GROWTH, NET PHOTOSYNTHESIS, AND PIGMENTATION OF *COLEUS* X *HYBRIDUS* AS AFFECTED BY PACLOBUTRAZOL

by

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Literature Review

Growth retardants are an important facet of florist crop production being used for height control. Limiting the size of bedding plants would be desirable because inclement weather in the spring months, which delays consumer demand, often results in excessively tall or poor quality bedding plants (Clifford and Lenton, 1980; ICI Americas Inc., 1985). The use of growth retardants would allow bedding plants to remain compact in the flat and extend the marketing period (Clifford and Lenton, 1980). Growth retardants are a tool used by bedding plant producers to predetermine the size of plants for many different uses.

Many growers use daminozide, chlormequat or ancymidol to reduce stretching and maintain good compact habit in the pack (Ball, 1985). Paclobutrazol, a relatively new growth retardant, has been used on a wide range of container-grown ornamentals, including begonia, impatiens, coleus, geranium, and vinca (ICI Americas Inc., 1985).

It has long been recognized that plant responses to growth retardants are generally the reverse of those elicited by exogenous gibberellins (GA). Conversely, retardant-induced inhibition of stem elongation can

usually be reversed by an appropriate application of GA (Clifford and Lenton, 1980; Quinlan and Richardson, 1984). There is direct evidence of growth retardants inhibiting GA biosynthesis but it seems that not all retardants block the biosynthetic pathway at the same point (Dicks, 1976). The simplest hypothesis is growth retardants have a primary site of action. At this site, numerous secondary biochemical changes will occur in an intact organism (Clifford and Lenton, 1980). Earlier studies with Fusarium moniliforme provided evidence that the growth regulator ACPC, chlormequat, and phosfon, but not daminozide, inhibited GA synthesis (Clifford and Lenton, 1980). The pathway is disrupted by blocking the formation of kaurene and stimulating production of transgeranylgeranyl. The active compound of paclobutrazol inhibits the oxidation of kaurene to kaurenoic acid, a cytochrome P 450 catalysed reaction taking place on ribosomes, thus in turn reducing the rate of cell division and expansion without causing any cytotoxicity (ICI Americas Inc., 1985).

Paclobutrazol (B-[(4-chlorophenyl)methyl]- α -(1,1-dimethylethyl)-1H-1,2,4-triazol-1-ethanol) displays activity on a wide selection of crops including deciduous trees and shrubs (ICI Americas Inc., 1985; Sterrett, 1985), fruit trees and vines (Dejong and Doyle, 1984;

Ahmedullah et al., 1986; Curry et al., 1987; Williamson et al., 1986; Stang et al., 1984; Embree and Craig, 1987), agronomic crops (Sankhla et al., 1985; Barrett and Bartuska, 1982), cereals and grass crops (Batch, 1981; Street et al., 1986; Watschke, 1981), and floriculture crops (Stamps and Henry, 1986; McDaniel, 1983; Shanks, 1980; ICI Americas Inc., 1985) such as begonia, cineraria, pot roses, Easter lily, petunia, and chrysanthemum.

ACPC has a relatively narrow spectrum of activity influencing the growth of only chrysanthemum, snapbean, rhododendron, and scarlet sage (Cathey, 1975). It does, however, allow application of low concentration with no detrimental effects on the plants.

Phosfon retards the growth of species responsive to ACPC but also petunia, marigold, Easter lily, coleus, euonymus, red maple, and mimosa (Cathey, 1961). An overtreatment of phosfon, however, results in permanent loss of chlorophyll near the main veins of all developing leaves and stems (Cathey, 1975).

Daminozide is active on a variety of plants as a foliar spray (Cathey, 1975), but inactive on geranium, carnation and holly. An increase in size and number of flowers in azaleas (Larson, 1983), total number of flowers in apples, peaches, pears, blueberries and bougaivillea was seen with daminozide foliar applications (McConnell

and Poole, 1981). Combination of chlormequat and daminozide in an foliar spray provided a greater effectiveness (Larson, 1980).

Ancymidol has extremely broad activity on plants (Cathey, 1975). Philodendron, devil vine, peperomia respond to ancymidol, as well as woody shrubs and trees. This broad range of activity makes ancymidol the most effective growth retardant for most foliage plants. Only slight activity is seen with use on impatiens, geranium and carnation. ACPC, phosfon, chlormequat, and daminozide were shown to be ineffective for controlling growth of Coleus blumei Benth. 'Lord Falmouth', however, ancymidol was effective (Cathey, 1975).

Paclobutrazol, one of the triazol derivatives, blocked the oxidation of kaurene to successive intermediates in the GA biosynthetic pathway in cell-free extracts from immature Cucurbita maxima endosperm (Hedden and Graebe, 1985; Izumi et al., 1985). The inhibitory activity of paclobutrazol can be reversed by GA (Quinlan and Richardson, 1984). The same effects were seen with ancymidol in the pathway of GA biosynthesis (Coolbaugh and Hamilton, 1976; Coolbaugh et al., 1978).

Paclobutrazol has exhibited little or no phloem mobility (Larson, 1985; ICI Americas, Inc., 1985). This compound is absorbed through stem tissue, leaves, and

roots (ICI Americas Inc., 1985). That which is taken up through stem and roots is transported in the xylem to the site of action in subapical meristems where persistent effects can be produced (ICI Americas Inc., 1985). Paclobutrazol was taken up by roots and transported in xylem through the stems and accumulated in leaves of apple seedlings (Wang and Steffens, 1985; Wang et al., 1986).

The effectiveness of a growth retardant is influenced by its method of application. Paclobutrazol can be effectively applied as a soil drench, stem application or injected into the tree, while foliar sprays are less effective (Larson, 1985; Wieland and Wample, 1985). Height may be regulated at different stages of growth. For maximum height control, daminozide is applied just before or at initiation of the first flowers (Cathey, 1975). Ancymidol is more effective in retardation of stem elongation if applied to plants in the vegetative state (Cathey, 1975). The vegetative growth of 'Concord' grapevines with trunk applications of paclobutrazol was controlled without any effects on yield, quality or cold hardiness of dormant buds (Ahmedullah et al., 1986). Paclobutrazol foliar sprays were not as effective as soil drench applications for controlling growth on potted chrysanthemums, however, height was controlled with soil drench applications (McDaniel, 1983). Marini (1987) found

paclobutrazol soil applications to be a promising method of controlling peach tree growth, however, a single application only controlled during the season following winter treatment. Paclobutrazol was effective as a soil drench or foliar application to reduce height of chrysanthemum (McDaniel, 1983; Shanks, 1980) and potted Bouvardia humboldtii (Wilkinson and Richards, 1987). Barrett and Bartuska (1982) found that regardless of application site, paclobutrazol treatment resulted in reduced st elongation compared to untreated bean and chrysanthemum. More retardation occurred, however, when the chemical was applied to the stem than when applied to the leaves.

Foliar applications of ancymidol, chlormequat, and daminozide are effective if foliage is sprayed to runoff, but paclobutrazol works best if spray applications are directed at stems (Moore, 1985). Soil moisture, air temperature, cultivar, watering and fertilizing schedules, freedom from pests, and use of surfactants are factors that can influence the effectiveness of growth retardant applications (Goulston and Shearing, 1985).

Paclobutrazol also promotes numerous adventitious roots at the soil level (Davis et al., 1985). This is believed to be the indirect result of the retardation of growth thereby increasing the partitioning of assimilates and/or hormones to the base of the cutting (Davis et al.,

1985; Wang and Steffens, 1985). Root:shoot ratios were increased in peach (Willamson et al., 1986), and in bean and chrysanthemum (Barrett and Bartuska, 1982), and in Bouvardia humboldtii the root:shoot ratio decreased (Wilkinson and Richardson, 1987). According to Wood (1984), paclobutrazol caused a leaf area reduction in young pecan seedlings and reduced the leaf area:leaf dry weight ratio, however, no significant difference was observed in the root:shoot ratio.

The uptake of paclobutrazol from a compost drench and transport in the xylem should lead to an even response provided that the compost is uniformly moist prior to treatment and application is applied evenly across the soil surface (Menhenett, 1984). Paclobutrazol was not effective when pine bark was included in the medium (Barrett, 1982), thus resulting in more water absorption by the bark than the plant roots. Similar results were observed when ancymidol was applied as a drench to growing medium containing pine bark humus (Larson, 1985).

Ancymidol and daminozide differ in uptake. Daminozide applications need to be applied to dry foliage of fully turgid plants and allowed to remain dry for 24 hours. Relative humidity plays an important role in determining the uptake of daminozide. Ancymidol applications, however, are taken up rapidly by plants,

even when the liquid was allowed to remain on the foliage for a brief time (Cathey, 1961). DeHertogh and Blakely (1976) reported that an ancymidol application by soil drench to dahlias was more effective than a foliar spray. Larson (1985) found that daminozide was effective only as a foliar spray, whereas chlormequat was effective when applied either as a soil drench or as a foliar spray.

The direct morphological effects of paclobutrazol are reduction in vegetative growth, leading to a more compact stem (Shanks, 1980; Barrett, 1982; McDaniel, 1983; Williamson et al., 1986; Wilkinson and Richards, 1987) increased and earlier flowering (ICI Americas Inc., 1985), and most importantly, improved coloration most notably darker green foliage (Sterrett, 1985; Sankhla et al., 1985; LeCain et al., 1986; Wample and Culver, 1983; Wood, 1984), and red color of poinsettia bracts (ICI Americas Inc., 1985; Wilfret, 1981). Daminozide changed the flower color of petunias, thereby changing the red-and-pink flowered cultivars to a grayish color and the violet flowered cultivars to pink, but the blue and purple flowers were not changed (Cathey, 1975).

Flower initiation is not an objective of foliage plant producers, but there are occasions (e.g. in breeding) when flowering is desired (Larson, 1985). Some of the general effects of paclobutrazol are an inhibition

of leaf and flower initiation, altered CO_2 assimilation and reduced subapical meristematic activity (ICI Americas Inc., 1985). A cessation of vegetative growth and initiation of flower buds occurs when azalea plants are treated with paclobutrazol, daminozide, ancymidol, phosfon, or chlormequat (Larson, 1985). In some plant species, there is a tendency for the inhibition of flower initiation, this can be an advantage for foliar types of bedding plants such as coleus where flowering is not desirable. Plant age and size also can influence the effectiveness of growth retardants (Larson, 1985). Plant size (canopy radius) was reduced by paclobutrazol which caused a greater flower density per canopy area and an increase in flower number per plant was observed over time (Stamps and Henry, 1986). Daminozide and chlormequat also indirectly influence flowering by retarding vegetative growth (Larson, 1985). Daminozide and phosfon were ineffective in promoting flowering of begonia, but plants treated with chlormequat flowered before untreated plants (Heide, 1969).

Paclobutrazol treatments have been shown to decrease leaf area of soybean (Sankhla et al., 1985), pear (Embree and Craig, 1987), apple seedlings (Wang and Steffans, 1985; Wang et al., 1986), and pecan seedlings (Wood, 1984). The reduction in leaf area of sunflower leaves was

characterized by a "wrinkled" appearance and a slight cupping of the leaf margins suggesting a greater growth of the adaxial than the abaxial tissues (Wample and Culver, 1983). Ancymidol applications resulted in few leaves that developed, which tended to be dark green and entire with crinkled indentations between the veins (Cathey, 1975). Although the leaf area of daminozide-treated maple and sycamore seedlings was less than untreated plants, height of sycamore was unaffected (Roberts and Domir, 1983).

In addition, paclobutrazol treatments on apple seedlings have increased total plant protein, root respiration and uptake of Mn^{+2} in leaves, N in roots, and N, Ca^{+2}, Mg^{+2}, Mn^{+2}, Zn^{+2}, and Cu^{+2} in stems (Swietlik and Miller, 1985).

Paclobutrazol has been shown to increase chlorophyll levels on a per gram fresh weight of leaf tissue basis (Wample and Culver, 1983; Wang and Steffens, 1985; Dejong and Doyle, 1984), hoever, in sunflower the darker green appearance of the foliage did not contain significantly higher amounts of chlorophyll per unit basis (Wample and Culver, 1983). Leaves developing under the influence of paclobutrazol apperared darker green than in untreated weeping fig (LeCain et al., 1986). McConnell and Struckmeyer (1971) noticed daminozide-treated marigolds appeared to have darker colored foliage 2 days after

plants were treated. Chlormequat application resulted in decreased height and darker green leaves of geranium seedlings (Semenuik and Taylor, 1970). Plants treated with chlormequat also had an increase in chlorophyll content and geranium leaves seemed greener than those from phosfon treatment (Armitage, 1984). Semenuik and Taylor (1970) had similar results with chlormequat, however, the net photosynthesis of hybrid geranium leaves were enhanced 2 to 3 days after treatment with chlormequat and remained elevated for at least 4 to 5 more days (Armitage and Vines, 1982). Other antigibberellins are reported to have similar effects (Wample and Culver, 1983; Ball, 1985; Nelson, 1985). According to Wood (1984), the increased leaf thickness of pecan seedlings was proportional to chlorophyll content and leaves from treated plants had a darker green coloration than those untreated and color was apparent within 2 weeks after treatment.

According to Crittendon and Kiplinger (1969), poinsettia plants treated with chlormequat and daminozide were darker green and contained more chlorophyll per square centimeter of fresh leaf tissue, however, there was no difference in total chlorophyll content between treated and untreated plants when it was determined on a unit fresh weight basis. There was a high

correlation between total chlorophyll and leaf color.

ACPC, phosfon, and chlormequat applied at effective concentrations promoted greening of foliage without altering leaf size or expansion rates (Cathey, 1985). Daminozide caused an intense greening of only the immature foliage with a thickened layer of palisade parenchyma (McConnel and Struckmeyer, 1971; Cathey, 1985). Sachs and Kofranek (1963) found that transverse cell division and expansion in the subapical tissues were stimulated by the retardants ACPC, phosfon, and chlormequat in chrysanthemum, resulting in stems that were thicker than the untreated plants. Halfacre and Barden (1968) observed that daminozide-treated leaves of apple seedlings were thicker as a result of longer palisade cells and a looser arrangement of the spongy mesophyll cells.

ACPC-treated plants were thicker due to 1 to 3 additional layers of spongy parenchyma and intercellular spaces (Halfacre and Barden, 1968). According to Sanderson et al. (1975), phosfon drenched and ancymidol drenched or sprayed Lilium longiflorum Thunb. cv. Georgia showed enlarged schlerenchyma cells and the enlargement was more pronounced in stem sections taken from the base of the plant than those taken in the middle. Cross section examination of leaves from ancymidol-treated plants showed all cells were smaller in size (Cathey,

1985). Shoub and DeHertogh (1974) found that cells in the stems of treated tulip plants were reduced in length and showed greater radial expansion. An increase in the number of palisade cells per unit area of chlormequat and daminozide-treated poinsettia leaves was due to the reduction in leaf area, not in actual increase in number of cell divisions in the palisade layer (Crittendon and Kiplinger, 1969).

Higher chlorophyll levels were observed in the second and third leaf pairs of paclobutrazol-treated sunflower plants, while net photosynthesis was reduced at the highest concentration, however, net photosynthesis of the third leaf pair from the cotyledons after 8 days, was significantly lower than that of untreated plants (Wample and Culver, 1983).

In apple seedlings, there was an increase in specific leaf weight with increasing paclobutrazol rates, therefore, net photosynthesis correlated positively with specific weight (Swietlik and Miller, 1985). This elevation of net photosynthesis rate per unit of leaf surface area was also seen associated with the leaf thickness of sugar beet (Swietlik and Miller, 1985). Soluble carbohydrate levels have increased in all parts of paclobutrazol- treated apple seedlings (Wang and Steffens, 1985) and increased soluble proteins have been found in

treated soybean plants (Sankhla and Davis, 1985).

Paclobutrazol-treated soybean plants showed a greater net photosynthesis rate by 14 days after treatment, particularly the first trifoliate, however, did not affect net photosynthesis when measured 7 days after treatment (Sankhla and Davis, 1985). Though pigment content declined in the primary and first trifoliate, chlorophyll and carotenoid content remained much greater in treated plants, whereas chlorophyll content increased in second trifoliate until 18 days then decreased thereafter (Sankhla and Davis, 1985). No significant effect of net photosynthesis was seen with young pecan seedlings, however, there was a trend for increased net photosynthesis rates with increasing levels of paclobutrazol (Wood, 1984).

Paclobutrazol is also known to inhibit abscisic acid biosynthesis in a suspension of fungus Cercospora rosicola (Norman et al., 1986) and sterol biosynthesis (Graebe, 1987). ACPC and chlormequat did not affect sterol metabolism in either Fusarium moniliforme or barley but ACPC did inhibit sterol biosynthesis in tobacco (Shive, 1976; Barnes et al., 1969). Cathey (1985) reported that any one chemical could not be formulated to retard growth of all plants.

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INFLUENCE OF PACLOBUTRAZOL ON QUALITATIVE CHANGES IN ANTHOCYANIDIN PIGMENTS OF COLEUS

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ABSTRACT

Foliar application of paclobutrazol on Coleus x hybridus 'Velvet Wizard and 'Jade Wizard' plants at different stages of develpment (sixth leaf-pair stage, 48 mg a.i./liter; eighth leaf-pair stage, 24 mg a.i./liter) were compared for qualitative changes in anthocyandins (ACY) in the leaves. Six ACY were isolated and identified using thin layer chromatography: pelargonidin, malvidin, peonidin, cyanidin, petunidin, and delphinidin. Paclobutrazol treatment did not result in a qualitative shift in ACY present, however over time there were changes in the number of ACY present.

INTRODUCTION

Paclobutrazol exhibits anti-gibberellin properties on a wide range of ornamental crops (Larson, 1985); ICI Americas, Inc., 1985). It is absorbed through stem tissue or roots and translocated in the xylem to subapical

meristems, the site of action. When apple seedlings are treated with paclobutrazol, distinct changes in leaf morphology (Wample and Culver, 1983), leaf color (LeCain et al., 1986, Ball, 1985; Nelson, 1985), and leaf area (Sankhla et al., 1985; Embree and Craig, 1987) occur being dependent upon the rate of chemical application (Clifford and Lenton, 1980; ICI Americas, Inc., 1985). The direct morphological changes are reduced vegetative growth, more compact stem, increased and earlier flowering, darker green foliage, and intensification of poinsettia bract coloration (ICI Americas, Inc., 1985; Wilfret, 1981; Ball, 1985; Nelson, 1985). There is a tendency towards the inhibition of flower initiation, which may be an advantage for foliar-types of bedding plants, such as coleus, where flowering is not desirable.

The anthocyanins are often responsible for the violet, blue or red color characteristics of many fruits, flowers and leaves, particularly in leaves during the autumn months (Witham and Blaydes, 1986). Vaccinium has particularly complex anthocyanins (ACY), with up to 15 reported for species in two sections of the genus (Ballington et al., 1987). Among those ACY are 3-monoglucosides of cyanidin (Cy), delphinidin (Dp), malvidin (Mv), peonidin (Pn), and petunidin (Pt). The pink-fruited genotypes of highbush blueberry (V.

corymbosum L.) also contained all 15 ACY but in smaller quantities (Ballington et al., 1987). Ethephon was seen to increase the amount of anthocyanin pigments and the degree of color in berries of both table and wine grapes (Weaver, 1978), perhaps causing a much more rapid rate of maturation.

Goldy et al. (1987) found that ACY were not limited to the fruit, but were also found in young shoots, tendrils, leaves, and leaf petioles. The five ACY pigments of muscadine grapes, identified through paper chromatography, were 3,5 diglucoside forms of Mv, Pn, Pt, Cy, and Dp (Goldy et al., 1987). Similar studies were investigated with Aquilegia flower pigments. A total of 13 anthocyanins were isolated and identified, the principal ACY pigments being 3-monoglucosides and 3,5 diglucosides of Cy, Dp, and pelargonidin (Pg)(Taylor, 1984).

The objective of this study was to observe the qualitative changes in the various anthocyanidins in leaves of Coleus x hybridus 'Jade Wizard' and 'Velvet Wizard' when plants of different stages of development were treated with paclobutrazol.

MATERIALS AND METHODS

Seeds of 'Jade Wizard' and 'Velvet Wizard' coleus were germinated under intermittant mist (6 seconds every 3

minutes) with a media temperature of 24°C. When the seedlings were large enough to handle, they were transplanted to individual 5.5-cm plastic pots filled with a soil: sphagnum peatmoss: perlite media (1:2:2, volume basis) amended with 0.29 kg P/m^3 superphosphate (0N-8P-0K). Pots were placed pot-to-pot on the bench. When plants were touching, they were transplanted into 10.5-cm plastic pots containing the same media and fertilizer amendments. Each pot received 1.5 grams of 14N-6P-12K Osmocote (Sierrablen Chemical Co., Milpitas, Calif.).

The plants were grown at 24°/15°C day/night temperatures and irrigated by hand as needed. The 2 cultivars were treated at the sixth leaf-pair stage with 48 mg active ingredient (a.i.) paclobutrazol/liter or at the eighth leaf-pair stage with 24 mg a.i./liter. Control plants were sprayed with water. Leaves were sampled before paclobutrazol treatment, and at 1, 3, 5, 7, 14, 21, and 28 days after treatment. One gram of fresh tissue from 4 plants in each of the 3 treatments from leaves positioned above the appropriate sixth and eighth leaf node was cut into small pieces. Leaf tissue was ground for 3 minutes in a mortar and pestle to which 10 ml of HCL (1% v/v) and 5 ml of ethanol was added. Plant material was refrigerated (4°C) prior to use to retard enzyme oxidation, a problem noted with phenolic plant

crude extracts (Harborne, 1973a). Buchner-funnel filtration was used for the original sample. Sediment was washed with 5 ml of ethanol, while mortar and pestle were washed with an additional 10 ml of ethanol. All filtrate were collected and stored under refrigeration at $4^{o}C$ prior to separation by thin-layer chromatography. Silica-gel GF Redi-plates (20 x 20 cm; Fisher Scientific) were heated at $75^{o}C$ for 30 minutes and then cooled prior to applying a 4-ul aliquot of the plant extract 2.5 cm from the plate edge. Plates were then placed vertically in a developing chamber filled to 1.25 cm depth with an n-butanol: acetic acid: water (5:1:4, volume basis) solvent. Filter paper soaked with the solvent and applied to the sides of the chamber provided an atmosphere of even relative humidity within the developing chamber. Pigments were recovered from the silica gel plates with 1% HCL in methanol, followed by centrifugation.

The absorption curve and maximum absorption spectrum, within the range of 500 to 600 nm, were measured and the pigment identified by comparing the Rf values with those reported by Harborne (1973a). Pelargonidin chloride, (Fluka Chemical Corporation, Ronkonkoma, New York) was included on the chromatograms. could be determined from Rf values.

RESULTS AND DISCUSSION

Six anthocyanidins were isolated and identified. According to Harborne (1973a), there are six anthocyanidins commonly isolated from plant tissue, the magenta colored Cy being by far the most common. These six anthocyanidins have a similar hydroxylation pattern and differ only in the number of hydroxyl groups attached to the B-ring. The number of B-ring hydroxyls present is correlated with color properties (Harborne, 1973b). Orange-red colors are due to Pg with one less hydroxyl group than Cy. The purple and red colors of mauve are due to Dp, which has one more hydroxyl group than Cy (Harborne, 1973a). Hydroxylation is thus a key factor in color production. Rf measurements are very important, particularly for anthocyanins, which cannot be characterized by melting point or elementary analysis (Harborne, 1973b).

Paclobutrazol treatment did not result in a qualitative shift in anthocyanidins present. However, over time, there were changes in the number of anthocyanidins present (Figures 1 and 2). This may be attributed to a shift in the degree of hydroxylation of the various anthocyanidins, thereby resulting in a change in color expression (Harborne, 1973a).

Leaf tissue from 'Velvet Wizard' harvested prior to treatment contained pigments Pg, Pn, Cy, Pt, and Dp; Mv

was not present. At days 1 and 3 after treatment, Pt and Pn were detected in 50% of the samples and Mv was present. By day 5, Pt was present in all samples, however, Pn was undetected in 50% of the samples. By day 7, Pn was still undetected and Mv was not detected in 50% of the samples. From day 14 on, all anthocyanidins were detected. 'Jade Wizard' lacked Mv and Pt over the length of the study.

Copigmentation of anthocyanin with flavones and the absorption of pigment complexes onto soluble polysaccharide present in the cell sap may be a factor, but synthesis in these conditions is probably due to the physiological changes involving carbohydrate metabolism as seen in leaves in the autumn (Harborne, 1973b). Findings by Marutani et al. (1987) suggest that the difference in color expression between the spathe and spadix of Anthurium amnicola, which contain the same pigments, show such modifications. Differences reported for the anthocyanidins in leaves of 'Jade Wizard' and 'Velvet Wizard' coleus may be due to pigment variations in different varieties (Asen et al., 1956). Factors influencing flower color are the kind and concentration of inorganic ions in the vacuole, (Paech, 1955) and pH, nature of aglycone, metal complexing, extent of glycosidation, concentration, and anthocyanin-phenol interactions (Timberlake and Bridle, 1975).

This study indicates that Pg is a pigment which may be responsible for the pink/red color of 'Velvet Wizard'. Harborne (1973a) indicated that the flower color of Pelargonium is due to the mixture of several anthocyanins thus giving a red/orange color when visualized under UV light (Tables 1 and 2). Pelargonidin chloride also showed a separation giving an orange/red mixture. Results from this study indicating Pg as a major leaf in Coleus x hybridus contrasts with Harborne (1973b) who states that Pg and Dp occur in many cyanic flowers, but are uncommon in pigmented leaves such as begonia and coleus which almost always contain cyanidin. The Rf values for Pg agree with those of Harborne (1973a) and Taylor (1984) and the Rf values for Pg and the reference pelargonidin chloride match to identify the pigment.

Stewart et al. (1980) reports that synthesis of anthocyanins and the flavonol share a precursor that can be increasingly controlled in flavonol synthesis when anthocyanin synthesis is suppressed. This supports the findings suggesting the ratio of anthocyanidins were shifted at the different harvest days. Stewart et al. (1980) also suggested that the change in bract color of poinsettia sports was a result of genetic change that suppressed the anthocyanin synthesis in the mutant epidermis.

Table 1. Properties of Coleus x hybridus anthocyanidins. Pg: pelargonidin; Mv: malvidin; Pt: petunidin; Pn: peonidin; Cy: cyanidin; and Dp: delphinidin.

Pigment	Rf in BAW[a]	Visible Color[b]	Color in UV light
Pg	84(80)	red orange(red)	bold pink red
Pn	75(71)	red purple(magenta)	bold lt blue
Cy	67(68)	red purple(magenta)	bold blue
Mv	58(58)	lt purple(purple)	faint red
Pt	53(52)	purple(purple)	lt blue
Dp	42(42)	blue purple(purple)	blue
Standard: Pelargonidin Chloride	85(80)		

[a]Values in parentheses by Harborne (1973a); BAW: butanol-acetic acid-water.
[b]Values in parentheses by Harborne (1973b).

Table 2. Spectral characteristics of Coleus x hybridus anthocyanidins. Pg: pelargonidin; Mv: malvidin; Pt: petunidin; Pn: peonidin; Cy: cyanidin; and Dp: delphinidin.

Pigment	Absorption maximum (nm)[a] in MeOH-HCl
Pg	520,530,535,545 (520)
Pn	530,545 (532)
Cy	535,550 (535)
Mv	540,560 (542)
Pt	545,555,560 (543)
Dp	535,545,575 (546)
Standard:[b]	
Pelargonidin chloride	(530)
Anthocyanidins:[c]	
Delphinidin derivatives	(535-545)
Cyanidin derivatives	(525-535)
Pelargonidin derivatives	(498-520)

[a]Values in parentheses by Harborne (1973a); MeoH-Hcl: methanol-hydrochloric acid.
[b]Values in parentheses by Fluka Chemical Corporation (1986).
[c]Values in parentheses by Harborne (1973b).

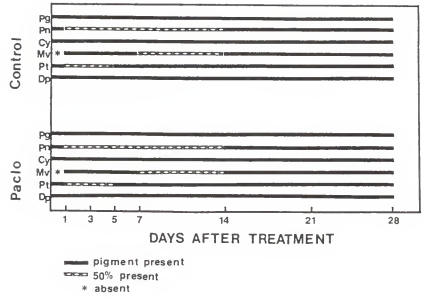

Figure 1. Distribution of anthocyanidins in 'Velvet Wizard' coleus over time. Pg: pelargonidin; Mv: malvidin; Pt: petunidin; Pn: peonidin; Cy: cyanidin; and Dp: delphinidin.

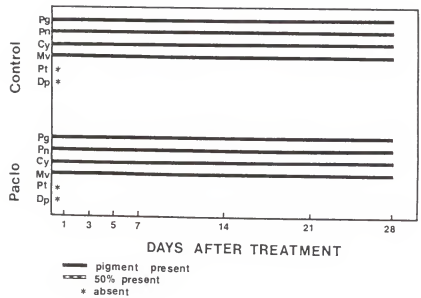

Figure 2. Distribution of anthocyanidins in 'Jade Wizard' coleus over time. Pg: pelargonidin; Mv: malvidin; Pt: petunidin; Pn: peonidin; Cy: cyanidin; and Dp: delphinidin.

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Chlorophyll concentration and net photosynthesis of coleus cultivars as influenced by paclobutrazol.

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Abstract. Coleus x hybridus 'Velvet Wizard' and 'Jade Wizard' were treated with paclobutrazol at either sixth leaf-pair stage at 48 mg a.i./liter or as eighth leaf-pair stage at 24 mg a.i./liter to determine the influence of paclobutrazol on net photosynthesis (Pn) and chlorophyll content. There was no effect on Pn due to paclobutrazol application. There was an effect on Pn though as light intensity increased over time. 'Velvet Wizard' had maximum Pn at 1848 micromols·m^{-2}·sec^{-1} whereas, 'Jade Wizard' exhibited minimum Pn at 1260 micromols·m^{-2}·sec^{-1}. Little influence on chlorophyll concentration was observed in the leaves of the 2 cultivars. However, 'Jade Wizard' exhibited a decrease in Pn and chlorophyll concentration over time. 'Velvet Wizard' plants exhibited greater chlorophyll a and b, and total chlorophyll concentration than the control plants at days 3 and 14 after treatment.

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Paclobutrazol, a relatively new growth retardant with potential benefits to the bedding plant industry, is active on a wide range of plant species such as ageratum, begonia, hydrangea, impatiens, geranium and vinca (ICI Americas. Inc., 1985). Plants treated with paclobutrazol show changes in leaf morphology (Wample and Culver, 1983), leaf color (LeCain et al., 1986; Ball, 1985; Nelson, 1985; ICI Americas, Inc., 1985), and leaf area (Sankhla et al., 1985; Embree and Craig, 1987; Wood, 1984; Wang and Steffens, 1985) depending on the application rate. Other responses to paclobutrazol applications include increased chlorophyll content (LeCain et al., 1986; Sankhla et al., 1985; Wample and Culver, 1983), enchanced bract coloration (ICI Americas, Inc., 1985; Wilfret, 1981) and earlier flowering (ICI Americas, Inc., 1985).

According to Wood (1984), paclobutrazol application resulted in increased leaf thickness which was proportional to increased chlorophyll content. Poinsettia plants treated with chlormequat and daminozide were also darker green and contained more chlorophyll per square centimeter fresh leaf tissue (Crittendon and Kiplinger, 1969). Sankhla et al. (1985) observed an inhibition of internode elongation, reduction in leaf enlargement, and enhanced chlorophyll content. Daminozide resulted in decreased net photosynthesis in apple (Halfacre and Barden, 1968). Net photosynthesis was determined to

be linearly proportional to the amount of chlorophyll, independent of leaf position on the stem (Sestak and Catsky, 1962). Armitage et al. (1984) found an increase in net photosynthesis on the second day with chlormequat-treated hybrid geranium and remained elevated for 5 more days, whereas net photosynthesis of plants treated with daminozide decreased on day 3 and remained constant throughout the length of the experiment. However, Sankhla et al. (1985) observed that paclobutrazol application did not affect net photosynthesis of soybean when measured 7 days after treatment, but by 14 days net photosynthesis of treated plants was much greater than that of controls particularly on the first trifoliate leaf. Greatest photosynthesis and chlorophyll content were found in the young but well developed leaves, i.e. the third and fourth from the apex (Sestak and Catsky, 1962). A further decrease in chlorophyll content paralleled the decrease in photosynthetic rate (Sestak and Catsky, 1962).

The objective of this study was to examine the relationship between chlorophyll content and net photosynthesis in leaves of Coleus x hybridus 'Jade Wizard' and 'Velvet Wizard' when plants of different developmental stages were treated with paclobutrazol.

Materials and Methods

Seeds of 'Jade Wizard' and 'Velvet Wizard' coleus were germinated under intermittant mist (6 seconds every 3 minutes) with a media temperature of 24°C. When the seedlings were large enough to handle, they were transplanted to individual 5.5-cm plastic pots filled with a 1 soil: 2 sphagnum peatmoss: 2 perlite media (volume basis) amended with 0.29 kg P/m^3 superphosphate (0N-8P-0K). Pots were placed pot-to-pot on the bench. When plants were touching, they were transplanted into 10.5-cm plastic pots containing the same media and fertilizer amendments. Each pot received 1.5 grams of 14N-6P-12K Osmocote (Sierrablen, Sierra Chemical Co., Milpitas, Calif.).

The plants were grown at 24°/15°C day/night temperatures and irrigated by hand as needed. The 2 cultivars were treated at the sixth mature leaf-pair stage with 48 mg active ingredient (a.i.) paclobutrazol/liter or at the eighth mature leaf-pair stage with 24 mg a.i./liter. Control plants at the sixth and eighth mature leaf-pair stage were sprayed with water. Measurements were made before paclobutrazol treatment, and at 3, 7, and 14 days after treatment for 'Velvet Wizard', whereas 'Jade Wizard' leaves were sampled before paclobutrazol treatment, and at 3, 7, 14, 21, and 28 days after treatment.

Net photosynthesis (Pn) was measured during noon with a battery-operated, portable infrared CO_2 analyzer (The Analytical Development Co. Ltd., Hoddesdon, England). Pn was measured on a standard leaf area basis of fully expanded leaves at the uppermost part of the plant (plants were well-watered 24 hours prior to measurement). Parkinson leaf chamber (The Analytical Development Co. Ltd., Hoddesdon, England) containing the leaf sample was positioned at a constant height above the greenhouse bench for all of the readings to ensure uniform light intensity. Relative humidity, air temperature, and photosynthetic active radiation (PAR; micromols$\cdot m^{-2} \cdot s^{-1}$) sensors were incorporated within the leaf chamber. The amount of fixed CO_2 was calculated by the difference between the inflow and outflow CO_2 levels of the leaf chamber. Comparison measurements for light radiation, relative humidity, and air temperature were taken with a steady state porometer (Li-Cor, Inc., Lincoln, Neb., Model No. LI-1600) to ensure the readings were comparable to the infrared CO_2 analyzer readings. Five readings were made on each of 3 plants in each treatment group per cultivar on each harvest day.

Leaf samples were harvested from the plants as soon as possible the following day for chlorophyll concentration analysis. Leaves were kept on the plants

until the time for chlorophyll extraction. One gram of fresh tissue from 4 plants in each of the 4 treatments from leaves positioned above the appropriate sixth and eighth node for the 2 cultivars was cut into small pieces and ground for 3 minutes in a mortar to which 40 ml of 80% (v/v) acetone was added. The chlorophyll extracts were refrigerated (4°C) immediately after Buchner-funnel filtration to prevent chlorophyll degradation. Buchner-funnel filtration was used for the original plant extract. Sediment was washed with 30 ml of 80% acetone and the mortar was washed with an additional 10 ml of 80% acetone. All filtrate was collected and stored under refrigeration at 4°C prior to the measurement of the absorption spectrum of chlorophyll a and chlorophyll b on a spectrophotometer. The final supernatant volume was brought up to 100 ml in a graduated cylinder by the addition of 80% acetone.

Chlorophyll content was read on a spectrophotometer (Beckman Instruments, Inc., Model 25, Fullerton, Calif.) set at 645, 652, and 663 nm. Chlorophyll concentration was calculated from the equation given by Witham and Blaydes (1986). For the chlorophyll extract absorption spectrum, optical density readings were taken at 20 nm intervals from 350 to 700 nm. At points of maximum absorption, additional readings were taken at 5 nm intervals.

Results and Discussion

From the analysis of covariance using light intensity as a covariant (Tables 1 and 2), it can be concluded that the application of paclobutrazol to either 'Velvet Wizard' or 'Jade Wizard' coleus plants does not influence Pn. However, as light intensity increased over the course of the experiment there was an influence on photosynthesis. 'Velvet Wizard' showed a significant positive response to light intensity with maximum photosynthesis occurring at 1848 micromols$\cdot m^{-2} \cdot sec^{-1}$; 'Jade Wizard' showed a significant negative response to light intensity with photosynthesis reaching a minimum rate at 1260 micromols$\cdot m^{-2} \cdot sec^{-1}$.

Armitage et al. (1984) reported an increase in Pn 2 to 3 days after chlormequat was applied to hybrid geraniums. The rate of Pn remained elevated for at least 4 to 5 more days. However, Sankhla et al. (1985) found no change in Pn of paclobutrazol-treated soybean plants until 14 days after treatment when there was an increase in Pn. An increase in Pn with increasing rates of paclobutrazol on young pecan seedlings has been reported (Wood, 1984). With these coleus cultivars, it would appear that available PAR has a greater influence on Pn than paclobutrazol application. The lack of response to paclobutrazol could be that under greenhouse conditions

where light is not limiting, photosynthesis could proceed. A reduction due to paclobutrazol-treatment may be detected if these plants were to be grown under reduced light levels since coleus is considered to be a plant which requires less light for growth than the hybrid geranium, soybean, or pecan.

There was little influence of paclobutrazol treatment on chlorophyll concentration in the foliage of 'Velvet Wizard' and 'Jade Wizard' coleus (Tables 3, 4 and 5). On the third day after treatment, 'Velvet Wizard' plants treated with paclobutrazol exhibited significantly greater chlorophyll a and b, and total chlorophyll concentration than the control plants. This was also seen on the 14th day for chlorophyll a and total chlorophyll concentration. There were no differences in chlorophyll concentration among paclobutrazol treatments for 'Jade Wizard' plants on any given harvest date. This would indicate that the red-pigmented cultivar, Velvet Wizard, is more sensitive to paclobutrazol than the green-cream variegated cultivar, Jade Wizard. Perhaps when plants with low chlorophyll content are treated with growth retardants, an increase in chlorophyll concentration results. However, when plants which are naturally green-pigmented cultivars are treated, due to a lower sensitivity to growth retardants, alterations in chlorophyll content may not be significant. Crittendon and Kiplinger (1969) found a high correlation

between total chlorophyll and leaf color of poinsettia cultivars treated with chlormequat and daminozide. With plants which usually exhibit only solid green foliage such as sunflower (Wample and Culver, 1983) there were no significant increases in chlorophyll content when paclobutrazol was applied to plants.

As leaves age, there is a decrease in chlorophyll and protein content, and Pn rate (Sestak and Catsky, 1962). Plant species will differ as to when, during leaf development, they exhibit maximal photosynthetic activity. With 'Jade Wizard', the green-cream variegated cultivar, there was a significant decrease in Pn over time when PAR was used as a covariant. A decrease in chlorophyll a concentration and total chlorophyll content over the first 14 days of the crop, though non-significant, was seen. 'Velvet Wizard' plants did not exhibit a significant reduction in chlorophyll a concentration; there appears to be a decrease in chlorophyll b though not significant. The reduction in chlorophyll a and total chlorophyll concentration for 'Jade Wizard' coleus plants paralleled the reduction in Pn. This helps to confirm the hypothesis that red-pigmented foliage which exhibit a positive response to increased levels of PAR may have an adaptive advantage at lower light levels since there is no change in chlorophyll content over time. This advantage could

possibly be enhanced with the application of paclobutrazol since on days 3 and 14 there was a significant increase in chlorophyll concentration.

Table 1. Analysis of covariance for 'Velvet Wizard' coleus testing the model: CO2=TRT LIGHT LIGHT2 where CO2 = net photosynthesis measured as fixed CO_2, TRT = paclobutrazol treatments, LIGHT = covariant of light intensity measured in micromols·m^{-2}·sec^{-1}, and LIGHT2 = light intensity squared.

Analysis of covariance

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	5	258.9196	51.7839	2.60
ERROR	42	838.0220	19.9529	PR > F
CORRECTED TOTAL	47	1096.9417		0.0392

R-SQUARE	C.V.	ROOT MSE	CO2 MEAN
0.236038	132.2537	4.4668	3.3775

SOURCE	DF	TYPE III SS	F VALUE	PR > F
TRT[Z]	3	77.8669	1.30	0.2868
LIGHT	1	182.7239	9.16	0.0042
LIGHT2	1	188.1244	9.43	0.0037

PARAMETER	ESTIMATE	T FOR H0: PARAMETER=0	PR > ^T^	STD ERROR OF ESTIMATE
INTERCEPT	-8.0426 B	-2.05	0.0467	3.9239
TRT 6 00	-0.3683 B	-0.20	0.8425	1.8426
6 12	2.8782 B	1.56	0.1270	1.8489
8 00	1.5034 B	0.81	0.4232	1.8590
8 06	0.0000 B	.	.	.
LIGHT	0.0307	3.03	0.0042	0.0101
LIGHT2	-0.000	-3.07	0.0037	0.0000

[Z] 6 00=sixth leaf-pair control; 6 12=sixth leaf-pair treated with paclobutrazol, 48 mg a.i.·$liter^{-1}$; 8 00=eighth leaf-pair control; and 8 06=eighth leaf-pair treated with paclobutrazol, 24 mg a.i.·$liter^{-1}$.

Table 2. Analysis of covariance for 'Jade Wizard' coleus testing the model: CO2=TRT LIGHT LIGHT2 where CO2 = net photosynthesis measured as fixed CO_2, TRT = paclobutrazol treatments, LIGHT = covariant of light intensity measured in micromols$\cdot m^{-2} \cdot sec^{-1}$, and LIGHT2 = light intensity squared.

Analysis of covariance

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	5	618.0377	123.6075	2.76
ERROR	66	2959.9810	44.8482	PR > F
CORRECTED TOTAL	71	3578.0186		0.0254

R-SQUARE	C.V.	ROOT MSE	CO2 MEAN
0.172732	189.5939	6.6969	3.5322

SOURCE	DF	TYPE III SS	F VALUE	PR > F
TRT[z]	3	139.8209	1.04	0.3811
LIGHT	1	225.1486	5.02	0.0284
LIGHT2	1	133.4275	2.98	0.0892

PARAMETER		ESTIMATE	T FOR H0: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE
INTERCEPT		17.2729 B	3.19	0.0021	5.4062
TRT	6 00	3.6419 B	1.59	0.1164	2.2891
	6 12	0.8215 B	0.36	0.7198	2.2803
	8 00	0.5388 B	0.24	0.8135	2.2744
	8 06	0.0000 B	.	.	.
LIGHT		-0.0280	-2.24	0.0284	0.0124
LIGHT2		0.00001	1.72	0.0892	0.0000

[z]6 00=sixth leaf-pair control; 6 12=sixth leaf-pair treated with paclobutrazol, 48 mg a.i.$\cdot$liter^{-1}; 8 00=eighth leaf-pair control; and 8 06=eighth leaf-pair treated with paclobutrazol, 24 mg a.i.$\cdot$liter^{-1}.

Table 3. Chlorophyll a concentration (mg chlorophyll/gram fresh tissue weight) for 'Velvet Wizard' and 'Jade Wizard' coleus plants treated with paclobutrazol at the sixth and eighth leaf-pair stage of development.

	Days after treatment				
Treatment	0	3	7	14	Mean[z]
'Velvet Wizard'					
600[y]	0.019[NS]	0.100b[x]	0.279[NS]	0.157bc[x]	0.139bc
612	0.039	0.223a	0.238	0.334a	0.210ab
800	0.119	0.074b	0.096	0.057c	0.087c
806	0.439	0.185a	0.226	0.311ab	0.290a
Mean[w]	0.154c	0.147c	0.210bc	0.215bc	
'Jade Wizard'					
600	0.408[NS]	0.445[NS]	0.182[NS]	0.147[NS]	0.296a
612	0.501	0.289	0.244	0.175	0.303a
800	0.537	0.315	0.102	0.181	0.284a
806	0.381	0.233	0.168	0.076	0.215ab
Mean[w]	0.457a	0.321b	0.174c	0.145c	

[z]Means for cultivar*treatment interaction followed by the same letter are not significantly different using an L.S.D., p=0.05.

[y]6 00=sixth leaf-pair control; 6 12=sixth leaf-pair treated with paclobutrazol, 48 mg a.i.·liter^{-1}; 8 00=eighth leaf-pair control; and 8 06=eighth leaf-pair treated with paclobutrazol, 24 mg a.i.·liter^{-1}.

[x]Means within days after treatment column for a given cultivar followed by the same letter are not significantly different using an L.S.D., p=0.05; NS means are not significantly different.

[w]Means for cultivar*days after treatment interaction followed by the same letter are not significantly different using an L.S.D., p=0.05; NS means are not significantly different.

Table 4. Chlorophyll b concentration (mg chlorophyll/gram fresh tissue weight) for 'Velvet Wizard' and 'Jade Wizard' coleus plants treated with paclobutrazol at the sixth and eighth leaf-pair stage of development.

	Days after treatment				
Treatment	0	3	7	14	Mean
'Velvet Wizard'					
600[z]	0.319[NS]	0.055[y]	0.328[NS]	0.303NS	0.251[NS]
612	0.309	0.314a	0.452	0.419	0.373
800	0.371	0.057b	0.180	0.081	0.172
806	1.282	0.237ab	0.440	0.333	0.573
Mean[x]	0.570ab	0.166c	0.350bc	0.284bc	
'Jade Wizard'					
600	0.353[NS]	0.615[NS]	0.454[NS]	0.155[NS]	0.394
612	0.205	0.777	0.466	0.371	0.455
800	0.218	0.709	0.259	0.293	0.370
806	0.307	0.619	0.993	0.042	0.490
Mean[x]	0.271c	0.680a	0.543ab	0.215c	

[z]6 00=sixth leaf-pair control; 6 12=sixth leaf-pair treated with paclobutrazol, 12 mg a.i.·liter^{-1}; 8 00=eighth leaf-pair control; and 8 60=eighth leaf-pair treated with paclobutrazol, 6 mg a.i.·liter^{-1}.

[y]Means within days after treatment column for a given cultivar followed by the same letter are not significantly different using an L.S.D., p=0.05; NS means are not significantly different.

[x]Means for cultivar*days after treatment interaction followed by the same letter are not significantly different using an L.S.D., p=0.07; NS means are not significantly different.

Table 5. Total chlorophyll concentration (mg chlorophyll/gram fresh tissue weight) for 'Velvet Wizard' and 'Jade Wizard' coleus plants treated with paclobutrazol at the sixth and eighth leaf-pair stage of development.

	Days after treatment				
Treatment	0	3	7	14	Mean
'Velvet Wizard'					
600[Z]	0.338[NS]	0.155b[Y]	0.607[NS]	0.461bc[Y]	0.390[NS]
612	0.348	0.542a	0.690	0.753a	0.583
800	0.490	0.131b	0.276	0.138c	0.259
806	1.721	0.422a	0.667	0.644ab	0.863
Mean[X]	0.724abc	0.313d	0.560bcd	0.499bcd	
'Jade Wizard'					
600	0.761[NS]	1.061[NS]	0.636[NS]	0.302[NS]	0.690
612	0.706	1.066	0.710	0.547	0.757
800	0.755	1.024	0.312	0.474	0.654
806	0.688	0.853	1.161	0.118	0.705
Mean[X]	0.728ab	1.001a	0.717abc	0.360cd	

[Z]6 00=sixth leaf-pair control; 6 12=sixth leaf-pair treated with paclobutrazol, 48 mg a.i.·liter^{-1}; 8 00=eighth leaf-pair control; and 8 06=eighth leaf-pair treated with paclobutrazol, 24 mg a.i.·liter^{-1}.

[Y]Means within days after treatment column for a given cultivar followed by the same letter are not significantly different using an L.S.D., p=0.05; NS means are not significantly different.

[X]Means for cultivar*days after treatment interaction followed by the same letter are not significantly different using an L.S.D., p=0.05; NS means are not significantly different.

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Appendix A

Preliminary screening of paclobutrazol rates at various stages of plant development

Seeds of 'Jade Wizard and Velvet Wizard' coleus were germinated under intermittant mist (6 seconds every 3 minutes) with a media temperature of 24°C. When the seedlings were large enough to handle, they were transplanted to individual 5.5 cm square plastic pots filled with a 1 soil: 2 sphagnum peatmoss: 2 perlite media (volume basis) amended with 0.29 kg P/m^3 superphosphate (0N-8P-0K). Pots were placed pot-to-pot on the bench. When plants were touching, they were transplanted into 10.5 cm round plastic pots containing the same media and fertilizer amendments. Each pot received 1.5 grams of 14N-6P-12K Osmocote (Sierrablen, Sierra Chemical Co., Milpitas, Calif.).

The plants were grown at 24°/15°C day/night temperatures and irrigated by hand as needed (Mastalerz, 1985). The 2 cultivars were treated at the sixth, eighth or tenth leaf-pair stage with foliar applications of paclobutrazol at 0.0, 12, 24, 36 and 48 mg active ingredient (a.i.) per liter. The control plants at the sixth, eighth, and tenth leaf-pair stage were sprayed with water only.

Plants were harvested every 7 days starting with the first sample taken just prior to paclobutrazol treatment

and continued for six weeks. The five paclobutrazol treatments were applied as single applications to the 3 stages of development.

At each sampling time, data collected included total leaf area (fully expanded and non-expanded leaves), measured on a Li-Cor 3100 area meter; number of leaves (fully expanded and non-expanded leaves); total shoot, leaf, stem and fresh and dry weights; plant height; number of nodes; and stem width.

The experiment was arranged as a completely ramdomized factorial design with cultivar (2 levels), growth stage (3 levels), and paclobutrazol rate (5 levels) as main effects with 3 single plant replications per treatment and at seven sample times per stage of growth level. The paclobutrazol treatment rate and plant growth stage which yielded the highest quality plant without detrimental visual growth effects and the one which did result in a poor quality plant, were used in subsequent studies on net photosynthesis, chlorophyll and anthocyanidin content, and internal anatomical changes.

'Velvet Wizard' plants at the sixth leaf-pair stage treated with either 36 or 48 a.i. paclobutrazol/liter exhibited little stem extension throughout the experiment (Fig.1). The lower paclobutrazol rates (12 and 24 mg a.i./liter) resulted in gradual height increase of

'Velvet Wizard' plants from day 7 through day 35, however, at 24 mg a.i./liter treatment rate, there was a significant height increase between day 28 and day 35. The control plants exhibited a significant increase in height from day 14 to day 28. Height increase of eight leaf-pair stage 'Velvet Wizard' plants for all paclobutrazol treatments was similar from day 0 through day 21. Between day 28 and day 35, there was a significant increase in height for paclobutrazol-treated plant for all rates. These plants were taller then the control and exhibited a growth spurt at the point the paclobutrazol residual was no longer effective. Treated 'Velvet Wizard' and 'Jade Wizard' plants exhibit constant height throughout the harvest days, however, there was a significant change in height for all paclobutrazol rates at days 14, 21, 28 and 35 after treatment compared to the control plants. There was no significant difference in height for the cultivars, however, there was a significant difference in height for the 3 stages of development. Little influence of paclobutrazol treatment was evident for both cultivars between the 2 lower rates (12 and 24 mg a.i./liter) and between the 2 higher rates (36 and 48 mg a.i./liter). However, 'Jade Wizard' at the sixth leaf-pair stage exhibited significant height increase for all paclobutrazol rates at day 21 to day 28. Eight leaf-pair stage 'Jade Wizard' plants exhibited significant stem

extension from day 14 throughtout the course of the experiment indicating minimal reduction of all paclobutrazol treatments.

The 3-way interaction among main effects of stage of development, paclobutrazol-application rate, and cultivar exhibited a significant increase in stem width over the 3 stages of development at the 5 treatments for both cultivars. 'Jade Wizard' plants have a smaller stem width throughout the experiment (Table 1). The area of mature, expanded-leaves (Table 2) decreased with increased paclobutrazol rates for both cultivars with 'Jade Wizard' plants having reduced leaf area. Total dry weight (Table 2) increased for both cultivars by day 35, however, 'Jade Wizard' plants had the greatest total dry weight content throughout the experiment. The fresh weight:dry weight ratio (Table 2) at the sixth and eighth leaf-pair stage for both cultivars decreased over the harvest days, but there was an increase in the fresh weight:dry weight ratio at the tenth leaf-pair stage throughout the experiment.

We chose the sixth leaf-pair stage at 48 mg a.i./liter and eighth leaf-pair stage at 24 mg a.i./liter based upon the fact that plants at the sixth leaf-pair stage produced wrinkled, cupped, smaller and necrotic leaves, stunted growth and distorted stems thus producing a poor quality plant. In contrast, plants at the eighth

leaf-pair stage were chosen for their good quality appearance due to the increased leaf area, uniform stems and upright habit. Most importantly, paclobutrazol treatment at the eighth leaf-pair stage of development exhibited a minimal reduction of height without detrimental effects on the overall growth of the plants.

Figure 1. Effect of paclobutrazol on height of Coleus x hybridus 'Velvet Wizard' at 3 stages of development. A. Six leaf-pair stage; B. Eight leaf-pair stage; C. Ten leaf-pair stage.

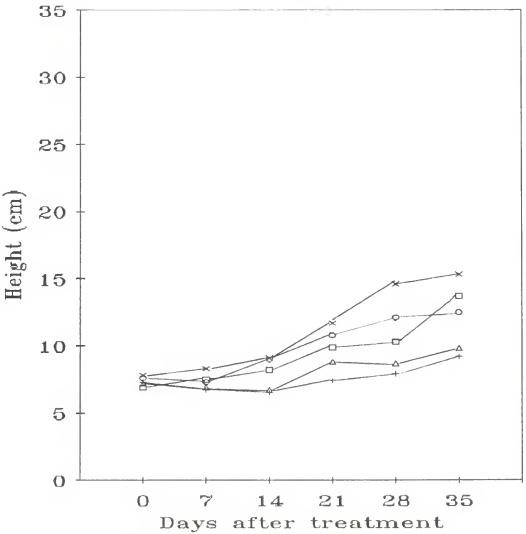
35
30
25
20
15
10
5
0
Height (cm)
0
7
14
21
28
35
Days after treatment
Stage 6
Control
12 mg a.i./liter
24 mg a.i./liter
36 mg a.i./liter
48 mg a.i./liter

Figure 1A.

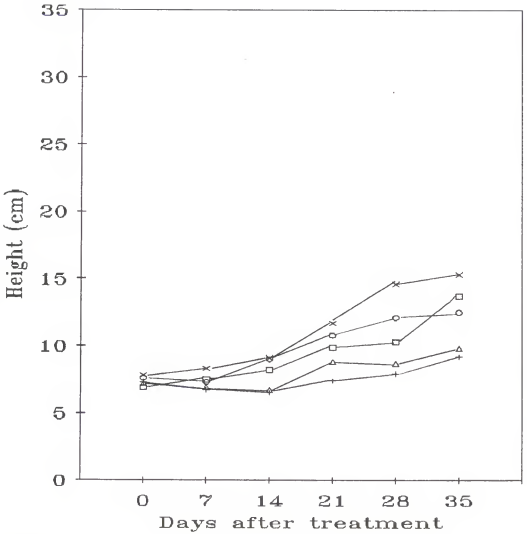
35
30
25
20
15
10
5
0
Height (cm)
0
7
14
21
28
35
Days after treatment
Stage 6
Control
12 mg a.i./liter
24 mg a.i./liter
36 mg a.i./liter
48 mg a.i./liter

Figure 1A.

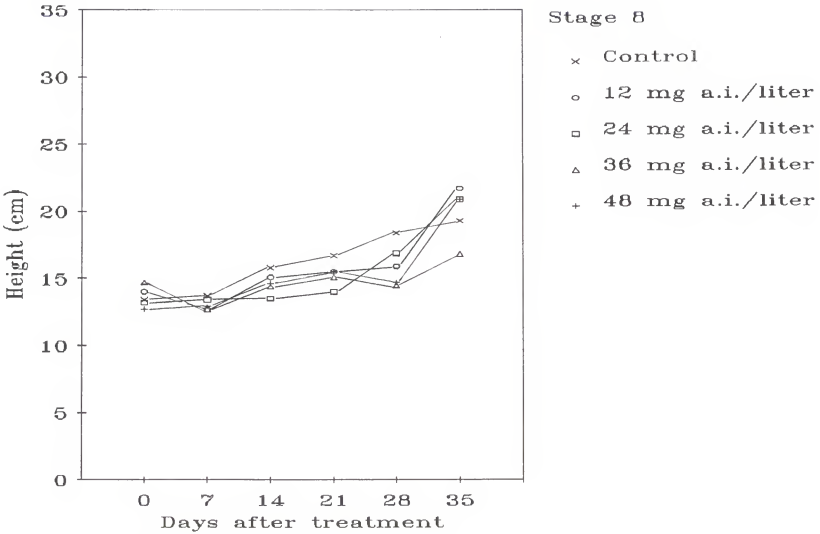
Stage 8
Control
12 mg a.i./liter
24 mg a.i./liter
36 mg a.i./liter
48 mg a.i./liter
Height (cm)
35
30
25
20
15
10
5
0
0
7
14
21
28
35
Days after treatment

Figure 1B.

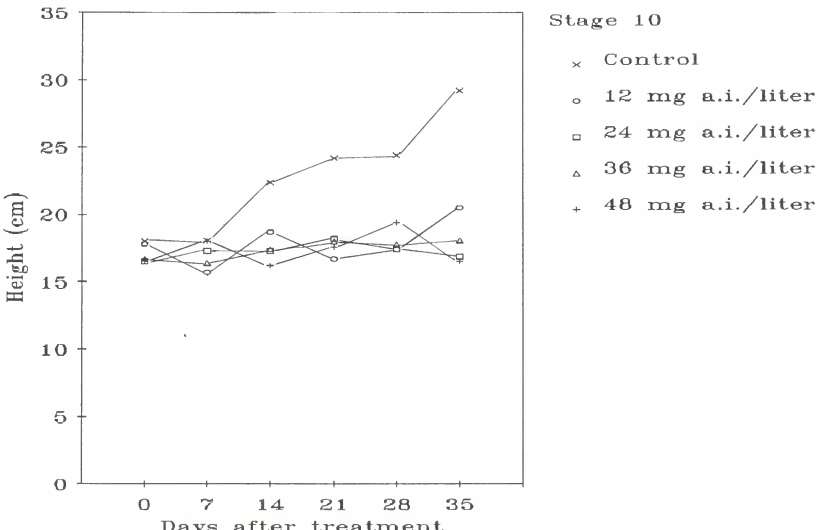
Stage 10
Control
12 mg a.i./liter
24 mg a.i./liter
36 mg a.i./liter
48 mg a.i./liter
Height (cm)
35
30
25
20
15
10
5
0
0
7
14
21
28
35
Days after treatment

Figure 1C.

Figure 2. Effect of paclobutrazol on height of Coleus x hybridus 'Jade Wizard' at 3 stages of development. A. Six leaf-pair stage; B. Eight leaf-pair stage; C. Ten leaf-pair stage.

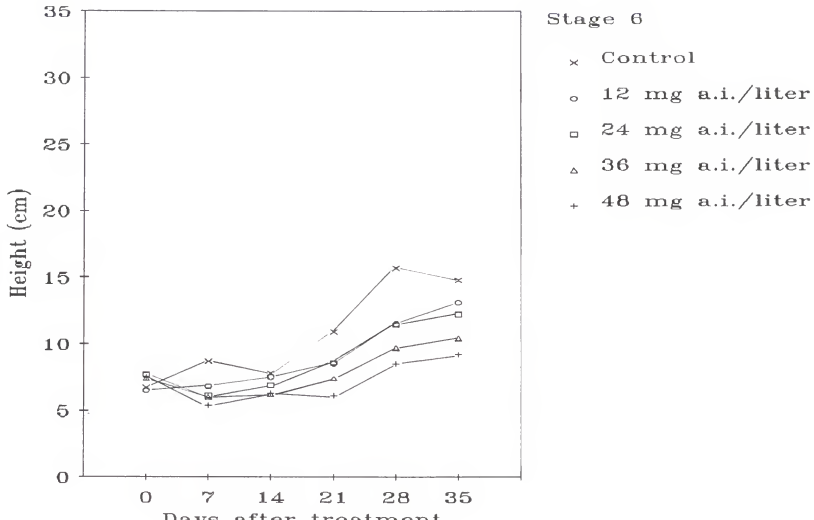
Stage 6
Control
12 mg a.i./liter
24 mg a.i./liter
36 mg a.i./liter
48 mg a.i./liter
Height (cm)
0
5
10
15
20
25
30
35
0
7
14
21
28
35
Days after treatment

Figure 2A.

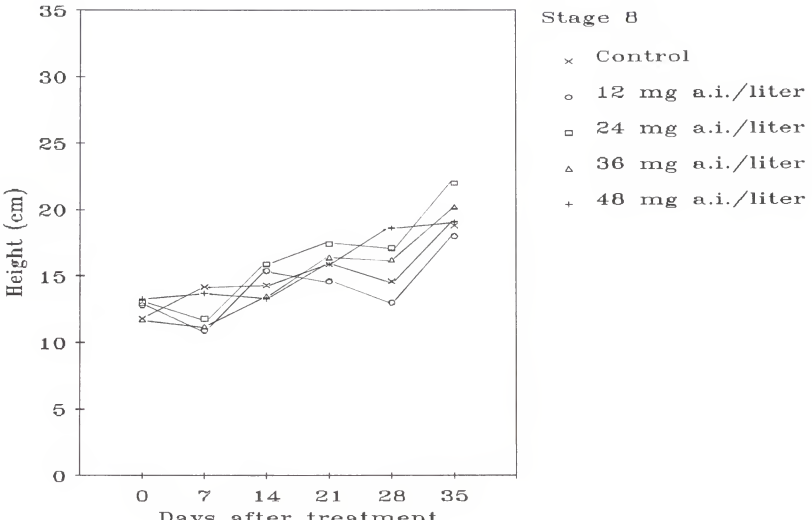
Stage 8
Control
12 mg a.i./liter
24 mg a.i./liter
36 mg a.i./liter
48 mg a.i./liter
Height (cm)
0
5
10
15
20
25
30
35
0
7
14
21
28
35
Days after treatment

Figure 2B.

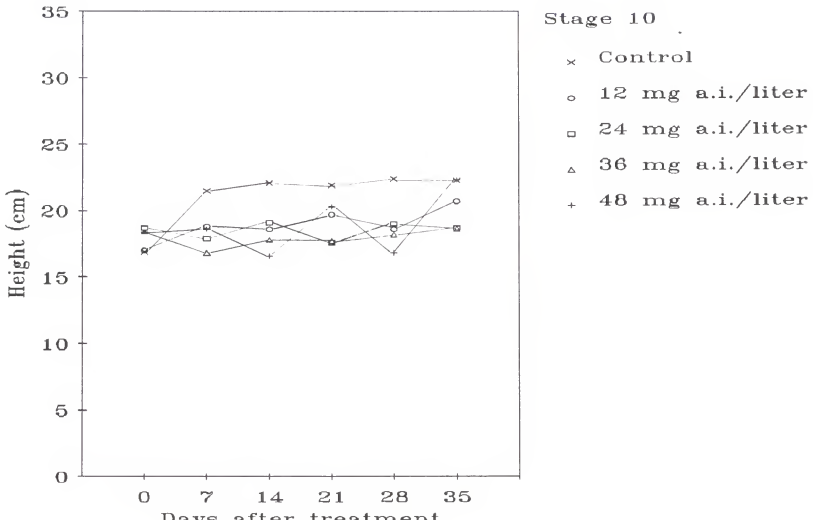
Stage 10
Control
12 mg a.i./liter
24 mg a.i./liter
36 mg a.i./liter
48 mg a.i./liter
Height (cm)
35
30
25
20
15
10
5
0
0
7
14
21
28
35
Days after treatment

Figure 2C.

Table 1. Interaction of stage of development, paclobutrazol application rate, and cultivar on stem width and surface area of mature leaves.

Paclobutrazol application rate (mg a.i./liter)	Stage of development (leaf-pair stage)		
	6	8	10
	Stem width (mm)		
'Velvet Wizard'			
0	6.64	7.86	8.53
12	6.46	7.91	8.49
24	6.57	7.49	8.27
36	6.13	7.61	8.23
48	6.42	7.41	8.67
'Jade Wizard'			
0	6.28	6.97	8.61
12	5.85	7.06	8.09
24	5.98	7.27	8.07
36	5.79	7.19	7.74
48	5.46	7.05	8.17

L.S.D. (0.05, 3-way interaction) = 0.31 mm

Table 1 (cont'd). Interaction of stage of development, paclobutrazol application rate, and cultivar on stem width and surface area of mature leaves.

Paclobutrazol application rate (mg a.i./liter)	Stage of development (leaf-pair stage)		
	6	8	10
	Area of mature expanded leaves (cm^2)		
'Velvet Wizard'			
0	515	783	1884
12	433	673	1113
24	396	627	1005
36	321	643	1091
48	314	614	1132
'Jade Wizard'			
0	466	651	1529
12	419	647	956
24	420	725	893
36	379	693	797
48	366	691	782

L.S.D. (0.05, 3-way interaction) = 99.5 cm^2

Table 2. Interaction of stage of development, harvest day, and cultivar on total dry weight, stem width, fresh weight:dry weight ratio, and leaf dry weight.

Harvest day	Stage of development (leaf-pair stage)		
	6	8	10
	Total dry weight (g)		
'Velvet Wizard'			
0	0.47	1.01	3.34
7	0.93	1.72	5.05
14	1.42	2.43	6.24
21	2.11	3.48	7.05
28	2.66	5.01	6.87
35	3.27	4.78	6.88
'Jade Wizard'			
0	0.63	1.34	4.11
7	1.12	1.90	5.82
14	1.66	2.95	6.34
21	2.26	3.83	7.27
28	3.22	5.29	6.55
35	4.00	6.33	6.85

L.S.D. (0.05, 3-way interaction) = 0.59 g

Table 2 (cont'd). Interaction of stage of development, harvest day, and cultivar on total dry weight, stem width, fresh weight:dry weight ratio, and leaf dry weight.

Harvest day	Stage of development (leaf-pair stage)		
	6	8	10
	Stem width (mm)		
'Velvet Wizard'			
0	4.40	6.68	7.85
7	5.86	7.36	8.40
14	6.11	7.70	8.33
21	7.06	7.82	8.62
28	7.39	8.18	8.47
35	7.85	8.19	8.95
'Jade Wizard'			
0	4.22	6.09	7.65
7	5.37	6.85	7.81
14	5.93	6.87	7.87
21	6.25	7.23	8.46
28	6.81	7.84	8.29
35	6.68	7.76	8.74

L.S.D. (0.05, 3-way interaction) = 0.34 mm

Table 2 (cont'd). Interaction of stage of development, harvest day, and cultivar on total dry weight, stem width, fresh weight:dry weight ratio, and leaf dry weight.

Harvest day	Stage of development (leaf-pair stage)		
	6	8	10
	Fresh weight:dry weight ratio (g)		
'Velvet Wizard'			
0	11.37	14.58	11.04
7	12.20	12.82	10.36
14	10.51	12.46	10.91
21	10.68	10.69	10.40
28	11.40	8.79	11.65
35	11.39	11.09	12.70
'Jade Wizard'			
0	13.11	13.90	10.20
7	10.90	12.80	9.55
14	9.87	11.47	10.32
21	9.35	9.79	10.79
28	9.67	8.52	11.74
35	8.57	9.64	12.58

L.S.D. (0.05, 3-way interaction) = 1.01 g

Table 2 (cont'd). Interaction of stage of development, harvest day, and cultivar on total dry weight, stem width, fresh weight:dry weight ratio, and leaf dry weight.

Harvest day	Stage of development (leaf-pair stage)		
	6	8	10
	Leaf dry weight (g)		
'Velvet Wizard'			
0	0.37	0.75	2.11
7	0.76	1.22	3.30
14	1.08	1.64	3.90
21	1.52	2.29	4.23
28	1.88	3.10	4.15
35	2.20	3.05	4.31
'Jade Wizard'			
0	0.52	1.02	2.23
7	0.93	1.39	3.39
14	1.31	1.99	3.44
21	1.71	2.50	3.83
28	2.35	3.21	3.36
35	2.79	3.90	3.59

L.S.D. (0.05, 3-way interaction) = 0.33 g

Table 3. Interaction of stage of development, paclobutrazol application rate, and harvest day on stem width, area of mature expanded leaves, fresh weight of leaves, and fresh weight of stems.

Paclobutrazol application rate (mg a.i./liter)	Harvest days 0	7	14	21	28	35
	Stem width (mm)					
6-leaf stage of development						
0	4.3	6.0	6.4	6.9	7.6	7.5
12	4.2	5.7	6.2	6.7	7.0	7.3
24	4.3	5.9	5.8	6.9	7.2	7.5
36	4.2	5.5	6.6	6.8	6.9	5.9
48	4.6	5.0	5.8	6.2	6.9	7.1
8-leaf stage of development						
0	6.3	7.1	7.1	7.8	8.2	8.1
12	6.6	7.2	7.7	7.7	7.9	7.8
24	6.4	7.1	7.4	7.4	8.0	8.0
36	6.3	7.3	7.3	7.7	7.8	8.0
48	6.3	6.8	7.0	7.2	8.1	8.0
10-leaf stage of development						
0	7.4	8.4	8.2	9.3	8.7	9.4
12	8.1	8.0	8.1	8.6	8.2	8.8
24	8.1	8.3	7.9	8.1	8.3	8.4
36	7.4	7.4	8.2	8.3	8.3	8.3
48	7.7	8.6	8.2	8.4	8.4	9.3

L.S.D.(0.05, 3-way interaction) = 0.54 mm

Table 3 (cont'd). Interaction of stage of development, paclobutrazol application rate, and harvest day on stem width, area of mature expanded leaves, fresh weight of leaves, and fresh weight of stems.

Paclobutrazol application rate (mg a.i./liter)	Harvest days 0	7	14	21	28	35
	Area of mature expanded leaves (cm^2)					
6-leaf stage of development						
0	155	330	416	527	640	874
12	158	295	362	463	609	672
24	158	274	331	459	575	651
36	169	255	297	355	485	541
48	174	220	304	366	452	525
8-leaf stage of development						
0	346	571	710	824	858	991
12	367	500	654	655	781	1002
24	400	509	663	712	760	1013
36	388	535	604	733	765	983
48	391	519	589	655	771	988
10-leaf stage of development						
0	782	1282	1693	1814	2110	2558
12	966	998	1072	1024	1279	866
24	720	936	961	1030	1044	1005
36	771	883	1060	1006	1030	913
48	687	1005	1058	1002	1037	952

L.S.D. (0.05, 3-way interaction) = 172.3 cm^2

Table 3 (cont'd). Interaction of stage of development, paclobutrazol application rate, and harvest day on stem width, area of mature expanded leaves, fresh weight of leaves, and fresh weight of stems.

Paclobutrazol application rate (mg a.i./liter)	Harvest days 0	7	14	21	28	35
	Fresh weight of leaves (g)					
6-leaf stage of development						
0	4.80	9.60	12.37	16.45	24.53	24.34
12	5.07	8.84	11.31	16.47	22.38	24.38
24	4.93	8.37	11.03	16.73	22.31	25.40
36	5.34	8.13	10.48	14.46	19.37	22.68
48	5.58	7.04	11.09	14.22	19.49	23.23
8-leaf stage of development						
0	9.92	16.01	21.10	24.35	26.71	36.24
12	10.43	14.31	20.80	21.55	28.42	35.00
24	11.00	14.26	23.30	22.65	26.43	36.17
36	10.84	15.27	19.96	23.41	26.52	36.51
48	10.99	14.40	18.81	21.59	26.15	35.96
10-leaf stage of development						
0	24.21	37.82	51.58	58.90	67.46	69.44
12	24.76	32.69	42.08	45.50	47.23	60.88
24	21.04	33.00	39.40	45.09	46.29	46.73
36	23.02	31.46	39.78	43.74	43.47	45.75
48	21.38	34.17	40.45	45.34	45.86	51.54

L.S.D. (0.05, 3-way interaction) = 5.02 g

Table 3 (cont'd). Interaction of stage of development, paclobutrazol application rate, and harvest day on stem width, area of mature expanded leaves, fresh weight of leaves, and fresh weight of stems.

Paclobutrazol application rate (mg a.i./liter)	Harvest days					
	0	7	14	21	28	35
	Fresh weight of stems (g)					
6-leaf stage of development						
0	1.54	3.68	5.04	7.04	12.97	15.09
12	1.45	3.31	5.02	6.38	9.30	12.66
24	1.34	3.14	4.02	6.75	9.38	11.12
36	1.60	2.63	3.56	5.25	6.92	9.04
48	1.87	2.43	3.39	4.64	6.35	8.95
8-leaf stage of development						
0	4.74	8.46	11.55	15.59	15.73	19.84
12	5.26	7.54	12.70	13.06	16.70	21.52
24	4.96	8.65	12.47	15.47	18.79	21.59
36	6.38	7.83	10.56	14.01	16.96	20.48
48	6.05	8.50	10.45	13.33	16.39	18.21
10-leaf stage of development						
0	17.48	21.91	27.04	31.92	37.81	39.78
12	16.28	19.60	26.34	25.57	24.81	30.75
24	13.16	19.30	20.97	27.16	24.76	26.38
36	17.47	18.06	22.57	22.84	24.45	26.01
48	15.26	19.80	21.56	26.76	25.27	31.47

L.S.D.(0.05, 3-way interaction) = 3.28 g

Table 4. Interaction of stage of development and harvest day on dry weight of stems.

Harvest day	Stage of development (leaf-pair stage)		
	6	8	10
	Dry weight of stems (g)		
0	0.10	0.29	1.55
7	0.18	0.50	2.09
14	0.34	0.88	2.62
21	0.56	1.26	3.13
28	0.82	2.00	2.96
35	1.14	2.08	2.91

L.S.D. (0.05, 2-way interaction) = 0.21 g

Table 5. Interaction of cultivar, paclobutrazol application rate and harvest day on surface area of mature leaves.

Paclobutrazol application rate (mg a.i./liter)	Harvest days 0	7	14	21	28	35
	Area of mature expanded leaves (cm^2)					
'Velvet Wizard'						
0	431	734	979	1117	1334	1768
12	482	579	672	761	851	1091
24	357	592	634	762	800	911
36	423	580	710	755	778	864
48	422	613	726	743	796	819
'Jade Wizard'						
0	424	721	900	993	1072	1181
12	445	595	671	698	757	877
24	495	554	669	705	786	868
36	461	535	597	641	742	762
48	412	549	574	607	710	824

L.S.D. (0.05,3-way interaction) = 140.72 cm^2

Table 6. Interaction of stage of development and paclobutrazol application rate on total dry weight.

Paclobutrazol application rate (mg a.i./liter)	Stage of development (leaf-pair stage)		
	6	8	10
	Total dry weight (g)		
0	2.21	3.33	6.96
12	2.14	3.33	6.14
24	2.09	3.60	5.66
36	1.72	3.32	5.41
48	1.73	3.12	5.98

L.S.D. (0.05, 2-way interaction) = 0.38 g

Table 7. Analysis of variance tables for preliminary screening of paclobutrazol rates at various stages of plant development.

Dependent variable: Height

Source	df	Sum of squares	Mean square	F-value
Model	179	12408.97	69.32	39.17
Error	360	637.20	1.77	PR > F
Corrected total	539	13046.17		0.0001

R-square	C.V.	Root M.S.E.	Height mean
0.95	9.2724	1.33	14.35

Source	df	Anova SS	F-value	PR > F
Cv	1	0.76	0.43	0.51
Stage	2	8949.22	2528.03	0.0001
Rate	4	496.69	0.15	0.0001
Harv	5	1571.21	177.54	0.0001
Cv*stage	2	27.91	7.89	0.0004
Cv*rate	4	52.46	7.41	0.97
Cv*harv	5	1.47	0.17	0.9747
Stage*rate	8	266.89	18.85	0.0001
Stage*harv	10	263.31	14.88	0.0001
Rate*harv	20	195.57	5.52	0.0001
Cv*stage*rate	8	50.02	3.53	0.0006
Cv*rate*harv	20	80.21	2.27	0.0016
Cv*stage*harv	10	45.30	2.56	0.0053
Stage*rate*harv	40	199.75	2.82	0.0001
Cv*stage*rate*harv	40	208.19	2.94	0.0001

Table 7(cont'd). Analysis of variance tables for preliminary screening of paclobutrazol rates at various stages of plant development.

Dependent variable: Area of mature expanded leaves

Source	df	Sum of squares	Mean square	F-value
Model	179	95360094.79	532737.96	22.97
Error	360	8350988.06	23197.19	PR > F
Corrected total	539	103711082.85		0.0001

R-square	C.V.	Root M.S.E.	Exparea mean
0.92	20.8103	152.31	731.88

Source	df	Anova SS	F-value	PR > F
Cv	1	766116.63	33.07	0.0001
Stage	2	46916178.90	1011.25	0.0001
Rate	4	7948991.39	85.67	0.0001
Harv	5	17409732.66	150.10	0.0001
Cv*stage	2	2139402.55	46.11	0.0001
Cv*rate	4	463339.61	4.99	0.0006
Cv*harv	5	589669.48	5.08	0.0002
Stage*rate	8	8446318.30	45.51	0.0001
Stage*harv	10	560994.73	2.42	0.0085
Rate*harv	20	3455195.64	7.45	0.0001
Cv*stage*rate	8	381959.99	2.06	0.0392
Cv*rate*harv	20	957288.43	2.06	0.0049
Cv*stage*harv	10	142428.98	0.61	0.8020
Stage*rate*harv	40	4300709.39	4.63	0.0001
Cv*stage*rate*harv	40	880768.12	0.95	0.5620

Table 7(cont'd). Analysis of variance tables for preliminary screening of paclobutrazol rates at various stages of plant development.

Dependent variable: Stem width

Source	df	Sum of squares	Mean square	F-value
Model	179	772.67	4.32	18.70
Error	360	83.11	0.23	PR > F
Corrected total	539	855.77		0.0001

R-square	C.V.	Root M.S.E.	Stemwd mean
0.90	6.6032	0.48	7.28

Source	df	Anova SS	F-value	PR > F
Cv	1	30.15	130.61	0.0001
Stage	2	410.25	888.57	0.0001
Rate	4	8.10	8.77	0.0001
Harv	5	212.53	184.13	0.0001
Cv*stage	2	2.05	4.44	0.0125
Cv*rate	4	1.83	1.98	0.0970
Cv*harv	5	1.28	1.11	0.3540
Stage*rate	8	7.34	3.97	0.0002
Stage*harv	10	47.46	20.56	0.0001
Rate*harv	20	9.46	2.05	0.0053
Cv*stage*rate	8	5.89	3.19	0.0016
Cv*rate*harv	20	5.09	1.10	0.3449
Cv*stage*harv	10	6.43	2.79	0.0025
Stage*rate*harv	40	13.84	1.50	0.0307
Cv*stage*rate*harv	40	10.05	1.19	0.2116

Table 7(cont'd). Analysis of variance tables for preliminary screening of paclobutrazol rates at various stages of plant development.

Dependent variable: Area of nonexpanded immature leaves

Source	df	Sum of squares	Mean square	F-value
Model	179	609123.85	3402.93	1.44
Error	360	848912.35	2358.09	PR > F
Corrected total	539	1458036.21		0.0019

R-square	C.V.	Root M.S.E.	Noxarea mean
0.42	83.0098	48.56	58.50

Source	df	Anova SS	F-value	PR > F
Cv	1	1687.84	0.72	0.3981
Stage	2	74487.72	15.79	0.0001
Rate	4	20454.55	2.17	0.0721
Harv	5	89224.04	7.57	0.0001
Cv*stage	2	8973.79	1.90	0.1507
Cv*rate	4	1972.35	0.21	0.9333
Cv*harv	5	19036.47	1.61	0.1553
Stage*rate	8	15715.11	0.83	0.5739
Stage*harv	10	41962.71	1.78	0.0628
Rate*harv	20	52812.89	1.12	0.3263
Cv*stage*rate	8	22478.62	1.19	0.3029
Cv*rate*harv	20	39255.90	0.83	0.6738
Cv*stage*harv	10	30892.20	1.31	0.2231
Stage*rate*harv	40	98262.55	1.04	0.4063
Cv*stage*rate*harv	40	91907.14	0.97	0.5186

Table 7(cont'd). Analysis of variance tables for preliminary screening of paclobutrazol rates at various stages of plant development.

Dependent variable: Total dry weight of leaves

Source	df	Sum of squares	Mean square	F-value
Model	179	2651.53	14.81	22.05
Error	360	241.85	0.67	PR > F
Corrected total	539	2893.37		0.0001

R-square	C.V.	Root M.S.E.	Ttldw mean
0.92	21.6665	0.82	3.78

Source	df	Anova SS	F-value	PR > F
Cv	1	19.07	28.39	0.0001
Stage	2	1531.09	1139.55	0.0001
Rate	4	29.64	11.03	0.0001
Harv	5	816.99	243.23	0.0001
Cv*stage	2	1.90	1.41	0.2452
Cv*rate	4	1.29	0.48	0.7508
Cv*harv	5	4.71	1.40	0.2229
Stage*rate	8	32.63	6.07	0.0001
Stage*harv	10	105.01	15.63	0.0001
Rate*harv	20	19.54	1.45	0.0945
Cv*stage*rate	8	9.54	1.77	0.0808
Cv*rate*harv	20	15.41	1.15	0.2996
Cv*stage*harv	10	14.59	2.17	0.0189
Stage*rate*harv	40	27.99	1.04	0.4068
Cv*stage*rate*harv	40	22.14	0.82	0.7693

Table 7(cont'd). Analysis of variance tables for preliminary screening of paclobutrazol rates at various stages of plant development.

Dependent variable: Fresh weight:dry weight ratio

Source	df	Sum of squares	Mean square	F-value
Model	179	1395.61	7.80	3.92
Error	360	715.42	1.99	PR > F
Corrected total		2111.03		0.0001

R-square	C.V.	Root M.S.E.	Ratio mean
0.66	12.76	1.41	11.05

Source	df	Anova SS	F-value	PR > F
Cv	1	62.87	31.64	0.0001
Stage	2	35.81	9.01	0.0002
Rate	4	34.46	4.33	0.0019
Harv	5	276.04	27.78	0.0001
Cv*stage	2	11.22	2.82	0.0608
Cv*rate	4	8.73	1.10	0.3575
Cv*harv	5	26.85	2.70	0.0206
Stage*rate	8	24.61	1.55	0.1394
Stage*harv	10	606.34	30.51	0.0001
Rate*harv	20	54.14	1.36	0.1377
Cv*stage*rate	8	11.97	0.75	0.6449
Cv*rate*harv	20	57.26	1.44	0.1002
Cv*stage*harv	10	80.73	4.06	0.0001
Stage*rate*harv	40	54.24	0.68	0.9299
Cv*stage*rate*harv	40	50.36	0.63	0.9605

Appendix B

Detailed methods of anthocyanidin extraction and analysis

Seeds of 'Jade Wizard' and 'Velvet Wizard' coleus were germinated under intermittant mist (6 seconds every 3 minutes) with a media temperature of 24°C. When the seedlings were large enough to handle, they were transplanted to individual 5.5-cm plastic pots filled with a 1 soil: 2 sphagnum peatmoss: 2 perlite media (volume basis) amended with 0.29 kg P/m^3 superphosphate (0N-8P-0K). Pots were placed pot-to-pot on the bench. When plants were touching, they were transplanted into 10.5-cm plastic pots containing the same media and fertilizer amendments. Each pot received 1.5 grams of 14N-6P-12K Osmocote (Sierrablen, Sierra Chemical Co., Milpitas, Calif.).

The plants were grown at 24°/15°C day/night temperatures and irrigated by hand as needed (Mastalerz, 1985). The 2 cultivars were treated at the sixth leaf-pair stage with 48 mg active ingredient (a.i.) paclobutrazol/liter or at the eighth leaf-pair stage with 24 mg a.i./liter. Control plants at the sixth and eighth leaf-pair stage were sprayed with water. Leaves were sampled before paclobutrazol treatment, and at 1, 3, 5, 7, 14, 21, and 28 days after treatment.

One gram of fresh tissue from 4 plants in each of the 4 treatments from leaves positioned above the appropriate sixth and eighth leaf node for the 2 cultivars was cut into small pieces and ground for 3 minutes in a mortar to which 10 ml of 1% HCL (v/v) in 5 ml of ethanol was added. Pigment plant extract was refrigerated (4°C) prior to use to prevent enzyme oxidation, a problem noted with phenol plant crude extracts (Harborne, 1973a).

A 15 cm qualitative filter paper (VWR Scientific Inc., San Francisco, Calif.) was soaked with distilled water and placed in a Buchner funnel to which the plant crude extract was added. Filtration was applied and continued until the crude extract was concentrated near dryness or until a precipitate began to form. The precipitate was washed with 5 ml of 95% ethanol and the mortar was washed with an additional 10 ml of 95% ethanol. All filtrate were collected and stored under refrigeration at 4°C prior to separation by thin layer chromatography. Silica-gel GF Redi-plates (20 X 20 cm; Fisher Scientific) were heated at 75°C for 30 minutes and then cooled prior to applying a 4-ul aliquot of plant extract 2.5 cm from the bottom of the plate. Sample spots were allowed to dry in between the additional seven 4-ul aliquot applications of plant extract. A 10-ul glass syringe (Hamilton Co., Reno, Nevada) was used for uniformity of sample applications. Eight different crude extracts were placed

at the marked origin, 2.5 cm from bottom of plate, at a distance of 2 cm from each other on the same plate. A 100 ppm standard marker, pelargonidin chloride, was applied to each plate. Caution was used to place the first extract and the last extract approximately 2.5 cm from the edge of the plate to prevent an "edge effect". A line was drawn across the plate 2.5 cm from the top to provide an even stopping point for the ascending solvent. Filter paper soaked with the solvent and applied to the sides of the chamber provided an atmosphere of even relative humidity within the developing chamber. At least 20 minutes was needed to saturate the chamber with the solvent vapors to prevent less tailing, a more even solvent front, and a faster moving solvent up the plate. Plates were then placed vertically in a rectangular developing chamber filled to 1.25 cm depth with an 5 n-butanol: 1 acetic acid: 4 water, (volume basis) solvent being careful not to let the plate touch the absorbent filter paper so siphoning would not occur. The plate was allowed to develop until the leading edge of the solvent migrating in an ascending direction reached the line drawn across the plate 2.5 cm from the top of plate. The plate was then removed from the chamber and allowed to dry. Freshly prepared solvent was used for each successive plate. The tank cover was kept on tight to prevent evaporation at the scratch mark and to stop capillary action.

The separated pigments were visualized under long wavelength UV light (254 nm) and marked round for identification according to Rf values. All Rf values were multiplied by 100 (hRf) to obtain whole numbers which provide better accuracy then Rf values:

$$hRf = \frac{\text{Distance of compound from origin}}{\text{Distance of solvent from origin}} \times 100$$

The value in the numerator was determined by measuring the distance from the origin to the center of the area of the pigment. Outlines of the marked pigments were transferred to tracing paper and kept for comparing pigments at different development stages.

Pigments separated at the same migration distance on the plate were scraped off with a spatula onto a sheet of weighing paper, placed into test tubes containing 2.5 ml of HCL (1% v/v) and 2.5 cm of methanol, shaken to disperse the pigment and centrifuged at 2500 rpm for 5 minutes to elute from absorbent. A blank was taken from a point at the same migration distance from the start as the sample spots. Eluted samples were placed in a 1 cm clean cuvette and absorption readings taken within the range of 500 to 600 nm in a spectrophotometer.

Other solvents were used to separate anthocyanidins were 85 formic acid: 9 conc HCL: 6 water, 14 ethyl acetate: 3 formic acid: 3 water, and 30 acetic acid: 3 conc HCL: 10 water (volume basis), however the results

pelargonidin. Perhaps this inconsistency was due to the degradation of the plant extracts. Eluted samples were placed in a 1 cm clean cuvette and absorption readings taken within the range of 500 to 600 nm in a spectrophotometer.

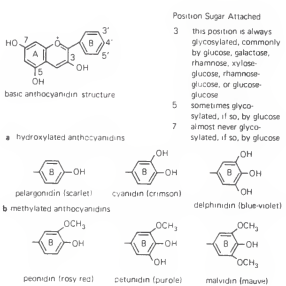

Figure 1. The basic anthocyanidin ring structure (from Salisbury, F.B. and C.W. Ross. 1978. Plant Physiology, 2nd edition. Wadsworth Publishing Company, Inc., Belmont, Calif.)

Appendix C

Internal Anatomy Study

Samples from the control, the sixth leaf-pair (48 mg a.i. paclaobutrazol/liter, foliar spray) and eighth leaf-pair (24 mg a.i. paclobutrazol/liter, foliar spray) treatments were taken for a leaf and stem anatomy. Leaf and stem samples positioned one node above the sixth and the eighth node on appropriate plants of 'Velvet Wizard' were taken 14 days after paclobutrazol-treatment and of 'Jade Wizard' were taken 21 and 28 days after paclobutrazol treatment. Plants used were also analyzed for chlorophyll concentration.

Sections near the center of each blade along the midrib were hand-sliced for cross section study. Observations of the spongy and palisade mesophyll and epidermis were made under a phase contrast microscope. Stem sections were hand-sliced using a razor blade. Stem radial sections were taken below, above and at the appropriate sixth and eighth leaf node. Observations of stem collenchyma, parenchyma and epidermis were made under a phase microscope.

Paclobutrazol-treated sixth leaf-pair plants from 'Jade Wizard' 21 days after treatment showed a single layer of epidermis with 4 collenchyma layers of uniform elliptical cells in cross sections whereas control plants

had a single layer of epidermis with only 2 collenchyma layers. Centripetally, both paclobutrazol-treatments produced large hexagonal parenchyma cells, however, the control plants exhibited irregularly shaped parenchyma.

No visual differences were seen in the eighth leaf-pair stem radial sections for the 2 cultivars but the xylem had a thicker appearance for the paclobutrazol-treated plants. No visual differences were evident in the eighth leaf-pair stem cross-sections, however, control plants appeared to have smaller parenchyma cells than in eighth leaf-pair paclobutrazol-treated plant stem cross-sections. No further work was done due to lack of visual differences among treatments.

Appendix D

Raw Data for Net Photosynthesis

Date listed in order of: treatment where 600 is the 6 leaf-pair stage control, 612 is the 6 leaf-pair stage treated with 48 mg a.i. paclobutrazol per liter, 800 is the 8 leaf-pair stage control, and 860 is the 8 leaf-pair stage treated with 24 mg a.i. paclobutraol per liter; harvest day; net photosynthesis measurement; and photosynthetic active radiation reading (micromols·m^{-2}·sec^{-1}.

'Velvet Wizard							
600	0	3.76	358	800	14	-0.83	1384
600	0	5.28	352	800	14	-7.30	1418
600	0	4.72	550	860	14	-0.48	1462
612	0	0.80	282	860	14	8.01	1256
612	0	11.85	482	860	14	0.05	1296
612	0	9.08	398	600	14	-1.42	1260
800	0	2.53	274	600	14	10.01	1138
800	0	0.59	422	600	14	2.24	1272
800	0	10.07	628	612	14	8.70	1066
860	0	1.86	768	612	14	5.73	1400
860	0	5.75	884	612	14	7.64	1042
860	0	-2.08	650				
600	3	-3.49	604	'Jade Wizard'			
600	3	-0.79	536				
600	3	-4.70	672	800	0	19.47	504
612	3	-0.86	528	800	0	9.89	502
612	3	-0.86	528	800	0	11.56	382
612	3	-0.86	528	860	0	-2.17	450
800	3	2.19	488	860	0	11.88	376
800	3	0.02	300	860	0	6.09	312
800	3	2.20	340	600	0	18.65	574
860	3	-1.77	320	600	0	8.24	438
860	3	-0.03	246	600	0	14.39	326
860	3	-0.22	342	612	0	18.00	550
600	7	2.55	564	612	0	4.33	286
600	7	6.83	816	612	0	5.77	716
600	7	6.89	666	600	3	-3.87	654
612	7	9.15	566	600	3	-0.69	1022
612	7	5.42	614	600	3	-4.79	952
612	7	9.62	500	612	3	-1.41	1010
800	7	5.05	438	612	3	-4.77	714
800	7	16.07	738	612	3	3.33	796
800	7	6.11	782	800	3	-2.35	700
860	7	1.86	926	800	3	-7.45	758
860	7	9.68	996	800	3	-3.86	756
860	7	6.33	792	860	3	2.69	638
800	14	-0.83	1384	860	3	0.86	432

'Jade Wizard' (continued)

860	3	5.24	566	600	21	13.02	1344
600	7	18.97	876	600	21	8.81	1364
600	7	13.34	970	600	21	1.27	1544
600	7	3.83	1218	612	21	-7.78	1444
612	7	2.10	640	612	21	2.95	1478
612	7	6.67	908	612	21	-3.70	1446
612	7	0.74	866	800	21	-0.28	1414
800	7	10.20	866	800	21	6.16	1284
800	7	-0.19	932	800	21	3.50	1446
800	7	7.95	1088	860	21	6.44	1334
860	7	9.98	820	860	21	4.80	1548
860	7	11.48	556	860	21	8.32	1562
860	7	10.12	528	800	28	3.38	1396
800	14	0.38	930	800	28	-8.46	1452
800	14	0.33	942	800	28	-8.44	1464
800	14	2.80	734	860	28	-6.50	1188
860	14	-0.87	814	860	28	-7.01	790
860	14	1.69	1018	860	28	-3.91	850
860	14	2.43	890	600	28	1.24	1024
600	14	8.28	846	600	28	0.77	786
600	14	6.44	1022	600	28	-5.60	1098
600	14	-3.68	934	612	28	1.78	1210
612	14	10.99	1058	612	28	-5.08	1078
612	14	10.14	906	612	28	-6.65	1196
612	14	12.14	666				

GROWTH, NET PHOTOSYNTHESIS, AND PIGMENTATION OF *COLEUS* X *HYBRIDUS* AS AFFECTED BY PACLOBUTRAZOL

by

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ABSTRACT

Bedding plants must remain compact in flats in order for growers to extend the marketing period. With the use of growth retardants, it is possible to control the height of bedding plants. Height of Coleus x hybridus 'Velvet Wizard' and 'Jade Wizard' have been controlled with the application of paclobutrazol.

Foliar sprays of paclobutrazol at 12, 24, 36, and 48 mg a.i./liter at each stage of development (sixth, eighth, and tenth leaf-pair stage) were tested for visual quality. A single application of paclobutrazol at the sixth leaf-pair stage (48 mg a.i./liter) and at the eighth leaf-pair stage (24 mg. a.i./liter) resulted in poor quality and good quality plants, respectively. These plants were used in successive net photosynthesis, chlorophyll and anthocyanidin studies.

Six floral anthocyanidins were identified in the leaves of 'Velvet Wizard' and 'Jade Wizard'. Pelargonidin, malvidin, petunidin, cyanidin, peonidin, and delphinidin were present, however, paclobutrazol treatment did not result in a qualitative shift in anthocyanidins (ACY). There were changes in the number of ACY present over time.

Paclobutrazol treatment had no effect on net photosynthesis (Pn) for the 2 cultivars, however, there

was a change in Pn as light intensity increased over time. 'Velvet Wizard' exhibited a positive response to light intensity with maximum Pn at 1848 micromols$\cdot m^{-2} \cdot sec^{-1}$ while 'Jade Wizard' exhibited a negative response with minimum Pn at 1260 micromols$\cdot m^{-2} \cdot sec^{-1}$. Little influence on chlorophyll concentration was observed in the leaves of coleus cultivars.

A decrease in Pn and chlorophyll concentration over time was seen in the green-cream variegated 'Jade Wizard' plants. At days 3 and 14 after treatment, 'Velvet Wizard' the magenta-colored cultivar, exhibited greater chlorophyll a and b, and total concentration than the control plants.

Internal anatomy study of stem and leaves from the 2 cultivars exhibited little visual differences among treatments.