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RESEARCH ARTICLE

The development of the radicular and vegetative systems of almond trees with different rootstocks following the application of biostimulants

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Abstract

Aim of study: Recently, the development of almond crops on a global scale has increased their area under cultivation. The demand for both plants and products that stimulate the growth of almond trees has therefore become increasingly necessary. Accordingly, in this project we have studied the response in the vegetative and root systems of almond trees with different rootstocks to varying inputs of several root stimulants.

Area of study: Valencia (Spain)

Material and methods: Several different organic biostimulants were studied in isolation, *i.e.* not combined with synthetic chemical fertilizers, in order to ascertain if chemical fertilizers could be at least partially replaced.

Main results: Good results were obtained by applying a biostimulant composed of organic matter rich in saccharides and carboxylates. Using an approach that enabled a distinguishing between them, plant radicular systems were shown to respond differently according to the biostimulant applied and the rootstock tested. The best results were obtained with a biostimulant composed of organic matter from corn hydrolysis and containing free amino acids and extracts from algae, as well as 0.07% zeaxanthins.

Research highlights: Although biostimulants are promoters of young almond tree growth, they should be applied to only partially replace chemical fertilizers. The present paper shows the importance of using an organic-origin biostimulant, as a complement to chemical nutrition.

Additional key words: growth promoters; fulvic acids; humic acids; amino acids; tryptophan; algae extracts

Abbreviations used: BS (biostimulant); OM (organic matter)

Authors' contributions: All authors conceived, designed and performed the experiments, analyzed the data, participated in the revision of scientific content, the drafting of the paper, and read and approved the final manuscript.

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Introduction

According to the Organization of the United Nations for Food and Agriculture (FAO), in 2017 the world production of almonds in the shell reached 2,239,697 tons, over a cultivated area of 1,925,887 hectares, and with an average yield of 1,163 kg per hectare (http://faostat3.fao.org/home/E). According to the International Nut and Dried Fruit Council (INC), the production of almonds in the shell has increased 25% in the past 10 years, with an annual increase of 5% (INC, 2019). Thanks to recent and continuing research findings about the beneficial impact of almond consumption on human health, almonds and other tree nuts have attracted considerable attention (Burns *et al.*, 2016). From 2004 to 2016, almond consumption doubled, reaching 160 g per person worldwide (INC, 2019).

In the past few years, the introduction of stricter environmental regulations and a change in mentality regarding the use of plant-protection products and fertilizers have stimulated interest in finding alternative methods of production and plant health (Wells *et al.*, 2003). According to the European Biostimulants Industry Council (2018), agricultural phytostrengtheners and biostimulants are attracting a considerable attention recently and are reporting one of the fastest growths in agriculture. Biostimulants may be an important and essential tool in the future due to climate change, in order to promote plant growth, crop yield, resistance to stress, and to serve as a stimulant to plant metabolism (Nardi *et al.*, 2016). According to Scaglia *et al.* (2017), biostimulants are classified according to five categories: microbial inoculants (Rouphael *et al.*, 2017), humic substances (humic and fulvic acids) (Olivares *et al.*, 2017), amino acids (Ertani *et al.*, 2009), and seaweed extracts (Khan *et al.*, 2009). Continuing this line of work, different root-system biostimulants have been developed in recent years (Apone *et al.*, 2010) in order to develop resistance to abiotic and biotic stressors factors (Deliopoulos *et al.*, 2010), and to improve crop quality. Phytostrengtheners extend the range of tools available to the modern farmer who wishes to reduce the environmental impact of their practices, which can in some cases improve the nutrient absorption capacity of plants, allowing them to prioritize the use of organic fertilizers.

Accordingly, it is important to study the influence of biostimulants on the vegetative growth of crops, and on their radicular systems. However, in the case of almond trees, it is important to verify the combined effects of the type of biostimulant and the rootstock used. This combination may provide important results for future agricultural practices in the almond sector, in addition to improving crop sustainability.

The aim of this work, therefore, is to study the response in the development of the vegetative and radicular systems of the rootstocks of almond trees following the input of various root biostimulants. This is of relevance to the tree nursery industry due to the importance of seedling growth. It also highlights the use of stimulants of organic origin (biostimulants) in their application, thereby encouraging a decrease in the use of traditional chemical fertilizers. Through a comparison of the effect of these biostimulants on plant vegetative and radicular development, the use of synthetic chemical treatments in almond trees may be reconsidered in the future, and perhaps partially replaced.

Material and methods

Samples and experimental design

For the present study, a total of 90 rootstocks were used: 30 from rootstock GF 677 (677), which results from the interbreeding of peach trees (*Prunus persica* L. Batcsh) with almonds trees (*Prunus dulcis* Miller), and obtained in France by the INRA (Institut National de la Recherche Agronomique) (Bernard & Grasselly, 1981); 30 from G×N rootstock Garnem® (GN), obtained by the Agricultural Research Service of the Government of Aragón (CITA-DGA), which were the result of crossing *P. dulcis* (cv. Garrigues) with *Prunus persica* (cv. Nemared) (Felipe, 2009); and 30 from rootstock ROOTPAC® R (RP-R), obtained in Spain from Agromillora Iberia, S.L., a natural hybrid between Myrobalan plum trees (*Prunus cerasifera* Ehr.) and almond trees (*P. dulcis* Miller). The rootstocks studied were two years old.

In quintuplicate (n = 5), we tested four commercial biostimulants and a nutrient solution *versus* a control treatment for each of the three rootstocks described (the control treatment received only the same amount of water supplied to the treatments tested). Although all these products are biostimulants, in this study we conducted a comparison without the combined influence of other fertilizer, as is commonly done in agricultural holdings. The composition of the four biostimulants and the nutritive solution used are given in Table 1.

The plant material used in the current work was under one year old and the stage corresponded to the moment where the first leaves begin to emerge and separate. The young plants was certified free from pests and diseases; it was collected from an authorized plant nursery, at phenological growth stage 10 on the BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) scale (Enz & Dachler, 1997).

The rootstocks were transplanted into 80 L pots, using a substrate prepared on request and based on a mixture of 25% silica, 38% vaporized peat, and 37% washed river sand. Five repetitions for each possible rootstock-treatment combination were carried out. Analyses were conducted at the Polytechnic University of Valencia (39° 38' 2" N, 0° 22 ' 29" W; height 4 m above sea level).

The trial lasted two years (July 2016 to July 2018). The irrigation dose was 40 L of water per month given by 40-minute irrigation on alternate days with a pressure-compensating and non-leakage dripper of 40 L/h flow rate and a uniformity coefficient of 85%.

Biostimulant treatments

Tests were planned by comparing the commercial biostimulants (the doses and applications of which followed the manufacturer's instructions) with a nutrient solution commonly used in the cultivation of young almond trees, and a control plant to which no external compounds were added, and which was irrigated with water only under the same conditions. The organic biostimulants applied are described as follows:

— Biostimulant 1 (BS1): composed mainly of organic matter (OM), humic extracts and fulvic acids, and applied by injection in the vicinity of the root;
— Biostimulant 2 (BS2): composed of OM from corn hydrolysis and containing free amino acids and extracts from algae and 0.07% zeaxanthins;
— Biostimulant 3 (BS3): composed of OM rich in saccharides and carboxylates;
— Biostimulant 4 (BS4): composed mainly of P and Mg, along with OM.

Table 1. Chemical compositions (%, w/w) of the biostimulants applied and the nutritive solution (B5).

BS1	
Total humic extract	25%
Fulvic acids	25%
Nitrogen (N)	4%
Phosphorus (P2O5)	0.5%
Potassium (K2O)	0.5%
Organic matter	45%
BS2	
"L" free aminoacids	4.7%
Nitrogen (N)	5.5%
Potassium (K2O)	1%
Organic matter	22%
Fe-HEDTA	0.5%
Weed extract	4%
Zeaxanthins	0.07%
BS3	
GABA	1%
Glutamic acid	1%
Tryptophan	1%
Carboxylates	4%
Macro and micro elements	2.8%
Saccharide mix	38%
BS4	
Nitrogen (N)	1%
Magnesium (MgO)	4%
Phosphorus (P2O5)	8%
BS5	
NO_{3-}	32.7%
$H_2PO_4^-$	4.1%
SO_4^{2-}	24.7%
HCO_3^-	1.2%
Cl^-	3.4%
NH_4^+	0.4%
K^+	11.7%
Ca^{2+}	15.2%
Mg^{2+}	5%
Na^+	1.6%

All four biostimulants were applied at a dilution of 8 cm^3 of product in 8 m^3 water.

In addition, another biostimulant was tested; this was characterized as BS5, or as nutritive solution. For the nutritive solution we drew up a preparation based on the extractions of almond trees obtained by Salazar & Melgarejo (2002). The formulation consisted mainly of N in the form of nitrate, K, Mg, Ca, and sulfate. This solution was applied to plants via a system of localized irrigation, with self-compensating emitters and non-drainage drippers of 4 L/h. The composition of the four applied biostimulants and the nutritive solution are given in Table 1.

The treatments were carried out on a weekly basis from the transplanting of the rootstock at phenologic stage 10 of the BBCH scale (Enz & Dachler, 1997) for stone-fruit trees, until the start date for measurements, which took place 20 weeks later.

Analysis of the vegetative system

The influence of the tested biostimulants on the vegetative system focused mainly on assessing total tree height, length and weight of the trunk, weight of leaves and young shoots, and the diameter of the graft zone between the aerial part and the root part.

Analysis of the root system

After eliminating the soil from the plants, the studied variables were the number of primary roots and the measurements for each of them, *i.e.* the diameter and the distance from the start of the root until the first junction 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, up to the first junction with a tertiary root (Mondragón-Valero *et al.*, 2017).

All measurements were always taken within 24 hours after plucking in order to avoid any drying of the aerial part or the root system. In the case of weight, roots were introduced in a Memmert model muffle at 38˚C until they stabilized to a constant weight, and weight was evaluated once they had dried.

Statistical analysis

An analysis of variance (ANOVA) with type III sums of squares was performed using the GLM (General Linear Model procedure) of the SPSS software package, vers. 21.0 (IBM Corporation, NY). The fulfilment of the ANOVA requirements, *i.e.* the normal distribution of the residuals and the homogeneity of variance, were evaluated by means of the Kolmogorov-Smirnov test with Lilliefors correction (if $n>50$), or the Shapiro-Wilk test (if $n<50$), and Levene's test, respectively. All dependent variables were analyzed using a one-way ANOVA with or without a Welch correction, depending on whether the requirement of the homogeneity of variances had been fulfilled. The main factor studied was the effect of rootstocks (GN, 677, and RP-R) and of the different biostimulants on the vegetative and radicular system variables of the almond trees studied. If a statistically significant effect was found, means were compared using Tukey's honestly significant difference multiple comparison test, or the Dunnett T3 test, depending on whether equal variances could be assumed. A two-way ANOVA was also performed to verify the combined effect of rootstock and biostimulants on the vegetative and radicular variables of young almond trees. All statistical tests were performed at a 5% significance level.

Results

Comparative study of the rootstocks

According to the results of our work regarding vegetative development, as presented in Table 2, the GN rootstock stands out as the most vigorous. Regarding vegetative growth in the control plants (without the application of biostimulants), the GN rootstock reported higher values for the majority of variables measured (except for trunk longitude and leaf weight; see Table 2 and Fig. 1).

Regardless of the biostimulant applied, the GN rootstock always produced a significant impact ($p<0.001$) on tree height (from 61.0 to 196.3 cm, control and BS5, respectively), compared to RP-R (from 57.6 to 170.5 cm, control and BS5, respectively) and 677 (from 46.8 to 174.9 cm, control and BS5, respectively) rootstocks under the same conditions (Table 2). The same observation was verified for trunk weight (from 12.3 to 108.1 g, control and BS5, respectively) and diameter of the grafted area (from 8.8 to 22.2 mm, control and BS5, respectively).

Regarding plant radicular development, we observed various types of roots in both annual and perennial plants, and were able to link these differences to wide variations in absorption and transfer capacity. In our study, rootstocks GN and RP-R (182.8 and 165.2 g with BS5, respectively) presented a higher root weight than rootstock 677 (120.1 g with BS5). The rootstock RP-R

Table 2. Vegetative system variables of different rootstocks treated with various biostimulants (n = 5; mean ± SD).

	RP-R	GN	677
	Tree height (cm)		
Control	57.6 ± 1.1 aB	61.0 ± 0.7 aC	46.8 ± 0.8 aA
BS1	97.8 ± 0.5 cA	121.0 ± 1.6 bC	111.9 ± 0.7 cB
BS2	108.7 ± 15.2 cA	149.7 ± 3.4 cC	128.2 ± 7.9 dB
BS3	80.0 ± 3.2 bA	121.5 ± 0.8 bC	98.3 ± 0.6 bB
BS4	60.2 ± 0.8 aB	69.2 ± 5.4 aC	48.0 ± 1.6 aA
BS5	170.5 ± 0.4 dA	196.3 ± 8.4 dB	174.9 ± 7.9 eA
	Trunk longitude (cm)		
Control	31.4 ± 8.0 bB	24.3 ± 0.3 aA,B	22.7 ± 0.5 aA
BS1	30.5 ± 0.4 bB	33.9 ± 0.8 b,cC	27.8 ± 2.4 a,bA
BS2	29.1 ± 4.3 bA	31.6 ± 0.5 b,cA	31.5 ± 3.4 bA
BS3	41.7 ± 3.7 cB	35.0 ± 0.2 cA	32.6 ± 0.4 bA
BS4	27.0 ± 0.3 a,bB	26.2 ± 0.1 aA	30.4 ± 0.4 bC
BS5	20.6 ± 0.4 aA	30.5 ± 5.0 bB	27.6 ± 5.5 a,bA,B
	Diameter of grafted area (mm)		
Control	7.7 ± 0.7 aA	8.8 ± 0.1 aB	8.0 ± 0.2 bA
BS1	13.0 ± 0.1 cB	14.1 ± 0.3 dC	10.8 ± 0.1 c,dA
BS2	13.4 ± 0.6 cB	13.7 ± 0.4 dB	11.4 ± 0.7 dA
BS3	9.4 ± 0.1 bA	11.3 ± 0.3 cC	9.8 ± 0.1 cB
BS4	8.8 ± 0.1 bB	10.0 ± 0.1 bC	6.7 ± 0.2 aA
BS5	21.3 ± 0.3 dB	22.2 ± 1.4 eB	17.6 ± 1.2 eA

In the same line, for each variable and treatment studied, mean values with different capital letters differed significantly ($p<0.05$). In the same column, for each variable and rootstock studied, mean values with different lowercase letters differed significantly ($p<0.05$).

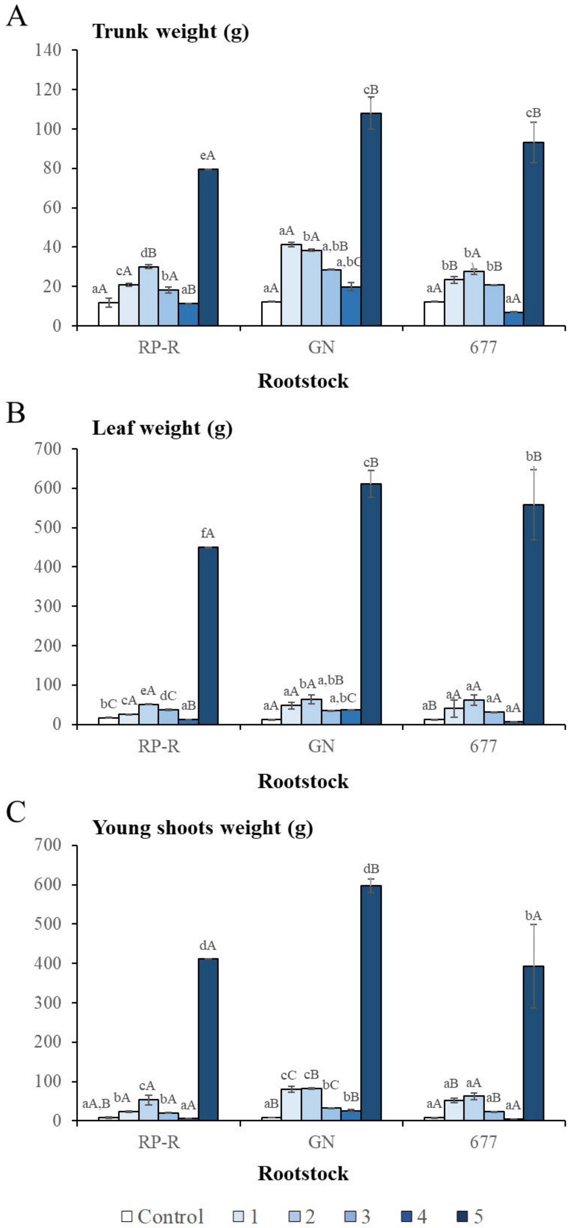

Figure 1. Trunk, leaf and young shoots weight of almond trees with different rootstocks treated with different biostimulants (n = 5; mean ± SD). In the same treatment, mean values with different capital letters differed significantly (p<0.05); In the same rootstock, mean values with different lowercase letters differed significantly (p<0.05).

showed greater maximum length of the root system (92 cm in control; Fig. 2), and as such, allowed for better in-depth exploration of the soil. In addition, this rootstock presented greater uniformity in the spatial distribution of its roots.

Based on the overall results obtained, rootstock GN proved to be more adequate for the development of both the vegetative (Table 2) and radicular systems (Table 3) of almond trees, although primarily the vegetative system. Therefore, it can be considered an appropriate choice for the cultivation of new almond orchards.

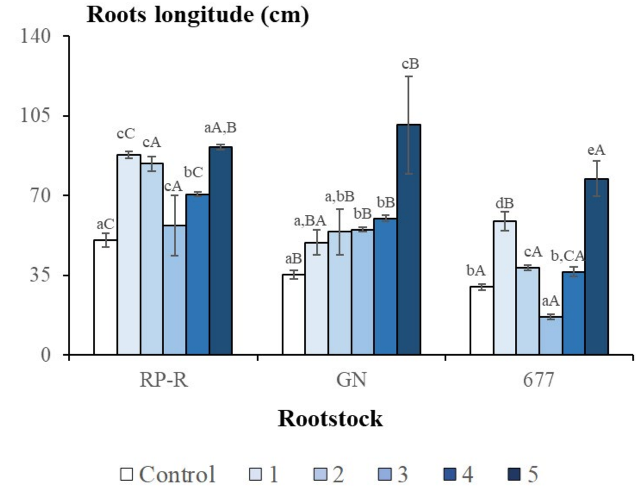

Figure 2. Roots longitude of almond trees with different rootstocks treated with various biostimulants (n = 5; mean ± SD). In the same treatment, mean values with different capital letters differed significantly (p<0.05); In the same rootstock, mean values with different lowercase letters differed significantly (p<0.05).

Characteristics of the vegetative system according to different biostimulants applied

The variables studied in the vegetative system of the plants are reported in Table 2. The results show that all treatments, except for those based on phosphite (BS4), produced trees of a height greater than the control sample, as well as higher values for average leaf weight, young shoots and trunks (Table 2 and Fig. 1).

The tested nutrient solution (BS5), rich in N, produced almond trees with an average height of 180 cm (in five months), three times that of the data collected from measurements of the control trees. Similar results were repeated when we compared the diameters in the graft area, depending on the applied treatment. The diameter of the trunk is an essential feature of nursery stock, since trunk thickness determines the appropriate time to proceed with the graft. BS3 managed to increase the average diameter in the possible area of graft, but to a lesser extent than BS1 and BS2 (which presented similar results), and with results much lower than those provided by the tested nutrient solution. Therefore, according to the results obtained, and by evaluating all the variables studied, BS2 appears to be a good option for improving the growth of young almond trees.

Characteristics of the radicular system according to different biostimulants applied

The applied biostimulants influenced the radicular system and the variables assessed in almond trees, in a way visible to the naked eye (see Fig. 3).

Table 3. Radicular system variables of different rootstocks (in cm) treated with various biostimulants (n = 5; mean value ± SD).

	RP-R	GN	677	RP-R	GN	677
	Distance of tap roots to first bifurcation			**Max. distance of tap roots to first bifurcation**		
Control	57.6 ± 1.1 aB	61.0 ± 0.7 aC	1.32±0.02 aA	3.87±2.39 aA,B	2.27±0.03 aA	5.33±0.07 a,bB
BS1	97.8 ± 0.5 cA	121.0 ± 1.6 bC	4.95±0.31 bB	10.8±0.96 cB	5.28±1.43 aA	13.2±2.52 c,dB
BS2	108.7 ± 15.2 cA	149.7 ± 3.4 cC	5.20±0.74 bB	8.15±1.58 b,cA	18.6±3.59 bB	8.51±0.08 b,cA
BS3	80.0 ± 3.2 bA	121.5 ± 0.8 bC	8.92±0.04 cC	9.41±3.93 cA	7.67±0.22 aA	22.0±0.09 eB
BS4	60.2 ± 0.8 aB	69.2 ± 5.4 aC	0.91±0.13 aA	5.11±0.06 a,bB	7.25±0.70 aC	1.25±0.06 aA
BS5	170.5 ± 0.4 dA	196.3 ± 8.4 dB	5.11±0.66 bB	8.16±0.09 b,cA	22.6±6.17 bB	16.8±8.29 d,eA,B
	Min. distance of tap root to first bifurcation			**Distance of lateral roots to first bifurcation**		
Control	0.30±0.17 b,cA,B	0.39±0.01 bB	0.20±0.01 aA	4.35±0.96 bA	5.84±0.04 a,bB	4.48±0.02 aA
BS1	0.22±0.03 a-cA	0.25±0.09 a,bA	1.62±0.45 bB	3.44±0.23 a,bA	3.63±0.27 aA	6.07±1.98 aB
BS2	0.32±0.08 cA,B	0.41±0.08 bB	0.22±0.02 aA	3.37±0.75a,bA	6.14±0.01 bB	4.28±0.57 aA
BS3	0.12±0.02 aA	0.11±0.02 aA	0.32±0.04 aB	2.55±0.25 aA	3.92±0.05 a,bB	5.86±0.07 aC
BS4	0.15±0.05 a,bA	1.12±0.07 cC	0.30±0.03 aB	3.46±0.06 a,bA	5.24±1.88 a,bA,B	5.63±0.06 aB
BS5	0.12±0.02 aA	0.33±0.18 bA	0.44±0.35 aA	3.64±0.21 bA	3.99±2.32 a,bA	4.46±1.86 aA
	Max. distance of lateral roots to first bifurcation			**Min. distance of lateral roots to first bifurcation**		
Control	11.6±1.56 aB	13.0±0.11 a,bB	10.1±0.05 aA	0.27±0.22 bA	0.18±0.02 a,bA	0.83±0.08 bB
BS1	15.9±0.42 aA	16.0±5.42 a,bA	17.5±1.11 bA	0.02±0.02 aA	0.57±0.48 b,cA	0.60±0.52 a,bA
BS2	25.9±10.8 bB	18.8±0.86 bA,B	14.7±3.03 bA	0.02±0.01 aA	0.70±0.13 cC	0.18±0.03 aB
BS3	13.9±1.89 aA	16.1±0.08 a,bB	16.1±0.09 bB	0.10±0.02 a,bB	0.04±0.03 aA	0.20±0.01 aC
BS4	10.0±0.09 aA	11.3±0.71 aB	14.0±0.27 bC	0.02±0.01 aA	0.28±0.36 a-cA	0.29±0.03 aA
BS5	16.2±0.07 aA	14.3±6.45 a,bA	15.0±3.06 bA	0.04±0.02 aA	0.18±0.02 aA	0.02±0.01 aB

In the same line, for each variable and treatment studied, mean values with different capital letters differed significantly ($p<0.05$). In the same column, for each variable and rootstock studied, mean values with different lowercase letters differed significantly ($p<0.05$).

Figure 3. Visual aspect of rootstock GF-677 treated with different biostimulants: A, control; B, BS1; C, BS2; D, BS3; E, BS4; F, BS5 or nutritive solution tested.

The results are presented in Table 3 and Figs. 4 to 6. In our study, only the treatments using BS1, BS2, and the tested nutrient solution, produced statistically significant increases in the total fresh weight of the root system (120.1, 165.2, and 182.8 g for the nutritive solution under 677, RP-R and GN rootstocks, respectively.

The distribution of roots can be affected by the input of fertilizers. In our study, we observed that the implementation of BS1 and BS3, and the tested nutrient solution, resulted in the development of a greater number of primary roots (Fig. 5) than in the control sample; in addition, all the products, except BS3, produced a significant increase in the number of secondary roots (Fig. 6). In contrast, the control treatment presented higher in-depth exploration of the soil (75.8, 76.2, and 92.0 cm for 677, GN, and RP-R, respectively) than BS11, BS2, BS4, and the tested nutrient solution (Fig. 2).

We must emphasize that the combination of rootstock and biostimulants influenced significantly all the variables studied comparatively to the control almond trees. We verified a $p<0.001$ for all the variables studied, except for the number of tap roots ($p=0.003$), but still significant.

Discussion

Comparative study of rootstocks

The results that were observed for the rootstocks point to the GN rootstock as being the most vigorous.

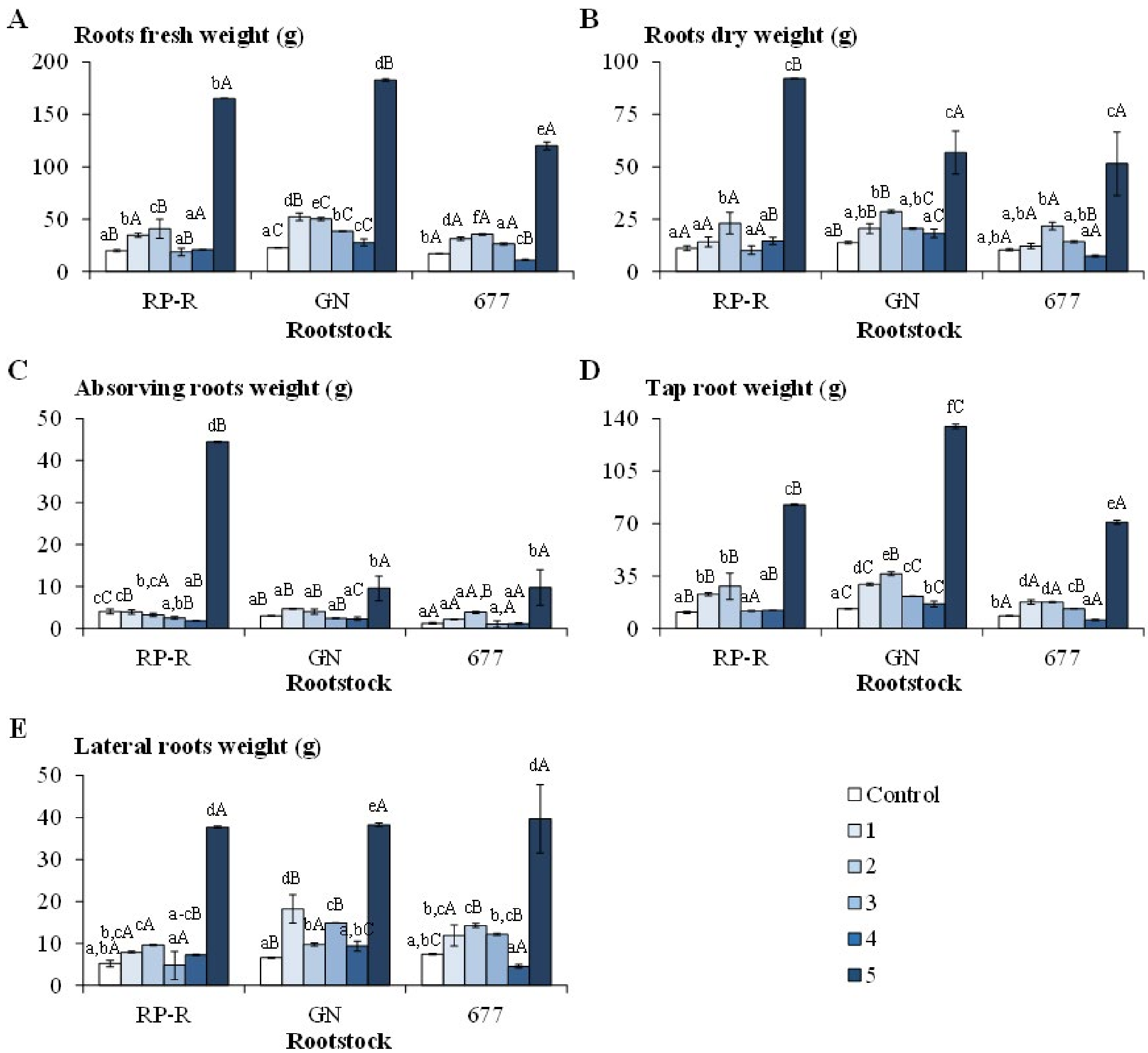

Figure 4. Weight variables of radicular system with different rootstocks treated with different biostimulants (n = 5; mean ± SD). In the same treatment, mean values with different capital letters differed significantly ($p<0.05$). In the same rootstock, mean values with different lowercase letters differed significantly ($p<0.05$).

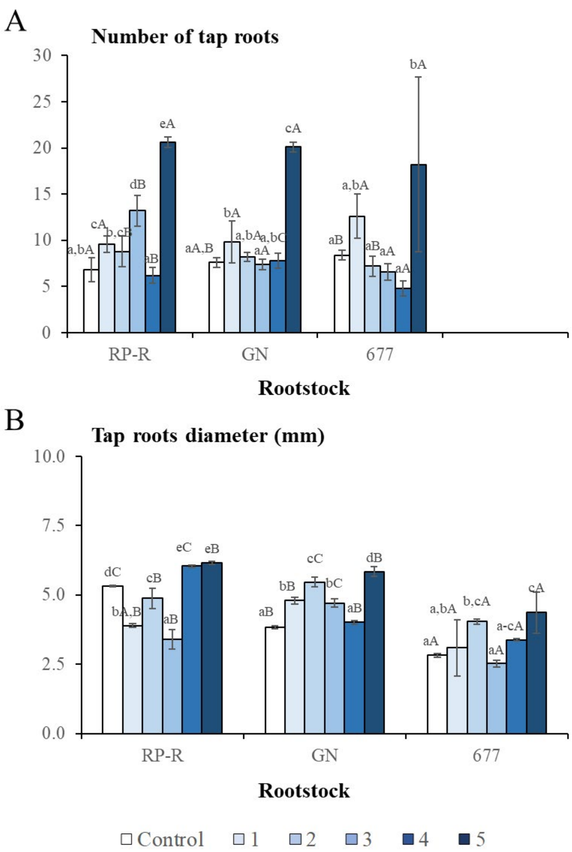

Figure 5. Number of tap roots (A) and their diameter (B) in almond trees with different rootstocks treated with different biostimulants (n = 5; mean ± SD). In the same treatment, mean values with different capital letters differed significantly ($p<0.05$). In the same rootstock, mean values with different lowercase letters differed significantly ($p<0.05$).

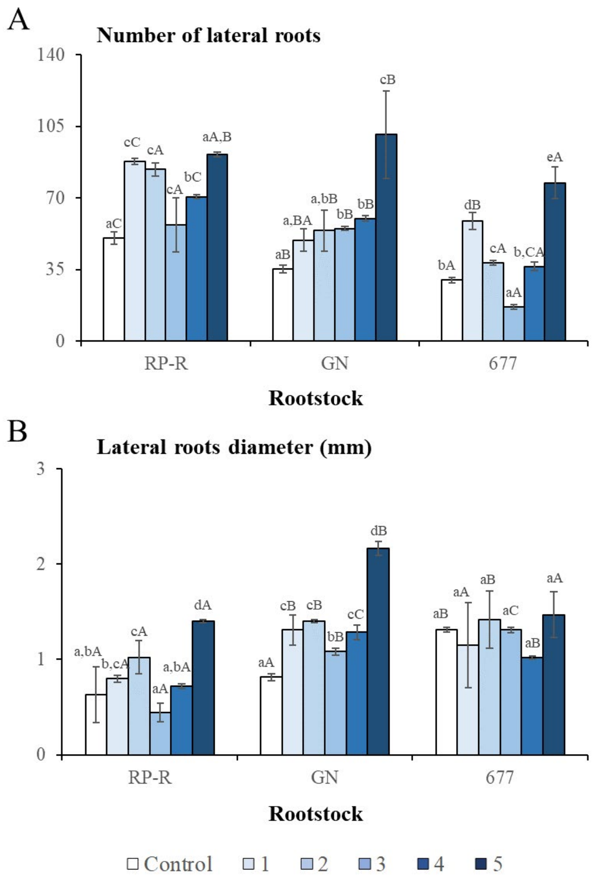

Figure 6. Number of lateral roots (A) and their diameter (B) inn almond trees with different rootstocks treated with different biostimulants (n = 5; mean ± SD). In the same treatment, mean values with different capital letters differed significantly ($p<0.05$). In the same rootstock, mean values with different lowercase letters differed significantly ($p<0.05$).

These results are consistent with those observed by Felipe (2009), who described this rootstock, prior to being grafted, as strong with upright growth. Sotomayor *et al.* (2008) concluded that, compared to 677, the GN rootstocks produced greater pruning weight and a larger number of young buds.

The different degrees of adaptability by rootstocks to the environment can be attributed partly to the depth that the root system can reach, its density, and its spatial distribution. This is why the best spatial distribution, *e.g.* for the roots of rootstock RP-R, resulted in greater soil exploration ability, at least under our study conditions; as such, this rootstock can be recommended for potted plants and for poor soil situations. It was also the most tolerant in terms of humidity levels.

The vigor conferred by the rootstock GF 677 is high (Espada *et al.*, 2013), providing to the young trees a faster fruiting and contributes for high yields (Bussi *et al.*, 1995) both under rain-fed and irrigated conditions. GF 677 rootstock has a good adaptation to limestone soils (Forcada *et al.*, 2012) and tolerates iron chlorosis (Moreno *et al.*, 2008). Nevertheless, this rootstock is one of the most sensitive to Meloidogyne nematodes (Vargas *et al.*, 1985).

GN rootstock vigor, production levels and entry into fruiting are very similar to GF 677 (Felipe, 2009). GN tolerates adequately drought conditions, although it is prepared to be implanted in irrigated conditions, with well-drained soils since it is a rootstock that is not very tolerant to radical asphyxiation (Gómez-Aparisi *et al.*, 2001). One of the main differences between GF 677 and GN is that GN presents resistance to main Meloidogyne nematodes.

RP-R is also a vigorous rootstock, its zero propensity to emit tiller offers significant savings in cultivation. RP-R has been shown to be highly productive in the US in different almond cultivars, especially with the 'Non Pareil' cultivar (Pinochet *et al.*, 2011). Its main advantage over the other two studied rootstocks lies in its adaptability to clayey soils where peach × almond hybrids present development problems. RP-R shows high tolerance to radical asphyxiation, iron chlorosis and active limestone and a moderate tolerant response to salinity (Pinochet, 2010).

Characteristics of the vegetative system treated with biostimulants

Regarding the vegetative system, our results show that fertilization based on OM and humic extracts, which are important components of BS1 and BS2 (Table 1), favored tree development. The promoting aspect of amino acids in growth is noted (BS2) and is recommended for their invigorating properties during critical periods of cultivation such as, in our case, budding and growth in the establishment of new plantations. In addition, recent studies of apple (Basak, 2008) and olive trees (Chouliaras *et al.*, 2009) point to a further development of the aerial part as a beneficial effect of the introduction of algae extracts. According to Pizzeghello *et al.* (2013), humic extracts are known to promote plant growth, by improving the bioavailability of important micronutrients in the soil, but also by acting in the metabolic pathways involved in specific physiological mechanisms. Furthermore, these authors also revealed that cytokinins are responsible for the biological effects of humic acids (Pizzeghello *et al.*, 2013). The same research group observed that biostimulants rich in amino acids improve enzyme activity, inducing the conversion of nitrate into organic N, thereby improving plant growth (Ertani *et al.*, 2009). Other important features of the results obtained with BS2 are the presence of algae extracts and zeaxanthins (Table 1). Algae extracts and seaweeds are recognized biostimulants in horticulture (Battacharyya *et al.*, 2015). Seaweed extracts possess a phytohormone-like activity, inducing plant growth at low concentrations (Battacharyya *et al.*, 2015). Furthermore, they also intervene in plant metabolism and physiology. Therefore, BS1 and BS2, with humic acid and amino acids in their compositions, achieved good vegetative growth rates for the variables evaluated.

The lack of results yielded by BS4 (the phosphorus-based product) contradicts the trials of Goss *et al.* (1993), which concluded that increases in P in the soil at a time prior to planting results in significant increases in the growth of the aerial part of the plant. One possible explanation for the lack of consistency in our results with those of the noted study may be the difference in the method and time of application of the product; in one case, 'pre planting', and in the other, 'post planting', in an already-established young plant.

BS5, rich in N (32.7% w/w; Table 1), caused a significant increase in tree height. Other variables were also considerably influenced by the application of this biostimulant. Based on the composition of Table 1 we can consider that the results obtained were related to the high concentrations of some elements. This fact was also verified in other studies, mainly considering N. Muhammad *et al.* (2009) studied the influence of increasing applications of N in almond trees and concluded that additional contributions of this element favored vegetative growth and productivity. Lopus *et al.* (2010) noted that N fertilization is key in the development of this tree. As in our work, Bi *et al.* (2003) proved in their study of nursery-stock almond trees that applications via fertigation and foliar N application favored greater vegetative development, evidenced by a greater number of buds and greater leaf mass.

Characteristics of the radicular system treated with different biostimulants

Our results regarding the radicular system are consistent with those obtained by Zhang & Ervin (2004), who also observed increases in the weight of the root of peach trees when a fertilizing solution was applied. Similarly, they detected significant increases in the mass of the root system when humic acids or algae extracts, or a combination of both treatments, were applied. There is vast bibliographic data that confirms the effect of humic and fulvic acids on root development and the proliferation of lateral roots. Recent studies show that treatments with seaweed extract improve the potential of root development in Arabidopsis (Rayorath *et al.*, 2008), and on the branching of the root system. Vernieri *et al.* (2005) prefer biostimulants as the elements that favor development, because they increase both the formation of lateral roots and the total size of the root system.

In the current research study, the control treatment presented higher in-depth exploration of the soil than the treatments with BS1, BS2, and BS4, and when compared to the tested nutrient solution. The data are consistent with Williams & Smith (1991), who explain that the number of roots is greatest in the layer of the most fertile soil. The implementation of all the treatments with the exception of BS4, induced a change in the distribution of roots, causing bifurcation of the primary to secondary roots to take place at a greater depth. This change in the distribution of the roots can possibly influence greater resistance to mild drought, since the absorption systems will be more effective at greater depth. Zhang & Ervin (2004) confirmed the effect of applying a combination of humic acids and extracts from algae on the increase of drought tolerance in beet plants. In fact, algae extracts affect the architecture of roots facilitating the uptake of nutrients by plants. Minor components of algae extracts, such as alginic acid, have the capacity to form a larger number of molecular cross-polymers, which improve the water retention capacity of the soil, and therefore stimulate radicular system growth and soil microbial activity (Chen *et al.*, 2003). There is enough evidence to indicate that specific amino acids and their derivatives and precursors can induce a defensive response in plants toward abiotic stressors factors such as drought and high temperatures (Apone *et al.*, 2010).

The organic stimulants applied were demonstrated to partially promote the growth of both the vegetative and radicular systems of almond trees, primarily in the combination of GN rootstock and BS2, composed of OM from corn hydrolysis and containing free amino acids and extracts from algae, as well as 0.07% zeaxanthins.

The organic stimulants applied on an individual basis at the tested dose are capable of substituting traditionally used chemical fertilizers or at least in reducing their use in fertigation programs for almond trees, principally in the primary developmental stages of a plantation. Furthermore, the biostimulants tested are compatible with organic agricultural practices. The application of organic stimulants can maintain good levels of productivity, without the need for significant applications of synthetic stimulants, thus representing good eco-friendly practice. Given the observed increase in the radicular system, and therefore in the absorptive capacity of young trees, the use of organic stimulants of quality is justified as a means for replacing chemical treatments. Nevertheless, and in line with the results obtained, the mixture of biostimulants with nutritive solutions and chemical fertilizers could give important results for the growth of almond trees. At the current stage, biostimulants cannot totally replace chemical fertilizers, but their contribution is important for the reduction of chemical inputs.

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