

EARLY GROWTH ANALYSIS OF HALF-SIB FAMILIES IN ASH (*FRAXINUS OXYPHYLLA* BIEB., *FRAXINUS OXYCARPA* WILLD.): CONSEQUENCE FOR EARLY SELECTION AND FOR BREEDING

Alessandro Camussi¹, Raffaello Giannini² & Federico Mattia Stefanini¹

¹ Institute of Silviculture, University of Florence, Via San Bonaventura 13, I-50145 Florence, Italy

² Institute of Forest Tree Breeding, C.N.R., Via Atto Vannucci 13, I-50134 Florence, Italy

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ABSTRACT

Natural populations of forest trees generally show a large amount of variability in many traits. Breeding programs need proper investigations on individuals with very favorable growth characteristics since the early stages of development. In the paper, several growth traits of a set of 22 half sib (HS) families of *Fraxinus oxyphylla* Bieb. (*Fraxinus oxycarpa* Willd.) are studied during the early years of growth in order to evaluate the stability of "height" under two silvicultural practices (*i.e.*, transplanting and not in the forest nursery). The statistical evaluation of HS families was performed using linear models on original variables and on the variables obtained as differences between subsequent years.

A large variability of traits among families is found and the influence of transplanting on the growth pattern of the plants is demonstrated. Very tall plants in early stages of development can be of practical interest in ash because their superiority tends to remain unchanged.

Key words: *Fraxinus oxyphylla*, linear model, growth analysis

INTRODUCTION

Natural populations of forest trees generally show a large amount of variability in many traits, allowing high potentiality in breeding and silvicultural practices (ZOBEL & TALBERT 1985; MÜLLER-STARCK *et al.* 1992).

Similar to many conifers and other outcrossed species, the presence of a relevant amount of variability can be seen and investigated in many broad leaf species starting from the first year of growth, even if a reduced number of plants is observed. Ash species are a typical example of the phenomenon, where populations at early stages of growth can be characterized by the presence of so tall individuals that we suggest to define them as "giant" (Fig. 1).

Many ash species and particularly *Fraxinus oxyphylla* Bieb. (*Fraxinus oxycarpa* Willd.) are economically important as regards high quality timber production.

Despite their economic relevance, few are the studies on the biological components of yield (COLLIN *et al.* 1995; MARINOV 1991).

For this reason the existence of individuals showing very favorable growth characteristics at first stages of development can be of the greatest interest in breeding strategies if this traits is further conserved in the following years of growth, being genetically controlled.

Moreover it is relevant also to know if the seedlings larger in height and in collar diameter are able to maintain their superiority even if standard cultivation techniques are applied, *i.e.*, without modifying the nursery practices to take into account for superior individual requirements as regards nutrient and water supplies.

In the paper several morphological traits of a set of 22 half sib (HS) families of *Fraxinus oxyphylla* are examined during the early years of growth, taking into account their variability and age to age stability parameters. The importance of trait stability is considered in the frame of choosing an optimized breeding strategy in early stages of development.

MATERIAL AND METHODS

During the fall of the 1989, seeds were collected from 22 single trees of natural stands of *Fraxinus oxyphylla* located in different areas of Tuscany and Emilia-Romagna regions of Italy (Fig. 2). After collection, the seeds were sowed in nursery and seedlings were cultivated with local standard practices.

At the end of the first growing season (1990), height and diameter were measured on individual plants within a random sample of at least 500 plants for each of the 22 HS families. Individual data were sorted and within each family two seedling sub-groups were distin-

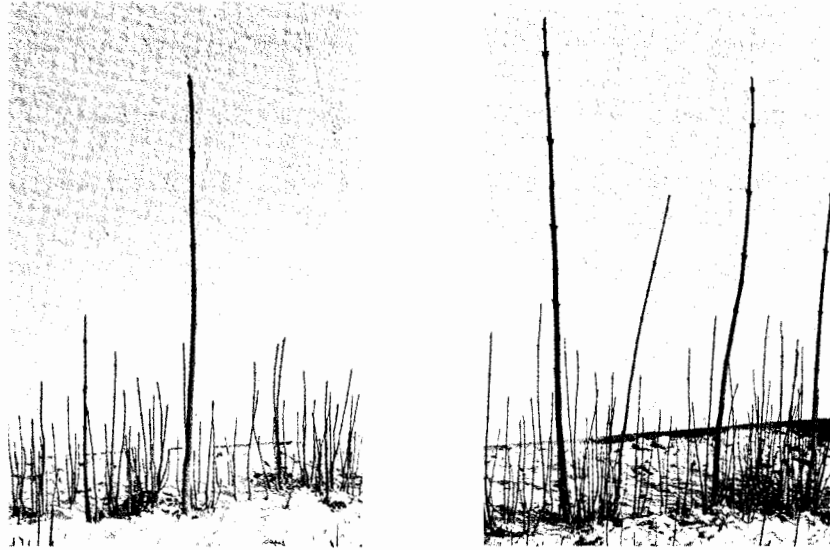


Figure 1 Typical example of "giant" seedlings

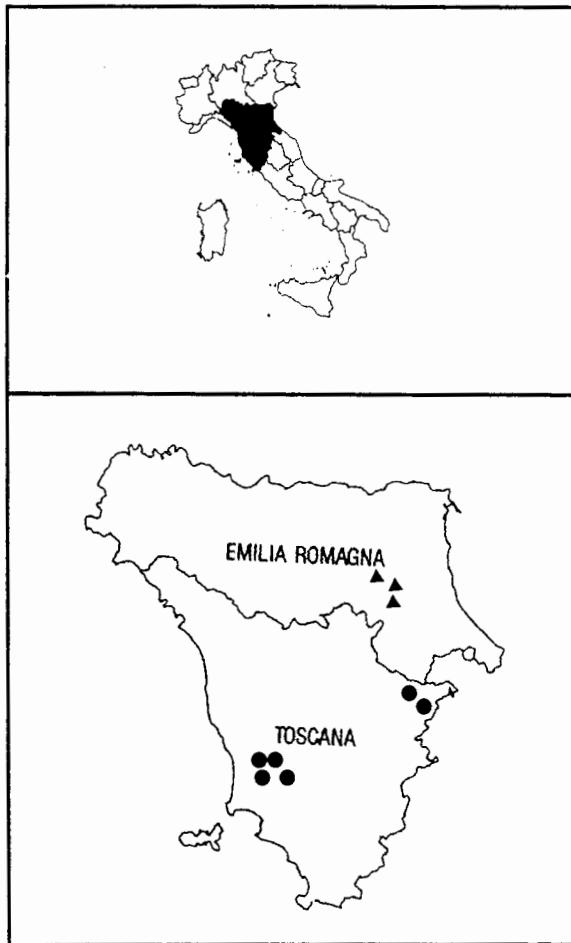


Figure 2 Location of sampled trees

guished: the 30 tallest plants (*P* – *plus*) and a representative sample of 30 plants of the smallest ones (*m* – *minus*). Extreme small individuals, not in conformity with minimal standards of sturdiness, were excluded from the sampling .

The sampled plants were transplanted in containers and cultivated in nursery up to the February of 1992. In order to evaluate the effect of transplanting from containers to forest, half of the *P* and *m* plants were transplanted in field following a complete randomized single tree plot design.

STATISTICAL METHODS

A table of mean values was obtained to describe average performances of HS families under different experimental conditions, such as sub-grouping, year of observation and transplanting effect. A table of mean differences between *Plus* and *minus* sub-groups is reported to point out the amount of changes occurring with time.

The following univariate linear model (SEARLE 1971) was applied to single plant data:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \alpha_i \cdot \beta_j + \gamma \cdot x_{ijk} + \epsilon_{ijk} \quad [1]$$

here y_{ijk} is the observation of plant k of the family i and assigned to the *P/m* (*Plus-minus*) subgroup j ; α_i is the effect of i -th HS family, β_j is the effect of j -th *P/m* sub-

grouping, $\alpha\beta_{ij}$ is the interaction term, γ is the regression parameter of the covariate X (the collars diameter of one-year old seedlings in 1990), and ϵ_{ijk} represents the residual. All the considered effects are fixed.

A modified model was applied to 1992 data. The linear model becomes:

$$y_{ijkw} = \mu + \alpha_i + \beta_j + \alpha_i\beta_j + \gamma \cdot x_{ijkw} + \delta_w + \alpha_i\delta_w + \beta_j\delta_w + \epsilon_{ijkw} \quad [2]$$

where δ_w represents the effect of transplanting. Second order interactions are considered.

An analysis of residuals (Studentized Residuals and Cook's Distance procedures) was performed to check the validity of model assumptions and other models were investigated to obtain an optimal fit for the distribution of residuals. Typical transformations (square root, log, etc.) and a weighted least square estimates approach (CARROLL & RUPPERT 1988) were not effective. Distributions of the exponential family (MCCULLAGH & NELDER 1989) in the generalized linear model

approach did not produce any sensible gain large enough to motivate an increase of model complexity.

The residuals within each year were considered to assess phenotypic stability, because they represent the amount of information that is not explained by the systematic causes of variation, as family and P/m sub grouping. Pearson moment correlations were calculated between years to estimate the age-age stability of individual performances.

Two multivariate linear models for the Repeated Measurements Analysis of Variance (COLE & GRIZZLE 1966) with fixed effects were applied to account for experimental sources of variation (family, Plus-minus sub-group, transplanting, time, interactions) and including plant diameter as covariate. Formally the profile analysis can be represented for height of 1990 by equation [1] where y is the difference between individual values 1991-1990, and by equation [2] where is the difference between individual values 1992-1991 (MORRISON 1967).

Data analysis was performed using SAS/STAT rel. 6.08 (SAS Institute, Cary, USA).

Table 1 Height mean values (cm) of *Plus* and *minus* subgroups within 22 HS families of *F. oxypylla*. 30 individuals in each subgroup were considered. The last two columns on the right report height mean values of *Plus* and *minus* sub-groups subdivided taking into account the absence or presence of transplanting

Year Family	1990		1991		1992 Plus group		1992 minus group	
	Plus group	minus group	Plus group	minus group	T	NT	T	NT
1	31.25	10.55	91.75	74.25	103.78	138.50	84.67	103.30
2	31.75	8.90	96.50	78.25	110.80	163.00	93.80	116.00
3	32.75	11.30	87.75	69.35	105.67	126.50	80.38	103.25
4	24.00	10.65	82.56	65.60	97.44	126.88	73.78	103.20
5	16.94	8.35	90.44	70.85	112.00	131.33	87.22	98.56
6	44.88	12.10	99.63	64.05	125.20	133.80	90.00	88.22
7	25.60	9.30	94.40	71.70	116.57	141.00	81.64	99.67
8	36.92	16.48	108.14	70.02	125.83	142.25	93.13	102.76
9	42.70	16.84	105.73	66.06	127.56	140.52	74.00	106.22
10	23.80	9.08	81.24	49.22	103.86	115.21	64.75	89.15
11	21.90	7.68	89.54	58.40	126.43	131.89	76.90	107.33
12	22.95	9.70	89.72	60.35	111.80	120.27	79.56	94.78
13	27.55	10.40	103.95	64.35	130.50	129.33	80.90	86.89
14	27.32	12.03	108.92	64.97	134.60	149.86	85.33	99.37
15	26.20	9.67	107.76	70.23	140.43	137.38	88.90	109.75
16	26.75	9.80	114.53	70.40	147.29	140.87	84.22	103.84
17	28.00	9.80	91.97	49.77	118.88	129.10	73.56	95.28
18	23.00	7.67	85.33	47.07	102.30	125.25	70.70	92.22
19	24.71	7.77	88.54	53.07	115.55	135.07	75.55	106.44
20	38.16	8.35	71.58	42.85	96.40	98.67	56.70	71.00
21	47.42	10.10	85.54	56.47	105.90	130.08	79.50	96.53
22	34.50	10.73	87.16	45.10	110.56	119.50	61.25	70.00
Mean	29.96	10.33	93.76	61.93	116.79	132.10	78.93	97.44

Table 2 Mean height value differences (cm) between *Plus* and *minus* groups within families. In 1992 mean values are classified as T = transplanted, NT = not transplanted

Family	Year	1990	1991	1992	
				NT	T
1		20.70	17.50	35.20	19.11
2		22.85	18.25	47.00	17.00
3		21.45	18.40	23.25	25.29
4		13.35	16.96	23.68	23.66
5		8.59	19.59	32.77	24.78
6		32.78	35.58	45.58	35.20
7		16.30	22.70	41.33	34.93
8		20.44	38.12	39.49	32.70
9		25.86	39.67	34.30	53.56
10		14.72	32.02	26.06	39.11
11		14.22	31.14	24.56	49.53
12		13.25	29.37	25.49	32.24
13		17.15	39.60	42.44	49.60
14		15.29	43.95	50.49	49.27
15		16.53	37.53	27.63	51.53
16		16.95	44.13	37.03	63.07
17		18.20	42.20	33.82	45.32
18		15.33	38.26	33.03	31.60
19		16.94	35.47	28.63	40.00
20		29.81	28.73	27.67	39.70
21		37.32	29.07	33.55	26.40
22		23.77	42.06	49.50	49.31
Mean		19.63	31.83	34.66	37.86

Table 3 Results of univariate ANOVA of height measurements of 22 HS families of *F. oxyphylla* including stem diameter as covariate

Source of variation	DF	Mean Square		
		1990	1991	1992
Diameter (<i>covariate</i>)	1	4380.8139***	14229.587***	6802.3483***
Family (F)	21	1246.4242***	5002.040***	3812.5045***
Sub-group (<i>Pm</i>)	1	17528.7802***	41471.904***	3187.3043***
F/ <i>Pm</i> interaction	21	470.4833***	802.730***	599.5138***
Transplanting (T)	1			8234.8712***
F/T interaction	21			592.3082**
<i>Pm</i> /T interaction	1			635.8863
Error 1990	1187	46.001		
1991	1186		17.405	
1992	996			284.156
R ²		0.74	0.70	0.64

Table 4 Results of the ANOVA of the height increments, obtained as difference between individual pairs of height values in successive years

Source of variance	DF	Height increment	
		Mean square	
		1990-1991	1991-1992
Mean	1	63810.9040***	23275.5640**
Diameter (<i>covariate</i>)	1	3129.5921***	345.1584
Family (F)	21	5304.1927***	707.6453**
Sub-group (<i>Pm</i>)	1	4783.7128***	1311.4377*
F/ <i>Pm</i> interaction	21	1463.5364***	230.0413*
Transplanting (T)	1		54092.0408**
F/T interaction	21		293.7692*
<i>Pm</i> /T interaction	1		150.5184
Error (1990-1991)	1186	187.6582	
Error (1991-1992)	996		149.5416

RESULTS

Mean heights of each sub-group in each family measured after 3 subsequent growing periods show that *P* individuals remain tall along their development in years (Table 1). The last two columns of the table classify the 1992 data by transplanting event. The superiority in mean height of *P* sub-groups tends to remain unchanged within the same subset of transplanted individuals, even if transplanting has induced an overall reduction growth rate.

Differences of family mean values between *P* and *m* groups in the considered years are reported in Table 2. A large variability of differences is shown among families, but 1992 data demonstrate the influence of transplanting on the growth pattern of the plants. Being reduced the difference in height between *P* and *m* subgroups in not transplanted plant, this stress source seems to be more effective on *m* group.

ANOVA results are reported in Table 3. All hypotheses of null effects of the different factors (families, sub-groups, interaction) were rejected with a very high significant level, including the linear effect of the covariate plant diameter.

The linear models applied to 1990 and 1991 data explained more than 70% (R^2) of the height variability among families and only 64% in 1992.

Two new auxiliary variables were calculated to verify null hypothesis about the effect of the sources of variation described above on the growth dynamics. These two height increments were obtained as differences of pairs of values measured in subsequent years

Table 5 Product-moment correlation coefficients (Pearson's *r*) between residuals from the linear models for height in the three considered years of growth. Within the correlation matrix, the second row indicates the probability of "no correlation" hypothesis, and the third row indicates the number of observations

	Year 1991	Year 1992
Year 1990		
correlation	0.1648	0.0497
probability	0.0001	0.1051
individuals	1231	1064
Year 1991		
correlation		0.6759
probability		0.0001
individuals		1064

(1991-1990 and 1992-1991) and analyzed by means of the same models in equations [1] and [2]. ANOVA results are reported in Table 4. All hypotheses of null effects of the different factors (families, sub-group, interaction) were rejected with a very high significant level, as regards the height increment 1990-1991. The ANOVA of second height increment (1991-1992) included also the transplanting effects and related interactions. Only the diameter and the interaction sub-groups by transplanting were not significant.

The stability of growth performances between years can be better assessed by means of age to age correlations. As single years data rejected all the hypotheses

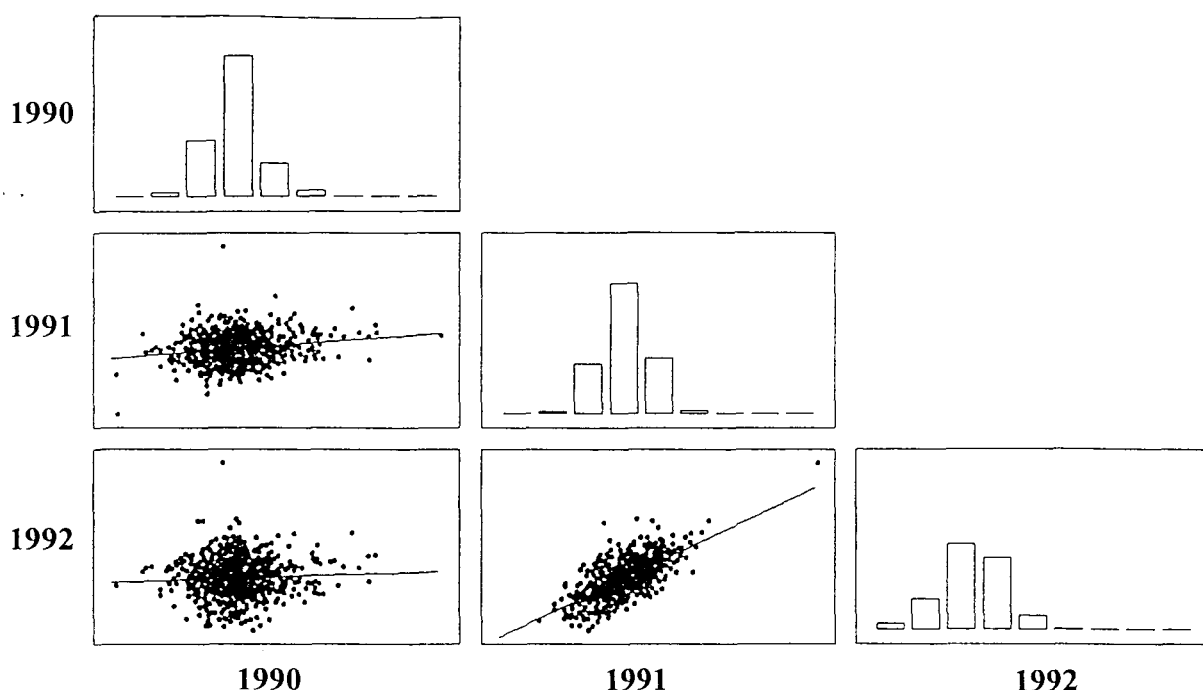


Figure 3 Graphical representation of the correlation patterns among residual from the adopted mean models in the years 1990, 1991, and 1992. The figures on the diagonal indicate the frequency plot of residuals within each year, meanwhile other plots include the correlations between residuals of each year

included in the ANOVA, the correlation analysis was performed on the residuals from the fitted models.

Pearson's product-moment correlations among residuals of different years are reported in Table 5, while the residual distributions are reported in Fig. 3. The lower correlation obtained between the 1990 and 1991 residuals suggests that phenotypic diversity of heights is not fully conserved, probably because of differences in growing performances. On the contrary the high correlation observed between values of 1991 and 1992 suggests a more powerful predictive ability, probably due to an increased stability in growth dynamics after the second year of growth.

The null hypotheses of no correlation was accepted only for the 1990 and 1992 data. The correlation between 1991 and 1992 heights received an higher significant value than the 1990–1991 one.

DISCUSSION

The existence of large genetic variability is a common result in most of the forest tree studies. The existence of very tall individuals can be often observed in nurseries in early stages of growing. In the ash families derived from sampling in different area in Central Regions of Italy, it seems a very common phenomena, in such extent to induce the definition of large seedlings as giants. Their presence in nurseries is generally underes-

timated because normal breeding practices impose the use of standard techniques as regards transplanting, container size and subsequent market requirements. There is consequently a lack of data on the future performances of such giant plants being they generally discarded by the practical nursery management. Our data suggest that giant plants can be of practical interest in ash, because their superiority tends to remain unchanged. The existence of significant age to age correlations is an index of a genetic basis of the observed early development characteristics, even if further sound investigations are to be planned.

The effect of sorting the plants according of their growing performance (the *Plus-minus* grouping) was important at least at family mean level. An uniform nursery practice, as usually applied, induces a sort of negative selection pressure that is expected to be strong against tallest individuals.

Transplanting shocks are a component of this pressure and new procedures are to be introduced to minimise its effect particularly for giant plants. If this point is solved, our findings suggest that a clonal selection procedure, taking into account also the family mean level, can be effective in ash, at least when large variability in progenies is present as in most of the families we have considered.

A large family effect was observed along with a different amount of variability within families for height

as demonstrated by the significant interactions. Many can be the explanations for such important differences among families, but mainly it is to be considered that the sampling strategy included mother plants growing in different and distant locations of Central Italy. The existence of individual variability among mother plants is an important finding as regards the possibility of an early selection procedure for growth performance in ash.

Finally the adopted statistical methodology was effective to study the variability in height and its time profile, even with a minimum increase of assumption fitting: it was demonstrated that an optimal exploitation of natural species for height characteristics in time should consider also the effects of families, nursery practice and the initial collar diameter.

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