South African Journal of Botany 87 (2013) 39–47

Contents lists available at SciVerse ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

# Ethylene- and dark-induced flower abscission in potted *Plectranthus*: Sensitivity, prevention by 1-MCP, and expression of ethylene biosynthetic genes

L.J. Rice [a], V. Soós [b], G.D. Ascough [a], E. Balázs [a,b], V. Ördög [a,c], C.S. Whitehead [d], J.F. Finnie [a], J. Van Staden [a,*]

[a] Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa
[b] Department of Applied Genomics, Agricultural Research Institute of the Hungarian Academy of Sciences, H-2462 Martonvársár, Brunszvik u. 2, Hungary
[c] Institute of Plant Biology, Faculty of Agriculture and Food Sciences, University of West Hungary, H-9200 Mosonmagyaróvár, Hungary
[d] Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, South Africa

ARTICLE INFO

*Article history:*
Received 6 December 2012
Received in revised form 13 March 2013
Accepted 13 March 2013
Available online 13 April 2013

Edited by MI Daws

*Keywords:*
ACC oxidase
ACC synthase
Potted *Plectranthus*

ABSTRACT

Prevention of ethylene- and shipping-induced flower abscission is necessary to maintain the quality of both cut flowers and potted plants during handling, transport and retail display. The aims of the present work were to determine the sensitivity of *Plectranthus* cultivars to applied ethylene, to alleviate ethylene- and shipping-induced flower abscission in intact potted plants using 1-methylcyclopropene (1-MCP), and to investigate the possible causes of dark-induced flower abscission. All cultivars were sensitive to ethylene in a concentration-dependent manner, and complete abscission occurred within 24 h with 1 and 2 μl $l^{-1}$ ethylene. Unopened buds were more sensitive to applied ethylene, and exhibited greater abscission than open flowers. Ethylene synthesis remained below detection limits at all time points under control and continuous dark conditions. Dark treatment significantly increased flower abscission in *Plectranthus* cultivars, and like ethylene-induced flower abscission, this could be prevented by continuous 1-MCP treatment. Gene expression of ethylene biosynthetic enzymes ACS and ACO was examined as possible causes for the accelerated flower abscission observed in plants kept in continuous darkness. Expression patterns of ACS and ACO varied between different cultivars of *Plectranthus*. In some cases, increased expression of ACS and ACO led to increased flower abscission. Gene expression was higher in open flowers when compared to unopened flowers suggesting a cause for the observed preferential shedding of open flowers in some cultivars. Although the cause of dark-induced abscission in *Plectranthus* remains elusive, it can be effectively controlled by treatment with 1-MCP.



## 1. Introduction

Abscission is the natural separation of organs from the parent plant (Patterson, 2001). In the natural environment it is an important process that enables plant survival. However, in agriculture and horticulture, abscission, particularly premature abscission, can lead to both crop and profit losses (Taylor and Whitelaw, 2001). This loss of quality is often attributed to the detrimental effects of ethylene ($C_2H_4$), a simple hydrocarbon plant hormone necessary and responsible for regulating many plant processes and responses to environmental stimuli (Johnson and Ecker, 1998).

In general, ethylene synthesis by plant tissues is low but increases at certain developmental stages (Jones and Woodson, 1999). Ethylene is active at very low concentrations, less than 1 μl $l^{-1}$ (Pech et al., 2004) and with the exceptions of fruit ripening and lateral root initiation, ethylene at high concentrations can be detrimental (Czarny et al., 2006) and even inhibitory to plant growth (Pierik et al., 2006). Ethylene biosynthesis is increased in response to a number of biotic and abiotic stresses including wounding, chilling, flooding, pathogen attack (Chang et al., 1993; Deikman, 1997), hypoxia (Wang et al., 2002), darkness (Mutui et al. (2007), shipping (Serek et al. (1998) and drought (Deikman, 1997). Under these conditions senescence and abscission are accelerated (Chang et al., 1993).

Ethylene biosynthesis follows a series of processes that occur from the reception of a stimulus by a plant, tissue or organ to the production of ethylene. The pathway follows the sequence: methionine → S-AdoMet (SAM) → 1-aminocyclopropane-1-carboxylate (ACC) → ethylene (Adams and Yang, 1979). Two enzymes in the pathway, ACC synthase (ACS) and ACC oxidase (ACO), are involved in regulating ethylene synthesis (Kende and Zeevaart, 1997; Pech et al., 2004; Tian and Lu, 2006). Increased transcription of ACS and ACO genes leads to an increase in ethylene biosynthesis (Woodson et al., 1992).

ACS is encoded by a medium-sized multigene family (Kende and Zeevaart, 1997; Barry et al., 2000; Wang et al., 2002; Ma et al., 2006; Tian and Lu, 2006), the members of which are expressed differentially in response to developmental, environmental and hormonal

*Abbreviations:* 1-MCP, 1-methylcyclopropene; ACC, 1-aminocyclopropane-1-carboxylate; ACS, 1-aminocyclopropane-1-carboxylate synthase; ACO, 1-aminocyclopropane-1-carboxylate oxidase.

* Corresponding author. Tel.: +27 33 260 5130.
*E-mail address:* rcpgd@ukzn.ac.za (J. Van Staden).

0254-6299/$ – see front matter © 2013 SAAB. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.sajb.2013.03.011

cues (Kende and Zeevaart, 1997). This enzyme is responsible for the conversion of SAM to ACC (Pech et al., 2004), and its function of regulating ethylene biosynthesis is controlled at both transcription and post transcription (Mutui et al., 2007).

ACO is encoded by a small multigene family (Kende and Zeevaart, 1997; Ma et al., 2006), which is also expressed differentially in response to both developmental and environmental factors (Clark et al., 1997). ACO catalyses the final step in the synthesis of ethylene, converting ACC to ethylene (Abeles et al., 1992; Kende and Zeevaart, 1997; Johnson and Ecker, 1998). The activities of ACS and ACO are both rate-limiting steps for ethylene production (Kende and Zeevaart, 1997).

There are several means of protecting plants from the detrimental effects of ethylene. These include irreversible non-competitive inhibition by silver ions through treatment with silver thiosulfate (Veen, 1979), preventing ethylene biosynthesis (Dostal et al., 1991) and the use of cyclopropenes. The latter have become widespread since Serek et al. (1994) demonstrated the effectiveness of 1-MCP (1-methylcyclopropene) in protecting potted plants from the effects of exogenously applied ethylene. Other compounds that are used include 2,5-NBD (2,5-norbornadiene), trans-cyclooctene, DACP (diazocyclopentadiene) and 3,3-DMCP (3,3-dimethylcyclopropene) (Sisler and Serek, 1999). These compounds compete with ethylene for the active site on the receptor and thus block ethylene action. In some cases, continuous exposure to the compound is needed since dissociation from the receptor occurs rapidly (for example, 2,5-NBD and transcyclooctene), but for 1-MCP, plants may be protected for as long as 12 d (Sisler et al., 1996).

*Plectranthus* is a genus with great potential as a potted plant, however this potential is compromised by shipping-induced flower abscission (Rice et al., 2011) limiting the commercialization and export potential of *Plectranthus*. A *Plectranthus* breeding project has resulted in a number of new cultivars, many of which are polyploids. Müller et al. (1998) found that different cultivars of miniature potted roses displayed differences in ethylene sensitivity and it is unknown whether this will be true for *Plectranthus*. Our previous research on cut flowers of three *Plectranthus* cultivars (P 96 04 06, P 00 06 03 and P 01 05 09) demonstrated that flower abscission was promoted by high concentrations of endogenously applied (ethephon) and exogenously applied (gaseous) ethylene. This could be prevented by cycloheximide and silver thiosulfate, but only limited control was obtained by 1-MCP (Ascough et al., 2006). Additionally, exposure to long periods of darkness increased flower abscission in intact potted plants of two cultivars (P 00 06 03 and P 01 05 09) (Ascough et al., 2008). In this study we aim to 1) characterize ethylene sensitivity at the whole-plant level using a range of concentrations of exogenously applied ethylene, 2) prevent ethylene- and dark-induced flower abscission using 1-MCP, and 3) investigate possible causes of dark-induced flower abscission by examining the effect of continuous darkness on gene expression of ACS and ACO in the abscission zone of unopened and open flowers. Four cultivars were selected for this study, some of which were suspected polyploids, in order to determine if increased ploidy affected the flower abscission process in any way.

## 2. Materials and methods

### 2.1. Plant material

Four cultivars of *Plectranthus* (P 00 06 03A, P 00 06 03, P 04 05 18 and P 08 05 06) were used in different parts of this study. For ethylene evolution studies, P 00 06 03 was used as a model plant. The four cultivars are the results of crosses from first generation parents *Plectranthus hillardiae* and *Plectranthus saccatus*. P 00 06 03A is the smallest of the four cultivars and has pink flowers. P 00 06 03 and P 08 05 06 are larger than P 00 06 03A. P 00 06 03 has pink flowers and P 08 05 06 has purple flowers. P 04 05 18 has pink flowers and is the largest of the four cultivars. Cuttings were planted in potting mix comprising of 83% compost, 16.72% bark, 0.14% LAN (limestone ammonium nitrate) and 0.14% 2:3:2 N:P:K fertilizer and were kept in a greenhouse under natural day length at UKZN, Pietermaritzburg (29° 37′ 28.8″ S, 30° 24′ 13.6″ E). Plants were watered every second day with approximately 10 ml of water and fertilized with a seaweed concentrate (Kelpak®) every three weeks.

### 2.2. Flow cytometry

Young leaves were collected from plants from each of the four *Plectranthus* cultivars. Leaves were collected from more than one plant so as to get a sample representative of the cultivar. Collected leaf material was immediately put onto ice.

Each sample, comprising approximately 300 mg was cut finely with a razor blade in 1 ml of LBO1 extraction buffer, containing 15 mM TRIS, 2 mM $Na_2EDTA$, 0.5 mM spermine.4HCl, 80 mM KCl, 20 mM NaCl, 15 mM β-mercaptoethanol, 0.1% (v/v)Triton X-100 and the pH adjusted to 7.5 (Doležel et al., 1989). To prevent browning, 10 μl DTT and PVPP (insoluble) were added to the sample prior to cutting. The suspension was filtered through a 50 μm mesh filter and stained with 500 μl propidium iodide. Fluorescence was measured using a Beckman Coulter Epics XL-MCL flow cytometer and total DNA content was compared with control data from a variety of known ploidy level. The diploid peak from the control diploid plants was set to channel 200.

### 2.3. Ethylene sensitivity

Sensitivity of plants to ethylene was determined by exposing plants to 0, 0.1, 0.25, 0.5, 1.0, or 2.0 μl $l^{-1}$ ethylene (Air Products, Johannesburg) for 4 d. Individual plants were transferred from the greenhouse to the laboratory and the number of inflorescences and flowers were recorded. They were placed inside sealed Perspex chambers (small: 288 × 438 × 250 mm (ca. 31.5 l), medium: 138 × 538 × 300 mm (ca. 70.7 l); large: 488 × 636 × 350 mm (ca. 108.6 l)) at 21 ± 1 °C under a 16-h photoperiod illuminated by overhead fluorescent tubes providing 12.6 μmol $m^{-2}$ $s^{-1}$ radiant flux density. Plants were observed every 24 h for 4 d and the number of flowers that had abscised was recorded. Abscised flowers were categorized as being either open at the time of abscission, or unopened (i.e. buds). At this time, plants were removed from the chambers and the chambers cleaned and vented. Plants were then replaced and the chambers resealed. Ethylene was injected using a syringe into the Perspex chambers daily after venting.

### 2.4. Ethylene evolution

Ethylene evolution by flowers was measured by gas chromatography as described by Whitehead and Nelson (1992). Four to six flowers or buds from control and dark-treated plants were placed in small glass vials, sealed with a rubber stopper and aluminium cap and allowed to incubate at 25 °C (either in the light or the dark) for 1 h. Samples were taken every 24 h for 4 d. Ethylene evolution was calculated as μl $g^{-1}$ $h^{-1}$.

### 2.5. Dark-induced flower abscission

Individual plants with at least three inflorescences were transferred from the greenhouse to the laboratory and the number of inflorescences and flowers recorded. At the start of the experiment approximately 20–25% of flowers on a plant were open, the rest were unopened. Flowers open sequentially from the base of the inflorescence upwards. Plants were placed inside sealed (gas-tight) impermeable Perspex chambers at 21 ± 2 °C and kept in darkness. Control plants were kept under a 16-h photoperiod illuminated by overhead fluorescent tubes providing 12.6 μmol $m^{-2}$ $s^{-1}$ radiant flux density. Plants were observed every 24 h for 4 d and the number of open and unopened flowers that had abscised recorded.

Total abscission was calculated as the cumulative number of (open + unopened) flowers abscised over 96 h, divided by the total number of flowers at the start. When dissecting this total abscission into open and unopened, care was taken to include in the calculation the number of unopened flowers that had opened during the 96 h (percentage conversion). Thus, the percentage of 'open' flowers that had abscised was calculated as the number of open flowers abscised divided by the (number of open flowers at the start + the number of unopened flowers that had opened). This explains why in some cases the sum of the flower abscission for open and unopened flowers is greater than the total abscission (Table 1).

### 2.6. 1-MCP treatment

To assess the effect of 1-MCP pre-treatment on ethylene- and dark-induced flower abscission plants in sealed chambers were exposed to 100 nl $l^{-1}$ 1-MCP either for a single 6 h pretreatment (1-MCP-eth1) (Serek et al., 1995), or continuously (1-MCP-eth2). This was performed by dissolving the desired amount of 1-MCP powder in 2 ml distilled water in a sealed pill vial. Once dissolved, the solution was poured onto filter paper discs within the Perspex chambers. The pill vial was left inside the chamber. Chambers were sealed immediately after 1-MCP was released. Following this pre-treatment, chambers were vented, resealed, and left as a control, placed in the dark, or ethylene (2 μl $l^{-1}$) was added. In one experiment, plants were completely defoliated by removing leaves with sharp scissors to determine the effects of reduced assimilate supply to the inflorescence. Flower abscission was recorded in the light every 24 h.

### 2.7. Cloning of ACC oxidase, ACC synthase and actin genes

For degenerate primer design, the corresponding Lamiales ACC synthase, ACC oxidase and actin sequences were collected, aligned with ClustalW and conserved and unique motifs used to design primer pairs manually. The following degenerate primers were used for cloning the corresponding sequences: ACOF: GDATTDCKSCHGAGTTTATGG and ACOR: TCGAAGTARGTCAGHAGRTTHTG; ACSF: GCDMTVTTTCAAGAYT ATCATG and ACSR: GARAYBARVCCRAARCTCGACAT; ACTINF: GAAAT YGTDAGGGACATCAAAGAGAAG and ACTINR: GCCACSACCTTRATCTTC ATGCTGC. Total RNA was extracted from leaf material from *Plectranthus* using the QIAGEN RNeasy Plant Mini Kit and TRIzol® (Invitrogen) and the samples were treated with DNase I (Invitrogen). cDNA was synthesized from the RNA using Oligo(dt) primers (Fermentas) and the RevertAid™ kit (Fermentas) in a GeneAmp® PCR System 9700 (Applied Biosystems). The cDNA was used as the template in a 3 step PCR using the Advantage® 2 enzyme (Clontech) and the degenerate primers. Samples were held at 72 °C for 3 min over 7 cycles. Step 2 was 30 s at 48 °C followed by 2 min at 72 °C over 35 cycles. Step 3 held the reaction at 67 °C for 7 min. The products of the PCR were run on a 1% agarose gel containing ethidium bromide. The bands viewed on the gel were excised and the fragments were extracted using the Agarose Gelextract Mini Kit (5′ PRIME). As the Advantage® 2 enzyme used in the PCR has a 5′ proof reading function which removes adenine from the DNA, an A overhang was added to the DNA strands using Taq Polymerase (Fermentas). DNA fragments were then ligated into pGEM T vectors (Promega) and then cloned into JM 109 competent cells (Promega).

The cloned cells were plated on LB plates supplemented with ampicillin, X-gal and IPTG and incubated overnight at 37 °C. White colonies were selected and inoculated into 2 ml of liquid LB broth. These cultures were incubated overnight on a shaker at 200 rpm at 37 °C. The DNA was extracted from overnight cultures using the QIAprep Spin Miniprep Kit (QIAGEN). The extracted DNA was sent for sequencing (ABI 3100, Applied Biosystems). From the sequences, specific primers for the genes of interest were designed by Primer3. The results of the ligation and cloning were checked by performing a restriction digest on the product of the miniprep using PstI and NcoI restriction enzymes. The products of the restriction digest were run on a gel and the banding patterns showed that the ligation and the cloning had been successful. Actin and ACO isolation resulted in one clone being detected, while ACS isolation yielded two clones, ACS2 and ACS5. ACS5 transcript abundance was not detectable during the pilot studies so we focused on ACS2. The sequences have been deposited in the GenBank under the following accession numbers: actin (JQ060954), ACO (JQ060955), ACS5 (JQ060956) and ACS2 (JQ060957).

### 2.8. Real-time PCR

For the real-time PCR gene expression assay, sequence specific primers were designed using the gene sequences cloned (ACOF: AGGCTGACCAAGGAGCACTA, ACOR: GAAAGTGCTTTCCCAATCCA; ACSF: CCCGACCCTAAAGCCGGGGA, ACSR: TACGCTGCCACGGTGTTCCG; ACTF: GTTCCAGCCATCGATGATCG, ACTR: CCCACCGCTCAGCACGATGT). Total RNA was extracted from the pedicles of open and unopened flowers from plants kept in the greenhouse, where they were exposed to light every day, and from plants which were kept in the dark for 24, 48, 72 and 96 h. RNA extraction and the reverse transcription procedures were the same as described above. Real-time PCR was performed with Applied Biosystems 7900 Fast System using SYBR Green detection chemistry (Applied Biosystems) and gene-specific primers. Real-time PCR data were obtained from two independent biological replicates and the reactions were performed in quadruplicate. The relative ratio of threshold cycle (Ct) values between the endogenous control and the specific gene were calculated for each sample and the expression level was normalized against the abundance of the actin transcript.

### 2.9. Statistical analysis

For statistical analysis, data were arcsine transformed and an analysis of variance (ANOVA) was carried out. Data were analysed using a Duncan's test at the 5% level in GenStat, 14th edition.

## 3. Results

### 3.1. Flow cytometry

Flow cytometry histograms (Fig. 1) show that P 00 06 03 is a diploid (peak at channel 200), while P 04 05 18 and P 08 05 06 are tetraploid (peak near channel 400). P 00 06 03A contained both 2n and 4n

**Table 1**
Flower abscission summary of four *Plectranthus* cultivars: effect of continuous darkness on total, open and unopened flower abscission (as a percentage of those available for abscission) and the conversion of flowers from unopened to open state (percentage of buds that opened during the 96 h treatment). All values are means ± se and represent cumulative abscission at 96 h. * denotes a significant difference ($P < 0.05$) from the control. Values in bold denote significant ($P < 0.05$) preferential abscission (open flowers in P 00 06 03 and unopened flowers in P 08 05 06).

| | Total | | Open | | Unopened | | Conversions | |
|---|---|---|---|---|---|---|---|---|
| | Control | Dark | Control | Dark | Control | Dark | Control | Dark |
| P 00 06 03A | 5.2 ± 3.5 | 19.5 ± 4.6* | 4.7 ± 2.5 | 31.8 ± 6.3* | 5.9 ± 5.0 | 15.9 ± 3.7 | 52.9 ± 17.0 | 37.5 ± 11.7 |
| P 00 06 03 | 9.9 ± 2.5 | 21.6 ± 3.8* | **17.7 ± 3.9** | **36.5 ± 6.1*** | 1.3 ± 0.6 | 2.2 ± 1.2 | 36.8 ± 7.9 | 34.0 ± 4.0 |
| P 04 05 18 | 10.4 ± 3.4 | 19.5 ± 2.5* | 7.6 ± 2.4 | 21.9 ± 3.5* | 10.0 ± 3.9 | 16.9 ± 4.2 | 43.2 ± 16.0 | 35.9 ± 5.9 |
| P 08 05 06 | 16.2 ± 6.2 | 41.5 ± 9.4* | 7.4 ± 4.4 | 19.7 ± 3.5* | **24.8 ± 9.3** | **75.0 ± 17.9*** | 29.4 ± 13.8 | 43.0 ± 13.3 |

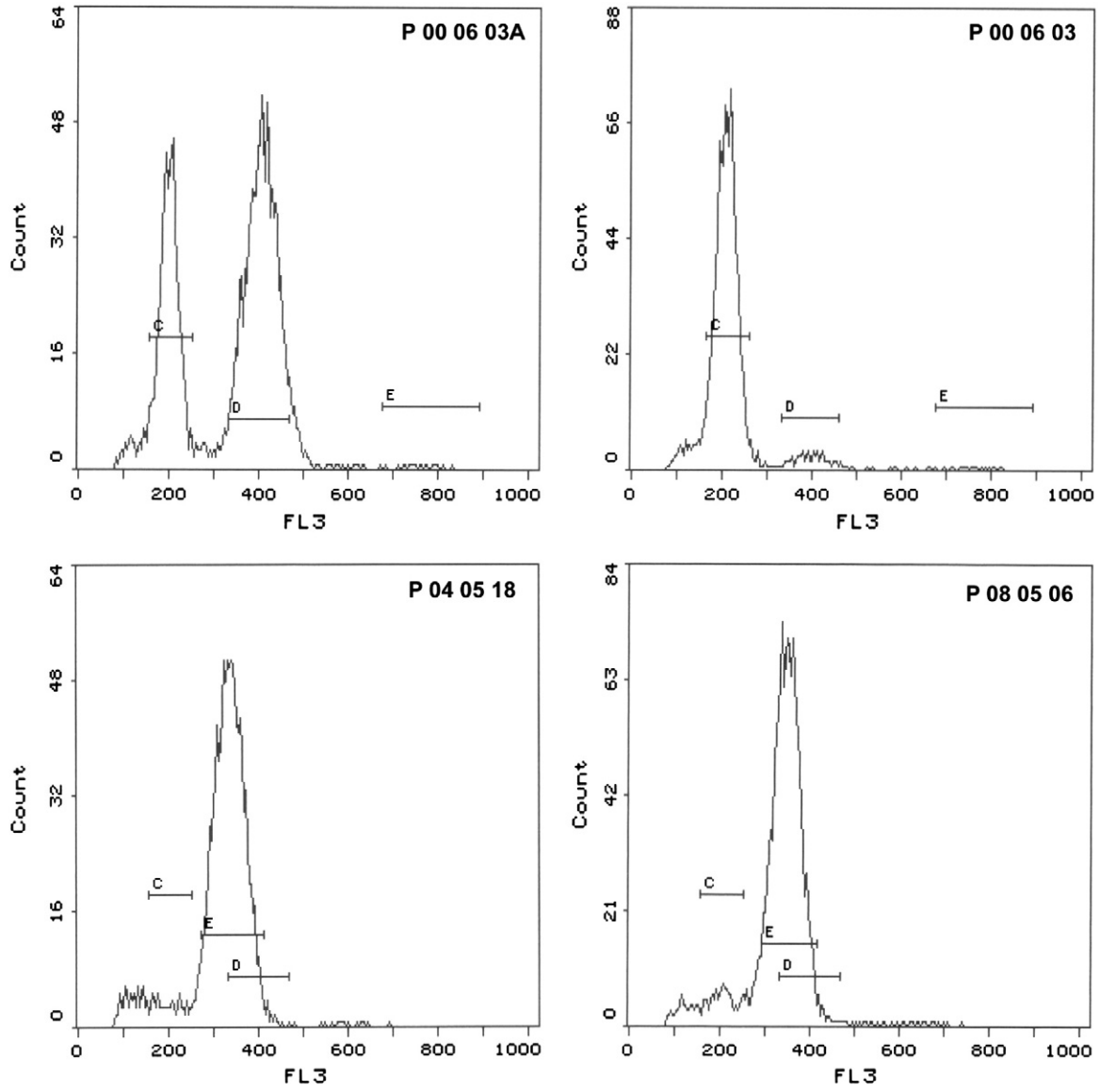


**Fig. 1.** Flow cytometry histograms of four *Plectranthus* cultivars, P 00 06 03A, P 00 06 03, P 04 05 18 and P 08 05 06. The diploid peak was set at channel 200.

nuclei, suggesting a diploid plant with endoreduplication (Smulders et al., 1994). Although each cultivar had individual responses to various ethylene concentrations, continuous darkness, 1-MCP treatment and gene expression of biosynthetic enzymes, specific differences could not be attributed to ploidy level alone. This suggests that an increase in gene dosage in tetraploid cultivars does not appear to play a significant deterministic role in the flower abscission process.

### 3.2. Ethylene sensitivity

All cultivars were sensitive to ethylene, with complete abscission occurring within 24 h when applied at 1.0 or 2.0 μl $l^{-1}$ (Fig. 2). Final abscission and rate of abscission decreased in a concentration-dependent manner. P 00 06 03A, P 04 05 18 and P 08 05 06 showed similar patterns of ethylene sensitivity. P 00 06 03 was more sensitive to ethylene than the other cultivars as there was significantly (71% ± 4.3 at 96 h) more flower abscission in this cultivar when treated with 0.1 μl $l^{-1}$ ethylene compared to the other three cultivars (23% ± 5.1; 17% ± 8.02; 17% ± 5.9 at 96 h). In all cultivars at low ethylene concentrations (0.1 and 0.25 μl $l^{-1}$), unopened buds were preferentially shed compared to open flowers (which were retained; data not shown).

### 3.3. Effect of continuous darkness on flower abscission

Continuous darkness significantly increased total flower abscission after 96 h in all four cultivars, with the effect being greatest in P 08 05 06 (Table 1). Similarly, abscission of open flowers (as a proportion of those available for abscission taking into account conversion), was significantly increased in all cultivars when exposed to continuous darkness. When considering unopened flowers (as a proportion of those available for abscission taking into account conversions) however, flower abscission was only significantly increased by darkness in P 08 05 06. In all cultivars, between 30 and 50% of all unopened flowers did open during the 96 h treatment, but no significant differences between plants under control or continuous dark conditions were observed (Table 1). Of particular interest, a significant preferential shedding of open flowers by P 00 06 03 and unopened flowers by P 08 05 06 in both control and dark conditions was noted.

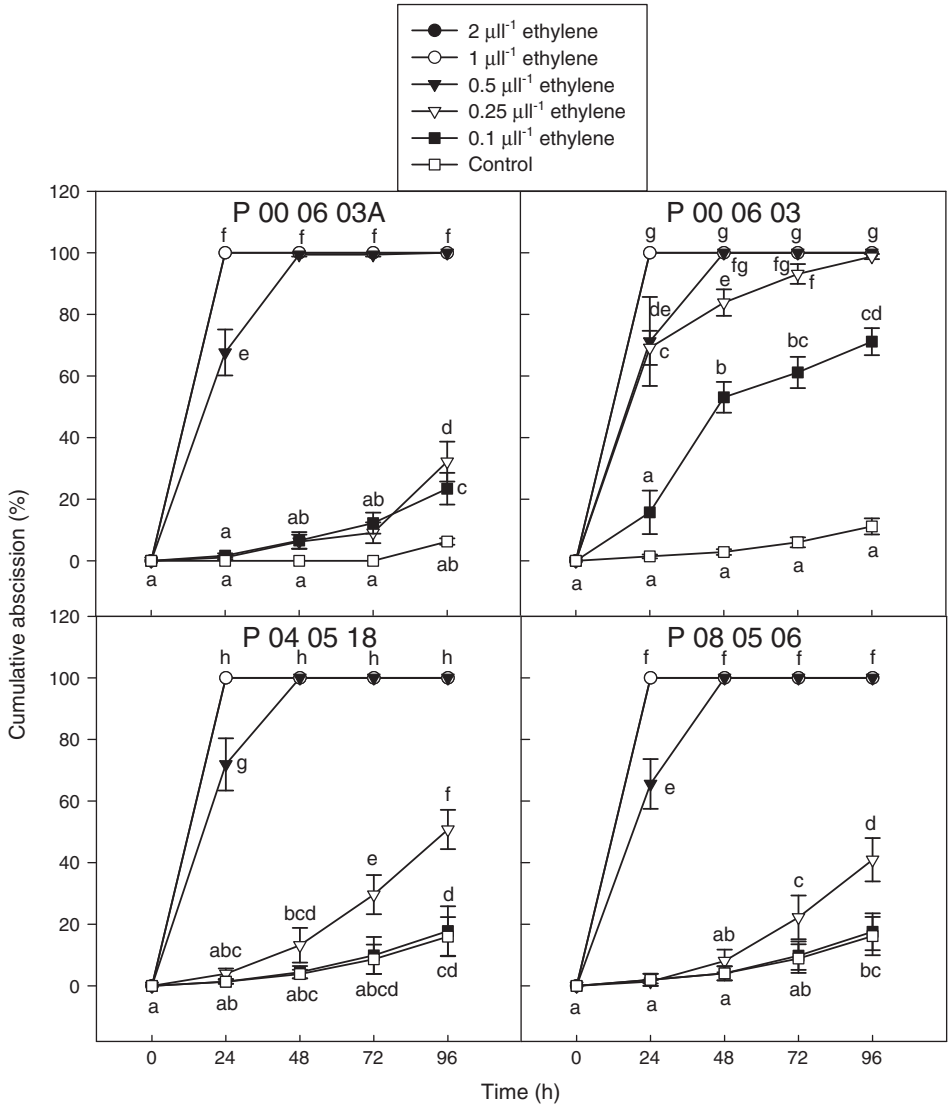


**Fig. 2.** Ethylene sensitivity of four *Plectranthus* cultivars in response to different concentrations of ethylene. Different letters indicate significant differences between treatments at a 5% level (ANOVA).

### 3.4. Ethylene evolution

No ethylene was detected in flowers or buds of control or continuous dark-treated plants at any time point. If ethylene was produced, it remained below the limit of detection (10 nl $l^{-1}$).

### 3.5. 1-MCP treatment

When pre-treated with 100 nl $l^{-1}$ 1-MCP for a single 6 h treatment, and subsequently exposed to 2 µl $l^{-1}$ ethylene (MCP-eth1), 100% of flowers from all cultivars abscised in 96 h (Fig. 3). This 6 h pre-treatment prevented flower abscission for the first 24 h and slowed the rate of flower abscission, but could not protect plants for the 96 h duration. However, continuous treatment with 1-MCP (MCP-eth2) reduced ethylene- and dark-induced flower abscission significantly in all four cultivars.

### 3.6. ACS and ACO expression levels

Transcript abundance of the ethylene biosynthetic enzymes ACS and ACO were investigated to determine if continuous darkness accelerated flower abscission through endogenous biosynthesis of ethylene. Open flowers of P 00 06 03A and P 00 06 03 had increased levels of ACS mRNA when put into the dark (Fig. 4). ACS mRNA levels remained high in open flowers while plants were kept in the dark. Unopened flowers of P 00 06 03A did not initially show a significant change in ACS mRNA levels when kept in the dark (Fig. 4), however the ACS mRNA levels were significantly higher in plants kept in the dark for 96 h than in plants kept in the light. Unopened flowers from P 00 06 03 did not show significant changes in ACS mRNA levels when put into the dark (Fig. 4).

Neither open nor unopened flowers from P 04 05 18 showed any significant change in ACS mRNA levels when put into the dark for up to 96 h (Fig. 4). Open flowers on P 08 05 06 did not initially show any change in ACS mRNA levels when put into the dark (Fig. 4). However, ACS mRNA levels were significantly lower at 48 h and 96 h dark exposure and lowest when plants were kept in the dark for 72 h. Unopened flowers in this cultivar showed an opposite trend. ACS mRNA levels increased when plants were placed in the dark for 24 h and continued to increase when plants were exposed to darkness for 48 h. ACS mRNA levels then decreased, to similar levels as in the control plants, after 72 h and 96 h in the dark.

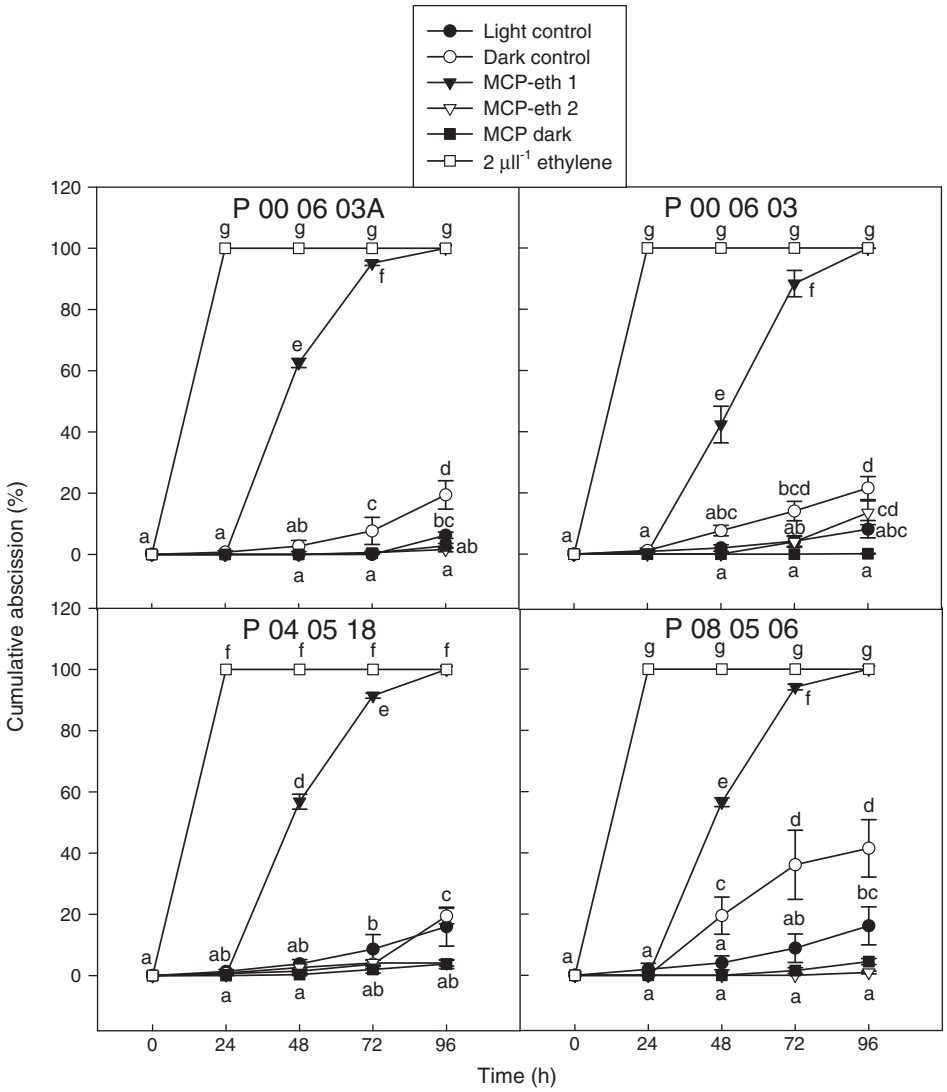


**Fig. 3.** The effect of 1-MCP treatments on *Plectranthus* flower abscission in the light and in the dark. Different letters indicate significant differences between treatments at a 5% level (ANOVA).

Open flowers on P 00 06 03A showed a considerable increase in ACO mRNA levels when plants were kept in the dark compared with those in the light (Fig. 4). Unopened flowers did not show significant changes in ACO mRNA levels when plants were put into the dark. Neither open nor unopened flowers from P 00 06 03 and P 04 05 18 showed significant changes in ACO mRNA levels when plants were placed in the dark (Fig. 4). Open flowers from P 08 05 06 did not show a significant change in ACO mRNA levels when kept in the dark for 24 h, 48 h and 72 h (Fig. 4). There was, however, a notable decrease in ACO mRNA levels of open flowers once plants had been exposed to darkness for 96 h.

## 4. Discussion

Flower abscission in all four cultivars of *Plectranthus* was promoted by applied ethylene (Fig. 2). In all cultivars, complete abscission occurred within 24 h if treated with 1.0 or 2.0 µl $l^{-1}$ ethylene. This places *Plectranthus* in the most sensitive category of pot plants with *Clerodendron thomsonii* and *Browallia speciosa* (Woltering, 1987). At the lowest concentration tested, 0.1 µl $l^{-1}$, abscission in all cultivars was significantly lower at all time points compared to higher levels of ethylene treatment. At this ethylene concentration, unopened flowers were preferentially abscised, suggesting that open and unopened flowers differ in their sensitivity to abscission-promoting signals, as was observed in *Phlox paniculata* (Porat et al., 1995) and *Dendrobium* (Uthaichay et al., 2007). Cultivar P 00 06 03 was most sensitive to ethylene. Similarly, in other species considerable variation has been observed with respect to cultivar sensitivity (Macnish et al., 2004).

No ethylene was detected by gas chromatography under control or continuous dark conditions. If ethylene was produced it may have been below the limit of detection of the GC used in this study (10 nl $l^{-1}$). It is possible that other plant parts, (e.g. peduncles) contributed to ethylene production under dark conditions, since in this study, ethylene production was only measured in open and unopened flowers.

The rapid promotion of flower abscission by applied ethylene was only inhibited if plants were continuously treated with 1-MCP (Fig. 3). While a 6 h pre-treatment with 1-MCP before ethylene exposure delayed abscission, it did not reduce the total flower abscission after 96 h. This could result from additional plant growth during the experiment, sub-optimal 1-MCP concentration, or from a rapid turnover of the receptor in mature flowers. In these instances, receptors are free to bind ethylene and transduce the abscission signal. Since 1-MCP is

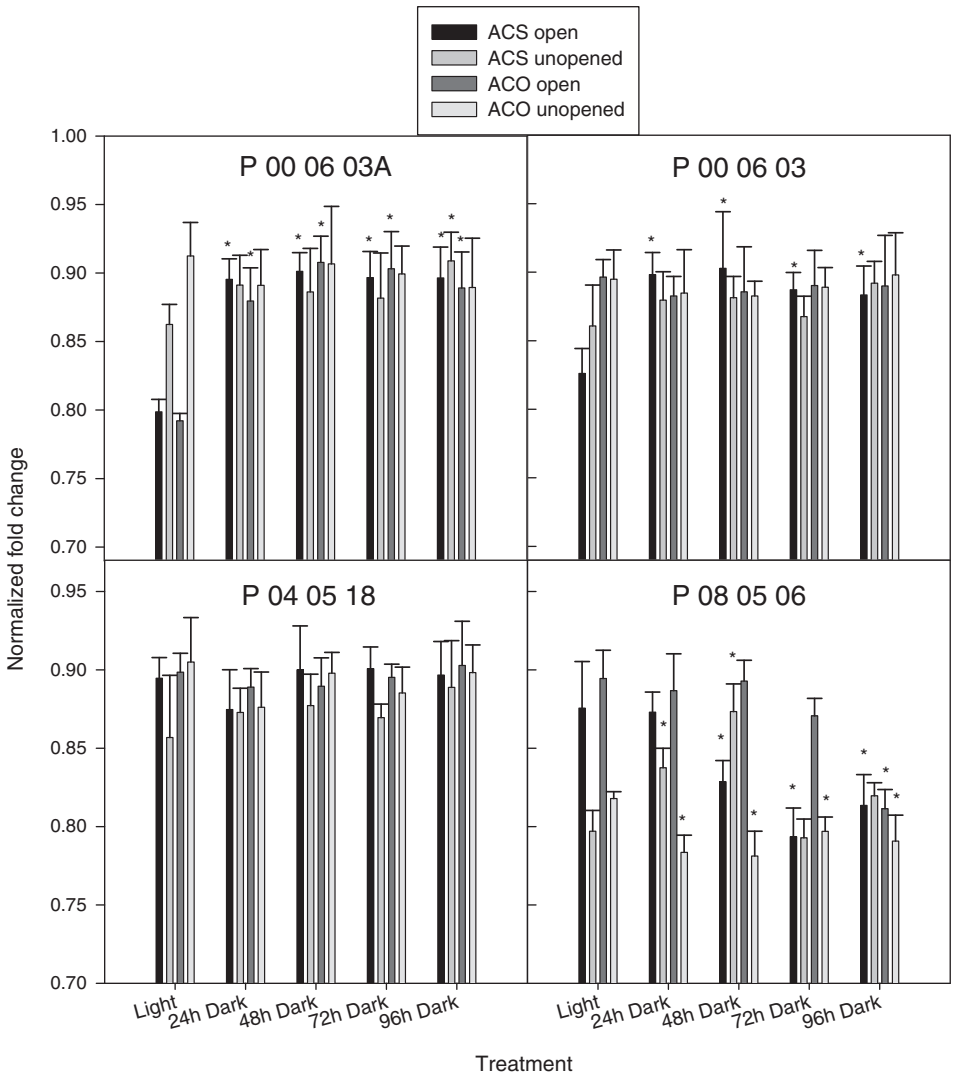


**Fig. 4.** ACS and ACO mRNA levels in pedicels of open and unopened *Plectranthus* flowers kept in 16 h light: 8 h dark, 24, 48, 72 and 96 h dark.* marks treatments which differ significantly from the light treatment.

a gas, it dissipates rapidly, and hence retreatment is required. While a soluble, systemic transportable inhibitor like STS would potentially be more effective by remaining in the plant and therefore binding new ethylene receptors as they are produced, STS was not considered in this study because of regulations regarding heavy metal use in agriculture and horticulture.

Prolonged periods of darkness (96 h) increased total flower abscission in all *Plectranthus* cultivars tested (Table 1). Abscission of open flowers was significantly increased by exposure to darkness in all cultivars, but only in P 08 05 06 was abscission of unopened flowers significantly increased. Continuously treating plants in the darkness with 1-MCP effectively controlled dark-induced flower abscission for 96 h. This implies that dark-induced flower abscission in *Plectranthus* is mediated by ethylene. However, flowers and buds of plants held in the dark for 96 h did not produce ethylene at detectable levels by gas chromatography as used in this study. Dark treatment caused increased ethylene production and increased the expression of two ACC synthase genes in *Pelargonium zonale* (Mutui et al., 2007), and it is possible that similar mechanisms operate in the dark-induced abscission of *Plectranthus*.

The two diploid cultivars (P 00 06 03A and P 00 06 03) showed similar trends in ACS mRNA levels when placed in the dark (Fig. 4). Increased ACS mRNA levels in open flowers indicate an increase in the transcription of the ACS genes and also an increase in the conversion of SAM to ACC, the first step towards increasing ethylene production. The synthesis of ACC synthase is induced by stress and a subsequent increase in the accumulation of ACC and a marked increase in stress ethylene production (Yang and Hoffman, 1984). These trends are consistent with studies on the effects of other stresses such as drought, chilling, freezing, wounding, high temperature and insect infestation on ethylene production (Czarny et al., 2006). Prolonged darkness would alter a plants physiology due to reduced carbohydrate production and altered phytochrome signalling. Since flowers are energetically "expensive" organs to produce and maintain, it may be beneficial to abscise flowers under stressed conditions ahead of other organs (for example leaves) which are necessary for plant survival.

Unopened flowers of P 00 06 03A and P 00 06 03 did not show a significant change in ACS mRNA levels and thus no notable change in abscission of unopened flowers occurred under dark conditions (Fig. 4). This may explain why P 00 06 03 preferentially sheds open flowers, and supports the hypothesis that flowers are shed when they become energetically expensive in comparison to opening a new flower (Ashman and Schoen, 1994).

Changes in ACO mRNA levels in P 00 06 03A (Fig. 4) are consistent with increased flower abscission of open flowers and retaining unopened flowers on the plant. The increase in ACS and ACO mRNA

levels (Fig. 4) indicate that the whole ethylene biosynthetic pathway has been upregulated when plants of this cultivar are placed in the dark. Increased ethylene production in senescing carnation flowers was accompanied by a significant increase in the abundance of mRNAs for ACC synthase and EFE (Woodson et al., 1992), or ACO.

ACS and ACO mRNA levels did not change significantly in open or unopened flowers of P 04 05 18 when plants were kept in the dark (Fig. 4). The rate of ethylene production in each organ depends at least partially on locally generated ACC (Clark et al., 1997). If there are not increased levels of ACC in the plant tissue then there is no need for increased expression of ACO genes. It is unusual, however, that there is no change in transcript abundance as flower abscission was observed by plants of this cultivar when kept in the dark.

The trends shown by both open and unopened flowers of P 08 05 06 (Fig. 4) are unique when compared to the other cultivars used in this study. At 96 h, ACS and ACO mRNA had decreased when plants were placed in the dark suggesting that ethylene production was downregulated in the dark at this point. In unopened flowers, ACS mRNA increased after 24 h, and again after 48 h, suggesting that ethylene production increases in unopened flowers when initially in the dark but then decreases. Although delayed by 24 h, this corresponds with the increased abscission of unopened flowers observed in the dark at 48 and 72 h (data not shown).

Although the dark-induced abscission in all cultivars could not be accounted for by differential expression of ACS and ACO genes, transcripts of these genes were nevertheless present, and hence ethylene biosynthesis could have continued. Alternatively, regulation of abscission may have occurred post-transcription, in which case differences in transcript abundance may not have been evident. Additionally, other members of the ACS and ACO multigene families may be involved, since these genes are differentially expressed in response to developmental, environmental and hormonal cues (Clark et al., 1997). Other hormones besides ethylene may play an important role, especially auxin. An *AUXIN RESPONSE FACTOR* (*ARF2*) identified in *Arabidopsis* was shown to regulate floral organ abscission via an ethylene-independent pathway (Ellis et al., 2005), while in *Cestrum elegans*, expression of auxin-responsive gene *CE-IAA5* after auxin application, increased abscission of unopened flowers, causing preferential shedding (Abebie et al., 2008). A further possibility is that a change in the pedicle tissue sensitivity to ethylene may occur. This has been observed in a number of other plant species (Campbell and Labavitch, 1991; Currv, 1991;; Tian et al., 1994 Sato-Nara et al., 1999).

## 5. Conclusions

*Plectranthus* plants are sensitive to exogenous ethylene in a concentration dependent manner, and ethylene-induced abscission can be partially or completely alleviated using 1-MCP with a 6 h pretreatment or continuous treatment respectively. Continuous dark conditions increased total flower abscission in all *Plectranthus* cultivars, with preferential shedding of open flowers and unopened flowers observed in different cultivars. Dark-induced abscission was alleviated by continuous 1-MCP treatment, implying ethylene regulation.

In some cultivars, abscission could be accounted for by changes in ACS and ACO expression, but in others no direct link was observed. In this study, the changes in expression of only one gene from each of these families was investigated, and it is possible that there were more marked changes in expression of other genes for these enzymes. Also, gene expression was limited to the abscission zones of the pedicels only, and possibly ethylene production is increased in other plant parts during prolonged periods of darkness. Lastly, dark-induced flower abscission in *Plectranthus* may also be regulated by other hormones such as auxin. Despite difficulty in determining the exact cause of dark-induced abscission, 1-MCP can be effectively used to control it for transportation purposes.

## Acknowledgements

*Plectranthus* cuttings were kindly provided by Dr G. Brits of Brits Nursery in Stellenbosch, South Africa. Funding was provided by The NRF/Hungarian Cooperative Programme to LJR, and by the Claude Leon Foundation to GDA.

## References

Abebie, B., Lers, A., Philosoph-Hadas, S., Goren, R., Riov, J., Meir, S., 2008. Differential effects of NAA and 2,4-D in reducing floret abscission in Cestrum (*Cestrum elegans*) cut flowers are associated with their differential activation of *Aux/IAA* homologous genes. Annals of Botany 101, 249–259.

Abeles, F.B., Morgan, P.W., Saltveit, M.E.J., 1992. Ethylene in Plant Biology. Academic Press, Inc., San Diego.

Adams, D.O., Yang, S.F., 1979. Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proceedings of the National Academy of Sciences of the United States of America 76, 170–174.

Ascough, G.D., Mtshali, N.P., Nogemane, N., Van Staden, J., 2006. Flower abscission in excised inflorescences of three *Plectranthus* cultivars. Plant Growth Regulation 48, 229–235.

Ascough, G.D., Rice, L.J., Van Staden, J., 2008. Considerations for evaluating flower abscission in potted plants with multiple inflorescences — *Plectranthus* as a case study. South African Journal of Botany 74, 753–756.

Ashman, T.L., Schoen, D.J., 1994. How long should flowers live? Nature 371, 388–391.

Barry, C.S., Llop-Tous, M.I., Grierson, D., 2000. The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. Plant Physiology 123, 979–986.

Campbell, A.D., Labavitch, J.M., 1991. Induction and regulation of ethylene biosynthesis and ripening by pectic oligomers in tomato pericarp discs. Plant Physiology 97, 706–713.

Chang, C., Kwok, S.F., Bleecker, A.B., Meyerowitz, E.M., 1993. *Arabidopsis* ethylene-response gene *ETR1*: similarity of product to two-component regulators. Science 262, 539–544.

Clark, D.G., Richards, C., Hilioti, Z., Lind-Iversen, S., Brown, K.M., 1997. Effect of pollination on accumulation of ACC synthase and ACC oxidase transcripts, ethylene production and flower petal abscission in geranium (*Pelargonium* X *hortorum* L.H. Bailey). Plant Molecular Biology 34, 855–865.

Currv, E.A., 1991. NAA-induced ethylene and ACC in 'delicious' spur tissues: changes with temperature and time. Journal of the American Society for Horticultural Science 116, 846–850.

Czarny, J.C., Grichko, V.P., Glick, B.R., 2006. Genetic modulation of ethylene biosynthesis and signaling in plants. Biotechnology Advances 24, 410–419.

Deikman, J., 1997. Molecular mechanisms of ethylene regulation of gene transcription. Physiologia Plantarum 100, 561–566.

Doležel, J., Binarová, P., Lucretti, S., 1989. Analysis of nuclear DNA content in plant cells by flow cytometry. Biologia Plantarum 31, 113–120.

Dostal, D.L., Agnew, N.H., Gladon, R., Weigle, J., 1991. Ethylene, simulated shipping, STS, and AOA affect corolla abscission of New-Guinea *Impatiens*. Hortscience 26, 47–49.

Ellis, C.M., Nagpal, P., Young, J.C., Hagen, G., Guilfoyle, T.J., Reed, J.W., 2005. *AUXIN RESPONSE FACTOR1* and *AUXIN RESPONSE FACTOR2* regulate senescence and floral organ abscission in *Arabidopsis thaliana*. Development 132, 4563–4574.

Johnson, P.R., Ecker, J.R., 1998. The ethylene gas signal transduction pathway: a molecular perspective. Annual Reviews of Genetics 32, 227–254.

Jones, M.L., Woodson, W.R., 1999. Differential expression of three members of the 1-aminocyclopropane-1-carboxylate synthase gene family in carnation. Plant Physiology 119, 755–764.

Kende, H., Zeevaart, J.A.D., 1997. The five "classical" plant hormones. The Plant Cell 9, 1197–1210.

Ma, N., Tan, H., Liu, X., Xue, J., Li, Y., Gao, J., 2006. Transcriptional regulation of ethylene receptor and *CTR* genes involved in ethylene-induced flower opening in cut rose (*Rosa hybrida*) cv. Samantha. Journal of Experimental Botany 57, 2763–2773.

Macnish, A.J., Irving, D.E., Joyce, D.C., 2004. Variation in ethylene-induced postharvest flower abscission responses among *Chamelaucium* Desf. (Myrtaceae) genotypes. Scientia Horticulturae 102, 415–432.

Müller, R., Andersen, A.S., Serek, M., 1998. Differences in display life of miniature potted roses (*Rosa hybrida* L.). Scientia Horticulturae 76, 59–71.

Mutui, T.M., Mibus, H., Serek, M., 2007. Influence of thidiazuron, ethylene, abscisic acid and dark storage on the expression levels of ethylene receptors (*ETR*) and ACC synthase (*ACS*) genes in *Pelargonium*. Plant Growth Regulation 53, 87–96.

Patterson, S.E., 2001. Cutting loose: abscission and dehiscence in *Arabidopsis*. Plant Physiology 126, 494–500.

Pech, J.-C., Latché, A., Bouzayen, M., 2004. Ethylene biosynthesis, In: Davis, P.J. (Ed.), Plant Hormones: Biosynthesis, Signal Transduction, Action!, 3rd edition.

Pierik, R., Tholen, D., Poorter, H., Visser, E.J.W., Voesenek, L.A.C.J., 2006. The Janus face of ethylene: growth inhibition and stimulation. Trends in Plant Science 11, 176–183.

Porat, N., Shlomo, E., Serek, M., Sisler, E.C., Borochov, A., 1995. 1-Methylcyclopropene inhibits ethylene action in cut phlox flowers. Postharvest Biology and Technology 6, 313–319.

Rice, L.J., Brits, G., Potgieter, C.J., Van Staden, J., 2011. *Plectranthus*: a plant for the future? South African Journal of Botany 77, 947–959.

Sato-Nara, K., Yuhashi, K.-I., Higashi, K., Hosoya, K., Kubota, M., Ezura, H., 1999. Stage- and tissue-specific expression of ethylene receptor homolog genes during fruit development in muskmelon. Plant Physiology 199, 321–329.

Serek, M., Sisler, E.C., Reid, M.S., 1994. A volatile ethylene inhibitor improves the postharvest life of potted roses. Journal of the American Society for Horticultural Science 572–577.
Serek, M., Sisler, E.C., Reid, M.S., 1995. Effects of 1-MCP on the vase life and ethylene response of cut flowers. Plant Growth Regulation 16, 93–97.
Serek, M., Prabucki, A., Sisler, E.C., Andersen, A.S., 1998. Inhibitors of ethylene action affect final quality and rooting of cuttings before and after storage. Hortscience 33, 153–155.
Sisler, E.C., Serek, M., 1999. Compounds controlling the ethylene receptor. Botanical Bulletin of Academia Sinica 40, 1–7.
Sisler, E.C., Dupille, E., Serek, M., 1996. Effect of 1-Methylcyclopropene and methylenecyclopropane on ethylene binding and ethylene action on cut carnations. Plant Growth Regulation 18, 79–86.
Smulders, M.J.M., Rus-Kortekaas, W., Gilissen, L.J.W., 1994. Development of polysomaty during differentiation in diploid and tetraploid tomato (*Lycopersicon esculentum*) plants. Plant Science 97, 53–60.
Taylor, J.E., Whitelaw, C.A., 2001. Signals in abscission. New Phytologist 151, 323–339.
Tian, Y., Lu, X.Y., 2006. The molecular mechanism of ethylene signal transduction. South African Journal of Botany 72, 487–491.
Tian, M.S., Downs, C.G., Lill, R.E., King, G.A., 1994. A role for ethylene in yellowing of broccoli after harvest. Journal of the American Society for Horticultural Science 19, 276–281.
Uthaichay, N., Ketsa, S., Van Doorn, W.G., 2007. 1-MCP pretreatment prevents bud and flower abscission in *Dendrobium* orchids. Postharvest Biology and Technology 43, 374–380.
Veen, H., 1979. Effects of silver on ethylene synthesis and action in cut carnations. Planta 145, 467–470.
Wang, K.L.-C., Hai, L., Ecker, J.R., 2002. Ethylene biosynthesis and signaling networks. The Plant Cell 14, S131–S151.
Whitehead, C.S., Nelson, R., 1992. Ethylene sensitivity in germinating peanut seeds: the effect of short-chain saturated fatty acids. Journal of Plant Physiology 139, 479–483.
Woltering, E.J., 1987. Effects of ethylene on ornamental pot plants: a classification. Scientia Horticulturae 31, 283–294.
Woodson, W.R., Park, K.Y., Drory, A., Larsen, P.B., Wang, H., 1992. Expression of ethylene biosynthetic pathway transcripts in senescing carnation flowers. Plant Physiology 99, 526–532.
Yang, S.F., Hoffman, N.E., 1984. Ethylene biosynthesis and its regulation in higher plants. Annual Review of Plant Physiology 35, 155–189.