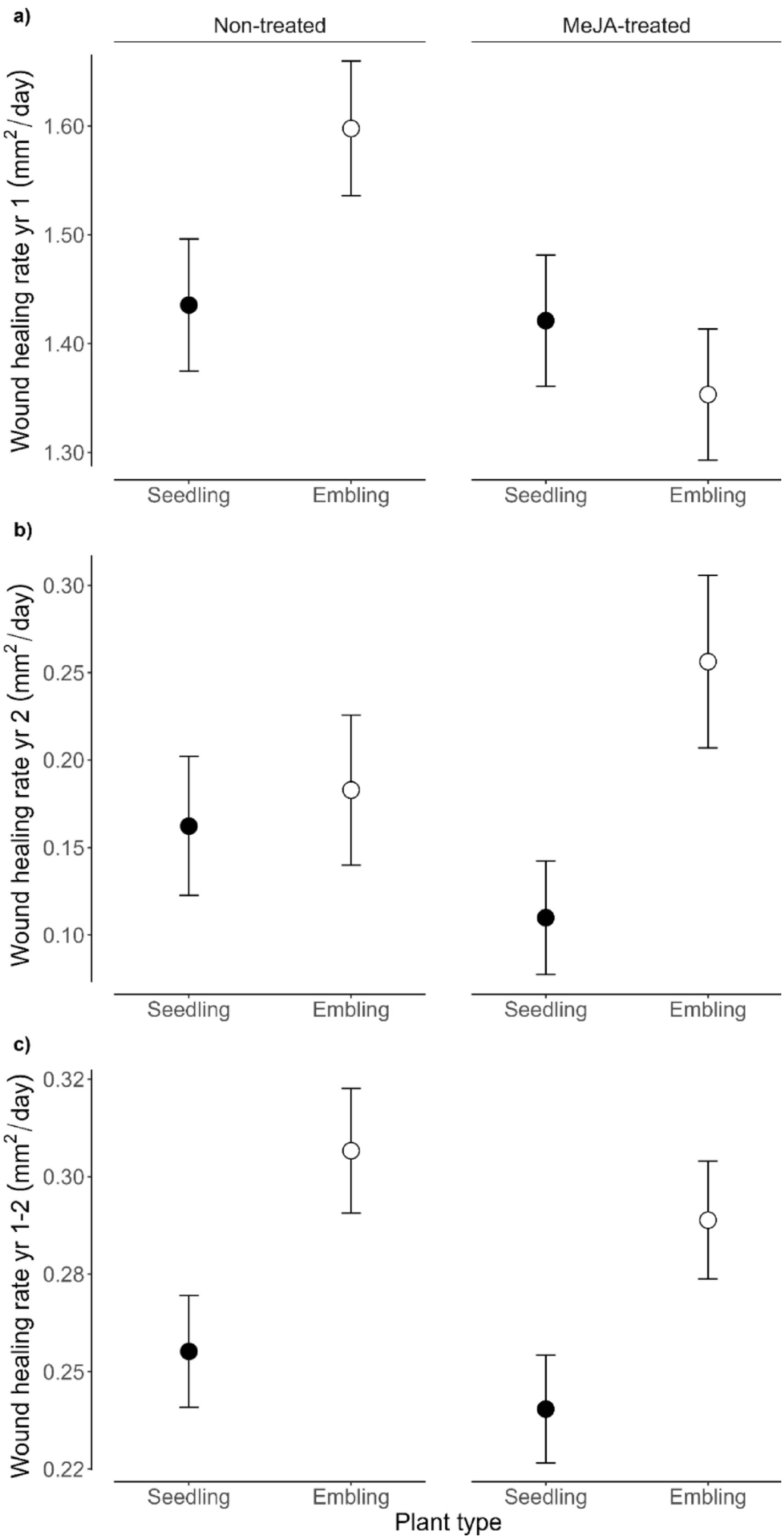

Table 3 Summary of results from a model examining the effects of plant type and MeJA treatment on wound size over time

Fixed effects	Wound size		
	F	df	*P*
Plant type	1.90	1	0.17
MeJA	16.73	1	**<0.0001**
$(\text{Time})^2$	3952.15	2	**<0.0001**
Initial wound size	31.16	1	**<0.0001**
Block	20.66	1	**<0.0001**
Plant type × MeJA	0.04	1	0.84
Plant type × $(\text{Time})^2$	0.31	2	0.73
MeJA × $(\text{Time})^2$	10.10	2	**<0.0001**
Plant type × MeJA × $(\text{Time})^2$	2.47	2	0.08
Random effects			Std. dev.
Plant id			20.08
Residual			12.28

F: F-value; *df*: degrees of freedom; *P*: *p*-value; plant type (seedlings and emblings of Norway spruce); MeJA (methyl jasmonate) treatment (10 mM or water). Wound size over time (mm^2) was analysed from two weeks after wounding until September 14, 2022, 12 weeks post-wounding (year 1). Initial wound size (at the day of wounding) and positional blocks in the greenhouse (plants were placed on two benches) were included as covariates. *Std. dev.*: standard deviation. Significant effects ($P<0.05$) are in bold

spruce emblings in the lab and field. In the field, non-treated emblings received ~30% less damage than non-treated seedlings. In contrast, in the lab, non-treated emblings experienced 51% higher damage than non-treated seedlings. Pine weevils were placed on plants and damage was measured 3 to 4 days later in the lab, while damage was measured 11 weeks later in the field. These results suggest that emblings may be slower at activating defense responses than seedlings, but once induced, they appear to be more effective at decreasing damage than seedlings. The benefits of the SE process may require a longer period to be observed (i.e., 3–4 days in the lab was not enough in Berggren et al. 2023), and indeed in the present experiment, the positive effects of SE on wound healing were observed later in year 1 and became even stronger in year 2.

The faster healing rate and greater frequency of complete wound closure observed in emblings may be associated with the SE process itself. In conifers, the process begins with the excision of the zygotic embryo from the megagametophyte located in a seed; subsequently, the embryo with or without the gametophyte, are placed in a medium containing hormones that enable the induction of SE. In angiosperm plants, stress responses associated with wounding have been documented to occur during SE induction (Rose et al. 2013; Elhiti and Stasolla 2022). For instance, reactive oxygen species (ROS) form rapidly after wounding and act as local and systemic signals (Rose et al. 2013), and genes such as *WOUND INDUCED DEDIFFERENTIATION 1* (*WIND1*) appear to be important for callus formation and cell proliferation during SE (Iwase et al. 2011).

SE may be influencing wound healing abilities in two ways. Firstly, it is possible that wounding-associated responses may have been primed by the process. Priming describes the ability of plants to respond faster and stronger to stimuli that they have previously encountered. Stress that occurs during the SE process may prime plants. For instance, a study in Maritime pine (*P. pinaster*) found that somatic embryos that had been exposed to greater temperatures during the SE process were more tolerant to heat stress as 2-year old emblings, than those emblings that had not been heat-exposed (Pérez-Oliver et al. 2021). In our experiment, once wound repair responses were activated in

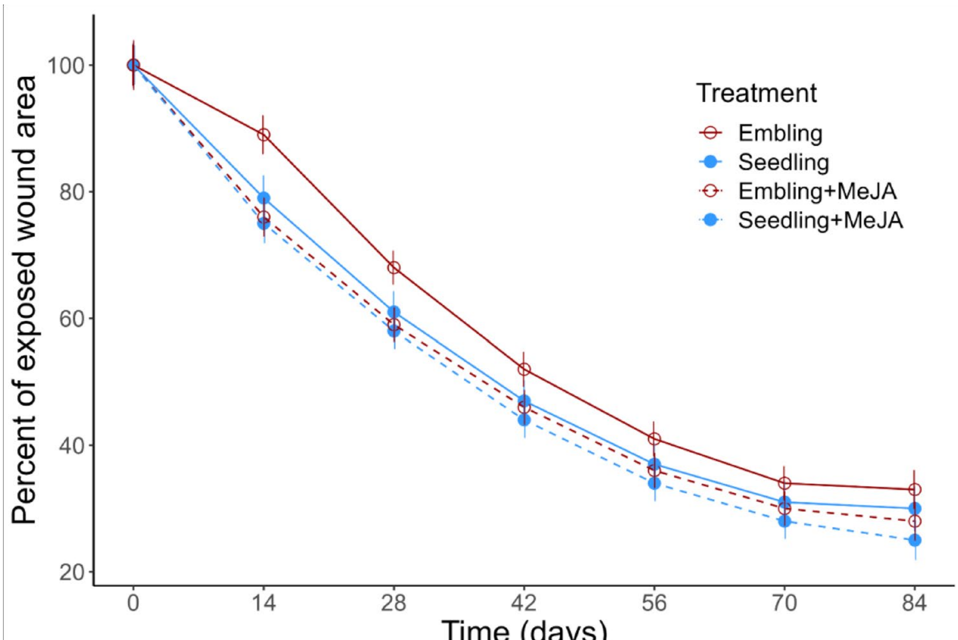

Fig. 4 Percentage of the estimated mean wound area (mm^2) that remained open (i.e., exposed xylem) over time during the first year (day 0 = Wounds were inflicted, day 84 = last wound size measurement year 1; plants were measured every 14 days) for Norway spruce (*Picea abies*) plants. Treatments represent plant types (seedlings– grown from seeds, or emblings– produced via somatic embryogenesis) treated or not with the plant hormone methyl jasmonate (MeJA, 10 mM or water applied once in June, 2022). Sample sizes were from top to bottom: non-treated emblings $n=37$, non-treated seedlings $n=40$, MeJA-treated emblings $n=39$, MeJA-treated seedlings $n=40$. Note that the y-axis does not start at zero

emblings, they appeared to be more efficient at healing than naïve non-primed seedlings. Secondly, not all tissue used in SE survives and becomes embryos; cell lines that do not respond to the process are selected away. Therefore, plants produced through SE may be individuals/genotypes that are inherently better at activating stress and repair mechanisms. These explanations are not necessarily mutually exclusive; SE may prime responses and unintentionally select for individuals that are well-suited to cope with stress. Our study does not allow us to pinpoint the exact reason, but it clearly showed that Norway spruce emblings exhibit superior wound healing and closure abilities relative to regular seedlings. It is important to note that we included a limited number of embling genotypes in our experiment, thus, studies with a greater number of genotypes should be conducted.

Effects of SE and MeJA together

Individually, SE and MeJA had opposing effects on stem wound healing in Norway spruce, which indicates that they can be used together to counteract the negative effects of each treatment alone. In our study, we found that MeJA-treated emblings enjoyed the benefits of treatment on onset of healing (Fig. 2A), suffered a somewhat slower healing rate in year 1 (Fig. 3A), but exhibited the highest healing rate and proportion of plants with complete wound closure in year 2 (Figs. 2B and 3B). From a plant protection and forest regeneration perspective, these effects are advantageous. As a possible plant type to use for re-planting forests, MeJA-treated emblings exhibit a better acute response to wounding/damage and far better in the long run than non-treated emblings, or treated and non-treated seedlings alone. Indeed, in a previous field study, we found that MeJA-treated emblings exhibited the greatest survival across three years and were most resistant to pine weevil damage (Berggren et al. 2023). Therefore, treated emblings do not only receive less insect damage, but are also better at recovering from it. These effects are desirable, as they increase the likelihood of successful forest regeneration and minimize economical costs due to plant losses.

Even though treated emblings had the highest healing rate the second year, it is important to note that regardless of SE and MeJA, the overall healing rate for all plants was lower the second compared to the first year (Fig. 3A vs. Figure 3B). It is possible that wound healing is prioritized early on after damage occurs to minimize the imminent threat of infection or structural damage. Once these threats are reduced, less resources are likely prioritized to healing, and it occurs more slowly without compromising other activities such as plant growth. In contrast to our results, a field study examining rockfall injuries in mature Norway spruce trees found that wound healing was greater the second compared

Table 4 Summary of results from models examining the effects of plant type and MeJA treatment on growth of wounded plants year 1 and 2

Fixed effects	Height						Diameter					
	Year 1			Year 2			Year 1			Year 2		
	χ^2	df	*P*	F	df	*P*	χ^2	df	*P*	F	df	*P*
Plant type	121.41	1	**< 0.0001**	28.14	1	**< 0.0001**	33.71	1	**< 0.0001**	13.17	1	**0.0004**
MeJA	0.19	1	0.67	0.49	1	0.48	0.22	1	0.64	4.75	1	**0.03**
Block	0.53	1	0.47	0.00	1	0.97	0.08	1	0.77	1.78	1	0.18
Plant type × MeJA	0.70	1	0.40	0.02	1	0.88	0.74	1	0.39	0.33	1	0.57

χ^2: Chi-square value; *df*: degrees of freedom; *P*: *p*-value; *F*: F-value; plant type (seedlings and emblings of Norway spruce); MeJA (methyl jasmonate) treatment (10 mM or water). Analyses of height (cm) and diameter (mm) were performed on growth increment measured as the difference between the start height or diameter (measured at the end of May 2022 (on the day before MeJA-treatment) and 2023) and the end height or diameter (measured in mid-September 2022 and 2023 (on the last day of the experiment)). Positional blocks in the greenhouse (plants were placed on two benches) were included as covariates. Significant effects ($P<0.05$) are in bold

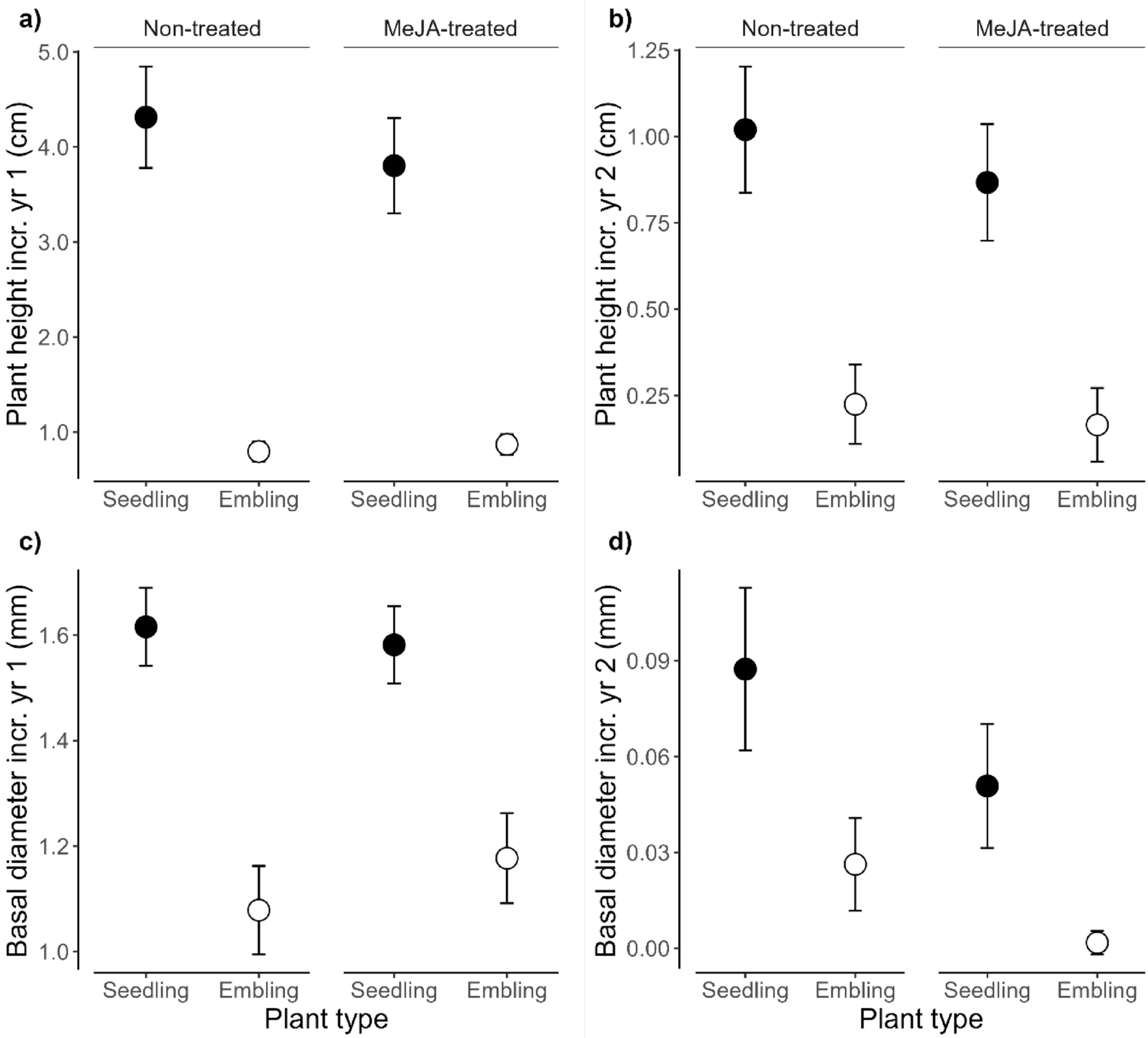

Fig. 5 Estimated means (±standard error) of Norway spruce (*Picea abies*) **a** year 1 and **b** year 2 height increment (cm), and **c** year 1 and **d** year 2 basal diameter increment (mm). Increment was calculated as the difference between the yearly start height or diameter (measured at the end of May 2022 (on the day before MeJA-treatment) and 2023) and the end height or diameter (measured in mid-September 2022 and 2023 (on the last day of the experiment)). Plant types consisted of wounded seedlings (grown from seeds) or emblings (produced via somatic embryogenesis) treated or not with the plant hormone methyl jasmonate (MeJA, 10 mM or water applied once in June, 2022). Sample sizes were from left to right: non-treated seedlings $n=40$, non-treated emblings $n=37$, MeJA-treated seedlings $n=40$, MeJA-treated emblings $n=39$. Note that the y-axes do not start at zero

to the first year post-wounding (Schneuwly-Bollschweiler and Schneuwly 2012). On the other hand, in the field, we have observed that many Norway spruce seedlings can completely close their wounds within the same growing season (K. Berggren, A. Puentes, personal observations). In the present experiment, plants were wounded only once, while in the field they receive multiple wounds from pine weevils throughout the growing season. Continuous wounding may lead to healing processes being more often triggered or activated, and thus a greater prioritization to healing occurs, compared to the effect of one wound in the lab experiment. A study where multiple wounds are inflicted throughout the growing season, after SE and MeJA treatment, would be of interest.

Growth of wounded and MeJA-treated emblings and seedlings

Lastly, we evaluated the effect of MeJA treatment and/or wounding on height and diameter growth of seedlings and emblings. We found that MeJA treatment affected diameter but not height growth (Fig. 5A/B/D and Table 4), and wounding had no effects on growth (Fig. S1A/C vs. S1B/D). On the other hand, emblings grew significantly less in both height and diameter across both years (Fig. 5 and S1A/C, and Table 4). Norway spruce emblings have been shown to grow less than seedlings in our previous two-year field experiment (Puentes et al. 2018). However, studies in other SE-propagated trees such as Interior spruce (*Picea glauca* (Moench) Voss × *Picea engelmannii* Parry), White spruce (*P. glauca*), Sawara cypress (*Chamaecyparis picifera*) and Yellow poplar (*Liriodendron tulipifera*) have not found major differences in growth between plant types across 1–2 growing seasons (Grossnickle and Major 1994; Lamhamedi et al. 2000; Ishii et al. 2001; Kim and Moon 2012). Thus, it appears that the effects of SE on growth may be species dependent. Our previous and present results suggest that the benefits conferred by SE (i.e., greater resistance and healing abilities) could come at the expense of reduced growth for Norway spruce plants. These effects may be transient and differences between emblings and seedlings may decrease with time. It should also be noted that the seedlings in our study originated from a more northern Finnish progeny than the emblings (i.e. their phenology and growth allocation differ), which may have influenced the differences in growth patterns.

Unlike our previous studies (Chen et al. 2023; Puentes et al. 2021), MeJA did not affect plant height but only diameter growth and only during the second experimental year. MeJA treatment has been shown to decrease height and diameter growth in several conifers (Heijari et al. 2005; Gould et al. 2008; Sampedro et al. 2011; Zas et al. 2014), and this is thought to occur due to a diversion of resource to stress/defense responses after treatment. In our experiment, we avoided applying MeJA on the newly-formed top shoots, which could have minimized its detrimental effects on height growth. On the other hand, wounding did not result in any major changes in growth relative to unwounded plants. In previous studies, wounding has been shown to have no effect on growth (Neely 1983; Vasaitis 2012; Schneuwly-Bollschweiler and Schneuwly 2012), while in other studies it seems to depend on the tree species (Vasiliauskas 2001). For Norway spruce seedlings, Chen et al. (2023) found that wounding and MeJA treatment together were more detrimental to basal diameter growth than these treatments alone. However, the authors inflicted two instead of one wound on seedlings. In our experiment, both height and diameter growth followed a pattern that is in line with the phenology of Norway spruce. Early in the season, plants invested more in height growth; height growth eventually slowed down and diameter growth accelerated later on (Fig. S1A/C and S1B/D).

Conclusion

We conclude that SE and MeJA treatment can affect healing of stem wounds in opposite ways. MeJA positively affects onset of healing but slows down healing rate and wound closure in the long run, and vice versa for SE. These individual effects can be taken advantage of in plant protection, as MeJA-treated emblings can rapidly start to repair wounds e.g., after insect feeding or mechanical damage during transport and achieve complete recovery after injury. Hence, the use of this seedling type can increase the likelihood of successful forest regeneration through planting, and reduce economic costs due to damage-related seedling losses.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10342-025-01795-0.

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Author contributions AP and KB conceived and designed the experiments. CB, HB, AT, YC, MT and UE took part in the experimental planning. MT and UE provided the plant material. AT, YC, and AP conducted the experiments and collected the data. KB compiled the data and conducted the statistical analyses. KB and AP wrote the manuscript with input from co-authors. All authors contributed to the manuscript and approved the submitted version.

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Data availability The datasets generated and analyzed in this study will be available from the corresponding author upon request.

Declarations

Competing interest The authors declare no competing interests.

Ethics approval Ethical review and approval was not required for the study, as no such permits are required for studies on insects in accordance with the local legislation and institutional requirements.

Consent for publication The authors agreed to publish the results described in this manuscript.

Consent to participate Not applicable.

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