

Rooting of two types of cuttings of fruit crops *Vaccinium floribundum* Kunth and *Disterigma alaternoides* (Kunth) Niedenzu (Ericaceae)

Enraizamiento de dos tipos de estacas de los frutales *Vaccinium floribundum* Kunth y *Disterigma alaternoides* (Kunth) Niedenzu (Ericaceae)

Stanislav Magnitskiy^{1,3}, Gustavo Ligarreto M.¹, and Héctor Orlando Lancheros²

ABSTRACT

Vaccinium floribundum Kunth and *Disterigma alaternoides* (Kunth) Niedenzu are two fruit-bearing species of the family Ericaceae, native to the Andean forest. Given very scarce knowledge on their ecophysiology, it is not known how to propagate these plants, which limits the possibility of introducing these into the commercial culture. The present study had an objective to evaluate the rooting potential of softwood and hardwood cuttings of *V. floribundum* and *D. alaternoides* using 50-400 mg L⁻¹ naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA) as rooting hormones in an organic-mineral medium. The softwood cuttings taken from the apical part of the branch had up to 47.0% rooting in *V. floribundum* and up to 77.2% in *D. alaternoides* at day 60, whereas hardwood cuttings taken from the base of the branch presented lower rooting values, among 8.1 and 24.0% in *V. floribundum* and among 6.8 and 34.5% in *D. alaternoides*. Softwood cuttings of *V. floribundum* y *D. alaternoides* treated with 400 mg L⁻¹ mg NAA remained viable up to 64 and 86%, while the rooting percentage was up to 44.4 and 77.2 %, and average number of roots was up to 8.4 and 6.3, respectively. The results of the study offer a base for development of the protocol for the vegetative propagation of these species.

Key words: propagation, mortiño, ovo, auxins, indole-3-butyric acid, 1-naphthaleneacetic acid.

RESUMEN

Vaccinium floribundum Kunth y *Disterigma alaternoides* (Kunth) Niedenzu son dos especies frutales de la familia Ericaceae, nativas del bosque andino. Dado el muy escaso conocimiento divulgado sobre su ecofisiología, no se conoce como propagar estas plantas, lo que limita la posibilidad de introducirlas como cultivo comercial. El presente estudio tuvo como objetivo evaluar el potencial de enraizamiento de las estacas jóvenes y semileñosas de *V. floribundum* y *D. alaternoides* utilizando 50-400 mg L⁻¹ del ácido naftalenacético (ANA) y del ácido indolbutírico (AIB) como hormonas enraizadoras en un sustrato orgánico-mineral. Las estacas jóvenes provenientes de primordios apicales de la rama tuvieron hasta 47,0% de enraizamiento en *V. floribundum* y hasta 77,2% en *D. alaternoides* al día 60, mientras que estacas semileñosas provenientes del tercio medio de la rama presentaron más bajas tasas de enraizamiento, entre 8,1 y 24,0% en *V. floribundum* y entre 6,8 y 34,5% en *D. alaternoides*. Las estacas jóvenes de *V. floribundum* y *D. alaternoides* tratadas con 400 mg L⁻¹ de ANA permanecieron viables hasta 64 y 86% respectivamente, mientras que el porcentaje de enraizamiento fue de 44,4 y 77,2% y el número promedio de las raíces fue de 8,4 y 6,3. Los resultados del estudio ofrecen una base para la elaboración del protocolo de la propagación vegetativa de estas especies.

Palabras clave: propagación, mortiño, ovo, auxinas, ácido indolbutírico, ácido naftalénacético.

Introduction

Vaccinium floribundum Kunth and *Disterigma alaternoides* (Kunth) Niedenzu are two fruit species of tribe Vaccinieae of family Ericaceae native to the Andean forest. In the neotropics, several fruit species of this family have a potential to be cultivated, such as *Vaccinium meridionale*, *Gaultheria anastomosans*, and *Macleania rupestris* that grow spontaneously in the north-western South America. Most of these species in the Andean region may be found in cold, wet and mountainous habitats located between 1,500

and 3,000 m a.s.l. (Luteyn, 2002) as shrubs, epiphytes and, occasionally, lianas (Gentry, 1996; Luteyn, 2002).

Vaccinium is the largest genus of the tribe Vaccinieae (Lens *et al.*, 2002). In case of *V. floribundum*, this plant has its natural distribution in Venezuela, Colombia, Ecuador, Peru (Luteyn, 2002), Costa Rica (Schneid *et al.*, 1996), and Bolivia (Sanjinés *et al.*, 2006). This fruit bearing plant may be encountered in páramos of the Andes from 2,700 (Salzer *et al.*, 2006) up to 4,000 m a.s.l. (Schick, 1993). *V. floribundum* is a shrub with height ranging among 2.0 and

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¹ Department of Agronomy, Faculty of Agronomy, Universidad Nacional de Colombia. Bogota (Colombia).

² Jardín Botánico de Bogotá José Celestino Mutis. Bogota (Colombia).

³ Corresponding author. smagnitskiy@unal.edu.co

3.5 m (Schick, 1993; Sanjinés *et al.*, 2006), although in the mountains of Cundinamarca dwarf plants from about 20 cm height could be found in a state of fruiting. The fruit of *V. floribundum* is a spherical berry of 5-12 mm in diameter, its color varies from blue and dark blue, with surface smooth and sometimes glaucous (Sanjinés *et al.*, 2006). In mountain forests of Colombia, this plant often grows in association with *V. meridionale* and *D. alaternoides* and could be distinguished from these species by lower size, leaves of dark green color with crenulate margins, dense branching, and berry of more acid flavor.

V. floribundum is a commonly used species, especially in Ecuador, to prepare marmalades, drinks and desserts (Luteyn, 2002; Vasco *et al.*, 2009). Its high content of anthocyanins, reaching 345 mg/100 g FW (Vasco *et al.*, 2009) as well as high accumulation of phenolics and vitamin C in fruits makes these stand out for their medicinal properties, mainly for treatment of cardiovascular problems and cancer (Schreckinger *et al.*, 2010).

In Colombia, *V. floribundum* is known under the names of agracejo, mortiño, mortiño de árbol (Antioquia), agraz, chivaco (Cundinamarca) (Cardozo *et al.*, 2009). It is one of five native species of the genus *Vaccinium* reported so far in Colombia: *V. meridionale*, *V. floribundum*, *V. corymbodendron*, *V. euryanthum*, and *V. singularis* (Salinas and Betancur, 2007). It is worth mentioning that *V. meridionale* and *V. floribundum* are traditionally considered the only two native species of this genus, whose fruits are edible (Schick, 1993), although the reports exist that *V. corymbodendron* also has edible fruits (Sierra *et al.*, 2005). *V. corymbodendron* grows in the same environments that *V. floribundum* and *V. meridionale* (Marcano-Berti, 2002) and is mistaken in its natural habitat with other Ericaceae species *Disterigma alaternoides* and *Gaultheria myrsinoides*. The confusion between the species comes from the lack of knowledge on the morphological differences between them in the field and the use of a common terminology, because the name “mortiño” in the Andean region is used for a wide variety of plants, including *V. corymbodendron* (Sierra *et al.*, 2005), *V. meridionale* (Schick, 1993), *V. floribundum* (Vasco *et al.*, 2009), *Hesperomeles* sp. (Gentry, 1996; Cardozo *et al.*, 2009) and other species.

The genus *Disterigma s.l.* holds up to 40 species, mainly small shrubs distributed in the forests of high mountains and páramos from southern Mexico (Wilbur, 1992) to Central America and northern South America (Pedraza-Peñalosa, 2009; Pedraza-Peñalosa, 2010). Information on the distribution, physiological and horticultural potential

of *D. alaternoides* is much more limited than that of *V. floribundum*. In South America, the plant grows spontaneously in Venezuela, Colombia, Ecuador, Peru, Guyana, and Bolivia at altitudes between 2,400 and 3,700 m a.s.l. (Smith, 1933), often in associations with other Ericaceae. It is a shrub that reaches up to 4.0 m height (Smith, 1933) with edible fruits of bitter taste characterized by a high content of flavonoids (Pino *et al.*, 2001). The fruits are rounded translucent berries with size between 5 and 12 mm in diameter and color ranging from white-pink to red. In Colombia, *D. alaternoides* is also known as *capulí silvestre* (Nariño), *uvo blanco*, and *ovo* grows in páramos of North Santander, Cundinamarca, Nariño (Smith, 1933), Chocó (Pino *et al.*, 2001), and Boyacá. Its use for food in Colombia appears to be limited and includes fresh fruit consumption as well as processed cakes and marmalades, the latter presentation is most commonly used modality in Sumapaz, rural town of Bogotá.

Given the lack of reported knowledge about the species under discussion, there is no reference to suggest the development of programs dedicated to the domestication of these species. In particular, it is not known how to propagate these plants, which limits the possibility of introducing these as commercial crops. For *V. floribundum*, some questions of propagation by cuttings (Noboa, 2010) and seeds (Cardozo *et al.*, 2009) were answered, the last one is slow, erratic and propagules are very non-uniform and apparently have high genetic diversity; there were, no published data found on the propagation of *D. alaternoides*. There is a need to develop a protocol for the propagation of these species. Taking into account that different ericaceous plants are often multiplied by cuttings, the present study was done to evaluate the potential of rooting of cuttings of two species of Ericaceae using indolebutyric and naphthaleneacetic acids as rooting hormones to provide a recommendation to potential farmers in the establishment of plants in culture selected for their production, fruit quality, and other desirable characteristics.

Materials and methods

The study was conducted in greenhouse under plastic cover at the Faculty of Agronomy, Universidad Nacional de Colombia, Bogota. The material of *V. floribundum* and *D. alaternoides* was obtained from branches cut from shrubs in Choachí, Cundinamarca. The plants of *V. floribundum* with an average height of 30 cm were obtained at the site located at coordinates 35° 17.2' W and 73° 59' 53.1" N, altitude 3,348 m a.s.l.; the plants of *D. alaternoides* with an average height of 1.30 m were obtained at the site located

at coordinates 36° 12.2' W and 73° 58' 02.2", altitude 3,200 m a.s.l. The vegetation at both sites corresponded to *páramo*, with the presence of low- and medium-sized shrubs of Ericaceae and Melastomataceae. Mother plants to select the cuttings were the adult ones, with the appearance of some fruits and without visual symptoms of nutritional deficiencies or phytosanitary problems. Orthotropic vegetative branches were used to prepare the cuttings with similar characteristics, with turgid leaves and of good vigor. For both species were used two types of cuttings: young taken from apical primordia and semi-hardwood taken from the middle third of the branch. The characteristics of the cuttings are presented in Tab. 1.

Rooting was carried out with different concentrations of auxins, indolebutyric acid IBA and naphthaleneacetic acid NAA. The hormone solutions were prepared taking 2,000 mg IBA (Sigma, Ontario, Canada) or 2,000 mg NAA (Sigma, Ontario, Canada) pure, diluted in 10 mL of 70% ethyl alcohol and adding distilled water to volume one liter. From these solutions, solutions of 0, 100, 400 and 800 mg L⁻¹ of IBA or NAA were prepared, doses that were mixed with industrial talc. The mode of application of the hormones was to perform a mixture of the solution of the hormone with talc as inert material in such a proportion to produce a paste containing 0, 50, 200 or 400 mg L⁻¹ IBA or NAA (Castrillón *et al.*, 2008). This method allows the hormone remaining at the base of the cutting any longer when compared with a dip of the cutting in hormone solutions, often of very high concentration (Castrillón *et al.*, 2008; Ávila Díaz-Granados *et al.*, 2009). Approximately 1 cm of the basal part of the cuttings were introduced into the mixture and allowed drying at ambient temperature for 1-2 min, then sown in plastic trays of 18 alveoli with diameter 8.0 cm and depth 8.0 cm. As rooting medium was used organically-mineral substrate composed of peat without mineral nutrients (Klasmann-Deilmann GmbH, Geeste-Germany) mixed in a 1:1 ratio with coal ash consisting of residues of medium structure. The mixture with peat present physical condition to maintain aeration and water retention in substrate avoiding problems of low water retention in the substrate of coarse ash or pure peat or poor drainage in the substrate of fine ash (Castrillón *et al.*, 2008).

In order to water the cuttings, water mist was supplied for 10 min twice a day during the first 30 d; after 30 d and until the end of the experiment the frequency of watering was once a day. The trays were kept covered with plastic after applying irrigation to reduce temperature changes on rooting bench and reduce transpiration by leaves/stem keeping alive the cuttings up to the time than these formed adventitious roots. A synthetic black mesh was installed providing 75% light on the bench, to reduce the intensity of solar radiation and to maintain high relative humidity during the rooting.

At 60 d, the following variables were measured: percentage viability of cuttings estimated by the presence of at least two leaves, percentage of cuttings rooted from total cuttings planted, number of adventitious roots, and average length of the roots on each cutting. We used a completely randomized design with 14 treatments, consisting of two hormones, IBA and NAA, three hormone concentrations and two types of cuttings, young and semi-hardwood, the control was distilled water. Each treatment consisted of 16 replicates. An analysis of variance was done using the statistical package SAS[®] version 9 and the differences between treatments were evaluated by Tukey test.

Results

From day 14 after start of the experiment some cuttings of *Vaccinium floribundum* and *Disterigma alaternoides* began to show symptoms of viability loss, recorded by leaf abscission. The fall of leaves was accompanied by necrosis of the cuttings that began in the basal end and was progressively moving toward the apex.

In general, the rooting of cuttings was more successful in *D. alaternoides* than in *V. floribundum* (Tabs. 2 and 3). *D. alaternoides* had adventitious root formation not only at the base of the cuttings, but also at the leaf base, which was not typical for *V. floribundum* (Fig. 1). When transplanting some control cuttings at day 60 in the pots filled with the same organic-mineral substrate, plants of *D. alaternoides* had intensive growth of new shoots within next 2 to 4 months, while the growth of *V. floribundum* growth was slow (data not shown).

TABLE 1. Morphological characteristics of cuttings of *V. floribundum* and *D. alaternoides*. *

State	<i>V. floribundum</i>				<i>D. alaternoides</i>			
	Length (cm)	Diameter (mm)	Number of leaves	Fresh weight (g)	Length (cm)	Diameter (mm)	Number of leaves	Fresh weight (g)
Young	6.4	1.5	16	0.53	6.4	2.0	14	0.62
Semi-hardwood	6.8	4.0	9.5	0.96	6.8	3.2	6	0.73

* Mean 20 units.

TABLE 2. Characteristics of rooting of cuttings of *V. floribundum*.

Treatments	Viability at 60 days (%)		Rooting (%)		Average number of roots		Average length of roots (cm)		
	Young	Semi-hardwood	Young	Semi-hardwood	Young	Semi-hardwood	Young	Semi-hardwood	
H ₂ O	40	54	29.8	17.2	5.2 ab	3.7 ab	1.2 b	1.7 bc	
50	42	60	32.1	8.1	6.2 bc	1.5 a	2.5 d	0.6 a	
IBA, mg L ⁻¹	200	41	48	25.6	24.0	7.0 bcd	4.8 b	1.7 bc	2.1 c
	400	32	46	26.0	12.7	4.0 a	3.5 ab	0.6 a	1.1 ab
50	35	62	31.8	19.3	8.2 cd	2.0 ab	3.7 e	1.5 abc	
NAA, mg L ⁻¹	200	56	56	47.0	22.9	7.0 bcd	4.0 ab	2.4 d	2.1 c
	400	64	56	44.4	17.8	8.4 d	3.3 ab	2.2 cd	1.2 abc

Means in the same column followed by the same letter are not significantly different according to Tukey test ($P \leq 0.05$).

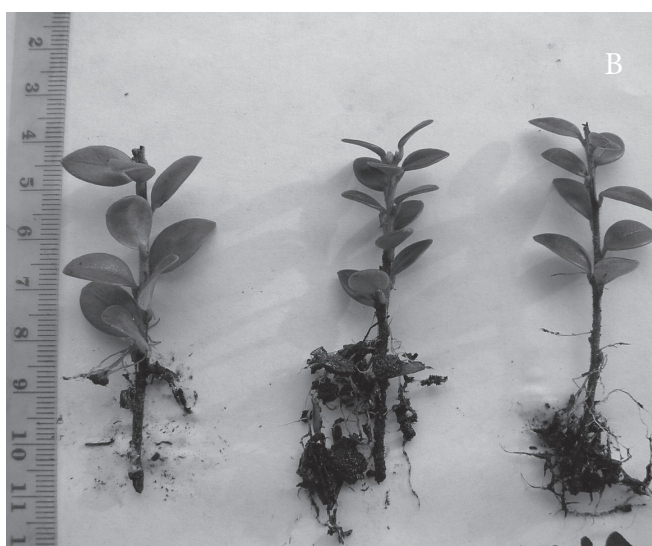


FIGURE 1. Rooting of young cuttings of *V. floribundum* (A) and *D. alaternoides* (B) after 60 days treated with 400 mg L⁻¹ NAA.

In *V. floribundum*, mortality rate of young cuttings was high; in most treatments, except NAA applications of 200 and 400 mg L⁻¹, for 60 days more than half of young cuttings died (Tab. 2). More lignified cuttings remained viable for longer than the tender cuttings, possibly due to a lower rate of drying off. Woody cuttings of *V. floribundum* thicker than those present in the study were viable for a longer time, they sprouted but did not emit roots (data not shown), the results were similar to those observed in *V. meridionale* (Ligarreto *et al.*, 2006) when highly lignified cuttings had very low rhizogenic activity. At the same time, the percentage of rooting was higher in young cuttings than in lignified ones (Tab. 2).

The application of NAA to the cutting base favored rooting of cuttings, increase of number and length of roots, while the IBA had a minor effect. Young cuttings of *V. floribundum* treated with 200 and 400 mg L⁻¹ NAA remained viable up to 56 and 64%, respectively, the rooting percentage was 47 and 44%, and the average number of roots was 7.0 and 8.4 that proves the usefulness of these treatments to propagate this species. The average length of roots increased significantly in young cuttings by treating these with 400 mg L⁻¹ NAA, whereas treatment with 400 mg L⁻¹ IBA did not cause the same effect (Tab. 2).

In case of *D. alaternoides*, it was shown higher percentage of viability of both types of cuttings compared with *V. floribundum* (Tabs. 2 and 3). By comparing the percentages of rooting of young and semi-hardwood cuttings, the first ones had higher rooting in *D. alaternoides*. The cuttings taken from the middle third of the branch remained viable for longer time than apical cuttings. Over 90% of young cuttings of *D. alaternoides* in all treatments, that remained

TABLE 3. Characteristics of rooting of cuttings of *D. alaternoides*.

Treatments	Viability at 60 days (%)		Rooting (%)		Average number of roots		Average length of roots (cm)	
	Young	Semi-hardwood	Young	Semi-hardwood	Young	Semi-hardwood	Young	Semi-hardwood
H ₂ O	72	63	64.3	24.0	4.3 ab	3.3 a	1.8 b	0.6 ab
50	64	79	61.0	29.7	4.7 abc	3.2 a	3.0 d	1.1 bc
IBA, mg L ⁻¹	200	78	64.3	17.7	6.6 c	4.0 ab	1.9 b	0.8 abc
	400	83	72	19.1	10.4 d	7.0 b	2.4 c	1.2 bc
NAA, mg L ⁻¹	50	61	60.6	6.8	3.4 a	2.0 a	0.6 a	0.2 a
	200	62	77	14.2	4.6 abc	4.5 ab	1.9 b	2.2 d
400	86	81	77.2	34.5	6.3 bc	7.5 b	2.4 c	1.3 c

Means in the same column followed by the same letter are not significantly different according to Tukey test ($P \leq 0.05$).

alive at 60 d after the start of the experiment, formed adventitious roots (Tab. 3). The application of rooting hormones (400 mg L⁻¹ NAA or IBA) increased the number of rooted cuttings and the number and length of roots formed, this effect was more evident in the young cuttings than in semi-hardwood cuttings.

Discussion

The results show that the multiplication of *Disterigma alaternoides* by cuttings has higher potential because it is rooted more easily than *Vaccinium floribundum*. The rate of woody plant regeneration after cutting as the cause of pruning, forest fires, etc., and the ability to produce adventitious roots are a feature of indirect activity of endogenous auxins (Hartmann *et al.*, 1996). Although these two species often form associations in páramos of the Andes, the results indicate that their rates of vegetative propagation in their natural habitat might be different.

Lignified cuttings of both species remained viable for more time than young cuttings, which could be attributed to the effect of their thickness. The lignified cuttings were thicker and should have contain more carbohydrate reserves to remain viable; additionally, a high degree of lignification might prevent rapid drying of the thick cuttings (Hartmann *et al.*, 1996), therefore, the young Ericaceae cuttings are more demanding in watering conditions than the lignified ones (Giroux *et al.*, 1999 and references therein). On the other hand, the rooting percentage was higher in young cuttings, with the presence of apical bud, as it was proven in other ericaceous (Gough, 1993; Lee *et al.*, 2004; Yong *et al.*, 2005). High percentage of rooting in young cuttings, especially *D. alaternoides*, might be due to endogenous au-

xin activity, taking into account that the apical and axillary buds are a source of auxin for callogenesis and rhizogenesis (Hartmann *et al.*, 1996). In this context, the length of cuttings may be important for rooting, with *Vaccinium angustifolium* cuttings of high number of nodes, 4 to 6, were more successful for rooting than short cuttings (Lee *et al.*, 2004). Moreover, the growth of adventitious roots in woody cuttings could be prevented by sclerenchyma (Hartmann *et al.*, 1996), a tissue whose formation tends to be increasing with the age of plant organs. For example, in hardwood cuttings of *Vaccinium corymbosum*, continuous layer of lignified pericycle fibers and the epidermis affected the emergence of adventitious roots that originated from the cambium and the phloem (Mahlstede and Watson, 1952).

Blueberries like other Ericaceae are typically propagated by stem cuttings (Horigome *et al.*, 2008, Trevisan *et al.*, 2008), although these are known to be very hardrooted when multiplied by this method. Their rates of rooting are low and time for emission of adventitious roots goes beyond 3 to 4 weeks (Gough, 1993), therefore, auxin applications tend to increase the percentage of rooting in cuttings (Debnath, 2007; Fischer *et al.*, 2008; Ávila Díaz-Granados *et al.*, 2009). Among auxins, IBA and NAA are used to induce rooting because these are non-toxic in a wide range of concentrations for a large number of species and chemically stable in contact with the substrate of propagation (Hartmann *et al.*, 1996). Auxin applications had more effect on rooting of apical cuttings than on those taken from the middle part of the branch, as was observed in *Vaccinium ashei* (Schuch *et al.*, 2007). Additional treatments to stimulate adventitious root formation in cuttings of ericaceous include: the increase of luminosity in the rooting chamber because darkness may reduce the percentage of rooting (Yong *et*

al., 2005, Damiani *et al.*, 2009), increase in the level of CO₂ in the rooting chamber between 0.1% (Prokaj *et al.*, 2004) and 5% (Biggs *et al.*, 1983) and warming the rooting bench (Horigome *et al.*, 2008; Luna, 2009). Other factors, whose effect could be evaluated for stimulating the rooting are the composition and characteristics of the substrate (Hoffmann *et al.*, 1995; Castrillón *et al.*, 2008), the type of cut made in the cutting (Trevisan *et al.*, 2008), the insertion depth of the cutting into the substrate (Horigome *et al.*, 2008), and the polarity of the cutting.

The use of cuttings for commercial propagation can significantly reduce the time Ericaceae require for the establishment of cultivated plants compared with other propagation methods, such as in vitro (Albert *et al.*, 2009), thus, the cuttings of *D. alaternoides* and *V. floribundum* as propagating material offer gain in time of culture establishment and fruiting. It is noteworthy that many neotropical Ericaceae in their natural habitat are propagated by rhizomes (genera *Lyonia*, *Pernettya*, *Tepuia*, and *Themistoclesia*) or adventitious roots that form along the stems (genus *Disterigma*) (Luteyn, 2002). In different ericaceous, extensive systems of rhizomes can form large clonal colonies, such as in *Disterigma empetrifolium*, while the stems of some species of the genera *Arbutus*, *Ceratostema*, *Comarostaphylis*, *Macleania*, and *Vaccinium* have lignotubers, in where meristematic areas are located with high capacity for regeneration after cuttage (Luteyn, 2002; Evans and Vander Kloet, 2010). The diversity of organs used for multiplication provides a possibility to use these materials for the propagation of the species studied. In practical terms, at 400 mg L⁻¹ NAA promises more than 44% (*V. floribundum*) and 77% (*D. alaternoides*) rooting of cuttings taken from apical branch primordia, which serves as a base for developing a protocol for vegetative propagation of these plants under greenhouse conditions.

Conclusions

The research results show that young cuttings have high rooting potential in *V. floribundum* and, especially, *D. alaternoides*, which may be useful as a method of vegetative propagation of these species. The percentage of rooting of semi-hardwood cuttings did not exceed 24.0 (*V. floribundum*) and 34.5% (*D. alaternoides*) at 60 d after starting the experiment and their use for propagation is limited. Exogenous auxin application to the base of young cuttings increased the viability, percentage of rooting, and number and length of roots formed. In practical terms, 400 mg L⁻¹ NAA promises more than 44% (*V. floribundum*) and 77% (*D. alaternoides*) rooting of cuttings taken from apical primordia of the branches.

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