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ORIGINAL PAPER

Effects of indole-3-butyric acid and age of stem cuttings on root morphology, growth, and survival of *Cornus sericea*

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Abstract Red osier dogwood (*Cornus sericea* L.), widely distributed throughout North America, is essential for wildlife, thus biodiversity. It is recommended for reclamation or revegetation of sites disturbed by oil and gas extraction because it tolerates a wide range of soil types and high pH levels. Since germination of this species is extremely difficult with long stratification requirements and poor germination, cuttings facilitate propagation. In this study, to develop techniques to propagate the species from stem cuttings, four concentrations of indole-3-butyric acid (IBA) and two cutting ages previous year's growth (PYG) and current year's growth (CYG) were investigated for survival and growth. After 4 months, survival rate, height growth, and shoot biomass from PYG cuttings were enhanced with IBA. In contrast, IBA did not affect these parameters in CYG cuttings. Root morphology was significantly affected by IBA concentration and cutting age. IBA, at higher concentrations, increased root surface and length. Total root surface area and length of PYG cuttings were increased, which may facilitate the absorption of essential resources and consequently increase growth. The results indicate that increasing the concentration of IBA may be an effective way to have better plant survival and growth of previous year's cuttings in red osier dogwood.

Keywords Vegetative propagation · IBA · Chronological age · *Cornus sericea* L. · Survival rate

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Introduction

Red osier dogwood (*Cornus sericea* L.) is a fast-growing, deciduous shrub native throughout North America and a vital food source for many wild animals, including ruminants and birds (Stevens and Dozier 2006). It thrives on different soil types and performs well in moderately wet soils (Smreciu et al. 2013). Species of *Cornus* are often referred to as dogwood due to their ability to develop hard woody stems (Dinda et al. 2016). The ability of red osier dogwood to tolerate a wide range of soil types and high pH levels enables resilience and a strong performance of the species on disturbed sites (Zhang and Zwiazek 2016). Moreover, it is often used in land reclamation due to its ability to minimize ion transport of either Na^+ or Cl^- from the roots to leaves, thereby limiting ionic toxicity in photosynthetic tissues (Renault et al. 2001; Davis et al. 2014). These characteristics allow red-osier dogwood to grow successfully on disturbed sites, and it has been widely used for reclamation of disturbed sites of oil and gas operations in Alberta, Canada (Smreciu et al. 2013).

While production from seeds may be economical (Hudson and Carlson 1998), a long period of stratification, a period of prolonged exposure to cold and moist conditions, and a low germination rate have been quite challenging in the propagation of the species. To overcome these issues, vegetative propagation by stem cuttings has been favored as an alternative method to seedling production (Stevens and Dozier 2006; Davis et al. 2014). One of the benefits of vegetative propagation is the potential to produce larger plants within a short time (Dumroese et al. 2009). Quicker results in larger plants by vegetative production can accelerate plant establishment, especially in reclamation of disturbed sites.

Although the use of cuttings is one of the easiest and most successful methods of propagation, a low adventitious rooting capacity could result in significant economic losses (de Klerk et al. 1999; Geiss et al. 2009). Adventitious root development is a critical stage of vegetative propagation, as these roots provide crucial functions, including water and nutrient acquisition, anchorage to a substrate, and storage of food reserves (Geiss et al. 2009). These functions facilitate the adaption of plants to the environment and permit further development. Despite recent progress in techniques of vegetative propagation, there is no universal method for all species that can produce optimum rooting capacity. Thus, it is important to develop suitable methods to successfully propagate different species from stem cuttings.

The formation of adventitious roots from cuttings is complex and success depends on multiple factors (Everett et al. 1978; Geiss et al. 2009). One of the most important factors influencing the rooting capacity is the growth stage of the cuttings (Porfírio et al. 2016). The position from which cuttings are taken influences rooting capacity and subsequent shoot growth and survival. It has been reported that rooting ability gradually decreases towards the upper part of the stem, and cuttings taken closer to older portions often root more successfully (Poethig 1990; Perry and Trueman 1999; Beyl and Trigiano 2011). However, the decreasing trend toward upper positions changes at apical seedling nodes, which possess the highest rooting capacity in some species (Hansen 1986; Wendling et al. 2015). The change in the rooting capacities of different parts of the plant could be attributed to structural changes of cuttings (Wendling et al. 2015) or to changes in the concentration of carbohydrates and levels of auxin that stimulates rooting in different species (Tsipouridis et al. 2003; Polat and Caliskan 2009; Hartmann et al. 2010).

Auxins promote adventitious root development of stem cuttings by promoting the formation of root primordia, and this is enhanced during rhizogenesis under an increased availability of auxin (Alvarez et al. 1989; Caboni et al. 1997; Frick and Strader 2018). Indole-3-butyric acid (IBA) is an important synthetic auxin that increases rooting percentage, quality, and vigor, thus it is widely used for vegetative propagation (Aminah et al. 1995; Hartmann et al. 2010; Beyl and Trigiano 2011). The average rooting time and the percentage of roots are closely related to auxin availability in the cutting base (Geiss et al. 2009). However, the optimal IBA concentration to promote adventitious root development can vary between species, the cutting type, and/or the age of the donor plant (Husen and Pal 2007; Husen 2012). Al-Saqri and Alderson (1996) found that rooting was enhanced in *Rosa × centifolia* L. after treatment of semi-hardwood cuttings with IBA. Some plants respond better under low concentrations of IBA (Aminah et al. 1995), whereas others show a significant effect on rooting ability only under higher concentrations (Azad et al. 2018). Porlingis and Therios (1976) found that the effect of IBA concentration on rooting percentage varied with the growth stage of the cuttings. Although numerous studies have examined the effects of IBA application on vegetative propagation, little information is available on its relationship to chronological age of cuttings, particularly in *Cornus* spp.

This study investigated the effects of different IBA concentrations on rooting of stem cuttings from 1 to 2-year-old-growth (previous year's growth) and current year's growth of *Cornus sericea* L. in order to develop effective techniques to propagate this species for use in reclamation of sites disturbed by oil and gas extraction.

Materials and methods

Plant material

Cuttings of red osier dogwood were collected near the town of Peace River, Alberta, Canada (altitude: 571 m, 56.23° N, 117.27° W) in early July 2018. The mean temperature of this area during the growing season (June–September) was 13.8 °C between 1981 and 2010 (https://climate.weather.gc.ca/climate_normals/index_e.html). Stem cuttings, approximately 40 cm in length were randomly collected from disease- and pest-free plants. Cuttings were harvested before noon when shoots were fully hydrated and stored in moist conditions at 5 °C prior to hormone treatments.

Preparation of cuttings and IBA application

Stems with a single node were cut into 10 cm sections using a sterilized razor blade and separated by their chronological age (previous year's growth (PYG) and current year's growth (CYG). PYG cuttings were taken from the position that contained approximately 1–2 years old stem, while the CYG cuttings were taken from the terminal position that was approximately 2-months- old. All leaves were removed from the cuttings and the base was cut diagonally to increase the surface area for rooting (Blythe et al. 2007). The basal end (2–3 cm) of each cutting was dipped in one of the concentrations of commercial rooting powder Plant-Prod® (Stim Root®#1 (0.1% IBA), Stim Root® #2 (0.4% IBA), or Stim Root® #3 (0.8% IBA); Premier Tech & Garden Inc, Brantford, ON, Canada), and inserted into the prepared growth media. Powder application has been commonly used for root-promoting chemical applications (Blythe et al. 2007; Beyl and Trigiano 2011). After treatments, the cuttings were planted in surface sterilized Styroblock™ (Beaver Plastics, Edmonton, AB, Canada) containers (72 cm × 14 cm × 24 cm) with a mixture of peat, perlite, and vermiculite (2:1:1 v/v). The surface of the growing media was covered with forestry sand (Target Products Ltd, Burnaby, BC, Canada) to minimize moss and algae development as well as to reduce surface evaporation. An automated overhead misting system (Cherry Creek Systems, Colorado Springs, CO, USA) 45 cm above the cuttings was used to irrigate consistently for 10 s at intervals of 15 min during full daylight in the greenhouse. The misting was set to keep the growing medium moist without causing leaching that would remove the growth hormone (IBA) from the system.

Experimental design

The experiment was carried out in a greenhouse at the Centre for Boreal Research, Peace River, AB, Canada with two treatment factors arranged in a completely randomized design. The first factor consisted of a control (0%) and three levels of indole-3-butyric acid (IBA) concentrations: 0.1% (Stim1), 0.4% (Stim2), and 0.8% (Stim3); the second was the chronological age of the cuttings, previous year's growth (PYG) and current year's growth (CYG). A total of 77 cuttings were used for each treatment combination, for a total of 616 cuttings (77 replications × 4 applications × 2 cutting ages).

Greenhouse environment controls

The experiment was carried out over four months from July to October 2018. The greenhouse was maintained at 22 °C (± 2) and relative humidity at 75% using the Argus Titan Environment Control System (Argus Controls Systems Ltd., Surrey, BC, Canada). All styroblocks were well-watered, with starter liquid fertilizer (Miracle Grow Quick Start) applied twice during the second and third weeks. After this establishment phase, the cuttings were fertilized twice a week with a fertilizer solution of each element (Table 1).

Data collection

After four months, cutting mortality was evaluated to determine the survival rate. Height of new growth and root collar diameter (RCD) were measured. Sample seedlings were harvested and separated into shoots and roots. Root morphology (root surface area, root lengths, and average root diameter) was determined using the Regent Instruments WhinRHIZO system (QC, Canada). The roots were placed in a tray of water and spread out for scanning and precise quantification of the different parameters by the software program. The roots were then scanned using a STD4800 scanner calibrated for image analysis and quantification. Shoot and root dry masses were determined after being oven-dried at 80 °C for 48 h.

Data analysis

The data were examined graphically for normality of distribution (probability plots of residuals) and homogeneity of variance (scatter plots) before being subjected to analysis of variance (ANOVA) using the R software (Version 3.6.3, R Development Core Team 2020). The effects of IBA concentration and chronological age were considered as "fixed effects" in the ANOVA. An effect was considered significant if $p < 0.05$. When ANOVA showed a significant ($p < 0.05$) IBA concentration effect or a significant interaction, Tukey's post hoc test was used to compare individual means.

Results

The mortality of PGY cuttings treated with IBA was lower than the controls (Fig. 1). The survival of PGY cuttings was generally higher with increasing IBA concentrations (control 49%, Stim1 65%, Stim2 58%, and Stim3 68%), although the difference was only statistically significant between the controls and the highest IBA concentration (Stim3), whereas CYG cuttings did not show any significant difference between IBA concentrations (Table 2; Fig. 2).

Height growth of PYG cuttings significantly increased with increases in IBA, whereas CYG cuttings did not show this (Table 2; Fig. 1). PYG Height growth increased by 1.3%, 19%, and 28%, respectively, with respect to controls. Although the height growth of CYG control cuttings was slightly higher than that of PYG controls, IBA treatment did not stimulate their growth.

Shoot biomass of PYG cuttings followed the same trend as height growth, but differences were only statistically significant between controls and the highest IBA concentration (Fig. 3a). Root biomass significantly increased with Stim3 compared to Stim2 (Fig. 3b-left). PYG cuttings had significantly higher root biomass than CYG cuttings (Fig. 3b-right). While PYG cuttings were

Table 1 The content and amount of fertilizer solution (Element/ Compound ($\times 10^{-6}$))

NO_3-N	NH_4-N	N	P	K	Ca	Mg	S	Fe	Zn	Cu	B	HCO_3	$CaCO_3$	Na
50.8	15.6	66.4	83.8	92.4	94.3	38.9	56.9	3.4	0.2	0.6	0.3	106.0	87.0	26.4

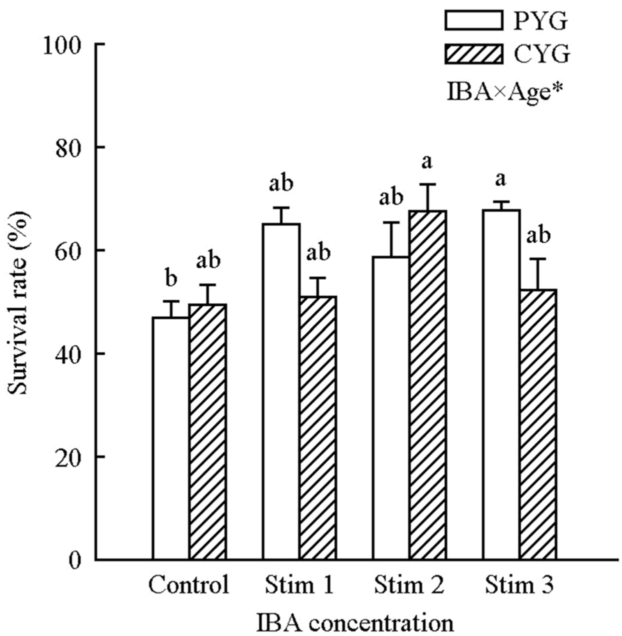

Fig. 1 Mean (+SE) survival rates of PGY and CYG cuttings treated with no IBA and with different concentrations, 0% (control), 0.1% (Stim1), 0.4% (Stim2) and 0.8% (Stim3); means with different letters are significantly different from each other based on Tukey's post hoc test ($p<0.05$); significance levels for the interactive effect of IBA and age were $p<0.05$; significance levels: $^*p<0.05$ indicated on the top

not affected by IBA, the root: shoot ratio of CYG cuttings was and Stim3 was significantly higher than the controls (Fig. 3c).

Total root surface area and total root length were significantly higher with the Stim3 treatment than with Stim1 and Stim2 (Table 3; Fig. 4a, b-left). Total root surface area and total root length significantly increased in PYG cuttings (Table 3; Fig. 4a, b-right).

Discussion

Interactive effect of IBA concentration and age on survival

The positive effect of IBA treatment on survival rate was expected, based on results from other studies (e.g., Aminah et al. 1995; Hartmann et al. 2010; Beyl and Trigiano 2011). Although the response to IBA levels was encouraging, the result varied with the age of the cuttings. The highest survival of the previous year's growth (PYG) cuttings (68%) was with IBA 0.8%, and the highest survival of the current year's growth (CYG) cuttings (67.5%) was with IBA 0.4%. The application of auxins such as IBA in the vegetative propagation of stem cuttings has a high potential as an enhancer of enzyme activities, which facilitates carbohydrate hydrolysis, thereby providing sufficient energy to support rooting (Morsink and Smith 1974; Gao and Zhao 2014). IBA constitutes an important hormone during rhizogenesis and for the initiation of root primordia (Caboni et al. 1997; Gao and Zhao 2014; Frick and Strader 2018). Our results indicate that the application of exogenous IBA increased rooting ability leading to enhancement of survival rates of both PYG and CYG cuttings.

It has been reported that rooting ability gradually increases towards the basal part of the crown/shoots, which may be attributed to higher carbohydrate accumulation (Poethig 1990; Perry and Trueman 1999; Hartmann et al. 2010; Beyl and Trigiano 2011). However, this trend is highly variable between and within species. For instance, cuttings from apical nodes have a higher rooting ability in *Schefflera arboricola* and *Corymbia torelliana* × *C. citriodora* subsp. *variegata* (hybrid family: 1CT2-013 × 1CV2-109), possibly due to the effect of auxin translocated from the apex (Hansen 1986; Wendling et al. 2015). Results of this study indicate that survival to IBA application on cuttings were highly responsive to the positions and chronological ages. The interactive effect may have been due to variations in the

Table 2 ANOVA results (F and p values) for the effects of IBA concentration, cutting age, and their interaction on survival rate, height, RCD, shoot, root biomass, and R:S ratio; treatments consisted of a control (0%) and three levels of IBA concentrations, 0.1% (Stim1), 0.4% (Stim2), and 0.8% (Stim3) and two different cutting ages (previous year's growth and current year's growth); numbers in bold are significant at < 0.05

Variable	IBA (DF = 3)		Age (DF = 1)		IBA × Age (DF = 3)	
	F	*p*	*F*	*p*	*F*	*P*
Survival rate	4.014	**0.013**	2.007	0.163	3.672	**0.018**
Height	7.145	**< 0.001**	16.221	**< 0.001**	3.810	**0.010**
RCD	2.227	0.085	2.958	0.086	2.138	0.095
Shoot biomass	2.760	**0.042**	5.353	**0.021**	3.105	**0.027**
Root biomass	2.695	**0.046**	3.867	**0.050**	0.277	0.842
R:S	2.938	**0.033**	1.066	0.303	3.575	**0.014**

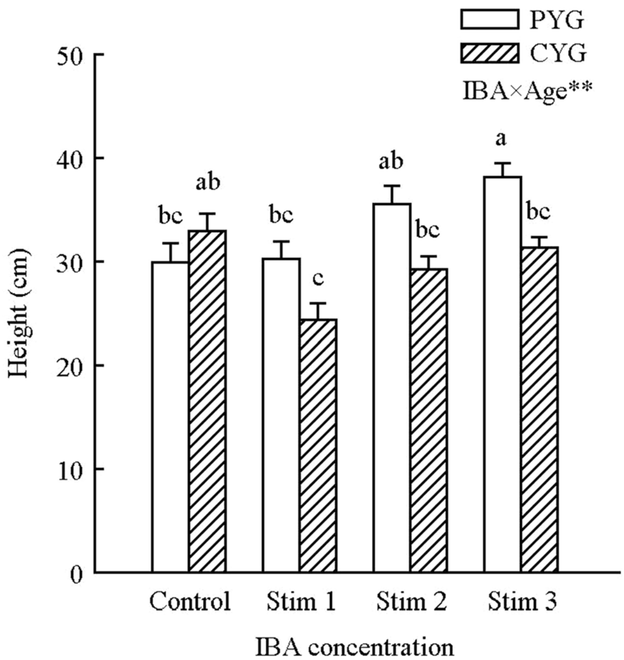

Fig. 2 Mean (+SE) height growth of PYG and CYG cuttings of controls and treated with different IBA concentrations, 0.1% (Stim1), 0.4% (Stim 2) and 0.8% (Stim 3). Means with different letters are significantly different based on Tukey's post hoc test ($p<0.05$). Significance levels: $^{**}p<0.01$ indicated on the top

physiological status of cutting tissues, level of assimilates, and/or stem structures and degree of lignification (Tsipouridis et al. 2003; Polat and Caliskan 2009; Hartmann et al. 2010). This study found a similar survival rate from the combination of IBA 0.8% and PYG (68%), and the combination of IBA 0.4% and CYG (67.5%). Survival rates can be improved by carefully selecting the IBA concentration for each age of cuttings.

Interactive effects of IBA concentration and age on growth and root morphology

Height growth of PYG cuttings significantly increased with increasing IBA, whereas it did not occur with CYG cuttings. The greater height growth with IBA application, particularly at the 0.8% concentration, is likely attributable to faster root primordial emergence and higher rooting percentage induced by the application of an exogenous hormone (Noor Camellia et al. 2009). Our results from root morphological analysis suggest that the highest root surface area was induced by the highest IBA concentration. Therefore, it may be assumed that increasing root surface area stimulated by IBA enabled plants to better absorb essential resources such as water and nutrients, leading to height growth enhancement (Frick and Strader 2018). Moreover, although a significant increase in PYG shoot biomass with increasing IBA concentration occurred in this present study, root-to-shoot ratios were unaffected, indicating that plants had adequate resources from the soil to support their shoot growth (Poorter et al. 2012).

In this study, although the difference was only statistically significant between the controls and the lowest IBA concentration (0.1%), height growth of CYG cuttings decreased with IBA application. The negative effects of IBA on rooting capacity and growth has been reported in several studies as a reduction in root and shoot development or defoliation of cuttings due to high doses of IBA (Trueman and Richardson 2008; Hung and Trueman 2012; Kilkenny et al. 2012). When plants already have optimum levels of endogenous auxin, additional applications raise concentrations to supra-optimal levels, and affect rooting capacity and shoot system development (Wendling et al. 2015). Similarly, cuttings that are closest to the most apical nodes have higher rooting capacity due to the effect of auxin translocated from the apex (Hansen 1986; Wendling et al. 2015). Taken together, it is possible that the optimum auxin levels of CYG may have been exceeded by IBA applications. Alternatively, the effect of IBA application may also be influenced by carbohydrate reserves in the cuttings used for propagation. Carbohydrates play a prominent role in rhizogenesis, as the metabolism of carbohydrates constitutes an essential source of energy required to initiate root development (Haissig 1974). As new growth cuttings tend to have higher auxin and lower endogenous carbohydrate levels than hardwood cuttings (Hartmann et al. 2010), the lower carbohydrates in CYG cuttings possibly affected their rooting capability, leading to decreases in height growth and shoot biomass. The root morphology also indicates a significant reduction of root surface area and length in CYG (current year's growth) cuttings, possibly resulting in lower absorption of essential resources, leading to lower growth and shoot biomass. Overall, the interaction between IBA concentration and chronological age suggests that high concentrations of exogenous IBA likely cause negative effects if applied to cuttings from the current year's growth. Increasing IBA concentration can only improve their growth if applied to cuttings from the previous year's growth.

In conclusion, IBA application increased survival rate and growth by improving the rooting capability of cuttings of the previous year's growth. However, although the survival rate improved with IBA application to the current year's growth cuttings, overall growth did not respond to IBA, possibly due to the already high concentration of endogenous auxin and the limited carbohydrate reserves in the cuttings. The most effective treatment combination for stem cutting propagation was the highest IBA concentration (0.8%) and cuttings from the previous year's growth, which resulted in the highest survival rate and the highest growth. It is, therefore, the combination of 0.8% IBA and cuttings from the previous

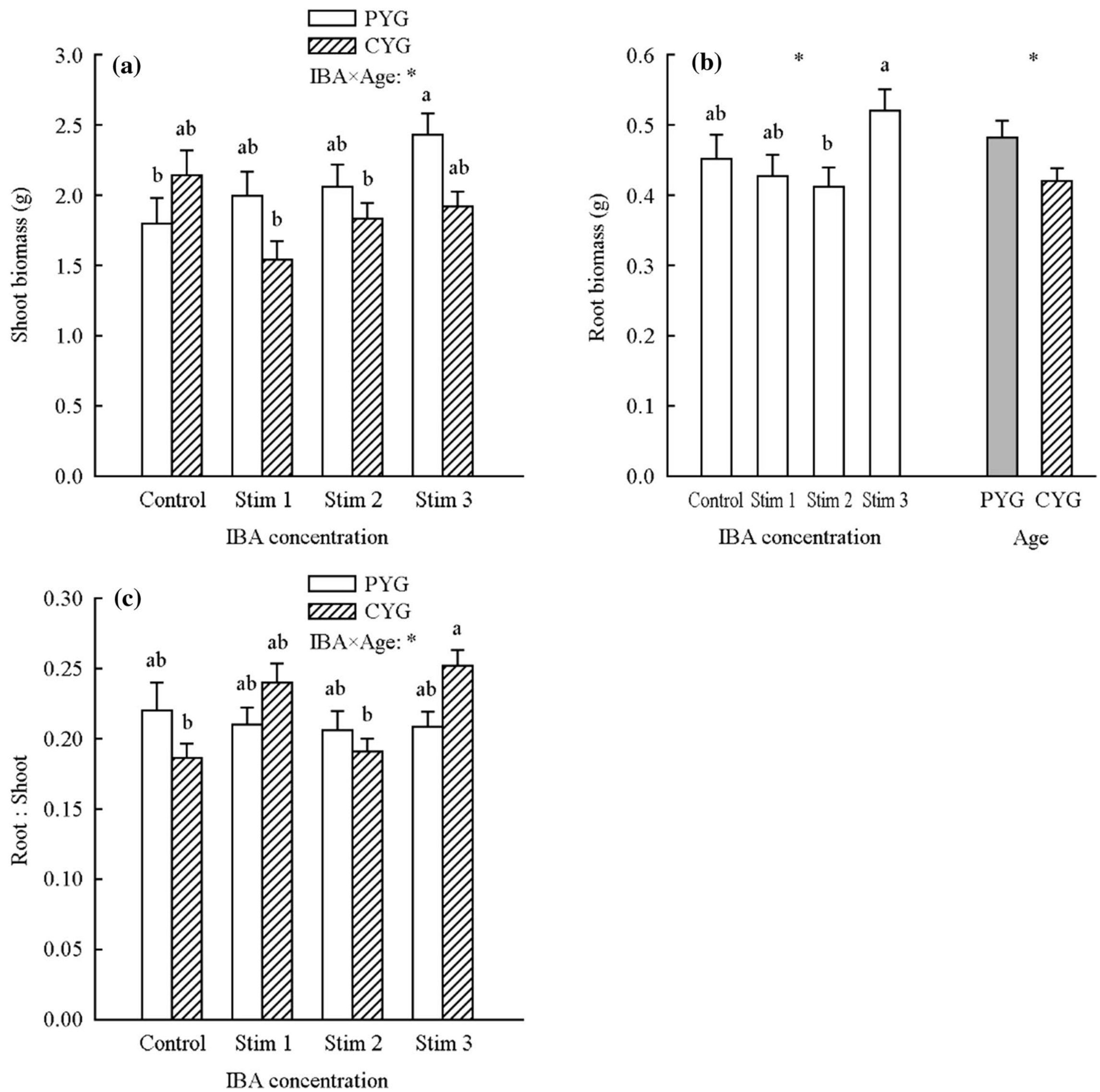

Fig. 3 Mean (SE+) **a** shoot biomass, **b** root biomass and **c** root: shoot ratio of PYG and CYG cuttings of untreated (controls) and treated with different IBA concentrations, 0.1% (Stim1), 0.4% (Stim2) and 0.8% (Stim3). **a** and **c** represent the interaction of IBA and age; for (**b**), data were pooled across (**b**-left) age (N = 154, 77 replications × 2 = 154) and (b-right) IBA concentration (N = 308, 77 replications × 4 = 308), because the 2-way interaction was not statistically significant. Means with different letters are significantly different based on Tukey's post hoc test ($p < 0.05$). Significance levels for the interactive effect of IBA and age are indicated with an asterisk (*$p < 0.05$)

Table 3 ANOVA results (F and p values) for the effects of IBA concentration, cutting age, and their interaction on total root surface, total root length, and average root diameter. The numbers in bold are significant at < 0.05

Variable	IBA (DF = 3)		Age (DF = 1)		IBA × Age (DF = 3)	
	F	*P*	*F*	*p*	*F*	*p*
Total root surface area	5.362	**0.001**	25.358	**< 0.001**	0.235	0.872
Total root length	6.042	**0.001**	29.625	**< 0.001**	0.426	0.735
Average root diameter	0.882	0.451	0.055	0.815	0.620	0.603

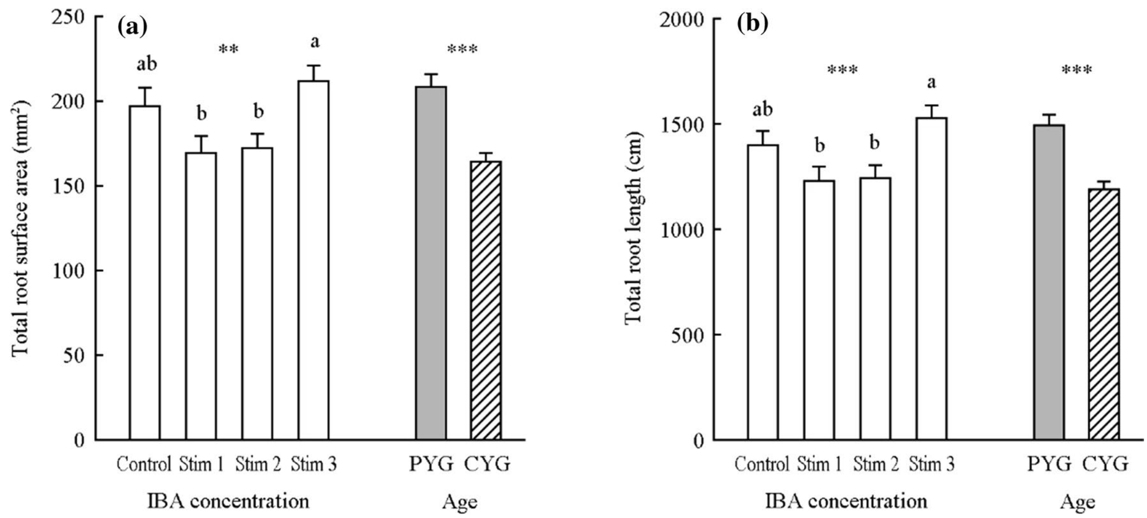

Fig. 4 Mean (+SE) **a** total root surface area and **b** total root length of PYG and CYG cuttings untreated (controls) and treated with different IBA concentrations, 0.1% (Stim1), 0.4% (Stim2) and 0.8% (Stim3). Data were pooled across **a** and **b**-left age (N = 154, 77 replications × 2 = 154) and **a** and **b**-right IBA concentration (N = 308, 77 replications × 4 = 308) because the 2-way interaction was not statistically significant. Means with different letters are significantly different based on Tukey's post hoc test ($p < 0.05$). Significance levels for the main effect of IBA and age are indicated with an asterisk (*); ** $p < 0.01$; *** $p < 0.001$)

year's growth to be used when propagating red osier dogwood from stem cuttings.

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Author contributions SI performed the statistical analysis and prepared the manuscript. CI planned and conducted the experiments. JMS contributed to manuscript writing and discussion of ideas. All authors have approved the manuscript.

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