

trees did not produce sufficient branching for the development of an adequate fruit bearing surface. The more vigorous individuals could be considered suitable for conventional dense planting.

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Cold Hardiness of Flower Buds from Selected Highbush Blueberry Cultivars (*Vaccinium australe* Small)¹

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Abstract. Flower bud hardiness of seven commercial highbush blueberry cultivars (*Vaccinium australe* Small) was determined from fall to spring for two consecutive years. Hardiness was expressed as T₅₀, estimated by Spearman-Kärber equations. The T₅₀ values for cultivars showed good agreement with spring field survival. Distal buds were less hardy than proximal buds on the same twig. The Average Exotherm (AET) methods were more variable than the T₅₀ method for determining flower bud hardiness.

Cold hardiness of highbush blueberry species (*V. australe* Small and *V. corymbosum* L.) has been of interest to workers since Coville's studies in 1910 (8). Theoretically, cold injury can occur during any season of the year. Except for rare summer freezes, however, injury has been observed only during the dormant season. The period of most frequent injury has varied with geographic location. Injury was most frequently observed in the fall in Minnesota (4), during the winter in New York and Washington (5, 13), and in the spring in Michigan and Massachusetts (1, 17, 18).

There is a need to evaluate cultivar differences in field hardiness so that more effective planting suggestions and breeding choices might be made. The results of controlled cooling studies to differentiate wood or flower bud hardiness among cultivars have varied. Work in Vermont on wood hardiness indicated that 'Collins', a cultivar not known for exceptional hardiness (9, 10) was harder than 'Jersey' and 'Bluecrop' (7). This conclusion was based on electrolyte leakage which was expressed as "index of injury". The relationship of "index of injury" to tissue browning or growth of the wood subsequent to stress was not reported. This relationship has been shown to be important (27) in determining the value of such a quantitative viability test. Minnesota workers evaluated differential thermal analysis as a means of determining wood hardiness among cultivars and found it unreliable, but good discrimination among cultivars was possible using tissue browning (23). In Washington electrical impedance was used to relate hardiness of unfrozen wood with the T₂₅ of wood and flower buds in controlled cooling studies (13). There was good

agreement between impedance and hardiness (T₂₅) from December through February but not in the fall or spring. To date no controlled cold stress research has been done on blueberries in Michigan, the leading producer of highbush blueberries (25).

In breeding for hardiness via interspecific crosses hardy germ plasm from other species, such as the Ashworth blueberry (*V. corymbosum* L.) (10, 11), the bogwhortle berry (*V. uliginosum* L.) (24), and the northern lowbush (*V. lamarckii* Camp) (19) have been used. A report (14) on the present status of breeding for hardiness in blueberry lists the main obstacle to progress as the inability to identify hardy germ plasm without "test" winters. In order to successfully screen for hardy material several questions must be answered: 1) what is (are) the critical tissue(s), 2) what is the best way to evaluate its (their) hardiness, and 3) what is the critical season of hardiness for the tissue(s) (28)? Our previous field observations indicated that flower buds were injured at higher field temp than wood.³ Therefore, we have attempted to answer these questions by studying the acclimation and deacclimation patterns of the flower buds of highbush blueberry for several commercial cultivars in Michigan.

Materials and Methods

Sampling and cooling techniques. In the 1972-73 season, three 3-node terminal stem pieces were used as the experimental unit. Enough material was collected, and stored at 0-2°C for no more than 12 hr to provide a field control and 8 to 10 cold stress treatments per rep per cultivar. Stem pieces were taped to aluminum foil strips and rolled into a bundle. A 26 gauge copper-constantan thermocouple was attached with tape to one stem piece in the center of the bundle. This foil roll contained all cultivars. Four such foil rolls (4 replicates) were placed in each vacuum flask. The thermocouple was connected to a potentiometer with a pen recorder which monitored temp decline in the foil roll. The 8 to 10 flasks were placed in a Revco "Ultralow" freezer set at -60°, which produced a cooling rate of 2-5°/hr. As the stems cooled, flasks were removed at 2.5° intervals within a predetermined temp stress range.

This procedure was modified for 1973-74 and two 2-node

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³Bittenbender, H. C. 1972. Unpublished winter injury cultivar ratings, 1969-1973, Michigan Blueberry Growers Association, Grand Junction, MI 49056.

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Table 1. T₅₀ values for 8 highbush blueberry cultivars at selected dates during 1972-73.

Location Cultivar	T ₅₀ (°C)							
	Sept. 15, 1972	Oct. 18, 1972	Oct. 28, 1972	Nov. 25, 1972	Dec. 17, 1972	Jan. 13, 1973	March 19, 1973	April 14, 1973
<i>Grand Junction</i>								
Jersey	-3.75	-7.29	-12.22	-24.72	-24.44	-24.37	-11.25	-7.50
Rubel	-4.31	-6.46	-12.22	-24.58	-24.31	-24.51	-9.58	-7.85
Northland	-4.58	-6.87	-11.32	-23.19	-22.92	-23.10	-9.03	-7.22
Berkley	-3.94	-6.32	-11.53	-21.32	-20.49	-19.93	-9.03	-4.51
Coville	-5.06	-7.01	-12.57	-21.18	-21.53	-21.04	-8.82	-5.21
Earliblue		-6.94	-12.43	-19.10	-22.92	-22.01	-8.23	-5.42
Elliot		-6.32	-12.36	-23.10	-23.54	-24.03	-10.63	-7.15
HSD 5% ^z	NS ^y	NS	NS	4.68	2.30	2.24	2.02	2.34
<i>South Haven</i>								
Bluehaven		-6.67	-10.00	-20.83	-21.81	-21.94	-8.61	-3.75
Jersey		-6.39	-11.32	-23.40	-24.24	-24.37	-9.58	-7.42
HSD 5%		NS	NS	1.23	NS	1.68	NS	2.80
<i>Both locations</i>								
Jersey, GJ		-7.29	-12.22	-24.72	-24.44	-24.37	-11.25	-7.50
Jersey, SH		-6.39	-11.32	-23.40	-24.24	-24.37	-9.58	-7.92

^zHSD for T₅₀'s within column.^yNo significant difference.

terminal stem pieces composed the experimental unit. The stem pieces were placed in stacked aluminum foil weighing pans rather than being rolled up in aluminum foil, and the flasks containing them were removed at 1°C intervals. A randomized complete block design was used to remove variation due to pan position within the flask.

After removal from the freezer, the tissue was allowed to thaw for 12 hr at ambient temp while still in the flasks. The foil rolls or pans were subsequently transferred to a 100% relative humidity chamber for 2 days after which the buds were evaluated visually with a binocular dissecting microscope for browning of the ovary, peduncle, and pith tissues at the base of the peduncle.

The hardiness of the buds stressed in this manner was reported as the T₅₀ using the Spearman-Kärber (S-K) equations. The "S" statistic which correlates with the slope of the temp-survival curve through the T₅₀ point, was also determined from the S-K equations (2).

Moisture content was measured (g H₂O/g dwt) based on 8 buds per rep. Buds were dried to constant weight at 65°C.

Data were analyzed by single factor analysis of variance, the means tested with Tukey's w procedure and simple correlation coefficients determined by the product moment method (26).

Cultivar study. Cultivars, selected to provide a range of hardiness based on known field performance in Michigan, included 'Jersey', 'Rubel', 'Coville', 'Berkeley', 'Earliblue', 'Elliot', 'Northland', and 'Bluehaven'. The hardiness of 'Jersey' was studied at two locations. 'Jersey' and 'Bluehaven' were grown at the first, a warm site which was located approximately 3 km east of Lake Michigan near South Haven (SH), Michigan. All cultivars were present at the second, colder site, which was a selection test planting at Grand Junction (GJ), Michigan, 16 km east of Lake Michigan and SH. 'Bluehaven' was not tested at GJ as it was severely winter injured each year resulting in insufficient sampling material.

Hardiness determinations (T₅₀) were initiated both years in

Table 2. T₅₀ values for 7 highbush blueberry cultivars at selected dates during 1973-74.

Location Cultivar	T ₅₀ (°C)									
	Sept. 23, 1973	Oct. 13, 1973	Nov. 3, 1973	Nov. 23, 1973	Dec. 19, 1973	Jan. 24, 1974	March 2, 1974	April 9, 1974	April 23, 1974	May 4, 1974
<i>Grand Junction</i>										
Jersey	-8.25	-7.85	-20.10	-25.25	-27.25	-24.94	-22.06	-10.75	-4.81	-4.94
Rubel	-7.64	-7.63	-19.31	-24.69	-25.94	-22.92	-20.81	-10.63	-5.63	-5.50
Northland	-7.99	-9.44	-20.66	-24.69	-25.50	-23.37	-22.06	-12.25	-4.88	-5.06
Berkley	-6.87	-8.06	-18.00	-24.10	-25.25	-23.06	-20.06	-11.50	-4.44	-5.00
Earliblue	-10.42	-12.31	-19.75	-24.56	-24.81	-23.00	-19.38	-8.75	-5.13	-5.31
Elliot	-8.47	-8.06	-20.44	-25.25	-25.94	-22.81	-21.62	-11.50	-5.75	-5.81
HSD .05 ^z	1.78	1.62	1.28	NS ^y	0.84	1.52	0.69	2.28	1.29	NS
<i>South Haven</i>										
Bluehaven	-8.13	-9.75	-18.44	-23.87	-25.81	-23.44	-18.00	-11.44	-4.63	-4.50
Jersey	-7.42	-10.50	-19.87	-25.25	-27.31	-25.06	-22.00	-11.00	-5.13	-4.63
HSD .05	NS	NS	NS	0.95	0.48	0.15	1.06	NS	NS	NS
<i>Both Locations</i>										
Jersey, GJ	-8.26	-7.75	-20.10	-25.25	-27.25	-24.94	-22.06	-10.75	-4.81	-4.94
Jersey, SH	-7.42	-10.50	-19.57	-25.25	-27.31	-25.06	-22.00	-11.00	-5.13	-4.63

^zHSD for T₅₀'s within column.^yNo significant difference.

September, and continued into mid-April 1973 and early May 1974. The field design was a completely randomized block with 4 bushes per rep and 4 reps. Freezer data were analyzed in 1972-73 as a completely randomized design and in 1973-74 as a randomized complete block because of cooling technique modifications. T₅₀ values for cultivars were compared at each sampling date by an HSD value. Daily air temp were recorded at both locations from September-May each year.

Effect of bud position within the bush. 'Jersey', 'Rancocas', and 'Berkeley' at GJ were studied the first year and 'Jersey' and 'Berkeley' the second year, to see if bud hardiness varied as a function of bud position within the bush. These cultivars were chosen because they represented a range of hardiness. The positions compared were: the top position of the bush above 1.9m from the ground and the north and south sides of the bush between 1.9 and 1m from the ground. The bushes averaged 2.5m in height.

Effect of bud position within a twig. Field survival of buds based on flowering of four cultivars, 'Jersey', 'Rubel', 'Berkeley', and 'Earliblue' was observed at East Lansing, 190 km east of Lake Michigan. Ten twigs were examined per cultivar per block on May 25, 1973. Buds were scored as number of live blossoms per node. A randomized complete block design with four blocks was used.

Exotherm studies. Average exotherm temp (AET) for excised florets was determined in January and April, 1974. A 3 mil copper-constantan thermocouple was inserted into the ovary of a floret, the floret was placed in a small vial to prevent dehydration, and cooled at 100–150°C/hr, as Graham⁴ showed that for florets AET was independent of cooling rate. The temp of the ovary was recorded with a potentiometer and pen recorder. The temp at which the heat of fusion was measured, was recorded as the exotherm temp and the average of three florets from a bud used as the AET. There was only one exotherm in the tissue evaluated. The bud material was taken from GJ and 2-node terminal stem pieces were used. The design was randomized complete block with 4 or 8 blocks. Means were tested using an HSD .05 value.

Results and Discussion

Observations of field controls in the spring of 1973 in a cultivar block at East Lansing indicated that the occurrence of peduncle browning had no negative effect on flower survival so long as ovary tissue survived. Therefore all T₅₀ values were based on ovary browning. In 1972-73 (Table 1) cultivar T₅₀'s did not differ significantly until November 25, at which time all cultivars except 'Earliblue' were at maximum hardiness. Dehardening commenced between January 13th and March 9th. The last hardiness determination was made April 14 and occurred 3 days after the only freeze of the dormant season causing field injury to buds. On April 14, 'Jersey', 'Rubel', 'Northland' and 'Elliot' were the most hardy and 'Earliblue', 'Coville', 'Berkeley', and 'Bluehaven' the least hardy. Location did not appear to influence 'Jersey' hardiness on any date.

'Earliblue', 'Northland', and 'Bluehaven' hardened earlier than other cultivars in the fall of 1973 (Table 2). Maximum hardiness was attained by all cultivars by end of Nov. as in the previous year, and spring dehardening again began in early March. In contrast with 1973, 'Berkeley' was as hardy as other cultivars in early April 1973 (Table 2), while in late April 'Berkeley' was the least hardy. There appeared to be a location difference on October 13th, 'Jersey' at SH being hardier.

Correlating cultivar hardiness between years showed significant correlation for winter and spring, $r = 0.74^*$ and 0.80^* respectively; however, the correlation for fall was not significant. This appears to be the result of the significant, early hardiness of 'Northland' and 'Earliblue' in the fall of 1973 (Table 1, 2). This suggests a cultivar x year interaction.

T₅₀ values correlated with percent field survival, $r = 0.97$,

Table 3. T₅₀ of highbush blueberry flower buds of different cultivars at nearest date vs. field survival after frosts at Grand Junction (GJ) and South Haven (SH), Michigan in the spring of 1973 and 1974. The T₅₀'s are laboratory hardiness assessments on April 14, 1973 and May 4, 1974. The field survival is based on the % of buds surviving -10°C on April 11, 1973 and -6°C on May 6, 1974.

Cultivar (Location)	1973		1974	
	T ₅₀	Field survival (%)	T ₅₀	Field survival (%)
Jersey (GJ)	-7.5	86	-4.9	99
Rubel (GJ)	-7.8	100	-5.5	88
Northland (GJ)	-7.2	84	-5.1	94
Berkeley (GJ)	-4.5	44	-5.0	92
Coville (GJ)	-5.2	53	-	-
Earliblue (GJ)	-5.4	53	-5.3	78
Elliot (GJ)	-7.2	80	-5.8	98
Bluehaven (SH)	-7.2	80	-5.8	98 ^Y
Jersey (SH)	-7.9	86	-4.6	99 ^Y
r ^Z	0.97**		-0.33 (ns)	

^ZCorrelation coefficient, r , for T₅₀ vs. % field survival, ** significant at 1%, n.s. = not significant.

^YExposed to -4°C rather than -6°C.

after the April 11th frost in 1973, the only damaging freeze in the 1972-73 dormant season (Table 3). However, in 1974 the field survival after the May 6th freeze did not correlate with the T₅₀ values on May 4. The lack of correlation between T₅₀ and field survival after May 6 may be due to several factors. The buds were in the pink stage and therefore quite large and the florets were spread apart. The freezing technique, in which the thermocouples for recording temp were attached to the stem and not inserted into the florets, may induce an error in measuring the actual temp of the florets at this stage of development. 'Earliblue', with the lowest field survival, was the most advanced, i.e. greatest floret development, and consequently had the greatest potential for error in hardiness determination. Perhaps the other cultivars rehardened in the two days between the hardiness determination and the -6°C frost on May 6, as frosts did occur during this period, but 'Earliblue' since it was so advanced could not reharden. Lastly, there was no significant difference among T₅₀'s on May 4 (Table 2), therefore the differences in T₅₀'s used in the correlation may be due to error alone.

Bud position within the bush. In the late fall of 1972 the buds on the north and south sides of the bushes were hardier than buds in the upper portion (Table 4). No difference was seen during the late winter or spring of that year or in any

Table 4. Effect of bud position on the T₅₀ of highbush blueberry flower buds.

Site on bush Cultivars	T ₅₀ (°C) of flowers			
	Sampling dates			
	Oct. 14, 1972	Nov. 11, 1972	Dec. 11, 1972	April 12, 1973
Berkley N side	-6.87	-17.78	-23.54	-6.25
top	-6.74	-17.36	-20.69	-6.04
S side	-7.36	-17.08	-21.81	-5.97
HSD 5%	NS ^Z	NS	2.50	NS
Rancocas N side	-7.22	-18.33	-26.04	-6.46
top	-6.60	-16.94	-24.58	-7.99
S side	-6.53	-18.19	-25.28	-6.74
HSD 5%	NS	1.18	NS	NS
Jersey N side	-7.92	-19.46	-26.74	-9.58
top	-7.64	-17.01	-24.93	-9.86
S side	-8.47	-18.75	-26.46	-9.79
HSD 5%	NS	2.35	1.03	NS

^ZNo significant difference.

season during 1973-74. Even though these differences in hardiness did not occur both years, it should be noted as a potential source of sampling error.

In a preceding paper (2) we showed that the "S" statistic of the S-K equations, a variance of the % survival about the T50 point, could be used to describe the slope of the temp-survival (T-S) curve of a cultivar. At that time we suggested that a cultivar with a larger S value (flatter slope) would be hardier at temp below its T50, than another cultivar with an equal T50 but steeper slope. No significant differences in the values of S were found among cultivars during any season. The S statistic was very sensitive to slight variation in cooling technique, which might account for the lack of difference among cultivar S values. A significant difference was shown for S between seasons in 1972-73 but not in 1973-74 (Table 5). The change in the T-S curve from steep in fall to flat in spring is in agreement with the change in the T-S curve between seasons reported for 'Elberta' peach flower buds (22).

Table 5. Change in S statistic among seasons between years using the average of 8 highbush blueberry cultivars.

Season	Changes in S statistic	
	1972-1973	1973-1974
Fall	0.156	0.241
Winter	0.238	0.191
Spring	0.258	0.228
HSD 5%	0.062	NS ^z

^zNo significant difference.

Bud position within a twig. Comparison of bud position on a stem piece indicated that distal buds are less hardy than proximal buds (Table 6). Whether the lesser hardiness of distal buds reflects the status of morphological development or some other factor such as moisture content is not clear. From these data it appears that cultivars with many flower buds per lateral, like 'Jersey', have an advantage over those with few. A consideration of this factor would, of course, be crucial in assessing field hardiness of a cultivar since a cultivar could potentially be hardier based on T50 evaluation and yet less productive because of differences in number and location of flower buds.

Table 6. Effect of bud position on survival of highbush blueberry flowers, May 25, 1973 at East Lansing, Michigan.

Bud position ^z	Mean no. live blossoms per node			
	Jersey	Rubel	Berkley	Earlblue
1	2.6	0.0	4.6	1.0
2	4.0	2.7	4.7	2.7
3	6.0	6.7	6.0	4.1
4	7.4	7.2	7.1	5.7
5	9.1	9.6	8.0	7.4

HSD 5% (within a column) = 2.9

^z1 = most distal, 5 = most proximal on five node stem.

Exotherm evaluation. The formation of ice in flower primordia or in blossoms has been shown to be lethal (6, 12, 15, 16). Exotherm analysis was investigated to determine if it were superior to T50 analysis. The results of controlled cooling experiments on Jan. 24 and April 24 (Table 7) indicated that for the same no. of reps the T50 analysis provided a smaller HSD value than did AET. There was no correlation between methods on either date ($r = .28$ and $.22$, respectively). Correlation between methods and moisture content (% H₂O dwt basis) were only significant for AET in Jan. ($r = .97$) and not in April. This suggests that the AET values reflect

Table 7. T50's, AET's, and moisture content for several cultivars on 2 dates in 1974.

Cultivar	T50(°C)			Moisture content of bud (g H ₂ O/g dwt)
	Method of Hardiness determination			
	T50°C	AET °C	AET °C	
<i>Jan. 24, 1974</i>				
Jersey	-24.92	-21.68	-23.36	125.5
Rubel	-22.62	-22.15	-23.29	122.4
Berkley	-23.05	-17.70	-18.78	135.0
Earlblue	-22.98	-19.82	-20.31	132.7
HSD 5%	0.89	3.32	2.06	6.4
No. of replicates	4	4	8	4
<i>April 24, 1974</i>				
Jersey	-4.81	-14.92		254.6
Rubel	-5.63	-13.67		232.6
Northland	-4.88	-13.22		265.8
Berkley	-4.44	-14.85		298.8
Earlblue	-5.13	-10.58		294.5
Elliot	-5.75	-14.02		239.4
HSD 5%	1.29	NS		17.3
No. of replicates (N)	4	4		4

differences in moisture content rather than hardiness per se. As reported elsewhere, there is a close relationship between moisture content and hardiness (21). Other factors such as temp operate independently of moisture status (3) and could lead to the greater variability of the AET method. These problems must be resolved before AET can be used with confidence for determining the hardiness of blueberry flower buds. Exotherm analysis is a rapid method and requires small amounts of material, 4 buds vs. 96 buds required using our techniques. Of course, considering the effect bud position on the stem and within the bush means that proper sampling would be very critical when using such small amounts of material.

Returning to the three questions raised earlier that must be answered to screen successfully for hardiness, we conclude that: 1) the ovaries are the critical cold sensitive tissue, 2) T50 analysis applied with a slow cooling procedure successfully discriminates between hardy and less hardy material, and 3) the critical hardiness season in Michigan is spring, April to May, based on observations from the literature and this study. When the hardiness relationship between flower buds of young plants and mature plants is known, a screening method using the T50 of flower buds from seedlings can be developed. Ideally, seedlings from crosses between hardy parents based on Stushnoff's hardiness criteria (28) would be cooled in April or May to two temp, above and below the T50 of some hardy standard such as 'Jersey' (20). The most hardy 5-10% would be saved for further selection based on other criteria. The acclimation-deacclimation pattern could then be studied using T50 analyses for the best selections.

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Aluminum Toxicity Symptoms in Peach Seedlings¹

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Abstract. ‘Elberta’ (*Prunus persica* (L.) Batsch) peach seedlings were grown in nutrient solutions for 27 days with aluminum concentrations of 0, 222, 666 and 2000 μM ; the 2000 μM concentration induced Al toxicity symptoms in leaves and severely restricted root growth. The early stage of Al toxicity was characterized by marginal chlorosis that later developed into necrotic areas that extended along the veins toward the midrib. Advanced stages of toxicity were characterized by collapse of the midrib, terminal dieback and defoliation of the seedlings which are typical symptoms of calcium deficiency in peaches. At high Al concentrations roots died back and new roots developed as irregularly shaped cylinders with constrictions and enlargements at the root apex.

Aluminum toxicity in annuals has been reported to induce a P deficiency (7, 4) and to reduce the uptake and utilization of Ca (8, 16). Evidently Al binds P on the root surface, cell wall, or in the free space of plant roots (15, 2, 3), which means that less P is available for metabolic activities within the cells. Aluminum at concn less than 10^{-4}N reduced both the rapid initial and linear absorption phase of Ca. The reduction in Ca uptake was accompanied by a reduction in Ca transport (9).

Aluminum toxicity has also severely restricted root growth in peach seedlings (10). In this study we determined the concn of Al in solution required to induce Al toxicity symptoms in ‘Elberta’ peach seedlings and the nature of the symptoms over a range of Al concn.

Materials and Methods

‘Elberta’ peach seedlings were germinated and grown to a height of 8 to 12 cm in the greenhouse in sand that had been washed with distilled water. They were transplanted into 4-liter plastic pots (2 plants/pot) containing the following nutrient

solution: 0.25 mM KH_2PO_4 , 0.5 mM $\text{Ca}(\text{NO}_3)_2$, 0.5 mM KNO_3 , 0.5 mM MgSO_4 , 0.5 mM NH_4NO_3 , 75 μM Fe DTPA (diethylene triaminepentaacetic acid), 46 μM B, 9 μM Mn, 0.8 μM Zn, 0.3 μM Cu, and 0.05 μM Mo.

Solution pH was measured daily and maintained at pH 4.0 by adding either HCl or NaOH. The vigorously aerated solutions were changed at 7-day intervals before variable treatments were begun and monitored between changes to prevent depletion of nutrients. The experiment was conducted during Dec. and Jan. with natural and supplemental light to provide a 14-hr day.

Seedlings were grown for 28–30 days and 10 seedlings were harvested to estimate growth before application of treatments,

Table 1. The effects of Al concn on growth of ‘Elberta’ peach seedlings.

Al concn (μM)	Increase terminal length (mm)	Increase in laterals (no.)	Increase trunk area (mm^2)	Dry wt increase (g/2 plants)			
				Leaves	Stems	Roots	Total
0	2953a ^z	11a	28.29a	10.72a	6.79a	3.72ab	21.22a
222	2798ab	11a	22.32b	8.05bc	5.45ab	2.98bc	16.48abc
666	2449ab	7ab	22.93b	9.59ab	5.69ab	4.37a	19.65ab
2000	1670b	5b	17.37c	6.64c	3.66b	2.68c	12.99c

^zMean separation within column by Duncan’s multiple range test, 5% level.

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