

Rooting 'Yoshino' Cryptomeria Stem Cuttings as Influenced by Growth Stage, Branch Order, and IBA Treatment

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Abstract. Stem cuttings of 'Yoshino' Japanese cedar [*Cryptomeria japonica* (L.f.) D. Don 'Yoshino'], consisting of tips (terminal 20 cm) of first-order laterals, distal halves (terminal 10 cm) of tips of first-order laterals, and proximal halves (basal 10 cm) of tips of first-order laterals, or tips (terminal 10 cm) of second-order laterals, were taken on four dates that represented four growth stages (softwood, semi-hardwood, hardwood, and pre-budbreak). The cuttings were treated with 0, 3000, 6000, or 9000 mg IBA/liter. Branch order affected all rooting measurements at each growth stage. Regardless of growth stage, tips of and proximal halves of first-order laterals containing lignified wood had the highest percent rooting, root count, total root length, root area, and root dry weight. Hardwood tips of and semi-hardwood proximal halves of first-order laterals exhibited the highest overall rooting (87%), followed by softwood proximal halves of first-order laterals (78%). Rooting of distal halves of first-order laterals and tips of second-order laterals never exceeded 55% and 34%, respectively, at any growth stage. IBA treatment influenced percent rooting, root count, total root length, root area, and root dry weight of semi-hardwood, hardwood, and pre-budbreak cuttings, except for root dry weight of semi-hardwood cuttings. IBA had no effect on softwood cuttings. Chemical name used: 1H-indole-3-butyric acid (IBA).

Japanese cedar or cryptomeria, a coniferous evergreen indigenous to Japan and southern China (Dirr, 1990), is a widely used timber species in the Far East. It also is considered a sacred tree in Japan with great landscape value (Creech, 1984). The species currently is gaining popularity not only in the northeastern United States but also in the hot and humid southeast. The tree thrives in rich, deep, acidic, moist soil but will tolerate heavy clay during dry and wet periods (Dirr, 1990; Tripp, 1993).

There are many cultivars of Japanese cedar that have a wide range of ornamental characteristics and uses. The popular cultivar Yoshino has a pyramidal form, reaching 15 to 18 m in height, with descending branches. 'Yoshino', which has no major insect or disease problems, grows rapidly and makes an excellent evergreen screen (Dirr, 1990; Tripp, 1993). In addition, the emerald green foliage exhibits little dieback or discoloration (Tripp, 1993).

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This particular cultivar is recommended as a replacement for Leyland cypress [*X Cupressocyparis leylandii* (A.B. Jacks and

Dallim.) Dallim. and A.B. Jacks], which has various disease and insect problems (Baker and Jones, 1987).

Although popularity and subsequent demand for 'Yoshino' cryptomeria and other cultivars of Japanese cedar have increased, little research has been reported in English-language journals on factors influencing propagation of the species and related cultivars by stem cuttings. For centuries, Japanese cedar has been propagated in Japan for forestry by seed and stem cuttings (Brix and van den Driessche, 1977; Ohba, 1993), and a body of practical knowledge on propagation and culture exists in the Far East. Unfortunately, this information appears not to be published in a retrievable form. If published, English translations of the articles are not available. Some research concerning stem-cutting propagation of the species has been conducted in the United States. Information regarding factors, such as growth stage (timing) and auxin treatment, have been published; however, much of this information is conflicting and needs to be resolved (Dirr and Heuser, 1987; Lahiri, 1975; Nakayama, 1978; Orndorff, 1974). In addition, tree forms of Japanese cedar exhibit a well-defined branch order (branch position), which may influence rooting. For some conifers, branch order is an important factor affecting adventitious rooting (Black, 1972; Bogdanov, 1984; Miller et al., 1982) and warrants study in Japanese cedar. Therefore, we investigated the effects of growth stage (timing), branch order, and IBA treatment on adventitious rooting of 'Yoshino' cryptomeria stem cuttings.

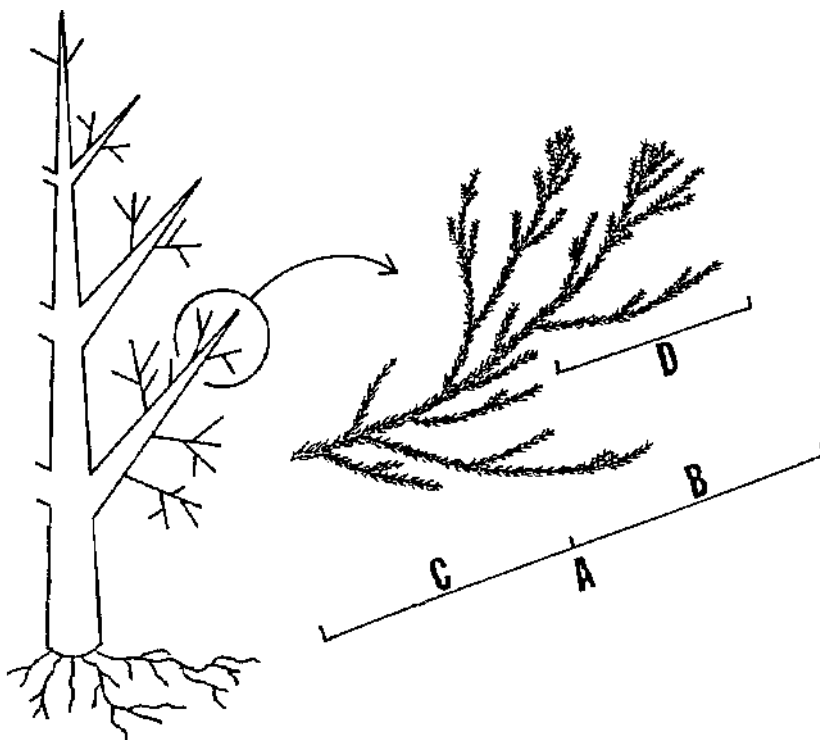


Fig. 1. Schematic of branch order illustrating locations on 'Yoshino' cryptomeria stock plants from which stem cuttings were taken. Following severance from the stock plants, the cuttings were trimmed further, resulting in (A) tips (terminal 20 cm) of first-order laterals, (B) distal halves (terminal 10 cm) of tips of first-order laterals, (C) proximal halves (basal 10 cm) of tips of first-order laterals, (D) tips (terminal 10 cm) of second-order laterals. Branches were pruned from the basal 4 cm of all cuttings before auxin treatment and subsequent insertion into the rooting medium.

Materials and Methods

Forty terminal cuttings, consisting of tips (terminal 25 cm) of first-order laterals with attached second-order laterals (Fig. 1) were taken from each of six, 10-year-old trees growing in the North Carolina State Univ. Arboretum, Raleigh, on four dates that represented specific growth stages: 7 Aug. 1992 (softwood), 6 Nov. 1992 (semi-hardwood), 15 Jan. 1993 (hardwood), and 12 Mar. 1993 (pre-budbreak). Shoot growth began ≈ 15 Apr. 1993. Cuttings were taken throughout the entire crown of each tree. The trees were growing under uniform fertility and had not been sheared.

As cuttings were collected, they were placed in plastic bags and transported to the Horticultural Science Greenhouses, Raleigh, N.C. After collection, all cuttings were pooled, randomized, and trimmed, resulting in the following groups: a) entire tips (terminal 20 cm) of first-order laterals, b) distal halves (terminal 10 cm) of tips of first-order laterals, c) proximal halves (basal 10 cm) of tips of first-order laterals, or d) tips (terminal 10 cm) of second-order laterals (Fig. 1). Before auxin treatment, lower branches, not needles, were pruned with hand shears from the basal 4 cm of each cutting. The basal 1 cm of each cutting then was treated with 0, 3000, 6000, or 9000 mg IBA/liter (reagent-grade IBA in 50% isopropanol) for 1 to 2 sec. The cuttings were air-dried for 15 min before insertion to a 4-cm depth into a raised greenhouse bench containing a nonheated medium of 4 peat : 3 perlite (v/v). The design within the propagation bed for each date (growth stage) was a randomized complete block with a factorial arrangement of treatments (four branch orders × four IBA levels), six blocks, and five cuttings per treatment per block.

Cuttings were maintained under natural photoperiod and irradiance with days/nights of 24 ± 4/16 ± 4C. Intermittent mist operated for 6 to 8 sec every 3.3 min from 7:00 AM to sunset. To control fungi, cuttings were sprayed initially and weekly thereafter alternating methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate (benomyl) and 3a, 4, 7, 7a-tetrahydro-2-[(trichloromethyl) thiol]-1H-isindole-1,3 (2H)-dione (captan) at 1.8 and 2.4 g/liter, respectively.

Cuttings were harvested after 12 weeks for each growth stage, and data were recorded on percent rooting, number of primary roots ≥ 1 mm in length, root area, total root length, and root dry weight (dried at 70C for 72 h). All data, except rooting percentage, were based on the actual number of cuttings that rooted (at least one primary root). Root area and total root length were measured using an image analyzer (Monochrome Agvision System 286 model; Decagon Devices, Pullman, Wash.). Data were subjected to analysis of variance and regression analysis. A linear contrast to test for differences between a pooled IBA treatment effect and nontreated cuttings (0 mg IBA/liter) also was conducted (SAS Institute, 1990).

Results and Discussion

Branch order affected all measurements of rooting for each growth stage, whereas IBA treatment affected all measurements for semi-hardwood, hardwood, and pre-budbreak cuttings, except for root dry weight of semi-hardwood cuttings. Auxin treatment had no influence on softwood cuttings (data not presented), results that are similar to those of Nakayama (1978). Waxman (1962) reported that stem cuttings taken during active growth (softwood) are composed of cells that are actively dividing and elongating and may contain high concentrations of root-promoting factors (e.g., auxin). These cells quickly initiate roots. Thus, softwood cuttings of 'Yoshino' cryptomeria may not have benefited from supplemental exogenous auxin. IBA × branch-order interactions were nonsignificant except for percent rooting of semi-hardwood cuttings and root count of hardwood and pre-budbreak cuttings.

Softwood and pre-budbreak tips of first-order laterals and proximal halves of first-order laterals had the highest percent rooting, followed by distal halves of first-order laterals and tips of second-order laterals (Table 1). Bases of both cutting types consisted of lignified wood, which may have contained various root-promoting factors (e.g., auxin synergists) not present in younger growth (Hartmann et al., 1990), resulting in increased rooting. Results were similar for hardwood cuttings, except tips of first-order laterals had the highest percent rooting of the four branch orders.

Similar to softwood and pre-budbreak cuttings, semi-hardwood tips of and proximal halves of first-order laterals had the highest percent rooting (Table 2). However, differences in percent rooting with distal halves varied with IBA concentration. In contrast to the other growth stages, semi-hardwood second-order laterals did not root.

Hardwood tips of first-order laterals (Table 1) and semi-hardwood proximal halves of first-order laterals treated with 3000 mg IBA/liter (Table 2) exhibited the highest rooting (87%), followed by softwood proximal halves of first-order laterals (78%) (Table 1), which illustrates that particular branch orders rooted well at several growth stages. Henry et al. (1992), working with eastern red cedar (*Juniperus virginiana* L.), reported maximum rooting with hardwood cuttings, whereas rooting of softwood cuttings was negligible. Reduced rooting with softwood cuttings of some coniferous species also has been reported by Hartmann et al. (1990). Rooting of distal halves of first-order laterals and tips of second-order laterals never exceeded 55% and 34%, respectively, at any growth stage (Tables 1 and 2). These findings agree with previous reports for conifers regarding the influence of branch order on rooting (Black, 1972; Bogdanov, 1984; Miller et al., 1982).

Recommendations regarding optimum IBA concentrations for rooting stem cuttings of Japanese cedar vary depending on whether solutions or auxin-talcum powder preparations are used (Dirr, 1990; Doran, 1957; Mitsch, 1975; Nakayama, 1978). Treatment with IBA

Table 1. Effect of branch order on overall percent rooting of 'Yoshino' cryptomeria stem cuttings taken at three growth stages.

Branch order ²	Growth stage		
	Softwood	Hardwood	Pre-budbreak
First-order (terminal 20 cm)			
Entire	75.0 a ^y	86.7 a	70.8 a
Distal half	50.0 b	55.0 c	45.8 b
Proximal half	78.3 a	71.7 b	73.3 a
Second order	34.2 c	12.5 d	20.4 c

²Entire = tips (terminal 20 cm) of first-order laterals, distal half = terminal 10 cm of first-order laterals, proximal half = basal 10 cm of first-order laterals, and second order = tips (terminal 10 cm) of second-order laterals.

^yMean separation within columns by least significant difference at $P \leq 0.05$.

Table 2. Effect of IBA concentration by branch order on percent rooting of semi-hardwood, 'Yoshino' cryptomeria stem cuttings.

IBA concn (mg·liter ⁻¹)	Branch order ²			
	First order (terminal 20 cm)			Second order
	Entire	Distal half	Proximal half	
0	33.3 ab ^y	20.0 bc	56.7 a	0.0 c
3000	66.7 ab	50.0 b	86.7 a	0.0 c
6000	66.7 a	---	70.0 a	0.0 b
9000	70.0 a	40.8 b	56.7 ab	0.0 c
Linear	*	---	NS	NS
Quadratic	NS	---	*	NS
IBA vs. control ^w	**	NS	NS	NS

²Entire = tips (terminal 20 cm) of first-order laterals, distal half = terminal 10 cm of first-order laterals, proximal half = basal 10 cm of first-order laterals, and second order = tips (terminal 10 cm) of second-order laterals.

^yMean separation within rows for an IBA concentration by least significant difference at $P \leq 0.05$.

^xData not available.

^wLinear contrast.

ns, *, **Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

at 3000 to 9000 mg·liter⁻¹, a range that encompasses the 4000 mg IBA/liter recommended by Doran (1957) for hardwood cuttings of Japanese cedar, produced about equal rooting with hardwood cuttings (Table 3). IBA treatment, which was an average of 3000, 6000, and 9000 mg·liter⁻¹ for hardwood cuttings (mean of 61%), increased percent rooting compared to the nontreated cuttings (mean of 43%).

Table 3. Effect of IBA concentration on overall percent rooting of 'Yoshino' cryptomeria stem cuttings taken at two growth stages.

IBA concn (mg·liter ⁻¹)	Growth stage	
	Hardwood	Pre-budbreak
0	43.3	33.3
3000	63.3	55.0
6000	56.7	60.8
9000	62.5	61.2
Linear	NS	**
Quadratic	NS	NS
IBA vs. control ²	**	***

²Linear contrast.

NS, **, ***Nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively.

Table 4. Effect of branch order on total root length and root dry weight of 'Yoshino' cryptomeria stem cuttings taken at four growth stages.

Branch order ^z	Growth stage			
	Softwood	Semi-hardwood	Hardwood	Pre-budbreak
<i>Total root length (cm)</i>				
First order (terminal 20 cm)				
Entire	34.9 a ^y	8.7 ab	39.4 a	39.8 a
Distal half	13.2 b	6.4 b	12.4 b	8.7 c
Proximal half	33.6 a	11.4 a	33.3 a	23.9 b
Second order	7.1 b	0.0 c	3.9 c	3.8 c
<i>Root dry wt (mg)</i>				
First order (terminal 20 cm)				
Entire	63.5 a	14.3 ab	64.2 a	97.0 a
Distal half	19.4 b	10.7 b	17.7 b	16.6 c
Proximal half	62.8 a	20.3 a	53.0 a	75.8 b
Second order	7.4 b	0.0 c	4.6 b	7.8 c

^zEntire = tips (terminal 20 cm) of first-order laterals, distal half = terminal 10 cm of first-order laterals, proximal half = basal 10 cm of first-order laterals, and second order = tips (terminal 10 cm) of second-order laterals.

^yMean separation within columns for a rooting measurement by least significant difference at $P \leq 0.05$.

Table 5. Effect of IBA concentration on total root length and root dry weight of 'Yoshino' cryptomeria stem cuttings taken at three growth stages.

IBA concn (mg·liter ⁻¹)	Growth stage		
	Semi-hardwood	Hardwood	Pre-budbreak
<i>Total root length (cm)</i>			
0	7.5	18.4	14.8
3000	7.7	23.7	18.0
6000	10.0	23.9	25.2
9000	11.1	32.4	25.1
Linear	*	*	NS
Quadratic	NS	NS	NS
IBA vs. control ²	NS	**	**
<i>Root dry wt (mg)</i>			
0	14.4	33.9	41.8
3000	13.8	37.6	54.9
6000	15.8	34.1	61.4
9000	18.0	50.4	60.6
Linear	NS	NS	NS
Quadratic	NS	NS	NS
IBA vs. control ²	NS	*	***

²Linear contrast.

NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

In contrast, percent rooting increased linearly with increasing IBA concentrations for pre-budbreak cuttings, suggesting that within the range of concentrations tested, 9000 mg IBA/liter was required to maximize rooting.

The effect of IBA on percent rooting of semi-hardwood cuttings depended on branch order. There was a linear increase in percent rooting with increasing IBA concentrations for tips of first-order laterals (Table 2). However, there was a quadratic response in percent rooting with a maximum at 3000 mg IBA/liter for the proximal halves of first-order laterals. Second-order laterals did not root regardless of auxin treatment. This result illustrates that the recommended IBA concentration should be based on growth stage and branch order. Henry et al. (1992) also reported that efficacy of applied auxin varied with time of year that eastern red cedar stem cuttings were collected.

Total root length and root area responded similarly to branch order and IBA treatment at all growth stages; therefore, only total root length data are presented. At all growth stages, total root length was generally greatest and about equal for tips of first-order laterals and

proximal halves of first-order laterals, followed by distal halves of first-order laterals and tips of second-order laterals (Table 4). Working with Norway spruce [*Picea abies* (L.) Karst.], Bogdanov (1984) reported similar results. Total root length within each branch order was similar at all growth stages, except for semi-hardwood cuttings, where overall total root length was reduced. Root dry weight responded similarly to total root length (Table 4). In contrast, McGuire (1987), working with two yew cultivars (*Taxus ×media* Rehd. 'Nigra' and 'Densiformis'), reported roots were longer for hardwood cuttings taken during late fall than for hardwood cuttings taken in early spring.

Total root length increased linearly with increasing IBA concentration for semi-hardwood and hardwood cuttings (Table 5). For pre-budbreak cuttings, IBA increased total root length compared to the nontreated cuttings. Similar to percent rooting, optimum IBA concentration for maximum total root length varied with growth stage. Total root lengths of semi-hardwood and hardwood cuttings were maximized at 9000 mg IBA/liter. Root dry weights of hardwood and pre-budbreak cuttings were increased by IBA compared to nontreated cuttings, suggesting that 3000 mg IBA/liter would be adequate to increase root dry weight. In general, total root length was maximized at a higher IBA concentration than root dry weight. Root length more accurately reflects the potential volume of soil that is accessible to a tree (Russell, 1977). Thus, root length may be a better indicator of future plant performance than root dry weight. Consequently, the higher IBA concentration may produce a higher quality cutting.

For softwood, semi-hardwood, and pre-budbreak cuttings, tips of and proximal halves of first-order laterals had the most roots, followed by distal halves of first-order laterals and tips of second-order laterals (Tables 6 and 7). These results agree with previous reports for other conifers regarding the influence of branch order on root count (Black, 1972; Bogdanov, 1984). For hardwood cuttings, the response varied with IBA concentration (Table 8). At 3000 and 9000 mg IBA/liter, tips of and proximal halves of first-order laterals had the most roots. At 6000 mg IBA/liter, tips of first-order laterals had more roots than the other branch orders. Tips of second-order laterals had the lowest root counts at all growth stages, but the data were not always significantly different from the distal halves.

Root count increased linearly with increasing IBA concentration for semi-hardwood cuttings (Table 6). Increased root counts with increasing auxin concentration has been demonstrated for many species (Bogdanov, 1984; Henry et al., 1992; Still and Zanon, 1991). The effect of IBA on root count of pre-budbreak and hardwood cuttings depended on branch order (Tables 7 and 8). Except for tips of second-order laterals, where IBA had no effect, there was a linear increase in root count with increasing IBA concentrations for all branch orders.

Table 6. Effect of branch order and IBA concentration on root count of 'Yoshino' cryptomeria stem cuttings taken at two growth stages.

Branch order ²	Growth stage	
	Softwood	Semi-hardwood
First order (terminal 20 cm)		
Entire	4.5 a ^y	3.9 a
Distal half	2.4 b	2.1 b
Proximal half	4.6 a	4.4 a
Second order	1.6 c	0.0 c
IBA concn (mg·liter ⁻¹)		
0	---	2.1
3000	---	2.6
6000	---	4.2
9000	---	5.2
Linear	---	***
Quadratic	---	NS
IBA vs. control ^x	---	***

²Entire = tips (terminal 20 cm) of first-order laterals, distal half = terminal 10 cm of first-order laterals, proximal half = basal 10 cm of first-order laterals, and second order = tips (terminal 10 cm) of second-order laterals.

^yMean separation within columns by least significant difference at $P \leq 0.05$.

^xLinear contrast.

ns, ***, NS nonsignificant or significant at $P \leq 0.001$, respectively.

Our results demonstrate that 'Yoshino' cryptomeria stem cuttings can be rooted at all growth stages; however, the branch order from which cuttings are prepared is critical in achieving high rooting percentages. Rooting, ranging from 75% to 78% for softwood cuttings and 72% to 87% for hardwood cuttings, can be realized by using the tips of and the proximal halves of first-order laterals containing lignified, hardened wood. Although distal halves of first-order laterals had lower rooting percentages, the fact that a certain percentage rooted illustrates that more rooted cuttings can be obtained by using terminal, 20-cm-long, first-order lateral cuttings, dividing them in half, and rooting both halves than by using intact, 20-cm-long cuttings. The resulting distal halves would probably need to be handled differently after rooting, because root quality would be lower. Optimal IBA concentration varies with branch order, growth stage, and rooting measurements. IBA increased all measurements of rooting for all growth stages except with softwood cuttings. In general, within the concentrations tested, 3000 mg IBA/liter was adequate for maximizing percent rooting and root dry weight; however, 9000 mg·liter⁻¹ maximized root length and

root count. Unlike many coniferous species, Japanese cedar stem cuttings exhibit no plagiotropic growth following rooting. Rooted cuttings from lateral branches grow orthotropically.

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Table 7. Effect of IBA concentration by branch order on root count of pre-budbreak 'Yoshino' cryptomeria stem cuttings.

IBA concn (mg·liter ⁻¹)	Branch order ²			
	First order (terminal 20 cm)			Second order
	Entire	Distal half	Proximal half	
0	4.2 a ^y	1.3 b	3.3 a	1.0 b
3000	7.1 a	2.6 b	5.6 a	1.5 b
6000	12.6 a	2.1 b	11.3 a	1.8 b
9000	13.2 a	2.7 b	10.6 a	1.4 b
Linear	***	*	***	NS
Quadratic	NS	NS	NS	NS
IBA vs. control ^x	**	**	**	NS

²Entire = tips (terminal 20 cm) of first-order laterals, distal half = terminal 10 cm of first-order laterals, proximal half = basal 10 cm of first-order laterals, and second order = tips (terminal 10 cm) of second-order laterals.

^yMean separation within rows for an IBA concentration by least significant difference at $P \leq 0.05$.

^xLinear contrast.

ns, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 8. Effect of IBA concentration by branch order on root count of hardwood 'Yoshino' cryptomeria stem cuttings.

IBA concn (mg·liter ⁻¹)	Branch order ²			
	First order (terminal 20 cm)			Second order
	Entire	Distal half	Proximal half	
0	4.2 a ^y	1.7 bc	3.0 ab	1.0 c
3000	6.4 a	2.9 b	8.3 a	1.0 b
6000	10.1 a	3.5 bc	5.7 b	1.4 c
9000	12.2 a	3.8 b	9.8 a	2.3 b
Linear	***	**	**	NS
Quadratic	NS	NS	NS	NS
IBA vs. control ^x	**	NS	**	NS

²Entire = tips (terminal 20 cm) of first-order laterals, distal half = terminal 10 cm of first-order laterals, proximal half = basal 10 cm of first-order laterals, and second order = tips (terminal 10 cm) of second-order laterals.

^yMean separation within rows for an IBA concentration by least significant difference at $P \leq 0.05$.

^xLinear contrast.

ns, **, *** Nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively.