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Irrigation Effects with Different Mixtures of Olive Mill Wastewater (OMW) and Clean Water on the Growth and Quality of Young Olive Plants cv. 'Ottobratica', in a Nursery

Gregorio Gullo[1*] • Valentino Branca[1] • Serafino Cannavò[2] • Antonio Dattola[1] • Rocco Zappia[1]

[1] Department of Agricultural and Forest System Management, University "Mediterranea"of Reggio Calabria, Feo Di Vito, 89060 Reggio Calabria, Italy
[2] Department of Agricultural-Forest Science and Technologies. University "Mediterranea" of Reggio Calabria, Feo Di Vito, Reggio Calabria, Italy

Corresponding author: * ggullo@unirc.it

ABSTRACT

This study examines the effects of olive mill wastewater (OMW) combined with clean water (CW) in a nursery of olive plants (*Olea europaea* L.). Four treatments (Control = 0%, T1 = 5%, T2 = 18%, T3 = 46% OMW) were compared. From July to November, the amount of OMW given to cv. 'Ottobratica' olive plants ranged from 0 ml in control plants to 1540 ml per plant in T3. The increase in shoot development was higher in T1 (6.25 cm) and in T2 (4.51 cm) compared to the control plants (1.96 cm). In T3 a decrease in shoot development (0.88 cm) was evident. Absorbent root length was statistically higher in T1 (157 m) than in T2 (85 m) and T3 (74 m), decreasing as the OMW% in the mixture increased. Stomatal conductance increased by 144% in T1, 35% in T2 and 42% in T3 compared to control plants. In T1, T2 and T3 the leaf area and stomatal conductance decreased with a reduction in length of the absorbent roots due to the higher OMW% in the mixture. The potassium level was highest in T1, but no significant differences were observed in the other treatments compared to the control. The total dry weight per plant increased by 28% in T1 and decreased ~12% in T2 and T3, compared to the control plants. OMW, if diluted, can be considered a valid fertilizer and interesting ecological and economic solution in olive nurseries.

Keywords: BOD_5, COD, pot, "rooted cutting"
Abbreviations: BOD_5, biochemical oxygen demand; **COD**, chemical oxygen demand; **CW**, clean water; **OMW**, olive mill wastewater; **T1**, 5% OMW treatment; **T2**, 18% OMW treatment; **T3**, 46% OMW treatment

INTRODUCTION

In Italy the tree nursery industry has an important role with a production of about 5 million plants per year. The production cycle of olive plants with agamic and gamic propagation is completed in a period which ranges from 24 to 36 months (Tattini 1993).

In order to achieve quality standards and competitive prices in a market economy, modern and innovative technologies are necessary both in the propagation and management of the plants within a minimum of environment pollution (Aqeel *et al*. 2007; Khatib *et al*. 2009) and with a reduction of fertilization costs.

For this reason, in the plant nursery industry great attention is given to the utilization of olive mill wastewater (OMW) (Ranalli 1992; Ranalli and Strazzullo 1995; Ranalli *et al.* 2003), which is difficult to discharge directly into water-beds.

The total quantity of organic and inorganic components in OMW depends on the type of oil press used (Cabrera *et al*. 1996). OMW is rich in potassium and contains relevant quantities of nitrogen, phosphorus and magnesium (Pacifico 1989; Paredes 1999; Sicari *et al*. 2005); therefore, it represents a potential alternative to traditional fertilization (Catalano and De Felice 1993). The organic substance of OMW includes sugar, tannins, polyphenols, polyalcohols, pectins and lipids.

The amount of organic substance favours the development of microflora which improves the chemico-physical properties of the soil (Perez and Gallardo-Lara 1987; Pagliai *et al*. 2001; Colucci *et al*. 2002; Brunetti *et al*. 2007). However, in OMW, particular attention must be given to the toxicological effects of natural organic compounds, such as the phenolic fraction (Roig *et al.* 2005), and chemical parameters such as biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) (Lanciotti *et al*. 2005; Papanikolaou *et al*. 2008). OMW has a COD value of 220 g l^{-1} and a BOD_5 value of about 100 g l^{-1} (Paraskeva and Diamadopoulos 2006).

The storage of OMW, over a long period of time, produces an increase in pH and a reduction in BOD_5 (Marrara *et al.* 2005), then can be utilized for irrigation, considering that the decomposition of organic compounds in OMW spread on the soil is attributable to the bacteria it contains.

Therefore, OMW can be regarded as a natural vegetal liquid soil conditioner and its application on soil, as a liquid fertilizer, could fulfil the dual objective of causing chemical and biological degradation and enriching the soil with organic substances and nutritional elements (Proietti and Nasini 2004). The possibility of spreading OMW onto soil (Bonari 1990; Bonari and Ceccarini 1991, 1993; Bonari *et al.* 1993; Rinaldi *et al*. 2003; Chartzoulakis *et al*. 2006) represents a valid alternative to depuration methods by discharging into water-beds (Saviozzi *et al*. 1991; Tamburino *et al.* 1999; Tomati 2001; Di Giovacchino *et al*. 2002).

However, there are contrasting opinions about the actual benefits or about the probable negative effects of the spreading of non-diluted OMW.

This study contains experiments and monitoring concerning the spread of diluted OMW on young olive plants in pots, during the last propagation phase in a nursery and, finally, the evaluation of its effects on the development of these plants.

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MATERIALS AND METHODS

The trial was conducted on young (age range 16-20 months) olive plants in the Calabria, South Italy, during 2006-2008.

Plant propagation

The olive plants were propagated and trained at the nursery. The vegetative segments used consisted of cuttings of cv. 'Ottobratica' and the rooted cuttings were obtained in a propagation greenhouse with a fog system on propagation beds under thermal control and a perlite substrate. The rooted cuttings were grown in small pots (7 cm × 7 cm × 7 cm), starting from the 4th rooting month and they were transplanted again, during the 13th month, into rigid pots (8-liters), with a substrate containing sandy soil (34%) and light peat (66%). In the nursery, the plants were subjected to normal treatments (fertilization and irrigation).

The plants were trained with a single axis method and the top remained upright, with secondary branches on it.

During the first 10 days of July 2007 (16th month), among the propagated young plants, 18 plants, uniform both in canopy height and axis (stem) diameter, were transferred into 14 liters rigid pots in order to facilitate the second phase of the trial. The pots were displayed on a polyethylene film in 4 rows of 20 plants, in open area.

OMW

The OMW was obtained from a continuous cycle oil press, which milled olives of cv. 'Carolea' (2006/2007 olive growing season), and was stored in an open air cistern of 200 m^3 for a period of 4 months (from November 2006 to February 2007). In this period the meteoric contribution was 0.32 m^3. At the beginning of the trial the OMW was analyzed; their composition is shown in **Table 1**.

Table 1 Parameters of the olive mill wastewater (OMW) used in the trial, obtained in a cycle continuous press from the Carolea cultivar.

pH	Dry matter (%)	Ash (%)	BOD_5 (mg l^{-1})	Total polyphenols (ppm)	COD (mg l^{-1})
5	0.89	0.37	2800	16.09	7300

Experimental design

Four treatments were compared. Each treatment corresponded to a 300 ml mixture of clean water (CW) with a percentage of OMW (Control = 0%, T1 = 5%, T2 = 18%, T3 = 46%). From the 5th of July to the 18th of November 2007, the amount of OMW given to the plants ranged from 0 ml per plant, in the control, to 1540 ml per plant, in T3 (**Table 2**).

During the entire trial period, the total quantity of OMW received by the plants was calculated:

1) per plant and per single irrigation;
2) per plant and per total irrigation period;
3) per hectare, assuming a layer of soil explored by roots of 0.50 m (**Table 2**).

Each week, in addition to the mixture (OMW+CW) or only CW (control), all the plants were irrigated twice with clean water (1 liter of water per plant and per irrigation). A pre-check of the application of the above-mentioned quantity does not lead to water loss due to percolation; the moisture of the soil remains at the bottom of the pot.

The plants were not given any fertilizer during this last phase in the nursery. At the beginning of the trial five plants per treatment were randomly marked and the height and diameter of the collet of the plant were measured.

From the 5th July to 23rd November 2007, shoot length was measured on 5 shoots randomly selected per plant and per treatment.

In July, August and September 2007, measurements of the stomatal conductance were carried out using an infrared gas analyzer (IRGA) CIRAS-1 PP System, Hitckin, UK, with a Parkinson cuvette, 1 h after the first of the two weekly irrigations with CW. Measurements were taken on 5 leaves per plant, randomly chosen, and on 5 plants per treatment.

Laboratory analysis of samples

In February 2008 five randomly selected plants were transferred to the lab and measurements were taken both on the epigeous and hypogeous portions. On the epigeous portion, dry weight of all the components of the plants (leaves, shoots and axis) were measured after drying at 70°C for 72 h. Leaf area was determined with an area meter LI-3100 (LI-COR, Lincoln, USA).

On the hypogeous portion, the structural roots were separated from absorbent roots. The absorbent roots have a diameter ≤ 2 mm, whereas the structural roots have a diameter ≥ 2 mm (Rieger 1995). The dry weight was measured after drying at 70°C for 72 h.

Root length is an important parameter for determination of the absorption of water and nutrients from soil. Absorbent roots are more involved in this process than structural roots (Barone and Di Marco 2003). Length of absorbent roots was measured by the line intersection method of Tennant (1975).

Foliar analysis was conducted on oven dried leaves. For nitrogen (N), phosphorus (P) and potassium (K) determination a sample of 1 g was digested in a diacid mixture of H_2SO_4 and $HClO_4$ (4: 1). 1 ml of digested solution was treated with Nessler's reagent and N content was determined spectrophotometrically (Shimadzu UV-Vis 2100, Japan) at 408 nm (Jackson 1967). P was determined colorimetrically by vanadomolybdo phosphoric yellow color method (Jackson 1967) and K was recorded using a flame photometer (Model PFP 7, Jenway, UK)

Data were subjected to analysis of variance (ANOVA) and treatment means were compared using Tukey's test at $P \leq 0.05$ (Sokal and Rohlf 1969).

RESULTS AND DISCUSSION

OMW

In all treatments, the quantities used, from a minimum of 120 m^3 ha^{-1} in T1 to a maximum of 1.120 m^3 ha^{-1} in T3 (**Table 2**), exceeded the limit of 80 m^3 ha^{-1} imposed by the current Italian legislation (G. U. Repubblica Italiana 1996) for the disposal on agricultural areas of OMW derived from continuous cycle oil press.

The BOD_5 and COD in OMW at the beginning of the trial reached 2.800 and 7.300 mg dm^{-3}, respectively (**Table 1**), whereas the values calculated in OMW treatments are shown in **Table 3**.

The BOD_5 and COD values appeared to be lower than those referred to in the literature for OMW obtained in an oil press similar to the one used in this experiment: Pacifico (1989) reported values of 54.1 g/l COD and 19.2 for the BOD_5; Proietti and Nasini (2004) indicate a range for 50-180 g/l for the COD and of 14-90 g/l for BOD_5; finally, Khatib *et al*. (2009) reported values of 98 g/l for the COD and 45 g/l for BOD_5.

Table 2 Quantity of olive mill wastewater (OMW) distributed in the different treatments.

Treatments	OMW distributed on each date (ml)	OMW distributed in the total trial (ml)	Percentage of OMW in 300 ml of the mixture (%)	OMW distributed during the trial (m^3ha^{-1})
Control	0	0	0	0
T1	15	165	5	120
T2	53	583	18	424
T3	140	1540	46	1120

Table 3 BOD_5, COD and polyphenols in the olive mill wastewater (OMW) in the different treatments.

Treatments	BOD_5 (mg dm^{-3})	COD (mg dm^{-3})	Polyphenols (ppm)
Control	0	0	0
T1	140	360	0.8
T2	500	660	2.88
T3	1290	1700	7.36

The low values we found are due to the storage of OMW that can be considered an effective pre-treatment method (Marrara *et al*. 2005).

Nevertheless, the BOD_5 and COD values are higher than the limits (BOD_5 40 mg/l; COD 160 mg/l) imposed by the Italian law (G.U. Repubblica Italiana 2006) for discharging OMW into water-beds.

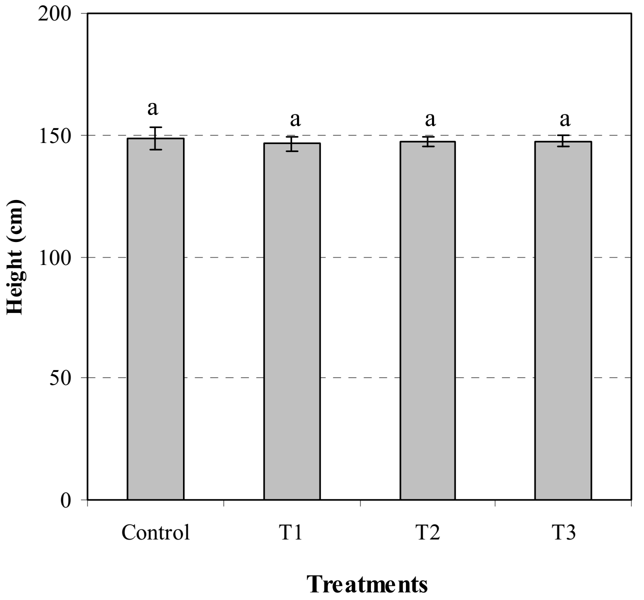

Fig. 1 Plant height of cv. 'Ottobratica' in different treatments at the beginning of the trial. Values represent mean ± Standard Error (SE). Different letters represent significant differences at $P \leq 0.05$.

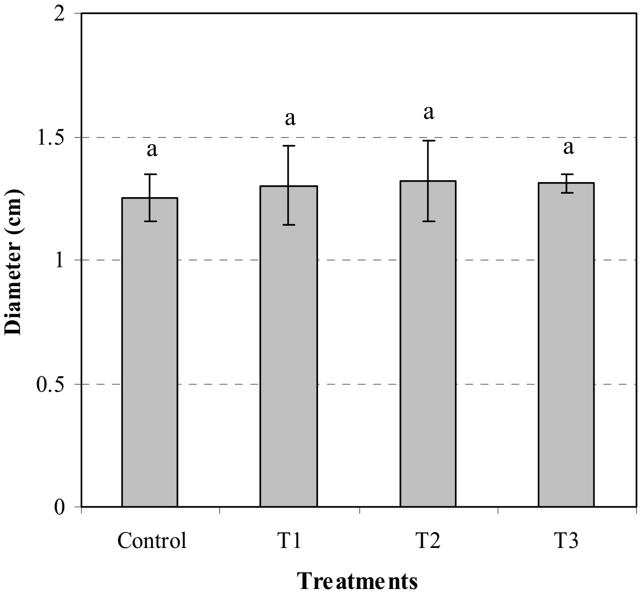

Fig. 2 Diameter of the collet in cv. 'Ottobratica' plants in different treatments at the beginning of the trial. Values represent mean ± Standard Error (SE). Different letters represent significant differences at $P \leq 0.05$.

Effects on growing shoots

At the beginning of the trial, the selected plants showed uniform height and diameter (**Figs. 1, 2**). 5% OMW caused the greatest lengthening of shoots (6.25 cm) compared to control plants (1.96 cm), with a maximum peak on October 12th, during the autumn development of shoots (**Table 4**).

In T2, the percentage of OMW used in the mixture (18%) caused an increase in the shoot length to 4.51 cm. However, the pattern of increase in shoot length of plants during the observation period appeared more irregular and as overall inferior to that in T1. In T3, negative effects of higher OMW% (46%) were observed in shoot development (**Table 4**). In this case the mean value of shoot development, compared to the control plants, was statistically lower than 55% (**Fig. 3**).

Phenols are considered the main compounds implicated in growth reduction in some Mediterranean crops, such as maize and wheat (El Hadrami *et al.* 2004). This was observed in T3, where the level of toxicity was so high that statistically reduced growth in the plants compared to the control.

Furthermore, the greater shoot length observed in T1 and T2 compared to the T3 could be due to the lower level of COD, BOD_5 and other substances, such as low-molecular-weight phenols (e.g. catechol and hydroxytyrosol) representing the fraction most toxic dell'OMW (Fiorentino *et al*. 2003). Ben Rouina *et al*. (1999) reported that OMW improves soil fertility that, few days after irrigation with OMW at low doses (4/6 liters in a pot with 16 kg of sandy soil), showed intense microbial activity. This could be one of the main factors that led to the growth of plants in treat-

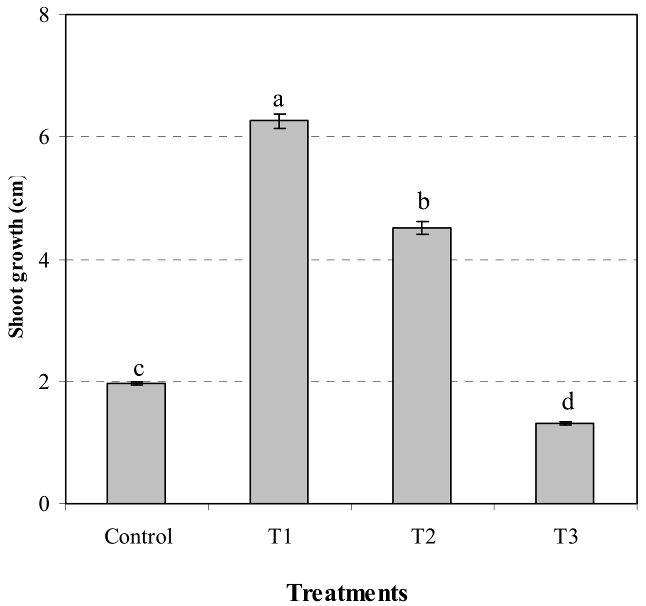

Fig. 3 Shoot growth in cv. 'Ottobratica' plants in different treatments. Values represent mean ± Standard Error (SE). Different letters represent significant differences at $P \leq 0.05$.

Table 4 Increase in shoot length (cm) in plants of cv. 'Ottobratica' in different treatments.

Date	Control	T1	T2	T3
20-Jul	0.14 c*	0.17 b	0.23 a	0.06 d
3-Aug	0.23 c	0.47 b	0.65 a	0.49 b
20-Aug	0.34 c	0.41 b	0.66 a	0.03 d
31-Aug	0.09 b	0.57 a	0.57 a	0.01 c
14-Sept	0.12 c	0.88 b	**1.10 a**	0.07 d
28-Sept	0.16 c	0.91 a	0.50 b	0.01 d
12-Oct	0.25 b	**1.25 a**	0.31 b	0.01 c
26-Oct	0.38 b	0.79 a	0.20 c	0.23 c
9-Nov	0.18 b	0.64 a	0.11 c	0.01 d
23-Nov	0.06 b	0.16 a	0.09 b	0.01 c

*Different letters within each row represent significant differences at $P \leq 0.05$.

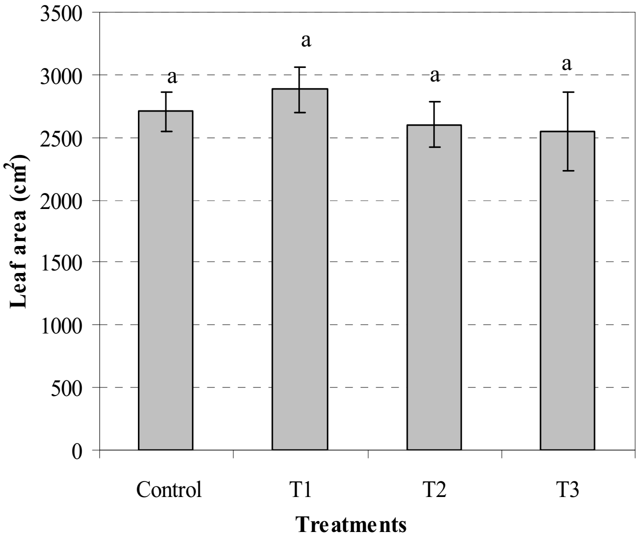

Fig. 4 Leaf area of cv. 'Ottobratica' plants in different treatments at the end of the trial. Values represent mean ± Standard Error (SE). Different letters represent significant differences at $P \leq 0.05$.

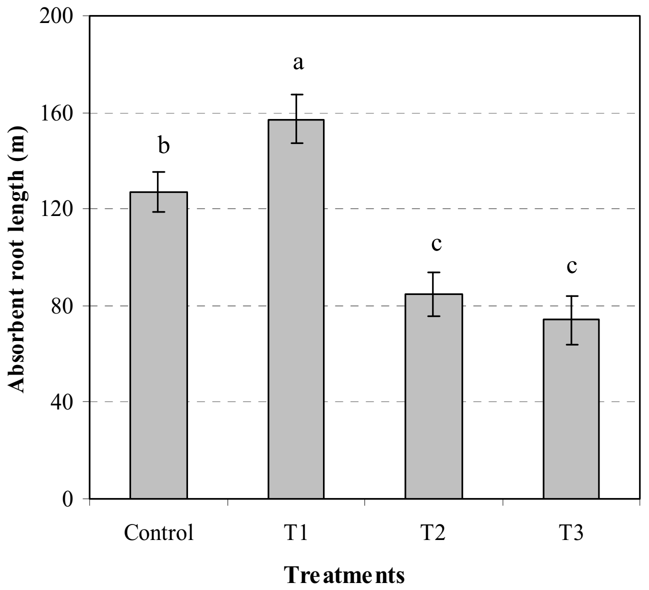

Fig. 5 Absorbent root length of cv. 'Ottobratica' plants in different treatments at the end of the trial. Values represent mean ± Standard Error (SE). Different letters represent significant difference at $P \leq 0.05$.

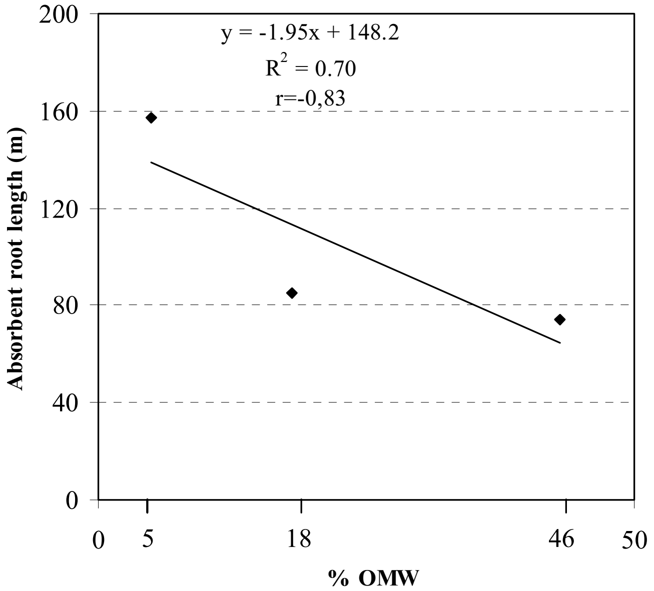

Fig. 6 Relation between absorbent root and percentage of OMW (5, 18, 46%) in cv. 'Ottobratica' plants.

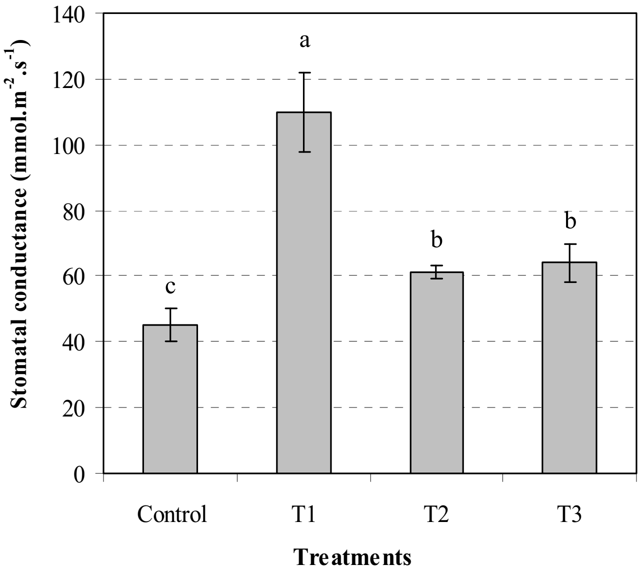

Fig. 7 Stomatal conductance (measured in July, August and September) of the leaves in cv. 'Ottobratica' in different treatments. Values represent mean ± Standard Error (SE). Different letters represent significant difference at $P \leq 0.05$.

Table 5 Nitrogen, phosphorus and potassium contents (%) in the leaves of cv. 'Ottobratica' in different treatments.

Treatments	N (%)	P (%)	K (%)
Control	1.65 a*	0.11 b	0.82 b
T1	1.52 a	0.16 a	1.46 a
T2	1.60 a	0.18 a	0.81 b
T3	1.65 a	0.11 b	0.59 c

*Different letters within each column represent significant differences at $P \leq 0.05$.

ments T1 and T2 compared to control.

T1 (5% OMW) and T2 (18% OMW) treatments promoted growth differently. In fact, in T2 longer shoots were observed in each treatment date, together with a faster growth peak (about a month earlier of T1) during autumn shoot development; after the peak, a toxic effect on shoot length was observed (**Table 4**). At the end of the trial shoot growth in T1 was higher than in T2.

Effects on leaf surface

No significant variation was observed after the analysis of the leaf area, although the mean value was higher in T1 (**Fig. 4**).

Regarding the content of principal macroelements in the leaves (**Table 5**), the N content in all treatments was similar to values published in literature: Tombesi (2002) shows how the optimum values of nitrogen in leaves (% dry matter) a range of 1.7-2.0%; optimum values for P a range of 1.7-2.0% and a range of 0.53-1.03% for K. Rotundo (2003) defines the appropriate values in the range of 1.8-2.2% (% dry matter).

The P and K levels were lower in T3 than in control, in T1 and T2 treatments. In T1 the potassium leaf content is very high and could be responsible for the higher degree of stomatal conductance observed (Peoples and Koch 1979; Singh and Sharma 1988). Outlaw (1983) reports that potassium is involved in the opening and closing processes of the stoma. Indeed, the opening and closing of guard cells is essentially a turgor driven response mediated by changes in solute concentration and the major solutes involved in stomatal movements is potassium. In the leaves of many plants very large amounts of K are accumulated, up to 6% of dry weight (Smith and Stewart 1990). This occurs through a

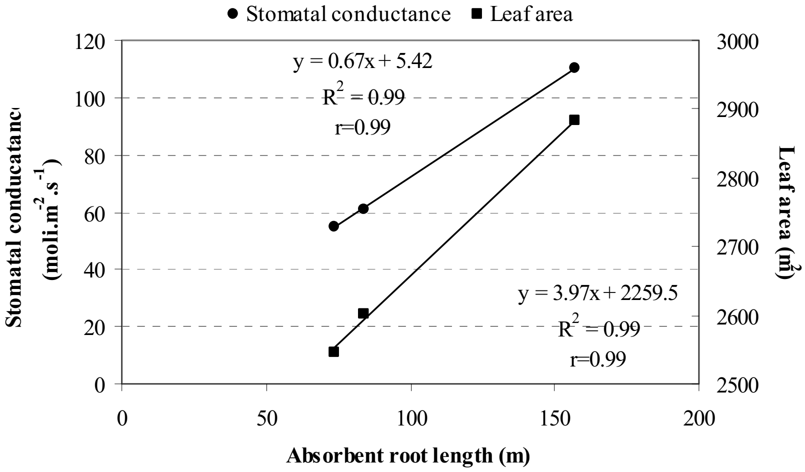

Fig. 8 Relation between stomatal conductance and absorbent root length and between leaf area and absorbent root length in cv. 'Ottobratica' plants treated with 5% (T1), 18% (T2) and 46% (T3) OMW.

Table 6 Dry matter (g) accumulation in the different portions of plants of the Ottobratica cultivar in the different treatments at the end of the trial.

Treatments	Leaf	Branch	Shoot	Stem	Structural root	Absorbent root
Control	64.68 a*	25.70 b	6.92 c	126.46 b	15.53 b	11.13 b
T1	65.75 a	31.43 a	9.70 a	175.21 a	16.20 a	29.59 a
T2	56.53 b	24.24 b	8.32 c	110.60 c	15.14 b	11.40 b
T3	55.20 b	21.61 c	6.53 c	104.45 d	12.60 c	8.69 c

*Different letters within each column represent significant differences at $P \leq 0.05$.

combination of high transpiration rates and lack of phloem connections which would allow the retranslocation of excess ions (Glatzel 1983).

Effects on root length

In T1 longer absorbent roots were observed than in control, in T2 and T3 treatments (**Fig. 5**). A decrease in root length with an increase in OMW% contained in the mixture was evident (**Fig. 6**).

Effects on stomatal conductance

The lowest values of stomatal conductance were detected in the control plants; stomatal conductance was significantly higher in T1 compared to other treatments (**Fig. 7**).

With increasing concentration of OMW a linear decrease in the values of stomatal conductance, leaf area and length of absorbent roots was observed (**Fig. 8**).

Effects on dry matter synthesized and accumulated by the plant

The productive potential of the tree can be expressed in terms of dry matter produced (Monteith 1977; Palmer 1981), value that reflect the plant efficiency.

The value of this parameter increased significantly in T1 (325 g) compared to the control plants (250 g) (**Fig. 9**). This value is probably linked to higher stomatal conductance in the leaves of the plants in this treatment (**Fig. 7**). In T2 and T3, a decrease of 10 and 13%, respectively, was observed compared to the control plants (**Fig. 9**).

The dry matter content was higher in T1 treatment, in both hypogeous and epigeous portion (**Table 6**). In particular, the largest percentage increase was observed in absorbent roots.

No significant differences were observed at the end of the trial between the pH of the substrate of the control plants (6.53) and T1 (6.23), while, compared to the control, T2 and T3 showed a reduction in pH (T2 = 5.5; T3 = 6).

Thus, if the OMW is adequately pre-treated (stored and diluted with irrigation water) and distributed with the fractioned irrigation techniques, it is possible to obtain the advantage of vegetative development of the plant while avoid-

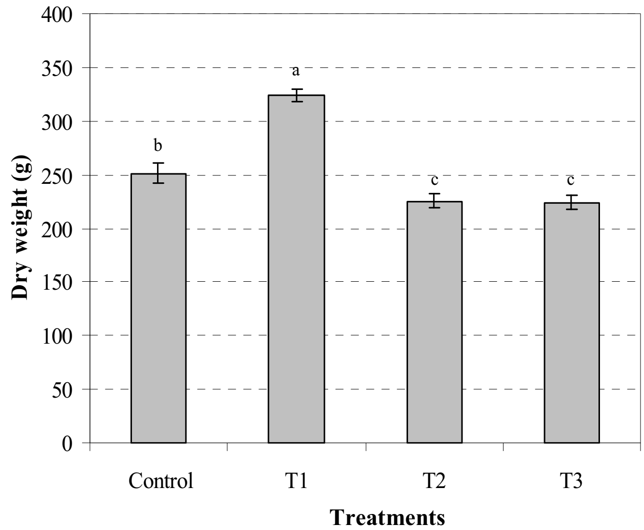

Fig. 9 Dry matter content in cv. 'Ottobratica' plants in different treatments at the end of the trial. Values represent mean ± Standard Error (SE). Different letters represent significant difference at $P \leq 0.05$.

ing symptoms of toxicity according to Briccoli Bati and Lombardo (1990).

CONCLUSION

During the final stage of propagation of "Ottobratica" olive trees in the nursery, the utilization of pre-treated OMW does not produce negative changes in the growth parameters, according to Proietti *et al*. (1988) and Gioffrè *et al*. (2005). In our experiments, the best performance occurred when the OMW concentration was 5%. In this case the concentration of polyphenols and BOD_5 and COD contents did not produce negative effects on growth, but a vegetative development was observed with an increase in dry matter. When OMW is used in a mixture of 18%, during the first doses there are evident advantages for the plants, but successive doses determine conditions of toxicity, which inhibit the development of the root system, according to Briccoli Bati and Lombardo (1990).

The highest K content in T1 treatment could be related to the highest stomatal conductance which promotes increased photosynthesis, which corresponds to a larger amount of dry matter and a greater shoot development.

The use of appropriately diluted OMW to irrigate young olive trees in pots (Lo Giudice 1993), which is an important feature of modern nurseries (Tattini 1993), can be considered an effective and valid fertilizer and an effective method for the discharging of OMW. The technique of plants grown in pots allows the use of larger quantities of OMW over the limits imposed by Italian law (G.U. Repubblica Italiana 1996).

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