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Floral Bud Cold Hardiness of Southern Highbush Blueberry (*Vaccinium corymbosum* L. interspecific hybrids) in Response to Late Season Fertilization

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Abstract

The objectives were to identify the effects of late season fertilization on southern highbush blueberry (SHB) dormant floral bud cold hardiness, flower timing, and plant nutrient uptake. Treatments included two fertilizers 10-10-10 (Super Rainbow) and 46-0-0 (urea) and two cultivars, 'Emerald' and 'Star', grown in pine bark media. There were four late season fertilization treatments: 1) August: 10-10-10 fertilization (28 g/container applied in August); 2) September: 10-10-10 fertilization (20 g and 10 g/container applied August and September, respectively); 3) October: 10-10-10 fertilization (10 g/container applied monthly; August-October); 4) Urea: 46-0-0 fertilization (3 g/container applied August and September). Dormant root and shoot tissue were analyzed for mineral nutrients. Floral bud tissue was freeze tolerance (FT) tested and floral bud stages were recorded from 24 Jan. to 14 Mar. The FT of 'Star' was unaffected by fertilization treatments. In contrast, 'Emerald' on 17 Jan. was hardiest in October treatment (at -12.0 °C) and on 8 Feb., the hardiest floral buds of 'Emerald' were in September treatment (at -13.5 °C). Regardless of fertilizer, the treatments were similar in N concentration with some variation observed between roots and shoots. In both cultivars, late fertilization increased root N concentration. All fertilizer treatments in 'Emerald' bloomed earlier than 'Star'. No significance was seen between treatments of bloom progression for 'Emerald'. Treatment October advanced bloom progression in 'Star'. This work demonstrates that urea and mid-October fertilization had no effect on floral bud cold hardiness and bloom timing was cultivar dependent.

Index Words: *Vaccinium corymbosum* interspecific hybrid, mineral nutrients, freeze tolerance, floral buds

Introduction

Blueberries (*Vaccinium* spp.) are grown in a wide range of climatic regions. Lowbush blueberries (*V. angustifolium*) are grown commercially in plant hardiness zones 4b (-31.7 to -28.9 °C) to 6a (-23.3 to 20.6 °C) in Maine. Highbush blueberries (*V. corymbosum*) grown commercially in Michigan are in plant hardiness zones 6a (-23.3 to -20.6 °C) to 6b (-20.6 to -17.8 °C), these zones are buffered climatically by Lake Michigan creating a lake-effect snow buffer. Southern highbush (SHB; *V. corymbosum* L. interspecific hybrids) are commercially grown in plant hardiness zones 8b (-9.4 to -6.7 °C) to 9a (-6.7 to -3.9 °C) in Georgia (USDA, 2017). Each species and cultivar of blueberry has chill requirements that are met within each plant hardiness zone. However, extreme low temperatures below the hardiness zone average and rapid drops in temperature may cause damage to floral buds and actively growing tissue.

Blueberry production in Georgia is in a sub-tropical climate, where blueberries are actively growing into late fall. Fertilization timing has been suggested to terminate from late August to early October to accommodate a 4 to 6 week interval before the first freezing event (Krewer and NeSmith, 2006). However, growers may apply fertilizer into mid-October to deplete on-farm stores or remedy nutritional deficiencies. Potentially, late season applications of N can stimulate growth resulting in increased sensitivity to freeze especially to floral buds.

Orchardists recognize that fertilization, especially N, is necessary to maintain plant health and increase yield. In Washington State, 'd'Anjou' pear (*Pyrus communis* L.) trees were fertilized with ammonium-nitrate (NH_4NO_3) at two rates (0.15 kg N $tree^{-1}$ or 0.45 kg n $tree^{-1}$) with two timings (late summer or late winter) over multiple years (Raese, 1997). The lower rate of N had lower vigor and growth than the late winter high rate application; however, yield was negatively affected by late winter low N rate application. Cold tolerance testing conducted on 1 to 2 year old shoots demonstrated that low rates of N were significantly hardier to early fall freezes; however, by late fall and into winter the cold tolerance was similar between the treatments (Raese, 1997). This work suggests low N rates negatively impact 'd'Anjou' pear and cold hardiness was not affected mid-winter regardless of timing or rate.

Further, cold tolerance testing on greenhouse grown bilberries (*V. myrtillus* L.) with N applications of 6.5 mmol m^{-2} ammonium nitrate with calcium hydroxide ($NH_4NO_3 + Ca(OH)_2$) and trace gas fumigation (CO_2 and O_3) showed N applications had increased cold hardiness, whereas the fumigation did not affect cold hardiness (Taulavuori et al., 1997). In another study, N was applied at 200 kg N $ha^{-1}yr^{-1}$ in an extended season to lingonberry (*V. vitis-idaea* L.) resulting in accelerated frost hardening (Taulavuori et al., 2001). From these studies, Taulavuori et al. (1997 and 2001) suggests that late season N fertilization accelerates frost-hardening in these ericaceous species.

Peach (*Prunus persica*), western red cedar (*Thuja plicata*), Douglas-fir (*Pseudotsuga menziesii*), Aleppo pine (*Pinus halepensis*), and Norway spruce (*Picea abies*) did not show sensitivity to cold from late season applications of N (Edgerton and Harris, 1950; Hawkins et al., 1995, Hellergren, 1981; Puertolas et al., 2005; Wiemken et al., 1996). However, newly rooted 'Bloodgood' Japanese maple (*Acer palmatum*) was sensitive to freezing injury with N applications (Stimart et al., 1995). The treated maple plants did not have any shoot growth when compared to unfertilized plants (Stimart et al., 1995). From the studies presented, there appears to be a species specific affect from N application in relation to cold tolerance. It should be noted that the above mentioned experiments were conducted in temperate climate, and the response to cold of blueberry grown in subtropical climate with late season N applications may not be predictably compared due to mild winter temperatures.

Blueberries thrive in acidic soils (pH 4.0-5.5) and require ammoniacal sources of nitrogen (Hanson, 2006). Plant growth and yield increases with increasing nitrogen in blueberry plants (Bryla and Machado, 2001; Finn and Warmund, 1997; Hanson and Retameles, 1992). Nitrogen fertilizer can be applied granularly or in a liquid solution with no significant impact to production (Williamson and Miller, 2009) and increasing the rate of nitrogen fertilizer in SHB 'Misty' and 'Star' improved production (Williamson and Miller, 2009). In South Georgia, growers are using rates of $\geq$112 kg N ha^{-1} in a season for SHB and commonly fertilize into mid-October to achieve this rate. The objectives of this study were to identify the effects of late

season fertilization on SHB dormant floral bud cold hardiness, flower timing, and plant nutrient uptake.

Methods and Materials

Plant material and fertilization. One gallon potted 'Star' and 'Emerald' plants were donated by Cornelius Farms, Manor, GA in April 2016. The plants had been fertilized via overhead fertigation at a rate of 50 ppm N with each irrigation. The plants were transported from Manor, GA to the University of Georgia's Alapaha Blueberry Research Station (31°20'43.9" N, 83°14'27.1" W). The pots were placed on a gravel pad with automated overhead irrigation. The irrigation was set for 30 min in the morning (0500) every other day with adjustments relevant to rain (summer of 2016 was in drought conditions in South Georgia). All floral buds, flowers and fruit were removed and fertilization commenced 5 May in Alapaha. Two fertilizers were used: 10-10-10 (Super Rainbow, Agrium, Denver, CO) and 46-0-0 (urea, Brownlee Farm Center, Tifton, GA). The plants were fertilized at rates of 28 g of 10-10-10 for three of the treatments and the fourth treatment was fertilized with 6 g of urea, these rates were applied to their respective treatment on 5 May,6 Jun., 5 Jul., and after transplant on 22 Jul.

By July 2016, many of the pots were root bound and all the plants were transplanted into 3 gallon pots. During transplanting, the roots were loosened before being placed into the 3 gal pots and were planted no more than 2.5 cm below the original soil line of the 1 gal pots. The media used from Cornelius Farms was milled pine bark (0.6 to 9.5 mm particle size) and the transplant pine bark were water soaked chips at 1 cm – 3 cm in size.

Late season fertilization treatments. The plants were randomized into 4 treatments of 20 plants per cultivar on the nursery pad, making a total of 160 plants, 80 per cultivar. The four treatments were for end date of fertilization on 23 Aug. (August), 15 Sept. (September), 15 Oct. (October) and urea (Urea) ending on 15 Sept. In South Georgia, urea is commonly used as an ammoniacal source in blueberry production and was included to observe response in the experiments. On 23 Aug., treatments August, September, and October received an application of 10-10-10 at 28 g, 20 g, and 10 g, respectively, and 3 g of urea was applied to the Urea treatment. On 15 Sept., treatments September, October and Urea received an application of 10 g (10-10-10), 10 g (10-10-10), and 3 g (urea), respectively. On 15 Oct., treatment October received an application of 10 g (10-10-10). The September, October, and Urea treatments were divided to deliver a similar concentration of N across the treatments and to avoid excessive fertilization. In November the plants were transferred to the University of Georgia's Griffin Campus (33°15'59.68" N 84°17'03.18" W) for further analyses.

Nutrient analysis. In early February 2017, four plants per cultivar and treatment were removed from pots and cleaned of soil media before separating roots from shoot material. Root material was determined as actively growing blueberry below the soil line. Roots and shoots were separated and placed in their respective labeled paper bag and set in a Grieve 13-261-27A forced air drying oven (The Grieve Corporation, Round Lake, IL) at 60 °C for 72h. The samples were analyzed for nitrogen (N), phosphorous (P), potassium (K) , magnesium (Mg), calcium (Ca), sulfur (S), boron (B), zinc (Zn), manganese (Mn), iron (Fe) , and copper (Cu) (Waters Agricultural Laboratories, Inc., Camilla, GA), where the dried material was ground to pass a 20-mesh screen, the samples were reduced to ash in a muffle furnace, acid digested, and measured by inductive coupled plasma spectrophotometer (ICP) coupled to a Digiblock 3000 (SCP

Science, Baie D'Urfé, Quebec, Canada). Nitrogen was determined through combustion of plant tissue using a LECO FP-428 N analyzer (LECO, ST. Joseph, MI, U.S.).

Controlled temperature plant thermal analysis (TA). Fresh samples were collected from random plants within each cultivar/treatment on three dates during the winter of 2016-17: 7 Dec. (162 accumulated chill units, CU), 17 Jan. (438 CU), 8 Feb. (553 CU). Chill units were calculated 0° C to 7.2° C from the University of Georgia Weather Network, Griffin, GA (www.weather.uga.edu). Leaves were removed from all collected shoots. Shoots were trimmed to 5 cm. A minimum of 16 buds per treatment/cultivar were grouped and placed in moistened tissue in freezer bags and sealed. Sample sets were stored at -2 °C overnight and run on a temperature program which decreased at 4°C h^{-1} in an ESPEC EY-101 (Tabai Espec Corp., Osaka, Japan) chamber. Removal rates occurred at every 3 °C from -3°C to -21°C by temperature probe placed in the same type of sample bag within the chamber next to the samples. Samples were removed and stored at 4 °C for a week. Following storage, the floral buds were bisected and viewed under a dissecting scope for percentage of bud necrosis. The lethal temperature at 50% (LT_{50}) floral bud necrosis was determined on a probit scale (Proebsting Jr and Mills, 1966).

Bloom observation. From the remaining plants, sixteen randomly selected branches from each treatment (four branches per plant, four plants per treatment) were tagged and labeled for bloom observation. Buds were counted and individually checked biweekly for bloom progression. Bloom progression was recorded according to the Michigan State University blueberry growth stages table: stage 1= tight/dormant bud; stage 2= bud swell with visible green tip; stage 3= budbreak signified by separation of bud scales; stage 4 = tight cluster, individual flower apices are visible; stage 5= early pink bud, individual floral apices appear pink and separated; stage 6 = late pink bud, flowers have elongated but corollas are still closed; stage 7 = early bloom, less than 50% of the corollas have opened; stage 8 = full bloom, greater than 50% of the corollas have opened; stage 9 = petal fall, greater than 50% of the corolla tubes have fallen off the flower; stage 10 = early green fruit, small pea size green fruit have expanded; stage 11 = late green fruit, expansion slows and fruit appear light green; stage 12 = fruit coloring, oldest fruit in the cluster change color to blue/pink. (Michigan State University, 2016).

The weighted average bloom stage per shoot was determined by calculating the summation of all buds at a designated bud stage and dividing by the total number of buds. Weighted averages of shoots were compared between plants within a date.

Statistical Analysis. TA temperature inflection points (LT_{50}) were determined using a logistic curve in JMP Pro (SAS, Cary, NC) version 13; PROC GLM, SAS 9.4 (SAS, Cary, NC). Root and shoot weight, nutrient concentration, LT_{50}, and bloom progression means were separated by Tukey honestly significant difference (HSD) at $P \leq 0.05$ using SAS 9.4 (SAS, Cary, NC).

Results and Discussion

Freeze tolerance (FT) LT_{50} was not affected by fertilization timing for both cultivars at the December sampling (Table 1). 'Emerald' had a greater sensitivity to FT in the August treatment for January testing. For 'Emerald', August treatment was 33% and 24% less freeze tolerant then the October and Urea treatments, respectively. By February, the hardiest 'Emerald' floral buds were observed in September treated plants at -13.5 °C with the other treatments at an

average of -10.0 °C (Table 1). For ‘Star’, the hardiness was not significantly different between the treatments with an average LT_{50} of -14.1 °C and -12.2 °C for January and February testing, respectively.

The hardiness differences observed in ‘Emerald’ probably are due to natural variation more than being affected by fertilization timing. The samples for nutrient analyses were collected while the plants were dormant in early February 2017 and the concentrations of N were not significantly different when comparing shoots and roots of the September treatment to the other fertilizer timings (Table 2). ‘Emerald’ needs a minimum of 100 hours of chill to flower (Lyrene, 2008). By January 17, the plants had received over 400 chill hours (Table 2) and floral buds were showing scale separation or stage 3 (Michigan State University, 2016) by 24 Jan. (data not shown). Bud tissue swelling suggested the plant was no longer dormant, which resulted in greater variability in LT_{50} compared to December testing.

Vaccinium vitis-idaea L. and *V. myrtillus* have both been observed to accelerate frost hardening with extended late season fertilization (Taulavuori et al., 2001; Taulavuori et al., 1997). Raese et al. (1997) determined that moderate rates of N (0.45kg N $tree^{-1}$) applied to ‘d’Anjou’ pear in late winter did not affect the mid-winter cold tolerance compared to low rates of N (0.15kg N $tree^{-1}$) or late summer fertilizer applications. Our observations did not show accelerated hardening but did demonstrate mid-winter hardiness was not affected by fertilizer timing in ‘Star’. The fertilization treatments did not significantly reduce cold tolerance of ‘Star’ floral buds suggesting late fertilization will not reduce yield from freeze damage to tight or stage 1 floral buds. Throughout the bloom timing observation period for ‘Star’, October treated plants were significantly advanced in development when comparing September and August fertilized plants. By the 7^{th} week of study, August treated plants reached bud stages 5 to 6, tight cluster/early pink bud; September treated plants reached stage 7, late pink bud; Urea and October treated plants reached stage 8, early bloom (Figure 1). Bloom timing for ‘Emerald’ was not affected by fertilization treatments (data not shown).

Root and shoot dry weights were not affected by fertilizer timing (data not shown) and the average shoot to root ratio for ‘Emerald’ and ‘Star’ was 1.19 and 0.83, respectively. The root and shoot analysis suggests that the plants were growing at similar rates and there were cultivar differences in growth. Further, dry masses and nutrients in shoot or roots did not show that fertilizer application timing has any effect on ‘Star’. ‘Emerald’ N uptake from urea was slightly lower when comparing October and August fertilization in root and shoots, respectively. However, the lower uptake did not affect bloom timing, which suggests levels were sufficient in the urea treated plants. Interestingly, urea treated plants did not appear to be affected by the lack of P or K fertilization when comparing the other treatments (Table 1).

This work demonstrates that late season fertilization did not increase the sensitivity of the cultivars tested to freeze, although Emerald did change in FT over time. Floral buds had LT_{50}s lower than historical levels of cold as seen in zones 8b (-9.4 to -6.7 °C) to 9a (-6.7 to -3.9 °C) where the majority of SHB production is in Georgia. Bloom timing progressed at a faster rate in late season October fertilized ‘Star’ and had no effect on ‘Emerald’. Nutrient concentrations had some variation between root and shoots; however, root and shoot dry weights were not significant within cultivar. In conclusion, late season fertilization was not injurious to southern highbush blueberry ‘Emerald’ and Star’.

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Table 1. Macro (nitrogen; N, phosphorous; P, potassium; K, magnesium; Mg, Calcium; Ca, and sulfur; S) and micro (boron; B, zinc; Zn, manganese; Mn, iron; Fe, and copper; Cu) nutrients analyzed from either roots or shoots of 'Star' and 'Emerald' southern highbush blueberry from final fertilization treatments using 10-10-10 or urea fertilizer: treatments; August, September, October, and Urea.

Treatment	Sample	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
		%						ppm				
						'Star'						
August	Shoot	0.94 bcd[z]	0.10 ab	0.25 a	0.12	0.68 a	0.09	16 a	54 a	172 a	94	4 a
	Root	0.81 d	0.08 b	0.23 bc	0.16	0.37 bc	0.11	10 bc	34 bc	132 ab	102	5 a
September	Shoot	0.99 abc	0.10 ab	0.30 ab	0.12	0.61 a	0.09	17 a	47 ab	132 ab	66	4 a
	Root	0.84 cd	0.11 ab	0.24 bc	0.15	0.32 c	0.10	9 bc	32 bc	94 bc	59	5 a
October	Shoot	1.08 ab	0.11 ab	0.30 ab	0.12	0.58 ab	0.09	16 a	46 ab	125 b	49	3 b
	Root	0.97 abcd	0.11 ab	0.24 bc	0.15	0.37 bc	0.11	13 ab	35 bc	71 cd	61	5 a
Urea	Shoot	1.13 a	0.13 a	0.29 ab	0.12	0.57 abc	0.10	15 a	42 abc	131 b	44	3 b
	Root	1.01 abc	0.11 ab	0.19 c	0.15	0.37 bc	0.10	7 c	28 c	47 d	94	5 a
						'Emerald'						
August	Shoot	1.53 a	0.12 ab	0.79 a	0.12	0.71 a	0.16 a	18 ab	54 a	149 ab	44	3 ab
	Root	0.89 c	0.07 b	0.18 b	0.09	0.17 c	0.06 c	8 d	35 b	115 bcd	31	2 b
September	Shoot	1.15 abc	0.08 b	0.23 b	0.11	0.77 a	0.09 abc	20 a	58 a	146 ab	89	4 a
	Root	1.10 abc	0.10 b	0.19 b	0.12	0.24 bc	0.08 bc	9 d	29 c	106 cd	64	3 ab
October	Shoot	1.36 ab	0.10 b	0.29 b	0.09	0.53 ab	0.08 bc	13 bcd	56 a	141 abc	53	2 a
	Root	1.56 a	0.17 a	0.26 b	0.12	0.23 bc	0.14 ab	10 cd	24 bc	115 bcd	64	4 a
Urea	Shoot	1.02 bc	0.10 b	0.31 b	0.10	0.60 a	0.10 abc	14 abc	58 a	161 a	77	2 b
	Root	1.12 abc	0.12 ab	0.20 b	0.14	0.27 bc	0.11 abc	8 d	17 c	93 d	57	3 ab

[z] Means followed by a different letter within a column and within a cultivar are significantly different at $P \leq 0.05$ according to Tukey HSD.

Table 2. Controlled temperature plant thermal analysis (TA) for floral bud tissue freezing of 'Emerald' and 'Star' as affected by timing of late season application N fertilizer treatments August, September, October, and Urea. TA was observed at three representative dates, 7 Dec (162 accumulated chill units, CU), 17 Jan (438 CU), and 8 Feb (553 CU). Chill units calculated - 0 °C to 7.2 °C from University of Georgia Weather Network (www.weather.uga.edu), Griffin, GA station. Lethal temperatures were reported at 50% floral bud necrosis (LT_{50}).

	7 Dec. 2016	17 Jan. 2017	8 Feb. 2017
	162 CU	438 CU	553 CU
		'Emerald' LT_{50}	
Aug	-17.4	-8.0 a[z]	-9.9 a
Sept	-15.4	-8.9 ab	-13.5 b
Oct	-17.5	-12.0 c	-10.7 a
Urea	-17.3	-10.5 bc	-9.5 a
		'Star' LT_{50}	
Aug	-15.3	-16.6	-11.3
Sept	-19.1	-12.0	-13.8
Oct	-15.0	-15.6	-10.7
Urea	-15.6	-12.1	-12.8

[z] Means followed by a different letter within a column and within a cultivar are significantly different at $P \leq 0.05$ according to Tukey HSD.

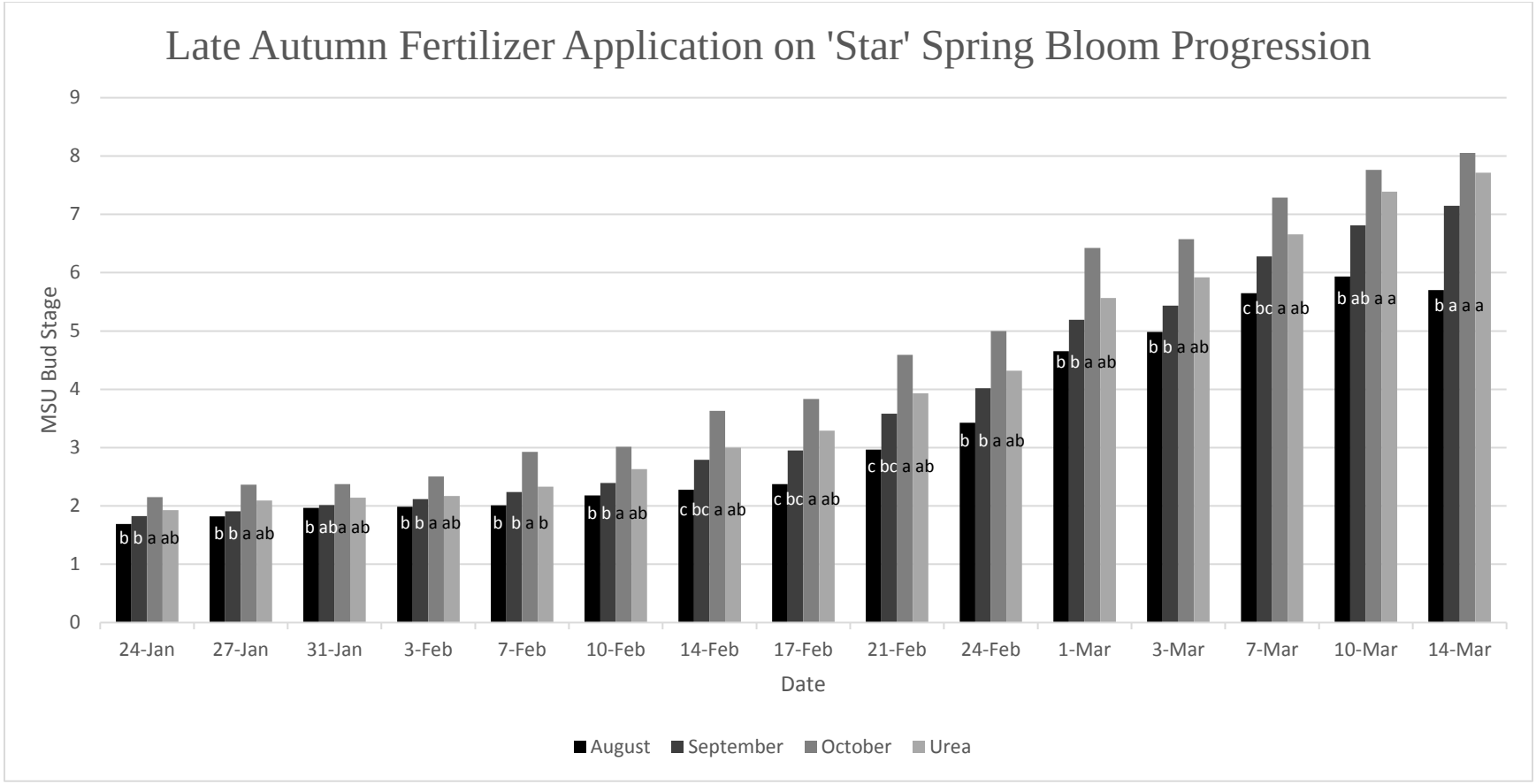

Figure 1. Bloom progression of 'Star' blueberry in response to the timing of late season N application treatments August, September, October, and Urea. Bloom progression was recorded according to the Michigan State University blueberry growth stages table: stage 1= tight/dormant bud; stage 2= bud swell with visible green tip; stage 3= budbreak signified by separation of bud scales; stage 4 = tight cluster, individual flower apices are visible; stage 5= early pink bud, individual floral apices appear pink and separated; stage 6 = late pink bud, flowers have elongated but corollas are still closed; stage 7 = early bloom, less than 50% of the corollas have opened; stage 8 = full bloom, greater than 50% of the corollas have opened; stage 9 = petal fall, greater than 50% of the corolla tubes have fallen off the flower; stage 10 = early green fruit, small pea size green fruit have expanded; stage 11 = late green fruit, expansion slows and fruit appear light green; stage 12 = fruit coloring, oldest fruit in the cluster change color to blue/pink (Michigan State University, 2016). Means followed by a different letter within a sample date are significantly different at $P \leq 0.05$ according to Tukey HSD.