HortScience 60(8):1350–1358. 2025. https://doi.org/10.21273/HORTSCI18531-25

Changes in Mineral Nutrient Content during the Growth, Maintenance, and Senescence Phases of Petunia Corolla Flower Development

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Keywords. cytokinins, ethylene, etr1-1, *Petunia ×hybrida*, SAG12-IPT

Abstract. **Changes in mineral nutrient content occur during the growth, maintenance, and senescence phases of petunia corolla development. Understanding the role of cultivar and phytohormones in nutrient remobilization is key in expanding our knowledge of flower senescence, an important physiological process in a number of crops and horticultural applications. The corollas of several wild-type and transgenic *Petunia ×hybrida* plants including V26, Mitchell Diploid (MD), SAG12-IPT, and CaMV35S*etr1-1* were collected in a time sequence from expansion through senescence and analyzed for fresh weight, dry weight, and macronutrient content. Changes in fresh weight, dry weight, and macronutrients were used to define and delineate phases in mobilization, homeostasis, and remobilization of nutrients. Corollas of the open-pollinated petunia varieties V26 and MD measured from corolla expansion through age-related senescence exhibited growth, maintenance, and senescence phases of development typical of climacteric flowers. SAG12-IPT, a transgenic cytokinin-overproducing plant, exhibited a prolonged corolla maintenance phase when compared with V26, possibly related to the sustained ability of the corollas to act as a sink tissue. The corollas of CaMV35S*etr1-1*, an ethylene-insensitive plant, showed altered patterns in dry weight, fresh weight, and macronutrient changes throughout development when compared with MD. These altered patterns suggested an important role for ethylene and cytokinins in all phases of corolla development.**

Flower development of petunia has been studied extensively from organogenesis through senescence (Gerats and Strommer 2009). As the reproductive organ, flowers contribute to fecundity and therefore the ecological fitness of plants (Ashman and Schoen 1994). In addition, flowers are also often produced for their ornamental value (Lone et al. 2025; Ma et al. 2018). As such, flower development—and senescence in particular—has been studied in detail to understand flower growth and development and ecological fitness of plants and to improve flower longevity (Jones et al. 2009; Lone et al. 2025; Ma et al. 2018; Parveen et al. 2023; Shibuya 2018). Studies on flower development often look at arbitrary stages or phases of development such as bud or cell division, open flower or cell expansion, senescing flower or cell shrinkage/death or narrow their focus on a specific stage of development such as senescence (Lone et al. 2025; Ma et al. 2018; Shibuya 2018; Trivellini et al. 2011). Some studies look at day-to-day or even hour-to-hour changes throughout flower development from flower bud formation and expansion through senescence (Shibuya 2018). Hourly observations are especially important in ephemeral flowers such as morning glory, iris, and daylily (Shibuya 2018). Those that monitor day-to-day or hour-to-hour changes often anchor observations to anthesis or time of pollination and set this day as time at hour 0 or day 0 (Shibuya 2018). Although many studies hint at a maintenance phase, often this phase is not explicitly studied (Ashman and Schoen 1994; Ma et al. 2018). Our study aims to (1) help establish clear universal terminology and language on the stages of flower development, (2) investigate the influence of ethylene and cytokinins on nutrient content of corollas throughout development, and (3) focus future research on the possibly important maintenance phase of development. Understanding nutrient remobilization from corollas will expand our knowledge of flower development and in turn help us target processes that can maintain the maintenance phase of flower development and therefore flower longevity, a key characteristic useful to a number of ornamental and other horticultural crops.

Despite some work on the growth and development of flowers including bud development and expansion, flower senescence has received the bulk of attention (Lone et al. 2025; Ma et al. 2018; Shibuya 2018). Senescence represents endogenously controlled degenerative processes leading to certain death (Bresson et al. 2018; Gan 2007; Leopold 1975; Miryeganeh and Hensel 2021). More specifically, senescence refers to those events that are part of a genetically based program leading to programmed cell death (PCD) (Miryeganeh and Hensel 2021; Rubinstein 2000; Wang et al. 2021; Xu and Hanson 2000). An important function of PCD, in addition to directing normal plant development, is to remobilize as much nutrition as possible to benefit the whole plant and conserve costly resources (Miryeganeh and Hensel 2021; Smart 1994). Flower senescence, ended by PCD, is a process that can be visibly recognized by morphological alterations such as petal wilting and color changes, although many endogenous changes precede these visible cues (Lin and Jones 2021). During flower development and senescence, a loss of dry matter in the petals or corolla occurs, most likely due to the hydrolysis of macromolecules for respiration, as well as the redistribution of a number of compounds to other floral organs or the remaining plant (Borochov and Woodson 1989; Jones 2013; Trivellini et al. 2011; Verlinden 2003).

Although proteins are degraded, the synthesis of new proteins including RNases, DNases, and transporters is necessary for petal senescence and nutrient remobilization to occur (Jones et al. 2009; Miryeganeh and Hensel 2021). Many genes with putative catabolic functions during corolla and petal development and senescence have been cloned and characterized from a number of plant species (Chapin and Jones 2009; Lone et al. 2025; Panavas et al. 1999; Rubinstein 2000; Shibuya 2018; Van Doorn 2003; Wang et al. 2013; Woodson 1994). Catabolism and remobilization of various nutrients, observed directly in only a few instances, therefore seem to be general and integral parts of a coordinated genetic program (Dar et al. 2021; Jones 2013; Trivellini et al. 2011; Verlinden 2003).

Petunia flowers, among others, are classified as climacteric based on the presence of an increased rate of ethylene production during flower senescence. Autocatalytic ethylene production has been demonstrated in *Petunia* corolla senescence, with or without pollination (Jones et al. 2009). Ethylene production plays a key role in the demise of the corolla and has been implicated in coordinated remobilization of nutrients from the corolla and petals of several plant species (Borochov and Woodson 1989; Dar et al. 2021; Jones 2013; Verlinden 2003; Wang et al. 2013). The perception of ethylene in these events is crucial (Dar et al. 2021). Genetic engineering with a mutant ethylene receptor, CaMV35S*etr1-1*, has produced ethylene-insensitive petunia (Gubrium et al. 2000; Wang et al. 2013). These transgenics show altered patterns of nutrient remobilization (Jones 2013).

Several other plant hormones have roles during flower development and senescence (Lone et al. 2025; Ma et al. 2018). Cytokinins have been shown not only to delay leaf

Received for publication 14 Feb 2025. Accepted for publication 4 Apr 2025.
Published online 15 Jul 2025.
We thank Dave Clark (University of Florida) for providing the SAG12-IPT and CaMVs35etr1-1 petunia seeds used in this study.
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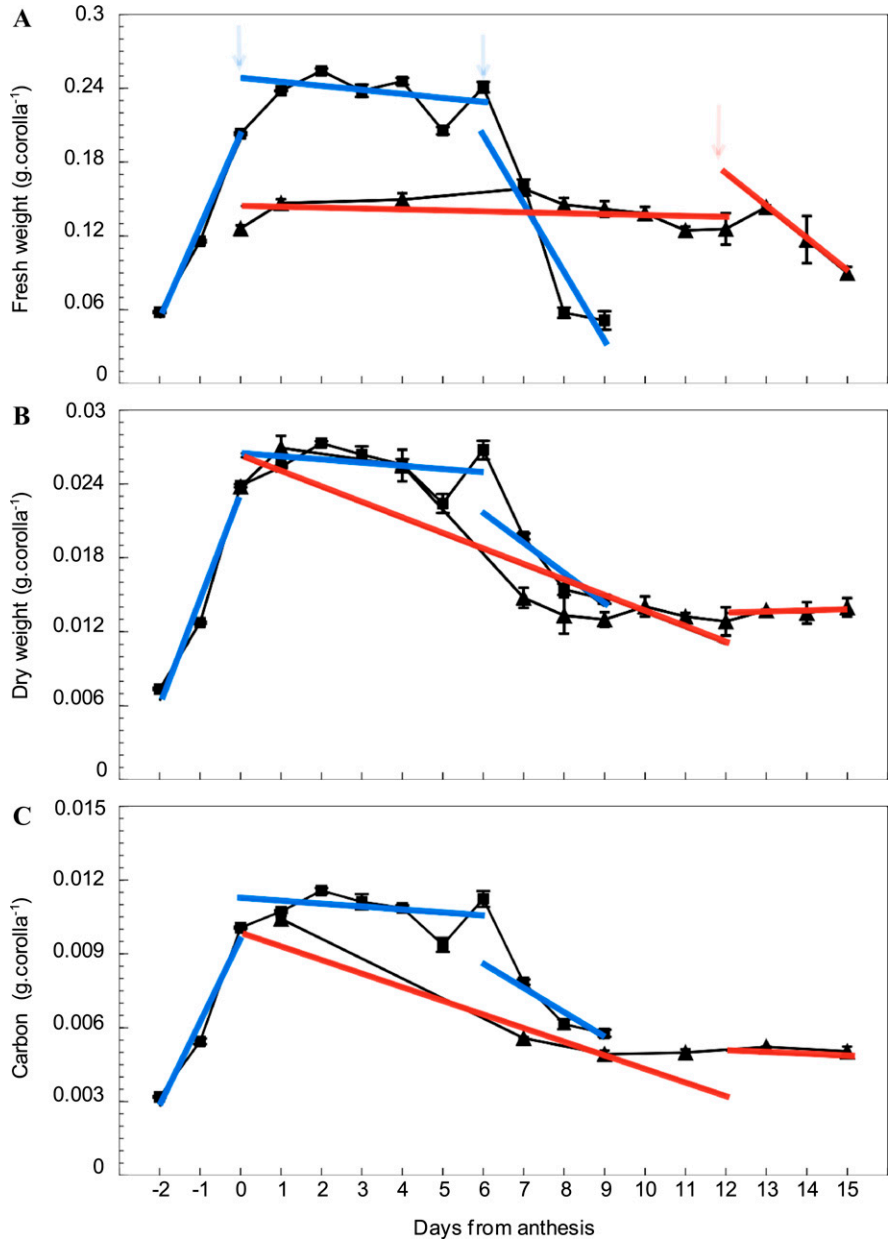

Fig. 1. Changes in fresh weight (**A**), dry weight (**B**), and carbon (**C**) content of corollas during flower development and senescence of MD (■) and CaMV35S*etr1-1* (▲). Error bars show the standard error of the mean (n = 3). Regression lines fitted to MD data are in blue, whereas the regression lines fitted to CaMV35S*etr1-1* are shown in red. Arrows indicate inflection points between the growth, maintenance, and senescence stages of development.

senescence (Dervinis et al. 1998; Richmond and Lang 1957) but also flower senescence (Chang et al. 2003; Khaskheli et al. 2018; Ma et al. 2018; Trivellini et al. 2015; Wu et al. 2017). Cytokinin concentration in roses (Mayak et al. 1972), carnation (Van Staden and Dimalla 1980), and *Cosmos sulfureus* (Saha et al. 1985) is greatest in young flowers and decreases during corolla opening and development. Exogenously supplied cytokinin suppresses senescence in cut carnation flowers (Van Staden et al. 1988) and delays senescence of *Petunia* corollas (Taverner et al. 1999; Trivellini et al. 2015; Wu et al. 2017). Transgenic petunias, transformed with SAG12-IPT, engineered to overproduce cytokinin, resulted in increased flower longevity most likely due to decreased ethylene sensitivity (Chang et al. 2003). In addition, links between ethylene and cytokinin content and signaling and their influence on flower development and senescence have been established (Khaskheli et al. 2018; Wu et al. 2017).

Few studies have addressed changes in mineral nutrient concentrations in individual floral organs (Jones 2013; Trivellini et al. 2011), instead focusing on nutrient concentrations of the whole flower as seen in studies of rose, *Juglans regia* L., and *Lupinus havardii* flowers (Drossopoulos et al. 1996; Picchioni et al. 2001; Tamimi et al. 1999). Remobilization of nitrogen, phosphorus, and potassium from *Petunia* corollas occurs before and during flower senescence in *Petunia* ×*hybrida*, while fresh weight and ethylene production followed patterns expected of climacteric flowers (Jones 2013; Verlinden 2003).

To establish detailed patterns of nutrient mobilization and remobilization during age-related development and senescence, we measured fresh weight, dry weight, and macronutrient (C, N, P, K, Ca, Mg, and S) content from a developmental series of corollas of two varieties of *Petunia* ×*hybrida:* Mitchell Diploid (MD) and V26. We also investigated the hormonal influence on changes in macronutrient contents from bud stage through senescence by comparing two transgenic lines of *Petunia* ×*hybrida,* one insensitive to ethylene and another overproducing cytokinins, to their wild-type counterparts. Our overall goal was to find key markers that could help us establish common language around flower development and help us identify key nutrient changes that indicate physiological changes in flowers.

Materials and Methods

Treatments and experimental design. Plants were grown at the West Virginia University greenhouse in Morgantown, WV, USA (lat. 39°39′N, long. 79°55′W). The greenhouse was an even-span structure composed of glass and a truss frame, with the ridge of the apex running East to West. MD and V26, referred to as wild type, and their respective transgenic counterparts, CaMV35S*etr1-1* in the MD background (line 44568) and SAG12-IPT in the V26 background (line I97-1-7), were grown in a replicated (15 pots each) completely randomized design (CRD). All seeds were obtained from Dr. David G. Clark (University of Florida, Gainesville, FL, USA). CAMV35S*etr1-1* (line 44568) has been described extensively before as an ethylene-insensitive petunia showing delayed corolla senescence (Clevenger et al. 2004; Gubrium et al. 2000; Jones 2013; Wilkinson et al. 1997). SAG12-IPT plants have also been described before and shown to contain the IPT transgene, overproduce cytokinins, reduce ethylene sensitivity, and confer extended flower longevity (Chang et al. 2003). Seeds from homozygous lines of the above described transgenics were sown separately in quarter flats using a peat-lite plug mix (Sunshine Mix #5; Sun Gro, Seba Beach, AB, Canada) and placed in a mist bed with 28 °C bottom heat to improve germination. When seedlings reached ∼1.5 cm in height, 45 plants per variety were transplanted into flats with cell packs using a peat-lite medium (Sunshine Mix #1; Sun Gro). These flats were moved into a glasshouse with day and night temperatures set at 21 and 18 °C, respectively. Once the plants reached ∼6 cm in height, they were transplanted into 25.4-cm pots, three plants per pot (pots were replicated 15 times per variety for a total of 60 pots). When adequate stem and root mass was produced, a final transplant was made to 11.37-L pots using the same medium.

Greenhouse management. The plants were grown under natural light conditions from January to May at the West Virginia University greenhouses in Morgantown, WV, USA,

and were fertilized at every watering with 250 mg·L^{-1} nitrogen based on a 20N–4.4P–16.6K fertilizer (Peters Professional 20-10-20; Peters, Fogelsville, PA, USA) based on preliminary work with *Petunia* plants at West Virginia University.

Tissue collection. The plants started flowering before the plants were transplanted into 11.37-L pots. We waited to collect flowers for our study until the plants were well-established after transplanting (3 weeks). To collect plant tissue for age-related senescence, 20 flowers, chosen at random for each of the 4 varieties (in a CRD), were tagged and emasculated immediately preceding anthesis to avoid accidental pollination. Every day thereafter, 20 additional flowers, also chosen at random, were tagged and emasculated until the flowers tagged on the first day were completely senesced. The final day of senescence varied for each petunia variety. All of the tagged flowers were harvested at this time and separated into corollas, sepals, pedicles, ovaries, and styles. Flowers at two bud stages were also collected before anthesis for each variety, except for the CaMV35S*etr1-1* plants due to a lack of plant material. Some time points could not be obtained because of difficulties maintaining CaMV35S*etr1-1* plants due to their extreme susceptibility to fungal pathogens. Fresh weights for each set of corollas were taken immediately before placing them in paper bags. The corollas were subsequently dried overnight in a 75 °C oven (Fisherbrand™ Isotemp™; Fisher Scientific, Pittsburgh, PA, USA). Upon removal from the oven the following day, a dry weight measurement was taken for each set of corollas before grinding them to a powder in a mortar with a pestle to pass a 40-mesh screen. The ground tissue was then wrapped in wax paper, placed back into the paper bags, and stored at −80 °C for later analysis. The above steps were repeated until three replications for each variety were completed (three replications with 20 flowers each).

Carbon, nitrogen, and sulfur analyses. Total carbon, nitrogen, and sulfur content for corollas from each of the three replications was measured using a LECO 2000 CNS carbon–nitrogen–sulfur analyzer (LECO Inc., Saint Joseph, MI, USA). For each variety and replication, 100 mg of previously dried, ground, and stored corolla tissue was weighed and placed in furnace boats for analysis. These steps were repeated until three carbon, nitrogen, and sulfur replications of each set of corollas were completed.

Phosphorus, potassium, calcium, and magnesium analyses. A total of 100 mg of dried and ground tissue was weighed and placed in 50-mL beakers. The tissue was subsequently digested in 5 mL of concentrated HNO_3 and 3 mL of 30% H_2O_2. The reaction was allowed to reach completion overnight. Next, the samples were filtered through Whatman no. 42 ashless filter paper, brought to a 50-mL total volume with double-distilled H_2O, and stored at −80 °C until further analysis. These steps were repeated until three replications per variety were complete. Phosphorus, calcium, and magnesium contents were measured on an inductively coupled plasma-optical emission spectrometer (ICP-OES) (Perkin-Elmer P400; Perkin-Elmer, Norwalk, CT, USA). Potassium content was measured on a Perkin-Elmer AAnalyst 100 atomic absorption spectrometer equipped with an autosampler.

Statistical analysis. Linear regressions were performed on corolla fresh weight, dry weight, and carbon content to evaluate absolute content, rate of changes in these measurements, and phases of corolla development (SAS software; SAS Institute, Cary, NC, USA). The regression coefficients were maximized by testing all combinations of break points to determine where statistically significant phase breaks in the data occurred. The statistical separation of these stages of development were confirmed with analysis of variance (ANOVA) (SAS software). The averages of data points in each phase of development (growth, maintenance, and senescence) were compared with ANOVA to show that the phases determined by visual and statistical analysis are indeed statistically different from each other.

Data availability statement. The data are available on request from the authors.

Results

Fresh weight, dry weigh, and carbon content. The fresh and dry weight of MD corollas increased sharply before anthesis (Fig. 1A and 1B, Table 1) and then remained relatively constant through day 6. A temporary decrease was observed at day 5 in both fresh and dry weights. The dry weight of CaMV35S*etr1-1* corollas was similar to MD corolla dry weight from anthesis through day 5. However, CaMV35S*etr1-1* diverged from MD patterns of changes in dry weight, showing a decline ∼1 to 2 d before the decline in dry weight observed in MD. From day 7, the dry weight of CaMV35S*etr1-1* remained at the same level through completed senescence (Fig. 1B). The total decline in dry weight following anthesis through senescence for both MD and CaMV35S*etr1-1* was about half of their respective dry weights at anthesis. V26 and SAG12-IPT fresh and dry weights increased rapidly before anthesis (Fig. 2A and 2B, Table 1). The rates of fresh

Table 1. Regression analysis of fresh weight, dry weight, and carbon content changes during the growth, maintenance, and senescence phases of MD, CaMV35S*etr1-1*, V26, and SAG12-IPT petunia corollas.

Plant	Measurements (statistics)[i]	Parameters	Growth	Maintenance	Senescence
Mitchell	Fresh weight (R^2 = 0.959; P = 0.0050; MSE = 0.022528)	y intercept	0.198041	0.249260	0.532378
		x slope	0.072441	−0.003522	−0.055270
	Dry weight (R^2 = 0.955; P = 0.0006; MSE = 0.001897)	y intercept	0.022882	0.026488	0.036987
		x slope	0.008237	−0.000247	−0.002550
	Carbon (R^2 = 0.956; P = 0.0005; MSE = 0.000806)	y intercept	0.009665	0.011234	0.014895
		x slope	0.003434	−0.000122	−0.001039
CaMV35S*etr1-1*	Fresh weight (R^2 = 0.712; P = 0.0022; MSE = 0.042372)	y intercept		0.144194	0.491684
		x slope		−0.000675	−0.026782
	Dry weight (R^2 = 0.841; P = 0.0015; MSE = 0.002527)	y intercept		0.026199	0.012055
		x slope		−0.001266	0.000120
	Carbon (R^2 = 0.739; P = 0.0005; MSE = 0.001465)	y intercept		0.009873	0.006199
		x slope		−0.000552	−0.000093
V26	Fresh weight (R^2 = 0.992; P < 0.0001; MSE = 0.012202)	y intercept	0.250734	0.264306	0.923928
		x slope	0.071882	0.004796	−0.098067
	Dry weight (R^2 = 0.988; P < 0.0001; MSE = 0.001561)	y intercept	0.032547	0.040151	0.033497
		x slope	0.009347	−0.000857	−0.000487
	Carbon (R^2 = 0.996; P < 0.0001; MSE = 0.000390)	y intercept	0.014392	0.017832	0.018828
		x slope	0.004095	−0.000458	−0.000794
SAG12-IPT	Fresh weight (R^2 = 0.909; P = 0.0011; MSE = 0.027944)	y intercept	0.166018	0.234942	0.406440
		x slope	0.045493	−0.002934	−0.028825
	Dry weight (R^2 = 0.920; P = 0.0003; MSE = 0.002967)	y intercept	0.023079	0.032822	0.033965
		x slope	0.007080	−0.000768	−0.001241
	Carbon (R^2 = 0.922; P = 0.0003; MSE = 0.000390)	y intercept	0.010061	0.014050	0.017264
		x slope	0.003081	−0.000271	−0.000748

[i] The values in parentheses represent the R^2 and P values for the combined linear regression of the growth, maintenance, and senescence phases of development.

MSE = Mean squared error.

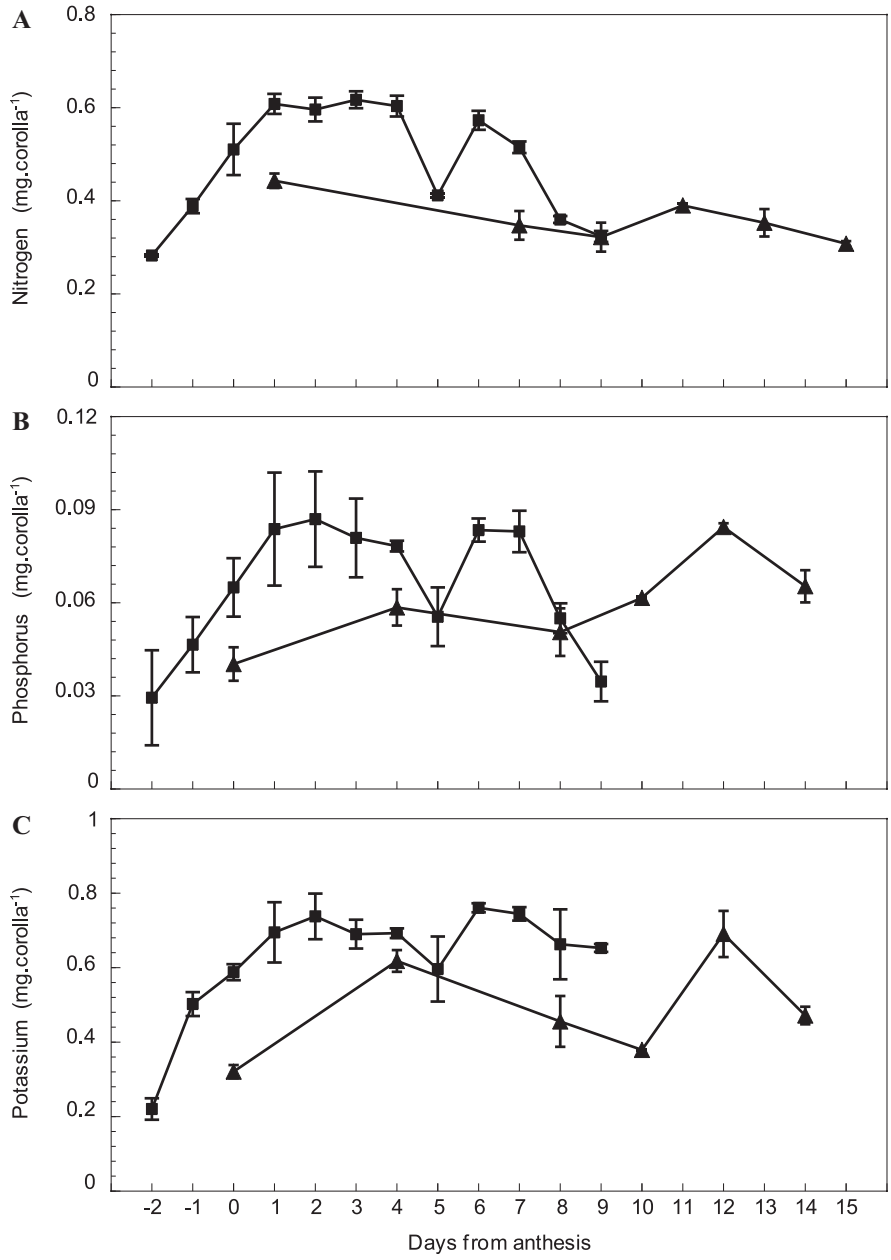

Fig. 2. Changes in nitrogen (**A**), phosphorus (**B**), and potassium (**C**) content of corollas during flower development and senescence of MD (■) and CaMV35S*etr1-1* (▲). Error bars show the standard error of the mean (n = 3).

weight and dry weight gains for V26 were slightly higher than for SAG12-IPT, resulting in higher fresh and dry weights at anthesis. Both fresh and dry weight then remained relatively constant until day 6 (Fig. 2A and 2B, Table 1).

SAG12-IPT fresh weight remained constant through day 9, ~2 d longer than V26. A slight decrease in SAG12-IPT fresh weight reminiscent of MD corolla patterns at day 8 was observed. The dry weight of V26 decreased steadily from day 1 through senescence, a more gradual decrease than observed in MD. Carbon content of all four petunia varieties closely mimicked changes in their respective dry weight (Figs. 1C and 2C). Carbon content at anthesis ranged from 38.5% for CaMVS35etr1-1 to 43.5% for MD.

Macronutrients. Changes in nitrogen, phosphorus, sulfur, and magnesium content of MD and V26 corollas were similar throughout corolla development and senescence (Figs. 2 and 3). Some of these elements increased in content up to 2 d after anthesis and continued to exhibit similar levels through day 6, except for a temporary decrease at day 5, which mimicked the changes observed in MD corolla fresh and dry weight. After day 6, there was a steady decrease in nitrogen, phosphorus, sulfur, and magnesium through senescence. Potassium followed a similar pattern in content changes to nitrogen, phosphorus, sulfur, and magnesium through day 6 (Fig. 3C). However, the decline observed in other nutrients cannot be seen in potassium content. Calcium increased nearly 4-fold from anthesis through day 9 (Fig. 5A). In CaMVS35*etr1-1* corollas, the only nutrients that showed a decrease in content, although small, were nitrogen and sulfur. Phosphorus, potassium, calcium, and magnesium showed either erratic patterns in nutrient content or a gradual increase through corolla development and senescence (Figs. 3B, 3C, 5A, and 5B). In V26, nitrogen, phosphorus, and sulfur content increased early in corolla development, followed by a slight decline through day 6 and a steep decline through senescence (Figs. 4A, 4B, and 6C). SAG12-IPT corollas showed lower peak levels of nitrogen, phosphorus, and sulfur than V26, the result of more gradual increases in these elements during early corolla development. However, final nitrogen, phosphorus, and sulfur content was lower in SAG12-IPT than V26 corollas. Calcium and magnesium content of V26 increased 6- and 2-fold, respectively, from anthesis through senescence (Fig. 6A and 6B). Calcium content of SAG12-IPT increased 4-fold from anthesis through senescence, whereas magnesium remained relatively constant (Fig. 6A and 6B). Potassium increased 7-fold from 3 d before anthesis through days 1 and 4 for V26 and SAG12-IPT corollas and remained at those levels through senescence (Fig. 4C). In SAG12-IPT, the accumulation of potassium to peak levels was delayed by 2 d in comparison with V26.

In our effort to establish that flower development can be discussed in terms of three developmental stages or phases, growth/expansion, maintenance, and senescence, we performed regression analysis on fresh weight, dry weight, and carbon content of the corollas. We clearly and objectively establish that phase breaks occur at two points during corolla development, between the growth/expansion phase and maintenance phase and the maintenance phase and senescence phase of development. The ANOVA used to help establish the statistical validity of these breaks did not allow us to confirm a growth/expansion phase separate from a maintenance phase in the CaMVS35*etr1-1* corollas. Hence, the lack of regression coefficients in Table 1 for the growth phase of CaMV35etr1-1 corollas.

Discussion

The results of our study indicate that development and senescence of the flowers of the wild-type petunia varieties used in our experiments, MD and V26, can be characterized by three distinct developmental phases: a growth phase, a maintenance phase, and a senescence phase. Clear statistical breaks can be observed between these phases supporting our contention that flower development can and should be discussed in terms of these three distinct developmental phases (Figs. 1 and 4, Table 1). Most research has discussed flower senescence in terms of arbitrary temporal divisions such as hours or days after anthesis or visible stages of petal development, as in carnation, rose, hibiscus, alstroemeria, petunia, morning glory, and others (Breeze et al. 2004; Khaskheli et al. 2018; Lone et al.

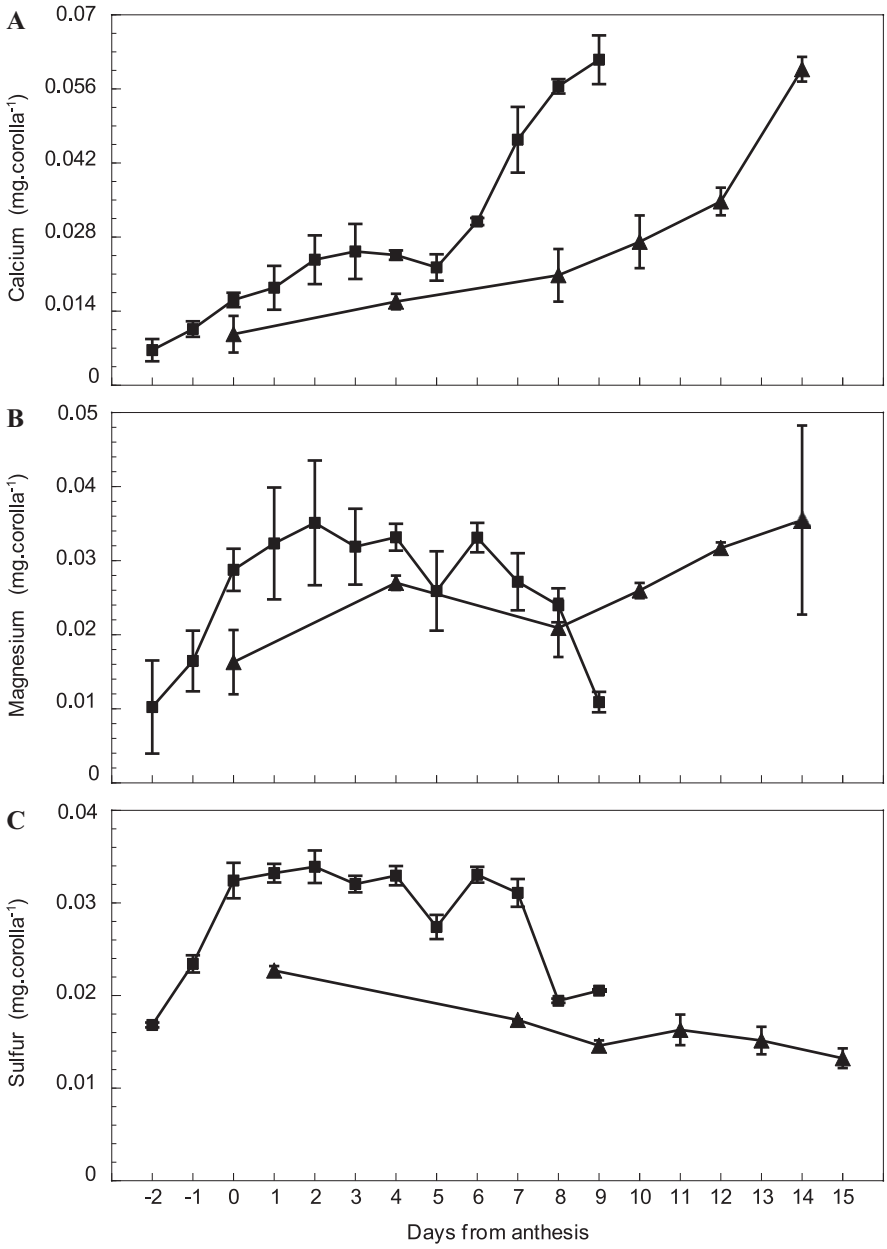

Fig. 3. Changes in calcium (**A**), magnesium (**B**), and sulfur (**C**) content of corollas during flower development and senescence of MD (■) and CaMV35S*etr1-1* (▲). Error bars show the standard error of the mean (n = 3).

2025; Nichols 1966; Shibuya 2018; Trivellini et al. 2011, 2015; Trupkin et al. 2019; Wu et al. 2017). Although valuable, the age-related changes in roses characterized flower development by growth and senescence phases but not a maintenance phase (Itzhaki et al. 1990). Trivellini et al. (2011) hinted at a maintenance phase in hibiscus by describing bud, open, and senescent flower stages of development. So did Ma et al. (2018) when they described cell division, cell expansion, and cell shrink/death stages of development. However, the necessity for the objective description of a maintenance phase independent of growing conditions during age-related senescence is supported by a widely accepted floral longevity model, which states that floral senescence should occur when the expected fitness gain per unit of floral maintenance investment diminishes to the point where it becomes more profitable to construct a new flower than to maintain an existing one (Ashman and Schoen 1994). The very small changes in dry weight and associated carbon content during the maintenance phase of corolla development are indicative of the fine balance between gains and losses in the corolla during this stage of development. The three developmental phases of corollas discussed here could also serve as the model for discussions of changes in styles and ovaries, because corollas are the best overall indicators of floral senescence due to their size, visible cues, and distinct ethylene climacteric.

Although the distinct phases we described have not been clearly defined before, work in monocarpic senescence has suggested an expansion, maturity, and senescence phase of leaf development (Gregersen et al. 2013). Senescence in this framework can show normal onset and normal kinetics of senescence or delayed onset with either normal or slow kinetics as observed in stay-green mutants or transgenic plants. Both transgenics in our study showed delayed senescence. However, SAG12-IPT petunias showed faster kinetics (fewer days in the senescence phase of development) than the corresponding wild-type V26 plants. CaMV35Setr1-1 plants, on the other hand, showed similar or normal kinetics in senescence as the senescence phase in the transgenics was similar in duration to the wild-type MD plants.

The significant changes in corolla fresh and dry weight we observed throughout flower development and senescence have previously and extensively been documented in petunia, hibiscus, orchid, day lily, and carnation (Borochov and Woodson 1989; Hew et al. 1989; Jones 2013; Rubinstein 2000; Trivellini et al. 2011; Verlinden 2003; Woltering 2017) and are considered to be universal changes associated with flower development and senescence (Borochov and Woodson 1989; Parveen et al. 2023; Rubinstein 2000; Woltering 2017). The initial increase in fresh weight during the growth phase is the result of a large volume of water and solutes, mainly carbon-based as seen in our study, moving into the expanding cells of the corolla. Interestingly, in CaMV35S*etr1-1*, an ethylene-insensitive petunia, the corollas take up less water during the growth phase than MD corollas while reaching similar dry weights at the beginning of the maintenance phase of development, suggesting a possible involvement of ethylene in corolla expansion. Although we did not measure cell size or corolla size, these observations are similar to those by Fomenkov et al. (2014), in a study in which they concluded that ethylene perception is needed for cell expansion. They concluded that cell size was significantly reduced in *Arabidopsis* etr1-1 mutants. Indeed, involvement of ethylene in rapid expansion of certain plant organs and tissues has been shown in number of plants (Dolan 1997; Fiorani et al. 2002; Kende 1993; Smalle et al. 1997) and has been specifically implicated in patterns of cell expansion in *Petunia* (Reale et al. 2002). The transgenic CaMVS35*etr1-1* corollas show limited changes in fresh weight but exhibit a rapid decrease in dry weight at about the same time as Mitchell corollas entered the senescence phase of corolla development. This may indicate that the respiratory climacteric, although not measured in this study but often observed concomitant with the ethylene climacteric, may be a separate event and can be uncoupled from ethylene production as has been observed in climacteric fruit (Bower

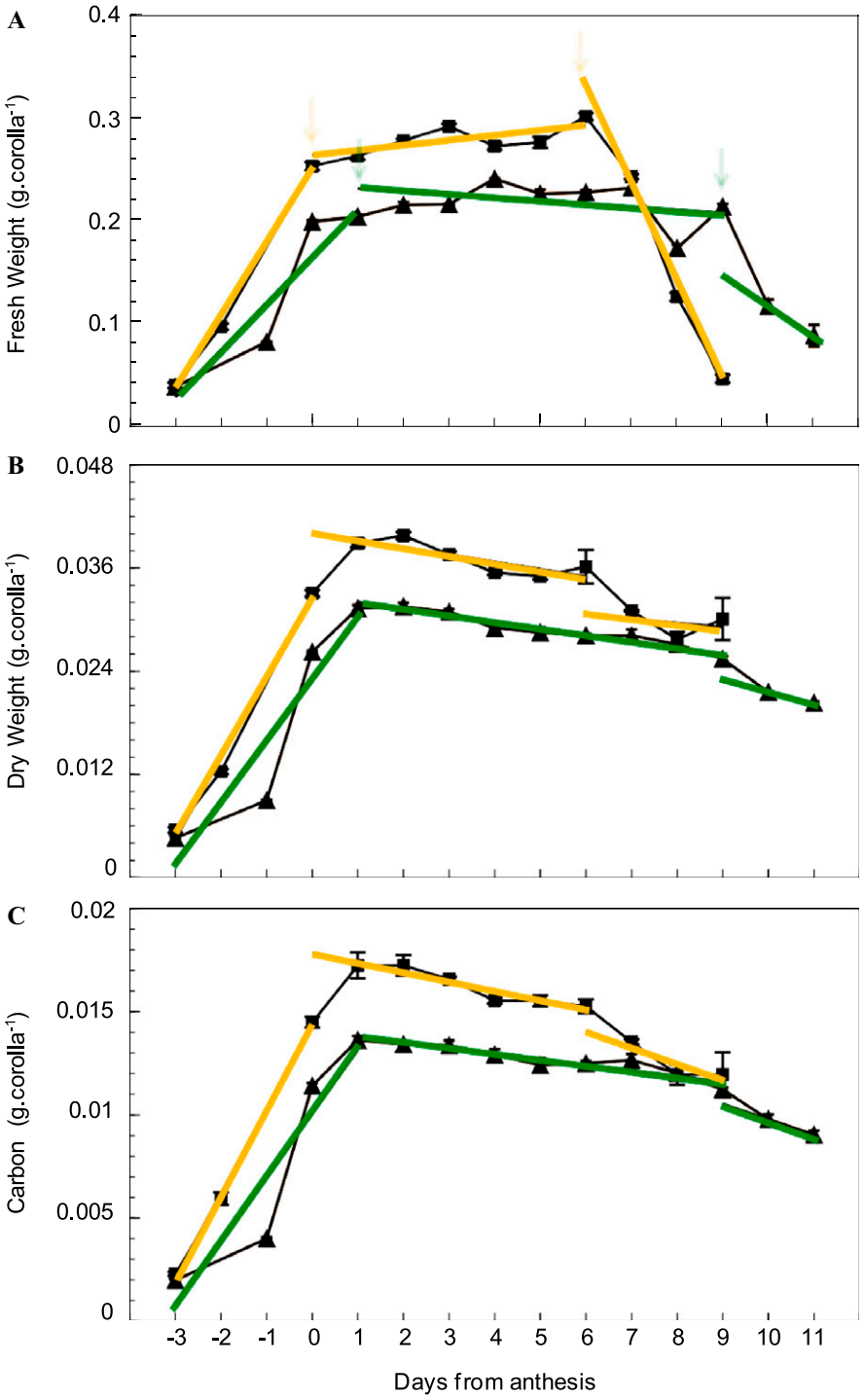

Fig. 4. Changes in fresh weight (**A**), dry weight (**B**), and carbon (**C**) content of corollas during flower development and senescence of V26 (■) and SAG12-IPT (▲). Error bars show the standard error of the mean (n = 3). Regression lines fitted to V26 data are in yellow, whereas the regression lines fitted to SAG12-IPT are shown in green. Arrows indicate inflection points between the growth, maintenance, and senescence stages of development.

et al. 2002; Shellie and Saltveit 1993). Alternatively, remobilization of carbon may be independent of ethylene. Our data, although indirectly, suggest that developmental factors in addition to ethylene may play a role in activating the respiratory climacteric or remobilization of carbon. However, a small and almost indistinguishable climacteric has been observed at the time of significant carbon losses in corolla development in the transgenic plants (Jones M, personal communication). Therefore, the small amount of ethylene still produced and possibly perceived by these corollas may trigger respiration or carbon remobilization but not the complete senescence program. Carbon losses in MD, CaMV35S*etr11*, and SAG12-IPT corollas occur at slightly lower rates than dry weight losses during the corolla maintenance and senescence phases of development (Table 1), a pattern previously demonstrated in MD. This observation is thought to be indicative of limited remobilization of carbon during the maintenance phase and the senescence phase of development when compared with other nutrients (Jones 2013; Verlinden 2003).

Nitrogen and phosphorus are highly mobile elements (Marschner 1995). Studies have shown movement of these elements from flower tissue of orchid, hibiscus, and petunia (Hew et al. 1989; Jones 2013; Trevillini 2011; Verlinden 2003) and from leaf tissue of soybean and *Arabidopsis* (Himelblau and Amasino 2001; Mauk and Noodén 1992). In this study, nitrogen is remobilized from corollas in V26, SAG12-IPT, MD, and CaMV35S*etr1-1* mostly during the senescence phase of development, although gradual changes can be observed during the maintenance phase of corolla development as well. In CaMV35S*etr1-1*, the decline in nitrogen content during the senescence phase is much less dramatic, indicating that ethylene production and perception is necessary for significant remobilization of nitrogen (Jones 2013). Phosphorus content, while showing clear patterns of remobilization in V26, SAG12-IPT, and MD, increased in CaMV35S*etr1-1* through senescence, again indicating a role for ethylene in remobilization of nutrients from the corolla. The extended flower longevity of SAG12-IPT corollas seems to be related to its ability to maintain carbon, nitrogen, and phosphorus content, most likely the result of the higher levels of cytokinins in the transgenic corollas and therefore a sustained ability to act as a sink, as observed in leaves (Smart 1994). Interestingly, feedback between cytokinin content and ethylene signaling has been established in roses, indicating that an interplay between plant growth hormones is integral to flower development and senescence (Khaskheli et al. 2018; Lone et al. 2025; Shibuya 2018).

Magnesium is considered to have low mobility in the phloem and is not remobilized from leaves of peas (Marschner 1995) and *Arabidopsis* (Himelblau and Amasino 2001). In contrast, magnesium remobilization from the leaves of soybean and wheat has been shown (Hocking 1994; Mauk and Noodén 1992). Jones (2013) showed a slight reduction in magnesium content in pollinated vs. unpollinated Petunia corollas. Here, we show a similar result for a developmental sequence of corollas of MD. Magnesium accumulated during growth of the corollas and was remobilized during the senescence phase. However, this was not the case for V26, SAG12-IPT, and CaMV35S*etr1-1* (Figs. 3B and 6B), echoing conflicting reports of magnesium mobility in leaves (Himelblau and Amasino 2001; Hocking 1994; Marschner 1995; Mauk and Noodén 1992). Magnesium was shown to accumulated in hibiscus petals during flower

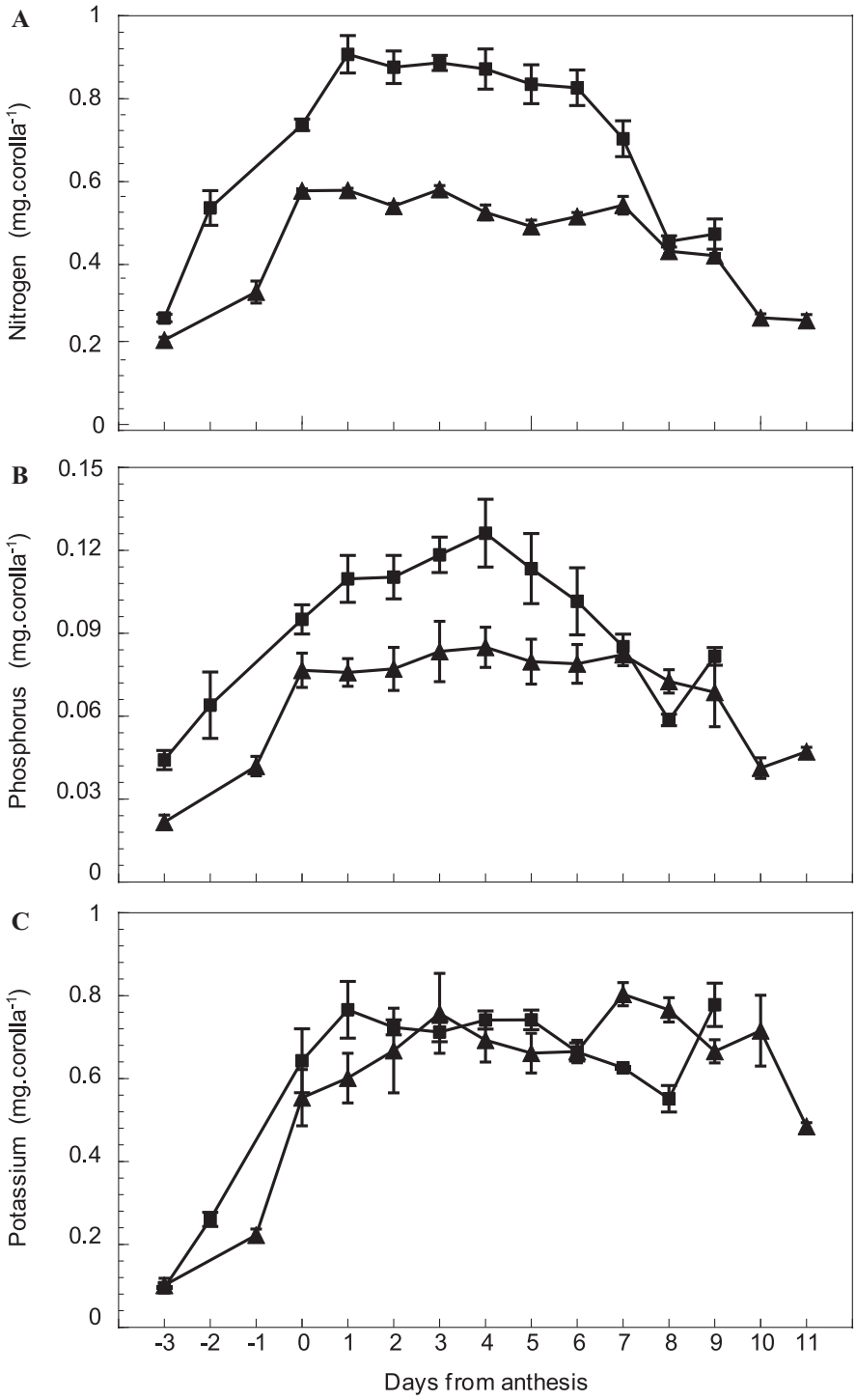

Fig. 5. Changes in nitrogen (**A**), phosphorus (**B**), and potassium (**C**) content of corollas during flower development and senescence of V26 (■) and SAG12-IPT (▲). Error bars show the standard error of the mean (n = 3).

expansion and decreased in concentration from open to senescent petal stage of development (Trivellini et al. 2011). Therefore, magnesium mobility appears to be tissue, species, and even cultivar dependent and may be influenced by ethylene and/or cytokinin production and perception.

Calcium is considered to be immobile in the phloem (Bukovac and Wittwer 1957), as shown in the senescing leaves of wheat and pea (Hocking 1994; Marschner 1995). Despite its apparent immobility, calcium was shown to remobilize from soybean leaves (Mauk and Noodén 1992). However, in contrast to our observations of magnesium mobility in MD, no remobilization was observed for calcium in any of the four *Petunia* corollas tested in this study. Work in hibiscus and on pollinated vs. unpollinated and attached vs. detached corollas of petunia confirmed this observation (Jones 2013; Trivellini et al. 2011). We did, however, show that calcium accumulated gradually from opening bud through senescence, most likely due to passive movement in the transpiration stream to and out of the corollas.

Interestingly, MD corollas exhibit a consistent decrease in fresh weight, dry weight, N, P, K, Mg, and S between days 4 and 6, immediately preceding the senescence phase (Figs. 1, 3, and 5). A similar temporary decrease can be seen in the styles of MD, also occurring between days 4 and 6 in fresh weight, dry weight, carbon, and nitrogen, although the decline is less pronounced compared with corollas (data not shown). The meaning of this temporary decrease is unclear and has not been suggested in previous studies. However, upon closer examination, this decrease, although much less pronounced, can be seen in one previous study (Verlinden 2003) and also in V26 fresh and dry weight. Senescence is "organized disorganization" (Solomos 1988), and perhaps to remobilize materials out (efflux) of senescing tissue, there has to be a movement of materials into (influx) the tissue. Although senescence is a degradative process, increases in protein synthesis and enzyme synthesis (Winkenbach and Matile 1970) and up-regulation of senescence–related genes are necessary for petal senescence (Breeze et al. 2004; Ma et al. 2018; Shibuya 2018; Trivellini et al. 2015; Wang et al. 2013; Woodson 1994; Woodson et al. 1992). It has also been suggested that the climacteric respiratory burst is necessary to produce ATP adequate for sustaining these processes (Solomos 1988), possibly creating a temporary influx of a number of different compounds. It should be noted that our study used attached flowers, and most studies on changes during flower development and senescence are carried out on cut flowers and therefore may show different patterns of development and senescence.

In conclusion, we have shown that petunia corolla development consists of growth, maintenance, and senescence phases of development. An influx of nutrients was observed at the end of the maintenance phase of corolla development that may indicate a heightened and temporary increase in metabolic rates. Ethylene was shown to play a role not only in remobilization of nutrients during the senescence phase but also during the growth phase of development. Increased flower longevity in senescence-associated cytokinin overproducing corollas was shown to be the result of a prolonged maintenance phase of development.

However, the overall results indicate that, when the absolute levels and the rate of changes in nutrient content of the four petunia varieties are compared, no apparent relationship can be established with flower longevity. Future studies could emphasize improving processes that sustain the maintenance

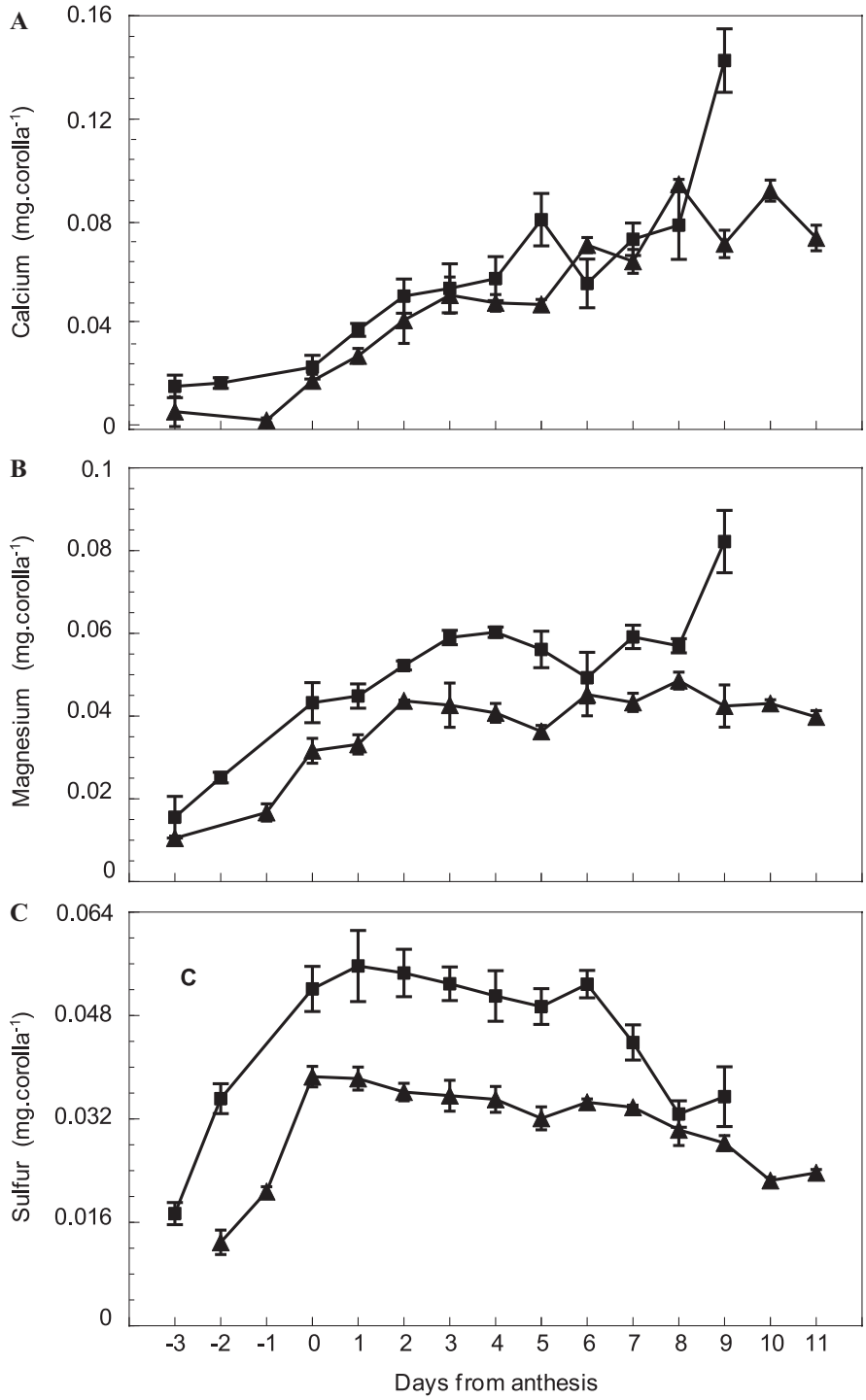

Fig. 6. Changes in calcium (**A**), magnesium (**B**), and sulfur (**C**) content of corollas during flower development and senescence of V26 (■) and SAG12-IPT (▲). Error bars show the standard error of the mean (n = 3).

phase of development instead of inhibiting the senescence phase of development.

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