Agronomy Research **19**(3), 1396–1405, 2021
https://doi.org/10.15159/AR.21.143

Slow-release fertilization and *Trichoderma harzianum*-based biostimulant for the nursery production of young olive trees (*Olea Europaea* L.)

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Received: May 8th, 2021; Accepted: July 30th, 2021; Published: October 5th, 2021

Abstract. Valorization of local olive cultivars is a key factor for the medium-term development strategy of the Italian olive agroindustry. This involves enhancements in both, cultural practices and nursery techniques. The aim of this research was the evaluation in nursery, on vegetative growth and root development of young olive plants, of two different treatments: substrate enrichment with Osmocote®, a slow-release fertilizer, and Trianum-P a *Trichoderma harzianum* based biostimulant applied as foliar spray. The trial was carried out on Rotondella and Salella, two autochthonous olive cultivars from the Campania region (southern Italy). Central axis height, number and length of lateral shoots, leaf number and trunk base diameter were monitored during the experiment. Eight months later, all plants were uprooted, and further parameters were measured: total leaf area, trunk cross sectional area (TCSA), fresh and dry weight of the leaves, shoots, trunk, roots, and total dry matter. The canopy/root ratio (C/R) was also determined. Overall, the Osmocote® treatment caused a significant increase in the vegetative growth parameters for both cultivars, with a more evident effect on the development of the canopy organs. The observation reported in the present work can represent a convenient piece of information particularly in relation to stress resilience in nursery production.

Key words: germplasm, nursery, Osmocote, *Trichoderma harzianum,* plant biostimulants.

INTRODUCTION

According to recent estimates, 95% of the olive trees cultivated worldwide are concentrated in the Mediterranean area (Catalano et al., 2019). Italy ranks second as the world largest producer of olives and exporter of olive oil, after Spain (FAOSTAT, 2010–2018). On the other hand, in terms of quality, Italy ranks first with 47 certified geographical indication (PDO - Protected Designation of Origin or PGI - Protected Geographical Indication). Furthermore, Italian extra virgin olive oil represent 40% of the certified quality labels produced in Europe (Sarnari, 2020).

Along with olive oil worldwide demand continuously increasing, the medium-term growth strategy for the Italian olive agroindustry will be based on the pursuing the constant enhancement of the products quality (Sarnari, 2020). This will stimulate olive grower to move toward modern production systems, improved pest management and updated cultural practices with a focus on the exploitation of the abundant patrimony of local genetic resources estimated to include more than 800 cultivars (Muzzalupo, 2012).

Olive cultivars neglected, maybe because undesirable characteristics such as low vigor, early and abundant production, high branching, resistance to low temperatures or to heat waves, salinity tolerance, adaptability to low pruning systems, etc., may play today a crucial role in matter of biodiversity, to meet the challenges of modern olive growing, improving olive and olive oil quality (Muzzalupo, 2012; Rosati et al., 2018a and 2018b). Within this framework, nurseries are the starting point for the propagation of high-quality olive plants, making use of the rich genetic diversity of the Italian olive germplasm. The development of innovative nursery technologies aimed to better support plant growth in the nursery and/or to improve recovery from the transplant shock when the young plants are transferred to the field, should be regarded as an important research approach in this sector.

In this context, a great interest is addressed to the use of innovative sustainable practices of plant raising. In the last decades, there has been an increasing attention for a more sustainable agriculture which could increase yields and product quality, while reducing the negative impact of agrochemicals on the environment (Ventorino et al., 2016; Almadi et al., 2020; Parfeniuk et al., 2020). All these points may be fostered using plant biostimulants (PB) (Ventorino et al., 2013; López-Bucio et al., 2015; Parillo et al., 2017). Plant biostimulants are defined as 'materials, other than fertilizers, that promote plant growth when applied in low quantities' (du Jardin, 2015; Rouphael & Colla, 2020). According to this definition, different types of PBs have constantly played an important role in agriculture: to cite just a few examples, mycorrhizal soil fungi that stablish symbiotic associations with most crop plants improving plant performance and soil health (Loit et al., 2018), nitrogen fixing rhizobia or humic substances have long been recognized as essential contributors to soil fertility (du Jardin, 2015). More recently, a number of biotic (Briccoli et al., 2009; Tataranni et al., 2009) or abiotic (Molina Soria, 2006) biostimulants have been reported to promote plant growth and defense against pathogens throughout the whole life cycle, from seed germination to plant maturity (Krouk et al., 2011) as well as increasing nutrient use efficiency and opening new routes of nutrients acquisition by plants therefore acting as biofertilizers (du Jardin, 2015).

Among the different categories of PBs, *Trichoderma* spp. are widely studied fungi and commonly used in agriculture as biological control agents (Harman et al., 2004; Prisa et al., 2013, Poveda & Baptista, 2021). *Trichoderma*-based preparations are marketed worldwide and utilized in crop protection against various plant pathogens (Woo et al., 2014). Moreover, *Trichoderma* spp. are known to improve plant resistance to environmental stresses such as salinity and drought by increasing the branching capacity of the root system, thus improving nutrient status and water acquisition (López-Bucio et al., 2015). The most common methods of application of *Trichoderma* are soil and foliar application. Soil is a repertoire of both beneficial and pathogenic microbes.

Delivering of *Trichoderma* spp. to soil will increase the population dynamics of augmented fungal antagonists and thereby would suppress the establishment of pathogenic microbes onto the infection court, this is the most effective method of application of *Trichoderma* particularly for the management of soil-borne diseases. The efficacy of biocontrol agents for foliar diseases is greatly affected by the fluctuation of microclimate. Phyllosphere is subjected to diurnal and nocturnal, cyclic and non-cyclic variation in temperature, relative humidity, dew, rain, wind and radiation. Hence, water potential of phylloplane microbes will be varying constantly. It will also vary between leaves or the periphery of the canopy and on sheltered leaves. Higher relative humidity could be observed in the shaded, dense region of the plant than that of peripheral leaves. Though foliar application of *Trichoderma* reduces the severity of diseases under field conditions, it is not technically feasible due to increased dosage and economy realized from the crop. Consequently, dosage and frequency of application has to be standardized based on the crop value, which could be as a reliable and practical approach (Kumar et al., 2014).

Based on the above knowledge, we conducted an experiment to compare the effects on the vegetative growth and root development of young olive plants raised in nursery of two different treatments, a substrate enrichment applying Osmocote®, a slow-release fertilizer (Poole & Conover, 1989) and a foliar spray, Trianum-P, a *Trichoderma harzanium*-based biostimulant (Barker, 1988). Two ancient olive cultivars Rotondella, an oil variety, and Salella a double purpose variety for both oil and table consumption, were selected for this study.

MATERIALS AND METHODS

Plant material and growth conditions

The trial was carried out in 2018 at the experimental site of the University of Naples in Portici (province of Naples, Southern Italy, 40°48'53.9"N, 14°20'52.8"E, 37 m a.s.l.), in a Mediterranean or Csa climate, according to Köppen classification (Peel et al., 2007). Two olive (*Olea europaea L.*) cultivars Rotondella, an oil variety, and Salella, a double purpose variety for both oil and table consumption, autochthonous of the Campania Region were chosen (Di Vaio et al., 2013b). For each cultivar, a total of thirty young plants obtained from semi-woody cuttings, uniform in size and vegetative activity, were transplanted in April into 3-liter square pots, containing a substrate obtained mixing equal volumes of peat, sand, and soil (1:1:1 v/v). Plants were grown in a nursery area, under UV stabilized high density polyethylene (PEHD) black shading net, 50% relative shading. Drip irrigation was automatically activated every two days with a flow rate of 2 liters per hour. Plants were irrigated with water equal to 100% of the evapotranspiration (ET) calculated by weight.

Experimental treatments

For each of the two cultivars, three treatments were compared: (1) untreated Control; (2) potting substrate enriched with 3 g per liter of Osmocote® 15/9/12, a slow-release granular fertilizer commercialized by ICL Specialty Fertilizers (ICL Italia Treviso srl, Italy); (3) foliar spray application of 'Trianum-P' by Koppert Biological Systems (Koppert Italia, Bussolengo (VR), Italy) at the dose of 50 mL per liter. According to the producer's description, 'Trianum-P' is a combination of spores and mycelium of saprophytic soil, mainly *Trichoderma harzianum*, with the addition of appropriate enzymatic mixtures to enhance its biological activity. The treatments were performed only once, at the beginning of the test (23 April) and each treatment was replicated on 10 potted plants.

Data collection

The effect of the experimental treatments on vegetative growth was assessed at monthly intervals during one whole growing season, starting from 23 April until the end of the vegetative growing season (30 November). At each sampling date, the following measurements were recorded: height of the primary axis; number and length of the lateral shoots; diameter at the stem base; number of leaves. At the end of the vegetative growing season the plants were uprooted, and the following measurements were recorded: leaf area; leaves, shoots, trunk, roots and total fresh and dry matter weight. The total leaf area per plant was measured using a bench top Li-Cor 3100 leaf area meter (LI-COR Inc. Nebraska USA), the trunk cross sectional area (TCSA) was calculated by standard formula ($girth^2/4\pi$). Following these measurements, the fresh weights were recorded for each individual replicate sample. Then the fresh samples were dehydrated under vacuum, in stove at 70°C until reached constant weight, consequently the dry weight of the samples was recorded. The canopy/root ratio (C/R) was also calculated.

Statistical analysis

The data of parameters analyzed were normally distributed and they were processed by analysis of variance (ANOVA) and mean separations were performed through the Duncan multiple range test ($P < 0.05$), using the XL-STAT version 2012 statistics software package (Addinsoft, Paris, France)

RESULTS AND DISCUSSION

Fig. 1 reports the height increase during the vegetative season for both cultivars. Overall, the control plants of cv. Rotondella (Fig. 1, A) showed a more vigorous growth over the season compared with cv. Salella in agreement with Di Vaio et al. (2013b) (Fig. 1, B). In fact, in this neutral condition the increase in height of cv. Salella moved from 22.40 cm, one month after the beginning of the test, to 51.90 cm, at the end of the growing season. Through the same time the cv. Rotondella increase was from 15 cm to 40.30 cm. The Osmocote treatment resulted in an enhanced shoot growth in both cultivars: the growth of the primary axis of the Osmocote treated trees was 19.1% taller for cv. Salella and 58.3% for cv. Rotondella. These results converge with previously published reports confirming the effectiveness of slow-release fertilizers on the growth of olive nursery plants (Fernandez-Escobar et al., 2004; Meddad-Hamza et al., 2010). In contrast, no significant differences in shoot height were recorded for the plants treated with Trianum-P. Our results do not confirm the observations by Hermosa et al., 2013 that reported enhances in plant growth based on similar *Trichoderma* spp. treatments.

The total leaf area of the Osmocote treated plants also resulted significantly larger than control in both cultivars (Fig. 2). In the case of cv. Salella (Fig. 2, A), at the last ampling date the total leaf area per plant was 573.34 cm^2 per plant for the control and 1,293.45 cm^2 per plant for the Osmocote treated plants, corresponding to 55.7%

enhancement of the surface. Similar effect was recorded for the cv. Rotondella (Fig. 2, B), leaf area was 123.1% compared to control.

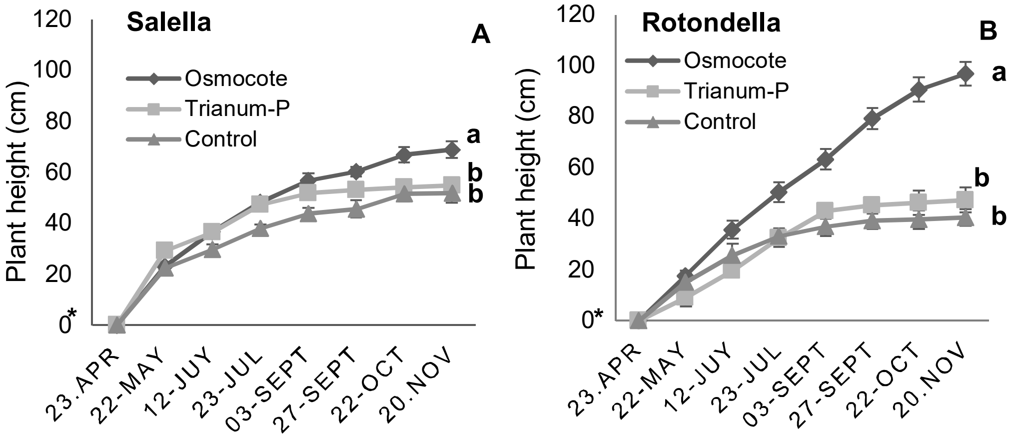

Figure 1. Effect of treatments on plant height increase of cvs Salella (A) and Rotondella (B) during the growing season (mean ± SE). Means with the different letters are significantly different using Duncan's test ($P < 0.05$). *Indicates the application of Osmocote® and *Trichoderma harzianum* (Trianum-P).

The Trianum-P treatment caused in cv. Salella 13.1% increase for leaf area compared to control, means were significantly different according to the Duncan's test at the 0.05% probability level. The leaf area increase resulting from *Trichoderma* treatments has previously documented in cucumber (Yedidia et al., 2001) and in tomato (Vinale et al., 2008; Azarmi et al., 2011). On the other hand, in the case of cv Rotondella the same Trianum-P treatment did not produce any significant difference in total leaf area compared to control.

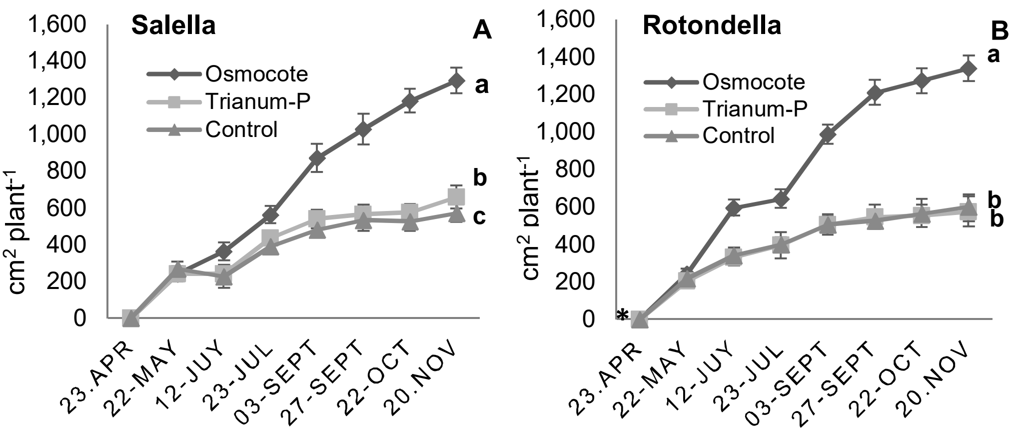

Figure 2. Effect of treatments on plant leaf area increase of cvs Salella (A) and Rotondella (B) during the growing season (mean ± SE). Means with the different letters are significantly different using Duncan's test ($P < 0.05$). *Indicate the application of Osmocote® and *Trichoderma harzianum* (Trianum-P).

As regards to TCSA (trunk cross sectional area), control plants of both cultivars showed similar increases:10.41 mm^2 for Salella and 10.68 mm^2 for Rotondella, at the end of the growing season (Fig. 3, A-B). Osmocote treated plants exhibited a larger growth, an average of 14.92 mm^2 TCSA for cv. Salella, corresponding to 30.2% increase compared to control. In the same experiment the cv. Rotondella plants had a final TCSA of 14.52 mm^2, 26.4% more than the respective control. Only a small, statistically non-significant, increase TCSA was recorded for the Trianum-P treatment in both cultivars.

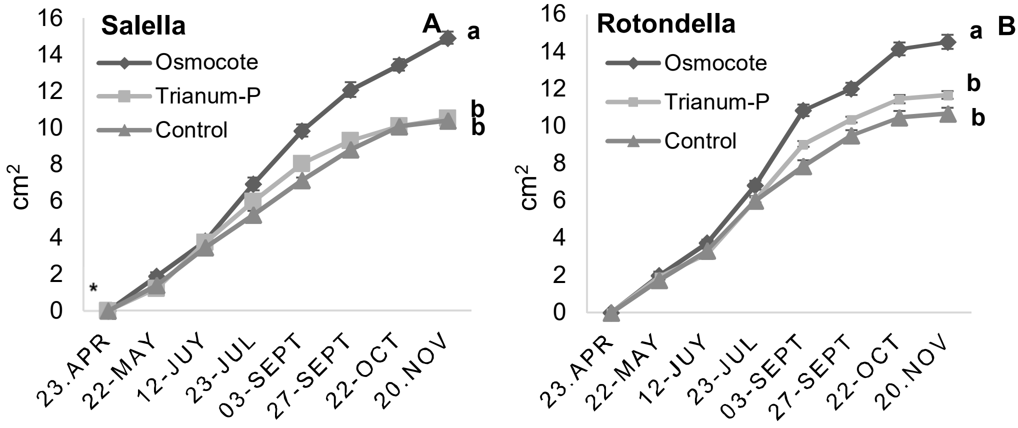

Figure 3. Effect of treatments on trunk cross sectional area (TCSA) increase of cvs Salella (A) and Rotondella (B) during the growing season (mean ± SE). Means with the different letters are significantly different using Duncan's test ($P < 0.05$). *Indicate the application of Osmocote® and *Trichoderma harzianum* (Trianum-P).

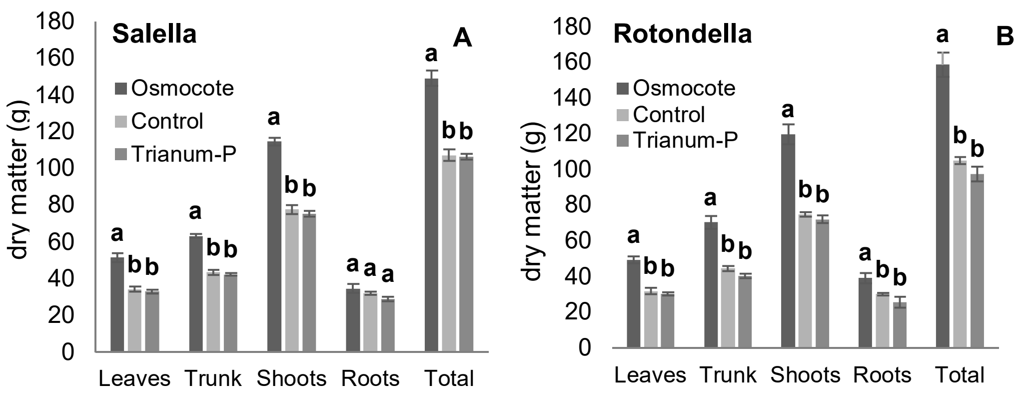

Figure 4. Effect of treatments on dry matter accumulation and partitioning (leaves, trunk, canopy, roots and total) of cvs Salella and Rotondella (mean ± SE). Means with the different letters are significantly different using Duncan's test ($P < 0.05$).

At the end of the growing season, the dry biomass data were recorded for each plant: leaves, trunk, shoots, and roots were weighed separately, Fig. 4. Compared to control, the supplement of Osmocote to the potting substrate significantly increased the final weight of each part of the plant, with the only exception of the root weight in cv. Salella.

In terms of total biomass, the dry weight was higher than control for both the cvs. Salella and Rotondella, 28.1% and 33.9% respectively. Although, other studies found that *Trichoderma* increased yield and total dry matter like in potatoes (Mohsin et al., 2010). Rakibuzzaman et al. (2021) reported an experimental settings where the increase in the total dry matter due to *Trichoderma* treatment can be attributed to the cumulative effect of increased leaf area index, increased nutrient uptake, and increased rate of photosynthesis.

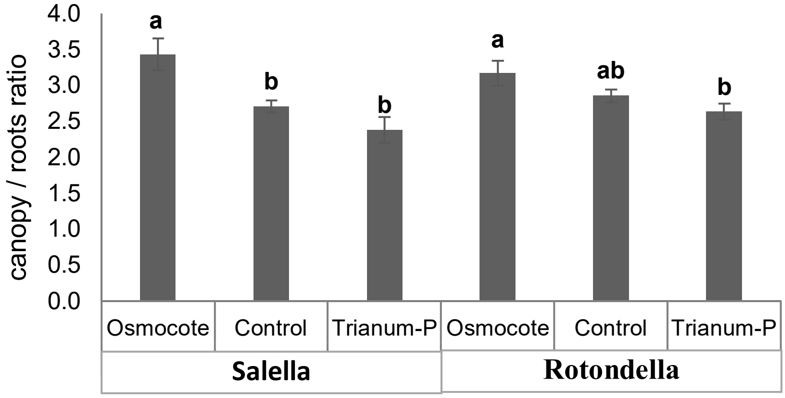

Figure 5. Effect of treatments on canopy/roots ratio of cvs Salella and Rotondella (mean ± SE). Means with the different letters are significantly different using Duncan's test ($P < 0.05$).

The canopy/root ratios, based on the dry mass data are presented in Fig. 5, reveal significant effects of the different treatments on both cultivars. Osmocote treatment favored the canopy growth in both cultivars and this effect was statistically significant in the case of the more vigorous cv. Salella (+21%). An analogous effect was reported by Worrall et al., 1987 in a previously published research. Conversely, the Trianum-P treatment resulted in a significantly lower canopy to root ratio compared with the Osmocote treatment (-30.6%), showing that this treatment favored the growth of the root system. A similar effect on the canopy to root ratio in olive plants, has also been reported as an effect of mycorrhizal inoculum (Citernesi et al., 1998; Meddad-Hamza et al., 2010) or water deficit as observed by Di Vaio et al. (2012 and 2013a) on Leccino and Racioppella cultivars. This effect can be interpreted as a reduction of the evapo- transpirant surface relative to the root surface, increasing the tolerance to drought conditions (Dichio et al., 2002). Our results diverge from previous studies showing a positive effect of *Trichoderma* treatment on the development of the plant root system (Tataranni et al., 2009; López-Bucio et al., 2015). Contrasting studies on the effects of *Trichoderma* are reported also in the bibliography, as some studies report the *Trichoderma* efficacy in symbiosis with mycorrhizal fungi, while others report inhibition of effects in the presence of these symbiotic fungi (Green et al., 1999).

CONCLUSIONS

Olive plants clearly responded to the substrate slow-release fertilizers application, in fact fertilized plants increased significantly in terms of leaf area per plant, central axis height, trunk diameter, and dry mass production compared to control plants; this product can improve the performance of young olive trees in the nursery.

Otherwise, plants treated with foliar spray containing a *Trichoderma harzianum* -based biostimulant, had a vegetative growth not statistically dissimilar to the control. Based on our results, we can say that a single application of foliar spray (*Trichoderma harzanium*) could be less effective compared to the direct effect on roots, when it is added to the potting substrate, where *Trichoderma* species behave as a free-living organism, common in soil and root ecosystems. Here it determines root colonization and, consequently, enhances growth and development, crop productivity, resistance to biotic and abiotic stresses, uptake and use of nutrients.

Numerous studies are available about *Trichoderma* application on the plant canopy, although not defined reliable protocols are available. This work represents a preliminary test of applications with products *Trichoderma*-based and slow-release fertilizer, on the two Campania autochthonous cultivars. Our results suggest a larger deepening, particularly in the use of the sprays products to improve the productive performance of nurseries and quality and quantity of young olive trees.

Author Contributions: Conceptualization, Claudio Di Vaio; methodology, Claudio Di Vaio, Stefano Conti, and Antonino Testa; data processing and software, Aurora Cirillo; validation, Claudio Di Vaio, Stefano Conti, and Antonino Testa; writing original draft preparation, Claudio Di Vaio, Stefano Conti, Antonino Testa, and Aurora Cirillo; writing review and editing, Claudio Di Vaio, Stefano Conti, and Antonino Testa; funding acquisition, Claudio Di Vaio. All authors have read and agreed to the published version of the manuscript.

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