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Effects of Foliar Application of Humic Acid and Gibberellic Acid on Mist-Rooted Olive Cuttings

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ABSTRACT

Humic substances, as part of humus-soil organic matter, are compounds arising from the physical, chemical and microbiological transformation (humification) of biomolecules. They are important because they constitute the most ubiquitous source of non-living organic materials that nature knows. In this study, the effect of humic acid (HA) (0, 0.25, 0.5, 1, 2%) and gibberellic acid (GA_3) (0, 200, 400 mg/L), when applied as a leaf spray individually or in combination, were investigated on the vegetative growth of mist-rooted olive (*Olea europaea* cv. 'Zard', a slow-growing cultivar) cuttings. Application of HA at 0.5, 1, or 2% could increase shoot length, elongate internodes and increase the fresh and dry weights of shoots, leaves, and roots. When HA was combined with GA_3, all these morphological parameters increased significantly more than in other treatments. The application of HA and GA_3 had the greatest effect on leaf surface area although the chlorophyll content decreased in treatments. The incorporation of HA and GA_3 significantly increased the soluble sugars and decreased the starch content relative to controls. The greatest nitrogen content was detected when 400 mg/L GA_3 with 0.5, 1 or 2% HA were applied. The effectiveness of HA might be related to its direct action through a hormone-like activity.

Keywords: humification, internode elongation, *Olea europaea*
Abbreviations: FA, fulvic acid; **GA_3**, gibberellic acid; **HA**, humic acid; **HS**, humic substance

INTRODUCTION

Humic substances (HSs) are organic matter formed during the physical, chemical, and microbiological transformation of dead animal and plant tissues (Nardi *et al.* 2002). HSs, the largest constituent of soil organic matter (~60%), are key components of the terrestrial ecosystem (Muscolo and Sidari 2007). HSs can be divided into three components: fulvic acids (FAs), humic acids (HAs) and humin. The most important part of HS is HA. HAs and FAs represent alkali-soluble humus fragments. HAs are commonly extracted using diluted alkali and precipitated with an acid; hence they are separated from soluble FAs (Peña-Méndez and Havel 2005; Canellas *et al*. 2008).

The main differences between HAs and FAs are: C distribution in the two humic fractions; HAs are slightly more aromatic than FAs although FAs are considerably richer in CO_2H groups; although HAs are richer in paraffinic C, they are poorer in carbohydrate-C than FAs (Nardi *et al.* 2002). The chemical composition of humic matter includes many aromatic rings that interact with each other and with aliphatic chains, giving rise to macromolecules with different masses (Baigorri *et al*. 2007). Considering that the genesis of HSs involves a combination of several reaction pathways and a wide variety of chemical binding systems, it is very difficult to define a clear concept based on their composition (Hayes 1997; Baigorri *et al*. 2007). HSs contain carbon, hydrogen, oxygen, nitrogen, and a small amount of sulfur. These elements are always present, regardless of their physical and geographic origin.

The beneficial effects of HSs on plant growth is possibly related to their increased fertilizer efficiency or reducing soil compaction, indirect effects, or improvement of overall plant biomass, a direct effect (Vayghan and Malcom 1985; Muscolo *et al*. 2005; Aguirre *et al*. 2009). Numerous papers in the literature have reported the impact of HSs on plant growth. Specific effects of HAs on plant growth include: a) solubilization of micronutrients (e.g. Fe, Zn, Mn), and some macronutrients (e.g. K, Ca, P), b) reduction of active levels of toxic elements, and c) enhancement of microbial populations (Vayghan and Malcom 1985). HAs are usually applied to the soil and favorably affect soil structure and soil microbial populations. Foliar sprays of HAs also promote growth in a number of plant species such as tomato, cotton and grape (Brownell *et al.* 1987; Fernández-Escobar 1996).

This paper studied the effects of a foliar-applied, commercial preparation of HAs made from Leonardite, and gibberellic acid (GA_3) on the growth of mist-rooted olive (*Olea europaea*) cuttings.

MATERIALS AND METHODS

Plant material and growth conditions

This experiment was carried out in a greenhouse. Mist-rooted 'Zard' olive cuttings, derived from mother plants approximately 25-years old, were transferred to 1.5-L plastic pots containing a mixture of sand and peat. These pots were placed in a greenhouse at 30/15°C (day-night) with a 14-h photoperiod under natural light.

Experimental conditions

After 2 weeks, each plant received one foliar application on the adaxial surface of HA at 0, 0.25, 0.5, 1 or 2% and GA_3 at 0, 200 or 400 mg/L, or in combination (all permutations were tested).

Measurements

There were four replications (plants) per treatment. Vegetative growth was determined one month after potting by measuring morphological variables (shoot and internode length, and shoot,

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leaf and root fresh and dry weight (FW and DW)) from harvested plants with the aid of a digital scale. Nitrogen (N) was determined by the Kjeldahl procedure (Fernández-Escobar 1996). Leaf area was determined by a delta-t-device. Soluble sugars and starch contents were determined by the method of Duboifh *et al.* (1956).

Statistical analyses

Data were statistically analyzed using MSTATC. Following analysis of variance, means were separated and significant differences were determined with Duncan's multiple range test at $P \leq 0.05$. The experiment was conducted only once.

RESULTS

Our results show that the application of HA at all concentrations improved the growth of olive cuttings. At higher concentrations of HAs, shoots were longer and internodes elongated more than control plants (**Figs. 1, 2**).

The incorporation of HA and GA_3 significantly increased shoot length and elongated internode more than controls. Longest shoots (58.97 cm) and elongation of internodes (2.47 cm) occurred with 400 mg/L GA_3 and 2% HA, combined.

HA decreased starch content resulting in an increase of soluble sugars. HA at 1 and 2% increased soluble sugars and decreased starch content, although there were no significant differences between these two concentrations. The incorporation of HA and GA_3 increased the soluble sugar and decreased the starch content significantly more than controls (sugar: 42.94 mg/g dry weight and starch: 130.9 mg/g dry weight). Although the greatest soluble sugar (223.3 mg/g dry weight) and lowest starch content (78.09 mg/g dry weight) occurred when 400 mg/L GA_3 was applied with 2% HA, the soluble sugar content showed no significant difference when 1% HA was used (**Figs. 3, 4**).

The application of HA and GA_3, either individually or in combination, increased the leaf areas of olive cuttings significantly more than control plants (**Fig. 5**): 400 mg/L GA_3 with 2% HA resulted in the greatest leaf area (313.3 cm^2) although the chlorophyll content decreased in this treatment.

No significant differences were found in N concentration when 400 mg/L GA_3 was combined with 0.5, 1 or 2% HA (**Fig. 6**). The lowest N concentration was found in control plants (1.15%).

Increasing the concentration of HA increased the shoots, root and leaf FW and DW more than the control. No significant differences in shoot and leaf FW were observed when HA was applied at 1 and 2%. Moreover, HA at 0.25, 0.5 and 1% showed no significant differences in root DW. The greatest shoot FW (12.33 g/plant), root FW (10.52 g/plant) and leaf FW (9.30 g/plant) occurred when 400 mg/L GA_3 + 2% HA was applied, but there were no significant differences when 1% HA was used instead (**Figs. 7-9**).

The greatest shoot (5.8g), root (3.58 g) and leaf (5.14 g) DW was also measured when 400 mg/L GA_3 was applied with 2% HA (**Figs. 10-12**).

DISCUSSION

A foliar application of HA increased the vegetative growth of olive cuttings (**Figs. 1, 2**). These results are in agreement with those reported for a wide number of plant species (Elgala *et al.* 1976; Rauthan *et al.* 1981; Dursun and Guvenc 1988; Chen and Aviad 1990; Fagbenro *et al.* 1993; David and Nelson 1994; Hartwigsen *et al.* 2000; Muscolo and Sidari 2007; Schmidt *et al.* 2007; Zandonadi *et al.* 2007). The positive influence of HA on plant growth and productivity, which seems to be concentration-specific, could be mainly due to the hormone-like activity of HA through its involvement in cell respiration, photosynthesis, oxidative phosphorylation, protein synthesis, and various enzymatic reactions (Chen and Aviad 1990; Muscolo and Sidari 2007). Although HA is known to evoke a plant's growth responses similar to those induced by plant hormones, it has not yet been conclusively proved whether or not HA contains hormone-like components. However, there are indications that they might (Chen and Inskeep 1992; Atiyeh and Lee 2002; Canellas *et al.* 2008).

The stimulative effect of HSs on plant growth has been related, at least in part, to the enhanced uptake of mineral nutrients. Increased uptake of macro- and micro-nutrients is influenced by HSs in different plant species (Lee *et al.* 1976; Rauthan and Schnitzer 1981; Chen and Aviad 1990; Fagbenro and Agboole 1993; Young and Chen 1997; Rupiasih *et al.* 2008). Tattini *et al.* (1991) reported increased N

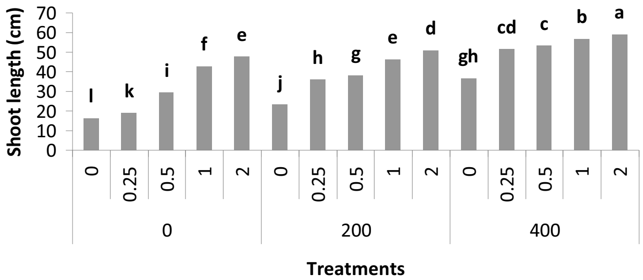

Fig. 1 Effect of the concentration of foliar-applied humic acid and gibberellic acid on shoot length (cm) of olive cuttings. Columns with the same letter do not differ significantly ($P \leq 0.05$).

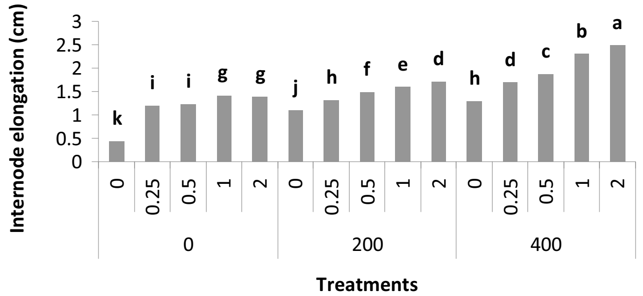

Fig. 2 Effect of the concentration of foliar-applied humic acid and gibberellic acid on internode elongation (cm) of olive cuttings. Columns with the same letter do not differ significantly ($P \leq 0.05$).

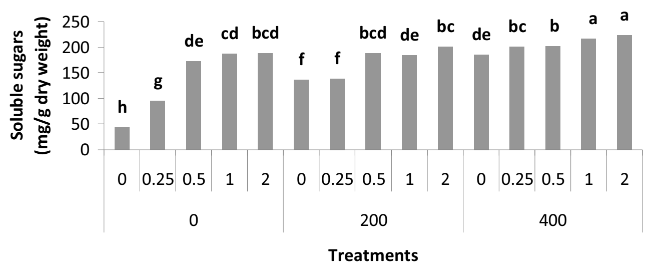

Fig. 3 Effect of the concentration of foliar-applied humic acid and gibberellic acid on soluble sugars (mg/g dry weight) of olive cuttings. Columns with the same letter do not differ significantly ($P \leq 0.05$).

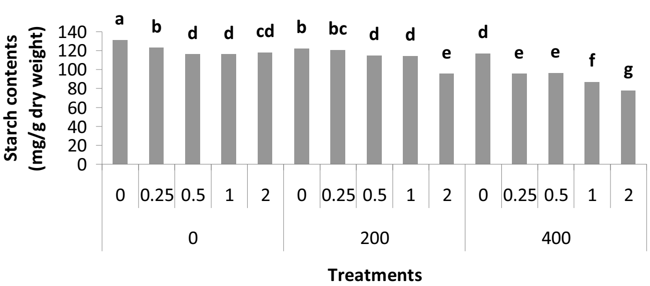

Fig. 4 Effect of the concentration of foliar-applied humic acid and gibberellic acid on starch contents (mg/g dry weight) of olive cuttings. Columns with the same letter do not differ significantly ($P \leq 0.05$)

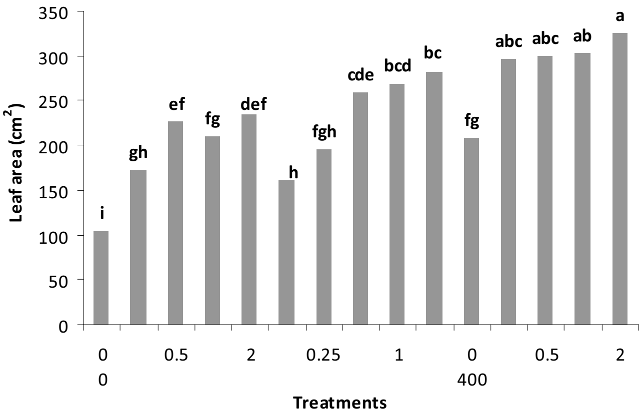

Fig. 5 Effect of the concentration of foliar-applied humic acid and gibberellic acid on leaf area (cm²) of olive cuttings. Columns with the same letter do not differ significantly ($P \leq 0.05$).

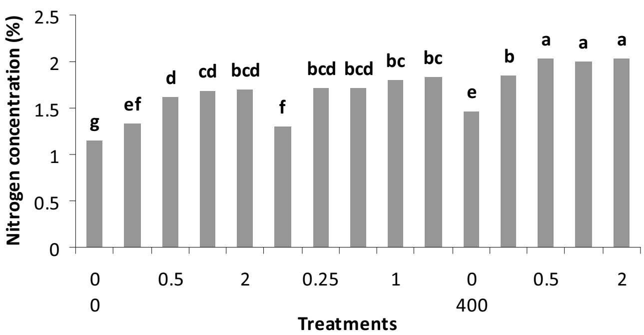

Fig. 6 Effect of the concentration of foliar-applied humic acid and gibberellic acid on nitrogen concentration (%) of olive plants. Columns with the same letter do not differ significantly ($P \leq 0.05$).

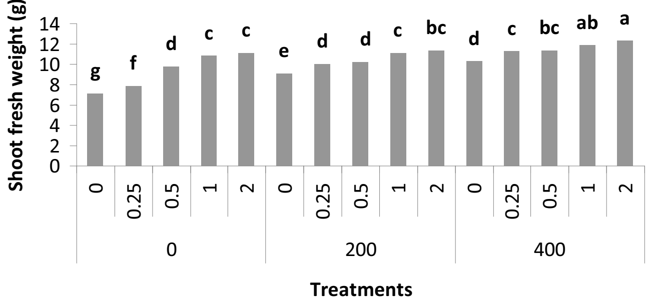

Fig. 7 Effect of the concentration of foliar-applied humic acid and gibberellic acid on fresh weight shoots (g) of olive plants. Columns with the same letter do not differ significantly ($P \leq 0.05$).

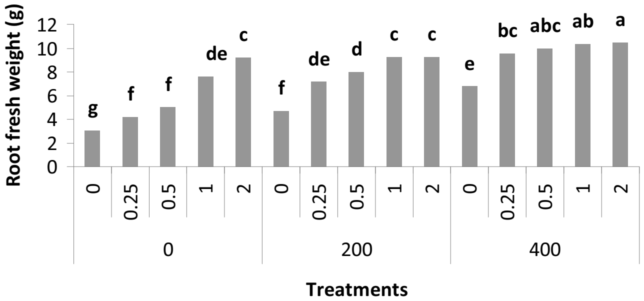

Fig. 8 Effect of the concentration of foliar-applied humic acid and gibberellic acid on fresh weight of roots (g) of olive cuttings. Columns with the same letter do not differ significantly ($P \leq 0.05$).

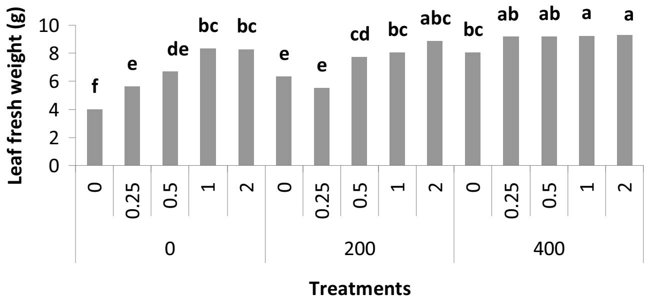

Fig. 9 Effect of the concentration of foliar-applied humic acid and gibberellic acid on fresh weight of leaves (g) of olive plants. Columns with the same letter do not differ significantly ($P \leq 0.05$).

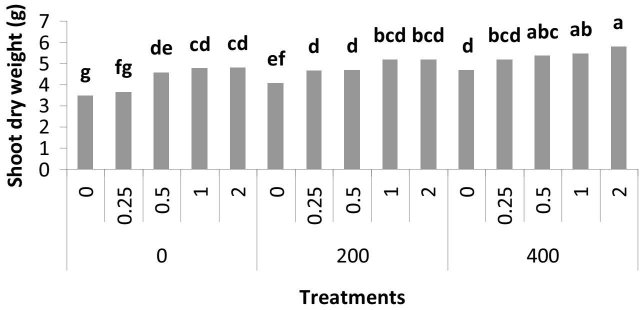

Fig. 10 Effect of the concentration of foliar-applied humic acid and gibberellic acid on dry weight of shoots (g) of olive plants. Columns with the same letter do not differ significantly ($P \leq 0.05$).

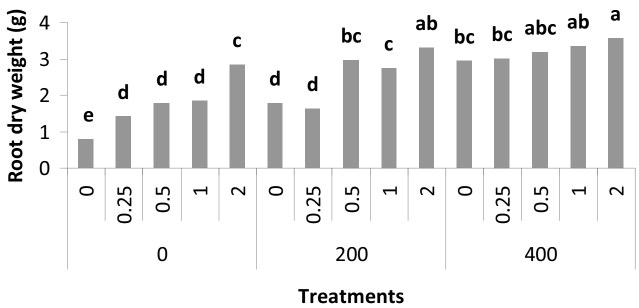

Fig. 11 Effect of the concentration of foliar-applied humic acid and gibberellic acid on dry weight of roots (g) of olive plants. Columns with the same letter do not differ significantly ($P \leq 0.05$).

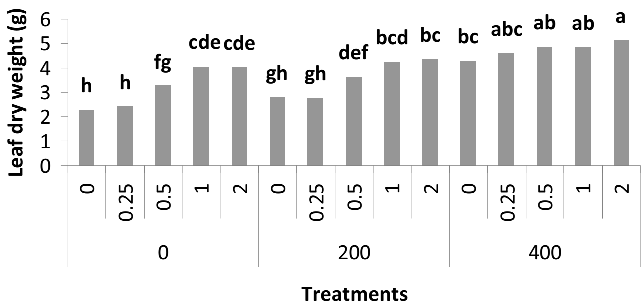

Fig. 12 Effect of the concentration of foliar-applied humic acid and gibberellic acid on dry weight of leaves (g) of olive plants. Columns with the same letter do not differ significantly ($P \leq 0.05$).

uptake by the roots of container-grown olive plants after the application of HA at 30-120 mg/pot; however, higher concentrations of HA decreased N uptake; this effect was observed when HA was applied to the soil or when mixed in nutrient solution. Aguirre *et al*. (2009) reported a close relationship between the effects of HSs on plant development and iron nutrition. HS may thus affect N leaf values by mechanism(s) other than the direct formation of complexes and chelates in soil (Odonnell 1973; Casenave de Sanfilippo *et al.* 1990).

Furthermore, application of HA and GA_3, either individually or combined, significantly elevated shoot, root and leaf FW and DW compared to controls. HA increased the proliferation of root hairs and enhanced root initiation (**Figs. 7-12**) (Chen and Adviad 1990; Schmidt *et al*. 2007).

Other positive effects of HA are by decreasing starch content and increasing soluble sugars (**Figs. 3, 4**). These changes may be mediated by variations in the activity in the main enzymes (Amylaze) involved in carbohydrate metabolism (Nardi *et al.* 2002). The chlorophyll content decreased due to the significant enlargement of leaf area (**Fig. 5**); the most prominent effect of HA in plant growth is in increasing the chlorophyll content which, in turn, could affect photosynthesis (Nardi *et al.* 2002). Spaccini *et al.* (2009) reported spectroscopic results that showed a concomitant entrapment in HA of bio-labile compounds, such as peptidic moieties.

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