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Cultural modification of Chrysanthemum Morifolium Ramat

Nadine A. Turner

To the Graduate Council:

I am submitting herewith a thesis written by Nadine A. Turner entitled "Cultural modification of Chrysanthemum Morifolium Ramat." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Landscape Architecture.

Gary L. McDaniel, Major Professor

We have read this thesis and recommend its acceptance:

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Gary L. McDaniel, Major Professor

We have read this thesis
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CULTURAL MODIFICATION OF *CHRYSANTHEMUM MORIFOLIUM* RAMAT.

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Nadine A. Turner

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ABSTRACT

The capacity of Chrysanthemum morifolium to produce a blue pigmentation through cultural modification involving a specific nutritional regime, soil pH conditions and metallic applications was explored. Two cultivars of cut chrysanthemum and five cultivars of garden chrysanthemum were used as plant material. Aluminum content of the leaves, stems and petals of the garden chrysanthemums was measured to test the ability of these plants to accumulate a metal.

No correlations were found between nutritional regime, soil pH conditions, metal application and aluminum content of the plant parts. None of the seven cultivars grown in pot culture showed any blue pigmentation in their petals. The chrysanthemum does not appear to be a metal accumulator and therefore is not a likely candidate for blueing through the use of cultural manipulation.

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CHAPTER I

INTRODUCTION

The chrysanthemum is an important florist crop, partly due to its long keeping quality. It also offers a wide variety of forms and colors to the florist trade and the public in general. These characteristics of the chrysanthemum indicate the benefits and desirable traits of this plant. As yet, there is no blue flowered chrysanthemum.

A blue cut chrysanthemum would be an asset to florists, since few species of blue flowers are commercially available. Those that are available do not exhibit the longevity of the chrysanthemum and their accessibility is more limited due to seasonal availability. This often necessitates the use of floral spray paint to obtain the blue colors that are needed to complete the color scheme of certain arrangements. Similarly, there is no pot chrysanthemum to provide a blue coloration when it is required by a particular decor. Nor is there a garden chrysanthemum available to color our gardens with blue. This would be very desirable, since there are so few blue flowering species of plants to choose from compared to those that offer white, red, orange, yellow, etc. In fact, the chrysanthemum would represent one of the few choices to provide blue flowers in our gardens in the late fall. These drawbacks indicate that a blue chrysanthemum could fill an obvious void that presently exists in the field of floriculture.

CHAPTER II

REVIEW OF LITERATURE

To a large extent, man is surrounded in nature by green. This necessarily imparts an aesthetically pleasing quality to colorful plants that provide a break in this overwhelming greenness. Those plants or plant parts that display a brightness and noticeable contrast to this vast greenness have always attracted man's interest. The various colors of flowers and fruits have largely fulfilled this role and make them an important element in man's environment.

This attractiveness of flowers has come to play an important role in directing horticultural practices and great efforts have been made for many years to produce more fascinating colors, color patterns or an increased number of colors available in a single species, which would then render that species more versatile.

We have become especially fascinated by species of plants whose flower colors alter during development, anthesis or by diurnal change. Differences in color due to geographical locations and environmental conditions have also proved intriguing. These observations of color alteration in nature preceded and were implemental in making the ability to control or change flower color an important element in the field of ornamental horticulture, and artificial alteration of flower color patterns came into practice (15).

An outstanding example of color variability is exhibited by Hydrangea macrophylla Thunb. In its native area where it has been cultivated for centuries, the hydrangea exhibited only a pink flower color. However, after it was introduced to England from China by Joseph Banks in 1790, reports of a color change from pink to blue began to appear in the literature. Intensive investigations ensued from this from 1800-1815. It was determined that differences in the components of the soil were responsible for the color change. Different types of soil were employed in an attempt to produce this color change from pink to blue. Initially, it was thought that iron (Fe) salts were responsible for this until it was found that it was the aluminum (Al) content in the soil that was causing the color change. This finding was later supported by the first cultural experiments in 1826 and again in 1892, when studies by plant physiologists began (1,18). The result of these investigations clearly showed that it was possible to alter or control the color of hydrangea cymes through cultural manipulation which could produce either pink or blue sepals. It was later found that at least three factors are influential in the sepal color of hydrangeas (3). These are:

1. The plant's capacity to absorb and accumulate aluminum.
2. Anthocyanin pigment type and concentration in the sepals.
3. The ratio between anthocyanin to the kaemferol glycosides.

Experiments were performed which clearly showed that the Al cation was involved in the formation of the blue coloration. When Al in the form of potash alum [$KAl(SO_4)_2$] and aluminum sulfate,

[$Al(SO_4)_3$] was tested, a positive effect on blueing was obtained. Aluminum sulfate sprayed several times onto the sepals of a pink hydrangea produced sepals with a clear blue color. When it was sprayed on before any color showed, a blue color developed as the sepal color began to appear. Aluminum sulfate injected into the stem caused blue portions to appear in direct correlation with the site of injection. It was determined that Al must be present in the sepals in the concentration of 21-24.6 ppm for blueing to occur (1,3).

Aluminum citrate can also be used to turn hydrangea sepals blue and this is a commonly used method in Europe. One way this can be done is by spraying a 5% solution combined with a surfactant 3 or 4 times beginning at the visible bud stage (21).

The aluminum experiments were also performed using sulfuric acid, ferrous sulfate, ferric sulfate, ferric chloride, salts of copper, magnesium and sodium, phosphates of iron, magnesia and caustic potash, but with negative results (1). Nickel, cobalt, charcoal, potassium sulfate, carbonate of potassium and iron filings also failed to produce a blue coloration (18).

The same anthocyanin pigment, delphinidin 3,5-diglycoside was found to be responsible for both the blue and the pink colors of the hydrangea sepals (2). From this, it was deduced that Al was in some way altering this anthocyanin. The discovery that a metal could be used to manipulate the color of flowers had an extreme impact on horticultural research and many experiments were performed using various metals, especially Al, on several plant species (18).

The Anthocyanins and Flower Color

Flower color production can be attributed to two groups of pigments (26):

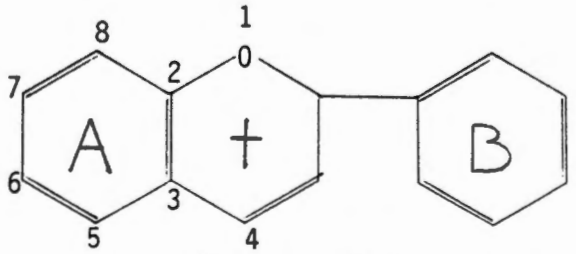
1. The fat-soluble carotenoids, which are contained in the plastids and produce the colors orange, yellow and ivory.

2. The water-soluble flavanoids, which are contained in the sap in the cell vacuoles and produce the colors from pink and red to purple and blue.

Of the 12 classes of compounds which make up the flavanoids, the anthocyanins are the most important and widespread groups of color pigments in plants and they are responsible for the majority of red and blue colors in flowers and fruits.

The term "anthocyanin" was first proposed in 1835 and simply represented the blue pigment of the cornflower. Since then, it has been recognized that the red and blue colors in plants are manifested by a single anthocyanin type (flavanoid), and the term is now used to include the whole group of similar pigments. Chemical, synthetic, biochemical and biogenesis studies have vastly increased our knowledge of these pigments.

As members of the flavanoid pigments, the anthocyanins have the basic structure of a flavone, which consists of two benzene rings (A&B) joined together by a 3 carbon link formed into a γ-pyrone ring, called 2-phenylbenzopyrylium.

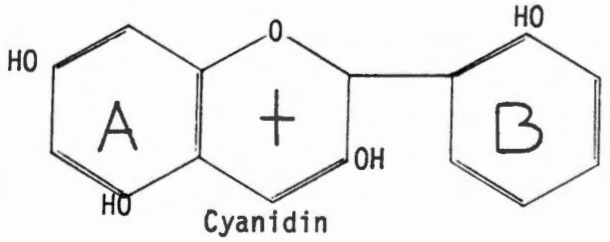


2-phenylbenzopyrylium

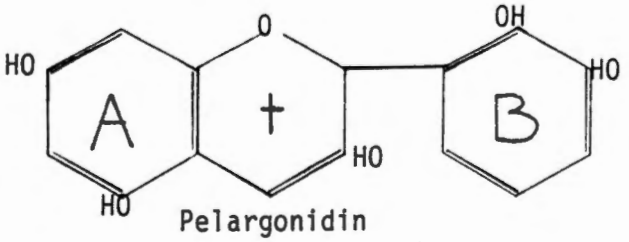
Anthocyanins may be considered derivatives of this flavylium. Within the anthocyanin class itself, the individual compounds are distinguished mainly by the number and orientation of OH and CH_3 groups substituted in the two benzene rings. The A ring may have OH groups substituted, usually at both the C3 and C5 positions, or rarely at the C7 position only. It is usually nonmethylated. The B ring may be substituted by one, two or three OH or CH_3 groups.

Hydroxylation patterns can be classified into the three basic pigment groups--cyanidin (Cy), pelargonidin (Pg) and delphinidin (Dp). These anthocyanidins are hydroxylated in the 3-, 5- and 7-positions in common. Their methylated derivatives commonly occur in nature also.

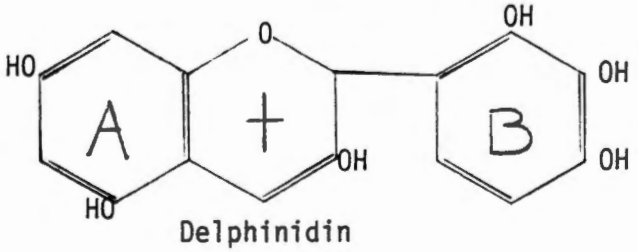
The three main anthocyanidins have the following structures (49):



Cyanidin



Pelargonidin



Delphinidin

The anthocyanins can be present in all organs of a plant and this varied distribution makes it difficult to form a unified hypothesis of their existence in the plant, as they can be localized both in plasma and membranes as well as in the vacuoles (15). However, histochemical determination of their presence is not difficult. Tissues containing anthocyanins are colored red in the presence of acid, but in neutral or alkaline solutions, they become blue. The oxidative process plays an important role in the formation of these pigments. All anthocyanins commonly found in nature have one or more of their OH groups joined to one or more units of sugar in the flower cells by a semi-acetal link (28). In this form, they are referred to as glycosides. Spectral measurements can determine the position of attachment of the sugars in the anthocyanin molecule. The sugar molecule imparts sap solubility and stability to light exposure and enzymatic attack. The sugars involved include monosaccharides, disaccharides and trisaccharides.

Apart from the sugar or sugars, the anthocyanins are referred to as anthocyanidins. Anthocyanins can be separated from their sugar components by boiling them for 3 minutes in 20% hydrochloric acid (HCl), however, they are not known to occur in nature without at least one attached sugar. Over 100 different aglycones have been isolated from plants, but only 11 of these occur commonly. Of these 11, six are anthocyanidins. These include the three main anthocyanidine and their derivatives, which differ in the number of their CH_3 subunits.

Three Main Anthocyanidins	Derivatives
cyanidin	petunidin
pelargonidin	peonidin
delphinidin	malvidin

The number and orientation of the OH and CH_3 groups present determines in part the color produced by the pigment and these anthocyanidins, singly or as mixtures, provide the entire range of colors from pink and scarlet to mauve, violet and blue. In general, the following pigments produce the colors listed below (49):

cyanidin - crimson, magenta
pelargonidin - pink, scarlet, crimson-red
delphinidin - mauve, blue

petunidin - purple
peonidin - rosy red
malvidin - mauve

Certain factors which influence the number of hydrogen ions released or acquired by the anthocyanins can alter this color scheme. An increased number of OH groups or a change in the sugar attachment from the 3- to the 3-, 5-sugar positions both induce a more intense blue tone. An increased number of CH_3 groups results in a more intense red coloration (15). Besides the number of OH and CH_3 groups and position of sugar attachment, all of which affect the structure of the pigment molecule, it has been determined that there are five factors which act to modify the intensity of the color exhibited. These are:

1. Concentration of pigment in the cell sap.
2. Acidity of the cell sap and growing medium.
3. Colloidal components of the cell sap which may afford stabilization of anthocyanins.

4. The presence of co-pigments.
5. The kind and concentration of inorganic ions in the cell vacuole.

Concentration of Pigment in Cell Sap

The concentration of anthocyanins varies over a wide range, from 0.01-15% of dry weight (35). Generally, there is a higher concentration of anthocyanin pigment in red, violet and purple flower parts than in blue colored parts. Robinson found that there was 6 to 7 times as high an anthocyanin concentration in the red as compared to the blue sepals of 'Merveille' hydrangea (3). An exception to the rule is the difference between the blue and magenta cineraria. The blue flowered strains contain more anthocyanin pigment than do the magenta flowers (15).

Usually more than one anthocyanin is present and in this case, the pigment that occurs in the higher concentration will have the greater influence on the actual flower color and the intensity of this pigment will be more pronounced. Also, any other types of pigments that may be present will modify the hue to a certain extent (35).

Color changes during maturation and anthesis of flowers are due to alterations in quantity of anthocyanins during developmental stages (24). Anthesis and a decrease in the extent of anthocyanin synthesis occur simultaneously. This concurrent lessening of the concentration of anthocyanin naturally lessens the intensity of the anthocyanin coloration in the flower petals (37).

Soil and Cell Sap Acidity

Soil pH. The acidity of the growing medium can be of prime importance in the production of blue pigments. This is because a lower soil pH favors metal uptake, while a higher soil pH tends to inhibit such action. Cymes of blue hydrangeas grown in a soil of pH 5.5 contain greater than 950 ppm Al, while those of mauve hydrangeas in pH 5.8-6.0 contain 200-950 ppm Al. Pink hydrangeas in pH 6.0-6.2 soil have less than 200 ppm Al (19). A soil pH of 6.2 was found to be the critical point in determining whether the hydrangea would produce blue or pink sepals. At this pH, nearly all soil types induced intermediate flower colors, with blue sepals produced at a soil pH of 6.0 and below and pink sepals produced at pH 6.4 and above (51).

Upon liming the soil, the concentration of soluble calcium (Ca), the base saturation and the cation exchange capacity (CEC) are all increased. This makes the independent effect of soil pH on plant uptake of metals complex because soil properties affecting uptake (exchangeable Ca, base saturation and CEC) are all influenced by pH. Studies using labelled isotopic strontium show that uptake of radiostrontium by plants is lower when the pH is 7.0 and greater than when the pH is below 5.0. However, this phenomenon is not attributed to the effect of the soil pH alone but is explained as resulting from the synergistic effects of the chemical properties of the soil. When the pH value is below pH 5.0, the exchange complex is dominated by H and Al cations that are more tightly adsorbed to exchange sites than strontium. Upon increasing the pH (liming),

H and Al cations are usually replaced by Ca. Base saturation is increased as is CEC. Base-unsaturated soils contain Al as their dominant exchangeable cation, which is much more difficult to displace from soil exchange sites than is Ca (23).

Cell sap acidity. The role of cell sap acidity in causing the blueing of flowers does not appear to be as evident as that of the growing medium. Some believe it to be extremely important while others dismiss it as being of little importance.

It was observed that anthocyanins are red under acidic conditions, violet under neutral and blue under alkaline conditions. This led to the deduction that the H ion concentration of the cell sap was the most determining factor influencing flower color variation. At a pH of 3.0 or less, the color of anthocyanins is determined by the degree of OH groups in the B ring which become methylated to produce a red color. An increase in pH from 3.0-4.0 produces a color change in anthocyanin solution from red to blue-violet. Some argued that such slight amounts of change in cell sap pH could not be such a major influential factor in flower coloration when it was found that the differences in pH values between red and blue flowers were very small, from 0.5-1.0 pH units (15). However, a 0.5 pH unit change, from 3.0-3.5, shifts an aluminum cyanidin 3-glucoside complex solution to its maximum wavelength absorption (515-545 nm) with a corresponding change in color from red to blue-violet (9). Similarly, an increase in the pH of larkspur epidermal tissue, from pH 5.5-6.6 as it ages, changes the color of many of the cells from moderate

reddish-purple to light purplish-blue (12). As the reddish-purple buds of morning glory mature into light blue flowers, the pH of the epidermal cells changes from 6.5-7.5 (14,43).

Another argument against the importance of pH is the fact that flowers of different species with similar cell sap pH may be of different coloration. This is exemplified by the cornflower and the red rose. They both have a cell sap of pH 4.8, yet the same pigment (Cy) produces a blue color in the cornflower and a red color in the rose (15). Also, examination of the cell sap from petals and fruits of 200 plants showed that the cell sap was always acidic (usually about pH 5.5) regardless of color (7). The occurrence of different flower colors with a similar cell sap pH suggested that cell sap reaction was not as important a factor in color variation as had been supposed.

The importance of cell sap acidity is more due to the interaction which occurs between anthocyanins and pH. Because they are electron deficient, anthocyanins are highly reactive and susceptible to attack by almost any nucleophilic reactant, including water and hydrogen peroxide. The rate of destruction that occurs from this reactant increases with higher cell sap pH. Under high pH conditions, anthocyanins lose a proton to form very unstable quinonoidal anhydro bases. These bases are colored, but they rapidly hydrate to yield fairly stable chromonols which are colorless (17). The actual pH range in which anthocyanins are colorless is pH 4.0-6.0. The pigmented cell sap of most flowers is pH 4.5-5.5. Within this range,

anthocyanins can be stabilized with metals and other substances that prevent discoloration and produce a blue color. For example, cyanidin-3-glucoside forms a blue colored complex with Al ions, with maximum formation at pH 5.5 (11).

Colloidal components of the cell sap which may afford stabilization of the anthocyanins. The condition or state of the pigment becomes very important, in that deviations in pH alone do not seem to adequately explain color variations in anthocyanin-containing tissues.

The Robinsons found that the cyanidin of the cornflower gave a more acidic reaction with blue litmus paper than that of the red rose. This led them to hypothesize that there may be a simultaneous presence of certain organic constituents in the cells of the cornflower which may modify the color of its anthocyanin-containing tissues (15). Further studies on the blue pigmentation "protocyanin" (cornflower) showed that it was a complex of cyanin, magnesium, iron and potassium in the ratio of 8:2:1:24 and also contained peptide, polysaccharide, and flavone-like material as an integral part of its structure. This would indicate that "protocyanin" does indeed require both metal chelation and the presence of a suitable polysaccharide and this agreed with the previously introduced concept by the Robinsons that the absorption of anthocyanins onto a polysaccharide was involved in the blueing of flowers in Centaurea and other plants (7).

The presence of co-pigments. The condition of co-pigmentation is extremely important and all common anthocyanins have been found

in a co-pigmented state. This condition causes a bathochromic shift in the absorption maximum towards the longer wavelengths and blue coloration. This bathochromic shift is influenced by concentration of the co-pigment. A change in the molar ratio of co-pigment to anthocyanin will produce a change in flower color. Different classes of co-pigments show various degrees of blueing and color enhancement (10). Anthocyanins can form complexes with such substances as flavones, flavonols, tannins, gallic acid, xylans, kaempferol and quercitin glycosides to modify color (15). This is substantiated by experiments which involve heating or adding alcohol to the loose co-pigment-:-pigment complex. Disassociation occurs and a corresponding reddening of color results. Upon cooling, the color reverts back to its original blue color (11).

It appears that co-pigmentation is unrelated to salt formation and that the mechanism is hydrogen bonding between the carbonyl group of the anthocyanin anhydro-bases and the aromatic OH groups of the flavanoids. Stabilization seems to be physical rather than chemical and chromatography shows that no new product is formed (17).

The presence of additional flavanoids in the same petals which contain anthocyanins can indicate a co-pigment complex. The blueness of Lathyrus hybrids is due to flavones acting as co-pigments in the presence of anthocyanins to produce a blueing effect (15). The mauve and purple color variations that have resulted from attempts to induce a blue rose are the result of cyanidin 3-,5-diglucoside of a crimson rose co-pigmented with large amounts of gallotannin.

The corresponding spectral shift is from 507-512 nm. Co-pigmentation is actually a widespread phenomenon and it may be that co-pigments exist for all types of anthocyanins. It has been shown to occur with all six glycosides of the commonly occurring anthocyanidins (49).

The Kind and Concentration of Inorganic Ions in the Cell Vacuole

Hydrangea research shows that nitrogen and phosphorus inhibit Al uptake and promote the production of pink sepals. Phosphorus alone has little effect on increasing pinkness but is more effective in conjunction with nitrogen (31, 41). Nitrogen in the form of NH_4^+ has a greater antagonism to Al uptake than does nitrogen in the form of NO_3^-(6). A high amount of potassium favors Al uptake and the production of blue sepals (4). The best fertilizer practices to produce pink flowers include the use of high nitrogen, medium phosphorus and low potassium, whereas the best blue flowers are produced by low nitrogen, low phosphorus and high potassium fertilization (39, 40,41).

Metal chelation of anthocyanins is an extremely important factor in blueing. Several metals have been implicated, such as Mg, Al, Fe and Mo. These metals act to chelate the anhydro bases of the anthocyanins in a stable quinoid structure at ph values of 2.8-6.0. This stable quinoid structure is responsible for imparting color. However, in order for metal chelation to occur, there must be a free ortho-dihydroxyl group present in the pigment. This condition is met in cyanidin, pelargonidin and delphinidin (17).

Electron spin resonance studies show that the metal becomes attached to the pigment through a covalent bond with the unpaired electron in the pyrylium ring, thereby stabilizing it (34).

Stabilization of the anthocyanin by the metal causes a bathochromic displacement so that the pigment absorption spectrum is shifted towards the longer wavelengths and a bluer coloration. Using the hydrangea as an example, the complex of Al and delphinidin-3-,5-diglycoside shifts the absorption maximum 40 nm toward the longer wavelengths. Actual absorption values are 550 nm for the red hydrangea and 590 nm for the blue hydrangea (2).

Of the five factors which are influential in flower color alteration, co-pigmentation and metal chelation are considered to be of prime importance. It has been suggested that metal chelation is actually necessary for co-pigmentation to occur. This is supported by the fact that certain organic compounds which commonly occur as co-pigments failed to form a complex with cyanidin 3-glucoside in solutions of pH 3-6.5 except in the presence of metal (Al) salts (29). Other factors that are of minor importance include light, temperature and possibly sugar as related to anthocyanin synthesis.

Light. Strong illumination is favorable to the formation of anthocyanns in chrysanthemums. Many experiments show a discontinuation of anthocyanin formation without illumination. However, some plants can produce anthocyanins in their vegetative root tip in the dark. Other plants require a photochemical as well as a darkness reaction for the formation of anthocyanins. There seems to be no

definite explanation for how light interferes with the synthesis of flavanoids (15).

In cultured Salpiglossis sinuata buds, the relative proportions of Dp and Cy changed with different light intensities with a lesser amount of Dp produced at a lower illumination. It is claimed that this occurs in intact flowers also. Possibly this phenomenon is because Dp and Cy have separate light-dependent pathways involved in the synthesis of the two anthocyanins (33).

Temperature. There have been contradictory reports concerning the relationship of temperature and anthocyanin synthesis, with some favoring a high temperature for its formation and others favoring a low temperature. It seems probable that all plants have an optimal temperature for anthocyanin formation, which probably coincides with the optimum temperature for metabolism. Chrysanthemums grown during the summer produce paler colors of red and purple and this has been related to a lesser availability of carbohydrates for synthesis of pigments because of their removal due to a higher respiration rate at higher temperatures. These smaller amounts of carbohydrates affect anthocyanins more than carotenoids (44). The paler color has also been attributed to an accelerated pigment decomposition at higher temperatures which may cause hydrolysis of the protective 3-glycoside linkage to give unstable anthocyanins or hydrolysis of the pyrylium ring. Some examples of how high temperatures can affect flower color are (15):

Primula sinensis rubra - inhibition of anthocyanin synthesis occurs and white flowers develop.
Calceolaria hybrida grandiflora - a similar situation occurs and yellow flowers are produced.
Dahlia variabilis - the yellow flowers normally produced become red in a hothouse at 30°C.
Chrysanthemum morifolium Ramat. - anthocyanin production of cultured florets is maximum at 15°C and florets are red. At 30°C anthocyanin synthesis ceases, carotenoids increase and the florets are bright yellow.

Sugar. Many attempts have been made to relate sugar to anthocyanin synthesis, but there is much disagreement on this. Experiments in which artificially applied sugar resulted in a reddening of cell sap would seem to corroborate the relationship of sugar to anthocyanin synthesis; however, no direct pathway linking the two has been proven. A close relationship has been found between anthocyanins and reducing sugars, and in the absence of sucrose, no anthocyanin was formed in Chrysanthemum morifolium. However, another study found no correlation between anthocyanins and sugar concentration at any stage of flower development, or anthesis. Sugar concentration was highest in November while that of anthocyanins was in October (37).

If there is any connection between sugar metabolism and anthocyanin formation, it is probably only of secondary importance and may follow the pattern of increased nutrition producing increased metabolism which increases anthocyanin synthesis (15).

CHAPTER III

MATERIALS AND METHODS

Experiment I

Chrysanthemum morifolium Ramat. cvs. 'Royal Purple' and 'Snow Purple' were used as experimental subject material. Five rooted cuttings of each cultivar were planted into crocks which contained sand that had been previously washed with 10% sulfuric acid to remove organic matter. These crocks were then arranged in a randomized complete block design on greenhouse benches. Plants were grown under long day conditions by lighting at night until shoots were pinched. When there were approximately 10 leaves below the central buds (May 31), terminals were soft pinched to promote lateral stem growth to obtain the maximum number of flowers per crock. The plants were then grown under short day conditions by covering them with shade cloth from 1700-0700 hours daily until the petals began to show color.

Three experimental parameters were used. These included:

1. Soil pH

   high level - pH 6.5 low level - pH 4.5

2. Nutritional Balance

   high N/low K (30-10-10) low N/high K (5-11-26)

3. Metal Application

   high level - 25 mg/l low level - 10 mg/l control - 0 mg/l

The treatments were applied in a 2 X 2 X 3 factorial design for each cultivar tested (Figure 1).

When the flowers had expanded, pigment color was read on a colorimeter, in order to determine flower color differences between treatments. A random sampling of 10 flowers was taken from each crock and color was measured using a Hunter Lab Color Difference Meter. Color was measured in terms of "a" and "b" readings, with "a" indicating the degree of redness on the positive side and blue-green on the negative side and "b" indicating the degree of yellow on the positive side and purple-blueness on the negative side. The "a" and "b" values represent hue, expressed as the angle, or arc cotangent a/b in the Hunter System (16).

Flowers were then placed individually into 250 ml beakers. The beakers were then filled with solutions containing the same concentrations as those used in sand culture (10 and 25 mg/l Al sulfate) until the flowers began to float. This was done to allow them to directly absorb the aluminum ions. The 25 mg/l solutions proved to be toxic, so this level was dropped down to 20 mg/l aluminum sulfate and concentrations of 10 and 20 mg/l aluminum sulfate were used to test the flowers' capacity to become blue. In all treatments, the flower color was observed and any visual changes were recorded daily.

Experiment II

An investigation was carried out to determine whether certain other metals of salts besides aluminum would either singly or

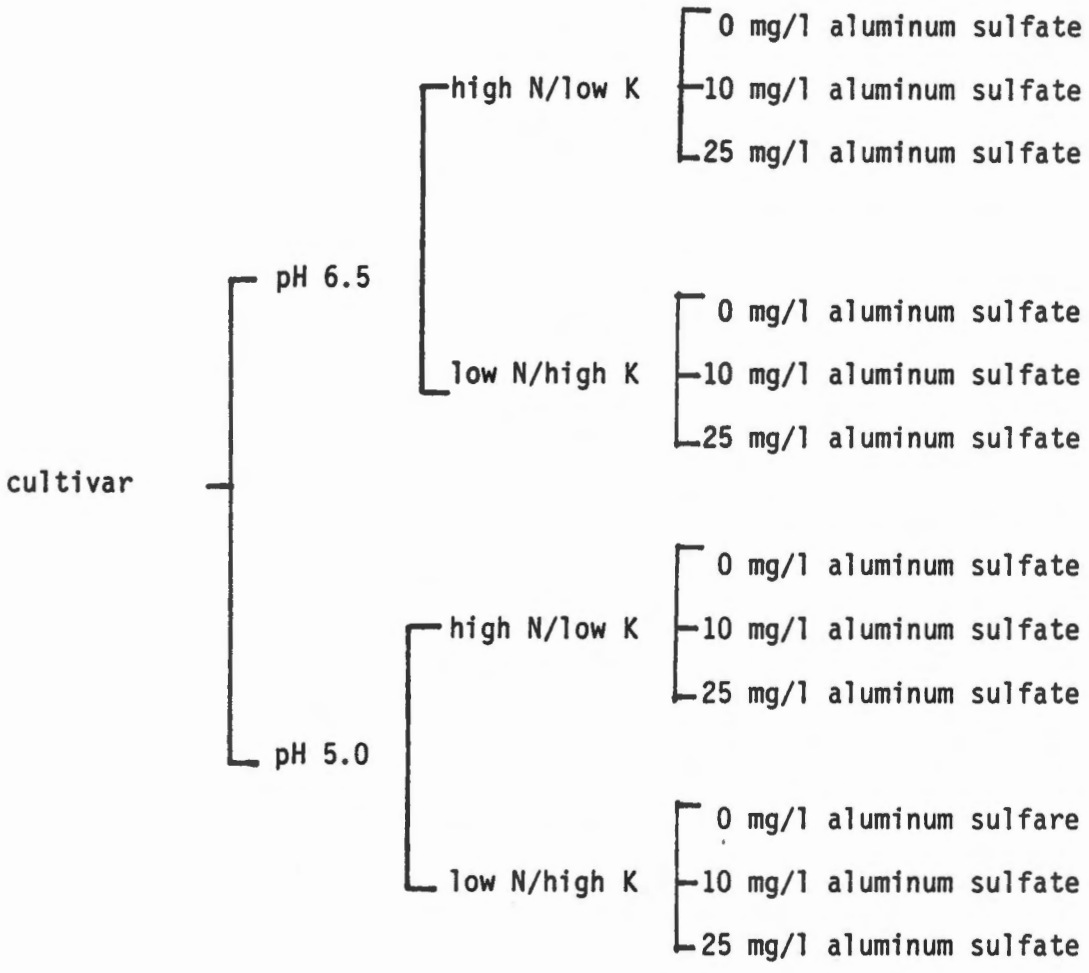


Figure 1. Treatments applied to cultivars.

collectively be instrumental in altering chrysanthemum flower color, as had been found for other species. The implicated elements or compounds are listed in Figure 2.

Consequently, six flowers from 'Royal Purple' and 'Snow Purple' controls were placed as before into individual 250 ml beakers which were then filled with the various solutions until the flowers began to float. This test period lasted 1 week and the solutions were changed every 2 or 3 days to prevent bacterial contamination. The solutions were in the concentrations of 20 and 25 mg/l of distilled water. The solutions were also applied as sprays to the chrysanthemum flowers while they were still on the plant and cut stems are also placed into the solutions. Whichever of these test solutions produced the most positive blueing effect was to be used for future experimentation.

Experiment III

A preliminary test was performed as described in Experiment II on two chrysanthemum garden cultivates, 'Lancer' (lavender) and 'Red Coat' (red), using only the solution which proved the most effective for producing a blue color in the previously tested flowers. This was done to see if garden chrysanthemums would respond positively to blueing. Five garden cultivars were then chosen as test material. These included: 'Grandchild,' 'Lancer,' 'Liberty,' 'Stardom,' and 'Mango.' These were placed in 15 cm pots with 4 or 5 rooted cuttings per pot. The pots were arranged on two greenhouse benches in a randomized complete block design. 'Grandchild,' 'Lancer' and

Single Metal Solutions

Aluminum Ammonium Sulfate	Ferric Chloride
Aluminum Sulfate	Ferric Sulfate
Aluminum Potassium Sulfate	Ferrous Sulfate
Magnesium Sulfate	Sodium Acetate
Ferrous Ammonium Sulfate	Sodium Citrate

Combined Metal Solutions

Ferric Chloride-Aluminum Potassium Sulfate
Ferric Chloride-Magnesium Sulfate
Ferric Sulfate-Magnesium Sulfate
Ferric Sulfate-Aluminum Sulfate
Ferric Sulfate-Magnesium Sulfate-Aluminum Potassium Sulfate
Ferric Ammonium Sulfate-Aluminum Potassium Sulfate

Ferrous Sulfate-Aluminum Sulfate
Ferrous Sulfate-Magnesium Sulfate-Aluminum Potassium Sulfate
Ferrous Sulfate-Sodium Acetate
Ferrous Sulfate-Sodium Citrate
Ferrous Sulfate-Magnesium Sulfate

Figure 2. Single and combined metallic solutions.

'Liberty' were used for pH treatment comparison and had two replicates per treatment. 'Mango' and 'Stardom' were used as a comparison of cultivar response to the treatments and single replicates were used. The treatment parameters were:

1. Soil pH levels.
2. N-P-K ratios.
3. Metal solution concentrations.

An acid soil pH is necessary for a plant to take up elements such as aluminum and iron from the soil. To examine this capacity in the chrysanthemum, half of the population was grown in a lesser acid medium (pH 6.5) and half in a more acid medium (pH 4.5). It was not necessary to modify the soil that was to be used for the lower pH, as the commercial potting medium (peat, vermiculite, perlite) was already at the desired level. Agricultural limestone was added to half of the soil to adjust it to pH 6.5. Since this limestone contained calcium carbonate ($CaCO_3$), gypsum ($CaSO_4$) was added in an equal amount to the less acidic soil mixture to provide an equivalent amount of calcium. The pH levels were monitored routinely to make sure they stayed constant during the course of this study.

The two nutritional regimes consisted of Peters' soluble liquid fertilizers 30-10-10 and 5-11-26. The high N/low K solution was used to inhibit metal uptake and promote pink flowers. The low N/high K solution was used to promote metal uptake and the production of blue flowers. Two levels of metallic salt solutions were applied

daily to the plants--a high level (25 mg/l) and a low level (20 mg/l) --based on hydrangea research. The fertilizer and metallic salt solutions were combined when applied to the plants. Controls received no metal application.

The plants were grown under the various conditions until their flowers unfolded. At this time, a random sampling of 10 flowerheads of approximately the same degree of expansion was collected from each pot. Flower color was determined by a Hunter Lab Digital Color Difference Meter (16). Subsequent to color readings, 25 flowers from each cultivar were placed in 20 and 25 mg/l Fe Sulfate-Al K Sulfate solutions to determine blueing capacity.

The stems, leaves and remaining flowers of all of the plants were collected and dried in a forced air oven at 75°C for 4 hours. The dried plant parts were ground separately in a Wiley Mill. Ground tissues were ashed in crucibles in a muffle furnace at 500°C for 4 hours in order to release any bound Al present in the tissue. Aluminum was then extracted from samples with 10 ml of 3N HCL. The acid extracts were then filtered and 5 cc of activated charcoal was added to each solution in order to remove color from the samples. Samples were again filtered and brought up to 100 ml volume with distilled water. A 4 ml aliquot of each was taken to be read for aluminum content in an Auto Analyzer using the Aluminon Colorimetric Method (16).

A separate sample of combined soil and roots was also taken from each pot for analysis. Fifty ml of 1 N KCL was added to each

5.0 gram sample of air dried soil and root mixtures to extract any aluminum present. These samples were shaken for 30 minutes and filtered, which reduced their original volume of 50 ml. The filtrate was acidified with HCL to pH 3.0 and refrigerated to prevent any growth of microorganisms. The filtrates were then brought back up to a 50 ml volume with distilled water and analyzed in the same way as the plant parts.

Cell sap of fresh leaves and stems of each chrysanthemum cultivar was measured for pH. Stem measurements included the basal, mid and tip regions. This was done by placing a pH electrode onto freshly cut surfaces of the leaves and stems.

A parallel experiment was done with 12 hydrangeas which were given the same treatments as the chrysanthemums. Production of blue florets of the hydrangeas would indicate that conditions were proper for metal uptake. Therefore, any lack of blueing of the chrysanthemums could then be attributed to the inability of the plants to mobilize aluminum.

CHAPTER IV

RESULTS

Experiment I

The "a" readings ranged from +12.79 in 'Snow Purple' treatment pH 6.5, low N/high K with 25 mg/l Fe Sulf-Al K Sulf to +14.47 in 'Royal Purple' treatment pH 4.5, low N/high K and 25 mg/l Fe Sulf-Al K Sulf. Readings were similar for both cultivars. The "b" readings ranged from -0.66 in 'Snow Purple' treatment pH 4.5, high N/low K and 0 mg/l Fe Sulf-Al K Sulf to -1.91 in 'Snow Purple' treatment pH 6.5, low N/high K and 25 mg/l Fe Sulf-Al K Sulf. Again, readings were similar for both cultivars. No trends were evident among treatments or cultivars.

The flowers placed into individual beakers containing the 10 and 25 mg/l Al Sulfate solutions (for direct absorbtion of the Al) did not exhibit any color change after 5 days of treatment. Black spotting of the petals occurred in all solutions and increased proportionally with increased concentration of the solutions.

Experiment II

Of the flowerheads that were placed into the various test solutions in the preliminary investigation, only those of 'Royal Purple' showed any capacity to change to blue petal color. The flowers of 'Snow Purple' either did not change color at all or they turned a yellow-green color.

Of the "Single Metal Solutions" tested, only ferrous sulfate caused any blue coloration. A minimal amount of blue or purple appeared in the outer petals in half of the flowers tested.

Four of the combined solutions caused blueing and produced the following results:

1. Ferrous Sulfate-Aluminum Potassium Sulfate--The amount of blueing ranged from a minimal amount to one third or two thirds of the flower. This solution produced the greatest number of blue flowers.

2. Ferrous Ammonium Sulfate-Aluminum Potassium Sulfate--A minimal amount of blueing occurred on a few of the flowers.

3. Ferrous Sulfate-Aluminum Ammonium Sulfate--Blueing ranged from a minimal to a small amount around the center of about half of the flowers tested.

4. Ferrous Ammonium Sulfate-Aluminum Sulfate--This solution produced an intensification of pink in most of the flowers tested, but it did produce purpleing in some of the flowers.

The solutions applied as sprays had no effect on coloration, nor was there any flower color change from the cut stems that were placed into the solutions.

Experiment III

The lavender-colored garden cultivar Lancer responded more positively to the metal solutions in the preliminary test than did the 'Royal Purple' or 'Snow Purple' cultivars, and began blueing

within 2 days. The other garden cultivar, 'Red Coat,' gave a negative response and no color difference occurred.

The "a" colorimetric readings ranged from +9.52 in 'Liberty' treatment pH 6.5, high N/low K and 25 mg/l Fe Sulf-A1 K Sulf to +19.56 in 'Stardom' treatment pH 6.5, low N/high K and 25 mg/l Fe Sulf-A1 K Sulf. 'Stardom' "a" readings were consistently higher than those of the other cultivars.

The "b" readings ranged from -1.31 in 'Grandchild' treatment pH 6.5, low N/high K and 20 mg/l Fe Sulf-A1 K Sulf to +8.90 in 'Mango' treatment pH 4.5, high N/low K and 25 mg/l Fe Sulf-A K Sulf. 'Mango' "b" readings were consistently higher than the other cultivars, while those for 'Grandchild' and 'Liberty' were consistently lower.

The "a" and "b" readings indicated no trends which could be correlated with pH, nutritional regime or metal application. Neither the readings showing the least or most amount of red or blue coloration were consistent with any one treatment. Some differences did occur among cultivars, with "a" or "b" readings being consistently lower or higher for all treatments. 'Grandchild' was the only cultivar for which negative "b" readings were obtained.

When the flowerheads were subsequently put into the solutions, 'Mango' and 'Lancer' were the only cultivars that showed any noticeable amount of blue, with coloration being greatest in the 25 mg/l solution.

Aluminum Content and pH

Leaf, stem and petal samples. Aluminum content ranged from 1.55 ppm in 'Stardom' petals at pH 6.5 and 25 mg/l Fe Sulfate-Al K Sulfate to 0.36 ppm of fresh weight in 'Mango' leaves at pH 5 and 0 mg/l Fe Sulfate-Al K Sulfate (Figures 3-9). No one cultivar consistently accumulated a greater or lesser amount of Al. Leaves and petals showed no consistent trends among cultivars except for a generally lower Al content with higher pH conditions. This is consistent with the evidence that a higher pH causes a lesser availability of metal to a plant. Incidence of a decrease in Al content occurred as often as did an increase in Al content with Al application. Those plant parts which initially contained the greatest amount of Al decreased the most in Al content with increased application of Al.

Stem Al content almost consistently increased with increased Al application, even when leaf and petal content decreased. This accumulation of Al in stem tissue is consistent with the evidence that Al is deposited in the stems when cell sap is not that of an Al accumulator. Although stem Al content usually increased with increased Al application, total Al content of the stems changed the least among plant parts.

Root-Soil Samples

The root-soil samples contained a greater amount of Al than did those of the leaves, stems and petals for all cultivars and treatments (Figures 7 and 8). A consistently greater amount of Al

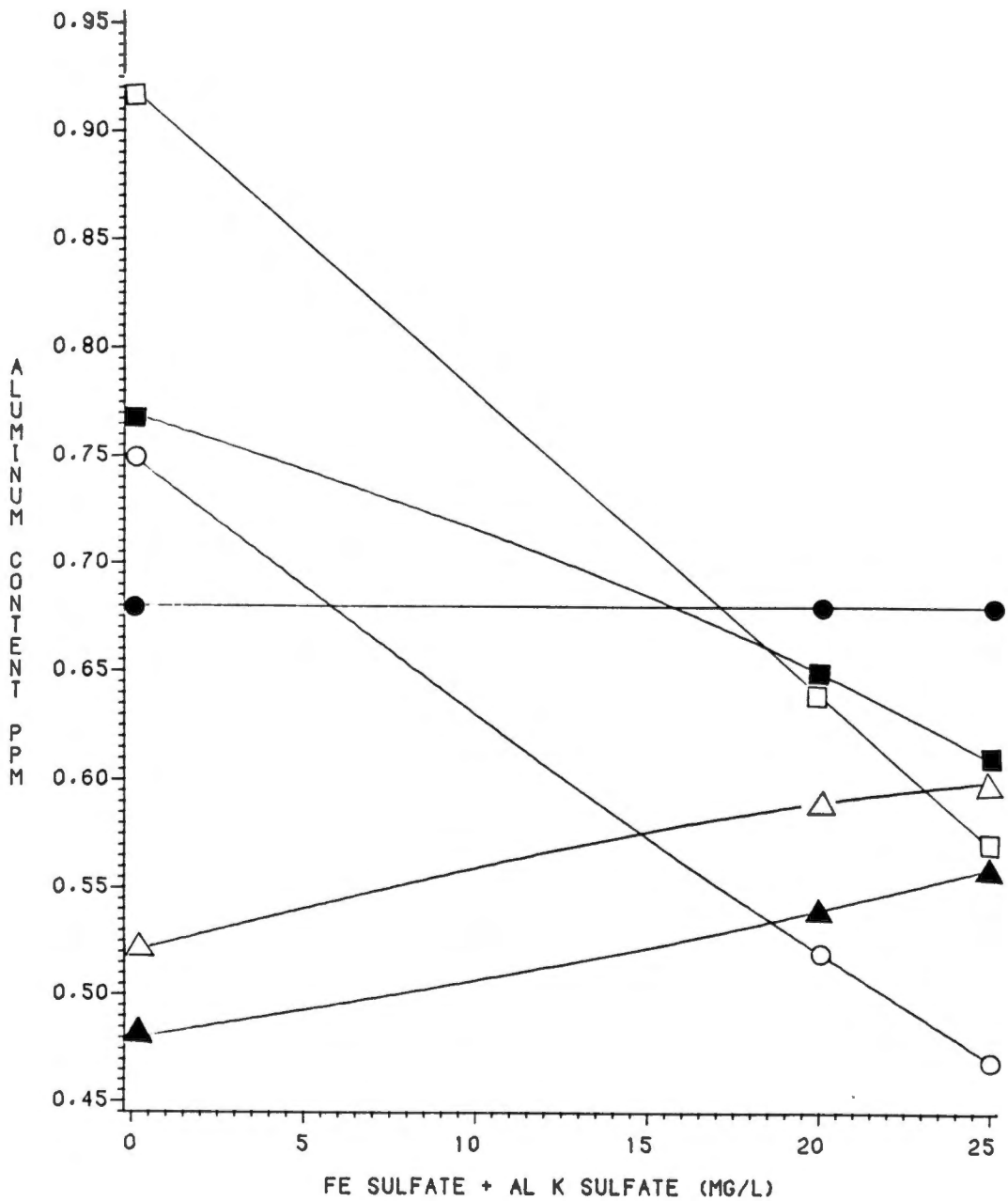


Figure 3. Aluminum content of Chrysanthemum morifolium 'Grandchild' grown in substrate of pH 4.5 and pH 6.5.

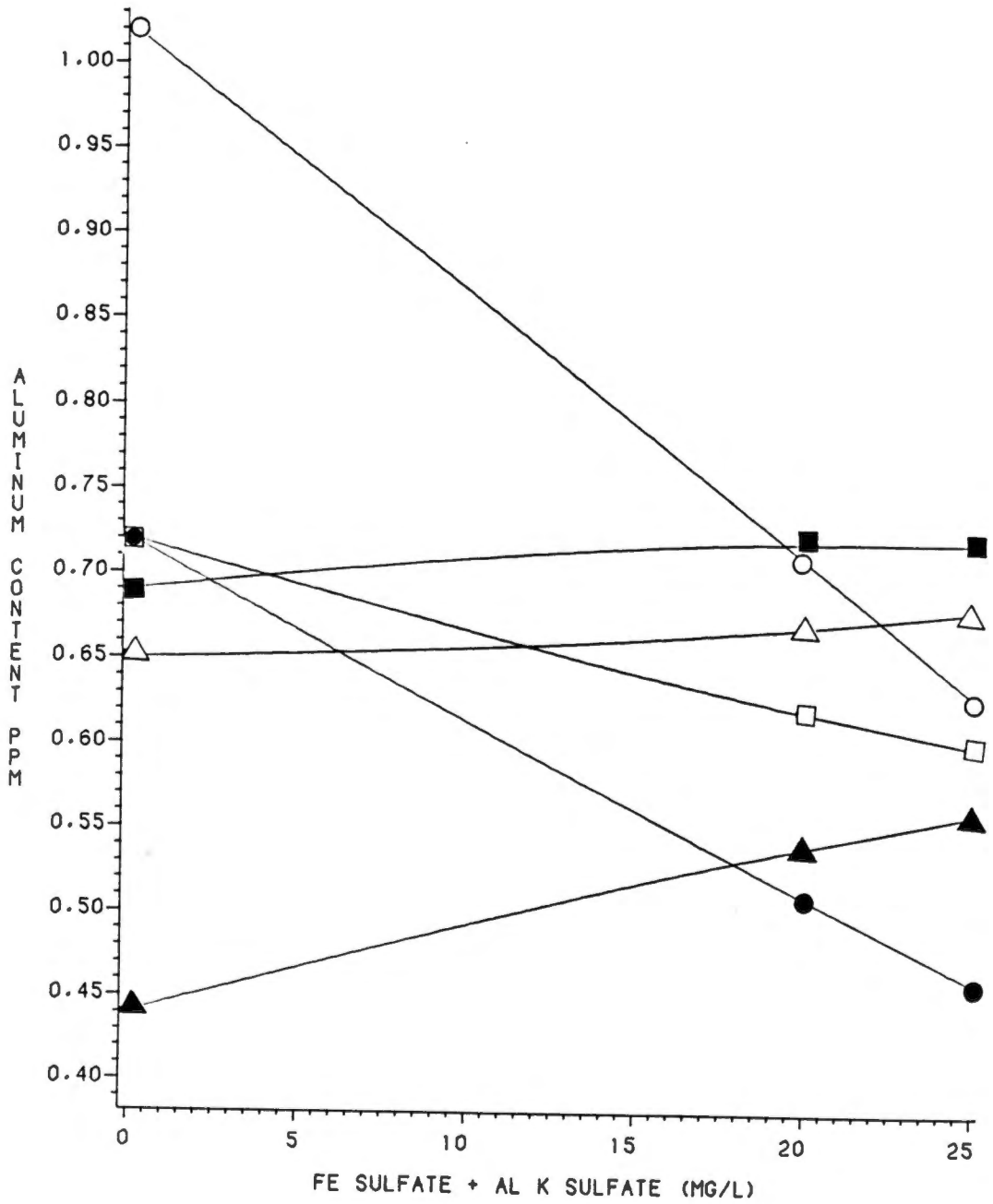
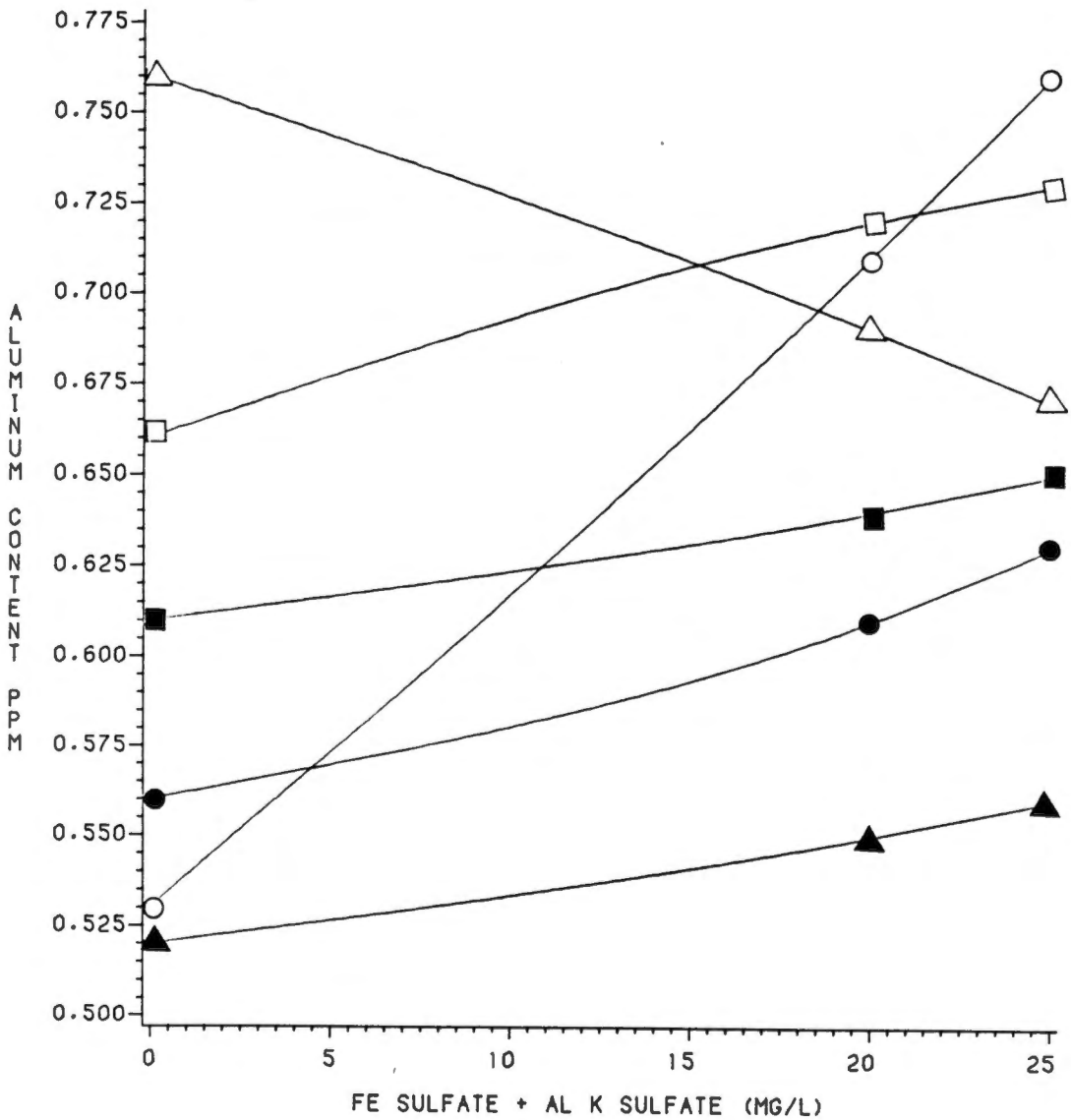
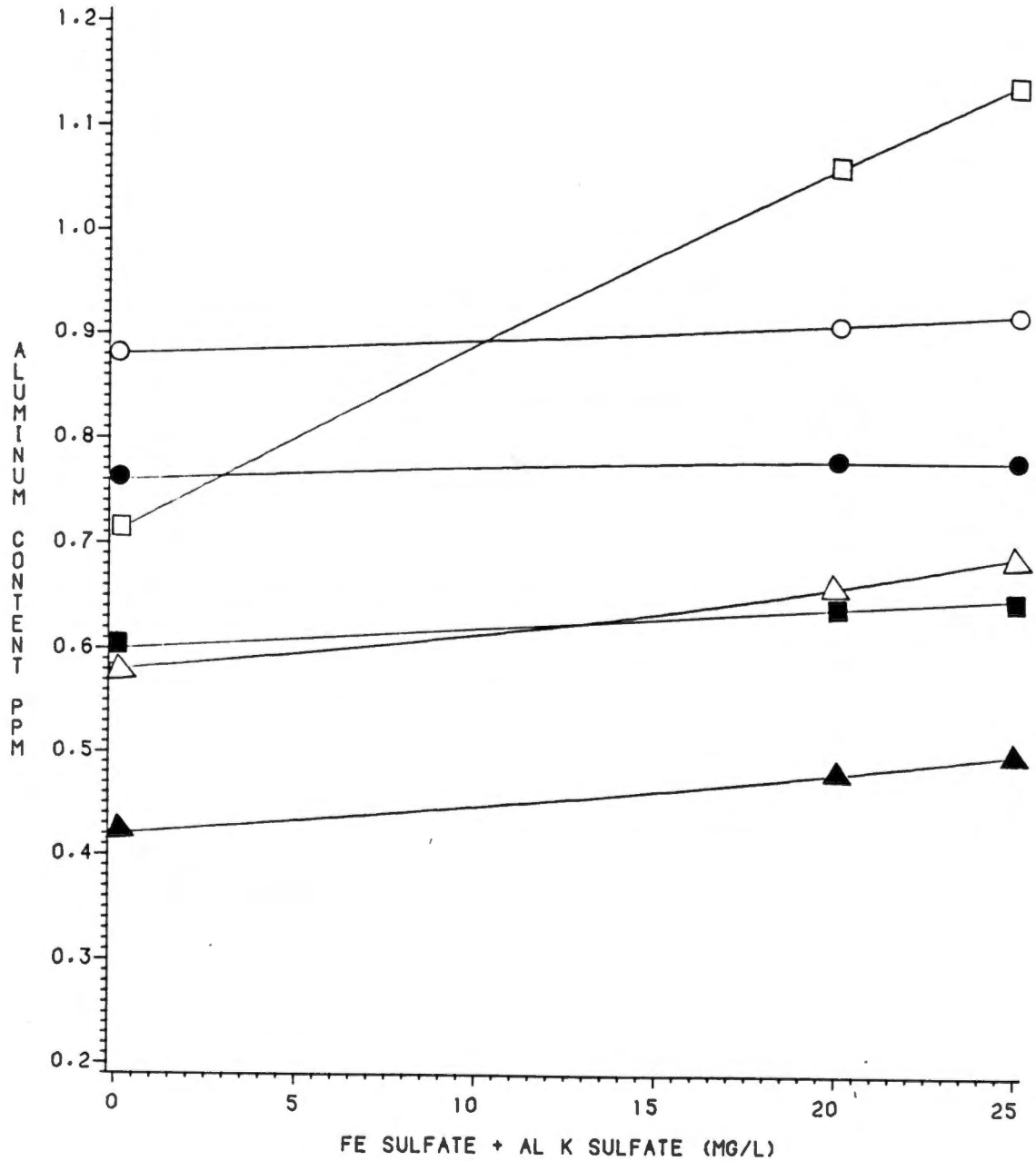


Figure 4. Aluminum content of Chrysanthemum morifolium 'Lancer' grown in substrate of pH 4.5 and pH 6.5.



LEGEND: Plant Part
○ Leaves, High pH
△ Stems, High pH
□ Petals, High pH
● Leaves, Low pH
▲ Stems, Low pH
■ Petals, Low pH

Figure 5. Aluminum content of Chrysanthemum morifolium 'Liberty' grown in substrate of pH 4.5 and pH 6.5.



LEGEND: Plant Part
○ Leaves, High pH
● Leaves, Low pH
△ Stems, High pH
▲ Stems, Low pH
□ Petals, High pH
■ Petals, Low pH

Figure 6. Aluminum content of Chrysanthemum morifolium 'Stardom' grown in substrate of pH 4.5 and pH 6.5.

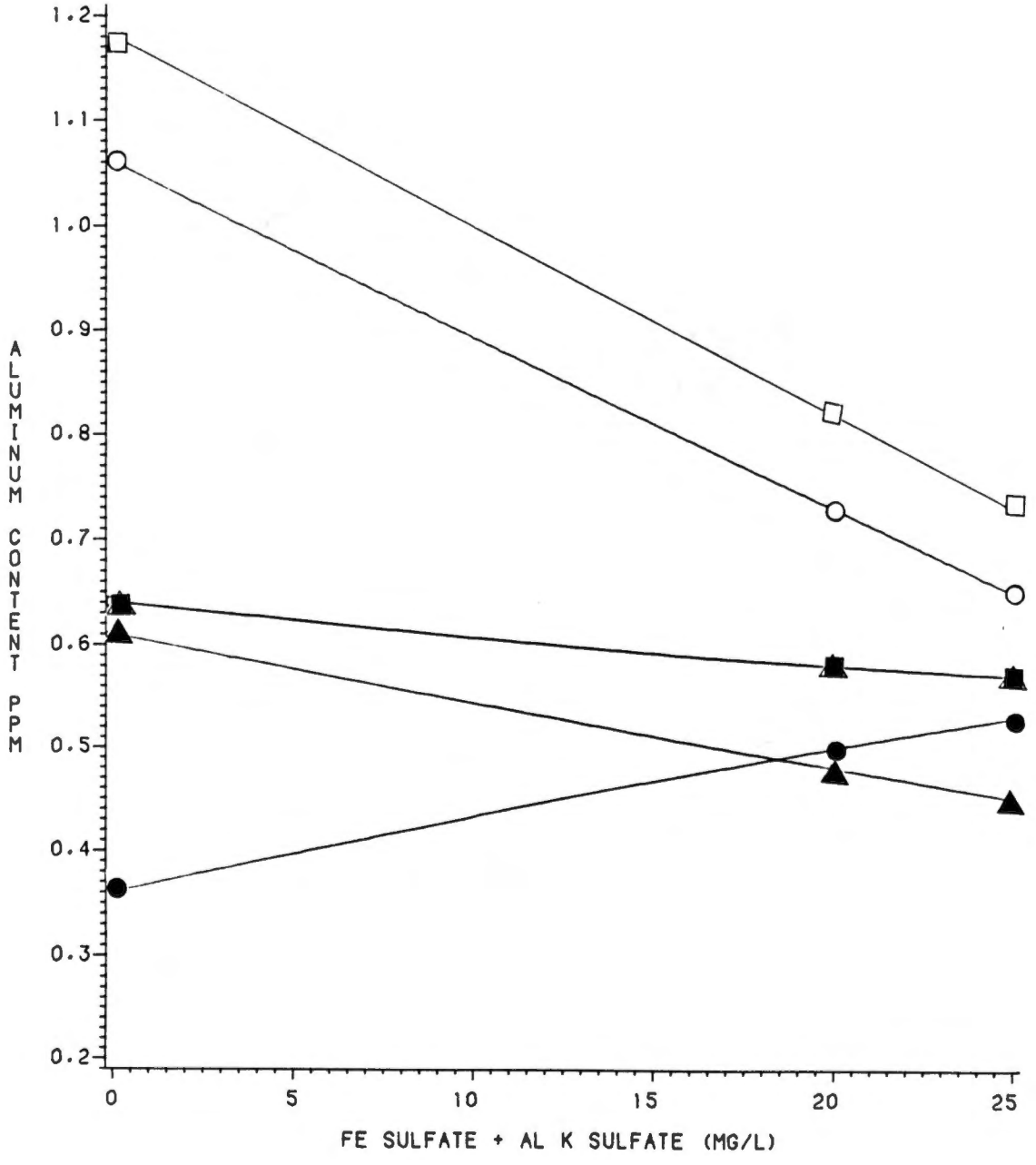


Figure 7. Aluminum content of Chrysanthemum morifolium 'Mango' grown in substrate of pH 4.5 and pH 6.5.

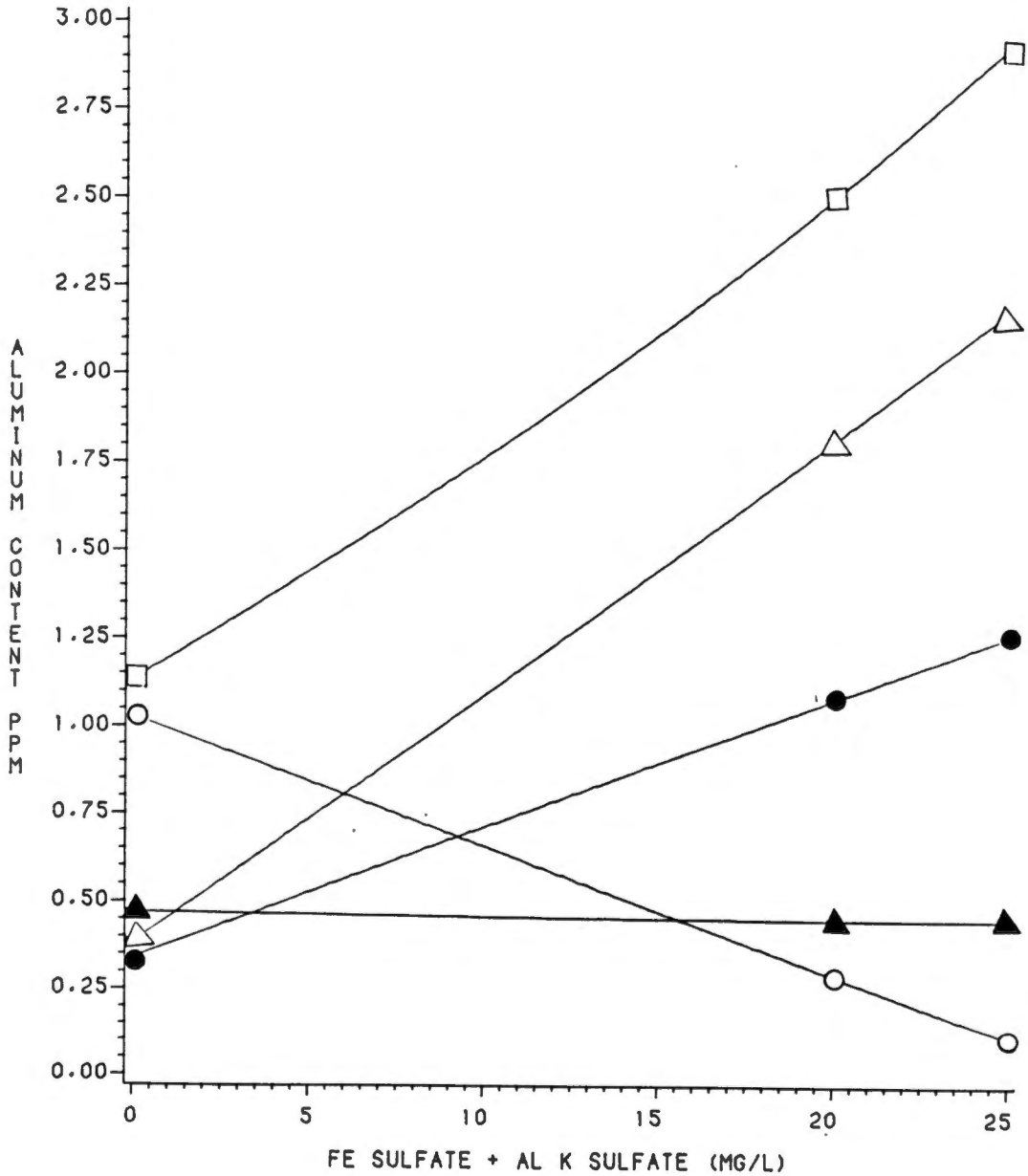


Figure 8. Aluminum content of five Chrysanthemum morifolium cultivars grown in substrate of pH 4.5.

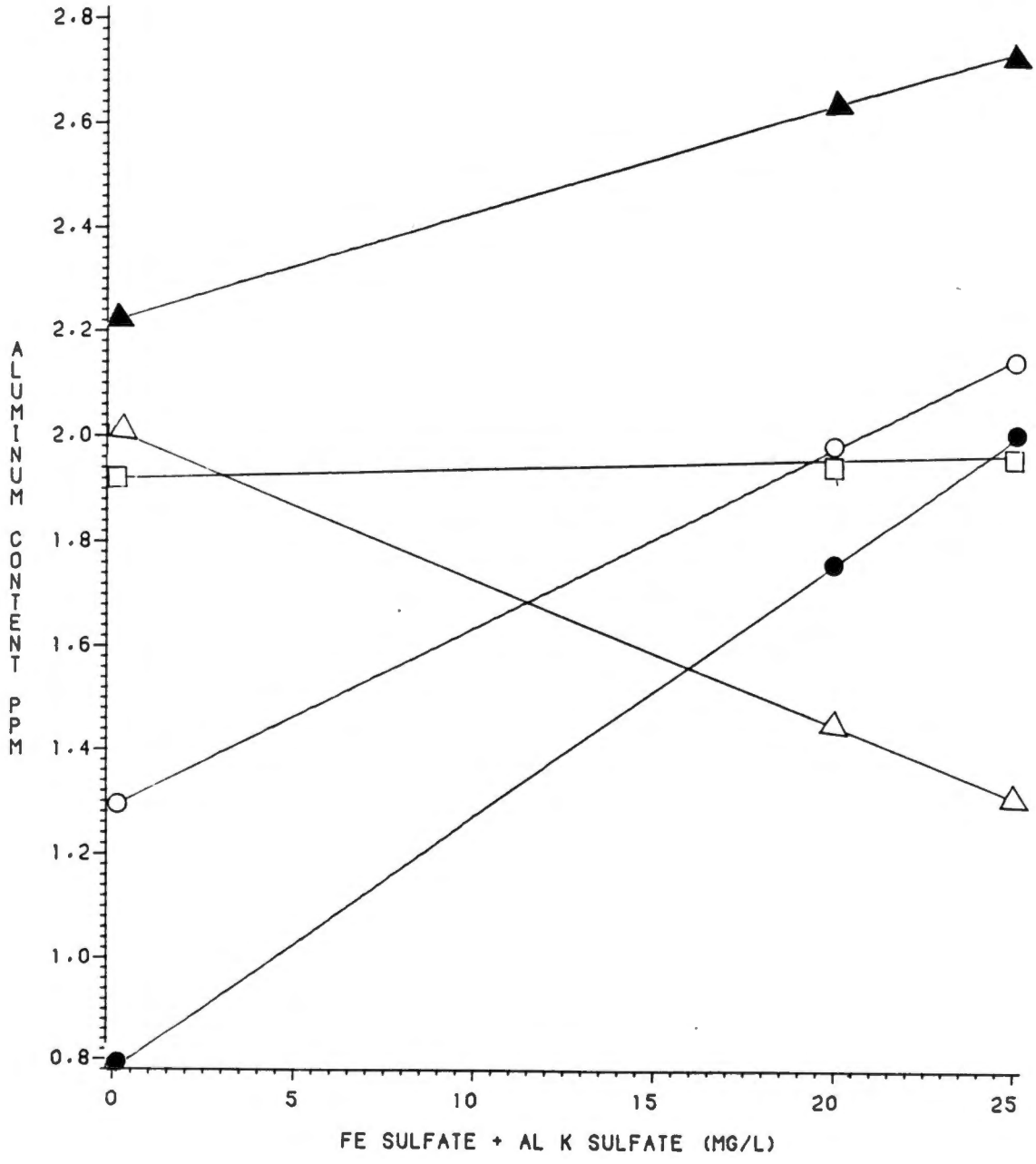


Figure 9. Aluminum content of five Chrysanthemum morifolium cultivars grown in substrate of pH 6.5.

occurred with lower pH conditions, probably due to more acidic soil conditions favoring metal accumulation.

Aluminum Content and Nutritional Regime

Leaf, stem and petal samples. Aluminum content ranged from 0.40 ppm for 'Liberty' leaves in the low N/high K treatment to 1.07 ppm for 'Lancer' leaves in the low N/high K treatment (Figures 10-16). Leaves and petals again were inconsistent in their response and no trends were evident between treatments or cultivars. Stem Al content in most cases rose with metal application regardless of nutritional treatment.

Root-soil samples. The only trend noted was a generally greater amount of Al with high N/low K treatments. Possibly because a high N nutritional regime inhibits Al accumulation in the plant, more Al remained in the soil to give these treatments a higher Al reading. Overall readings between cultivars were similar for both nutritional regimes.

Since all plant parts showed an initial amount of Al present in them (at 0 mg/l Fe Sulf-Al K Sulf), some amount of Al must have been present in the growing medium previous to application treatments. In some cases, there was a lesser amount of Al accumulated in the metal application treatments than the controls. This occurred more in the lower pH condition, possibly as a result of a greater amount of Al in the soil medium causing Al toxicity, which in turn caused stunted root growth and a subsequent decrease in Al uptake.

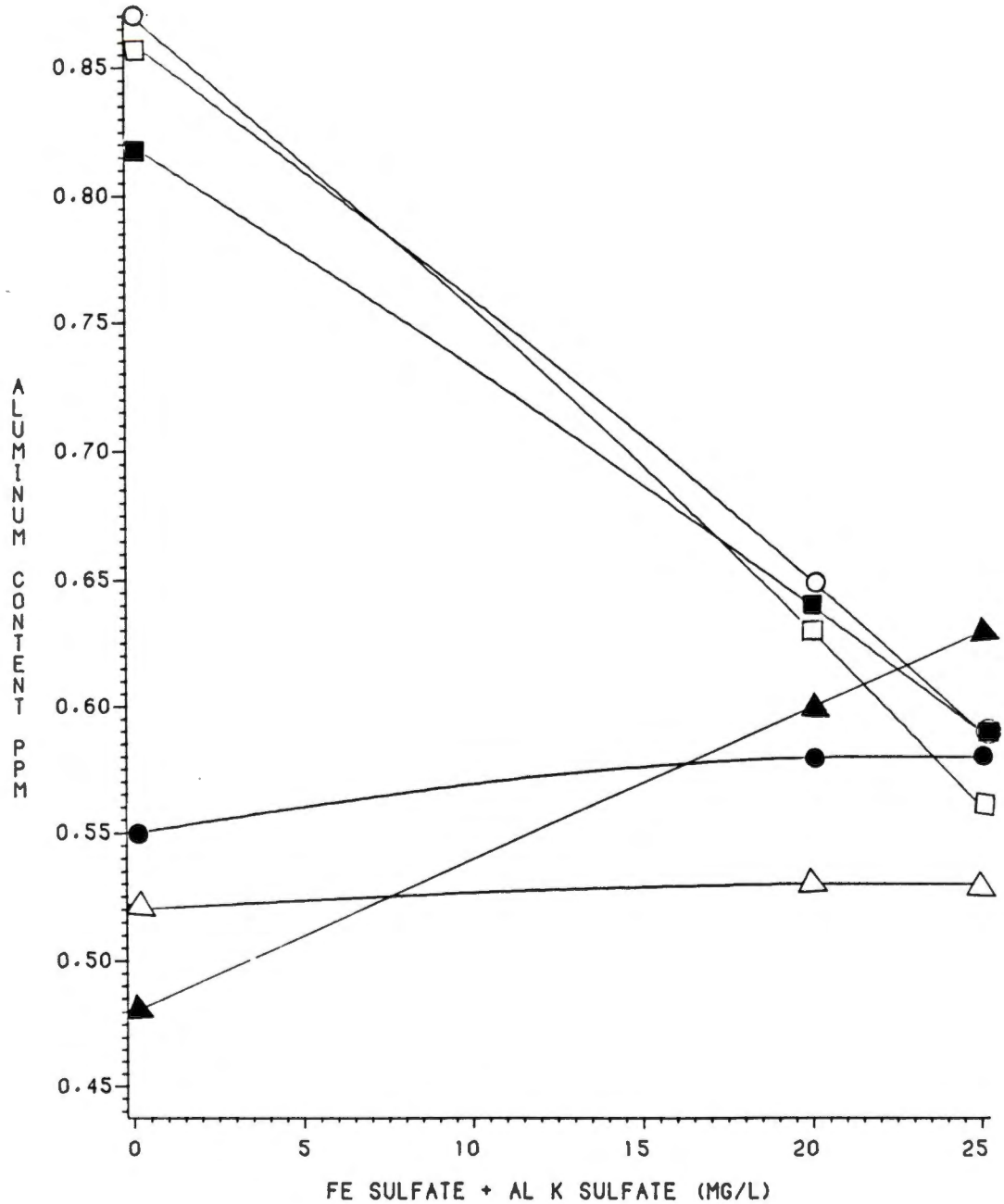


Figure 10. Aluminum content of Chrysanthemum morifolium 'Grandchild' grown in substrate with high nitrogen and high potassium nutritional regime.

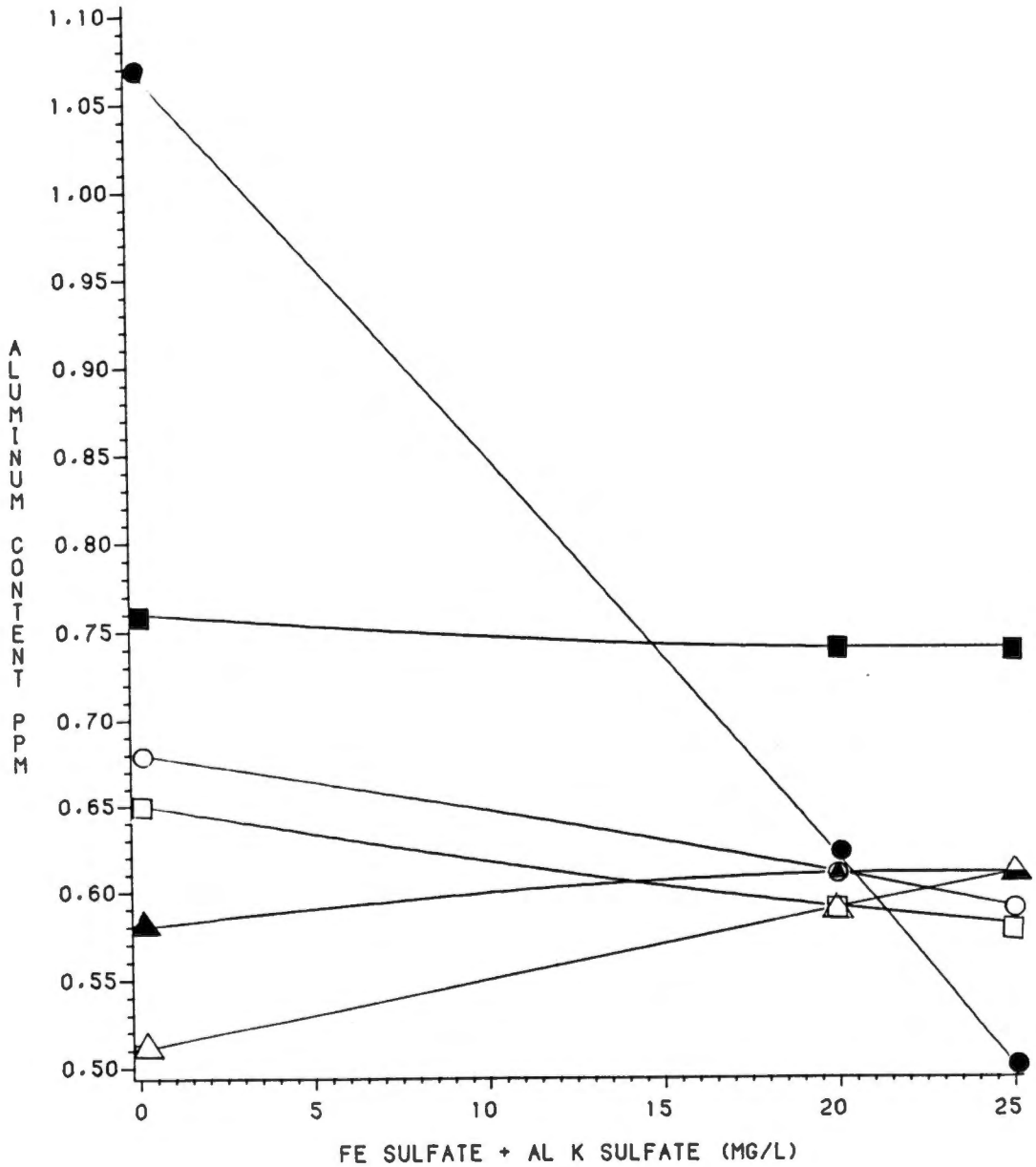


Figure 11. Aluminum content of Chrysanthemum morifolium 'Lancer' grown in substrate with high nitrogen and high potassium nutritional regime.

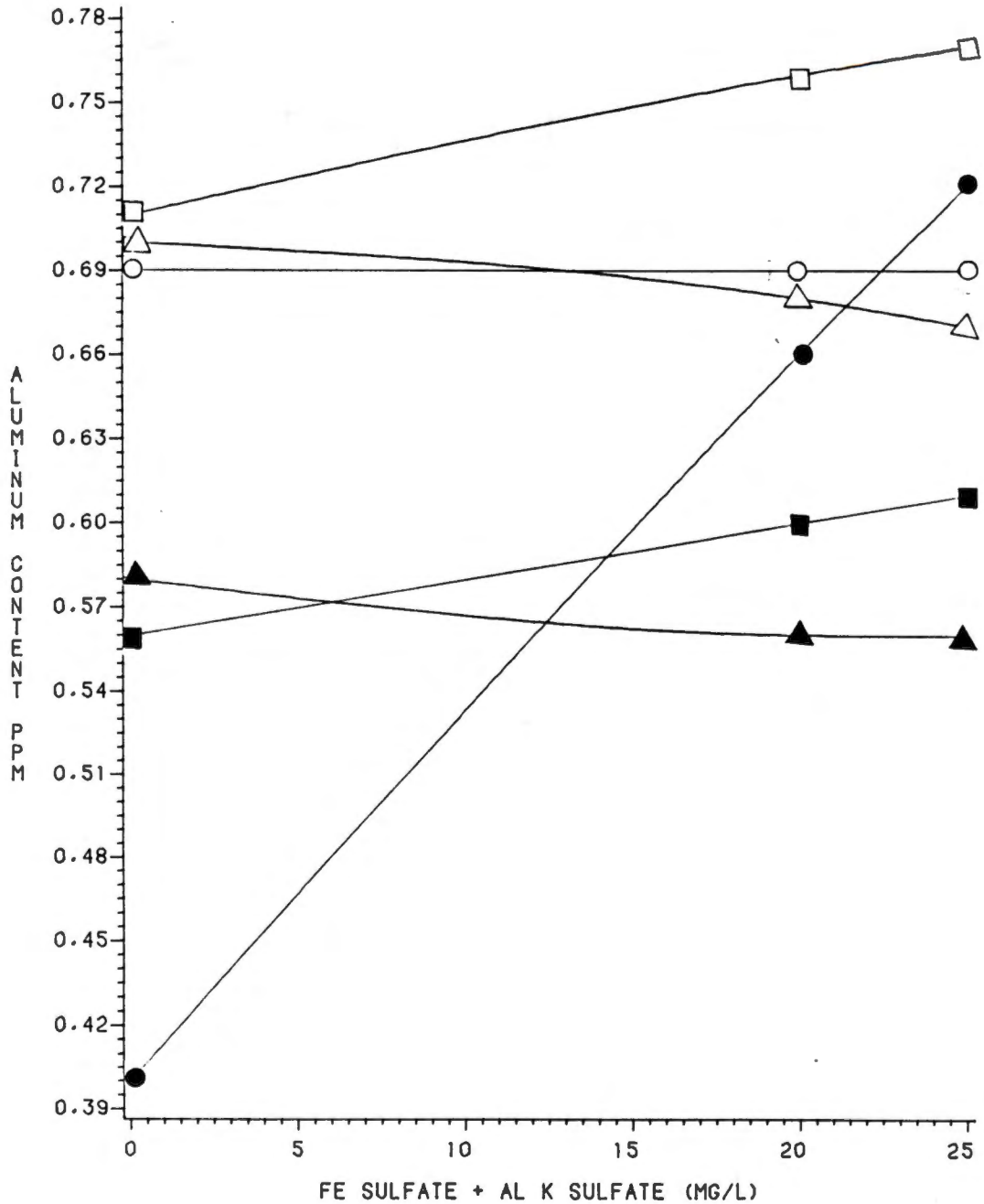
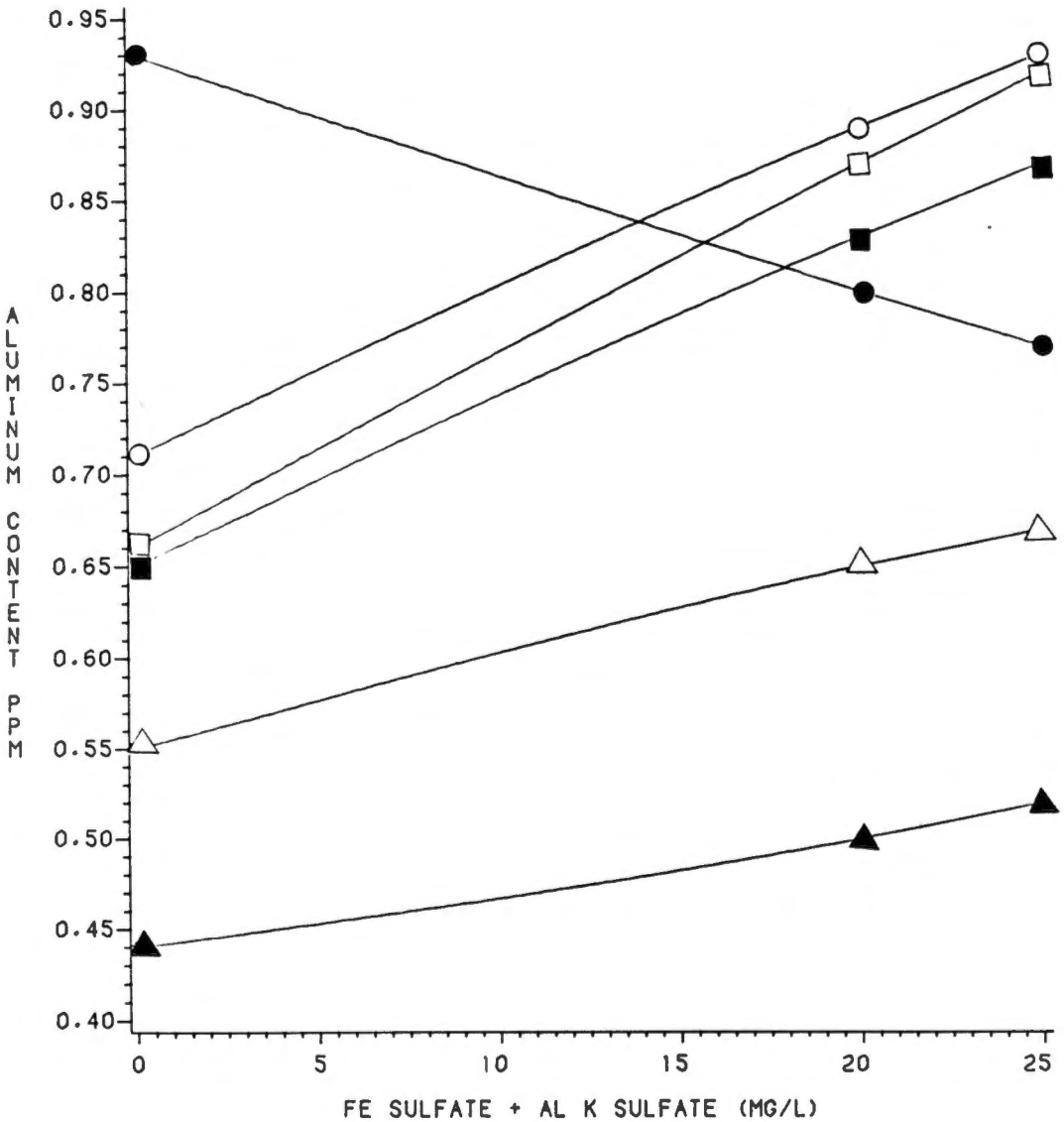


Figure 12. Aluminum content of Chrysanthemum morifolium 'Liberty' in substrate with high nitrogen and high potassium nutritional regime.



LEGEND: Plant Part

○ Leaves, High N	● Leaves, High K
△ Stems, High N	▲ Stems, High K
□ Petals, High N	■ Petals, High K

Figure 13. Aluminum content of Chrysanthemum morifolium 'Stardom' grown in substrate with high nitrogen and high potassium nutritional regime.

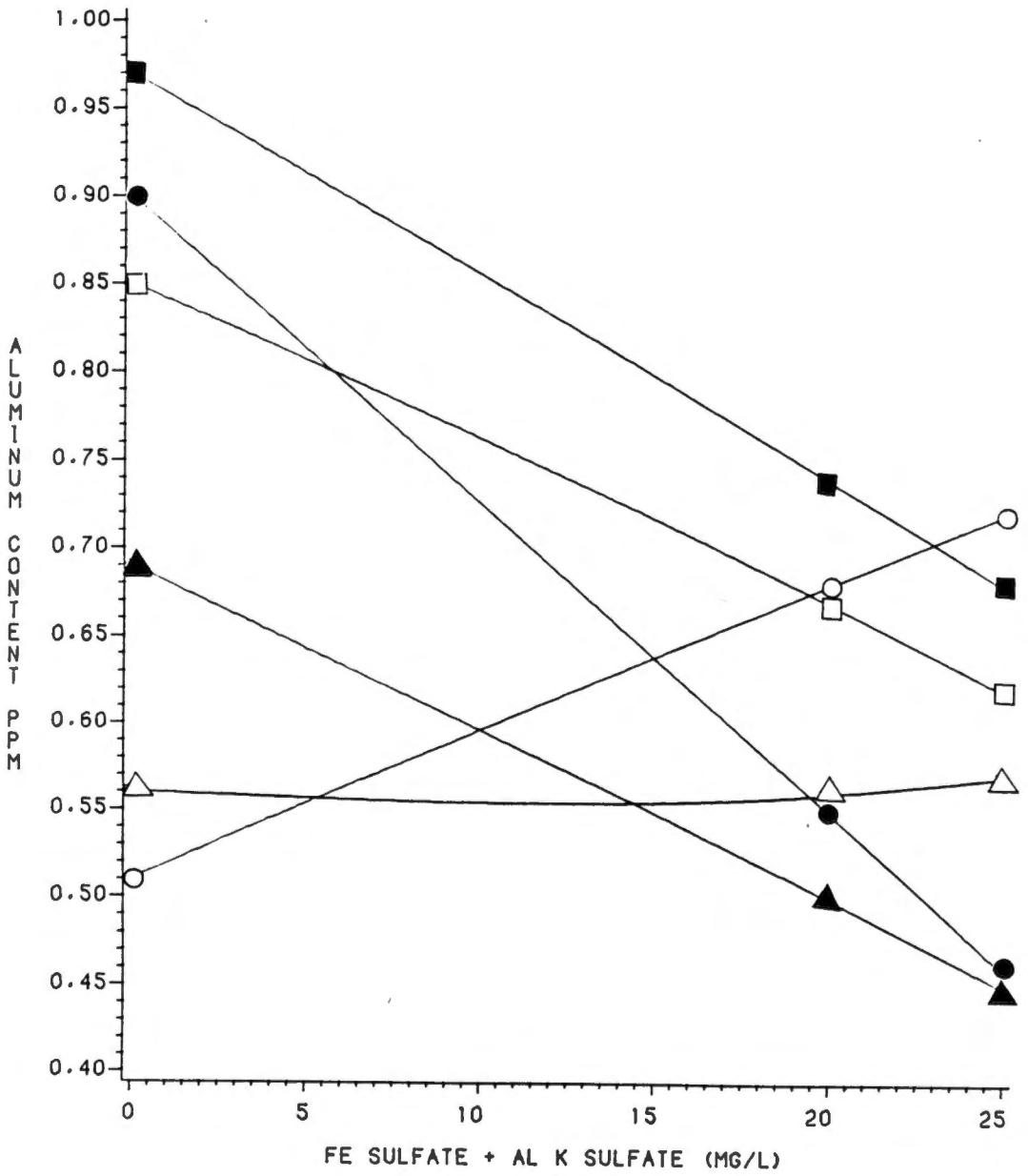


Figure 14. Aluminum content of Chrysanthemum morifolium 'Mango' grown in substrate with high nitrogen and high potassium nutritional regime.

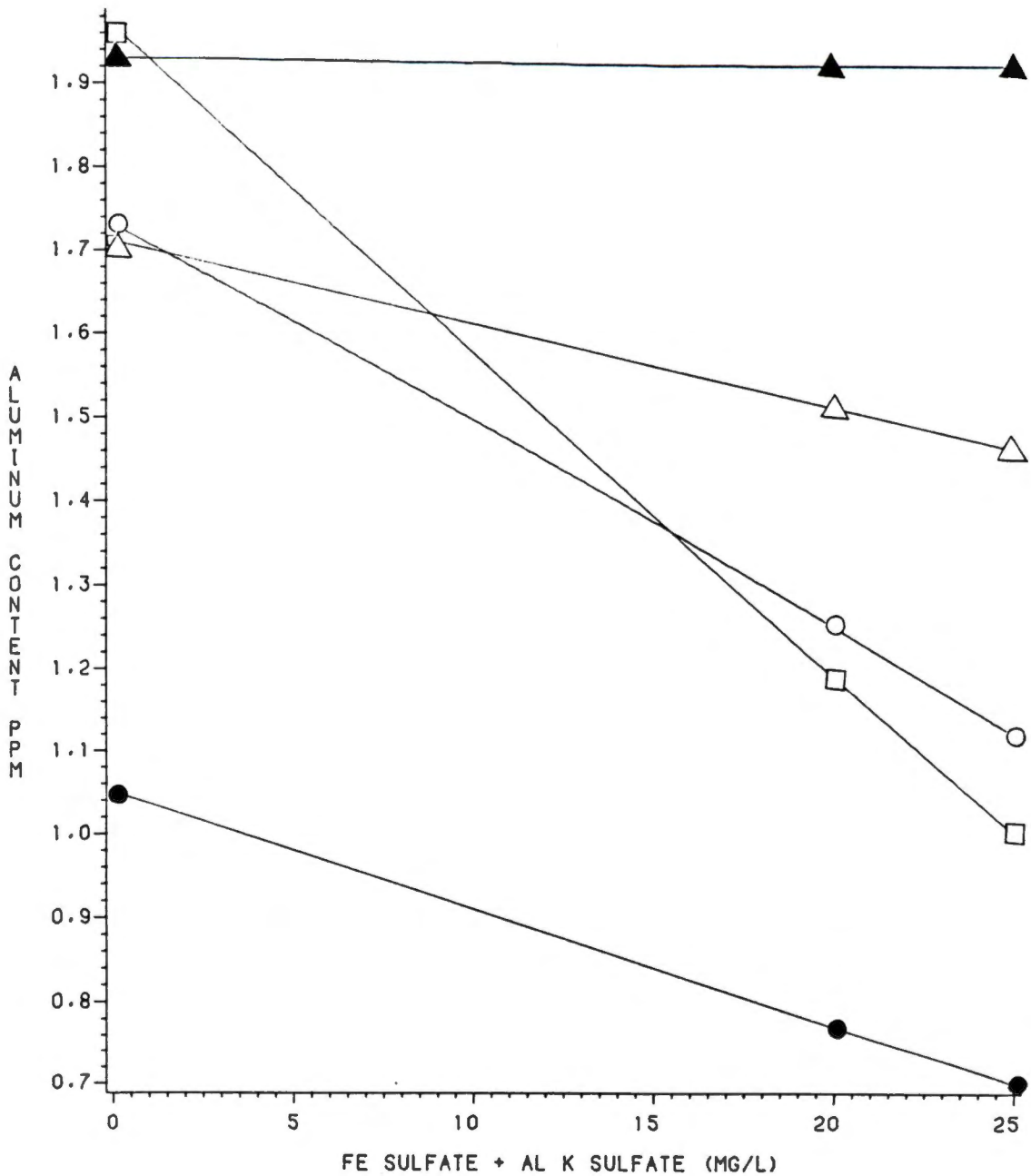


Figure 15. Aluminum content of five Chrysanthemum morifolium cultivars grown in substrate with high nitrogen low potassium nutritional regime.

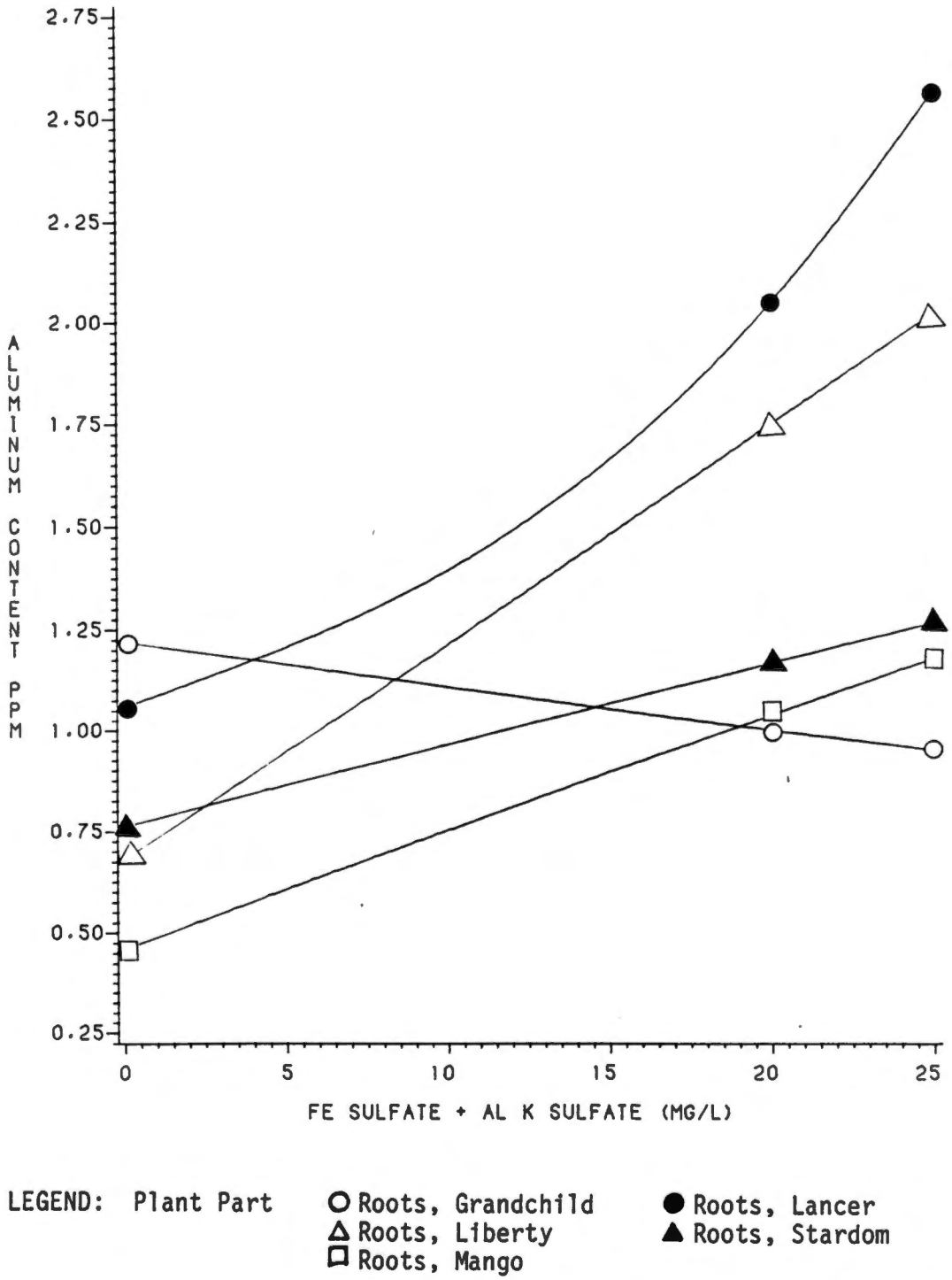


Figure 16. Aluminum content of five Chrysanthemum morifolium cultivars grown in substrate with low nitrogen high potassium nutritional regime.

Cell sap pH. Cell sap pH of the leaves and stems ranged from pH 5.4 to pH 6.5. Average pH readings for the leaves were: pH 5.9 for 'Grandchild,' pH 5.8 for 'Lancer,' pH 5.8 for 'Liberty,' pH 5.8 for 'Stardom' and pH 5.9 for 'Mango.' Average pH readings for the stems were: pH 5.9 for 'Grandchild,' pH 5.8 for 'Lancer,' pH 5.8 for 'Liberty,' pH 5.8 for 'Stardom' and pH 5.9 for 'Mango." These pH readings indicate that the cell sap pH of these chrysanthemum cultivars was less acidic than that of Al "accumulators." A favorable cell sap pH range is 3.6-5.2 if the Al is not to precipitate as a phosphate in the root and stem tissue long before the leaves are reached (37). However, the fact that a similar amount of Al was found in all chrysanthemum plant parts indicates that it was translocated throughout the plant and all of it did not remain in the roots and stems. This would suggest that the cell sap pH of the chrysanthemum may not be the most critical factor in determining its ability to accumulate Al.

Parallel experiment. In the parallel experiment, the hydrangeas that were given the same Fe Sulfate-Al K Sulfate treatments as the chrysanthemums produced blue cymes. The controls, which were not given any metal applications at all, produced pink cymes. These results indicate that the amount of Fe and Al used in these experiments was sufficient to cause blueing in a plant that is capable of taking up and accumulating Al and has a pigment that is capable of complexing with a metal.

CHAPTER V

DISCUSSION AND SUMMARY

Discussion

The similar colorimetric readings and low Al contents of the different plant parts of the chrysanthemum suggest that the cultivars used in these experiments are not likely candidates for blueing by cultural manipulation which involves the application of metallic solutions.

Most plants take up Al in minute quantities and accumulation seems to be restricted to certain species of plants. "Non-accumulator" plants, even when grown in a very acid environment, such as volcanic soil (pH 1.0), rather than taking up Al, concentrate it in or on their roots. The azalea concentrates 90% of the Al it takes up in the roots without any detrimental effects to the plant. This suggests that there is a mechanism which restricts Al entry into the cell metabolism of the root. This Al tolerance may be due to selective binding of metal in the freespace of the root with the cell wall acting as a selective barrier to toxic levels of metals in the root environment. The cell membrane may be differentially permeable to Al ions among species. Altering the concentration of Al around the roots of wheat significantly affects its sensitivity to it by modifying the exclusion properties of the membrane with respect to Al. It has been suggested that the site of exclusion is the plasmalemma (36).

It is quite possible that an exclusion mechanism similar to that of other plants is used by the chrysanthemum to prevent toxic levels of Al from entering its internal metabolism. The fact that the plants that did not receive the metal solutions had a similar amount of Al in them (which must have been obtained from the growing medium) would substantiate the existence of such a mechanism. It suggests that the chrysanthemum will only take up a certain amount of Al no matter how much is available to it. The greater amount of Al in the acidic root-soil samples (pH 4.5) was probably caused by an increased amount of Al available due to more acidic soil conditions.

Metal "accumulating" plants tolerate large amounts of Al, which may even be necessary for some of them, but for most plants, Al toxicity is more of a problem (32). It is possible that certain enzymes in Al tolerant plants may function in the presence of high heavy metal concentrations which would severely inhibit enzyme systems in nontolerant plants, meaning they may contain aluminum-tolerant enzyme systems. However, such systems have not been identified.

Another factor that can affect Al accumulation is the internal buffering system of a plant. It seems that plants with a predominantly inorganic buffering system are more sensitive to Al than those with an organic acid buffer system which can internally chelate Al to prevent it from interfering with phosphorus metabolism and maintain a high foliar content in these species. In the latter case, the plant tissue pH is usually less than 5.3 (36). The leaf and stem

cell sap pH of the chrysanthemum plant tissue ranged from pH 5.4-6.4, suggesting an inorganic buffering system. This may have restricted accumulation of Al in the leaves and petals of the plants, even though it was translocated to these parts.

Several screening tests and observations have been devised to distinguish Al tolerant species of plants and the implementation of these would provide a convenient tool in eliminating all unlikely blueing candidates. This would help to determine which chrysanthemums would be more likely to respond in a positive way to blueing techniques.

Five characteristics have been distinguished which occur almost universally among aluminum-accumulating plants (20):

1. An arborescent habit.
2. Leathery leaves of fine, prominent reticulate venation.
3. Conspicuous inflorescences.
4. A tendency to grow only in acid soils, since Al is essential to them.
5. A comparatively low cell sap acidity range, from pH 3.6-5.2.

Most screening tests involve subjecting the plant to Al stress (36):

1. The first obvious evidence of the presence of excess Al is the slowing of root elongation within 2 to 3 hours. Different varieties of plants differ significantly in their ability to initiate root elongation after the Al stress is removed. Among wheat cultivars, there are four distinct classes in tolerance and ability to recover. These are based on the Al concentration necessary to irreversibly inhibit root growth. This suggests that Al tolerance is qualitatively inherited and may make it possible to determine which species of

chrysanthemum were more tolerant and therefore more likely to accumulate Al or other metals.

2. Some divalent cations can protect the roots from Al toxicity and resultant reduction in root growth, such as Ca+ and Mg+. Therefore, by varying the concentrations of Ca+ and Mg+ in screening solutions, a wide range of Al stress capacity could be obtained. In corn, both Ca+ and Mg+ were used and the response seemed to be nonspecific to cations in general.

3. Since Al can tie up phosphorus in acid soils, inbred plants can be ranked with respect to Al tolerance using phosphorus deficiency symptoms and phosphorus contents of the tops of plants as a criteria of Al tolerance. Plants that were more tolerant of Al would contain more phosphorus in their plant tops.

4. Another test could involve the effect of various salts on pigment extracts for a positive response in color change.

Application of some of these tests in relation to chrysanthemum blueing may be valuable, given the wide choice of cultivars available to choose from and the fact that some of the cultivars in this study blued in solution while others did not. This suggests that the internal mechanisms and metabolism of pigmentation may differ among cultivars.

Two remaining methods that could be utilized in producing blue chrysanthemum flowers would include genetic techniques and tissue culture, as pigment synthesis can be modified by both breeding and biotechnical means.

Genetics and breeding. Genetics is the most determining factor in anthocyanin synthesis and as such becomes an extremely important factor when considering the manipulation of flower color. Genes appear to control all aspects of anthocyanin pigmentation, including the type of anthocyanin synthesized, the amount produced, the distribution (localization and pattern) and structural modifications of anthocyanins through metal chelation, co-pigmentation and conversion of pH of the cell sap. Studies show that these genes follow certain rules regarding the dominance of certain characteristics over others (15). Experimentation with the poinsettia indicates there are three general classes of genes which are involved in the inheritance of pigments and their synthesis. They control:

1. Chemical structure.
2. General production.
3. Distribution in flower.

Differences within a single color class (reds of poinsettia) suggest that a fourth class of genes controls color through controlling the state of the pigment in the living cell (pH, metal chelation, and co-pigmentation) (8).

It seems that relatively few genes are involved in the biosynthesis of flavanoids. Some Anthirrinum majus mutant genotypes indicate genetic blocks may occur in the pigment pathway, thus preventing the production of the flavanoid in spite of the presence of the necessary gene. However, the introduction of certain precursors can override the genetic block and allow anthocyanin production. They can also initiate new pigments foreign to the species (48).

Dihydroquercitin (DHQ), dihydrokaempferol (DHK), and dihydromyrecitin (DHM) are the flavanonol precursors that can be administered to acyanic flowers of a certain genotype to overcome the necessary genetic blockage so that anthocyanins can be produced. Natural precursors from macerated and ground flowers have also initiated the synthesis of anthocyanins (47).

Double pigmentation can be produced by a single flower when DHQ is introduced to an unblocked pink anthirrinum (Pg formed naturally, magenta Cy induced). Similarly, it is possible to produce the synthesis of both Cy and Dp in the same plant, Primula obconica, from only DHQ. DHM can initiate the formation of purple Dp, which would not normally be present.

Other precursors can induce pigmentation in acyanic flowers due to their chemical structure (e.g., naringenin). The various precursors can be tested on cut flowers with good synthesis being obtained within a day or two (46).

The important aspect of all of this is that perhaps if a white chrysanthemum were given the right precursor, it would be capable of producing a purple or blue Dp.

Care should be given to selecting appropriate inheritable traits in breeding programs involving the alteration of flower color. One factor that should be realized is that the inheritance of co-pigments is as important as the inheritance of anthocyanin type, since co-pigmentation can have such a strong influence on flower color (10).

Within the boundaries of available germplasm, desired colors can be obtained by recombining factors controlling pH, anthocyanin concentration and molar ratio of co-pigment to anthocyanin. Bluer shades could be obtained by increasing both the pH and the molar ratio of co-pigment to anthocyanin. The various flavanoid co-pigments exhibit different co-pigment effects, some producing some bluer anthocyanin co-pigment complexes than others; therefore, if possible, structure should be related to co-pigment effects for proper selection.

The structure of the anthocyanin is also important. Breeding toward higher pH's with glycosides of the most hydroxylated anthocyanins available would again produce bluer shades (13). Pigment concentration is another factor that should be considered, since it is those flowers within a species that have a lesser amount of anthocyanin that become blue more readily, even though the pH of the pink or red and blue flowers may be the same. This approach, therefore, bears some importance in a search for blueing techniques (3).

A number of both natural and synthetic intergeneric hybrids among grasses have been reported, with the hybrids in some cases having more vigor than their parents (38). Possibly this approach could be utilized in producing a blue chrysanthemum by crossing it with a blue-flowered species of a related genera (e.g., aster).

Tissue culture. Tissue culture removes anthocyanin production from the restrictions that genetics places on it and it can override the limitations of pot culture. Red cabbage, when grown in a Knopp's

solution plus 0.01% aluminum nitrate, was able to produce a blue anthocyanin. This effect, however, was never obtained in pot culture (20).

It has been shown that not only can acyanic species produce anthocyanin given the proper environmental conditions, but that cyanic species can add another pigment to the one they are already producing. Several specific conditions that are necessary for anthocyanin synthesis have already been established for both acyanic and cyanic species. These include a salt-nutrient medium rich in phosphates and inorganic nitrogen, auxin (preferably NAA) and kinetin, as well as a combination of high light intensity and cool temperature.

Cy is considered to be a biosynthetically key anthocyanin for the synthesis of Dp, and it is conceivable that steps leading to hydroxylation of Cy to Dp may be modified under the conditions of in vitro culture. Callus cultures of both apple and rose cotyledons produce Cy equally in their peel and petal tissues. However, Dp occurred as an added pigment in the latter tissue, even though Dp is absent in the genus Rosa. Its identity has been unequivocally determined (although in small amounts) and chelation by a metal salt is suggested due to difficulty in extracting it from callus tissue. This production of Dp suggests that cultural conditions may stimulate further hydroxylation of Cy. Dp, as well as Cy, has also been produced in tissue culture in the marigold, a compositae. Thus the pigment Dp has been formed under appropriate conditions in the family to which the chrysanthemum belongs, where this pigment does not normally occur (26).

Tissue culture can also alter the amount of anthocyanin produced. Experiments with Impatiens balsamina indicated that both the upper limit of anthocyanin synthesis and the relative pigment content of the flowers are governed by the same gene. However, culturing appears to remove the restrictions on the relative amount of anthocyanin that is produced in the petals, suggesting that genetic control for this lies elsewhere in the plant (30,45).

This data suggests that callus tissue of various species has the complete machinery for de novo synthesis of anthocyanin pigments under proper conditions (44). That factors other than genetic control play an important role in anthocyanin synthesis is evidenced by the dissimilar structural patterns of pigments between in vitro cultures and their parent plants. Determination of these factors could lead to the production of new color pigmentation for certain species (27). The chrysanthemum is certainly an eligible candidate for this, since Dp has already been produced in a composite (marigold) that normally does not produce this pigment (26).

It is unlikely that the practice of feeding precursors in tissue culture would prove to be very beneficial in producing a blue chrysanthemum, as the effect of such feeding would be lost when the plants were placed into pot culture. Also, these precursors are often only available in small amounts. However, this does not detract from the importance of such research, as it demonstrates that factors inhibiting the production of blue pigmentation can be overcome when genetic blocks are overcome. Biotechnical procedures

involving gene transplant in tissue culture could possibly lead to blue pigmentation in a plant where it would not normally occur. Protoplast fusion makes possible to the breeder a genetic variability that may not be possible by any other means (42).

Summary

Five cultivars of garden chrysanthemums were grown under specific cultural conditions to induce blueing of their pink flower petals. These conditions included an acid growing medium (pH 4.5), a low N/high K nutritional regime and 20 and 25 mg/l Fe Sulf-Al K Sulf metal applications. Controls were grown in a less acid medium (pH 6.5) with a high N/low K nutritional regime and no metal applications. Flower color was measured as degree of hue by a Hunter Digital Color Difference Meter (16). Flower color readings were similar among treatments and cultivars and petal color remained pink regardless of treatment. Al content of the leaves, petals, stems and root-soil combinations of each plant were measured as ppm of dry weight using the Aluminon Colorimetric Method (23). Al content readings of the leaves, stems and petals showed that these plant parts accumulated Al only in very minute quantities for all treatments and cultivars. Root-soil samples showed a slightly higher Al content but no trends were noted. Cell sap pH of the leaves and stems was measured, and it was found that the cell sap pH of the cultivars was less acidic than that of an Al accumulating plant. It was concluded that the chrysanthemum is not an Al accumulator and,

therefore, not a likely candidate for blueing by cultural modification using metal application in pot culture. Growing methods involving genetics and tissue culture are suggested as an alternative method for producing a blue petalled chrysanthemum.

REFERENCES

REFERENCES

1. Allen, R. C. 1931. Factors influencing the flower color of hydrangeas. Proc. Amer. Soc. Hort. Sci. 28:410-12.

2. Asen, et al. 1957. Effect of aluminum on absorption spectra of anthocyanins and favanoids from sepals of Hydrangea macrophylla var. Merveille. J. Amer. Soc. Hort. Sci. 70:478-81.

3. Asen, et al. 1957. Anthocyanins and other phenolic compounds in red and blue sepals of Hydrangea macrophylla var. Merveille. J. Amer. Soc. Hort. Sci. 69:561-69.

4. Asen, et al. 1959. Effect of various concentrations of nitrogen, phosphorous and potassium on sepal color of Hydrangea macrophylla. J. Amer. Soc. Hort. Sci. 73:496-98.

5. Asen, et al. 1960. Color of Hydrangea macrophylla sepals as influenced by the carry-over effects from summer applications of N, P and K. J. Amer. Soc. Hort. Sci. 76:631-36.

6. Asen, et al. 1962. Sepal color of Hydrangea macrophylla as influenced by source of nitrogen available to the plants. J. Amer. Soc. Hort. Soc. 82:504-07.

7. Asen, S., and L. Jurd. 1967. The constitution of a crystalline, blue cornflower pigment. Phytochemistry 6:577-84.

8. Asen, et al. 1969. Relation of flower color to optical-density spectra of intact tissue of anthocyanin extracts. Amer. J. Bot. 56:227-31.

9. Asen, et al. 1969. Absorption spectra and color of aluminum-cyanidin 3-glucoside as influenced by pH. Phytochemistry 8:653-39.

10. Asen, et al. 1971. Co-pigment effect of quercitin glycosides on absorption characteristics of cyanidin glycosides and color of red wing azalea. Phytochemistry 10:171-75.

11. Asen, et al. 1972. Co-pigmentation of anthocyanins in plant tissues and its effect on color. Phytochemistry 11:1139-44.

12. Asen, et al. 1975. Anthocyanins, flavanoid copigments, and Phytochemistry 14:2677-82.

13. Asen, S. 1976. Known factors responsible for infinite flower color variations. Acta Horticulturae 63:217-23.

14. Asen, et al. 1977. Anthocyanins and pH involved in the color of 'Heavenly Blue' morning glory. Phytochemistry 16:118-19.

15. Blank, F. 1947. The anthocyanin pigments of plants. Bot. Rev. 13:241-317.

16. Bertsch, P. M., et al. 1981. Automated aluminum analysis with the aluminon method. Soil Sci. Soc. Amer. J. 45:666-67.

17. Chen, Lao-Jer, and Geza Hrazdina. 1981. Structural aspects of anthocyanin-flavanoid complex formation and its role in plant color. Phytochemistry 20:297-303.

18. Chenery, E. M. 1937. The problem of the blue hydrangea. J. Roy. Hort. Soc. 62:304-06.

19. Chenery, E. M. 1948. Aluminum in plants and its relation to plant pigments. Ann. Bot. 12:121-36.

20. Chenery, E. M. 1948. Aluminum in the plant world. Rev. Bulletin 2:173-83.

21. Chisholm, Nerine. 1962. Chemicals give precise color control in hydrangeas. Acta Horticulturae 40:128-29.

22. Connors, et al. 1923. Color control in forcing hydrangeas. J. Amer. Soc. Hort. Sci. 20:282-88.

23. Francis, C. W. 1978. Radiostrontium movement in soils and uptake in plants. U.S. Dept. of Energy, pp. 83-84.

24. Hagen, C. W., Jr. 1966. The differentiation of pigmentation in flower parts. I. The flavanoid pigments of impatiens balsamina genotype ♀♀HHP^{r}P^{r} and their distribution within the plant. Amer. J. Bot. 53(1):46-54.

25. Hagen, C. W., Jr. 1966. The differentiation of pigmentation in flower parts. II. Changes in pigments during the development of buds in impatiens balsamine genotype ♀♀HHP^{r}P^{r}. Amer. J. Bot. 53(1):54-60.

26. Harborne, J. B. 1965. Flavanoids: Distribution and contribution to plant color. Chemistry and Biochemistry of Plant Pigments. T. W. Goodwin, ed. Academic Press, NY, pp. 247-76.

27. Ibrahim, et al. 1971. Formation of anthocyanins in callus tissue cultures. Lloyodia 34(2):175-81.

28. Jurd, L. 1962. Spectral properties of flavanoid compounds. Chemisry of Flavanoid Compounds. T. A. Geissman, ed. The Macmillan Co., NY, pp. 107-55.

29. Jurd, L., and S. Asen. 1966. The formation of metal and "co-pigment" complexes of cyanidin 3-glucoside. Phytochemistry 5: 1263-71.

30. Klein, A. O., and C. W. Hagen, Jr. 1961. Anthocyanin production in detached petals of impatiens balsamina L. Plant Physiology 36(1):1-8.

31. Link, et al. 1952. Experiments on fertilizer levels for greenhouse hydrangeas. J. Amer. Soc. Hort. Sci. 60:449-58.

32. Miller, L. P. 1964. The role of minerals in phytochemistry. Phytochemistry 3:29-30.

33. Miller, et al. 1967. Anthocyanin synthesis in Salpiglossis sinuata. Amer. J. Bot. 54(9):1163-70.

34. Ôsawa, Y., and Norio Saitô. 1968. Electron spin resonance studies on anthocyanins. Phytochemistry 7:1189-95.

35. Paech, K. 1955. Color development in flowers. Ann. Rev. Plt. Phys. 6:273-98.

36. Rhue, R. D., and C. D. Grogan. 1977. Differential aluminum tolerance in crop plants. Agron. J. 69:755-60.

37. Rutland, R. B., and K. W. Seawright. 1973. Anthocyanins in flowers of Chrysanthemum morifolium Ramat. during anthesis in relation to sugar content. J. Amer. Soc. Hort. Sci. 98(1):74-77.

38. Sadasivaiah, R. S., and J. Weijer. 1981. Cytogenetics of some natural intergeneric hybrids between Elymus and Agropyron species. Can. J. Cytol. 23:131-40.

39. Shanks, et al. 1950. A preliminary study on the mineral nutrition of hydrangeas. J. Amer. Soc. Hort. Sci. 56:457-65.

40. Shanks, J. B., and C. B. Link. 1951. Additional experiments on the mineral nutrition of hydrangeas. J. Amer. Soc. Hort. Sci. 58:329-31.

41. Shanks, J. B., and C. B. Link. 1953. Fertilization during the growing and forcing periods on growth and flowering of hydrangeas. J. Amer. Soc. Hort. Sci. 62:471-73.

42. Sprague, G., F. Alexander, and J. W. Dudley. 1980. Plant breeding and genetic engineering: A perspective. Bioscience 30(1):17-21.

43. Stewart, et al. 1975. Microspectrophotometric measurement pH and pH effect on color of petal epidermal cells. Phytochemistry 14:937-42.

44. Stickland, R. G. 1972. Changes in anthocyanin, carotenoid, chlorophyl, and protein in developing florets of the chrysanthemum. Ann. Bot. 36:459-69.

45. Stickland, R. G. 1974. Pigments produced by cultured florets of Chrysanthemum morifolium. Ann. Bot. 38:1-6.

46. Stickland, R. G., and B. J. Harrison. 1976. Pathways of color. J. Roy. Hort. Soc. 101:326-28.

47. Stickland, R. G., and B. J. Harrison. 1977. Precursors and genetic control of pigmentation. I. Induced biosynthesis of pelargonidin, cyanidin and delphinidin in Antirrhinum majus. J. Heredity 39(3):108-12.

48. Stickland, R. G., and B. J. Harrison. 1977. Precursors and genetic control of pigmentation. II. Genotype analysis of pigment controlling genes in acyanic phenotypes in Antirrhinum majus. J. Heredity 39(3):112-15.

49. Swain, T. 1965. Nature and properties of flavanoids. Chemistry and Biochemistry of Plant Pigments. T. W. Goodwin, ed. Academic Press, NY, pp. 211-21.

50. Van Buren, J. P. 1974. Color of anthocyanin solutions expressed in lightness and chromaticity terms. J. Fd. Sci. 39:325-28.

51. Weiler, T. C. 1980. Hydrangeas. Introductory Floriculture. R. A. Larsen, ed. Academic Press, pp. 354-69.

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