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Additional Information

1	Physical mechanisms produced in the development of nursery almond trees (Prunus dulcis
2	Miller) as a response to the plant adaptation to different substrates
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#### 27 Abstract

Aims: The aim pursued in this work is to compare the changes induced in the plant of young
 almond tree by two different culture media, analysing how those changes are interrelated with
 a better adaptation of the plant to the open field.

Methods: Two different rootstocks, GF 677 and GxN rootstock Garnem<sup>®</sup> (GN), were tested in two types of growing media: substrate 1, consisting on a substrate prepared on request and based on a mixture of 25% silica, 38% vaporized peat and 37% of washed river sand and substrate 2, based on cocopeat with coarse particle size (10-25 mm). All plants received the same nutritive solution during the analysis. Twenty weeks after the plantation trees were uprooted and several parameters were recorded in both vegetative and radicular systems to observe the impact of the substrates.

**Results**: The results of this study indicated that the use of different substrates produces statistically significant changes not only in root development and distribution but also in the vegetative growth. Plants grown under cocopeat substrate presented, among others, further development of the trunk and an increase in the total fresh weight of the radicular system produced mostly by a massive increase of absorbing roots, while plants under substrate 1 presented greater root system longitude.

44 **Conclusions**: The selection of an appropriate substrate in the nursery of almond trees is a key 45 factor in the early development of the young tree. Knowledge about root growth and root 46 architecture during the first stages of development would help nursery industry to determine 47 which should be the most suitable substrate regarding later field adaptation, survival and plant 48 performance, focusing on the soil and climatic characteristics of the final destination of the 49 plant.

50 **Keywords:** cocopeat; peat; field adaptation; vegetative system; radicular system.

## 52 **1. Introduction**

The cultivation of seedlings in container has large advantages over traditional field crop (Gilman & Beeson, 1996). These include a better preservation of the root system during the transplantation process (Thomas, 2000), a better field establishment (Mathers et al., 2005; Gilman, 2001), a lower cost of labour and lower investment in the acquisition of land by the nurseryman (Beeson et al., 2004; Whitcomb, 1984) and a greater availability of plant number in the market (Mathers et al., 2007).

In recent years, soilless cultivation techniques have been widely studied and implemented in the Mediterranean countries in the production of ornamental plant (Raviv & Heinrich, 2007; Garcia-Gomez et al., 2002) and even in the production of horticultural (Schwarz, 2012; Voogt & Sonneveld, 2001) or forestry plants (Guérin et al., 2001; Peñuelas & Ocaña, 2000) but nevertheless there is little information about these techniques in the nursery of fruit species because plants are usually sold bare root.

Generally, roots account for 15-30% of the total tree biomass (Persson, 2013). Despite their importance, root systems have received limited attention in ecological studies. Understanding and predicting ecosystem behaviour requires an accurate knowledge of growth strategies of plant roots and their distribution (Persson, 2000). Numerous observations have indicated that a healthy root system is necessary to secure vigorous growth, knowledge about root growth and root architecture during the first stages of development will ensure survival and good plant performance (Abad et al, 2004; Jacobs et al, 2005).

The use of different substrates both organic and inorganic allows the plant a better nutrient absorption and further optimization in water and oxygen retention (Verdonck et al., 1981). The properties of the different materials used as culture media have direct and indirect effects on plant growth and future productivity, hence the choice of a suitable substrate is essential in plant development. (Abad et al., 2004; Loggiodice et al., 2009). 77 World production of almonds in shell (Prunus dulcis Miller) reached, according to the Organization of the United Nations for Food and Agriculture (FAO) 2,917,894 tonnes in 2013, in 78 a cultivated area of 1,637,245 hectares (FAOSTAT, 2016). Almonds are attracting a lot of 79 80 attention in the last years mainly due to the continuous reports of their healthy beneficial 81 properties (Burns et al. 2016). From 2004 to 2013 almonds consumption increased 71.1% to 135 82 g per world habitant in 2012 (INC, 2013). From the period from 2000 to 2012 almonds with shell 83 production increased 103%, with an annual growth of 6.4%, and an annual price increase of 7,5% 84 and, as a consequence, the demand of young almond trees has grown notably in the last few 85 years. As it occurred previously with other ornamental or horticultural crops it seems necessary 86 to focus on improving the nursery of almond trees to meet the increasingly world demand. The 87 aim pursued in this work is to compare the changes induced in the plant of young almond tree 88 by two different culture media, analysing how those changes are interrelated with a better 89 adaptation of the plant to the open field.

90 **2. Materials and methods** 

## 91 2.1 Samples and treatments

92 This analysis was conducted in the Polytechnic University of Valencia (39° 38' 2" N, 0° 22
93 ' 29" W; height 4 m.a.s.l.).

94 The plant material used in the tests is certified material, free from pests and diseases, 95 from an authorized plant nursery in phenological state 10 of the BBCH scale. A total of 48 96 rootstocks were used, of which 24 individuals were rootstock GF 677 (677) which comes from 97 the interbreeding of peach tree (*Prunus persica* L. Batcsh) by almond tree (*Prunus dulcis* Miller) 98 obtained in France by the INRA (Institut National de la Recherche Agronomique) (Bernhard and 99 Grasselly, 1981) and 24 individuals were GxN rootstock Garnem® (GN) obtained by the 100 Agricultural Research Service of the Government of Aragón (CITA-DGA) which were the result of 101 the crossing of a hybrid between Prunus dulcis (cv. Garrigues) and Prunus persica (cv. Nemared) 102 (Felipe, 2009).

The rootstocks were transplanted to 80 liters pots. Two types of growing media were used: substrate 1, consisting on a substrate prepared on request and based on a mixture of 25% silica, 38% vaporized peat and 37% of washed river sand and substrate 2, based on cocopeat with coarse particle size (10-25 mm). We carried out twelve repetitions of each of the possible rootstock-substrate combinations

108 The irrigation dose was 40 liters of water per month distributed in a 40 minute irrigation 109 on alternate days with a pressure-compensating and non-leakage dripper of 4 L/h flow rate and 110 a uniformity coefficient of 85%.

For the nutritive solution we established a preparation based on the extractions of young almond trees obtained by Salazar and Melgarejo (2002). The formulation consists mainly of nitrogen in the form of nitrate, potassium, magnesium, calcium and sulfate. This solution was applied to plants with a system of localized irrigation. The treatments were carried out on a weekly basis from the transplant of the rootstock in phenologic state 10 of the scale BBCH for stone fruit trees until the tearing date which took place 20 weeks after.

117 **2.2. Evaluated parameters** 

## 118 2.2.1. Analysis of the vegetative system

The influence of the tested substrates in the vegetative system focuses mainly on the length and weight of the trunk and the diameter of the graft zone as a reference between the aerial and the root parts. Total tree height and weight of the leaves and young shoots was consider irrelevant as we are working with rootstocks that will be subsequently pruned to be grafted.

124 All of the measurements were always taken in fresh within 24 hours after the plucking 125 in order to avoid drying of the aerial part or the root system.

126 2.2.2. Analysis of the root system

127 After eliminating the soil in the plants, the studied parameters have been the number 128 of main roots and measurement, for each one of them, its diameter and the distance from the start of the root until the first bifurcation with a secondary root. The number of secondary roots
was also counted, and we measured each one's diameter and the distance from the start of the
lateral root until the first bifurcation with a tertiary root.

In the case of the weight, roots were introduced in a Memmert model muffle at 38 °C
until they stabilized to constant weight, and the weight was evaluated once they dried.

#### 134 **2.3 Statistical analysis**

135 An analysis of variance (ANOVA) with Type III sums of squares was performed using the 136 GLM (General Linear Model procedure) of the SPSS software, version 21.0 (IBM Corporation, 137 New York, U.S.A.). The fulfilment of the ANOVA requirements, namely the normal distribution 138 of the residuals and the homogeneity of variance, were evaluated by means of the Kolmogorov-139 Smirnov with Lilliefors correction (if n>50) or the Shapiro-Wilk's test (if n<50), and the Levene's 140 tests, respectively. All dependent variables were analyzed using a one-way ANOVA with or 141 without Welch correction, depending on whether the requirement of the homogeneity of 142 variances was fulfilled or not. The main factor studied was the effect of substrate on the 143 vegetative and radicular development parameters of the almond trees studied. If a statistical 144 significant effect was found, means were compared using Tukey's honestly significant difference 145 multiple comparison test or Dunnett T3 test also depending on whether equal variances could 146 be assumed or not. All statistical tests were performed at a 5% significance level.

- 147 **3. Results and discussion**
- 148

#### **3.1.** Comparative study of the rootstocks

According to the obtained results, in terms of vegetative development, as presented in Table 1, the GN rootstock stands out in our study as the most vigorous, this result is consistent with what was observed by Felipe (2009) who describes the rootstock, before being grafted, as strong with an upright growth, and Sotomayor et al. (2008) who concludes that compared with the 677, rootstocks GN produce greater weight of pruning and a greater number of fresh buds.

Trunk longitude (cm)		Trunk weight (g)	Diameter of grafted area (mm)	
GF 677	35.82±12.74 a	89.56±12.61 a	16.77±2.01 a	
GN Garnem	32.07±9.50 a	105.76±14.66 b	21.38±3.01 b	

157 Regarding the radicular development, we observed various types of roots in both annual 158 and perennial plants and we can link these differences with wide variations in the absorption 159 and transfer capacity (Clarkson, 1996). In our study, rootstocks GN presented a higher total root 160 weight than the rootstock GF 677 (Table 2). There were no statistical differences in the average 161 number of tap and lateral roots between the two rootstocks but rootstock GN presented a 162 bigger root diameter for both kind of roots.

163 The rootstock GF 677 showed greater maximum length of the root system and therefore 164 greater in-depth exploration but both, GF 677 and GN, presented uniformity in the spatial 165 distribution of their roots. The adaptability to the environment of the different rootstocks can 166 partly be attributed to the depth that the root system can reach, its density and its spatial 167 distribution (Castle and Krezdorn, 1997).

**Table 2.** Radicular parameters of the different rootstocks (n = 24; mean value ± standard
deviation; P <0.05).</li>

	Roots fresh weight (g)	Tap roots weight (g)	Lateral roots weight (g)	Absorbing roots weight (g)	Root system longitude (cm)	
GF 677	144.08±33.84 a	65.61±12.36 a	47.09±6.48 b	31.37±4.40 a	35.82±12.84 b	
GN	<b>GN</b> 186.34±26.10 b 115		115.27±30.16 b 36.50±5.93 a		32.07±9.50 a	
	Average number of tap roots	Average number of absorbing roots	Average tap roots diameter (mm)	Average absorbing roots diameter (mm)	Average distance of tap roots to first bifurcation (cm)	
GF 677	22.71±2.55 a	137.35±11,8 a	4.11±1.17 a	1.16±0.27 a	6.40±2.33 a	
GN	28.66±2.64 a	132,46±10.36 a	4.94±1.55 ab	1.88±0.54 b	6.43±3.10 a	
	Maximum distance of tap roots to first bifurcation (cm)	Minimum distance of tap root to first bifurcation (cm)	Average distance of lateral roots to first bifurcation (cm)	Maximum distance of lateral roots to first bifurcation (cm)	Minimum distance of lateral roots to first bifurcation (cm)	
GF 677	25.74±8.51 a	3.80±1.90 a	4.80±2.42 a	22.24±14.07 a	0.23±0.26 a	
GN	26.49±17.49 a	7.95±3.95 ab	4.53±2.79 a	23.46±28.23 a	0.09±0.13 a	

## 3.2. Characteristics of the vegetative system under different substrate cultivation

The parameters studied in the vegetative system of the plants are reported in Table 3. The obtained results showed that substrate 2 based on cocopeat produced a higher and heavier trunk than substrate 1 nevertheless, both substrates have led to similar diameter of the grafted area. The diameter of the trunk is an essential feature in nursery of rootstocks since the trunk thickness determines the appropriate time to proceed with the graft (Estaún et al., 1999).

176 **Table 3.** Vegetative parameters according to the type of substrate studied (n = 24; mean value

177 ± standard deviation; P < 0.05).

	Trunk longitude (cm)	Trunk weight (g)	Diameter of grafted area (mm)
Substrate 1	29.87±0.70 a	94.97±10.3 a	19.38±2,27 a
Substrate 2	50.56±1.61 b	105.23±26.97 b	18.95±4.89 a

178

#### **3.3.** Characteristics of the radicular system under different substrate cultivation

The ability of a plant to produce different types of roots is an inherent aspect of its plasticity which has important adaptive characteristics (Barlow, 1993; Bell & Lechowicz, 1994). Variation in traits among multiple components of plant root systems affect the capability of these plants to deal with their complex environments (Caldwell, 1994; Fitter, 1994).

As the individual parts of a root system develop at different microsites, under different internal and environmental conditions, variations in growth and physiological characteristics among them should be expected (Waisel & Eshel, 2002).

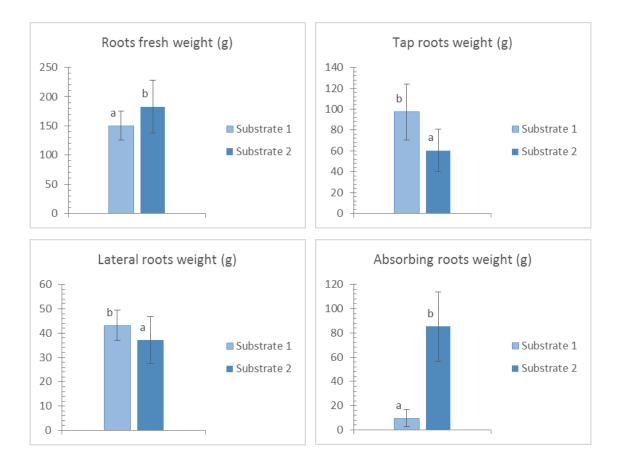
The assessed parameters in the radicular system are presented in Figures 1 and 2 and in Table 4. In our study, the use of cocopeat as substrate for young almond trees originated an increase in the total fresh weight of the radicular system. However this weight difference generated between substrates is highly localized and we can find statistical differences between the two substrates according to the root architecture (Figure 1). When separating the radicular system into tap, lateral and absorbing roots, we observe that trees under substrate 1 presented a higher weight of tap and lateral roots but substrate 2 generated five times more weight of
absorbing roots. Such absorbing roots are less sensitive to gravity and extend the root system
horizontally. They branch as much as the taproot or even more, and increase the specific root
density at the upper soil layers exploiting the most fertile portions of soil (Waisel & Eshel, 2002).

The number and spatial distribution of structural roots are important traits for tree stability (Dupuy et al., 2005). We found in our study that cocopeat substrate promoted the proliferation of tap and lateral roots to the extent that the number of tap or lateral roots in substrate 2 triplicated the ones found in substrate 1 (Figure 2). Abundant production of laterals is highly important for root growth in heterogeneous media. It affects the nutrient supply of the plants, the allocation of assimilates, the production of growth substances (McCully & Mallett, 1988) and the anchorage of the tree in the ground (Stokes, 2002).

204 Root strength varies enormously inter and intra species but also within the same root 205 system and may depend on the mechanical role attributed to the root.

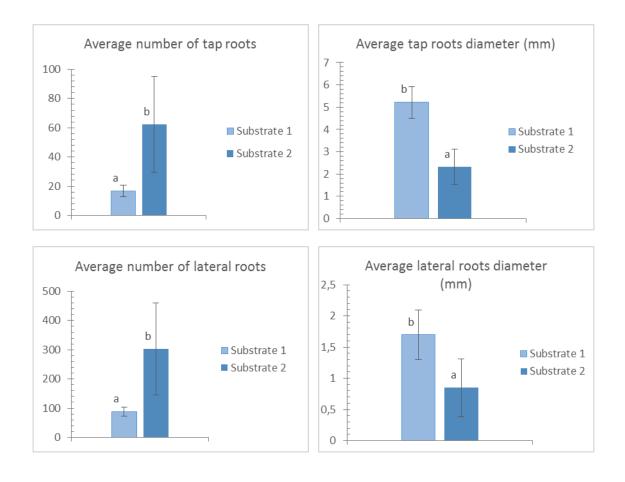
206 Commandeur and Pyles (1991) consider tensile strength to be the most important factor 207 governing soil stabilization and fixation. O'Loughlin & Watson (1979) found that tensile strength 208 decreases with increasing root diameter in roots of Pinus radiata D. Don. and attributed this 209 phenomenon to differences in root structure, with smaller roots possessing more cellulose than 210 older roots and being cellulose more resistant than lignin in tension. In our study of young 211 almond trees, roots developed on substrate 1 obtained greater diameters than roots developed 212 on substrate 2 (Figure 2) and this significant difference could perhaps influence the mechanical 213 resistance of the root system and permit a better adaptation of the trees develop with substrate 214 2 to open field. Contrary to the increase in tensile strength with decreasing root size, 215 compression and bending strength decrease with increasing root size (Stokes & Guitard, 1997). 216 Dickman and Pregitzer (1992) found that the thinner the diameter of a root, the shorter is its life 217 span. In peach, roots  $\leq$  0,25 mm in diameter have a median life span of 77 days while roots 218 classed between 0,5 and 1,7 mm lived more than 370 days (Wells et al., 2002). Similar results 219 were found by Wells and Eissenstat (2001) in apple tree roots. Roots also differ in the structure 220 of various mature cells, ion selectivity of fine roots is much better because of the smaller gap 221 between the mature endodermis and the tips. Plant strategy may follow one of two alternatives 222 for root architecture: production of long, strong, fast growing roots, thus sacrificing some 223 selective capability or production of fine slow growing roots with the gain of a better control of 224 ion movement into tops (Waisel & Eshel, 2002). Ion content seems to be determined not only 225 by the physiological traits of the roots but also by the ratio of long to short ones.

226 As Table 4 shows, in our study, the trees grown in substrate 1 presented greater root 227 system longitude. Radicular systems developed under substrate 1 were average 20 cm longer 228 than those developed under cocopeat substrate. However, the average distance of tap roots to 229 first bifurcation occurs deeper in roots under substrate 2. This same trend can be observed on 230 the average distance of lateral roots to first bifurcation. No differences between substrates were 231 found in the minimum distance to first bifurcation in both tap and lateral roots. Long roots and 232 their branches facilitates an efficient system for soil exploration and provides a long-lasting 233 structure from which the short roots can proliferate when conditions are favorable (Persson, 234 2000). The efficiency of root penetration depends on soil conditions as well as on degree of 235 suberization and mycorrhizal infection, soil mechanical resistance reduces the rate of root 236 penetration especially in dry or compacted soils (Masle, 2002).



**Figure 1.** Weight parameters of radicular system with different substrates (n = 24; mean value  $\pm$ 

240 standard deviation; P <0.05).



244

246 Figure 2. Physical parameters measured in the radicular system with different substrates (n =

247 24; mean value ± standard deviation; P <0.05).

248

- **Table 4.** Length radicular parameters according to the type of substrate studied (n = 24; mean
- 250 value ± standard deviation; P < 0.05).

251

	Root system longitude (cm)	Distance of tap roots to first bifurcation (cm)	Maximum distance of tap roots to first bifurcation (cm)	Minimum distance of tap root to first bifurcation (cm)	Distance of lateral roots to first bifurcation (cm)	Maximum distance of lateral roots to first bifurcation (cm)	Minimum distance of lateral roots to first bifurcation (cm)
Substrate 1	68.82±7.59 b	4.94±0.45 a	21.34±7.40 a	0.51±0.26 a	3.80±1.90 a	14,22±4.76 a	0.12±0.06 a
Substrate 2	44.12±11.74 a	11.79±2.95 b	46.23±21.27 b	0.50±0.63 a	7.95±3.95 b	47.09±33.64 b	0.18±0.33 a

252

### **4.** Conclusions

In conclusion, the obtained data showed that GN Garnem stands out as a vigorous rootstock with a higher total root weight compared to GF 677 while GF 677 presented greater maximum length of the radicular system.

The results of this study indicated that the use of different substrates produce statistically significant changes not only in root development and distribution but also in the vegetative growth being both factors of extreme importance in the improvement of the nursery processes.

In our work, cocopeat substrate produced a higher and heavier trunk but no differences were found in the diameter of the grafted area as to what the use of substrate refers. Cocopeat also generated an increase in the total fresh weight of the radicular system produced mostly by a massive increase of absorbing roots that could probably enhance the adaptation of the young plant to open field. Although the number of tap and lateral roots in substrate 1 (composed of silica, peat and sand) was lower than in substrate 2, substrate 1 obtained a greater weight of both tap and laterals that could be explained by an enlargement of their diameter.

The selection of the substrate affects as well the architecture of the root system. Trees grown under substrate 1 presented greater root system longitude permitting deeper soil exploration which is suitable for young trees that will be later established in non-irrigated conditions. The average distance of tap and laterals to first bifurcation occurred however deeper when using substrate 2.

The choice of an appropriate substrate in the nursery of almond trees is a key factor in the early development of the young tree. Knowledge about root growth and root architecture during the first stages of development would help nursery industry to determine which should be the most suitable substrate regarding later field adaptation, survival and plant performance, focusing on the soil and climatic characteristics of the final destination of the plant.

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