The Differences in Exit Propagation via Cuttings for *Cistus ladanifer* and *Cistus* × *cyprius*

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***Abstract*— In Mediterranean ecosystems, reforestation and the cultivation of aromatic species from the *Cistus* genus are vital for restoring degraded soils and producing essential oils. Propagation via cuttings is crucial for preserving local genetic material, but a clear methodology is often lacking. This study evaluated the optimal conditions for the vegetative propagation of *Cistus ladanifer* L. and *Cistus* × *cyprius* Lam. from cuttings, with the goal of maximizing both the rooting rate and root length. Cuttings from both species were collected across four seasons (winter, spring, summer, and autumn) and were subjected to four different doses of IndoleButyric Acid (IBA) in four distinct greenhouse temperatures. The results from statistical analyses (ANOVA) demonstrated that the time of year is the most significant factor. The highest rooting percentages and longest roots were obtained from cuttings collected in winter and autumn, while summer proved to be the worst period. A dose of 750 mg/l of IBA was found to be most effective for promoting root growth. Additionally, higher cultivation table temperatures, up to 32 °C, favored greater root length. In conclusion, this study provides a clear methodology: for successful propagation, it is recommended to take cuttings in autumn or winter, treat them with 750 mg/l of IBA, and cultivate them at 32 °C.**

Keywords-Cistus; rooting; cutting propagation; cultivation conditions; aromatic shrubs.

I. INTRODUCTION

In Mediterranean areas, reforestation and forest cultivation have become more important given the impact of climate change and wildfires on soil loss and habitat degradation [1]. Efforts to restore degraded ecosystems by means of forest cultivation and restoration are vital to maintaining rural and natural ecosystems and ensuring the profitability of primary activity in rural areas. Thus, trees and shrubs are being grown for restoration and forest cultivation. Nonetheless, while the origin of specimens for agricultural production might not be crucial, the biodiversity and ensuring the local origin of implanted specimens are essential in forest cultivation.

The use of local genetic material in both reforestation and forest cultivation is essential to ensure that planted individuals are well adapted to the specific edaphoclimatic conditions of the area [2]. This impacts both the survival rate of reforested specimens and the productivity of forest cultivation. Moreover, using local individuals helps prevent the genetic contamination of native populations, thereby safeguarding their evolutionary potential and the species' genetic integrity.

Besides the use of forestry species for restoration, some of them can potentially be exploited for commercial purposes. In the case of aromatic shrubs, the extraction of essential oils has become a valuable resource. Some Mediterranean species currently exploited for their essential oil and other subproducts, are from the *Salvia, Cistus*, and *Thymus* genera. The propagation of some species can become challenging, since this propagation in natural conditions is linked to the occurrence of wildfires. These are known as pyrophytic species; examples are *Cistus ladanifer* L. and *Cistus laurifolius* L, among others [3]. *C. ladanifer* is an important species for its essential oil production [4]. The most effective way of artificially propagating the local specimens is the use of cuttings. Moreover, propagating the exploited individuals by seeds does not ensure that new individuals share the same traits as the original one.

Even though there are multiple benefits of using cuttings as a strategy for propagation, the success of the cutting depends on multiple factors. Some authors indicated that cutting propagation moment, the planting moment, might even interfere in the production of essential oil [5]. Propagation success is commonly evaluated based on the rooting rate, expressed as the proportion of cuttings that formed roots under controlled conditions. Some of these factors can be extrinsic, such as environmental temperature, photoperiod, or the inclusion of growth regulator hormones. In contrast, other factors are intrinsic and directly related to the plant physiology when the cutting collection occurs. In fact, differences among species' ecology can generate the fact that the best conditions for a given species' propagation differ from those for other species.

The aim of this paper is to evaluate the best conditions for propagating specimens of *C. ladanifer* and *C.* × *cyprius* for production purposes. To do so, cuttings were propagated at four different times of the year. Individuals of both species are included in this study to assess if there are differences between them. To homogenise the environmental conditions, propagation was conducted under stable temperatures.

The main challenge for this research is the limited natural distribution of *C.* × *cyprius*. Besides, the lack of previous studies of cutting propagation success in *C. ladanifer* and *C.*

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× cyprius populations in Spain, jointly with diverse results in other populations, poses a scenario with multiple factors to be studied.

The rest of the paper has been structured as follows; Section 2 outlines the related work. The materials and methods are described in Section 3. Then, Section 4 discusses the obtained results, indicating the best conditions to propagate individuals of both species. Finally, the conclusions are summarised in Section 5.

II. Related Work

In this section, we summarise current efforts comparing the best conditions for propagating Mediterranean species through different methods.

A recent study conducted by Kostas et al. [6] in Greece with *Rosmarinus officinalis* L. indicates rooting success across all seasons (April, July, October and January) from 7 different locations. Currings were grown in controlled conditions, and potassium salt Indole-3-butyric acid (K-IBA) was used. The results indicate that when no K-IBA was used, success is strongly conditioned by the season, reaching the best success in October with a success rate of about 40 %. When K-IBA was used, success reached 80 % in October. There is a high variability in rooting success among locations and seasons.

A similar study was performed by Scaltrito et al. [7] in Italy with *Salvia* 'Farina Silver Blue' and 'La Siesta'. They evaluated the success rate of cuttings under an aeroponics system. Spraying interval and the IBA dose were the evaluated factors, while the root length and root diameters were the evaluated parameters. Their results indicated that propagation by cuttings in an aeroponic system is possible and has a high success rate with a spraing interval of 10 minutes and with 1g/L of IBA. Results are similar for both cultivars in terms of root length but strongly differ in root diameter.

A recent study, conducted on specimens of the genus *Cistus,* was presented by K. Ioannidis and Koropouli [8] with *Cistus creticus* L. (rockrose). In this case, in vitro culturing was evaluated. They determined that the origin of plant material does not impact the success of propagation. The maximum success in routing reached 98.61 % using an enriched medium. Other authors have assessed the in vitro propagation of *C. ladanifer* to culture tissues from leaf and stem explants [9]. Finally, Boukili et al. [10] in Morocco have assessed the in vitro propagation of a given ornamental variety of *C. ladanifer*. They have used explants from seed germination and from wild plants in the field. Their results indicate a low caulogenic response for explants from wild plants. High success was achieved using microcuttings derived from shoots regenerated through micropropagation.

Some authors pointed out that the best moment for *C. ladanifer* propagation is during autumn [11], but no data is provided to support this affirmation, and no information on the percentage of routing success has been reached. In addition, no details of an effective method for cutting propagation have been provided.

As far as we are concerned, no clear methodology was found for cutting propagation of *C. ladanifer* or *C. × cyprius*. Using in vitro cultivation has also been challenging and relies on seeds, which do not ensure maintaining the genetic traits of parental plants. Therefore, the obtention of a method to effectively propagate individuals for both production and reforestation purposes is needed.

III. Materials and Methods

This section describes the origin of plant material and the methods and materials for plant propagation by cuttings.

A. Sampled Area Description

The plant material was sampled at the Sierra Norte of the Community of Madrid (Spain), where specimens of *C. ladanifer* were collected at Berzosa del Lozoya and *C. × cyprius* at Bustarviejo.

Both sites are characterized by an altitude over 1200 meters above sea level, the climate is continental (average temperature of 10.4 ºC and annual precipitation of 520 mm, typically distributed along spring and autumn). The area has very stony soil, which is classified as Inceptisols/Entisols [12]. Dominant vegetation includes forests of oaks and pine, alongside a rich understory of Mediterranean scrub, mainly the *Cistus* genus.

B. Sample Collection

Throughout the hydrological year (winter, spring, summer and autumn), fifty plants from both specimens were collected in order to cover their entire phenological spectrum. This corresponded to the months of December, March, June and September.

The collection was carried out manually by selecting cuttings that had sprouted during the year. The cuttings were 15–20 cm long, with 2–3 whorls kept and the leaves removed to prevent further water loss. The sampling locations of the individuals were geolocated so that they could be reproduced in future. On the same day, the cuttings were transferred to IMIDRA and stored at 4–6 °C until the following day.

C. Treatmeants

Heated tables were prepared at 20, 24, 28 and 32 °C in a greenhouse with a perlite substrate prior to the cuttings being placed on them (Figure 1). Four treatments of IndoleButyric Acid (IBA), a synthetic hormone used as a rooting promoter, were also applied at concentrations of 0, 750, 1500 and 3000 mg/l. The day after collection, the cuttings were immersed in the solution for two minutes and immediately placed on the substrate.

D. Cultivation and Measures

The greenhouse tables were covered with plastic (Figure 1d) and irrigated every two hours for two minutes to maintain a saturated atmosphere. Fungicide treatments were applied as needed.

Four months after planting, the success of the rooting process was evaluated. The number of cuttings that had rooted was determined by measuring the length of the root. Those that had not rooted were differentiated according to whether they had continued to grow after planting or had died since being cut.

Other measures were made, but not included in this work.

E. Statistical Analysis

The differences in rooting and root length success among the various treatments were assessed using variance analysis (ANOVA). The Least Significant Difference was used to obtain a multiple-range group test. Statgraphics Centurion XVIII was employed.

The factors studied are: species (*C. ladanifer* and *C. × cyprius*), season (autumn, winter, spring, summer), IBA dosses (0, 750, 1500, and 3000 mg/L), and temperature (20, 24, 28, and 32 ºC).

IV. RESULTS

This study analyses the influence of the cutting season, growth temperature and rooting hormone dose applied, both quantitatively and qualitatively, in the *C. ladanifer* variety and its hybrid, *C. × cyprius*. The quantitative analysis is based on measuring the success rate of rooting for the total number of cuttings planted (6,385), while the qualitative analysis is based on measuring the root length obtained for the 2,119 cuttings that showed roots.

A. Successful rooting

The analysis considered three scenarios: the presence of roots, the absence of roots, and the death of the cutting. The species with the lowest mortality rate and the highest number of individuals with developed roots is considered to be the best for cuttings. Figure 2a shows that there are no graphical differences in mortality between species with similar rates: 9 % for *C. ladanifer* and 8 % for *C. × cyprius*. However, there is greater success in rooting (19 %) for *C. × cyprius* compared to 15 % for *C. ladanifer*.

Regarding the season for cutting, there is a clear difference, with winter and autumn being the best periods for cutting (see Figure 2b), since *Cistus* plants are dormant at these times and do not produce any vegetative growth. Both periods show low mortality rates among the cuttings, with the winter period standing out with zero mortality and a higher number of individuals with roots. In contrast, during spring and summer, mortality rates reach 8 % of individuals. Furthermore, summer is clearly the worst time to carry out these grafting tasks, as only 1 % of individuals had roots.

Figure 1. Greenhouse tables: (a) setting up; (b and c) heating system for 20, 24, 28 and 32 °C; (d) winter cuttings on perlite substrate.

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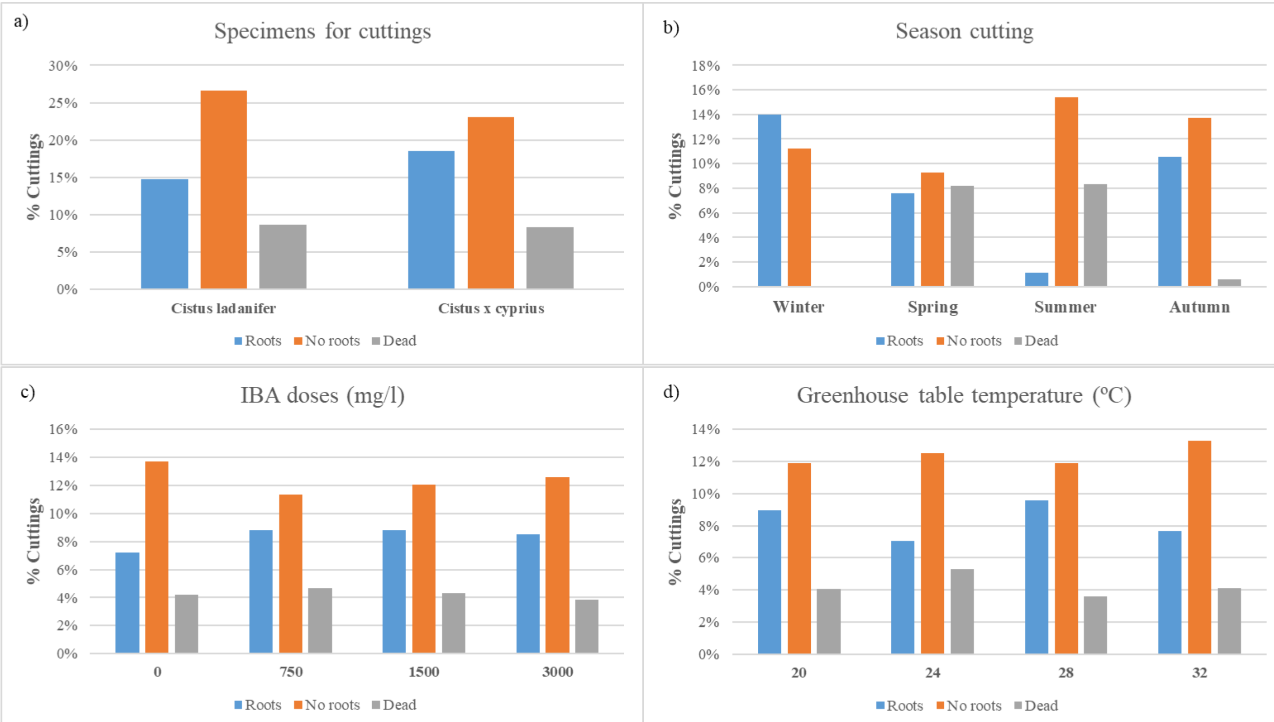

Figure 2. Rooting Success by (a) specimens of *C. ladanifer* and *C. x cyprius*, (b) season cutting, (c) IBA doses and (d) greenhouse table temperature.

Regarding the effect of applying IBA (Figure 2c) or varying the temperature conditions of the table (Figure 2d), neither seems to significantly affect rooting success. However, a slight increase in root presence is observed when IBA is applied compared to when it is not.

B. *Root length*

Following, the differences in root length are analysed. First of all, the effects of different factors are presented. Then, some images are provided to evidence the encountered differences.

Root lengths ranged from 0.5 to 45 cm, with an average of 11.6 cm ± 6.4 and a median of 11.0 cm for the 2,119 individuals with roots (see Table I and Figure 3). ANOVA was performed on these to determine the most effective treatment combinations for taking cuttings from *C. ladanifer* and *C. x cyprius*. The analysis confirmed that there were significant differences in root length depending on the time of year that cuttings were taken. Winter resulted in the longest roots, with average length of 13.9 cm, followed by autumn with an average length of 11.2 cm (Figure 3b). However, there were no differences in root length between species (Figure 3a).

TABLE I. ROOT LENGTH BY ALL ROOTED INDIVIDUALS AND BY THE FACTORS CONSIDERED. THE LETTERS SYMBOLIZE GROUPS OF SIGNIFICANCE.

		N	Avg ± SD	Median
Total Rooted		2119	11.6 ± 6.4	11.0
Specimens	*C. ladanifer*	935	$11.4^{a} \pm 6.4$	10.5
	C. × cyprius	1184	$11.7^{a} \pm 6.4$	11.5
Season	Winter	887	$13.9^{a} \pm 6.3$	14.0
	Spring	483	$8.7^{b} \pm 5.7$	8.0
	Summer	73	$6.4^{c} \pm 5.6$	5.0
	Autumn	676	$11.2^{d} \pm 5.8$	10.5
IBA (mg/l)	0	454	$11^{a} \pm 6.1$	10.5
	750	562	$12.5^{b} \pm 6.7$	12.0
	1500	561	$11.1^{a} \pm 6$	10.0
	3000	542	$11.7^{a} \pm 6.6$	11.0
Temp. (ºC)	20	566	$10.9^{a} \pm 5.2$	11.0
	24	451	$11.2^{ab} \pm 6$	11.0
	28	612	$11.9^{bc} \pm 6.9$	10.5
	32	490	$12.3^{c} \pm 7.2$	12.0

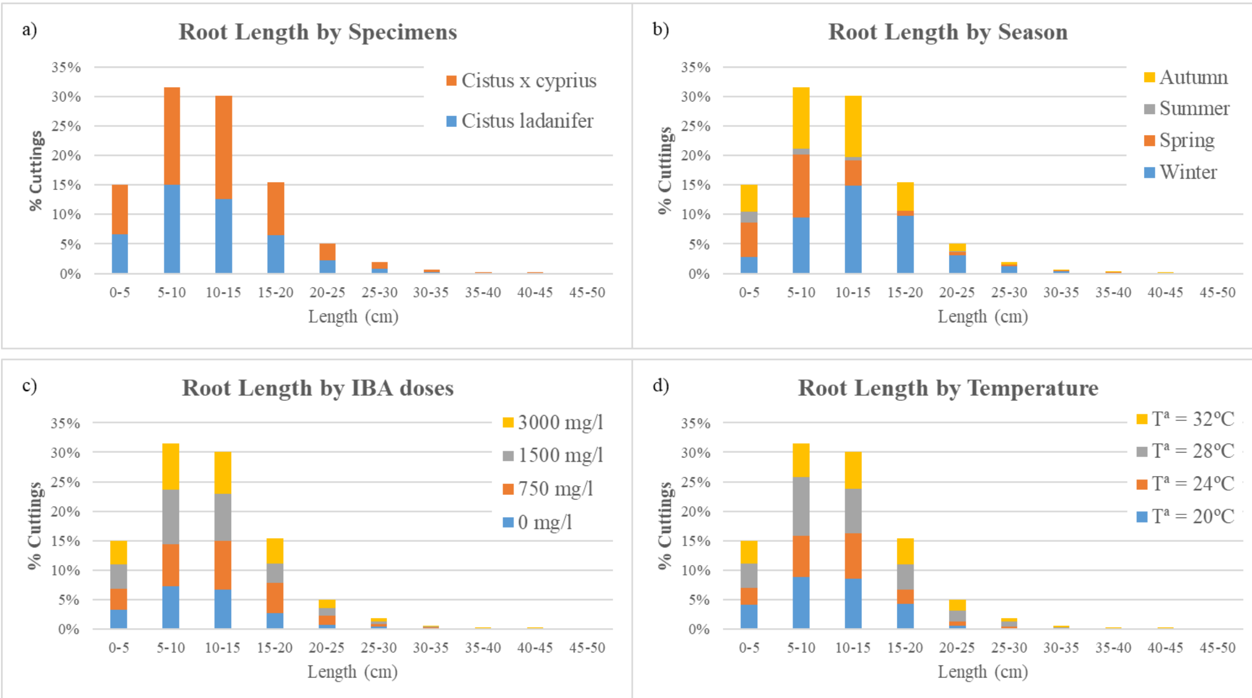

Figure 3. Rooting Length by (a) specimens of *Cistus ladanifer* and *Cistus* x *cyprius*, (b) season cutting, (c) IBA doses and (d) greenhouse table temperature.

The IBA dose applied (Table I, Figure. 3c) only shows differences for the 750 mg/l dose with a root length of ~1.5 cm greater than the other doses considered (Figure 4). This suggests that the crop's capacity to absorb the hormone is exceeded at higher doses, resulting in waste.

The temperature of the greenhouse table (Table I, Figure 3d) shows an upward trend in root length as the temperature increases, with differences appearing when the distance between them exceeds 4°C. Thus, the 32°C table stands out with a greater root length (12.3 ±7.2 cm).

Multifactorial ANOVA analysis shows that the only significant factor affecting root length is the season in which the cuttings are taken. However, several significant interactions were identified, indicating that the combined effect of IBA treatment and temperature on root growth varies substantially depending on the season. Furthermore, when cuttings are taken in spring, it is observed that the C. ladanifer variety has longer roots, and the interaction between temperature and IBA dose is maintained. In this case, however, the recommended temperature would be 24 °C, with the dose remaining at 750 mg/l.

Figure 4. *Cistus ladanifer* spring cuttings roots on 24 ºC table with (a) no, (b) 750 mg/l, (c) 1500 mg/l and (d) 3000 mg/l of IBA doses.

V. Discussion

Finally, a discussion comparing the obtained results with existing literature is provided.

Compared with existing studies, the obtained root success was considerably lower [6][8]. In [6], the success rates range from 40 to 80 % for *Rosmarinus officinalis* and in [8] the success rate exceeds 98 % for *Cistus criticus*. Other authors have also reported variable success rate with *Myrtus communis L*. ranging from maximum rates of 43% for white myrtle to 76 % for black myrtle [13] or *Juniperus sabina L.* with maximum rates of 60 % [14] or Salvia fruticosa Mill. with maximum rooting success of 80 % [15]. For *C. ladanifer* and *C. × cyprius*, the success rate never reached 40 %. Nonetheless, the used species are different from those in the aforementioned papers. Thus, differences in routing success might be due to the different growing conditions and physiology of different species. There is no data about routing success in previous work conducted with the used species (*C. ladanifer* and *C. × cyprius*).

Concerning the best conditions for cutting, our results indicate that the best moment for propagation of *C. ladanifer* is in both winter and autumn, which is partially aligned with [6] and [11]. The highest success and longest roots were achieved with 0.75 g/l of IBA, which is similar to the conclusions of [7]. The maximum rate was reached with 1 g/l [14] and with 0.5 g/L in [13]. Thus, our results in terms of the effects of the analysed factors are aligned with existing literature.

VI. Conclusion and Future Work

In this paper, the success of cutting propagation, in terms of rooting, of two Mediterranean species has been evaluated. Research has shown that the rooting capacity of both species remains consistent, with the exception of spring rooting. In this season, *C. ladanifer* exhibits a marginally enhanced response, as indicated by an increase in root length.

The best season for cutting is autumn and winter, preferably winter, applying a hormonal treatment of 750 mg/l IBA and maintaining the greenhouse table temperature at 32 ºC. If cutting is carried out in spring, the table temperature can be reduced to 24 ºC. Cuttings should be avoided during the summer months.

In future work, the evaluation of other aspects, such as flower development of the cuttings and their adaptation and survival in field conditions, will be conducted. Moreover, intermediate doses of IBA will be studied. Finally, efforts to conduct in vitro propagation to reach higher success will be considered.

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