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Original article

Increase of flowering in *Pinus nigra* Arn subsp *salzmannii* (Dunal) Franco by means of heteroplastic grafts

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Summary – Behaviour of black pine (*Pinus nigra* subsp *salzmannii*) ramets grafted in 1987 on *P nigra* and *P brutia* rootstocks was compared in a clonal seed orchard located in Guadalajara (Spain). Graft take percentage was lower on average and more variable between clones for interspecific unions than for intraspecific ones; however, later field survival was rather similar. Between 1990 and 1994, heteroplastic grafts displayed a female flowering ranging from four to 13 times higher than homoplastic ones depending on the year, and with 5 years of age, interspecific grafts produced male strobili, nearly absent in the second group. Although seed production is still scarce, due to lack of pollination, heteroplastic ramets seem to yield bigger cones with more sound seeds per cone. Significant differences between both types of grafts were observed regarding stem diameter, branching pattern, apical dominance and crown width. Differentiation of lateral shoots, both floral and vegetative, was higher in heteroplastic grafts, resulting not only in more branches, but also in more strobili of both sexes per branch. Heteroplastic ramets displayed a marked tendency to lose apical dominance, thus supporting their higher number of shoots since the number of branches per whorl is equal in both groups. These results suggest that utilization of *P nigra* grafts on *P brutia* rootstocks, in similar site conditions, may be a helpful tool to obtain more precocious and abundant fruit yields than those derived from the more common use of homoplastic grafts.

seed orchard / flowering / heteroplastic graft / *Pinus nigra* / *Pinus brutia*

Résumé – Accroissement de la floraison chez *Pinus nigra* Arn subsp *salzmannii* (Dunal) Franco au moyen d'hétérogreffes. Une étude comparée du comportement de ramets de pin noir (*Pinus nigra* ssp *salzmannii*) greffés en 1987, sur des porte-greffe de *P nigra* et *P brutia*, a été menée dans un verger à graines clonal situé à Guadalajara (Espagne). Le pourcentage de reprise des greffes est plus faible en moyenne, et présente une plus forte variabilité entre les clones pour les greffes interspécifiques que pour les greffes intraspécifiques. Cependant, leur survie ultérieure est à peu près équivalente. Entre 1990 et 1994, nous avons observé une floraison femelle de 4 à 13 fois plus importante

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(selon l'année) chez les hétérogreffes que chez les homogreffes (fig 1). Cinq ans après transplantation, les hétérogreffes forment des strobiles mâles, alors que ceux-ci sont quasiment absents chez les homogreffes (tableau II). Bien que la production de graines soit peu abondante, en raison d'une faible pollinisation, les ramets hétéroblastisques semblent produire de plus gros cônes (tableau III) contenant plus de graines pleines par cône. Des différences significatives ont été enregistrées entre les deux types de greffe, en ce qui concerne le diamètre du tronc, la ramification, la dominance apicale et la largeur de la couronne. La différenciation de rameaux latéraux, à la fois floraux et végétatifs, est plus forte chez les hétérogreffes, avec non seulement plus de branches, mais aussi plus de strobiles des deux sexes par branche (tableau III). Alors que le nombre de rameaux par verticille est le même dans les deux groupes, les ramets hétéroplastiques présentent un plus grand nombre total de rameaux, dû à une forte tendance à la perte de dominance apicale (tableau IV). Ces résultats suggèrent que l'emploi d'hétérogreffes de *P nigra* sur *P brutia* peut constituer un outil de choix pour l'obtention d'une production plus précoce et plus abondante de graines que celle obtenue par les homogreffes.

verger à graines / floraison / hétérogreffe / *Pinus nigra* / *Pinus brutia*

INTRODUCTION

Natural stands of Spanish black pine (*Pinus nigra* Arn subsp *salzmannii* (Dunal) Franco, present in Spain and the south of France) are the most evolved forest cover in a number of calcareous mountain areas in the eastern half of the Iberian peninsula (Sánchez Palomares et al, 1990; Regato, 1992). In addition, certain provenances yield the best wood among Spanish pines (García and Guindeo, 1988), which increases interest in the conservation and improvement of these genetic resources. In Spain, 370 000 ha were afforested with black pine between 1940 and 1982. Nevertheless, one of the main handicaps for the utilization of this species rests on the difficulty in obtaining seed of good genetic quality, owing to the generally scarce fructification, with marked periodicity of seed crops in natural stands (Ruiz de la Torre, 1971).

For several decades, seed orchards have been planted in some countries of central and southern Europe, where this species has been extensively used for afforestation in continental and sub-Mediterranean areas. In France, Corsican pine seed orchards (*P nigra subsp laricio*) were installed between 1976 and 1981, with annual seed productions of about 20 kg/ha (Arbez, 1987). In Greece, too, seed orchards of black pine from the Peloponesos have been planted; some studies of these having been published by Matziris (1978, 1989).

In Spain, the first *P nigra* seed orchard was installed between 1987 and 1989 as part of the network of clonal seed orchards established by the state forest administration (ICONA), including all Spanish *Pinus* species (Pardos and Gil, 1986). Most ramets of this orchard are grafts on black pine itself, but some others are interespecific grafts on *P brutia* rootstocks, the latter displaying obvious differences from the rest of the ramets regarding their general aspect as well as the flowering of both sexes.

Precedents of heteroplastic grafting in *P nigra* are quite old. In Fontainebleau Forest (France), specimens of this species grafted on *Pinus sylvestris* have been living since the 14th century in healthy condition (Bouvarel, 1960). This same scion-rootstock combination was utilized in the former USSR in order to propagate black pine in regions colder than its natural area (Nitikin, 1963). On the contrary, Monteuuis and Barnéoud (1991) recommended the use of *P nigra* as the rootstock for grafting Scots pine in order to increase the general vigour of the plant, particularly on calcareous soils.

The influence of graft rootstock may become an important tool in breeding programmes of certain forest species (Melchior, 1987), but the information available for these species is scarce compared with the knowledge related to fruit trees, extensively reviewed by Hartmann and Kester (1975). Some of the most prominent applications of rootstock effect are the increment of flowering and the adaptation to different environments; thus, in Spain, grafting of *P pinea* on *P halepensis* has been succesfully employed in order to increase the yield of edible seeds on calcareous soils in the Mediterranean region (Catalán, 1990). There exist precedents of utilization of known specific or clonal rootstocks in seed orchards: in genus *Pinus,* Ahlgren (1972) found important differences in the shape and number of flowers produced by intra- and interspecific graft combinations; similarly, Schmidtling (1973, 1983) reported distinct responses on flowering, graft take and survival of homo- and heteroplastic pine grafts.

In this paper, the influence of *P brutia* rootstocks on the flowering of *P nigra* grafts belonging to different clones in a seed orchard is studied in relation to the morphological differences of the ramets.

MATERIALS AND METHODS

The seed orchard is located near Guadalajara, 70 km northeast of Madrid. Its geographical position is 3°9'E and 41°37'N, 685 m in altitude. Dryness of the site is remarkable, with an annual rainfall as low as 300 mm, with the maximum in the fall, which makes summer watering indispensable. Annual mean temperature is 16 °C, with high summer maxima (average of maxima being 32.4 °C). The soil is clayey, formed from tertiary calcareous deposits. The orchard comprises 56 clones (arranged in 18 randomized blocks, spaced 5 x 5 m) coming from Serranía de Cuenca-Alta Alcarria, a subregion included in the Sistema Ibérico provenance region (Catalán et al, 1991). Black pine natural stands live in this region under a Mediterranean-continental climate, ranging from subhumid to humid depending on the zones, with 850 mm of mean precipitation and a mean annual temperature close to 10 °C. The ortets are located between 990 and 1 500 m of altitude, and were selected according to phenotypic characteristics relevant to growth and straightness.

In 1987, 40 clones of black pine were tip-grafted on *P nigra* (30 grafts per clone) and *P brutia* rootstocks (from five to ten grafts per clone). All the grafts were made under the same conditions by non-specialized workers. From 1990, flowering was assessed in a population of 19 clones, constituted by 30 heteroplastic ramets and 228 homoplastic ones, transplanted to the orchard during the first winter after grafting.

Analysis of the variation of female flowering (FF) in relation to clone and rootstock species was undertaken with data from 1993, the best flowering year to date. In the case of male flowers, the highest number of ramets with strobili was observed in the year 1994. Male flowering (FM) was assessed as the number of male floral shoots, identifiable early in March. In January 1995, fruit cones of homo- and heteroplastic grafts corresponding to three clones were collected, weighted and measured (cl: cone length; cw: cone width). Seeds were extracted, determining the percentage of sound seed. The lack of pollination in the orchard and scarcity of cones in homoplastic grafts, did not allow further statistical analyses related to seed production.

The study of tree structure was carried out in 1993, restricted to the eight clones with a minimum of two interspecific grafts in the orchard. The sampling population consisted of the heteroplastic grafts (19 individuals) and an equal number – randomly selected – of homoplastic ones for each clone, in order to facilitate later analyses. The following parameters were measured in each ramet: crown width (CD, measured on NS and EW axes), height increment in 1993 (HI), needle length (NL, mean length of five needles taken from the top of terminal shoot), stem diameter (SD, measured above graft union), number of total branches (TB), number of branches of first, second and third order (B1, B2, B3) in the upper four whorls and number of branches in the first upper whorl (BW1). Loss of apical dominance in each ramet (LAD) was estimated using a subjective scale from 0 to 3, roughly representing the number of times apical shoot was bifurcated.

Two factor (clone and rootstock species) analyses of variance (ANOVA), based upon a fixed effects model, were undertaken for all the variables studied. In the cases where homogeneity of the variance was not fulfilled, even after having transformed the variable, components of variance were analyzed by means of the Brown-Forsythe statistical test (1974), although this is less powerful than the classic ANOVA. Influence of rootstock species on the frequency of the different degrees of lack of apical dominance (LAD) was evaluated with a Pearson's χ^2-independence test.

Male flowering was assessed only to a descriptive level, owing to the limited number of ramets bearing male strobili. Relationships between all the form and branching variables and female and male flowering were determined with the help of a correlation matrix. All analyses were carried out with BMDP (1990) statistical software.

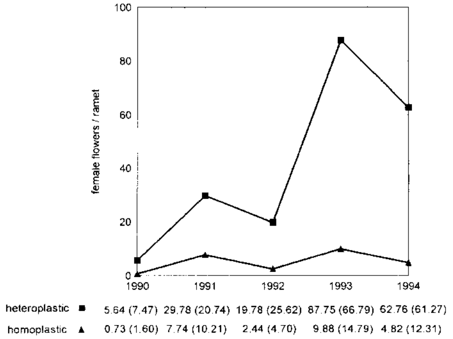

Fig 1. Evolution of female flowering of hetero and homoplastic ramets. Mean values (standard deviation).

RESULTS

Success of heteroplastic grafting was highly variable between clones, while in homoplastic unions clonal differences were less pronounced. This difference is partially explained by the lower number of interspecific grafts made per clone. Average take percentage was 23% on *P brutia* and 56% on *P nigra;* later survival of successful grafts after being transplanted to the orchard, was very similar for both rootstock species (92.3% and 98.1%, respectively), and no failures have been observed since 1991, 4 years after planting. To date, symptoms of delayed incompatibility have not been detected in heteroplastic ramets, graft union being identifiable only by the different bark appearance.

Female flowering in this seed orchard has shown sharp fluctuations in the period of study (fig 1), which may be due to climatic causes as well as to the species' characteristic masting. Superiority of heteroplastic grafts is evident: the number of strobili per ramet ranges from four times higher in 1991, up to 13 times in 1994, with an average of more than eight times. In the same figure it may be observed that higher production of female strobili in grafts on *P brutia* is accompanied by increased precocity and less pronounced interannual fluctuations on a percentage basis; thus, decreases found in 1992 and 1994 were about half of the reduction suffered by homoplastic ramets. Statistical analyses reveal significant differences between grafts of the different rootstocks, and no significant differences either for clonal effect or clone x rootstock species interaction (table I).

Male flowering also indicates a possible positive effect of *P brutia* rootstock (table II). It is remarkable that, while heteroplastic grafts bore male strobili since the age of 5 years, increasing year by year, in homoplastic grafts, values are practically null for the period of study.

Table I. Brown-Forsythe (1974) test for female flowering of 1993.

Source of variation	*DF*	*F*	
Clone	18, 3	4.97	ns
Rootstock species	1, 3	176.99	***
Interaction (C x S)	18, 3	2.94	ns

DF: degree of freedom; F: value of F statistics; ns: not significant; *** significant at 99.9% level.

Table II. Male flowering per ramet and type of graft given in means ± (standard deviations).

Graft	*1992*	*1993*	*1994*
Heteroplastic	2.03 (7.80)	8.73 (27.53)	10.33 (19.72)
Homoplastic	0.10 (1.11)	0.02 (0.27)	0.16 (1.07)

Table III. Parameters descriptive of shape, mean values ± (standard deviations) for hetero- and homoplastic grafts.

Variable	*Homoplastic grafts*	*Heteroplastic grafts*	*Signification level (grafts)*	*Signification level (clone)*
FF 1993	9.88 (14.79)	87.75 (66.79)	***	ns
FM 1994	0.16 (1.07)	10.33 (19.72)	–	–
CD (m)	1.265 (0.383)	1.734 (0.484)	**	*
HI (cm)	39.15 (14.50)	42.31 (11.3)	ns	ns
NL (mm)	17.44 (2.24)	19.61 (2.9)	**	**
SD (cm)	7.05 (1.921)	11.97 (2.342)	***	ns
BW1	4.52 (1.02)	4.15 (1.38)	ns	ns
B1	17.36 (3.48)	22.73 (8.99)	*	ns
B2	41.26 (22.37)	62.57 (36.04)	*	ns
B3	5.00 (10.60)	11.58 (12.80)	ns	ns
TB	70.15 (39.58)	143.52 (76.46)	***	ns
LAD	0.00 (0.00)	1.15 (1.21)	***	ns
FF/(B1 + B2)	0.16 (0.18)	0.99 (0.93)	***	ns
cl (mm)	55.31 (7.39)	67.44 (4.60)	***	ns
cw (mm)	22.41 (3.23)	30.36 (1.86)	***	**

FF: female flowers; FM: male flowers; CD: crown diameter; HI: height increment; NL: needle length; SD: stem diameter; BW1: branches in the first whorl; B1, B2 and B3: branches of first, second and third order; TB: number of total branches; LAD: loss of apical dominance, cl: cone length; cw: cone width; *** significant at 99.9% level; ** significant at 99% level; * significant at 95% level; ns: not significant.

In relation to cone size, it can be observed in table III how cones of heteroplastic ramets are significantly longer and wider than those belonging to homoplastic grafts of the same clones. The analysis of variance showed a slight clonal influence in cone width. On the other hand, although the number of seeds extracted per cone was similarly low in both types of grafts, the percentage of sound seeds was higher in heteroplastic ramets.

Homoplastic and heteroplastic ramets displayed remarkable morphological differences, as indicated by mean values of the parameters included in table III. Values are higher for *P brutia* grafts for all the variables analyzed, except for the number of branches of the first whorl. Results obtained for the rest of the parameters related to branching pattern are clear: heteroplastic grafts displayed a higher number of branches of the three orders, so their crowns are more densely ramified. This circumstance is confirmed by the presence, not included in the table, of fourth-order branches exclusively in three heteroplastic ramets corresponding to different clones. In table IV, the differ-

Table IV. Number of ramets with different levels of loss of apical dominance (LAD) for homo- and heteroplastic grafts.

LAD	*Homoplastic graft*	*Heteroplastic graft*
0	19	8
1	0	4
2	0	3
3	0	4

O: single terminal leader; 1: bifurcated main stem; 2: several codominant leaders; 3: absence of apical dominance. Probability of H_0 (frequencies independent of type of graft) = 0.0014.

ent frequencies of LAD values for both types of grafts are shown. Rootstock influence in the observed frequencies is clearly significant (P (H_0) = 0.0014); grafts on *P brutia* rootstock have a much higher tendency to lose apical dominance, which corresponds to their higher branching density and crown width, since the number of branches per whorl (table III) is equal to that of grafts on *P nigra*.

Values of the calculated variable FF/(B1 + B2) also scored lower in homoplastic ramets, which bore six times fewer female strobili per shoot of the first and second order.

Analyses of variance revealed that the rootstock effect fails to be significant for height increment, number of branches in the upper whorl and number of third-order branches. On the other hand, a significant clonal influence has been detected for crown width and needle length; the interaction clone x rootstock was not significant in any case.

Table V gathers correlation coefficients between form variables of the ramets and flowering of both sexes. The number of female strobili was found to be related to the variables concerning the size of vegetative structures: crown and stem diameters, needle length and number of total branches. The low correlation between female flowering and current height growth, as well as with branches of first, second and especially third order is noticeable, whereas correlation with lack of apical dominance is highly significant. This last parameter displayed also a clear relationship with the number of male strobili, unlike the rest of the variables, which were poorly correlated with this trait. Absence of correlation between female flowering and number of third-order branches gives power to the variable FF/(B1 + B2) as an indicator of flowering density in the crown.

DISCUSSION

Differences in initial graft success could suggest a certain degree of incompatibility in heteroplastic grafts. However, their satisfactory later performance seems to indicate that lower percentage is due to mechanical factors such as the *P nigra* buds (scions) being notably bigger than the shoots of *P brutia* rootstocks. The taxonomic proximity of scion and rootstock in interspecific grafts, both species being included in subsection *sylvestres* (Little and Critchfield, 1969; Schirone et al, 1991), may explain the lack of delayed incompatibility. Moreover, regarding observations by other authors (Corti, 1968; Jakovleva, 1970), pines from the *halepensis* group used as the rootstocks, display good graft compatibility with a great number of species, not necessarily those that are genetically close.

Grafts of black pine on *P brutia* showed a more abundant and precocious flowering than homoplastic grafts of the same clones. This behaviour of heteroplastic ramets in relation to flowering – and presumably to seed yield – runs in parallel with that observed by Holzer (1970) in *Pinus cembra* on *P griffithii* and *P sylvestris* rootstocks, and by Schmidtling (1973) in *Pinus taeda* on *P virginiana*, even when the differences observed in the case of *P nigra* are

Table V. Correlations between morphological characteristics of grafts and female and male flowering.

	Loss of apical dominance (LAD)	*Crown diameter (CD)*	*Stem diameter (SD)*	*Current height growth (HI)*	*Needle length (NL)*	*No of branches (1st order) (B1)*	*No of branches (2nd order) (B2)*	*No of branches (3rd order) (B3)*	*No of total branches (TB)*
LAD		0.498**	0.564***	0.093	0.411*	0.509**	0.490**	0.530***	0.582***
CD			0.761***	0.546***	0.710***	0.474**	0.801***	0.532***	0.714***
SD				0.414**	0.525***	0.463**	0.658***	0.551***	0.788***
HI					0.401*	0.205	0.497**	0.401*	0.507**
NL						0.369*	0.484**	0.371*	0.443**
B1							0.661***	0.397*	0.388*
B2								0.671***	0.748***
B3									0.708***
FF 93	0.759***	0.670***	0.681***	0.186	0.574***	0.333*	0.383*	0.274	0.570***
FM 94	0.603***	0.338*	0.385*	–0.018	0.137	0.010	0.092	0.281	0.378

FF: female flowers; FM: male flowers. *** Significant at 99.9% level; ** significant at 99% level; * significant at 95% level.

much more impressive. Results reveal, in addition to a higher flowering each year studied, a moderation of masting effect induced by the rootstock of *P brutia*. This is shown by less pronounced interannual percentage oscillations of flowering in heteroplastic than in homoplastic grafts.

The absence of differential response between clones, with respect to their rootstock species, suggests the possibility of extending the utilization of heteroplastic grafts to a wide number of genotypes. The scant clonal influence on flowering is particularly striking, mainly because these effects are often a notorious problem in seed orchards (Sweet, 1975).

Results of morphological study reveal a higher development of vegetative structures – either macroblasts or needles – in heteroplastic ramets, as well as a marked tendency to lose apical dominance. Some form traits induced by *P brutia* rootstock have shown correlations with flowering: wide crown, long needles, dense branching and reduced apical dominance. The relationship between production of strobili and needle length was formerly pointed out by Ahlgren (1972) in different interspecific scion–rootstock combinations of *Pinus*, which he attributed to a higher level of photosynthesis in branches. However, these traits of heteroplastic grafts mentioned hitherto are nearly opposite to those upon which ortet selection, aimed at the improvement of timber production, was based. This opposition between characteristics desirable for genetic improvement and those related to an acceptable fruit yield was demonstrated in *P sylvestris* by Nikkanen and Velling (1987) and, as mentioned by Giertych (1987), the development of techniques for getting round this problem is one of the biggest challenges to the productivity of seed orchards.

Bigger size and branching density of the crown in heteroplastic grafts under study do not by themselves explain the differences in strobili bearing, which has been underlined by heavier flowering density in these ramets, represented by the variable FF/(B1 + B2). Thus, the influence exerted by *P brutia* rootstock on graft flowering may have a physiological cause, a reasonable supposition being the effect of a growth factor synthetized in the root (Bonnet-Masimbert and Zaerr, 1987).

The proven superiority of heteroplastic grafts for a *P nigra* seed orchard in similar conditions would advance its productivity by several years, and lead to more abundant and regular crops. On the other hand, absence of pollen formation in seed orchards is one of the main causes of production delay (Giertych, 1987), so the precocity in male flowering is particularly interesting for seed orchard management. It must be borne in mind, however, that lower take percentage in interspecific grafts may require a greater number of grafts at this stage of the improvement programme, and, even if symptoms of incompatibility have not been revealed up till now, we cannot dismiss the shortening of yield-life in grafts on *P brutia* in comparison with those made on *P nigra*.

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