Tree Genetics & Genomes (2017) 13: 32
DOI 10.1007/s11295-017-1111-6

ORIGINAL ARTICLE

Clonal consistency of wood technological properties in canker-resistant *Cupressus sempervirens* clones at two contrasting sites

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Received: 25 August 2016 / Revised: 15 January 2017 / Accepted: 18 January 2017 / Published online: 2 February 2017

Abstract *Cupressus sempervirens* L. (Mediterranean cypress) has been traditionally used as a multipurpose tree. In the past, its wood was extensively used as a highly durable raw material in the Mediterranean, but nowadays, production of cypress wood is constrained by the lack of exploitable woods and plantations and by the spread of bark canker. In this study, the wood properties of canker-resistant clones specifically meant for timber production were assessed in two different locations. The aim was to evaluate the effect of genotype and environment on physical and mechanical properties of wood and its bonding quality. Four ramets of each of 10 clones were sampled in both sites, wood density, shrinkage, hardness, and the bonding strengths when glued together with two different adhesives were determined, and clonal repeatabilities and genetic correlations were also estimated. Clonal consistency for wood traits was moderate to high within and across environments, far higher than for growth- and morphological traits. Indications are that selection based exclusively on tree height would result in a lower wood density and hardness. Bonding strength reflected the combination of the material properties and the selected adhesive: For adhesive M (polyvinyl acetate dispersion), it was negatively correlated with density, whilst for adhesive E (emulsion polymerization isocyanate), it was not as influenced. Thus, even favouring higher-density wood (and therefore not only hardness but also higher shrinkages), adhesive E would give excellent bonding.

Keywords Wood density · Shrinkage · Hardness · Mediterranean cypress · Clonal repeatability · Gluability · Bonding

Introduction

Mediterranean cypress (*Cupressus sempervirens* L.) has been traditionally employed for several uses: as an ornamental and landscape tree, for windbreaks and hedges, for soil protection, and as a source of durable wood for a range of uses. In ancient times, the native cypress woods in the eastern part of the Mediterranean (Turkey, Greece, Crete, Rhodes, etc.) were intensively exploited as a valuable raw material for constructing ships, buildings and furniture (Xenopoulos et al. 1990; Farjon 2005).

Technological properties highly valued by the wood industry such as fine texture, easy workability, low shrinkage, stability, high natural durability, good mechanical and aesthetic features have contributed to the favourable reputation of cypress wood. It is now mainly used for exterior and interior woodwork, including door and window frames, furniture, joinery and turnery (Hosseini Hashemi and Kord 2011; Okino et al. 2010; Paraskevopoulou 1991; Thibaut et al. 1999).

Due to the lack of a reliable supply of raw material, there is no longer a viable market for cypress wood. In central Italy, a poor supply of wood is available from sanitation cuttings in ornamental plantations affected by cypress canker (*Seiridium cardinale*) (Puleri and Toccafondi 2003), a pandemic disease

Communicated by R. Burdon

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which has caused severe economic losses in woods, ornamental plantations and nurseries in the course of the last 50 years throughout the Mediterranean, limiting the cultivation of this tree (Panconesi 1990; Graniti 1998; Danti et al. 2013, 2014). For this reason and also due to the lack of suited varieties, cypress plantations dedicated to timber production have rarely been established.

A long-term breeding program, started in the 1970s at CNR-IPSP of Florence, led to the selection of a series of canker-resistant clones of *C. sempervirens*; some of which were patented between 1990 and 2010 and largely sold as ornamental varieties (Raddi and Panconesi 1994; Panconesi and Raddi 1991; Danti et al. 2006, 2012). Other canker-resistant *C. sempervirens* var. *horizontalis* clones have been obtained, and their use as multiclonal plantations aimed at erosion control and soil protection has recently been evaluated in two EU Interreg projects 'Cypmed' (2002-02-4.1-I-039) and 'Medcypre' (2004-04-4.13-I-015).

The var. *horizontalis* has always been generally preferred to var. *sempervirens* (with a fastigiate crown architecture and a typical columnar habit) for timber production due to the single stem, the lower number of branches, and wider angle of branch insertion in the trunk (fewer and smaller knots). Therefore, considering the high potential production (up to 8–9 m^3 ha^{-1} $year^{-1}$; Danti et al. 2007), we evaluated canker-resistant clones of var. *horizontalis* for use in plantations dedicated to wood production. The clones were chosen mainly for single stems without forks.

Using the same material, namely 25-year-old trees of 10 clones, Nocetti et al. (2015) reported an analysis focused on growth and morphological traits: Trunk diameter and tree height, branch characteristics (size and insertion angle), crown width and stem form (taper, ellipticity and circularity) were measured and compared in trees planted in two contrasting locations. Due to the phenotypic plasticity of the species (Santini and Camussi 2000), growth traits and crown morphology were shown to be highly influenced by the environment. Despite this, the effect of genotype was relatively high supporting the effectiveness of selection for additional traits. The most promising trait was the branch insertion angle which showed a high repeatability, whilst stem-form traits showed the weakest clonal effects. The branch insertion angle is an important trait since it 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) will result in smaller knots. As a general conclusion, the study suggested selection based on tree height rather than trunk diameter, since the former reduced the adverse consequences on morphological traits (Nocetti et al. 2015). Similar findings were reported for *Pinus radiata*, for which height tended to be in markedly more favourable genetic correlations with other traits than diameter (Burdon et al. 1992).

However, due to the marked phenotypic plasticity exhibited by most cypress clones growing in contrasting sites, the study of the environmental effect and the environment × genotype interactions for the clonal properties is of great interest. Study of the clonal consistency of wood characteristics is important for evaluating the potential of this species for the genetic improvement of wood quality (Paraskevopoulou 1991). Evidence of stability might favour the use of clonal plantations, indicating that a similar product could be more generally achieved in other sites (Baltunis and Brawner 2010; Baltunis et al. 2013). Therefore, in this second paper, the same 10 clones have been analysed with regard to their technological properties, specifically, consistency of wood traits across environments and their relationships with growth.

The assessment included wood density and linear and volumetric shrinkage variables. Wood density is often considered to be the most important property of wood, being generally related to the other physical and mechanical properties, and used as a global indicator of wood quality (Zobel and van Buijtenen 1989). Shrinkages are associated with the drying process during manufacturing and to the response of the solid-wood product to changing of relative humidity. It is well-known that wood shrinks or swells according to the ambient moisture levels with different amounts in the different directions (anisotropy). Indeed, the ratio between tangential and radial shrinkages is an important indicator of possible deformation during desorption and absorption of water (Hai et al. 2009; Pliura et al. 2005).

Beside physical properties, some mechanical characteristics, such as wood hardness and glue-bond performance, were included in the analysis. Hardness is a valuable property for solid-wood products (Negri et al. 1995), and bonding quality is a crucial for woodworks that are glued to make reconstituted elements (glued and laminated frame elements, laminated solid-wood panels, etc.).

The study aimed at (1) evaluating the environmental and genetic influences on wood traits of cypress clones, (2) comparing the performance in terms of wood quality of the same clones in two different growth conditions, (3) examining the performance of different genotypes in terms of real technological performance of the material, and (4) adding information on the relationships between growth and wood traits of cypress clones.

Materials and methods

Plant material

The plant material was described in Nocetti et al. (2015). It consisted of 10 *C. sempervirens* var. *horizontalis* clones, which were previously tested for resistance to cypress canker. The clones were part of two clonal trials established by the

Institute for Sustainable Plant Protection (CNR-IPSP) in two different locations in central Italy: Roselle (province of Grosseto) and Cannara (province of Perugia). Roselle was characterized by a lower altitude (5 m a.s.l. in respect to 190 m a.s.l of Cannara), a lower annual precipitation (650 mm against 874 mm), and a higher mean annual temperature (14.8°C against 13.7°C). The prolonged summer drought at Roselle and the lower winter temperatures at Cannara could be considered to be the main limiting factors for cypress growth (more details on the two sites can be found in Nocetti et al. 2015).

At the two locations, clones were first phenotypically chosen for suitability to timber production when they were 25 years old; in particular, the var. *horizontalis* habit was required, as well as single-stemmed trees without forks, but no wood properties were taken into account. In the two trials, clones had been previously selected only for canker resistance, and several habits were represented, mostly multi-stemmed and aimed at ornamental use. Accordingly, 10 clones (out of 50) with 4 ramets per clone were sampled per site and analysed.

Growth and wood traits

Before the trees were cut down to collect material for laboratory observations, dendrometric parameters as well as morphological characteristics were measured. The results for these traits have been covered by Nocetti et al. (2015). Here, only the two main growth traits have been considered, insofar as they are correlated to the wood properties of the clones: stem diameter at breast height (DBH) and total tree height (H).

After felling, a 1.2-m long log was collected 50 cm above the ground level from each tree and transported to the laboratory, where it was immediately processed. From each log, a 20-cm thick disc was cut and used to produce specimens for the determination of the physical properties of wood. Wood density, linear and volumetric shrinkages and ratio of tangential to radial shrinkage were determined in defect-free specimens (20 × 20 × 30 mm^3) cut from the four radial directions of the discs; the heartwood/sapwood classification (detected visually, mainly for colour difference) as well as the radial position were recorded for each specimen. To determine the wood density, maximum shrinkage and dimensional stability, specimen weight and dimensions in the three axes (radial, tangential and longitudinal) were first measured in the green state (soon after felling), measured after conditioning at 65% RH and 20°C and finally oven-dried. Basic wood density (BA) was calculated as the ratio of oven-dry weight to fresh volume, radial (RS), tangential (TS) and volumetric shrinkage (VS) were calculated as the percentage of the difference between oven-dried measures and green measures, and the tangential/radial shrinkage ratio (T/R) was the ratio between tangential and radial shrinkage. About three specimens from each of the four radii per tree were obtained, with a total of 940 specimens for physical determination. The final sample for the physical characterization was quite balanced with about 480 specimens (about 200 from sapwood and 280 from heartwood) for Cannara and about 460 (250 from sapwood and 210 from heartwood) for Roselle.

The remaining parts of the logs were used to evaluate wood hardness and bonding quality; two timber properties deemed to be important based on the common uses of cypress wood. However, in this case, only a subsample of six clones was used, chosen according their mean basic density: two clones of low, two of medium and two of high density.

Wood hardness was determined by means of the Brinell method (EN 1534 2002) on approximately 50-mm long specimens per tree. A 10-mm diameter steel ball was pressed to the tangential surface of the specimen applying a force of 1 kN, maintained for 25 s. After the withdrawal of the indenter, the Brinell hardness (HB) was calculated (in MPa) by measuring the residual indentation (two diameters, one along and one across the grain), according to Eq. 1:

$$HB = \frac{2F}{\pi D\left(D-\sqrt{D^2-d^2}\right)} \tag{1}$$

where F is the maximum load applied, D is the diameter of the steel ball and d is the diameter of residual indentation (average of the two measurements).

Bonding quality was assessed by measuring the shear strength of glue lines. Firstly, boards were cut from the remaining 1-m long logs, and the boards were then conditioned at 65% RH and 20°C, and then cut again to produce strips 20 mm thick and 40 mm wide. The strips were glued in couples on their radial face. A few minutes before the adhesive was applied, the wooden surface was sandpapered (grit 150).

Two different types of adhesives were tested:

1. Product M: a commercial polyvinyl acetate (PVAc) dispersion in water, belonging to the durability class D3 as defined in EN 204 (2002; the reference standard for the non-structural-grade wood adhesives). Polyvinyl acetate is the monocomponent adhesive class most widely used in the wood industry for the manufacture of solid-wood panels, wood frames and similar non-structural products.
2. Product E: a commercial vinyl acetate-ethylene copolymer to which an isocyanate hardener has been added, thus enabling the product to reach the durability class D4 (EN 204 2002). This class of bi-component products are known as emulsion polymerization isocyanates (EPI) and are mostly used in window manufacture.

For both adhesives, the following gluing parameters were used: open time, 2–3 min; closed assembly time, 20 min; pressure, 0.7 bar; wood moisture content, 12%; and environmental temperature and relative humidity during gluing, 24°C and 50%, respectively. The adhesive quantity spread on each wooden face was 150 g/mm^2 for M and 120 g/mm^2 for E, according to the manufacturer's instructions.

Thirty days after gluing, the assessment was performed following the provisions of standard EN 13354 (2009). The specimens had the shape and dimensions shown in Fig. 1, each specimen having a shearing area of 20 ± 1 × 25 ± 1 mm^2; they were subjected to pre-treatment 1 as described in EN 13354 (2009), i.e. vacuum impregnation in water for 30 min followed by water immersion for 24 h (whole process carried out at 20 ± 3°C), and then tested wet applying the shearing force on the glue line (Fig. 1).

For each adhesive, about 16–18 specimens per tree were prepared and tested wet, with a total of around 1600 specimens. Moreover, 20 additional specimens per each adhesive were prepared with the material coming from Roselle, and they were tested in standard humidity conditions (20°C and 65% r.h., nominal moisture content 12%), without pre-treatment.

The shear strength (f_v) was calculated from the following equation:

$$f_v = \frac{F}{A} \quad (2)$$

where F is the maximum load of the test piece and A is the shear area.

Statistical analysis

Data were analysed according to the following linear models for a combination of the two sites (3) and for separate analysis of each individual site (4). For the physical properties of wood (i.e. density, shrinkages and shape factor), the variability due to the specimen position in heartwood or sapwood area was included in the analysis (model 3b and 4b), because of its significance in explaining variability of these traits (Nocetti et al. 2010).

Due to the specimen preparation, the hardness assessment was performed on sapwood specimens only, while as concerns bonding quality the distinction between heartwood and sapwood could not be taken into account; therefore, in these cases, the models 3a and 4a were used.

$$Y_{ijk} = m + S_i + C_j + C_jS_i + \varepsilon_{ijk} \quad (3a)$$

$$Y_{\mathrm{injk}} = m + S_i + HS_n + C_j + C_jS_i + \varepsilon_{\mathrm{injk}} \quad (3b)$$

where Y_{ijk}/Y_{injk} is the observed phenotypic value of the kth ramet from the jth clone in the ith site for the nth heartwood/sapwood position (when present), 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, HS_n is the effect due to the heartwood/sapwood position (when assessable) and $\varepsilon_{ijk}/\varepsilon_{injk}$ is the random error;

$$Y_{jk} = m + C_j + \varepsilon_{jk} \quad (4a)$$

$$Y_{njk} = m + HS_n + C_j + \varepsilon_{njk} \quad (4b)$$

where Y_{jk}/Y_{njk} is the observed phenotypic value of the kth ramet from the jth clone for the nth heartwood/sapwood position (when present), m is the general mean, C_j is the effect due to the jth clone, HS_n is the effect due to the heartwood/sapwood position (when assessable) and $\varepsilon_{jk}/\varepsilon_{njk}$ is the random error.

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

Estimation of genetic parameters

Mixed model analysis and the calculation of the variance component for random effects were performed by means of

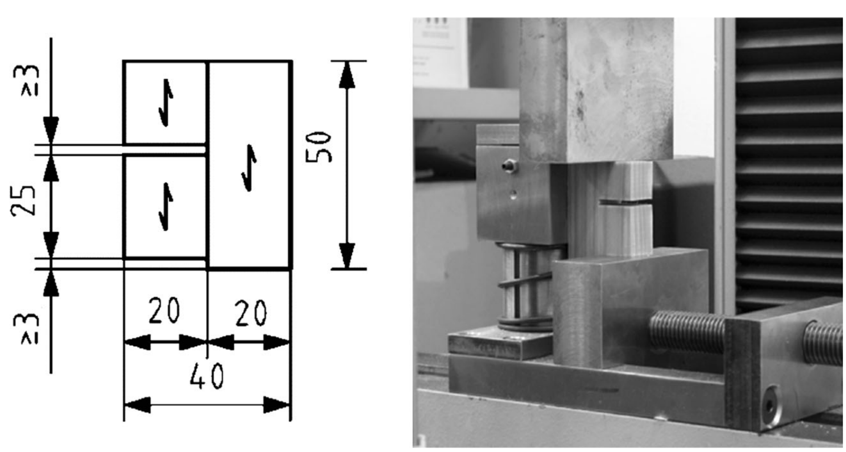

Fig. 1 Specimen shape and dimension and test arrangement for the bonding quality evaluation according to the standard EN 13354 (2009)

package lme4 (Bates et al. 2014) for R software (R Core Team 2014). The significance of fixed and random effects were verified with likelihood ratio tests.

For the joint analysis of the two sites, the variance components of random effects were derived from the model (3). All terms were considered random, except for location and heartwood/sapwood status, which were considered fixed.

The estimated genotypic (clonal) variance component ($\hat{\sigma}_c^2$) was expressed as the proportion of estimated total phenotypic variance of all random effects ($\hat{\sigma}_c^2$, $\hat{\sigma}_{SxC}^2$ site-clone interaction, and $\hat{\sigma}_e^2$ residual variance) (cf Zhang et al. 2003; Pliura et al. 2007).

For the separate analysis of individual sites, the variance components of random effects were derived from the model (4), where heartwood/sapwood position was considered as a fixed effect. The repeatability of clonal means ($\hat{R}_c^2$) within a site, the individual-tree clonal repeatability ($\hat{R}_b^2$), the standard errors (SE) for repeatability estimations (Becker 1984) and the coefficient of genotypic variation were estimated using the formulae of Nocetti et al. (2015).

Across sites (a fixed effect), the estimated repeatability of clone means across the sites ($\hat{R}_c^2$) and individual-tree clonal repeatability ($\hat{R}_b^2$) across sites were calculated as detailed in Nocetti et al. (2015).

Genetic correlations between the same traits 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 (Burdon 1977).

Finally, trait–trait phenotypic and genetic correlation were estimated using a suite of R codes (META-R) for analysing multi-environment trials (Alvarado et al. 2015).

Results

Summary statistics of growth and wood traits are shown grouped by site in Table 1. In Roselle, trees exhibited a lower growth rate (smaller diameter and height) compared to Cannara (Nocetti et al. 2015). Basic density was significantly higher in Roselle, while wood shrinkage and hardness were on average slightly higher in Roselle, but the differences between the two locations were not significant (see below).

As concerns the bonding quality, the shear strength obtained for adhesive E was generally far higher than achieved by adhesive M for wood from both sites. This difference was not observed on the samples tested without undergoing the pretreatment with water and those derived from Roselle: Measurements gave values of 11.7 N/mm^2 (±1.23) for the adhesive E and 10.9 N/mm^2 (±1.43) for the adhesive M; this difference was not statistically significant.

Additionally, comparing the results observed in the glued wood specimens that were tested when wet between the two sites, they were conflicting for the same adhesive: The shear strength of glue lines for adhesive E was higher in Roselle than in Cannara; on the contrary, it was slightly lower in Roselle for adhesive M.

Finally, for all the traits, the coefficients of phenotypic variation were comparable between the two sites, even if slightly smaller in Roselle. Lower variation compared with the other traits was observed for basic density and volumetric shrinkage.

Clonal consistency within and across sites

The statistical analysis and the estimation of repeatability values were performed considering the two sites separately (Table 2) and together (Table 3). Results for DBH and H have already been shown and discussed (Nocetti et al. 2015); here, they are shown only for comparison with wood traits.

The analysis carried out separately at each site (Table 2) indicated a substantial consistency between the two locations regarding wood traits. The repeatability values were moderate to high: At Roselle, T/R showed the highest value (46.1), whereas at Cannara, the highest value was noticed for BD. Moreover, for SS-E, the clone effect was significant at Cannara (but it showed the lowest repeatability), but no difference among clones was observed at Roselle for this trait, whilst the results for SS-M were in line with the findings of the other wood traits. The coefficients of genotypic variation were low to moderate, but slightly higher at Cannara.

The combined analysis indicated a significant ($p < 0.05$) site effect for growth traits, basic density and bonding quality, but not for linear and volumetric shrinkages, shape factor and hardness. The other effects, heartwood/sapwood position when assessable, and clone and site × clone interaction, were significant too.

The estimated variance component due to clone was relatively high for T/R and BD, in that the individual-tree repeatability was relatively high for these traits. Shape factor (T/R) also showed relatively high repeatability of clone means, very similar to BD. The smallest such values were observed for bonding quality, with the shear strength of adhesive E (SS-E) close to zero.

The estimated percentage of variance due to the interaction site × clone was much lower in the wood traits compared with growth. Nevertheless, the variance due to error accounted for well over half the variation for all growth and wood traits (Table 3).

The role of the environment in generating interaction was also estimated by means of Type-B genetic correlations

Table 1 Means, range, and observed individual-tree coefficients of phenotypic variation ($\widehat{CV}_p$) of all measured traits of 10 *C. sempervirens* clones, grouped by site (Roselle and Cannara)

Trait	Acronym	Roselle			Cannara		
		Mean	Range	$\widehat{CV}_p$ (%)	Mean	Range	$\widehat{CV}_p$ (%)
Diameter at Breast Height (cm)	DBH	19.3	15.0–27.0	12.6	25.0	19.7–30.0	10.0
Height (m)	H	10.5	7.5–12.5	9.3	17.7	14.9–20.0	7.8
Basic density (kg/m^3)	BD	461	378–574	6.9	429	329–546	8.3
Radial shrinkage (%)	RS	3.4	1.6–5.2	13.3	3.2	1.4–6.5	16.8
Tangential shrinkage (%)	TS	5.6	2.6–7.5	10.1	5.4	2.6–8.7	10.7
Volumetric shrinkage (%)	VS	9.1	5.6–11.4	7.5	8.7	5.8–12.2	8.7
Tangential/radial shrinkage ratio (−)	T/R	1.7	1.1–3.0	16.7	1.8	0.7–4.0	20.5
Hardness (MPa)	HB	21.3	14.2–31.9	17.4	19.8	10.5–30.1	25.1
Shear strength of glue line – E (MPa)	SS-E	5.8	3.5–8.2	13.7	4.8	2.1–7.1	19.7
Shear strength of glue line – M (MPa)	SS-M	1.9	1.3–2.8	13.7	2.2	1.2–3.2	18.5

(Tables 3 and 4), which were not calculated for traits that did not show significant clone effect in one or both sites (SS-E in separate analysis) (Table 2). The correlations for wood traits were positive and very high; the lowest value of 0.76 was registered for HB, but it was not significantly different from 0 ($p > 0.05$). The significance against the null hypothesis of +1 was also checked for the Type-B genetic correlations: None of them resulted significantly different from 1 ($p > 0.05$).

Then, it was observed that the significance of genotype × environment interaction was due to small changes in the relative rankings of few genotypes in the two locations. As an example, the Fig. 2 represents the performance of the 10 clones in the two sites for BD.

Correlations between traits and selection efficiency

Estimated phenotypic and genetic correlations between traits are presented in Table 5; genetic correlations for SS-E were not reported because of the very low variance due to genotype observed for this trait.

It is known that genetic correlations are even more difficult to estimate precisely than repeatabilities, and they required very large experiments with high numbers of genotypes involved (White et al. 2007). Therefore, due to the rather small number of clones investigated and the low repeatabilities of growth variables, the observations below should be considered as indicative, referring just to positive or negative relationships.

Table 2 Results from the mixed linear model separately by site for individual traits: significance of fixed effect (HS = heartwood/sapwood position); estimated variance components as percentage of the total variances for clone effect ($\hat{\sigma}_c^2$), genotypic variation ($\widehat{CV_G}$) and estimated repeatabilities ($\hat{R}_b^2$ = individual-tree clonal repeatability; $\hat{R}_c^2$ = repeatability of clone means; SE = approximate standard error)

Trait	Roselle						Cannara					
	HS	Clone		$\hat{R}_b^2$	$\hat{R}_c^2$ ± SE	$\widehat{CV_G}$	HS	Clone		$\hat{R}_b^2$	$\hat{R}_c^2$ ± SE	$\widehat{CV_G}$
	Sig.	$\hat{\sigma}_c^2$ (%)				(%)	Sig.	$\hat{\sigma}_c^2$ (%)				(%)
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
BD	***	29.2	***	0.29	0.62 ± 0.15	3.7	***	49.8	***	0.50	0.80 ± 0.09	5.9
RS	***	34.9	***	0.35	0.68 ± 0.13	7.9	***	32.5	***	0.33	0.66 ± 0.14	9.6
TS	***	28.4	***	0.28	0.61 ± 0.15	5.4	***	27.6	***	0.28	0.60 ± 0.15	5.6
VS	***	22.3	***	0.22	0.53 ± 0.17	3.6	***	25.9	***	0.26	0.58 ± 0.16	4.4
T/R	***	46.1	***	0.46	0.77 ± 0.10	11.3	***	40.8	***	0.41	0.73 ± 0.12	13.1
HB	–	29.9	***	0.30	0.63 ± 0.20	9.5	–	48.1	***	0.48	0.79 ± 0.13	17.4
SS-E	–	0.9	ns	–	–	–	–	11.1	***	0.11	0.33 ± 0.24	12.5
SS-M	–	16.7	***	0.17	0.44 ± 0.24	8.7	–	22.4	***	0.22	0.54 ± 0.22	12.2

ns not significant

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

Table 3 Results from joint linear mixed model for traits measured on 10 *C. sempervirens* clones at the two sites combined: significance of fixed effects (site and HS); estimated variance components for random effects ($\hat{\sigma}^2$) as percentage of total variation, and estimated repeatabilities ($\hat{R}^2_b$ = individual-tree clonal repeatability; $\hat{R}^2_c$ = repeatability of clone means; SE = standard error)

Trait	Site Sign.	HS Sign.	Clone $\hat{\sigma}^2_c$ (%)		Site X $\hat{\sigma}^2_{SxC}$ (%)	Clone	Error $\hat{\sigma}^2_e$ (%)	$\hat{R}^2_b$ ± SE	$\hat{R}^2_c$ ± 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
BD	***	***	32.1	**	7.5	***	60.4	0.32 ± 0.14	0.74 ± 0.10
RS	ns	***	23.4	**	8.0	***	68.9	0.23 ± 0.13	0.65 ± 0.12
TS	ns	***	24.7	***	3.1	*	72.2	0.25 ± 0.13	0.70 ± 0.11
VS	ns	***	18.1	**	5.8	***	76.1	0.18 ± 0.12	0.59 ± 0.13
T/R	ns	***	35.0	***	4.6	***	60.4	0.35 ± 0.14	0.78 ± 0.09
HB	ns	–	26.2	***	15.4	**	58.4	0.26 ± 0.18	0.64 ± 0.17
SS-E	***	–	0.1	***	5.9	**	94.1	0.00 ± 0.08	0.01 ± 0.09
SS-M	**	–	15.3	***	5.2	***	79.6	0.15 ± 0.15	0.55 ± 0.18

ns not significant

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

Generally, wood traits were poorly correlated with growth; the few significant correlations noticed were negative, with the exception of the relationship between DBH and SS-M, which was positive.

Among wood traits, BD was positively correlated both with shrinkage and hardness, and negatively or not correlated with bonding quality (SS-E and SS-M) and T/R, respectively. Linear shrinkages (RS and TS) seemed to be independent of each other but showed a positive autocorrelation with VS. Also, a negative autocorrelation was noticed between RS and T/R.

HB was positively correlated with BD and shrinkages, but not with T/R, whilst SS-M and SS-E were negatively correlated both with BD, shrinkages and HB.

The comments above were noticed at both sites (details not shown).

Tentative calculations of the efficiency of indirect selection (details not shown) indicated that any wood trait had an evident advantage of direct selection compared to what could be achievable by indirect selection. The only exception could be BD in respect to HB and SS-M: a selection based on higher BD could result in an effective improvement for HB and a substantial loss of SS-M.

Table 4 Estimated genetic intra-trait, between-sites correlations for the 10 *C. sempervirens* clones (Type-B)

Trait		
BD	0.95	***
RS	1.02	***
TS	0.88	**
VS	0.98	***
T/R	1.00	***
HB	0.76	ns
SS-M	1.09	*

ns not significant

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

Discussion

Technological characterization of wood across the two sites

The technological properties observed in the two clonal trials revealed a low–medium density wood, with low shrinkage and high dimensional stability. Since the trees were younger than the rotation age for a commercial plantation, some

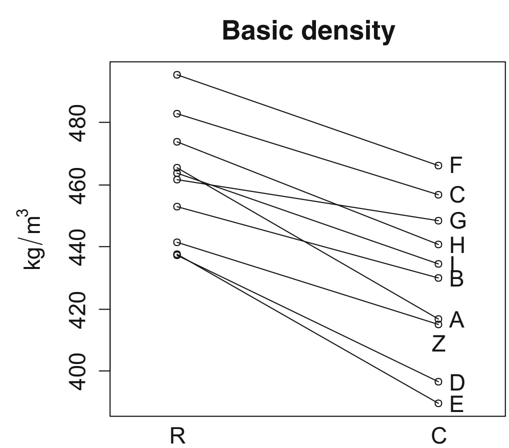

Fig. 2 Performance of the 10 clones in the two sites (*R* Roselle, *C* Cannara) for basic density

Table 5 Phenotypic (below the diagonal) and genetic (above the diagonal) inter-trait correlations combined across the two sites (Cannara and Roselle) for each trait of 10 *C. sempervirens* clones

Trait	DBH	H	BD	RS	TS	VS	T/R	HB	SS-E	SS-M
DBH		0.58	−0.54	−0.29	−0.74 *	−0.75 *	−0.21	−1.00 ***	–	0.87 *
H	0.40		−0.36	−0.72 *	−0.31	−0.59	−0.52	−0.69	–	0.81
BD	−0.28	−0.30		0.59	0.72 *	0.84 **	−0.08	1.00 ***	–	−0.91 *
RS	0.06	−0.55	0.64 *		0.23	0.76 *	−0.79 **	0.96 **	–	−1.00 ***
TS	−0.47	−0.15	0.66 *	0.19		0.81 **	0.42	1.00 ***	–	−0.65
VS	−0.31	−0.39	0.86 **	0.71 *	0.83 **		−0.19	1.00 ***	–	−0.86 *
T/R	−0.35	0.43	−0.11	−0.76 *	0.48	−0.09		0.12	–	0.39
HB	−0.31	−0.61	0.99 ***	0.85 *	0.82 *	0.94 **	0.06		–	−1.00 ***
SS-E	−0.07	0.17	−0.85 *	−0.98 ***	−0.37	−0.68	0.55	−0.78		1.00 ***
SS-M	0.17	0.50	−0.94 **	−0.93 **	−0.50	−0.74	0.36	−0.89 *	0.94 **	

*Significant at 5% level; **significant at 1% level; ***significant at 0.1% level; otherwise not significant $p > 0.05$)

differences in technological properties compared with the harvested material can be expected, due to both the process of heartwood formation and the known radial variability of wood. However, a comparison with previous observations (see below) on cypress wood revealed no marked discrepancies. For *C. sempervirens* in the Mediterranean, Thibaut et al. (1999) reported the following mean values: 420 kg/m^3 for basic density, and 3.1%, 5.9% and 9.1% for radial, tangential and volumetric shrinkages, respectively. The same species in three natural populations of Greece was characterized by a basic density ranging from 447 to 510 kg/m^3 (Paraskevopoulou 1991). In Iran, basic density ranged between 410 and 460 kg/m^3, while radial, tangential and volumetric shrinkages varied between 2.55 and 3.88, 4.70 and 7.21, and 7.72 and 11.00%, respectively (Hosseini Hashemi and Kord 2011).

The values observed for wood hardness are difficult to compare with those reported in the literature, since hardness can be assessed using a wide range of techniques (Doyle and Walker 1985). However, the clones tested here ranked among the average of similar cypress species (Kothiyal et al. 1998; Okino et al. 2010).

Comparisons of bonding quality can be performed with technical standardization of solid-wood panels (EN 13353 2011). According to the standard, the lower 5th percentile of the shear strength on glue line should not be less than 2.5 N/mm^2 for single-layer solid-wood panels and not less than 1.2 N/mm^2 for multi-layer panels. These requirements were met by both adhesives in multi-layer panels, but only by adhesive E for the single layer; the 5th-percentile for adhesive E was 4.5 N/mm^2 for Roselle sample and 3.4 N/mm^2 for Cannara, while for M, it was 1.5 and 1.6 N/mm^2 for Roselle and Cannara, respectively. In this sense, the adhesive M appeared to not be suitable in assemblies obtained by gluing two elements/lamellae (and characterized by a single bond surface, instead of two).

The loss of performance of both products (M and E) when tested wet compared to the dry-test values is associated with the decrease of all mechanical properties of wood with increasing moisture content. This decrease was more evident for adhesive M compared to E. Indeed, the two products are chemically different, and hence their setting mechanism is also different. In product M, the adhesive polymer is already formed and dispersed in water; after the application, water is removed by both diffusion (in wood) and evaporation, and a continuous film is then formed. In contrast, in product E, the added hardener is able to crosslink the prepolymer, by means of a chemical reaction, and this makes the final adhesive stronger and more resistant to both water and temperature.

Clonal consistency and repeatability of wood traits

Unlike growth traits, wood properties were rather consistent across the two environments. Significant site effects in the combined analysis were observed only in basic density and bonding quality. In accord with previous results on other species (for example Pliura et al. (2007); Zhang et al. (2003) and Zhang et al. (2012) on poplar; Nocetti et al. (2010) on wild cherry), the influence of the environment on wood traits was far less important compared with on growth rates.

As confirmation of the clonal consistency of wood traits, the percentage of variation due to the genotype × environment interaction was small, far lower than that due to the clone effect. Also, the inter-site genetic correlations were strongly positive. White et al. (2007), explaining the possible reasons of significant genotype × environment interaction, reported two possible effects: the rank-change interaction, that is when the relative ranking of clones changes in the different environments, or the presence of different among-clones variability of performance even if rankings remain constant, this was called scale-effect interaction. Looking at the clonal performance in the present trials, the small genotype × environment effect could be ascribed to changes of the rankings of a few clones across the two locations.

Many of the studies analysing wood traits focused on density, concluding that trees had minor phenotypic plasticity for this character (Pliura et al. 2007; Zhang et al. 2012; Wu et al. 2008), even if a clear main effect of site on basic density is also widely recognized in the literature (Auty et al. 2014; Kimberley et al. 2016). Here, cypress clones were also very consistent across different environments in terms of wood density, shrinkage, dimensional stability, hardness and attitude to bonding. This is even more significant if it is compared to the marked plasticity for growth and other morphological traits observed for the same cypress clones in the same trials (Nocetti et al. 2015) and the ability of this species to tolerate a wide variety of climates and edaphic conditions.

Thus, the repeatability values for wood traits (particularly the clonal means) were rather high, with the exception of the performance of adhesive E (SS-E). The values observed ranged from 0.55 for adhesive M to 0.78 for dimensional stability in the combined analysis, and when considered by site, the results were similar, with values ranging from 0.44 again for adhesive M in Roselle to 0.80 for basic density in Cannara, indicative of a high effect of genotype on the wood traits. Repeatabilities were then comparable with what reported for *Pinus radiata* in New Zealand and Australia (Wu et al. 2008), eucalyptus in Brazil (Botrel et al. 2007), poplar in Canada (Huda et al. 2014; Pliura et al. 2007; Zhang et al. 2003; Zhang et al. 2012) and China (Wu et al. 2013), and acacia in Vietnam (Hai et al. 2009). Only in a study on *C. lusitanica* in Tanzania, the 32 families sampled did not differ significantly for basic density (Mugasha et al. 1997).

Despite the satisfactory values of repeatabilities and the relatively low influence of the environment on wood traits, most of the variation in these properties represented residual error. The variance due to error represented not only differences among ramets within clone and site but also the intra-tree variability, one of the main sources of variation for wood traits (Pliura et al. 2007; Zhang and Jiang 1998). In the present study, it was taken into account partially with the fixed effect HS, which was highly significant for all the traits where it was assessable. Previous works (Nocetti et al. 2010; Rink and Phelps 1989) already underlined the importance of heartwood and sapwood identification when dealing with genetic studies on wood. However, the within-tree variation can be due to other factors too, such as cambial age and ring width. While an exploratory analysis of variance showed heartwood formation to be far more important than cambial age in explaining the variation observed in density and shrinkages (details not shown), the residual variance might still accommodate cambial age, as well as other factors that cannot be separated in the analysis.

The findings outlined above indicate that selection for individual wood traits (except SS-E) would be very effective. However, the genetic gain might be lower than expected because of the low genetic variability of wood properties (Zhang et al. 2003). Some authors consider the coefficient of variation to be more meaningful when evaluating the genetic variation and the response to selection, because it is independent of the residual variance and the scale effects (Pliura et al. 2007). In the present experiment, the coefficients of genotypic variation were quite low, and slightly higher in Cannara, but in line with other observations for wood traits (Cornelius 1994; Wu et al. 2013).

Correlations between traits

Although indicative only, the analysis of the phenotypic and genotypic relationships between traits revealed adverse correlation between wood properties and growth. In particular, wood density seemed to be negatively influenced by a faster growth rate. The same was observed for *C. sempervirens* in Greece (Paraskevopoulou 1991), where wider rings were associated with a lower wood density, as well as in other conifers (e.g. *Pinus radiata*, Wu et al. 2008).

Density has been often considered as the most important trait to be included in improvement programmes when wood properties have to be taken into account. It is easy to measure and can be considered as a global indicator of wood quality (Hosseini Hashemi and Kord 2011; Huda et al. 2014; Rozenberg et al. 2001; Wu et al. 2008). In the present study, density was positively correlated both with linear and volumetric shrinkage and with hardness; no significant correlations were observed with the dimensional stability, whilst negative correlations were observed with bonding performance, mainly in the case of the adhesive M.

In a previous work focusing on growth and morphological traits of cypress clones (Nocetti et al. 2015), the authors pointed out how a selection based on tree height should be preferred to avoid negative consequences on stem form and branch dimension. This same selection strategy seems appropriate but should be combined with wood traits too.

High-density wood is usually preferred for solid-wood products; therefore, if the end-product is sawn timber, higher wood density should be favoured (Paraskevopoulou 1991). Hardness is also important for high-value interior joinery, furniture or window frames. Selecting for higher density would result in higher shrinkage, but the dimensional stability would not be negatively affected. Dimensional stability is crucial not only for the workability and drying of raw material but also for the response of the end-product to any change of ambient moisture levels that may induce distortion and warping (Huda et al. 2014).

In this context, the bonding performance deserves particular attention, since it can be explained by a combination of wood properties and adhesive characteristics. When adhesive

M was used, a negative relationship was observed between wood density and the shear strength of the glue line.

Conversely, adhesive E was more independent of the physical properties of the material, and this could also explain the lack of clonal discrimination. The differences found for the two adhesives can be explained by their different interactions with the wood. In particular, for product M, the performance is largely determined by its ability to penetrate inside the wood, which is lower in high-density material. Conversely, the penetration depth is less important for adhesive E, which utilizes additional types of chemical-physical interactions with the support (e.g. hydrogen bonds in addition to mechanical interlocking).

Therefore, by favouring higher density during selection and the use of adhesives like E, it should be possible to achieve good results in terms of both wood quality (higher hardness and good dimensional stability) and bonding performance.

Conclusions

The key wood traits, those considered to be important for the use of wood as solid timber, unreconstituted or glued, have been studied in cypress clones previously selected for resistance to cypress canker disease and intended for timber production in plantations. Despite the spread of bark canker, selection of cypress genotypes for wood production seems very promising.

The estimates of genetic parameters assessed the consistency of wood quality across environments. Considerable variation was evident among clones in several wood properties which included basic density, shrinkages and hardness.

These clonal variations in wood properties were generally much more consistent across the two contrasting environments than clonal variations for growth. This is very important, considering the plasticity of Mediterranean cypress regarding growth and adaptation to different climates and soils. The natural distribution of *C. sempervirens*, and the vast area where it was introduced since ancient time (and now naturalized) (Caudullo and Della Rocca 2016; Caudullo and de Rigo 2016), shows its adaptability and the possibility of cultivation throughout the Mediterranean area. Nevertheless, growth traits, and hence yields, may vary markedly among sites.

The performance of a timber plantation will reflect three main factors: the environment, the genotype and the cultural practises. The last-mentioned will be an important further step in following up the present research, while the performance of genotypes and the interrelationships among traits represent useful bases for future selection strategies.

Evidence for clonal correlations between traits was very limited but included indications of a negative association between wood density and growth rate, and positive associations between basic density, shrinkage and hardness. Selection based exclusively on growth rate could result in a decrease in wood density as well as hardness.

Finally, the study of the gluability aimed at analysing the performance of different genotypes in a real technological process. The results derived from the combination of raw material properties and the adhesive used. In particular, performance of adhesive type M (for which the penetration in wood is crucial) was negatively correlated with density: the higher the density, the worse the bonding performance. The use of the adhesive type E was not as strongly influenced by the density, thus favouring higher-density wood (and therefore also hardness) and providing excellent performances for bonding.

Acknowledgements 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.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Data archiving statement The cypress clones in the study belong to the germplasm collection and breeding programme of IPSP-CNR and are identified in the archive of mother plants of the Institute as accessions: PM 37, PM 38, PM 43, PM 45, PM 160, PM 239, PM 304, PM 736, PM 740 and PM 820. Phenotype data are currently being submitted to TreeGenes database, and accession numbers will be supplied once available.

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