Effect of Root-promoting Products in the Propagation of Organic Olive (*Olea europaea* L. cv. Cornicabra) Nursery Plants

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Abstract. **Olive cuttings root well using synthetic auxin indole-3-butyric acid (IBA). However, European and North American regulations do not allow the use of synthetic products to obtain organic vegetative propagation materials. In this work, we evaluated different products that could replace IBA in the propagation of olive cv. Cornicabra leafy-stem cuttings. In 2003, six products with a known auxin effect were assessed: IBA, algae extract, brewer's yeast, a bed of sunflower seed, seaweed dry extract (Sm-6 Organico™), and an extract of macerated seeds (Terrabal Organico™). The basal end of cuttings was treated with one of these products and placed on a mist bed with basal temperature control. After 2 months, rooting percentage, number of roots per cutting, number of cuttings with callus formation, and number of cuttings with basal thickening were determined. No significant differences were found in rooting percentage or number of roots per cutting between IBA and Terrabal Organico™ and Sm-6 Organico™. These last products had significantly higher percentage of rooted cuttings without callus formation than IBA. In 2004, a new trial was conducted in which seven treatments were evaluated: IBA applied for 7 s; Terrabal Organico™ applied for 1, 4, and 8 h; and Sm-6 Organico™ applied for 1, 4, and 8 h. No significant differences in rooting percentage or number of roots per cutting were observed between IBA and Terrabal Organico™ applied for 1 h, whereas all the Sm-6 Organico™ treatments had significantly lower rooting percentages than IBA. Both rooting percentage and the percentage of rooted cuttings without callus development decreased significantly as treatment duration with Terrabal Organico™ increased. Therefore, Terrabal Organico™ could produce a toxic effect on cuttings when treatment duration is increased. Thus, Terrabal Organico™ could be a valid alternative to IBA in the propagation of organic olive plants of cv. Cornicabra when applied to the basal end of cuttings for 1 h.**

Spain has the largest area of cultivated olive trees in the world (International Olive Council, 2007). Traditionally, olive cultivars were vegetatively propagated by rooting 0.4- to 0.6-m hardwood cuttings. However, this method requires a lot of vegetative material, making genetic and sanitary control difficult. Formation pruning is also expensive because the use of wood cuttings promotes a shrub-like growth. Later, Hartmann (1946) developed a technique for olive tree propagation by leafy-stem cuttings. This technique uses 1-year-old stems, which have a lower natural rooting capacity than hardwood cuttings, and obtains many olive plants from the same tree. This method is based on the application of root-promoting products, which stimulate adventitious root formation in small cuttings with leaves retained at the upper end. The cuttings are then placed in a mist bed, which keeps a thin film of water on the leaves, thereby reducing leaf temperature through evaporative cooling and transpiration and avoiding leaf dehydration. Root formation is stimulated by the application of bottom heat (23 to 25 °C) and coarse mineral components (perlite). Rooting success mainly depends on two factors: cultivar and hormone–nutrition balance (Hartmann, 1946; Hartmann et al., 2002; Khabou and Trigui, 1999). A callus is commonly formed on the basal end of cuttings placed for rooting. It is an irregular mass of parenchyma cells that commonly develops at the basal end of a cutting placed under environmental conditions favorable for rooting (Hartmann et al., 2002). Its occurrence is the result of its dependence on internal and environmental conditions. Roots frequently emerge through the callus so that callus formation is essential for rooting in some species.

In the mid-1930s, auxins were found to stimulate root growth (Thimann and Went, 1934). Zimmerman and Wilcoxon (1935) demonstrated that the most reliable plant growth regulators in stimulating root production in cuttings were indole-3-butyric acid (IBA) and α-naphthaleneacetic acid compared with indole-3-acetic acid (IAA), the most abundant endogenous auxin in plants. IBA is considered the best synthetic auxin because it is nontoxic to plants over a wide concentration range. Furthermore, it is a relatively stable compound whose shelf life can be extended by darkness and refrigeration. This well-known plant growth regulator, which acts on cell division and elongation, is used to stimulate root formation in cuttings of herbaceous and woody plants (Hartmann et al., 2002; Wiesman and Lavee, 1995; Zimmerman and Wilcoxon, 1935). In olive propagation from cuttings, IBA has been found to be the best root-promoting compound (Hartmann, 1946). However, as a synthetic product, IBA is not permitted in organic agriculture.

Organic or biological agriculture defines an agrarian system whose main objective is to obtain high-quality products while preserving the environment and land fertility. European regulation CE 1452/2003 and the U.S. regulation, The Organic Foods Production Act of 1990, as amended, 7 U.S.C. 6501 et seq., allow the use of nonorganic vegetative propagation materials during a transitory period if producers are unable to obtain organic vegetative propagation materials from nurseries. However, there are natural auxin sources such as germinating seeds, fungi, and algae extract, which could be used to obtain organic plants. As far back as the Middle Ages, cuttings were enclosed with germinating seeds to stimulate root formation (Audus, 1953). Auxins were assessed for the first time by Went (1928) in oat stem development from experiments initiated in 1880. Weaver (1972) later found that seeds produce auxins when they germinate. Fungi synthesize auxins (Gruen, 1959; Thimann, 1935) and have proteins, carbohydrates, lipids, minerals, and vitamins (Nagar-Legmann and Margalith, 1987; Pacheco et al., 1997). Algae contain IAA (Ashen et al., 1999; Mowat, 1965), protein, lipids, and carbohydrates (Lavens and Sorgeloos, 1996). Ishikawa et al. (2007) found that yeast extract had a positive effect on root growth in *Glehnia littoralis* by acting as a nutrient source and an elicitor. The rooting of cuttings is improved with mineral nutrients and vitamins (Hartmann et al., 2002).

However, there is currently no information on how natural auxin sources could be used to replace IBA in the organic propagation of olive nursery plants. The only work we know that tried to find organic products to replace IBA in semihardwood olive cuttings was conducted by Suárez et al. (2002). They did not obtain higher rooting percentages with any of the products than when no treatment was applied. They tested different commercial products such as Auxym Òligo (ANE, Lleida, Spain) (commercial organic product from tropical plants extracts with amino acids, humic substances, nutritional elements, auxins, and cytokinins), roots (commercial organic product with humic extract of peat, algae extracts, vitamins, myoinositol, and other metabolites), and Micor+AA (commercial organic product with polysaccharids and natural alcohol enriched with microelements).

The aim of this work was to evaluate the effect of products with natural auxins authorized in organic agriculture on the propagation of cv. Cornicabra olive cuttings: a bed of sunflower seeds (SEE), brewer's yeast (YEA), algae extract (ALG), and the two commercial products Sm-6 Organico™ (Sm-6; Plymag, Inc., Alicante, Spain) (dry extract of seaweed) and Terrabal Organico™ (Terrabal; Plymag; extract of macerated seeds).

Material and Methods

This trial was conducted in a nursery located at the Technological Transference Center La Isla in Arganda del Rey (Madrid) (lat. 40°18′49″ N, long. 3°29′52″ W). The olive cultivar tested was 'Cornicabra', the second most important in Spain with more than 270,000 ha of cultivated areas (Barranco et al., 2005). Semihardwood cuttings of cv. Cornicabra are considered moderately easy to root by Wiesman and Lavee (1995) and easy to root by Del Río and Caballero (2005). In Jan. 2003 and 2004, 15-cm semihardwood cuttings with two pairs of leaves were collected from a 40-year-old commercial organic olive orchard. After the treatments were applied to the basal end, cuttings were inserted in greenhouse benches containing perlite and kept under mist (80% to 90% relative humidity) and high basal temperature (24 °C). The intermittent mist system sprayed a film of water over and around the cuttings.

In 2003, seven treatments were evaluated in a complete randomized block design with three replications for each treatment. Each treatment consisted of 30 cuttings per experimental unit and all the determinations were carried out on the cuttings placed in the internal part of the block (12 control cuttings).

For the IBA treatment, the basal end of cuttings was dipped in 3000 ppm IBA for 7 s following the recommendations of De Oliveira et al. (2003), IBA powder (Rootone™ F; Compo, Barcelona, Spain) was diluted in a 10% distilled water and 90% alcohol solution.

For no treatment (NT), cuttings were set to root with any root-promoting product.

For the ALG treatment, the basal end of cuttings was dipped in an alcoholic solution (25%) of alga *Fucus* extract 1:1 (g·mL^{-1}) (Eladiet, Barcelona, Spain) for 7 s. This organic product was developed for human medical use, especially to control obesity and toning muscular fiber.

For the YEA treatment, the basal end of cuttings was dipped in yeast powder (*Saccharomyces cereviseae*) (Eladiet).

For the SEE treatment, the basal end of cuttings was placed in the propagation bed with four to five sunflower seeds (L'orto Biologico, Salerno, Italy).

For the Sm-6 treatment, the basal end of cuttings was directly dipped in a liquid preparation of Sm-6 Organico™ for 1 h 20 min. Sm-6 Organico™ is a mixture of *Ascophyllum nodosum*, *Fucus serratus*, *Laminaria hyoborea*, and *Laminaria digitata* algae dry extract (30% w/v). It has growth hormones and essential nutrients (citoquinines, betaines, glycine, aminobutyric acid, aminovaleric acid, and minerals).

For the Terrabal treatment, the basal end of cuttings was dipped in the bionutrient Terrabal Organico™ for 1 h 20 min. Terrabal Organico™ is obtained from a soluble fraction of an extract of macerated cereal seeds. It has soluble proteins, amino acids, vitamins, nitrogen, phosphorus, and potassium.

Both Sm-6 Organico™ and Terrabal Organico™ are products authorized in organic agriculture as a biostimulant for horticultural crops. It can be applied by foliar spray or irrigation.

The two treatments that obtained the best rooting results in the 2003 experiment were chosen for the 2004 experiment: Terrabal Organico™ and Sm-6 Organico™. Seven treatments were evaluated in a randomized four-block design: IBA applied for 7 s; Terrabal Organico™ applied for 1, 4, and 8 h; and Sm-6 Organico™ applied for 1, 4, and 8 h.

Each treatment consisted of 25 cuttings per experimental unit and all the determinations were carried out on the cuttings placed in the internal part of the block (nine control cuttings).

In both years, the experiment started in January and the control cuttings were taken out of the mist bed after 2 months. Number of roots per cutting, number of cuttings with callus formation, and number of cuttings with basal thickening were counted. Callus formation and basal thickening were considered the beginning of the rooting process. In commercial nurseries, cuttings with callus formation and with basal thickening are usually put in the propagation bed again. Rooting percentage was calculated as the number of cuttings with at least one root. Percentage of rooted cuttings without callus formation and percentage of cuttings without roots, callus, or basal thickening, that is, without any signs of rooting, were also calculated.

Analysis of variance was carried out using the software package MSTAT-C v.2.1.0. (Michigan State University, 1988) and differences between means of each treatment were analyzed by the Duncan multiple range test ($P \leq 0.05$). Percentage data were transformed before analysis of variance using the arcsine square root transformation method to ensure that the data were normally distributed.

Results and Discussion

In this work, we evaluated the effect of natural root-promoting products authorized by organic agriculture on leafy-stem cuttings of the Cornicabra olive cultivar. We try to prove the effect of these products on the rooting percentage of cuttings and to compare them with the results obtained with IBA treatment. The organic products or treatments applied to the basal end of cuttings with a known auxin effect were germinating seeds, fungi, and algae. Wiesman and Lavee (1995), who obtained a rooting percentage of 37% in cv. Cornicabra cuttings treated with 0.8% IBA talc powder, considered this cultivar moderately easy to root. However, in a study carried out by Del Río and Caballero (2005), a rooting percentage of 70% was obtained when cuttings were dipped in 3000 ppm IBA for 5 s. In our study, the percentage of rooted cuttings in the IBA treatment ranged between 47% and 54% (Tables 1 and 2). Differences in rooting percentage could be the result of factors that determine rooting (wood characteristics) and inherent differences in the trial (temperature and humidity) (Hartmann et al., 2002).

Despite the rooting characteristics of the Cornicabra cultivar, we found that only 6% of the cuttings rooted when no treatment was applied (Table 1). Considering that the

Table 1. Effect of treatments on percentage of rooted cuttings, number of roots per cutting, percentage of rooted cuttings without callus formation, and percentage of cuttings without roots, callus, or basal thickening in 2003 in olive cuttings cv. Cornicabra.

Treatment	Rooted cuttings (%)	Number of roots per cutting	Rooted cuttings without callus (%)	Cuttings without roots, callus, or basal thickening (%)
IBA	47.2 a[z]	2.86 ab	16.6 b	16.6 c
NT	5.5 c	0.05 b	2.7 b	60.8 a
ALG	36.0 ab	3.25 ab	8.3 b	47.6 ab
YEA	36.1 ab	2.36 ab	33.3 ab	50.0 a
SEE	8.3 bc	0.22 b	2.7 b	39.2 abc
Sm-6	58.0 a	4.89 a	58.3 a	25.0 bc
Terrabal	56.0 a	5.42 a	55.8 a	41.6 ab

[z]Means within columns followed by different letters were significantly different at $P \leq 0.05$ using Duncan's test.

IBA = indole-3-butyric acid; NT = no treatment; ALG = algae extract; YEA = yeast; SEE = sunflower seeds; Sm-6 = Sm-6 Organico™; Terrabal = Terrabal Organico™.

Table 2. Effect of treatments on the percentage of rooted cuttings, number of roots per cutting, percentage of rooted cuttings without callus formation and percentage of cuttings without roots, callus, or basal thickening in 2004 in olive cuttings cv. Cornicabra.

Treatment	Rooted cuttings (%)	Number of roots per cutting	Rooted cuttings without callus (%)	Cuttings without roots, callus, or basal thickening (%)
IBA (7 s)	53.7 a[z]	6.1 a	11.1 b	19.4 c
Terrabal (1 h)	38.9 ab	4.0 ab	31.5 a	47.2 b
Terrabal (4 h)	13.0 cd	1.2 bc	13.0 b	74.1 a
Terrabal (8 h)	3.7 d	0.4 c	5.6 b	81.5 a
Sm-6 (1 h)	29.6 bc	2.9 bc	15.7 b	48.1 b
Sm-6 (4 h)	20.4 cd	2.1 bc	13.0 b	55.6 b
Sm-6 (8 h)	24.1 bc	2.7 bc	19.4 ab	50.9 b

[z]Means within columns followed by different letters were significantly different at $P \leq 0.05$ using Duncan's test.

IBA = indole-3-butyric acid; Terrabal (1 h), Terrabal (4 h), Terrabal (8 h) = Terrabal Organico™ applied 1, 4, and 8 h, respectively; Sm-6 (1 h), Sm-6 (4 h), Sm-6 (8 h) = Sm-6 Organico™ applied 1, 4, and 8 h, respectively.

economic threshold for cutting olive propagation is estimated ≈20% rooting (Wiesman and Lavee, 1995), hardwood material should be used in the new organic orchard if no organic product is found to promote rooting in olive cuttings. However, it is difficult to control the genetic and sanitary quality of hardwood material as a result of the large amount of material required. Besides, the cost of formation pruning is expensive because this material has a shrub-like growth.

In the 2003 trial, the organic treatments applied to the basal end of cuttings were fungi extracts (YEA), algae extracts (ALG and Sm-6), germinating seeds (SEE), and seed soluble extract (Terrabal). Significantly higher rooting percentages were obtained in the IBA (47%), Terrabal (56%), and Sm-6 (58%) treatments than in the NT (6%) and SEE (8%) treatments (Table 1). The number of roots per cutting was also significantly higher in the Sm-6 and Terrabal treatments than in the NT and SEE treatments. No significant differences were observed in percentage of rooted cuttings or in number of roots per cutting among the IBA, Sm-6, and Terrabal treatments. The percentage of rooted cuttings without callus formation was significantly higher in the Sm-6 and Terrabal treatments than in NT, IBA, ALG, and SEE treatments. The NT, ALG, YEA, and Terrabal treatments had the highest percentages of cuttings with no signs of rooting. These percentages were significantly higher than in the IBA treatment. SEE was the only treatment in which no significant increase in the percentage of rooted cuttings was observed compared with the NT treatment. This could be the result of water consumption of the sunflower seedlings, which may have reduced the amount of water available for the cuttings. The IBA and Sm-6 treatments had the lowest percentages of cuttings without roots, callus, or basal thickening that is no signs of rooting.

In the 2004 trial, the Sm-6 Organico™ and Terrabal Organico™ treatments with treatment durations of 1, 4, and 8 h were compared with the IBA treatment (Table 2). Mean rooting percentage was significantly higher in the IBA treatment than in the Terrabal (4 h), Terrabal (8 h), Sm-6 (1 h), Sm-6 (4 h), and Sm-6 (8 h) treatments and not significantly different from Terrabal (1 h). Number of roots per cutting was significantly higher in the IBA treatment than in the Terrabal (4 h), Terrabal (8 h), Sm-6 (1 h), Sm-6 (4 h), and Sm-6 (8 h) treatments. Besides, both mean rooting percentage and the number of roots per cuttings decreased significantly as the duration of the Terrabal treatment increased. However, in the Sm-6 treatments, treatment duration produced no significant effect on mean root number. Treatment duration with Terrabal significantly decreased the percentage of rooted cuttings without callus development, whereas treatment duration with Sm-6 produced no significant effect. The lowest percentage of cuttings without any signs of rooting was obtained in the IBA treatment (19%). The percentage of cuttings without any signs of rooting was significantly lower in Terrabal (1 h; 47%) than in Terrabal (4 h; 74%) and Terrabal (8 h; 81%). On the other hand, treatment duration with Sm-6 produced no significant effect on the percentage of cuttings without any signs of rooting.

No significant differences were found in rooting percentage or in number of roots per cutting between IBA and Terrabal (1 h) in 2004 (Table 2). However, Terrabal Organico™ seems to have some component from macerated seeds that is phytotoxic for cuttings, because rooting percentage and number of roots per cutting decreased when the length of the treatment with this product increased from 1 h. This may be the result of the high level of phenolic compounds in the extract (Faivre-Rampant et al., 2002). Terrabal Organico™ does not seem to promote callus development on the basal end of cuttings as IBA does (Tables 1 and 2). This could be beneficial for certain species in which excess callusing may hinder rooting (Hartmann et al., 2002). The success of Terrabal Organico™ in rooting olive cuttings could be the result of its nutrient and hormone composition. It has been reported that the combination of nutrients with hormones improves the rooting of cuttings (Wiesman and Lavee, 1995) as well as growth and development (Wiesman et al., 2002). Thus, the results of this study indicate that Terrabal Organico™, applied for 1 h, could be an adequate root-promoting product in propagating organic olive cv. Cornicabra cuttings in accordance with current regulations on the organic production of vegetative propagation materials. Therefore, further research should be carried out to evaluate the effect of different treatment durations and concentrations on rooting as well as the rooting effect of this product in other species and cultivars. Besides, the influence of this product on shoot growth should also be evaluated, because some products have been found to positively affect root formation but negatively affect leaf growth (Ishikawa et al., 2007).

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