

Article

Influence of Plant Biostimulant as Technique to Harden Citrus Nursery Plants before Transplanting to the Field

María R. Conesa ¹, Pedro J. Espinosa ², Diego Pallarés ² and Alejandro Pérez-Pastor ^{3,*}

¹ Irrigation Department, CEBAS-CSIC, P. O. Box 164, 30100 Murcia, Spain; mrconesa@cebas.csic.es

² Marketing Department, FMC Agricultural Solutions, 28046 Madrid, Spain; pedro.espinosa@fmc.com (P.J.E.); diego.pallares@fmc.com (D.P.)

³ Department of Plant Production, Technical University of Cartagena (UPCT), Paseo Alfonso XIII, 48. ETSIA, 30203 Cartagena, Murcia, Spain

* Correspondence: alex.perez-pastor@upct.es; Tel.: +34-968-327035

Received: 10 July 2020; Accepted: 29 July 2020; Published: 31 July 2020



Abstract: The supply of commercial plant biostimulants (PB) for sustainable agriculture is currently very broad but also confusing, as there is little information on their use to mitigate the negative effects of water stress on plants growing in areas of water scarcity. The issue addressed in this article deals with the effects of Amalgerol[®], a PB and soil conditioner mainly based on seaweed extracts (SWEs), on the water relations and the growth patterns of mandarin trees grown in pots and their response to a subsequent period of water stress compared with un-treated plants. When the SWE treatment accumulated 75 mL of product, plants exhibited an increase in vegetative growth and higher values of gas exchange rate, with 57% higher substrate microbiological activity than un-treated plants. After this, the irrigation was completely suppressed in all plants until a mean threshold value of -1.6 MPa of midday stem water potential was reached, and it was then reestablished after 7 consecutive days. The un-treated plants showed a higher level of water stress, around 0.4 – 0.7 MPa, compared to the treated ones, recovering at least three days after irrigation recovery. Furthermore, the presence of mycorrhized roots was 60% higher than un-treated plants, which resulted in greater resistance to water stress. The use of Amalgerol[®] resulted in a good complement for mineral plant fertilization in semi-arid agrosystems, where water resources are limited, allowing the hardening of citrus nursery plants, which can contribute to their more efficient field transplantation in water scarcity areas.

Keywords: Amalgerol[®]; mycorrhized roots; seaweed extracts; soil microbiological activity; vegetative growth; water deficit

1. Introduction

Optimal crop development demands a high application of mineral fertilizers and pesticides. However, it has been demonstrated that the uncontrolled use of them produces harmful effects on the environment and human health, as their contribution may contaminate the soil and groundwater, reduce biodiversity, increase soil/water salinity, and also diminish bio-resources such as energy and freshwater [1–3]. This situation makes necessary the introduction of alternatives that manage the drawbacks derived from the fertilization of crops in agriculture.

Spain is currently the sixth-largest producer of citrus crops in the world and the main producer in the Mediterranean areas, where the predominant climatic conditions are high evaporative demand and low rainfall, which is typically below 250 mm per year [4]. This is important because the agricultural industry is the largest user of freshwater withdrawals for irrigation, accounting for 70% of all water

use globally [5]. Mandarin trees have become one of the most important crops in Southeastern Spain and now represent around 35% of the national production of citrus fruit, most of which is exported. In spite of this, the Spanish citrus grower faces the need to maintain market competitiveness by reducing costs and increasing product quality in a situation of water shortage, both in quantity as well as in quality [6].

The use of plant biostimulants has considerably increased in recent years. A plant biostimulant (PB), first mentioned by [7], is any substance or microorganism applied to crops with the objective of enhancing nutrition efficiency, abiotic stress tolerance, and/or crop quality traits, regardless of its nutrient content [2]. Therefore, PBs are considered to be among the most innovative and promising solutions for improving the sustainability and profitability of sustainable organic agriculture [8], for their positive effects not only on plant growth and nutrition but also on abiotic stress tolerance [3]. Furthermore, the use of PBs may constrain the negative effects of (i) salinity [9,10]; (ii) chilling temperatures [3,11]; (iii) excess moisture [12]; (iv) pests and diseases [13]; (v) pruning [14]; (vi) plant transplant [15], and (vii) postharvest storage [16].

To some extent, plant biostimulants are available in many formulations, with variable ingredients, but are generally classified into three main groups based on their modes of action [2,7], including humic substances and products that contain hormones and amino acids [17]. Moreover, commercial products should be developed to guarantee greater yield and yield stability, in order to meet the increasing food global demand [18]. Lastly, it is important to highlight that PBs operate through different mechanisms to fertilizers, regardless of the presence of nutrients in the commercial products [19].

Despite the considerable increase in the use of PBs in agriculture, the information obtained until now is scarce and of limited reliability for many fruit crops worldwide. In this sense, the industry and companies that manufacture PBs have taken part in creating associations, such as the European Biostimulant Industry Council (EBIC), which promotes the contribution of plant biostimulants for making agriculture more sustainable and resilient. Particularly, Amalgerol[®] is a physical liquid formulation mainly based on seaweed extracts (SWEs), aside from the essential oils and fatty acids in oil/water emulsion.

The role of SWEs in agriculture—for example, in enhancing yield and fruit quality, stimulation of plant growth and disease management—has been reviewed by [20]. Commercial formulations of SWEs may promote fine roots, allowing the plant to increase water and nutrient uptake and extraction [3]. Bioactive compounds present in SWEs enhance the performance of crops/plants under abiotic stresses and include cytokinins [21,22], betaines [23], phenolics [24], and other hormone-like substances [25]. Moreover, traces of nutrients are present in the extracts. Nevertheless, until now, the influence of SWE application to crops in order to minimize the losses caused by abiotic stresses such as water storage has been poorly addressed.

Under water stress conditions, mycorrhized roots have been associated with a better plant water status by improved host nutrition, particularly higher *p* uptake, whereas gas exchange is regulated through hormonal signals or by lowered leaf osmotic potential for greater turgor maintenance [26]. Meanwhile, microbiological activity also plays a significant role in the alleviation of drought stress in plants, inducing the accumulation of osmolytes and antioxidants, the upregulation or downregulation of stress responsive genes, and alterations in root morphology [27]. This work aimed to assess the ability of citrus nursery plants to harden by the application of the plant biostimulant Amalgerol[®] based on SWE. The mechanisms developed by treated plants to improve the suitability for transplanting to the field were studied. In this sense, water relations, vegetative growth patterns, and soil fertility were compared with untreated plants to ascertain any differences in the behavior of citrus nursery plants during tolerance to drought as well as during recovery.

2. Materials and Methods

2.1. Experimental Nursery Conditions

A pot experiment was conducted from March to May 2016 with 40 young mandarin plants (*Dancy tangerine* × *Clementine* (*Citrus clementine* Hort. Ex Tanaka × *Citrus reticulata* Blanco), grafted onto *Citrus macrophylla*, in a climate-controlled greenhouse of a commercial nursery (Viveros Caliplant, S.L) located in Pozo Aledo (San Javier, Murcia, Spain). The cylindrical pots had a volume of 25 L (35 × 28 × 25 cm) and contained a substrate of coconut fibers + 50 g of horse organic matter. Water and air content at container capacity of the substrate and the available water were 73%, 24%, and 39%, respectively. The bulk density was 0.11 g cm⁻³ whereas the carbon-to-nitrogen (C/N) ratio was 80. At the beginning of the experiment, the selected plants were homogeneous in size. The drip-irrigation and fertilization management were the same for all plants in the experiment, using the standard mineral fertilization program of the nursery's criteria. The experimental layout consisted of four replicates per treatment with 5 plants each ($n = 20$ per treatment), which were arranged in a randomized complete block design.

2.2. Pre-Conditioning Period

The plant biostimulant (PB) used in this study was Amalgerol® (FMC Agricultural Solutions) which contains seaweed extracts (SWEs), essential oils, and fatty acids in an oil/water emulsion.

Two treatments were assessed: (i) control plants (untreated, hereafter), corresponding to those plants that did not receive PB applications, and (ii) plant biostimulant (SWE, hereafter), corresponding to those plants that received 0.025 L plant⁻¹ in each PB application, that were applied on 11-March (first application, 1A); 22-April (second application, 2A); and 15-May (third application, 3A), respectively.

These applications were done manually in each pot with a 60 mL syringe, applying 25 mL, as recommended by the manufacturer, in each pot and mixing with freshwater until obtaining the field capacity of the substrate in every pot. Therefore, during this period, plants in the SWE treatment group received a total of 75 mL of product.

2.3. Stress and Recovery Periods

After the preconditioning period, stress and recovery periods were imposed in all plants, in the two treatments studied:

Stress:

For 7 consecutive days (from 16-May to 22-May), the irrigation was completely suppressed in all the plants in the experiment. Every 2–3 days, the midday stem water potential (Ψ_s) was assessed until a mean threshold value of -1.6 MPa was reached, as indicated by [28], to avoid a proliferation of water sprouts.

Recovery:

Once the threshold value of Ψ_s was reached, the irrigation was completely recovered to initial levels. This period had a duration of 7 consecutive days (from 23-May to 29-May). It should be noted that the plant measurements were taken with the same periodicity as the stress period.

2.4. Measurements

2.4.1. Soil Respiration

Before each PB application and during the stress/recovery irrigation periods, measurements of soil respiration (R_s , mmol m⁻² s⁻¹) were taken in two pots per replicate ($n = 8$ per treatment) using a portable gas exchange closed system CIRAS-2® (PP Systems, Hitchin, Hertfordshire, UK), which incorporates an infrared gas analyzer (IRGA: CIRAS-2) and a soil chamber SRC-1 with a measuring area of 78 cm² and a volume of 1170 cm³.

2.4.2. Microbiological Activity

Microbiological activity, expressed as colony forming units (CFUs) of actinomycetes, was determined before each PB application in 2 samples per replicate ($n = 8$ per treatment). Each sample was collected after PB application. Results were provided by an external and accredited laboratory (FITOSOIL Laboratorios S.L. Spain).

2.4.3. Vegetative Growth Patterns

During the experiment, the vigor was visually estimated using a 5-point hedonic scale representing the intensity of vigor: 1 (very low), 2 (low), 3 (medium), 4 (high), and 5 (very high). All plants had a value of 1 at the beginning of the experiment. Canopy diameter in the north-south and east-west orientations, plant height, and shoot length were also determined with a tape measure. Trunk diameter was obtained with a digital caliper (Mitutoyo, CD-15D) around 10 cm above the substrate surface. Each measurement was carried out biweekly in 3 plants per replication ($n = 12$ per treatment).

At the end of the experiment, plants were divided into roots, stems, and leaves, oven-dried at 60 °C for 48 h, and then weighed for biomass, expressed as % of dry matter.

2.4.4. Plant Water Status

Plant water status was evaluated before each PB application and during the stress/recovery irrigation periods, on two leaves per replicate ($n = 8$), by measuring midday stem water potential (Ψ_s , MPa) using a pressure chamber (Soil Moisture Equip. Crop. Model 3000, 153 Santa Barbara, CA, USA) at 12:00 solar time. Leaves were fully expanded and selected at random from the middle third of the shoots, covered with aluminum foil bags for at least 2 h prior to the measurements, following the recommendations of [29].

2.4.5. Gas Exchange

Gas exchange parameters were determined from a similar number and type of leaf and on the same days as Ψ_s was measured, using a portable gas exchange system, CIRAS-2 (PPSystems, Hitchin, Hertfordshire, UK). Leaf net photosynthesis (P_n , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) were measured at 10:00 solar time, fixing a photosynthetic photon flux density (PPFD) $\approx 1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ above the photosynthesis light saturation intensity for citrus leaves [6], near constant ambient CO_2 concentration ($C_a \approx 350 \mu\text{mol mol}^{-1}$) and leaf temperature ($T_{\text{leaf}} \approx 30 \text{ }^\circ\text{C}$). Intrinsic water use efficiency (WUE) was calculated as the ratio between P_n and g_s ($\mu\text{mol mol}^{-1}$).

2.4.6. Percentage of Mycorrhizal Colonization

Mycorrhizal colonization was studied at the end of PB applications, following the gridline intersect method [30]. Roots of four plants per treatment (one per replicate) were (i) washed with water, (ii) cleared in 10% KOH, (iii) in HCl_2N for 10 min, and (iv) staining with 0.05% trypan blue dissolved in lactic acid (v/v) [31]. After this, 100 fragments of roots per each repetition, 1 cm in length ($n = 400$ per treatment), were observed under an Olympus microscope CX21 (Microscope Company Inc., New York, USA). The percentage of mycorrhizal colonization was calculated as the ratio of infected roots with respect to the total roots observed.

2.4.7. Statistical Analysis

Data were analyzed using the SPSS 20 (IBM, Armonk, NY, USA). Means were compared with the least significant difference test at a confidential level of 95% ($\text{LSD}_{0.05}$).

3. Results

3.1. Vegetative Growth Patterns

Figure 1 shows the seasonal evolution of vigor following a 1–5 hedonic scale. The maximum significant differences of about 15% were found after the second PB application. At this time, a mean average increase of 10% was observed in plants that received the SWE treatment (Figure 1B).

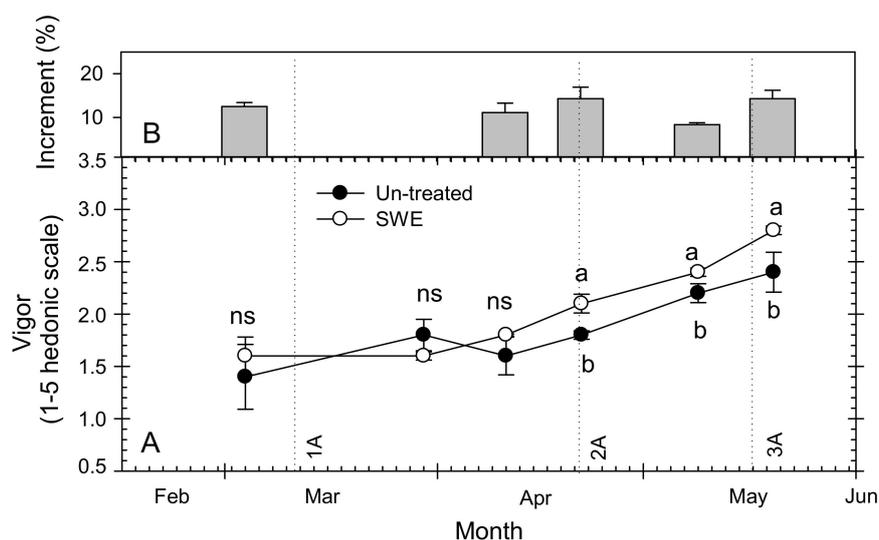


Figure 1. Plant vigor evolution in each treatment: SWE (○) and untreated (●) (A). Increase in the values obtained in SWE plants with respect to those that were untreated (B). Values are means \pm SE of four replications ($n = 12$ per treatment). Means followed by different letters indicate significant differences between treatments according to $LSD_{0.05}$ test. ns: not significant.

Significant differences in shoot length between both treatments were only detected at the end of the experiment (Figure 2). Indeed, shoots from the untreated plants showed a compensatory growth with respect to plants that received the SWE treatment, as a result of typical plant development. In this regard, it should be noted that commercial nursery plants are grown for up to 30–45 days before they are sold. Then, the commercial viability of SWE plants corresponds to the first two PB applications (from March to mid-April), where the differences between both treatments are noticeable.

Canopy diameter evolution, particularly in the E-W direction, showed lower values in the SWE treatment plants (Figure 3b). However, these plants showed an increase in vigor, indicating that the vegetative growth of SWE plants was more concentrated in the canopy than in the shoot's tips. It also suggests an increase in the water sprout growth inside the canopy. No significant differences were noticed in the trunk diameter from both types of plants. Lastly, it is worth noting that the significant differences found between both treatments were minimized with the development of the crop.

Regarding the biomass of the plants, expressed as a percentage of dry matter, while the average values of the leaves and the roots were around 65% and 55%, respectively, the stem was the only part that showed significant differences between treatments, being 21% higher in the treated plants (data not shown).

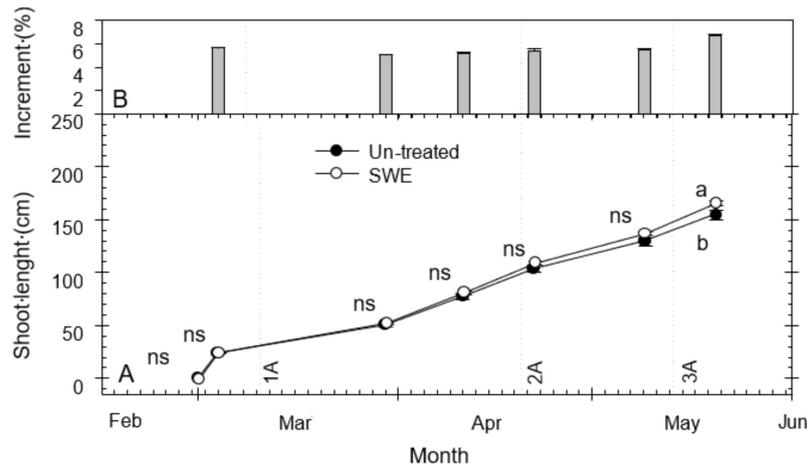


Figure 2. Shoot length evolution in each treatment: SWE (○) and untreated (●) (A). Increase in the values obtained in SWE plants with respect to those that were untreated (B). Values are means \pm SE of four replications ($n = 12$ per treatment). Means followed by different letters indicate significant differences between treatments according to $LSD_{0.05}$ test. ns: not significant.

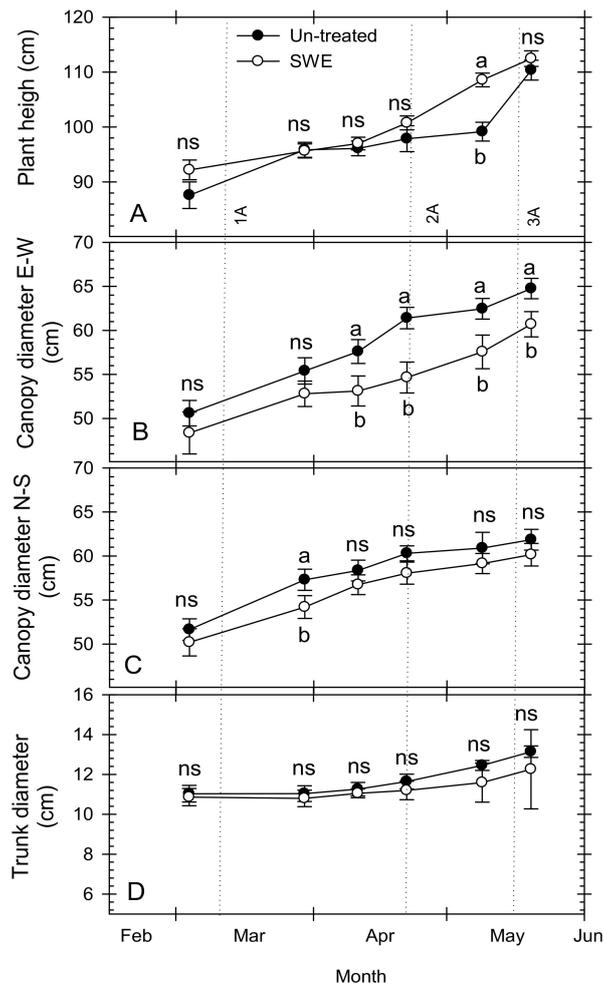


Figure 3. Plant height (A), canopy diameter at east-west direction (B), canopy diameter at north-south direction (C), and trunk diameter (D) evolution in each treatment: SWE (○) and untreated (●). Values are means \pm SE of four replications ($n = 12$ per treatment). Means followed by different letters indicate significant differences between treatments according to $LSD_{0.05}$ test. ns: not significant.

3.2. Plant and Soil Water Relations

After having accumulated 75 mL of product, plants that received SWE treatment had the highest values of soil respiration (R_s), with a mean difference of around 50% compared with untreated plants (Figure 4a). R_s is directly related to the microorganisms present in the soil. Therefore, the results agree with the microbiological activity reported in Figure 5. In this sense, Figure 6 shows a strong relationship when R_s and the microbiological activity are compared ($r^2 = 0.91$; $p < 0.001$). However, during the stress/recovery periods, no significant differences were observed between both treatments. It is important to note that during the stress periods, R_s values from all plants exhibited a sharp drop, reaching values close to zero. During the recovery period, there was a slight increase in R_s values, but it was insufficient to bring these up to the initial levels of R_s (Figure 4a).

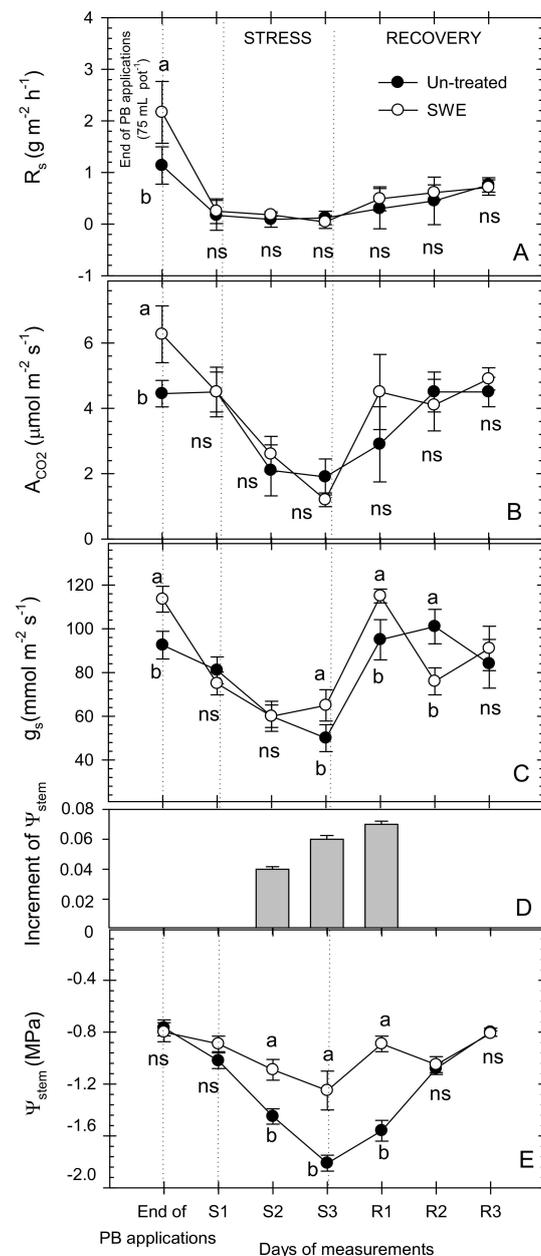


Figure 4. Seasonal evolution of soil respiration (R_s , A), leaf net photosynthesis (P_n , B), stomatal conductance (g_s , C), increase in midday stem water potential (C) of the values obtained in SWE plants

with respect to those that were untreated, and midday stem water potential (Ψ_s , D) in each treatment: SWE (\circ) and untreated (\bullet). Values are means \pm SE of four replications ($n = 8$ per treatment). Means followed by different letters indicate significant differences between treatments according to $LSD_{0.05}$ test. ns: not significant. S1: 17-May; S2: 19-May; S3: 22-May; R1: 24-May; R2: 26-May; R3: 29-May.

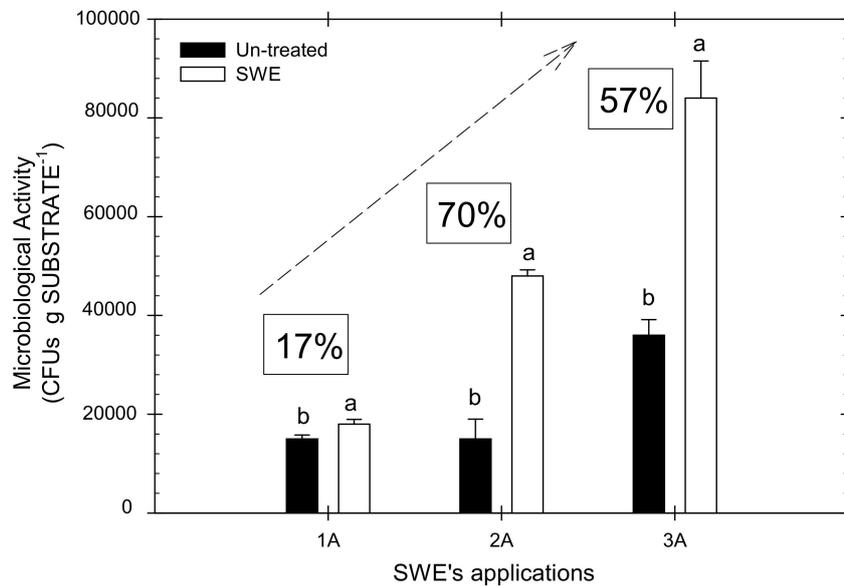


Figure 5. Microbiological activity quantified as colony forming units (CFUs) of actinomycetes in each treatment: SWE (white bars) and untreated (black bars). Each bar is mean \pm SE of four replications ($n = 8$ per treatment). Means followed by different letters indicate significant differences between treatments according to $LSD_{0.05}$ test. ns: not significant.

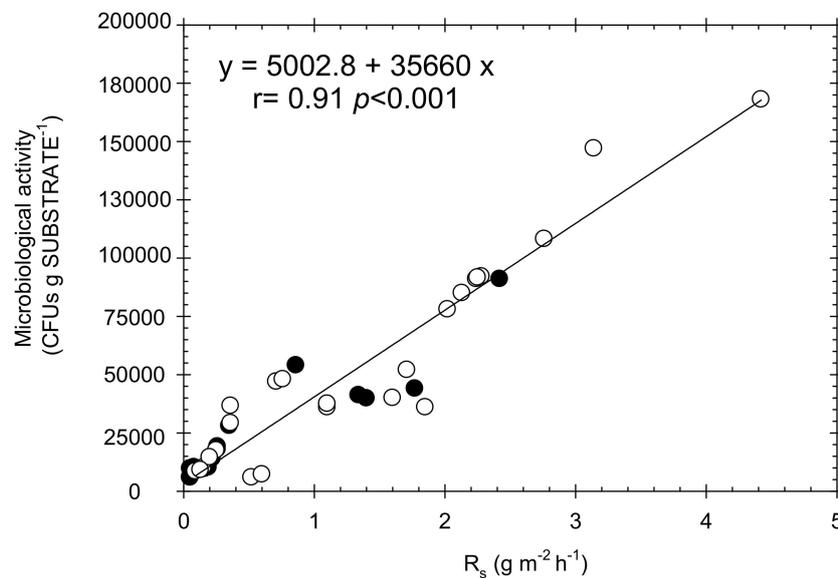


Figure 6. Linear relationship between microbiological activity and soil respiration (R_s) assessed before every PB application in each treatment SWE (\circ) and untreated (\bullet). Each point corresponds to the value reached in each determination ($n = 8$ samples per treatment and PB application).

The photosynthetic capacity was higher (by around 32%) in those plants that received PB applications in respect to untreated plants after having accumulated 75 mL of product (Figure 4b). Values of net photosynthesis (P_n) were 6.62 and 4.45 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in SWE and untreated plants,

respectively. The behavior of stomatal conductance (g_s) was as for P_n (Figure 4c). At the end of the PB applications, g_s was 18.5% higher in the SWE treatment. A similar result was also observed in the water use efficiency (WUE), with significant differences between both treatments at this time (Figure 7). As expected, the gas exchange parameters (P_n , g_s , and WUE) decreased during the stress period in both treatments. All plants had a plateauing effect as a result of limited stomatal opening (Figure 4e). During the recovery period, all gas exchange parameters increased, demonstrating that the negative influence of the water stress can be reversed.

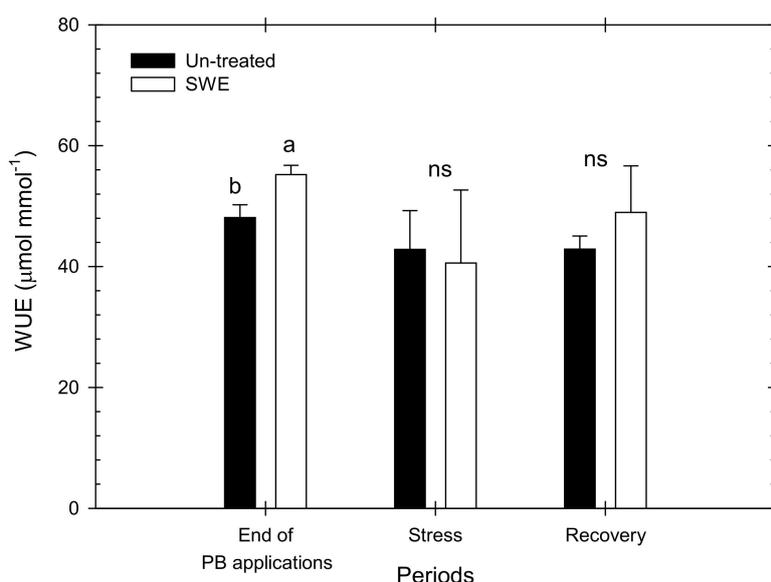


Figure 7. Water use efficiency (WUE) after having accumulated at the end of PB applications, stress and recovery periods in each treatment: SWE (white bars) and untreated (black bars). Each bar is mean \pm SE of four replications ($n = 8$ per treatment). Means followed by different letters indicate significant differences between treatments according to $LSD_{0.05}$ test. ns: not significant.

The stem water potential (Ψ_s) that determined the duration of the stress period showed a better water status in the PB treatment (Figure 4e), showing a maximum increase in Ψ_s of about 0.7 MPa, as compared to the control treatment (Figure 4d). Therefore, the SWE treatment had a higher resistance to drought after receiving 75 mL of product. The suppression of irrigation induced a decrease in the Ψ_s values, with a minimum of -1.81 MPa in the untreated plants. Furthermore, this measurement was significantly influenced by the plant's defoliation, which was lower in the plants treated with PB (data not shown). It is also interesting to note that the control treatment took 3 days to achieve Ψ_s values similar to the SWE treatment.

3.3. Microbiological Activity

Soil microbiological activity clearly increased after PB application in the SWE treatment (Figure 5). Just after one PB application, the soil microbiological activity increased by 17% compared with the untreated plants. At the end of the PB applications, when the treatment plants had accumulated 75 mL of product, soil microbiological activity was 57% higher than the untreated plants.

3.4. Mycorrhized Roots

At the end of experiment, the percentage of mycorrhized roots evaluated showed values of 24% and 64% in the untreated and SWE plants, respectively. The total colonization of roots from the SWE treatment was 60% higher than untreated plants. Thus, the PB applications in the SWE treatment group increased the amount of mycorrhized roots which were naturally present in the soil substrate.

4. Discussion

Applications of Amalgerol[®], mainly based on SWEs, are able to harden citrus nursery plants by increasing their tolerance to water stress before being transplanted to field conditions, an important issue in areas suffering from water scarcity. This effect was quantified by an improvement of 0.4–0.7 MPa in Ψ_s values of SWE treatment with respect to those plants that were untreated (Figure 4d,e). This improvement was motivated by the increased amount of mycorrhized roots in SWE plants, 60% higher than untreated plants, and therefore by the increased substrate microbiological activity, which may have promoted higher water uptake for improving drought tolerance.

As verified, plants treated with SWEs can maintain their water status to levels corresponding to well-irrigated citrus trees [6]. The authors of [11] reported an improvement in plants' tolerance after the foliar application of SWEs because the extract formulation caused a reduction in the leaf osmotic potential. The accumulation of K⁺ is an essential step in protecting against both ionic and osmotic stress and may contribute to tolerance. In this sense, the authors of [32] reported that under osmotic stress conditions, drought-sensitive species scavenge active oxygen at different rates by increasing the permeability of protoplast membranes, whereas an osmotic-tolerant species maintains better membrane integrity. Moreover, the authors of [33] reported that the cytokinins present in the SWE could mitigate stress-induced free radicals by direct scavenging and by preventing reactive oxygen species (ROS) formation by inhibiting xanthine oxidation.

Furthermore, gas exchange dynamics increased in plants subjected to PB application when they had accumulated 75 mL of product (Figure 4b,c). The increase in the photosynthetic capacity has been related to a reduction in the leaf chlorophyll content [11,34]. This behavior has been shown to be largely due to an increase in the biogenesis of the chloroplasts, a reduction in chlorophyll degradation, and a delay in senescence [34]. As SWEs promote endogenous cytokinin synthesis and impart protective effects on chloroplasts, the physiological processes of the plant are improved by cytokinin compounds [33,34]. The authors of [19] also indicated that improved chlorophyll biosynthesis (higher soil plant analysis development (SPAD) index) associated with SWEs improved the yield of baby spinach.

However, during the suppression of irrigation (water stress period), both treatments exhibited a strong drop in the gas exchange values, although to a lesser extent in SWE plants (Figure 4b,c). The authors of [35] reported better water relations and increased WUE under irrigation at 50% restoration of evapotranspired water in orange trees subjected to drought stress and treated with commercial SWEs. However, as a result of the degree of water stress imposed, the SWE treatment did not offset the physiological behavior (Figure 4), indicating that water losses were regulated via transpiration in response to extreme water deficit [6,36]. The authors of [20] reported that, due to the complex metabolic pathways involved in stress tolerance, limited success had been achieved in generating stress-tolerant crops through genetic engineering.

During early recovery in the post-stress periods, SWE-treated plants had significantly higher stomatal conductance, as observed by [20]. In this sense, the authors reported that the extracts from *Ascophyllum nodosum* were able to alleviate drought stress in soybean by regulating the expression of genes involved in abscisic acid (ABA) biosynthesis and reactive oxygen species (ROS) detoxification. The authors of [37] also found an increase in WUE and improvement in the recovery of drought-wilted plants that received SWE applications. In our study, WUE also increased in the SWE treatment (Figure 7), meaning that carbon fixation was higher than loss due to transpiration [38]. Consistent with an improved stomatal response, it was also observed that K⁺ and Ca²⁺ fluxes at the stomatal level were higher in plants treated with SWEs, which could favor osmotic adjustment [3].

An increase in vegetative growth was observed in our study, shown as enhanced vigor, shoot length, plant height (Figures 1, 2 and 3a,b), and stem dry weight. These results were in agreement with the outcomes of other experiments conducted with SWE on apples [39], due to reversing the drought stress effects on the plant, enhancing their growth and leaf density [40,41]. The authors of [33] reported that *Arabidopsis thaliana* plants treated with different *Ascophyllum nodosum* extracts (a kind of SWE)

with 1 g L^{-1} showed growth enhancement effects as compared to the untreated plants 3 weeks after treatment. Other experimental evidence on the plant growth promoted by PB applications based on SWE were reviewed by the authors of [20,42].

At the end of the PB applications, the microbiological activity was 57% higher in SWE treatment than those plants that were untreated (Figure 5). This was also strongly related to soil respiration (R_s) obtained from the soil substrate (Figures 4a and 6). Furthermore, changes in root architecture by an increase in mycorrhized roots have been observed (Figure 8). The production of mycorrhiza affects the microbial population in the rhizosphere directly or indirectly through changes in the composition and quantity of root exudates [43]. Lastly, the colonization roots result in further soil exploration by new roots, leading to an increase in water uptake by the roots, thus improving the plant water status, as occurred in our study [44]. It is important to note that R_s values suffered an important drop during the water stress because the decrease in soil moisture promoted anaerobic conditions which limited aerobic respiration [45], and this effect was slightly alleviated with the recovery of irrigation.

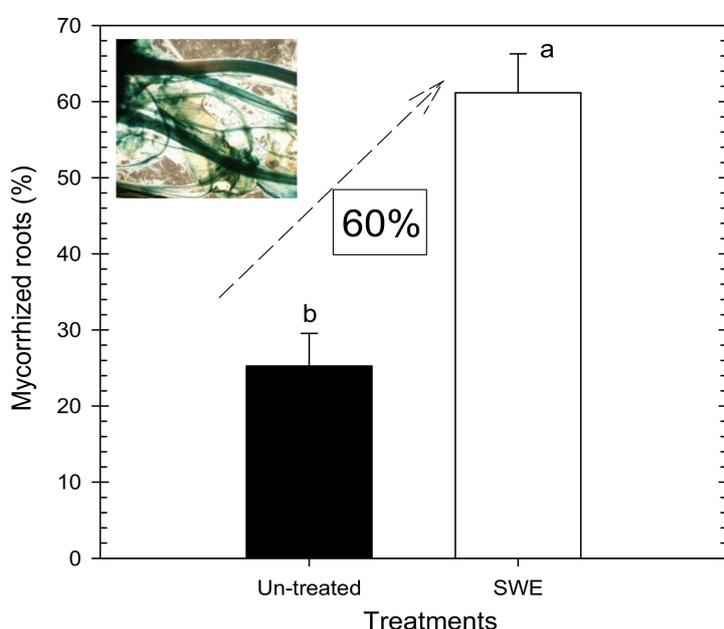


Figure 8. Mycorrhized roots (%) in untreated (black) and SWE (white) plants. Values are means \pm SE from four replications ($n = 20$ per treatment). Means followed by different letters indicate significant differences between treatments according to $\text{LSD}_{0.05}$ test. ns: not significant. The picture represents an example of the mycorrhized roots observed.

5. Conclusions

Two main benefits arising from the commercial PB Amalgerol[®] are considered in this study: (i) the improvement in water status of around 0.4–0.7 MPa and (ii) the positive influence on the physiological parameters and plant soil capacity. An increase in the values of P_n , g_s , and WUE in treated plants was reported. Moreover, this behavior was also conditioned by R_s , since the PB application promoted and encouraged the proliferation of the soil microbial community and the increase of mycorrhized roots. Therefore, this is the mechanism by which treated plants increased their water stress tolerance, improving their uptake of water and nutrients, ultimately maintaining the plant water status at optimum levels and promoting plant growth. The use of the PB Amalgerol[®] may be recommended as a technique to harden nursery plants before transplanting them to the field, due to its role in constraining drought stress effects, an important issue especially in the context of future climate change.

Author Contributions: Conceptualization, M.R.C. and A.P.-P.; methodology, M.R.C. and A.P.-P.; validation, P.J.E. and D.P.; formal analysis, M.R.C.; investigation, M.R.C. and A.P.-P.; resources, M.R.C.; data curation, M.R.C.; writing—original draft preparation, M.R.C.; writing—review and editing, M.R.C., P.J.E., D.P., and A.P.-P.; visualization, M.R.C.; supervision, A.P.-P.; project administration, A.P.-P. All authors have read and agreed to the published version of the manuscript.

Funding: This study was included in the R&D activities of the agreement between UPCT and the company FMC Agricultural Solutions (CATEDRA FMC-UPCT) and funding by the FMC Agricultural Solutions.

Acknowledgments: The authors thank the company VIVEROS CALIPLANT, S.L., for the use of the nursery and facilities to carry out the study. M.R. Conesa also thanks Sergio Navarrete from its technical department for his help in nursery determinations. M.R. Conesa acknowledges the postdoctoral financial support received from the Juan de la Cierva program (FJCI-2017-32045).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Calvo, P.; Nelson, L.; Kloepper, J.W. Agricultural uses of plant biostimulants. *Plant Soil* **2014**, *383*, 3–41. [[CrossRef](#)]
2. Du Jardin, P. Plant biostimulants: Definition, concept, main categories and regulation. *Sci. Hortic.* **2015**, *196*, 3–14. [[CrossRef](#)]
3. Van Oosten, M.J.; Pepe, O.; De Pascale, S.; Silletti, S.; Maggio, A. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. *Chem. Biol. Technol. Agric.* **2017**, *4*, 5. [[CrossRef](#)]
4. Fereres, E.; Soriano, M.A. Deficit irrigation for reducing agricultural water use. *J. Exp. Bot.* **2007**, *58*, 147–159. [[CrossRef](#)]
5. Fischer, E.M.; Seneviratne, S.I.; Luthi, D.; Schar, C. Contribution of land-atmosphere coupling to recent European summer heat waves. *Geophys. Res. Lett.* **2007**, *34*, L06707. [[CrossRef](#)]
6. Conesa, M.R.; de la Rosa, J.M.; Fernández-Trujillo, J.P.; Domingo, R.; Pérez-Pastor, A. Deficit irrigation in commercial mandarin trees: Water relations, yield and quality responses at harvest and after cold storage. *Span. J. Agric. Res.* **2018**, *16*, e1201. [[CrossRef](#)]
7. Zhang, Z.; Schmidt, R.E. Biostimulating turfgrasses. *Ground Maint.* **1997**, *1*, 15–32.
8. Povero, G.; Mejia, J.F.; Di Tommaso, D.; Piaggese, A.; Warrior, P.A. Systematic approach to discover and characterize natural plant biostimulants. *Front. Plant Sci.* **2016**, *7*, e435. [[CrossRef](#)]
9. Gómez-Bellot, M.J.; Ortuño, M.F.; Nortes, P.A.; Vicente-Sánchez, J.; Martín, F.F.; Bañón, S.; Sánchez-Blanco, M.J. Protective effects of *Glomus iranicum* var. *tenuihypharum* on soil and *Viburnum tinus* plants irrigated with treated wastewater under field conditions. *Mycorrhiza* **2015**, *25*, 399–409. [[CrossRef](#)]
10. Acosta-Motos, J.R.; Ortuño, M.F.; Bernal-Vicente, A.; Díaz-Vivancos, P.; Sánchez-Blanco, M.J.; Hernández, J.A. Plant Responses to Salt Stress: Adaptive Mechanism. *Agronomy* **2017**, *7*, 18. [[CrossRef](#)]
11. Mancuso, S.; Azzarello, E.; Mugnai, S.; Briand, X. Marine bioactive substances (IPA extract) improve ion fluxes and water stress tolerance in potted *Vitis vinifera* plants. *Adv. Hortic. Sci.* **2006**, *20*, 156–161.
12. Augé, R.M. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* **2001**, *11*, 3–42. [[CrossRef](#)]
13. Xavier, L.J.C.; Boyetchko, S.M. Arbuscular mycorrhizal fungi as biostimulants and bioprotectants of crops. *Appl. Mycol. Biotech.* **2002**, *2*, 311–340. [[CrossRef](#)]
14. Baños, H.L.; Alemán, J.; Martínez, M.A.; Ravelo, J.; Surís, M.; Miranda, I.; Rodríguez, H. Evaluación del efecto de bioestimulantes sobre la brotación de *Murraya paniculata* L. antes y después de la poda. *Cultiv. Trop.* **2009**, *30*, 36–39.
15. Fraser, G.A.; Percival, G.C. The influence of bioestimulants on growth and vitality of three urban tree species following transplanting. *Arboric. J.* **2003**, *27*, 43–57. [[CrossRef](#)]
16. Soppelsa, S.; Kelderer, M.; Casera, C.; Bassi, M.; Robatscher, P.; Andreotti, C. Use of Biostimulants for Organic Apple Production: Effects on Tree Growth, Yield, and Fruit Quality at Harvest and During Storage. *Front. Plant Sci.* **2018**, *9*, 1342. [[CrossRef](#)]
17. Parrado, J.; Bautista, J.; Romero, E.J.; García-Martínez, A.M.; Friaza, V.; Tejada, M. Production of a carob enzymatic extract: Potential use as a biofertilizer. *Biores. Tech.* **2008**, *99*, 2312–2318. [[CrossRef](#)]

18. Roupshael, Y.; Franken, P.; Schneider, C.; Schwarz, D.; Giovannetti, M.; Agnolucci, M.; Deascale, S.; Bonini, P.; Colla, G. Arbuscular mycorrhizal fungi act as bioestimulants in horticultural crops. *Sci. Hortic.* **2015**, *196*, 91–108. [\[CrossRef\]](#)
19. Roupshael, Y.; Giordano, M.; Cardarelli, M.T.; Cozzolino, E.; Mori, M.; Kyriacou, M.; Bonini, P.; Colla, G. Plant- and Seaweed-Based Extracts Increase Yield but Differentially Modulate Nutritional Quality of Greenhouse Spinach through Biostimulant Action. *Agronomy* **2018**, *8*, e126. [\[CrossRef\]](#)
20. Shukla, P.S.; Mantin, E.G.; Adil, M.; Bajpai, S.; Critchley, A.T.; Prithiviraj, B. *Ascophyllum nodosum*-based biostimulants: Sustainable applications in agriculture for the stimulation of plant growth, stress tolerance and disease management. *Front. Plant Sci.* **2019**, *10*, e655. [\[CrossRef\]](#)
21. Brain, K.R.; Chalopin, M.C.; Turner, T.D.; Blunden, G.; Wildgoose, P.B. Cytokinin activity of commercial aqueous seaweed extract. *Plant Sci. Lett.* **1973**, *1*, 241–245. [\[CrossRef\]](#)
22. Zhang, X.; Ervin, E.H. Cytokinin-containing seaweed and humic acid extracts associated with creeping bentgrass leaf cytokinins and drought resistance. *Crop Sci.* **2004**, *44*, 1737–1745. [\[CrossRef\]](#)
23. Mackinnon, S.; Hiltz, D.; Ugarte, R.; Craft, C.A. Improved methods of analysis for betaines in *Ascophyllum nodosum* and its commercial seaweed extracts. *J. Appl. Phycol.* **2009**, *22*, 489–494. [\[CrossRef\]](#)
24. Fan, D.; Kandasamy, S.; Hodges, D.M.; Critchley, A.T.; Prithiviraj, B. Pre-harvest treatment of spinach with *Ascophyllum nodosum* extract improves post-harvest storage and quality. *Sci. Hortic.* **2014**, *170*, 70–74. [\[CrossRef\]](#)
25. Hamza, B.; Suggars, A. Biostimulants: Myths and realities. *TurfGrass Trends* **2001**, *8*, 6–9.
26. Sánchez-Día, M.; Honrubia, M. Water relations and alleviation of drought stress in mycorrhizal plants. In *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystem*; Gianinazzi, S., Schüepp, H., Eds.; ALS Advances in Life Sciences; Birkhäuser: Basel, Switzerland, 1994; pp. 176–178. [\[CrossRef\]](#)
27. Vurukkonda, S.S.K.P.; Vardharajula, S.; Shrivastava, M.; Ali, S.K.Z. Enhancement of drought stress tolerance in crops by plants growth promoting rhizobacteria. *Microb. Res.* **2016**, *184*, 13–24. [\[CrossRef\]](#)
28. Pérez-Pastor, A.; Domingo, R.; Torrecillas, A.; Ruiz-Sánchez, M.C. Response of apricot trees to deficit irrigation strategies. *Irrig. Sci.* **2009**, *27*, 231–242. [\[CrossRef\]](#)
29. Hsiao, T.C. Measurements of plants water status. In *Irrigation of Agricultural Crops. Agronomy Monograph. No. 30.*; Stewart, B.A., Nielsen, D.R., Eds.; American Society of Agronomy: Madison, WI, USA, 1990; pp. 243–277.
30. Giovanetti, M.; Mosse, B. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* **1980**, *84*, 489–500. [\[CrossRef\]](#)
31. Phillips, J.; Hayman, D. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **1970**, *55*, 158–161. [\[CrossRef\]](#)
32. Rajabpoor, S.; Kiani, S.; Sorkheh, K.; Tavakoli, F. Changes induced by osmotic stress in the morphology biochemistry, physiology, anatomy and stomatal parameters of almond species (*Prunus L. spp*) grown in vitro. *J. For. Res.* **2014**, *25*, 523–534. [\[CrossRef\]](#)
33. Khan, W.; Rayirath, U.P.; Subramanian, S.; Jithesh, M.N.; Rayorath, P.; Hodges, D.M.; Critchley, A.T.; Craigie, J.S.; Norrie, J.; Prithiviraj, B. Seaweed extracts as biostimulants of plant growth and development. *J. Plant Growth Regul.* **2009**, *28*, 386–399. [\[CrossRef\]](#)
34. Battacharyya, D.; Babgohari, M.Z.; Rathor, P.; Prithiviraj, B. Seaweed extracts as biostimulants in horticulture. *Sci. Hortic.* **2015**, *196*, 39–48. [\[CrossRef\]](#)
35. Spann, T.M.; Little, H.A. Applications of a commercial extract of the brown seaweed *Ascophyllum nodosum* increases drought tolerance in container-grown ‘Hamlin’ sweet orange nursery trees. *HortScience* **2011**, *46*, 577–582. [\[CrossRef\]](#)
36. Pérez-Pastor, A.; Ruiz-Sánchez, M.C.; Conesa, M.R. Drought stress effect on woody tree yield. In *Water Stress and Crop Plants: A Sustainable Approach*, 1st ed.; Ahmad, P., Ed.; John Wiley & Sons Ltd.: Oxford, UK, 2016; Chapter 22; Volume 2, pp. 356–374. ISBN 9781119054368. [\[CrossRef\]](#)
37. Neily, W.; Shishkov, L.; Nickerson, S.; Titus, D.; Norrie, J. Commercial extracts from the brown seaweed *Ascophyllum nodosum* (Acadian®) improves early establishment and helps resist water stress in vegetable and flower seedlings. *HortScience* **2010**, *45*, S234.
38. Ehlenringer, J.R.; Cook, C.S. Photosynthesis in *Encelia farinosa* Gray in response to decreasing leaf water potential. *Plant Physiol.* **1984**, *75*, 688–693. [\[CrossRef\]](#)

39. Spinelli, F.; Giovanni, F.; Massimo, N.; Mattia, S.; Guglielmo, C. Perspectives on the use of a seaweed extract to moderate the negative effects of alternate bearing in apple trees. *J. Hortic. Sci. Biotech.* **2009**, *17*, 131–137. [[CrossRef](#)]
40. Xu, C.; Leskovar, D.I. Effect of *A. nodosum* extracts on spinach growth, physiology and nutrition value under drought stress. *Sci. Hortic.* **2015**, *183*, 39–47. [[CrossRef](#)]
41. Goñi, O.; Quille, P.; O’Connell, S. *Ascophyllum nodosum* extract biostimulants and their role in enhancing tolerance to drought stress in tomato plants. *Plant Physiol. Biochem.* **2018**, *126*, 63–73. [[CrossRef](#)]
42. Verkleij, F.N. Seaweed extracts in agriculture and horticulture: A review. *Biol. Agric. Hortic.* **1992**, *8*, 309–324. [[CrossRef](#)]
43. Fernández, F.; Juárez, J. Procedimiento de Obtención de un Agente Micorrizógeno. Patente Europea No: ES20110030566 20110411, 13 September 2012. [[CrossRef](#)]
44. Gómez-Bellot, M.J.; Ortuño, M.F.; Nortes, P.A.; Vicente-Sánchez, J.; Sánchez-Blanco, M.J. Mycorrhizal euonymus plants and reclaimed water: Biomass, water status and nutritional responses. *Sci. Hortic.* **2015**, *186*, 61–69. [[CrossRef](#)]
45. Zhao, C.; Miao, Y.; Yu, C.; Zhu, L.; Wang, F.; Jiang, L.; Hui, F.; Wan, S. Soil microbial community composition and respiration a long an experimental precipitation gradient in a semiarid steppe. *Sci. Rep.* **2016**, *6*, e24317. [[CrossRef](#)] [[PubMed](#)]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).