Characterization of *Hydrangea macrophylla* Cultivars by the Anthocyanin Content in their Sepals[1]

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Abstract

In order to ascertain the role of anthocyanin content on the color and brightness of *Hydrangea macrophylla* sepals, anthocyanin contents of different colored sepals were measured for numerous commercial cultivars. Anthocyanin contents were primarily determined by extraction of the pigment, then measurement by differential spectrophotometry. Concentrations ranged from about 25 to over 400 µg delphinidin-3-glucoside per g of fresh sepal, with the magnitude roughly proportional to the perceived intensity of sepal coloration for that *Hydrangea macrophylla* cultivar. However, the anthocyanin content was independent of the sepal color, being the same for red, purple, or blue sepals of the same cultivar. Even though significant sepal-to-sepal variation in color intensity existed within a single inflorescence, the extractable anthocyanin content was constant for a specific cultivar. Accordingly, *Hydrangea macrophylla* cultivars were classified in terms of their color brightness or anthocyanin content in sepals as blush (very light colored, 25 to 60 $\mu g \cdot g^{-1}$), cold-hardy (light colored, 80 to 120 $\mu g \cdot g^{-1}$), classic (medium colored, 140 to 190 $\mu g \cdot g^{-1}$), vivid (deep colored, 230 to 270 $\mu g \cdot g^{-1}$), or vibrant (very deep colored, >300 $\mu g \cdot g^{-1}$) at peak bloom. The anthocyanin content of the sepals steadily increased as the inflorescence approached peak bloom, remained constant for a week or more, then decreased. The cultivar-dependent anthocyanin contents of the sepals can be used, in part, to rationalize the relative bluing capability of the various *Hydrangea macrophylla* cultivars.

Index words: anthocyanin, hydrangea, sepals, cultivar classifications, bluing, delphinidin-3-glucoside.

Species used in this study: *Hydrangea macrophylla*.

Significance to the Nursery Industry

The brightness of the red, purple, and blue colors in the sepals of inflorescences on *Hydrangea macrophylla* cultivars is controlled by the anthocyanin content unique to each cultivar. This study has quantified the anthocyanin content of each cultivar, and has thus categorized the cultivars in terms of the brightness or intensity of color in their sepals. If one assigns a scale from very light colored to very deep colored for sepal brightness, examples of representative cultivars range from the very light colored of 'Blushing Bride' and 'Regula', to the light colored of 'Penny Mac' and 'Endless Summer', to the medium colored of 'Red Star' and 'Blue Danube', to the deep colored of the 'Hamburg' and 'Pia', and finally to the very deep colored of 'Forever Pink' and 'Kardinal'. Sepals of a specific cultivar show the same color intensity or anthocyanin content regardless of its red, purple, or blue color. This quantification allows nurseries to market specific *Hydrangea macrophylla* cultivars by the plant's color intensity of sepals, as customer or landscaper preferences for hydrangea inflorescences may range from pale or pastel color intensity to dark or vibrant brightness.

The anthocyanin content of the sepals may also, in part, help define the threshold aluminum content of soil needed to change the sepals of *Hydrangea macrophylla* inflorescences from red to blue. Gardeners want to avoid adding more aluminum sulfate than necessary for sepal bluing, because of potential toxicity to neighboring plants. Bluing requires the aluminum content in the sepals to be in a specific molar excess of the anthocyanin content (12, 27). Indeed, this study argues that different cultivars may require different levels of aluminum sulfate to change sepals from red to blue depending on their anthocyanin content.

Introduction

Hydrangea macrophylla is a landscape shrub that continues to grow in popularity with the introduction of commercially-available remontant and cold-hardy cultivars, as well as cultivars with interesting or novel sepal colors. With a profusion of color from its many and showy inflorescences, *Hydrangea macrophylla* is often the focal point

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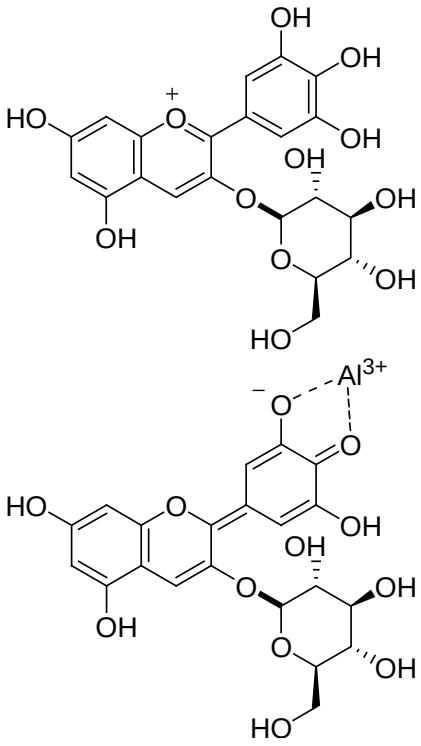

Fig. 1. Red flavylium cation of delphinidin-3-glucoside (TOP) and the aluminum complex with delphinidin-3-glucoside in one resonance form of its blue quinoidal base anion (BOTTOM).

of many summer gardens. Gardeners can even control the color of these inflorescences, as the sepal colors on many *Hydrangea macrophylla* cultivars are sensitive to the soil pH. The sepals comprising the inflorescences are red for plants grown in basic soils, but blue for those in acidic soils. Soils of intermediate pH can lead to a continuous spectrum of sepal colors from lavender to purple to violet, enhancing the charm of *Hydrangea macrophylla* with its color variability.

The pigment providing the red color to sepals of all *Hydrangea macrophylla* cultivars is the anthocyanin delphinidin-3-glucoside (34). Sepals change color to blue when aluminum, as Al^{3+}, complexes with this anthocyanin (34). Only in acidic soil is aluminum mobile, allowing Al^{3+} to be transported through the roots to the sepals of the hydrangea. Accordingly, although the sepal color of *Hydrangea macrophylla* acts as an *in situ* soil pH indicator, the sepal colors are merely reflecting the availability of Al^{3+} to the plant.

At a cellular level, the vacuoles of the red sepals contain delphinidin-3-glucoside in its red flavylium cationic form. In the presence of Al^{3+}, the flavylium cation loses two hydrogen ions and transforms to the blue quinoidal base anion of the delphinidin-3-glucoside, which then forms a complex with the Al^{3+} (Fig. 1). This primary complex then acts as a template for stacking either another flavylium cation, which results in a bathochromic shift of its absorbance and enhances the bluing, or a co-pigment (27). The Al^{3+} content has to be in an appropriate excess over the delphinidin-3-glucoside for this bluing mechanism to be operational (27).

Although the role of Al^{3+} in the bluing of *Hydrangea macrophylla* sepals has been established (14), some have questioned whether aluminum is the sole cause for bluing (21). For example, the vacuolar pH (35) and co-pigment profiles (12) for red sepals differ from those of blue sepals. Other studies (2, 24) have also reported that the concentration of delphinidin-3-glucoside is lower in blue than in red sepals. Nevertheless, recent studies (12) have measured about the same concentrations of this anthocyanin in both red and blue sepals. In order to model this red to blue transition, yet other studies (14, 27) have assumed the concentration of delphinidin-3-glucoside to be about the same in sepals of both colors.

A preliminary study (21) has identified unique anthocyanin contents in sepals of specific cultivars of *Hydrangea macrophylla*, leading to the potential to classify the various hydrangea cultivars by their sepals' anthocyanin content. Such a systematic ordering of the *Hydrangea macrophylla* cultivars with respect to delphinidin-3-glucoside content in their sepals may define a subsequent ordering of these cultivars with respect to ease of bluing. The cultivars with lower anthocyanin contents might be expected to be easier to change their sepal color from red to blue than those with higher anthocyanin contents, as less Al^{3+} would be necessary to have an appropriate molar excess of Al^{3+} over the anthocyanin.

The principal objective of this study was to determine the delphinidin-3-glucoside content of red, blue, and purple sepals of representative *Hydrangea macrophylla* cultivars in order to ascertain whether specific cultivars or particular colors can be characterized by their sepals' anthocyanin contents. In addition, we investigated certain characteristics of the sepal coloration; that is, the variation of anthocyanin content from sepal-to-sepal within an inflorescence as well as the changes in anthocyanin content with bloom stage.

Materials and Methods

Most *Hydrangea macrophylla* cultivars were obtained from Hydrangeas Plus® (VanHoose Enterprises LLC, Aurora, OR), although some were acquired from Wayside Gardens (Hodges, SC) and the Center for Applied Nursery Research (Dearing, GA). Some inflorescences were harvested from stock planted in containers using Sta-Green Nursery Blend Tree and Shrub Planting Mix (soil pH ≈ 7.0). Sepal colors were adjusted by chemical additives such as lime (20 g Ca per kg dry soil, soil pH ≈ 7.5), aluminum sulfate (650 mg Al per kg dry soil, soil pH ≈ 6.0; 1300 mg Al per kg dry soil, soil pH ≈ 5.2), and equimolar aluminum sulfate – citric acid mixes (soil pH ≈ 0.5 unit lower than that with just aluminum sulfate additions). Other inflorescences were harvested from mature *Hydrangea macrophylla* shrubs of various ages grown in the gardens (soil pH ≈ 6.0 to 6.5) at BackCountry Research (Rockbridge County, VA). Osmocote® was used as a fertilizer for all hydrangea plants, whether grown in container or garden. Samples of inflorescences were obtained during the summers of the seven-year period from 2004 through 2010.

The extractable anthocyanin content of *Hydrangea macrophylla* sepals was determined by a procedure (21) based on a standard method of differential spectrophotometry (10). After the anthocyanin was extracted into an acidified methanol solution, the peak and baseline absorbances (at 533 nm and 700 nm, respectively) of extract aliquots in two pH buffers (of pH 1 as 0.025 M KCl and of pH 4.5 as 0.400 M $NaC_2H_3O_2$) were measured. This differential absorbance was then related to the anthocyanin content, expressed as

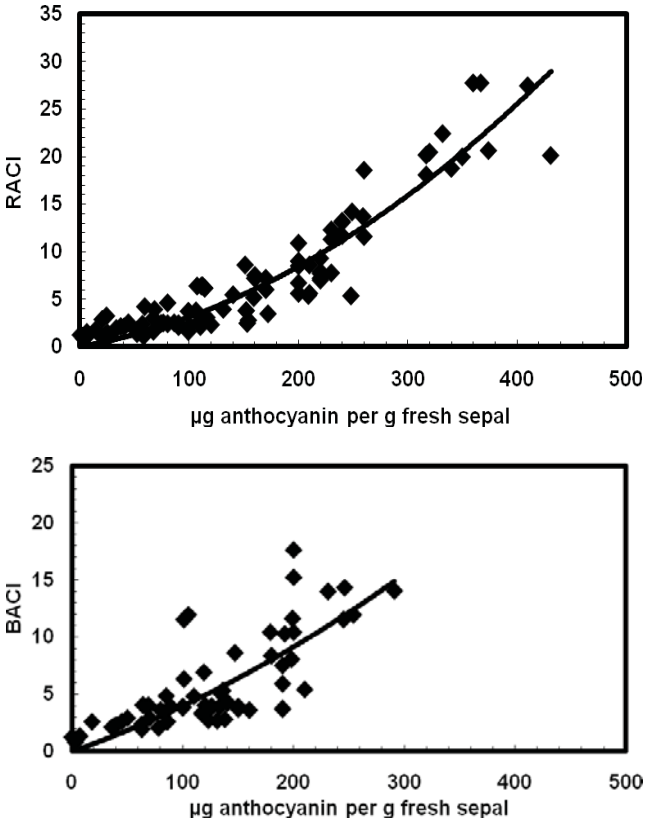

Fig. 2. Red anthocyanin content index, RACI, for red sepals (TOP), and blue anthocyanin content index, BACI, for blue sepals (BOTTOM) calibrated to the extractable anthocyanin content of *Hydrangea macrophylla* sepals. RACI and BACI are unitless absorbance indices. Trend lines are drawn as a guide to the eye.

µg delphinidin-3-glucoside per g of fresh sepal. Extraction efficiency of the anthocyanin from the sepals was assumed to be 100%, with the assumption confirmed by standard delphinidin and cyanidin additions. The extractable anthocyanin content was always determined from sepals of inflorescences harvested at the peak of blooming (maximum color intensity), or alternatively defined as stage III blooms (31) or a fully-open flower (26). Peak bloom was ascertained by visual observation.

Prior to extracting the anthocyanin from the sepals, the anthocyanin contents of *Hydrangea macrophylla* sepals were also determined using field-portable CCM-200 Chlorophyll Content Meters (OptiSciences, Tyngsboro, MA), appropriately adapted to measure the relative anthocyanin content (28). Two separate meters, one measuring the red anthocyanin content index (RACI) and one the blue anthocyanin content index (BACI), were calibrated to the extractable anthocyanin content of red and blue sepals, respectively (Fig. 2). Whereas a prior study (28) showed linear calibrations, the cumulative results in Fig. 2 indicate some curvature in these relationships. Nevertheless, the calibrations provide confidence not only in the reported determinations by the extraction/measurement methods but also that the meters are capable of accurate non-destructive measurements of the anthocyanin content of *Hydrangea macrophylla* sepals. Accordingly, the calibrated meters have been used in the field in this and prior studies to rapidly measure anthocyanin contents (28).

Results and Discussion

Extractable anthocyanin content. Table 1 summarizes the extractable anthocyanin contents of the sepals for selected *Hydrangea macrophylla* cultivars with red/pink, purple, and blue inflorescences. For a specific cultivar, the sepals' anthocyanin content did not vary with plant source, whether grown in a container or in the garden, For example, the anthocyanin content was 110 ± 20 µg·g^{-1} fresh sepal for four 'Endless Summer' inflorescences harvested from pots during summer 2010, and 115 ± 20 µg·g^{-1} fresh sepal for seven 'Endless Summer' inflorescences harvested from the garden during the same time period. The soil pH only controlled the color of the sepal, not the anthocyanin content, regardless of the

Table 1. Extractable anthocyanin content (µg delphinidin-3-glucoside per g of fresh sepal) of sepals for *Hydrangea macrophylla* cultivars. The number of separate inflorescences analyzed of a specific color is provided in parentheses. The average value includes analyses of all colors. Average standard deviation in all analyses is ± 20 µg·g^{-1}. Variation from inflorescence-to-inflorescence of a particular cultivar is about the same as the year-to-year variation shown in Table 2.

	[anthocyanin], µg·g^{-1} fresh sepal			
Cultivar	**Pink-red**	**Purple**	**Blue**	**Avg.**
Lanarth White	—			4
——— blush (very light colored) ↓ ———				
Blushing Bride[z]	27 (1)			27
Regula[z]	50 (2)			50
VanHoose White[z]	50 (1)			50
Lilacina[z]	60 (3)			60
——— remontant and cold-hardy (light colored) ↓ ———				
Penny Mac	70 (13)	90 (3)	100 (8)	80
Nikko Blue	80 (8)	100 (1)	80 (6)	80
Dooley	70 (1)	—	120 (2)	100
Endless Summer™	115 (18)	90 (11)	100 (17)	105
David Ramsey	100 (1)	—	110 (4)	110
General Vicomtesse de Vibraye	80 (1)	120 (5)	130 (8)	120
All Summer Beauty	100 (4)	—	130 (8)	120
Forever and Ever	120 (2)	—	—	120
——— classic (medium colored) ↓ ———				
Bottstein	140 (5)	—	—	140
Blue Danube	140 (13)	190 (8)	150 (9)	160
Red Star	160 (2)	160 (1)	—	160
Blauer Zwerg	190 (5)	—	120 (1)	180
Mathilda Gutges	200 (3)	150 (1)	200 (1)	190
Tovelit	190 (3)	—	—	190
——— vivid (deep colored) ↓ ———				
Eisvogel	—	—	210 (2)	210
Alpengluhen	230 (2)	—	—	230
Hamburg	230 (1)	220 (2)	250 (1)	230
Pia	240 (4)	—	—	240
Masja	270 (4)	230 (3)	—	250
Enziandom	270 (2)	—	270 (2)	270
——— vibrant (very deep colored) ↓ ———				
Marechal Foch	340 (3)	260 (4)	—	300
Leuchtfeuer	330 (4)	—	—	330
Forever Pink	360 (6)	—	—	360
Glowing Ember[y]	420 (5)	—	—	420
Kardinal	420 (2)	410 (3)	480 (2)	430
Monteforte Pearle[y]	700 (5)	—	—	700

[z]Pink blush phase of 'white' inflorescence.

[y]Measured by RACI calibrated to extractable content.

Table 2. Anthocyanin content for the sepals of three cultivars of *Hydrangea macrophylla* measured over seven years of harvesting inflorescences. The average of the values for the columns may not agree with the average reported in Table 1, because both extracted and *in situ* measurements are included in this table and the number of measured inflorescences varied from year-to-year.

	[anthocyanin], μg·g^{-1} fresh sepal		
Year	Penny Mac	Endless Summer	Blue Danube
2004	75 ± 15	90 ± 20	145 ± 20
2005	80 ± 10	95 ± 15	180 ± 20
2006	75 ± 15	110 ± 10	170 ± 15
2007	—	105 ± 20	180 ± 20
2008	80 ± 15	100 ± 20	180 ± 30
2009	80 ± 20	90 ± 20	155 ± 30
2010	85 ± 15	115 ± 25	175 ± 20

cultivar. The sepal anthocyanin content was also independent of the vendor. Likewise, the anthocyanin contents of sepals for a specific cultivar at peak bloom were similar over the seven summers of measurements (Table 2).

Only a few quantitative analyses of the anthocyanin content of *Hydrangea macrophylla* sepals have been previously reported. For example, the anthocyanin content of 'Merveille' sepals was reported to be about 2.8 mg anthocyanin per g of dry sepal (2). In order to afford comparison of this previous study to the results reported in Table 1, we determined that an average *Hydrangea macrophylla* sepal lost 87.5 ± 1.9% of its mass upon drying, allowing conversion of this determination to an equivalent 350 μg anthocyanin per g of fresh sepal. Others reported that the sepals on 'Monteforte Pearle' (1) and 'Todi' (2) have almost twice the anthocyanin content as the sepals of 'Merveille'. An indirect measurement of the anthocyanin content of 'Monteforte Pearle' sepals by calibrated RACI values in this study showed an equivalent 700 μg anthocyanin per g of fresh sepal. Thus, the 'Monteforte Pearle' results reported in the previous study (1) and the results reported in Table 1 are in reasonable agreement, providing further confidence in the reported measurements.

The sepals' anthocyanin content strongly depends on the *Hydrangea macrophylla* cultivar. Ordering of cultivars with respect to anthocyanin content of sepals is consistent with visual characterization of the inflorescence's color intensity and consistent with previous qualitative observations (2). In essence, the genetic composition of each cultivar controls the inherent anthocyanin content of the sepals, which in turn controls the observed color intensity of the cultivar's inflorescence.

Anthocyanin contents among red, purple, and blue sepals were similar for the same cultivar with neither a systematic increasing nor decreasing trend (Table 1). In most cases, the anthocyanin contents for all colors overlap within experimental error. Evidently, the amount of anthocyanin controls only the intensity of the sepal color rather than the hue or color itself.

Other factors such as climate, temperature, fertilizer (for example; nitrogen availability), and sunlight may also affect the anthocyanin content of the sepals of a specific *Hydrangea macrophylla* cultivar. However, these variables were kept relatively constant in this study, although the contents reported in Table 1 are specific to the Rockbridge County VA (USA) climate. That anthocyanin contents were similar over the seven summers of this study (Table 2) provides evidence for minimal effect of these environmental factors.

Characteristics of anthocyanin content. Interestingly, the sepals within a single inflorescence varied greatly in anthocyanin content. Such analyses had at least 10 to 30% standard deviation, representative of the variation in the color intensities of the individual sepals. Even though an inflorescence appeared to be a homogeneous color, upon closer inspection sepal-to-sepal variation was visually obvious. An example of the actual heterogeneous nature of a typical inflorescence is shown in Fig. 3. Despite this sepal-to-sepal variation in anthocyanin content noted in the inflorescences in numerous cultivars, the average anthocyanin content for a particular cultivar always tended to be constant.

Anthocyanin content of the sepals of a blue 'Penny Mac' inflorescence changed as a function of its stage of blooming (Fig. 4), similar to a red 'Masja' inflorescence followed in a previous study (28). *Hydrangea macrophylla* blooming stages have been defined previously (31). The anthocyanin content of the sepals first rises in the inflorescence's approach (stages I and II) to full bloom, remains relatively constant for about a week or so at peak bloom (stage III), and then steadily falls once the inflorescence passes its prime (stage IV). The results of this study are consistent with other reports (33) of a threefold decrease in the anthocyanin content of the sepals from stage III to stage IV blooms. The exact length of time for which *Hydrangea macrophylla* remains at peak anthocyanin content is likely dependent on environmental conditions such as sun and rain. Table 1 lists anthocyanin contents of the sepals when the *Hydrangea macrophylla* inflorescences were at peak bloom.

Classification of Hydrangea macrophylla *cultivars.* The *Hydrangea macrophylla* cultivars are different in terms of their inherent, genetically-controlled delphinidin-3-glucoside

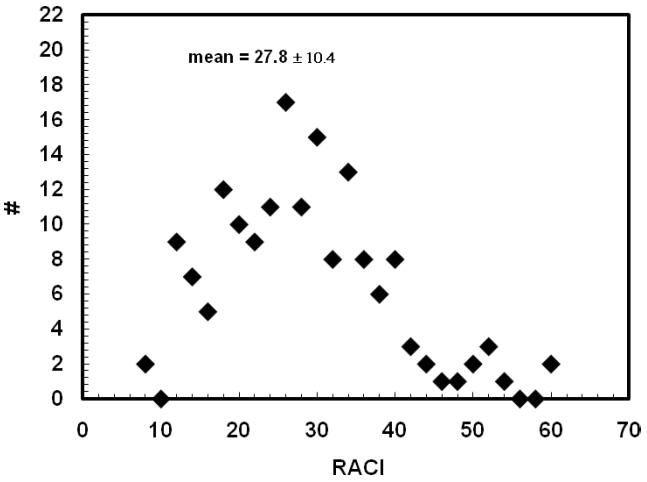

Fig. 3. Heterogeneity in anthocyanin content of the sepals in a single inflorescence of 'Forever Pink' at peak bloom. The number of sepals with a specific RACI (red anthocyanin content index) is plotted as a function of that RACI (with a value of ±1) for the sepals. The RACI mean value for the sepals of this particular 'Forever Pink' inflorescence represents an extractable anthocyanin content of ≈400 μg·g^{-1} fresh sepal.

content in sepals (Table 1). That one can classify the cultivars by their sepals' anthocyanin content is somewhat expected, especially in light of fruit and vegetable cultivars being categorized similarly. For example, the various cultivars of blueberries (6, 22), raspberries (7, 13), potatoes (4, 25), and cherries (9) have all been shown to have unique anthocyanin contents.

Even if environmental factors such as light intensity and quality (17), temperature (8, 29), magnesium content (18), and water availability affect the exact anthocyanin content (19) of the hydrangea sepals, the ordering of the cultivars as shown in Table 1 would be expected to remain about the same. However, the anthocyanin content of some floral cultivars of *Hydrangea macrophylla* has been shown to be unresponsive to such environmental effects (30). In addition, changes in anthocyanin content of *Hydrangea macrophylla* sepals for specific cultivars were unrelated to changes in nitrogen, potassium, and phosphorous content of the soil (2). Thus, anthocyanin data in Table 1 may be equally valid for other growing environments and climates.

Suggested classifications of the *Hydrangea macrophylla* cultivars in terms of sepal anthocyanin content or intensity of color are the 'blush' (very light colored) cultivars with 25 to 60 µg delphinidin-3-glucoside per g fresh sepal, the 'remontant and cold-hardy' (light colored) cultivars with 80 to 120 $\mu g \cdot g^{-1}$, the 'classic' (medium colored) cultivars with 140 to 190 $\mu g \cdot g^{-1}$, the 'vivid' (deep colored) cultivars with 230 to 270 $\mu g \cdot g^{-1}$, and the 'vibrant' (very deep colored) cultivars with 300 and higher µg delphinidin-3-glucoside per g fresh sepal. Such categories allow quantification and description of the intensity of the sepal coloration of the various cultivars.

Within the limits of the sample pool of this study, the remontant (blooms form on new wood) and cold-hardy cultivars always tended to possess the lowest sepal anthocyanin contents of the cultivars with red or blue sepals. Thus, this study may provide a basis for breeding programs trying to enhance the intensity of the coloration, and thus the sepal anthocyanin content, of the remontant cultivars (11). That all remontant and cold-hardy cultivars have similar anthocyanin contents of their sepals is evidence that all are probably genetically related (20). Further, that 'Penny Mac', 'Dooley', and 'Nikko Blue' form one sub-group and 'Endless Summer' and "David Ramsey' another sub-group in terms of sepal anthocyanin content is consistent with their genetic relationships (16, 23). However, the representative cultivars in the classic, vivid, and vibrant classifications of sepal color intensity and anthocyanin content do not appear to directly correspond to their reported genetic relationships (23).

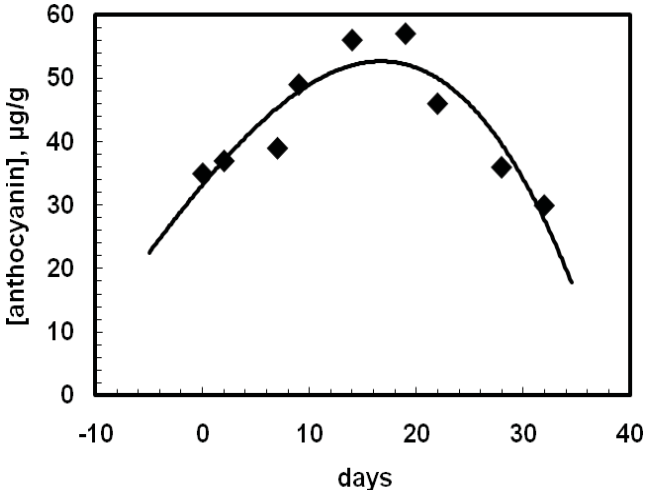

Fig. 4. Anthocyanin content of blue sepals of an inflorescence from 'Penny Mac' measured with a calibrated field-portable meter as a function of bloom stage. Measurements were taken during summer 2006 in the gardens of BackCountry Research (Rockbridge County VA, USA). Zero time was defined to be when sufficient sepals reached a size of 7 mm so that measurements could be taken, with days measured from this start time. Trend line is drawn as a guide to the eye.

Hydrangea cultivars and bluing of sepals. Those cultivars that are anecdotally resistant to sepal bluing tended to have the higher anthocyanin contents. For bluing to occur, Al^{3+} has to be in molar excess of anthocyanin in the sepals (27, 31). For example, sepals on cultivars such as 'Forever Pink' and 'Kardinal' may be difficult to change to blue because ten times more aluminum on a molar basis must be incorporated in the plants to achieve the same aluminum excess over anthocyanin in the sepals than do cultivars such as 'Penny Mac' and 'Nikko Blue'. In fact, the sepals of all remontant and cold-hardy cultivars should be relatively easy to change to blue because of their low anthocyanin content. The ease of bluing of the sepals of these cultivars appears to be generally consistent with observations in the field.

However, the process of sepal bluing may be less than straightforward. For example, 'Marechal Foch', 'General Vicomtesse de Vibraye', 'Domotoi', 'Enziadom', and 'Nikko Blue' are all reported to be easy to change from red to blue with aluminum sulfate (3, 15, 32) despite their wide variation in anthocyanin content. The cultivars with a higher anthocyanin content in their sepals, nevertheless, result in more intensely colored blues. Just as each *Hydrangea macrophylla* cultivar has its own unique sepal anthocyanin content, perhaps each likewise has its own unique sepal co-pigment content or profile which also contributes to the cultivar's bluing capability (12). Alternatively, the cultivars with sepals that are easier to change to blue may accommodate the uptake of aluminum more easily from the soil, similar to differential uptake of metal ions by olive cultivars (5). Thus, the list established in Table 1 may be less than a perfect match for bluing capability, as other factors must also be considered; but, the classifications provide a first approximation in bluing capability.

Anthocyanin content and bloom stage. The anthocyanin content of *Hydrangea macrophylla* sepals increased to peak bloom, then decreased. The length of the peak flowering or stage III is shown in Fig. 4. The anthocyanin content meter can readily be used to non-destructively monitor this length of peak bloom (28). Interestingly, the Al^{3+} content continues to steadily increase through the bloom stages for blue sepals but remains a constant low value for red sepals (31). Accordingly, the molar ratio of Al^{3+} to anthocyanin is continuously changing with bloom stage. This change may help explain why pink sepals fade to blue as the bloom stage advances to stage IV with the concomitant production of an even richer crescendo of colors.

In summary, the anthocyanin content of the *Hydrangea macrophylla* sepals is a measure of the sepals' color bright-

ness, regardless of the red, purple, or blue coloration. For a particular inflorescence, the anthocyanin content of the sepals steadily increases until peak bloom, then stays constant for more than a week, and finally steadily decreases after peak bloom. Even at peak bloom, there exists a heterogeneous distribution among sepal color intensities, but on the average the anthocyanin content for a specific *Hydrangea macrophylla* cultivar is constant. The anthocyanin content is used to categorize the cultivars in several groups according to the lightness or deepness of their sepal colors.

Literature Cited

1. Asen, S., N.W. Stuart, and H.W. Siegelman. 1959. Effect of various concentrations of nitrogen, phosphorus and potassium on sepal color of *Hydrangea macrophylla*. Proc. Amer. Soc. Hort. Sci. 73:495–501.

2. Asen, S., N.W. Stuart, and A.W. Specht. 1960. Color of *Hydrangea macrophylla* sepals as influenced by the carry-over effects from summer applications of nitrogen, phosphorous, and potassium. Proc. Amer. Soc. Hort. Sci. 76:631–636.

3. Bowman, D.P. 2001. Hydrangeas. Friedman/Fairfax Publishers, New York.

4. Brown, C.R., D. Culley, M. Bonierbale, and W. Amoros. 2007. Anthocyanin, carotenoid content, and antioxidant values in South American potato cultivars. HortScience 42:1733–1736.

5. Chatzistathis, T., I. Therios, and D. Alifragis. 2009. Differential uptake, distribution within tissues, and use efficiency of manganese, iron, and zinc by olive cultivars Kothreiki and Koroneiki. HortScience 44:1994–1999.

6. Connor, A.M., C.E. Finn, and P.A. Alspach. 2005. Genotypic and environmental variation in antioxidant activity and total phenolic content among blackberry and hybridberry cultivars. J. Amer. Soc. Hort. Sci. 130:527–533.

7. de Ancos, B., E. Gonzalez, and M.P. Cano. 1999. Differentiation of raspberry varieties according to anthocyanin composition. European J. Food Res. Technol. 208:33–38.

8. Dela, G., E. Or, R. Ovadia, A. Nissim-Levi, D. Weiss, and M. Oren-Shamir. 2003. Changes in anthocyanin concentration and composition in 'Jaguar' rose flowers due to transient high-temperature conditions. Plant Sci. 164:333–340.

9. Gao, L. and G. Mazza. 1995. Characterization, quantification, and distribution of anthocyanins and colorless phenolics in sweet cherries. J. Agric. Food Chem. 43:343–346.

10. Giusti, M.M. and R.E. Wrolstad. 2001. Characterization and measurement of anthocyanins by UV-visible spectroscopy. p. F1.2.1–F.2.13 *In*: Current Protocols in Food Analytical Chemistry (R.E. Wrolstad, ed.). John Wiley & Sons, New York.

11. Griffith, M., J. Beasley, and M. Dirr. 2007. Breeding and introducing new plants for tomorrow's markets. Accessed July 14, 2010. www.plantintroductions.com/companyinformation.html.

12. Ito, D., Y. Shinkai, Y. Kato, T. Kondo, and K. Yoshida. 2009. Chemical studies on different color development in blue- and red-colored sepal cells of *Hydrangea macrophylla*. Biosci. Biotechnol. Biochem. 73:1054–1059.

13. Kassim, A., J. Poette, A. Paterson, D. Zait, S. McCallum, M. Woodhead, K. Smith, C. Hackett, and J. Graham. 2009. Environmental and seasonal influences on red raspberry anthocyanin antioxidant contents and identification of quantitative traits loci (QTL). Molec. Nutr. Food Res. 53:625–634.

14. Kondo, T., Y. Toyama-Kato, and K. Yoshida. 2005. Essential structure of co-pigment for blue sepal-color development of hydrangea. Tetrahedron Lett. 46:6645–6649.

15. Lawson-Hall, T. and B. Rothera. 2001. Hydrangeas: A Gardeners' Guide. Timber Press, Portland, OR.

16. Lindstrom, J.T., M.C. Pelto, and M.A. Dirr. 2003. Molecular assessment of remontant (reblooming) *Hydrangea macrophylla* cultivars. J. Environ. Hort. 21:57–60.

17. Lu, Y., J. Du, J. Tang, F. Wang, J. Zhang, J. Huang, W. Liang, and L. Wang. 2009. Environmental regulation of floral anthocyanin synthesis in *Ipomoea purpurea*. Molec. Ecology 18:3857–3871.

18. Nissim-Levi, A., S. Kagan, R. Ovadia, and M. Oren-Shamir. 2003. Effects of temperature, UV-light and magnesium on anthocyanin pigmentation in cocoplum leaves. J. Hort. Sci. Biotechnol. 78:61–64.

19. Oren-Shamir, M. 2009. Does anthocyanin degration play a significant role in determining pigment concentration in plants? Plant Sci. 177:310–316.

20. Orozco-Obando, W., G.N. Hirsch, and H.Y. Wetzstein. 2005. Genotypic variation in flower induction and development in *Hydrangea macrophylla*. HortScience 40:1695–1698.

21. Pharr, K.E., K.M. Mayhew, and H.D. Schreiber. 2006. Anthocyanin content in hydrangea sepals. J. Undergrad. Chem. Res. 5:49–53.

22. Prior, R.L., G. Cao, A. Martin, E. Sofic, J. McEwen, C. O'Brien, N. Lischner, M. Ehlenfeldt, W. Kait, G. Krewer, and C.M. Mainland. 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. J. Agric. Food Chem. 46:2686–2693.

23. Reed, S.M. and T.A. Rinehart. 2007. Simple sequence repeat marker analysis of genetic relationships within *Hydrangea macrophylla*. J. Amer. Soc. Hort. Sci. 132:341–351.

24. Robinson, G.M. 1939. Notes on variable colors of flower petals. J. Am. Chem. Soc. 61:1606–1607.

25. Rodriguez-Saona, L.E., M.M. Giusti, and R.E. Wrolstad. 1998. Anthocyanin pigment composition of red-fleshed potatoes. J. Food Sci. 63:458–465.

26. Schmitzer, V., R. Veberic, G. Ostere, and F. Stampar. 2010. Color and phenolic content changes during flower development in groundcover rose. J. Amer. Soc. Hort. Sci. 135:195–202.

27. Schreiber, H.D., A.M. Swink, and T.D. Godsey. 2010. The chemical mechanism for Al^{3+} complexing with delphinidin: A model for the bluing of hydrangea sepals. J. Inorg. Biochem. 104:732–739.

28. Schreiber, H.D. and N.A. Wade. 2007. Field-portable analysis of anthocyanin concentration in sepals of *Hydrangea macrophylla*. HortScience 42:1323–1325.

29. Shaked-Sachray, L., D. Weiss, M. Reuveni, A. Nissim-Levi, and M. Oren-Shamir. 2002. Increased anthocyanin accumulation in aster flowers at elevated temperatures due to magnesium treatment. Physiol. Plant. 114:559–565.

30. Stiles, E.A., N.B. Cech, S.M. Dee, and E.P. Lacey. 2007. Temperature-sensitive anthocyanin production in flowers of *Plantago lanceolata*. Physiol. Plant. 129:756–765.

31. Toyama-Kato, Y., K. Yoshida, E. Fujimori, H. Haraguchi, Y. Shimizu, and T. Kondo. 2003. Analysis of metal elements of hydrangea sepals at various growing stages by ICP-AES. Biochem. Eng. J. 14:237–241.

32. Van Gelderen, C.J. and D.M. van Gelderen. 2004. Encyclopedia of Hydrangeas. Timber Press, Portland, OR.

33. Yoshida, K., D. Ito, Y. Shinkai, and T. Kondo. 2008. Change of color and compounds in sepals of chameleon hydrangea during maturation and senescence. Phytochem. 69:3159–3165.

34. Yoshida, K., M. Mori, and T. Kondo. 2009. Blue flower color development by anthocyanins: from chemical structure to cell physiology. Nat. Prod. Rep. 26:857–964.

35. Yoshida, K., Y. Toyama-Kato, K. Kameda, and T. Kondo. 2003. Sepal color variation of *Hydrangea macrophylla* and vacuolar pH measured with a proton-selective microelectrode. Plant Cell Physiol. 44:262–268.