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# Use of an Organic Fertilizer Also Having a Biostimulant Action to Promote the Growth of Young Olive Trees

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**Abstract:** In 2019–2020, trials were carried out with the aim of evaluating the possibilities of using an organic fertilizer, also having a biostimulant action, for promoting the growth of young olive trees. The experiments were implemented using both potted and field-grown trees. The effects of the organic fertilizer were evaluated with respect to trees treated with a chemical fertilizer; for potted trees a control without any fertilization was also used. Therefore, the compared treatments were: organic fertilization vs. chemical fertilization or no fertilization in the experiment with potted trees; organic fertilization vs. chemical fertilization in the experiment with field-grown trees. Non-fertilized potted trees had the lowest growth. The organic fertilizer, with respect to the chemical one, determined higher growth in both potted and field-grown trees (+22–29% of the increment of the trunk cross sectional area). In potted trees, it determined a rapid elongation of the stem (+30% of the increment of tree height with respect to chemically fertilized trees) and this likely favored the development of a higher number of leaves, which, together with an increase in their photosynthetic activity (in August, +27% with respect to chemical fertilized trees), created conditions for higher assimilate production and in turn greater tree growth. Trees in the field showed that the greater growth was not obtained at the expenses of reproductive growth, which increased to the same degree as the vegetative growth. Overall, the results support the biostimulant action of the organic fertilizer and indicate the possibility of its use to improve the growth of young olive trees.

**Keywords:** biostimulants; *Olea europaea* L.; organic fertilizer; photosynthesis; plant nutrition



**Citation:** Mazeh, M.; Almadi, L.; Paoletti, A.; Cinosi, N.; Daher, E.; Tucci, M.; Lodolini, E.M.; Rosati, A.; Famiani, F. Use of an Organic Fertilizer Also Having a Biostimulant Action to Promote the Growth of Young Olive Trees. *Agriculture* **2021**, *11*, 593. https://doi.org/10.3390/agriculture11070593

Academic Editor: Claudio Marzadori

Received: 1 June 2021
Accepted: 15 June 2021
Published: 26 June 2021



## 1. Introduction

Fertilization is an important cultural practice with the aim of creating and maintaining optimal soil fertility, in order to ensure both good vegetative growth and constant and high yields [1,2]. Depending on the fertilizer used, this practice can positively affect all or part of the characteristics of soil fertility. Amendments, such as manure or composts, are supplied in large quantities (the concentration of nutrients is low) and have positive effects on the physical, chemical and biological characteristics of the soil [2]. Only in particular situations can these amendments cause negative effects, such as addition of heavy metals and nitrate leaching [3]; however, these inconveniences can be avoided with accurate choice and correct use of amendments. Chemical fertilizers and concentrated organic fertilizers, which have a relatively high concentration of nutrients, only affect the chemical aspects of fertility, by ensuring the availability of necessary nutrients to plants, for optimum vegetative and reproductive growth [4].

Both organic and chemical fertilizers benefit olive (*Olea europaea* L.) trees. Chemical fertilizers are usually cheaper, contain higher amounts of nutrients, and are less bulky than organic fertilizers [5]. Concentrated organic fertilizers, with respect to chemical ones, ensure slow release of nutrients that provides a more efficient supply to trees throughout the entire vegetative season [4]. Both chemical and concentrated organic fertilizers are easy to apply. Chemical fertilizers are not allowed in organic farming.

The three most important nutrients for plants, and olive is not an exception, are nitrogen, phosphorus, and potassium [2]. Among them, nitrogen produces the most obvious effects on both vegetative and reproductive growth as well as on assimilate production (photosynthesis) [6].

Several kinds of fertilizers, both chemical and organic, can be found on the market. Comparative evaluations about their effectiveness and how they can be best used in different cultivation situations could be beneficial. However, their comparison is particularly difficult because their compositions always differ. An acceptable comparison could be made by ensuring a similar supply of the nutrient that has the greatest impact on the plant: nitrogen.

In recent years, there has been increasing interest in the use of biostimulants, which have been defined as any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance, and/or crop quality traits, regardless of its nutrient content [7].

Several physiological and molecular mechanisms are reported to be involved in the biostimulant action of these products, but beneficial effects on the plant cannot be generalized for all crops [8] so that methods, time, and rate of application must be determined for each species, also considering the different cultural and environmental conditions.

Very few studies have been carried out to evaluate the possibilities of using concentrated organic fertilizers and biostimulants on olive to increase vegetative and/or reproductive growth and/or olive oil quality. Molina Soria [9] reported no significant effects of biostimulants on the growth of young olive trees, while Saour [10] found a positive effect on the growth of young plants of a combination of biostimulant/kaolin particle film and Porras-Soriano et al. [11] reported that inoculating olive plantlets with mycorrhizal fungi increased plant growth and their ability to uptake nitrogen, phosphorus, and potassium from both non-saline and saline media. Moreover, Almadi et al. [12] found that a biostimulant based on complexes of natural amino acids was able to promote olive tree growth, and Roussos et al. [13] reported that the addition of organic fertilizer to inorganic fertilizer led to greater canopy density and higher yield. Trials carried out with adult olive trees showed that biostimulants can improve fruit characteristics and yield [14–17]. The effects of biostimulants on olive oil quality depended on the biostimulant used [14,16,18,19], and in some cases, no effects were observed [17]. Recently, a study found that a biostimulant could be used to increase the resistance of olive plants to severe salt stress conditions [20].

Increasing the rate of growth of young olive trees is a very important goal for both nurseries and olive farms. In nurseries, this would reduce the time required to obtain ready-for-transplanting plantlets. In farms, it would reduce the time required for trees to reach full maturity and bear fruit production. Both objectives are important because of the increasing demand for olive oil on a worldwide scale. Furthermore, the large number of old traditional orchards needing renovation or replacement has stimulated the establishment of new olive orchards to increase olive oil production.

The aim of this study was to evaluate the possibilities of using an organic fertilizer, also reported by the producer to have a biostimulant effect, for promoting the growth of young olive trees. This was done using both potted and field trees. In the former, the effects on some physiological parameters were also evaluated, while in the latter effects on reproductive activity were also assessed.

## 2. Materials and Methods

In order to evaluate the possible biostimulant effects of the organic fertilizer, and distinguish it from the mere nutritional effect, the chemical control treatments were supplied with the same amount of nitrogen as the organic treatments. Nitrogen form and timing were also planned to replicate, as much as possible, the same conditions as for the organic fertilizer. As detailed in the discussion section, this allows to estimate/evaluate the possible additional biostimulant effect of the organic fertilizer.

### *2.1. Application of the Organic Fertilizer/Biostimulant to Young Potted Olive Trees*

The experiment was carried out in 2019, by using 1-year-old potted olive trees of the cultivar Leccino. The substrate in the pots was constituted by 2/3 of pozzolan ($v/v$) and 1/3 ($v/v$) of peat. Trees were kept outdoors under natural conditions. Pots were drip-irrigated to ensure optimal water availability during the experimental period. An organic fertilizer (Grena olivo special, GRENA, San Bonifacio Verona, Italy), also reported by the producer to have a biostimulant effect, containing 9% nitrogen, was applied to the substrate in each pot at the dosage of 60 g per tree (=5.4 g of nitrogen per tree). Seven percent of the nitrogen contained in the organic fertilizer is in the ammoniacal form; the remaining 2% is in an organic form.

Moreover, a chemical fertilizer, namely urea, was supplied at each pot at weekly intervals with fertigation from May to September, at a dosage that ensured an amount of total urea nitrogen equal to that provided with the organic fertilizer: 21 administrations with 0.559 g of urea per tree, solubilized in 300 mL of water at each time, for a total of 11.74 g of urea per tree in the period May-September, which is equal to 5.4 g of nitrogen per tree (urea fertilizer contains 46% of nitrogen, all in the ureic form).

In this way, the supplied nitrogen was the same for the two treatments.

Unfertilized trees were used as general control.

For each treatment, six trees were used as replicates. Tree growth was monitored by measuring stem diameter, 5 cm above the collar, and total tree height, periodically, from the beginning (May) to the end of the experiment (November).

In August, photosynthetic rates were measured on fully developed leaves, using the ADC-LCA3 device (Analytical Development Co., Hoddesdon, Herts, UK).

### *2.2. Application of the Organic Fertilizer/Biostimulant to Young Olive Trees in the Field*

The experiment was carried out in 2020, in a young olive orchard with 3-year-old trees of the cultivar Moraiolo. Trees were spaced m 6 × 5. The orchard has drip irrigation (2 emitters per tree, each supplying 4 L of water $h^{-1}$). The orchard is located in central Italy, near Spello (PG).

At the beginning of March, trees were fertilized by spreading the same organic fertilizer/biostimulant as for the potted experiment (Grena olivo special, GRENA, San Bonifacio Verona, Italy). The fertilizer was applied under the drip emitters, close to the trunk, at the dosage of 3 kg per tree (=270 g of nitrogen per tree).

Trees fertilized with chemical fertilizers according to the practices normally used for commercial olive growing in the area were used as control. At the beginning of April, 0.82 kg per tree of Nitrophoska Special 12-12-17 (=98 g of nitrogen, 98 g of phosphorous and 139 g of potassium per tree), was applied. Moreover, trees received 150 + 94 g per tree of urea distributed on the soil at mid-April and mid June, and a total of 130 g per tree of urea by fertigation, which was applied every 7–14 days, from the beginning of May to the end of June, for a total of 270 g of nitrogen per tree.

Both treated and control trees received the same amount of total nitrogen.

For each treatment, three replicates of 10 trees each were used.

The growth of the trees was recorded by measuring, periodically, every 1–1.5 months, the diameter of the trunk. Olive yield was determined at harvest, in December. At harvest, one hundred olives per replicate were used to determine fruit fresh and dry weights, by weighing them before and after drying at 105 °C in a ventilated oven. The same olives, before drying them, were used to determine the level of pigmentation, by using the "Jaèn pigmentation index", which ranges from 0 to 7, with 0 corresponding to green olives and 7 corresponding to olives with superficial pigmentation on 100% of the epicarp and 100% pigmentation of the pulp [21,22]. Pulp (epicarp + mesocarp) firmness was determined with a hand-held dynamometer with a 1 mm plunger (Effe.gi, Ravenna, Italy) on samples of 100 olives per replicate. Oil content was determined with an InfraAlyzer apparatus (SpectraAlyzerZeutec BRAN + LUEBBE, Rendsburg, Germany), using one sample of olives per replicate, and expressed on both FW (fresh weight) and DW (dry weight) basis.

The yield efficiency of the trees was calculated as the ratio between the yield (g per tree) and the trunk cross sectional area (g of olives or oil $mm^{-2}$ of trunk cross sectional area) or the increment of trunk cross sectional area in 2020 season.

### 2.3. Statistical Analysis

Data are presented as means ± standard error or were statistically analyzed by ANOVA according to a completely randomized design and the averages were compared by using the Student–Newman–Keuls Test. The kind of treatment, with no fertilization and chemical or organic fertilization in the experiment with potted trees, and with chemical or organic fertilization in the experiment with field grown trees, was the predictive variable. Parameters reported in Figure 3 were the response variables for the experiment with potted trees and those reported in Tables 1 and 2 were the response variables for the experiment in the field.

## 3. Results

### 3.1. Application of the Organic Fertilizer/Biostimulant to Young Potted Olive Trees

Both chemical and organic fertilization significantly increased the growth of the trees with respect to the control (unfertilized trees) (Figures 1 and 2). The organic fertilizer determined a higher growth than the chemical one, by determining a higher increment of both the trunk cross sectional area (+22%) and tree height (+30%) (Figures 1 and 2). Differences between the chemically treated trees and the organically treated ones became evident about two months after the beginning of the experiment in terms of increase in height of the stem, and about three months later in terms of increment in stem diameter.

In August, the leaves of trees treated with organic fertilizer showed a higher photosynthesis than those of trees treated with chemical fertilizer and trees used as control (Figure 3).

### 3.2. Application of the Organic Fertilizer/Biostimulant to Young Olive Trees in the Field

Organic fertilized trees had greater trunk growth than the control (Figure 4). Differences became evident in the summer (July) and then increased up to the end of the season, when the increment of the trunk cross sectional area of treated trees was about 29% greater than that of the control.

Although not statistically significant, the yield of treated trees (kg of olives or oil per tree) was higher than that of control trees (Table 1). Treated and control trees had a similar yield efficiency, expressed as the ratio between the yield and the trunk cross sectional area at the end of the 2020 season, in terms of both olives and oil (Table 1). The yield efficiency was still similar for the different treatments when expressed as the ratio between the yield and the increase in the trunk cross sectional area obtained in the 2020 season.

Fruit characteristics, that is fresh weight, pigmentation index, flesh firmness and oil and water contents, were not significantly different between treated and control trees (Table 2).

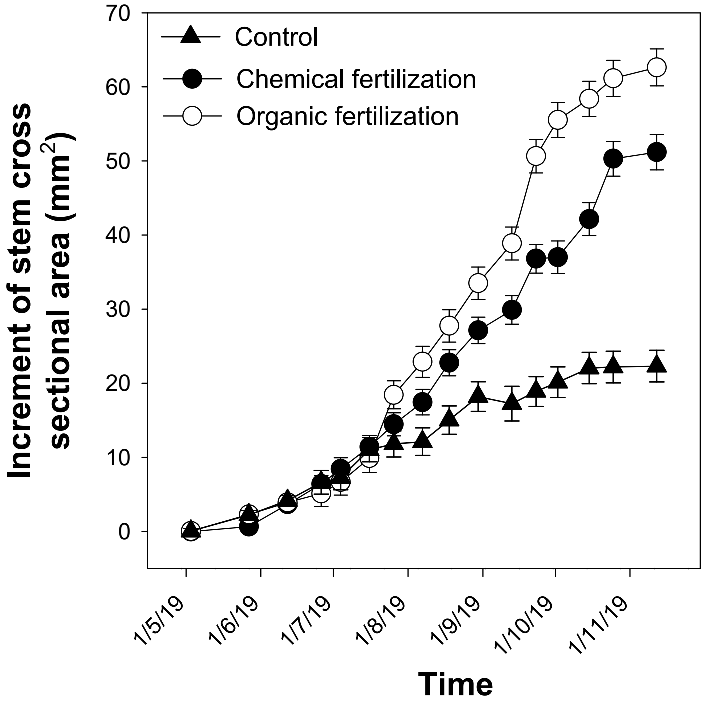


**Figure 1.** Increment of stem cross sectional area of chemically or organically fertilized potted trees and of unfertilized ones (control) during the vegetative season of 2019. Bars represent standard errors ($n = 6$).

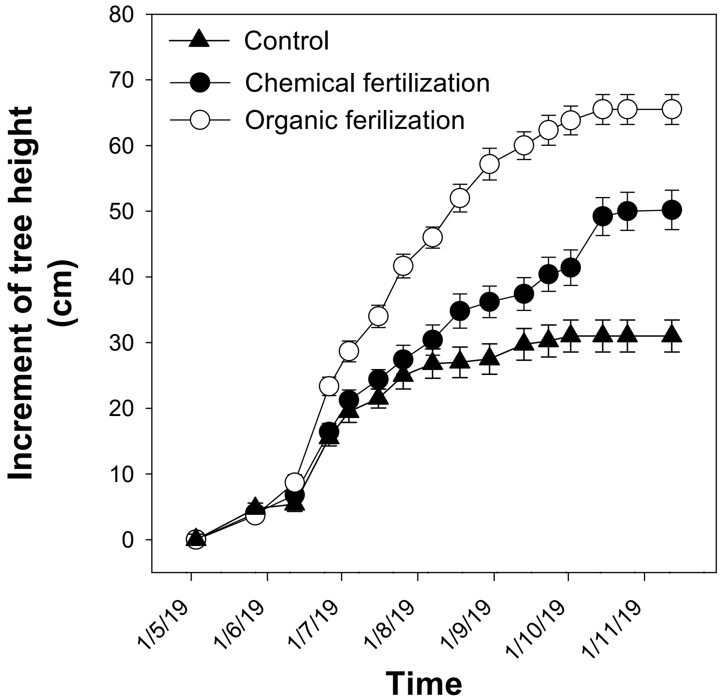


**Figure 2.** Increase in height of chemically or organically fertilized potted trees and of unfertilized ones (control) during the vegetative season of 2019. Bars represent standard errors ($n = 6$).

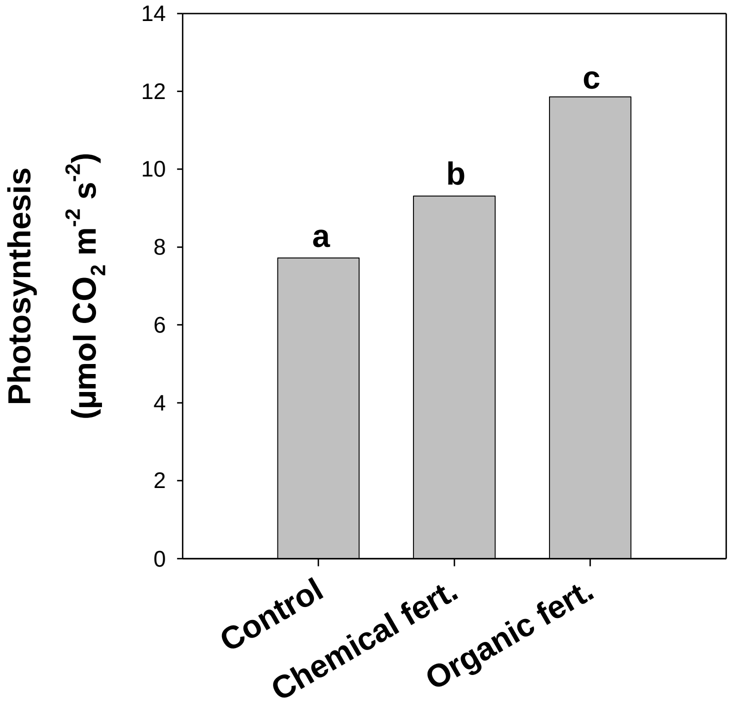


**Figure 3.** Photosynthetic activity of leaves of chemically or organically fertilized potted trees and of unfertilized ones (control) measured in August 2019. Means accompanied by different letters are significantly different at $p < 0.05$ ($n = 6$).

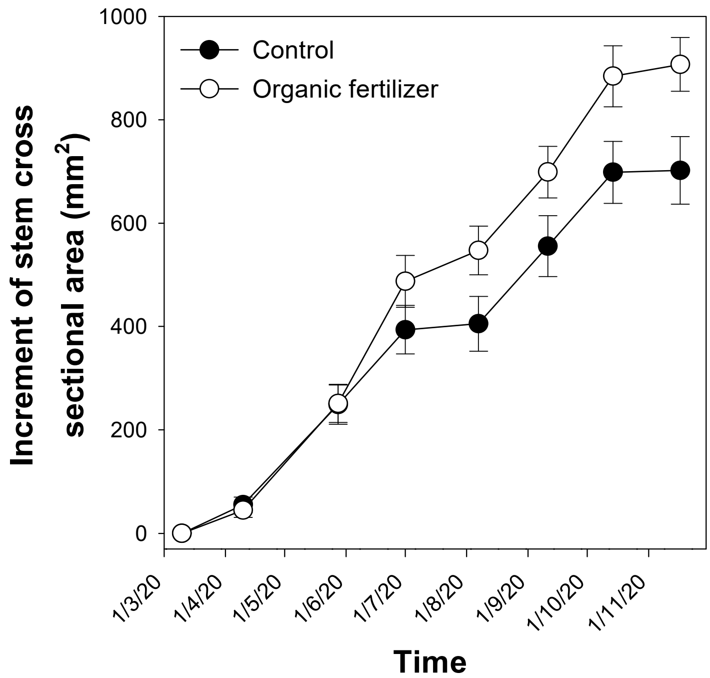


**Figure 4.** Increment of the trunk cross sectional area of organically fertilized trees (organic fertilizer) and of chemically fertilized ones (control) in the field during the vegetative season of 2020. Bars represent standard errors ($n = 3$).

**Table 1.** Tree yield and yield efficiency of treated (organic fertilizer) and control (chemical fertilizer) trees in the field [1].

| Treatment | Yield (kg Olives Tree$^{-1}$) | Yield (kg Oil Tree$^{-1}$) | Yield Efficiency [2] (g Olives mm$^{-2}$) | Yield Efficiency [2] (g Oil mm$^{-2}$) | Yield Efficiency [3] (g Olives mm$^{-2}$) | Yield Efficiency [3] (g Oil mm$^{-2}$) |
|---|---|---|---|---|---|---|
| Control | 3.80 a | 0.66 a | 2.19 a | 0.38 a | 5.24 a | 0.95 a |
| Organic fertilizer | 4.63 a | 0.78 a | 2.32 a | 0.39 a | 5.44 a | 0.88 a |

[1] In each column, means followed by the same letter are not significantly different at $p < 0.05$. [2] Expressed as the ratio between the yield and the trunk cross sectional area at the end of 2020 season. [3] Expressed as the ratio between the yield and the increment of the trunk cross sectional area obtained in the 2020 season.

**Table 2.** Fruit characteristics at harvest of treated (organic fertilizer) and control (chemical fertilizer) trees in the field [1].

| Treatment | Fresh Weight (g) | Pigmentation Index (0–7) | Flesh Firmness (g) | Water Content (%) | Oil Content (% F.W. [2]) | Oil Content (% D.W. [2]) |
|---|---|---|---|---|---|---|
| Control | 1.99 a | 4.01 a | 181.7 a | 59.9 a | 17.5 a | 43.5 a |
| Organic fertilizer | 1.95 a | 3.51 a | 211.3 a | 62.2 a | 16.8 a | 44.4 a |

[1] In each column, means followed by the same letter are not significantly different at $p < 0.05$. [2] F.W = fresh weight; D.W. = dry weight.

## 4. Discussion

Both experiments showed significant positive effects of the organic fertilizer on the growth of young olive trees.

There is abundant evidence that nitrogen can greatly increase the vegetative and reproductive activity of olive trees [2]. Recent studies have shown a robust positive response of olive tree performance to nitrogen fertilization [23–25]. Since chemically and organically fertilized trees received the same amount of nitrogen, the greater growth of organically treated trees must have been due to the form of nitrogen applied and/or the biostimulant action.

The organic fertilizer contained 9% nitrogen, 7% of which was in the ammoniacal form, and the remaining 2% as the organic form. In the soil solution, nitrogen is primarily present as nitrate and ammonium ions [5]. Ammonium has a positive charge, and this allows it to be retained in the negatively charged clay minerals and organic matter in the soil. It is then gradually transformed into the nitric form, which is the form most absorbed by plants. Nitrate is very soluble and can easily migrate to the roots through the soil solution, but it can also be easily leached [5]. Nitrogen in organic fertilizers is tied up in proteins/amino acids, which in the soil are broken down by microbes to form nitrate and ammonium which are absorbed by the roots and translocated into the plant [4]. When nitrogen is supplied as urea, it must be transformed into the ammoniacal form and then into the nitric one to be taken up by plants. Because urea is very soluble, it is subjected to leaching before being transformed to ammoniacal nitrogen. The organic fertilizer used, containing ammoniacal and organic forms of nitrogen was most likely able to ensure a slow and continuous release of nitrogen, that is, an efficient nitrogen nutrition to olive trees. However, urea is also a long-lasting fertilizer, because when transformed to the ammoniacal form it is retained by the soil. Moreover, in both experiments it was applied gradually through fertigation, which minimizes the loss of nitrogen by leaching, because the trees can absorb it as needed [26]. Moreover, in the experiment in the field, nitrogen was applied gradually also on the soil. Therefore, both fertilization treatments (chemical and organic ones) ensured an efficient supply of nitrogen.

The organic fertilizer also contained 1% phosphorous (P) and 1% potassium (K), but the amounts of these nutrients supplied to the trees was lower than that supplied in the control, in the field, with Nitrophoska (organic fertilizer 30 g per tree of both phosphorus and potassium; Nitrophoska 98 g per tree of phosphorous and 139 g per tree of potassium). Therefore, it is unlikely that the positive effects of the organic fertilizer on tree activity were due to P and K. Moreover, the organic fertilizer also contained micronutrients, such as boron, iron, manganese, copper and zinc, which could have contributed to its effects. However, they usually do not produce showy increases in plant growth.

The organic fertilizer has also been reported to have a biostimulant action due to the high content of amino acids and protein and humic and fulvic acids. Indeed, amino acids and peptide mixtures, like the ones in the organic fertilizer can play multiple roles as biostimulants on plant growth [7]. Effects include modulation of N uptake and assimilation, hormonal-like activities, chelating, and complexing activities able to improve the availability and uptake of nutrients by roots, antioxidant activity and mitigation of environmental stress [6]. In addition, also the content of humic substances, such as humic and fulvic acids, may confer a biostimulant action [7]. It has been reported that most biostimulant effects of humic substances refer to the improvement of root nutrition, through different mechanisms: increased uptake of macro- and micronutrients, hormonal effects and stress protection [7]. These effects of biostimulants produce changes on the physiology of the plants, such as, among others, an improvement of photosynthetic activity [8]. This agrees with the highest values of photosynthesis showed by trees treated with the organic fertilizer in our study. Similar results were also observed in another study on young olive trees where protein hydrolysates were used [12]. In addition, biostimulants determined higher values of photosynthesis also in broccoli [27].

By considering the significant increases in plant growth determined by the organic fertilizer with respect to the chemical one, both in potted and field-grown trees, its nutritional characteristics described above in comparison with fertilization with Nitrophoska and urea, and the positive effects on photosynthesis, it seems very likely that a significant part of the effects determined by the organic fertilizer was due to its biostimulant properties.

In the field, where trees also produced fruit, the greater tree growth was not obtained at the expense of reproductive growth, which also tended to be enhanced, both in terms of olives and oil. In this regard, it is important to note that the competition between vegetative and reproductive activities causes reproductive growth to be negatively related to vegetative growth. This has been observed in both young and adult olive trees [28–31], and it is due to the fact that reproductive growth is a very expensive process [32,33]. However, our results indicate that the organic fertilizer had a direct, positive effect on tree growth; that is, its action was not due to an increased partitioning of assimilates to the vegetative growth at the expenses of the reproductive one (fruits). This was also demonstrated by the fact that, with respect to the control, the application of the organic fertilizer did not alter the ratio between yield and the increment of the trunk cross sectional area (Table 1), indicating that both activities increased to the same degree.

The positive effects of the organic fertilizer/biostimulant on tree growth reported in our experiments are in line with those obtained in young olive trees with biostimulants by Saour [10] and, more recently, by Almadi et al. [12]. In addition, also the good effects on reproductive growth of the trees are consistent with other studies where positive effects of biostimulants on olive/oil yields were observed [12,14–16]. With the use of biostimulants, good results, in terms of plant growth and yield, have been also shown in other cultivated species [34–40].

Altogether, the results indicate that the treatment with organic fertilizer/biostimulant created conditions for greater vegetative growth. This was likely mediated by the increase in photosynthetic activity of leaves. As a result of this, the trees treated with the organic fertilizer/biostimulant developed a larger canopy which was able to produce a larger amount of assimilates, further increasing tree growth. It is also important to consider that higher growth determines a higher sink/source ratio which stimulates greater photosynthesis [41,42], creating a virtuous mechanism. Similar general results in olive were also obtained by using a biostimulant based on protein hydrolysates by Almadi et al. [12].

## 5. Conclusions

The organic fertilizer was very effective in promoting the growth of both potted and field-grown young olive trees, determining greater growth than urea, most likely also due to its biostimulant action.

Treatments with the organic fertilizer/biostimulant determined a rapid elongation of the stem of the trees, and this likely favored the development of a larger number of leaves. This, together with the fact that it appeared to improve the photosynthetic activity of the leaves, created better conditions for higher assimilate production and in turn greater tree growth.

The experiment in the field showed that the greater growth determined by the organic fertilizer/biostimulant was not obtained at the expenses of the reproductive activity, which increased to the same extent as the vegetative growth.

Overall, the results indicate the possibility of using the organic fertilizer/biostimulant to improve the growth of young olive trees, both in the nursery and in the field, resulting in an improvement of assimilate production and, consequently, tree growth.

**Author Contributions:** Conceptualization, F.F., M.M., L.A., A.P. and M.T.; methodology, all authors; observations and analysis, M.M., L.A., A.P., N.C. and E.D.; validation, all authors; data curation M.M., L.A. and A.P.; interpretation of results, all authors; writing—original draft preparation, F.F., M.M., L.A., A.P. and M.T.; critical revision of the work, N.C., E.D., E.M.L. and A.R.; writing—review and editing, F.F. and M.M.; supervision, F.F.; funding acquisition, F.F.; final approval, all authors. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Grena s.r.l., Porcilana, San Bonifacio (VR), Italy, as part of an agreement with the "Dipartimento di Scienze Agrarie, Alimentari e Ambientali-Università degli Studi di Perugia—Italy" for carrying out research activities for the definition of sustainable and innovative nutritional protocols able to favor the growth of young olive trees and the vegetative-reproductive activities of mature olive trees (Title of the research: "Sviluppo di schemi innovativi per l'uso di fertilizzanti organici e biostimolanti in olivicoltura").

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Acknowledgments:** This paper represents the publication of a part of the thesis "Use of biostimulants to promote growth of young olive trees" of Mona Mazeh, CIHEAM Bari, Collection "Master of Science. Mediterranean Organic Agriculture", n. 910, 2020.

**Conflicts of Interest:** The authors declare no conflict of interest. The funder had no role in the design of the study, in the collection, analyses, and interpretation of data and in the writing of the manuscript and agreed to publish the results of the research.

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