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ORIGINAL PAPER

Resin yield response to different tapping methods and stimulant pastes in *Pinus pinaster* Ait

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Abstract

Selecting the best resin tapping method and stimulant paste in the resin tapping process is crucial. In timber-oriented rainy Atlantic pine forests of north-west Spain, the interest in resin tapping is raising but information on the best tapping methods and pastes is still lacking. In this study, an appropriate experimental design used on five representative plots of *Pinus pinaster*, allowed us to explore the resin productive differences between two tapping methods (traditional Spanish method and circular groove) and three stimulant treatments (control, Ethephon and ASACIF). The use of a standardized measure of resin yield allowed to adequately compare methods differing in groove length. Results indicated that the standard resin yield was 1.43 times greater with the traditional method than with the circular groove method. The two stimulant pastes drastically increased resin yield (up to sixfold) in all sites and for all tapping methods. The effectiveness of the paste was also influenced by the tapping method, obtaining greater increases in resin yield after the application of stimulant paste in trees with the circular groove method. Resin yield was only slightly related to the dasometric variables and varied among test sites when no pastes were used, but differences among sites disappeared when stimulant pastes were used. Our results contribute to the understanding of the factors involved in resin performance and the technological development of the sector.

Keywords *Pinus pinaster* · Tapping methods · Stimulant paste · Constitutive defences · Inducibility of defences · Standard resin yield

Introduction

In the Pinaceae family, resin is the tree's main defence against biotic challenges (Phillips and Croteau 1999). Resin is constitutively produced in all tree tissues providing a physical and chemical barrier to invaders (Luan et al. 2022). In addition, resin production is highly inducible in response to biotic stimuli, with large increases in production after mechanical injury or attacks by pathogens and insects (Lombardero et al. 2000, 2006; Kim et al. 2010). Pine resin is a viscous and sticky substance formed by a complex mixture of volatile and non-volatile terpenes with a wide variety of potential uses in different industrial sectors (Neis et al. 2019b; Demko and Machava 2022). For centuries, humans have taken advantage of resin properties by harvesting this non-wood forest product from living pine trees (Soliño et al. 2018; Cunningham 2009, 2012). Nowadays, resin is tapped from trees by applying repeated mechanical wounds enhanced by acid-based stimulant pastes and collecting the resin flowing from the wounds in open or closed recipients (Sharma et al. 2018).

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Maritime pine (*Pinus pinaster*) is the resinous species with the greatest presence in southern Europe and northern Africa. It ranges from southern France, through the whole Iberian Peninsula, northern Italy, Corsica and northern Tunisia, Algeria and Morocco (Caudullo et al. 2017). Spain is one of the countries where maritime pine is one of the most representative forest tree species in terms of both timber volume ($153 \cdot 10^6$ m^3, 14% of total timber volume) and surface area (1.1 Mha, 5.35%) (MITECO 2019).

Maritime pine has been resin-tapped for long in Spain, mainly in Segovia (Sebastián and Uriarte 2003; Pinillos et al. 2009; Rodríguez-García et al. 2014), a province located in the Spanish Meseta Central, which consists of a sandy plateau surrounded by several mountain chains. After a pronounced crisis in the Spanish resin sector in late past century (Pinillos et al. 2009), the increasing demand from the industry for alternative renewable bioproducts has prompted the reactivation of the sector since 2000 (Soliño et al. 2018).

In addition to the resin-tapping activities already existing in the Spanish Meseta Central, new initiatives are currently being taken to promote this activity throughout the country, even in areas where resin production had not been previously tested (Martínez 2016). Specifically, resin yield is seen as an attractive complementary activity in the timber-oriented maritime pine forest of northwest Spain (Gómez-García et al. 2017; Zas et al. 2020a, b; Touza et al. 2021), not only because of the potential contribution to profitability (Martínez et al. 2019) but also because of the multiple and valuable ecosystem services that resin tapping provides (Demko and Machava 2022; Soliño et al. 2018). With vast extensions of maritime pine (> 400,000 ha) (MAGRAMA 2012) and high net primary production (Martins et al. 2009), Atlantic pine forests may have large potential for resin yield. However, maritime pine forests in these areas are markedly different (e.g. higher growth potential, lower temperature oscillation, higher precipitation, lower summer drought) from those of the Meseta Central where knowledge on resin yield has been produced (Benito-Garzón et al. 2011). Whether the technological advances made in traditional areas are transferable to Atlantic areas remains to be adequately tested.

In the Iberian Peninsula, resin has been traditionally extracted following the method presented in Rodríguez-García et al. (2016), which consist in the periodical execution of horizontal striped wounds ("grooves") on the main trunk moving upwards, and the application of a strip of stimulant paste (typically including sulfuric acid) on the upper-inside border of each groove. Although information is still limited, this method implies large traumatisms on the trees and may likely impact wood production and wood quality (Génova et al. 2014; Rodríguez-García et al. 2016). In timber-oriented forests, such as those of the Atlantic regions, there is a need to find out alternative methods to make resin tapping compatible with obtaining quality timber. This is a rare topic in the literature, as normally in the most productive countries, the aim is not to make these two activities compatible, but rather to maximise one or the other. In addition, in traditional tapping, resin is collected in open pots which may be easily filled with water in rainy weathers. Besides the operational complications for separating resin and water, the rain water diminishes the volume capacity of the pots and favors resin leakage. Alternative methodologies that prevent water contamination are thus required in Atlantic regions where annual precipitation may more than triplicate that on Central Spain (Serrano-Notivoli et al. 2018).

One of the alternative and most promising closed-bottling methodologies is the mechanised circular notching extraction method (Pinillos et al. 2009). This method consists of making circular holes by means of a battery-powered screwdriver in the trunk, thus avoiding the debarking process and reducing the operator's workload. A cylindrical plastic device, introduced in the hole, connects to a plastic bag where the resin is stored (Martínez et al. 2021). Another advantage of this method is that it does not allow water to get into the resin containers. Finally, storage in bags makes the harvested resin easier to transport through the pine forest, an advantage that is particularly relevant in pine forests such as the Atlantic ones, which usually have steep slopes. These methods are still under development, and its efficiency has been not formally compared with other methods yet.

Since early times, different chemicals stimulants are used to increases the resin yield (Parham 1976). These stimulants allow to (i) enhance and extend the wounding effects (Neis et al. 2018), (ii) induce the defensive machinery of the tree to stimulate resin yield (Neis et al. 2018, 2019a) and (iii) avoid resin crystallization lengthening the period during which resin flow remains active (de Oliveira Junkes et al. 2019; Michavila et al. 2021). Many of the most widely used stimulants includes corrosive acids such sulfuric acid as the main principle active (da Silva Rodrigues et al. 2011; Michavila et al. 2021). Over time, the form of application of stimulants has changed (formerly sprayed as an aerosol and currently in paste form) (Zamorano and Solís 1974) and the proportions of acids is trying to be reduced, as they are highly corrosive and impose hazards to the field workers. Other types of stimulants with alternative active components (e.g. ethylene, paraquat, auxin, fungal treatments, metal cofactors of terpene synthases) are continuously appearing (Rodrigues-Corrêa and Fett-Neto 2012, 2013). Several studies have explored the effectiveness of different pastes applied in resin tapping (de Oliveira Junkes et al. 2019; Neis et al. 2018; da Silva Rodrigues et al. 2011; Rodrigues and Fett-Neto 2009) but little effort has been paid to explore the suitability of a specific paste depending on the geographical area where it is applied. Whether the effectiveness of the different stimulants is context dependent remains to be tested before generalising their use to a specific biogeographical region.

Currently, in *P. pinaster*, the most commonly used stimulants are based on sulfuric acid (Vázquez-González et al. 2021). Other stimulants based on different phytohormones (e.g. Ethephon, salicylic acid, methyl jasmonate) have been also shown to enhance resin yield in this species (Michavila et al. 2021; Vázquez-González et al. 2022) but formal comparisons of the effectiveness increasing resin yield of different stimulants under different environmental conditions are still lacking. In addition, the effectiveness of the chemical stimulants may largely differ depending on whether the extraction procedure uses closed or open recipients to collect the resin, something that has remain elusive in previous investigations.

After the resurgence of the interest for resin tapping in the Spanish *P. pinaster* forests in recent years, it is highly desirable to move towards the technicalisation and professionalisation of the sector. In particular, optimizing the tapping methodology (extraction procedure and stimulant pastes) emerges as one the main topics that remain to be fine-tuned according to the environmental and silvicultural particularities of the tapped stands. The objectives of this work carried out in maritime pine forests across an environmental gradient from Atlantic areas to the Mediterranean Meseta Central of the Iberian Peninsula were (1) to compare the effectiveness in terms of resin yield of the Spanish traditional method for resin tapping and the emergent circular groove methodology, (2) to compare the effectiveness of the two most promising stimulate pastes (Ethephon and ASACIF), (3) test how dendrometry affects resin yield as a function of tapping method and stimulant paste and (4) to study the efficiency during the tapping season of the two methods and the two stimulant pastes.

Material and methods

Study area and data acquisition

Therefore, the present study was carried out in five stands of maritime pine (*P. pinaster*) located in Galicia, Asturias and Castilla y León: Culleredo, Pantón, Godos, Barcia and Coca (Fig. 1). The test sites were representative stands of two of the most potential resin-producing areas of Spain: the Northwest and the Meseta Central. The area of study followed a marked climatic gradient from the humid and thermal Atlantic climates of the northwest coastal areas to the drier and more continental areas of Central Spain (Table S1). The five sites (n_{total} = 433 trees) were pure adult regular pine forests between 35 and 40 years, in which resin tapping had not been carried out before. Average normal diameter at breast height in the five stands was greater than 25 cm, the threshold upon which resin extraction is allowed in the area.

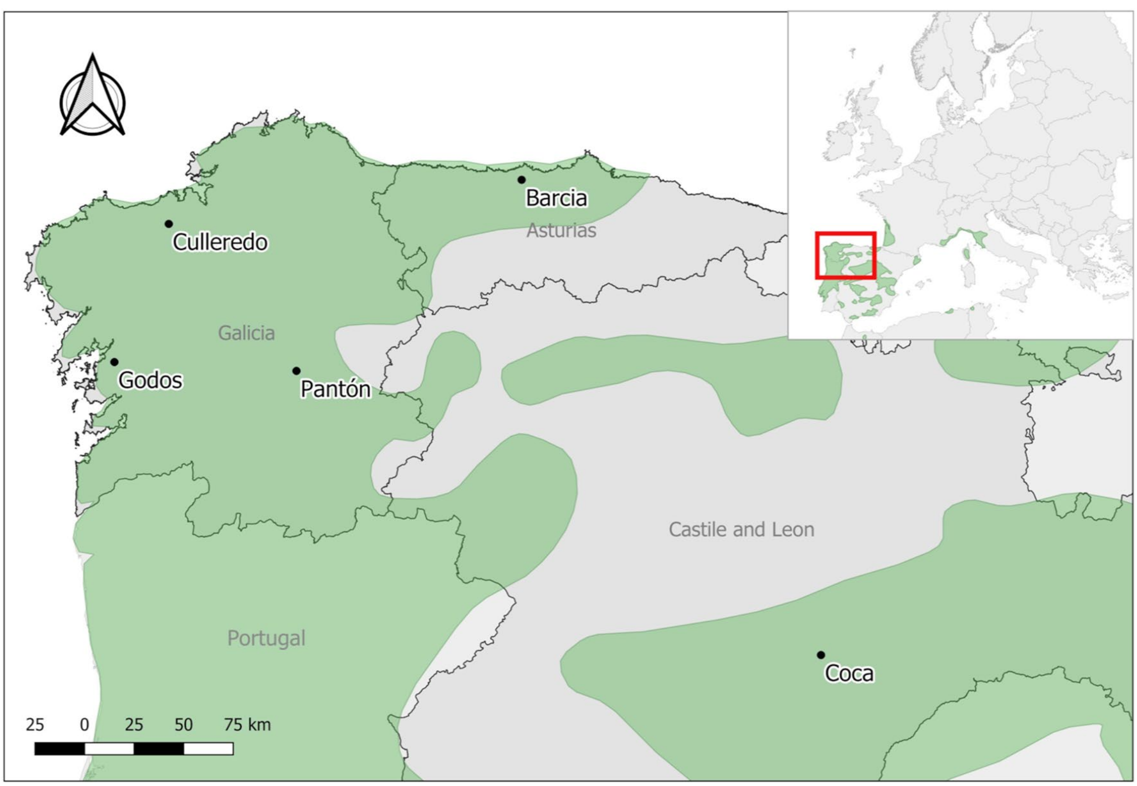

Fig. 1 Study area and spatial distribution sampling plots. Green area is the distribution range zone delimited by Caudullo et al. (2017)

In each of the stands, 90 trees with similar dendrometric characteristics between them were selected and separated into three blocks of 30 trees according to topography and environmental particularities. Within each block, 6 groups of five contiguous trees were made, and treatments allotted randomly to each group. A total of six treatment, corresponding to the combination of three stimulant pastes (Control, Ethephon and ASACIF), and two different extraction methods (traditional and circular) were tested between June and November 2021. Some of the trees sampled were written off because they died during the tapping period.

The first tapping method was the traditional method used in the Iberian Peninsula (Rodríguez-García et al. 2016). In this method, after removing most of the bark from the area to be resin-tapped during the whole campaign, a strip of phloem 2–3 cm wide and, 16 cm long was removed manually every two weeks moving upward. Resin flowing from the practiced wounds was collected in 2L plastic open pots (Fig. 2a). Starting at a height of approximately 20 cm from the ground, a total of 8 groves were made in each tree. The second method was the circular groove (Pinillos et al. 2009), which is a mechanised method in which circular wounds of 5 cm in diameter (15.7 cm in perimeter) were made every 2 weeks with the aid of a battery-powered screwdriver. Successive wounds were spaced 2–3 cm upward or lateral from previous wounds. Specifically-designed plastic devices were introduced within the practiced holes and the resin collected in closed plastic bags (Fig. 2b). The total number of grooves was the same than in the other method. Grooves of the two methods were done simultaneously within each site.

For each of the two extraction methods, different stimulant pastes were applied either in the inner-upper border of the horizontal grooves or in the inner contour of the circular holes. Three different treatments were considered, a control without stimulant paste and two commercial pastes that have shown promising results in previous experiments (Michavila et al. 2021; Gómez-García et al. 2022), the Ethephon (8% Ethephon(60% v/v), 14% sulfuric acid (50% v/v), 55% distilled water, 1.7% polysorbate, 1% cetyl alcohol, 4% vaseline, 5.5% silica, 10.8 sawdust) (Gómez-García et al. 2022) and the salicylic paste ASACIF (1% salicylic acid, 25% sulfuric acid (96% v/v), 5% propylene glycol, 19% wheat straw, 50% distilled water) (Michavila et al. 2021). The production per tree was weighed each time a new groove was made with a scientific scale calibrated in decigrams. Due to inconveniences during the weighing of the intermediate grooves, especially in the Coca site, there were values of these weighings that were not registered at the moment of making the new groove and were added to subsequent weighings. Periodical yields were summed up to obtain the resin yield per tree across the experimental campaign.

Before resin tapping, diameter at 1.30 m from the ground (diameter at breast height, dbh), the height to the tree's apex (total height, h_t) and the height to the insertion of the first live branch into the stem (h_{flb}) of all experimental trees were measured. The slenderness and volume of each tree were also calculated. The slenderness was calculated as the relation between the total height and the diameter at breast height. The regional formulas of the IV National Forest Inventory of Spain were used to estimate volumes (MAGRAMA 2012).

Fig. 2 Resin tapping methods used in the study, **a** traditional extraction method and **b** circular groove extraction method. Source: FORESIN and Inés

Standard resin yield

To ensure reproducibility and homogeneity in comparisons between resin extraction methods (which differ slightly in the length of the practiced strips), resin yield was adjusted according to the length of the strips of each method. The standard resin yield (SRY, i.e. the resin yield per unit of strip length) was estimated as:

$$\text{SRY} = P_t / \sum_{i=1}^{n} L_i \quad \left(g\ \text{cm}^{-1}\right)$$

$$L_{\text{traditional}} = 2 \cdot \text{dbh}/2 \cdot \text{arcsen}\left(\frac{p/2}{\text{dbh}/2}\right) = \text{dbh} \cdot \text{arcsen}(p/\text{dbh})$$

$$L_{\text{circular}} = 2 \cdot \pi \cdot \sqrt{\frac{L^2_{\text{taditional}} \cdot p^2}{2}}$$

where P_t is the total resin yield of the tree (g), L_i is the length of each strip as a function of tree diameter (cm), n the number of strips within the season, dbh is the diameter at breast height and p is the theoretical groove length (in the case of circular groove was the diameter).

Statistical analysis

Before choosing the tests to be used to analyse the differences between methods, pastes and sites and the correlations between the SRY and the dasometric and estimated variables, we verified that the assumptions of normality and homogeneity of variance of the parametric versions of the tests were fulfilled in any case. The Shapiro–Wilk test was used for checking normality and the Levene test (normal data) or Fligner Killeen test (non-normal data) for homogeneity of variance.

In order to test for statistical differences in central tendency between the SRY of the two extraction methods a Mann–Whitney U test was performed, this test is a non-parametric version of the 2-sample t-test to compare two independent groups. Comparisons of the SRY accumulated up to the eighth groove between methods and stimulant pastes were made using the Welch one-way ANOVA, which evaluates the differences among three or more independently sampled groups, with a slight deviation from normality and unequal variances. To test for statistically significant differences between the SRY of the different plots, two tests were used, Fisher's one-way ANOVA, when the data had a normal distribution, and Kruskal–Wallis one-way ANOVA, when the data did not have a normal distribution, in all cases the assumption of homoscedasticity was met. The Spearman's correlation test was used to calculate the correlations between the SRY and the dasometric variables and those estimated on the basis of them; this test is a non-parametric statistical measure of the strength of the association between two variables. The non-parametric Friedman rank sum test, which is the alternative to repeated-measures ANOVA when the assumptions are not fulfilled, was employed to determine whether there were statistically significant differences within the periodic SRY of each of the methods and pastes (the Coca plot was not included in this analysis because the first two periodic yields were accumulated in the third).

The post-hoc tests employed were the Games-Howell test for the Welch one-way ANOVA, Student's t-test for the Fisher's one-way ANOVA, Dunn test for the Kruskal–Wallis test and the Durbin-Conover test for the Friedman rank sum test.

The significance level used in all cases was 95%. All statistical analyses were performed with version 4.2.2 of the statistical software R (R Core Team 2022) and the "ggstatsplot" package (Patil 2021) was used to perform the comparisons between and within groups.

Results

Comparation between resin tapping methods

The Mann-Whithney test showed that there were statistically significant differences between the SRY obtained by the traditional and the circular groove methods (Fig. 3). The median of the SRY was 1.43 times higher in the traditional method than in the circular groove method. Furthermore, the observed effect size (Glass rank biserial coefficient) of -0.35 was medium according to Vargha and Delaney (2000).

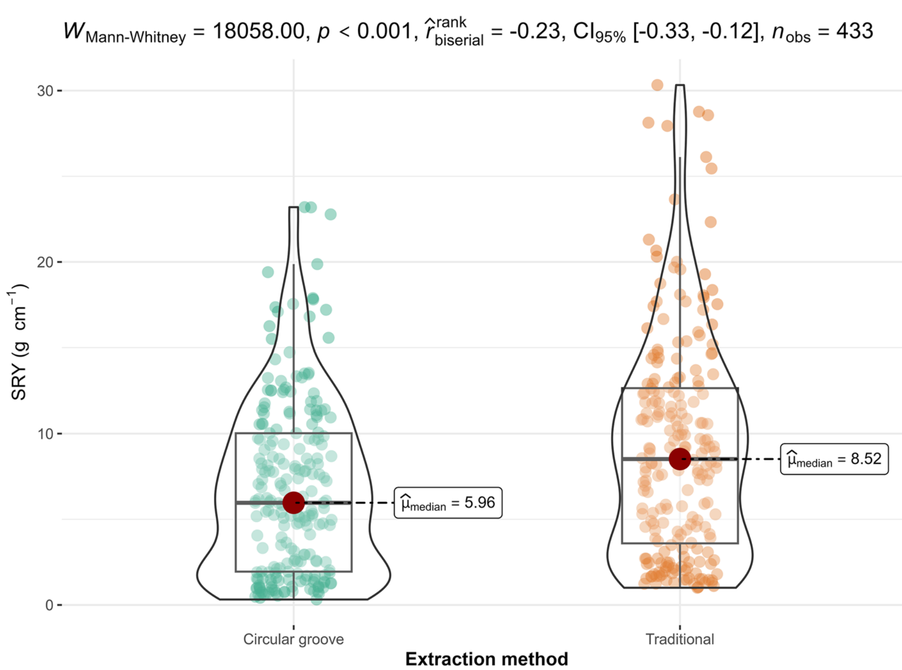

Fig. 3 Results of the non-parametric Mann–Whitney test, carried out on the SRY data to check if there were statistically significant differences between the productions obtained by the two different methods. $W_{\text{Mann–Whitney}}$: Mann–whitney test result; p: p-value; $\hat{r}^{\text{rank}}_{\text{biserial}}$: Glass rank biserial coefficient; $CI_{95\%}$: confidence intervals of Glass rank biserial coefficient; n_{obs}: number of observations

As there were differences between the median yields of the two methods, the performance of the pastes was analysed for each of the extraction method separately.

Comparation between stimulant pastes

For both tapping methods, the Welch test revealed significant differences between the SRY of the control trees and those with stimulant paste applied (Fig. 4). There was no significant difference between the pastes in both cases. The size effect of the test (rank epsilon squared) was similar in both cases (0.63 and 0.60) and can be qualified as large according to Field (2013).

Comparing the medians showed in Fig. 4b, in the circular groove the Ethephon paste yields 5.98 times more SRY than the control, while SRY with the ASACIF paste was 6.26 times higher. In the traditional method, the efficiency of the Ethephon and ASACIF pastes was relative lower

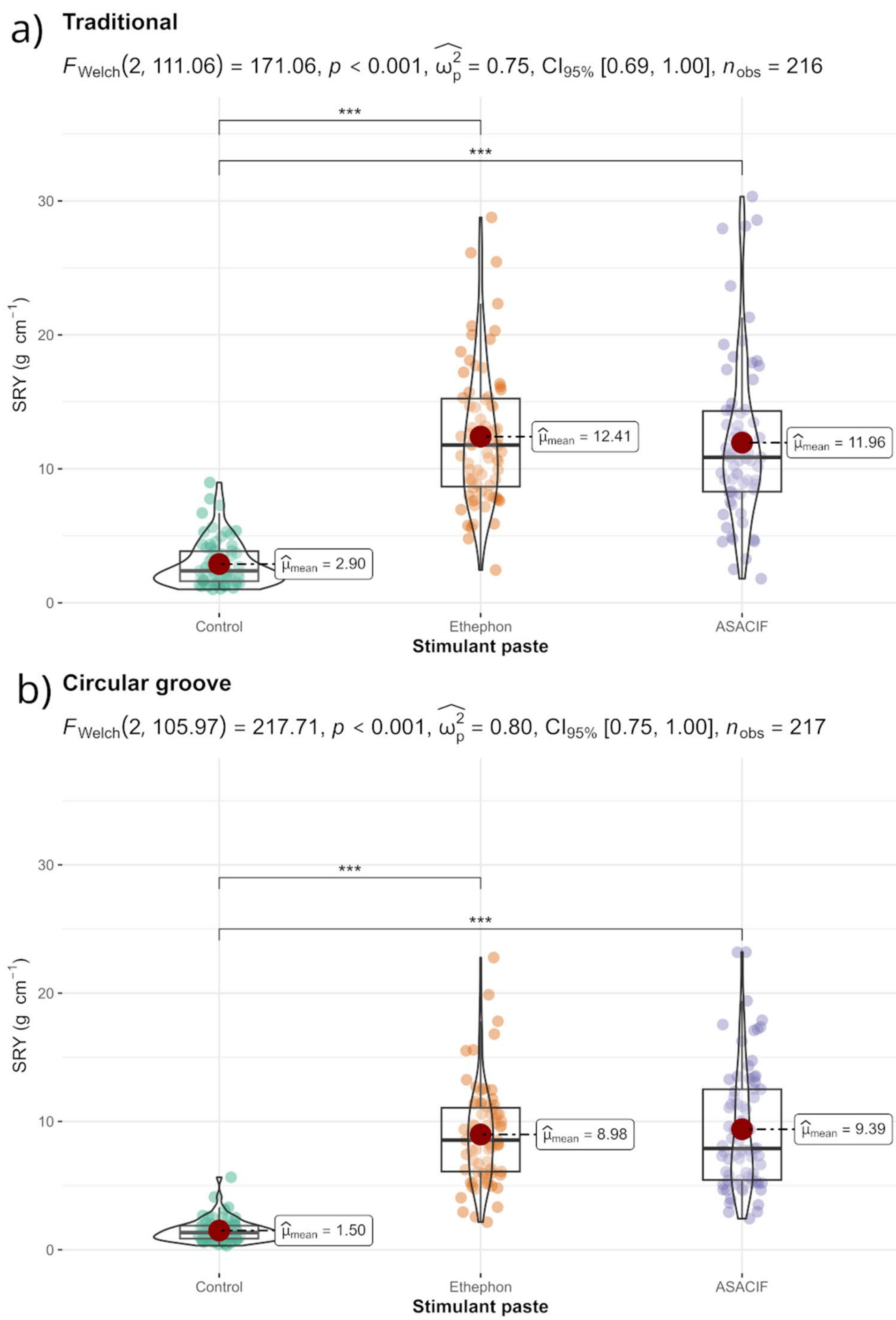

Fig. 4 Results of the Welch non-parametric tests performed on the SRY data of the cumulative production values with the pastes and the control up to the eighth strip for each extraction method, **a** traditional and **b** circular groove. F_{Welch}: Welch test result; p: *p*-value; $\widehat{\omega}_p^2$: rank epsilon squared coefficient; $CI_{95\%}$: confidence intervals of rank epsilon squared coefficient; n_{obs}: number of observations

(4.27 and 4.12 times higher than the control, respectively) (Fig. 4a).

Effects of tapping methods and pastes on inter-site variation in SRY

Statistically significant differences and large effect sizes in SRY among plots were observed when the traditional method was used (Fig. 5). Resin tended to be higher in the plot located in Coca, especially in control trees tapped without stimulant paste (Fig. 5a). In the case of the circular groove tapping method, no significant differences in SRY was observed between any of the plots irrespective of the stimulant paste (Fig. 5d, f).

Dendrometry effects in SRY depending the tapping methods and pastes

Correlations between the SRY and dendrometric variables were mostly not statistically significant, only between the traditional tapping method and control trees the total height and slenderness had low negative significant correlation values (Table 1).

Trend in SRY during the season

Standardized resin yield after each groove showed significant temporal variation across the tapping season irrespective of the tapping method and the stimulant paste used (Fig. 6). In general, SRY after each groove tended to increase along the tapping campaign, with this relative

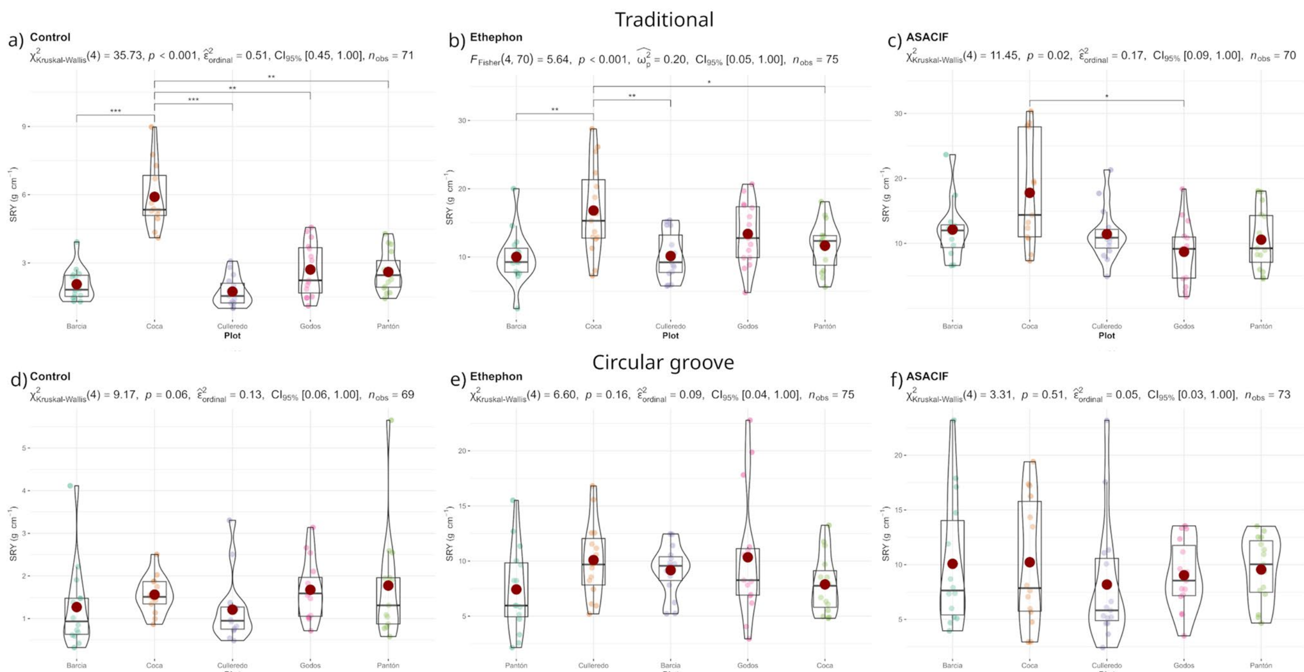

Fig. 5 Results of the Kruskal–Wallis non-parametric tests performed on the standard yield data of the cumulative production values in the study plots. The scale on which the graphs are represented is not the same for control and stimulant pastes. $\chi^2_{Kruskal\text{-}Wallis}$: Kruskal–Wallis test result; F_{Fisher}: Fisher test result; p: p-value; $\widehat{\omega_p^2}$: rank epsilon squared coefficient; $\widehat{\epsilon}^2_{ordinal}$: epsilon squared coefficient; $CI_{95\%}$: confidence intervals size effect coefficient; n_{obs}: number of observations

Table 1 Spearman correlations values between resin yield and dendrometry variables. dbh: diameter at breast height; ht: total height; h_{flb}: height to the insertion of the first live branch into the stem; V: tree volume; Values in bold have p-value < 0.05

Resin tapping method	Paste	*dbh*	h_t	h_{flb}	Slenderness	V
Traditional	Control	0.11	**−0.3**	−0.27	**−0.34**	0.14
	Ethephon	0.07	−0.18	−0.18	−0.2	0.07
	ASACIF	0.19	0.04	0.03	−0.19	0.23
Circular groove	Control	−0.03	−0.08	−0.17	−0.11	−0.04
	Ethephon	0.07	−0.18	−0.08	−0.2	0.07
	ASACIF	0.13	0.14	−0.01	−0.05	0.16

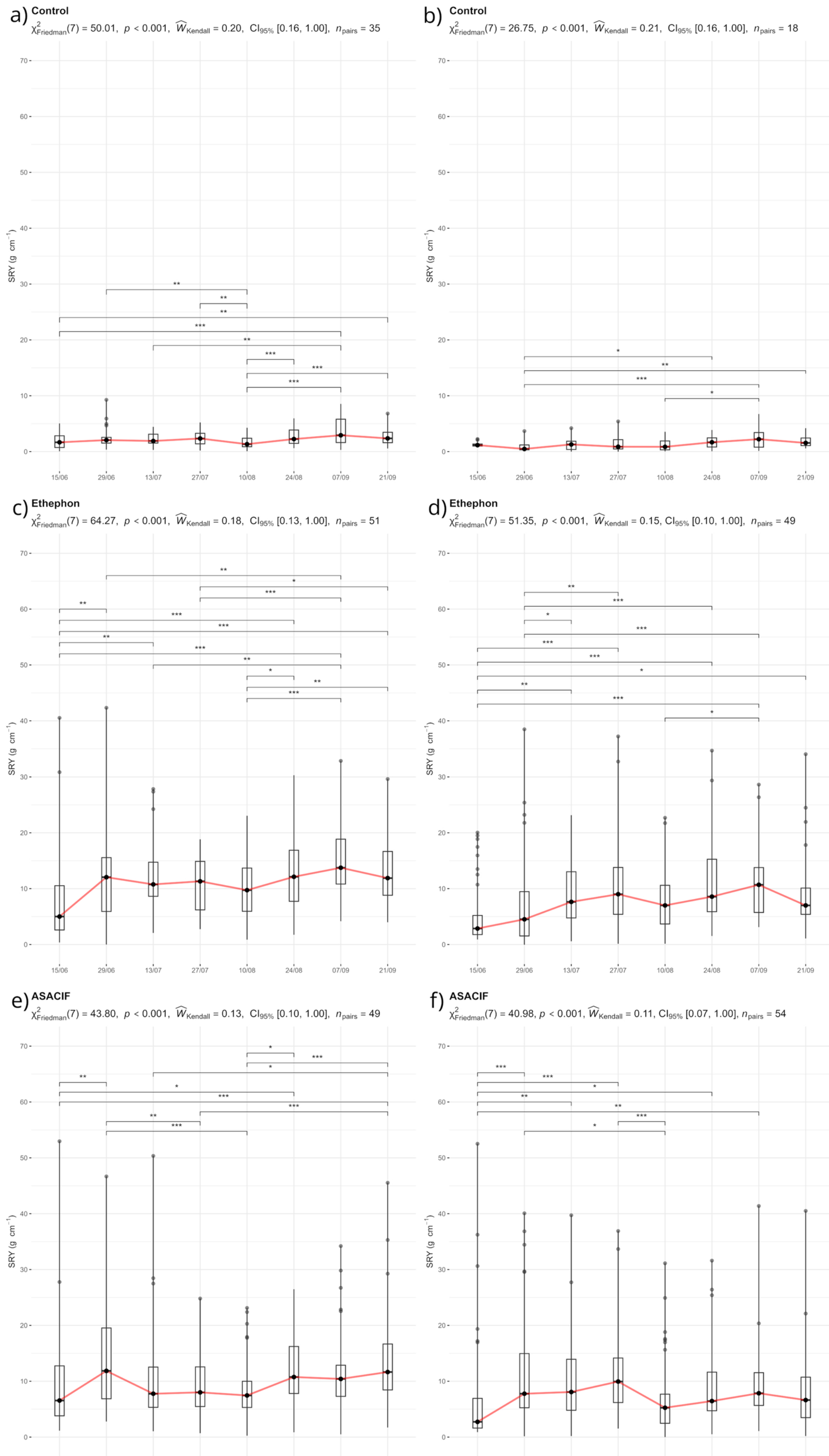

Fig. 6 Results of the non-parametric Friedman test, carried out on the complete trends in standard yields for each of the methods and stimulant paste to check if there were statistically significant differences. **a**, **c** and **e** traditional tapping method; **b**, **d** and **f** circular groove tapping method. $\chi^2_{Friedman}$: Friedman test result; p : p – value; $\widehat{W}_{Kendall}$: Kendall coefficient, reports the effect size; $CI_{95\%}$: effect size confident interval; n_{pairs} : number of pairs used in th test.

increase varying depending on the tapping method and the stimulant paste used, with the last groove decreasing in most cases. Judging from the effect sizes (Kendall's W coefficient), temporal trends were slightly more pronounced in the control treatment than in trees tapped with stimulant pastes (Fig. 6).

Discussion

Our study obtains clear and sharp results on the influence of tapping methods and stimulant pastes on resin yield of maritime pine trees across an environmental gradient in Spain. An appropriate experimental design and the use of a standardized measurement of resin yield per unit of strip length make the results obtained from different tapping methods, pastes and sites comparable. Understanding how the tree reacts to different stimulants and tapping methodologies is fundamental for optimizing tapping extraction protocols during the tapping season.

Tapping methods

The traditional tapping method produced higher SRY than the circular groove method, regardless of the chemical stimulant applied and the environmental characteristics of the test site (Fig. 3). These results were in line with those obtained by Pinillos et al. (2009), who found, in average, 1 kg per tree more resin using the traditional method than the circular groove method. Higher SRY in the traditional method could be because the number of axial resin ducts cut per unit of strip length was likely higher in the horizontal strips of the traditional method than in the circular strips of the circular groove method, in which axial ducts are exposed to a greater extent in the upper and lower parts of the notch than on the sides. In addition, the bottom part of the circular notch could produce less resin than the upper part of the notch, just as the downward methods produce less than the upward methods (Rodríguez-García et al. 2016).

Differences in the mean SRY between the two tapping methods was greater in control trees (1.93 fold change) than in trees tapped with stimulant (1.38 with Ethephon and 1.27 with ASACIF). This may be because the application of the stimulant paste in a closed environment, such as that of the circular groove method, could favour or sustain during a longer time the effect that the paste has on the tree. Another effect of the circular groove tapping method is that equalizes the standard yields between the different locations and masks the differences in SRY that appears in the traditional method between the Coca plot and some of the other locations, especially when no stimulants were used (Fig. 5).

Pastes performance

Results also clearly demonstrate a huge effect of the two stimulant pastes increasing resin yield, with the effectiveness of the different treatments varying depending on the tapping method used and the plot. These results were in line with other works reporting a positive effect of the application of chemical stimulants on resin yield (Rodrigues et al. 2008; Liu et al. 2022; Neis et al. 2018) and with the expected influenced of the environmental conditions in the resources allocated to defence (Zas et al. 2020b; Vázquez-González et al. 2019).

The extraction method affected the performance of the stimulant pastes, but it can further accentuate the effect of a specific paste. This can be seen by comparing the average yields per paste and method (Fig. 4). The ASACIF paste gives a slightly higher SRY with the circular method than the Ethephon stimulant paste, whereas the opposite is true with the traditional method. This may be due to the fact that the ASACIF paste reacts slightly better to the closed circular device used in the circular notching method.

The improvement in resin yield obtained in our work (ranging from 4.12 to 6.26 times the yield obtained by the control trees depending on the method and the paste) (Fig. 4) was greater than the reported by Neis et al. (2018) and Liu et al. (2022) in *P. elliottii* and *P. elliottii* × *P. caribaea* (2.15 and 2.14 fold change relative to control trees, respectively). This difference may be due to the fact that most of the pines used in this study were timber-oriented and therefore fast-growing. Following the Resource Availability Hypothesis (RAH), fast-growing trees tend to produce less constitutive resin than those with slower growth rates but may produce greater amount of induced defences (Endara and Coley 2011). According to this idea, in the present study, constitutive resin production (i.e. that produced by control trees with no stimulant paste) was 2.2 and 3.4 times higher in the less favourable site of Coca than in the other timber-oriented and fast growing stands. This trend is consistent, for example, with the negative relationship between growth potential and constitutive resin production observed across populations of *P. pinaster* (Zas et al. 2020b). Resources available in the Coca plot are much more limiting for tree growth (poorer soil and greater water deficit) than in the rest of the plots, thus increasing the availability of carbohydrates for defence (Hood and Sala 2015).

When stimulant paste was applied to the timber-oriented trees in the favourable sites, resin yield was much more similar to that of the resin-oriented and resource-limited site of the Meseta Central (Coca). This result can again be explained by the RAH, which predicts that induced defences are favoured under abundance of resources due to lower tissue replacement costs (Endara and Coley 2011). Therefore, as higher resource availability and higher growth rates of the Atlantic timber-oriented plots may have favoured greater responses to the application of stimulant paste, thus reducing the difference between the yields of the plots.

Dendrometry effects in SRY as a function of tapping methods and pastes

The correlations show that the dendrometric variables h_t and tree slenderness have a slight negative correlation with SRY when the traditional method is used on control trees. When one of the stimulating pastes or the circular groove method is used, the values of the correlations become non significant, indicating that the resin production in a tree stimulated or tapping by the circular groove method does not depend to a great extent on the dendrometric variables or the volume.

The significant correlations obtained could indicate that trees with a smaller height and higher *dbh* in relation to their total height produce a higher amount of resin when no chemical stimulant is used in the traditional method. These results are in agreement with those obtained by Zas et al. (2020a), they reported that tree size does not contribute significantly to explain resin yield and slenderness has a negative effect on resin production. The negative effect of slenderness can be explained as a function of stand density, according to previous studies, at lower densities, resources for growth and defence are greater, increasing resin production (McDowell et al. 2007; Rodríguez-García et al. 2014, 2015; Hood and Sala 2015; Miina et al. 2020).

Seasonal trend in SRY

The temporal trends of the SRY after each groove through the season varied depending on the tapping methods and the stimulant paste used. For the control and Ethephon pastes, regardless of the method utilised, the trends peaked in late summer, around 100 days after the start of wounding. These results were similar to the results obtained in other studies (Zas et al. 2020a; Touza et al. 2021), but deviations from this general pattern have also been reported (Rodríguez-García et al. 2016). The decrease of the SRY observed in the fifth groove coincides with the maximum average temperature (Fig. S1) and the minimum values in the accumulated precipitation during the period between grooves (Fig. S2). These two phenomena have likely increased the water deficit, which could be the reason for the decrease of resin production as previous studies have shown that extreme water deficits can reduce resin yield (Lombardero et al. 2000; Turtola et al. 2003; Rodríguez-García et al. 2015; Neis et al. 2018; Hood and Sala 2015). The ASACIF positive trend at the end of the season was uncommon when compared to other papers (Rodríguez-García et al. 2016; Touza et al. 2021; Zas et al. 2020a), as resin production normally decreases in autumn when temperatures start to decrease, due to the seasonal component of the resin (Hood and Sala 2015; Neis et al. 2018; Rodrigues-Corrêa and Fett-Neto 2013).

Conclusions

Pine resin is one of the main non-wood forest products that can be carried out alongside timber production and that is gaining relevance in Spain today's social context. In order to fine-tune resin taping exploitations, it is crucial to know the responses of pine trees to different extraction methods and stimulant pastes. In this study, we found that the extraction method was one important factor influencing the quantity of resin yield, with the traditional method being more productive than the circular groove. It is important to note that the use of a standardized measure of resin yield adjusted to the length of the inflicted groove allowed to adequately compare the different tapping methods. The application of stimulant pastes was another factor that drastically increased to resin production in all sites and tapping methods. However, no significant differences were observed in the efficacy increasing resin yield of the two tested pastes (ASACIF and Ethephon). Another main result of our study was the positive effect of the closed extraction device utilised in the circular groove method, increasing the effect of the pastes on the trees. Furthermore, there was no clear evidence that the dasometric variables alone were able to explain resin production. Finally, important differences in resin yield were observed between sites when no stimulants were used but these differences tend to disappear when trees were tapped with stimulant pastes. Consistent with theoretical predictions on plant defence investment, constitutive resin yield was maximized in the harder environment of Central Spain while response to stimulants seem to be greater in the milder Atlantic sites. This study represents a further step in the standardisation, understanding and comparison of factors influencing resin yield in pine forests in Northwest and Central Spain. Future research should focus on evaluating the effect generated by the combination of the pastes with the rest of the factors that intervene in the resin yield (climatic, edaphic, genetic factors or a combination of all of them) and continue developing final production models with new methodologies.

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Competing interests The authors declare no competing interests.

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

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