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RESEARCH

Resin tapping of Atlantic pine forests: towards an optimized use of stimulant pastes over the season

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Abstract

Pine resin is a valuable non-wood forest product with an increasing interest in multiple industrial sectors. Resin-tapping activities also provide valuable ecosystem services in timber-oriented and highly productive pine forest of Atlantic regions, where little previous experience in resin-tapping is available. The objectives of this study were to determine the efficiency of different stimulant pastes and its variation with pine species, site conditions, seasonality and frequency of tapping interventions. We conducted parallel experiments using both conventional and micro-tapping techniques in mature pine stands in NW Spain. We tested four stimulants (control and Zeta, Cunningham and Salicylic pastes) and two groove frequencies (every 2 or 3 weeks). All stimulant pastes significantly increased resin yield compared to the control, being resin stimulation highly consistent across years, sites and species. In conventional resin tapping, resin yield was maximized with the Cunningham and Salicylic pastes while in micro-tapping Salicylic was the most outstanding stimulant treatment. According to the rapid decay of resin flow after wounding, total resin yield decreased with more spaced grooves. However, the reduction was low, and the global efficiency of the tapping operations are likely maximized with grooves applied every three weeks, which would allow increasing the number of tapped trees. Micro-tapping techniques were valuable for screening stimulant pastes and anticipating variation among sites in resin production. Altogether, the Salicylic paste is recommended, especially at the beginning of the resin campaign, when the effect of the pastes was maximized, and if tapping is conducted using closed atmospheres and containers.

Keywords Resin yield · Micro-tapping · *Pinus pinaster* · *Pinus radiata* · Groove frequency · Stimulant paste

Introduction

Pine resin is one of the most important non-timber forest products with multiple uses in a vast array of industrial sectors (Neis et al. 2019b). Exploited by humans since ancient times, resin tapping has been a main activity in many rural areas worldwide (Rodrigues-Correa et al. 2013), and has driven the relationships between locals and pine forests since the prehistory until the emergence of the oil industry and its highly-competitive derivatives in the XX century (Ortuño Perez et al. 2013; Palma et al. 2016b). Nowadays, the current crisis of fossil fuels and the urgent needs for decarbonizing the global economy are relaunching again the pine resin sector as a source of renewable and sustainable bioproducts that may substitute petroleum derivatives in many industrial processes (Rodrigues-Correa et al. 2012).

The interest for resin tapping is not limited, however, to the production of a bioresource, but also to the provision of valuable ecosystem services that the resin tapping activities entail, including (i) the reduction of risk of forest fires (Solino et al. 2018), (ii) the production of intermediate incomes that may alleviate the delays of forest profitability (Susaeta et al. 2014), (iii) the creation of rural employment to fight against the social draining of rural areas (Ortuño Perez et al. 2013), and (iv) the contribution to a healthful silviculture in harmony with rural development (Palma et

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al. 2016a). Indeed, resin tapping is beginning to be seen as an attractive tool to revert the progressive global decay that are suffering many pine forests as a consequence of climate change, rising sanitary risks and fluctuating timber markets (Solino et al. 2018; Touza et al. 2021).

Particularly, the resin tapping sector is progressively recovering in the Iberian Peninsula, where it has been a main activity in the past, mainly on low productivity and harsh environments of the Central Plateau (Rodriguez-Garcia et al. 2016). The current resurgence is even prompting the introduction of resin tapping activities on timber-oriented and highly-productive pine forests of the Atlantic areas (Gómez-García et al. 2022; Zas et al. 2020a). Little experience on resin production is available for this type of pine forests that had no history of resin tapping in Spain. Atlantic forests, however, differ from those traditionally tapped in a number of climatic, environmental and silvicultural factors that may be relevant for resin production and specifically for fine-tuning the exploitation protocols for resin tapping (Zas et al. 2020a).

In the Iberian Peninsula, resin tapping is traditionally performed applying periodical stripped wounds (grooves) followed by a strip of stimulant paste in the upper-inside border of the wound (Rodriguez-Garcia et al. 2016). Every two weeks from ca. May to October, grooves are renewed moving upwards. The resin flowing from the xylem and phloem exposed tissues flows out and is collected in pots sited at the bottom of the first groove. Up to date the stimulant paste more frequently used in the Iberian Peninsula is the "white paste", also called ZETA, a paste composed by sulfuric acid thicken with gypsum (79% sulfuric acid 45% v/v, 21% plaster) (Zamorano 1983). By digesting adjacent tissues, the stimulant paste magnifies the physical extent of the wounds, contributing also to delay resin crystallization allowing resin flow to keep active for longer periods (Rodrigues-Corrêa and Fett-Neto 2012).

Recent research on plant defenses have make huge progress in the understanding of how plants are able to perceive biotic aggressions and react to them eliciting a wide battery of physical and chemical responses that enhance the defensive machinery of the plant and prevent the progression of the aggressor (see review for trees in Eyles et al. 2010). The activation of these induced defenses is known to be mediated by different plant phytohormones such as salicylic acid, jasmonic acid and ethylene that act as signals of the damage and promote the physiological rearrangements in surrounding and distal tissues (Jones and Dangl 2006). Pines also respond to traumatisms increasing resin production (their main defensive mechanism against biotic aggressions) in preexisting resin ducts and differentiating new resin canals, named traumatic resin ducts (Vázquez-González et al. 2020). The exogenous application of these hormones to pine trees also triggers induced responses similar to those that occur after physical of biotic damage (Moreira et al. 2012), and this has been the foundation for the formulation of new stimulant pastes aimed to activate the defensive machinery of the pines trees, which translates to the production of more resin (Fuller et al. 2016; Rodrigues-Corrêa and Fett-Neto 2012). A number of patents of new formulations for resin tapping stimulation have emerged in recent years, some of which are starting to be used in commercial resin tapping in different countries. Among them, the "Cunningham" paste (based on sulfuric acid and ethephon or CEPA (2-chloroethylphosphonic acid), an ethylene-releasing agent widely used in subtropical *Pinus elliotii* forests), and the Asacif paste (based on sulfuric acid and salicylic acid), specifically developed for tapping southern coastal European pine forests, have shown promising results (Michavila et al. 2021) and are timidly bursting in the Iberian resin sector (Gómez-García et al. 2022). However, little is still known about whether the relative efficiency of these alternative formulations may vary depending on the pine species, the environmental conditions (Neis et al. 2018) and the moment of application within the season (but see Lukmandaru et al. 2021).

Besides selecting the best stimulant, optimizing exploitation requires also maximizing the production in relation to the labor costs (Justes and Solino 2018). Profitability of resin tapping is quite limited and depends mainly on the resin yield of individual trees and the effectiveness of resin tapping operations, which is directly related to the frequency of interventions on each tree (Touza et al. 2021). Groove frequency largely varies across countries, and this variation is probably related to both environmental and silvicultural factors, but also to manpower costs. In Spain, conventional resin tapping is performed through biweekly interventions (Rodriguez-Garcia et al. 2016), but to our knowledge, this periodicity has little experimental support, and may be questioned (Touza et al. 2021; Zas et al. 2020a). As labor costs are a major critical point in the Spanish resin sector, optimizing the frequency of interventions emerge as a key point for optimizing management plans. Whether this frequency should be adjusted to each pine species, the environmental conditions and the stimulant paste used are questions that remain to be answered.

Testing pastes and resin management protocols are hard tasks that require large experimental efforts through long periods (Junkes et al. 2019). Developing alternative experimental protocols for accelerating and minimizing the costs of evaluations is, thus, a desired goal for experimental resinosis. Recent research has demonstrated that micro-tapping (i.e. assessment of resin flow from small wounds (ca. 1–2 cm^2) in a short period of time (ca. 24 h or a few days) is a valuable technique for predicting resin yield of individual trees or stands (Neis et al. 2019a; Zas et al. 2020a). Similar protocols for fast assessment of resin production have shown promising results for prospecting new stimulant pastes (Junkes et al. 2019), although the correspondence of the relative efficiency of different stimulants between micro-tapping and conventional protocols remains to be tested. As resin flow from micro-tapping is collected in closed cup vials while open pots are used in conventional tapping, evaporation, oxidation and pollution in the later may distort the relationship among both techniques (Zas et al. 2020a). Distortion may also occur due to different crystallization rates as a consequence of different wound sizes and oxidative environments (Cabaret et al. 2019).

Aiming to adjust resin tapping operations to the particular conditions of the Atlantic pine forest of Northwest Spain, in the present study we experimentally tested (i) the efficiency of different stimulant pastes for enhancing resin production, (ii) whether this efficiency varies depending on the tapped pine species, the site conditions and the time of the year within the season, (iii) the effect of different frequencies of tapping interventions for each of the tested stimulant pastes, and (iv) whether fast micro-tapping techniques may anticipate the results obtained through long-term conventional experimentation. To this end, we conducted parallel experiments across two consecutive campaigns through both conventional and micro-tapping techniques in six mature pine stands scattered in the region of Galicia (NW Spain). The experiments included 4 plots of *Pinus pinaster* and 2 plots of *P. radiata*, the two main pine species dominating the forested area in the region. We hypothesized that (i) the best stimulant past could differ depending on the species, the environment and the time of the year, (ii) the optimal frequency of tapping operations may varied depending on the stimulant paste and the time of the year and (iii) micro-tapping techniques will anticipate results obtained through conventional tapping.

Materials and methods

Experimental design

The effect of different stimulant pastes and groove frequencies were tested in six mature stands of either Maritime pine (*Pinus pinaster* Ait.) or Monterrey pine (*P. radiata* D. Don) sited in Galicia (NW Spain) (Fig. 1a, Online Resource 1). Experiments sited in Baroña (BARO) and Pantón (PANT) included each two experimental sites, one of each species, while those in Culleredo (CULL) and Mondoñedo (MOND) only included *P. pinaster*. In all sites, two parallel experiments were carried out, one using the conventional resin tapping method typically used in Spain (Rodriguez-Garcia et al. 2016) and the other using the micro-tapping techniques described in Zas et al. (2020a). In CULL, MOND and PANT sites, the experiments were conducted in two successive resin campaigns on the same experimental trees (2020 and 2021), while the experiments in BARO were only done in 2021. In all cases, experiments followed a randomized complete block design with three blocks. Healthy and previously untapped trees were randomly assigned to the treatments within blocks. All experimental trees were measured at the beginning of the experiment (March 2020 or 2021) for total height and breast height diameter.

All locations used in this study were situated in NW Spain that according to Köppen-Geiger climate classification has a temperate humid climate type *Csb* (Beck et al. 2018). Mean annual temperature ranged from 10 to 14 °C and annual precipitation varied from 800 to 1400 mm in inland areas (MOND and PANT) and from 1200 to 2000 mm in coastal areas (BARO and CULL) (Online Resource 1). The MOND site was the colder site while the PANT site had the driest summers and the highest continentality. Predominant soil parent materials in the studied stands are the igneous rock granite, although metamorphic rocks (quartzite and schist) also appear (Online Resource 1). Soil temperature ranges from 8 to 15 ºC (mesic) except for the *P. pinaster* stand in Baroña that fluctuates from 15 to 22 ºC (thermic). Soil moisture regime is udic (common in humid climates, where the stored moisture plus rainfall is equal to, or surpasses, the evapotranspiration in summer) in all location except in PANT, which is xeric (stronger Mediterranean influence, showing warmer and drier summers).

Conventional resin tapping

In both campaigns, conventional tapping was carried out performing periodical 12-cm wide and 2–3 cm high stripped wounds ("grooves") every two or three weeks moving upwards. A strip of stimulant paste was applied in the upper-inside border of each wound, and resin flowing from the wounds collected in 2 L plastic open pots sited at the bottom of the first groove (Online Resource 2a).

In the 2020 campaign (only in sites CULL, MOND and PANT), four stimulant treatments were tested: (i) ZET, the traditional "white" or "Zeta" paste (79% sulfuric acid 45% v/v, 21% plaster) commonly used in central Spain, (ii) CUN, the Brazilian, "Ethephon" or "Cunningham" paste (14% sulfuric acid 50% v/v, 8% ethephon 60% v/v, 55% distilled water, 1.7% polysorbate, 1% cetyl alcohol, 4% vaseline, 5.5% silica, 10.8% sawdust) commonly used in subtropical pine forests, (iii) SAL, the salicylic or "Asacif®" paste (25% sulfuric acid 96% v/v, 1% salicylic acid, 50% distilled water, 5% propylene glycol, 19% wheat straw), that is starting to be used in Atlantic pine forests in NW Spain, and

(iv) CTR, a control treatment without stimulant paste. The CTR, SAL and ZET treatments were applied using biweekly grooves while the CUN treatment was applied every three weeks. The first groove was done at the beginning of May and the last at mid-October. Thirteen grooves were done in the case of CTR, SAL and ZET treatments, while 9 grooves were done in the case of the CUN treatment. Each block included 35 trees, with each paste applied to 10 randomly selected trees per block, and 5 trees received the CTR treatment.

In the 2021 campaign, the three previously described pastes (CUN, SAL and ZET) were factorially combined with two alternative frequency applications (every 2 or 3 weeks). For this purpose, the 10 trees per block assigned to each stimulant paste in the preceding campaign were split into two groups of 5 trees, each tapped following one of the two tested frequencies. The CTR treatment was only tapped following biweekly grooves. In the sites also tapped in the 2020 campaign, twenty new previously untapped trees (5 per treatment, all tapped following biweekly grooves) were included in each site to test the effects of the previous tapping campaign. In addition, two new experimental sites (*P. pinaster* and *P. radiata* in Baroña) were added to the series. These sites followed the same design except that all trees in the experiment were never tapped before. In all sites, grooves started at early-May and ended at the mid-October, with 13 and 9 grooves applied every 2 and 3 weeks, respectively.

In both campaigns and for all treatments, every pot was weighted (0.1 g accuracy) before each periodical groove. Grooves carried out every 2 and 3 weeks coincided in time in four moments (mid-Jun, late-July, mid-September and late-October) within each season. Accumulated resin production within these periods was calculated for analyses of resin production dynamics along the tapping season. Total resin production across each campaign, which also included the resin production of a further "blank groove" in all treatments applied at the end of each campaign and the collection of all the resin attached to the trunk that has not flowed to the pot, was also determined.

Micro-tapping

In September of both years, coinciding with the lasts grooves of conventional-tapping, parallel experiments testing for the effects of the stimulant pastes were conducted using micro-tapping techniques. These experiments were conducted on different trees sited close to the conventional-tapping experiments. In each site (4 sites in 2020 and 6 sites in 2021) 48 trees were selected and measured as described before. Three environmentally homogeneous blocks of 16 trees were differentiated and trees within blocks were randomly assigned to one of the four stimulant treatments described before (CTR, CUN, SAL and ZET, with 4 trees per treatment and block). Micro-tapping was performed following Zas et al. (2020a). Briefly, after demarking the bark in a small window of 10 × 10 cm at 50 cm above the ground, a 1.5-cm diameter disk of the remaining bark, phloem and cambium was retired with the aid of an arch punch and a hammer, avoiding damage to the xylem. A bead of the corresponding stimulant paste (~ 0.5 g) was then applied to the inner circumference of the wound. Holes were then capped with specifically-designed plastic devices, on which pre-weighted 50-ml Falcon® plastic vials were screwed (Online Resource 2b). In order to explore the dynamics of resin flow after wounding, vials were replaced periodically. Resin flowing from the wounds was determined gravimetrically (0.01 g accuracy) at days 1, 2, 4, 8, 16, 24 and 30 after wounding in the 2020 campaign, and at days 1, 2, 4, 11 and 24 in the 2021 campaign. The rate of resin flow (g of resin per day) for each period was then determined and used as the dependent variable for temporal dynamic analyses. Accumulated resin flow at the last assessment day in each campaign was also determined.

Statistical analyses

Total resin production in each campaign (both through conventional tapping and micro-tapping) was analyzed by fitting mixed models with treatments (i.e. the combination of the stimulant paste and the grove frequency), test sites (the location combined with the species), and their interaction as fixed factors, and blocks within sites as random factors. Individual tree diameter was also included in the models as a fixed covariate. As large differences in residual variance were observed between the control and the three stimulation treatments, heterogeneous residual variances between these two groups of treatments were allowed. Akaike information criteria (AIC) confirmed the superiority fit of this type of heterogeneous variance models compared to other structure aggrupations. The dependent variables were square-root transformed to achieve residual normality.

For those sites with representation of the two pine species (PANT and BARO), in order to test for differences between species and for variable effects of the treatments depending on the species, a similar mixed model was used considering the pine species, the treatments and their interaction as the fixed factors. These models were fitted separately for each location.

Effect of previous tapping on resin yield of the 2021 campaign was analyzed in those sites tapped in the two campaigns (CULL, MOND and PANT), using only trees tapped every 2 weeks (common frequency for both previously untapped and tapped trees). A similar mixed model to those described before but including now the factor *tapped* (two levels: previously untapped or tapped), the treatment, the site, and all the corresponding interactions was fitted.

Temporal resin production along the conventional resin tapping campaign and along time after wounding in micro-tapping was analyzed fitting repeated measures mixed models in which the treatment, the site, their interaction and the covariation with tree diameter acted as across subject factors, and the time factor (either the common periods for grooves applied every 2 and 3 weeks in conventional tapping or the days after wounding in micro-tapping) and all its interaction with the main factors acted as repeated correlated measures within each subject (i.e. within each individual tree). The covariance structure for the repeated measures was selected based on Akaike information criteria, which was interpreted in the smaller-is-better form. In all cases a first autoregressive structure was selected. Again, heterogeneous residual variances (control vs. the three pastes) were allowed, and the dependent variable was square root transformed before analyses.

In all fitted models, least square means of fixed effects were obtained and compared through Tukey's multiple comparison tests. Relationships between campaigns were explored through linear regression analyses on the least square means for total resin at the treatment by site interaction level. Relationships between conventional tapping and micro-tapping was assessed in a similar way, but in this occasion we explored all possible combinations between the periods within the conventional resin campaign and the days after wounding in micro-tapping. These regressions were carried out using the least square means estimated at the site x time, the treatment x time and the site x treatment x time levels within the repeated mixed models described before.

For micro-tapping analyses, and in order to explore when differences among treatments and sites are maximized, simple-effects tests partitioning the time by treatment and time by site interactions for each day after wounding were performed. The larger the F-ratio of these tests the greater the differences between treatments and sites, respectively.

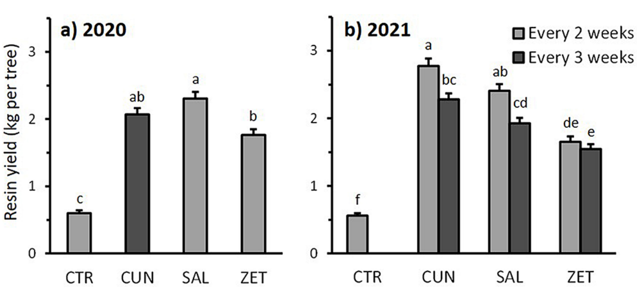


Fig. 1 Total resin yield (kg per tree) through conventional resin tapping in the **a** 2020 and **b** 2021 campaigns as a function of the stimulant treatment used (CTR: Control, CUN: Cunningham, SAL: Asacif and ZET: Zeta) and the frequency between consecutive grooves (every two and three weeks). Note that different treatments were tested in each campaign. Least square means ± SE are shown. Different letters within each campaign

Results

Conventional resin tapping

Average resin yield per tree varied between 1.2 and 2.3 kg depending on the campaign and the test site. Treatments had a large effect on total resin yield in both campaigns, but the effect was contingent on the test site (Online Resource 3). Overall, all stimulant pastes significantly increased resin yield in relation to the control treatment irrespective of the groove frequency (Fig. 1).

Resin yield was maximized with the CUN and SAL treatments (with no significant differences between them) and under biweekly grooves (Fig. 1). Resin yield also varied depending on the test site (Online Resource 3), with resin yield being higher in PANT and CULL, intermediate in BARO and lower in MOND in both campaigns (Online Resource 4a). Despite site also modulated the effect of treatments, variation among treatments were fairly consistent across sites and species (Online Resource 5). Thus, the site by treatment interaction appeared to be more a question of scale rather than to relevant changes between the relative performances of the treatments across sites. Separated analyses of only those sites where the two species were assessed, revealed little overall differences in resin yield between species (1.7 and 1.6 kg per tree in average in the 2020 campaign and 1.9 and 1.7 kg per tree in the 2021 campaign in *P. pinaster* and *P. radiata*, respectively), a significant treatment by species interaction ($F_{3, 180} = 3.4$, $p = 0.020$ and $F_{6, 370} = 3.2$, $p = 0.004$ for the 2020 and 2021 campaigns, respectively) and no significant site by species interaction ($p > 0.05$). Despite the significant treatment by species interaction, the relative performance of the treatments was fairly consistent across the two species (Online Resource 5), suggesting that the interaction was again more a question of scale.

Treatment and site effects were highly consistent between the two consecutive tapping campaigns, as revealed by a close relationship between least square means at the site by treatment interaction level (Fig. 2a). Resin yield was, in general, higher in the 2021 than in the 2020 campaign (Fig. 2a). This result seemed to be due to the responses to previous tapping as resin yield in the 2021 campaign of previously untapped trees was significantly lower than those that had been tapped before (Online Resource 4b). The effect of previous tapping was similar across sites ($F_{3,232}$=1.5, $p = 0.205$) and across treatments ($F_{3,232}$=1.2, $p = 0.323$).

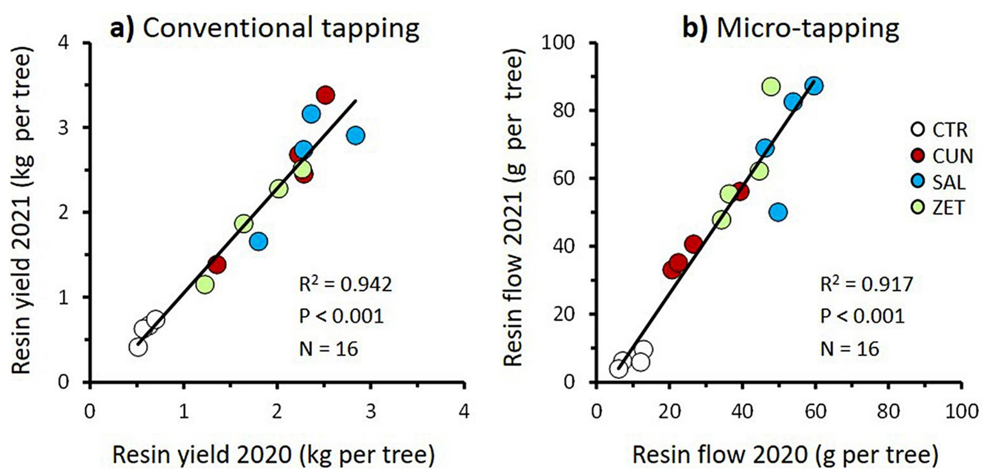


Fig. 2 Relationships between resin production in the 2020 and the 2021 campaigns using **a** conventional resin tapping and **b** micro-tapping procedures. Each point represents the least square mean (as derived from the corresponding mixed models) for each of the four stimulant treatments (CTR, CUN, SAL, ZET) in each of the four test sites (those with two assessed resin tapping campaigns: CULL, MOND, PANT with *P. pinaster* and PANT with *P. radiata*). Coefficients of determination (R^2), associated probability values (P) and sample sizes of the regression analyses (N) are shown

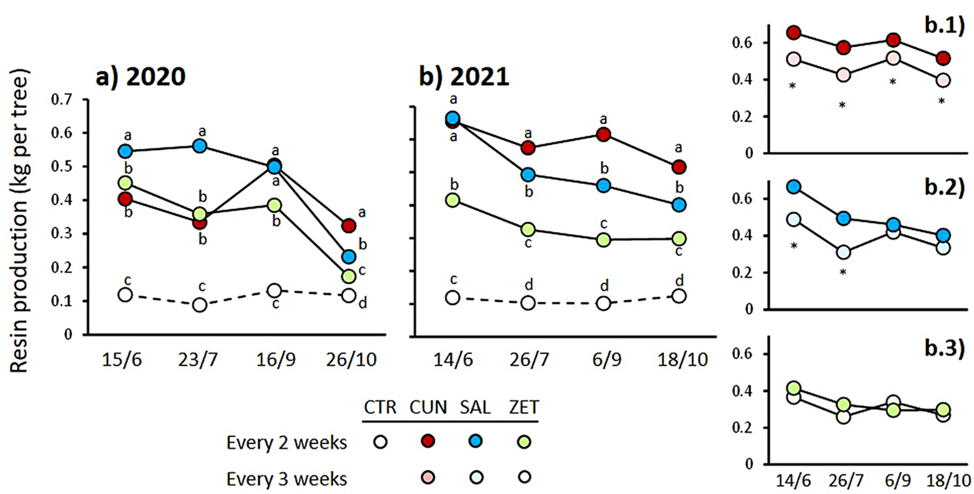


Fig. 3 Resin production (in kg per tree) along the tapping season for the **a** 2020 and **b** 2021 campaigns using conventional resin tapping procedures. In **a** and **b**, data from biweekly grooves using the four stimulant treatments (CTR, CUN, SAL and ZET) are shown. Measurements were done approximately every 1.5 months. Different letters within each assessment date denote significant ($P < 0.05$) differences among treatments. For the 2021 campaign, panels **b1**, **b2** and **b3** show the differences in temporal resin production between grooves applied every 2 and 3 weeks for each stimulant paste (**b1** CUN, **b2** SAL, **b3** ZET). For each assessment date, significant differences ($P < 0.05$) between the two tested frequencies are indicated with an asterisk. Least square means as derived from the repeated measures mixed models are shown

Resin production was highly variable along the season (significant Time effect in Online Resource 6), with a general trend of decreasing resin production as the tapping season progressed in all treatments except the control (Fig. 3). Importantly, the time by treatment interaction (Online Resource 6) indicated that the relative effects of the different treatments varied depending on the moment of the season. In both campaigns, the relative performance of the SAL stimulant paste was higher at the beginning of the season, while that of the CUN paste tended to increase at the end of the tapping period (Fig. 3b). In the case of the CUN paste, resin production through biweekly grooves was always higher than that produced through grooves applied every 3 weeks (Fig. 3b1). However, these differences were only significant in the first half of the tapping period for the SAL paste (Fig. 3b2), while no significant differences between the two frequencies were observed in the case of the ZET paste (Fig. 3b3).

Micro-tapping

Despite the small size of the practiced wounds, the resin flow collected at the end of the experimental micro-tapping period (30 and 24 days in 2020 and 2021, respectively)

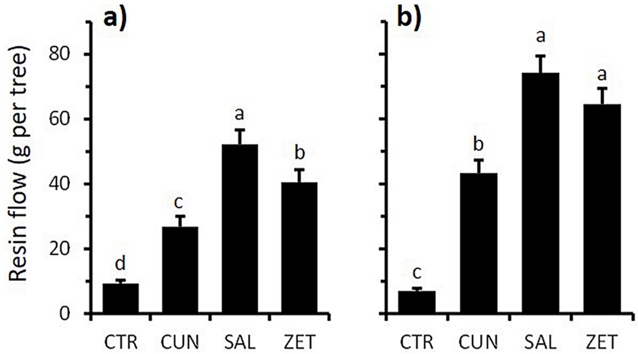


Fig. 4 Resin flow through micro-tapping (in g per tree) in the **a** 2020 and **b** 2021 campaigns as a function of the stimulant treatment used (CTR: Control, CUN: Cunningham, SAL: Asacif and ZET: Zeta). Least square means ± SE of accumulated resin flow 24 and 30 days after the initial wound are shown for the 2020 and 2021 campaigns, respectively. Different letters within each campaign denote significant differences ($P < 0.05$)

was considerable, averaging between 25.4 and 37.5 g per tree depending on the site. Total resin production estimated by micro-tapping techniques was highly influenced by the stimulant paste used, and the responses to the different treatments were highly consistent across sites (see non-significant Site x Treatment effects in Online Resource 7). Overall, all stimulant pastes increased resin flow in relation to the control treatment, with resin flow being maximized with the SAL stimulant paste, followed by the ZET and the CUN treatments (Fig. 4). Again, the results were highly consistent across the two resin campaigns (Fig. 2b).

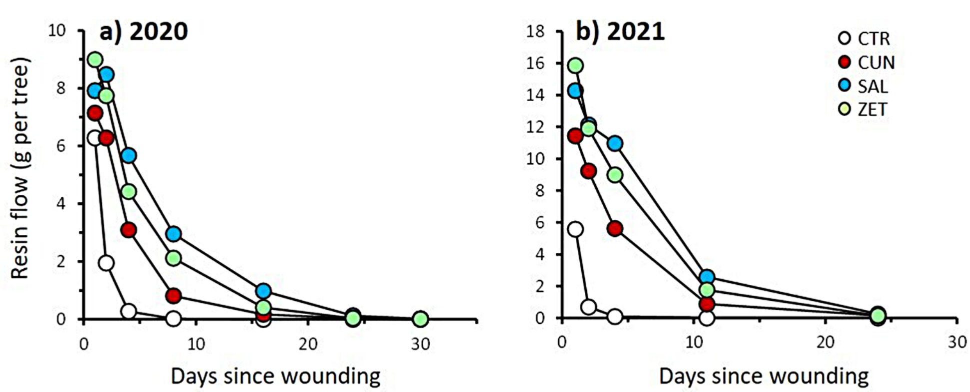
Repeated measures analyses indicated that the rate of resin flow rapidly decreased with time since wounding (Online Resource 8, Fig. 5). This exponential decay was, however, contingent on the treatment and the test site (see significant interactions in Online Resource 8). In comparison with the control treatment, all stimulant pastes prolonged the time during which resin flow remains active, but the SAL treatment maintained higher resin flow rates throughout longer periods than the other treatments (Fig. 5). The rhythm of decay of resin flow rate also varied across sites (Online Resource 8), and this effect was reflected in the amplitude of the differences in resin flow among sites, which was highly variable depending on the time since wounding. Judging from the F-ratios of the test of simple effects for partitioning the time by site interaction, differences in resin flow between sites were maximized at day one after wounding, decreasing thereafter (Online Resource 9c,d). On the contrary, the magnitude of the treatment effect showed a marked peak at day four after wounding (Online Resource 9a,b), suggesting that more time is required for maximizing the differences between treatments.

Relationships between conventional tapping and micro-tapping

The results on the effects of the different treatments and the variation among sites obtained through conventional tapping and through micro-tapping were positively related, but the strength of the relationships were, in general, low (Fig. 6). Specifically, the coefficients of determination of the relationships between conventional tapping and micro-tapping were highly variable depending on the level at which the relationship was explored (sites, treatments or site-treatment effects) and both the period within the season considered in conventional tapping and the time since wounding considered in micro-tapping. In general, all relationships improved at increasing time since wounding in micro-tapping (Fig. 6, left tables). The relationships at the site level (i.e. the consistence between site variation) were maximized for the relationships with the last period of conventional tapping (October, $R^2 = 0.648$), which coincided with the time when micro-tapping was performed (Fig. 6a). On the contrary, the relationships at the treatment level were maximized for the relationships with resin production at the beginning of the conventional resin tapping period ($R^2 = 0.691$, Fig. 6b). An intermediate situation was observed at the site by treatment level ($R^2 = 0.473$, Fig. 6c).

Relationships between resin flow through micro-tapping and total resin yield across the whole resin campaign were significant at the treatment and site by treatment levels, with close to half of the variation in total resin yield explained

Fig. 5 Resin flow (in g per tree) at different days since wounding in the **a** 2020 and **b** 2021 micro-tapping campaigns as a function of the stimulant treatment used (CTR: Control, CUN: Cunningham, SAL: Asacif and ZET: Zeta). Least square means derived from the repeated measures mixed models are shown



by the micro-tapping resin flow estimated at day 24 after wounding (Fig. 6).

Discussion

Stimulant pastes

The results from both the conventional-tapping and the micro-tapping experiments clearly demonstrate a huge effect of the stimulant pastes increasing resin production of both maritime pine and radiata pine adult trees in comparison with control trees tapped with no stimulant paste (up to 10.4 fold change). The role of the active principles of the three stimulant pastes used here (namely sulfuric acid, salicylic acid and ethylene) in pine resinosis has been demonstrated before in different pine species and has been related to (i) the physical magnification of the mechanical wound, (ii) the induction of reactive oxygen species, (iii) the elicitation of resin production as part of the induced defensive responses of pine trees to external stimuli, and/or (iv) the reduction of the crystallization rates allowing resin to flow for longer periods (Junkes et al. 2019; Lukmandaru et al. 2021; Michavila et al. 2021).

In conventional tapping, the CUN and SAL stimulant pastes performed better than the traditional ZET paste, with this result being fairly consistent across campaigns, sites and species. The three studied stimulants include sulfuric acid in their formulation but the CUN and SAL pastes reduce its concentration and add a second active principle (ethylene and salicylic acid, respectively). Combining different principles may favor triggering multiple pathways of resin induction (Lukmandaru et al. 2021; Moungsrimuangdee et al. 2022; but see Rodrigues-Corrêa and Fett-Neto 2012; Rodrigues et al. 2008), while reducing the amount of sulfuric acid is highly desirable to contain environmental and human-health hazards (Michavila et al. 2021), and to reduce the impact of tapping on wood properties (Missio et al. 2017). Based on these considerations, the CUN and SAL stimulant pastes should be preferred in these Atlantic timber-oriented pine forests.

The relative superiority of the different pastes differed, however, when assayed through conventional tapping or micro-tapping. CUN and SAL were superior in

a) Relationship at the Site level

Conventional tapping period	Days since microtapping 1	2	4	24
Whole Campaign	0.02	0.06	0.12	0.23
Mid Jun	0.02	0.01	0.00	0.05
End of July	0.02	0.05	0.10	0.20
Mid September	0.08	0.14	0.19	0.26
End of October	0.28	0.35	**0.50**	**0.65**

a) Relationship at the Treatment level

Conventional tapping period	Days since microtapping 1	2	4	24
Whole Campaign	0.27	0.45	0.47	**0.52**
Mid Jun	0.40	**0.59**	**0.63**	**0.69**
End of July	0.24	0.43	0.46	**0.56**
Mid September	0.12	0.27	0.28	0.33
End of October	0.39	0.46	0.45	0.36

a) Relationship at the Site x Treatment level

Conventional tapping period	Days since microtapping 1	2	4	24
Whole Campaign	**0.11**	**0.23**	**0.33**	**0.44**
Mid Jun	0.07	**0.17**	**0.30**	**0.46**
End of July	**0.13**	**0.26**	**0.35**	**0.47**
Mid September	0.09	**0.20**	**0.26**	**0.31**
End of October	**0.12**	**0.21**	**0.29**	**0.30**

Coefficient of determination (R^2)

0.0 0.7

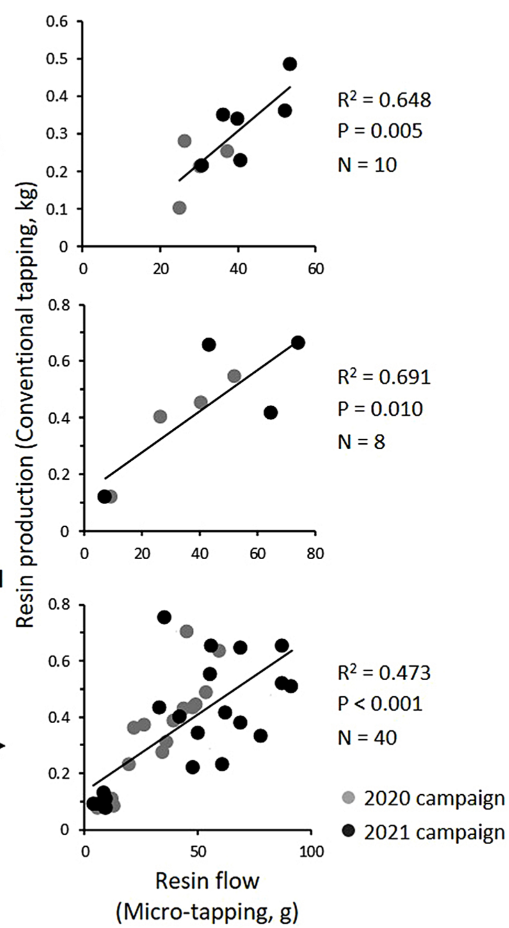


Fig. 6 Relationships between resin flow assessed by micro-tapping and resin production through conventional resin tapping at **a** the Site level ($N=10$, 6 sites assessed in 2021 and 4 sites assessed in 2020), **b** the Treatment (four stimulants assessed in two consecutive campaigns, $N=8$) and **c** the Site x Treatment interaction (a total of 40 combinations across the two resin campaigns). Tables in the left panels show the coefficient of determination (R^2) as a function of the days since wounding in micro-tapping (columns: resin flow in 1, 2, 4 and 24 days since wounding) and the period of conventional resin tapping assessment (4 moments along the season (approximately every 1.5 month, plus the total resin production through the whole campaign). Scatter plots in the right show the relationship between micro-tapping and conventional tapping for selected cases of study. Dots in these figures are least square means as derived from the corresponding mixed models. The coefficient of determination (R^2), associated p values and sample size (N) are shown. Only biweekly grooves were considered for conventional tapping

conventional tapping, with CUN tending to surpass SAL, while in micro-tapping SAL always produced the highest yields and CUN the lowest. A number of explanations may be behind this apparent incongruence. (1) Experimentation through conventional tapping may be strongly affected by different operational problems such as pot losses, water contamination, resin overflow, or accumulation of impurities. However, the high correspondence between the two conventional campaigns and the reasonably repeatability among sites rules out this hypothesis. (2) Environmental or dendrometric differences between experiments could have affected the results since conventional or micro-tapping experiments were independent and included different trees. However, within each site, the two experiments were adjacent (same environment) and no significant differences in tree diameter and height were observed (data not shown). (3) The effect of the stimulant pastes might differ when applied on open or closed wounds as different oxidative atmospheres may likely alter the rate of crystallization and sealing of the wounds as well as the volatilization of the active principles of the stimulants. Particularly, the CUN paste is composed by Ethephon that release ethylene, a gaseous phytohormone with a well-known role in the stimulation of resin production (Junkes et al. 2019), but that, at high concentrations, may be ineffective or even detrimental (Wolter and Zinkel 1984). It is possible, thus, that undesirable effects of this compound could be favored in closed wound conditions, where higher ethylene concentrations may accumulate. (4) The size of the wound may also interfere as may determine the obturation rate and thus the facility of resin to flow out (Rodrigues et al. 2008). (5) In conventional tapping, at least part of the volatile fraction may have evaporated from the open containers while in micro-tapping, both fractions are likely retained (Cabaret et al. 2018). Whenever the stimulants differently affect the two fractions (Liu et al. 2022), discrepancies in the relative performance of different stimulants can occur between the two assessment techniques.

Taking altogether, differences in the relative performance of the different stimulant pastes between conventional tapping and micro-tapping seem to be related in some way to whether open or closed atmospheres and recipients are used. Following this argument, which should nevertheless be formally tested in further studies, the SAL stimulant paste should be preferred if tapping is performed in closed containers. In addition, it is important to note that Ethephon or CEPA is the most expensive component in the CUN paste, and is highly problematic for resin workers' health and for the environment (Neis et al. 2018). This has prompted an important research effort to develop other lower-cost and environmentally-safe stimulants with similar or enhanced performance (Rodrigues-Corrêa and Fett-Neto 2012). More specifically, our results showing that the salicylic acid-based paste consistently increased resin yield in adult maritime pine trees are in agreement with other studies in slash pine (Neis et al. 2018; Rodrigues-Correa et al. 2011, 2013) and maritime pine (Michavila et al. 2021). The use of the SAL paste is thus recommended to optimize the resin production in the Atlantic pine forests, although there is likely room to fine-tune its formulation.

Site and species variation

Although theoretically all pine species are susceptible to be used for resin extraction, the quality and quantity of the resin they produce may largely differ (Rubini et al. 2022). According to Rodrigues-Corrêa and Fett-Neto (2012), the resin of *P. radiata* is among the ones with superior quality for the chemical industry (high concentration of pinenes), but rates of production are low. In our study, resin production of *P. radiata* was comparable to that of *P. pinaster*. Then this species can be an interesting alternative for resin production in Atlantic conditions of NW Spain, where it occupies vast extensions. Nevertheless, other operational costs must be taken into account. For example, *P. radiata* trees have a thicker and harder outer bark than *P. pinaster*, and this may increase the labor costs, as bark must be manually removed before the beginning of the tapping season.

Within each species, variation among testing sites was also important. In Maritime pine, resin production was maximized in PANT and CULL, two contrasting environments, and was minimal in MOND, the coldest and wettest site. This pattern partially agrees with previous studies positively relating resin production with temperature and water deficit (Rodríguez-García et al. 2015; Zas et al. 2020b). However, with our limited number of sites, no clear geographical or environmental gradient can be depicted.

Despite the large variation between sites and species, it is important to note that the effect of the different stimulant pastes was fairly consistent, suggesting that no adjustments of the recommended tapping protocols seem to be needed.

Timing of operations

Overall, biweekly resin-tapping was most productive than tapping every 3 weeks, with differences being more evident for those stimulant pastes and periods with larger effects on resin production. This result is consistent with the exponential decrease of resin flow obtained in the micro-tapping experiments, where more than 95% of the accumulated resin flow is produced during the first week after wounding, a result that agrees with previous findings (Zas et al. 2020a). However this does not imply that the optimal periodicity of grooves is every two weeks as the global efficiency of the tapping operations would not only depend on the resin produced per tree but also on the number of trees that a worker can manage, and this number would be higher with more spaced grooves (Touza et al. 2021). Assuming that the number of trees that a resin-worker can process with grooves applied every three weeks is 1.5 times greater than that using biweekly grooves, the relative efficiency of the tapping operations will be even greater with a three-week frequency, especially at the end of the campaign. Besides saving considerable labor costs, other substantial benefit of decreasing the groove frequency is that fewer wounds are inflicted to the tapped trees annually, and this may result in lower impacts in wood quality, tree growth and tree health (Touza et al. 2021).

As observed elsewhere, resin production was variable along the tapping campaign (Rodrigues-Correa and Fett-Neto 2012; Rodríguez-García et al. 2015). Previous studies indicates that variation in resin production among grooves may be affected not only by the seasonal weather variation (Neis et al. 2018; Zas et al. 2020b) but also by the accumulation of induced responses to successive wounds (Touza et al. 2021). In temperate climates such as that of northwest Spain, both factors point to an increase of resin production along the tapping season up to a maximum, that typically occurs in late summer (Rodríguez-García et al. 2015; Zas et al. 2020a). Here, however, resin production tended to decrease along the resin campaign, at least when stimulant pastes were used. Despite resin production may be induced in response to previous grooves, the increase of resin production can exhaust the resin accumulated in the resin duct web, diluting the induction effect as time progress. This pattern is consistent with the reduction of resin production observed only in trees tapped using stimulant paste but not in control (and less productive) trees. Altogether, these considerations suggest that patterns of resin production along the tapping season may be differentially altered depending on the initial effects on resin production of the applied treatments, which may cause variable rhythms of exhaustion of the resin accumulated in the resin duct web. This may explain why the effects of the stimulant pastes obtained with micro-tapping are better related with the early effects of the treatments in conventional tapping (before any disturbance), a result that is consistent with some kind of legacy effects of previous grooves on subsequent patterns of resin production, as it has been suggested in previous studies (Touza et al. 2021).

Importantly, we found that the stimulant effect of the pastes on resin production depended on the moment of the season. SAL stimulant paste was more effective at the beginning of the season, while CUN paste performed better at the end of the tapping period. These results are consistent with the previously mentioned exhausting effect so that, the better the early efficiency of a treatment the greater the reduction of resin production through time. The variable efficiency of the different pastes along the season could justify different recommendations depending on the moment. For example, based on the results presented here, the SAL stimulant paste should be preferred in the early season and the CUN paste in the late season. This recommendation would nevertheless need support from further studies addressing the alternative use of different pastes throughout the whole tapping season. In previous studies effectiveness of resin stimulation tend to be higher in the faster growing season (Rodrigues-Correa and Fett-Neto 2012).

Micro-tapping as a tool for screening stimulant pastes and for exploring variation among sites

Previous studies proved that micro-tapping is a useful procedure to predict resin yield of mature trees (Zas et al. 2020a), or to assess different stimulant pastes, identify new adjuvants or select high resin yielders using young saplings (Junkes et al. 2019; Michavila et al. 2021; Neis et al. 2019a; Vázquez-González et al. 2021). In the present study, we demonstrate that micro-tapping of adult trees is also a convenient procedure to inform about the efficacy of different stimulant pastes. However, the predictive power of this technique was not especially high (close to half of the variation among treatments remained unexplained) and significant discrepancies between the results obtained with conventional tapping and micro-tapping were observed. An important part of these discrepancies likely arise from comparing a closed- and an open-cup tapping procedure (see discussion above), so the predictive value of the micro-tapping techniques are expected to increase for anticipating treatment efficiency of commercial tapping methods using closed recipients.

Another important concern for resin tapping management is to explore variation in resin productivity among sites and to anticipate the potential for resin tapping of a given pine stand. In this work, we demonstrate that micro-tapping can be useful for this purpose. Despite the very few studied sites, resin flow estimated by micro-tapping 24 days after wounding was significantly correlated with resin yield at the end of the conventional tapping season (October groove), when micro-tapping was accomplished. Again, the coefficient of determination was relatively low. However, it is important to highlight the huge amount of effort and resources that can be saved using the micro-tapping techniques compared to the time-consuming and costly conventional tapping. Whenever some variation can be predicted, the micro-tapping methodology will be useful. As the predictive value of this technique depended on the period of conventional resin tapping with which it was compared, an important point that requires further attention is to determine the optimum moment within the season in which micro-tapping maximize prediction. Micro-tapping evaluations completed at the beginning of the season could be, for example, more efficient to anticipate the results obtained by conventional tapping through the whole campaign, but this remains to be tested.

Conclusions

Resin extraction can not only contribute to the profitability of timber-oriented Atlantic pine forests, but also facilitate their management and conservation through the provisioning of multiple ecosystem services (Palma et al. 2016a; Touza et al. 2021). This study consistently shows that resin yield of both maritime pine and radiata pine was significantly increased by the application of stimulating agents such as Salicylic acid (in SAL paste), Ethephon (in CUN paste) or sulfuric acid (present in all pastes tested at different concentrations). Based on the relative efficiency of the different treatments, the SAL and CUN stimulant pastes are recommended. In order to reduce operational costs and increase the safety of resin workers, the SAL paste should be preferred, especially at the beginning of the resin campaign, and if tapping is conducted using closed atmospheres and containers. Another important recommendation to optimize the resin-tapping procedure in Atlantic pine forests is to decrease the groove frequency to every three weeks, since the small observed losses in resin yield will be likely compensated by the increase of the number of trees that a worker can manage. The large consistence of the observed results among species, sites and resin campaigns adds support to these recommendations, which could be crucial to surpass the profitability threshold of resin-tapping activities. Finally, we showed that micro-tapping allows to predict at least part of the variation in resin production among treatments, species and sites. Incongruences between results obtained through micro-tapping and conventional tapping were likely due to comparing a closed- and an open-cup tapping procedure. Despite these discrepancies, being and easy, rapid and cost-saving technique, micro-tapping in adult trees emerge as a highly useful tool for experimentation in resin production.

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Author contributions RZ, EM, RT conceived the idea, designed the experiment and provided the founds. All authors helped in field assessments but GB, DF and RT performed most of the resin sampling. RZ analyzed the data and RZ and ML leaded the writing, and all authors contributed to the writing by successive rounds of revision.

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Declarations

Competing interests The authors declare no competing interests.

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