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ORIGINAL RESEARCH PAPER

Rooting and growth of root cuttings of two old rose cultivars 'Harison's Yellow' and 'Poppius' treated with IBA and biostimulants

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Abstract

Propagation by root cuttings is an easy and low-cost method for plant taxa with an ability to produce rhizomes or suckers. This research examined the possibility of using root/rhizome cuttings in the propagation of two difficult-to-root old rose cultivars, 'Harison's Yellow' and 'Poppius'. A plant-based preparation (Root Juice), titanium (Tytanit), and IBA (Chryzotop Green 0.25% IBA, Rhizopon AA 020 XX 2.00% IBA) were tested as rooting and growth enhancers for thick, medium, and thin rhizome/root cuttings. Additionally, observations were made to identify the site of the initial root and shoot formation. Shoots appeared before roots, without polarity. The visible swellings differentiating in new root/shoot buds in these two rose cultivars were placed along the rhizome. The primordia of root and shoot buds were situated near the pith rays and the vascular cambial zone. The trial reported here showed significant effects of the thickness of root cuttings and the preparations used in terms of rooting success and growth characteristics. Medium-sized cuttings of rose 'Harison's Yellow' (45.0%), and thin cuttings of 'Poppius' (74.3%) achieved the highest rooting percentages. The most effective treatment was with Chryzotop Green, but Root Juice 0.01% and Tytanit 0.04% ('Poppius') and 0.02% (both cultivars) also had positive activity. Root Juice and Tytanit can be suggested for rooting cuttings of these roses as eco-friendly preparations.

Keywords

shrub roses; rhizogenesis; rhizomes; suckers; titanium

Introduction

The roses (*Rosa* spp.) have been known for thousands of years as plants connected with many areas in the life people. The genus *Rosa* comprises a great number and differentiation of diverse taxa. The cultivar 'Harison's Yellow', was introduced ca. 1830 in the USA. *Rosa foetida* Herrm. and *R. spinosissima* L. could be its parentage [1]. The cultivar 'Poppius' was bred by Carl Stenberg before 1872 [1,2]. This is a hybrid Spinosissima originating from *R. pendulina* L. and a seedling of *R. spinosissima* L. [2]. They are both recommended by the Swedish Rose Society for the northern Swedish climate according to their frost resistance [2,3] and their ornamental value observed in Poland is high [4].

To maintain the biodiversity of rose cultivars, modern plant production requires different but easy, quick, cheap, eco-friendly, and effective methods of vegetative propagation excluding the most commonly used budding method, because of the possibility of extensive root growth, needed however, for example, for shrub ground cover roses. Roses growing without the share of a rootstock are easier to cultivate, because there is no need to remove the so-called "wild" layers. Propagation methods currently used are cuttings, suckers, layers [5], and micropropagation [6], but in the case of the old roses (groups occurring before 1867 [5]) and cultivars of species the standard methods often fail [7,8]. These difficult-to-root roses of different origin can be propagated by rhizome/root cuttings, e.g., for *R. nitida* Willd. [9]. Rhizome cuttings of roses should be treated in the same way as root cuttings. Rhizomes are underground dorsiventral stems growing horizontally under the surface of the soil [10]. However, the main requirement for this is sufficient ability to produce the rhizomes [9,10], because the low efficiency of stock plants can limit the possibility of using this method on a large scale [5,9]. Moreover, the roots, layers, or rhizomes should be of a proper quality including thickness and structure [10]. Adventitious root or shoot formation needed for root cuttings is dependent on plant cell dedifferentiation. Shoot cuttings form only adventitious roots [9].

Indole-3-butyric acid (IBA) is the one of the most effective rooting stimulators and is also applied commercially to improve the quantity and quality of ornamental plants [5,9,10] from cuttings, including rose hardwood [11,12] and softwood cuttings [7,13]. Experiments have shown the positive effect on the rooting percentage [11] and number of young roots in roses [7]. However, these plant growth preparations are unable to improve the rooting percentage of difficult-to-root taxa. Any improvements can only be by changing the methods or conditions [9].

The chemicals used in plant production are restricted by edicts from the European Union (1991-07-15, No. 91/41/EEC; 2009/128/WE, *Official Journal the European Union*). The use of products of natural origin and biostimulants are recommended by the National Organic Program USDA and Organic Materials Review Institute (OMRI). Organic and eco-friendly products contain many different and unknown compounds and their activity on plant biological processes is being constantly a subject of ongoing experiments [14]. Root Juice is an organic product (Tab. 1) which enhances the growth of adventitious and existing roots on cuttings and during replanting. This preparation is recommended to stimulate root growth in plant production [15]. It has been shown that Root Juice used in rooting cuttings of the difficult-to-root Gallica rose 'Duchesse d'Angoulême' inhibited the ageing processes in leaves [16].

The element titanium (Ti) is the subject of much research for crops, vegetables, and fruit plants as it has been shown to have an important positive beneficial effect on their growth and development, increasing biomass and crop yields [17]. The activity of titanium is connected with its supporting role in some enzyme activities, nutrient uptake, chlorophyll biosynthesis, and even increasing the intensity of photosynthesis [18]. Furthermore, titanium-ascorbate reduces the negative effect of heavy metal damage [19] and has a balancing effect on element contents (e.g., Mg, Fe, Mn, and Zn) [20]. Titanium dioxide nanoparticles (TiO_2NPs) have been shown to increase the drought tolerance of *Linum usitatissimum* L. [21] and cold tolerance in chickpea [22]. However, higher concentrations can provoke unfavorable reactions in plants [17], but the potential for toxicity in tissues is not clearly determined [20]. Commercial titanium preparations are applied to the soil or directly on to leaves as a foliar spray [23].

The present trial was designed to investigate the possibility of using rhizome cuttings for the propagation of difficult-to-root old rose cultivars 'Harison's Yellow' and 'Poppius' [8]. This method could be an effective and alternative to stem cuttings. The eco-friendly plant origin preparation Root Juice (a combination of humic acid and algae), titanium (Tytanit 0.8% Ti), and standard commercial rooting powders (Chryzotop Green 0.25% IBA and Rhizopon AA 020 XX 2% IBA) were tested as rooting and growth enhancers. Additionally, the observations included identifying the site of initial root and shoot bud formation.

Material and methods

The trial was conducted at the Polish Academy of Sciences Botanical Garden – Center for Biological Diversity Conservation in Powsin (52.11° N, 21.10° E) in the years 2014–2015. Rhizomes of the two cultivars of old roses chosen, 'Harison's Yellow' (hybrid Foetida, G. F. Harison 1824) and 'Poppius' (hybrid Spinosissima, Carl Stenberg, Sweden 1872), were dug from the National Collection of Rose Cultivars PAS CBDC in Powsin, on December 1–15, before the soil was frozen. Rhizome/root cuttings 9–10 cm long were prepared and sorted into three size classes, according to their thickness, depending on the cultivar:

- 'Harison's Yellow': thick ø 8–10 mm, medium ø 6–7 mm, thin ø 4–5 mm;
- 'Poppius': thick ø 10–11 mm, medium ø 7–9 mm, thin ø 5–6 mm.

The cuttings were mixed with Kaptan 50WP (50% captan) fungicide to protect from fungal diseases and then tied into loose bundles (10–25 pieces/bundle). The bundles were then laid vertically in plastic crates filled with a mixture of slightly moist peat and sand (1:2). The crates were kept in a dark stockroom at a temperature of 3–5°C. Plastic trays were filled with peat (Karaska, Poland) and sand (Vistula River) mixture 1:1, pH 6.0–6.5. The cuttings were planted out on March 10–15, when the first buds were visible on the root cuttings. They were placed horizontally in the plastic trays at a spacing of 10 × 5–8 cm apart and then covered with 1.0–2.0 cm of the growth medium. All cuttings were subsequently treated with the four preparations according to the scheme in Tab. 1.

The plastic trays were maintained in a container nursery and watered manually according to demand. The temperature data for the PAS Botanical Garden in the years 2014–2015 in the months of rooting the cuttings are shown in Fig. 1. The cuttings were covered with row cover (density 17 g/m^2) and shade netting (40%, density 38 g/m^2) until May 1. They were protected from fungal diseases every 10–14 days, alternately with the fungicide preparations Amistar 250 SC (azoxystrobin 250 g dm^{-3}), Previcur Energy 840 SL (propamocarb 47.28%, fosetyl 27.65%) and Score 250SC (difenoconazole 250 g dm^{-3}). Ekolist fertilizer (0.01%) (Ekoplon S.A., Poland) was used as a foliar spray twice, on June 1 and July 1.

Observations of the site of root and shoot formation

Untreated cuttings were selected for observations on root and shoot formation. They were observed on the first days of root and shoot formation visible externally. The parts of the root cuttings with visible buds were cut and stabilized in a mixture of ethyl alcohol and glycerine (v:v 1:1). They were then serially sectioned transversely. Sections with a thickness of 30–100 µm were prepared using a SLIDE 4003E sledge microtome (pfm medical AG, Germany) and stained with a solution of safranin and observed under an Olympus Vanox AHBT3 (Olympus America Inc., New York, USA) microscope with a digital camera attachment linked to a computer.

Growth characteristics

The rooted cuttings were dug up after 18 weeks (in the first days of August), counted (%), and the root systems scored on a scale of 1–5 for degree of rooting: 1 – no roots (only survived); 2 – a few roots, 1–10 cm long; 3 – 6–15 roots >10 cm; 4 – 10–15 long, well-developed, branched roots; 5 – roots forming a root ball. Root lengths were measured from the origin of the root primordium of the longest root, and the shoot length from the base of the young shoot to the apical meristem of the longest shoot. The number of shoots was also counted. All leaves on the shoot contributing to total leaf area were scanned with a leaf area meter (AM 300, ADC BioScientific Ltd., Hoddesdon, UK).

Statistical analysis

The trial was arranged in a randomized block design and consisted of four replicates each with 10 cuttings. Three hundred sixty cuttings were thus planted for each cultivar. All percentage values were transformed using the arcsin(x)1/2 function according to Bliss [24] to normalize the data. Data were then analyzed by ANOVA (two-way analysis of variance: thickness of cuttings, treatment; and three-way: thickness of cuttings, treatment, year) procedures followed by post hoc Tukey's tests. STATISTICA 10 software (Statsoft Polska, Kraków, Poland) was used for all statistical analyses.

Tab. 1 The scheme and content of the preparations used in the trial.

No.	Treatment		Preparation	Components
1	Control		-	-
2	0.1%	Watering cuttings (10 mL per cutting) after planting, 14 and 28 days	Root Juice (BioBizz, the Netherlands)	Combination of humic acid and algae, containing: N (0.1%), P_2O_5 (0.1%), K_2O (0.1%), Mg (0.03%), Fe (0.013%), Mn (0.002%), Zn (0.004%), B (0.025%), Cu (0.001%)
3	0.2%			
4	0.4%			
5	0.01%	Watering cuttings (10 mL per cutting) after planting, then spraying after 28 and 42 days	Tytanit (Intermag, Poland)	Titanium (Ti) 0.8% (8.5 g dm^{-3})[1]
6	0.02%			
7	0.04%			
8	Before rooting, the 1/3 basal part of the cuttings were dipped in preparation and this part placed horizontally in tray at the base		Chryzotop Green (Rhizopon, the Netherlands)	0.25% indole-3-butyric acid (IBA)
9			Rhizopon AA 020 XX (Rhizopon, the Netherlands)	2.00% indole-3-butyric acid (IBA)

[1] https://intermag.pl/uprawa-roslin/produkt/tytanit

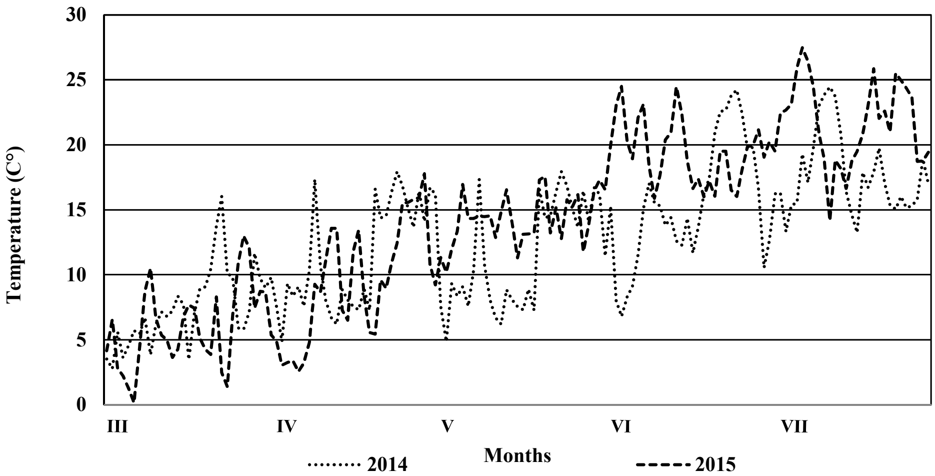

Fig. 1 The 24 temperatures in the years 2014–2015 for the months of the rooting of cuttings (March–July) at the Polish Academy of Sciences Botanical Garden – Center for Biological Diversity Conservation in Powsin.

Results

The root and shoot formation

The rhizomes of 'Harison's Yellow' and 'Poppius' in December did not have visible buds or bumps showing possible developing adventitious roots or shoots required for root cutting propagation. Adventitious buds were observed on root cuttings in the spring before planting over the whole length of their surfaces. These buds developed in shoots before root appearance to preserve the polarity. The wounds had not yet been scarred over completely with callus (Fig. 2A,B). The visible swellings differentiating in adventitious root or shoot tissues in both cultivars were positioned radially in cross section to all the tissues of the vascular system (Fig. 3). The typically forming of the shoot and root buds were connected with widening of the pith rays and subtending trace visible on the sections (Fig. 3A–D). These buds were also revealed as swellings and were located in the pericycle and near the vascular cambial zone without subtending any traces as adventitious buds visible in longitudinal sections (Fig. 2C).

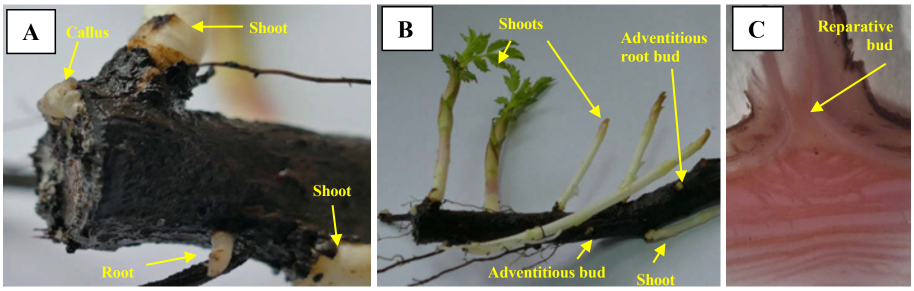

Fig. 2 The buds' appearing was not connected with the wound place, the shoots developed before roots, and the buds grew at the beginning without polarity in 'Harison's Yellow' (**A,B**) and reparative buds in 'Poppius' (**C**).

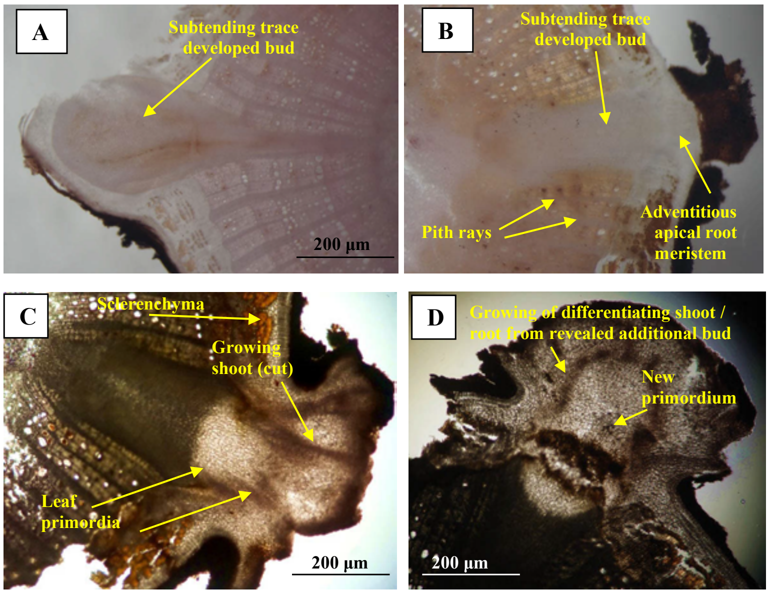

Fig. 3 The transversal sections of swelling on the root cuttings in *Rosa* 'Harison's Yellow' (**A,C,D**) and 'Poppius' (**B**).

Percentage rooting

The root cuttings of 'Harison's Yellow' rooted at a higher percentage in 2015 compared to 2014 (Tab. 2). In the case of rose 'Poppius', the year of the trial did not affect the rooting percentages (Tab. 3). However in the control cuttings of rose. 'Harison's Yellow', the thickness did not influence the rooting percentage but the thin root cuttings of rose 'Poppius' had the highest rooting ability of 90.0% (Tab. 2, Tab. 3).

The preparations used for the improvement of the rooting percentage showed different activity, dependent on the thickness of the cuttings. Considering the mean of all the thicknesses of the cuttings, spraying with 0.02% Tytanit contributed to an increase in 'Harison's Yellow' (61.7%) compared to the control (19.2%), IBA, and Root Juice treatments. However, the use of IBA contributed to an increase in the mean rooting percentage in this cultivar (Chryzotop Green 49.6%, Rhizopon AA 020 XX 53.3%), nearly twice that of the control. Root Juice (0.01%), Tytanit, and Chryzotop Green did not further influence the increase in the rooting percentage of thick cuttings (Tab. 2). In the case of rose 'Poppius', the thin root cuttings had the highest rooting ability in the control and treated with 0.01% Root Juice. Decreases or similar results to the control (73.8%) were obtained after the use of the all preparations for the thick cuttings. In the case of the medium cuttings, increases in rooting percentages were obtained after the use of 0.01% Root Juice, Tytanit and IBA (Tab. 3).

Tab. 2 The percentage (%) of rooted root cuttings of rose Harison's Yellow's.

	Thickness of root cuttings									
	Thick		Medium		Thin		Mean			
Treatment	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**Thick**	**Medium**	**Thin**	**Mean**
Control	17.5 $^{\text{lm 1}}$	25.0 $^{\text{ij}}$	10.0 $^{\text{o}}$	15.0 $^{\text{m}}$	22.5 $^{\text{ij}}$	25.0 $^{\text{i}}$	21.3 $^{\text{GHI 2}}$	12.5 $^{\text{I}}$	23.8 $^{\text{GHI}}$	19.2 $^{\text{D 3}}$
Root Juice 0.1%	22.5 $^{\text{ij}}$	35.0 $^{\text{h}}$	37.5 $^{\text{g}}$	47.5 $^{\text{f}}$	12.5 $^{\text{o}}$	15.0 $^{\text{m}}$	28.8 $^{\text{FGH}}$	42.5 $^{\text{EF}}$	13.8 $^{\text{HI}}$	28.3 $^{\text{C}}$
Root Juice 0.2%	15.0 $^{\text{m}}$	22.5 $^{\text{ij}}$	22.5 $^{\text{ij}}$	30.0 $^{\text{h}}$	30.0 $^{\text{h}}$	40.0 $^{\text{g}}$	18.8 $^{\text{HI}}$	26.5 $^{\text{F–I}}$	35.0 $^{\text{EFG}}$	26.8 $^{\text{C}}$
Root Juice 0.4%	15.0 $^{\text{m}}$	20.0 $^{\text{k}}$	15.0 $^{\text{m}}$	20.0 $^{\text{j–l}}$	10.0 $^{\text{o}}$	17.5 $^{\text{lm}}$	17.5 $^{\text{HI}}$	17.5 $^{\text{HI}}$	13.8 $^{\text{GHI}}$	16.3 $^{\text{E}}$
Tytanit 0.01%	12.5 $^{\text{o}}$	25.0 $^{\text{i}}$	15.0 $^{\text{m}}$	25.0 $^{\text{ij}}$	17.5 $^{\text{lm}}$	22.5 $^{\text{ijk}}$	18.8 $^{\text{HI}}$	20.0 $^{\text{GHI}}$	18.8 $^{\text{HI}}$	19.2 $^{\text{D}}$
Tytanit 0.02%	57.5 $^{\text{e}}$	75.0 $^{\text{b}}$	90.0 $^{\text{b}}$	95.0 $^{\text{a}}$	30.0 $^{\text{h}}$	25.0 $^{\text{i}}$	66.3 $^{\text{BC}}$	91.3 $^{\text{A}}$	27.5 $^{\text{FGH}}$	61.7 $^{\text{A}}$
Tytanit 0.04%	17.5 $^{\text{lm}}$	22.5 $^{\text{ijk}}$	72.5 $^{\text{c}}$	77.5 $^{\text{c}}$	47.5 $^{\text{f}}$	52.5 $^{\text{ef}}$	18.8 $^{\text{HI}}$	75.1 $^{\text{B}}$	50.0 $^{\text{DE}}$	47.8 $^{\text{B}}$
Chryzotop Green	17.5 $^{\text{lm}}$	25.0 $^{\text{ij}}$	55.0 $^{\text{ef}}$	67.5 $^{\text{d}}$	67.5 $^{\text{b}}$	65.0 $^{\text{d}}$	21.3 $^{\text{GHI}}$	61.3 $^{\text{BCD}}$	66.3 $^{\text{BC}}$	49.6 $^{\text{B}}$
Rhizopon AA 020 XX	55.0 $^{\text{e}}$	62.1 $^{\text{d}}$	52.5 $^{\text{ef}}$	65.5 $^{\text{d}}$	40.0 $^{\text{g}}$	45.0 $^{\text{f}}$	58.8 $^{\text{CD}}$	58.8 $^{\text{CD}}$	42.5 $^{\text{EF}}$	53.3 $^{\text{B}}$
Mean	25.6 $^{\text{D 3}}$	34.4 $^{\text{C}}$	41.2 $^{\text{B}}$	48.8 $^{\text{A}}$	30.8 $^{\text{B}}$	33.9 $^{\text{C}}$	30.0 $^{\text{B}}$	45.0 $^{\text{A}}$	32.4 $^{\text{B}}$	

[1] Different letters indicate significant interactions between treatment, thickness, and year of cuttings, according to Tukey's HSD test ($p \leq 0.05$).
[2] Different letters indicate significant interactions between treatment and thickness of cuttings, according to Tukey's HSD test ($p \leq 0.05$).
[3] Mean values marked with the same capital letters in a column or row do not differ significantly at $p \leq 0.05$ according to Tukey's HSD test.

Tab. 3 The percentage (%) of rooted root cuttings of rose 'Poppius'.

	Thickness of root cuttings									
	Thick		Medium		Thin		Mean			
Treatment	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**Thick**	**Medium**	**Thin**	**Mean**
Control	75.0 $^{\text{d 1}}$	72.5 $^{\text{de}}$	50.0 $^{\text{i}}$	47.5 $^{\text{ij}}$	92.5 $^{\text{a}}$	87.5 $^{\text{ab}}$	73.8 $^{\text{BCD 2}}$	48.8 $^{\text{GH}}$	90.0 $^{\text{A}}$	70.8 $^{\text{AB 3}}$
Root Juice 0.1%	70.0 $^{\text{e}}$	70.0 $^{\text{e}}$	60.0 $^{\text{g}}$	62.5 $^{\text{fg}}$	92.5 $^{\text{a}}$	92.5 $^{\text{a}}$	70.0 $^{\text{CDE}}$	61.3 $^{\text{EF}}$	92.5 $^{\text{A}}$	74.6 $^{\text{A}}$
Root Juice 0.2%	80.0 $^{\text{c}}$	80.0 $^{\text{c}}$	40.0 $^{\text{k}}$	47.5 $^{\text{ij}}$	60.0 $^{\text{g}}$	62.5 $^{\text{fg}}$	80.0 $^{\text{BC}}$	43.8 $^{\text{H}}$	61.3 $^{\text{EF}}$	61.7 $^{\text{B}}$
Root Juice 0.4%	65.0 $^{\text{f}}$	62.5 $^{\text{fg}}$	50.0 $^{\text{i}}$	47.5 $^{\text{ij}}$	77.5 $^{\text{cd}}$	72.5 $^{\text{de}}$	63.8 $^{\text{E}}$	48.8 $^{\text{GH}}$	75.0 $^{\text{BCD}}$	62.5 $^{\text{B}}$
Tytanit 0.01%	65.0 $^{\text{f}}$	72.5 $^{\text{de}}$	65.0 $^{\text{e}}$	65.0 $^{\text{e}}$	60.0 $^{\text{g}}$	67.5 $^{\text{ef}}$	68.8 $^{\text{CDE}}$	65.0 $^{\text{DE}}$	63.8 $^{\text{E}}$	65.8 $^{\text{AB}}$
Tytanit 0.02%	57.5 $^{\text{gh}}$	62.5 $^{\text{fg}}$	70.0 $^{\text{e}}$	70.0 $^{\text{e}}$	67.5 $^{\text{ef}}$	70.0 $^{\text{e}}$	60.0 $^{\text{EFG}}$	70.0 $^{\text{CDE}}$	68.8 $^{\text{CDE}}$	66.3 $^{\text{AB}}$
Tytanit 0.04%	70.0 $^{\text{e}}$	72.5 $^{\text{de}}$	82.5 $^{\text{bc}}$	87.5 $^{\text{ab}}$	72.5 $^{\text{de}}$	75.0 $^{\text{d}}$	71.3 $^{\text{CDE}}$	85.0 $^{\text{AB}}$	73.8 $^{\text{BCD}}$	76.7 $^{\text{A}}$
Chryzotop Green	67.5 $^{\text{ef}}$	67.5 $^{\text{ef}}$	75.0 $^{\text{d}}$	75.0 $^{\text{d}}$	77.5 $^{\text{cd}}$	82.5 $^{\text{bc}}$	67.5 $^{\text{DE}}$	75.0 $^{\text{BCD}}$	80.0 $^{\text{BC}}$	74.2 $^{\text{A}}$
Rhizopon AA 020 XX	52.5 $^{\text{hi}}$	50.0 $^{\text{i}}$	87.5 $^{\text{ab}}$	87.5 $^{\text{ab}}$	57.5 $^{\text{gh}}$	70.0 $^{\text{e}}$	51.3 $^{\text{FGH}}$	87.5 $^{\text{A}}$	63.8 $^{\text{E}}$	67.5 $^{\text{AB}}$
Mean	66.9 $^{\text{B 3}}$	67.8 $^{\text{AB}}$	64.4 $^{\text{B}}$	65.6 $^{\text{B}}$	73.1 $^{\text{A}}$	75.6 $^{\text{A}}$	67.4 $^{\text{B}}$	65.0 $^{\text{B}}$	74.3 $^{\text{A}}$	

[1–3] See Tab. 2 for notes.

The root system of cuttings

The applied preparations had a differential action on the rooting process in both cultivars according to the thickness of the cuttings but they all resulted in an improvement in the degree of rooting or the length of the roots produced. The highest degree of rooting was obtained in 'Harison's Yellow' with medium and thin cuttings, and for thin cuttings in 'Poppius'. The mean length of the roots of the three thicknesses of cuttings was similar in 'Harison's Yellow' and higher for the medium and thin cuttings in 'Poppius' (Tab. 4, Tab. 5).

The degree of rooting of rose 'Harison's Yellow' cuttings was higher in the control, and after the use of Chryzotop Green, Tytanit at 0.02% and 0.04% for all three thicknesses. It decreased after the use of Rhizopon AA 020 XX for the thick and the thin cuttings. The use of Root Juice 0.02% resulted in a decrease in the degree of rooting in the thick cuttings in 'Poppius' compared with an increase in the medium cuttings and when using Root Juice 0.04% for the thin ones (Tab. 4). The use of Root Juice 0.04%, Tytanit, and IBA caused an increase in the length of the roots of the thick cuttings of 'Poppius'. In contrast, the length of roots of thick and medium cuttings in 'Harison's Yellow' decreased after the use of Root Juice 0.04% (Tab. 5).

Tab. 4 The degree of rooting of rooted root cuttings (2–5) of two old shrub roses. The rooting degree is specified on a valuation scale (1–5): 1 – no roots (only survived); 2 – a few roots, 1–10 cm long; 3 – 6–15 roots >10 cm long; 4 – 10–15 cm long, well developed, branched roots; 5 – roots forming a root ball.

Treatment	Thickness of root cuttings: Thick	Medium	Thin	Mean
'Harison's Yellow'				
Control	4.50 a–e [1]	4.50 a–e	4.78 ab	4.62 AB [2]
Root Juice 0.1%	4.69 a–c	4.04 ef	4.87 ab	4.38 BC
Root Juice 0.2%	4.30 b–f	4.69 a–c	4.65 a–d	4.59 AB
Root Juice 0.4%	4.20 c–f	4.10 d–f	4.50 a–e	4.23 C
Tytanit 0.01%	3.87 f	4.10 d–f	4.50 a–e	4.20 C
Tytanit 0.02%	4.62 a–d	4.53 a–e	4.65 a–d	4.58 AB
Tytanit 0.04%	5.00 a	4.58 a–e	4.78 ab	4.79 A
Chryzotop Green	4.83 ab	4.67 a–c	4.74 a–c	4.71 A
Rhizopon AA 020 XX	3.26 g	4.72 a–c	3.03 g	3.71 D
Mean	4.25 B	4.53 A	4.46 A	
'Poppius'				
Control	4.00 d–i	4.07 c–g	4.24 b–f	4.12 BC
Root Juice 0.1%	4.06 c–g	4.24 b–f	4.02 d–h	4.09 BC
Root Juice 0.2%	3.58 j	4.51 ab	4.33 a–d	4.04 CD
Root Juice 0.4%	4.11 c–g	4.05 d–h	4.67 a	4.32 A
Tytanit 0.01%	3.67 ij	4.17 b–f	4.51 ab	4.11 BC
Tytanit 0.02%	3.71 h–j	4.41 a–c	4.49 ab	4.23 AB
Tytanit 0.04%	4.17 b–f	3.90 f–j	4.3 b–e	4.11 BC
Chryzotop Green	3.97 e–i	4.22 b–f	4.5 ab	4.24 AB
Rhizopon AA 020 XX	3.77 g–j	3.78 g–j	4.26 b–e	3.92 D
Mean	3.90 E	4.12 B	4.36 A	

[1] Different letters indicate significant interactions between treatment and thickness of cuttings, according to Tukey's HSD test ($p \leq 0.05$; two-way ANOVA).
[2] Mean values marked with the same capital letters in a column or row do not differ significantly at $p \leq 0.05$ according to Tukey's HSD test.

Tab. 5 The length of roots (cm) of rooted root cuttings of two old shrub roses.

Treatment	Thickness of root cuttings			Mean
	Thick	Medium	Thin	
'Harison's Yellow'				
Control	13.41 a–h 1	11.50 b–h	10.04 e–i	11.52 BC 2
Root Juice 0.1%	15.53 a–c	10.23 d–i	16.50 a	12.93 B
Root Juice 0.2%	15.65 a–c	14.16 a–e	15.75 ab	15.17 A
Root Juice 0.4%	7.80 hi	8.05 hi	16.60 a	9.92 C
Tytanit 0.01%	14.31 a–e	6.85 i	13.71 a–f	11.58 BC
Tytanit 0.02%	12.40 a–h	10.97 c–i	13.99 a–e	12.19 BC
Tytanit 0.04%	15.58 a–c	11.38 b–i	13.75 a–f	12.72 B
Chryzotop Green	12.17 a–h	12.42 a–h	9.00 g–i	11.20 BC
Rhizopon AA 020 XX	9.71 e–i	14.81 a–d	9.32 f–i	11.40 BC
Mean	12.78 A	11.76 A	11.90 A	
'Poppius'				
Control	7.02 k	10.34 h–j	11.41 f–i	9.65 C
Root Juice 0.1%	7.58 k	12.41 d–g	10.76 g–j	10.22 C
Root Juice 0.2%	7.41 k	17.14 a	12.03 d–h	11.11 B
Root Juice 0.4%	9.29 j	12.46 d–g	15.00 b	12.44 A
Tytanit 0.01%	9.76 ij	14.23 bc	13.39 b–d	12.44 A
Tytanit 0.02%	10.82 g–j	14.78 b	12.81 c–f	12.92 A
Tytanit 0.04%	10.51 h–j	11.57 e–i	15.00 b	12.34 A
Chryzotop Green	11.32 f–i	12.70 c–f	12.95 c–f	12.37 A
Rhizopon AA 020 XX	9.11 j	10.85 g–j	12.33 b–e	11.15 B
Mean	9.10 B	12.74 A	12.87 A	

[1,2] See Tab. 4 for notes.

The above-ground part of cuttings

The application of the preparations had different results for the above-ground parts in growth attributes with respect to the thickness of the cuttings. The number of shoots in the root cuttings of both cultivars was similar for all three thicknesses. The preparations did not increase this number in 'Harison's Yellow' and the thick and medium cuttings in 'Poppius' (Tab. 6). The lengths of the shoots in both cultivars were significantly higher for the medium thickness cuttings. In the thick and medium sets of the 'Poppius' cuttings all the preparations caused an increase in shoot lengths. However for 'Harison's Yellow', after the use of 0.02% and 0.04% Tytanit and IBA preparations, the cuttings of all three thickness showed an increase in shoot length (Tab. 7). For both cultivars, leaf areas were highest for the thick root cuttings. IBA, Tytanit (0.02 % and 0.04 %), and Root Juice (0.4%) all increased this in thick and medium cuttings of both cultivars (Tab. 8).

Discussion

Adventitious roots arise in underground stems and old roots, and also on twigs, branches, leaves, and aerial stems, as opposed to developing from the embryo [25]. These root primordia do not form during normal early plant development. Adventitious roots on cuttings of a few species have been shown to be a result of a reaction to injury [9]. The process of roots developing on rhizomes of 'Harison's Yellow' and 'Poppius' visible as swellings on the surface suggest a reaction to excision from the stock plant, where the

Tab. 6 The number of shoots of rooted root cuttings of two old shrub roses.

Treatment	Thickness of root cuttings			Mean
	Thick	Medium	Thin	
'Harison's Yellow'				
Control	1.58 ab 1	1.33 ab	1.64 ab	1.56 AB 2
Root Juice 0.1%	1.69 ab	1.19 b	1.12 b	1.34 AB
Root Juice 0.2%	1.20 b	1.50 ab	1.20 b	1.30 AB
Root Juice 0.4%	1.30 ab	1.30 ab	1.17 b	1.27 B
Tytanit 0.01%	1.50 ab	1.50 ab	1.25 ab	1.40 AB
Tytanit 0.02%	1.62 ab	1.85 ab	1.50 ab	1.72 A
Tytanit 0.04%	2.08 a	1.54 ab	1.56 ab	1.62 AB
Chryzotop Green	1.92 ab	1.5 ab	1.91 ab	1.68 AB
Rhizopon AA 020 XX	1.49 ab	1.55 ab	1.53 ab	1.52 AB
Mean	1.60 A	1.55 A	1.55 A	
'Poppius'				
Control	1.38 a–d	1.28 b–d	1.26 b–d	1.31 A
Root Juice 0.1%	1.50 ab	1.41 a–d	1.37 a–d	1.42 A
Root Juice 0.2%	1.25 b–d	1.49 a–c	1.25 b–d	1.30 A
Root Juice 0.4%	1.48 a–c	1.28 b–d	1.42 a–d	1.40 A
Tytanit 0.01%	1.27 b–d	1.39 a–d	1.57 a	1.41 A
Tytanit 0.02%	1.46 a–c	1.27 b–d	1.28 b–d	1.33 A
Tytanit 0.04%	1.41 a–d	1.17 d	1.36 a–d	1.30 A
Chryzotop Green	1.24 b–d	1.22 cd	1.37 a–d	1.28 A
Rhizopon AA 020 XX	1.29 b–d	1.31 a–d	1.43 a–d	1.34 A
Mean	1.36 A	1.30 A	1.36 A	

[1,2] See Tab. 4 for notes.

site of such an injury has not been covered completely with callus. Root and shoot bud formation on root cuttings of *Sassafras albidum* (Nutt.) Ness have been reported to include two types of buds: additional and reparative. The additional buds form during the early growth of roots, but the reparative form de novo in response to, e.g., injuries or other disturbances, where the bud traces are absent or not to the centre of a root [26]. This type of reparative bud is visible on longitudinal sections in roses revealed also as swellings sited in the pericycle and near the vascular cambial zone.

It was observed that the young shoots in both rose cultivars developed before the roots to preserve polarity. The same mode of development has been observed in *Detarium macrocarpum* Harms, where the new root meristem formed more slowly than the adventitious shoot buds [27]. However, in the case of most species, a well-developed root system forms by the time the first shoots appear [9]. A lack of polarity was described in the case of *Rosa nitida* [10], but most species form roots at the distal end (morphological bottom part) and shoots at the proximal (morphological top part) of the root which has been shown to be related to auxin transport [9,10,28].

Subtending trace adventitious buds were observed in *S. albidum* [26] and this observation is similar in both rose cultivars investigated in this study, where the formation of the adventitious shoot or root buds is also connected to the widening of the pith rays. Newly-forming organs are sited along the medullary rays and on all the tissues lateral to the vascular system. The visible subtending wedge-shaped traces of adventitious roots in roses suggest that the additional roots tend to be endogenous in origin and to form during early growth, as in *S. albidum* [26]. The bud traces are completed by groups of isodiametric or radially-oriented xylem parenchyma cells and are produced by the vascular cambium as in the Australian tree *Eucalyptus regnans* F. Muell. [29].

Tab. 7 The length (cm) of shoots of rooted root-cuttings of two old shrub roses.

Treatment	Thickness of root cuttings			Mean
	Thick	Medium	Thin	
'Harison's Yellow'				
Control	12.12 d–f 1	18.03 a–e	6.73 gh	10.87 B 2
Root Juice 0.1%	14.39 c–f	12.09 d–f	22.01 ab	14.41 A
Root Juice 0.2%	16.10 b–f	16.01 b–f	14.48 c–f	15.36 A
Root Juice 0.4%	10.01 f–h	4.58 h	19.80 a–c	10.18 B
Tytanit 0.01%	6.32 gh	6.16 gh	12.12 d–f	8.59 B
Tytanit 0.02%	17.89 a–e	17.90 a–e	10.72 e–h	16.72 A
Tytanit 0.04%	21.81 a–c	16.90 b–f	16.68 b–f	17.45 A
Chryzotop Green	24.84 a	15.79 b–f	19.49 a–d	17.90 A
Rhizopon AA 020 XX	5.80 gh	18.45 a–d	5.48 gh	10.17 B
Mean	13.82 B	15.77 A	14.10 B	
'Poppius'				
Control	10.83 jk	13.85 f–j	12.15 i–k	12.08 B
Root Juice 0.1%	14.71 d–i	16.88 b–f	13.28 g–j	14.70 A
Root Juice 0.2%	9.90 k	23.72 a	14.10 f–i	14.37 A
Root Juice 0.4%	14.23 e–j	17.27 b–e	12.80 g–k	14.43 A
Tytanit 0.01%	12.12 i–k	15.50 c–h	15.99 b–g	14.52 A
Tytanit 0.02%	13.18 g–j	18.32 bc	13.78 f–j	15.23 A
Tytanit 0.04%	19.00 b	16.71 b–f	12.42 i–k	16.04 A
Chryzotop Green	16.11 b–g	17.68 b–d	11.60 i–k	15.05 A
Rhizopon AA 020 XX	16.24 b–g	16.65 b–f	10.98 jk	14.85 A
Mean	13.87 B	17.20 A	12.95 C	

[1,2] See Tab. 4 for notes.

The success of propagation by root cuttings is influenced by many factors, e.g., the phenological period, the length of cold storage, the condition of the donor plants [9,10], and anatomical structure [26,28]. Cultivars 'Harison's Yellow' and 'Poppius' had a great ability at rooting from the rhizome, but they both displayed different abilities at different thicknesses of cuttings. The control cuttings of rose 'Harison's Yellow' rooted at a similar percentage, whereas the thin root cuttings of 'Poppius' had the highest rooting ability of 90.0%. In the case of aspen, the rooting percentage was not seen to be affected by the length of the root cuttings [30]. However, this result could be related to the greater content of available sugars in thick and long root cuttings [31].

The positive effects of titanium [14,18,19,23] or preparations of plant origin [13,16] have also been reported by many authors in the cultivation or production of ornamental plants. IBA is often used as a rooting enhancer for stem cuttings in roses and other ornamental plants [8–12,16]. In this research, IBA preparations, Tytanit, and Root Juice showed different activities in the quality and quantity of rooted cuttings produced. The mean values of all the thicknesses tested for rooting percentage of rose 'Harison's Yellow' were significantly higher by application of 0.02% Tytanit compared to the control, IBA and Root Juice treatments. However, the use of IBA increased the means of the control treatment. Biostimulants containing nutrients can cause a positive reaction in the effectiveness of rooting for roses as has been shown in the one-bud stem cuttings of *Rosa beggeriana* 'Polstjärnan' and *R. helenae* 'Semiplena' which have higher contents of total carbohydrates in the leaves and stems after the use of Root Juice. However, the rooting percentage was higher only in 'Semiplena' [13]. The results for rooting percentages in root cuttings of cultivars 'Harrison's Yellow' and 'Poppius' seem to indicate this method to be effective. The control hardwood cuttings of both cultivars

Tab. 8 The means of total leaf area (cm²) of rooted root cuttings of two old shrub roses.

Treatment	Thickness of root cuttings: Thick	Medium	Thin	Mean
'Harison's Yellow'				
Control	84.54 hi 1	56.80 kl	47.55 l	62.96 C 2
Root Juice 0.1%	94.56 fgh	63.89 jk	55.69 kl	71.38 BC
Root Juice 0.2%	110.25 a–e	90.51 ghi	60.23 kl	87.00 ABC
Root Juice 0.4%	122.78 a	96.35 e–h	65.85 jk	94.99 AB
Tytanit 0.01%	118.99 ab	110.23 a–e	78.59 ij	102.60 A
Tytanit 0.02%	119.56 ab	112.36 a–d	97.68 d–h	109.87 A
Tytanit 0.04%	98.56 d–h	113.63 abc	98.99 c–h	103.73 A
Chryzotop Green	106.23 b–f	89.69 ghi	60.45 kl	85.46 ABC
Rhizopon AA 020 XX	100.26 c–g	85.67 ghi	59.98 kl	81.97 ABC
Mean	106.19 A	91.01 B	69.45 C	
'Poppius'				
Control	115.56 ghi	105.68 hij	97.99 j	106.41 C
Root Juice 0.1%	120.36 gh	98.57 j	101.23 ij	106.72 C
Root Juice 0.2%	158.36 bcd	108.56 hij	99.56 j	122.16 BC
Root Juice 0.4%	163.54 bc	127.97 fg	109.56 hi	133.69 ABC
Tytanit 0.01%	156.52 bcd	145.63 de	103.57 i	135.24 ABC
Tytanit 0.02%	168.56 ab	140.65 ef	110.27 hij	139.83 AB
Tytanit 0.04%	180.37 a	158.63 bcd	120.59 gh	153.20 A
Chryzotop Green	165.23 bc	160.59 bcd	95.40 j	140.41 AB
Rhizopon AA 020 XX	150.63 cde	170.39 ab	95.62 j	138.88 AB
Mean	153.24 A	135.19 B	103.75 C	

[1,2] See Tab. 4 for notes.

rooted in open fields showed a low rooting percentage (4.0% and 18.0%, respectively) and did not root in the greenhouse environment. Chryzotek beige 004 XX (IBA 0.4%) provoked an increase in the rooted cuttings only in 'Poppius' in the open field situation (38.0%) [8]. However, the favorable effect of IBA in rooting percentage in roses has been shown in a few studies, including in the difficult-to-root Damask rose [7]. The percentage from hardwood cuttings achieved for *Rosa dumalis* Bechst. increased with the use of higher concentrations of IBA in aqueous solution (0.15%, 0.35%, and 0.50%) [11]. It was found that the root cuttings of 'Harison's Yellow' and 'Poppius' trialed in the work reported here had a higher quality and degree of rooting compared to the results for hardwood cuttings reported elsewhere. The method for evaluation in these two trials employed a similar scale [11]. In this research, the preparations used exhibited different activity in the degree of rooting and the length of the roots generated and also in the characteristics of the above-ground parts in both roses. In the case of one bud leaf cuttings of *Rosa beggeriana* 'Polstjärnan' and *R. helenae* 'Semiplena', the degree of rooting was not reported to be increased by the use of the Root Juice [13]. The latter research also showed no significant effect from the use IBA on root length of *R. canina* softwood cuttings [32] and in the hybrid tea rose 'Dalas' [33]. However, the use of IBA solutions did improve the quality of the *R. dumalis* hardwood cuttings, and a higher concentration resulted in a greater number and length of their roots [11]. Furthermore, the hardwood cuttings of rose 'Harison's Yellow' had both longer roots and shoots when they were treated with IBA [4]. In the case of *Detarium microcarpum* Guill. & Perr., longer and wider diameter root cuttings had a higher sprouting efficiency in rooting and produced the largest, most vigorous sprouts [27].

Conclusions

Propagation by root cuttings seems to be a cheap option and does not require special equipment [10]. The results reported here for two old rose cultivars confirm this expectation and suggest that root cuttings are a more effective method for propagation than by hardwood cuttings. This method can be recommended for roses with an ability to produce rhizomes. The use of the eco-friendly (Root Juice, Tytanit) and IBA formulations (Chryzotop Green 0.25% IBA, Rhizopon AA 020 XX 2.00% IBA) had a varied influence on the rooting process and their effectiveness in increasing rooting percentage and quality of rooted cuttings. However, after analyzing the results presented here, the use of Root Juice and Tytanit can be suggested. The additional beneficial effects on rooting of rose stem cuttings were demonstrated from their containing biologically-active ingredients and growth promoters [13,16]. The use of these biostimulants to replace other chemicals in plant cultivation is recommended world-wide and can be used for rooting the root cuttings of roses.

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Ukorzenianie i wzrost sadzonek korzeniowych róż historycznych 'Harison's Yellow' i 'Poppius' traktowanych IBA i biostymulatorami

Streszczenie

Rozmnażanie przez sadzonki korzeniowe jest prostą i niskokosztową metodą reprodukcji taksonów roślin posiadających zdolność tworzenia odrostów lub rozłogów korzeniowych. Badania miały na celu określenie możliwości rozmnażania przez sadzonki korzeniowe dwóch trudno ukorzeniających się róż historycznych: 'Harison's Yellow' i 'Poppius'. Sadzonki korzeniowe o trzech grubościach (grube, średnie i cienkie) traktowano preparatami wspomagającymi ukorzenianie i wzrost: na bazie ekstraktów roślinnych (Root Juice; BioBizz Worldwide, Holandia), zawierających tytan (Tytanit; Intermag, Polska) i IBA (Chryzotop Green 0,25% IBA; Rhizopon AA 020 XX 2,00% IBA; Rhizopon BV, Holandia). Ponadto obserwowano miejsce formowania się korzeni i pędów u sadzonek. Odnotowano, że pędy wyrastają zanim pojawią się korzenie, nie wykazując biegunowości. Pędy i korzenie powstawały w miejscu zgrubień widocznych wzdłuż sadzonek. Zawiązki korzeni i pędów pojawiały się w pobliżu promieni rdzeniowych i strefy kambium waskularnego. Niniejsze badania wykazały istotny wpływ grubości sadzonki i użytych preparatów na proces ukorzeniania i wzrost sadzonek. Najwyższy procent ukorzenionych sadzonek uzyskano dla sadzonek średniej grubości u róży 'Harison's Yellow' (45,0%) i cienkich u 'Poppius' (74,3%). Najbardziej efektywnym okazał się preparat Chryzotop Green, przy czym wysoką efektywność otrzymano także stosując Root Juice 0,01% i Tytanit 0,04% ('Poppius') i 0,02% (obie odmiany). Oba powyższe przyjazne środowisku preparaty mogą być stosowane do ukorzeniania sadzonek róż.