Changes in Mineral Nutrient Concentrations in *Petunia* Corollas during Development and Senescence

Sven Verlinden¹

Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26505

Additional index words. ethylene, flower, micronutrients, macronutrients, *Petunia ×hybrida*

Abstract. To observe changes in the nutritional status of corollas during development and senescence, *Petunia ×hybrida* cv. Mitchell corollas were analyzed for macronutrient and micronutrient content, dry weight, fresh weight, and ethylene production. Carbon content decreased at slightly lower rates than dry weight during corolla development between anthesis and senescence, while fresh weight and ethylene production followed patterns expected of climacteric flowers. Nitrogen, phosphorus, and potassium content declined during development. Both phosphorus and potassium content gradually declined throughout development with overall losses of about 75% and 40%, respectively. Nitrogen content declined 50% during development but losses occurred only during the final stages of senescence. No significant changes were observed in sulfur, calcium, magnesium, and micronutrient content of the corollas during development. Most elements were present in much lower concentrations in corollas than in leaves. The concentrations of calcium, magnesium, and manganese were about 1-, 5-, and 15-fold lower in corollas than in leaves, respectively. Results indicate that remobilization of selected macronutrients from corollas occurred before and during senescence. Taken together with the presence of low concentrations of macronutrients, my data support the contention that petunia corollas are nutritionally inexpensive and therefore easily disposable organs.

Petal development in higher plants is terminated by pollination-induced or age-related petal or corolla senescence. The regulation of petal or corolla senescence is thought to facilitate the ordered breakdown and remobilization of cellular constituents to other plant and flower organs (Borochoy and Woodson, 1989; Rubinstein, 2000). Flower senescence in many plants, including *Petunia ×hybrida*, is associated with a significant increase in production of ethylene, which plays a regulatory role in the demise of petals or corollas in several plant species (Borochoy and Woodson, 1989; Reid and Wu, 1991). Numerous cellular components, especially membranes, break down during petal senescence. Changes in membranes occurring throughout flower development have been studied extensively (Adam et al., 1983; Borochoy et al., 1984; Brown et al., 1987; Brown et al., 1991; Itzhaki et al., 1990). Macromolecules such as starch, proteins, and nucleic acids also break down during this time (Borochoy and Woodson, 1989). Increased levels of catabolic enzymes, senescence-related mRNAs, and catabolic end products have all been observed before and during senescence (Bialeski and Reid, 1992; Bialeski, 1995; Lawton et al., 1990; Leshem, 1987). However, few studies have actually shown remobilization of the products of these catabolic reactions although remobilization of sugars, 1-aminocyclopropane carboxylic acid (ACC) (the ethylene precursor), and phosphate to other parts of the plant has been observed

or inferred from other observations (Fobel et al., 1987; Nichols and Ho, 1975; Woltering et al., 1995). In addition several studies have also reported increased electrolyte leakage due to increased membrane permeability (Kende and Hanson, 1975; Rubinstein, 2000; Woodson and Borochoy, 1989).

Few studies have addressed changes in mineral nutrient concentrations, a good indication of overall changes in flower tissue from anthesis through flower senescence. The research that has been carried out on this subject generally focuses on the whole flower and not the corolla or petals (Drossopoulos et al., 1996; Tamimi et al., 1999).

The objectives of this study were two-fold. First, I wanted to identify changes, if any, in macronutrient and micronutrient concentrations during corolla development through flower senescence. Changes in element concentrations before and during flower senescence may clarify if flower senescence is a programmed recycling process. Second, I wanted to compare the nutritional status of the corolla with that of leaves, to gain insight into the nutritional expense to the plant associated with disposing of the corolla.

Materials and Methods

Plant material and weight measurements. Thirty-six *Petunia* (*Petunia ×hybrida* cv. Mitchell) plants were grown under greenhouse conditions. Day and night temperatures were set at 21 and 18 °C, respectively. Plants were grown under natural light conditions from January until May in 12 pots (25 cm diameter) containing three plants each in a

peat-lite media (Sunshine Mix #1, Sun Gro Horticulture, Bellevue, Wash.). 'Mitchell' is an open-pollinated, white variety of the common garden petunia. The plants were fertilized at every watering with 150 mg·L⁻¹ nitrogen based on a 20N–4.4P–16.6K fertilizer (Peters Professional 20–10–20, Fogelsville, Pa.). Eighteen flowers, chosen at random, were tagged and emasculated one day prior to expected anthesis, to avoid accidental pollination, thereby decreasing the longevity of a subset of flowers. Every day thereafter, 18 additional flowers, chosen at random, were tagged and emasculated until the flowers tagged on the first day were completely senesced (≈8 d after anthesis). The corollas of all flowers were harvested at this time, grouped in three samples of six flowers, dried overnight in an 80 °C oven, ground in a mortar with a pestle to pass a 40-mesh screen, and stored at –20 °C for later analysis. The only exception to this procedure was the tissue used for ethylene measurements (see below). Fresh weights were taken immediately before drying. Dry weights were measured after the tissues were ground to a powder.

Recently expanded leaves were harvested at the same time corollas were collected. Three samples of six leaves each, chosen at random, were removed from the plants and processed immediately as described for corollas.

Ethylene measurements. Six corollas were labeled and harvested as described above. Immediately after harvest the corollas, two per sample, were enclosed in three 50-mL vials. After a 30-min incubation at room temperature, a 1-mL headspace sample was taken from each vial and injected into a gas chromatograph (Varian, Walnut Creek, Calif.) equipped with a flame ionization detector and a Haysep R column.

Carbon, nitrogen, and sulfur analyses. Total carbon, nitrogen, and sulfur were analyzed on a LECO 2000 CNS carbon-nitrogen-sulfur analyzer (LECO, Mich.). One hundred mg of previously dried and ground tissue was weighed and placed in furnace boats for analysis.

Phosphorus, potassium, calcium, magnesium, and microelement analyses. One hundred mg of dried and ground tissue were weighed and used for microwave digestion. The tissue was placed in digestion vessels to which 5 mL of concentrated HNO₃ and 3 mL of 30% H₂O₂ were added. The reaction was allowed to subside overnight. The samples were digested in a CEM MDS-2000 microwave (CEM, N.C.), filtered through Whatman no. 42 ashless filter paper, brought to 50 mL total volume with ddH₂O, and stored at –20 °C until further analysis. Concentrations of phosphorus, calcium, magnesium, boron, iron, manganese, copper, zinc, and aluminum were measured on a PerkinElmer P400, an inductively coupled plasma-optical emission spectrometer (Perkin Elmer, Norwalk, Conn.). Potassium concentrations were measured on a PerkinElmer 5000 atomic absorption spectrometer equipped with an autosampler (Perkin Elmer).

Statistical analyses. Statistical analyses were carried out with the Analyst software package, part of the SAS system for Windows

Received for publication: 26 Dec. 2001. Accepted for publication 9 Apr. 2002.

¹Assistant Professor.

(SAS Inc., Cary, N.C.). Linear, quadratic, and cubic relationships were assessed at 5%, 1%, 0.1%, and 0.01% level of significance. In the event that an equal level of significance was obtained (P value), a 0.10 or greater increase in R^2 was used to determine if the next highest order polynomial would be applied.

Results

Ethylene production. Ethylene production from corollas remained low but detectable from 1 day through 5 days after anthesis (Fig. 1A).

A typical ethylene climacteric was observed from day 6 to day 8.

Dry and fresh weight. After an one day increase in fresh weight immediately following anthesis, the fresh weight of the corollas remained constant through day 6 (Fig. 1B). Fresh weight decreased by one third during the ethylene climacteric, which included wilting and inrolling of the corollas. The dry weight of the corollas also declined about one third following anthesis but the loss was progressive through senescence (Fig. 1C). Dry weight losses occurred at a rate of 0.0013 g/day.

Carbon. The amount of carbon in the corollas decreased by one half from anthesis through senescence (Fig. 1D). Carbon losses occurred progressively, in a similar pattern to losses in dry weight, and at a rate of 0.0011 g/day.

Changes in macronutrients and micronutrients. Nitrogen, phosphorus, and potassium contents of corollas declined from anthesis through senescence (Fig. 2). However, the pattern of change in nitrogen content was different from that for phosphorus and potassium contents. After a brief decline in

Fig. 1. Changes in ethylene production (A), fresh weight (B), dry weight (C), and carbon content (D) of petunia corollas from anthesis through senescence. Each point is the mean of three, six corolla replications. Carbon content is expressed on a per corolla basis. Vertical bars represent $\pm$ standard error (SE) ($n=3$). Regression analysis shows a significant linear decline in dry weight ($P = 0.0042$) and carbon content ($P < 0.0001$) during development.

Fig. 2. Changes in nitrogen, phosphorus, and potassium content of petunia corollas from anthesis through senescence. Each point represents the mean of three, six corolla replications. All numbers are expressed on a per corolla basis. Vertical bars represent $\pm$ standard error (SE) ($n=3$). Regression analysis shows a significant linear decline in phosphorus ($P = 0.0005$) and potassium ($P = 0.0032$) content and a cubic relationship in the decline in nitrogen content ($P = 0.0112$).

nitrogen content after anthesis, nitrogen content remained constant through day 6. Nitrogen content decreased by 50% during the ethylene climacteric. Phosphorus and potassium content declined progressively by 75% and 35%, respectively, from anthesis through senescence. The rates at which phosphorus and potassium were lost from the corollas were 0.0068 mg/day and 0.0313 mg/day, respectively. Sulfur, calcium, and magnesium content remained constant from anthesis through development (data not shown). Among micronutrients no significant change occurred in boron, copper, iron, manganese, or zinc content during corolla development (data not shown).

Recently expanded leaves had nitrogen, phosphorus, potassium, sulfur, calcium, and magnesium concentrations that were much higher than those found in the corollas of flowers at anthesis (Fig. 3). Concentrations of boron, copper, and zinc were the same in both leaves and corollas (Fig. 4).

Discussion

Significant losses in fresh and dry weight (Fig. 1) are universal changes associated with petal senescence (Borochoy and Woodson, 1989; Rubinstein, 2000). The loss of fresh weight results from decreased water uptake and significant loss of water during the ethylene climacteric in petals, which is typically marked by a loss of cellular compartmentalization. The gradual decrease in dry weight is thought to result from tissue respiration and remobilization of cellular constituents (Borochoy and Woodson, 1989). In petunia corollas, as in those of many other species, the loss of dry mass was independent of the ethylene climacteric (Fig. 1, A and C) (Borochoy and Woodson, 1989).

Carbon loss from the corollas occurred at slightly lower rates than dry weight losses, 0.0011 g/day vs. 0.0013 g/day, respectively. Since the rate of carbon loss almost equaled that of dry weight loss, most carbon losses were probably due to respiration. Remobilization of sugars, amino acids, phospholipids, and nucleotides, should produce a much greater rate of dry weight loss due to the removal of additional nitrogen, oxygen, or phosphorus in the molecules. Losses of both nitrogen and phosphorus during this time were small (Fig. 2). However, flowers that are pollinated or treated with ethylene before natural senescence occurs may show very different patterns of nutrient changes (Borochoy and Woodson, 1989).

Direct and indirect evidence exists for the breakdown of a number of cellular constituents including many carbon-containing molecules during flower senescence (Rubinstein, 2000). Several studies have addressed the question of catabolism in flower tissues (Borochoy and Woodson, 1989; Rubinstein, 2000), however very few have demonstrated actual transport from the corolla or petals. My observations of decreasing nitrogen and phosphorus content show that nitrogen and phosphorus were remobilized during corolla development and senescence. However, the data do not support significant remobilization of carbohydrates as seen in carnation, daylily,

and Ipomoea tricolor (Bielecki, 1995; Borochoy and Woodson, 1989; Wiemken-Gehrig et al. 1974). Emasculation of the flowers may have played a role in this observation, but flower longevity was unaffected (data not shown).

Phosphorus content decreased progressively during corolla development. During flower development activity of enzymes involved in the catabolism of phospholipids increases before and during senescence (Borochoy and Woodson, 1989; Rouet-Mayer et al., 1992; Rubinstein, 2000). Phospholipid synthesis at the same time decreases during senescence (Rubinstein, 2000). In addition,

RNAse activity increases during senescence, resulting in the loss of total RNA from petals (Matile and Winkenbach, 1971; Woodson, 1987). Changes in membranes occur throughout flower development, not only during senescence (Rubinstein, 2000). The decrease in phosphorus content observed in this study can therefore be interpreted as the result of catabolism of cellular constituents that remobilized phosphorus. Processes associated with protein and nucleic acid catabolism are most likely also responsible for the decrease, albeit smaller, in nitrogen content observed in this study during days 7 and 8. However, this decline in nitrogen content seemed to be

Fig. 3. Comparison of macronutrient concentrations in petunia corollas (one day after anthesis) and recently expanded leaves. Each bar represents three replications. Replications consisted of six corollas or leaves. All numbers are expressed on a dry weight basis. The vertical bars represent $\pm$ standard error (SE) (n=3).

Fig. 4. Comparison of micronutrient concentrations in corollas (one day after anthesis) and recently expanded leaves. Each bar represents three replications. Replications consisted of six corollas or leaves. All numbers are expressed on a dry weight basis. The vertical bars represent $\pm$ standard error (SE) (n=3).

more closely associated with the ethylene climacteric than with the gradual decline in phosphorus concentration, which appeared to be unrelated to ethylene. The decline in potassium, the most mobile cellular cation, during flower development, is most likely attributable to its ability to balance charges of components being exported from the corolla.

The calcium concentration in mature leaves was ≈ 10 -fold higher than the calcium concentration in corollas (Fig. 3). Calcium has been implicated in many studies as an agent delaying senescence and increased postharvest quality of a number of plant tissues, including flowers (Mills and Jones, 1996; Torre et al., 1999). Calcium is thought to affect flower longevity through its stabilizing effect on membranes (Leshem, 1987). Treatment of petals with calcium has been shown to significantly prolong the postharvest life of rose flowers (Torre et al., 1999), suggesting a role for calcium in flower maintenance.

In conclusion, all macronutrients were present in lower concentrations in corollas than in leaves, most likely due to the limited photosynthetic capabilities of corollas. This makes disposing of corollas, through abscission, after remobilization of a significant portion of the macronutrients, a relatively nutritionally cheap process. Indeed, the showy parts of a flower have been shown to function only as long as a competitive advantage can be obtained from maintaining the flower in anticipation of pollination. Disposal in favor of a new flower that can be pollinated is an intricate part of maintaining a competitive advantage for plants (Ashman and Schoen, 1994, 1997). A nutritionally cheap organ may fit well in this strategy of survival. Additional research should be carried out to establish the differences in remobilization of nutrients from the petals and corollas of other species.

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