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Effects of the times of application of a soil humic acid on berry quality of table grape (*Vitis vinifera* L.) cv Italia

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Abstract

Humic acids are the most active components of soil organic matter and have been shown to have an hormone-like activity thus stimulating plant growth. The objective of this work was to verify the quantitative and qualitative yield responses of the table grape cv. Italia after the application of a humic acid at various phenological stages. The humic acid used in this study has been extracted from a clay soil of the Apulia region and was applied at a concentration of 100 mg L^{-1} in four different times: pre-bloom (I), full-bloom (II), fruit set (III) and veraison (IV) and in two years, 2007 and 2008. The following parameters were measured at harvesting: berry size, °Brix, pH and titratable acidity. Finally, the °Brix/titratable acidity ratio has been calculated. Soil Plant Analysis Development (SPAD) readings were performed every 30 days up to harvesting time on the leaves of the middle shoots. No statistical differences were observed between the two years for all the parameters examined. The humic acid applied at full-bloom (II) induced a significant increase of berry size (width and weight) and a significant improvement of the other quality parameters (titratable acidity and °Brix/titratable acidity) with respect to the control. This study confirmed that humic acids, if applied at full-bloom, can induce significant increase of qualitative and quantitative parameters in table grape and can find a positive application in an organic and sustainable viticulture.

Additional key words: foliar sprays, hormone-like activity, overhead system («tendone»), phenological stages, SPAD.

Resumen

Efectos de los tiempos de aplicación de un ácido húmico del suelo sobre la calidad de las bayas de uva de mesa (*Vitis vinifera* L.) cv Italia

Los ácidos húmicos son los componentes más activos de la materia orgánica del suelo y se ha demostrado que tienen una actividad similar a las hormonas, estimulando así el crecimiento de las plantas. El objetivo de este trabajo fue verificar los incrementos en el rendimiento cuantitativo y cualitativo de la uva de mesa cv. Italia después de la aplicación de un ácido húmico en diferentes etapas fenológicas. El ácido húmico se extrajo de un suelo arcilloso de la región de Apulia y se aplicó a una concentración de 100 mg L^{-1} en cuatro momentos diferentes: pre-flor (I), plena floración (II), fruto (III) y envero (IV), y en dos años, 2007 y 2008. Se midieron los siguientes parámetros en la época de la cosecha: tamaño de la baya, °Brix, pH, acidez titulable y ratio °Brix/acidez titulable. Cada 30 días hasta la época de cosecha se realizaron lecturas SPAD sobre las hojas de los brotes medios. Para todos los parámetros examinados no se observaron diferencias estadísticas entre los dos años. El ácido húmico aplicado en plena floración (II) indujo un aumento significativo del tamaño de las bayas (anchura y peso) y una mejora significativa de los demás parámetros de calidad (acidez titulable y °Brix/acidez) con respecto al control. Este estudio confirmó que los ácidos húmicos, si se aplican en plena floración, pueden inducir un aumento significativo de los parámetros cualitativos y cuantitativos en la uva de mesa y se puede encontrar una aplicación positiva en una viticultura ecológica y sostenible.

Palabras clave adicionales: actividad similar a las hormonas, aplicaciones foliares, etapas fenológicas, sobrecarga del sistema («tendone»), SPAD.

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Abbreviations used: CEC (cation exchange capacity), HA (humic acid), HA-S (soil humic acid), HS (humic substances), RT (room temperature), SPAD (soil plant analysis development), TA (titratable acidity).

Introduction

Humic acids (HAs) are the main fractions of humic substances (HS) and the most active components of soil and compost organic matter. HAs have been shown to stimulate plant growth and consequently yield by acting on mechanisms involved in: cell respiration, photosynthesis, protein synthesis, water and nutrient uptake, enzyme activities (Vaughan and Malcolm, 1985; Albuzio *et al*., 1986; Chen and Aviad, 1990; Concheri *et al*., 1994; Nardi *et al*., 1996; Chen *et al*., 2004). This action of HAs has been demonstrated to be dose dependent and particularly effective in a low concentration range (Chen and Aviad, 1990). In particular, optimal concentrations able to affect and stimulate plant growth have been generally found in the range of 50-300 mg L^{-1}, but positive effects have been also exerted by lower concentrations (Chen *et al*., 2004). A distinction on the effects of HAs should be made between indirect and direct effects on plants growth. Indirect effects are mainly exerted through properties such as: enrichment in soil nutrients, increase of microbial population, higher cation exchange capacity (CEC), improvement of soil structure; whereas direct effects are various biochemical actions exerted at the cell wall, membrane or cytoplasm and mainly of hormonal nature (Varanini and Pinton, 2001; Chen *et al*., 2004). The hormone-like activities of HAs is well documented in various papers, in particular auxin-, cytokinin- and gibberellin-like effects (O'Donnell, 1973; Cacco and Dell'Agnola, 1984; Casenave de Sanfilippo *et al*., 1990; Piccolo *et al*., 1992; Pizzeghello *et al*., 2002) and, further, the presence of plant hormone-like substances in HAs has been recently demonstrated, in particular the high content of indolacetic acid in a humic fraction isolated from forest soils (Muscolo *et al*., 1998; Nardi *et al*., 2000).

Studies related to the effects of HAs on plant growth have been generally conducted in controlled environments, such in the case of herbaceous species grown hydroponically or on specific substrates (Malik and Azam, 1985; Lulakis and Petsas, 1995; Loffredo *et al*., 1997; Ferrara *et al*., 2001; Atiyeh *et al*., 2002). Few researches deal with foliar applications of HAs in the open field, in species such as strawberry (Neri *et al*., 2002), rice (Tejada and González, 2004) and durum wheat (Delfine *et al*., 2005). Applications of HAs to fruit species are very scarcely reported in the literature and some investigations have been conducted in olive (Fernández-Escobar *et al*., 1996), lemon (Sánchez-Sánchez *et al*., 2002) and apple (Neilsen *et al*., 2005). In the case of the effects of HAs in grape (*Vitis vinifera* L.), few studies have been performed in wine grapes (Brownell *et al*., 1987; Vercesi, 2000), table grape (Sánchez-Sánchez *et al*., 2006) and grapevine rootstocks (Zachariakis *et al*., 2001). A variability of results has been observed in all these studies, which can be attributed to the variable sources of HAs used, the various concentrations tested, the different times of application, etc.

Actually, responses of table grape cultivars to the foliar application of HAs are still limited, and in a recent paper (Ferrara and Brunetti, 2008) positive effects of foliar applications of two HAs on qualitative and quantitative parameters of 'Italia' table grape have been clearly observed. In order to better understand the action of HAs, the main objective of this work was to individuate the optimal time for a foliar application of a soil HA, chemically and spectroscopically characterized, in order to optimize the quantitative and qualitative yield responses of the table grape cv. Italia.

Material and methods

Humic acid origin and properties

The HA used in this work was obtained from a clay soil of the Apulia region (HA-S). The HA-S was isolated according to the method proposed by the International Humic Substances Society (IHSS) (MacCarthy and Rice, 1985). Briefly, a 0.1 M $Na_4P_2O_7$ and 0.1 M NaOH solution was added to 2-mm sieved soil sample using a ratio extractant:sample = 10:1. The mixture was shaken mechanically in N_2 gas atmosphere for 24 h at room temperature (RT, 20 ± 2°C). The supernatant solution was then separated from the residue by centrifugation at 9,600 g for 30 min. The extraction procedure was repeated three times on the residue that was finally discarded. The combined alkaline supernatant was acidified with 6 M HCl to pH~1, allowed to stand for 24 h in a refrigerator, and then centrifuged at 30,400 g for 20 min. The HA-S precipitate was purified by repeating three times the following steps: (a) dissolution in a minimal volume of the alkaline extractant; (b) centrifugation as above; (c) removal of the residue; (d) acidification of the recovered alkaline supernatant with 6 M HCl to a pH~1; (e) standing the suspension for 24 h at RT; and (f) final centrifugation as above. The centrifuged HA-S was recovered with distilled water, and then dialyzed against distilled water using a membrane having a molecular weight cutoff of 6-8 kDa, until the dialysis water gave a negative Cl^- ion test with $AgNO_3$. Finally, the dialyzed HA-S was freeze-dried, and stored at RT in plastic vials placed in a desiccator containing P_2O_5. The HA-S was finally analyzed (Chen *et al.*, 1977; Brunetti *et al.*, 2007) and data are shown in Table 1.

Field experiments

The experiment was carried out in Apulia (Southern Italy) in the years 2007 and 2008. The trial was performed in a commercial table grape vineyard located near Castellaneta Marina, in the Taranto province, on 72 'Italia' table grape grapevines. All the grapevines were grafted onto 1103 P (*V. berlandieri* × *V. rupestris*), spaced 2.5 × 2.5 m, trained to a overhead system («tendone») and drip irrigated (2,000-2,200 m^3 ha^{-1}). Fertilizers addition (N 140 kg ha^{-1}, P 60 kg ha^{-1}, K 170 kg ha^{-1}), pest control and other vineyard operations (leaves removal, bunches and berries thinning) were conducted in the most appropriate way. The soil type was a sandy clay loam and the general properties are shown in Table 2. A randomized block design was used with three blocks and eight treatments, and each treatment in the block consisted of three grapevines. Each treatment consisted of: (a) H_2O plus wetting agent, used as control; (b) HA-S at a concentration of 100 mg L^{-1} in four times: pre bloom (I), full bloom (II), fruit set (III) and veraison (IV). The pH of all solutions used was about 7.2, as the water normally used in the vineyard for irrigation and pesticide applications. The 72 vines were sprayed with

Table 1. Elemental composition and atomic ratios, extraction yield, ash content, acidic functional group contents and E_4/E_6 ratios (ratio of absorbance at 465 and 665 nm) of the soil humic acid (HA-S)

	HA-S		HA-S
C[a]	52.46	O/C	0.51
H[a]	5.77	Yield[b] (%)	0.4
N[a]	5.40	Ash[b] (%)	6.9
S[a]	0.70	Total acidity[c]	5.0
O[a]	35.66	Carboxyl (COOH)[c]	2.7
C/N	11.33	Phenolic OH[c]	2.2
C/H	0.76	E_4/E_6	7.4

[a] C, H, N, S, and O are the elemental composition in % (w/w) on a moisture- and ash-free basis. [b] On moisture-free basis. [c] Carboxyl is the charge density (meq g^{-1} C) at pH 8.0; phenolic is two times the change in charge density (meq g^{-1} C) between pH 8.0 and pH 10.0. On moisture- and ash-free basis.

Table 2. Physical and chemical properties of the soil at the experimental field site

Sand (g kg^{-1})	551.90	Na (cmol kg^{-1})	0.82
Silt (g kg^{-1})	170.60	K (cmol kg^{-1})	1.17
Clay (g kg^{-1})	277.50	Ca (cmol kg^{-1})	12.40
$CaCO_{3\ active}$ (g kg^{-1})	6.00	Mg (cmol kg^{-1})	3.75
pH_{H_2O}	7.84	C/N	8.25
EC (dS m^{-1})	0.32	K/Mg	1.01
Cl^- (g kg^{-1})	0.19	Ca/Mg	5.46
CEC (cmol kg^{-1})	10.84	Mn_{ava} (mg kg^{-1})	11.07
C_{org} (g kg^{-1})	5.70	B_{sol} (mg kg^{-1})	0.67
OM (g kg^{-1})	8.74	Cu_{ava} (mg kg^{-1})	4.22
N_t (g kg^{-1})	0.61	Zn_{ava} (mg kg^{-1})	1.72
P_2O_5 (mg kg^{-1})	78.28	Fe_{ava} (mg kg^{-1})	4.60

the HA-S in the four phenological stages by using a manual pump with care to wet whole leaves and bunches.

A sample of 27 bunches per treatment was picked at harvest from the middle shoots, collected in plastic bags and stored in a portable ice box to be carried in the laboratory for the subsequent determinations. The mean of 9 bunches was considered as a replicate.

The effect of HA-S on chlorophyll content was determined in the field with a SPAD-502-meter (Minolta Camera Co., Osaka, Japan). For this determination, three fully-expanded leaves opposite to or above the first bunch of the middle shoots were used for each grapevine and nine SPAD readings were averaged for each leaf to represent one observation. The SPAD readings were performed every month, beginning from May (full bloom), on the leaves of the primary shoots. Only the last measurement (August) was performed on the leaves of the lateral shoots.

Laboratory determinations

The 27 bunches per treatment collected in the field and carried to the lab were subjected to the following determinations, according to the AOAC (1990): a) diameter, length and weight of each berry; b) total soluble solids (°Brix); c) pH; d) titratable acidity (TA, as g tartaric acid per 100 mL juice) and the ratio °Brix/TA was finally calculated.

Statistical analysis

Prior to analysis, data were subjected to Levene's test (homogeneity of variance) and Lilliefors' test (normal distribution). Successively, analysis of variance

(ANOVA) was performed at the 0.05 P and 0.01 P levels and the mean values obtained for the different treatments were statistically compared to the control treatment by using the Dunnett's test.

Results and discussion

SPAD

A general slight increase in SPAD values was observed in almost all the treatments (Fig. 1). These results are in agreement with previous results (Ferrara and Brunetti, 2008) using two HAs, from a soil and a olive pomace compost, when various foliar applications were performed. In this previous work, highly significant correlations were found between SPAD values and nitrogen content ($R^2 = 0.87$) and SPAD values and chlorophyll content ($R^2 = 0.65$) in the leaves. Results obtained in the current experiment seem to suggest that even one application of the HA-S was able to increase SPAD values and consequently nitrogen and chlorophyll contents in the table grape leaves. The HA-S probably caused an increase in the synthesis of the chlorophyll (Vaughan and Malcolm, 1985; Nardi *et al*., 1996) and/or delayed chlorophyll degradation in the two different types of leaves, primary and lateral shoot leaves.

Yield and fruit quality

The application of HA-S caused a significant increase in berry size. In particular, HA-S applied at full bloom (II) significantly increased width and weight of berries collected at harvest (Fig. 2) with respect to the control treatment. These results are in agreement with data reported in our previous paper (Ferrara and Brunetti, 2008) and by other authors using commercial HAs (Sánchez-Sánchez *et al*., 2006). The increase in berry size as a consequence of HA-S application at full bloom is probably ascribed to the uptake of mineral nutrients by the grapevines, but the possible hormone-like activity of the HA-S (*i.e.,* auxin-, gibberellin- and cytokinin-like activity) should also be taken into consideration. Similar results are often obtained when synthetic hormones (gibberellins) are applied in seedless table grape cultivars, and this action of the HA-S could show a possible application of this natural organic compound as an alternative to various chemical molecules. However, positive effects of HAs are generally higher on roots than on shoots and fruits of various species (Chen and Aviad, 1990; Tattini *et al*., 1990) and at concentrations in the range of 50-300 mg kg^{-1} (Chen *et al*., 2004) or even higher in the case of chicory plants (Valdrighi *et al*., 1996). However, the hormone-like activity of HAs is more concentration-specific and higher concentration (200-500 mg kg^{-1}) seem to be more effective on the upper part of the plants (Atiyeh *et al*., 2002). Great increase of the yields (from 3 up to 70%) were reported for various wine grapes cultivars in California (Brownell *et al*., 1987) after the applications of two leonardite extracts. However, no details on the chemical properties of the two extracts are reported in the paper.

With regards to the qualitative parameters, application of HA-S generally caused a slight increase in total soluble solids (°Brix) in almost all the samples. In the case of the TA, HA-S (II) induced a significant decrease

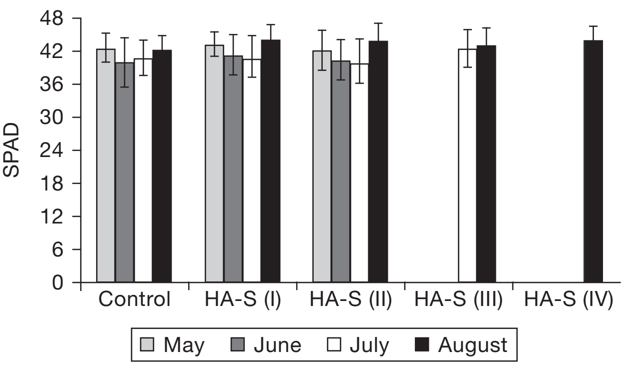

Figure 1. Effects of foliar sprays with a soil humic acid (HA-S) at a concentration of 100 mg L^{-1}, in four different times (I, pre bloom; II, full bloom; III, fruit set and IV, veraison), on SPAD values. Standard deviations are also indicated.

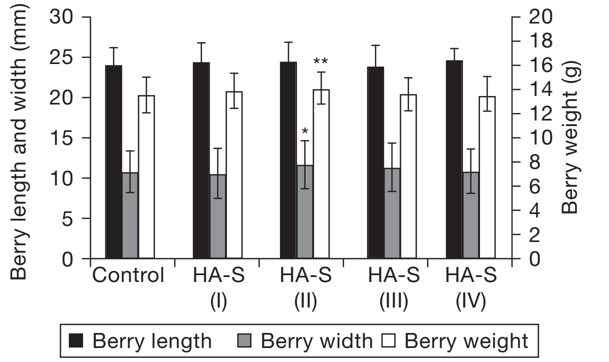

Figure 2. Effects of foliar sprays with a soil humic acid (HA-S) at a concentration of 100 mg L^{-1}, in four different times (I, pre bloom; II, full bloom; III, fruit set and IV, veraison), on berry length, width and weight. Standard deviations are also indicated. *, ** Statistically different at 0.05 and 0.01 *P* respectively according to the Dunnett's test.

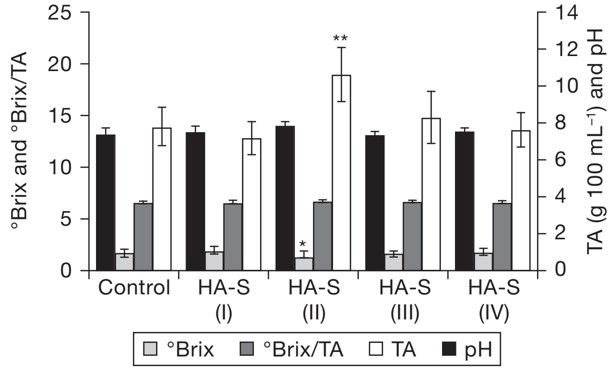

Figure 3. Effects of foliar sprays with a soil humic acid (HA-S) at a concentration of 100 mg L^{-1}, in four different times (I, pre bloom; II, full bloom; III, fruit set and IV, veraison), on °Brix, titratable acidity (TA), °Brix/TA and pH. Standard deviations are also indicated. *, ** Statistically different at 0.05 and 0.01 *P* respectively according to the Dunnett's test.

of tartaric acid and also a significant increase of the °Brix/TA ratio (Fig. 3) with respect to the control treatment. The improvement of quality parameters (°Brix, TA), by using the HA-S, is in agreement with what reported in 'Chardonnay' and 'Barbera' (Vercesi, 2000) and in table grape (Ferrara and Brunetti, 2008) with various HAs, applied in commercial products or in purified form, respectively. The slight increase of °Brix and the statistically significant reduction of TA are noteworthy results, and HAs may be applied in order to hasten ripening and/or to obtain more uniformly ripened bunches in some table grape cultivars.

Conclusions

Results obtained in the present work confirmed that a soil humic acid was able to produce some positive effects in table grape cv. Italia. In particular, significant increases in berry size and a significant reduction of titratable acidity have been observed when the humic acid was applied at full bloom with respect to the control treatment. Finally, in a sustainable viticulture the application of organic products can be a noteworthy alternative to chemicals and foliar spray applications of these products can have prospects for a possible economical and sustainable use.

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References

ALBUZIO A., FERRARI G., NARDI S., 1986. Effects of humic substances on nitrate uptake and assimilation in barley seedlings. Can J Soil Sci 66, 731-736.

AOAC, 1990. Official methods of analysis, 15th ed. Association of Official Analytical Chemistry, Arlington, Virginia, USA.

ATIYEH R.M., LEE S., EDWARDS C.A., ARANCON N.Q., METZGER J.D., 2002. The influence of humic acids derived from earthworm-processed organic wastes on plant growth. Bioresource Technol 84, 7-14.

BROWNELL J.R., NORDSTROM G., MARIHART J., JORGENSEN G., 1987. Crop responses from two new leonardite extracts. Sci Total Environ 62, 491-499.

BRUNETTI G., SENESI N., PLAZA C., 2007. Effects of amendment with treated and untreated olive oil mill wastewaters on soil properties, soil humic substances and wheat yield. Geoderma 138, 144-152.

CACCO G., DELL'AGNOLA G., 1984. Plant growth regulator activity of soluble humic complex. Can J Soil Sci 64, 225-228.

CASENAVE DE SANFILIPPO E., ARGÜELLO J.A., ABDALA G., ORIOLI G.A., 1990. Content of auxin-, inhibitor- and gibberellin-like substances in humic acids. Biol Plantarum 32, 346-351.

CHEN Y., AVIAD T., 1990. Effects of humic substances on plant growth. In: Humic substances in soil and crop sciences: selected readings (MacCarthy P., Clapp C., Malcolm R.L., Bloom P.R., eds). Am Soc Agron, Madison, WI, USA. pp. 161-186.

CHEN Y., SENESI N., SCHNITZER M., 1977. Information provided on humic substances by E_4/E_6 ratios. Soil Sci Soc Am J 41, 352-358.

CHEN Y., DE NOBILI M., AVIAD T., 2004. Stimulatory effects of humic substances on plant growth. In: Soil organic matter in sustainable agriculture (Magdoff F., Weil R.R., eds). CRC Press, NY, USA. pp. 103-129.

CONCHERI G., NARDI S., PICCOLO A., RASCIO N., DELL'AGNOLA G., 1994. Effects of humic fractions on morphological changes related to invertase and peroxidase activities in wheat seedlings. In: Humic substances in the global environment and implications on human health (Senesi N., Miano T.M., eds). Elsevier Sci, Amsterdam, The Netherlands. pp. 257-262.

DELFINE S., TOGNETTI R., DESIDERIO E., ALVINO A., 2005. Effects of foliar application of N and humic acids on growth and yield of durum wheat. Agron Sustain Dev 25, 183-191.

FERNÁNDEZ-ESCOBAR R., BENLLOCH M., BARRANCO D., DUEÑAS A., GUTÉRREZ GAÑÁN J.A., 1996. Response of olive trees to foliar application of humic substances extracted from leonardite. Sci Hortic 66, 191-200.

FERRARA G., BRUNETTI G., 2008. Foliar applications of humic acids in *Vitis vinifera* L. cv Italia. J Int Sci Vigne Vin 42, 79-87.

FERRARA G., LOFFREDO E., SENESI N., 2001. Antimutagenic and antitoxic actions of humic substances on seedlings of monocotyledon and dicotyledon plants. In: Humic substances: structures, models and functions (Ghabbour E.A., Davies G., eds). Royal Soc Chem Press, Cambridge, UK. pp. 361-371.

LOFFREDO E., SENESI N., D'ORAZIO V., 1997. Effects of humic acids and herbicides, and their combinations on the growth of tomato seedlings in hydroponics. J Plant Nutr Soil Sci 160, 455-461.

LULAKIS M.D., PETSAS S.I., 1995. Effect of humic substances from vine-canes mature compost on tomato seedling growth. Bioresource Technol 54, 179-182.

MacCARTHY P., RICE J.A., 1985. Spectroscopic methods (other than NMR) for determining functionality in humic substances. In: Humic substances in soil, sediment and water: geochemistry, isolation, and characterization (Aiken J.R., McKnight D.M., Warshaw R.L., eds). Wiley-Intersci, USA. pp. 527-559.

MALIK K.A., AZAM F., 1985. Effect of humic acid on wheat (*Triticum aestivum* L.). Environ Exp Bot 25, 245-252.

MUSCOLO A., CUTRUPI S., NARDI S., 1998. IAA detection in humic substances. Soil Biol Biochem 30, 1199-1201.

NARDI S., CONCHERI G., DELL'AGNOLA G., 1996. Biological activity of humus. In: Humic substances in terrestrial ecosystems (Piccolo A., ed). Elsevier, NY, USA. pp. 361-406.

NARDI S., PIZZEGHELLO D., RENIERO F., RASCIO N., 2000. Chemical and biochemical properties of humic substances isolated from forest soils and plant growth. Soil Sci Soc Am J 64, 639-645.

NEILSEN G.H., HOGUE E.J., NEILSEN D., BOWEN P., 2005. Postbloom humic- and fulvic-based zinc sprays can improve apple zinc nutrition. HortScience 40, 205-208.

NERI D., LODOLINI E.M., SAVINI G., SABBATICI P., BONANOMI G., ZUCCONI F., 2002. Foliar application of humic acids on strawberry (cv. Onda). Acta Hortic 594, 297-302.

O'DONNELL R.W., 1973. The auxin-like effects of humic preparations from leonardite. Soil Sci 116, 106-112.

PICCOLO A., NARDI S., CONCHERI G., 1992. Structural characteristics of humic substances as related to nitrate uptake and growth regulation in plant systems. Soil Biol Biochem 24, 373-380.

PIZZEGHELLO D., NICOLINI G., NARDI S., 2002. Hormone-like activities of humic substances in different forest ecosystems. New Phytol 155, 393-402.

SÁNCHEZ-SÁNCHEZ A., SÁNCHEZ-ANDREU J., JUÁREZ M., JORDÁ J., BERMÚDEZ D., 2002. Humic substances and amino acids improve effectiveness of chelate FeEDDHA in lemon trees. J Plant Nutr 25, 2433-2442.

SÁNCHEZ-SÁNCHEZ A., SÁNCHEZ-ANDREU J., JUÁREZ M., JORDÁ J., BERMÚDEZ D., 2006. Improvement of iron uptake in table grape by addition of humic substances. J Plant Nutr 29, 259-272.

TATTINI M., CHIARINI A., TAFANI R., CASTAGNETO M., 1990. Effect of humic acids on growth and nitrogen uptake of container-grown olive (*Olea europea* L. 'Maurino'). Acta Hortic 286, 125-128.

TEJADA M., GONZÁLEZ J.L., 2004. Effect of foliar application of a byproduct of the two-step olive oil mill process on rice yield. Eur J Agron 21, 31-40.

VALDRIGHI M.M., PERA A., AGNOLUCCI M., FRASSINETTI S., LUNARDI D., VALLINI G., 1996. Effects of compost-derived humic acids on vegetable biomass production and microbial growth within a plant (*Cichorium intybus*)-soil system: a comparative study. Agr Ecosyst Environ 58, 133-144.

VARANINI Z., PINTON R., 2001. Direct *versus* indirect effects of soil humic substances on plant growth and nutrition. In: The rhizosphere: biochemistry and organic substances at the soil-plant interface (Pinton R., Varanini Z., Nannipieri P., eds). Marcel Dekker Inc, NY, USA. pp. 141-157.

VAUGHAN D., MALCOLM R.E., 1985. Influence of humic substances on growth and physiological process. In: Soil organic matter and biological activity (Vaughan D., Malcolm R.E., eds). Martinus-Nijhoff, Boston, MA, USA. pp. 37-75.

VERCESI A., 2000. Concimi organici a terreno e foglie in viticoltura. L'Informatore Agrario 6, 83-89. [In Italian].

ZACHARIAKIS M., TZORAKAKIS E., KRITSOTAKIS I., SIMINIS C.I., MANIOS V., 2001. Humic substances stimulate plant growth and nutrient accumulation in grapevine rootstocks. Acta Hortic 549, 131-136.