Tree Genetics & Genomes (2015) 11: 73
DOI 10.1007/s11295-015-0900-z

ORIGINAL PAPER

Genetic growth parameters and morphological traits of canker-resistant cypress clones selected for timber production

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Received: 27 January 2015 / Revised: 2 June 2015 / Accepted: 9 June 2015 / Published online: 27 June 2015

Abstract Common cypress is widespread throughout the Mediterranean area, where since ancient times, it has been used as a multipurpose tree. In the past, cypress woods were also greatly exploited as a source of strong and durable timber, but nowadays, the availability of cypress timber is extremely limited. The creation of new cypress plantations with properly selected genotypes could sustain a valuable market for high-quality timber. In this study, ten 25 year-old *Cupressus sempervirens* var. *horizontalis* canker-resistant clones grown in two plantations located in central Italy were assessed for timber production. The aim of the work was to evaluate the genetic and environmental influences on growth and morphological traits for each clone. Growth traits, branch characteristics (size and insertion angle) and heartwood content offered potential in the selection for timber quality and quantity, while the selection of clones based upon stem form and branch number did not seem easily practicable. Selection for growth should be based on tree height since it results in fewer adverse effects on other properties such as stem form and branching. Insertion angle resulted the most interesting trait, as it showed very high repeatability values and favourable genetic correlations. Heartwood content correlated positively with growth traits, so it may be indirectly favoured in the course of future improvements in clone productivity on the basis of growth. The marked effect exerted by site on all growth traits underlines the importance of identifying suitable locations for cypress plantations.

Keywords *Cupressus sempervirens* · Stem form · Branch traits · Clonal repeatability · Genetic correlations · Genetic gain

Communicated by Z. Kaya

Topical Collection on *Disease Resistance*

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Introduction

Since the 1990s (Reg. CEE 2080/92), the planting of trees on agricultural land has been an important component of the Common Agricultural Policy (CAP) aimed at obtaining both economic (shortage of timber production, surplus of agricultural production, need to create new sources of income) and environmental benefits (biodiversity and landscape conservation, soil erosion control and CO_2 capture). Common cypress (*Cupressus sempervirens* L.) is widespread throughout the Mediterranean area as a native or naturalised species. The cypress is an important component of Mediterranean culture, being used as a multipurpose tree since ancient times for its valuable timber and for ornamental, symbolic and landscape uses, such as soil protection and as windbreaks (Andreoli and Xenopoulos 1990; Xenopoulos et al. 1990; Della Rocca et al. 2007). Common cypress tolerates different pedoclimatic conditions, growing under Mediterranean climates from sea level up to 2000 m a.s.l. on a wide variety of bedrock formations and soil types, even when poor, dry, calcareous or clayey (Zohary 1973; Gellini and Grossoni 1996).

Two morphological varieties of common cypress are known: *C. s.* var. *sempervirens* L. (sin. var. *stricta* Aiton var. *pyramidalis* Nyman, var. *fastigiata* Hansen) which is characterised by fastigiated habit (main branches growing

upward and inserted in the trunk with a narrow angle) and a columnar crown, probably selected for ornamental planting in ancient times (Bolotin 1964; Makkonen 1968) and *C. s*. var. *horizontalis* (Mill.) Aiton. which has a broader crown habit with the main branches inserted in the trunk at a 60–90° angle. The latter variety is widely represented in the wild common cypress populations growing in Turkey, Cyprus, Rhodes and Crete (Gellini and Grossoni 1996; Farjon 2005). The var. *horizontalis* has always been generally preferred over var. *sempervirens* for timber production due to the single stem, the lower number of branches and wider angle of branch insertion in the trunk (lower knots and lower knot size). Since the second half of the last century, the use and cultivation of cypress has been limited by the spread of the bark canker, a pandemic disease due to the pathogenic fungus *Seiridium cardinale* (Wag.) Sutton and Gibson, which in the Mediterranean basin has caused heavy damage and losses in woods, plantations, gardens, parks and nurseries (Graniti 1998; Danti et al. 2013), thus negatively affecting the landscape. As part of an integrated control strategy against the disease, a genetic improvement programme of cypress was started in the 1970s by the Institute for Sustainable Plant Protection (IPSP-CNR) (Raddi et al. 1984; Santini and Di Lonardo 2000; Danti et al. 2006, 2012, 2013).

Several canker-resistant clones have been selected by IPSP-CNR. Among these varieties, six *C. sempervirens* clones were patented in 1990 and successfully sold for ornamental purposes, while others have been used in multiclonal plantations in European projects aimed at protecting the land against natural risks (Panconesi and Raddi 1991; Danti et al. 2006, 2012). At present, no further selection or use of cypress clones resistant to canker have been implemented in timber production. Nonetheless, cypress wood has always been appreciated for its technological properties such as fine texture, high natural durability as well as good mechanical and aesthetic traits which make it suitable for joinery, buildings, furniture and uses indoors and outdoors. Production of house doors and windows with cypress wood is typical of central Italy (Andreoli and Xenopoulos 1990).

Common cypress is also characterised by a high potential production, being able to achieve yields as high as 8–9 mc/ha/year when planted in favourable sites (Andreoli and Xenopoulos 1990; Danti et al. 2007), although for hydrogeological purposes, most woods were planted on poor sites where growth is strongly limited. Currently, in Mediterranean countries, there is not a real market of cypress wood, mainly due to a lack of plantations assigned to timber production. So the current supply is poor and limited to some regions of central Italy, where it derives mainly from sanitation cuttings of cankered plantations or from cuttings of small groups of trees. Industrial exploitation of cypress wood would be possible if a constant supply of the raw material was ensured. The aim of this study is to therefore evaluate the use of selected cypress genotypes in plantations assigned to the production of high-quality wood (arboriculture).

In this study, 25-year-old common cypress clones of suitable size for a technological evaluation aimed specifically at timber production were taken into consideration. In particular, ten clones of *C. sempervirens* var. *horizontalis*, evaluated as resistant to cypress canker in previous trials, were characterised in two contrasted plantations of central Italy. This study aimed at (1) evaluating the genetic and environmental influences on growth and morphological traits of each clone, (2) exploring the relationships between growth and quality traits and (3) giving useful information to develop appropriate selection strategies for high-quality timber production.

Materials and methods

Plant material

The material used in this study consisted of common cypress clones, which have been previously assayed for resistance to bark canker (*S. cardinale*). The trials were established in two experimental sites (Table 1), in central Italy. On each site, common cypress (*C. sempervirens* L.) clones were planted in 1985 at 3×3 m spacing, and no thinning was applied until the time of survey. For each clone, ramets were obtained through scions collected in December-January from wild vigorous and healthy ortets selected in central Italy in woods, groves or ornamental plantations and grafted on *C. sempervirens* seed rootstocks grown in small pots containing a mixture of peat, compost and perlite (ratio 3:1:1 in volume, respectively). Grafts were kept in a greenhouse until the end of spring, and they were then transplanted into larger pots

Table 1 Characteristics of the two *C. sempervirens* clonal plantations

Site	Roselle	Cannara
Location	lat. 42° 48′ N, long. 11° 05′ E	lat. 43° 00′ N, long. 12° 37′ E
Altitude a.s.l.	5 m	190 m
Soil	Alluvial, sandy loam, well drained	Alluvial, reclaimed but clayey
Mean annual T	14.8 ° C[a]	13.7 ° C[b]
Total annual precipitation	650.2 mm[a]	874 mm[b]
Spacing	3×3 m	3×3 m
Age at sampling	25	25
Sampled clones	10	10
Ramets per clone	4	4

[a] Weather station of Grosseto airport (average 1961–1990)

[b] http://it.climate-data.org/location/115157/

(4–5 dm^3) and kept for 2 years in a shading tunnel before further transplanting in the field.

The bark-canker response of these clones was previously evaluated through artificial inoculations performed on trunks of 4-year-old grafted plants growing at the two sites with a standard *S. cardinale* isolate (ATCC 38654), and their response was subsequently monitored for 6 years (Tab. 2). A standard procedure was followed for both execution of artificial inoculations and evaluation of the progress of cankers, as described by Danti et al. (2006). A score ranging from 1 to 3 with 0.3 gradations was assigned to each inoculated ramet, where 1 represented complete healing of the necrotic lesion, 2 represented small cankers without resin and forming scars, and 3 represented actively growing cankers with resin exudation, stem deformation and eventually dieback due to the girdling of the stem. In 2012, 25 years after planting, a preliminary screening of phenotypic traits was carried out to choose the clones more suited to timber production, based on the following: (1) a fair resistance to cypress canker; (2) the var. *horizontalis* habit; (3) single-stemmed trees without forks. Finally, ten clones all coming from central Italy were identified and 4 ramets per clone per site were sampled and analysed (Table 2).

Growth and morphological traits

Trees were cut down to perform measurements and to collect material for laboratory observations. For each tree, the following dendrometric parameters were measured: stem diameter at the base, stem diameter at breast height, i.e. at 1.3 m in height (DBH), stem diameter at 4 m in height; total tree height (H); crown diameter (CD); number (Bn), diameter (Bd) and insertion angle (Ba) of all the branches inserted on the trunk between 0.5 and 1.5 m from the ground. From these data, stem volume (VOL) and taper (TF) were inferred; the latter calculated as the difference between the diameters measured at the base and at 4 m in height, divided by the distance between the two measurements.

From each tree, a 2-cm thick cross section was taken from the stem at breast height and scanned in the laboratory, to evaluate the regularity of the trunk shape and the heartwood/sapwood extension by means of an image analyser software (ImageJ, http://rsb.info.nih.gov/ij/). Specifically, the traits measured were as follows: ellipticity (EL), that is the difference between the maximum and minimum orthogonal diameters under bark divided by the maximum diameter and expressed as percentage; circularity (CI), the ratio of the cross area to the area of a circle having the same circumference; and the percentage area of heartwood (HW) in the total cross area.

Table 2 List of the ten *C. sempervirens* clones used in the present study and their respective mean bark canker resistance score

Clone	Mother plant[a]	Bark canker resistance score (mean)	
		Roselle (GR)	Cannara (PG)
1	PM 37	1.4	1.0
2	PM 43	1.4	1.0
3	PM 45	1.0	1.0
4	PM 38	1.3	1.0
5	PM 160	2.0	1.4
6	PM 239	2.0	2.4
7	PM 304	1.4	1.4
8	PM 736	2.3	1.4
9	PM 820	1.6	3.0
10	PM 740	1.4	1.4

[a] Number assigned by CNR–IPSP to the ortets listed in the book of mother plants

Statistical analysis

Data were analysed according to the following linear model for the two sites combined (1) and for separate analysis of individual site (2):

$$Y_{ijk} = m + S_i + C_j + C_jS_i + \varepsilon_{ijk} \quad (1)$$

where Y_{ijk} is the observed phenotypic value of the kth ramet from the jth clone in the ith site, m is the general mean, S_i is the effect due to the ith site, C_j is the effect due to the jth clone, C_jS_i is the effect due to the interaction between the jth clone and the ith site and ε_{ijk} is the random error;

$$Y_{jk} = m + C_j + \varepsilon_{jk} \quad (2)$$

where Y_{jk} is the observed phenotypic value of the kth ramet from the jth clone, m is the general mean, C_j is the effect due to the jth clone and ε_{jk} is the random error.

Assumptions of normal distribution and variance homogeneity were tested for each trait by using Shapiro-Wilk test and Levene test, respectively. Ellipticity, circularity and heartwood area (traits expressed in terms of percentage) were arcsin transformed to obtain normal distributions.

Estimate of genetic parameters

Mixed model analysis and calculation of variance component for random effects were performed by means of package lme4 (Bates et al. 2014) for R software (R Core Team 2014). The significance of fixed and random effect was verified with likelihood ratio tests.

For the joint analysis of the two sites, the variance components of random effects were derived from the model (1). All

terms were considered random, except for location, which was considered fixed.

The genotypic (clonal) variance component (σ_c^2) was expressed as ratio of total phenotypic variation of all random effects (σ_{SxC}^2 site-clone interaction; σ_e^2 residual variance) (Zhang et al. 2003; Pliura et al. 2007). The repeatability of clone means across the sites, (R_c^2) was calculated as follows for each trait:

$$R_c^2 = \frac{\sigma_c^2}{\sigma_c^2 + \frac{\sigma_{SxC}^2}{q} + \frac{\sigma_e^2}{rq}} \quad (3)$$

where q and r are the number of locations and the number of replications per clone per environment, respectively.

The individual-tree clonal repeatability (R_b^2) across sites was estimated as:

$$R_b^2 = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_{SxC}^2 + \sigma_e^2} \quad (4)$$

For the separate analysis of individual sites, the variance components of random effects were derived from the model (2). The repeatability of clonal means (R_c^2) within a site was calculated using the following formula:

$$R_c^2 = \frac{\sigma_c^2}{\sigma_c^2 + \frac{\sigma_e^2}{r}} \quad (5)$$

where r is the replications per clone.

The individual-tree clonal repeatability (R_b^2) was estimated using the equation:

$$R_b^2 = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_e^2} \quad (6)$$

The standard errors (SE) for repeatability estimations were calculated with the following formula (Becker 1984):

$$\mathrm{SE}(R^2) = \sqrt{\frac{2(1-R^2)^2\left[1+(k-1)R^2\right]^2}{k(k-1)(N-1)}} \quad (7)$$

where R^2 is the repeatability estimate; k is the coefficient associated with the variance due to clonal variation; and N is the number of clones tested.

The coefficient of genotypic variation was calculated as follows:

$$CV_G = \frac{\sqrt{\sigma_c^2}}{\overline{X}}\ 100 \quad (8)$$

where $\overline{X}$ is the phenotypic mean of the trait. In the same way, the coefficient of phenotypic variation was obtained from the phenotypic variance, estimated as $\sigma_p^2 = \sigma_c^2 + \sigma_e^2$.

Genetic correlations between the same trait assessed in different locations (Type B correlations, r_B) were estimated from the measurements on different ramets of the same clone planted in different sites using the following formula (Burdon 1977):

$$r_B \frac{r_{p\,(x1,x2)}}{\sqrt{R_{c(x1)}^2 R_{c(x2)}^2}} \quad (9)$$

where $r_{p(x1,x2)}$ is the phenotypic correlation coefficient between clone means estimated between trait x measured in site 1 (x_1) and the same trait measured in site 2 (x_2); $R_{c(x1)}^2$ and $R_{c(x2)}^2$ are the clonal mean repeatability of trait x at site 1 and 2, respectively.

Genotypic correlation coefficients ($r_{G(x,y)}$) between traits at each site (inter-trait clonal correlations) were estimated utilising the following formula (Becker 1984):

$$r_{G\,(x,y)} = \frac{\sigma_{c(x,y)}}{\sqrt{\sigma_{c(x)}^2 \sigma_{c(y)}^2}} \quad (10)$$

where $\sigma_{c(x,y)}$ is the clone covariance component between trait x and y; $\sigma_{c(x)}^2$ is the clone variance component for the trait x and $\sigma_{c(y)}^2$ for the trait y. The standard errors of genotypic correlations were estimated using the following equation (Falconer 1981):

$$\mathrm{SE}(r) = \frac{1-r^2}{\sqrt{2}} \sqrt{\frac{SE\left(R_x^2\right) SE\left(R_y^2\right)}{R_x^2 R_y^2}} \quad (11)$$

where r is the estimated genetic correlation, R_x^2 is the repeatability estimated for trait x and R_y^2 for trait y; $\mathrm{SE}\left(R_x^2\right)$ is the standard error of R_x^2 and $\mathrm{SE}\left(R_y^2\right)$ is the standard error of R_y^2.

The phenotypic correlations of clonal means for each pair of traits at each site were calculated using the Pearson correlation coefficient.

In order to evaluate the effect of selection for one trait to another, the indirect selection efficiency between traits was estimated as the following, assuming equal intensity of selection for both traits (i_x=i_y):

$$E_{ind} = \frac{R_x}{R_y} r_{G(x,y)} \quad (12)$$

where R_x and R_y are the square roots of the repeatability of clonal means of trait x on which selection is made and y on which the indirect effect is evaluated, respectively; r_{xy} is the genotypic correlation between trait y and trait x (Falconer 1981).

Table 3 Means, standard error (SE) and coefficients of phenotypic variation (CV_p) of all measured traits of ten selected *C. sempervirens* clones, grouped by site

Trait	Acronym	Roselle Mean±SE	CV_p (%)	Cannara Mean±SE	CV_p (%)
Diameter at breast height (cm)	DBH	19.3±0.4	12.6	25.0±0.4	10.0
Height (m)	H	10.5±0.2	9.3	17.7±0.2	7.8
Stem volume (dm^3)	VOL	148.8±5.8	24.8	384.1±14.1	23.3
Crown diameter (m)	CD	3.5±0.1	24.5	3.7±0.1	14.4
Branch number (−)	Bn	20.0±1.0	32.2	16.7±0.7	26.8
Branch diameter (mm)	Bd	20.0±0.3	41.7	24.1±0.5	51.9
Branch angle (°)	Ba	76.0±0.5	18.2	69.8±0.4	16.6
Taper (cm/m)	TF	2.9±0.1	27.5	1.5±0.1	44.8
Ellipticity (%)	EL	8.3±0.7	50.7	11.1±0.7	41.4
Circularity (−)	CI	0.96±0.00	2.3	0.94±0.00	3.1
Heartwood area (%)	HW	28.7±1.6	35.6	45.1±1.7	24.0

Results

Basic statistics and differences between sites

Descriptive statistics, mean values, standard errors and coefficients of phenotypic variation for each parameter measured or calculated are listed in Table 3. The results are shown for each clonal trial individually.

Trees generally showed a higher growth at Cannara: in fact DBH, H and VOL showed higher mean values compared to trees at Roselle. Also, the CD was higher at Cannara than at Roselle, but the difference between the two sites was not statistically significant (Table 4). At Cannara, there were fewer but larger branches with slightly smaller insertion angles than branches in Roselle; the stem showed a lower TF but higher EL and smaller CI; the HW was significantly larger.

The coefficients of phenotypic variation were generally higher in the less productive Roselle site (except for Bd, TF and CI).

The joint analysis for the two sites combined (Table 4) showed significant site effect for all traits except for CD and CI.

Clonal variation, repeatability and clonal stability

The results of the analysis for the two combined sites are reported in Table 4. The clone effect was always significant except for EL. The component of variance due to the clone effect was the highest for CD (32.5 %) and HW (32.6 %), followed by H (31.2 %) and Ba (30.6 %); therefore, these traits had the highest estimated individual-tree repeatability (0.33 for CD and HW, 0.31 for H and Ba). Height, CD and HW also showed the highest clonal repeatability (0.62, 0.60 and 0.67, respectively).

The 'site × clone' interaction was significant for almost all the studied traits, excluding Bn, TF, EL and CI. Highly significant values were shown by CD and Ba, for which the variance due to interaction was the highest (34.1 and

Table 4 Results from joint linear mixed model for each trait of ten selected *C. sempervirens* clones at the two sites combined (Cannara and Roselle): significance of fixed effect (site); variance components for random effects as percentage of the total variation and estimated repeatabilities (R_b^2 = individual-tree clonal repeatability; R_c^2 = repeatability of clone means; SE = standard error)

Trait	Site Significance	Clone σ_c^2	(%)	Site × σ_{SxC}^2	Clone (%)	Error σ_e^2 (%)	R_b^2 ± SE	R_c^2 ± SE
DBH	***	12.9	***	25.3	*	61.8	0.13±0.10	0.39±0.14
H	***	31.2	***	27.6	**	41.2	0.31±0.14	0.62±0.13
VOL	***	15.5	***	27.8	**	56.8	0.15±0.11	0.42±0.14
CD	ns	32.5	***	34.1	***	33.4	0.33±0.14	0.60±0.13
Bn	*	3.6	*	16.8	ns	79.6	0.04±0.08	0.16±0.11
Bd	**	19.9	***	27.1	*	53.1	0.20±0.12	0.50±0.14
Ba	*	30.6	***	48.7	***	20.7	0.31±0.14	0.53±0.14
TF	***	18.0	**	13.5	ns	68.5	0.18±0.12	0.54±0.14
EL	**	4.1	ns	0.0	ns	95.9	–	–
CI	ns	19.4	***	18.7	ns	61.9	0.19±0.12	0.53±0.14
HW	***	32.6	***	20.4	*	47.0	0.33±0.14	0.67±0.12

ns not significant

*significant at 5 % level; **significant at 1 % level; ***significant at 0.1 % level

Table 5 Results from the mixed linear model: variance component as percentage of the total variance for clone effect, genotypic variation (CV_G) and estimated repeatabilities for each trait of ten selected *C. sempervirens* clones at the two sites separately (Cannara and Roselle). R_b^2 = individual-tree clonal repeatability; R_c^2 = repeatability of clone means

Trait	Roselle Clone σ_c^2	(%)	R_b^2	R_c^2	CV_G (%)	Cannara Clone σ_c^2	(%)	R_b^2	R_c^2	CV_G (%)
DBH	39.8	**	0.40	0.73±0.12	8.1	36.7	*	0.37	0.70±0.13	6.2
H	16.9	ns	–	–	–	78.3	***	0.78	0.94±0.03	7.2
VOL	36.5	*	0.37	0.70±0.13	15.2	44.4	**	0.44	0.76±0.11	15.8
CD	76.4	***	0.76	0.93±0.04	22.1	39.4	**	0.39	0.70±0.13	9.1
Bn	20.3	ns	–	–	–	20.4	ns	–	–	–
Bd	46.1	**	0.46	0.77±0.10	11.0	47.4	**	0.47	0.78±0.10	13.4
Ba	84.5	***	0.85	0.96±0.02	12.5	65.5	***	0.65	0.88±0.06	7.4
TF	53.2	***	0.53	0.82±0.08	20.5	1.6	ns	–	–	–
EL	5.6	ns	–	–	–	0.0	ns	–	–	–
CI	21.3	ns	–	–	–	47.9	**	0.48	0.79±0.10	5.2
HW	53.8	***	0.54	0.82±0.08	27.8	52.3	***	0.52	0.81±0.09	19.4

ns not significant

**significant at 1 % level; ***significant at 0.1 % level

48.7 %, respectively), while for all the other traits, the variance ranged between 18.7 and 27.8 %.

The variance due to error (i.e. differences among ramets within clones and within site) accounted for most of the variation for all traits, ranging from 41.2 % (H) to 95.9 % (EL). However, for the percentage of variance due to error was as low as 20.7 and 33.4 % in the case of Ba and CD, respectively.

The analyses carried out separately at each site resulted in some notable differences between the two locations: the clone effect for H and CI was highly significant at Cannara (it showed the highest repeatability), but no difference among clones was observed at Roselle (Table 5). Similarly, the clone effect for TF was highly significant at Roselle but not significant at Cannara. For the other considered traits, the results were generally consistent between the two sites: for Bn and EL the clone effect was never significant; while for Ba, the clone was the most important effect at both sites (84.5 % of total variance at Roselle and 65.5 % at Cannara).

Genetic correlations and selection efficiency

The Type B (inter-site) genetic correlations were not calculated for traits which did not show significant differences among clones in one or both sites. Generally, correlations were positive and moderate; the highest values were obtained for CD and HW (Table 6). Phenotypic and genetic correlations between traits are presented in Table 7. For some traits, different correlations could be noticed in the two sites: Bd showed contrasting correlations with growth traits, positive in Roselle and negative in Cannara, while for other traits, the relationships in both sites were similar. There were strong positive autocorrelations between DBH/H and VOL because VOL was calculated from these parameters. However, growth traits correlated differently with the traits describing stem form. A positive and strong genotypic correlation was noted between DBH/VOL and TF, strong and negative between DBH/VOL and CI. This suggests that higher diametric growth will result in a lower quality stem form. Height (H) was significantly correlated with VOL at Roselle and with both VOL and DBH at Cannara, where H also negatively correlated with Bd (Table 7).

Crown and branch characteristics did not reveal any clear correlations with the other traits, both with growth characters or with stem form. However, a positive but moderate correlation was found between CD and Ba and a moderate/strong negative correlation between Bd and Ba. Finally, HW showed positive correlations with growth traits but no further clear trends with the other traits were found.

Indirect selection efficiency calculated for different selection criteria (Table 8) confirmed the previous observations.

Table 6 Genetic intra-trait correlations (Type B) of ten selected *C. sempervirens* clones

Trait		
DBH	0.34	**
H	–	
VOL	0.55	***
CD	0.68	***
Bn	–	
Bd	0.45	***
Ba	0.46	***
TF	–	
CI	–	
HW	0.62	***

** significant at 1 % level; *** significant at 0.1 % level

Table 7 Phenotypic (below the diagonal) and genetic (above the diagonal) inter-trait correlations at individual site (Cannara and Roselle) for each trait of ten selected *C. sempervirens* clones. Standard error in brackets

Trait	DBH		H		VOL		CD		Bd		Ba		TF		CI		HW	
Roselle																		
DBH			–		0.98	(0.00)	0.06	(0.06)	0.67	(0.06)	−0.04	(0.04)	0.93	(0.01)	–		0.72	(0.04)
H	0.40	**			–		–		–		–		–		–		–	
VOL	0.96	***	0.51	**			0.15	(0.06)	0.58	(0.07)	0.05	(0.04)	0.85	(0.03)	–		0.82	(0.03)
CD	0.17		0.14		0.21				0.16	(0.05)	0.48	(0.02)	−0.24	(0.04)	–		0.56	(0.03)
Bd	0.51	***	0.18		0.45	**	0.17				−0.18	(0.04)	0.87	(0.02)	–		−0.04	(0.08)
Ba	−0.11		−0.20		−0.10		0.40	*	−0.21				−0.36	(0.03)	–		0.33	(0.03)
TF	0.63	***	0.03		0.55	***	−0.16		0.55	***	−0.28				–		0.11	(0.07)
CI	−0.28		−0.03		−0.27		0.16		−0.50	***	0.26		−0.29				–	
HW	0.49	**	0.18		0.52	***	0.43	**	0.09		0.20		0.11		−0.17			
Cannara																		
DBH			0.43	(0.04)	0.88	(0.03)	0.44	(0.11)	−0.52	(0.08)	−0.18	(0.08)	–		−0.70	(0.06)	0.14	(0.10)
H	0.25				0.84	(0.01)	−0.11	(0.06)	−0.97	(0.00)	0.44	(0.03)	–		0.12	(0.04)	0.25	(0.04)
VOL	0.86	***	0.57	***			0.22	(0.10)	−0.91	(0.02)	0.11	(0.07)	–		−0.38	(0.08)	0.37	(0.08)
CD	0.21		0.06		0.16				−0.22	(0.11)	0.28	(0.08)	–		−0.60	(0.06)	−0.21	(0.10)
Bd	−0.32	*	−0.56	***	−0.44	**	0.03				−0.73	(0.03)	–		0.24	(0.08)	−0.20	(0.08)
Ba	0.05		0.30		0.13		0.09		−0.63	***			–		−0.24	(0.06)	−0.02	(0.06)
TF	0.41	***	0.01		0.14		−0.04		−0.12		0.16				–		–	
CI	−0.54	***	0.05		−0.37	*	−0.30		0.15		−0.13		−0.41	**			0.16	(0.08)
HW	0.12		0.10		0.22		−0.12		−0.08		−0.11		0.07		0.06			

( ) not significant

*significant at 5 % level; **significant at 1 % level; ***significant at 0.1 % level

Table 8 Efficiency of indirect selection among growth and morphological traits at each site (Cannara and Roselle)

Selection trait	Selection efficiency on:								
	DBH	H	VOL	CD	Bd	Ba	TF	CI	HW
Roselle									
DBH		–	1.00	0.06	0.65	−0.04	0.87	–	0.68
H	–		–	–	–	–	–	–	–
VOL	0.97	–		0.13	0.54	0.04	0.78	–	0.76
CD	0.07	–	0.18		0.17	0.47	−0.26	–	0.60
Bd	0.70	–	0.60	0.14		−0.16	0.85	–	−0.04
Ba	−0.05	–	0.06	0.48	−0.20		−0.39	–	0.35
TF	0.99	–	0.92	−0.23	0.90	−0.34		–	0.11
CI	–	–	–	–	–	–	–		–
HW	0.77	–	0.89	0.53	−0.04	0.30	0.12	–	
Cannara									
DBH		0.37	0.84	0.44	−0.49	−0.16	–	−0.66	0.13
H	0.50		0.93	−0.12	−1.06	0.45	–	0.13	0.26
VOL	0.92	0.76		0.23	−0.90	0.10	–	−0.37	0.36
CD	0.44	−0.09	0.21		−0.21	0.25	–	−0.56	−0.20
Bd	−0.55	−0.88	−0.92	−0.24		−0.68	–	0.24	−0.20
Ba	−0.21	0.43	0.12	0.31	−0.77		–	−0.25	0.02
TF	–	–	–	–	–	–		–	–
CI	−0.74	0.11	−0.38	−0.65	0.24	0.23	–		0.16
HW	0.15	0.23	0.39	−0.22	−0.20	0.02	–	0.16	

The predicted gain obtainable with direct clonal selection was generally higher (except in very few cases) than that achievable with indirect selection. Selection based on DBH had a rather high and positive effect on TF and a negative effect on CI. Selection for H could result in a positive effect on both VOL and DBH, although the gain for the latter was the half than that obtainable with a direct selection, also avoiding negative effects on stem form. Selecting for higher H seemed to reduce Bd with an estimated efficiency that was as equal as that due to a direct selection and to have also positive effects on Ba. Finally, selection for higher Ba will result in lower branch diameters.

Discussion

Differences between sites

Growth traits of the ten selected *C. sempervirens* clones proved to be mostly influenced by site effect. Generally, the site effect revealed the response of trees to different climatic and edaphic conditions. Although in the present study, no specific analysis was done to study such effects on the tree growth, and some conclusions could be drawn. The lower growth rate at Roselle may be due to the prolonged summer drought, which induced quiescence on cypress trees, as evidenced by the annual rings in the trunks at Roselle (data not shown). Similar findings were reported by Giannini and Raddi (1992), who studied 50 Mediterranean cypress clones (including the clones considered here) at the two same locations. They cited the low winter temperatures at Cannara and the summer drought at Roselle as the main limiting environmental factors to cypress growth, but their results were contrary to those of the present study: the authors found significant differences between the two sites as regards trunk diameter and total height but with Roselle showing higher mean values. The discrepancy could be due to the different age of the trees sampled (1 to 5 years old against 25 years old in our study). The prolonged summer drought at Roselle was probably more limiting on adult trees, which, on the other hand, better withstood the lower winter temperatures of Cannara.

Contrasting tree development of *C. sempervirens* clones (height, crown diameter and shape) related to different site conditions was also reported by Santini and Camussi (2000). The same authors underlined a marked phenotypic plasticity of cypress for growth and crown shape, thus questioning the advisability of selecting a genotype grown in a certain environment and then using it under very different conditions.

Clonal variation and repeatability

As consequence of the high differences between the two sites, the repeatability values of the studied traits in the joint analysis were generally low to moderate. Estimations of repeatability

for each site were moderate to high, comparable with previous height and diameter measurements in *C. sempervirens* (Giannini and Raddi 1992). For example, low to moderate (individual) or moderate to high (clonal means) genetic control for height and stem form was observed in clones of yellow cypress (*C. nootkatensis*) in British Columbia (Baltunis et al. 2013). Narrow-sense heritability in a progeny test on *C. macrocarpa* Hartw. ex Gordon was also low to moderate for growth traits, branching and stem straightness (Gea and Low 1997). Furthermore, moderate narrow-sense heritability of growth and branch size was also estimated for *C. lusitanica* Mill. in New Zealand for (Dungey et al. 2013), and low values were reported in a progeny test in Costa Rica (Cornelius et al. 1996). Utilising an identical approach to our and previous studies, Cornelius (1994), reviewed several research papers regarding the estimation of heritability in forest trees, concluded that both growth and form traits seem to be under weak to moderate genetic control. Critically, height tends to have higher heritability than diameter and volume. In our study, strong repeatability was estimated for H at Cannara, while it did not permit effective discrimination of the clones at Roselle. Other studies observed that in poorer sites, the rate of variance due to environmental factors could be higher than in richer locations, where, conversely, the variance due to genotype effects was higher allowing differentiation of the clones (Muranty et al. 1998; Pliura et al. 2007; Zhang et al. 2012). In our experiment, H appeared to be the trait most strongly affected by differences between the sites.

In terms of stem form, the estimation of clonal variation produced no significant (EL) or conflicting results (TF and CI); therefore, direct selection on the basis of these traits does not seem to be easily practicable. No potential for selection could be ascribed to the number of branches, as clone effect for this trait was never significant in the single site analyses. On the contrary, high repeatability values were estimated for both Ba and Bd (at both sites), suggesting that these parameters offer a high potential for selection. These results are not surprising, especially with regard to Ba, due to the existence of many intermediate forms between the two definite morphological varieties of *C. sempervirens* (var. *sempervirens* and var. *horizontalis*) that are distinguished by crown habit and angle of branching.

Genotype × Environment interaction

Due to the significant site × clone interaction, the clone effect was lower in the combined analysis when compared to the single site analysis suggesting that selection of traits across environments may be problematic. However, the component of the variance observed due to the site × clone interaction that might have resulted from differences between sites generally depends upon whether environment is considered a fixed or a random effect in the test. Rather, this does not affect the calculation of genetic correlations between environments, thus allowing a more precise detection of genotype x environment interactions (Burdon 1977). Type B genetic correlations were subsequently calculated for traits showing significant clone effect at a site level, consistently resulting in positive but moderate clonal effects.

The common cypress study of Giannini and Raddi (1992) observed genetic correlations for plant height and diameter ranging from 0.70 to 0.81 and a variance component due to site × clone interaction of approximately 10 % that remained constant over the 5-year duration of the experiment. In the same species, Santini and Camussi (2000) reported significant site × clone interaction (for both height and diameter) across six Mediterranean locations, but they did not calculate genetic correlations. In yellow cypress clones, the estimated genetic correlations across seven sites were 0.80±0.04 for H and 0.83 ±0.05 for tree form (Baltunis et al. 2013), while the correlation for diameter growth of *C. lusitanica* between two sites was 0.91 (Dungey et al. 2013). A high correlation between two sites for growth traits were also found in seventy-six families of *C. macrocarpa* in New Zealand (Gea and Low 1997).

In our experiment, the comparative growth of the *C. sempervirens* clones was not as stable across different environments as found in other studies; the use of clones in an environment different to that where they were selected may therefore be questionable. To ensure the success of a plantation, better knowledge of specific environmental factors and their interaction with the most desirable traits would be extremely valuable. Nevertheless, considering the significant positive genetic correlations detected in our experiment, the percentage of variance due to the site × clone interaction is not sufficient to justify a site-specific clone development.

Genetic correlations between traits

Larger DBH values were related to poorer stem forms (i.e. higher TF and lower CI). The occurrence of unfavourable genetic correlations implies the worsening of stem form if stem quality is not considered and selection is performed on the basis of DBH. In contrast, H appeared to be related with lower TF and higher CI; therefore, selection for tree height, as opposed to tree diameter, might have a smaller negative effect on stem form.

In the context of branch and crown morphology, Bn resulted poorly influenced by genotype, and it therefore could be recommended to modify this characteristic applying careful and punctual cultural practices (pruning). On the other hand, artificial selection to improve branch size, angle and crown dimension offered some promise. Selection to increase Ba will result in smaller branch size and a wider crown. Furthermore, Ba demonstrated a positive correlation with H and negative relationship with DBH but not as strong as that observed between DBH and Bd. Branching closer to perpendicularity to

the stem will be positive for future timber products (smaller knots) but also favourable to stem form and reduce susceptibly to bark canker. Gea and Low (1997) considered acute-angled branching in *C. macrocarpa* to be associated with the stem fluting of older trees that was more conducive to canker infection.

Finally, since HW was positively correlated with growth traits, its assessment is not required in any selective improvement programme. The direct selection of varieties on the basis of HW content would result in a much higher gain, but the measurement of this attribute in standing trees is not as simple, practicable or cost effective as the survey of DBH or H.

Conclusions

Tree selection for timber production should involve both quantitative (e.g. growth rate) and qualitative evaluation. In this study, cypress clones previously selected for bark canker resistance were assessed for traits related to timber production. Suitable habits were selected and evaluated in order to establish a pool of cypress clones to be used for high-quality arboriculture plantations. The clones were sampled to quantify the genetic parameters of those traits that are relevant to the characterisation of the technological quality of timber and to examine their performance in two contrasting sites. Low stem taper, as well as high circularity and low ellipticity of the cross section, will result in easier processing, with higher yields and better quality of the sawn material. In the same manner, branch diameter and insertion angle will influence the technological quality of the final product in terms of knot size; thus, small branches with a wider insertion angle (direction perpendicular to the stem) are favourable. Due to the increasing cost of pruning, branch number and size are also an important economic trait. Furthermore, the proportion of heartwood in the cross section is a valuable trait, especially cypress heartwood that is widely appreciated for its natural durability against decay and insects.

The genetic parameters related to the abovementioned traits were calculated on ten 25-year-old cypress clones that were of a suitable size for a technological evaluation and suitable for timber production. Information on performance of genotypes in different growth conditions, as well as the estimation of genetic correlations between traits interesting by technological point of view, could be useful for the future use and further development of these and other cypress clones aimed at high-quality timber production.

In summary, a strong potential for selection was observed in growth traits, branch characteristics (size and insertion angle) and heartwood content, while direct selection for stem form and branch number did not seem easily practicable. Insertion angle was the most interesting trait since it showed very high repeatability values and favourable genetic correlations. It was negatively correlated with branch size (a wider insertion angle will result in smaller branches) and positively with tree height. Heartwood content correlated positively with growth traits and would be indirectly favoured when selective improvement is based on growth parameters. Selection on the basis of trunk diameter could result in a worse stem form, but this does not seem to occur when selecting by tree height.

Therefore, for *C. sempervirens*, selection for growth should be based on tree height; even if this leads to lower gains in diameter growth, it results in fewer adverse effects on other properties such as stem form and branching. Unfortunately, tree height has been shown to be strongly dependent on the site, so that in the poorest location, the clone effect was overwhelmed by the environment.

The marked effect exerted by site on all growth traits shows that the site selection is fundamental when planting *C. sempervirens* clones when the production of timber is the main goal. A deeper knowledge of the climatic and edaphic conditions affecting cypress growth would be helpful to guarantee the success of future plantations.

Acknowledgments The authors gratefully thank: the IPSP and IVALSA technical teams (Paolo Burato, Paolo Pestelli, Giovanni Torraca and Luciano Scaletti) for their assistance during field and laboratory work, Dr. Matthew Haworth for critical reading of the manuscript and the Tuscany Region (ARSIA) which funded this study.

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