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Improved Propagation and Growing Techniques for Oleander Nursery Production

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Abstract: In the first trial, we examined rooting of stem cuttings in relation to number of nodes and indole-3-butyric acid (IBA) treatment in several *Nerium oleander* clones grown in Sicily. In a second trial, we tested the effect of different forcing dates and shading on oleander plants for gardens and natural landscapes. Three- and four-node cuttings, ranging in length from 10 to 14 cm, were significantly superior to two-node cuttings (8–10 cm long) in terms of rooting percentage and number of roots per cutting. The application of IBA improved rooting percentage and root number as compared to untreated control. Irrespective of IBA, rooting percentages ranged from 94% in clone 1 to 52% in clone 4. Shaded plants forced in October were significantly higher than those forced in November and in December. Beginning of flowering was delayed in unforced plants. Plants forced in October flowered significantly sooner (first decade of March) than unforced ones (first decade of May) and reached complete flowering almost two months earlier (last week of March).Shading had little effect on plants forced in October and in November as compared to unshaded plants in terms of start of flowering, but it slightly hastened beginning of flowering of December forced plants as compared to their unshaded counterparts.

Keywords: rooting; cutting; forcing; oleander; shading

1. Introduction

Nerium oleander L. (oleander) is a shrub native to northern Africa and the Mediterranean region grown for flowers and evergreen foliage. The long flowering period extending from early spring through late fall and the appealing flower display make oleander a valuable ornamental plant and one of the best shrubs for landscaping and xero-gardening projects in semi-arid environments [1]. The species has also become an important plant for flowering pots [2,3].

Implementing propagation methods to enhance transplant success, establishment, and post-plant maintenance is a major objective for plant nurseries involved in the production of shrubs to be used for gardens and natural landscapes in regions with a Mediterranean climate. In this regard, the production of oleander rooted cuttings with a well-developed root system is fundamental for successful transplanting and establishment in the field. By growing plants in a greenhouse or other protected facilities, plant nurseries force plants to bloom out of season. Ornamental shrubs with colors create a great amount of impulse buying from the average garden plant consumer because a flowering plant provides immediate gratification and guarantees that the consumer is getting what they paid for [4]. Based on these considerations, reducing the time span between propagation and flowering by individuating opportune forcing and shading techniques induces oleander to bloom out of season and improves its marketability for garden plantings.

According to Hartmann [5], oleander cultivars are clonally propagated by leafy cuttings, which root easily if taken from rather mature wood during the summer and treated with a 3000 ppm indole-3-butyric acid (IBA) quick-dip. Toogood [6] affirms that to produce a oleander flower plant in 2 years, leafy cuttings or semi-hardwood cuttings can be rooted directly in 3–6 weeks and that bottom-heat at 12–20 °C enhances rooting. Dirr [7] suggests to root cuttings collected from mature wood in late July or August after a 3000 ppm IBA application. Ochoa et al. [8,9] report that basal temperatures ≥25 °C favor rooting and root quality of semi-hardwood oleander cuttings, and that cuttings taken from the basal part of the stem produce larger root growth, although more roots with a more homogeneous length distribution were obtained from distal cuttings. However, to our knowledge, no published data is available concerning the influence of the morphological characteristics of the cuttings on adventitious root formation. Therefore, in the first series of experiments our objective was to examine rooting of stem cuttings in relation to number of nodes and IBA treatment in several *Nerium oleander* clones grown in Sicily.

Although there have been several studies undertaken to improve the suitability of this species as a potted plant, especially through the use of plant growth regulators, there are no reports concerning the effects of shading and diverse forcing date on oleander nursery production. Therefore, in the second experiment we tested the effect of different forcing dates and shading on oleander production.

2. Materials and Methods

The research was conducted at the experimental field of the Department of Agricultural, Food and Forest Sciences of Palermo (SAAF), at Marsala, Trapani Province (longitude 12°26′ E, latitude 37°47′ N, altitude 37 m above sea level (asl) in the north-western coast of Sicily (Italy).

2.1. Propagation Treatments

In the first experiment, distal 30-cm-long stem cuttings were harvested on 23 March 2018, from actively growing oleander stock plants. Plants were of cutting origin and from a 10-year-old Sicilian unnamed clone characterized by a red corolla (clone 1). Hardwood stem cuttings were collected from the previous year's growth cycle in the middle of the crown of the stock plants. Cuttings were stored overnight at 6 °C. The next day, the terminal shoot was removed and cuttings were cleaned and defoliated prior to planting. The cuttings were trimmed into three types: (1) two-node cuttings (length 8–10 cm); (2) three-node cuttings (length 10–12 cm); (3) four-node cuttings (length 12–14 cm). Average diameter of the cuttings ranged from 10 to 13 mm. All cuttings had the first cut made 10 mm above the distal node and the second cut made 20 mm below the proximal node. To verify the cutting response to exogenous auxin, cuttings were dipped to a 2.0 cm depth in a 0.3% indole-3-butyric acid (IBA) solution for 30 s. Reagent grade IBA (Sigma Aldrich, Italy) was dissolved in 90% ethanol, then brought to a final concentration of 6% ethanol and 94% distilled water (*v*/*v*). Untreated cuttings were dipped in distilled water (control). After 30 and 45 d, data were recorded as percent survival, percentage of cuttings rooted, number of roots per cutting, and length of the 6 longest roots. Growth resulting from sprouting of the buds present on the original cutting material was rated by measuring shoot length after 30 and 60 d.

In the second experiment, distal 30-cm-long hardwood stem cuttings were harvested on 30 March 2018, from actively grown stock plants of five Sicilian unnamed oleander clones characterized by a red (clone 1), pink (clone 2), white (clone 3), salmon (clone 4), and yellow (clone 5)corolla (Figure 1).

Cuttings were collected as described in the first experiment, and stored overnight at 6 °C. The next day, three-node cuttings (length 10–12 cm) were prepared as described in the first experiment. To verify the cutting response to exogenous auxin, half of the cuttings were dipped to a 2.0 cm depth in a 0.3% IBA solution for 30 s, whereas the remainder were dipped in distilled water (control). After 30 and 45 days, data were recorded as percentage of cuttings rooted, number of roots per cutting, and length of the 6 longest roots. Growth resulting from sprouting of the buds present on the original cutting material was rated by recording shoot length and number of leaves per rooted cutting after 30 and 60 d.

Figure 1. *Nerium oleander* clones tested.

In the two experiments, propagation was performed in an unheated greenhouse covered with clear polyethylene (PE) and external 70% shade-cloth. Single cuttings were stuck to each plastic pot (diameter 12) containing a peat:perlite (2:1/*v*:*v*) media. Air temperature in the greenhouse was 18–23 °C during the day and 14–16 during the night. Rooting medium temperature was 18–22 °C during the day and 14–18 °C during the night. Intermittent mist operated daily for 30 s every 2 h from 8:30 A.M. to 6:00 P.M.

2.2. Forcing and Shading Treatments for Plant Nursery Production

In this experiment, three-year-old plants of a Sicilian unnamed clone characterized by a red corolla (Clone 1) were used. These plants were originally from cuttings and were grown in round plastic pots (20 cm diameter) filled with a commercial peat-perlite substrate mix (VIGORPLANT, SER FS V10-18, Italy) 80:20 (*v*/*v*). The substrate mix used had the following properties: pH 5.5, electric conductivity 0.20 dS^{m-1}, (dry) bulk density 95 kg m^{-3}, total porosity 94%, NH_4 220 mg kg^{-1}, NO_3 833 mg kg^{-1}, P 40 mg kg^{-1}, K 631 mg kg^{-1}. In March 2018, to obtain homogenous plants the material plants were pruned to five branches. Seven days before the beginning of the experiment, plants were fertilized with a water soluble fertilizer (20-20-20) at 2 gL^{-1}. In the first week of June 2018, to verify the plant response to shading, half of the plants were placed into a shade house (Aluminet TM 30% shade cloth), whereas the remaining were left outside. To test the effect of different forcing times, three forcing dates were selected: 1 October, 1 November, and1 December. For each shading treatment (shading vs.no shading), half of the plants were transferred into ethylene vinyl acetate (EVA)-covered forcing tunnels and half of the plants were left outside. The experimental plants were watered as necessary to ensure that the plants would not be exposed to drought stress. Maximum, minimum, and average temperature were monitored during the forcing periods (Figure 2).

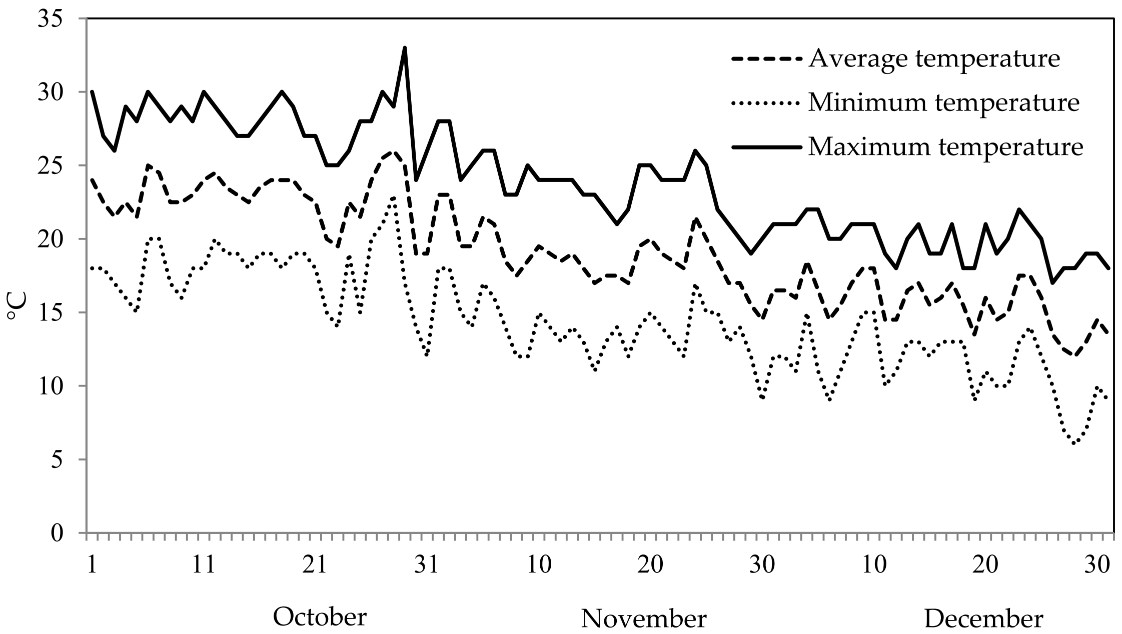

Figure 2. Maximum, minimum, and average temperatures during the forcing periods.

In order to assess the ornamental quality of oleander plants, data were collected on plant height at beginning of flowering, number of days from first of January to beginning of flowering, and number of days from first of January to full flowering.

2.3. Statistical Analysis

In the first propagation experiment, three levels of node number (two-, three-, and four-node cuttings) in combination with IBA concentrations of 0 and 0.3% were tested in a two-factor, randomized complete block design with 4 blocks and 96 cuttings per IBA treatment, 24 per block. Data were subjected to a two-way analysis of variance ANOVA. In the second propagation experiment a 5 × 2 (clones × IBA) factorial set of treatments within a complete randomized block design was used with 4 blocks and 96 cuttings per IBA treatment. Data were subjected to a two-way ANOVA. In the forcing experiment, 2 levels of shading (shading vs. unshaded), in combination with 3 forcing times plus the not-forced treatment were tested in a 2 × 4 factorial set of treatments within a randomized complete block design with 4 blocks and 100 plants per treatment. Data were subjected to a two-way ANOVA.

All data were statistically analyzed using the Statistical Package for Social Science (SPSS) software version 14.0 (StatSoft, Inc., Chicago, IL, USA). Mean separation was assessed by Tukey Honest Significance Difference (HSD) test. Percentage data were subjected to arcsin transformation before ANOVA analysis ($Ø = \arcsin (p/100)^{1/2}$).

3. Results

3.1. Propagation Treatments

In the first experiment, overall percent survival was 97% to 98%; no significant effects of node number and IBA treatment were found on cutting survival (data not shown). Rooting percentage at 30 days after cutting insertion into the rooting medium was significantly affected by node number and IBA treatment (Table 1). Regardless of auxin treatment, three- and four-node cuttings gave a significantly higher rooting percentage as compared to two-node cuttings. Independently of node number, percentage of rooting increased from 83% in untreated cuttings to 93% in cuttings exposed to IBA. However, no significant interaction was found between node number and IBA exposure. Node number did not affect the number of roots per cutting 30 days after planting regardless of IBA treatment (Table 1), whereas root number per cutting at 45 days averaged over IBA was significantly higher in three and four node cuttings as compared to two node cuttings. Irrespective of node level, IBA significantly increased the number of roots per cutting from 7 to 10 and from 16 to 23 after 30 and 45 days, respectively. No significant interaction was found between node number and IBA exposure in terms of root number.

Table 1. Effects of number of nodes per cutting and indole-3-butyric acid (IBA) on rooting and shoot length of *Nerium oleander*.

Treatments	Rooting (%)		No. of Roots Cutting^{-1}				Root Length (cm)		Shoot Length (cm)		
			at 30 d		at 45 d		at 30 d	at 45 d	at 30 d		at 60 d
Node Number (N)											
2	87.7	b [z]	7.8		15.9	b	11.8	12.0	6.8		8.5
3	94.1	a	8.8		22.3	a	10.4	12.2	7.0		8.9
4	91.9	a	8.9		21.1	a	12.2	14.0	8.2		8.6
IBA											
0 ppm	83.5	b	6.9	b	16.4	b	10.4	13.1	5.9	b	8.4
3000 ppm	93.0	a	10.0	a	23.2	a	12.5	12.4	8.1	a	8.8
Significance											
N	** [y]		NS		**		NS	NS	NS		NS
IBA	*		**		***		NS	NS	***		NS
N × IBA	NS		NS		NS		NS	NS	NS		NS

[z] Data within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey HSD Test. [y] The statistical significance is designated by asterisks as follows: *, at $p \leq 0.05$; **; at $p \leq 0.01$; ***, at $p \leq 0.001$; NS = not significant.

Node number and IBA treatment did not significantly affect root length eitherat 30 or45 d from planting in the rooting medium (Table 1). The length of the shoots resulting from sprouting of the buds present on the original cutting material averaged over IBA was not influenced by node number after either 30 or60 days from planting (Table 1). After 30 days, shoots developed from cuttings exposed to IBA were significantly longer than those grown from untreated ones regardless of the node number. There was no significant interaction between node number and IBA exposure in terms of root length.

In the second experiment, there were significant effects of auxin treatment, tested clones, and their interaction for rooting and shoot growth of the cuttings (Table 2).

Regardless of IBA treatment, clone 1 (red) had the highest rooting percentage (94%), followed by clone 2 (pink), clone 3 (white), and clone 5, whereas, clone 4 (salmon) showed the lowest rooting (52%). Rooting percentage averaged over clones accounted for 78% and 80% in untreated and IBA treated cuttings, respectively. The ANOVA revealed a significant effect of the interaction between clones and IBA (Tables 2 and 3). Rooting percentages ranged from 96% in clone 1 (red color) cuttings exposed to IBA to 48% and 56% in clone 4 cuttings exposed and not exposed to IBA, respectively.

Table 2. Effects of indole-3-butyric acid (IBA) on rooting and growing of three-node cuttings of several clones of *Nerium oleander*.

Treatments	Rooting (%)		No. of Roots Cutting^{-1}				Root Length (cm)			Shoot Length (cm)			No. of Leaves Cutting^{-1}			
			at 30 d		at 45 d		at 30 d		at 45 d	at 30 d	at 60 d		at 30 d		at 60 d	
Clones																
Clone 1	93.8	a [z]	8.8	ab	22.3	a	10.4	b	12.1	7.0	8.8	b	16.3	b	28.9	a
Clone 2	90.1	b	10.2	a	14.2	bc	14.7	a	13.5	8.2	10.4	a	19.0	ab	22.4	b
Clone 3	87.5	b	10.2	a	16.5	b	11.6	ab	12.4	9.5	11.0	a	22.1	a	23.0	b
Clone 4	52.1	d	6.3	b	7.3	d	8.4	b	13.3	7.4	8.8	b	15.8	b	23.7	b
Clone 5	71.8	c	7.4	ab	9.3	cd	11.4	ab	10.8	8.9	9.8	ab	19.0	ab	22.9	b
IBA																
0 ppm	78.3		7.3	b	12.9	b	11.8		12.3	7.6	9.4	b	19.5		26.3	a
3000 ppm	79.8		9.9	a	15.3	a	10.8		12.5	8.8	10	a	17.4		22.0	b
Significance																
Clones	***[y]		*		***		**		NS	NS	***		**		***	
IBA	NS		**		*		NS		NS	NS	**		NS		***	
Clones × IBA	**		NS		NS		NS		NS	NS	NS		*		**	

[z] Data within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey HSD Test. [y] The statistical significance is designated by asterisks as follows: *, at $p \leq 0.05$; **; at $p \leq 0.01$; ***, at $p \leq 0.001$; NS = not significant.

Table 3. Effects of the interaction clones and IBA on rooting and growing of three-node cuttings of several clones of *Nerium oleander*.

Treatments	Rooting (%)		No. of Leaves Cutting^{-1} at 30 Days		No. of Leaves Cutting^{-1} at 60 Days	
Clone 1 × 0 ppm	87.5	c [z]	14.5	c	28.6	a
Clone 1 × 3000 ppm	95.8	a	18.0	ab	29.2	a
Clone 2 × 0 ppm	91.7	b	21.8	ab	24.9	b
Clone 2 × 3000 ppm	92.7	b	16.3	b	19.9	c
Clone 3 × 0 ppm	85.4	c	23.5	a	24.2	b
Clone 3 × 3000 ppm	89.6	bc	20.8	ab	21.8	bc
Clone 4 × 0 ppm	56.3	e	19.0	ab	27.6	a
Clone 4 × 3000 ppm	47.9	f	12.5	c	19.8	c
Clone 5 × 0 ppm	70.8	d	18.5	b	26.3	b
Clone 5 × 3000 ppm	72.9	d	19.5	b	19.5	c

[z] Data within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey HSD Test.

Irrespective of the auxin treatment, 30 days after cutting insertion in the rooting medium, clone 2 (pink) and clone 3 (white) gave the highest number of roots per cutting (10.0 roots), followed by clone 1 (8.8 roots), clone 5 (7.4 roots), and clone 4 (6.3 roots) (Table 2). Data expressed as number of roots per cutting 45 days after planting, regardless of IBA, revealed that clone 1 had the highest score because it almost tripled root number. Data from the remaining treatments supported the trend established at 30 days.

Irrespective of the clones, IBA exposure significantly increased root number as compared to the control, both at 30 and 45 days after planting. There was no significant interaction between node number and IBA exposure in terms of root number, either at 30 or 45 days after planting.

Data on root length at 30 d averaged over IBA showed significant effects of clone only. Clone 2 had the longest roots, followed by clone 3 and clone 5. Irrespective of the clones, IBA treatment had no effect on root length. No significant interaction was found between IBA and clones in terms of root length. There were no significant effects of auxin treatment, tested clones, and their interaction for root length at 45 days.

Treatments had no significant effects on shoot length after 30 days from planting (Table 2). However, after 60 days from planting, irrespective of IBA, shoot length was significantly affected by clones. Clone 2 (pink) and clone 3 (white) had the longest shoots, whereas clone 1 (red) and clone 4 (salmon) had the shortest ones (Table 2). Shoot length averaged over clones was higher in IBA treated cuttings as compared to the untreated ones.

Irrespective of the auxin treatment, clones significantly influenced number of leaves per cutting^{-1} at 30 days from planting (Table 2). Clone 3 (white) had the highest number of leaves and clones 1 and 4 the lowest. On the contrary, regardless of the clones, IBA did not significantly affect number of leaves at 30 days from planting. A significant interaction was found between clones and IBA in terms of number of leaves per cutting after 30 days (Table 3); the highest number of leaves per cutting was observed in clone 3 and clone 2 cuttings in absence of IBA, followed by clone 3 cuttings exposed to IBA.

There were highly significant effects of tested clones, auxin treatment, and their interaction for number of leaves per cutting after 60 days from planting (Table 2). Regardless of IBA, clone 1 had the highest number of leaves (29 leaves), followed by clone 4 (24 leaves). Number of leaves per cutting averaged over clones was higher in IBA-treated cuttings as compared to the untreated ones.

Regarding the interaction, the highest number of leaves per cutting was observed in clone 1, either in the presence or in absence of IBA treatment, whereas the lowest value was detected in the combination of clone 5 with IBA (Table 3).

3.2. Forcing and Shading Treatments

There were significant effects of forcing dates, shading, and their interaction for plant height (Table 4).

Table 4. Effects of the interaction forcing and shading on plant height, beginning of flowering, and full flowering of *Nerium oleander* pot plants.

Treatments	Plant Height (cm)		Beginning of Flowering (Days from 1 January)		Full Flowering (Days from 1 January)	
Not-forced × U	82.3	d [z]	122.7	a	142.0	a
October forcing × U	73.0	e	69.0	e	85.0	g
November forcing × U	95.0	c	78.3	d	112.7	e
December forcing × U	97.7	c	111.7	b	130.0	c
Not-forced × S	98.0	c	120.0	a	136.0	b
October forcing × S	116.3	a	71.0	e	96.7	f
November forcing × S	103.0	b	79.3	d	95.0	f
December forcing × S	105.0	b	99.0	c	117.0	d
Significance						
Forcing (F)	***y		***		***	
Shading (S)	**		*		NS	
F × S	***		*		***	

[z] Data within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey HSD Test. [y] The statistical significance is designated by asterisks as follows: *, at $p \leq 0.05$; **; at $p \leq 0.01$; ***, at $p \leq 0.001$; NS = not significant.

Plants exposed to shading from June to September and transferred into the tunnels on October 1 were significantly higher than shaded plants forced on November 1 and December 1. These plants in turn were significantly higher than their unshaded counterparts. Plants in all other treatments were significantly shorter (73 to 98 cm in height).

Regardless of shading, forcing hastened beginning of flowering, as plants transferred into the tunnels in October, November, and December flowered significantly earlier than unforced ones. Regarding the interaction, both shaded and unshaded plants forced in October began to flower 71 and 70 days after January 1, respectively, whereas shaded and unshaded plants forced in November began to flower 9 and 8 days later, respectively. Plants in the remaining plots began to flower significantly later.

Irrespective of shading, forced plants reached full flowering significantly earlier than unforced ones. There was a significant interaction between forcing and shading in terms of full flowering. Unshaded plants forced in October reached the full flowering phase 11 and 10 days earlier than shaded plants transferred into the tunnels in October and in November, respectively. Plants in the remaining treatments reached full flowering significantly later.

4. Discussion

In the first experiment of the present study, three- and four-node cuttings, ranging in length from 10 to 14 cm, were significantly superior to two-node cuttings (8–10 cm long) in rooting percentage and number of roots per cutting. Our results agree with those obtained by Smalley and Dirr [10], Henry et al. [11], Hinesley et al. [12], and Caruso and Iapichino [13], who reported that longer and multi-node cuttings positively affect root count in *Acer rubrum*, *Juniperus virginiana*, *Chamaecyparis thyoides*, and *Plumeriarubra*, respectively, as compared to shorter cuttings. The superior efficacy of longer cuttings in inducing higher rooting performance as compared to shorter cuttings might be attributed, as suggested by Beyl et al. [14] in a study on *Actinidia arguta*, to their higher carbohydrate reserves.

In our study, the application of IBA improved rooting percentage and root number as compared to untreated control. IBA has been reported to increase in vivo and in vitro adventitious root formation in many species, including vegetables, perennials, and ornamental shrubs [15–26].

In the second experiment in the absence of IBA, clones 1, 2, and 3 had a rooting percentage ranging from 85% to 92%, whereas in clones 4 and 5, rooting did not exceed 70%. These findings partly agree with those obtained by Ochoa et al. [8,9], who reported that in the absence of auxin treatments rooting percentage of semi-hardwood oleander cuttings can be higher than 80%. We also found that exposing clones to IBA resulted in an increase in rooting capacity for clones 1, 2, and 3, but had no effect on clones 4 and 5, whose rooting capacity either declined or remained unchanged. This different response among clones confirms that rooting ability of cutting in oleander is influenced by genotype, as reported for other woody species, such as (*Corylusavellana*) and olive (*Olea europaea*) [25,26].

Shaded plants forced in October were significantly higher than those forced in November and in December, which in turn where higher than plants of the other treatments.

Oleander plants forced in October flowered significantly sooner (first decade of March) than unforced ones (first decade of May) and reached complete flowering almost two months earlier (last week of March). Beginning of flowering was delayed in unforced plants. Shading had little effect on plant forced in October and in November as compared to unshaded plants, in terms of beginning of flower, but it slightly hastened beginning of flowering in December forced plants as compared to their unshaded counterparts.

5. Conclusions

According to our findings we suggest that the use of three- and four-node cuttings together with IBA basal dip may be beneficial to propagators wishing to produce oleander with a well-developed root system and capable of achieving rapid establishment in the field. The greater root systems of three- and four-node IBA-treated rooted cuttings as compared to untreated ones facilitated the uptake of water and nutrients and provided more carbohydrate reserves to support early spring sprouting and faster top growth in the subsequent growing season. Shading (from June to September) prior to forcing promoted vigorous growth and taller plants as compared to no shading. Shading might also save a considerable amount of water during the driest months, especially in Mediterranean areas where water is a scarce resource. Finally, by forcing oleander to flower earlier, growers can supply blooming plants to the market when they would naturally still be vegetative. Overall, this would provide nurseries involved in ornamental plant production with a great marketing tool. Therefore we can conclude that the use of appropriate plant propagation material and techniques together with opportune shading and forcing might be beneficial to Sicilian plant nurseries involved in oleander production.

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