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Influence of propagation techniques on growth and yield of olive trees cultivars 'Carolea' and 'Nocellara Etnea'

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Abstract

The vegetative and productive performance of micro-propagated olive plants in comparison with grafted and own-rooted plants was evaluated in southern Italy. Two cultivars of *Olea europaea* L. 'Carolea' and 'Nocellara Etnea', were planted in 1997 at two-row spacings: 6 m × 3 m and 6 m × 6 m. Percentage of flower bud differentiation, fruit weight, yield, pruning material and trunk diameter were measured on each plant of the experimental plot. Plants came into flowering the second year after planting with significant differences between cultivars. Micro-propagated 'Nocellara Etnea' plants came into bearing as early as the second year, whereas grafted plants had no bearing. Eight years after planting, cumulated yield of 'Nocellara Etnea' plants was almost double as compared to the 'Carolea' plants. Yield from micro-propagated plants was slightly higher with respect to grafted plants in 'Nocellara Etnea', but fruit weight was significantly lower. Micro-propagated 'Carolea' plants have shown a similar percentage of flower buds but a very low cumulated yield in the period of the trial, due to poor fruit set. In general, vegetative growth was significantly higher on plants with lower crop level. Our results have shown that micro-propagated plants did not exhibit any juvenile trait as, for instance, delay in flowering. In vitro propagation can thus be a rapid and a powerful olive propagation technique. Further investigations are however necessary to check if the main phenological differences observed (average fruit weight and poor fruit set) are somehow due to genetic modifications induced by in vitro propagation.

Keywords: *Olea europaea* L.; In vitro propagation; Own-rooted plants; Grafted plants; Oil content

1. Introduction

Over the last years, the Italian olive growing has been experiencing deep changes in its production system. In the nursery sector, own-rooted plants of most cultivars are now available together with the traditional grafted plants. At present, about 60% of marketed olive plants come from own-rooted cuttings. Own-rooted plants have some advantages over the grafted ones, i.e, they: (i) provide genetically homogeneous material; (ii) require shorter stay in the nursery; (iii) have lower production costs.

Thanks to the intense research activity of these last years, micro-propagated plants have been obtained also for olive (Rugini, 1984; Rama and Pontikis, 1990; Cozza et al., 1997; Leitão et al., 1997; Briccoli Bati et al., 1999; Chaari-Rkhis et al., 1999; Dimassi, 1999; Roussos and Pontikis, 2002; Zuccherelli and Zuccherelli, 2002; Santos et al., 2003).

Micro-propagation has a number of advantages over grafting and own-rooting: (i) the production of genetically uniform and pathogen-free plant material in a short time; (ii) the possibility of propagating cultivars difficult to obtain through own-rooted cutting; (iii) the possibility of exporting in vitro material more rapidly with no obligation to have a long quarantine period; (iv) the possibility of scheduling plantlet production closer to the market demand.

Though successfully applied in different public laboratories and on various cultivars this technique is not widely diffused in nurseries yet, especially because of the high cost of some products required during the in vitro maintenance phase of explants. Notwithstanding, Zuccherelli and Zuccherelli (2002) have recently reported that different olive cultivars can be mass in vitro-propagated.

Knowledge on the behaviour of micro-propagated olive plants in the field is still scarce if compared to that on plants

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from grafted or own-rooted cuttings (Briccoli Bati et al., 2002; Leva et al., 2002). The preliminary results of these experiments indicate that, differently from other species where relatively important epigenetic variations of the starting cultivars have emerged (Rani and Raina, 2000), micro-propagation techniques applied to olive tend to maintain the genetic, physiological and phenological characteristics of the mother plants as early as at the acclimatization phase (Santos et al., 2003; Brito et al., 2003).

The aim of this work was to assess, over a relatively long period, the main vegetative and productive characteristics of two olive cultivars ('Carolea' and 'Nocellara Etnea'), the former being obtained by grafting, micro-propagation and own-rooting, the latter – due to its very poor rooting ability – only through grafting and micro-propagation.

2. Materials and methods

2.1. Plant material

Two cultivars of *Olea europaea* L. were used: 'Carolea' and 'Nocellara Etnea'. All the plant material was obtained from one single mother plant for each variety.

2.1.1. Grafted plants

Own-rooted clonal cuttings of *Olea europaea* L. var. *oleaster* Hoffmanns & Link, were obtained by taking fruiting shoots from one single mother plant. The shoots were collected at the vegetative resumption (March), this being a time that previous experiments proved to be the best to obtain the highest rooting percentage of olive cuttings.

From the medial portion of each fruiting shoot, a cutting of five nodes was obtained. The three basal nodes were defoliated, whereas the leaves of the two apical nodes were maintained. The basal part of the cuttings was soaked for 5 s in a hydroalcoholic solution of indole-3-butyric acid (IBA, Sigma Aldrich), at 1 mg L^{-1}. Immediately after, they were left to root in '*agriperlite*'® on a bench heated at 23 °C and covered with plastic film. A mist nozzle was installed on the bench to keep air relative humidity close to saturation (RH > 90%) and prevent dehydration of the cutting. After approximately 3 months, the own-rooted cuttings were transplanted into pots of 1.5 L with a mixture of soil and peat moss (1:2, v/v) for hardening. Later, once the plants exhibited 10–15 cm growth of the new shoot, they were transferred into 3 L containers. At this stage, the soil and peat moss mixture was 2:1 (v/v).

Grafting was performed in the subsequent spring, using uninodal mature scion of 'Carolea' and 'Nocellara Etnea' cultivars. After grafting, plants were kept in the greenhouse until planting in the field.

2.1.2. Own-rooted plants

Own-rooted plants of cv. 'Carolea' were obtained following the same procedure previously described for obtaining the clonal rootstock of *Olea europaea* L. var. *oleaster*. Therefore, also in this case, the fruiting shoots were taken early at the vegetative resumption. Cutting being rooted, the plantlets were transplanted twice and grown in the greenhouse until planting in the field.

2.1.3. In vitro plants

Olive micro-propagated plants were obtained through the following steps.

- Obtaining explants: early at the vegetative resumption of 1993, some fruiting shoots of the two cultivars were taken and uninodal cuttings to be introduced in vitro were obtained from the medial part of each.
- Establishment of explants: the leaves of single node explants were excised and the explants were thoroughly washed under running tap water overnight. The explants were then vacuum disinfected in a 2‰ (v/v) solution of Mercury chloride for 10 min, with periodical agitation, followed by rinsing three times with sterile bi-distilled water. The in vitro culture of olive explants was initiated into test tubes filled with 5 mL initial medium, as described by Rugini (1984), lacking growth regulators and supplemented with 20 g L^{-1} mannitol as carbon source. The medium was adjusted to pH 6.0 before adding '*phytagel*™' (Sigma Aldrich) at 2.5 g L^{-1} as gelling agent, and autoclaved at 121 °C for 16 min. Cultures were incubated at 25 ± 1 °C with a 16 h photoperiod provided by cool white fluorescent lamps (40 μmol m^{-2} s^{-1}).
- Multiplication of explants: after 20 days, the explants exhibiting no contamination were shifted on a proliferation medium derived from the olive medium (Rugini, 1984) modified using half concentration of macro-elements, D-mannitol (30 g L^{-1}) and 4 mg L^{-1} *trans*-zeatin (Sigma Aldrich). Every month the explants were sub-cultured into the same proliferation fresh medium.
- Shoot rooting: after 4 years in proliferation medium, the explants with three or four nodes were transferred into root inducing medium that was half-strength proliferation medium, devoid of *trans*-zeatin and containing 160 mg L^{-1} 1 putrescine dihydrochloride (Rugini, 1988) and 2 mg L^{-1} IBA. Rooting took place after 4 weeks.
- Acclimatation: the rooted plantlets were acclimatized on ''coco-pot'' in transparent plastic chamber under controlled environmental conditions (25 °C temperature, RH at 90%, photoperiod of 16 h per day as above). After 1 month, the plantlets were transplanted into pots of 1.5 L containing a mixture of soil and peat moss (1:2, v/v) and placed in the greenhouse. The second transplant in pots of 3.0 L took place 3 months later, in the same way as own-rooted and grafted plants.

In all the adult phase plants thus obtained, the root system was of equal age, whereas the top was 1 year younger in the grafted plants. Nevertheless, plantlets exhibited homogeneous vegetative development after planting.

2.2. Site, planting description and cultural practices

The trial was carried out from 1997 through 2004 near Metaponto, southern Italy (latitude N40°24′, longitude E16°41′).

The climate of the area was classified as warm semi-arid, with an average annual rainfall of 600 mm mostly concentrated in the October–February period, and a monthly average temperature ranging from 7.8 to 25.6 °C.

The soil of the experimental field is silty-clay, with groundwater at about 2.00 m depth. In this area, typically silty-clay soil has sufficient amounts of P, K, Ca and Mg, and very small quantities of organic matter and total nitrogen. From the hydrological point of view, these soils have 16% available water (on dry weight basis and calculated as the difference between the values of soil water content at −0.03, and −1.5 MPa) and 1.25 t m^{-3} soil bulk density (Lacertosa et al., 1998).

Two-year-old olive trees were planted late in winter 1997. Two planting spacings, 6.0 m × 3.0 m and 6.0 m × 6.0 m, and two repetitions per spacing were adopted. Each variety and each type of propagation material were randomly arranged within every planting spacing. The split-plot experimental design was adopted with the planting spacings as the main plot and each variety/propagation material combination being represented by 12 trees. In total, 72 plants represented 'Carolea' and 48 plants 'Nocellara Etnea'. Rows were North–South oriented.

Plants were free-vase trained with an averaged trunk height of about 0.5–0.7 m and 3–5 primary branches inclined at about 45° and oriented in different directions, surrounding an open space in the central part of the canopy.

During the first growing season, plants with a good number of lateral shoots at about 0.5–0.7 m from the soil surface were left to grow freely, while lateral shoots situated below 0.5 m were removed. In order to have a well-balanced canopy, when necessary, vigorous shoots and watersprouts were weakened by bending or twisting, or completely eliminated. The main stem was headed during the first growing season or the subsequent years (the second or the third) when the primary branches were definitively chosen. Primary branches were selected from laterals that were at least 0.05 m apart vertically and oriented in different directions to avoid overlapping and mutual shading. When necessary, the different orientations were obtained by binding the laterals to a trainer.

During the first and the third year after planting, pruning also eliminated the overlapping branches or shoots, the excessively low-hanging shoots, the shoots growing upright in the central or in the inner part of the canopy, and the suckers arising from the trunk and the stump.

From the fifth year after planting, primary branches were headed back to a lateral shoot or branch to decrease the height and lateral expansion of the canopy; overcrossing shoots and watersprouts from the central part of the canopy were removed, suckers arising from the trunk and the stump were also eliminated, exhausted shoots were eliminated and secondary and tertiary branches were renewed.

Normally, summer and dormant pruning was performed every 2 years. Summer pruning was normally performed in July or the first week of August; at these stages all strong shoots (watersprouts type) arising from the upper side of the selected scaffolds or from the renewal branches were eliminated since they tended to block light penetration through the canopy. Dormant pruning was normally performed in January. All exhausted fruiting shoots growing in the shaded part of the canopy were eliminated; crown, trunk and stump suckers were eliminated and fruiting shoot selection was also performed.

With respect to the normal pruning methods usually adopted in the area, we tried to prune the trees without eliminating too much foliage.

Weed growth was controlled by regular shallow tillage; pest and disease control was applied according to the regional service recommendations for commercial olive grove.

The entire plot, of approximately 8000 m^2, was irrigated using a localized system (microjets discharging 80 L h^{-1} over 1 m radius). Meteorological variables were measured by a standard weather station placed close to the trial field. Reference evapotranspiration (ETo) was calculated using Hargreaves equation; irrigation volume and scheduling were determined using a simplified soil water balance. Crop evapotranspiration (ETc) was calculated with the two steps procedure. Following Pastor and Orgaz (1994), the monthly crop coefficient (K_c) for olive orchards having 60% ground cover was: 0.50, 0.50, 0.65, 0.60, 0.55, 0.50, 0.45, 0.45, 0.55, 0.60, 0.65 and 0.50 from January through December. No reduction of K_c for immature stand was made.

Fertilizers were ground applied on a per-tree basis in March, at the beginning of the annual cycle, and in May, at flowering (Xiloyannis et al., 2002). The units of applied nitrogen are given in Table 1.

2.3. Measurements and statistical analysis

2.3.1. Vegetative growth

On each tree of the trial, the trunk diameter was measured at harvest, at 0.40 m from the soil surface. The pruned material was also weighed on each tree of the plot just after pruning. The summer and winter pruned material was added up. The total weight was submitted to the analysis of variance.

2.3.2. Reproductive measurements

Olives were harvested when the cultivar 'Carolea' was at veraison. Olive yield was measured on each tree. Fruit fresh weight was measured on 4 samples of 25 fruits randomly selected from the total fruits harvested per each spacing/variety/propagation material combination.

The start of flowering was measured on all the plants of the trial in the second and the third year after planting, through

Table 1
Annual crop evapotranspiration (ETc), rainfall, seasonal irrigation volume and unit of nitrogen in the period of the trial

	Years after planting							
	I	II	III	IV	V	VI	VII	VIII
ETc (mm)	606	630	633	657	659	625	650	626
Rainfall (mm)	652	470	316	368	320	683	583	730
Seasonal irrigation volume (mm)	Not meas.	85	102	180	300	116	164	300
Nitrogen (g $tree^{-1}$)	14	72	108	133	162	194	245	288

counting the number of fruiting shoots with flowers out of the total number of fruiting shoots present on the plant. Arbitrarily, we considered as ''flowered plant'' those plants presenting a percentage of flowering in fruiting shoots greater than 50%. Whereas, from the third year after planting on, flower buds were determined on 10 one-year-old shoots per tree, randomly selected around the crown; all the trees of the trial were taken into account for this determination. The percentage of flower buds was obtained as a ratio between the total number of buds on the fruiting shoots and the number of flower buds.

2.3.3. Fruit and leaf analysis

Oil content was determined on a sample of about 100 fruits per each plot, by extracting dry material with 40–60 °C petroleum ether using a Soxhlet apparatus. Olives were dried at 70 °C in a ventilated oven until a constant weight was measured in two successive weighing measurements. Olives were ground in a mortar, the paste was weighed and analyzed by Soxhlet apparatus (Donaire et al., 1977).

The nutritional status of the olive plants was determined at 4-year intervals (2000 and 2004) by leaf analysis of a sample of about 100 leaves per plot. Fully-expanded, mature leaves from the middle portion of non-bearing, current season shoot were collected in July for mineral nutrients analysis. Leaves were collected in paper bags and stored in a portable ice basket. Once in the laboratory, they were washed with 0.03% Triton X-100, rinsed in deionized water, dried at 80 °C for 48 h, ground and stored until analysis. Nitrogen was determined by the Kjeldahl procedure.

Other elements (P, K and B) were determined using an inductively coupled plasma-mass spectrometer (Agilent technologies series 7500) after mineralization of 300 mg of sample dissolved in 6 mL of HNO_3 and 2 mL of H_2O_2 in a micro-wave oven (Milestone srl, Bergamo, Italy).

3. Statistical analysis

All the collected data were analyzed through the analysis of variance, comparing the means with the Holm–Sidak test reported in the statistical package Sigmastat 3.0.1 (SPSS, Inc.).

In this work, the ''planting density'' factor was not considered in the statistical analysis of results.

4. Results

Over the 8 years of the trial, the ETc pattern was practically constant, while the erratic amount and distribution of rainfall resulted in different seasonal irrigation volumes that ranged between 85 mm the second year after planting and 300 mm the fifth and the eighth year after planting (Table 1).

4.1. The effects of the cultivar

Between the two cultivars, differences on the number of trees with flowers were observed only in the second year after planting, while the third year almost all plants exhibited a quite high number of flowers (Fig. 1). From the third to the eighth year after planting, the number of flower buds was statistically different on the fourth, the seventh and the eighth year after planting. 'Nocellara Etnea' has shown a higher number of trees with flowers in the second year after planting and, with the exception of the third and the fourth year after planting, a higher number of flower buds as compared to 'Carolea' (Fig. 2). However, the average value of flower buds was almost similar and equal to 50.75% on 'Carolea' trees and to 52.48% in 'Nocellara Etnea' (Table 2).

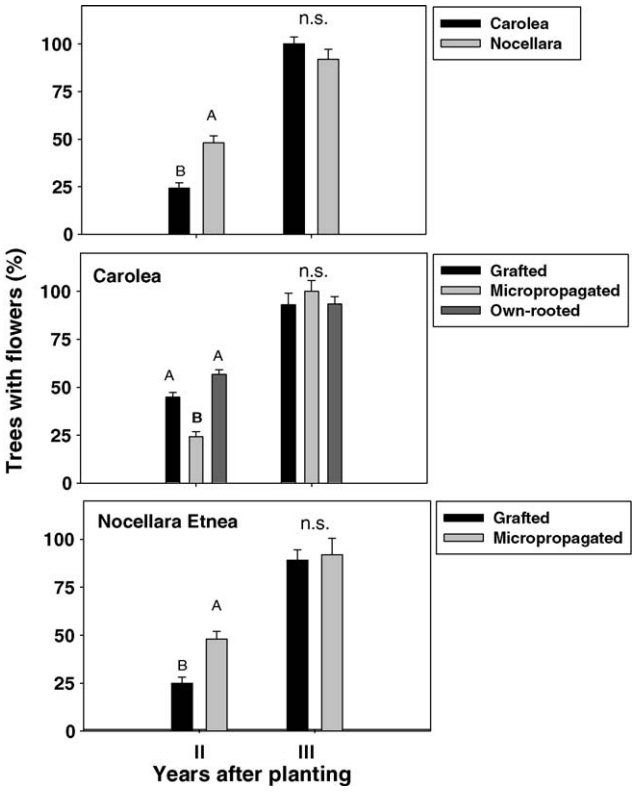

Fig. 1. Percentage of olive trees with flowers in the second and third years after planting on 'Carolea' ($n = 72$ plants) and 'Nocellara Etnea' ($n = 48$ plants). Trees with flowers were those having a number of flowered shoots equal to at least 50% of the total 1-year-old shoots of the tree. 'Carolea' trees were obtained by own-rooting, grafting and micro-propagation techniques. 'Nocellara Etnea' trees were obtained by grafting and micro-propagation techniques. Both cultivars were present with 24 plants per propagation method. Different capital and small letters represent statistical differences at $p < 0.01$ and 0.05, respectively, n.s. means non-significant differences.

Olive bearing started in the second year after planting with 0.15 kg tree^{-1} yield on 'Nocellara Etnea' and 0.005 kg tree^{-1} on 'Carolea'; the differences were statistically different at $p < 0.001$. Maximum yield in the period of the trial was measured on the fifth year after planting both for 'Carolea' and 'Nocellara Etnea'. 'Nocellara Etnea' yielded 10.02 kg tree^{-1}, while 'Carolea' yielded less than 5.89 kg tree^{-1} (Fig. 3). After 8 years, cumulated yield was 29.76 kg tree^{-1} in 'Nocellara Etnea' and 17.12 kg tree^{-1} in 'Carolea' (Table 2).

The cultivar also has an effect on oil content, both on dry and fresh weight basis, and on fruit weight. 'Carolea' has a significantly higher oil content and fruit weight than 'Nocellara Etnea' (Table 3).

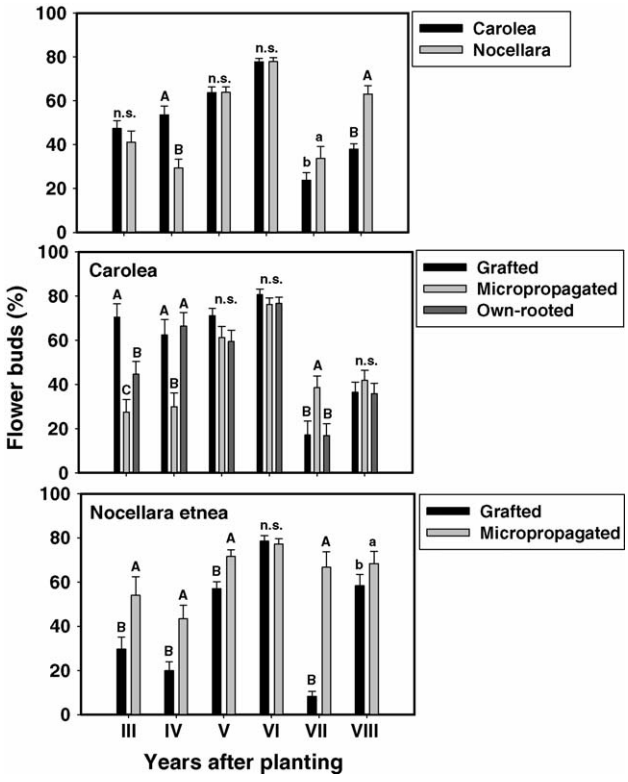

Fig. 2. Percentage of flower buds per shoot from the third to the eighth year after planting on 'Carolea' ($n = 72$ plants) and 'Nocellara Etnea' ($n = 48$ plants) obtained with different propagation method. Both cultivars were present with 24 plants per propagation method. Different capital and small letters represent statistical differences at $p < 0.01$ and 0.05, respectively, n.s. means non-significant differences.

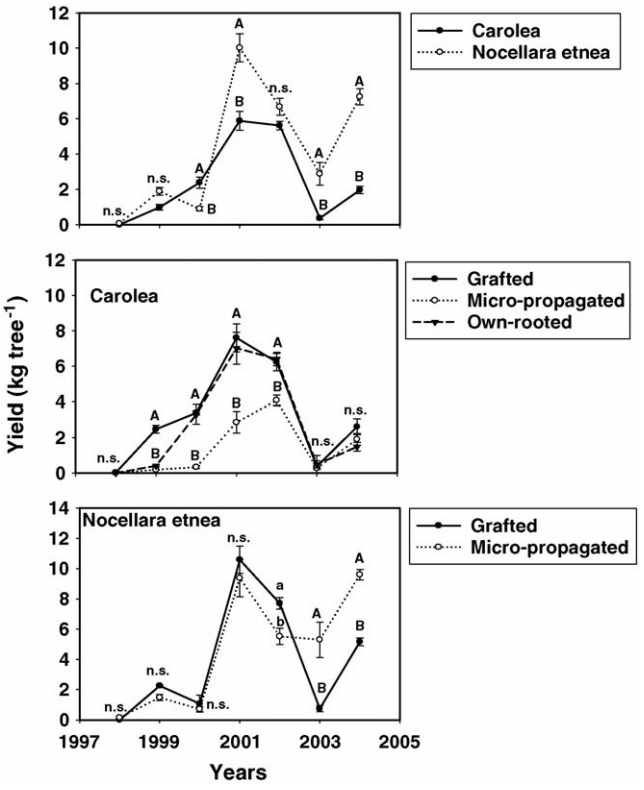

Fig. 3. Yield per plants from the second to the eighth year after planting on 'Carolea' ($n = 72$ plants) and 'Nocellara Etnea' ($n = 48$ plants) obtained with different propagation methods. Yield was measured at harvest on all trees of the trial. Both cultivars were present with 24 plants per propagation method. Different capital and small letters represent statistical differences at $p < 0.01$ and 0.05 respectively, n.s. means non-significant differences.

The trunk diameter was not statistically different in the first year after planting but was significantly greater in 'Carolea' than in 'Nocellara Etnea' the following years. As a matter of fact, the trunk diameter increased by about 3 cm year^{-1} in 'Carolea' plants and about 2 cm year^{-1} in 'Nocellara Etnea' plants (Fig. 4). The data on pruning material equally confirmed the higher vegetative growth as measured through the trunk diameter. 'Carolea' showed significantly higher pruning material in the fifth and seventh year after planting with respect to 'Nocellara Etnea' (Fig. 5). The cumulated amount of pruning material at the end of the seventh year was statistically different between the two cultivars, i.e.: 38.88 kg tree^{-1} in 'Carolea' plants and 30.46 kg tree^{-1} in 'Nocellara Etnea' (Table 2).

In 2000 no differences in leaf N, P and K content were observed, while in 2004 the leaf content of B was significantly higher in 'Carolea' with respect to 'Nocellara Etnea' (Table 4). Moreover, the leaf mineral content determined in 2004 was statistically higher than 4 years before.

Table 2
Average flower buds, cumulated yield and pruning material over 8 years (1997–2004) of 'Carolea' and 'Nocellara Etnea' obtained with different propagation techniques

Cultivar[a]	Type of plants[b]	Flower buds (%)	Yield (kg tree^{-1})	Pruning weight (kg tree^{-1})
Carolea		50.75 ± 1.61	17.12 ± 0.86 B	38.88 ± 2.30 A
Nocellara Etnea		52.48 ± 2.29	29.76 ± 1.55 A	30.46 ± 1.55 B
Carolea	Grafted	56.40 ± 2.57 A	22.67 ± 1.31 a	28.69 ± 2.89 c
	Micro-propagated	45.90 ± 3.35 B	9.60 ± 0.83 c	40.82 ± 4.33 b
	Own-rooted	49.99 ± 2.20 B	19.08 ± 0.95 b	47.12 ± 3.78 a
Nocellara Etnea	Grafted	41.45 ± 2.05 B	27.52 ± 2.04	37.88 ± 3.17 A
	Micro-propagated	63.52 ± 2.78 A	32.00 ± 2.29	23.04 ± 2.19 B

Flower buds data are the average for the period 1999–2004, while yield and pruning weight are cumulated values over the same period. Different capital and small letters represent statistical differences at $p < 0.01$ and 0.05, respectively. Non-significant differences were not reported.

[a] Data are average ± standard error of 72 single values for Carolea and 48 single values for Nocellara Etnea.

[b] Data are average ± standard error of 24 single values.

Table 3
Some fruit characteristics of 'Carolea' and 'Nocellara Etnea' obtained with different propagation techniques

Cultivar[a]	Type of plants[b]	Oil content		Fruit weight (g)
		(% Dry weight)	(% Fresh weight)	
Carolea		48.76 ± 0.47 A	18.01 ± 0.23 A	5.99 ± 0.18 A
Nocellara Etnea		45.56 ± 0.76 B	17.28 ± 0.36 B	4.86 ± 0.24 B
Carolea	Grafted	48.22 ± 0.74 b	18.18 ± 0.40 A	6.21 ± 0.30 A
	Micro-propagated	48.66 ± 0.76 ab	17.56 ± 0.40 B	6.08 ± 0.31 B
	Own-rooted	49.40 ± 0.68 a	18.30 ± 0.39 A	5.68 ± 0.30 B
Nocellara Etnea	Grafted	46.30 ± 0.92 A	18.09 ± 0.46 A	5.83 ± 0.32 A
	Micro-propagated	44.79 ± 1.10 B	16.47 ± 0.52 B	3.96 ± 0.33 B

Data for oil content are the average of 5 years, while data for fruit weight are the average of 4 years. Different capital and small letters represent statistical differences at $p < 0.01$ and 0.05, respectively.
[a] Data are average ± standard error of 45 single values for Carolea and 30 single values for Nocellara Etnea.
[b] Data are average ± standard error of 15 single values.

4.2. The effects of propagation techniques on 'Nocellara Etnea'

In the second year after planting, flowering was observed in 48% of micro-propagated plants and 25% of grafted ones. This difference was statistically significant. Whereas, in the third year after planting, almost all the plants exhibited abundant flowering without statistical differences between differently propagated plants (Fig. 1). The percentage of flower buds, observed from the third year after planting on, was significantly higher in micro-propagated plants as compared with the grafted ones (Fig. 2). On average, in the years of the trial, the micro-propagated plants had a flower bud percentage equal to 63.52% as compared to 41.45% of grafted plants (Table 2).

Micro-propagated plants came into bearing as early as at the second year, whereas grafted plants had no bearing. However, from the third to the sixth year, amounts of olives were greater on grafted plants although the differences were statistically

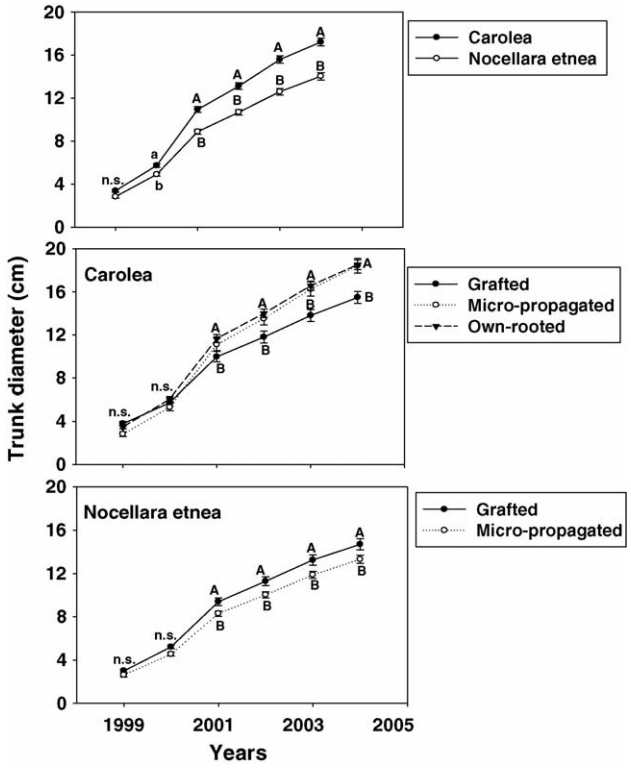

Fig. 4. Trunk diameter measured at harvest in 'Carolea' ($n = 72$ plants) and 'Nocellara Etnea' ($n = 48$ plants) obtained with different propagation methods. Trunk diameter was measured at harvest on all trees of the trial. Both cultivars were present with 24 plants per propagation method. Different capital and lower case letters represent statistical differences at $p < 0.01$ and 0.05, respectively, n.s. means non-significant differences.

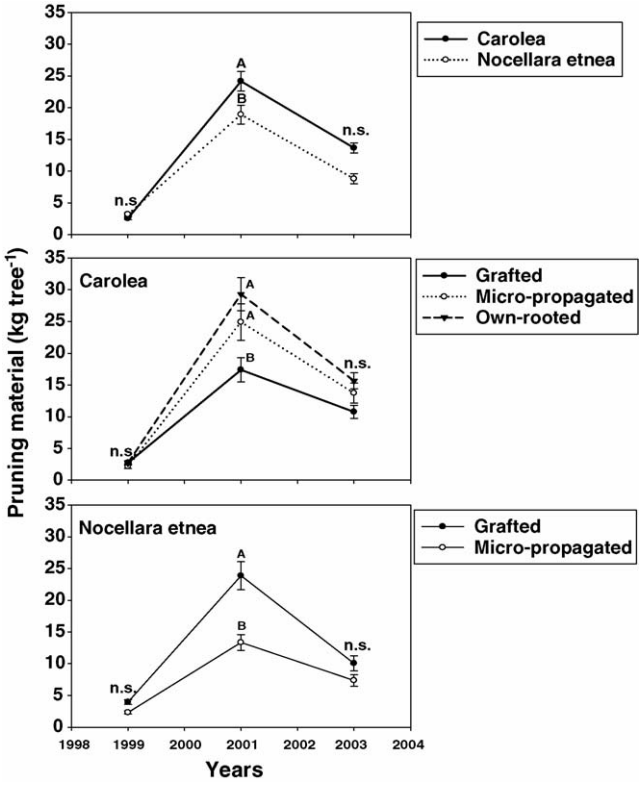

Fig. 5. Vegetative material removed by pruning in 'Carolea' ($n = 72$ plants) and 'Nocellara Etnea' ($n = 48$ plants) obtained with different propagation methods. Both cultivars were present with 24 plants per propagation method. Pruning was performed twice every 2 years: in summer and in winter; the weight of the pruned material was measured both on each tree of the trial and each pruning event, the sum of the two events are presented in the graph. Different capital and small letters represent statistical differences at $p < 0.01$ and 0.05, respectively, n.s. means non-significant differences.

Table 4
Leaf N, P, K, and B concentration of 'Carolea' and 'Nocellara Etnea' obtained with different propagation techniques

Cultivar[a]	Type of plants[b]	N (%)		P[c] (%)		K (%)		B (ppm)	
		2000	2004	2000	2004	2000	2004	2000	2004
Carolea		1.85 ± 0.06	2.14 ± 0.03	0.11	0.12	0.90 ± 0.02	0.93 ± 0.02	15.5 ± 0.23	17.0 ± 0.28 a
Nocellara Etnea		1.85 ± 0.02	1.97 ± 0.04	0.11	0.12	0.94 ± 0.03	0.92 ± 0.03	15.4 ± 0.47	15.8 ± 0.45 b
Year (p)		<0.001		0.030		n.s.		0.012	
Cultivar × year (p)		n.s.		n.s.		n.s		n.s.	
Carolea	Grafted	1.71 ± 0.07 B	2.17 ± 0.54 A	0.12 A	0.13 a	0.88 ± 0.02 b	0.96 ± 0.02 a	16.0 ± 0.07	17.1 ± 0.54
	Micro-propagated	2.07 ± 0.55 A	2.22 ± 0.16 A	0.11 B	0.11 b	0.98 ± 0.01 a	0.98 ± 0.03 a	14.9 ± 0.54	16.8 ± 0.16
	Own-rooted	1.77 ± 0.05 B	2.04 ± 0.75 B	0.10 B	0.13 a	0.85 ± 0.02 b	0.86 ± 0.05 b	15.9 ± 0.05	17.1 ± 0.75
Year (p)		<0.001		0.003		n.s.		0.002	
Cultivar × year (p)		<0.001		0.014		n.s.		n.s.	
Nocellara Etnea	Grafted	1.88 ± 0.03	1.90 ± 0.06 b	0.10 B	0.11	0.90 ± 0.01	0.92 ± 0.03	16.3 ± 0.49 a	14.9 ± 0.30 b
	Micro-propagated	1.82 ± 0.01	2.04 ± 0.02 a	0.12 A	0.12	0.98 ± 0.04	0.91 ± 0.05	14.5 ± 0.31 b	16.7 ± 0.35 a
Year (p)		0.007		n.s.		n.s.		n.s.	
Cultivar × year (p)		0.020		n.s.		n.s.		0.001	

Different capital and small letters represent statistical differences at $p < 0.01$ and 0.05, respctively, n.s. means non-significant differences. Non-singificant differences were not reported.

[a] Data are average ± standard error of nine single values for Carolea and six single value for Nocellara Etnea.

[b] Data are average ± standard error of three single values.

[c] Standard error being less than 0.005 was not reported.

significant only in the sixth year ($p < 0.05$), whereas in the seventh and the eighth year, the micro-propagated plants produced a significantly higher ($p < 0.001$) amount of olives than the grafted plants (Fig. 3). The cumulated yield in the eighth year after planting was statistically similar and equal to 32.00 kg tree^{-1} of olives in the micro-propagated plants and 27.52 kg tree^{-1} of olives in the grafted ones (Table 2).

The in vitro plants of 'Nocellara Etnea' exhibited significantly smaller fruits (averaged from the third to the eighth year) than the corresponding grafted plants, 3.96 ± 0.33 and 5.83 ± 0.32 g fruit^{-1} respectively (Table 3). The minimum value of the average fruit weight was recorded for both the types of plants in the eighth year from planting (3.37 ± 0.34 g fruit^{-1} in micro-propagated plants and 3.81 ± 0.18 g fruit^{-1} in the grafted ones, with non significant differences). Maximum values were observed in the seventh year from planting (4.33 ± 0.08 g fruit^{-1}) for the micro-propagated plants and in the third year from planting (7.38 ± 0.22 g fruit^{-1}) for grafted plants.

Also oil content of drupes from micro-propagated plants was significantly lower ($p < 0.01$).

Vegetative development as determined through the trunk diameter, showed a smaller diameter growth in the micro-propagated plants than in grafted ones (Fig. 4).

As for pruning wood, the amount of plant material removed from the grafted plants was almost double as compared to the micro-propagated plants (Fig. 5 and Table 2).

Some statistical differences (generally at $p < 0.05$) were observed in leaf mineral content between micro-propagated and grafted plants. Four years after planting, micro-propagated plants have shown a higher level of phosphorous (at $p < 0.01$), and a lower level of boron, while 8 years after planting, grafted plants have shown a lower concentration of nitrogen and boron with respect to the micro-propagated ones (Table 4).

4.3. The effects of propagation techniques on 'Carolea'

In 'Carolea' cultivar, in the second year after planting, flowering occurred on 24% of micro-propagated plants, 45% of grafted plants and 58% of the own-rooted ones. In the third year, practically all the plants exhibited flowers (Fig. 1).

As for the percentage of flower buds, in the third year, the grafted plants exhibited a statistically higher percentage than the two other types of plants. Whereas, in the fourth year from planting, the percentage of flower buds of own-rooted and grafted plants was similar and significantly higher than the micro-propagated plants. In the fifth, sixth and eighth year, no significant difference was observed between the different types of compared plants, only in the seventh year from establishment the micro-propagated plants had a higher percentage of flower buds than the own-rooted and grafted plants (Fig. 2).

Bearing was practically zero on all the plants of the compared treatments in the second year after planting (Fig. 3). In 'Carolea', olive bearing started in the third year after planting on grafted plants, whereas yield was very low on the two other types of plants. From the fourth year after planting on, the grafted and own-rooted plants produced practically the same amounts of olives, whereas the yield levels of the micro-propagated plants were significantly lower ($p < 0.001$). In the eighth year, cumulative yield was 9.60 kg tree^{-1} in micro-propagated plants, 22.67 kg tree^{-1} in grafted plants and 19.08 kg tree^{-1} in own-rooted plants.

The weight of drupes (the average from the third through to the eighth year) was not significantly different between the two types of plants (Table 3). Oil content from drupes of micro-propagated plants was significantly different at $p < 0.001$ with respect to the grafted or own-rooted plants only when measured on fresh weight basis (Table 3).

As for the trunk diameter, the own-rooted and micro-propagated plants showed values practically similar to each other but significantly higher ($p < 0.01$), by about 20%, with respect to the grafted ones (Fig. 4). Also for pruning wood, cumulated wood removal in the eighth year after planting was significantly higher at $p < 0.05$ in own-rooted plants (47.12 kg tree^{-1}) with respect to micro-propagated (40.82 kg tree^{-1}) and grafted plants (28.69 kg tree^{-1}). Also the difference between the micro-propagated plants and the grafted plants was significantly higher (Table 2 and Fig. 5).

Four years after planting, leaf N and K concentration of micro-propagated plants was significantly higher as compared to grafted and own-rooted plants ($p < 0.01$ and $p < 0.05$, respectively for N and K), while leaf P concentration was lower at $p < 0.01$ in micro-propagated and own-rooted with respect to grafted plants. Eight years after planting, leaf N and K concentration was significantly lower in own-rooted plants with respect to micro-propagated and grafted plants. Leaf P concentration was statistically ($p < 0.05$) lower in micro-propagated plants as compared to the other two propagation methods (Table 4).

5. Discussion

To the best of our knowledge, this was the first trial where two olive cultivars, 'Carolea' and 'Nocellara Etnea' obtained by grafting, own-rooting and in vitro micro-propagation, were compared in field conditions over 8 years after planting. Also Leva et al. (2002) have reported some results of a field trial were in vitro micro-propagated and grafted olive plants where compared, but they set up the trials 1 year later and have reported only the results of the first three growing seasons. Our findings indicate that, despite the prolonged in vitro proliferation phase (more than 4 years) of explants, micro-propagated plants exhibited no juvenile traits and came into bearing as early as the second year after planting. Olive yield was comparable in grafted and micro-propagated plants of 'Nocellara Etnea', while it was significantly lower in micro-propagated plants of 'Carolea' with respect to grafted and own-rooted plants. Leva et al. (2002), by comparing grafted and micro-propagated plants of the cultivar Maurino, did not observe any juvenile trait both for the coming into bearing (their plants start bearing the second year after planting too) and for leaf characteristics. Moreover, they did not observe any variation in the amplification pattern of DNA or in the architecture of the canopy (number of lateral shoots, number of nodes per shoot and shoot length).

Differently from what was observed by Leva et al. (2002) with cultivar 'Maurino' only, our results show some differences between cultivars. For instance, micro-propagated 'Nocellara Etnea' plants showed greater flower bud differentiation, except the sixth year, with respect to the grafted plants. Whereas 'Carolea' micro-propagated plants, until the fourth year after planting, have shown significantly lower ($p < 0.001$) values of flower buds than the grafted and own-rooted plants (Fig. 2 and Table 1).

Also Rugini et al. (1995) have reported that micro-propagated plants of 'Canino' abundantly flowered as early as after 18–20 months of age, whereas those of the cultivar 'Dolce di Agogia' delayed flowering thus exhibiting a varietal component on the start of flowering also in micro-propagated plants. Though referred only to potted plants, their observations also agree with our data as for homogeneity in vegetative and production pattern. Under the conditions of the trial, the two compared cultivars did not always show significant differences in the percentage of flower buds. But 'Carolea' produced a significantly smaller amount of olives than 'Nocellara Etnea' 4 years out of 7. Such smaller olive yield can be attributed both to the cytoplasmic male sterility of 'Carolea' (Cavallotti et al., 2003) and to morphological sterility effects, like pistil abortion which is widely diffused in 'Carolea' cultivar (Iannotta et al., 1999), rather than to poor cross-pollination. As a matter of fact, some experimental data (Iannotta et al., 1996) indicate that numerous cultivars, 'Nocellara Etnea' being one of them, are good pollinators of 'Carolea'. Moreover, the presence of other traditional olive groves nearby (100–200 m) the experimental field would have guaranteed enough pollen for the two cultivars. In fact, Galán et al. (2004) were able to capture pollen grains of *Olea europaea* from olive groves distributed within a radius of 100 km surrounding the site where the pollen trap was located. We still need to check if micro-propagation in 'Carolea' cultivar could emphasize morphological sterility of the flower organs since the yield performance of 'Carolea' micro-propagated plants was particularly negative.

Our results have highlighted an effect of the propagation technique on the fruit size. In particular, in the two cultivars, the micro-propagated plants gave fruits of a significantly lower average weight ($p < 0.001$) than the grafted plants. In a characterization study of 25 cultivars of Sicilian olives, Barone et al. (1995) have reported an average fruit weight of 4.95 ± 1.41 g fruit^{-1} for 'Nocellara Etnea', which is practically intermediate between the value we measured on micro-propagated plants (3.96 ± 0.33 g fruit^{-1}) and the one of the grafted plants (5.83 ± 0.32 g fruit^{-1}). Conversely, for 'Carolea' other authors have reported an average fruit weight varying between 3.3 and 4.5 g fruit^{-1} (Inglese et al., 1999a,b). It is thus quite difficult to state that the differences recorded on the average weight of the fruit of 'Nocellara Etnea' are attributable to the effect of the propagation technique or to the greater olive yield observed in micro-propagated plants especially in the last 2 years. In fact, some authors have reported smaller fruits in rubus micro-propagated plants (Swartz et al., 1983), whereas Inglese et al. (1999a), referring to olive tree, have reported a significant decrease in the average fruit weight (from 4.3 ± 0.5 to 3.6 ± 0.7) with the increase in the number of fruits per shoot. Moreover, Lavee and Wodner (2004) have shown, on cvs. Barnea and Manzanillo, that olive fresh weight can be about 100% lower in high yield trees than low yield ones.

Oil content of drupes was another yield component exhibiting significant differences. Probably, the differences in this parameter are not so much attributable to the plant propagation techniques but to the influence of a number of factors like: variety, leaf/fruit ratio, canopy/root ratio, assimilate availability (Inglese et al., 1999a; Proietti, 2003; Lavee and Wodner, 2004).

In general, vegetative and reproductive activity in fruit trees takes place simultaneously and, in many circumstances, the available resources are not sufficient to sustain the two processes at a potential level. In particular, the presence of fruits exerts a strong competition with shoot growth. The different production levels recorded for the two cultivars could then account for the greater vegetative growth, expressed as trunk diameter and vegetative plant material removed by pruning, of 'Carolea' as compared to 'Nocellara Etnea'. The same remarks may apply when referring to grafted and micro-propagated plants of 'Nocellara Etnea', whereas the grafted plants of 'Carolea' have shown reduced vegetative activity as compared to the own-rooted and micro-propagated plants. Finally, the vegetative and production performance observed in our trial seems not to differ from what was observed by other authors who have reported comparable values of increase in trunk diameter, amount of pruning material and yield (Nuzzo et al., 1997; Palliotti et al., 1999).

Leaf N, P and K concentrations were 30%, 1% and 15% higher, respectively, while boron concentration was 16% lower than the sufficiency (ha un senso specifico o è superfluo?) threshold indicated by some authors for olive trees (Fernández-Escobar et al., 1999; López-Granados et al., 2004). However, our leaf N and K concentration was very close to the optimal concentration reported by Bongi and Palliotti (1994), and leaf boron concentration was approximately 10% higher than the deficiency threshold reported by the same authors.

6. Conclusions

For modern olive growing, the availability of genetically and sanitary certified plantlets is a basic condition for establishing new olive groves or renewing old ones. Micro-propagation applied to olive tree can be a great opportunity both to nurserymen and farmers. The former would succeed in having ''mass scale production'' of genetically homogeneous and virus-free plantlets, thus facilitating trade even with far-off countries. Farmers could obtain best quality and relatively low price nursery material for establishing olive groves.

From an agronomic point of view, our findings prove that the micro-propagation technique does not change the vegetative and productive characteristics of the olive cultivars it was applied to.

In agreement with what was observed by other authors (Inglese et al., 1999a,b; Palliotti et al., 1999; Leva et al., 2002; Lavee and Wodner, 2004), the results from this trial highlight the major role of the varietal component, the environmental or cultural practices in the vegetative-production expression of the plants obtained through different propagation techniques.

In particular, results were positive for 'Nocellara Etnea' where yield per plant was significantly higher than grafted plants, to the detriment of the fruit size that still remains, however, in the typical range of the cultivar.

Instead, yield data of the micro-propagated cultivars of 'Carolea' were not equally satisfactory and in 8 years they have produced less than 10 kg of olives per plant. In this case, further research work should investigate if such poor yield is effectively due to epigenetic variations occurred during the long in vitro staying of the explants that have in some way modified the functionality of ovaries, or to environmental or cultural factors.

The application of a propagation technique as powerful as in vitro propagation that allows obtaining as many as 200.000 and more new plants from one explant after 12 sub-cultures only, raises some problems related to genetic variability as well as to the definition of typicality of the oil as we know it today. In particular, if we consider that high intra-varietal genetic variability is present in olive (Carriero et al., 2002), equally evident in the corresponding oil quality, we may risk losing most of such variability both in terms of genetics and typicality.

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