understood. It has been reported that K fertilization did increase titratable acids in peaches (5) and in apples (2, 3) but not in sweet cherries (4). Soluble solids were not affected by N or K. The large seasonal difference in soluble solids (11.8-18.8%) is likely due to the size of the crop since soluble solids appear to be higher in a low crop situation.

Leaf and fruit composition. Only 3 nutrient elements (N, K and Mn) were affected by treatments (Table 5). The initial levels of leaf N (2.38-2.52%) and K (3.20-3.42%) were considered adequate for plums in Western New York. The increase of leaf N the first year and fruit N the third year, by either higher rates of N or higher rates of N combined with K, was statistically significant when compared to the no N treatments. The K level of leaf and fruit was not affected by fertilizer treatments until the third year. Ammonium nitrate favors accumulation of Mn in the leaves. Similar effects on peaches have been reported (8, 9). Mineral content of all elements is lower in fruit than in leaves. The leaf analysis indicates that maintaining an adequate level of leaf K alone did not improve yield unless the leaf N was also maintained sufficiently high. Leaf K less than 2.0% was considered to be the threshold for developing deficiency symptoms in plums (6, 7). The results of the present study suggest that the leaf K should be maintained above 2.0% and the level of leaf N should be maintained not less than 2.1% for optimum production of 'Stanley' prunes.

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Anthocyanin in Flowers of *Chrysanthemum morifolium* Ram. During Anthesis in Relation to Sugar Content[1]

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Abstract. Anthocyanin concentration decreased and sugar concentration increased in petals of *C. morifolium* cv. Orchid Queen during petal expansion in a greenhouse from the day of first coloration until full bloom. No correlation was found between either total sugar or reducing sugar and anthocyanin concn. Dry wt % decreased also during petal expansion so that sugar was an increasing proportion of the dry matter. Anthocyanin was at higher concn in petals expanding in Oct. than in July or Nov., but reducing sugar was higher in Nov. and total sugar was higher in Nov. and July than in Oct. The possibility of insufficient sugar substrate for anthocyanin synthesis under these conditions is discounted.

Intense coloration in red chrysanthemum flowers occurs under cool temp and bright light intensity. Similar climatic conditions favor anthocyanin accumulation in apple fruit (9, 14) and *Spirodela* leaves (12). Anthocyanin accumulates in fruit tissues under conditions that favor sugar production, and a relationship between sugar concn and anthocyanin synthesis has been noted since 1899 (2). Several tissues have been shown to redden after infusion of sugar (2, 3, 4, 12, 13). Although a direct pathway for synthesis of anthocyanin from sugars has not been proven, Thimann et al. (13) did find a close relationship between reducing sugars and anthocyanin. We attempted to determine if a correlation between reducing sugars or total sugars and anthocyanin could be found in red chrysanthemum flowers at any stage of anthesis from time of first coloration until pollen shed and if the more intense pigmentation in autumn relative to summer was associated with higher sugar content of petals.

Materials and Methods

Plants of cv. Orchid Queen were grown in a glasshouse in Athens, Georgia, in which max summer temp were moderated by a fan-pad evaporative cooling system and winter temp were 15°C night/20-25°C day. Shading by titanium oxide paint on the glass was provided from May 20 until Sept. 15. Standard commercial cultural procedures were followed: photoperiods controlled according to schedules for cut flowers, high fertility (150 ppm N and 56 ppm K continuous injection of water), peat-amended clay soil. The plants and flowers were of size and quality to meet upper commercial grades. The pink blossoms of 'Orchid Queen' are sensitive to climatic factors for color intensity.

Plantings were made on May 11, July 27, and Aug. 31, 1968 and subjected to short photoperiods so that flowers would be at optimum stage for harvest on Aug. 3, Oct. 19, and Nov. 16. Petals became visible and first developed color on July 16, Oct. 1, and Nov. 7, respectively. On those dates and every third day thereafter until pollen shed, 7 blossoms were taken from each of 7 plants and were analyzed for anthocyanin and sugar content. Anthocyanin was determined by boiling 0.5 g samples of outer ray petals in 1% HCl-methanol for 2 min and letting stand for 4 hr. Filtration through glass fiber filters using additional

[1]Received for publication January 17, 1972.

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HCl-methanol gave complete extraction. Anthocyanin concn in fresh petal tissue was calculated by reading transmittance (T) of extract of standard volume on a B/L Spectronic-20 colorimeter and converting to cyanidin equivalent from a curve relating concn of cyanidin with T.

The Shaffer-Somogyi method (7) was used to determine reducing and total sugars. The tissue sample was the entire petallage at early sampling dates and 4 g of petals from a wedge-shaped section of the inflorescence at more advanced stages of expansion.

Means were computed for each constituent at each time of sampling and were plotted on linear graph paper with tissue concn as ordinate and time as abscissa.

Results and Discussion

The concn of anthocyanin in petals was highest in the earliest stage of anthesis and declined as petals expanded (Fig. 1A). The concn decreased rapidly during the first 6 days of petal expansion in July, probably because petals increased in fresh wt more rapidly than anthocyanin was synthesized, as observed in roses (1). The concn continued to decrease after the 6th day, but the decline was more gradual. Fresh wt increase was linear during the entire 16 days of petal expansion, so the rate of anthocyanin synthesis possibly increased after the 6th day. The rate of synthesis, however, was never sufficient to stabilize the concn of anthocyanin in the expanding petals. Furthermore, the decline in anthocyanin concn during the latter phase of expansion was due partly to fading. A visual reduction in intensity of color occurred after full expansion of the outermost petals. By the 20th day, the detectable anthocyanin had been lost and the flowers were white or only very slightly pink.

While anthocyanin concn decreased, sugar concn was steadily and rapidly increased. The anthocyanin concn decreased by a factor of 6 (from 0.29 to 0.05 mg/g petal) in 16 days, while reducing sugar increased by a factor of 2.5 (6 to 15 mg/g) and total sugar increased by a factor of 5 (7 to 35 mg/g). Furthermore, a correlation between anthocyanin concn and sugar concn was not found on any day of sampling.

The flowers that bloomed in Oct. had much more pigment, initially 2.21 mg/g, but similarly experienced a decrease in anthocyanin concn and a reduction in color intensity during petal expansion (Fig. 1B). Total and reducing sugars both increased to a max on the 16th day and remained essentially constant thereafter.

A similar pattern of rapid decline in anthocyanin and increase in sugar concn in expanding petals was also observed in Nov. (Fig. 1C). The highest sugar concn were found in Nov. (50 mg total and 32 mg reducing sugar/g petal), but the anthocyanin concn was only 0.23 mg/g on the day of highest sugar (18th). In Oct. that concn of anthocyanin was found on the 17th day when total sugar was only 30 mg/g and reducing sugar was 24 mg/gl.

Weather conditions were more favorable for anthocyanin synthesis (1, 12) and accumulation in flowers (6, 10, 11) during Oct. than in July. Maximum temp were 20 to 28°C during the first 15 days and only 15 to 22°C thereafter. Night temp were 15°C except for a few nights early in the period when they were as warm as 18°C. Sunlight was reduced or excluded by cloud cover on 11 days. By contrast, in July, day temp were often warmer than 35°C and were generally above 28°C for most of the day. Night temp ranged between 22 and 25°C. A significant cloud cover formed during only 6 days. Temperatures were

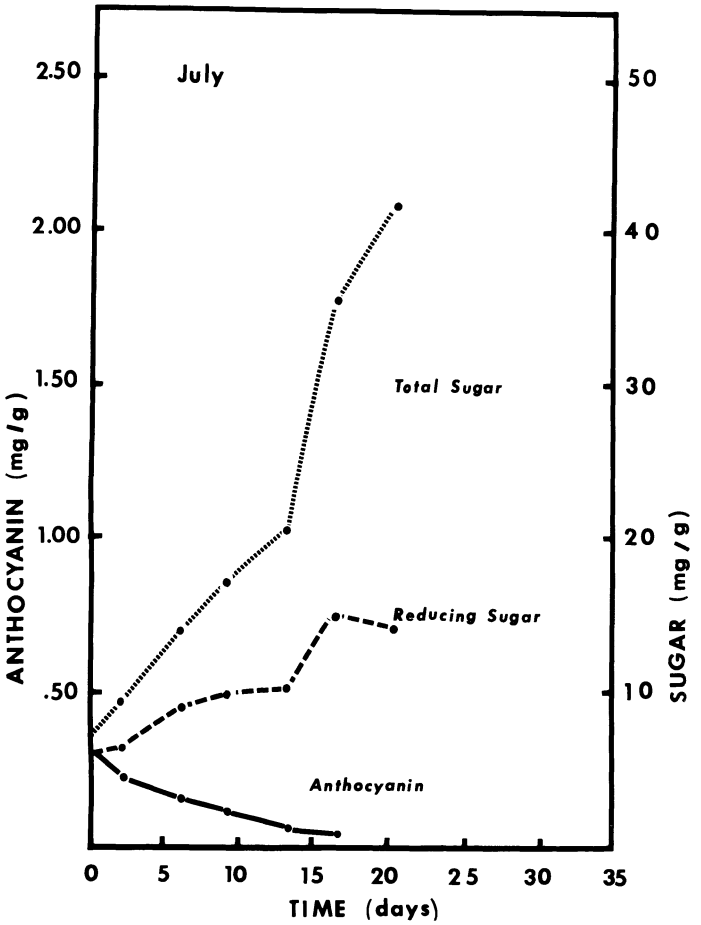

Fig. 1 A. Changes in concn of anthocyanin, reducing sugars and total sugars in fresh petal tissue of *C. morifolium* Ram. cv. Orchid Queen from the day of first visible color until pollen shed in (A) July, (B) Oct., and (C) Nov.

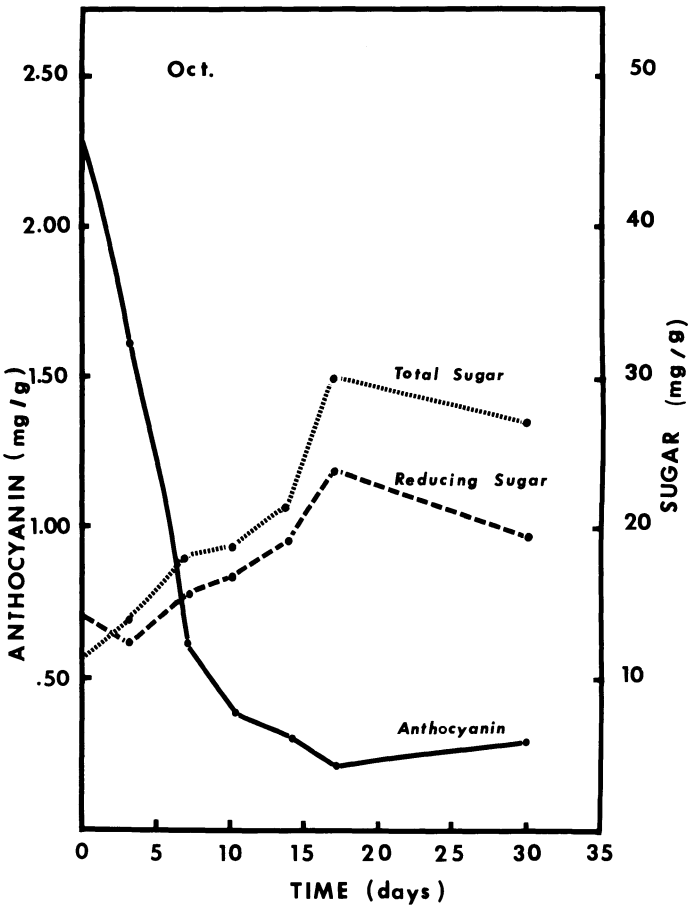

Fig. 1 B.

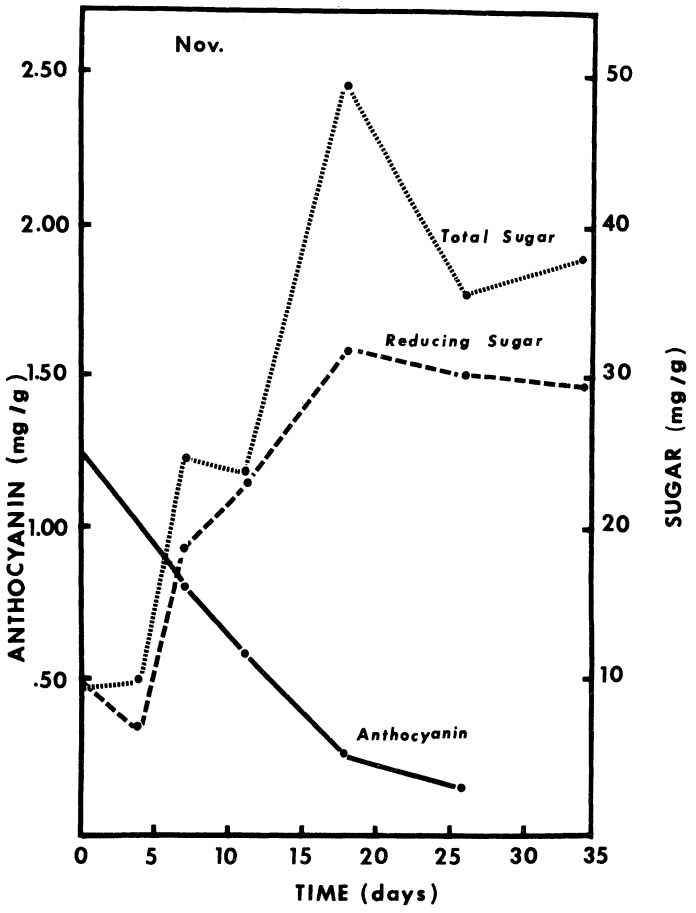

Fig. 1 C.

most favorable for anthocyanin synthesis in Nov. (10°C night and 15 to 22°C day). Sunlight, however, was totally clouded on 16 days. The low light intensity of Nov. possibly limited anthocyanin synthesis, and the excessively high radiant energy in July might have caused photodegradation. During Oct. an ideal combination of temp and light intensity produced the highest anthocyanin concn.

A conclusion cannot be drawn, however, that the reason for highest anthocyanin in Oct. was the availability of a rich sugar substrate for anthocyanin synthesis. Both total and reducing sugars were higher in Nov. than in Oct., and total sugar was higher in July than in Oct. Total dry matter concn of the petal tissue decreased as petals expanded in a manner similar to the decrease in anthocyanin concn (Fig. 2). The consistency of the patterns of increasing concn of sugars and decreasing concn of anthocyanin and total dry matter during anthesis in each period of observation is a basis for questioning an insufficiency of sugar as the factor limiting anthocyanin synthesis in this flower.

A simple correlation of sugar content with anthocyanin concn did not exist at any stage of anthesis during any of the 3 observation periods. Frey-Wyssling and Blank (5) concluded similarly that anthocyanin synthesis in red cabbage seedlings was not directly related to sugar content, but only a general relationship pertained. Thimann et al. (13) also found no linear correlation of anthocyanin concn with sucrose in *Spirodela* tissue fluids. A valid interpretation of their data is that a min concn of sucrose is required for max anthocyanin production. They found an increase in anthocyanin 100 to 800 units as sucrose in the sap increased from .003 to .005. No further increase in anthocyanin was observed at higher sucrose concn. The relationship between reducing sugars and anthocyanin in *Spirodela* tissue was closer than that of sucrose and anthocyanin. Even so, anthocyanin increased as hexoses increased in the range 0.002 to .005 M, asymtoped at .006 M, and did not increase further even at .015 M.

In experiments in which sucrose has been supplied in the culture medium for plant parts the optimum ambient sucrose concn was reported to be about 0.05 M (approx 1.7%) for *Spirodela* (13), strawberry leaf disks (3), and *Impatiens* petals (8). In those reports, only slightly higher sucrose concn caused reduced growth of the excised plant organs and reduced anthocyanin concn. Growth was probably inhibited by the high osmotic potential of sucrose solutions at the higher concn. Other means of inhibiting growth of *Spirodela* caused an increase in anthocyanin in the leaf (13). Anthocyanin increased more in apple skins in 0.06 M sucrose solutions when urea was not supplied than when urea was included and protein synthesis was stimulated (4).

In the chrysanthemum flowers we studied, total sugar concn in petal tissue was above 0.05 M (17 mg/g) except during the first 8 days of anthesis in July and first 6 days in Oct. and Nov. Reducing sugar was also greater than 0.05 M after the 10th day of anthesis in Oct. and 7th day in Nov., but was always less than 0.05 M in July. The conclusion should not be drawn that a critical concn of reducing sugars is .05 M, however, because the highest anthocyanin concn were found during the earliest stages of anthesis when reducing sugar was least (less than .02 M). A possible conclusion is that environmental conditions must exert a direct influence on the secondary metabolic systems responsible for anthocyanin synthesis and not on the primary metabolic processes that control the production and conservation of sugars. Another possibility is an environmentally influenced degradation of anthocyanin in the expanding petals that accounts for loss of the pigment after its synthesis.

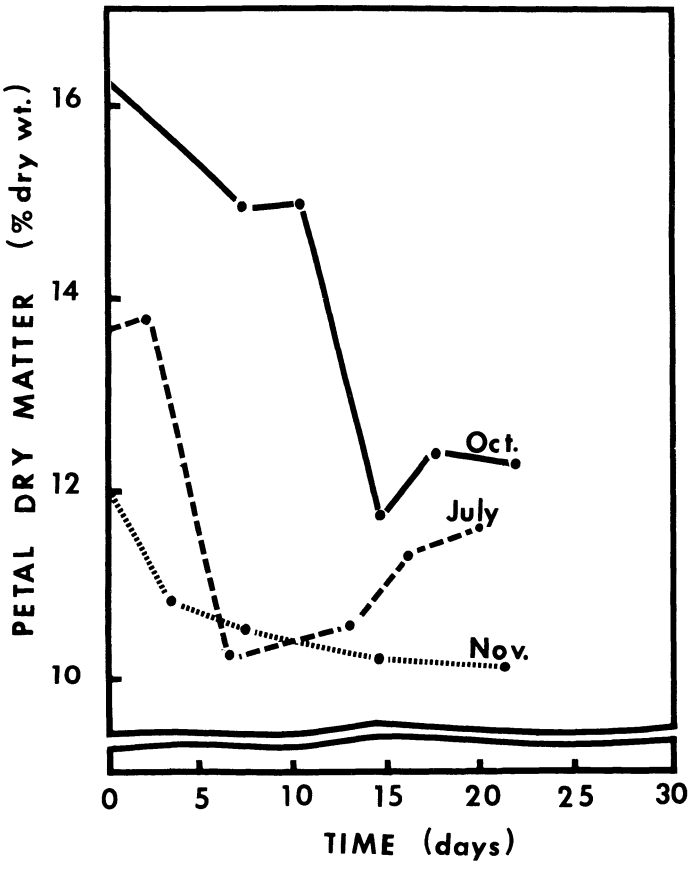

Fig. 2. Decreases in dry wt percentage of petals of *C. morifolium* Ram. cv. Orchid Queen during anthesis in July, Oct. and Nov.

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Effects of Growth-Regulators on Cellulase, Polygalacturonase, Respiration, Color, and Texture of Ripening Tomatoes[1]

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Abstract. Changes in firmness, protein, color, respiration, cellulase, and polygalacturonase were followed during maturation and ripening of tomatoes, *Lycopersicon esculentum* L., on the plant and in detached fruit allowed to ripen at 20°C. Cellulase activity in the young fruit increased steadily during the maturation period. Cellulase activity in detached fruit ripened at 20°C increased rapidly during the onset of ripening and reached a higher level than in fruit ripened on the plant. Polygalacturonase activity was not detectable in developing fruit until after the fruit had initiated ripening. Polygalacturonase activity in detached fruit ripened at 20°C did not appear until the onset of the climacteric and then increased rapidly. This corresponded to the polygalacturonase activity in fruit allowed to ripen on the plant.

The changes during ripening appeared to follow a pre-determined pattern. Grow-regulating substances only moderately affected the onset of the climacteric rise, but markedly influenced the time interval to reach the climacteric peak. They also markedly affected the rate of the normal sequence of changes during ripening. Such changes as softening, color formation, and enzyme activities of cellulase and polygalacturonase were accelerated by ethephon and SADH and delayed by gibberellic acid and indoleacetic acid. Gibberellic acid suppressed polygalacturonase activity. After 14 days polygalacturonase activity in the control fruit was 25 times greater than in fruit treated with gibberellic acid. Cellulase activity in gibberellic acid treated fruit increased steadily during this period. The loss of firmness in treated fruit suggests that softening is initiated by action of cellulolytic enzymes and that pectinolytic enzymes are involved in subsequent changes in texture.

The role of specific enzymes in the textural changes of fruits is still unresolved. The use of tomato fruit for studying these enzymatic systems during ripening has several advantages. Tomatoes are characterized by a climacteric rise (a rapid increase in respiration at maturity) which delineates cellular activities associated with growth and maturation and those of ripening and senescence (3, 19). The changes in the fruit after ripening is initiated proceed very rapidly, and these changes occur whether the fruit is left on the plant or removed and allowed to ripen off the plant. Furthermore, a tomato is composed principally of relatively large flesh cells. Whittenberger and Nutting (45) and Cocking and Gregory (7) have observed microscopically that the cells become separated and that their walls become progressively thinner during ripening. The softening of fruit during ripening has been attributed to the solubilization of the pectin materials by pectinases. The implication of a close link between firmness of fruit and polygalacturonase activity has been demonstrated by Hobson with tomatoes (24), Reymond and Phaff with avocadoes (38), and Patterson et al. with cranberries (34).

It has also been suggested by Hall (17) and Dickinson and McCollum (11) that cellulases might contribute to softening of tomato fruit. However, Hobson (25) reported that, although the activity of cellulases increases with normal ripening, the loss in firmness during this period is directly controlled by pectinases.

The recent findings that growth-regulating substances can affect cell wall degrading enzymes are extremely significant, particularly in respect to their roles in the life of plants and plant organs. Several studies (8, 9, 33, 43) have reported the effects of growth-regulating substances on polygalacturonase,

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