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Research paper

Growth performance of selected eucalypt hybrid clones for SRWC in central and southern Italy

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Abstract - Eucalypt short-rotation woody crop (SRWC) is becoming an attractive option for energy biomass in Mediterranean dry environments. The present study is aimed at assessing growth performance of selected eucalypt hybrid clones for SRWC in three Italian sites (Massama, Sardinia; Mirto, Calabria; Rome, Latium) compared with *Eucalyptus camaldulensis*, the most commonly cultivated eucalypt species in Italy. The study identified eucalypt clones with stable and high performance between several alternatives. Results pointed out the declining growth performance observed in the second rotation compared with the first cycle. This is due to the cultivation model, age rotation and harvesting method adopted, which negatively affect the available soil nutrients' content. The clone/site interaction as for basal area growth at the three investigated sites, suggests a significantly different clones' performance among sites. Viglio and Velino clones showed the best overall performance and are suggested to be used over the large scale SRWC in central and southern Italy.

Keywords - agroforestry, bioenergy, Eucalyptus, clones, Italy

Introduction

Eucalypt was cited, for the first time in Italy, by Graefer (1803, in Agostini 1953) in the "Reggia di Caserta" royal botanical garden. By that time eucalypts spread, as ornamental tree and botanical curiosity, in gardens and parks of southern Italy. In central Italy, the first plantations were established by Trappist Fathers in the second part of XIX century near Rome and in Maremma (southern Tuscany). In the same period, plantations were established in Sicily and Sardinia respectively by Railway State Company and Mine Company for sleepers and mine supports (Gemignani 1988). Between 1920 and 1950, big works of marsh reclamation were made, and eucalypts were planted for shelterbelts in Sardinia (Arborea), Latium (Agro Romano and Pontino) and Apulia (Pavari et al. 1941, Bassi 1951, Giuliani et al. 1951, Susmel 1951).

Large reforestation programs took place in southern Italy during the 1950s for soil protection purposes and especially during the 1980s for the production of paper mill raw material. The most used eucalypts species were *Eucalyptus globulus* Labill. ssp. *bicostata*, *E. globulus* Labill. ssp. *globulus*, *E. occidentalis* Endl, *E.* x *trabutii* M. Vilm., *E. camaldulensis* Dehnh. and *E. viminalis* Labill. The area of eucalyptus plantations in mid 1980s was estimated at 72,000 hectares, of which 54,000 ha in pure stands and 18,000 ha in mixed stands (Boggia 1987). Productivity varies depending on the species and sites. As an example, *E. globulus* ssp *globulus* and *E. occidentalis* productivity range respectively between 10 and 35 m^3 ha^{-1} $year^{1}$ (Avanzo 1964, Ciancio et al. 1981, Bronzi et al. 1987) and between 3 and 8 m^3 ha^{-1} $year^{1}$ (Gemignani 1988). Cultivation density averaged 1,100 to 1,600 plants per hectare with 3 x 3 m or 3 x 2 m spacing. The most used rotation ages encompass 10-12 years for 3-4 harvests.

Eucalypt short rotation woody crops (SRWC) is becoming an attractive option for energy biomass in Mediterranean dry ecosystems. Research programs have been activated to evaluate the feasibility of this option (Facciotto et al. 2007, Facciotto et al. 2009), to producing ligno-cellulosic biomass in very short time (2-3 years). Uses of Eucalypt biomass include energy production as alternative method to the conventional ones that use fossil fuel (coal and oil) originating greenhouse gases (Dallemand et al. 2008). Two cultivation models were identified as the most productive and suitable options for central and southern Italy: (i) 5,000-5,500 plants ha^{-1} for 3-4 rotation cycles lasting 2-3 years and (ii) 1,100-1,600 plants ha^{-1} for 2 rotation cycles lasting 5-6 years. Deep soils, irrigation or a superficial water table during dry periods and fertilization especially

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Table 1 - Selected soil characteristics at the three experimental fields in central and southern Italy.

Site	Mirto, Calabria			Rome, Latium			Massama, Sardinia		
	lat. 39°36' (N) long.16°46' (E) elev.10 m			lat. 41°55' (N) long.12°21' (E) elev. 81 m			lat. 39°57' (N) long. 08°36' (E) elev.15 m		
Soil depth (cm)	0-30	30-60	60-90	0-30	30-60	60-90	0-30	30-60	60-90
Sand (%)	30.00	64.09	25.00	63.00	65.00	66.00	50.07	64.64	56.18
Loam (%)	61.47	35.97	59.49	16.00	16.00	14.00	11.87	9.97	12.40
Clay (%)	18.53	19.94	20.51	21.00	19.00	20.00	38.05	25.39	31.42
pH in H2O	7.62	8.03	8.01	7.50	7.70	7.70	7.88	8.03	8.28
Organic matter (%)	5.57	0.92	0.51	1.03	0.22	0.22	0.94	0.36	0.44
Total Ca (%)	3.96	3.14	2.53	Trace	Trace	Trace	8.19	13.57	17.61
Active Ca (%)	0.12	1.47	0.48	-	-	-	2.86	3.65	4.19
Total N (%)	-	-	-	0.06	0.02	0.02	0.08	0.04	0.04
P (ppm)	4.00	2.00	2.00	8.00	5.00	5.00	-	-	-
K (ppm)	31.00	49.00	63.00	622.00	641.00	649.00	-	-	-
Mg (ppm)	-	-	-	490.00	510.00	575.00	-	-	-
Na (ppm)	-	-	-	251.00	375.00	391.00	-	-	-

at the planting time and after the harvest, are being considered necessary to get valuable growth rates (Facciotto et al. 2007, Facciotto et al. 2009). *Eucalyptus camaldulensis* Lake Albacutya is, at the moment, the best choice for eucalypt plantations in Mediterranean climate in central and southern Italy (Gemignani 1968, Lacaze 1970). Mughini (1997, 2000) described a few eucalypt hybrid clones selected for SRWC with higher growth rate than *E. camaldulensis*.

Based on these premises, the aim of this paper is to compare the growth performance of selected clones with *E. camaldulensis* in three sites of central and southern Italy (Rome, Latium; Massama, Sardinia; Mirto, Calabria). A clonal test to 5,500 plants ha^{-1} and 1,600 plants ha^{-1} was established at all sites (including *E. camaldulensis* as a control). Sectional Area (SA) and percent Survival (S) to the end of each rotation cycle were evaluated as growth performance indicators.

Materials and methods

Study sites

The climate of the three experimental sites is typically Mediterranean. A dry period of 3-4 months (June-September) was observed with summer rainfall averaging 59, 54 and 12 mm in Mirto, Rome and Massama (1961-1990). The three sites received 585 mm, 840 mm and 616 mm of rainfall concentrated in autumn-winter time. Summer water deficit limits plant growth if irrigation is not provided. Selected soil characteristics at the three sites are shown in Table 1.

Planting materials

The eucalypt hybrid clones used in the experimental fields are reported in Table 2. *E. camaldulensis* Lake Albacuya, the most common eucalypt species cultivated in Italy was used as control. *E. camaldulensis x E. globulus* subsp *bicostata*, *E. camaldulensis x E. viminalis*, *E. camaldulensis x E. grandis* hybrid clones were selected during the 1990s for biomass plantations. These clones were obtained by controlled cross pollinations using *E. camaldulensis* mother trees, selected in the Lake Albacutya provenance seed orchard (established near Rome), and pollens collected from the best trees of the provenance tests of *E. viminalis* and *E. globulus* subsp *bicostata*. *E. grandis* pollens were collected from Morocco secondary provenances. Among the 900 hybrid seedlings obtained by the above mentioned crosses, 100 clones were selected on the base of rooting ability (> 90%). Clones were tested in optimum, cold and drought conditions and the best 15 were selected for growth performance. The planting materials used in the three experimental fields were 6-8 months years-old rooted cuttings and seedlings respectively for hybrid clones and *E. camaldulensis* (Mughini 1997, 2000).

Crop method

Three experimental plots were established in spring 2004. Before planting, chemical weeding using glyfosate was performed, after soil plowing at 30-40 cm depth. Each plant was fertilized with 40 g of slow-release fertilizer (9 + 20 + 8 + 3.0 MgO + 0.1 B). Chemical weeding was applied after plantation using Oxyfluorfen (0.50-0.70 liter ha^{-1} active ingredi-

Table 2 - Control and eucalypt hybrid clones used at the three experimental fields.

Clone/control	Species/hybrid
E. cam	*E. camaldulensis*
14	*E. camaldulensis x E. globulus ssp bicostata*
20	*E. camaldulensis x E. globulus ssp bicostata*
22	*E. camaldulensis x E. globulus ssp bicostata*
40	*E. camaldulensis x E. globulus ssp bicostata*
44	*E. camaldulensis x E. globulus ssp bicostata*
45	*E. camaldulensis x E. globulus ssp bicostata*
13a	*E. camaldulensis x E. globulus ssp bicostata*
Velino	*E. camaldulensis x E. globulus ssp bicostata*
Viglio	*E. camaldulensis x E. globulus ssp bicostata*
65	*E. camaldulensis x E. grandis*
81	*E. camaldulensis x E. viminalis*

ent). Until the rotation age, the soil was disked 2-3 times during the growing season (10 cm depth) for weed control and to prevent fire risk.

Experimental design

Plants (rooting cuttings and seedlings) were planted at two densities: (i) 5,500 plants ha^{-1} and (ii) 1,600 plants ha^{-1}. In the first case, the inter-rows distance was 3 m and the intra-row distance between plants was 0.60 m, the elementary plot for each accession (clone or control) being made by 20 plants in a single row. In the second case, the inter-rows distance was 3 m and the intra-row distance between plants was 2 m, the elementary plot for each accession being made by 6 plants in a single row. Each test was established with completely randomized design with each accession replicated 5 times as a minimum.

Statistical analysis

Only tests providing complete datasets collected at the end of the rotation cycle were performed. In each test, the diameter at breast height (dbh) and S of all the plants were measured each year in autumn. Dbh was measured with a digital caliper taking two orthogonal measurements. Dbh was converted to SA (cm^2) of stem/shoot using the equation $\pi(dbh/2)^2$. Plot SA for each accession was derived by summing the SA of every plant in each plot. The plot accession S was calculated for each plot. Plot accession SA and S were analyzed through ANOVA with the aim to verify: (i) accession x rotation interaction (SA and S accessions at the end of first and second rotation cycle of Mirto site 5,500 plants ha^{-1}); (ii) accession x site interaction (SA and S accessions at the end of the first rotation cycle in the three tests with 5,500 plants ha^{-1}) and (iii) SA and S accessions at Mirto site with 1,600 plants ha^{1}. Data were tested for normality and homoschedasticity of residuals.

Results and Discussion

Accession x rotation interaction

Differences between SA accessions and rotations (Table 3) are significant ($p < 0.01$) while accession x rotation interaction was found not significant. S differed significantly between accessions ($p < 0.01$). Results suggest that percent survival did not influence SA in the first and second rotation. On the contrary, results indicate that accessions show different performances being quite stable in the two rotations. SA differed however between the first and second rotation; the SA of the first rotation was found greater than the second rotation (427.6 cm^2 and 335.4 cm^2, respectively). This pattern was reported also by FAO (1979) and Mora et al. (2000) and clashes with what observed for poplar and willow SRWC, SA increasing with rotation (Paris et al. 2011). Species, pedo-climatic conditions, cultivation models and harvesting system may influence this pattern.

Limiting factors to growth rate in the second rotation in Mirto are the cultivation model, rotation age and harvesting system which reduce the available nutrients in the soil. In the adopted cultivation model, plants were fertilized only at the planting time but not in the following years and after harvest. The harvesting system used for SRWC (modified forage harvester) collects the whole plant, including branches, leaves and bark. This operation removes, according to Mora et al. (2000) more than 70% of the nutrients. Moreover, according to a recent study carried out in clonal plantations in Congo (Laclau et al. 2000), the rotation age used in our tests (2 years) coincides with the period where nutrients are supplied by uptake from soil reserves, preventing the intense nutrients recycling that characterizes the period from the third year to the rotation age. As a matter of fact, a rotation age of 7-10 years is recommended in conventional plantations (660 to 1,600 plants ha^{-1}) to maintaining soil fertility, whilst the most suggested harvesting system removes only the logs leaving bark, branches and leaves in the field (Laclau et al. 2003).

In Italy, this cropping system cannot be implemented in SRWC with 2-3 years rotation age since fertilization is an expensive operation and cultivation is no profitable without public incentives (Facciotto et al. 2003, Sperandio 2004, Manzone et al. 2009). In northern Europe irrigation with urban pre-treated waste-water was introduced to address this problem in poplar and willow SRWC (2-3 years rotation age) (Dallemand et al. 2008, Dimitrou et al. 2005). The same method was successfully applied to

Table 3 - Combined ANOVA for stem/shoot sectional area (SA) and percent survival (S) between accessions and rotations (first and second rotation) and between accessions and sites in the sites with 5,500 plants ha^{-1} (ns: not significant, ** $p < 0.01$).

Variable		SA	S			SA	S
Source of variance	df	MS	MS	Source of variance	df	MS	MS
accessions	11	58,187.8**	740.883**	accessions	11	26,257.2**	537.54**
rotations	1	18,6819.7**	309.375ns	sites	2	893,579.8**	14,779.31**
accessions x rotations	11	9,701.06ns	40.057ns	accessions x sites	22	18,277.90**	252.612**
residual	64	19,877.93	113.67	residual	116	5,837.85	115.78

Table 4 - ANOVA for stem/shoot sectional area (SA) and percent survival (S) in the first rotation of accessions at 1,600 plants ha^{-1} density.

Variable		SA	S
Source of variance	df	MS	MS
Accessions	11	3,998.367**	761.720**
Residual	32	729.258	110.240

eucalypt plantations in Northern Africa countries, Australia and USA (Myers et al. 1998, Rockwood et al. 2004).

Accession x site interaction

A significant difference in percent survival between accessions and rotations was found, it suggesting that S could influence SA performance between sites. This is especially true in the case of Rome site, where S is only 61.7% (Table 4). The SA average (Table 5) is similar in Massama and Rome (189 cm^2 and 169 cm^2, respectively) and higher at Mirto (427.6 cm^2) with a statistically significant difference ($p < 0.01$). The difference should be due to: (i) the presence of a superficial water table (1-2 m depth) in Mirto and (ii) the severe galls makers insects (*Ophelimus maskelli* Ashmead and *Leptocybe invasa* Fisher & La Salle) attacks occurred in 2004 and 2005 (Arzone et al. 2000, Viggiani et al. 2001 and 2002, Bella et al. 2002, Bagnoli et al. 2004, Laudonia et al. 2004, Laudonia 2006, Protasov et al. 2007).

Some clones showed SA lower than *E. camaldulensis* (clone 13a in Massama; clone 45 in Rome; clones 81, 13a, 65 and 45 in Mirto). These results clash with those obtained in a previous study (Mughini 2000). The accession x site interaction suggests that clones and the control show interactive performance with environmental conditions. The same results were obtained in Portugal (*E. globulus* clones), Morocco (*E. grandis*, *E. tereticornis*, *E. rudis x E. camaldulensis*, *E. grandis x E. camaldulensis* clones) and Brazil (Borralho et al. 1992, Nunes et al. 2002, Belghazi et al. 2008).

In Mirto, the difference in the SA observed between accessions at the first rotation was significant (Table 6). The observed SA ranged between 376.98 cm^2 (clone 22) and 2020.18 cm^2 (clone 14). Only clone 22 showed a SA value lower than the control.

Selection of the most productive clone

The more productive and stable clone at all sites was identified as the best clone. Clone x site interaction may indicate a separate site selection, which can be assumed in the case of different economic importance of eucalypt raised in the three areas. Clones showing a good overall performance across sites should be selected with practical advantages in breeding programs and nursery and commercial management (Borralho et al. 1992, Nunes et al. 2002). Stable clone selection has been adopted recently in Spain in the case of poplar clones selection for SRWC (Sixto et al. 2011). The following methodology was adopted to select the most stable and productive clone: (i) the five clones showing higher SA than the control at all sites; (ii) the five clone and control performances observed at each site to analyze phenotypic plasticity and (iii) the two most suitable clones were identified.

Viglio and Velino were identified as the most productive and stable clones. The same results were

Table 5 - Means and standard error (in brackets) of stem/shoot sectional area (SA) of *Eucalyptus* clones and the control (*E. camaldulensis*) tested at the three experimental fields in central-southern Italy.

Site	Massama (Sardinia)	Rome (Latium)	Mirto (Calabria)		
Age	R3S3[1]	R3S3	R2S2	R4S2	R6S6
Plants density per hectare	5,500	5,500	5,500	5,500	1,600
Clone	cm^2	cm^2	cm^2	cm^2	cm^2
22	155.42 (21.51)	142.65 (57.57)	428.13 (56.09)	357.67 (170.40)	376.98 (90.63)
E. cam	150.89 (52.24)	107.48 (4.76)	358.40 (29.72)	273.98 (32.50)	537.69 (74.79)
81	180.71 (39.65)	173.64 (33.12)	277.42 (37.56)	247.05 (37.24)	608.27 (34.10)
13a	148.95 (23.31)	150.23 (45.77)	313.06 (17.43)	338.88 (31.72)	861.95 (163.64)
65	320.92 (29.90)	246.00 (25.16)	323.41 (8.51)	276.13 (29.72)	869.01 (166.10)
45	221.76 (28.91)	98.31 (30.68)	308.02 (52.36)	218.63 (48.98)	889.44 (80.10)
20	166.26 (13.38)	200.74 (37.21)	523.22 (49.13)	291.40 (39.54)	1053.58 (218.96)
40	156.54 (17.71)	170.43 (29.62)	594.85 (35.00)	492.71 (94.12)	1108.56 (169.63
44	163.42 (19.44)	182.42 (21.06)	466.87 (57.99)	367.83 (71.13)	1244.62 (226.65)
Velino	195.46 (39.35)	203.87 (39.21)	499.39 (55.05)	320.97 (57.86)	1267.98 (238.20)
Viglio	279.95 (46.95)	207.74 (26.81)	523.22 (114.20)	336.62 (159.54)	1456.87 (483.00)
14	181.38 (48.53)	159.54 (23.31)	510.91 (49.77)	456.38 (144.53)	2020.18 (270.29)
Average	189.11 (10.54)	168.91 (10.30)	427.60 (19.78)	335.44 (26.00)	1015.67 (80.80)
Velino *vs E.cam*[2]	29.54 %	89.68 %	39.34 %	17.15 %	135.82 %
Viglio *vs E. cam*[3]	85.54 %	93.28 %	45.99 %	22.86 %	170.95 %

[1] R3S3:roots 3 years old and stem/shoot 3 years old; [2] Velino *vs E.cam:* % of SA of Velino greater than *E. camaldulensis;* [3] Viglio *vs E.cam:* % of SA of Velino greater than *E. camaldulensis*.

Table 6 - Mean and standard error (in brackets) of percent survival of Eucalyptus clones and the control (*E. camaldulensis*) tested in central and southern Italy.

Site	Massama (Sardinia)	Rome (Latium)	Mirto (Calabria)		
Age	R3S3[1]	R3S3	R2S2	R4S2	R6S6
Plants density (per hectare)	5,500	5,500	5,500	5,500	1,600
Clone	%	%	%	%	%
22	88.75 (5.15)	48.75 (8.00)	75.00 (6.45)	67.50 (7.50)	44.33 (5.67)
E. cam	75.00 (9.79)	62.50 (1.44)	97.50 (1,44)	97.50 (1,44)	95.80 (3.29)
81	93.33 (1.67)	70.00 (2.24)	81.25 (7.74)	77.50 (8.78)	83.25 (4.25)
13a	97.50 (1.71)	70.00 (10.80)	98.75 (1.25)	98.75 (1.25)	100.00 (0.00)
65	95.00 (2.04)	62.50 (4.33)	82.50 (2.50)	80.00 (0.00)	91.50 (8.50)
45	96.00 (2.92)	42.00 (6.44)	72.50 (9.24)	66.25 (11.06)	70.75 (7.88)
20	98.33 (1.05)	53.75 (7.47)	95.00 (2.04)	80.00 (4.56)	91.50 (4.91)
40	100.00 (0.00)	63.00 (7.00)	91.25 (2.39)	91.25 (1.25)	91.50 (4.91)
44	91.67 (6.41)	72.50 (6.61)	88.75 (4.27)	88.75 (5.15)	87.25 (4.25)
Velino	96.25 (2.39)	77.50 (4.33)	93.75 (2.39)	93.75 (2.39)	95.75 (4.25)
Viglio	93.75 (2.39)	60.00 (4.56)	80.00 (5.00)	75.00 (0.00)	75.00 (8.00)
14	93.00 (4.36)	60.00 (8.90)	95.00 (3.54)	90.00 (7.07)	83.25 (6.74)
Average	93.77 (1.33)	61.67 (2.16)	88.18 (1.83)	84.43 (2.25)	85.41 (2.51)

[1] R3S3: roots 3 years old and stem/shoot 3 years old

found also considering separately the performance of the first rotation for the three tests with a density of 5,500 plants ha^{-1}. Notably, the identified clones do not represent the best choice in all tests; clone 14 performs better than Viglio and Velino at Mirto (in both first and second rotation with both 5,500 plants ha^{-1} and 1,600 plants ha^{-1}).

Conclusions

The present study identified eucalypt clones with stable and high performance between several alternatives. Results pointed out the declining growth performance observed in the second rotation compared with the first rotation. This result depends on cultivation model, age rotation and harvesting method adopted, which negatively affect the available nutrients in the soil. The significant clones/control x site interaction for SA performance at the 5,500 plants ha^{-1} sites suggests that some clones showed a different performance among sites. This result is important for clone selection. Viglio and Velino clones showed the best overall performances and are suggested to be used over the large scale SRWC in central and southern Italy.

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