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Biotechnical Characteristics of Root Systems in Erect and Prostrate Habit *Rosmarinus officinalis* L. Accessions Grown in a Mediterranean Climate

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Rosmarinus officinalis L. is a shrub species typically found in the Mediterranean Basin area. Studies carried out in Sicily on the biodiversity of the genus Rosmarinus found only one species (*Rosmarinus officinalis* L.) with varying morphology (erect habit and prostrate habit). The species does not require high input, managing to thrive even in marginal areas, and is a medicinal and aromatic species of great agronomic and economic interest, being one of the top 20 species most-used in Italy and with highest wholesale revenues. Studies carried out on the species in Italy are recent, as is the whole medicinal and aromatic plants sector, and have mostly regarded agrotechniques. This study shows the results of initial observations carried out in Sicily on the biotechnical characteristics of the root system of disetaneous rosemary accessions (erect habitus and prostrate habitus) grown in the same soil.
Results show that the species adapts well to soil bioengineering requirements; young plants also showed better root system tensile strength than older plants.

1. Introduction

Rosmarinus officinalis (Lamiaceae) is a perennial shrub native to the Mediterranean area and is widely distributed in many parts of the world. From an ecological point of view, the species is found in areas with an elevation ranging from 0 to 800 m above sea level and up to 1500 m above sea level only in Mediterranean regions (Napoli et al., 2010). The species prefers temperate or warm-temperate climates and adapts to different types of substrate, although finding limestone substrates the most favourable. Studies carried out in Sicily on the biodiversity of the genus Rosmarinus found only one species (*Rosmarinus officinalis* L.) with varying morphology (erect habit and prostrate habit). The species does not require high input, managing to thrive even in marginal areas, and is a medicinal and aromatic species of great agronomic and economic interest (Leto et al., 2013, Tuttolomondo et al., 2014, Tuttolomondo et al., 2015). Due to its morphological and phenological characteristics, it is of considerable interest for the creation of shrubby areas or flowering hedges in gardens or for landscaping and environmental restoration. The species is also known for its antioxidant properties (Napoli et al., 2015).
Recent studies concerning the effects of *Rosmarinus officinalis* and other aromatic shrubs on reducing soil erosion (Bochet et al., 2006, Casermeiro et al., 2004, Durán et al., 2007) have focused primarily on the influence of plant cover, whereas much less attention has been paid to the effects of plant roots on water erosion processes. It is, therefore, important to study the influence of root systems on soil loss (Gyssels et al., 2003, De Baets et al., 2007b). Knowledge of the relative efficiency of the different components of the plants is of great importance for the selection of natural species able to combat soil erosion in the Mediterranean region (Bochet et al., 1998).
The aim of this study was to evaluate the biotechnical characteristics of the root system of two types of rosemary: erect habitus and prostrate habitus and two growth development stages: 12 years and 18 years from planting.

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2. Experimental

2.1. Plant material and agronomical management

Tests were carried out in February 2017 at the University of Palermo experimental farm (N 38 ° 06'26.2 '', E 13 ° 20'56.0 '', 31 m a.s.l.) using two *Rosmarinus officinalis* plots of differing ages (12 and 18 years). Both plots were grown in sandy clay loam soil types (Aric. Regosol, 54% sand, 23% silt and 23% clay) with a pH of 7.6, 14 g kg^{-1} organic matter, 3.70% of active calcareous, 1.32% total nitrogen, 18.1 ppm assimilable phosphate, 320 ppm assimilable potassium. A plot layout of 1.5 m row length and 1.5 m inter-row space was adopted using two biotypes of rosemary (erect habitus and prostrate habitus) agamically propagated, both of which grow wild on the island of Sicily. Agronomic management included 2 supplementary irrigation applications only during the first 6 months of planting, for both of the rosemary biotypes (1999 and 2005), and manual weed control. The climate is Mediterranean with mild, humid winters and hot, dry summers. In 2016, average annual rainfall was 539 mm, the maximum average temperature was 27.0 °C and the minimum average temperature was 10.6 °C.

2.2. Sampling and morphometric analysis

In the experiment four plant were used, one by habitus (prostrate, erectus) and age (12 and 18 years). The plants need for morphological analysis and root tensile strength tests were extracted by hand, carefully excavating around the plant in order to cause as little damage to the root system as possible. As soon as a depth was reached in which there was no longer any root system, the clod of earth was isolated, detached and measured. The soil/root clods were shaken to remove the soil and cleaned with a pressure wash, taking care to avoid damage to the root system. The roots underwent a further washing in the laboratory and the sample was checked to ensure it had not been damaged before analysis began.
Diameter measurements (maximum and average root diameters) were taken by directly measuring (using a digital caliper with accuracy to two decimal places) the root sections at fixed distances from the neck; root number N was measured by counting the roots in the system extracted, in addition to the portion of roots which remained in the soil (following excavation and collected through sifting of the soil). Root development (cm), root wet weight (g) and above-ground biomass production (divided into stems (kg) and leaves (kg) was also determined.
Before plant extraction, each sample was measured for root system depth (cm), root expanse (cm), plant height (cm), plant diameter (cm) and number of main branches.

2.3. Determination of tensile strength force

Root tensile strength was determined in the Hydrology laboratory of the SAF Department using traction testing with a digital Sauter FH500 force gauge for tension tests of up to 500 N and resolution of 0.1 N, fixed to a Sauter TVM-N motorized vertical test stand.
Tensile strength tests were carried out on roots with a free root length of 15 cm, uniform diameter as far as possible, and no knots or anomalous growth features. The root diameter was measured in three different points on each root in order to find the average diameter D; this was then used as a reference value. The sample was fixed with double-sided adhesive, medium-grit sandpaper inside a clamp.
Fixed at a test speed of 7mm/minute, traction force/elongation data were collected for each test, recorded by the gauge until breaking point for each sample was reached. Tensile strength (Tr [MPa]), was calculated using the ratio of tensile force Fr [N] over root section area, supposing a circular root section:

$$\mathrm{Tr(D)} = \frac{\mathrm{Fr}}{(\pi \mathrm{D}^2/4)} \quad (1)$$

Break strength (Tr) is that of the maximum force (Fmax) recorded before rupture.

Only those tests which produced continual and increasing stress-strain curves were taken into consideration and showing with no signs of slipping from the clamps. (Pirrera et al., 2016).

3. Results and discussion

3.1 General plant properties

Tables 1 and 2 contain summary data of the plant parts (aboveground, belowground) of *R. officinalis* (RSM) samples taken from the two growth habitus types (prostrate (p) and erect (e) for each of the two plot years (1999, 2005)).

Table 1 – Aboveground plant properties

	Aboveground Height (cm)	Aboveground expanse (cm)	Main branches (n)	Average (CV) branch diameter (cm)	Stem fresh weight (kg)	Leaf fresh weight (kg)	Leaf incidence (%)
Year 1999							
RSM (e)	180	100	11	1.67 (4.5)	15.80	4.00	20.20
RSM (p)	100	260	15	1.52 (5.3)	5.40	4.30	44.32
Year 2005							
RSM (e)	190	140	25	2.01 (1.2)	15.00	4.35	22.48
RSM (p)	195	230	22	1.60 (7.7)	16.00	14.37	47.31

From the data in Table 1, it is clear that the aboveground height is similar in all of the samples, with the exception of RSM (p) 1999, the aboveground bush expanse is greater in the prostrate habit samples whilst the main branch number was greater in the younger samples for both RSM (e) and RSM (p) from 2005. The leaf incidence (%) of total biomass was similar for both growth habits and decreased with the age of the plant.

Table 2 – Belowground plant properties

	Root depth (cm)	Root expanse (minor diameter) (cm)	Root fresh weight (g)	Root development (m)	Max. root diameter (mm)	Average (CV) root diameter (mm)	RD (kg/m^3)	RLD (m/m^3)
Year 1999								
RSM (e)	48	86	1172	140.8	24.8	1.78 (1.40)	3.15	505
RSM (p)	35	117 (95)	1017	127.1	10.5	1.14 (1.15)	2.50	416
Year 2005								
RSM (e)	30	101 (85)	408	155.1	5.4	0.81 (0.90)	1.61	767
RSM (p)	40	122 (92)	1542	146.8	14.8	0.96 (1.61)	3.28	417

Data from table 2 show that the greatest root expanse was found in prostrate growth habits; the expanse is oval shaped, becoming more elongated than in the erect growth habit. The root system is small (ranging from 30-48 cm) which gives a heart type, squashed, semi-spherical morphology (Kokutse et al. 2006).
Morphological analysis of the root system is described by the root density RD[kg/m^3], which is given by the ratio between dry root mass and the root soil clod cylinder volume and root length density RLD [m/m^3] (Smit et al., 2000) defined as the ratio between root development and the root soil clod volume of the sample.

3.2. Root diameter distribution

The root diameter composition is shown in Figures 2 and 3.

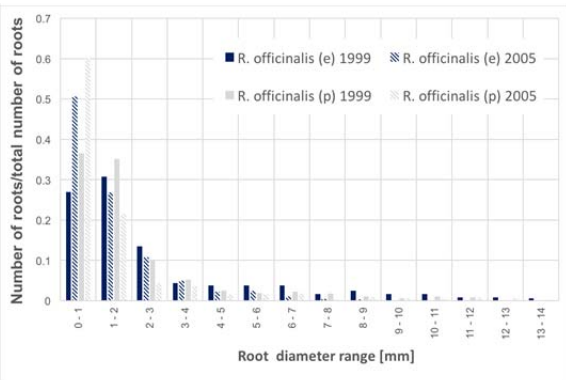


Figure 2 – Diameter distribution of the samples

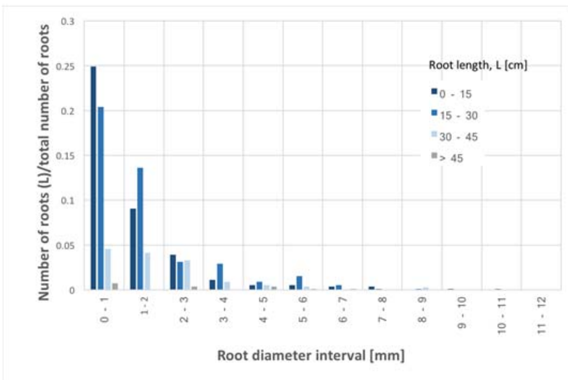


Figure 3a – Diameter distribution grouped by 4 categories of root length of RSM (e) 2005 samples

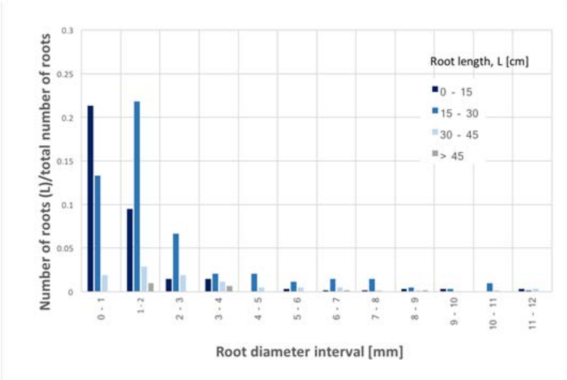


Figure 3b - Diameter distribution grouped by 4 categories of root length of RSM (p) 1999 samples

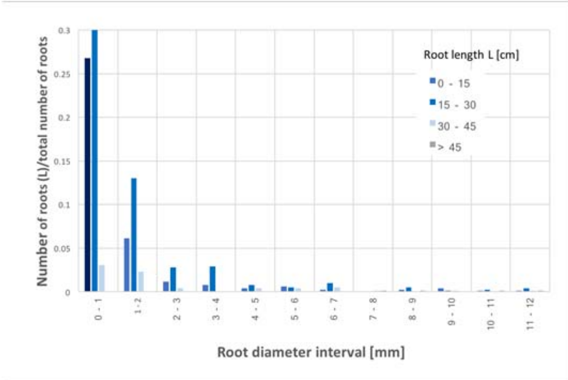
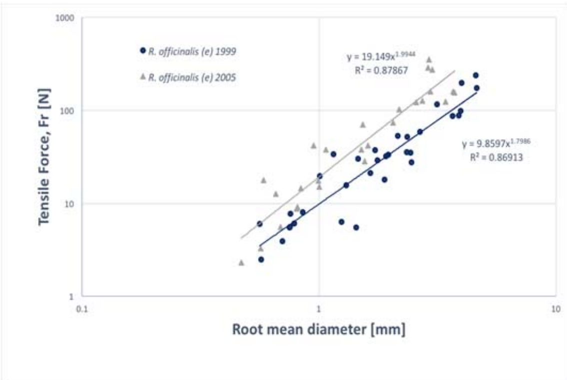


Figure 3c - Diameter distribution grouped by 4 categories of root length of RSM (p) 2005 samples

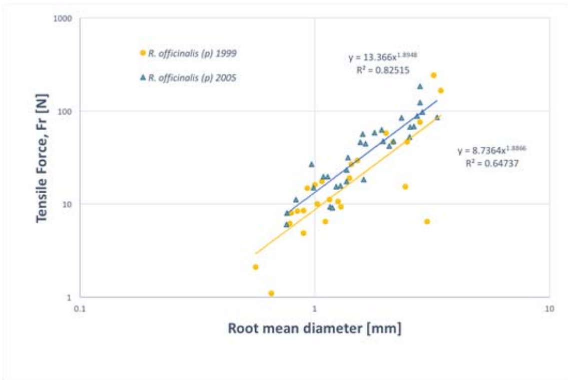
Figure 2 shows a comparative analysis of the distribution of average diameters of the 4 samples; Figures 3a, 3b and 3c shows the diameter distribution grouped according to root length of 3 of the 4 samples (*R. officinalis* (e) 2005, *R. officinalis* (p) 1999, *R. officinalis* (p) 2005)

3.3 Tensile strength test results

Results of the tensile strength tests are shown in Figures 4 and 5.

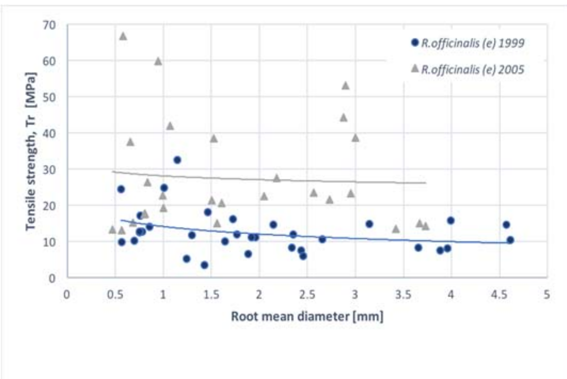


(a)

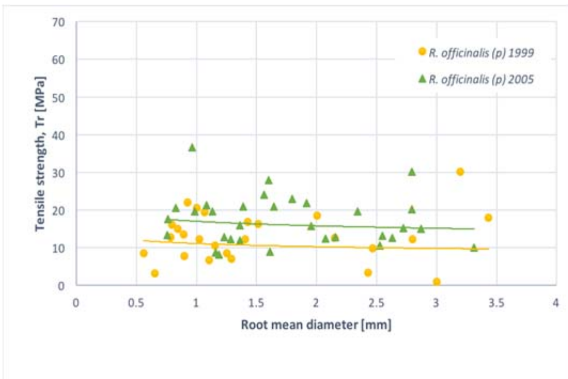


(b)

Figure 4 - Tensile force (Fr) at failure vs. Root diameter (D) relationship for RSM erect (Figure 4a) and prostrate (Figure 4b).



(a)



(b)

Figure 5 - Root tensile strength (Tr) vs. Root diameter (D) relationship for R. officinalis erect (Figure 5a) and prostrate (Figure 5b)

As is clear from Figures 4a and 4b, test results for tensile break force (Fr [N]), as a function of the root mean diameter (D [mm]), were fairly linear for all of the samples, using the equation:

$$Fr = a\ D^b \qquad (2)$$

with exponent *b* varying little (1.80-1.99) both for growth habit type and planting date. Parameter *a in (2)*, however, was found to vary both as regards growth habit and plot age; there seems to be a clear fall in the coefficient as the age of the plant increases. In other words, it appears that, at least as regards the number of samples in the study and in relation to the plant ages observed, aging leads to a reduction in root system resistance. Within the same age group, however, erect growth habit plants showed greater resistance to stress.

In terms of tensile strength Tr [MPa], the trends in Figure 5 expressed by interpolated law of potential type, for both of the growth habits, show very little variation in tensile strength over mean root diameter D; however, both growth habits show a reduction in resistance values between the younger and older plants. This is coherent with the previous force tests. The samples comparison has also been performed in terms of distribution, as shown in the box-plot in Figure 6 showing quartiles, averages, maximum and minimum values and outliers of the 4 tensile strength test samples. It is clear from this last diagrams the loss of tensile strength with age.

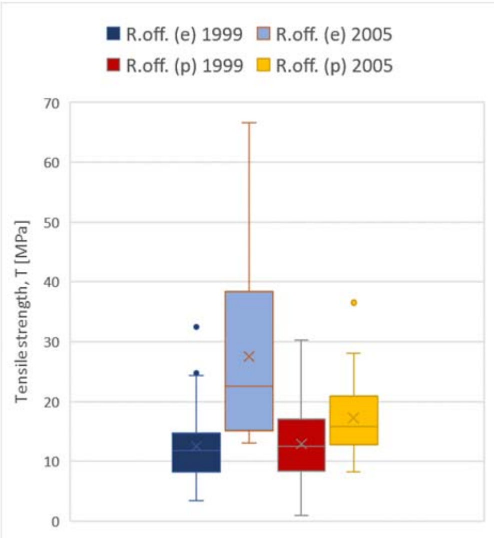


Figure 6 - Box-plot diagram for the 4 root tensile strength samples

4. Conclusions

Results show good plant cover capacity of the species, independent of age and best in the prostrate growth habit biotype compared to the erect type. Regarding the aboveground plant portion, leaf biomass as a percentage of total biomass was found to be similar between the two growth habits but was found to decrease with plant age. From measurements taken on the root system, for both years of observations, it is clear that root development remains within a depth of 50 cm and that horizontal development is greater in the prostrate growth habit biotype. Root breaking resistance, however, was found to be greater in both of the younger biotypes. Younger plants of the species, therefore, at least based on these initial observations, would seem to perform better.

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