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Humic substances and modes of application in the rooting of semi-hardwood cuttings of two olive cultivars

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Abstract

Olive propagation by the cutting method demands the study of several techniques due to the low percentage of rooting. This study aimed to evaluate the rooting of semi-hardwood cuttings of olives using humic substances and their modes of application. Two experiments were carried out in a hoop house built under a black screen in the Professor Mário César Lopes seedling nursery, belonging to the Western Paraná State University. The first experiment evaluated the rooting of cuttings of the cultivars Maria da Fé and Ascolano in different humic substances and a control. The experimental design was randomized in blocks in a 2×5 factorial arrangement (cultivar x humic substances), with three replications of 25 cuttings per experimental unit. The second experiment consisted of the rooting of the two cultivars using three modes of application of the commercial product SoloHumics® and a control. The experimental design was randomized in blocks in a 2×4 factorial scheme (cultivar x mode of application), with three replications and 25 cuttings per plot. Data were collected for phytotechnical evaluations after 75 days. Humic substances did not influence a higher acquisition of rooted cuttings and the quality of adventitious roots emitted in both cultivars. The application using irrigation and spraying for the cultivar Maria da Fé and spraying for Ascolano 315 were more efficient when using humic substances aiming at their propagation by cuttings.

Keywords: asexual propagation, *Olea europaea* L., organic fertilizers

Introduction

The commercial production of olive (*Olea europaea* L.) seedlings by the asexual propagation using the semi-hardwood cutting technique is the most used among all the existing methods (Micheli et al., 2019). This technique shows intrinsic (time of year, mother plant, hormonal balance, and phenolic compounds) and extrinsic factors (light, substrate temperature, and humidity) that are involved and influence the rhizogenesis process of cuttings (Fachinello et al., 2013; Porfirio et al., 2016).

Growth regulators are essential in olive cuttings, and the most common is the indole butyric acid (Oliveira et al., 2010), an auxin that has a consistent effect in inducing adventitious rooting in cuttings of different species (Porfirio et al., 2016; Vieira Neto et al., 2011). The percentage of rooting of cuttings is still low even using this growth regulator, in addition to being variable, depending on the cultivar (Oliveira et al., 2012; Penso et al., 2016; Inocente et al., 2018). Some cultivars such as Mission and Frantoio obtained 30% rooting of cuttings in the study carried out by Villa et al. (2017), who tested the rooting of cuttings of 10 olive cultivars in different substrates. Therefore, studies with other treatments are necessary to optimize this process (Dalla Rosa et al., 2018).

Therefore, it was observed that in the soil organic matter some substances are present that may be able to promote direct effects on plant growth in addition to providing nutrients through mineralization, called humic substances (Canellas et al., 2015). Humic substances are condensed organic compounds produced by microbial action during the degradation process and are constituted by the fractions humic acids, fulvic acids and humines (Baldotto & Baldotto, 2014).

Relating humic substances to rooting, two studies analyzing these substances indicate that they can

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influence the increase and growth of roots in a way similar to that promoted by auxin, as they actively participate in the auxin biosynthesis pathway (Elena et al., 2009; Trevisan et al., 2011).

The availability of nutrients is also related to the cutting process. They are normally absorbed by the roots of olive trees, but as the stake does not have these structures, other forms of application and absorption can be tested (Araya, 2008).

In this context, this study aimed to evaluate the rooting of semi-hardwood cuttings of olive cultivars using humic substances and their modes of application.

Material and Methods

Two experiments were conducted from November 2018 to January 2019, in the seedling nursery of the Protected Cultivation and Biological Control Station "Professor Mário César Lopes". The Station is located at the geographic coordinates of latitude 24° 32' 22" S, longitude 54° 03' 24" W and altitude of 420 m and belongs to the "Núcleo de Estações Experimentais" of the Universidade Estadual do Oeste do Paraná (Unioeste), *Campus* Marechal Cândido Rondon, PR.

The experiments were carried out under conditions of a black screen with 30% of solar radiation retention and a masonry bed measuring 5 × 0.80 m filled with medium-sized washed sand. A low tunnel with dimensions of 5.0 × 0.80 m and 0.55 m in height was built in the bed using posts and a wire structure, covered with a 150-micron transparent plastic canvas. A sprinkler irrigation system with intermittent nebulization, with activation from 7:00 to 18:00 h, was installed inside the tunnel to keep high the relative air humidity and sand moisture.

Semi-hardwood branches of olive trees of the cultivars Maria da Fé and Ascolano were collected from 5 plants with 4 years of age located at the Experimental Farm "Professor Doutor Antônio Carlos dos Santos Pessoa" at Unioeste, *Campus* Marechal Cândido Rondon and immediately taken to the seedling nursery to make the piles. These were prepared with a length of 12 cm and a diameter of 4 mm, keeping two pairs of leaves at the top and a straight cut at the base, just below the bud.

Experiment I

The cuttings were treated with 3000 mg L^{-1} of indolebutyric acid (IBA) (Penso et al., 2016), having their base immersed in the solution for 15 seconds and arranged in a 2 x 3 cm spacing in the masonry bed containing sand, according to the substances used, and with 5cm of the length introduced into the substrate.

Before placing the olive stakes in the beds, five spaces were separated (dimensions of 0.50 x 0.80 m each), avoiding contact between the applied substances [S1 = commercial product SoloHumics® (CP); S2 = humic acid (HA); S3 = fulvic acid (FA); S4 = peat residual water (PRW) and S5 = control (C) without product, only water]. The substances S1 to S4 were diluted in water, forming 2 L of solution in the final volume and applied on the sand by means of irrigation with a watering can.

As each substance had a different formulation, a concentration of 2500 mg L^{-1} of active ingredient was established for all, with a volume of irrigated area of 0.032 m^3, resulting in the use of 10 mL of S1, 10 mL of S2, 50 mL of S3 and 30 mL of S4.

The commercial product SoloHumics® was acquired from the manufacturing company, having in its composition humic substances, such as humic acid (25%), fulvic acid (5%) and organic matter (60%). The other substances came from the separation of the commercial product, presenting 1.30% of active ingredient and provided by the Federal University of Lavras (UFLA).

The management practices used during the conduction of the experiment consisted of only the opening of the tunnel for observation and control of the irrigation operation. The percentage of rooted, dead, callused, and sprouted cuttings, the number and size of shoots (cm), longest root length (cm), and the number of roots were evaluated after 75 days of experimentation. A digital caliper was used to measure the lengths.

The experimental design was randomized in blocks in a 2×5 factorial scheme (olive cultivars x humic substances + control), with three replications and 25 cuttings per experimental unit.

For data normality, the Shapiro-Wilk test was used, where only the variables rooted cuttings, calloused and number of roots did not need to be transformed to (Y+1.0)^0.5. The data obtained were subjected to analysis of variance and the means compared with the Tukey test, at 5% error probability, using the SISVAR statistical program (Ferreira, 2019).

Experiment II

The second experiment consisted of the rooting of the two olive cultivars using three modes of application of the commercial product SoloHumics®, that is, immersion of cuttings, spraying, and irrigation, in addition to a control treatment.

After the cuttings were made, the immersion and spraying solutions were prepared, where the final solution of each presented 2500 mg L^{-1} of active ingredient, using 10 mL of the commercial product. The immersion treatment stakes were immersed in the solution for 1 h

30 min and later, all the stakes were treated with indole butyric acid (IBA) at a concentration of 3000 mg L^{-1} (Penso et al., 2016), based on immersed in the solution for 15 seconds, and immediately after being taken to the sand bed, arranged in a spacing of 5 x 3 cm and 5 cm of their buried length.

For the treatment, the commercial product was purchased from the company responsible for the manufacture and its composition contains the humic substances humic acid (25%), fulvic acid (5%) and organic matter (60%). For the cuttings that received the treatment by spraying the leaves, they initially received treatment with indole butyric acid (IBA) at a concentration of 3000 mg L^{-1} and were placed in the sand bed. Subsequently, the product solution was prepared at the same concentration of active ingredient as in the immersion. The solution was placed in a manual sprayer and applied to the leaves of the cuttings, the process being repeated every 7 days, totaling 11 foliar applications. The irrigation treatment was applied directly to the rooting substrate before the cuttings treated with IBA and the control received only the treatment with IBA.

The experimental design consisted of randomized blocks in a 2×4 factorial scheme (cultivar x mode of application + control), with three replications of 25 cuttings per experimental unit.

The tunnel was opened during the experiment for observation and control of the irrigation operation. Irrigation was performed by a sprinkler system with intermittent nebulization, activated every 30 minutes for 3 minutes from 7 am to 6 pm to maintain humidity. The data were collected 75 days after the experiment was set up and the percentage (%) of rooted, dead, callused, and sprouted cuttings, the number and size of shoots (cm), longest root length (cm), and the number of roots were evaluated. A digital caliper was used to measure the lengths.

Subsequently, the data were submitted to the Shapiro-wilk normality test, where only the variables of number of roots and rooted cuttings did not receive a (Y+1.0)^0.5 transformation. The data underwent analysis of variance and the means were compared by the Tukey test, at 5% error probability, by the SISVAR program (Ferreira, 2019).

Results and Discussion

Experiment 1

The analysis of variance showed a significant interaction between treatments using humic substances and olive cultivars for the percentage of rooted cuttings (**Table 1**). The means of rooting (%) for the cultivar Maria da Fé did not differ statistically from each other regarding the treatments, but the cultivar Ascolano 315 presented the highest percentages of rooting in the control and when treated with humic acids.

Table 1. Rooted cuttings (%) and root length (cm) as a function of cultivars and treatments with humic Substances. Unioeste, *Campus* Marechal Cândido Rondon, PR. 2022

Treatments	Rooted cuttings (%)	
	Maria da Fé	Ascolano 315
CP	14.0 aA*	5.3 bA
HA	9.67 aA	7.3 aA
FA	9.00 aA	2.6 bA
PRW	12.6 aA	6.0 bA
C	9.00 aA	8.0 aA
CV(%)	27.26	
	Root length (cm)	
CP	2.72 aA*	2.55 abA
HA	2.04 abA	2.21 abA
FA	1.79 bB	2.90 aA
PRW	2.13 abA	2.30 abA
C	2.25 abA	2.09 bA
CV(%)	13.60	

*Lowercase letters statistically differ from each other in the column and uppercase letters in the row, by Tukey's test, at a 5% error probability. CP = commercial product, HA = humic acids, FA = fulvic acids, PRW = peat residual waster, C = control, CV = coefficient of variation.

The treatments showed no statistical difference between the means of rooting when comparing the cultivars. The interaction cultivar x treatment for the mean of the longest root length showed that almost all treatments with humic substances showed superiority, except for Maria da Fé and Ascolano 315 under fulvic acid and control, respectively.

A difference was observed between the means of the cultivars only when fulvic acid was applied to Ascolano 315, showing the highest value, and differing from the others.

The percentage of callused cuttings also showed a significant interaction between cultivar and treatment with humic substances. The highest means for callused cuttings in the cultivar Maria da Fé were observed when using humic acid and peat wastewater, which also showed the same superiority for the cultivar Ascolano 315, which, added to the commercial product and control treatments, did not differ statistically from each other (**Table 2**).

The cultivar Maria da Fé presented the highest means among the cultivars for all treatments, not differing from those obtained with Ascolano 315 for the commercial product, humic acids, and control.

The number of shoots was another variable that also showed a significant interaction between factors. Only the treatments PRW had a difference among the cultivars, resulting in a higher mean for Ascolano 315 compared to the mean obtained for Maria da Fé. The

Table 2. Callused cuttings (%) and number of shoots as a function of cultivars and treatments with humic substances. Unioeste, *Campus* Marechal Cândido Rondon, PR. 2022

	Callused cuttings (%)	
Treatments	Maria da Fé	Ascolano 315
CP	5.00 bcA*	3.67 aA
HA	8.67 aA	1.33 abA
FA	4.67 bcA	1.00 bB
PRW	6.33 abA	3.33 abB
C	3.00 cA	2.67 abA
CV(%)	23.92	
	Number of shoots	
CP	1.59 aA*	1.45 abA
HA	1.52 aA	1.46 abA
FA	1.51 aA	1.58 abA
PRW	1.44 aB	1.79 aA
C	1.52 aA	1.28 bA
CV(%)	11.00	

* Lowercase letters statistically differ from each other in the column and uppercase letters in the row, by Tukey's test, at a 5% error probability. CP = commercial product, HA = humic acids, FA = fulvic acids, PRW = peat residual waster, C = control, CV = coefficient of variation.

cultivar Maria da Fé showed no significant difference between its means for the number of shoots, regardless of the treatment used in the cuttings.

The PRW treatment provided the highest average number of shoots together with the CP, HA and FA treatments, which did not differ from the control treatment with the lowest average in the Ascolano 315 cultivar.

The cultivar Ascolano 315 had the highest means for the number of roots than the cultivar Maria da Fé (**Table 3**). The opposite was observed for the percentage of sprouted cuttings, in which Maria da Fé presented the highest value. Shoot length did not present a significant difference between the means of the cultivars.

Table 3. Sprouted cuttings (%), number of roots and bud length (cm) of the cultivars Maria da Fé and Ascolano 315. Unioeste, *Campus* Marechal Cândido Rondon, PR. 2022

Olive cultivars	SC (%)	NR	LS (cm)
Maria da Fé	2.27 a*	7.36 b	1.30 a
Ascolano 315	2.10 b	10.44 a	1.25 a
CV (%)	24.02	26.07	6.99

*Lowercase letters differ statistically from each other in the column, by Tukey's test, at a 5% error probability. SC = sprouted cuttings, NR = number of roots, LS = length of shoots, CV = coefficient of variation

The number of roots also showed a significant effect for treatments (**Table 4**), with the highest mean when using the commercial product, although not statistically different from HA, FA, and the control. Similarly, the shoot length in the treatments was also not significant.

Experiment 2

The analysis of variance showed a significant interaction between olive cultivar and mode of product application only for the percentage of rooted cuttings. The percentage of rooting for the cultivar Maria da Fé was higher in the treatment with irrigation and application

Table 4. Number of roots and length of shoots (cm) as a function of treatments with humic substances. Unioeste, *Campus* Marechal Cândido Rondon, PR. 2022

Treatments	NR	SL (cm)
CP	11.87 a*	1.29 a
HA	8.03 ab	1.27 a
FA	9.32 ab	1.30 a
PRW	7.47 b	1.25 a
C	7.83 ab	1.25 a
CV(%)	26.07	6.99

*Lowercase letters differ statistically from each other in the column, by Tukey's test, at a 5% error probability. NR = number of roots, SL = shoot length, CP = commercial product, HA = humic acids, FA = fulvic acid, PRW = peat residual waster, C = control, CV = coefficient of variation.

by spraying, with the immersion treatment showing the lowest value.

The same behavior was observed for the cultivar Ascolano 315, which had the lowest percentage of rooting when the immersion treatment was applied. The other treatments showed the highest rooting rates, not differing statistically from each other (**Table 5).**

The percentage of sprouted cuttings presented a difference between cultivars. In this case, the cultivar Maria da Fé had the highest means (**Table 6**). The number of roots and the percentage of callused cuttings presented a significant effect on both factors, with the cultivar Ascolano 315 showing a higher number of roots relative to Maria da Fé, but the opposite behavior was observed for the percentage of callused cuttings, in

Table 5. Rooted cuttings (%) depending on the cultivars and treatments used. Unioeste, *Campus* Marechal Cândido Rondon, PR. 2022

	Olive cultivars	
Treatments	Maria da Fé	Ascolano 315
Pulverization	11.33 abA*	5.33 abB
Immersion	6.00 cA	2.67 bA
Irrigation	14.00 aA	5.33 abB
Control	9.00 bcA	8.00 aA
CV(%)	25.26	

* Lowercase letters statistically differ from each other in the column and uppercase letters in the row, by Tukey's test, at a 5% error probability. CV = coefficient of variation

Table 6. Number of roots, calloused cuttings (%) and sprouts, length of shoots and number of shoots of the cultivars. Unioeste, *Campus* Marechal Cândido Rondon, PR. 2022

Olive cultivars	NR	CC (%)	SC (%)	LS (cm)	NB
Maria da Fé	7.97 b*	2.01 a	2.05 a	1.30 a	1.48 a
Ascolano 315	10.61 a	1.55 b	1.46 b	1.21 a	1.37 a
CV (%)	15.45	17.17	25.83	11.13	15.32

*Lowercase letters differ statistically from each other in the column, by Tukey's test, at a 5% error probability. NR = number of shoots, CC = calloused cuttings, SC = sprouted cuttings, LS = length of shoots, NB = number of shoots, CV = coefficient of variation.

which Maria da Fé had the highest means.

Regarding the modes of application, the highest number of roots was observed only in the treatment with substrate irrigation, whose mean was 11.88 roots per cutting (**Table 7**). Moreover, the variable percentage

Table 7. Length of the largest root, number of roots, percentage of calloused cuttings, number of shoots and length of shoots in the treatments. Unioeste, *Campus* Marechal Cândido Rondon, PR. 2022

Treatments	LLR (cm)	NR	CC (%)	NS	LS (cm)
Pulverization	2.29 ab*	8.24 b	1.34 c	1.44 a	1.25 a
Immersion	2.28 ab	9.21 b	1.53 bc	1.35 a	1.20 a
Irrigation	2.63 a	11.88 a	2.29 a	1.52 a	1.29 a
Control	2.07 b	7.83 b	1.95 ab	1.40 a	1.25 a
CV(%)	13.88	15.45	17.17	15.32	11.13

*Lowercase letters differ statistically from each other in the column, by Tukey's test, at a 5% error probability. LLR = length of the largest root, NR= number of roots, CC = calloused cuttings, NS = number of shoots, LS = length of shoots.

of callused cuttings presented the highest means in the treatments with irrigation and control.

The longest root length had a significant effect only for the factor application treatment, with almost all of them presenting the highest means, except the control. The other variables did not show a significant effect for the cultivar regarding the modes of application of the commercial product.

Experiment 1

The percentage of rooted cuttings was low compared to other studies found in the literature, as studied by Penso et al. (2016) where he presented results above 40% rooting and in Villa et al. (2017) with percentages above 78% in sand-rooted cuttings.

Working with the rooting of olive cuttings of the cultivar Cornicabra, Centeno & Gómez-del-Campo (2008), obtained a higher percentage of rooting (57%) in cuttings treated with an organic biostimulant. These organic substances are capable of inducing the formation of adventitious roots by activating the auxin biosynthesis pathway complex (Trevisan et al., 2011).

The different olive cultivars have different behaviors from each other and between cultivation sites (Vieira Neto et al., 2010). Therefore, interference may occur at the time of cuttings removal, as cuttings collected in periods of high temperature are not ideal for seedling formation (Penso et al., 2016). The percentages of rooting in studies carried out with olive cuttings at the same experimental site, but collected in mid-September (Dalla Rosa et al., 2018; Ritter et al., 2021), ranged from 37 to 72% in the cultivar Maria da Fé.

The percentages of rooting of the cultivars Maria da Fé and Ascolano 315 showed the same behavior, that is, no statistical difference was observed between them, which may be a consequence of their genetic characteristics.

Among the various olive cultivars that exist, it is possible to classify them into groups of high, medium and low rooting potential, even with the interaction of intrinsic and extrinsic factors. Although the cultivars studied in this work showed a low percentage of rooting of cuttings, they are classified as cultivars with high potential for seedling formation through cuttings (Oliveira et al., 2012).

The cultivar Ascolano 315 showed an affinity with humic acid, being the only treatment, in addition to the control, which stood out for the percentage of rooting. The ability of humic substances to promote plant growth and induction of adventitious roots has been reported in the literature (Nardi et al., 2009; Canellas & Olivares, 2014; Shen et al., 2017).

Thus, the treatments with humic substances were not efficient for the olive cultivars Maria da Fé and Ascolano 315, as the control with water showed the same superiority values.

When the percentage of callogenesis is observed, it is verified in both cultivars that there was a stimulus for cell differentiation, whose difference was more accentuated for some treatments in relation to others within each cultivar. However, such stimulus also serves as a parameter that may have adventitious roots, as the stimulus to differentiation is perceived by callogenesis, even if these do not become roots. With this, it can be seen here that the witness also indicated that there is no need to apply humic substances in order to obtain lower averages, since it is desirable here since they compete for the same reserves directed to rooting.

The cutting uses reserves of photoassimilates accumulated inside it to produce shoots and these same reserves are used in the process of developing adventitious roots (Taiz et al., 2017).

The number of shoots can be an intrinsic characteristic of each cultivar, as well as its relationship with the used treatments. These shoots can even be beneficial in the rooting process, increasing the photosynthetic area of the plant in formation and the synthesis of plant regulators occurs in buds and new leaves (Vignolo et al., 2014). Thus, the non-statistical difference for rhizogenesis may reflect the similarity between the number of shoots.

Experiment 2

Applications via irrigation and spraying showed a higher percentage of rooting for the cultivar Maria da Fé, which may be due to the availability of nutrients for rooting and rapid treatment of plant regulator in the cuttings. In most species in horticulture, the application of humic substances via foliar spray is efficient for root growth due to the photosynthetic efficiency that the product can provide (Colla & Rouphael, 2015).

The immersion treatment showed several lower averages among some variables, which may occur due to the fact of direct exposure for a long time or high concentration of the product in the solution, causing an opposite and inhibitory effect to the desired one (Ruzzi & Aroca, 2015).

The number of roots influences the initial development of the seedling, as the roots perform nutrient absorption and in greater quantities increase this absorption. The same condition of number of roots of this experiment was observed by Silva et al. (2012), where the Ascolano 315 cultivar presented a higher number of roots than Ascolano 315.

The same authors also corroborate the results of the percentage of calloused piles, where Maria da Fé had a higher rate, compared to Ascolano 315, in this analyzed variable. Likewise, Dalla Rosa et al. (2018) working with cuttings of the cultivars Maria da Fé, Arbequina and clone 2, obtained superior results with the first in the variable percentage of calloused cuttings.

For the length of the largest root to obtain larger sizes when applying treatments, it can be justified by the supply of nutrients from the product applied, and sand as a rooting substrate is considered inert in terms of availability and mineral nutrients (Zeist et al., 2015).

The treatment with substrate irrigation may have stood out in terms of the number of roots, as it provided nutrients that the control does not provide, and the cuttings were immediately treated with the plant regulator and placed in the rooting bed. The rapid treatment allows the cutting to be in immediate contact with the regulator and the product that is in the substrate before the high production of phenolic compounds, substances abundant in the olive tree (Erbay & Icier, 2010).

Humic substances have been shown to be efficient in the formation of roots and plant growth, but more study and research is still needed in different forms of application and doses in the rooting process and may become a widespread practice in the agronomic environment.

Conclusion

Humic substances did not influence a higher acquisition of rooted cuttings and the quality of adventitious roots emitted in both cultivars. The application using irrigation and spraying for the cultivar Maria da Fé and spraying for Ascolano 315 were more efficient when using humic substances aiming at their propagation by cuttings.

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