

## ABSTRACT

LANDIS, HUNTER GLENN. Aluminum Effect on Hydrangea Plant Tissue Nutrient Concentrations and Sepal Coloration. (Under the direction of Dr. Brian E. Whipker).

Florists' hydrangeas [*Hydrangea macrophylla* subsp. *Macrophylla* var. *macrophylla* (Thunb.)] are a significant crop to the U.S. greenhouse industry due to their vibrant sepal colors of pink, blue, red or white. The pink or blue hydrangea cultivars contain the anthocyanin pigment (delphinidin-3-glucoside) that gives the sepal its color. The natural color of the pigment is a pink or red depending on the cultivar when aluminum (Al) is not supplied. To produce blue sepals, the plants are drenched with  $\text{Al}_2(\text{SO}_4)_3$  (AS) and a low dose of phosphorus.

Applications of AS can result in a decrease of the substrate pH and affect nutrient uptake by the hydrangea. Specific leaf tissue concentrations for pink and blue cultivars have not been developed. To determine nutrient tissue concentrations 'Early Blue', 'Hor Tivoli', 'Jip', and 'Mathilda Gutges' were treated with 0 g (pink) and 12 or 15 (blue) of AS. Leaf tissue concentrations were analyzed from weeks 5 through 8 of production. The blue hydrangeas generally had higher concentrations of S, Fe, Mn, Zn, B, Cu, and Al, while the pink plants had higher concentrations of N, P, Ca, and Mg. The results of this project provide nutrient tissue ranges for florists' hydrangeas specifically for plants being managed for pink or blue sepals. The different nutrient concentrations among cultivars also suggest that nutrient uptake in hydrangeas varies. This is especially evident with leaf tissue Al concentrations in blue hydrangeas, and lab recommendations appear to require customization by cultivar.

The drench application of AS can be time consuming and labor intensive for growers. Therefore, two studies conducted to consider alternatives to the existing drench method, 1) applying AS in constant liquid fertilization and 2) incorporating a slow release coated polymer into the substrate. To complete the first study 4 hydrangea cultivars were treated with 0, 9, 12,

and 15 g of AS in constant liquid feed fertilization. The results of this study found that applying AS through a constant liquid fertilization increased blue sepal colorations in all 4 cultivars while pH and EC levels were within acceptable parameters. To complete the slow release coated polymer application study ‘Early Blue Rose’ hydrangeas were treated with 0, 5, 10, 20, and 40 grams of slow release  $\text{Al}_2(\text{SO}_4)_3$  (15% Al) incorporated into the substrate. The use of a slow release coated polymer aluminum sulfate applied at these rates did not produce the desired blue sepals on ‘Early Blue Rose’ hydrangea.

The effect of Al applications to control sepal color of potted hydrangea has been examined considerably with a focus on blue or pink sepals. As the effect of Al on plants is cultivar specific, this study was conducted to determine the effect of Al application doses on the sepal color of two red hydrangea cultivars. In year 1, ‘Hot Red’ plants were treated with 0, 1.2, 1.8, 2.4, and 3.0 g/L of  $\text{Al}_2(\text{SO}_4)_3$  (AS) at each irrigation throughout the entire production process. In year 2, ‘Curly Sparkle’ were treated with 0, 9, 12, and 15 g of AS as a constant liquid feed over a five-week period (weeks 1-5) when the plants required irrigation. Applying AS contributed to increasing the level blue sepal coloration of red hydrangeas ‘Hot Red’ and ‘Curly Sparkle’. The highest concentration of Al in the leaf and sepal tissue generally corresponded with greater blue sepal coloration in both cultivars, although the highest AS dose did not consistently produce the most blue sepal coloration. Therefore, growers should determine the level of desired blueness desired when determining the dose of AS to be applied to red colored hydrangeas.

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Aluminum Effect on Hydrangea Plant Tissue Nutrient Concentrations and Sepal Coloration

by  
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## **DEDICATION**

To my wonderful family for their support and encouragement throughout this process.

## **BIOGRAPHY**

Hunter Landis began his agricultural experience on his family's dairy farm in Farmville, Virginia. He holds a Bachelor of Science in Business Administration from Bridgewater College and a Master of Agricultural Education from Clemson University. He worked as an Agronomist for the North Carolina Department of Agriculture & Consumer Services while completing a Master of Science in Horticulture at North Carolina State University. Hunter recently accepted the position of Director of State Farm Agribusiness with the Virginia Department of Corrections and will complete his M.S. in Horticulture in May 2019.

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## **Chapter 1: Leaf Tissue Nutrient Ranges for Blue and Pink Hydrangeas**

## Leaf Tissue Nutrient Ranges for Blue and Pink Hydrangeas

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### Abstract

Florists' hydrangeas [*Hydrangea macrophylla* subsp. *Macrophylla* var. *macrophylla* (Thunb.)] are a significant crop to the U.S. greenhouse industry because of their vibrant sepal colors of pink, blue, red or white. The pink or blue hydrangea cultivars contain the anthocyanin pigment delphinidin-3-glucoside that gives the sepal its color. The natural color of the pigment is pink or red depending on the cultivar when aluminum (Al) is not supplied. To produce blue sepals, the plants are fertilized with  $\text{Al}_2(\text{SO}_4)_3$  (AS) and a low dose of phosphorus. Applications of AS can result in a decrease of the substrate pH and affect nutrient uptake by the hydrangeas, but a set of specific leaf tissue values for pink and blue cultivars is lacking. Specific leaf tissue concentrations for pink and blue cultivars have not been developed. To determine nutrient tissue concentrations 'Early Blue', 'Hor Tivoli', 'Jip', and 'Mathilda Gutges' were treated with 0 g (pink) and 12 or 15 (blue) of AS. Leaf tissue concentrations were analyzed from weeks 5 through 8 of production. The blue hydrangeas generally had higher concentrations of S, Fe, Mn, Zn, B, Cu, and Al, while the pink plants had higher concentrations of N, P, Ca, and Mg. The results of this project provide nutrient tissue ranges for florists' hydrangeas specifically for plants being managed for pink or blue sepals. The different nutrient concentrations among cultivars also suggest that nutrient uptake in hydrangeas varies. This is especially evident with leaf tissue

Al concentrations in blue hydrangeas, and lab recommendations appear to require customization by cultivar.

## **Introduction**

Florists' hydrangeas [*Hydrangea macrophylla* subsp. *Macrophylla* var. *macrophylla* (Thunb.)], native to Japan, are a significant crop to the U.S. greenhouse industry. They are produced from early spring to late fall for their vibrant sepal color (Bailey, 1989). The bright sepal colors can be shades of pink, blue, red or white (Bailey, 1989; Dole and Wilkins, 2005).

To begin the production process of florists' hydrangeas, vegetative hydrangea cuttings are taken from stock plants and rooted from early spring to summer. Young plants are pinched during the growing process to produce multiple lateral shoots. In early fall the plants are defoliated and placed in cold storage to provide a vernalization period (Weiler, 1980). These rooted hydrangeas are kept in cold storage for 6 to 8 weeks at 4 to 11°C (Shanks, 1985). A relative humidity of 20-60% should be maintained and direct airflow should be avoided to prevent plant desiccation or disease (Bailey, 1989). In early winter plants are taken out of cold storage and placed in a greenhouse to force into flowering. Hydrangeas are generally transplanted from 10 cm to 15 cm pots and grown at temperatures of 16 to 17°C night temperature during the forcing process (Dole and Wilkins, 2005). The time to complete forcing process is dependent on temperature and cultivar, but most cultivars will be at full bloom between 10 and 14 weeks (Bailey, 1989).

The pink or blue hydrangea cultivars contain an anthocyanin pigment that gives the sepal its color. The specific anthocyanin found in hydrangeas is delphinidin-3-glucoside (Takeda et al., 1985). The natural color of the pigment is a pink or red depending on the cultivar when

aluminum (Al) is not supplied. When supplemental Al applications are made it will bond with the anthocyanin resulting in the sepal having a bluish hue and the mechanics of this biochemical interaction has been extensively investigated (Chen et al., 2015; Okada and Okawa, 1974; Schreiber et al. 2011; Yoshida et al., 2008).

There are several fertilizer regime options for growers producing florists' hydrangeas. The specific regime will be dependent on the desired color of the hydrangea sepals (Bailey, 1989; Dole and Wilkins, 2005; Landis and Whipker, 2017). Hydrangeas are typically produced in 15-20 cm pots (Bailey, 1989). Growers who are producing pink hydrangeas should maintain a pH of 6.0 to 6.5, use a fertilizer with 25N-4.4P-8.3K, and not provide supplemental Al. To produce blue flowers, a fertilization plan requires up to 16 g  $\text{Al}_2(\text{SO}_4)_3$  (AS) for subirrigation and 20 g for overhead irrigation, a fertilizer with 25N-2.2P-24.9K, and a pH of 5.5 (Bailey 1989; Dole and Wilkins, 2005). Blom and Piot (1992) determined that Al applications provided earlier (weeks 2 through 5) in the greenhouse production cycle were more effective at producing blue sepals versus later (weeks 6 through 9) applications.

While Al can be toxic to some plants and inhibit root growth (Delhaize and Ryan, 1995; Havlin et al., 2013), hydrangeas are considered tolerant to Al (Chen et al., 2015) and can accumulate up to 3,000 ppm in the leaf tissue (Havlin et al., 2013). Growers should still be careful with Al applications as tolerance to high concentrations of Al is cultivar specific (Epstein and Bloom, 2005) and roots are sensitive to salt injury (Dole and Wilkins, 2005).

While Al is applied to enhance blue sepal color, it can also influence nutrient concentrations in the plant. High Al levels can inhibit uptake of calcium (Ca) and magnesium (Mg) (Havlin, et al., 2013). The applied Al will decrease the substrate pH (Shanks et al., 1950) which can increase the availability of micronutrients to toxic concentrations in many plant

species (Mengel and Kirkby, 2001). Iron (Fe) and manganese (Mn) toxicity was found in seed geraniums when pH was below 5.5 (Gibson et al., 2007). Soil pH from 5.0 to 5.4 resulted in boron (B) toxicity in peas and barley (Gupta and Macleod, 1981). Zinc (Zn) was also found to be toxic to several *Brassica* species in acidic soil (Fornes et al., 2009). The concentrations of N, P, and K are generally lower in plant tissue at higher pH as greater plant growth will contribute to a diluted tissue concentration. Available Al in the substrate may also have an antagonistic effect on P, K, Mg, Ca, and Cu concentrations in the plant (Bryson and Mills, 2014).

Plant tissue analysis is a valuable tool for growers to monitor the efficacy of their nutrient management program (Bryson and Mill, 2014; Havlin et al., 2013). While there are currently fertilizer recommendations (Bailey 1989; Blom and Piot, 1992; Dole and Wilkins, 2005; Shanks et al. 1950) specific to blue or pink plants, the available hydrangea nutrient tissue sufficiency ranges (Bryson and Mills, 2014) are not differentiated for blue or pink plants. Given the difference in fertilization practices used by greenhouse growers to produce the two different sepal colors and its impact on nutrient availability, there is a need for more refined leaf tissue nutrient standards. The purpose of this study was to determine the nutrient concentration in the leaves among hydrangea cultivars managed for both pink and blue sepals.

## **Materials and Methods**

### **Controlled Greenhouse Study.**

Vernalized, one year old, 'Early Blue', 'Hor Tivoli', 'Jip', and 'Mathilda Gutges' hydrangea plants were used in this experiment and transplanted 21 January 2018 (week 0). The 'Early Blue' and 'Hor Tivoli' were transplanted from 10 cm diameter pots into 15 cm diameter plastic pots. The 'Jip', and 'Mathilda Gutges' were transplanted from 15 cm diameter pots into



20 cm diameter plastic pots. Pots were filled with a soilless substrate composed of sphagnum peat moss amended with 20% (v/v) horticultural grade perlite (Sun Gro Horticulture, Agawam, MA). The pH was adjusted to 5.5 with the addition of 2.2 kg dolomitic lime (Rockydale, Roanoke, VA) and 600 g of wetting agent (Aquatrol, Paulsboro, NJ) per m<sup>3</sup>. We treated plants with 0 g of AS (Fischer Scientific, Fair Lane, NJ) to produce pink sepals and 12 or 15 g doses to produce blue sepals. The two doses used to produce blue sepals were based on current production recommendations (Bailey, 1998; Dole and Wilkins, 2005). The AS was applied as either a constant liquid feed of 10 applications in 150 ml aliquots made over a five-week period (weeks 1-5) when the plants required irrigation or through three drench applications of 200 ml aliquots applied over a three-week period (weeks 2-4). The 0 g plants were treated with the same volume of water without Al during weekly applications. Plants receiving Al through the constant liquid feed were laid out in a complete randomized block design and plants receiving Al drench were completely randomized. There were six replications of constant liquid feed applications and five replications of each drench application. Plants were drip irrigated and fertilized with a constant liquid fertilizer at 150 mg·L<sup>-1</sup> N derived 13N–0.9P–10.8K Cal-Mag (Everris, Marysville, OH) in order to provide a complete nutrient containing fertilizer. Plants were grown at 22 °C day/18 °C night air temperatures.

### **Substrate Sample Collection and Analysis**

Substrate leachate was collected in a pour-thru solution extraction method (Cavins et al., 2005) from each replicate for each treatment. The pH and electrical conductivity (EC) were measured every week for the 11-week production cycle after all AS applications had been

completed (Week 5). The pH and EC values of the pour-through extracts were measured using a portable pH/EC meter (Hanna HI9813-6; Hanna Instruments, Woonsocket, RI).

### **Tissue Sample Collection and Nutrient Analysis**

For plant tissue analysis, the most recent mature leaves were harvested from each stem (Week 5) and the leaves were consolidated into single-plant replicates for each treatment rate. Leaves were initially immersed into deionized (DI) water, then immersed in a solution of 0.5 N HCl for 1 min. and again immersed in DI water. Samples were dried (12–24 hr.) at 80 °C, then processed through a stainless-steel grinder (Wiley Mini-Mill; Thomas Scientific; Swedesboro, NJ) with a 20-mesh (1 mm) screen (Campbell and Plank 1992). Total N concentration was determined by oxygen combustion gas chromatography with an elemental analyzer (NA1500s2; CE Elantech Instruments; Lakewood, NJ) (AOAC 1990; Campbell 1992) on a 5-7 mg aliquot of the dried and ground sample. Results are expressed in percent (%) on a dry-weight basis. Total concentrations of P, K, Ca, Mg, S, Fe, Mn, Zn, B, and Al were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (Spectro Arcos EOP and Arcos II EOP, Spectro Analytical: A Division of Ametek; Mahwah, NJ), after closed-vessel Nitric acid (HNO<sub>3</sub>) digestion in a microwave digestion system (MARS 6 Microwaves; CEM Corp.; Matthews, NC). Total P, K, Ca, Mg, and S are expressed as a percentage (%) and Fe, Mn, Zn, B, and Al are expressed in parts per million (ppm) on a dry-weight basis.

## **Sepal Color Reading and Analysis**

The sepal color (hue) value was determined at full bloom for two sepals on three different blooms for each replicate using a handheld colorimeter (PCE-RGB2 Color Meter; PCE Instruments, Meschede, Germany).

## **Experimental Arrangement and Statistical Analysis**

The experiment contained a factorial arrangement with cultivar (4 levels), color (2 levels), and sampling week (4 levels) as factors. To determine if significant interactions were present among these factors (cultivar, color, and week) for plant tissue concentration of N, P, K, Ca, Mg, S, Fe, Mn, Zn, B, and Al, SAS PROC GLM (version 9.4; SAS Inst. Inc., Cary, NC) was used. The factor interactions were also analyzed for leachate pH and EC using the same program. The sepal color (hue) was analyzed using PROC GLM. Tukey's honestly significant differences (HSD) test at  $P \leq 0.05$  was used to determine the difference in hue for each cultivar between pink plants treated with 0 g of AS and blue plants treated with 12 or 15 g of AS.

## **Results**

### **Sepal Color**

The sepal color (hue) value was analyzed by cultivar as sepal colors varied among cultivars (Bailey, 1989). Plants treated with 0 g AS were pink while plants receiving 12 g or 15 g of AS were considered blue (Fig. 1). There was significant difference in the hue mean between blue and pink plants for each cultivar (Fig. 2). Based on the significant difference of hue between pink and blue plants, the pH, EC, and nutrients concentrations data was pooled for plants receiving 12 g and 15 g AS.

## **Substrate pH and EC**

While there was a significant interaction among the sepal color, cultivar and weeks collected ( $P \leq 0.05$ ) for the leachate pH, there was a greater interaction between color and week ( $P \leq 0.0001$ ). There was no significant interaction among the sepal color, cultivar and weeks collected for EC, but the interaction between sepal color and week collected was significant ( $P \leq 0.0001$ ). The pH was lower for blue plants each week (Fig. 3A). The lower pH for blue plants was likely due to the early season AS applications (Shanks et al., 1950). The pH increased over time for both blue and pink plants as the acidic effects of AS applications was most likely countered by the basic 13N–0.9P–10.8K Cal-Mag fertilizer. The EC was higher for blue plants each week and decreased over time (Fig. 3B). The higher EC levels found in the blue plants was also likely due to the fertilizer salts provided by the AS applications.

## **Tissue Nutrient Concentration**

Leaf tissue nutrient concentrations for N, P, K, Ca, and S (Table 1) and Fe, Mn, Zn, B, and Al (Table 2) were significant at the sepal color x cultivar x weeks interaction. While there was no color x cultivar x week interaction for Mg and Cu, both nutrients were significant ( $P \leq 0.05$ ) at the color x week interaction (Table 3).

## **Nitrogen**

While the N concentration was within the sufficiency range (2.24%-5.60%) reported by Bryson and Mills (2014) for blue and pink plants, the concentration of N was lower in the blue than the pink hydrangeas at each week for all cultivars (Table 1). There was an overall trend of N concentration declining over the growing period in both blue and pink hydrangeas among all

cultivars. This is likely due to the dilution of N in the plants as plant size increase over time (Bryson and Mills, 2014).

## **Phosphorus**

The P concentration in pink and blue plants for all cultivars was below the current ranges reported by Bryson and Mills (2014) of 0.25%-0.70% for most weeks. The blue hydrangeas had lower P concentrations than pink hydrangeas at each week (Table 1). A low P fertilization program was used in this study to aid in the induction of blue color (Bailey, 1989). The P that was provided was likely bound to the Al supplied to the blue plants and restricting the P from translocating throughout the plant (Bryson and Mills, 2014). While blue plants for most cultivars had P concentrations at or below the deficiency concentration of 0.09% reported in ‘Supreme Rose’ hydrangeas by Bailey and Hammer (1988), no P deficiency symptoms as reported by Bailey and Hammer (1988) were observed in this study. The lowest P concentration for all blue plants of 0.08% was found in ‘Early Blue’ at weeks 6 and 7 and in ‘Jip’ at week 7. The pink ‘Early Blue’ had the highest P concentration (0.27%) at weeks 7 and 8.

## **Potassium**

The K concentration for all pink and blue plants was within the sufficiency range (2.20%-7.80%) reported by Bryson and Mills (2014). ‘Early Blue’ was the only cultivar where pink plants had higher K concentration each week (Table 1). The K concentration was higher in the pink plants at week 5 for ‘Hor Tivoli’, ‘Jip’, and ‘Mathilda Gutges’, but was higher in the blue plants at week 8 for these cultivars. These results are similar to those of Shanks et al. (1950) where K concentration was higher at the end of the season in plants treated with AS.

## **Calcium**

The low end of the Ca sufficiency range of 0.60%-2.00% reported by Bryson and Mills (2014) is marginally below the previously report deficiency concentration of 0.61% in ‘Supreme Rose’ hydrangeas (Bailey and Hammer, 1988). The Ca concentration for this study was lower in the blue plants than the pink plants for all cultivars during each week (Table 1). The Al applied to the blue plants, and the lower corresponding substrate pH (Fig. 3A), likely inhibited the uptake of Ca into the leaf tissue (Havlin et al., 2013). ‘Jip’ had the lowest Ca concentration of all cultivars for blue (0.56%) and pink (0.59%) at week 5. Both concentrations are marginally below the sufficiency values (Bryson and Mills, 2014) and deficiency values (Bailey and Hammer, 1988) previously reported. Despite the leave tissue Ca concentrations being low, no Ca deficiency symptoms reported by Bailey and Hammer (1988) were observed in this study.

## **Magnesium**

The Mg concentration was within the sufficiency range of 0.22%-0.61% (Bryson and Mills, 2014) in both blue and pink plants. The blue plants had a lower Mg concentration than the pink plants each week (Table 3). Magnesium uptake was likely inhibited by the Al (Havlin et al., 2013) in the blue plants and the corresponding effect on Mg uptake at the lower pH (Bryson and Mills, 2014). The Mg concentration in the blue plants increased from week 5 to 6 and then decreased from week 6 to 8. The concentration of Mg in the pink plants increased from week 5 to 6 and decreased from week 7 to 8.

## **Sulfur**

The blue plants had greater S concentration in the leaf tissue than the pink plants (Table 1). The AS applied to blue plants would have contributed to the higher S concentration. The S concentration for all blue plants was within the sufficiency range of 0.20%-0.70% (Bryson and Mills, 2014) each week while the mean S concentration for pink 'Early Blue', 'Hor Tivoli', and 'Jip' were below this range for at least one of the weeks sampled. The blue 'Early Blue', 'Hor Tivoli', and 'Mathilda Gutges' all exhibited a decrease in leaf tissue S concentrations over time.

## **Iron**

The concentration of Fe was higher in the blue hydrangeas for all cultivars at each week (Table 2). The higher Fe in the blue plants is likely due to the lower substrate pH (Fig. 3A) (Mengel and Kirkby, 2001) as a result of the AS applications (Shanks et al. 1950). The blue plants for all cultivars had Fe concentration within the Bryson and Mills (2014) sufficiency range of 50-300 ppm. The pink 'Early Blue', 'Hor Tivoli', and 'Mathilda Gutges' were below this range for at least one week. The lowest concentration of Fe (41.5 ppm) was found in the pink 'Mathilda Gutges' at week 8 and the highest Fe concentration of 101.3 ppm was found in the same cultivar of blue plants at week 5. In most cultivars the Fe concentration decreased over time for both blue and pink plants.

## **Manganese**

The Mn concentration was higher in the blue plants than the pink plants at each week for all cultivars (Table 2). The higher Mn in the blue plants was likely due to increased Mn availability in the substrate as a result of lower substrate pH (Fig. 3A) (Mengel and Kirkby,

2001) caused by the AS applications (Shanks et al., 1950). The Mn concentration decreased over time in the blue plants for most cultivars. The Mn became less available to the plants as substrate pH increased over time (Fig. 3A). The ‘Mathilda Gutges’ had the highest Mn for blue plants at week 5 of 83.5 ppm while the ‘Early Blue’ had the highest Mn of pink plants during the same week of 55.1 ppm. There was an increase of Mn at each week for the blue and pink ‘Jip’. The Mn concentration was within the sufficiency range of 38–300 ppm (Bryson and Mills, 2014) for all blue plants during each week. The Mn concentration for the pink ‘Mathilda Gutges’ and ‘Hor Tivoli’ were below the Bryson and Mill (2014) range for at least two weeks and was the lowest in the pink ‘Hor Tivoli’ (26.9 ppm) at week 6.

## **Zinc**

The leaf tissue Zn concentration was higher in the blue plants for most weeks in all cultivars (Table 2). The lower substrate pH for the blue plants (Fig. 3A) likely increased the Zn availability in the substrate (Mengal and Kirkby, 2001) resulting in the higher Zn tissue concentrations. The Zn concentration decreased over time for the pink and blue ‘Hor Tivoli’, ‘Jip’ and ‘Mathilda Gutges’, and the blue ‘Early Blue’. The concentration of Zn was within the sufficiency range 20 -200 ppm (Bryson and Mills, 2014) each week for all blue and pink cultivars except pink ‘Hor Tivoli’.

## **Boron**

The leaf tissue B concentration was higher in the blue plants than the pink plants for all cultivars in most weeks (Table 2). Boron was likely more available to the blue plants at the lower substrate pH (Fig. 3A) (Mengal and Kirkby, 2001) due to the acidic AS applications



(Shanks et al., 1950). The B concentration increased in the pink and blue plants over time for all cultivars. All B mean concentrations were within the sufficiency ranges 20-50 ppm (Bryson and Mills, 2014) and above the previously reported deficiency concentration of 23 ppm (Bailey and Hammer, 1988).

## **Aluminum**

There are currently no recommended Al concentrations for hydrangeas in the vegetative growth stage. Blom and Piot (1992) report a mean of 1960 ppm Al in the most recent mature leaf tissue of blue ‘Mathilda Gutges’ and ‘Brestenburg’ hydrangeas at full bloom. The Al concentrations found in this study were higher in all the blue plants during each week than in the pink plants. The Al concentration increased in blue plants for most cultivars from weeks 5 to 7. The Al in the blue ‘Jip’ increased from week 7 to 8, while Al decreased in the blue plants of all other cultivars from week 7 to 8. The variability of Al was much greater in the blue plants for all cultivars as the standard deviations were higher in the blue plants than the pink plants for all cultivars at each week. The lowest Al concentration for blue plants of 195.9 ppm occurred in ‘Hor Tivoli’ at week 8 while the highest concentration of 1153.5 ppm was found in the blue ‘Jip’ at week 8. As expected, the overall leaf tissue Al concentrations were lower in the pink plants because Al was not applied.

## **Copper**

The concentration of Cu for blue and pink plants was within the sufficiency range (1-25 ppm) reported by Bryson and Mills (2014) (Table 3). The blue plants had higher Cu concentrations each week than the pink plants. The higher Cu in blue plants was likely due to

lower pH (Fig. 2A) in the blue plants (Mengel and Kirkby, 2001). The Cu decreased over time in both blue and pink plants.

## **Discussion**

These results provide nutrient tissue ranges for florists' hydrangeas specifically for plants being managed for pink or blue sepals. The different nutrient concentrations among cultivars also suggest that nutrient uptake in hydrangeas is cultivar specific. This is especially evident with leaf tissue Al concentrations in blue hydrangeas, and lab recommendations appear to require customization by cultivar. The blue hydrangeas generally had higher concentrations S, Fe, Mn, Zn, B, Cu, and Al while the pink plants had higher concentrations of N, P, Ca, and Mg. The general sufficiency range for Ca and P can be expanded as the mean Ca and P concentrations were below the current sufficiency ranges (Bryson and Mills, 2014) for at least one cultivar of blue and pink plants. Future experiments to determine nutrient deficiency and toxicity concentration differences between blue and pink hydrangeas would be beneficial to provide a wider set of data to aid in nutrient disorder diagnostics.

## Literature Cited

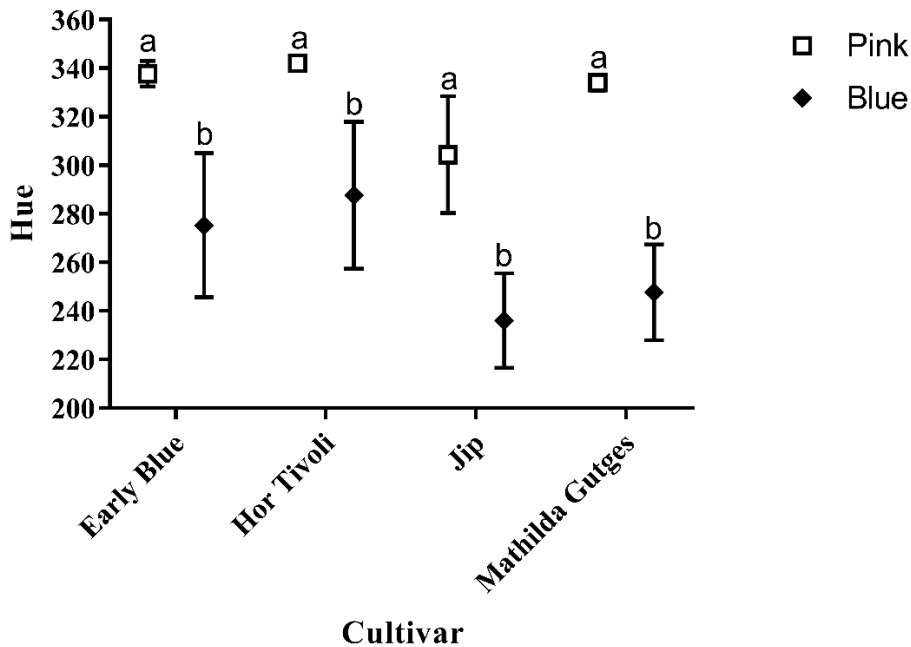
- Association of Official Analytical Chemists [AOAC]. 1990. Official methods of analysis. 15th ed. Arlington, Va: AOAC International, p.341.
- Bailey, D.A. 1989. Hydrangea production. Growers handbook series, vol 3. Timber Pres, Portland, OR.
- Bailey, D.A. and P.A. Hammer 1988. Evaluation of nutrient deficiency and micronutrient toxicity symptoms in florists hydrangea. *J. Amer. Soc. Hort. Sci.* 113.3: 363-67.
- Blom, T.J. and B.D. Piott, 1992. Florists' hydrangea blueing with aluminum sulfate applications during forcing. *HortScience* 27.10: 1084-1087.
- Bryson, G.M., and H.A. Mills. 2014. Plant analysis handbook IV: a guide to sampling, preparation, analysis, interpretation and use of results of agronomic and horticultural crop plant tissue. Micro-Macro, Athens, GA.
- Campbell C.R. 1992. Determination of total nitrogen in plant tissue by combustion. In: Plank C.O., editor. Plant analysis reference procedures for the southern region of the United States. Athens (GA): Georgia Cooperative Extension Service. p 20–22. Southern Cooperative Series Bulletin 368.
- Campbell C.R. and C.O. Plank. 1992. Sample preparation. In: Plank CO, editor. Plant analysis reference procedures for the southern region of the United States. Athens (GA): Georgia Cooperative Extension Service. p 1–12. Southern Cooperative Series Bulletin 368.
- Cavins, T.J., B.E. Whipker, and W. Fonteno. 2005. Timing of PourThru affects pH, electrical conductivity, and leachate volume. *Comm. Plant Soil Sci.* 36:1573-1581.

- Chen, H.C., Lu., and H. Jiang, and J. Peng. 2015. Global transcriptome analysis reveals distinct aluminum-tolerance pathways in the Al-accumulating species *Hydrangea macrophylla* and marker identification. *Plos One*, vol. 10, no. 12, doi:10.1371/journal.pone.0144927.
- Delhaize, E., and P.R. Ryan. 1995. Aluminum toxicity and tolerance in plants. *Plant Physiol.* 107:315–321.
- Dole, J.M., and H. F. Wilkins. 2005. Floriculture: principles and species. Pearson Prentice Hall, Saddle River, NJ.
- Epstein E., and A.J. Bloom. 2005. Mineral nutrition of plants: principles and perspectives. Sinauer Associates, Sunderland, MA.
- Fornes, F., R.G. Fuente, R.M. Belda, and M. Abad. 2009. ‘Alperujo’ compost amendment of contaminated calcareous and acidic soils: Effects on growth and trace element uptake by five *Brassica* species. *Biosource Technol.* 100:0960-8524.
- Gibson J.L., D.S. Pitchay, A.L. Williams-Rhodes, B.E. Whipker, P.V. Nelson, and J.M. Dole. 2007. Nutrient deficiencies in bedding plants. Ball Publishing. Batavia, IL.
- Gupta, U.C. and J.A. Macleod. 1981. Soil and boron as influenced by soil pH and calcium sources on podzol soils. *Soil Sci.* 31:0038-075.
- Havlin, J., S.L. Tisdale, W.L. Nelson, and J.D. Beaton. 2013. Soil fertility and fertilizers, 8<sup>th</sup> ed. Pearson Prentice Hall. Saddle River, NJ.
- Landis, H. and Whipker, B.E. 2017. Nutrient management strategies for ensuring blue coloration of greenhouse hydrangeas. *e-GRO Alert* 6.4 (2017): 1-6.
- Mengel K. and E.A. Kirkby. 2001. Principles of plant nutrition. Kluwer Academic Publishers. Dordrecht, The Netherlands.

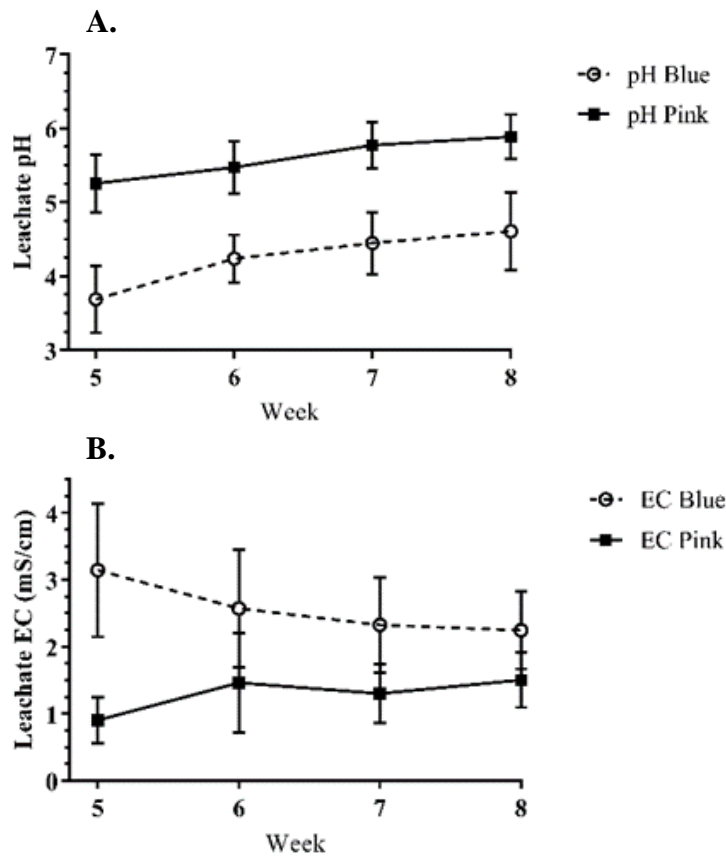
- Okada, M., and K. Okawa. 1974. The quantity of aluminum and phosphorus in plants and its influence on the sepal color of *Hydrangea macrophylla*. J. Jpn. Soc. Hort. Sci. 42: 361-70.
- Schreiber, H. D., A.H. Jones, A.M. Lariviere, K.M. Mayhew, J.D. Cain, 2011. Role of aluminum in red-to-blue color changes in *Hydrangea macrophylla* sepals. BioMetals. 24:1005–1015., doi:10.1007/s10534-011-9458-x.
- Shanks, J. B., J.R. Haun, and C.B. Links, 1950. A preliminary study on the mineral nutrition of hydrangeas. Proc. Amer. Soc. Hort. Sci. 56:457-565.
- Takeda, K., M. Kariuda, and H. Itoi. 1985. Blueing of sepal colour of *Hydrangea macrophylla*. Phytochemistry 24:2251-2254.
- Weiler, T. C. 1980. Hydrangeas, p. 353–372. In: R.A. Larson, (ed.). Introduction to floriculture, Academic Press, New York.
- Yoshida, K., D. Ito, Y. Shinkai, and T. Kondo. 2008. Change of color and components in sepals of Chameleon hydrangea during maturation and senescence. Phytochemistry, 69:3159–3165. doi:10.1016/j.phytochem.2008.02.024.



**Figure 1.** ‘Jip’ hydrangeas treated with 0 g  $\text{Al}_2(\text{SO}_4)_3$  were classified as pink while plants receiving 12 g or 15 g of  $\text{Al}_2(\text{SO}_4)_3$  had blue sepals.



**Figure 2.** Sepal color (hue) means for pink (treated with 0 g  $\text{Al}_2(\text{SO}_4)_3$ ) and blue (treated with 12 or 15 g  $\text{Al}_2(\text{SO}_4)_3$ ) plants of four florists’ hydrangea cultivars. Lower case letters indicate significant differences among cultivar mean hue values, with lower hue values corresponding to greater blue color. Means with different letters are significantly different at  $P \leq 0.05$ .



**Figure 3.** Pour-thru lechate means of (A) pH and (B) electrical conductivity (EC) for florists' hydrangeas managed for pink (treated with 0 g  $\text{Al}_2(\text{SO}_4)_3$ ) or blue (treated with 12 or 15 g  $\text{Al}_2(\text{SO}_4)_3$ ) sepal color. The lechate solution pH and EC were collected and analyzed from weeks 5 through 8 of production. The interaction between color and the week sampled for pH and EC was significant at  $P \leq 0.0001$ .

**Table 1.** Leaf tissue macronutrient concentration means and standard deviations for four cultivars of florists' hydrangeas managed for blue (treated with 12 or 15 g Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>) or pink (treated with 0 g Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>) sepal color. The leaf tissue nutrient concentrations were collected from weeks 5 through 8 of production.

		<b>N</b>		<b>P</b>		<b>K</b>		<b>Ca</b>		<b>S</b>	
		%									
<b>Cultivar</b>	<b>Week</b>	<b>Blue</b>	<b>Pink</b>	<b>Blue</b>	<b>Pink</b>	<b>Blue</b>	<b>Pink</b>	<b>Blue</b>	<b>Pink</b>	<b>Blue</b>	<b>Pink</b>
<b><i>'Early Blue'</i></b>	<b>5</b>	2.70 ± 0.21	3.16 ± 0.18	0.10 ± 0.01	0.21 ± 0.01	2.21 ± 0.17	2.40 ± 0.16	0.69 ± 0.07	0.93 ± 0.09	0.43 ± 0.12	0.21 ± 0.02
	<b>6</b>	2.39 ± 0.12	3.27 ± 0.21	0.08 ± 0.02	0.24 ± 0.02	2.31 ± 0.19	2.62 ± 0.32	0.68 ± 0.06	0.98 ± 0.09	0.42 ± 0.11	0.21 ± 0.01
	<b>7</b>	2.41 ± 0.15	3.31 ± 0.20	0.08 ± 0.02	0.27 ± 0.03	2.30 ± 0.21	2.50 ± 0.27	0.65 ± 0.10	0.96 ± 0.12	0.44 ± 0.18	0.20 ± 0.01
	<b>8</b>	2.54 ± 0.22	3.26 ± 0.37	0.11 ± 0.02	0.27 ± 0.02	2.28 ± 0.26	2.62 ± 0.29	0.66 ± 0.07	1.06 ± 0.15	0.29 ± 0.08	0.19 ± 0.03
<b><i>'Hor Tivoli'</i></b>	<b>5</b>	3.23 ± 0.31	3.80 ± 0.36	0.15 ± 0.03	0.24 ± 0.04	2.63 ± 0.29	2.78 ± 0.30	0.69 ± 0.11	0.86 ± 0.12	0.31 ± 0.03	0.19 ± 0.03
	<b>6</b>	3.03 ± 0.34	3.44 ± 0.22	0.13 ± 0.03	0.23 ± 0.02	2.56 ± 0.29	2.81 ± 0.26	0.68 ± 0.10	0.90 ± 0.19	0.31 ± 0.11	0.18 ± 0.02
	<b>7</b>	2.73 ± 0.22	3.40 ± 0.31	0.09 ± 0.02	0.20 ± 0.03	2.17 ± 0.24	2.58 ± 0.22	0.59 ± 0.11	0.90 ± 0.17	0.23 ± 0.08	0.16 ± 0.02
	<b>8</b>	2.83 ± 0.36	3.33 ± 0.43	0.09 ± 0.02	0.20 ± 0.03	2.79 ± 0.39	2.61 ± 0.38	0.74 ± 0.15	0.89 ± 0.16	0.26 ± 0.09	0.17 ± 0.02
<b><i>'Jip'</i></b>	<b>5</b>	3.04 ± 0.27	3.39 ± 0.42	0.13 ± 0.01	0.18 ± 0.03	2.48 ± 0.18	2.55 ± 0.25	0.51 ± 0.08	0.59 ± 0.07	0.39 ± 0.08	0.23 ± 0.03
	<b>6</b>	2.58 ± 0.23	2.98 ± 0.20	0.09 ± 0.01	0.18 ± 0.02	2.87 ± 0.28	2.77 ± 0.30	0.53 ± 0.10	0.66 ± 0.09	0.44 ± 0.06	0.22 ± 0.01
	<b>7</b>	2.44 ± 0.20	2.96 ± 0.42	0.08 ± 0.01	0.17 ± 0.03	2.91 ± 0.32	2.51 ± 0.23	0.56 ± 0.09	0.82 ± 0.16	0.43 ± 0.04	0.22 ± 0.03
	<b>8</b>	2.64 ± 0.27	3.19 ± 0.30	0.09 ± 0.02	0.18 ± 0.03	3.44 ± 0.40	2.65 ± 0.37	0.70 ± 0.11	0.95 ± 0.14	0.45 ± 0.08	0.18 ± 0.01



**Table 1** (continued).

<b><i>Mathilda Gutges</i></b>	<b>5</b>	2.86 ± 0.27	3.20 ± 0.28	0.15 ± 0.04	0.24 ± 0.03	2.36 ± 0.21	2.39 ± 0.27	0.83 ± 0.12	0.97 ± 0.05	0.33 ± 0.05	0.20 ± 0.04
	<b>6</b>	2.50 ± 0.21	2.91 ± 0.30	0.12 ± 0.01	0.23 ± 0.02	2.45 ± 0.19	2.37 ± 0.25	0.79 ± 0.12	1.01 ± 0.13	0.34 ± 0.04	0.22 ± 0.02
	<b>7</b>	2.60 ± 0.25	2.89 ± 0.36	0.12 ± 0.02	0.23 ± 0.03	2.39 ± 0.13	2.30 ± 0.30	0.79 ± 0.11	1.04 ± 0.10	0.32 ± 0.03	0.24 ± 0.02
	<b>8</b>	2.45 ± 0.15	2.82 ± 0.13	0.12 ± 0.04	0.24 ± 0.01	2.44 ± 0.14	2.42 ± 0.14	0.83 ± 0.17	1.01 ± 0.05	0.27 ± 0.04	0.23 ± 0.02
<b>Significance<sup>†</sup></b>		*	*	*	***	*	***				

<sup>†</sup> \*, \*\*, or \*\*\* indicates statistically significant based on a cultivar x week x color interaction among sample means based on *t* test at  $P \leq 0.05$ ,  $P \leq 0.01$ , or  $P \leq 0.001$ , respectively.

**Table 2.** Leaf tissue micronutrient concentration means and standard deviations for four cultivars of florists' hydrangeas managed for blue (treated with 12 or 15 g Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>) or pink (treated with 0 g Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>) sepal color. The leaf tissue nutrient concentrations were collected from weeks 5 through 8 of production.

Cultivar	Week	Fe		Mn		Zn		B		Al	
		Blue	Pink	Blue	Pink	Blue	Pink	Blue	Pink	Blue	Pink
		ppm									
<i>'Early Blue'</i>	<b>5</b>	69.6	54.6	79.4	55.1	31.0	24.8	30.4	26.8	744.5	85.5
		±	±	±	±	±	±	±	±	±	±
	12.2	5.0	16.9	12.1	3.7	3.0	2.6	2.6	283.4	31.8	
	±	±	±	±	±	±	±	±	±	±	
<b>6</b>	66.6	56.1	84.9	58.7	27.6	22.1	32.5	31.5	860.3	90.1	
	±	±	±	±	±	±	±	±	±	±	
11.3	5.7	14.1	15.7	4.2	2.1	2.9	4.2	249.4	49.4		
±	±	±	±	±	±	±	±	±	±		
<b>7</b>	67.6	54.4	74.0	50.9	24.7	22.4	34.2	28.9	917.5	33.0	
	±	±	±	±	±	±	±	±	±	±	
12.8	5.9	15.5	7.4	4.4	2.9	3.8	3.0	445.7	14.7		
±	±	±	±	±	±	±	±	±	±		
<b>8</b>	58.8	48.2	70.7	65.3	20.2	24.9	35.6	34.5	679.0	18.5	
	±	±	±	±	±	±	±	±	±	±	
18.1	10.5	14.5	9.9	3.2	3.0	5.7	4.2	250.8	8.0		
±	±	±	±	±	±	±	±	±	±		
<i>'Hor Tivoli'</i>	<b>5</b>	65.3	53.3	45.7	32.5	31.0	25.8	29.6	23.4	198.4	6.5
		±	±	±	±	±	±	±	±	±	±
	19.2	11.1	9.3	11.3	5.7	4.0	4.3	4.4	182.6	5.6	
	±	±	±	±	±	±	±	±	±	±	
<b>6</b>	59.4	47.5	43.1	26.9	28.1	19.4	30.9	24.8	217.0	2.8	
	±	±	±	±	±	±	±	±	±	±	
12.6	6.3	13.8	9.2	4.7	3.7	4.6	4.3	181.1	2.2		
±	±	±	±	±	±	±	±	±	±		
<b>7</b>	57.9	49.6	39.4	33.3	22.8	17.6	28.5	31.0	222.0	3.3	
	±	±	±	±	±	±	±	±	±	±	
22.2	6.5	9.4	10.8	3.8	4.0	4.7	6.2	179.6	2.1		
±	±	±	±	±	±	±	±	±	±		
<b>8</b>	56.5	43.7	43.2	24.1	23.2	16.4	39.1	31.2	195.9	4.7	
	±	±	±	±	±	±	±	±	±	±	
13.8	7.2	14.5	8.2	3.8	3.3	8.0	8.2	167.5	1.9		
±	±	±	±	±	±	±	±	±	±		
<i>'Jip'</i>	<b>5</b>	71.9	66.2	63.3	40.4	42.1	37.0	25.3	23.5	751.4	217.2
		±	±	±	±	±	±	±	±	±	±
	13.3	10.5	12.7	8.5	7.2	3.8	3.0	2.8	230.7	107.2	
	±	±	±	±	±	±	±	±	±	±	
<b>6</b>	69.8	63.3	66.1	44.4	44.1	33.2	31.1	28.6	877.8	200.1	
	±	±	±	±	±	±	±	±	±	±	
11.5	5.9	8.3	9.0	7.7	5.3	3.6	2.3	253.1	68.4		
±	±	±	±	±	±	±	±	±	±		
<b>7</b>	65.4	60.9	72.6	51.4	37.1	30.9	31.2	28.5	914.5	188.9	
	±	±	±	±	±	±	±	±	±	±	
12.9	12.2	19.7	10.0	4.1	2.6	4.1	4.9	203.7	79.1		
±	±	±	±	±	±	±	±	±	±		
<b>8</b>	83.7	56.5	91.3	64.8	39.3	27.3	43.3	33.8	1153.	159.0	
	±	±	±	±	±	±	±	±	±	±	
26.4	7.3	16.6	22.8	9.1	3.7	7.9	5.1	360.2	47.7		
±	±	±	±	±	±	±	±	±	±		

**Table 2** (continued).

<b>'Mathilda Gutges'</b>		101.3	59.6	83.5	40.0	29.3	21.1	29.1	24.2	389.8	1.3
		±	±	±	±	±	±	±	±	±	±
	<b>5</b>	22.5	11.8	13.2	6.9	8.7	2.6	2.3	2.6	170.3	0.7
		±	±	±	±	±	±	±	±	±	±
	<b>6</b>	93.2	47.4	79.9	36.1	26.4	20.9	31.7	27.0	539.8	0.9
		±	±	±	±	±	±	±	±	±	±
		14.5	5.6	11.4	7.3	4.1	2.9	2.6	2.1	280.7	1.8
		±	±	±	±	±	±	±	±	±	±
	<b>7</b>	79.5	46.9	75.4	37.3	26.7	20.5	31.0	28.5	678.6	0.7
		±	±	±	±	±	±	±	±	±	±
		14.7	4.3	6.2	3.8	3.1	1.5	2.3	2.3	301.5	0.8
		±	±	±	±	±	±	±	±	±	±
	<b>8</b>	69.3	41.5	71.0	40.9	22.4	20.5	34.5	34.7	527.2	5.5
		±	±	±	±	±	±	±	±	±	±
		10.3	2.8	8.2	7.0	3.0	2.9	3.5	2.0	173.2	3.9
		±	±	±	±	±	±	±	±	±	±
<b>Significance<sup>‡</sup></b>		**		*		***		***		**	

<sup>‡</sup> \*, \*\*, or \*\*\* indicates statistically significant based on a cultivar x week x color interaction among sample means based on *t* test at  $P \leq 0.05$ ,  $P \leq 0.01$ , or  $P \leq 0.001$ , respectively.

**Table 3.** Leaf tissue concentration means and standard deviations of magnesium (Mg) and copper (Cu) of four cultivars of florists' hydrangeas managed for blue (treated with 12 or 15 g Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>) or pink (treated with 0 g Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>) sepal color. The leaf tissue nutrient concentrations were collected from weeks 5 through 8 of production.

Week	Mg		Cu	
	%		ppm	
	Blue	Pink	Blue	Pink
<b>5</b>	0.32 ± 0.07	0.34 ± 0.06	5.55 ± 2.28	4.75 ± 2.13
<b>6</b>	0.33 ± 0.08	0.36 ± 0.07	4.99 ± 1.93	4.05 ± 1.93
<b>7</b>	0.31 ± 0.08	0.36 ± 0.06	4.64 ± 1.85	3.52 ± 1.66
<b>8</b>	0.29 ± 0.06	0.35 ± 0.06	4.96 ± 1.75	3.42 ± 1.58
Significance†	*		*	

† \*, \*\*, or \*\*\* indicates statistically significant based on a week x color interaction among sample means based on *t* test at  $P \leq 0.05$ ,  $P \leq 0.01$ , or  $P \leq 0.001$ , respectively.

**Chapter 2: Enhancing Blue Hydrangea Sepal Coloration by Applying Aluminum Sulfate  
Through Constant Liquid Fertilization Application**

# Enhancing Blue Hydrangea Sepal Coloration by Applying Aluminum Sulfate Through Constant Liquid Fertilization Application

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## Abstract

The vibrant color produced on hydrangea (*Hydrangea macrophylla*) sepals make it significant crop to the U.S. greenhouse industry. When aluminum is supplied blue sepals are produced as the Al binds an anthocyanin, while pink sepals are formed when no Al is supplied. The typical production practice used to induce blue sepals is by applying  $\text{Al}_2(\text{SO}_4)_3$  (AS) via several substrate drenches. The drench application process can be time consuming and labor intensive for growers and there are no recommended doses for Al applied through a constant liquid fertilization available. This study determined the effect of applying AS in constant liquid fertilization on sepal color and substrate leachate pH and EC for 4 hydrangea cultivars. The 4 hydrangea cultivars were treated with 0, 9, 12, and 15 g of AS. The results of this study found that applying AS through a constant liquid fertilization increased blue sepal colorations in all 4 cultivars while pH and EC levels were within acceptable parameters.

**Keywords** sepal color, aluminum sulfate, *Hydrangea macrophylla*

## Introduction

Florists' hydrangeas [*Hydrangea macrophylla* subsp. *Macrophylla* var. *macrophylla* (Thunb.)], native to Japan, are a significant crop to the greenhouse industry due to their vibrant sepal color (Bailey, 1989). The bright sepal colors can be shades of pink, blue, red or white

(Bailey, 1989; Dole and Wilkins, 2005). Pink or blue hydrangea cultivars contain an anthocyanin pigment (delphinidin-3-glucoside) that gives the sepal its color (Takeda et al., 1985). Hydrangea sepals are generally pink when aluminum (Al) is not supplied. When supplemental Al applications are made it will bind with the anthocyanin resulting in the sepal to develop a bluish hue and the mechanics of this biochemical interaction has been extensively investigated (Chen et al., 2015; Okada and Okawa, 1974; Schreiber et al. 2011; Yoshida et al., 2008).

There are specific fertilizer regimes that growers adhere to depending on the desired sepal color (Bailey, 1989; Dole and Wilkins, 2005; Landis and Whipker, 2017). Growers producing pink hydrangeas should maintain a pH of 6.0 to 6.5, use a fertilizer with 25N-4.4P-8.3K, and no Al should be supplied. To produce blue sepals, growers should apply up to 20 g  $\text{Al}_2(\text{SO}_4)_3$  (AS) split over three to four weekly applications. They will also use a fertilizer with 25N-2.2P-24.9K and maintain a pH of 5.5 (Bailey, 1989; Dole and Wilkins, 2005). Applying Al earlier (weeks 2 through 5) in the greenhouse production cycle was found to be more effective at producing blue sepals versus later (weeks 6 through 9) applications (Blom and Piot, 1992). The AS recommendation above is based on applying the AS as a drench. This process can be labor intensive for growers.

The applied Al will decrease the substrate pH (Shanks et al., 1950) which may cause a toxicity due to increased availability of micronutrients (Mengel and Kirkby, 2001). High levels of Al can also be toxic to some plants and inhibit root growth (Havlin et al., 2013). Although hydrangeas are considered tolerant to Al (Chen et al., 2015) the roots are sensitive to salt injury (Dole and Wilkins, 2004), which may result from AS drench applications.

Although studies have been conducted to find alternatives to AS drench application (Opena and Williams, 2002; Owen et al., 2013; Stoven and Owen, 2008), no results have been published indicating the effect of applying AS through a constant liquid fertilization. Therefore, this study was conducted to determine the effect of applying AS through a constant liquid fertilization on hydrangea sepal color.

## **Materials and Methods**

### **Controlled greenhouse study**

Vernalized, one year old, 'Early Blue', 'Hor Tivoli', 'Jip', and 'Mathilda Gutges' hydrangea plants were used in this experiment and transplanted 21 January 2018 (week 0). The 'Early Blue' and 'Hor Tivoli' were transplanted from 10 cm diameter pots into 15 cm diameter plastic pots. The 'Jip' and 'Mathilda Gutges' were transplanted from 15 cm diameter pots into 20 cm diameter plastic pots. Pots were filled with a soilless substrate composed of sphagnum peat moss amended with 20% (v/v) horticultural grade perlite (Sun Gro Horticulture, Agawam, MA). The pH was adjusted to 5.5 with the addition of 2.2 kg dolomitic lime (Rockydale, Roanoke, VA) and 600 g of wetting agent (Aquatrol, Paulsboro, NJ) per m<sup>3</sup>. We treated plants with 0, 9, 12, and 15 g of AS (Fischer Scientific, Fair Lane, NJ). The AS was applied as a constant liquid fertilization of 10 applications in 150 ml aliquots made over a five-week period (weeks 1-5) when the plants required irrigation. The 0 g plants were treated with the same volume of water without Al during weekly applications. The experiment was laid out in a complete randomized block design with 6 single pot replicates for each treatment. All plants were drip irrigated and fertilized with a constant liquid fertilizer at 150 mg·L<sup>-1</sup> N derived 13N-0.9P-10.8K Cal-Mag



(Everris, Marysville, OH) in order to provide a complete nutrient containing fertilizer. Plants were grown at 22 °C day/18 °C night air temperatures.

### **Substrate sample collection and analysis**

Substrate leachate was collected in a pour-thru solution extraction method (Cavins et al., 2005) from each replicate for each treatment. The pH and electrical conductivity (EC) were measured from weeks 5 through 8 of the 11-week production cycle. The pH and EC values of the pour-thru extracts were measured using a portable pH/EC meter (Hanna HI9813-6; Hanna Instruments, Woonsocket, RI).

### **Tissue sample collection and nutrient analysis**

For plant tissue analysis, leaves and sepals were sampled at full bloom (Week 11). The most recent mature leaves were harvested from each stem and the leaves were consolidated into single-plant replicates for each treatment rate. Leaves and sepals were initially rinsed with deionized (DI) water, then washed in a solution of 0.5 N HCl for 1 min. and again rinsed with DI water. Samples were dried (12–24 hr.) at 80 °C, then processed through a stainless-steel grinder (Wiley Mini-Mill; Thomas Scientific; Swedesboro, NJ) with a 20-mesh (1 mm) screen (Campbell and Plank 1992). Total concentrations of Al were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (Spectro Arcos EOP and Arcos II EOP, Spectro Analytical: A Division of Ametek; Mahwah, NJ), after closed-vessel Nitric acid (HNO<sub>3</sub>) digestion in a microwave digestion system (MARS 6 Microwaves; CEM Corp.; Matthews, NC).

## **Sepal color reading and analysis**

The sepal color (hue) value was determined at full bloom for two sepals on three different blooms for each replicate using a handheld colorimeter (PCE-RGB2 Color Meter; PCE Instruments, Meschede, Germany).

## **Statistical analysis**

The leaf Al concentration, sepal Al concentration, plant dry weight, and sepal color (hue) were analyzed using PROC GLM (version 9.4; SAS Inst. Inc., Cary, NC). Tukey's honestly significant differences (HSD) test at  $P \leq 0.05$  was used to determine the differences among mean values. Cultivars were analyzed separately for color and Al concentrations (Bailey, 1989) as the plant response to Al is cultivar specific (Epstein and Bloom, 2005).

## **Results**

### **Sepal color**

The application of AS as a constant liquid fertilization had a significant effect on sepal color (hue) ( $P \leq 0.0001$ ) for each cultivar. For each cultivar the application of AS as a constant liquid fertilization increased the level of blueness in sepal coloration (Fig. 1-3). For each cultivar the plants treated with 0 g of AS had significantly different hue values from all other treatments (Table 1). The hue value for plants treated with 9, 12, and 15 g of AS were similar the 'Early Blue' and 'Jip'. 'Hor Tivoli' developed more blue sepal coloration in plants treated with 15 g of AS and the hue value for these plants were significantly different from all other treatments. The 'Mathilda Gutges' treated with 15 g of AS had the most blue sepal hue value (240.4) and was significantly different from plants treated with 12 g.

## **Growth**

Applying AS as a constant liquid fertilization had a significant effect on plant dry weight ( $P \leq 0.001$ ) for each cultivar (Table 1). Plant growth was inhibited due to the AS applications and dry weight values were lower in plants treated with 9, 12, and 15 g of AS compared to plants receiving 0 g for all cultivars. The dry weights were similar for plants treated with 9, 12, and 15 g of AS, but the dry weights for these plants were significantly lower than plants receiving 0 g for each cultivar.

## **Sepal tissue aluminum concentration**

The effect of AS application as constant liquid fertilization had a significant effect on concentration of Al in the leaf tissue for all cultivars (Table 1). The Al concentration in leaves for 'Early Blue' and 'Jip' cultivars was significantly higher in plants treated with 9, 12, and 15 g of AS than plants treated with 0 g. The highest concentration of Al was found in the leaves of 'Hor Tivoli' plants treated with 15 g of AS and was significantly different from leaf Al concentration of plants treated with 0 g. The highest Al concentration in the leaves was found in 'Mathilda Gutges' plants treated with 15 g of AS. The Al concentration in the leaves of plants treated with 15 g of AS was significantly higher than plants treated with 0 and 9 g. All 'Jip' plants treated with AS had significantly higher leaf Al concentrations than the untreated controls, and the levels >1000 ppm were higher than any of the other cultivar tested.

### **Leaf tissue aluminum concentration**

The effect of AS application as constant liquid fertilization had a significant effect on concentration of Al in the leaf tissue for ‘Jip’, and ‘Mathilda Gutges’ ( $P \leq 0.0001$ ), Early Blue ( $P \leq 0.0002$ ), and ‘Hor Tivoli’ ( $P \leq 0.003$ ) (Table 1). The Al concentration in leaves for ‘Early Blue’ and ‘Jip’ cultivars was significantly higher ( $P \leq 0.05$ ) in plants treated with 9, 12, and 15 g of AS than plants treated with 0 g. The highest concentration of Al was found in the leaves of ‘Hor Tivoli’ plants treated with 15 g of AS and was significantly different from leaf Al concentration of plants treated with 0 g ( $P \leq 0.05$ ). The highest Al concentration in the leaves was found in ‘Mathilda Gutges’ plants treated with 15 g of AS. The Al concentration in the leaves of plants treated with 15 g of AS was significantly higher than plants treated with 0 and 9 g ( $P \leq 0.05$ ).

### **Substrate leachate pH and EC**

There was significant AS dose\*week sampled interaction ( $P \leq 0.05$ ) for both leachate pH and EC (Fig. 4 A and B). The pH was lower in the plants treated with 15 g of AS compared to other treatments, while plants receiving 0 g of AS had the highest pH for each week. The lower pH values were likely due the acidifying effect of AS application (Shanks et al., 1950). The pH generally increased over time for all treatments, which was to be expected because the final AS applications occurred during week 5. The EC values were higher each week in plants treated with 9, 12, or 15 g AS than plants receiving 0 g AS. The EC displayed a major decrease between weeks 5 and 6 for plants treated with 9, 12, and 15 g of AS. The higher EC at week 5 was likely due to recent and finale AS application and the decline reflects the discontinuation of the AS applications.

## **Conclusion**

The application of AS through a constant liquid fertilization enhanced blue sepal coloration among all cultivars. The higher Al concentrations in the sepal and leaf tissue correlated with bluer sepal coloration. These results are similar to AS drench application results (Blom and Piot, 1992). While higher AS applications inhibited growth this may not be problematic as some growers generally use plant growth regulators in hydrangea production (Bailey, 1989). The higher EC and lower pH values found in week 5 for plants treated with 9, 12, and 15 g of AS would suggest growers end AS applications at week 5 or provide a lower continual dose of AS over a longer time if AS application continues past week 5. Constant liquid fertilization of AS through the irrigation system resulted in enhanced blue coloration of hydrangeas. This production practice is less labor intensive than applying AS as a drench and offer a method for growers to still achieve blue coloration at lower cost.

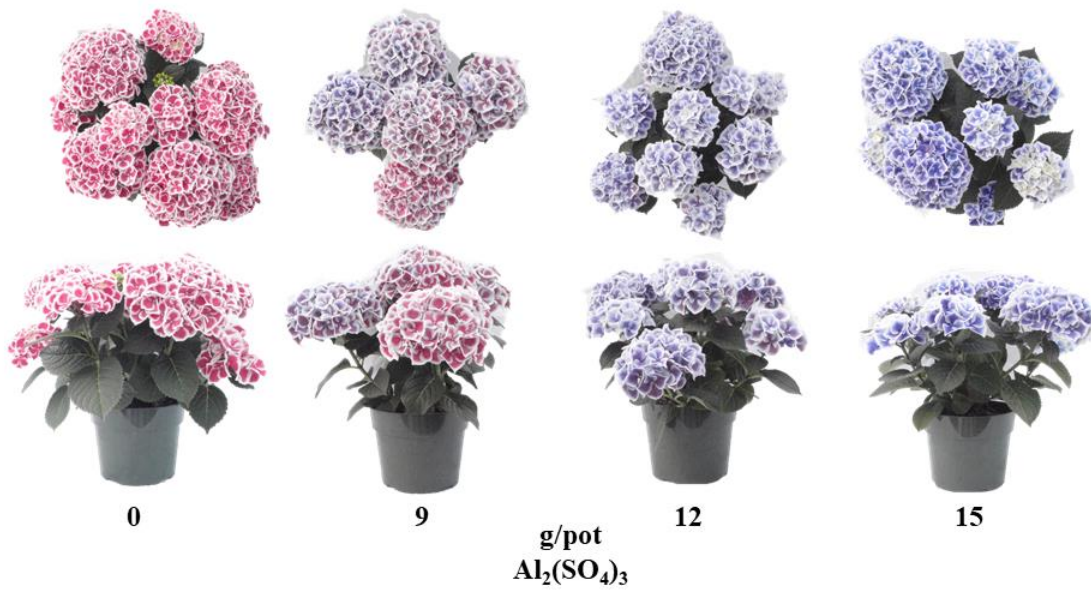
## **Acknowledgements**

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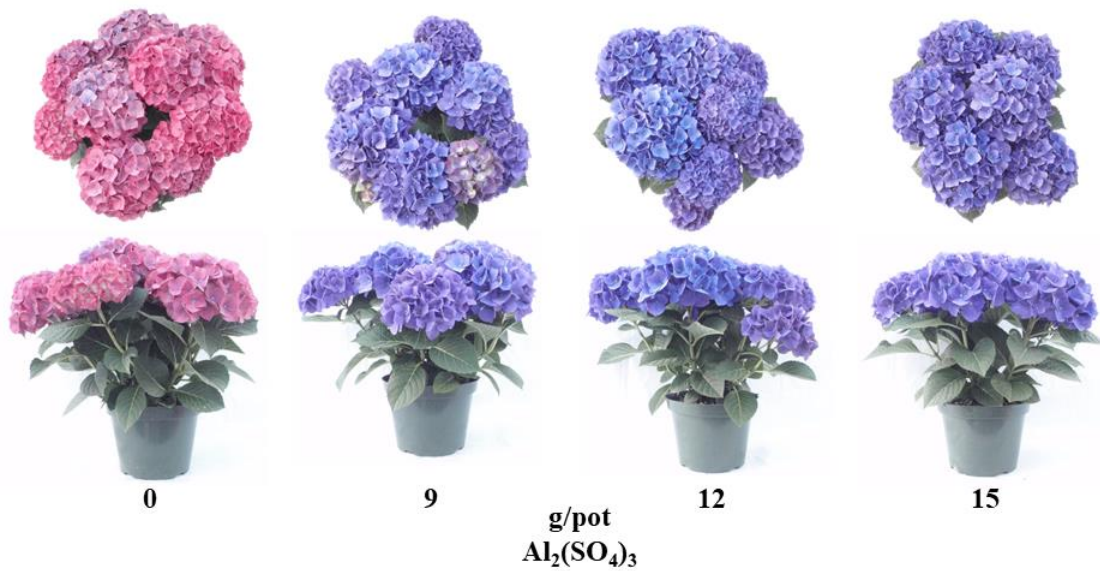
## Literature Cited

- Bailey, D.A. 1989. Hydrangea production. Growers handbook series, vol. 3. Timber Pres, Portland, OR.
- Blom, T.J. and B.D. Piott. 1992. Florists' hydrangea blueing with aluminum sulfate applications during forcing. *HortScience* 27.10: 1084-1087.
- Campbell C.R. and C.O. Plank. 1992. Sample preparation. In: Plank CO, editor. Plant analysis reference procedures for the southern region of the United States. Athens (GA): Georgia Cooperative Extension Service. p 1–12. Southern Cooperative Series Bulletin 368.
- Cavins, T.J., B.E. Whipker, and W. Fonteno. 2005. Timing of PourThru affects pH, electrical conductivity, and leachate volume. *Comm. Plant Soil Sci.* 36:1573-1581.
- Chen, H.C., Lu., and H. Jiang, and J. Peng. 2015. Global transcriptome analysis reveals distinct aluminum-tolerance pathways in the Al-accumulating species *Hydrangea macrophylla* and marker identification. *Plos One*, vol. 10, no. 12, doi:10.1371/journal.pone.0144927.
- Dole, J.M., and H. F. Wilkins. 2005. Floriculture: principles and species. Pearson Prentice Hall, Saddle River, NJ.
- Epstein E., and A.J. Bloom. 2005. Mineral nutrition of plants: principles and perspectives. Sinauer Associates, Sunderland, MA.
- Havlin, J., S.L. Tisdale, W.L. Nelson, and J.D. Beaton. 2013. Soil fertility and fertilizers. Pearson Prentice Hall. Saddle River, NJ.
- Mengel K. and E.A. Kirkby. 2001. Principles of plant nutrition. Kluwer Academic Publishers. Dordrecht, The Netherlands.

- Okada, M., and K. Okawa. 1974. The quantity of aluminum and phosphorus in plants and its influence on the sepal color of *Hydrangea macrophylla*. J. Jpn. Soc. Hort. Sci. 42: 361-70. Web.
- Opena G.B. and K.A. Williams. 2002. Use of precharged zeolite to provide aluminum during blue hydrangea production. J. of Plant Nutrition, 26:9,1825-1840, DOI: 10.1081/PLN-120023286
- Owen, J.S. Jr., K.A. Williams, H.M. Stoven, J. Craver, J. Brindley. 2013. Bluing of hydrangea 'Endless Summer' sepals is influenced by timing of aluminum sulfate drenches or aluminum chelate foliar sprays in three different locations and production systems. ASHS Annual Conference Poster Board #020
- Shanks, J. B., J.R. Haun, and C.B. Links. 1950. A preliminary study on the mineral nutrition of hydrangeas. Proc. Amer. Soc. Hort. Sci. 56:457-565
- Schreiber, H. D., A.H. Jones, A.M. Lariviere, K.M. Mayhew, J.D. Cain. 2011. Role of aluminum in red-to-blue color changes in *Hydrangea macrophylla* sepals. BioMetals. 24:1005–1015., doi:10.1007/s10534-011-9458-x.
- Stoven H.M. and J.S. Owen Jr.. 2008. Comparison of substrate amendments for the adjustment of hydrangea (*Hydrangea macrophylla* (THUNB.) SER 'Bailmer', Endless Summer®) flower color. Proc. So. Nur. Res. Conf. 53: 32-35.
- Takeda, K., M. Kariuda, and H. Itoi. 1985. Blueing of sepal color of *Hydrangea macrophylla*. Phytochemistry 24:2251-2254.
- Yoshida, K., D. Ito, Y. Shinkai, and T. Kondo. 2008. Change of color and components in sepals of Chameleon hydrangea during maturation and senescence. Phytochemistry, 69:3159–3165. doi:10.1016/j.phytochem.2008.02.024.



**Figure 1.** The influence of increasing concentrations of constant liquid fertilization  $\text{Al}_2(\text{SO}_4)_3$  on 'Hor Tivoli' hydrangea plant growth and sepal color.

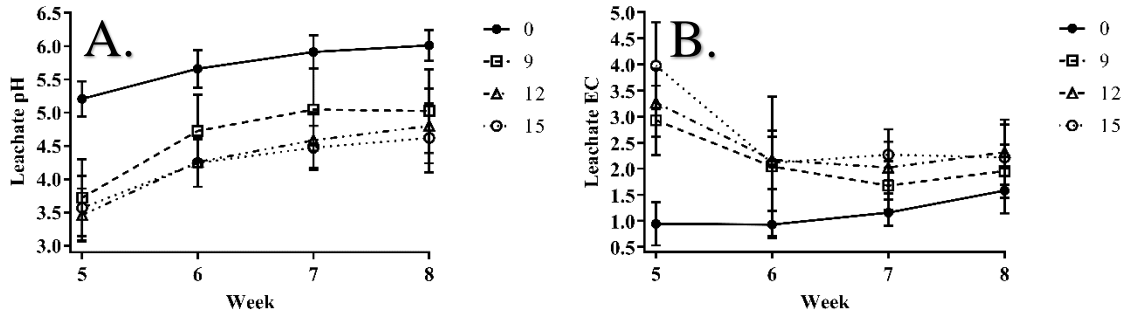


**Figure 2.** The influence of increasing concentrations of constant liquid fertilization  $\text{Al}_2(\text{SO}_4)_3$  on 'Jip' hydrangea plant growth and sepal color.





**Figure 3.** The influence of increasing concentrations of constant liquid fertilization  $\text{Al}_2(\text{SO}_4)_3$  on 'Mathilda Gutges' hydrangea plant growth and sepal color.



**Figure 4.** The influence of increasing concentrations of constant liquid fertilization  $\text{Al}_2(\text{SO}_4)_3$  on leachate pH and EC for hydrangea. There was a significant week\* $\text{Al}_2(\text{SO}_4)_3$  dose interaction ( $P \leq 0.05$ ) for pH and EC.

**Table 1.** The influence of increasing  $\text{Al}_2(\text{SO}_4)_3$  constant fertilization on sepal color, plant growth, leaf and sepal tissue Al concentration at full bloom of four hydrangea cultivars.

Parameter	$\text{Al}_2(\text{SO}_4)_3$ Dose (g/pot)	Cultivar			
		‘Early Blue’	‘Hor Tivoli’	‘Jip’	‘Mathilda Gutges’
Sepal Color (Hue)	0	337.6 a <sup>y</sup>	341.9 a	314.0 a	334.1 a
	9	293.1 b	293.7 b	234.3 b	247.1 bc
	12	280.1 b	280.9 b	233.1 b	251.3 b
	15	278.2 b	260.8 c	236.3 b	240.4 c
	Significance <sup>z</sup>	***	***	***	***
Dry Weight (g)	0	49.5 a	49.5 a	51.4 a	98.2 a
	9	27.6 b	27.6 b	38.3 b	78.8 b
	12	27.9 b	26.9 b	36.7 b	70.2 b
	15	24.5 b	24.5 b	39.3 b	66.5 b
	Significance	***	***	***	***
Sepal Al (ppm)	0	24.6 a	35.9 a	75.4 a	13.1 a
	9	250.9 b	160.3 ab	525.5 b	280.7 bc
	12	255.2 b	192.0 ab	494.2 b	247.3 b
	15	280.7 b	320.0 b	459.0 b	324.5 c
	Significance	***	**	***	***
Leaf Al (ppm)	0	29.4 a	8.4 a	144.7 a	8.3 a
	9	566.8 b	191.1 a	1308.8 b	568.8 b
	12	698.7 b	303.3 ab	1242.5 b	700.5 bc
	15	780.7 b	566.5 b	1368.3 b	898.2 c
	Significance	**	**	***	***

<sup>z</sup> \*, \*\*, or \*\*\* indicates statistically significant differences in sample based on *t* test at  $P \leq 0.05$ ,  $P \leq 0.01$ , or  $P \leq 0.001$ , respectively.

<sup>y</sup> Lower case letters signify significant differences among all  $\text{Al}_2(\text{SO}_4)_3$  concentrations. Means with different letters are significantly different at  $P \leq 0.05$ .

### **Chapter 3: Incorporating Slow Release Coated Polymer Aluminum Sulfate into the Substrate to Produce Blue Hydrangeas**

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## **Incorporating Slow Release Coated Polymer Aluminum Sulfate into the Substrate to Produce Blue Hydrangeas**

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### **Abstract**

Hydrangeas (*Hydrangea macrophylla*) contain an anthocyanin pigment within the sepal that naturally produces a pink color. In the presence of aluminum (Al) the pigment will bind with the Al producing a blue or purple color. The current method for producing blue hydrangeas is to apply 4 weekly applications of aluminum sulfate ( $\text{Al}_2(\text{SO}_4)_3$ ) as a liquid drench. The purpose of this study was to determine if a slow release coated polymer  $\text{Al}_2(\text{SO}_4)_3$  could be used to produce the desired blue hydrangeas as a standalone alternative to  $\text{Al}_2(\text{SO}_4)_3$  drench applications. To complete this study 'Early Blue Rose' hydrangeas were grown in 15 cm pots with 0, 5, 10, 20, and 40 grams of slow release  $\text{Al}_2(\text{SO}_4)_3$  (15% Al) incorporated into the substrate. There were 5 replications of each treatment. The substrate pH and EC were monitored every other week by pour-thru extraction, as well as the leaf tissue nutrient concentrations. Plants were visually analyzed for marketable color at the end of the study. The untreated and the plants that received 5 g of  $\text{Al}_2(\text{SO}_4)_3$  produced bright pink sepals. The plants that received 10 g of  $\text{Al}_2(\text{SO}_4)_3$  displayed a mix of pink and purple sepals and had optimal plant growth. Plants treated with 20 g of  $\text{Al}_2(\text{SO}_4)_3$  displayed primarily purple sepals and growth was inhibited. The highest application of 40 grams of  $\text{Al}_2(\text{SO}_4)_3$  inhibited plant growth and produced necrosis on most of the leaves. The use of a slow release coated polymer aluminum sulfate applied at these rates did not produce the desired blue hydrangea. Based on this study the use of the slow release coated

polymer aluminum sulfate at these specific concentrations with ‘Early Blue Rose’ hydrangeas should not be used as the sole method of Al application to produce blue sepals.

**Keywords:** slow release fertilizer, substrate, anthocyanin, aluminum, *Hydrangea macrophylla*

## **Introduction**

Greenhouse growers begin the process of forcing vernalized hydrangeas in the winter to market the crop for spring holidays. Plants are moved from cold storage into the greenhouse at this time and the forcing cycle last 10-14 weeks depending on the cultivar. Non-white hydrangeas contain an anthocyanin pigment that will cause the sepal to be either blue or pink depending on the concentration of aluminum (Al) in the leaf tissue (Bailey, 1989). Higher levels of Al in the tissue will produce blue sepals while low Al will produce red or pink sepals. Therefore, growers manage the substrate to ensure a high or low level of Al depending on the desired color (Bailey, 1989).

Some hydrangeas are pretreated with Al prior to being placed in cold storage. Both pretreated and untreated hydrangeas can be managed to produce blue sepals. The general practice is to make four weekly aluminum sulfate ( $\text{Al}_2(\text{SO}_4)_3$ ) applications as a drench in the first five weeks after removal from cold storage. The current recommended rate is 1.2 to 1.8 kg of  $\text{Al}_2(\text{SO}_4)_3$  per 100 L of water (Dole and Wilkins, 2005). Over application of  $\text{Al}_2(\text{SO}_4)_3$  can cause stunted growth and phytotoxicity to the plant, while under application may produce an undesirable color (Landis and Whipker, 2017).

Applying Al to the substrate does not guarantee that the plant will produce blue sepals, due to potential interaction with other substrate properties. For instance, substrate pH, as well as the

level of phosphorus (P) and potassium (K) in the substrate impact the availability of Al and the final color of the sepal. Growers who are producing pink hydrangeas maintain a pH of 5.8-6.2, and supply higher levels of P and low levels of K. When blue hydrangeas are desired a pH of 5.2-5.5 is recommended and lower levels of P and higher levels of K are provided to the plant (Landis and Whipker, 2017). The practice of applying  $\text{Al}_2(\text{SO}_4)_3$  in weekly intervals can be labor intensive and can produce unmarketable plants if not done correctly. The purpose of this study was to determine if a one-time application of a slow release polymer coated  $\text{Al}_2(\text{SO}_4)_3$  could produce the desired blue hydrangea.

## **Materials and Methods**

### **Controlled greenhouse study**

Vernalized, 1 year old, 'Early Blue Rose' hydrangea plants were transplanted from 10-cm diameter pots into 15-cm diameter plastic pots. Pots were filled with a soilless substrate composed of sphagnum peat moss amended with 20% (v/v) horticultural grade perlite (Sun Gro Horticulture, Agawam, MA). The pH was adjusted to 5.5 with the addition of 2.2 kg dolomitic lime (Rockydale, Roanoke, VA) and 600 g of wetting agent (Aquatrol; Paulsboro, NJ) per  $\text{m}^3$ . In addition, 0, 5, 10, 20, or 40 grams per pot of slow release  $\text{Al}_2(\text{SO}_4)_3$  (15% Al; Florikote Sapphire 90-day Polymer Coated  $\text{Al}_2(\text{SO}_4)_3$ ; Florikan, Sarasota, FL) was incorporated into the substrate. Florikan recommends a dose of 10 grams per pot. The rates for this study were determined as 0x, .5x, 1x, 2x, and 4x the recommended rate. There were 5 replications of each treatment. Plants were fertilized at a rate of  $150 \text{ mg}\cdot\text{L}^{-1}$  N using a liquid fertilizer 13 N-2  $\text{P}_2\text{O}_5$ -13  $\text{K}_2\text{O}$  Cal-Mag (Everris, Marysville, OH) in order to provide a complete nutrient feed. The plants were irrigated as needed with a drip system utilizing injectors. Plants were grown at 22 °C day/18 °C night air

temperatures.

### **Sample collection and preparation**

Substrate nutrient content samples were collected every other week for the 10 week production cycle beginning two weeks after the  $\text{Al}_2(\text{SO}_4)_3$  had been incorporated into the substrate. Substrate nutrient data was collected in a pour-thru solution extraction method (LeBude and Bilderback, 2009) from all five replicates for each treatment. The solution samples were frozen after collection and thawed to room temperature prior to analysis. Total concentrations of P, K, and Al were determined on ~10 mL homogenized sample, filtered using acid washed filter paper (Laboratory Filtration Group, Houston, TX) with Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (Spectro Arcos EOP and Arcos II EOP, Spectro Analytical: A Division of Ametek; Mahwah, NJ) (Donohue and Aho 1992; adapted USEPA 2001). The pH values of the pour-through extracts were measured using a hydrogen electrode (Orion 920A; Thermo Fisher Scientific; Beverly, MA). The electrical conductivity (EC) values were measured using a conductivity meter (Orion 550A; Thermo Fisher Scientific; Beverly, MA). Results of EC are expressed as mS/cm.

For plant tissue analysis, the most recent mature leaves were harvested from each stem and the leaves were consolidated into single-plant replicates for each treatment rate. At the final harvest, sepals were also collected from each plant for tissue analysis. Leaves and sepals were initially rinsed with deionized (DI) water, then washed in a solution of 0.5 N HCl for 1 min and again rinsed with DI water. Prior to analysis, samples were dried (12–24 hr) at 80 °C, then processed through a stainless steel grinder (Wiley Mini-Mill; Thomas Scientific; Swedesboro, NJ) with a 20-mesh (1 mm) screen (Campbell and Plank 1992). Total concentrations of P, K,



and Al, were determined by digesting a 0.5 g dried/ground subsample with 10 mL 15.6N HNO<sub>3</sub> for 30 minutes in a closed-vessel microwave digestion system (MARS 6 Microwaves; CEM Corp.; Matthews, NC) (Campbell and Plank 1992). The sample volume was brought to 50 mL with deionized water and then filtered through acid-washed filter paper (Laboratory Filtration Group, Houston, TX) prior to measurement. Elements were quantified by ICP-OES as previously described. Elements are expressed either as a percentage (%) or in parts per million (ppm) [as milligrams per kilogram (mg/L)] on a dry-weight basis.

In order to determine how growth was impacted by treatments, the height, two diameters, and whole plant dry weights were measured at week 10. The data was analyzed using PROC MIXED and PROC GLM using SAS (version 9.2; SAS Institute, Cary, NC).

## **Results and Discussion**

There was a negative correlation between increasing Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> concentration and all plant growth measurements (Table 1; Fig. 1). Plant heights were similar for the 0 g and 5 g treatments. Plants that were treated with 10 g were 20% shorter than the plants receiving 0 g. Plant canopy diameters were significantly less than the controls at rates  $\geq 5$  g Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>. Plants treated with 5 g of Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> had an average diameter 13% smaller than the untreated plants. The plant dry weights were also significantly less than the controls at rates  $\geq 5$  g Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>. The plants treated with 5 g had 13% smaller dry weights than the untreated plants.

There was a positive correlation between increasing Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> application rates and the Al tissue concentration at week 10 (Table 1). There was no significant difference in the Al tissue concentrations among the 0 g, 5 g, and 10 g treated plants. There was significant difference in Al tissue values at the rates of  $\geq 20$  g. The mean Al tissue values for the 20 g at week 10 were

1191.6 ppm (ranged from 585 to 2360 ppm) and for 40 g were 1612.6 ppm (ranged from 931 to 2790 ppm). These levels were slightly lower than levels reported by Blom and Piott (1992) of 1250-1960 ppm Al for producing blue sepals. The 20 g and 40 g rates did not consistently produce the desired blue sepal colors, while causing significant plant stunting and occasional plant death. All other nutrient levels in the leaf tissue were within the recommended ranges reported by Bryson et al. (2014) (Data not shown), except for lower phosphorus (P) and potassium (K) levels. Low P was intentionally supplied to the plant to avoid interference with Al uptake.

There was a positive correlation between increasing  $\text{Al}_2(\text{SO}_4)_3$  application rates and the Al sepal concentration. The sepal Al concentration levels were not significantly different between the 0 g and the 5 g. Sepal Al concentrations in plants treated with 10 g and 20 g  $\text{Al}_2(\text{SO}_4)_3$  were 197.2 ppm and 370.2 ppm, respectively (Table 1). While a deep blue color was not consistently produced at these rates, these sepal tissue Al concentrations did correspond with findings that >100 ppm Al produce blue sepals (Toyama-Kato et al., 2003). This would suggest that Al sepal concentration is not reliable indicator of sepal color for this variety when using this product.

There was a positive correlation between  $\text{Al}_2(\text{SO}_4)_3$  and pour-through solution EC (Fig. 2). The product released relatively high rates of Al in the first four weeks and the soluble Al levels decreased significantly thereafter (Fig. 3). The rapid Al release caused a marked increase in EC levels for the 20 g and 40 g treatments. The elevated EC levels caused observable root burn on the plants and contributed to plant stunting and phytotoxicity.

A visual observation of the sepal colors at full bloom revealed the following information. The plants receiving 0 g and 5 g produced bright pink sepals while the 10 g produced multicolor

pink and purple sepals. The plants receiving 20 g produced purple sepals and the 40 g produced the bluest sepal, but resulted in nonmarketable low quality plant.

## **Conclusion**

The product released Al early in the production cycle causing the pH to decrease below the desired range and the EC to exceed safe levels for plants. Incorporation of the recommended rate did not produce the desired color, while the increased rate resulted in stunting and phytotoxicity (Fig. 4). The incorporation of Florikote Sapphire Polymer Coated Aluminum Sulfate is not recommended as a method to produce deep blue robust hydrangeas with this variety. This product could be used to produce multi-color sepals at the 10 g rate when growers have a market for a multi-color hydrangea (Fig. 5).

## **Acknowledgements**

We would like to thank the Agronomic Division of the North Carolina Department of Agriculture and Consumer Services and the Fred C. Gloeckner Foundation for their support of this research project.

## Literature Cited

- [AOAC] Association of Official Analytical Chemists (1990). Official methods of analysis. 15th ed. Arlington, Va: AOAC International, p.341.
- Bailey, D. (1989). Hydrangea production. Portland, Ore.: Timber Press, pp.1-24.
- Ball, V. (1998). Ball Redbook. Batavia, Ill.: Ball Publishing, pp.553-558.
- Blom, T. and Piott, B. (1992). Florists' Hydrangea Blueing with Aluminum Sulfate Applications during Forcing. HortScience, 27(10), pp.1084-1087.
- Bryson, G.M. and H.A. Mills. (2014). Plant analysis handbook IV. Micro-Macro Publ., Athens, GA.
- Campbell C.R. 1992. Determination of total nitrogen in plant tissue by combustion. In: Plank CO, editor. Plant analysis reference procedures for the southern region of the United States. Athens (GA): Georgia Cooperative Extension Service. p 20–22. Southern Cooperative Series Bulletin 368.
- Campbell C.R. and Plank C.O. (1992). Sample preparation. In: Plank CO, editor. Plant analysis reference procedures for the southern region of the United States. Athens (GA): Georgia Cooperative Extension Service. p 1–12. Southern Cooperative Series Bulletin 368.
- Dole, J. and Wilkins, H. (2005). Floriculture principles and species. 2nd ed. New Jersey: Prentice Hall, pp.598-602.
- Donohue S.J., Aho D.W. (1992). Determination of P, K, Ca, Mg, Mn, Fe, Al, B, Cu, and Zn in plant tissue by inductively coupled plasma (ICP) emission spectroscopy. In: C.O. Plank, editor. Plant analysis reference procedures for the southern region of the United States. Athens (GA): Georgia Cooperative Extension Service. p 34–7. Southern Cooperative Series Bulletin 368.

- Eaton A.D., Clesceri L.S., Rice E.W., Greenberg A.E., editors. (2005). SM 4500 H+B: pH value in water by potentiometry using a standard hydrogen electrode. In: Standard methods for the examination of water and wastewater. 21st ed. Washington (DC): Public Health Association, Water Environment Federation, and American Water Works Association. p. 1368.
- Kempers A.J., Luft A.G. (1988). Re-examination of the determination of environmental nitrate by reduction with hydrazine. *Analyst* 113: pp.1117–20.
- Krom M. 1980. Spectrophotometric determination of ammonia: a study of modified Berthelot reaction using salicylate and dichloroisocyanurate. *Analyst* 105: pp.305–16.
- LeBude, A. and Bliderback, T. (2009). The Pour-Thru Extraction Procedure: A nutrient Management Tool for Nursery Crops. North Carolina Cooperative Extension.
- Landis, H., and Whipker B.W. "Nutrient Management Strategies for Ensuring Blue Coloration of Greenhouse Hydrangeas." *E-GRO Alert* 6.4 (2017): 1-6.
- Skalar Analytical B.V. (1995). Issue 6. Nitrate + Nitrite. In: The SAN<sup>++</sup> segmented flow analyzer water analysis. Breda (The Netherlands): Skalar Analytical B.V. pp. 190–2.
- Skalar Analytical B.V. (1995). Issue 6. Ammonia. In: The SAN<sup>++</sup> segmented flow analyzer water analysis. Breda (The Netherlands): Skalar Analytical B.V. pp. 73–5.
- Skalar Analytical B.V. (1995). Issue 6. Chloride. In: The SAN<sup>++</sup> segmented flow analyzer water analysis. Breda (The Netherlands): Skalar Analytical B.V. pp. 103-105.
- Toyama-Kato, Y., Yoshida, K., Fujimori, E., Haraguchi, H., Shimizu, Y. and Kondo, T. (2003). Analysis of metal elements of hydrangea sepals at various growing stages by ICP-AES. *Biochemical Engineering Journal*, 14(3), pp.237-241.

[USEPA] United States Environmental Protection Agency. (1983). Method 120.1. Conductance (specific conductance,  $\mu\text{mhos/cm}$  at 25 °C). In: Methods for chemical analysis of water and wastes. Cincinnati (OH): USEPA Office of Research and Development. EPA/600/4-79-020.

[USEPA] United States Environmental Protection Agency. (2001). Method 200.7. Trace elements in water, solids, and biosolids by inductively coupled plasma–atomic spectrometry, revision 5.0. Cincinnati (OH): USEPA Office of Research and Development. EPA-821-R-01-010.

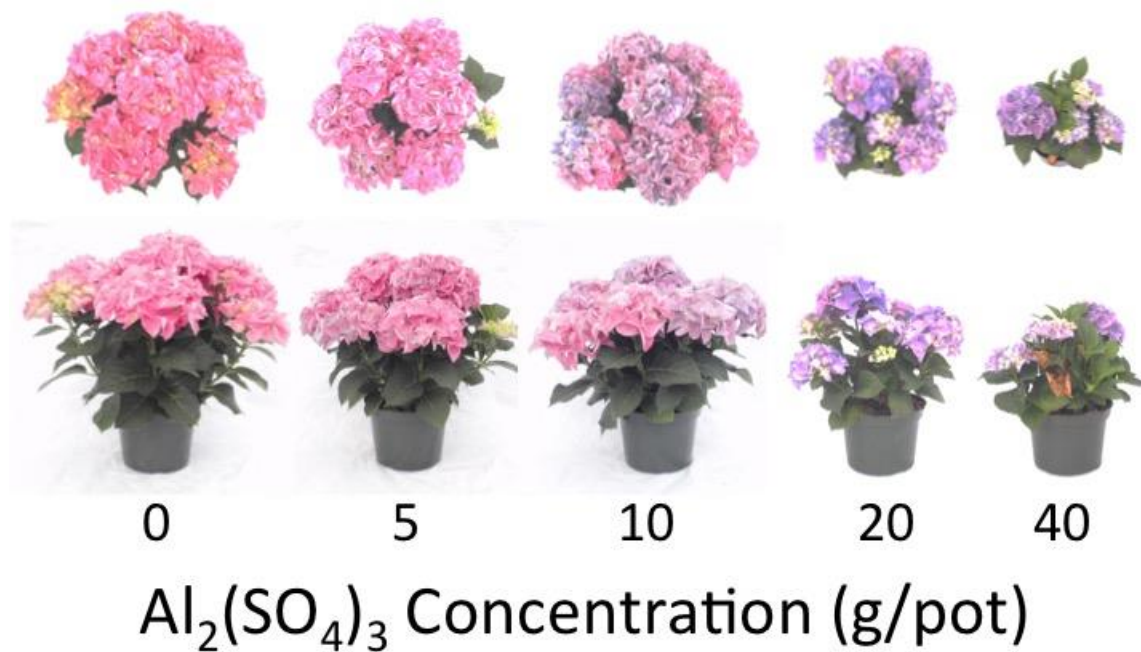
Zall D.M., Fisher D., Garner M.Q. (1956). Photometric determination of chloride in water. Anal Chem 28: pp. 1665–8.

**Table 1.** Influence of slow release  $\text{Al}_2(\text{SO}_4)_3$  applications on hydrangea plant size values and leaf tissue and sepal Al concentrations.

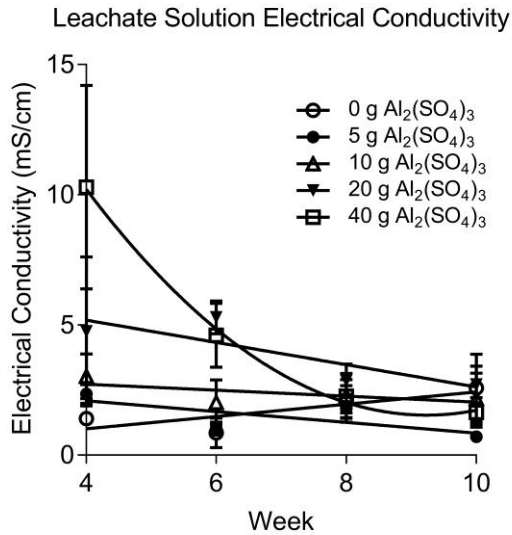
Concentration of $\text{Al}_2(\text{SO}_4)_3$ (g)	Plant Height (cm)	Plant Diameter (cm)	Plant Dry Weight (g)	Leaf Tissue Al Concentration (mg/kg)	Sepal Al Concentration (mg/kg)
0	27.1±1.9 <sup>2</sup>	39.6± 2.6a	118.8±1.8a	3.2±.99a	7.4±2.8a
5	25.5±.99a b	34.4± 2.3b	103.2±2.4b	53.4±25a	55.7±23ab
10	21.8±1.7b	33.0± 2.5bc	98.8±3.2bc	272.6±134.9a	197.2±87.2bc
20	20.2±1.9b	29.2± 2.9cd	87.6±4.3cd	1191.6±719.3b	370.8±164c
40	15.5±2.8c	25.9± 3.8d	77.6±3.6d	1612.6±771.8b	336.4±85.6c
Significance <sup>1</sup>	***	***	***	**	***
R <sup>2</sup>	0.83	0.87	0.87	0.54	0.76

<sup>1</sup> \*, \*\*, or \*\*\* indicates statistically significant differences between sample means based on *F* test at  $P \leq 0.05$ ,  $P \leq 0.01$ , or  $P \leq 0.001$ , respectively. NS (not significant) indicates the *F* test difference between sample means was  $P > 0.05$ .

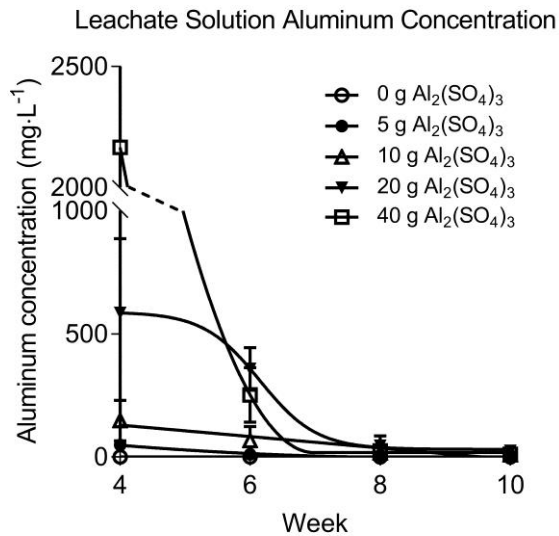
<sup>2</sup> Lower case letters signify significant differences among all  $\text{Al}_2(\text{SO}_4)_3$  concentrations. Means with different letters are significantly different at  $P \leq 0.05$ .



**Figure 1.** The influence of increasing concentrations of substrate applied slow release  $\text{Al}_2(\text{SO}_4)_3$  on hydrangea plant growth and flowering.



**Figure 2.** The influence of increasing concentrations of substrate applied slow release  $\text{Al}_2(\text{SO}_4)_3$  on the substrate solution electrical conductivity (EC) levels over time for hydrangeas. Each data point is an average of five replicate measurements.



**Figure 3.** The influence of increasing concentrations of substrate applied slow release  $\text{Al}_2(\text{SO}_4)_3$  on the substrate solution Al concentrations levels over time for hydrangeas. Each data point is an average of five replicate measurements.





**Figure 4.** Phytotoxicity which results when an excessive ( $\geq 20$  g/pot) amount of slow release  $\text{Al}_2(\text{SO}_4)_3$  was incorporated into the substrate. (Left: untreated plant; Right: 40 g/pot of  $\text{Al}_2(\text{SO}_4)_3$  added)



**Figure 5.** Multi-colored sepal colors resulted when 10 g/pot of slow release  $\text{Al}_2(\text{SO}_4)_3$  was incorporated into the substrate.

## **Chapter 4: Aluminum Sulfate Applications Intensifies Red Hydrangea Sepal Coloration**

## **Aluminum Sulfate Applications Intensifies Red Hydrangea Sepal Coloration**

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### **Abstract**

Hydrangeas (*Hydrangea macrophylla*) contain an anthocyanin pigment within the sepal that naturally produces a pink color. In the presence of aluminum (Al) the pigment will bind with the Al producing a blue or purple color. As the effect of Al on plants is cultivar specific, this study was conducted to determine the effect of Al application doses on the sepal color of two red hydrangea cultivars. In year 1, 'Hot Red' plants were treated with 0, 1.2, 1.8, 2.4, and 3.0 g/L of  $\text{Al}_2(\text{SO}_4)_3$  (AS) applied as a constant liquid feed at each irrigation throughout the entire production process. In year 2, 'Curly Sparkle' were treated with 0, 9, 12, and 15 g of AS. The AS was applied as a constant liquid feed of 10 applications in 150 ml aliquots made over a five-week period (weeks 1-5) when the plants required irrigation. Applying AS increased the level blue sepal coloration of red hydrangeas in both "Hot Red" and 'Curly Sparkle'. The highest concentration of Al in the leaf and sepal tissue generally corresponded with greater blue sepal coloration in both cultivars, although the highest AS dose did not consistently produce the most blue sepal coloration. The desired plant size should also be considered as the application of AS and had a negative impact on plant dry weight for both cultivars. The application of AS affected the plant dry weight, substrate pH and EC for both cultivars as well as tissue nutrient concentration in the 'Curly Sparkle'. Therefore, growers should determine the level of blueness desired when deciding the dose of AS to be applied to red colored hydrangeas.

**Keywords:** substrate, anthocyanin, aluminum, *Hydrangea macrophylla*

## **Introduction**

Florists' hydrangeas [*Hydrangea macrophylla* subsp. *Macrophylla* var. *macrophylla* (Thunb.)], native to Japan, are a significant crop to the greenhouse industry. They are produced for their vibrant sepal colors of pink, blue, red, or white from early spring to late fall (Bailey, 1989; Dole and Wilkins, 2005). The pink, red, or blue hydrangea cultivars contain an anthocyanin pigment (delphinidin-3-glucoside) that gives the sepal its color (Takeda et al., 1985). The natural color of the pigment is a pink or red depending on the cultivar when aluminum (Al) is not supplied. When supplemental Al applications are made it will bond with the anthocyanin causing the sepal to have a bluish hue and the mechanics of this biochemical interaction has been extensively investigated (Chen et al., 2015; Okada and Okawa, 1974; Schreiber et al., 2011; Yoshida et al., 2008).

The effect of Al applications to control sepal color of potted hydrangea has been examined considerably with a focus on blue or pink sepals (Blom and Piot, 1992; Owen et al., 2013; Opena and Williams, 2002; Shanks et al., 1950; Stoven and Owen, 2008), but minimal focus has been on red hydrangeas. Kodama et al. (2016) found an increase in blue color when red hydrangeas were grown in acidic soil compared to alkaline soils. The Al applied to enhance sepal will also decrease the substrate pH (Shanks et al., 1950) which affects nutrient availability (Havlin et al., 2013) as well nutrient tissue concentrations in the plant (Bryson and Mills, 2014). As the effect of Al on plants is cultivar specific (Epstein and Bloom, 2005), it is important to have a better understanding of Al effect on specific hydrangea cultivars. This study was conducted to determine the effect of Al application doses on the sepal color two red hydrangea cultivars.

## Materials and Methods

### Controlled greenhouse study

There were two experiments conducted with different red cultivars of vernalized, one year old, hydrangeas. The ‘Hot Red’ cultivar was transplanted 28 April 2017 (week 0) as a preliminary study and ‘Curly Sparkle’ was transplanted 21 January 2018 (week 0) as a follow up study. Both cultivars were transplanted from 10 cm diameter pots into 15 cm diameter plastic pots filled with a soilless substrate composed of sphagnum peat moss amended with 20% (v/v) horticultural grade perlite (Sun Gro Horticulture, Agawam, MA). The pH was adjusted to 5.5 with the addition of 2.2 kg dolomitic lime (Rockydale, Roanoke, VA) and 600 g of wetting agent (Aquatrol, Paulsboro, NJ) per m<sup>3</sup>. The ‘Hot Red’ plants were treated with 0, 1.2, 1.8, 2.4, and 3.0 g/L of Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> (AS) (Fischer Scientific, Fair Lane, NJ) applied as a constant liquid feed at each irrigation throughout the entire production season. There were 5 replicates of each treatment and the experiment was completely randomized. ‘Curly Sparkle’ plants were treated with 0, 9, 12, and 15 g of AS. The AS was applied as a constant liquid feed of 10 applications in 150 ml aliquots made over a five-week period (weeks 1-5) when the plants required irrigation. The 0 g plants were treated with the same volume of water without Al during weekly applications for both cultivars. There were 6 single pot replicates of ‘Curly Sparkle’, and the study was laid out in a complete randomized block design. Both cultivars were drip irrigated and fertilized with a constant liquid fertilizer at 150 mg·L<sup>-1</sup> N derived 13N–0.9P–10.8K Cal-Mag (Everris, Marysville, OH) in order to provide a complete set of nutrients. Plants were grown at 22 °C day/18 °C night air temperatures.

### **Substrate sample collection and analysis**

Substrate leachate was collected in a pour-thru solution extraction method (Cavins et al., 2005) from each replicate for each treatment. The pH and electrical conductivity (EC) were measured at the final harvest for 'Hot Red' and weeks 5 through 8 for 'Curly Sparkle'. The pH and EC values of the pour-through extracts were measured using a portable pH/EC meter (Hanna HI9813-6; Hanna Instruments, Woonsocket, RI).

### **Tissue sample collection and aluminum analysis**

For plant tissue analysis, the most recent mature leaves were harvested from each stem at final harvest for 'Hot Red' and weeks through 8 for 'Curly Sparkle'. Sepals were collected at final harvest for both cultivars. Tissue samples were consolidated into single-plant replicates for each treatment dose. They were initially rinsed with deionized (DI) water, then washed in a solution of 0.5 N HCl for 1 min. and again rinsed with DI water. Samples were dried (12–24 hr.) at 80 °C, then processed through a stainless-steel grinder (Wiley Mini-Mill; Thomas Scientific; Swedesboro, NJ) with a 20-mesh (1 mm) screen (Campbell and Plank 1992). The leaves and sepals for the 'Hot Red' were analyzed for Al and a complete nutrient analysis was completed for the 'Curly Sparkle'. Total N concentration was determined by oxygen combustion gas chromatography with an elemental analyzer (NA1500s2; CE Elantech Instruments; Lakewood, NJ) (AOAC 1990; Campbell 1992) on a 5-7 mg aliquot of the dried and ground sample. Total concentrations of P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, and Al were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (Spectro Arcos EOP and Arcos II EOP, Spectro Analytical: A Division of Ametek; Mahwah, NJ), after closed-vessel Nitric acid

(HNO<sub>3</sub>) digestion in a microwave digestion system (MARS 6 Microwaves; CEM Corp.; Matthews, NC).

### **Sepal color reading and analysis**

The sepal color (hue) value was calculated for ‘Hot Red’ based on the measured L\* (level of lightness), a\* (level of redness), and b\* (level of blueness) values. The L\*a\*b\* values were measured at full bloom for three sepals on three different blooms for each replicate of the ‘Hot Red’ using a handheld colorimeter (ColorTec-PCM; Pittsford, NY). Hue values were determined on two sepals on three different blooms for each replicate of the ‘Curly Sparkle’ using a handheld colorimeter (PCE-RGB2 Color Meter; PCE Instruments, Meschede, Germany).

### **Experimental arrangement and statistical analysis**

The sepal and leaf tissue Al were analyzed using SAS PROC GLM (version 9.4; SAS Inst. Inc., Cary, NC) for both cultivars. The ‘Hot Red’ experiment was completely randomized. The experiment with ‘Curly Sparkle’ contained a factorial arrangement with AS dose (4 levels), and sampling week (4 levels) as factors. To determine if significant interactions were present among these factors (AS dose, and week) for leachate pH and EC and leaf tissue Al concentration PROC GLM was used. The sepal color (hue) for both cultivars was analyzed using PROC GLM. Tukey’s honestly significant differences (HSD) test at  $P \leq 0.05$  was used to determine the difference in hue among different AS treatments. The HSD was used to determine any significant differences among blocks for the ‘Curly Sparkle’ experiment. There were no blocks that were significantly different from all other blocks for the hue data. Therefore, data among blocks was pooled.

## Results

### Sepal color

The effect of AS applications on ‘Hot Red’ (Table 1) sepal color was not significant for hue values, but  $a^*$  (level of redness) and  $b^*$  (level of blueness) values were both significant ( $P \leq 0.001$ ). Higher  $a^*$  values indicate greater redness while lower  $b^*$  values indicate greater blueness. The sepals displayed a decrease in redness (lower  $a^*$  values) as AI dose increased and an increase in blueness (lower  $b^*$  values) as AI dose increased (Fig. 1). The plants treated with 0 g had the highest level of redness (41.9) and lowest level of blueness (84.6) (Fig. 1). The plants treated with 2.4 g/L had the greatest blueness level (11.4) and was significantly different from all other treatments ( $P \leq 0.05$ ).

The effect on hue value due to AS applications was significant ( $P \leq 0.0001$ ) for ‘Curly Sparkle’ (Table 2). The hue for ‘Curly sparkle’ had the reddest sepals with 0 g (345.3), while the 9 g and 15 g had sepals with the most blue coloration (Fig. 2) with hue values of 304.0 and 305.7 respectively. The 9 and 15 g hue values were similar to each other and both were significantly different from plants treated with 0 g.

### Plant dry weight

The effect on dry weight was significant due to AS applications on both cultivars of red hydrangeas, ‘Hot Red’ ( $P \leq 0.05$ ) (Table 1) and ‘Curly Sparkle’ ( $P \leq 0.001$ ) (Table 2). ‘Hot Red’ plants treated with 0 g/L had the highest dry weight, while plants treated with 1.2, 1.8, 2.4, and 3.0 g/L were smaller. There was negative correlation between AS application and dry weight for ‘Curly Sparkle’. The ‘Curly Sparkle’ plants treated with 9, 12, and 15 g of AS were similar to each other and significantly smaller than plants treated 0 g of AS.



### **Sepal tissue aluminum concentration**

There was a positive correlation between AS application and sepal Al concentration for ‘Hot Red’ plants (Table 1). Plants treated with 2.4 and 3.0 g/L had the highest Al concentration in the sepal of 620.2 and 658.6 ppm respectively. The sepal Al concentrations for the plants treated with 2.4 g/L and 3.0 were similar to each other and significantly different from all other treatments. The effect of AS applications on sepal Al concentration was also significant for ‘Curly Sparkle’ ( $P \leq 0.001$ ) (Table 2). The Al concentration in the sepal tissue was highest for the 9 g (248.0 ppm), but was similar to the 12 (183.8 ppm) and 15 g (207.3 ppm). The Al concentration in the sepal of plants treated with 0 g (12.1 ppm) was significantly different from all other treatments.

### **Leaf tissue aluminum concentration**

There was a positive correlation between AS application and leaf Al concentration for the ‘Hot Red’ (Table 1). Plants treated with 2.4 and 3.0 g/L had the highest Al concentration in the leaves of 589.4 and 646.0 ppm respectively. The leaf Al concentrations for the plants treated with 2.4 g/L and 3.0 were similar to each other and significantly different from all other treatments. The effect of AS applications on leaf Al concentration was also significant for ‘Curly Sparkle’ ( $P \leq 0.001$ ) (Table 2). The Al concentration in the leaf tissue was highest for the 9 g (592.8) but was similar to the 12 (374.0 ppm) and 15 g (406.8 ppm). The Al concentration in the leaves of plants treated with 0 g (8.94 ppm) was significantly different from all other treatments.

## **Leachate pH and EC**

There was a negative correlation between AS applied and leachate pH for the ‘Hot Red’ (Table 1). The lowest pH values were found in plants treated with 1.8 (3.58), 2.4 (3.56), and 3.0 (3.50) g/L. These values were similar to each other and significantly different from all other treatments. The effect of AS application on EC was not significant for ‘Hot Red’ and values ranged from 3.13 to 4.50 (Table 1). The pH and EC were collected from weeks 5 through 8 of production for ‘Curly Sparkle’. There was a significant interaction between AS dose and the week the leachate was collected ( $P \leq 0.0001$ ) for both pH and EC. The pH was lower in plants treated with 9, 12, and 15 g of AS (Fig. 3A) due to the acidifying effect of AS applications (Shanks et al., 1950). The EC was higher with increasing doses of AS (Fig. 3B) and decreased over time in these treatments. The higher EC likely contributed to the lower plant dry weights (Table 2).

## **Leaf tissue nutrient concentration**

The concentration of nutrients was determined in the leaves for ‘Curly Sparkle’ during weeks 5 through 8 of production. There was no significant week\*dose interaction for all nutrient, but dose (Table 2) and week (Table 3) were significant as separate variables. The N, P, K, Ca, Mg, S concentrations were significantly higher in plants treated with 0 g AS than plants treated with 9, 12, and 15 g (Table 2). The micronutrients were generally higher in plants treated with 9, 12, and 15 g than plants treated with 0 g. The micronutrients were likely more available to the plants due to lower pH (Fig. 3A) (Havlin et al., 2013). The Zn, Cu, and Al concentrations were similar in the 9, 12, and 15 g and were significantly higher than plants treated with 0 g of

AS (Table 2). The N, P, S, Fe, Zn, and Cu concentrations generally decreased in the leaf tissue over time. The K, Ca, Mg, Mn, B, and Al generally increased over time (Table 3).

## **Conclusion**

Applying AS contributed to increasing the level blue sepal coloration of red hydrangeas in both “Hot Red” and ‘Curly Sparkle’ hydrangeas. The highest concentration of Al in the leaf and sepal tissue generally corresponded with greater blue sepal coloration in both cultivars. The highest AS application dose did not consistently produce the most blue sepal coloration in both cultivars. Therefore, growers should determine the level of desired blueness when determining the dose of AS to be applied. The desired plant size should also be considered as the application of AS and had a negative impact on plant dry weight for both cultivars. The application of AS affected the substrate pH and EC for both cultivars as well as tissue nutrient concentration in the ‘Curly Sparkle’. The pH, EC, and tissue concentrations can be used to monitor plant nutrition by growers producing these cultivars.

## **Acknowledgements**

We would like to thank the Agronomic Division of the North Carolina Department of Agriculture and Consumer Services and the Fred C. Gloeckner Foundation for their support of this research project.

## Literature Cited

- Association of Official Analytical Chemists [AOAC]. 1990. Official methods of analysis. 15th ed. Arlington, VA: AOAC International, p.341.
- Bailey, D.A. 1989. Hydrangea production. Growers handbook series, vol 3. Timber Pres, Portland, OR.
- Blom, T.J. and B.D. Piott, 1992. Florists' hydrangea blueing with aluminum sulfate applications during forcing. HortScience 27.10: 1084-1087.
- Bryson, G.M., and H.A. Mills. 2014. Plant analysis handbook IV: a guide to sampling, preparation, analysis, interpretation and use of results of agronomic and horticultural crop plant tissue. Micro-Macro, Athens, GA.
- Campbell C.R. 1992. Determination of total nitrogen in plant tissue by combustion. In: Plank CO, editor. Plant analysis reference procedures for the southern region of the United States. Athens (GA): Georgia Cooperative Extension Service. p 20–22. Southern Cooperative Series Bulletin 368.
- Campbell C.R. and C.O. Plank. 1992. Sample preparation. In: Plank CO, editor. Plant analysis reference procedures for the southern region of the United States. Athens (GA): Georgia Cooperative Extension Service. p 1–12. Southern Cooperative Series Bulletin 368.
- Cavins, T.J., B.E. Whipker, and W. Fonteno. 2005. Timing of PourThru affects pH, electrical conductivity, and leachate volume. Comm. Plant Soil Sci. 36:1573-1581.
- Chen, H.C., Lu., and H. Jiang, and J. Peng. 2015. Global transcriptome analysis reveals distinct aluminum-tolerance pathways in the Al-accumulating species *Hydrangea macrophylla* and marker identification. *Plos One*, vol. 10, no. 12, doi:10.1371/journal.pone.0144927.
- Dole, J.M., and H. F. Wilkins. 2005. Floriculture: principles and species. Pearson Prentice Hall, Saddle River, NJ.

- Epstein E., and A.J. Bloom. 2005. Mineral nutrition of plants: principles and perspectives.  
Sinauer Associates, Sunderland, MA.
- Havlin, J., S.L. Tisdale, W.L. Nelson, and J.D. Beaton. 2013. Soil fertility and fertilizers.  
Pearson Prentice Hall. Saddle River, NJ.
- Kodama, M., Y. Tanabe, and M. Nakayama, 2016. Analyses of coloration-related components in  
hydrangea sepals causing color variability according to soil conditions. Hort. J.  
85:10.2503/hortj.MI-131.
- Okada, M., and K. Okawa. 1974. The quantity of aluminum and phosphorus in plants and its  
influence on the sepal color of *Hydrangea macrophylla*. J. Jpn. Soc. Hort. Sci. 42: 361-  
70. Web.
- Opena G.B. and K.A. Williams, 2002. Use of precharged zeolite to provide aluminum during  
blue hydrangea production. J. of Plant Nutrition, 26:9,1825-1840, DOI: 10.1081/PLN-  
120023286
- Owen, J.S. Jr., K.A. Williams, H.M. Stoven, J. Craver, J. Brindley. 2013. Bluing of hydrangea  
'Endless Summer' sepals is influenced by timing of aluminum sulfate drenches or  
aluminum chelate foliar sprays in three different locations and production systems. ASHS  
Annual Conference Poster Board #020
- Shanks, J. B., J.R. Haun, and C.B. Links, 1950. A preliminary study on the mineral nutrition of  
hydrangeas. Proc. Amer. Soc. Hort. Sci. 56:457-565
- Schreiber, H. D., A.H. Jones, A.M. Lariviere, K.M. Mayhew, J.D. Cain, 2011. Role of aluminum  
in red-to-blue color changes in *Hydrangea macrophylla* sepals. BioMetals. 24:1005-  
1015., doi:10.1007/s10534-011-9458-x.

- Stoven H.M. and J.S. Owen Jr., 2008. Comparison of substrate amendments for the adjustment of hydrangea (*Hydrangea macrophylla* (THUNB.) SER 'Bailmer', Endless Summer®) flower color. Proc. So. Nur. Res. Conf. 53: 32-35.
- Takeda, K., M. Kariuda, and H. Itoi. 1985. Blueing of sepal color of *Hydrangea macrophylla*. Phytochemistry 24:2251-2254.
- Yoshida, K., D. Ito, Y. Shinkai, and T. Kondo. 2008. Change of color and components in sepals of Chameleon hydrangea during maturation and senescence. Phytochemistry, 69:3159–3165. doi:10.1016/j.phytochem.2008.02.024.

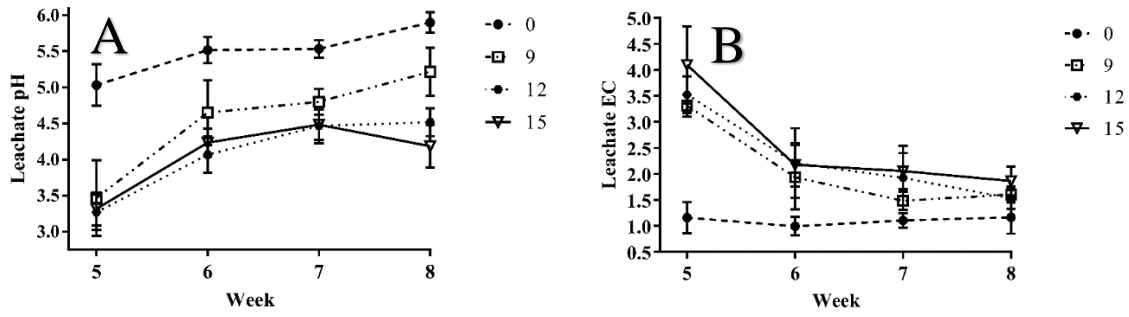


**Figure 1.** The influence of increasing concentrations of constant liquid feed  $\text{Al}_2(\text{SO}_4)_3$  on red hydrangea ‘Hot Red’ plant growth and sepal color.



**Figure 2.** The influence of increasing concentrations of constant liquid feed  $\text{Al}_2(\text{SO}_4)_3$  on red hydrangea 'Curly Sparkle' plant growth and sepal color.





**Figure 3.** The influence of increasing concentrations of constant liquid feed  $\text{Al}_2(\text{SO}_4)_3$  on leachate pH and EC for red hydrangea ‘Curly Sparkle’. There was a significant week\* $\text{Al}_2(\text{SO}_4)_3$  dose interaction ( $P \leq 0.0001$ ) for pH and EC.

**Table 1.** The influence of increasing  $\text{Al}_2(\text{SO}_4)_3$  constant fertilization on ‘Hot Red’ hydrangea sepal color, plant growth, leaf and sepal tissue Al concentration, pH and electrical conductivity at final harvest.

Variable	$\text{Al}_2(\text{SO}_4)_3$ Dose (g/L)					Significance <sup>z</sup>
	0	1.2	1.8	2.4	3.0	
Sepal Color (Hue)	13.8 ab <sup>y</sup>	11.0 b	12.7 b	33.6 a	11.9 b	NS
a*	84.6 a	75.6 b	70.0 c	67.4 d	67.8 d	***
b*	20.7 a	14.7 b	15.6 b	11.4 c	14.2 b	***
Dry Weight (g)	24.0 a	17.4 b	18.2 ab	19.9 ab	18.5 ab	*
Sepal Al (ppm)	9.6 a	237.6 b	396.6 c	620.2 d	658.6 d	***
Leaf Al (ppm) Week 11	2.5 a	97.9 ab	248.2 b	589.4 d	646.0 d	***
Leachate pH	4.68 a	3.82 b	3.58 c	3.56 c	3.50 c	***
Leachate EC	3.13 a	4.24 ab	3.96 ab	4.50 b	3.13 a	NS

<sup>z</sup> \*, \*\*, or \*\*\* indicates statistically significant differences in sample means based on *t* test at  $P \leq 0.05$ ,  $P \leq 0.01$ , or  $P \leq 0.001$ , respectively. NS (not significant) indicates the *t* test difference between sample means was  $P > 0.05$ .

<sup>y</sup> Lower case letters signify significant differences among all  $\text{Al}_2(\text{SO}_4)_3$  concentrations. Means with different letters are significantly different at  $P \leq 0.05$ .

**Table 2.** The influence of increasing  $\text{Al}_2(\text{SO}_4)_3$  dose of on ‘Curly Sparkle’ hydrangea sepal color, plant growth, leaf and sepal tissue Al concentration at final harvest, and leaf tissue nutrient concentration during production.

Variable	$\text{Al}_2(\text{SO}_4)_3$ Dose (g/pot)				Significance <sup>z</sup>
	0	9	12	15	
<b>Harvest</b>					
Sepal Color (Hue)	345.3 a <sup>y</sup>	304.0 c	320.6 b	305.7 c	***
Dry Weight (g)	38.95 a	24.05 b	23.24 b	27.90 b	***
Sepal Al (ppm)	12.1 a	248.0 b	183.8 b	207.3 b	***
Leaf Al (ppm)	8.9 a	592.8 b	374.0 b	406.8 b	***
<b>Production</b>					
N (%)	3.34 a	2.78 b	2.82 b	2.88 b	***
P (%)	0.22 a	0.09 c	0.10 bc	0.12 b	***
K (%)	2.79 a	2.58 b	2.57 b	2.70 ab	***
Ca (%)	0.80 a	0.60 b	0.58 b	0.59 b	***
Mg (%)	0.33 a	0.23 b	0.24 b	0.25 b	***
S (%)	0.19 a	0.21 b	0.23 b	0.23 b	***
Mn (ppm)	34.0 a	36.3 ab	40.9 bc	43.8 c	***
Zn (ppm)	23.0 a	31.9 bc	29.0 b	33.2 c	***
Cu (ppm)	4.7 a	6.6 c	6.0 bc	5.4 ab	***
B (ppm)	35.1 ab	33.6 b	33.2 b	38.2 a	**
Al (ppm)	21.1 a	341.3 b	375.3 b	314.6 b	***

<sup>z</sup>\*, \*\*, or \*\*\* indicates statistically significant differences in sample based on *t* test at  $P \leq 0.05$ ,  $P \leq 0.01$ , or  $P \leq 0.001$ , respectively.

<sup>y</sup> Lower case letters signify significant differences among all  $\text{Al}_2(\text{SO}_4)_3$  concentrations. Means with different letters are significantly different at  $P \leq 0.05$ .

**Table 3.** The plant tissue nutrient concentrations over time for red hydrangea ‘Curly Sparkle’.

Element	Week				Significance <sup>z</sup>
	5	6	7	8	
N (%)	3.10 a <sup>y</sup>	2.95 ab	3.00 a	2.77 b	***
P (%)	0.15 a	0.13 b	0.12 b	0.14 ab	***
K (%)	2.64 a	2.71 a	2.57 a	2.72 a	*
Ca (%)	0.57 a	0.60 ab	0.66 b	0.75 c	***
Mg (%)	0.25 ab	0.24 a	0.29 c	0.27 bc	***
S (%)	0.24 a	0.20 b	0.24 a	0.19 b	***
Fe (ppm)	54.4 a	42.8 bc	44.5 b	40.0 c	***
Mn (ppm)	33.4 a	38.3 ab	38.7 b	44.5 c	***
Zn (ppm)	34.1 a	25.5 b	30.7 a	26.4 b	***
Cu (ppm)	6.5 a	5.9 ab	5.1 b	5.3 b	***
B (ppm)	29.3 a	32.4 a	36.4 b	42.0 c	**
Al (ppm)	227.6 a	231.7 a	323.7 a	269.2 a	*

<sup>z</sup> \*, \*\*, or \*\*\* indicates statistically significant differences in sample means based on *t* test at  $P \leq 0.05$ ,  $P \leq 0.01$ , or  $P \leq 0.001$ , respectively.

<sup>y</sup> Lower case letters signify significant differences among all  $\text{Al}_2(\text{SO}_4)_3$  concentrations. Means with different letters are significantly different at  $P \leq 0.05$ .