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EVALUATION OF HIGH RESIN YIELDERS OF *PINUS PINASTER* AIT.

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ABSTRACT

The resin yield superiority of 51 selected high resin yielding candidate trees of *Pinus pinaster* Ait. in Central Spain, was evaluated over two years. The resin yield of each selected individual was compared to the yield of 10 control trees. Micro-chipping evaluations were carried out in a clonal bank to determine the clonal heritability and the correlation between the resin yield of the ortets and their ramets. The selection of candidate trees in the forest was quite effective, in comparison to that of the control trees (average production of 7.2 kg/tree/year in selected trees, 3.7 kg/tree/year in control trees). The candidate trees had slight differences in diameter, height, crown size and tree stocking when compared to control trees. However, there was no correlation between resin yield superiority and any of these morphological traits. Trees were selected over the large area in the forest and, to standardise the resin yield under different ecological conditions, the selection intensity of each candidate tree with respect to its ten control trees was computed. Eleven candidate trees (21 %) yielded a selection intensity of over 3.3 (one tree selected from more than 800 trees) out of which three obtained a selection intensity greater than 4 (1 in 10,000 trees). In terms of actual resin production, ten trees produced more than 10 kg/tree/year, out of which one tree produced 25.1 kg/year. Strong correlation was recorded between the selection intensity and resin yield of the high yielders, indicating the superiority of the candidate trees, but not with the mean production of the control trees. A moderate correlation r = 0,63) was recorded between the resin yield of the ortets with their micro-chipped clones, and high clonal heritability ($h^2 = 0.5$) was obtained. These results indicate that the obtained high selection intensity of the high yielding trees was strongly influenced by genetic factors, and therefore, indicate the possibility of breeding for this trait in *Pinus pinaster*.

Key words: *Pinus pinaster*, selection, high resin yielder, selection intensity, resin tapping.

INTRODUCTION

Non-timber secondary forest products such as oleoresin, gum, cork, edible fruit, mushrooms, and pharmaceuticals (from leaves, fruit, roots etc. of different trees) play an important role in the national economy of many countries. Research on sustainable forestry and development programmes are being stepped up in order to ensure that these renewable resources continue to be available for use by current and future generations.

One of the forest resources that have undergone an intensive development programme is resin, one of the oldest renewable forest products. Resin extractives are used extensively in the paper, soap, pharmaceutical and paint industries. Resin has been produced in a number of countries, with the main resin producers until the mid-1960's being the U.S.A. (with 50 % of global production), the former USSR, Portugal, Spain and Greece. But currently, China and other mainly developing countries (Indonesia, Brazil, India, Argentina etc.) are replacing the traditional major suppliers of resin, and China is producing one third of the global resin supply (ZHANQIAN 1999).

Thirty-one pine species are tapped for resin production; of these, 7 species including *P. pinaster* (maritime pine) supply the largest portion of resin in the world market (MCREYNOLDS *et al.* 1989). *P. pinaster* produces resin not only high in quantity but also in quality (COPPEN & HONE 1995). *P. pinaster* is commercially tapped in Spain, Portugal and South Africa.

Resin yielding ability varies among and within pine species. High and low yielding trees are found throughout the natural distribution of pine species and this variation enables genetic improvement programmes on resin yielding trees.

Genetic improvement to increase resin yield was initiated in 1945 (DORMAN 1945). It has been shown that resin yield is a highly heritable trait and important genetic gains can be obtained from the selection of the high resin yielders (MERGEN *et al.* 1955; SQUILLACE & DORMAN 1959 and GODDARD & PETERS 1965). Similar improvement programmes have been extended to different pine species of the main resin producing countries: *P. caribaea* (ALVAREZ 1982), *P. elliottii* (GARRIDO *et al.* 1986 a), *P. roxburghii* (SEHGAL & CHAUHAN 1995), *P. pinaster* (GIL 1998) etc.

Genetic inheritance of monoterpene composition has been investigated in different pine species. MARPEAU *et al.*, (1998) confirmed that the resin composition of *P. pinaster* is linked with a biosynthetic process and is controlled by genetic factors. The relative proportions of constitutive monoterpenes are strongly inherited, little influenced by environmental traits (MICHELOZZI *et al.* 1998) and have been used for many years as biochemical markers in forest genetics (HANOVER 1992 and PLOMION *et al.* 1996). These studies have direct implications for all genetic studies of resin yield and quality in these species.

Tapping of resin in Spain started in the 19th century and was an important production activity until 1970. Peak resin production was recorded in 1961 with 56,000 metric tonnes of resin being produced. However, resin production was drastically reduced in the 1970s, largely due to the international crisis in this sector. In the mid-1990s, resin tapping was reintroduced gradually in different pine forests of the country, and now many of the abandoned stands are tapped again. At present the output of resin is about 3,575 tonnes per year (MAPA 1999), with 95 % of the production coming from *P. pinaster*. The quality of resin obtained from *Pinus pinaster* is important for the economy of many resin industries in Spain.

Due to the enormous socio-economical importance of resin production, various efforts have been made to improve resin production and productivity. Genetic improvement of resin yield is usually combined with research to improve timber quality (SQUILLACE 1965 & ALVAREZ 1982). In addition, research is underway into new methods of resin tapping and collection (HODGES & JOHNSON 1997; LAPORTERIE 1999). A research project into the mechanisation of resin tapping to make this sector more profitable (LAPORTERIE 1999) is being financed by the European Community and is currently undergoing field testing in pine forests.

In 1994, a programme aimed at the genetic improvement of resin yield from *P. pinaster* started in Central Spain. The programme began with the identification and selection of high yielding trees throughout the natural pine forests used for resin tapping in the province. The selection was based on the information provided by the Forestry Development Agents and resin producers who had been working for several years with these trees. A group of 143 phenotypically selected high resin yielding trees were therefore used for the breeding population of the programme. From this population, two progeny trials (from open pollinated seeds of 118 families) and a clonal bank (59 clones with 225 ramets) have been established (PRADA *et al.* 1997; GIL 1998).

From the breeding population, 51 genotypes were selected as an evaluation population on which to concentrate different, but interrelated, research activities: controlled resin tapping of the selected candidate trees, cross-pollination, micro-chipping evaluation and chemical composition studies (TADESSE *et al.*, 2001 a).

The main objectives of the present field evaluation are therefore, to determine the effectiveness of the selection, comparing the resin yielding ability of the selected high resin yielding trees with control trees of the same stand chosen for their similar values for different morphological traits; to determine the correlation between resin yields of different years of resin production and evaluate the correlation between resin yield of the selected high yielding trees (ortets) and resin yield of their micro-chipped clones at the clonal bank. These evaluations will provide the best possible trees for further genetic improvement and also for future improved plantations.

MATERIAL AND METHODS

Study area and selection of candidate trees

The study area is located in pure and relatively homogenous *P. pinaster* natural stands of Central Spain (province of Segovia), one of the major resin tapping regions in the country. The area is characterised by the complete absence of significant slopes, a relatively uniform soil texture and a high percentage of sand at the higher soil horizon.

The 51 individuals of the evaluation population which are distributed in different resin producing pine stands of the province, were selected to carry out the present study. Ten control trees were selected for each candidate tree in the same stand and with similar values for morphological traits (diameter at breast height, total height, crown size etc.). Consequently, each experimental plot consisted of 11 trees. All trees in the plot were marked and numbered to facilitate further evaluation work.

Controlled resin tapping and micro-chipping evaluations

The resin tapping work was carried out over 2 normal tapping seasons (1998 and 1999), starting in May and finishing in October of both years. All trees were tapped using the bark chipping method. All streaks had the same height and depth, and sulphuric acid stimulant was applied after each streak to all of the evaluated trees.

The resin yield of each individual tree was carefully scaled by determining resin weight in the field every 3 to 4 weeks, when pots had become full of resin (hereafter referred to as "partial production").

Additionally, other tree traits have been recorded such as diameter at breast height, total height, crown size (width and length), and tree stocking (estimated by the distance to the 5th nearest tree) for all candidate and control trees.

Part of the programme has been to carry out micro-chipping studies in the clonal bank (grafts of the tapped candidate trees, or ortets). The micro-chipping study was carried out in the year 2000, with 17 clones and 4 ramets per clone (on average) and for a total of 69 ramets. The selected clones are those whose ortets belongs to the 51 evaluated candidate trees and had more than 2 ramets per clone in the clonal bank (TADESSE *et al.*, 2001 b).

The micro-chipping consisted on the tapping of an area of one square inch (2.5 cm by 2.5 cm) above the graft union, every two weeks. At each tapping face, sulphuric acid stimulant was applied (GARRIDO *et al.* 1986 b). Six tappings were made during the 3 months of June, July and August.

Data analysis

The *t*-test was used to examine the presence of significant differences between candidate trees and their control trees, with respect to their morphological traits. The Pearson correlation coefficient between annual resin yield and tree morphological traits was also computed.

The superiority of the candidate trees with respect to their control trees was evaluated by the difference with respect to the average yield of the ten control trees. Due to the fact that the candidate trees were selected in quite diverse ecological and geographical sites, the superiority of the candidate trees was standardised with respect to its controls by computing the selection intensity (difference in standard deviation units, between the value of the selected individual and the mean of its control trees). The selection intensity was calculated based on the Chaudhry-Ahmad Probability Density Function (CHAUDHRY & AHMAD 1993) which has been reported to fit well to resin yield distributions of *P. pinaster* in the present study region (NANOS *et al.*, 2000). The Chaudhry-Ahmad Probability Density Function for the random variable *x*, is given by the formula:

$$f(x) = 2(a/\pi)^{1/2}\exp(2a\mu_o^2)\exp(a\mu_o^2((x/\mu_o)^2 + (\mu_o/x)^2))$$

where: x is the resin production (kg), μ_0 the location parameter of the distribution that coincides with the mode and $a\mu_0{}^2$ the shape parameter.

For every experimental plot, maximum likelihood was used for parameter estimation. The expected proportion of trees that produce more than the selected individual was then computed by the integration of the density function as follows:

$$SI = 1 - \int_0^s f(x)dx$$

where: $f(x)$ is the Chaudhry-Ahmad function and s the resin production of the high yielder.

The corresponding selection intensity was then derived from the selection pressure (ratio of no. of selected trees) of an equivalent normal distribution.

Simple regression was used to determine the relationship between the selection intensity and the resin yield of both the control trees and the high yielders. The resin yield of the high yielders was log-transformed to achieve normality.

Analysis of variance (GLM procedure) was used to determine the mean difference of resin yield among clones.

Table 1. Components of variance for resin yield

Source of variation	Mean square of the variance
Among clones	$\sigma_w^2 + k\sigma_d^2$
Within clones	σ_w^2

The clonal heritability for resin yield was estimated with the formula (FALCONER & MACKAY 1996):

$$h^2 = \frac{V_G + V_{EG}}{V_P} = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_{w/k}^2}$$

where: V_G is the genetic variance, V_{EG} is the general environmental variance, V_P is the phenotypic variance, σ_c^2 is the clonal variance and $\sigma_{w/k}^2$ is the variance error.

Simple regression was used to determine the relationship between the resin yield of the ortets (selection intensity of each candidate tree) and their micro-chipped ramets.

RESULTS

Resin production of the control trees has a mean value of 3.7 kg/tree/year over the two years, with a large correlation for individual yield production for the two years ($r = 0.799$, $\alpha = 0.05$). Partial productions are highly correlated with the annual production of the same year, with the most important contribution being that of the mid-summer period (which corresponds to the production of July). The correlation with the total production of the other year is clearly lower. Differences between sites are expressed by the high variability among mean values of the plots (minimum of 2.2 kg/tree/year, maximum of 6.2 kg/tree/year).

In comparison to mean production, actual resin production of the evaluated candidate trees is higher, with a mean value of 7.2 kg/tree/year (minimum of 3.5 kg/tree/year, maximum of 25.1 kg/tree/year). Ten candidate trees (20 %) produced more than 10 kg/tree of resin in each year. One of the candidate trees yielded an average of 25.1 kg/year. Due to the environmental variability affecting the mean values, comparison with control trees of the same plot give a more accurate idea of the superiority of the trees. 24 candidate trees (47 %) produced twice as much resin as their corresponding control trees, and only one candidate tree had a smaller production than the mean of the candidate trees.

Selection intensity values obtained by considering a Normal distribution and by the best fitted function of the Chaudhry-Ahmad Probability Density Function, are highly correlated (r = 0.98). The normal distribution usually over-estimates the selection intensity of the candidate trees (slope of the regression line of the two values: 1.4). Therefore, the value computed from the Chaudhry-Ahmad Probability Density Function has been used.

From the 51 evaluated candidate trees, 28 trees (55 %) obtained a selection intensity of 2 or more (1 tree selected from 20 trees), out of which eleven trees (22 %) yielded a selection intensity of over 3.3 (1 in 800 trees). Three trees obtained a selection intensity greater than 4 (1 in 10,000 trees). Only one candidate tree yielded below the mean of its control trees. A strong correlation (r = 0.75, $p < 1$ %) has been recorded between selection intensity and resin production of the high resin yielders. However, due to the environmental influence on the mean production of the plot, resin production can not be considered to be a good predictor of superior production.

No correlation is observed between selection inten

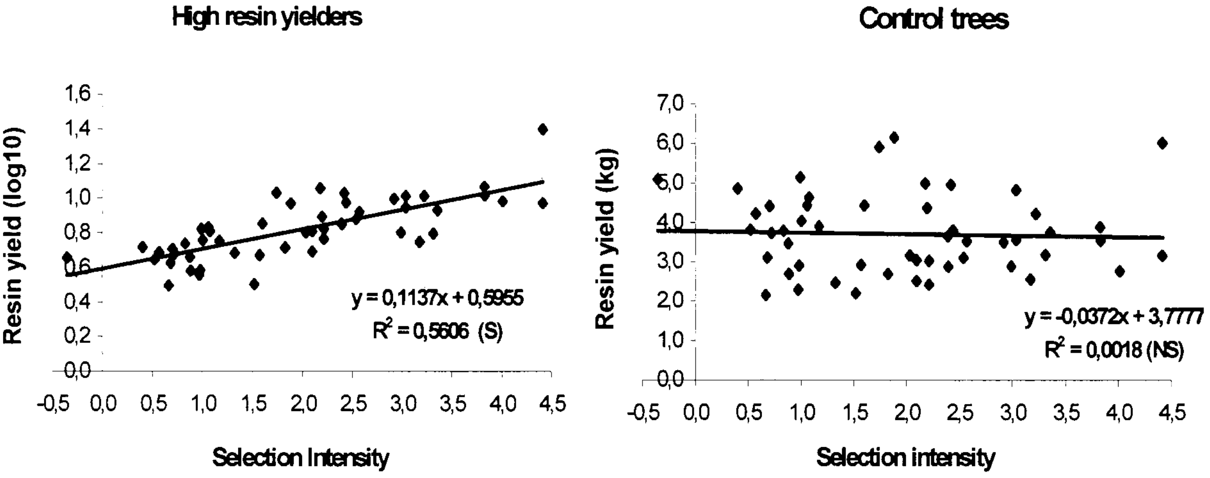

Figure 1. Simple regression between selection intensity and resin yield of the high yielders (left) and the mean production of the control trees (right). Note: (S) significant at 95 % and (NS) non-significant.

Table 2. Comparisons on the resin yield and morphological traits of the candidate trees and their control trees.

Morphological parameters	Mean		t value	P value	Pearson correlation coefficients
	Candidate trees	Control trees			
Resin yield	7.2	3.7	8.2	<0.0001	0.55*
Diameter (cm)	51.1	45.4	6.7	<0.0001	0.75**
Tree height (m)	14.1	15.1	-2.64	0.01	0.49**
Crown height (m)	8.9	7.6	3.59	0.0008	0.25
Crown diameter (m)	9.9	6.7	10.4	<0.0001	0.31*
Density (trees/ha.)	90	136	-4.5	0.0003	0.45*

Note: * and ** significant to confidence levels of 95 % and 99 % respectively.

sity of the candidate trees and the mean yield of the control trees (Figure 1).

Candidate trees, in comparison to their control trees, had slightly lower tree stocking, smaller heights and slightly larger diameters and crown sizes (Table 2). The positive significant correlation of diameters, tree heights and tree stocking between the candidate trees and their control trees demonstrated the homogeneity of these traits in each evaluated plot.

These slight differences do not affect resin production, as a significant correlation was not found between selection intensity and either the measured morphological traits of candidate trees or the tree stocking where the candidate trees were located (Table 3).

There are strong and moderately strong correlations respectively between different morphological traits of the candidate trees: tree height and crown height, diameter and crown diameter, whilst there is a negative correlation between tree stocking and diameter and crown diameter. However, no correlation was found between morphological traits and resin production. From the micro-chipping evaluation of the clonal bank, a moderately strong correlation was recorded between the resin yield of the ortets (evaluated in the present work) and their micro-chipped ramets ($r = 0.63$ significant to a confidence level of 95 %) (Figure 2). From the 17 clones, 2 of them were not acceptable for regression analysis; the ortet of the first rejected clone was not properly evaluated in the field and the coefficient of variation for the second clone was very high. Significant differences in resin yield among clones were found, and a high clonal heritability value ($h^2 = 0.5$) for resin yield was calculated.

DISCUSSION

Different important and interrelated aspects have to be considered during the selection and field evaluation of resin production: the superiority of selected individuals compared to control trees growing under the same conditions has to be established using the proper evaluation method (selection intensity), the correlation between resin yield from the same trees in different production years has to be assessed, and the effectiveness of field evaluation of ortets as opposed to using micro-chipped resin yield of their ramets has to be determined.

The present field evaluations revealed that 55% of the evaluated candidate trees responded positively and have shown superiority over trees of normal production with which they were compared, and there was a moderately strong correlation between the field evaluation of the ortets and the micro-chipped evaluation of their ramets, thus demonstrating the effectiveness of the selection and evaluation methods used.

Strong correlation is obtained between total produc-

Table 3. Correlation between selection intensity and the measured morphological characters of the candidate trees.

	Selection intensity	Resin yield	Diameter	Tree height	Crown height	Crown diameter
Resin yield	0.75**					
Diameter	0.031	0.128				
Tree height	0.064	0.021	0.393*			
Crown height	0.017	0.046	0.427*	0.875**		
Crown diameter	0.052	0.244	0.645**	0.308*	0.390*	
Density	–0.073	–0.252	–0.504**	–0.098	–0.178	–0.402*

Note: * and ** significant to confidence levels of 95 % and 99 % respectively.

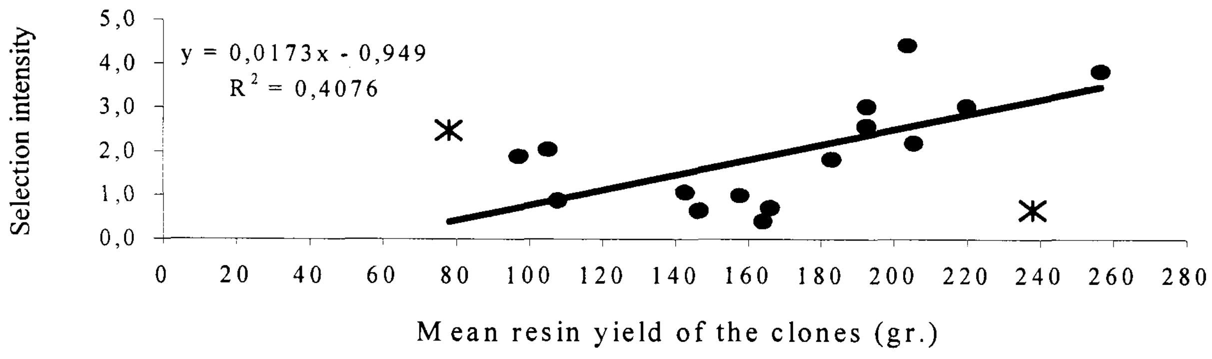

Figure 2. Simple regression of selection intensity of the evaluated candidate trees and the mean resin yield of their micro-chipped ramets.

tion of the two years ($r = 0.799$, significant to a confidence level of 95 %) and among partial and total production of both years. Similar results of correlations were reported by KOSSUTH (1984) with a coefficient of correlation of $r = 0.76$ between the yield of the first 4 streaks of trees and total yield over a 4-year-period in *P. elliottii*. Thus, the positive correlation between partial and total productions and between different production years can be used to predict long term production of specific resin producing areas.

The average production of resin in the present region is estimated to be around 3.5–3.8 kg/tree/year. The mean resin yield of all candidate trees and the control trees was 7.2 kg, and 3.7 kg respectively and therefore, the great majority of the evaluated candidate trees have yielded above the mean yield of the region. CATALÁN (1963) has also reported maximum resin yields up to 36 kg/tree/year from the same species in the same region than the present work. Hence, these results from high resin yielding candidate trees of *P. pinaster* confirm the species' potential in a continued breeding program. SEHGAL *et al.*, (1994) reported similar findings after evaluating high resin yielders of *P. roxburghii*, the other high yielding pine species, with a recorded maximum yield of 9.5 kg/tree/year.

The absence of correlation between selection intensity and the mean production of the control trees, yet the strong correlation between selection intensity and resin production of the high resin yielders, and the high clonal heritability of resin yield demonstrate that the obtained high resin yield was influenced, fundamentally, by genetic factors. This is in accordance with the conclusions reached by MERGEN *et al.*, (1955) and KOSSUTH (1984) that resin yield is under strong genetic control. BURCZYK *et al.*, (1998) also suggested that the intensity of resin production is influenced by a relatively low number of genes with major effects, and the identification of loci for a quantitative trait using molecular markers could be helpful during selection in early life stage.

Moderately strong correlation is recorded between the resin yield of the ortets and their micro chipped clones ($r = 0.63$). This result agrees well with the correlation reported by KRAUS (1965) with $r = 0.661$, significant to a confidence level of 99 % and SQUILLACE & GANSEL (1974) with $r = 0.61$. The strong correlation of these two types of evaluations (field and clonal) confirm the effectiveness of field evaluation of the candidate trees and moreover, guarantees the possible use of field evaluation results to rank and select superior individuals for the breeding programme.

In the present study, a high clonal heritability value ($h^2 = 0.5$) is estimated in the micro-chipped clones of *P. pinaster*. Clonal heritability could be determined by the regression coefficient of resin yield of ortets and ramets. However, in this case the contrasting environmental factors of each different experimental unit, would bias the estimation. This result is comparable with that obtained in other pine species: 0.45–0.9 (SQUILLACE & BENGSTON 1961); 0.38–0.52 (GARRIDO *et al.* 1986a); 0.67 (ALVAREZ *et al.* 1987); and 0.37 (PSWARAYI *et al.* 1996).

As many researchers have pointed out, the best strategy for increasing resin production is to select high yielding trees. The inheritance of gum yielding ability (MERGEN 1954), and the important increment in resin yield with the cross breeding of high yielding parents (MCREYNOLDS & GANSEL 1985) confirm the possibilities for continued work on the genetic improvement programme for resin production. In some pine species selections of high resin yielders have provided substantial improvement in height and diameter growth (GANSEL 1965; SQUILLACE 1965, and SHIMIZU 1980) and improved seed yield and quality (KHIROV 1972) due to genetic correlation between resin yield and these traits.

These field evaluations, the selection of superior genotypes and the further genetic improvement programme for resin yield will improve and promote sustainability in the silvicultural sector; and at the same time will ensure high-grade seed sources (in quantity and quality) and improved growth rate of the plantations. Moreover, the progress of the breeding strategies and the introduction of mechanised resin tapping methods with improved resin collection technique in France and Portugal (LAPORTERIE 1999) will be essential to the renewal of resin tapping and make pine forests more economically viable in the Mediterranean region.

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