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Does the Physiological Age of Stock Plant Material Affect the Uptake of Indole-3-Butyric Acid (IBA) in Leafy Cuttings of *Prunus subhirtella* 'Autumnalis'?

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Abstract: The aim of this study was to investigate the uptake of exogenously added indole-3-butyric acid (IBA) as an adventitious root (AR) inducer. The concentration of indole-3-butyric acid (IBA) in leafy cuttings of *Prunus subhirtella* 'Autumnalis' from stock material of different physiological ages was analyzed at three evaluation time points (1, 4, 24 h). We examined three stock plants: a physiologically mature stock plant (approximately 60 years old); physiologically rejuvenated plants (16 years old) that were previously propagated by cuttings from semi-mature stock material; and in vitro juvenile stock material. For IBA to effectively induce AR formation, it must first be converted into the active hormone indole-3-acetic acid (IAA). The total concentration of IAA in the first 24 h after cutting was evaluated according to the physiological age of the stock material and the IAA concentration in the cuttings according to the evaluation time points was assessed. We also examined rooting success and the quality of the adventitious root system depending on the physiological age of the stock material. High performance liquid chromatography coupled with mass spectrometry (HPLC-MS/MS) was used to identify and quantify the phytohormones. The difference in rooting and quality of the developed AR was observed between semi-mature stock and mature stock material. Cuttings from semi-mature plants rooted in 95.00 ± 5.00% of cases, while cuttings from mature stock plants only rooted in 68.33 ± 4.09%. Successfully rooted cuttings from mature stock material were accompanied by callus formation, which was significantly lower in cuttings from semi-mature stock material. Our results show that the interaction between physiological age and time after severance had no effect on IBA concentration in the cuttings (p = 0.907). Duration of time elapsed since severing (evaluation time points) had a significant effect on the uptake of IBA (p = 0.002 **) and IAA (p = 0.0009 ***) in the cuttings. Physiological age had a significant effect on IAA concentration in the early stages of AR formation in the cuttings (p = 0.038 *). Our results suggest that the dynamics of other endogenous phytohormones and the physiological state of the stock plant are also important for AR formation, as the proportion of successfully rooted cuttings from semi-mature stock material was significantly higher than the proportion of successfully rooted cuttings from mature stock material, irrespective of IBA uptake in stock material of different ages.

Keywords: adventitious roots; auxin; ornamental cherry; phytohormones; vegetative propagation

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1. Introduction

Propagation by cuttings is the most important and most frequently used method of vegetative propagation in ornamental horticulture [1]. The formation of adventitious roots (AR) is a crucial step in the propagation of cuttings of economically important horticultural and woody species [2]. Recent studies on various plants show that complex interactions between many hormones occur during the formation of ARs [3–6]. Among these hormones, auxin plays a central role in stimulating the formation and development of ARs and is involved in almost all interactions with other phytohormones [3–6]. The AR process

and the physiological stages of rooting are correlated with changes in endogenous auxin concentration, which is known to trigger AR formation. The most common natural form of auxin is indole-3-acetic acid (IAA). A high rooting rate is usually associated with a high endogenous auxin concentration at the beginning of the rooting process [5–8]. In the propagation of *Pisum sativum*, Rasmussen et al. [5] found that the IAA concentration immediately after cutting is much higher in the cutting bases of juvenile material than in mature plants. At 6 h after cutting, the IAA concentration peaked in the younger tissues and decreased to the background concentration by 24 h after propagation [5]. This early IAA peak was not observed in the older cuttings, which indicates that the auxin level does not increase as much as in younger cuttings and that the peak value is reached much later in the older cuttings [5]. The formation of ARs is of great practical importance in horticulture, but many important plant species, especially those belonging to woody species, are difficult to root [9]. Since auxins have been shown to be effective in AR formation, exogenous application of auxin is used to promote rooting of cuttings in several plant species [10–13]. The most commonly used exogenous auxin is indole-3-butyric acid (IBA). After IAA, IBA is the most frequently occurring natural auxin. Compared to other auxins, IBA has a higher root induction capacity and greater light- and temperature-stability [9]. IBA was originally thought to be a synthetic auxin compound used primarily as a rooting agent, but it was later discovered to be an endogenous compound in several plants. IBA is structurally similar to IAA except for the side chain, where IAA has two carbons and IBA has one side chain with four carbons. In studies where endogenous IBA has been detected, its content is often low and difficult to detect, being much lower than that of IAA [14,15]. The proportion of IBA in the total auxin content varies from species to species, indicating different IBA metabolism in different plants [14]. Thus, the physiological effects of IBA treatment are probably caused by IBA-derived IAA and not by the IBA molecule itself. For IBA to be an effective inducer of root initiation, it would first have to be converted into the active hormone IAA. The main mechanism by which IBA influences plant development is the reduction of its carbon side chain to convert to IAA [14,16]. Since root formation is a complex process involving hormonal, developmental and environmental factors, the ability to convert the applied IBA into the active hormone IAA is a crucial step without which hormone treatment is ineffective. IBA can be converted to IAA by the removal of two methylene units from the side chain through the multi-step process of β-oxidation in the peroxisomes [16]. In cuttings, the effect of IAA in the area of root development in a short period of time, directly after wounding, is decisive for the successful development of the AR [6]. The 72 h period after the start of propagation is divided into three phases: the induction phase, the root initiation phase and the root growth and development phase. The presence of auxin IAA at the base of the cutting is crucial within 24 h of the start of propagation [17]. A high auxin content is required for a successful induction phase and has an inhibitory effect in the root growth and development phase. However, even in the induction phase, the IAA concentration must be optimal for successful development of the AR formation. It is the rate of auxin metabolism that can explain the differences in rooting success between different species [18,19]. Phytohormones, together with many other internal and external stimuli, co-ordinate and control every step of the formation of ARs [4]. External stimuli such as light, wounding, and temperature influence this complex process through interaction with auxin [9]. The process of physiological aging in woody plants is another very important factor affecting ARs [7]. The internal physiological status of a stock plant plays a crucial role in the process of vegetative propagation. Aging has a negative effect on rooting ability in the process of vegetative propagation [5,7,20]. Various reports have shown that physiologically younger propagation material is more suitable for successful adventitious rooting, as this age effect is very strong [5,7,21].

Prunus subhirtella ($2n = 16$, diploid) family Rosaceae, genus *Prunus* L., section Cerasus, is an ornamental cherry most commonly found in horticulture. It originates from Transcaucasia, Asia Minor and Persia and is used as a rootstock for cherries due to its winter hardiness and has some potential for genetic improvement of cherries [22]. *Prunus*

subhirtella 'Autumnalis' is considered to be a plant that is easily propagated [20]. It is generally known that the physiological condition of the stock material plays a decisive role in rooting success. If a low level of free basal IAA is a limiting factor for rooting, one might expect that exogenous basal IAA, or other synthetic auxins would stimulate rooting. In cuttings treated with synthetic auxins such as IBA, the IAA concentration at the base of the cuttings may rise temporarily and then fall again before rooting, sometimes within 24 h [19]. The following experiment was carried out to test whether the uptake of exogenously applied IBA in the first 24 h after cutting is the same in all physiologically different old stock plants and whether there are noticeable differences in the quality of the newly developed adventitious root system depending on the physiological age of the stock plant. Pacurar et al. [9] reported that exogenously supplied IBA accumulated only in cuttings of the easy-to-root cultivar for several days after severance, which may indicate that the difficult-to-root cultivars are unable to properly hydrolyze IBA conjugates during the appropriate steps of AR formation. Since the physiological status of the stock plant has a decisive influence on the success of adventitious root formation, the uptake of IAA was also tested. The dynamics of IBA and IAA in cuttings from different starting materials were studied during the first 24 h after the exogenous addition of IBA. The period of 24 h after separation from the stock plant is important because a high endogenous auxin concentration is required during the induction phase of AR formation [20].

2. Material and Methods

2.1. Sample Preparation

The experiment was conducted at the Biotechnical faculty (46°3′4″ N; 14°30′18″ E) of the University of Ljubljana (Ljubljana, Slovenia) in a greenhouse with an automatically controlled fogging system that enabled a humidity of 98–100%. A high-pressure fogging system allows the cuttings to develop both roots and above-ground parts in one growing season. There was a natural photoperiod as it occurs and changes in the northern hemisphere during the growing season from June to November (46°3′4″ N; 14°30′18″ E). The substrate used for the experiment was a mixture of peat and sand in a 1:1 ratio. The substrate was fertilized with Osmocote Exact (16 (N) – 9 (P) – 12 (K) + 2 (MgO) + trace elements) fertilizer (ICL). We used 2 g of fertilizer per liter of substrate, which was mixed until the fertilizer was evenly distributed.

The propagation beds in which the cuttings were placed were divided into 12 plots with four replicates for each treatment (physiological age of the stock plant). In each plot 28 cuttings were used. For the evaluation of IBA concentration, 12 cuttings were removed from each plot on the same day of the experiment (three cuttings per evaluation time: immediately after separation from the stock plant (control), after 1 h, after 4 h and after 1 day), and the remaining 16 cuttings were left in the substrate to evaluate the rooting quality in the fall, at the end of the growing season in November. The experiment was designed as a two-factorial design with four replicates, with the physiological age of the stock plants and the time after severance being the selected factors. The replicates were distributed according to the simple random sampling. Each plot represented a single experimental replicate.

In our experiment, three types of stock plants of the Japanese ornamental cherry *Prunus subhirtella* 'Autumnalis' were used for cuttings: mature, semi-mature and juvenile. The mature stock trees were about 60 years old, both rejuvenated stock plants were 16 years old. In 2002, the mature trees were propagated in vitro and propagated by cuttings. In vitro propagation and propagation by cuttings from mature stock plants served as a rejuvenation method for physiologically mature stock material. Both the in vitro propagated plants and the plants obtained from cuttings were planted out in the experimental field of the Biotechnical faculty (Ljubljana, Slovenia) in spring 2003. The plants were grown as stock plants in the following years. This means that the semi-mature and young stock plants were pruned every spring, while the mature stock plants were not pruned, but only the dry branches were cut off. The different types of stock plants served as experimental varieties.

Leafy cuttings were harvested in summer in June. The shoots of the current year (diameter 3–5 mm) were used from each type of stock plant, whereby the upper part of a cutting was shortened to a length of 10 cm and the lower 2–3 leaves were removed. A mixture of peat and sand in a 1:1 ratio was used as the substrate for the experiment. Before insertion into the substrate, their bases were treated with 5000 ppm IBA (10 mg of a mixture of 0.5% indole-3-butyric acid (IBA) and 10% Captan (Captra)). The production of the hormone powder begins with the weighing of the hormone IBA (>95% purity). As the hormone is not soluble in polar solvents, it is dissolved in a small amount of acetone. When the hormone is dissolved, water is slowly added to prevent precipitation. Captan is then added, followed by the slow addition of talcum powder as a carrier for the mixture. We dip the basal parts from 0.5 to 1 cm deep into the mixture for three to five seconds. Then we insert them into the substrate (~2 cm). The IBA mixture was not added to the cuttings bases of the control observations, as the purpose of the control was only to check whether IBA was endogenously present in *Prunus subhirtella* 'Autumnalis'. Three cuttings from each plot (12 cuttings per physiological age of the stock material) were removed from the substrate at three evaluation time points: 1, 4, 24 h later. Samples were washed, so there was no substrate or IBA mixture residues, placed in liquid nitrogen and stored at −20 °C for further analysis.

2.2. Evaluation of the Root Formation

The quality of the root system of cuttings growing until the end of the season (120 days) was evaluated using a scheme (Figure 1). The quality of the newly formed adventitious root system was determined using grades 1–6, with grade 1 being the lowest and grade 6 being the highest. The criteria for a particular classification are described in the legend of Figure 1. The number of main roots, length of the root bush, number of newly formed shoots, and their lengths were also observed and measured.

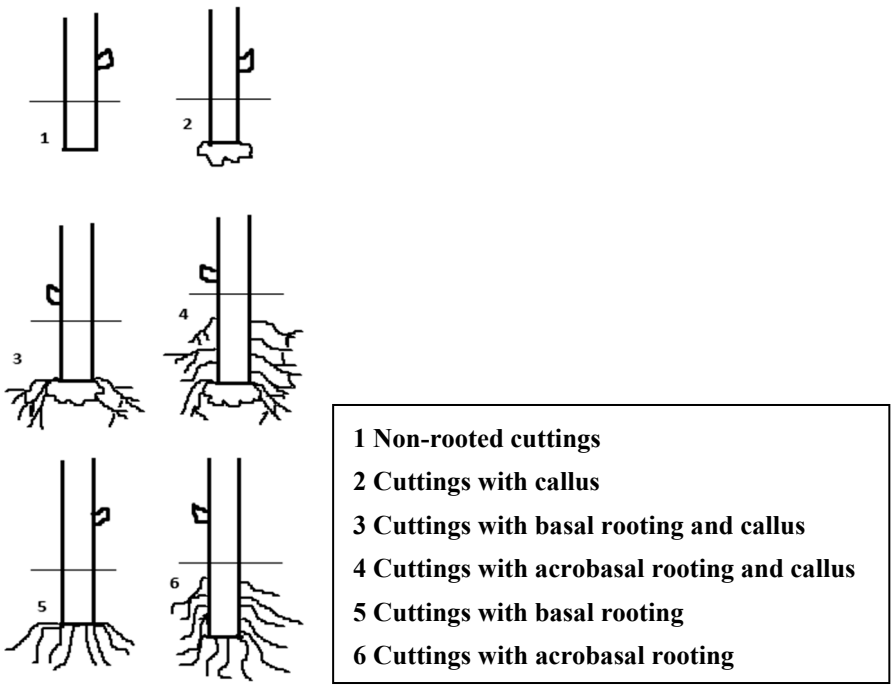


Figure 1. Scheme for evaluating the quality of the newly formed adventitious root system.

2.3. Phytohormone Extraction and Analyses

IBA and IAA were extracted according to the modified method of Osterc and Stampar (2011) [7]. The samples (leafy cuttings, frozen in liquid nitrogen and stored at −20 °C) were ground to a fine powder using a mortar and pestle in liquid nitrogen. Then, 250 mg of the sample was extracted with 1 mL of 100% acetone and 4 mL of double-distilled water

adjusted to pH 3.5 with formic acid. The samples were put on ice shaken for 1 h (Unimax 1010 shaker, Heidolph, Schwabach, Germany). Then, the samples were extracted using a vacuum separator (Supelco, Merck KgaA, Darmstadt, Germany). Strata C18-E columns (pore size 55 µm, retention capacity 500 mg, tube size 6 mL; Phenomenex, Torrance, CA, USA) were used for auxin purification. To activate the columns to an acidic pH, they were first washed with 2 mL of 100% acetone followed by 2 mL of double-distilled water with the addition of formic acid (pH 3.5). During the activation of the columns, the samples were kept in the refrigerator. The samples were run through the columns after that. After the samples were run through, the columns were washed with another 2 mL of double-distilled water. Due to the acidic environment in the cartridge filler, the auxins bound to the filler, so the eluates were discarded. The auxins were finally washed from the cartridge by rinsing the cartridges with 2 mL of 80% acetone in double-distilled water. The solution was filtered into the vials and the samples were stored at −20 °C until further analysis.

IBA was analyzed using a UHPLC system (Dionex UltiMate 3000; Thermo Scientific, Waltham, MA, USA) coupled with a tandem mass spectrometer (LTQ XL; Thermo Scientific, Waltham, MA, USA) according to the modified Almeida Trapp method (2014) [23]. The C18 column (150 × 4.60 mm; 3 µm column; Gemini; Phenomenex, Torrance, CA, USA) was used to separate IBA and IAA, and the temperature was set at 25 °C. The following solvents were used for the analysis: Solvent A, 0.1% formic acid with 3% acetonitrile in double-distilled water ($v/v/v$); and Solvent B, 0.1% formic acid with 3% double-distilled water in acetonitrile ($v/v/v$). The elution profile was: 0–10 min, 42–55% B; 10–13 min, 55–100% B; 13–15 min 100% B; 15.1–20 min 42% B and at 20–23 min the reconditioning of the column was performed. The flow rate of the mobile phase was 0.6 mL/min. The injection volume was 20 µL. IBA was identified by coupling the UHPLC system with a tandem mass spectrometer (LTQ XL; Thermo Scientific, Waltham, MA, USA) using heated electrospray ionization. The mass spectrometer was operated in negative ion mode for the identification of IBA and IAA. The following parameters were used for auxin identification: capillary temperature, 200 °C; capillary voltage, 8.0 V; sheath temperature, 200 °C; sheath gas, 30 arbs; ion spray voltage, 4.5 kV; tube lens, −82 V; and auxiliary gas, 10 arbs. After determining the retention time and identifying typical fragmentation patterns of the quantification standards, these selected compounds were identified by selected reaction monitoring (SRM). The transitions with the optimized parameters and retention times are listed in Table 1.

Table 1. SRM transitions for phytohormone quantifications.

Phytohormone	Retention Time (min)	Pseudomolecular Ions (*m*/*z*) $[M-H]^-$	Fragmentation Pattern (Relative Peak Intensity %)
IBA	7.87	202	158 (100), 184 (9.67), 115 (0.34)
IAA	5.50	174	130 (100)

Indole-3-butyric acid (IBA), Indole-3-acetic acid (IAA).

2.4. Chemicals

Indole-butyric acid and indole-3-acetic acid (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) were used to identify and quantify the phytohormone. For extraction and further analysis, we used acetone (Merck KgaA, Darmstadt, Germany), formic acid (Kemika d.d., Zagreb, Croatia), and acetonitrile (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

2.5. Statistical Analysis

Data were collected using Microsoft Excel 2016 and statistically analyzed using R commander (package Rcmdr) version 2.3.0. One-way analysis of variance (ANOVA) was performed. For pairwise post-hoc multiple comparisons, package 'emmeans' and Tukey tests were performed. All data are presented as means ± standard errors (SE). Statistical means were calculated at a 95% confidence level to determine significance of differences ($p < 0.05$).

3. Results

3.1. Phytohormone Dynamics in Cuttings

The cutting bases were treated with exogenously applied IBA using the quick-dip method before being placed in the propagation beds for rooting. Cuttings taken directly from the stock plant without IBA treatment served as a control treatment for endogenous IBA concentration in *Prunus subhirtella* 'Autumnalis'. We tested whether the physiological age, the evaluation time after exogenous IBA application and their interaction influenced the uptake of IBA in the cuttings. In the control observations (without exogenously added IBA), IBA was not found, or the concentration was too low to detect it regardless of the physiological age of the stock material. Our results show that the interaction between physiological age and time after severance has no effect on IBA concentration in IBA cuttings ($p = 0.907$). There were also no significant differences in IBA concentration between observations at other evaluation times (after 1, 4, 24 h) (Figure 2). In a next step, we tested whether physiological age or time after cutting influenced IBA or IAA concentration in the cuttings. Our results showed that physiological age had no significant effect on IBA uptake at the early stages of AR formation in the cuttings ($p = 0.667$), as shown in Figure 3a, but affected IAA concentration ($p = 0.038$ *). IAA concentration was significantly higher in cuttings from juvenile stock material (433.40 ± 82.51 ng g^{-1}) than in mature stock material (174.26 ± 32.33 ng g^{-1}) (Figure 3b). The time of evaluation had a significant effect on the uptake of IBA ($p = 0.002$ **) and IAA ($p = 0.0009$ ***) in cuttings. The concentration of IBA was significantly higher 1 h (159.94 ± 128.27 ng g^{-1}) and 4 h (216.99 ± 60.41 ng g^{-1}) after severance compared to the control observation. At 24 h (130.50 ± 27.35 ng g^{-1}), the IBA concentration was not significantly different from the other sampling time points, as shown in Figure 4a. The IAA concentration was significantly higher 4 h (461.32 ± 103.29 ng g^{-1}) and 24 h (467.74 ± 71.38 ng g^{-1}) after severance than 1 h after severance (90.89 ± 12.60 ng g^{-1}) (Figure 4b).

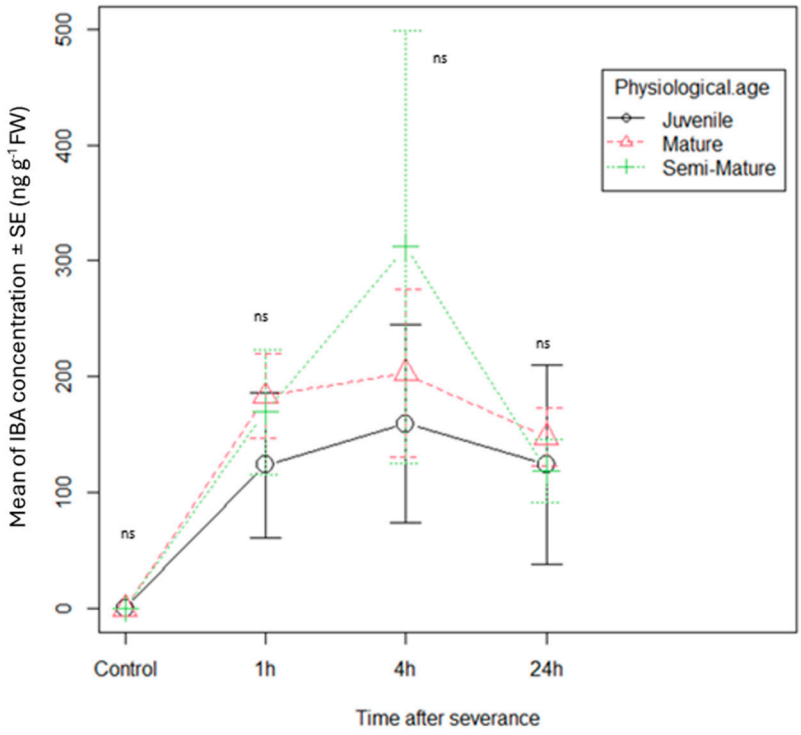
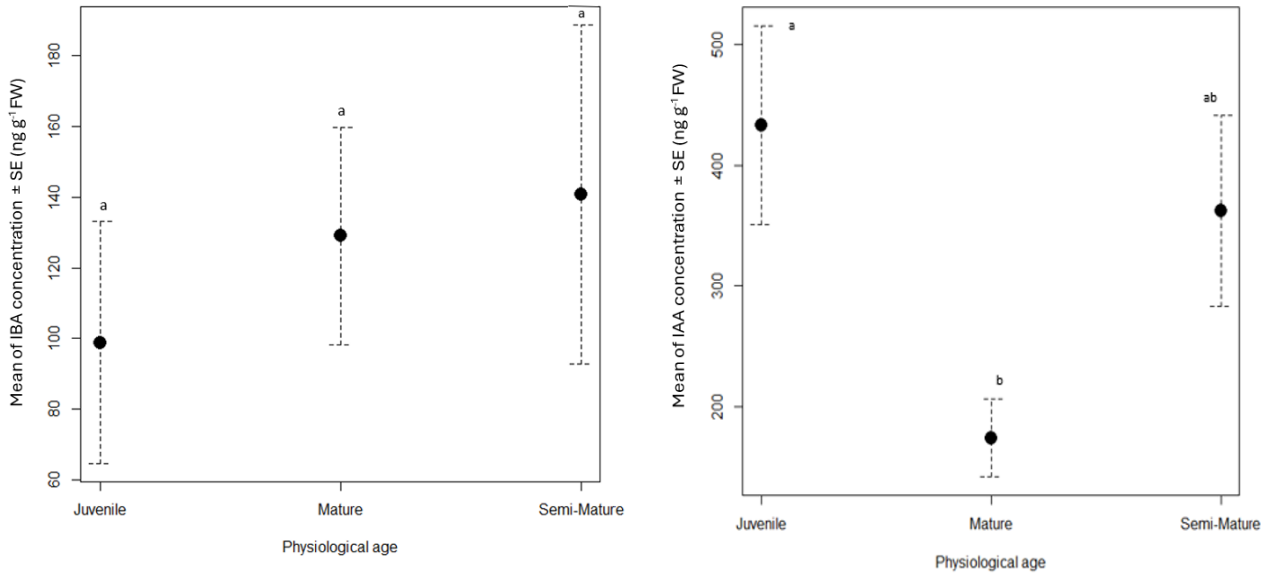
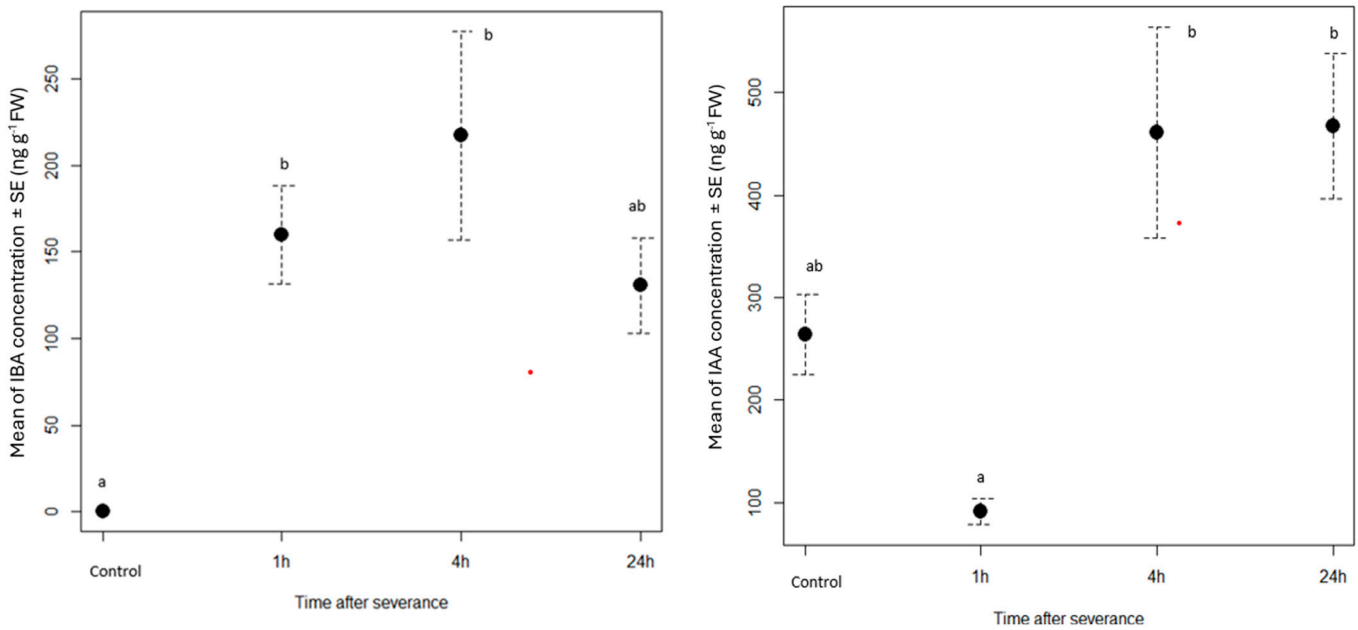


Figure 2. IBA concentration depending on the time of evaluation and physiological age of stock plant of *Prunus subhirtella* 'Autumnalis'. Means ± standard errors are shown. Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'ns' 1. Mature: stock trees about 60 years old, semi mature: mature stock trees rejuvenated by cuttings, juvenile: mature stock trees rejuvenated in vitro.



(**a**) (**b**)

Figure 3. IBA (**a**) and IAA (**b**) concentration depending on the physiological age of stock plant of the cuttings of *Prunus subhirtella* 'Autumnalis'. Means ± standard errors are shown. The different lowercase letters indicate significant difference ($p < 0.05$). Mature: stock trees about 60 years old, semi mature: mature stock trees rejuvenated by cuttings, juvenile: mature stock trees rejuvenated in vitro.



(**a**) (**b**)

Figure 4. IBA (**a**) and IAA (**b**) concentration depending on the time after severance of *Prunus subhirtella* 'Autumnalis' cuttings. Means ± standard errors are shown. The different lowercase letters indicate significant difference ($p < 0.05$).

3.2. Evaluation of Newly Formed Adventitious Root System

The percentage of successfully rooted cuttings, cuttings with callus, cuttings with acrobasal or basal rooting and cuttings that rooted but failed were evaluated (Table 2 and Figure 5). The number of main roots, the number of newly formed shoots and their length, and the length of root bush were also analyzed (Table 2 and Figure 5). The difference in the percentage of successfully rooted cuttings, cuttings with acrobasal rooting and cuttings

with callus formation according to the different observations was noted. The percentage of successfully rooted cuttings from semi-mature stock material was significantly higher at 95.00 ± 5.00% compared to mature cuttings with 68.33 ± 4.09% rooting. The percentage of successfully rooted cuttings from juvenile stock material did not differ significantly from that of semi-mature and mature stock material (78.33 ± 9.57%). The formation of acrobasal roots was also higher in cuttings from semi-mature stock material (44.17 ± 13.70%) than in cuttings from mature stock material (10.28 ± 4.09%). Callus formation was more frequent in mature stock material (52.92 ± 12.14%) than in semi-mature stock material (12.08 ± 2.99%). The evaluation of the above-ground parts of the cuttings revealed no significant differences between the observations. There were also no differences in the number of main roots and the length of the root bush between the stock plants of different physiological ages. Semi-mature cuttings showed the best results, with the highest number of successfully rooted cuttings, with acrobasal roots. Callus formation, which is considered an undesirable characteristic of a cutting, was also observed less frequently than in cuttings from mature stock material.

Table 2. Evaluation of the newly formed adventitious root system according to physiological age.

Physiological Age of Stock Plant	Successfully Rooted Leafy Cuttings ± SE (%)	Rooted Cuttings but Failed ± SE (%)	Cuttings with Basal Root Development ± SE (%)	Cuttings with Acrobasal Root Development ± SE (%)	Cuttings with Callus Formation ± SE (%)
Juvenile	78.33 ± 9.57 ab	20.00 ± 8.16 a	76.25 ± 1.25 a	23.75 ± 1.5 ab	23.13 ± 5.38 ab
Semi-Mature	95.00 ± 5.00 b	12.50 ± 7.98 a	53.75 ± 15.70 a	44.17 ± 13.70 b	12.08 ± 2.99 a
Mature	68.33 ± 4.09 a	21.67 ± 3.19 a	89.72 ± 4.09 a	10.28 ± 4.09 a	52.92 ± 12.14 b
Physiological age of stock plant	Number of main roots ± SE	Length of the root bush ± SE (cm)	Length of the newly formed shoots ± SE (cm)	Number of the newly formed shoots ± SE	
Juvenile	5.9 ± 0.20 a	25.26 ± 1.21 a	96.67 ± 9.53 a	4.50 ± 0.33 a	
Semi-Mature	6.80 ± 0.43 a	21.65 ± 0.89 a	99.49 ± 12.78 a	6.02 ± 0.68 a	
Mature	6.01 ± 0.45 a	23.37 ± 1.28 a	72.86 ± 9.81 a	3.92 ± 0.78 a	

Mature: stock trees about 60 years old, semi mature: mature stock trees rejuvenated by cuttings, juvenile: mature stock trees rejuvenated in vitro. The different lowercase letters in the same columns indicate significant difference ($p < 0.05$).

(**a**) (**b**) (**c**)

Figure 5. Leafy cutting in propagation bed during growing season (**a**) successfully rooted leafy cutting after 60 days (**b**) acrobasal root formation after 120 days (**c**).

4. Discussion

The ornamental cherry *Prunus subhirtella* 'Autumnalis' is considered easy to successful propagate [7] compared to some other woody plants, such as species of the genus *Castanea*, which are difficult to root by cuttings [24]. Our study also yielded generally good results in the rooting of *Prunus subhirtella* 'Autumnalis'. Cuttings from mature stock material rooted with a success rate of 68.33%, while rooting success was significantly higher with semi-mature material at 95.00%. Mature stock trees were about 60 years old, mature stock trees rejuvenated by cuttings (16 years old) served as semi-mature material and mature stock trees rejuvenated in vitro (16 years old) served as juvenile material. The reduced rooting ability of cuttings due to maturation is a major problem for the efficiency of asexual propagation [21]. Callus formation often occurs with species that are difficult to root or with insufficient stock material [25], which was also observed in our results, where callus formation was significantly higher in cuttings from mature stock material with a lower rooting percentage. In horticultural practice, acrobasal rooting is more desirable than basal rooting, as the rooted cuttings are easier to dig up and the newly formed roots are less damaged during digging and transplanting. In cuttings from mature stock material, the percentage of acro-basal root formation was significantly lower compared to semi-mature stock material. The number of main roots, the length of the root bush, the number and length of newly formed shoots and the percentage of rooted but failed cuttings did not differ significantly between the physiological ages of the stock material. This could indicate that if cuttings from physiologically old material can successfully form ARs, the under- and above-ground parts of the cuttings in the later growth phase do not differ from cuttings from physiologically younger material. This is also indicated by the result that the proportion of cuttings from physiologically mature material that successfully rooted and—later in growing season—failed does not differ from the proportion of rooted and failed cuttings from physiologically younger material. Our results show that the main difference between physiologically mature and semi-mature material is the difficulty of rooting. The difficulty of rooting was also associated with callus formation. Babashpour et al. [26] found no difference in rooting success independent of the concentration of exogenously applied IBA in semi-hardwood cuttings of *Bougainvillaea* sp., while Camellia et al. [27] and Khandakera et al. [28] reported that rooting success improved with increasing IBA concentration in *Syzygium samarangense* and *Jatropha curcas* [27,28]. To stimulate better rooting of mature stock material, a higher auxin concentration could be used (>5000 ppm) or the auxin used (IBA) could be replaced by another auxin, which is also often used exogenously to improve rooting, such as 1-naphthaleneacetic acid (NAA) or IAA.

No differences in IBA uptake were found according to physiological age and time of evaluation. There were also no differences in total IBA uptake (24-h period) between physiologically different ages. The only factor that influenced IBA concentration in the cuttings was the time of evaluation. Our results show that the IBA concentration in the cuttings differs from the control only after 1–4 h of exogenous application, and after 24 h there are no differences from the control treatment. The IAA concentration increases in the period of 4–24 h, indicating that the conversion of IBA to IAA by peroxisomal β-oxidation [29] already begins at this point. Compared to the control treatment, a significantly lower IAA concentration was observed after 1 h, which is consistent with the results of Osterc et al. [20] who also report a decrease in IAA concentration 1 h after wounding of *Prunus subhirtella* 'Autumnalis'. It would be advisable to further investigate the dynamics of IAA formation in the first hour after tissue wounding. There were no differences in IBA uptake in cuttings, regardless of the physiological age of the stock plant. The total concentration of IAA was significantly higher in juvenile stock material than in physiologically mature material during the first 24 h. Ford et al. [19], hypothesized that auxin metabolism explains the differences between easy and difficult-to-root plant species. Auxin metabolism could be faster in difficult-to-root plant species than in easy-to-root plants, resulting in lower basal auxin levels. De Klerk et al. [18], find that high auxin levels are required for a successful induction phase but are inhibitory during the growth phase, suggesting that auxin degra-

dation is essential, as high auxin concentrations inhibit root elongation and promote cell differentiation. The best rooting was expected in juvenile material, but our results do not show this. This could indicate that the auxin concentration is too high in juvenile material and inhibits rooting in later stages of rooting. This could also indicate that basal IAA is not the only factor influencing rooting success. Ford et al. [19], found a correlation between rooting ability and the ability to transport basipetal auxin. The results of our experiment on rooting ability are not in agreement with the results of Osterc et al. [30], who found better rooting of juvenile material compared to mature stock material in *Prunus subhirtella* 'Autumnalis'. Since the same ornamental cherry stock plant material as Osterc et al. [30] was used, it can be assumed that juvenile plant material loses its rooting ability after a certain period.

The rooting results were best with semi-mature material and callus formation was lower than with mature material. This indicates that the initial basal concentration of IAA is not the only determinant of rooting ability, but that other factors such as other phytohormones, light, temperature, minerals, and the physiological state of the plant are also important factors that stimulate AR formation [5,9]. There are a variety of factors that, together with their interactions, contribute to the difference between difficult-to-root and easy-to-root species or stock material, and there is probably no single limiting factor. The importance of basipetal auxin transport for rooting may be related to, among other factors, the accumulation of auxin in certain tissues at a given time for the initiation of rhizogenesis [19]. Although cuttings from mature stock were dipped in IBA, rooting success did not increase because the auxin may not have accumulated in the correct tissues. Ford et al. [19] report that experiments with basal application of radioactive IAA have shown that most of the labelling can be found in the leaves within a few hours. It can be assumed that the concentration of basal IAA in the semi-mature stock material and its accumulation were just right, resulting in better rooting.

Since there was no difference in IBA uptake in cuttings from mature and semi-mature stock plants, but the percentage of successfully rooted cuttings was significantly different, it can be assumed that the ability to accumulate exogenously applied IBA is not the crucial point that could explain the mentioned differences in rooting ability of differently aged propagation material. Other endogenous phytohormones may influence the ability to form ARs. It is assumed that endogenous IAA is synthesized in the apical parts of the plant stem and transported downwards via the polar transport pathway [5]. The differences in rooting success between cuttings of different physiological ages can therefore rather be explained by different levels of endogenous IAA in the cuttings and perhaps additionally by their ability to accumulate exogenously applied auxin and to convert it into different metabolites after 24 h. Removing the bark can also help to ensure that the bark is not a physical obstacle to the root growth of the older shoots. The selection of younger shoots as propagation material is crucial to better rooting success. The IBA concentration should be analyzed after a longer period than 24 h, since the first difference was observed after 24 h. In this case, it would be easier to summarize IBA conversion to IAA. Understanding the environmental and endogenous factors that influence the success of AR formation is key to maximizing the yield of vegetative propagation. This applies both to plants that are notoriously difficult to root and to plants where reduced rooting success is due to physiological aging. The most important endogenous factor influencing ARs is the concentration and ratio of various phytohormones. Auxins play the main role in the formation of ARs. For this reason, the exogenous application of auxin is used to promote rooting [9,10]. It is important to know whether the exogenous addition of auxins is useful in horticultural practice, regardless of the physiological age of the stock material. Basal dipping in IBA may therefore not result in sufficient auxin levels in the specific tissues for root initiation in ornamental cherry, which is consistent with the results of Ford et al. [19]. However, some cuttings root easily, while others are recalcitrant even when growth regulators are used. Successful propagation of cuttings depends on many factors, such as the physiological age of the cutting, the propagation environment and the concentration of the auxin used [31].

5. Conclusions

Among the auxins, indol-3-butyric acid is generally the most commonly used for better rooting. It is known for its long retention time at the application site and promotes rooting of many tree species. Our results show that there is no difference in the uptake of exogenously applied IBA in the first 24 h after cutting in all physiologically different old stock plants. There was also no difference in the total uptake of IBA between physiologically different stock material. Our results suggest that the only factor influencing IBA concentration is the time elapsed since the severance. Differences in IBA concentrations between IBA control and 1 h after severance, and between the control and 4 h after severance. This result shows that after 24 h, decreases in IBA concentration were observed. The IAA concentration was significantly higher in cuttings from juvenile stock material than in mature stock material. Our results suggest that the dynamics of other endogenous phytohormones are much more important for the formation of ARs, as the proportion of successfully rooted cuttings from semi-mature stock material was significantly higher than the proportion of successfully rooted cuttings from mature stock material. Successfully rooted cuttings from mature stock material were accompanied by callus formation, which was significantly lower in cuttings from semi-mature stock material. Although *Prunus subhirtella* 'Autumnalis' is considered easy to propagate, it is advisable to use juvenile stock material for successful propagation and rooting because rejuvenated stock material has better rooting potential, less frequent occurrence of callus, and significantly higher acrobasal rooting, which is advantageous in horticultural practice. To maximize the yield of vegetative propagation, further investigations are needed. For better understandings of IBA-to-IAA conversion, IBA concentration should be analyzed after a longer period than 24 h. Since the uptake of IBA varies from plant species to plant species, different concentrations of exogenously added IBA, as well as other hormones and compounds that stimulate AR development, should be investigated in physiologically differently aged stock material.

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