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A Biostimulant Based on Protein Hydrolysates Promotes the Growth of Young Olive Trees

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Abstract: Experiments were carried out in 2018 and 2019 to evaluate the effects of a biostimulant of animal origin, with a hormone-like action, on the growth and physiology of young olive trees. The biostimulant, consisting of a complex of natural amino acids (glycine, proline, and hydroxyproline, etc.), was applied by fertigation to both potted (one-year-old) and field-grown (2 years after transplanting) young olive trees. The control consisted of trees treated with an amount of urea nitrogen equal to the total nitrogen supplied with the biostimulant. Potted trees treated with the biostimulant showed greater growth than the control soon after treatment and up to the end of the vegetative season. Generally, this was associated with higher leaf photosynthetic rates and stomatal conductance. At the end of the experiment, all tree parts (roots, stem, shoots, and leaves) of the treated trees had greater biomass than the control. The trunks of field-grown trees treated with the biostimulant had greater growth with respect to the control. Overall, the results indicate the possibility of using the biostimulant based on complexes of natural amino acids to promote the growth of young olive trees.

Keywords: assimilate partitioning; biostimulants; *Olea europaea* L.; protein hydrolysates; olive tree growth

1. Introduction

A biostimulant is any substance or microorganism applied to plants with the aim of enhancing nutritional efficiency, abiotic stress tolerance, and/or crop quality traits, regardless of its nutrient content [1]. Plant biostimulants are also designated in commercial products containing mixtures of such substances and/or microorganisms [1].

Several biostimulants are based on amino acids (of plant and animal origin), peptides, vitamins, enzymes, substances with hormone-like effect (deriving from algal extracts), antioxidants, humic and fulvic acids, silicon and other minerals, and some strains of microorganisms [1,2]. Biostimulants act by regulating/improving the physiological processes of plants, in order to make them more efficient, in terms of vigour, tolerance/resistance to abiotic and biotic stresses, and quantity and quality of the yield obtained [1].

Various studies have been carried out on the effect of biostimulants on plant growth and production [3–6]. However, very few studies have evaluated the use of biostimulants on olive trees [7–12]. As far as young trees are concerned, Molina Soria [8] reported no significant effects of biostimulants on their growth, whereas Saour [9] found that the use of a combination of biostimulant/kaolin particle film enhanced growth, resulting in the production of higher quality olive seedlings. In adult olive trees, biostimulants were reported as being able to improve the yield and fruit characteristics [7,10], to replace fertilizers of inorganic origin [11] and to have positive effects on oil quality [12].

The worldwide increase in olive oil consumption has stimulated efforts to establish new plantations, mainly intensive (300–400 trees/ha) or superintensive (>1200 trees/ha), with the former realizable with all cultivars and the latter requiring low-vigour compact cultivars [13–16]. This also implies the need to increase the production of trees in the nurseries. Given this situation, speeding up the growth of young olive trees, both in nurseries and in the field, is a very important target as it reduces the time required for nurseries to obtain trees ready for transplanting and, in the field, the time for trees to reach the adult stage, and hence full production.

The purpose of this study was to evaluate the effects of a biostimulant on the growth and physiology of young olive trees. In particular, a biostimulant consisting of a complex of natural amino acids, supplied through fertigation, was applied to both potted and field-grown young olive trees. The biostimulant used is permitted in organic farming.

2. Materials and Methods

2.1. Application of the Biostimulant to Young Potted Olive Trees

The experiment started at the end of May 2018 and was carried out using 1-year-old potted olive trees of the cultivar Leccino. Before starting the experiment, the diameter of the stem was measured 5 cm above the collar. At the beginning of the experiment, a biostimulant based on protein hydrolysates (Sinergon 3000, now commercialized as Sinergon Bio, Cifo, Bologna, Italy) was applied as a solution (25 g/L) to the substrate in the pots and was repeated 4 times at weekly intervals. The volume of the solution used per application was 300 mL/pot. At the same time, a solution of urea (4.83 g/L) was applied to the control trees, by using a volume of 300 mL/pot. As the biostimulant and urea contained 8.9% and 46% nitrogen, respectively, the amount of nitrogen supplied to the trees was the same and equal to 0.67 g/tree for each application.

For each treatment, six replications each consisting of a single tree were used. From the beginning to the end of the experiment (May to November), tree growth was recorded by measuring stem diameter. In addition, from June to August, photosynthetic activity and related parameters (stomatal conductance and internal CO_2 concentration) were measured on fully developed leaves, every 1–3 weeks, using the ADC-LCA3 device (Analytical Development Co., Hoddesdon, Herts, UK).

At the end of the experiment, plants were destructively harvested to determine the biomass (dry weight) of the various components of the trees (roots, stem, shoots and leaves) [17,18].

2.2. Application of the Biostimulant to Young Olive Trees in the Field

The trial was carried out in 2019 in central Italy, locality "San Fortunato" near Spello (PG), in a young olive orchard established in the autumn of 2016. The orchard consisted of trees of the cultivar Moraiolo, spaced 6 × 5 m, with drip irrigation (2 emitters/tree, each supplying 4 L of water h^{-1}). Three replicates of 10 trees each were used. At the beginning of April, all the trees were fertilized by spreading 0.5 kg/tree of Nitrophoska Special 12-12-17, corresponding to 60 g of nitrogen, 60 g of phosphorous and 85 g of potassium per tree. In addition, the control received 100 g/tree of nitrogen as urea by fertigation, whereas the treated trees received urea + biostimulant by fertigation. Fertigation was applied every 7–10 days, from the beginning of May to the end of June. All trees received the same amount of total nitrogen, but in the treated trees, 6.7 g of nitrogen was applied as a biostimulant based on protein hydrolysates (Sinergon 3000), which contains 8.9% nitrogen. The biostimulant was applied 3 times, at the beginning, mid and end of May, at the rate of 10 + 10 + 5 kg/ha (corresponding to a total dose of 75 g/tree = 6.7 g of nitrogen/tree).

The growth of the trees was monitored by measuring the diameter of the trunk every 1–1.5 months. Moreover, the olive yield was determined at harvest (in December) and samples of olives were collected in order to determine fruit characteristics, such as pigmentation, fresh and dry weight and oil content.

Fruit weight, both fresh (FW) and dry (DW), was determined on samples of 100 olives per replicate by weighing them before and after drying to a constant weight in a ventilated oven at 105 °C. This also allowed to determine the fruit water content. Pigmentation was determined on samples of 100 olives per replicate using the "Jaèn pigmentation index", ranging from 0 to 7, with 0 indicating green olives and 7 indicating olives with superficial pigmentation on 100% of the epicarp and 100% pigmentation of the pulp [19,20]. Oil content was determined using an InfraAlyzer apparatus (SpectraAlyzerZeutec BRAN + LUEBBE, Rendsburg, Germany) both on fresh and dry weight on one sample per replicate.

The yield efficiency of the trees was determined as the ratio of the yield (g/tree) to the trunk cross section (g of olives or oil/mm^2 of trunk cross section).

2.3. Statistical Analysis

Data are presented as means ± standard error and/or were statistically analysed by ANOVA according to a completely randomized design and the averages were compared by the Student–Newman–Keuls Test.

3. Results

3.1. Application of the Biostimulant to Young Potted Olive Trees

Compared to the control, trees treated with the biostimulant had greater growth, determined by measuring the stem diameter, soon after application of the treatments (Figure 1). From the beginning to the end of the experiment, both control and treated trees grew continuously, but differences increased continuously up to end of the experiment (Figure 1).

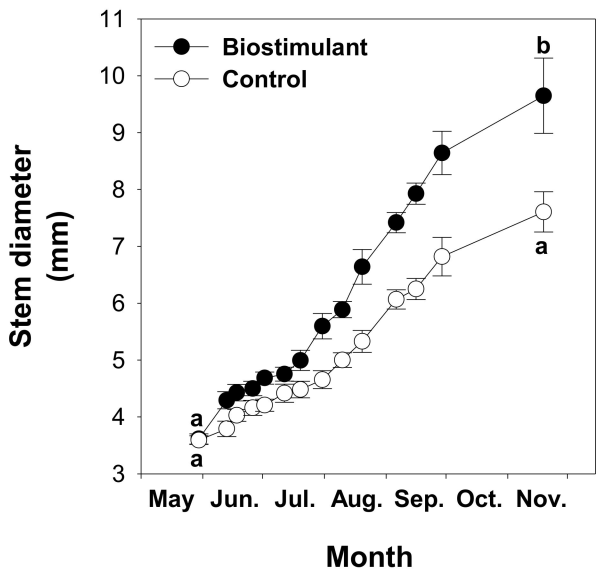

Figure 1. Diametral growth of the stem of treated and control potted trees during the vegetative season of 2018. Bars represent standard errors. At the beginning and at the end of the experiment, means accompanied by different letter are significantly different at $p < 0.05$.

During the first 2 months of the experiment, on average, the photosynthetic rate of the leaves of treated trees was higher than that of control trees (Figure 2). This was associated with higher values of stomatal conductance and tendentially lower, statistically different at the end of June, values of internal CO_2 concentration in treated trees (Figure 2).

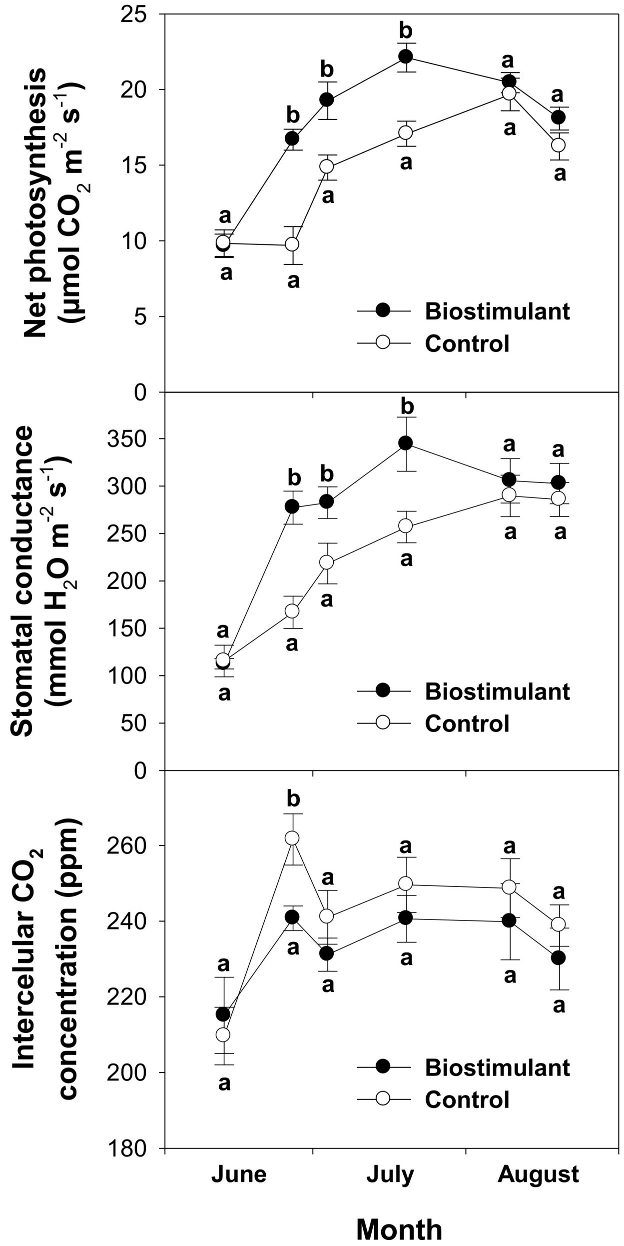

Figure 2. Photosynthetic activity, stomatal conductance and intercellular CO_2 concentration of the leaves of treated and control potted trees. Bars represent standard errors. For each date of measurement, means accompanied by different letter are significantly different at $p < 0.05$.

At the end of the experiment, the trees were destructively harvested in order to determine the biomass (dry weight) of the various parts (roots, stem, shoots, and leaves). Treated trees had a higher total biomass than the control (Figure 3). All the components of the trees (roots, main stem, shoots, and leaves) were positively affected by the application of the biostimulant; however, the effect on roots was not statistically significant (Figure 3).

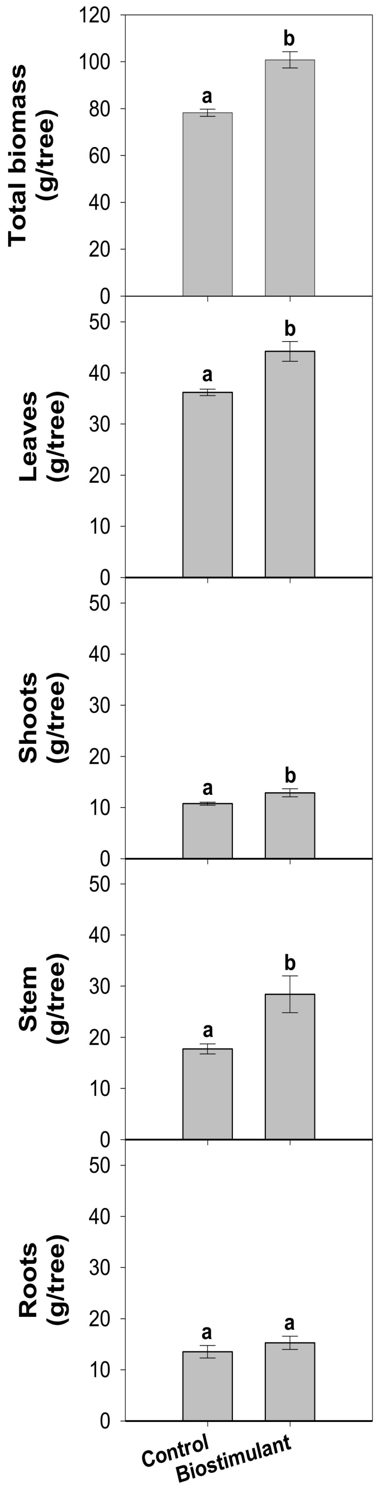

Figure 3. Total biomass (dry weight) at the end of the vegetative season and of the different components, such as roots, stem, leaves, and branches, of pot treated and control trees. Bars represent the standard error. Means accompanied by different letters are significantly different at $p < 0.05$.

3.2. Application of the Biostimulant to Young Olive Trees in the Field

Treated trees had greater trunk growth with respect to the control (Figure 4). Differences between treated trees and the control increased throughout the season (Figure 4). The trunk cross section area of control trees increased by about 470 mm^2, whereas treated trees increased by about 565 mm^2.

This indicates that the addition of the biostimulant to fertigation resulted in a 20% increase in the trunk cross section area increment with respect to the control.

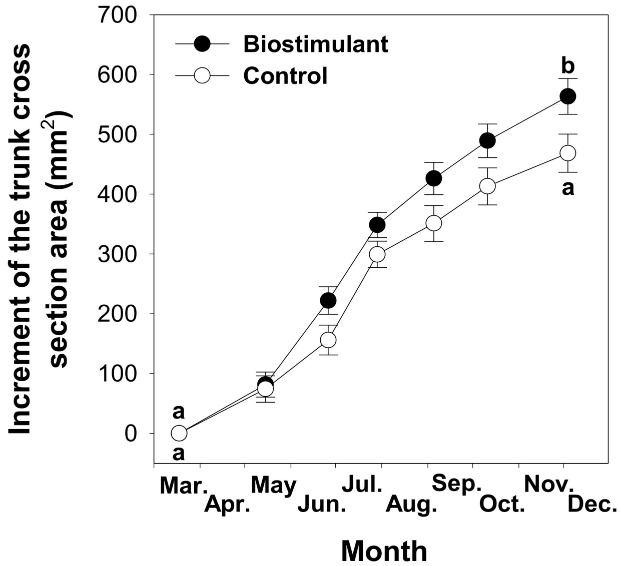

Figure 4. Increment of growth of the trunk cross section area of treated and control trees in the field during the vegetative season of 2019. Bars represent standard errors. At the beginning and at the end of the experiment, means accompanied by different letters are significantly different at $p < 0.05$.

The yield of treated trees, even though the difference was not statistically significant, tended to be higher than that of the control, both when expressed in terms of olives/tree and, especially, when expressed as oil/tree (Table 1). Treated trees also tended to have a higher yield efficiency when yield was expressed as oil (Table 1). Fresh weight, pigmentation index and oil content of fruits were substantially similar in treated and control trees (Table 2).

Table 1. Tree yield and yield efficiency of young treated and control trees in the field.

Treatment	Yield (g olives/tree)	Yield (g oil/tree)	Yield Efficiency (g olives/mm^2)	Yield Efficiency (g oil/mm^2)
Control	514 a	123 a	0.52 a	0.124 a
Biostimulant	636 a	165 a	0.56 a	0.146 a

In each column, means followed by the same letter are not significantly different at $p < 0.05$.

Table 2. Fruit characteristics at harvest of young treated and control trees in the field.

Treatment	Fresh Weight (g)	Pigmentation Index (0–7)	Water Content (%)	Oil Content (% F.W.)	Oil Content (% D.W.)
Control	2.2 a	4.6 a	53.2 a	24.1 a	51.5 a
Biostimulant	2.3 a	4.3 a	52.1 a	26.1 a	54.5 a

In each column, means followed by the same letter are not significantly different at $p < 0.05$.

4. Discussion

The results of both experiments showed a significant effect of the biostimulant in promoting the growth of young olive trees. Although the biostimulant was applied at the beginning of the vegetative season (spring), the effects lasted until the end of the growing season (autumn) (Figures 1 and 4).

The fact that the control received the same amount of nitrogen as the treated trees, demonstrates that the biostimulant had the ability to stimulate tree growth which was independent of (and in addition to) a mere effect of nitrogen fertilization. In fact, the positive effects were obtained with small amounts of biostimulant per tree. These amounts were about 30 g/tree in the pot experiment and 75 g/tree in the field experiment, corresponding to a nitrogen supply of 2.67 g/tree and 6.7 g/tree, respectively. In the latter case the amount of nitrogen was only about 4% of the total nitrogen applied (160 g/tree), indicating that the impact in terms of nitrogen supply was negligible. This result is in line with the first definition of biostimulant by Kauffman et al. in 2007 [21] as "materials, other than fertilisers, that promote plant growth when applied in low quantities".

The biostimulant used consisted of a complex of natural amino acids (such as glycine, proline, and hydroxyproline) of animal origin. These substances have been reported to have a hormone-like action on plant physiology and positive effects on plant metabolism during possible stress conditions [1,22]. Our results support such positive action on the physiology of olive trees. However, the precise mechanisms of these actions remain unclear.

In the field investigation, trees also produced fruit. Reproductive growth proportionally reduces vegetative growth in both young [15,23] and mature [24] olive trees. Indeed, it is a high assimilate demanding activity [25]. Therefore, the greater vegetative growth with the biostimulant could have been due to reduced flowering and/or fruit set and growth. On the contrary, the greater tree growth of treated trees was accompanied by a tendency of higher reproductive growth, especially when expressed in terms of oil production. This implies that the biostimulant induced greater growth directly and not indirectly via increased partitioning towards vegetative growth. The positive effects on vegetative growth are in agreement with those obtained by Saour [9], who used another kind of biostimulant than was used in this study. Also, the lack of negative effects on reproductive growth is in line with studies showing positive effects of biostimulants on olive yield and fruit oil content [7,10]. In general, positive effects of biostimulants on growth and yield (quantity and quality) have also been obtained in many other agricultural and horticultural crops [3–6,26–34].

The direct positive effect of the biostimulant on plant growth appeared to be related to increased leaf photosynthesis. In broccoli, the use of biostimulants increased stomatal conductance and the photosynthetic rate [35]. Increased photosynthesis was associated with increased stomatal conductance as is usually the case [36–38]. However, despite higher stomatal conductance, intercellular CO_2 concentration (Ci) was lower, suggesting that the biostimulant enhanced photosynthesis directly and not indirectly via increased stomatal aperture (otherwise Ci would have been higher). Therefore, it appears that the biostimulant acted directly on the photosynthetic biochemistry, similar to what a nitrogen fertilization does [39]. However, considering that the amount of nitrogen was the same in treated and control trees, the stimulation of photosynthesis must have been related to mechanisms different from a mere nitrogen supply, unless the different forms of nitrogen (i.e., complex of natural amino acids) were used by the plant with much greater efficiency. For example, by more efficient uptake by the roots, or to a reduced metabolic cost for the plant when using such forms. These aspects need further investigations.

By the end of the growing season, the greater growth of trees treated with the biostimulant was likely due to the fact that larger trees intercept more light, and thus increase their source activity. Once the trees treated with the biostimulant become larger than control trees (i.e., due to enhanced photosynthesis) and develop greater leaf area, they intercept more light than control trees, thus increasing their source activity and resulting in greater growth. Additionally, greater vegetative and reproductive growths imply stronger sink activity, which in turns could stimulate photosynthesis. Indeed, in several studies, treatments that created conditions of a higher sink/source ratio also induced higher net photosynthesis [15,40,41]. All of this might explain sustained greater tree growth even well after (i.e., in the autumn) the time when the biostimulant was applied (spring) or the time when photosynthesis equalized between treatments (August). Therefore, the biostimulant probably had an immediate and lasting direct effect on photosynthetic rates, resulting in an increasingly larger canopy, which in turn intercepted more light, further increasing growth compared to the control.

5. Conclusions

The results indicate the possibility of using biostimulants based on complexes of natural amino acids to promote the growth of young olive trees. The biostimulant used in this paper increased the growth of young olive trees. It acted by stimulating physiological processes, that is, by improving assimilate production through photosynthesis.

The results point out that the biostimulant used may be utilized both in nurseries and in young olive orchards to promote tree growth. This is very important, because speeding up the development of young plants makes it possible to reduce the time spent in nurseries and that needed in the field to reach the adult phase and, hence, full production. This is particularly important in olive, as this time can be particularly long.

The positive effects on both vegetative and reproductive activities shown in this study also suggest a potential positive use on adult trees, where it is essential to maintain a balance between these two activities.

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