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Quantitative measurements of root growth on buried stems of four euryoecious species adequate for soil bioengineering in Portugal and Mediterranean region

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ABSTRACT: The paper describes adventitious rooting tests for four species of the Portugal mainland flora with a wide territorial distribution (Fraxinus angustifolia, Sambucus nigra, Rosmarinus officinalis, and Viburnum tinus). The objective of the study is to verify the suitability of the roots and buried stems of these species to soil reinforcement, in order to apply these results to soil bioengineering and ecological restoration projects in Mediterranean environment. Five experimental plots, with different soil characteristics, were settled in Lisbon and Santo Tirso (Porto district), simulating a live crib wall structure. Small rooted plants were installed in individual bags to make their extraction easier and to have the same available soil volume. After 26 months a destructive analysis was carried out, obtaining measurements of root systems and above ground elements, namely geometric dimensions and mass in term of dry weight. All species showed a positive response to stem burying, as all live plants produced adventitious roots on the buried stems and new roots sprouted from the original root-ball. Relevant quantities of adventitious and new roots were able to reinforce soil. These species, that showed ability to be used in any soil bioengineering project in Mediterranean environment, had already been used in Portugal in soil bioengineering projects carried out near Lisbon. Significant dependencies of plant parameters on plot or on species factors were found, as well as significant correlations were detected between above ground and below ground parameters of new roots.

Keywords: Adventitious roots; slope stabilization; soil bioengineering; Mediterranean climate; mainland Portugal flora

1 INTRODUCTION

Soil Bioengineering (SB) is still an underused technique in Mediterranean regions. In this region few species of plants were tested to be used where water availability is limited.

Living materials for SB projects - plants transplanted or propagated by cuttings or seeds - can have severe problems with long summer aridity. In Portugal, SB has neither a large diffusion nor an effective application and most of the known species used in SB projects in Southern Europe do not belong to the Portuguese flora.

Extensive application of SB projects in the Vesuvius National Park, in Southern Italy, clearly showed how willow cuttings did not survive to the summer dryness (Bifulco, 2001). Nevertheless, willow cuttings are the main living material used to implement SB project in alpine and central Europe (Schiechtl, 1992) and in North America (USDA-NRCS, 1992;). On the Vesuvius slopes, the problem was solved in 1999 using whole plants - instead of cuttings - placed in the ground with buried stems in subhorizontal position – like cuttings, to stimulate a greater root mass growth. However, in spite of successes, generating adventitious root along the stem is not a common feature for trees and shrubs, as they have to support a change in the stem anatomy.

Used as plants rather than cuttings, *Fraxinus ornus* L., *Coronilla emerus* L., *Colutea arborescens* L., and *Ligustrum vulgare* L., gave the best results in SB projects carried out between 1999 and 2005 on Vesuvius slopes (Bifulco, 2011). In order to broaden the range of species of flora of mainland Portugal, to be used in SB projects, it was necessary to test their adventitious rooting capacity as cuttings or as plants installed with buried stems. Previous research (Bifulco and Rego, 2013) characterized a considerable collection of autochthone species with wide territory distribution. Within that collection, *Fraxinus angustifolia* Vahl, *Sambucus nigra* L., *Rosmarinus officinalis* L., and *Viburnum tinus* L. resulted as the best candidates to test.

The assessment of root behaviour of the four species is fundamental for the understanding of their suitability to be used in any SB projects. In the case of positive results, these four species, which can be defined euryoecious because of their wide territorial distribution, will be the best according to what Schiechtl suggests to be used in SB projects (1973). This important information allows us to go further overcoming present limitations to perform SB projects due to scarcity of suitable known species in conditions to be used on slopes far from water streams.

2 MATERIALS AND METHODS

We chose five sites where to install the plants, in order to repeat the test in different conditions. The main different characteristic of each one was its soil origin. Experimental plots with calcareous and basaltic soils were placed in Lisbon, Tapada da Ajuda, together with a third one, in a substrate of peat and vermiculite, out of the forest nursery. The other experimental plots were located in Santo Tirso, the Great Porto area, one in granitic soil, Monte Padrão, and the last one in the courtyard of Resinorte facilities, in schist soil. Concerning the plants, 1 year old, they were produced in small pots (17 cm diameter, 29 cm height) by Lisbon University, ISA forest nursery, in a substrate of peat and vermiculite. We designed the plant layout to simulate their spatial arrangement in a live crib wall. To achieve that arrangement, we put the plants inside permeable rectangular bags (50 cm x 80 cm), woven with plastic fibres, which, once filled with plant and soil, assumed a cylindrical shape. Bags with plants were positioned almost horizontally into four rows, each row with four bags, one above the other and in each horizontal line the four tested species. We filled the bags burying as more stems as possible; all the present stems were buried with their leaves on. In each plot we laid the 16 bags in excavations carried out in small slopes, or next to vertical surfaces, no space left between the bags. As in a live crib wall, the rows of bags were placed slightly tilted upward and with a slightly sloping block face, allowing light arrival to the plants in lower position. Finally, the block of bags was covered with a 5 cm layer of soil and the plants were irrigated, as usual in SB project, with 100 l of water. 26 months after the bags with the plants were extracted. Each bag was opened to remove the plant stems and roots. The roots were washed in water and photos of the whole plant on a white board were taken. The above ground fraction was separated from buried fraction, and both stored in paper bags. Afterward, in the laboratory, buried stems with adventitious roots were separated by cutting from the initial root system. We also cut the new roots sprouting from the original root-ball. All the material was photographed and stored in paper bags. After the first series of manual measurements and root scanning, all the parts were dried at 80ºC for 48 hours. For each plant we prepared three types of measurements: *(i)* the first manual measurements, made on the above ground part - at extraction stage - and on buried stems, before drying them; *(ii)* with scanner and image analysis software, made on roots and buried stems; and *(iii)* calculations combining main measurements to obtain ratios and aggregate dimensions. The first manual measurements were used to determine: above ground crown height (AGH) and diameter (AGD), above ground number of stems (AGS), above ground weight (AGW), buried stem number (BSN), total length of all buried stems (BSTL), number of first order adventitious roots on the stems (ARFO), length of the longest adventitious root (ARLL), diameter at the original collar (OSD), length of the longest root sprouting from root-ball (NRLL), weight of the adventitious roots including buried stems (ARW), weight of roots in the volume of the original root-ball (ORW), weight of the new roots sprouting from the original root-ball (NRW). We used a scanner workstation with software for image analysis. That tool was used to determine for each plant: *(i)* adventitious root (AR acronym prefix) on stem, including the buried stems, and *(ii)* new roots (NR acronym prefix) sprouting from original root-ball: root total length (ARTL and NRTL), root average diameter (ARAD and NRAD), root projected area (ARPA and NRPA), root surface area (ARSA and NRSA), root volume (ARVO an NRVO). Some derived parameters were computed. Visual evaluation of adventitious root distribution along the stem (ARD) was performed; three classes were used: regular, irregular, and shallow. Some remarks must be made on the determination of root length. Root length is difficult to measure when dealing with extensive root systems. Since most of the root length is associated with both not straight and fine roots, it is extremely difficult to have an accurate estimate of total root length from the shrubs or trees collected in the field. Therefore, root length was determined mainly for comparison purposes and it was strictly interpreted on a relative basis and not as absolute values. The first investigation on the relationships between variables was performed by computing Pearson's correlations between all continuous variables, using data of all the plants alive after the experiment. Afterwards, these data were used to perform a Principal Component Analysis (PCA). The principal components (PC's) extracted by the analysis allowed the understanding of the main variables responsible for the variation found and their relationship with the species and plots. The significance of the relationship between the survival of the initial plants with species and plots was evaluated by a Chi-Square test. After, to understand the relationship between continuous variables with plots and species, we performed exploratory full factorial univariate Analyses of Variance (ANOVAs) for all variables. As no important interactions between species and plots were detected, univariate ANOVAs taking into account independently the two factors were performed.

3 RESULTS

Plants of two plots in Tapada da Ajuda died after the drought occurred in whole Portugal the first year within the highest severity drought classes, severe and extreme. In the third plot, where plants were installed in a substrate of peat and vermiculite, damages were limited to those on the superior row and lateral sides of the plot. In the experimental plots of Tapada da Ajuda 3 Forest Nursery and Santo Tirso - Resinorte, the general look of living plants was very good. The plants of Santo Tirso - Monte Padrão presented a smaller development. All living plants presented adventitious roots on buried stems, and new roots sprouting from the original root-ball. No leaves were found on the buried stems. All the *F. angustifolia* plants presented a polycormic shape, even with only one buried stem. Each plant had approximately a volume of 55 litres of soil available in the bag. In many cases the root development was such that relevant quantities of roots were found intertwined with bag plastic fibres, and in some cases, particularly for *S. nigra*, the roots went out of the bag going into adjacent bags or into the soil behind or below the installation. All roots outside the bags were discarded. A quantitative description of the characteristics of all plants grouped by species is presented in the next table 1.

Tab.1 Descriptive parameters (mean ± SE) of the living plants distributed by species

Variable	Units	*F. angustifolia*	*V. tinus*	*R. officinalis*	*S. nigra*
N		11	10	10	6
Above-ground					
AGH	cm	68.0 ± 14.0	77.6 ± 16.3	80.1 ± 15.1	79.2 ± 24.7
AGD	cm	49.6 ± 11.0	50.6 ± 7.2	68.5 ± 14.5	44.1 ± 12.4
AGS		2.1 ± 0.4	4.0 ± 0.9	5.7 ± 1.4	2.8 ± 0.5
AGW	g	36.6 ± 11.1	87.5 ± 31.3	104.9 ± 36.8	20.9 ± 8.2
Manual measurements on buried part					
BSN		1 ± 0.0	4.5 ± 0.8	2.5 ± 0.5	1.8 ± 0.5
BSTL	cm	32.9 ± 2.7	134.3 ± 21.8	61.5 ± 13.2	58.4 ± 26.7
ARFO		46.8 ± 24.3	60.9 ± 10.9	89.5 ± 14.0	9.2 ± 1.3
ARLL	cm	23.0 ± 3.0	29.6 ± 4.1	33.8 ± 4.2	28.3 ± 4.9
NRLL	cm	32.5 ± 3.7	36.2 ± 4.5	31.3 ± 3.3	8.8 ± 2.3
OSD	mm	11.5 ± 1.7	11.7 ± 1.7	10.9 ± 2.0	27.4 ± 4.9
ORW	g	28.9 ± 3.6	34.6 ± 8.4	13.4 ± 2.5	13.2 ± 2.5
ARW	g	30.4 ± 7.1	38.2 ± 8.6	20.5 ± 4.5	11.3 ± 3.2
NRW	g	6.7 ± 1.4	5.4 ± 1.3	2.3 ± 0.5	5.4 ± 2.9
AR versus NR					
ARTL	cm	735.7 ± 214.3	1072.2 ± 431.5	1158.5 ± 334.6	218.4 ± 51.4
NRTL	cm	1156.5 ± 253.8	1619.2 ± 481.3	1104.5 ± 414.2	684.9 ± 273.9
ARAD	mm	1.7 ± 0.4	2.4 ± 0.5	1.0 ± 0.1	2.3 ± 0.2
NRAD	mm	0.8 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	1.4 ± 0.3
ARPA	cm^2	76.8 ± 17.6	116.9 ± 22.3	84.8 ± 14.9	44.0 ± 10.4
NRPA	cm^2	83.8 ± 17.0	99.2 ± 24.5	58.5 ± 26.0	65.8 ± 22.3
ARSA	cm^2	241.3 ± 55.4	367.3 ± 70.1	265.1 ± 47.1	153.5 ± 45.1
NRSA	cm^2	263.3 ± 53.4	311.5 ± 77.1	183.8 ± 81.8	206.9 ± 70.0
ARVO	cm^3	8.2 ± 1.6	14.9 ± 2.0	5.9 ± 1.1	8.4 ± 2.1
NRVO	cm^3	5.9 ± 1.6	5.3 ± 1.1	3.0 ± 1.6	6.7 ± 3.0
Computed variables					
AGHI	%	353.0 ± 93.2	417.1 ± 108.7	417.1 ± 109.6	428.1 ± 164.5
TLAN	cm	1603.1 ± 380.7	2556.5 ± 844.1	1894.9 ± 583.6	804.3 ± 300.1

Significant correlation coefficients (R) among the variables, with their corresponding statistical significance were found.

The results of the Principal Component Analysis (PCA) showed that the two first components extracted explained 47.8% of the total variance. In that diagram on the two first axes, it was possible to recognize associations with species and plots. So, as a general summary of this analysis, we can detect and interpret two main axes in the PCA diagram: one, from low-left to upper-right, showing from more root to more shoot biomass, the second, from lower-right to upper-left, showing a trend from root lengths to root diameters.

The interpretation of the same two axes of the PCA diagram can also be performed by the association with the original variables. In that diagram it was possible to recognise groups of clearly associated variables, as for example height, diameter and weight of the above ground part, or associated because of their computational method, like the length of adventitious and new roots from root-ball and their sum. On the other hand, similar scores for variables of adventitious roots (AR acronym prefix) and new roots from root-ball (NR acronym prefix), not initially expected, give interesting new perspectives for the understanding of the experiment results.

From the analysis of the ANOVAs for all continuous variables of the surviving plants, statistically significant relationships ($p<0.05$) were obtained for the variables:

- Significant p values for Plot: *(Above-ground)* AGH, AGD, AGW; (*New root from root-ball)* NRLL, NRTL, NRAD; (*Adventitious root)* ARW;

- Significant p values for Species: (*Adventitious root)* ARAD, ARVO; (*Buried stem)* BSN, BSTL.

4 DISCUSSION

The analysis on living plants gave significant results about quantitative measurements and correlations, giving important contributions with new information, rare to find in literature. Adventitious roots grew on buried stems of all living plants. That occurred on the new stems (*R. officinalis* and *S. nigra*), on the stems already present in the nursery plants of *V. tinus, R. officinalis* and *S. nigra*, and on the single stem of *F. angustifolia* plants. All *F. angustifolia* specimens presented only one below ground stem, but multiple shoots above ground like a shrub. It was concluded from the study that all 4 species are adequate to be used in SB projects taking into account: (*i*) the soil volume available for each plant, approximately 55 litres; (*ii*) the measured values of root total length and volume; (*iii*) the number of first order adventitious roots and (*iv*) their average diameter. ANOVA on variables shows that some plant dimensions are also significantly dependent on the plot factor: (*i*) crown height and height increase; (*ii*) above ground plant crown diameter and weight; (*iii*) adventitious roots weight on buried stems; (*iv*) collar diameter: (*v*) length of the longest root, and (*vi*) total length of roots sprouting from the root-ball, and (*vii*) their average diameter. These results confirm the previous speculation that the plant development depends on plot conditions. On the other hand, the number of buried stems for each plant and their total length, the volume of adventitious roots on buried stems and their average diameter are significantly dependent on the species factor. With these results, we can speculate that the main characteristics of adventitious roots on buried stems are depending on species. The correlations found allow us to speculate that, for the tested species, the above ground plant shape has dimensions depending on the local conditions, but with similar proportions; furthermore, the above ground height is correlated with adventitious roots and new roots from root-ball roots weights, and weight of adventitious and weight of new from root-ball roots are correlated too. On this basis, looking at plant above ground appearance, we can infer about their below ground conditions.

For those four species, which are adequate to be installed horizontally and with buried stems, we can conclude that the increase of their above ground parts corresponds to the development of both adventitious roots on the stem and new roots from the root-ball. Adventitious roots frequently (73.0%) have a regular distribution along the buried stem. When irregular, as with *F. angustifolia* (18.9%), the highest density was nearby the root-ball. Only a few plants (8.1%) presented a shallow root distribution. It seems that using plants instead of cuttings, in a SB technique like the live crib wall, prevents the problems due to a shallow distribution of cutting roots. Observed data are useful for Portugal mainland, but can also be used in other regions, as the tested species also part of the flora of the western basin of Mediterranean, with *S. nigra* having a wide distribution throughout Europe.

5 CONCLUSIONS

The performed measurements allowed an analytical study of the species. These four tested species, *Fraxinus angustifolia* Vahl*, Sambucus nigra* L.*, Rosmarinus officinalis* L.*, and Viburnum tinus* L., euryoecious and useful for SB projects, are the best choice following the advices of Schiechtl (1973) and can be used not only in large areas of Portugal to implement SB projects, but also in Mediterranean region. We can conclude that some main factors - (i) spatial distribution and density of adventitious roots along the buried stems, (ii) relevant values of adventitious roots on buried stems and further development of roots from ball-root, (iii) their density referred to soil volume available - are sufficient to show adequacy of those species to use in SB projects to consolidate soils with their roots and their own buried stems.

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