Flower abscission in potted *Plectranthus*

Laura Jane Rice

FLOWER ABSCISSION IN POTTED *PLECTRANTHUS*

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Submitted in fulfillment of the academic requirements for the degree of Doctor of Philosophy

in the

Research Centre for Plant Growth and Development

School of Life Sciences

University of KwaZulu-Natal

Pietermaritzburg

January 2013

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Flower Abscission in Potted *Plectranthus*

I, **LAURA JANE RICE**
Student Number: **203507134**

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Thesis title: **Flower Abscission in Potted *Plectranthus***

Regular consultation took place between the student and us throughout the investigation. We advised the student to the best of our ability and approved the final document for submission to the College of Agriculture, Engineering and Sciences Higher Degrees Office for examination by the University appointed Examiners.

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DR G.D. ASCOUGH

Flower abscission in potted *Plectranthus.*

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Abstract

Transport and post-harvest handling of flowers both cut and potted is one of the greatest challenges in the horticulture industry (REDMAN *et al.*, 2002). Ethylene-induced flower abscission is responsible for the loss of crops (KIM *et al.*, 2007). Flower abscission is greater when plants are transported (ABEBIE *et al.*, 2005). This limits the sale of flowers and potted plants to areas close to the site of production and prevents export opportunities. South Africa is home to many spectacular species with great horticultural potential (RICE *et al.*, 2011). Unfortunately however, development of a number of these species for export is difficult due to transport-induced flower abscission. Transport-induced flower abscission is a problem experienced by Dr Gert Brits, a breeder of *Plectranthus* in Stellenbosch in South Africa.

In this study a number of Dr Brits's *Plectranthus* varieties were used as model plants to understand the process of transport-induced flower abscission and develop a protocol for the prevention of such abscission.

Flow cytometry was used to determine the ploidy levels of each of the varieties. It was important to be aware of this during the experiments as varieties with different ploidy levels have been reported to behave differently under stressful environmental conditions. Of the eight varieties examined, three were diploid (2n), one was triploid (3n), three were tetraploid (4n) and one was a mixopliod (2n/4n) variety.

To determine the effects of packaging plants during transport and the effects of darkness on flower abscission, plants were packaged into perspex chambers and kept either in a 16 h photoperiod or in darkness for 96 h. Every 24 h the number of open and unopened flowers that had abscised was recorded. Both packaging and darkness increased flower abscission of open and unopened flowers in all eight varieties. Four varieties preferentially abscised open flowers; while the remaining four preferentially abscised unopened flowers.

All eight varieties were exposed to different concentrations of ethylene (0, 0.1, 0.25 0.5, 1 and 2 $\mu l l^{-1}$) to determine their level of ethylene sensitivity. All of the *Plectranthus* varieties were determined to be extremely sensitive to ethylene. With 100% flower abscission occurring within 24 h at 1 and 2 $\mu l l^{-1}$ in all varieties.

In order to determine what internal changes were causing this increase in flower abscission under these conditions, the changes in the expression of key ethylene biosynthetic enzymes, cytokinin content and carbohydrates in the flowers were examined.

ACS and ACO are the two key enzymes in the ethylene biosynthetic pathway (JOHNSON & ECKER, 1998). Changes in the levels of mRNAs coding for these two enzymes were examined when plants were packaged and put into the dark. In general there was an upregulation of the ethylene biosynthetic pathway and in turn this may have increased ethylene production by the plants under simulated transport conditions. However, the changes were not large enough to be solely responsible for the increased flower abscission observed under simulated transport conditions.

The concentrations of 43 cytokinins were measured in pedicle tissue from plants which had been kept in the dark for 0, 24, 48, 72 and 96 h. Of the 43 cytokinins measured 21 were below the level of detection. Concentrations for the remaining 22 cytokinins at each of the time points were examined and it was found that in general cytokinin concentrations increase when plants are packaged and put into the dark. DHZ-type cytokinins remained stable during the 96 h continuous dark monitoring period, with most of the changes observed in the tZ and iP types. Peaks in cytokinin concentrations are often followed by an increase in flower abscission, indicating that an increase in cytokinin concentrations may be one of the factors causing the increase in transport-induced flower abscission

Only glucose and fructose were detected in peduncle tissue. Changes in glucose and fructose over 24 h in the greenhouse and over 0, 24, 48, 72 and 96 h in simulated transport conditions were measured. During the day, glucose and fructose levels increased towards the afternoon and evening and decreased in the early morning. This is consistent with studies conducted on other species (ALONI *et al.*, 1996). When plants were put into the dark, glucose and fructose levels increased slightly at 24 h and then decreased to levels similar to those measured in control plants. Although there were changes in glucose and fructose level in simulated transport conditions, they were very slight and it is unlikely that these changes are not responsible for the transport-induced flower abscission.

These results suggest that the observed transport-induced flower abscission is the result of increased cytokinin concentrations and expression of ACO and ACS genes when plants are packaged and put into the dark. These changes in turn cause an increase in ethylene production by the plants, and the build-up of ethylene in the transport container causes flowers to abscise. Ethylene perception by the plant is the step which could be targeted to prevent flower abscission.

A number of ethylene antagonists block the ethylene receptors in the plant and in so doing prevent the receptors from binding ethylene and transducing the abscission signal. 1-MCP is one such ethylene antagonist. To test whether 1-MCP could be used for the prevention of flower abscission in *Plectranthus*, plants were placed in sealed perspex chambers in the light and in the dark and treated with 100 nll^{-1} 1-MCP for a single 6 h treatment, or for 6 h every day prior to continuous exposure to ethylene. 1-MCP treatment greatly reduced ethylene- and transport-induced flower abscission when plants were treated continuously, but reduced flower abscission for the first 24 h when pre-treated with a single 6 h exposure to 1-MCP.

Transport-induced flower abscission in *Plectranthus* is the result of exposure to ethylene. The increase in ethylene production by the plants in transport conditions is likely due to an upregulation of the ethylene biosynthetic pathway and an increase in cytokinin concentrations

or movement in the pedicle tissue. This transport-induced flower abscission can be prevented by continuous treatment with 100 nll^{-1} 1-MCP during the transport period. By using 1-MCP plants can be transported for up to 4 d and the opportunity for export is made possible.

Acknowledgments

I would like to thank the following people without whom this project and thesis would never have been possible.

Firstly to Dr Gert Brits, thank you for all the cuttings you provided me with. The plants which grew from these formed the foundations of the project. You were so kind in harvesting, packaging and couriering them whenever required.

To Dr Glendon Ascough. This project was your baby and you were very generous to hand it over. Thank you for all your help with the flow cytometry, carbohydrates and the early parts of this study. You have counted many a flower and credit must go to you for your huge contribution to this project.

To Prof van Staden and Prof Finnie, thank you for your guidance and advice over the years. Prof, thank you for the hours that you put in for your students. You really care about all of us and I really appreciate your commitment. Prof Finnie, you have been involved in my academic life since my first year at university. Thank you for your enthusiasm and guidance throughout these studies.

Thank you to Dr Vili Soós and Prof Ervin Balázs who hosted me at their research laboratory in Hungary. You were both so generous of your time and expertise. I loved my time working with you and I learnt so much from both of you.

Prof Miroslav Strnad and Dr Karel Doležal are thanked for their help in measuring cytokinin concentrations.

Prof Charles Whitehead is thanked for supplying the 1-MCP used in this study and for his time spent training me on the ethylene GC.

Next to my parents. You have supported me, more than just financially, for longer than I am sure you bargained on. Without your generosity, love and gentle pushes this would not have been possible. You were also very willing lab assistants! To my brothers, thanks for being the amazing people that you are.

To Cameron, who has tirelessly helped to water plants, harvest samples, cart plants in and out of the dark, count flowers, grind samples, package samples for travel, read and edit chapters. Thank you for sticking with me when this project went on for a lot longer than expected. I love you very much.

To my partner in crime Lee Cheesman. I literally could not have done this without you. It was a privilege to have you along for the ride. I will always cherish the memories that we made in Pietermaritzburg.

Thank you to members of the Research Centre, in particular Lee Warren, Judy Magnussen, Gary Stafford, Marnie Light, Heino Papenfus, Manoj Kulkarni, Maya Chinsamy, Halima Abdillahi and Rofhiwe Mulaudzi. The support and friendship in the Centre is amazing and this makes it a very special place to work.

To all my other friends and family who have offered words of encouragement along the way, it's always good to know that someone is cheering you on. Thank you.

List of Abbreviations

°C	degrees centigrade
µl	microlitre
$\mu l l^{-1}$	microlitres per litre
µm	micrometers
$\mu mol\, m^{-1}\, s^{-1}$	micromoles per meter per second
1-MCP	1-methylcylopropene
2,4-D	2,4-dicholorophenoxyacetic acid
ABA	abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
ACO	ACC oxidase
ACS	ACC synthase
AIB	α-aminoisobutyric acid
ANOVA	analysis of variance
AOA	aminooxyacetic acid
AVG	aminoethoxyvinyl glycine
BAP	6-Benzylaminopurine
BAP5'MP	6-Benzylaminopurine-5'-monophosphate
BAP9G	6-Benzylaminopurine-9-glucoside
BAPR	6-Benzylaminopurine riboside
cDNA	complementary DNA
cm	centimeters
cv	cultivar
cZ	*cis*-zeatin
cZ9G	*cis*-zeatin-9-glucoside
cZOG	*cis*-zeatin-*O*-glucoside
cZR	*cis*-zeatin riboside
cZR5'MP	*cis*-zeatin riboside-5'-monophosphate
cZROG	*cis*-zeatin-*O*-glucoside riboside
d	days
DACP	diazocyclopentadiene
DHZ	dihydrozeatin
DHZ9G	dihydrozeatin-9-glucoside
DHZOG	dihydrozeatin-*O*-glucoside
DHZR	dihydrozeatin riboside
DHZR5'MP	dihydrozeatin riboside-5'-monophosphate
DHZROG	dihydrozeatin-*O*-glucoside riboside
DNA	deoxyribonucleic acid
DPSS	1,1-dimethyl-4-(phenylsulfonyl)semicarbazide
DTT	Dithiothreitol

h	Hours
IAA	indole-3-acetic acid
iP	N^6-isopentenyladenine
iP9G	N^6-isopentenyladenine-9-glucoside
iPR	N^6-isopentenyladenosine
iPR5'MP	N^6-isopentenyladenosine-5'-monophosphate
IPTG	isopropylthio-β-galactoside
l	Litres
LB	Lysogeny broth
MET	Methionine
mg ml^{-1}	miligram per millilitre
mm	Millimetres
mM	Millimolar
mRNA	messenger RNA
mT	*meta*-topolin
mT9G	*meta*-topolin-9-glucoside
mTOG	*meta*-topolin-*O*-glucoside
mTR	*meta*-topolin riboside
mTR5'MP	*meta*-topolin-5'-monophosphate
mTROG	*meta*-topolin-*O*-glucoside riboside
NAA	1-naphthaleneacetic acid
NBD	2,5-norbornadiene
nll^{-1}	nanolitres per litre
oT	*ortho*-topolin
oT9G	*ortho*-topolin-9-glucoside
oTOG	*ortho*-topolin-*O*-glucoside
oTR	*ortho*-topolin riboside
oTR5'MP	*ortho*-topolin-5'-monophosphate
oTROG	*ortho*-topolin-*O*-glucoside riboside
PCR	polymerase chain reaction
PG	polygalacturonase
pmol/g	picomoles per gram
pT	*para*-topolin
pTOG	*para*-topolin-*O*-glucoside
pTR	*para*-topolin riboside
pTR5'MP	*para*-topolin-5'-monophosphate
pTROG	*para*-topolin-*O*-glucoside riboside
PVPP	polyvinylpolypyrrolidone
RNA	ribonucleic acid
rpm	revolutions per minute
SAM	*S*-adenosyl-methionine
STS	Silverthiosulfate
TDZ	thidiazuron

tZ	*trans*-zeatin
tZ9G	*trans*-zeatin-9-glucoside
tZOG	*trans*-zeatin-*O*-glucoside
tZR	*trans*-zeatin riboside
tZR5'MP	*trans*-zeatin riboside-5'-monophosphate
tZROG	*trans*-zeatin-*O*-glucoside riboside
UPLC	ultra performance liquid chromatography
X-gal	5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside

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Conference Contributions

Rice, L.J., Ascough, G.D., Finnie, J.F. and Van Staden, J. 2010. DNA fingerprinting of *Plectranthus* plants for protection of cultivar registration. 36th Annual Conference of the South African Association of Botanists (SAAB). 11-15 January, Potchefstroom.

Publications

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

Rice, L.J., Soós, V. Ascough, G.D, Balázs, E., Ördög, V., Whitehead, C.S., Finnie, J.F., Van Staden, J. 2013. Ethylene- and dark-induced flower abscission in potted *Plectranthus*: sensitivity, prevention by 1-MCP, and possible roles of expression of ethylene biosynthetic genes. South African Journal of Botany. **87**: 39-47.

Chapter 1

Literature Review

1.1 Introduction

Ornamental horticulture is an important sector of horticulture, and floriculture is a major area of ornamental horticulture (CHANDLER & LU, 2005). Floriculture encompasses both cut flowers and potted plants. One of the major obstacles in this industry is the transport and postharvest handling of both potted and cut flowers (REDMAN *et al.*, 2002). The longevity of flowering potted plants is often cut short by ethylene-induced flower abscission (KIM *et al.*, 2007). Shipping-induced abscission is a problem in many horticultural plant species (ABEBIE *et al.*, 2005) as it reduces the quality and retail value of the plant. Extensive research has been carried out to find methods of preventing crop loss during transport.

Plectranthus is a popular potted and garden plant and many varieties have been bred for the potted plant industry (BRITS & LI, 2008). However, suppliers of potted *Plectranthus* have problems during transportation of the plants. Transport conditions induce flower drop, resulting in a product of inferior quality. In many species ethylene is the main cause of shipping-induced abscission but this may be counteracted by ethylene antagonists (BUNYA-ATICHART *et al.*, 2006). The susceptibility of flowers to ethylene is a major setback for producers of many ornamental plants (SRISKANDARAJAH *et al.*, 2007).

Traditional potted plants are purchased when they are in or almost in full flower (REID *et al.*, 2002). They are expected to last up to a month indoors and are then discarded. The presence of flowers determines the value of flowering potted plants. Without flowers the plants are not as attractive and will not provide the instant colour that is their appeal.

1.2 Horticulture in South Africa

The vast floral diversity in South Africa provides many opportunities for horticultural development. A number of South African plant species have achieved international fame and can be found in gardens and homes around the world. VAN JAARSVELD (2006) lists proteas, aloes, mesembs, clivias, gladioli, freesias, Barberton daisies and arum lilies as some of our international superstars. This, however, is only a minute fraction of the estimated 23000 flowering species that grow in South Africa (VAN JAARSVELD, 2006). There are still many wild species with potential that have not yet been explored. One genus, however, has attracted the attention of breeders and growers. These are the *Plectranthus* or spurflowers.

1.2.1 Plectranthus

Plectranthus is a widespread and widely used genus. Belonging to the Lamiaceae family there are approximately 300 species of *Plectranthus* from tropical Africa, Asia and Australia (LUKHOBA *et al.*, 2006). The majority are native to Africa with 44 occurring naturally in South Africa. *Plectranthus* is the largest genus in the Lamiaceae family in South Africa (HANKEY, 2001).

Many species are used in traditional medicines in Africa, South America and Asia. Nine species are edible. The tubers, roots and leaves are eaten as a vegetable in Africa and Malaysia. Three species are used as food additives and five species are used as fodder for sheep, goats, cattle and camels. The wood from *Plectranthus insignis* is used for building huts and for firewood (LUKHOBA *et al.*, 2006).

Twenty species are important in the horticultural trade as potted house plants, rock garden plants and boundary markers (LUKHOBA *et al.*, 2006) (Table 1.1). The colourful blooms and textured leaves make for very attractive house and garden plants (LUKHOBA *et al.*, 2006; BRITS & LI, 2008). Their flowers vary from shades of purple, blue, pink to white. Many species of *Plectranthus* are found in gardens and homes around the world, “attracting growing attention internationally” (BRITS & LI, 2008). A small group of *Plectranthus* plants are compact with decorative leaves and large flowers which make these ideal for use as potted plants (LUKHOBA *et al.*, 2006; BRITS & LI, 2008).

Table 1.1: Horticultural uses of southern African *Plectranthus* species.

Species	Species name with authority & recent synonym	Horticultural use	Reference
Plectranthus aliciae	*Plectranthus aliciae* (Codd) Van Jaarsv. & T.J.Edwards [=*Plectranthus madagascariensis* (Pers.) Benth. var. *aliciae* Codd]	Gardens	(JOFFE, 2001)
		Groundcover	(JOFFE, 2001)
		Hanging baskets	(JOFFE, 2001)
		Potted plant	(VAN JAARSVELD, 2006)
		Rockeries	(VAN JAARSVELD, 2006)
Plectranthus ambiguus	*Plectranthus ambiguus* (Bolus) Codd	Container	(POOLEY, 1998) (VAN JAARSVELD, 1987, 2006)
		Gardens	(VAN JAARSVELD, 1987) (PIENAAR, 1985, 1991)
		Groundcover	(POOLEY, 1998) (VAN JAARSVELD, 1987, 2006) (PIENAAR, 1984)
		Hanging baskets	(VAN JAARSVELD, 1987, 2006)
Plectranthus amboinicus	*Plectranthus amboinicus* (Lour.) Spreng.	Rockeries	(VAN JAARSVELD, 2006)
Plectranthus barbatus[1]	*Plectranthus barbatus* Andrews	Gardens	(VAN JAARSVELD, 2006) (PIENAAR, 1984)
Plectranthus brevimentum[2]	*Plectranthus brevimentum* T.J.Edwards		

Table 1.1 continued

Plectranthus caninus	*Plectranthus caninus* Roth	Bushveld gardens	(VAN JAARSVELD, 2006)
Plectranthus ciliatus	*Plectranthus ciliatus* E.Mey. ex Benth.	Container	(POOLEY, 1998)
			(VAN JAARSVELD, 1987, 2006)
		Garden	(VAN JAARSVELD, 1987)
		Groundcover	(POOLEY, 1998)
			(VAN JAARSVELD, 1987, 2006)
		Hanging baskets	(VAN JAARSVELD, 1987, 2006)
Plectranthus cylindraceus	*Plectranthus cylindraceus* Hochst. ex Benth.	Bushveld Gardens	(POOLEY, 1998)
			(VAN JAARSVELD, 2006)
		Pot plant	(VAN JAARSVELD, 2006)
		Rockeries	(VAN JAARSVELD, 2006)
Plectranthus dinteri	*Plectranthus dinteri* Briq.	Bushveld gardens	(VAN JAARSVELD, 2006)
		Rockeries	(VAN JAARSVELD, 2006)
Plectranthus dolichopodus	*Plectranthus dolichopodus* Briq.	Gardens	(VAN JAARSVELD, 2006)

Table 1.1 continued

		Potted plant	(VAN JAARSVELD, 2006)
Plectranthus ecklonii	*Plectranthus ecklonii* Benth.	Gardens	(POOLEY, 1998) (LUKHOBA *et al.*, 2006) (VAN JAARSVELD, 1987, 2006) (PIENAAR, 1984, 1985, 1991) (JOFFE, 2001)
Plectranthus elegans	*Plectranthus elegans* Britten		
Plectranthus elegantulus	*Plectranthus elegantulus* Briq.	Garden	(LUKHOBA *et al.*, 2006) (VAN JAARSVELD, 1987)
		Groundcover	(VAN JAARSVELD, 1987, 2006)
		Potted plant	(VAN JAARSVELD, 1987, 2006))
		Hanging baskets	(VAN JAARSVELD, 1987, 2006)
Plectranthus ernstii[3]	*Plectranthus ernstii* Codd	Bonsai	(POOLEY, 1998) (VAN JAARSVELD, 2006)
		Container	(POOLEY, 1998)
		Hanging baskets	(VAN JAARSVELD, 1987)
		Pot plant	(VAN JAARSVELD, 2006)
		Rockeries	(VAN JAARSVELD, 2006)

Table 1.1 continued

Plectranthus esculentus[4]	*Plectranthus esculentus* N.E.Br.	Bushveld gardens	(VAN JAARSVELD, 2006)
Plectranthus fruticosus	*Plectranthus fruticosus* L'Hér.	Gardens	(POOLEY, 1998) (LUKHOBA *et al.*, 2006) (VAN JAARSVELD, 1987, 2006) (PIENAAR, 1984, 1985, 1991) (JOFFE, 2001)
		Hanging baskets	(VAN JAARSVELD, 1987)
Plectranthus grallatus	*Plectranthus grallatus* Briq.	Gardens	(VAN JAARSVELD, 2006)
Plectranthus grandidentatus	*Plectranthus grandidentatus* Gürke	Gardens	(VAN JAARSVELD, 1987)
		Groundcover	(POOLEY, 1998) (VAN JAARSVELD, 2006)
Plectranthus hadiensis	*Plectranthus hadiensis* (Forssk.) Schweinf. ex Spreng.	Bushveld gardens	(POOLEY, 1998) (VAN JAARSVELD, 1987, 2006)
Plectranthus hereroensis	*Plectranthus hereroensis* Engl.	Bushveld gardens	(VAN JAARSVELD, 1987, 2006)
Plectranthus hilliardiae	*Plectranthus hilliardiae* Codd	Gardens	(POOLEY, 1998)
		Groundcover	(VAN JAARSVELD, 1987)

Table 1.1 continued

		Potted plant	(VAN JAARSVELD, 1987, 2006) (PIENAAR, 1984)
Plectranthus laxiflorus	*Plectranthus laxiflorus* Benth.	Forest gardens	(VAN JAARSVELD, 2006)
Plectranthus lucidus	*Plectranthus lucidus* Van Jaarsv. & T.J.Edwards	Groundcover	(VAN JAARSVELD, 2006)
Plectranthus madagascariensis	*Plectranthus madagascariensis* (Pers.) Benth.	Garden	(LUKHOBA *et al.*, 2006)
		Groundcover	(VAN JAARSVELD, 1987) (PIENAAR, 1991) (POOLEY, 1998) (VAN JAARSVELD, 1987, 2006) (PIENAAR, 1984)
Plectranthus malvinus[5]	*Plectranthus malvinus* Van Jaarsv. & T.J.Edwards	Forest gardens	(VAN JAARSVELD, 2006)
		Hanging baskets	(VAN JAARSVELD, 2006)
Plectranthus mirabilis	*Plectranthus mirabilis* (Briq.) Launert	Bushveld gardens	(VAN JAARSVELD, 2006) (VAN JAARSVELD, 2006)
Plectranthus mutabilis	*Plectranthus mutabilis* Codd	Gardens	(VAN JAARSVELD, 1987)

Table 1.1 continued

		Bushveld gardens	(VAN JAARSVELD, 2006)
Plectranthus mzimvubuensis[6]	*Plectranthus mzimvubuensis* Van Jaarsv.	Bushveld gardens	(VAN JAARSVELD, 2006)
Plectranthus neochilus	*Plectranthus neochilus* Schltr.	Groundcover	(POOLEY, 1998)
			(VAN JAARSVELD, 1987, 2006)
		Bushveld gardens	(VAN JAARSVELD, 1987, 2006)
		Rockeries	(VAN JAARSVELD, 1987)
Plectranthus oertendahlii[6]	*Plectranthus oertendahlii* T.C.E.Fr.	Bushveld gardens	(VAN JAARSVELD, 1987, 2006)
		Container	(POOLEY, 1998)
			(VAN JAARSVELD, 1987, 2006)
		Groundcover	(VAN JAARSVELD, 2006)
		Hanging baskets	(VAN JAARSVELD, 2006)
			(VAN JAARSVELD, 1987)
		Potted plants	(VAN JAARSVELD, 2006)
Plectranthus oribiensis[6]	*Plectranthus oribiensis* Codd	Subtropical garden	(POOLEY, 1998)
			(VAN JAARSVELD, 2006)

Table 1.1 continued

		Rockeries	(VAN JAARSVELD, 2006)
Plectranthus pentheri[7]	*Plectranthus pentheri* (Gürke) Van Jaarsv. & T.J.Edwards	Bushveld gardens	(VAN JAARSVELD, 2006)
		Rockeries	(VAN JAARSVELD, 2006)
Plectranthus petiolaris	*Plectranthus petiolaris* E.Mey. ex Benth.	Bushveld gardens	(VAN JAARSVELD, 2006)
		Groundcover	(VAN JAARSVELD, 2006)
Plectranthus porcatus[8]	*Plectranthus porcatus* Van Jaarsv. & P.J.D.Winter	Bushveld gardens	(VAN JAARSVELD, 2006)
Plectranthus praetermissus[8]	*Plectranthus praetermissus* Codd	Groundcover	(VAN JAARSVELD, 2006)
		Hanging basket	(VAN JAARSVELD, 1987, 2006)
		Potted plant	(VAN JAARSVELD, 1987, 2006) (PIENAAR, 1984)
Plectranthus psammophilus[5]	*Plectranthus psammophilus* Codd	Bushveld gardens	(VAN JAARSVELD, 2006)
Plectranthus purpuratus	*Plectranthus purpuratus* Harv.	Bushveld gardens	(VAN JAARSVELD, 1987, 2006)
		Groundcover	(VAN JAARSVELD, 2006)
		Hanging baskets	(VAN JAARSVELD, 2006)

Table 1.1 continued

		Rockeries	(VAN JAARSVELD, 2006)
Plectranthus ramosior	*Plectranthus ramosior* (Benth.) Van Jaarsv. [=*Plectranthus madagascariensis* (Pers.) Benth. var. *ramosior* Benth.]	Rockeries	(VAN JAARSVELD, 2006)
Plectranthus reflexus[5]	*Plectranthus reflexus* Van Jaarsv. & T.J.Edwards	Subtropical gardens	(VAN JAARSVELD, 2006)
Plectranthus rehmannii	*Plectranthus rehmannii* Gürke	Subtropical gardens	(VAN JAARSVELD, 2006)
Plectranthus rubropunctatus	*Plectranthus rubropunctatus* Codd	Forest gardens	(VAN JAARSVELD, 2006)
		Groundcover	(VAN JAARSVELD, 2006)
Plectranthus saccatus	*Plectranthus saccatus* Benth.	Container	(POOLEY, 1998)
			(JOFFE, 2001)
		Garden	(JOFFE, 2001)
			(VAN JAARSVELD, 1987)
			(PIENAAR, 1985)
		Hanging baskets	(VAN JAARSVELD, 1987)
		Groundcover	(VAN JAARSVELD, 1987, 2006)
			(PIENAAR, 1984)
			(JOFFE, 2001)
		Potted plant	(VAN JAARSVELD, 2006)

Table 1.1 continued

Plectranthus sanguineus	*Plectranthus sanguineus* Britten	Potted plant	(VAN JAARSVELD, 2006)
		Rockeries	(VAN JAARSVELD, 2006)
Plectranthus spicatus	*Plectranthus spicatus* E.Mey. ex Benth.	Bushveld gardens	(POOLEY, 1998)
			(VAN JAARSVELD, 2006)
		Potted plants	(VAN JAARSVELD, 2006)
Plectranthus strigosus	*Plectranthus strigosus* Benth.	Groundcover	(VAN JAARSVELD, 1987)
		Hanging baskets	(VAN JAARSVELD, 1987, 2006)
		Rockeries	(VAN JAARSVELD, 1987, 2006)
		Subtropical gardens	(VAN JAARSVELD, 1987, 2006)
Plectranthus stylesii[2]	*Plectranthus stylesii* T.J.Edwards	Rockeries	(VAN JAARSVELD, 2006)
		Subtropical gardens	(VAN JAARSVELD, 2006)
Plectranthus swynnertonii	*Plectranthus swynnertonii* S.Moore	Forest gardens	(VAN JAARSVELD, 2006)
		Groundcover	(VAN JAARSVELD, 2006)
Plectranthus tetensis	*Plectranthus tetensis* (Baker) Agnew	Bushveld gardens	(VAN JAARSVELD, 2006)
		Groundcover	(POOLEY, 1998)

Table 1.1 continued

Plectranthus tetragonus	*Plectranthus tetragonus* Gürke	Bushveld gardens	(VAN JAARSVELD, 2006)
Plectranthus unguentarius	*Plectranthus unguentarius* Codd	Bushveld gardens	(VAN JAARSVELD, 2006)
Plectranthus venteri[6]	*Plectranthus venteri* Van Jaarsv. & Hankey	Bushveld gardens	(VAN JAARSVELD, 2006)
		Rockeries	(VAN JAARSVELD, 2006)
Plectranthus verticillatus	*Plectranthus verticillatus* (L.f.) Druce	Container	(VAN JAARSVELD, 2006)
		Garden	(LUKHOBA *et al.*, 2006)
			(VAN JAARSVELD, 1987)
		Groundcover	(POOLEY, 1998)
			(VAN JAARSVELD, 1987, 2006)
			(JOFFE, 2001)
		Hanging baskets	(POOLEY, 1998)
			(VAN JAARSVELD, 1987)
			(JOFFE, 2001)
Plectranthus woodii	*Plectranthus woodii* Gürke *Plectranthus hadiensis* (Forssk.) Schweinf. ex Spreng. var. *woodii* (Gürke) Codd	Rockery	(VAN JAARSVELD, 2006)
Plectranthus xerophilus	*Plectranthus xerophilus* Codd	Bushveld gardens	(VAN JAARSVELD, 2006)

Table 1.1 continued

Plectranthus zuluensis	*Plectranthus zuluensis* T.Cooke	Container	(POOLEY, 1998) (VAN JAARSVELD, 1987) (JOFFE, 2001)
		Garden	(COE, 2001) (VAN JAARSVELD, 1987) (JOFFE, 2001)
		Groundcover	(VAN JAARSVELD, 2006)
		Subtropical garden	(VAN JAARSVELD, 2006)

[1] Not native to South Africa; [2] Critically rare; [3] Near Threatened; [4] Data deficient-insufficient information; [5]Possibly threatened; [6] Rare; [7] Data deficient-Taxonomically problematic; [8] Vulnerable.

1.3 Flower abscission

Abscission is broadly defined as the "process of cutting off or violent separation" (SEXTON & ROBERTS, 1982). In botanical terms it is the process which regulates the detachment of organs from the main body of a plant (PATTERSON & BLEECKER, 2004). The term may however be applied to three mechanisms in the plant (SEXTON & ROBERTS, 1982), the most widely debated of which involves the dissolution of the wall between adjacent cells at the point of detachment (SEXTON & ROBERTS, 1982; VAN DOORN & STEAD, 1997) resulting in the shedding of organs from the parent plant (VAN DOORN & STEAD, 1997; TAYLOR & WHITELAW, 2001). Senescence is the wilting or withering of plant parts, usually petals, flowers or leaves (VAN DOORN & WOLTERING, 2008), rather than the detachment of the plant part. This term is often confused with abscission. Abscission is a natural process in the development of a plant and may involve the loss of leaves, petals, sepals, stamens, style, entire flowers, inflorescences and fruit (ASCOUGH *et al.*, 2005).

A progression of biochemical events leads to the breakdown of the cell walls in one or two rows of cells on either side of the detachment site (SEXTON & ROBERTS, 1982). These areas in the plant are known as abscission zones. These cells are morphologically distinct from others in the tissue before abscission occurs (TAYLOR & WHITELAW, 2001), suggesting that they are important in understanding abscission. In flowers the abscission zone is most often at the base of the corolla (SANKHLA *et al.*, 2005). In *Plectranthus* however, there are two abscission zones at which flowers detach from the plant (unpublished observations). The first seems to be at the base of the corolla, the corolla abscises leaving the ovary and sepals attached to the plant. The second abscission zone is behind the sepals, as the whole flower including the corolla abscises.

Abscission may take place as part of the development of the plant or as a response to environmental factors or stresses (TAYLOR & WHITELAW, 2001). Flowers are abscised following pollination and fruits are abscised when they are ripe. The abscission of leaves in temperate plants allows them to survive through winter (TAYLOR & WHITELAW, 2001). In the horticultural sector, flower abscission can

lead to significant loss of quality, yield (MARCELIS *et al.*, 2004; ASCOUGH *et al.*, 2006; ASCOUGH *et al.*, 2008) and ultimately crops (TAYLOR & WHITELAW, 2001). Shipping of both cut and potted plants often results in flower abscission and a product which is not wanted by the retailer. A method for preventing abscission, specifically shipping-induced flower abscission, in commercial species is important. In order to achieve this, flower abscission and the factors that control and affect it, must be understood.

There are a host of factors, both internal and external, which effect flower abscission. These include temperature (ALONI *et al.*, 1996; BENO-MOUALEM *et al.*, 2004; MARCELIS *et al.*, 2004; ASCOUGH *et al.*, 2005), light intensity and photoperiod (TAYLOR & WHITELAW, 2001; ASCOUGH *et al.*, 2005), limited water supply (MARCELIS *et al.*, 2004), carbohydrates (ALONI *et al.*, 1996), gene expression and enzymes (TAYLOR & WHITELAW, 2001), as well as plant hormones (MARCELIS *et al.*, 2004).

1.3.1 Factors effecting flower abscission

1.3.1.1 Plant Growth Hormones

It is well known that ethylene plays a major role in abscission, as a result ethylene will be discussed separately from the other plant growth hormones. It has been shown that hormones other than ethylene play a role in regulating abscission (PATTERSON, 2001). Hormone levels and the sensitivity of plants to hormones can induce flower abscission (SANKHLA *et al.*, 2005), and the interaction between ethylene and other hormones (auxins, gibberellic acid and ABA) is also important (PIERIK *et al.*, 2006). Next to ethylene, auxin is the hormone that is reported on most widely in abscission studies.

Although abscission is regulated by ethylene, auxin is also one of the main regulators (BUNYA-ATICHART *et al.*, 2006), and fine tunes abscission regulation (SANKHLA *et al.*, 2003). The application of auxin reduces flower abscission in some species (ASCOUGH *et al.*, 2005; MEIR *et al.*, 2006).

The balance between auxin and ethylene is important in flower abscission (ALONI *et al.*, 1996; ABEBIE *et al.*, 2005; ABEBIE *et al.*, 2008). For example, a decrease in auxin and an increase in ethylene initiated cell separation in the abscission zone of *Cestrum elegans* cut flowers (ABEBIE *et al.*, 2008).

This balance can determine when and from where abscission will take place (TAYLOR & WHITELAW, 2001). The timing of abscission is affected by increased ethylene sensitivity, and auxin is responsible for this sensitivity of the abscission zone. A polar IAA (indole-3-acetic acid) flux through the abscission zone makes it insensitive to ethylene (MEIR *et al.*, 2006; ABEBIE *et al.*, 2008). MEIR *et al*. (2006) found that deblading the leaves of *Mirabilis jalapa*, removing the source of auxin (IAA), caused the abscission zone to become sensitive to ethylene and abscission followed shortly after. It is thought that IAA controls the sensitivity of the abscission zone to ethylene by regulating the genes which control abscission zone sensitivity (MEIR *et al.*, 2006).

Exogenous auxin applied to plants can reduce abscission. Certain auxins are more effective in counteracting abscission than others. 2, 4-D (2,4-dicholorophenoxyacetic acid) in combination with STS (silverthiosulfate) reduced shipping-induced abscission in *Cestrum elegans*, while a similar treatment using NAA (1-naphthaleneacetic acid) had no effect on abscission (ABEBIE *et al.*, 2005). This may be due to differences in the polarity of transport of different auxins within the plant (ABEBIE *et al.*, 2005) and the accumulation of different auxins in different areas of the plant (ABEBIE *et al.*, 2008).

The differential accumulation of auxin in the abscission zone results in differential activation of auxin-induced genes. For example, 2,4-D increased gene expression of several auxin responsive genes in the abscission zone to a greater extent than NAA did (ABEBIE *et al.*, 2008). Auxin also affects the transport of hydrolytic enzymes within the plant. The movement of polygalacturonase (PG), which is involved in cell wall degradation, is hindered in the presence of auxin. The same effect is seen on β-1,4 endoglucanases, responsible for cell separation (MEIR *et al.*, 2006).

Abscission is also delayed by the application of cytokinins to the plant (ASCOUGH *et al.*, 2005). It is thought that cytokinins act in two ways to delay abscission. Firstly the presence of cytokinin is related to a decrease in ethylene production (ZIESLIN & GOTTESMAN, 1983). Secondly, cytokinins lower the buildup of ABA (abscisic acid) and reduce the sensitivity to ethylene, thereby delaying flower abscission (CHANG *et al.*, 2003).

The majority of research on the roles of cytokinins in abscission focuses on fruit abscission (MARCELIS *et al.*, 2004) particularly in soybean (CROSBY *et al.*, 1981; CARLSON *et al.*, 1987; NAGEL *et al.*, 2001). The commercial importance of soybean has led to research around preventing pod abortion, thereby increasing fruit set using exogenous cytokinin applications (NAGEL *et al.*, 2001). There is a gap in the literature with regard to changes in endogenous cytokinins during the process of abscission.

Flower abscission in *Capsicum annuum* was completely suppressed when treated with cytokinin in combination with gibberellic acid (WIEN & ZHANG, 1991). However in a study involving *Begonia*, gibberellic acid has no effect on abscission (HÄNISCH TEN CATE & BRUINSMA, 1973) suggesting that gibberellic acid had different effects in different species.

The literature is contradictory on the role of ABA in flower abscission. Although the name suggests otherwise, PATTERSON (2001) believes that ABA plays only a minor role in flower abscission. ABA increases the levels of ACC in the plant (PATTERSON, 2001). As a result there is an increase in the conversion of ACC to ethylene and an increase in ethylene production. There is evidence that ABA increases flower abscission (SWANSON *et al.*, 1975), and conflicting evidence that it has no effect on flower abscission (PORTER, 1977). In cocoa flowers, abscission was induced by ABA (ANEJA *et al.*, 1999). The extent to which ABA affects abscission thus remains unclear.

1.3.1.1.1 Ethylene and the ethylene biosynthetic pathway

Structurally the simplest of the plant hormones, ethylene is a gaseous alkene (C_2H_4) (ALTMAN & SOLOMOS, 1995; JOHNSON & ECKER, 1998). It was first discovered in the 1800's when streets were illuminated with gas lamps. The trees growing around the lamps showed an increase in defoliation (PODD, 2000). In later years it was shown that the gas from the lamps was ethylene (PODD, 2000).

Ethylene plays a role at every stage of a plant's life (JOHNSON & ECKER, 1998). From germination (CZARNY *et al.*, 2006) where ethylene plays a role in the establishment of the hypocotyl; through plant growth and development, where it plays a protective role; through flower and fruit development (CZARNY *et al.*, 2006) to flower abscission and fruit ripening. It is essential for plant growth, development and survival (CZARNY *et al.*, 2006). Ethylene can promote growth and inhibit growth (PIERIK *et al.*, 2006), depending on the concentration and plant organ under examination.

Important as it is to plants, ethylene at high levels is usually detrimental to the plant (CZARNY *et al.*, 2006). Ethylene accelerates flower and corolla abscission (BROWN, 1997), and in some cases it induces flower abscission (ASCOUGH *et al.*, 2006). However it is not always the primary factor or even necessary for abscission to occur (BROWN, 1997).

The triple response shown by dark-grown seedlings when subjected to ethylene is useful in determining sensitivity to ethylene. Seedlings show exaggerated curvature of the apical hook, radial swelling of the hypocotyl, as well as inhibited elongation of the hypocotyl and root (JOHNSON & ECKER, 1998). Seedlings which do not display these traits are said to be insensitive to ethylene. Ethylene sensitivity can differ among cultivars (BUNYA-ATICHART *et al.*, 2006), for this reason it was important to test more than one variety of *Plectranthus* with regard to ethylene sensitivity.

Ethylene insensitive species do not show increased flower abscission in the presence of exogenous ethylene. Ethylene insensitivity can be obtained by mutations to the ethylene induced genes (PATTERSON & BLEECKER, 2004). Ethylene sensitivity of

flowers can change as flowers develop, the abscission zone of flower buds in *Dendrobium* is sensitive to ethylene while the abscission zone of open flowers is not (BUNYA-ATICHART *et al.*, 2006). This suggests that the opening of flowers causes them to become insensitive to ethylene (BROWN, 1997).

The first step in the synthesis of ethylene is the conversion of MET (methionine) to SAM (*S*-adenosyl-methionine), which is then converted by ACC synthase to ACC (1-aminocyclopropane-1-carboxylic acid) (JOHNSON & ECKER, 1998; PIERIK *et al.*, 2006; SEREK *et al.*, 2006). Next ACC oxidase converts ACC to ethylene (JOHNSON & ECKER, 1998; SEREK *et al.*, 2006; PIERIK *et al.*, 2006). Ethylene is perceived by ethylene receptors (e.g. ETR1, ETR2, ERS1, ERS2 and EIN4), to which ethylene binds (PIERIK *et al.*, 2006). It is at the receptor level that the ethylene responses can be targeted and those such as flower abscission can be prevented (SISLER & SEREK, 1999; SEREK *et al.*, 2006; SISLER, 2006). It is thought that there is a metal involved in binding. Ethylene must first bind to a metal and withdraw ions from the metal before it binds to the receptor (SISLER & SEREK, 1999).

It is interesting that the leaves and stems of *Plectranthus barbatus* are used to make bananas ripen quickly (LUKHOBA *et al.*, 2006). This suggests that they readily produce ethylene. This ethylene could build up during transport and cause flowers to abscise.

1.3.1.2 Carbohydrates

Sugars, produced through photosynthesis, are the source of energy in a plant. Sugars therefore, have a marked effect on many of the processes occurring within the plant. Depletion of their sugar reserves will result in the death of the plant (REID *et al.*, 2002).

Within a plant there are regions known as the source (assimilate supply) and the sink (assimilate demand) (MARCELIS *et al.*, 2004). The mature leaves, as the site of assimilate production are the source, while flowers and flower buds, requiring assimilates, are a sink. The source strength and availability of carbohydrates in the plant has an effect on flower and flower bud abscission (ALONI *et al.*, 1996;

MARCELIS *et al.*, 2004) and also on the display life of potted plants (REID *et al.*, 2002).

When assimilate supply is low there is competition between flowers and the adjacent developing leaves (ALONI *et al.*, 1996). As flowers are an energetically expensive organ to produce and maintain, these will be shed in order to allow assimilates to be used in the leaves.

Low light conditions will decrease photosynthesis (REID *et al.*, 2002), which in turn will decrease the assimilate supply within the plant and the source strength (MARCELIS *et al.*, 2004). A decrease in photosynthesis will therefore cause an increase in abscission (ALONI *et al.*, 1996). The photosynthetic rate of plants indoors is too low to produce enough carbohydrates. REID *et al*. (2002) showed that potted hibiscus plants growing indoors produced less than half the carbohydrates needed to sustain the plants and produce flowers. When photosynthesis is low a high percentage of flowers are shed, as observed in *Dendrobium* species (BUNYA-ATICHART *et al.*, 2006).

Sugars have been shown to counteract the effects of ethylene during shipping and improve postharvest display life in phlox flowers (SANKHLA *et al.*, 2005). Exactly how they counteract ethylene action is still unknown, however, there are a number of levels at which sugar could affect ethylene.

Sugar has a series of effects at the cell wall level (O'DONOGHUE *et al.*, 2002) which affect abscission. Sugar is involved in the activation of the enzymes responsible for cell wall synthesis (O'DONOGHUE *et al.*, 2002). While sugar starvation enhances the action of enzymes which breakdown proteins and lipids (VAN DOORN, 2004), the main constituents of cell membranes, sugars may protect the cell walls and cell membranes of cells in the abscission zones.

Sugars may play a role in the sensitivity of the abscission zone. BUNYA-ATICHART *et al*. (2006) suggest that the insensitivity of some plant species to ethylene may be due to a buildup of carbohydrates in the abscission zones. There is a buildup of carbohydrates in the abscission zones of open flowers of *Dendrobium*, but not in the

abscission zones of closed buds (BUNYA-ATICHART *et al.*, 2006). This could explain the difference in sensitivity of open flowers and closed buds observed by these authors, where closed buds were preferentially abscissed.

Sugar has an influence on ethylene production (O'DONOGHUE *et al.*, 2002; SANKHLA *et al.*, 2005). However, it is not yet known exactly how sugar effects ethylene production. It counteracted the effects of ethylene and ACC on plants, shown as the prevention of flower abscission, (SANKHLA *et al.*, 2005), suggesting that it may block the conversion of ACC to ethylene by the enzyme ACC oxidase.

Other enzymes are also affected by an excess or lack of sugar. When photosynthesis decreases, sugar starvation induces the expression of senescence associated genes (VAN DOORN, 2004). Sugar starvation may have similar effects on the enzymes involved in abscission.

Many studies indicated that a lack of carbohydrates increases abscission, however in previous studies on *Plectranthus*, low photosynthesis resulting in a decrease in carbohydrates did not increase abscission (ASCOUGH *et al.*, 2006). This may however not be the case for all varieties of *Plectranthus*.

1.4 Control of flower abscission

As flower abscission is such a problem for producers of ornamental crops, methods to prevent flower abscission have been widely researched. Particular attention has been paid to shipping- and ethylene-induced flower abscission.

1.4.1 Physiological control of flower abscission

There are a number of compounds which inhibit the action and the negative effects of ethylene. These include STS (silverthiosulfate), 1- MCP (1-methylcyclopropene), AOA (aminooxyacetic acid), AIB (α-aminoisobutyric acid), AVG (aminoethoxyvinyl glycine), DPSS (1,1-dimethyl-4-phenilsulfonylsemicarbazide), TDZ (thidiazuron) and DACP (diazocyclopentadiene) (ICHIMURA *et al.*, 2002). They may be used in the commercial sector to prevent flower abscission especially during transportation.

The most commonly used, especially during transport, are STS and 1-MCP (ICHIMURA *et al.*, 2002; PHILOSOPH-HADAS *et al.*, 2005). 1-MCP binds to ethylene receptors, thereby preventing ethylene binding to its receptor and having an effect on the plant (WATKINS, 2006). 1-MCP competes with ethylene for receptor sites and has a higher affinity for binding to the receptor sites than ethylene. 1-MCP reduces the conversion of ACC to ethylene, reducing ethylene production by plants (UTHAICHAY *et al.*, 2007) STS alters the structure of the receptor site preventing ethylene from binding to the receptor (ROBERTS *et al.*, 1995).

The shelf life of a number of horticultural species has improved by the use of 1-MCP (Table 1.2). 1-MCP has been reported to effectively prevent petal and flower abscission in simulated transport experiments (JONES *et al.*, 2001; SANKHLA *et al.*, 2003; PHILOSOPH-HADAS *et al.*, 2005; KIM *et al.*, 2007; UTHAICHAY *et al.*, 2007) and so may be used as a powerful tool for preserving the quality of potted plants and facilitating their prolonged transport (PHILOSOPH-HADAS *et al.*, 2005).

Each compound has advantages and disadvantages to their use. In *Geraniums*, STS predisposed plants to *Phythium* root rot (HAUSBECK *et al.*, 1987). STS contains silver which is a heavy metal, an environmental pollutant (SISLER & SEREK, 1997; ICHIMURA *et al.*, 2002; KIM *et al.*, 2007) and toxic to plants at certain concentrations (PHILOSOPH-HADAS *et al.*, 2005). This means that, when using STS, there is a narrow range of effective, non-toxic concentrations which may be applied to the plant (JONES *et al.*, 2001). 1-MCP is non-toxic, odourless (SISLER & SEREK, 1997) and easier to administer. However, STS remains in the plant for longer than 1-MCP.

The gaseous nature of 1-MCP causes the unbound compound to diffuse out of the plant tissue after treatment (KIM *et al.*, 2007). STS on the other hand is sprayed onto the plant (ROBERTS *et al.*, 1995) and remains in the plant and is mobile within the plant (ICHIMURA *et al.*, 2002), allowing for STS to bind to new receptors synthesized during plant growth and development. STS provides longer-lived protection.

Table 1.2: The use of 1-MCP in preventing flower abscission in horticulturally important species showing the most effective concentrations and treatment duration.

Plant Species	Concentration	Treatment duration (h)	Reference
Alstroemeria spp	20 nl l^{-1}	6	(SEREK *et al.*, 1995b)
Antirrhinum majus	20 nl l^{-1}	6	(SEREK *et al.*, 1995b)
Begonia x *elatior* 'Najada' and 'Rosa'	5 or 20 nl l^{-1}	6	(SEREK *et al.*, 1994, 1995a)
Begonia x *tuberhybrida* 'Non-Stop'	5 or 20 nl l^{-1}	6	(SEREK *et al.*, 1994, 1995b)
Boronia heterophylla	10 nl l^{-1}	12	(MACNISH *et al.*, 1999)
Campanula carpatica 'Dark Blue'	20, 50 or 100 nl l^{-1}	6	(SISLER & SEREK, 1999)
C. carpatica 'Dark Blue' and 'Blue Clips'	20, 50 or 100 nl l^{-1}	6	(SEREK & SISLER, 2001)
C. medium 'Champion Pink'	800 nl l^{-1}	4	(BOSMA & DOLE, 2002)
Cassinia adunca	10 nl l^{-1}	12	(MACNISH *et al.*, 2000)
Ceratopetalum gummiferum	10 nl l^{-1}	12	(MACNISH *et al.*, 2000)
Chamelaucium uncinatum	10 nl l^{-1}	12	(MACNISH *et al.*, 2000)
C. uncinatum	200 nl l^{-1}	6 or 13	(SEREK *et al.*, 1995b)
Consolido ambigua	20 nl l^{-1}	6	(SEREK *et al.*, 1995b)
Cymbidium sp	0, 0.1, 1 or 10 µl l^{-1}	20	(SEREK *et al.*, 2006)
Dendranthema grandiflorum 'Coral Charm'	200 nl l^{-1}	6	(SEREK *et al.*, 1998)
Dendrobium 'Karen'	0, 100, 200, 300, 400 or 500 nl l^{-1}	4	(UTHAICHAY *et al.*, 2007)
Dianthus barbatus	20 nl l^{-1}	6	(SEREK *et al.*, 1995b)
D. caryophyllus 'Sandra'	0.6, 1.7, 3.3, 5.8 or20 nl l^{-1}	6	(SEREK *et al.*, 1995b, a)
D. caryophyllus 'White Sim' or 'Sandra'	5 nl l^{-1}	12	(SISLER *et al.*, 1996)
Epipremnum pinnatum	200 nl l^{-1}	6	(MULLER *et al.*, 1997)
Eriostemon scabe	10 nl l^{-1}	12	(MACNISH *et al.*, 2000)
Grevillea 'Kay Williams'	10 nl l^{-1}	12	(MACNISH *et al.*, 2000)
Grevillea 'Misty Pink'	10 nl l^{-1}	12	(MACNISH *et al.*, 2000)
Gypsophila paniculata 'Perfecta', 'Gilboa', and 'Golan'	200 nl l^{-1}	24	(NEWMAN *et al.*, 1998)
Hibiscus rosa-sinensis	200 nl l^{-1}	6	(REID *et al.*, 2002)
H. rosa-sinensis	200 nl l^{-1}	6	(SEREK *et al.*, 1998)
Ixora coccinea 'Big Red'	100 nl l^{-1}	8	(MICHAELI *et al.*, 1999)
Kalanchoe blossfeldiana 'Tropicana'	5 or 20 nl l^{-1}	6	(SEREK *et al.*, 1994; PORAT *et al.*, 1995)

Table 1.2 continued

Kalanchoe 'Alexandria', 'Debbie', 'Caroline', 'Jaqueline', 'Nadia', 'PaleJaqueline', and 'Simone'	200 nl l^{-1}	6	(SEREK & REID, 2000)
K. blossfeldiana	0.5, 2.5, 5 or 10 nl l^{-1}	6	(SISLER & SEREK, 1999)
Leptospermum petersonii	10 nl l^{-1}	12	(MACNISH *et al.*, 2000)
L. scoparium	10 nl l^{-1}	12	(MACNISH *et al.*, 2000)
Lilium 'Mona Lisa'	500 nl l^{-1}	18	(CELIKEL *et al.*, 2002)
Lilium 'Stargazer'	500 nl l^{-1}	18	(CELIKEL *et al.*, 2002)
Lilium 'Cordelia' and 'Elite'	150 nl l^{-1}	6	(ELGAR *et al.*, 1999)
L. longiflorum 'Lorena'	150 nl l^{-1}	6	(ELGAR *et al.*, 1999)
Lupinus havardii 'Texas Sapphire'	450 nl l^{-1}	12	(PICCHIONI *et al.*, 2002)
Matthida incana	20 nl l^{-1}	6	(SEREK *et al.*, 1995b)
M. incana	500 nl l^{-1}	6	(CELIKEL & REID, 2002)
Metrosideros collina	0, 1.5, 15, or 150 nl l^{-1}	6	(SUN *et al.*, 2000)
Ozothammus diosmifolius	10 nl l^{-1}	12	(MACNISH *et al.*, 2000)
Pelargonium x *hortorum*	0.1 or 1.0 nl l^{-1}	3, 6, 12 or 24	(JONES *et al.*, 2001)
P. zonale 'Isabel'	200 nl l^{-1}	6	(SEREK *et al.*, 1998)
P. peltatum 'Pink Blizzard'	1 nl l^{-1}	2	(CAMERON & REID, 2001)
Penstemon 'Firebird'	20 nl l^{-1}	6	(SEREK *et al.*, 1995b)
Petunia hybridia 'Pink Cascade'	150 nl l^{-1}	6	(SEREK *et al.*, 1995c)
Phalaenopsis 'Herbert Hager'	250 nl l^{-1}	6	(PORAT *et al.*, 1995)
Phalenopsis 'Sogo'	500 ppt	12	(SUN *et al.*, 2009)
Phlox paniculata 'Rembrandt'	25, 250 or 500 nl l^{-1}	6	(PORAT *et al.*, 1995)
Platysace lanceolata	10 nl l^{-1}	12	(MACNISH *et al.*, 2000)
Rosa 'Royal' and 'Sunset'	100 nl l^{-1}	6	(SEREK *et al.*, 1996)
Rosa 'Vanilla' and 'Bronze'	200 nl l^{-1}	6	(MULLER *et al.*, 1999)
R. hybrida 'Samantha'	0.5 or 2 ppm	12	(MA *et al.*, 2006)
R. hybrida 'Victory Parade'	5 or 20 nl l^{-1}	6	(SEREK *et al.*, 1994, 1995a)
Schlumbergera truncata 'Dark Marie'	20, 50 or 100 nl l^{-1}	6	(SEREK & SISLER, 2001)
Telopea 'Shady Lady'	10 nl l^{-1}	12	(MACNISH *et al.*, 2000)
Thryptomene calycina	10 nl l^{-1}	12	(MACNISH *et al.*, 2000)
Tulipa gesneriana 'Apeldoorn'	1 nl l^{-1}	16	(DE WILD *et al.*, 2002)
Verticordia nitens	10 nl l^{-1}	12	(MACNISH *et al.*, 2000)
Zieria cytisoides	10 nl l^{-1}	12	(MACNISH *et al.*, 2000)

It has been reported that 1-MCP had no effect on the performance of certain plants after transport (KIM *et al.*, 2007), and so it does not improve the display life of plants.

On the contrary it was noted that 1-MCP prevented the effects of ethylene for up to seven days after ethylene treatment in *Dendrobium* (UTHAICHAY *et al.*, 2007) and 12 days in carnations (SISLER & SEREK, 1997).

Although 1-MCP binds irreversibly to the ethylene receptor (ICHIMURA *et al.*, 2002), further growth of the plant produces new ethylene receptor sites. This means that a single 1-MCP treatment will not protect the plant indefinitely. This is not a problem in cut flowers such as carnation that exhibit little or no growth after retail, but must be considered in studies involving potted plants.

Antagonists of ethylene action such as 1-MCP prevent abscission during shipping, but only reduced ethylene sensitivity obtained through breeding improves the quality during the entire post production period (KIM *et al.*, 2007). STS has been banned in several countries due to its toxicity (SRISKANDARAJAH *et al.*, 2004). It would therefore be favorable to render the plant insensitive to ethylene rather than use chemicals such as 1-MCP and STS. Ethylene insensitivity can also be achieved through gene transfer methods (CHANDLER & LU, 2005), such as genetic transformation which is an alternative to traditional breeding methods (SRISKANDARAJAH *et al.*, 2004; SRISKANDARAJAH *et al.*, 2007).

1.4.2 Genetic control of flower abscission

Desirable genes can be incorporated into the genome of a plant through breeding or alternatively by gene transfer methods. The latter requires less time and is more precise (CHANDLER & LU, 2005). Certain traits which have been impossible to include in plants through breeding have been easily introduced into the plant's genome by genetic transformation (SRISKANDARAJAH *et al.*, 2004; SRISKANDARAJAH *et al.*, 2007). Engineered traits are those which are valuable to either the consumer or the producer (TANAKA *et al.*, 2005).

Gene transfer methods include: microprojectile bombardment or biolistic transformation; infiltration with *Agrobacterium tumefaciens* or *in planta* transformation; sonication-assisted *Agrobacterium*-mediated transformation; electroporation; direct DNA uptake mediated by electroporation or polyethylene

glycol treatment; and *Agrobacterium*-mediated transformation (CHANDLER & LU, 2005).

In the horticulture sector, *Agrobacterium*-mediated transformation is used to incorporate desirable genes into crops. Ethylene sensitivity may be reduced by genetic transformation (KIM *et al.*, 2007). More than 20 floral crops have been successfully transformed (CHANDLER & LU, 2005). Ethylene insensitivity has been gained in carnation (BOVY *et al.*, 1999), *Nemesia strumosa* (CUI *et al.*, 2004), *Campanula carpatica* (SRISKANDARAJAH *et al.*, 2004) through transformation mediated by *Agrobacterium tumefaciens*. This has successfully prevented ethylene induced abscission. In one transformed species, flowers remained on the plant for 27 d after exposure to ethylene, while flowers on the wild type plant abscised within 3 d of ethylene exposure (SRISKANDARAJAH *et al.*, 2007).

The allele that confers ethylene insensitivity is the *etr1-1* allele (BOVY *et al.*, 1999; SRISKANDARAJAH *et al.*, 2004; SRISKANDARAJAH *et al.*, 2007), which is a mutant *ERS1* gene (SRISKANDARAJAH *et al.*, 2007). There are four *etr1* mutant alleles. They contain a missense mutation near the amino terminus of the predicted protein (CHANG *et al.*, 1993). The *etr1-1* allele is dominant (SRISKANDARAJAH *et al.*, 2007) and so confers ethylene insensitivity in heterologous individuals (BOVY *et al.*, 1999). This allele comes from *Arabidopsis thaliana* (BOVY *et al.*, 1999) which is insensitive to ethylene.

In *A. thaliana* the *etr1-1* allele is under the control of its own promoter, CaMV 35S (BOVY *et al.*, 1999). However when this allele was first incorporated into plants, it was found that it not only affected ethylene mediated processes in the flowers but also in the rest of the plant. It is therefore important for the mutant allele to be under the control of an abscission- (SRISKANDARAJAH *et al.*, 2007) or a flower–specific promoter (BOVY *et al.*, 1999). This prevents the mutant allele from affecting any plant process other than abscission.

Obtaining transgenic plants which are insensitive to ethylene is favorable over the use of antagonists of ethylene action as transgenic plants have been shown to perform better than plants treated with STS under shipping conditions (BOVY *et al.*, 1999).

STS is also costly (SRISKANDARAJAH *et al.*, 2004) and not environmentally friendly (SRISKANDARAJAH *et al.*, 2007). Genetic transformation is an exciting means to obtain ornamental crops with a greatly enhanced shelf-life (SRISKANDARAJAH *et al.*, 2004).

1.5 Aims

Transport-induced flower abscission is a problem which affects many different varieties of South African plants. A comprehensive understanding of the triggers of flower abscission during transport will aid in developing a protocol to control transport-induced flower abscission. This work aims to examine a number of the factors which influence transport-induced flower abscission in order to understand the endogenous changes which occur. Using what is learned about the endogenous changes it is hoped that a method for reducing or preventing transport-induced flower abscission may be developed.

Chapter 2

Plectranthus as a potted plant

2.1 Introduction

Plectranthus is considered the largest genus in the Lamiaceae in southern Africa, with approximately 53 southern African species described to date (CODD, 1975, 1985; VAN JAARSVELD & EDWARDS, 1991; VAN JAARSVELD & HANKEY, 1997; VAN JAARSVELD & EDWARDS, 1997; EDWARDS *et al.*, 2000; VAN JAARSVELD & VAN WYK, 2004; WINTER & VAN JAARSVELD, 2005; EDWARDS, 2005). Despite this large number of species, relatively few are popular as ornamental plants in South Africa. Nevertheless the number of species that are being introduced into gardens is steadily increasing.

Plectranthus is a horticulturally important genus of predominantly herbaceous plants that is becoming increasingly popular in indigenous landscaping in South Africa. Some species are suitable as shrubs or may be pruned into hedges; some make good groundcovers. In addition there are good species for rockeries and succulent gardens, and a number of species thrive in large containers, pots and hanging baskets. Most species are easily grown from cuttings or seed and require little maintenance other than a need for pruning at the end of the flowering season (VAN JAARSVELD, 2006), making them a welcome addition to any garden.

Species of *Plectranthus* have been grown in hanging baskets in Europe for decades and one such species, *P. oertendahlii* T.C.E.Fr. ('Swedish Ivy'), was named from a cultivated plant in 1924 despite having an unknown wild origin. It has been in cultivation in Sweden for over 100 years. A specimen collected from Oribi Gorge, on the KwaZulu-Natal South coast of South Africa, by L. Britten in 1936, remained unidentified until 1974. Material collected from Oribi Gorge by H. Nicholson in 1971/72 was identified in 1973 and finally the mystery was solved (CODD, 1977). It is thought that plants made their way to Scandinavia via the Hermannsburg Mission

Society in southern KwaZulu-Natal, or via a Swedish surveyor working at Uvongo (VAN JAARSVELD, 2006).

A continuous demand for novelty has become axiomatic in the field of flower market development. In Europe the cooler climate favours mostly new kinds of potted plants whilst elsewhere new types of both garden and potted subjects are sought. The contribution of South Africa's spectacular wildflowers to world floriculture spans several centuries and has yielded major new flower crops to world floriculture – e.g. *Gerbera* L.*, Gladiolus* L.*, Pelargonium* L'Her. ex Aiton. The herbaceous *Plectranthus* species is a fairly new resource in the continued exploitation of wildflower diversity.

Plectranthus make very good candidates for development as potted plants as they occur naturally on semi-dry forest floors and enjoy shade. They thus fulfil all the requirements of indoor plants (VAN JAARSVELD, 1987). From an aesthetic side *Plectranthus* are compact plants with attractive, often coloured, leaves and brightly coloured flowers (BRITS *et al.*, 2001).

2.2 Breeding in *Plectranthus*

The wide variety in terms of flower and leaf colour offered by *Plectranthus* makes it an exciting genus for breeding projects. These projects were focused on enhancing the attractive qualities of the genus. The continued demand for new and exciting plant varieties has meant that breeding work on *Plectranthus* has continued and will continue in the future.

2.2.1 Previous breeding projects

Breeding work on *Plectranthus* in South Africa has been predominantly carried out at Kirstenbosch in Cape Town. The focus of the *Plectranthus* breeding programs has been aimed at semi-shade garden plants and flowering potted plants (VAN JAARSVELD, 1987).

Plectranthus first attracted the attention of Ernst Van Jaarsveld in 1974 while he was working as horticulturist at Kirstenbosch Botanical Garden in Cape Town, South

Africa (VAN JAARSVELD, 2006; RICE *et al.*, 2011). While at Kirstenbosch Van Jaarsveld released a number of *Plectranthus* ecotypes under their botanical variety names or more commercially acceptable popular names as new horticultural varieties (RICE *et al.*, 2011). Van Jaarsveld's initial breeding work was aimed at producing new varieties for gardens. Examples of these are 'King Goodwill' and 'Nkandla' which are varieties of *Plectranthus saccatus* (VAN JAARSVELD, 1994). The discovery of a spectacular *Plectranthus hilliardiae* specimen in the Transkei, prompted Van Jaarsveld to hybridize the plant with *Plectranthus saccatus* (RICE *et al.*, 2011). Further work on this particular hybridization by Roger Jacques, also a horticulturist at Kirstenbosch lead to the development of 'Mona Lavender' in 2002 (VAN JAARSVELD, 2006). 'Mona Lavender' is the most popular commercial *Plectranthus* in South Africa to date and marked the beginning of *Plectranthus* breeding in South Africa (RICE *et al.*, 2011).

After the success of 'Mona Lavender', protea breeder, Gert Brits became interested in breeding *Plectranthus* as potted plants (VAN JAARSVELD, 2006; RICE *et al.*, 2011). Brits screened the genus for species which would perform well as potted plants. The following *Plectranthus* types were chosen in accordance with potted and garden plant industry standards, for further work:
P. ambiguus, P. hilliardiae, P. reflexus and *P. saccatus* were selected for their larger flowers; *P. ciliatus, P. ecklonii* 'Tommy', *P. saccatus* Benth. var. *longitubus* Codd 'King Goodwill' (VAN JAARSVELD, 1994) and *P. verticillatus* were selected for their white flower colour; *P. ecklonii* 'Erma', *P. fruticosus* and *P. verticillatus* 'Pink Surprise' were selected as pink flower varieties; *P. ambiguus, P. ecklonii* 'Medley Wood', *P. hilliardiae, P. praetermissus, P. saccatus* and *P. zuluensis* were the selected blue flower varieties; *P. hilliardiae, P. oertendahlii, P. praetermissus* and *P. verticillatus* were selected for their compact growth habit; *P. hilliardiae, P. oertendahlii* and *P. praetermissus* were selected for their decorative leaves (MILLER & MORGAN, 2000); *P. ernstii* Codd, *P. neochilus* Schltr., *P. purpuratus* Harv. and *P. verticillatus* were selected for their tolerance to full sunlight.

In 2001 Brits released a series of varieties called 'Cape Angels' (VAN JAARSVELD, 2006). The 'Cape Angels' series includes varieties with pink (Figure 2.1), blue, purple and white flowers and is well known (VAN JAARSVELD, 2006).

Figure 2.1. 'Cape Angels Pink'.

2.2.2 Current breeding projects

Gert Brits continues to breed *Plectranthus* varieties for release as potted plants at his nursery in Stellenbosch, South Africa and new varieties are undergoing testing in South Africa, Europe and in the U.S.A. (RICE *et al.*, 2011). Recently the Keith Kirsten Horticultural International Group bought the rights to work on a number of Brits's varieties. They are in the process of introducing 5 new *Plectranthus* varieties in South Africa. The series of varieties has been named Jazz-It-Up® (Figure 2.2). These all have large flowers and it is hoped that they will be a milestone in *Plectranthus* breeding. VAN JAARSVELD (2006) believes that there is endless scope for breeding in this genus towards both potted and garden plants.

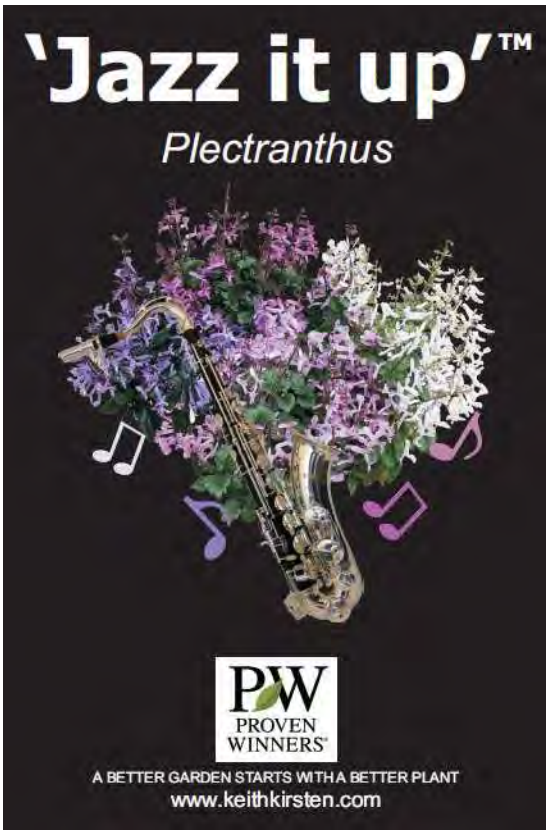

Figure 2.2. Promotional poster for the new 'Jazz it up' series of *Plectranthus* varieties.

2.3 Work on flower abscission in *Plectranthus*

Previous work has been carried out on flower abscission in *Plectranthus*. ASCOUGH *et al*. (2006) investigated flower abscission in excised inflorescences of *Plectranthus*. The study concluded that flower abscission in this genus was most likely attributed to endogenous ethylene production. They also found that flower abscission could not be controlled by ethylene antagonists used to control flower abscission in other plant species. This was very important preliminary work, however *Plectranthus* are sold as potted plants and not cut flowers and so it was not clear if the results from this study would apply to potted *Plectranthus* plants. In a later study ASCOUGH *et al*. (2008) worked on potted *Plectranthus* to develop a set of equations useful when investigating flower abscission in potted plants with multiple inflorescences.

2.4 Introduction to *Plectranthus* varieties used in this study

2.4.1 Plant material

Cuttings of eight *Plectranthus* varieties were generously provided by Dr G Brits of Brits Nursery in Stellenbosch. Cuttings were potted in potting mix comprising of 83% compost, 16.72% bark, 0.14% LAN and 0.14% 2:3:2 fertilizer and were kept for 60 d in the mist house at 22°C and 85% humidity at the University of KwaZulu-Natal, Pietermaritzburg (Figure 2.3). Once cuttings had formed and established a root system, individual plants were transferred to pots, 12 cm in diameter, and kept in the greenhouse under natural day length (Figure 2.4). Plants were watered every second day with approximately 10 ml of water and fertilized with a 1% seaweed concentrate (Kelpak®) every three weeks. Once a year, after flowering, plants were pruned and re-potted to encourage new growth.

Figure 2.3. *Plectranthus* cuttings during a 60 d rooting period in the mist house.

Figure 2.4. Potted cuttings in the greenhouse.

2.4.2 Descriptions of varieties

Eight different varieties of *Plectranthus* were used in this project. All varieties were supplied by Brits Nursery in Stellenbosch, South Africa and all are a product of the breeding endeavours of Dr Gert Brits. The different varieties are all very closely related as six of the eight varieties have the same first generation parents, *Plectranthus hilliardiae* and *Plectranthus saccatus*. One variety comes from a cross between two individuals of *Plectranthus saccatus*, and the other is the result of a natural mutation of *Plectranthus hilliardiae*. Individuals from these two species that showed favourable characteristics were used in crosses, the progeny of which were kept as a variety, or put though further crosses to obtain polyploids.

2.4.2.1 P 00 06 03

P 00 06 03 (Figure 2.5) is the result of a hybridization between *Plectranthus hilliardiae* and *Plectranthus saccatus*. This is a medium-sized variety (Figure 2.5a) with pink flowers (Figure 2.5d). This variety has a fairly compact growth habit (Figure 2.5b). Leaves are covered in small hairs and the abaxial surface is lighter than the adaxial surface (Figure 2.5c). Veins on the abaxial side of the leaf are dark pink in

colour. Plants reach a height of up to 32.6 cm with the largest leaves measuring 4.3 x 6.4 cm. Flowers are 25.81±1.56 mm long when fully open and pedicels are 9.10±0.51 mm long. Petals have dark pink spots (Figure 2.5e).

2.4.2.2 P 00 06 03A

P 00 06 03A (Figure 2.6) is the result of breeding *Plectranthus hilliardiae* and *Plectranthus saccatus*. This particular variety comes from one plant that emerged from this cross. The plant had larger flowers than the other plants in the F1 generation and was selected for this feature. This is a small and compact variety (Figures 2.6a & b) with pink flowers (Figure 2.6 d). The insides of the petals are scattered with many small dark pink spots (Figure 2.6e). Much like P 00 06 03 the leaves of this variety have a lighter abaxial surface with pink venation (Figure 2.6 c). The plants reach a height of 21.2 cm with the largest leaves measuring 5.5 x 6.7 cm. When fully open flowers are 32.04±0.81 mm in length and the pedicels are 9.36±0.74 mm long. This variety is not registered and as a result, it has not been released into the market.

2.4.2.3 P 05 04 08

P 05 04 08 (Figure 2.7) is the result of a second generation cross from parents *Plectranthus hilliardiae* X *Plectranthus saccatus*. It is thought that the second generation cross resulted in polyploidization forming an allotetraploid. This is a medium sized variety (Figure 2.7a), plants measure up to 36 cm in height, with a compact growth habit (Figure 2.7b). The flowers are dark pink (Figure 2.7d) and measure 24.49±0.48 mm in length when they are fully opened. Flowers are supported by pedicels which measure 8.61±0.60 mm in length. The petals at the opening to the flower have a few darker pink spots on them (Figure 2.7e). The leaves have a dark adaxial surface and a lighter abaxial surface with dark pink venation and pink colouring around the veins (Figure 2.7c). The largest leaves on the plants are 5.0 x 5.9 cm in size. This variety is registered as Jazz-It-Up® (4) and is in the process of being introduced into the market.

2.4.2.4 P 04 05 18

As is the case for P 05 04 08, P 04 05 18 (Figure 2.8) was formed by a second generation cross. The first generation cross was between parents *Plectranthus hilliardiae* X *Plectranthus saccatus*. This was followed by polyploidization and then a second generation cross between (*Plectranthus hilliardiae* X *Plectranthus saccatus*) (4n) X (*Plectranthus hilliardiae* X *Plectranthus saccatus*) (4n). This variety is thought to be an allotetraploid. This variety is quite tall (Figure 2.8a), reaching 38.6 cm, with a fairly compact growth habit (Figure 2.8b). The flowers are large and light pink (Figure 2.8d) and measure 29.89±2.01 mm when fully open. Pedicels are 10.42±0.59 mm long. The petals at the entrance to the flower are uniform in colour and are not spotted like in other varieties (Figure 2.8e). The largest leaves on the plant reach 6.0 x 6.0 cm in size. The abaxial surface of the leaves has a light pink colouring and the veins are also light pink in colour (Figure 2.8c). This variety is also a member of the Jazz-It-Up® series and will soon be released into the market as Jazz-It-Up® (3).

2.4.2.5 P 08 05 06

This variety (Figure 2.9) is the result of a third generation cross. The first generation cross was between *Plectranthus hilliardiae* X *Plectranthus saccatus*. The final cross was between P 00 06 03A (also used in this study) x P 05 03 07A (a natural polyploid sport of the variety P 05 03 07). This variety has medium sized plants (Figure 2.9a), reaching 33.5 cm in height. They have quite a messy growth habit (Figure 2.9b). The flowers are purple (Figure 2.9d) and when fully open they measure 33.89±1.37 mm long and the pedicels measure 10.20±0.52 mm long. Petals at the entrance to the flower have dark purple spots on them (Figure 2.9e). The leaves have a dark green adaxial surface and a dark purple abaxial surface (Figure 2.9c). The veins on the abaxial side are also dark purple in colour. The largest leaves on the plants measure 5.6 x 8.4 cm. This variety has not yet been registered or released.

2.4.2.6 P 00 06 07

P 00 06 07 (Figure 2.10) is the result of a cross between *Plectranthus hilliardiae* (4n) X *Plectranthus hilliardiae* (2n). This cross was planned to obtain a triploid (3n). The

plants are quite short (27 cm) (Figure 2.10a) and have a compact growth habit (Figure 2.10b). Leaves are large, shiny and dark green (9.5 x 6 cm). The abaxial leaf surface is lighter green with a slight purple colour in parts (Figure 2.10c). The veins are dark purple. Flowers are large and purple (Figure 2.10d) and when fully opened they are 24.30±1.51 mm long. Pedicles measure 9.50±0.51 mm. Petals at the entrance to the flower have dark purple spots on them (Figure 2.10e). This variety is registered as Cape Angels® Purple and is available to the public.

2.4.2.7 P 01 05 09

This variety (Figure 2.11) comes from a natural sport of *Plectranthus hilliardiae*. These are small (27 cm high) (Figure 2.11a) compact plants with a quite messy growth habit (Figure 2.11b). The leaves are dark green and very shiny on the adaxial surface while the abaxial surface is light green with light purple veins (Figure 2.11c). Leaves measure up to 5.8 x 3.7 cm in size. Flowers are purple (Figure 2.11d) and when completely open they are 22.49±1.75 mm long. Flowers are attached to pedicels measuring 8.11±0.18 mm. The petals at the opening to the flower are spotted with dark purple (Figure 2.11e). This variety has not yet been registered or released into the market.

2.4.2.8 P 96 04 06

P 96 04 06 (Figure 2.12) is the only variety involved in this study with white flowers (Figure 2.12d). Flowers measure 29.90±2.13 mm when completely opened and the petals are plain white with no markings (Figure 2.12e). Pedicels are 11.2±0.66 mm in length. Plants grow up to about 40 cm tall (Figure 2.12a) in a compact fashion (Figure 2.12b) and have small leaves (3.5 x 5 cm). Leaves have a light green adaxial surface and a yellow-green abaxial surface (Figure 2.12c). This variety resulted from a cross between *Plectranthus saccatus* X *Plectranthus saccatus*. Named, Vivid white, P 96 04 06 is not yet registered or available on the market.

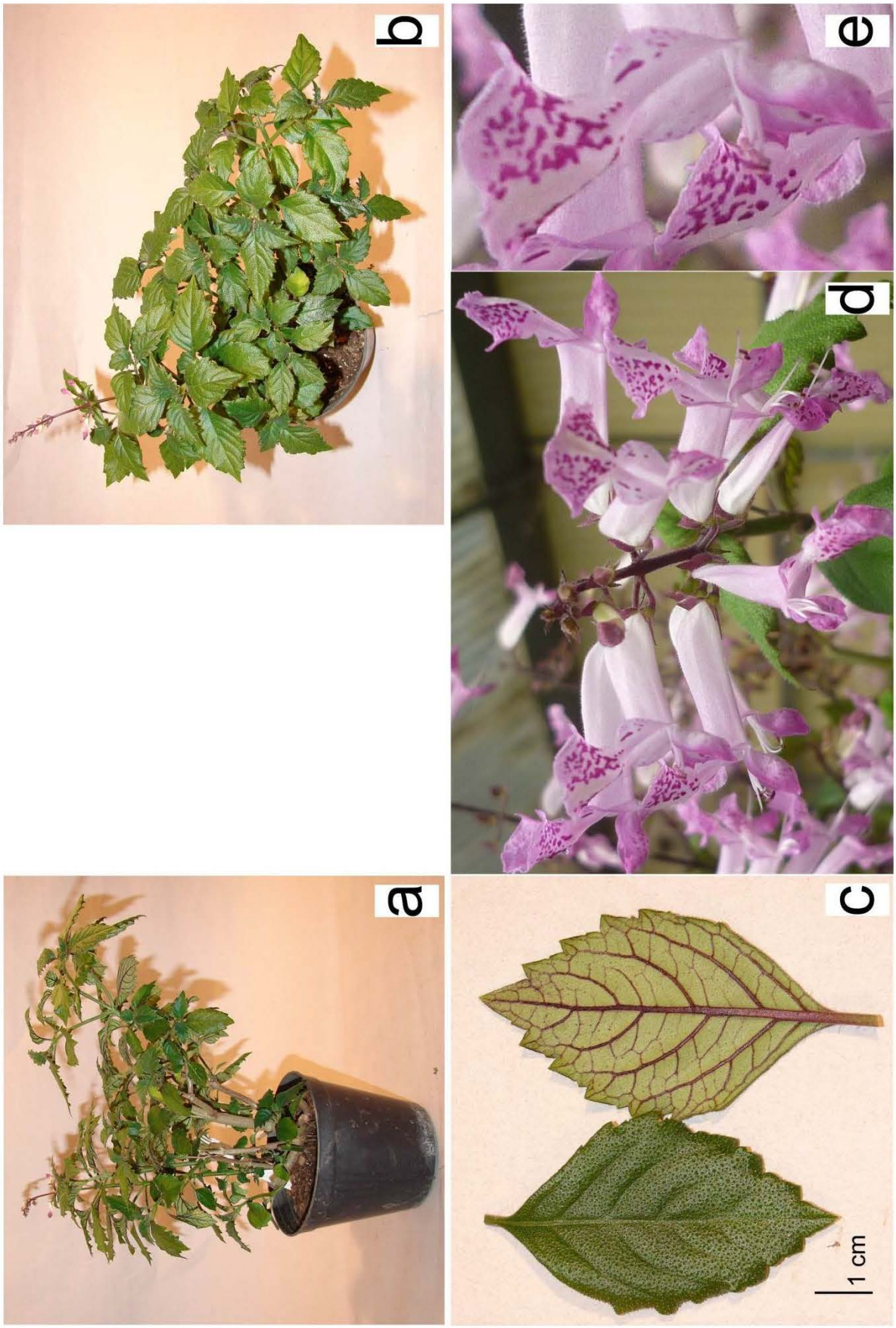

Figure 2.5. P 00 06 03, side view of potted plant (a), top view of potted plant (b), abaxial and adaxial leaf surface (c), inflorescence (d), petals (e).

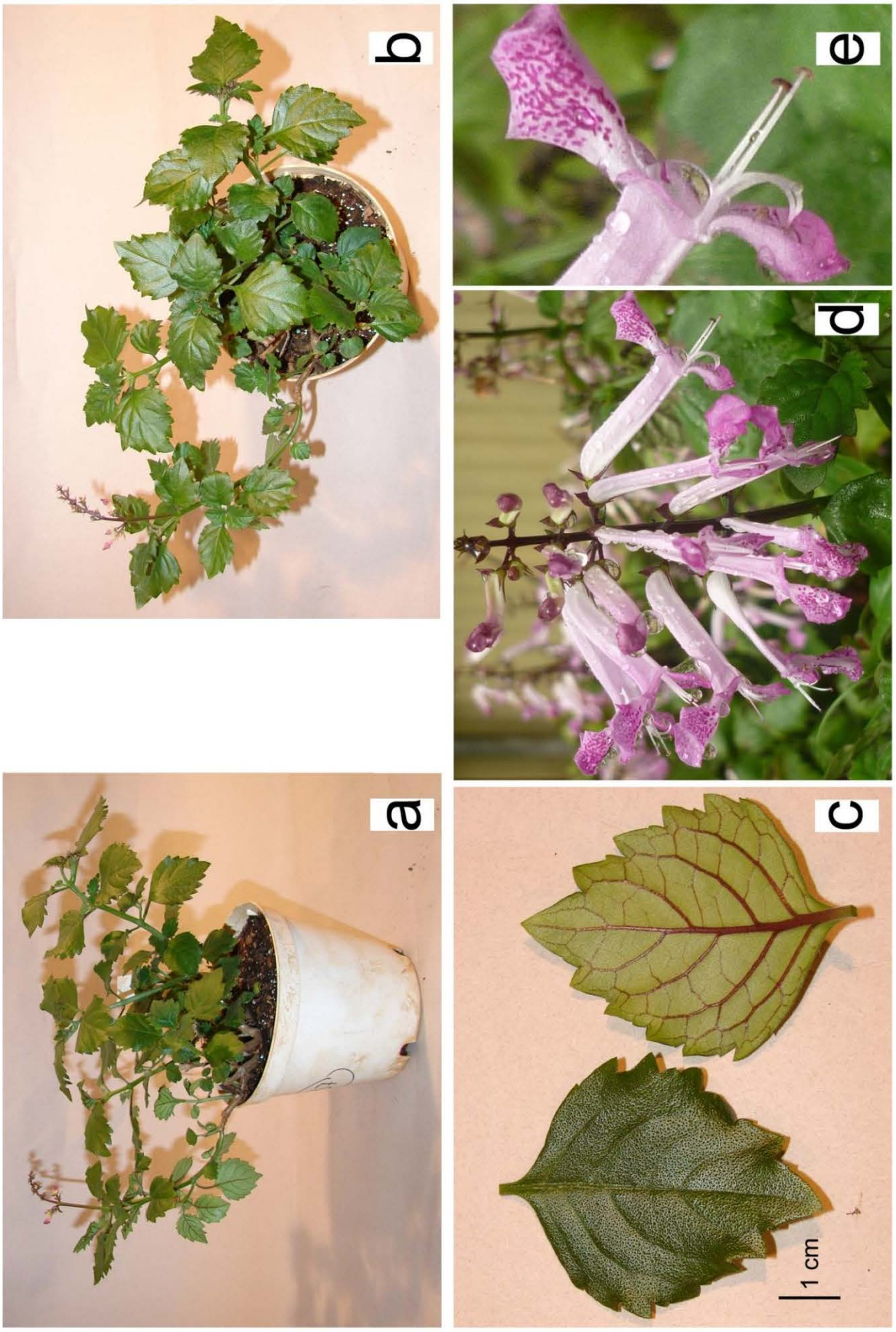

Figure 2.6. P 00 06 03A, side view of potted plant (a), top view of potted plant (b), abaxial and adaxial leaf surface (c), inflorescence (d), petals (e).

Figure 2.7. P 05 04 08, side view of potted plant (a), top view of potted plant (b), abaxial and adaxial leaf surface (c), inflorescence (d), petals (e).

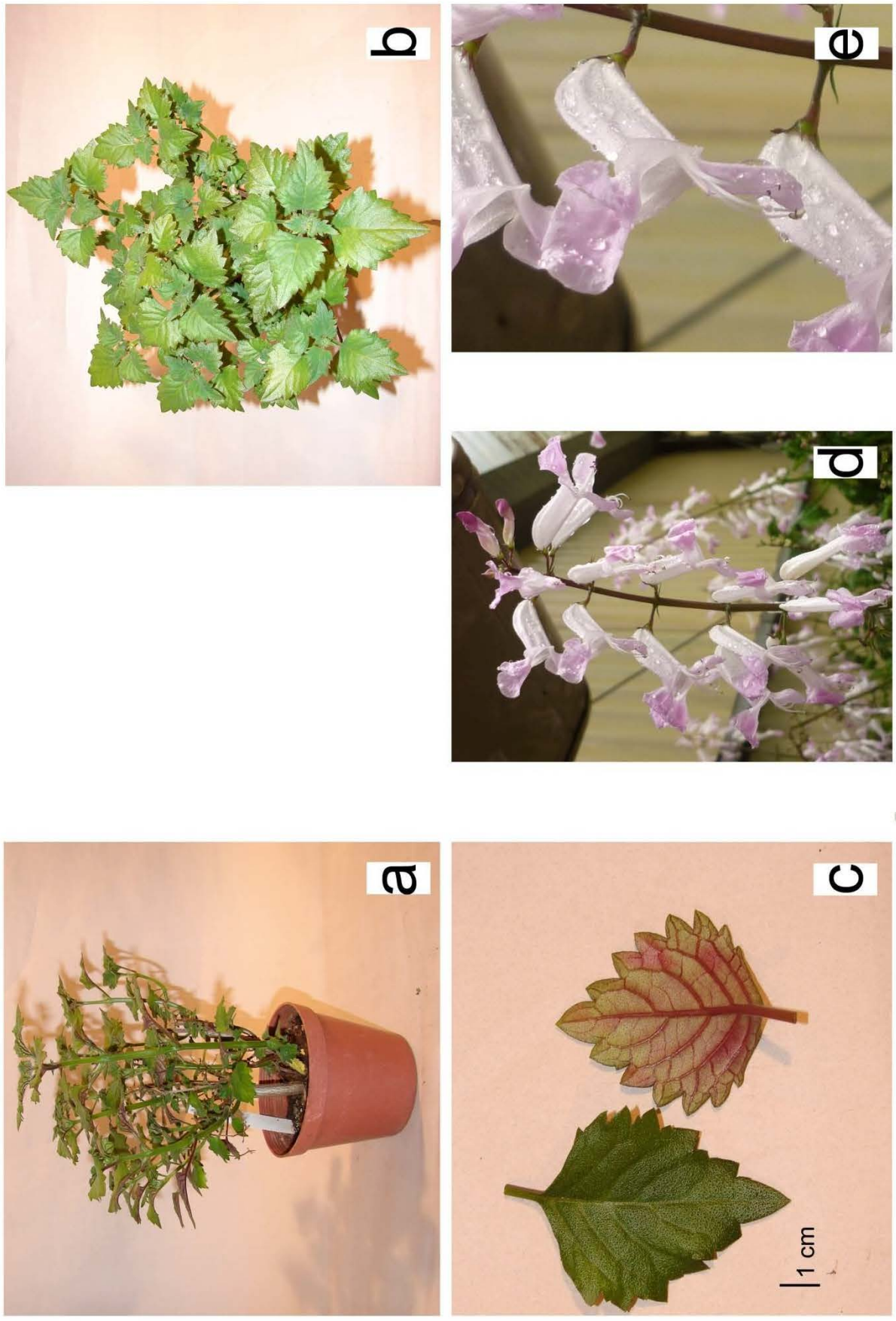

Figure 2.8. P 04 05 18, side view of potted plant (a), top view of potted plant (b), abaxial and adaxial leaf surface (c), inflorescence (d), petals (e).

Figure 2.9. P 08 05 06, side view of potted plant (a), top view of potted plant (b), abaxial and adaxial leaf surface (c), inflorescence (d), petals (e).

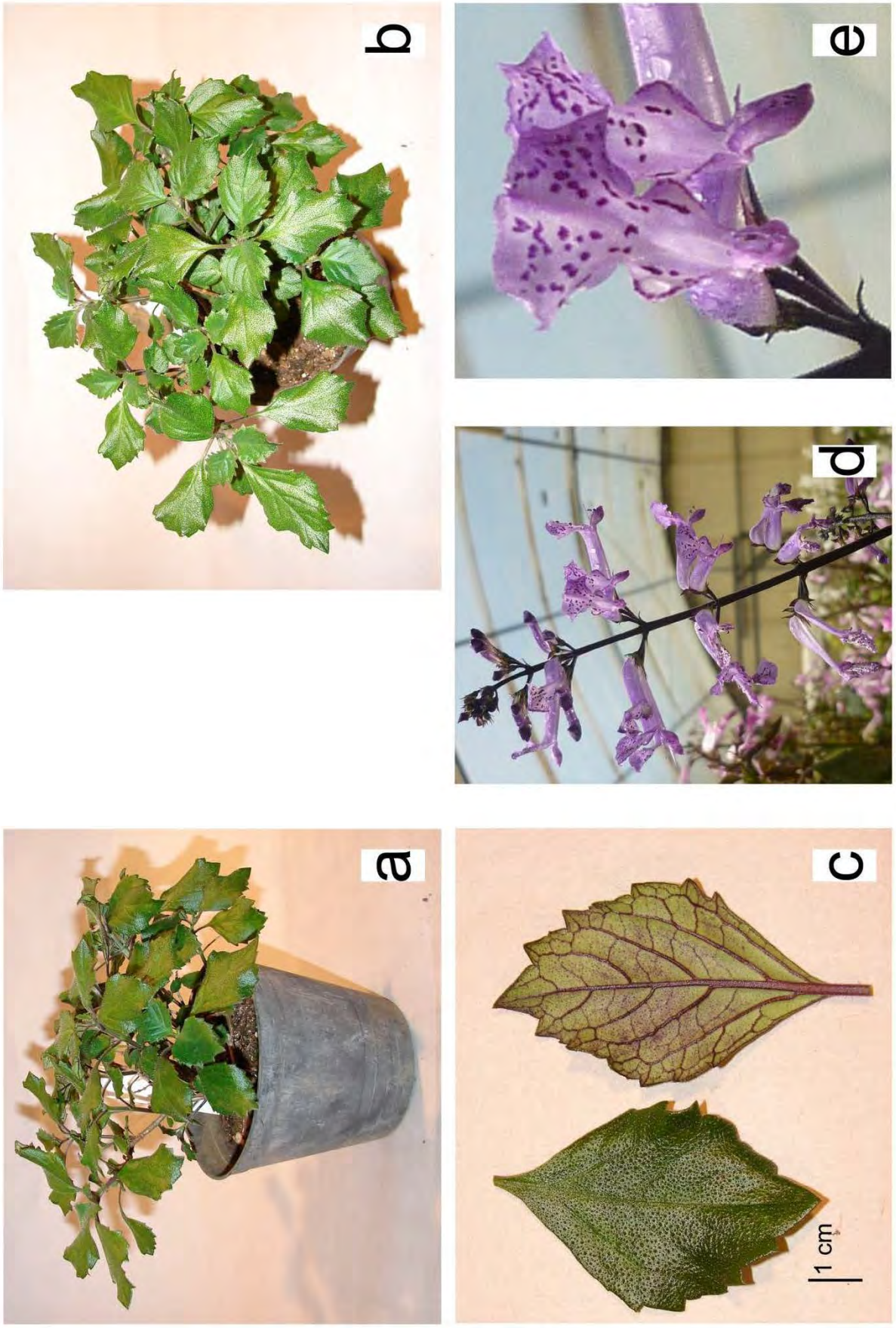

Figure 2.10. P 00 06 07, side view of potted plant (a), top view of potted plant (b), abaxial and adaxial leaf surface (c), inflorescence (d), petals (e).

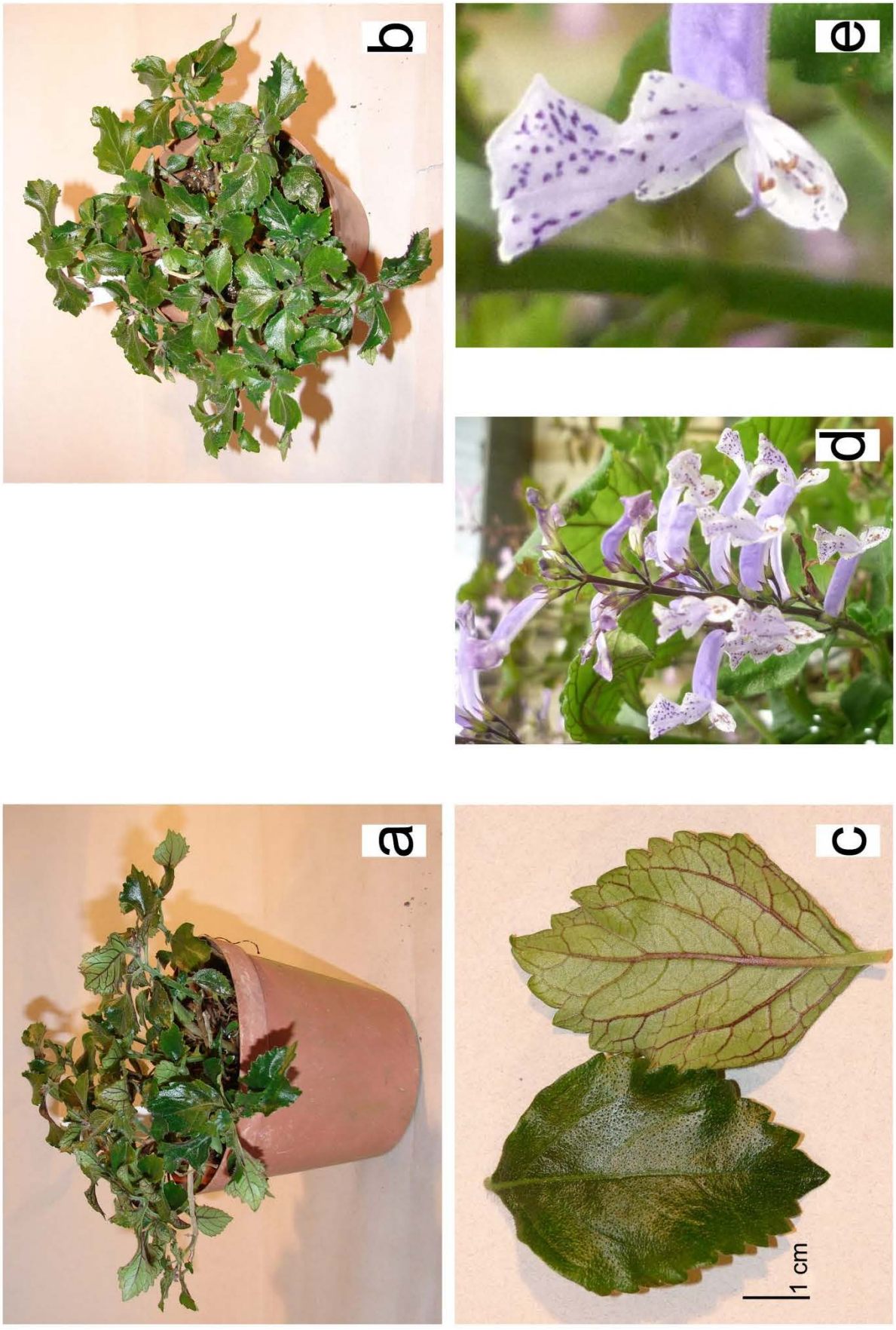

Figure 2.11. P 01 05 09, side view of potted plant (a), top view of potted plant (b), abaxial and adaxial leaf surface (c), inflorescence (d), petals (e).

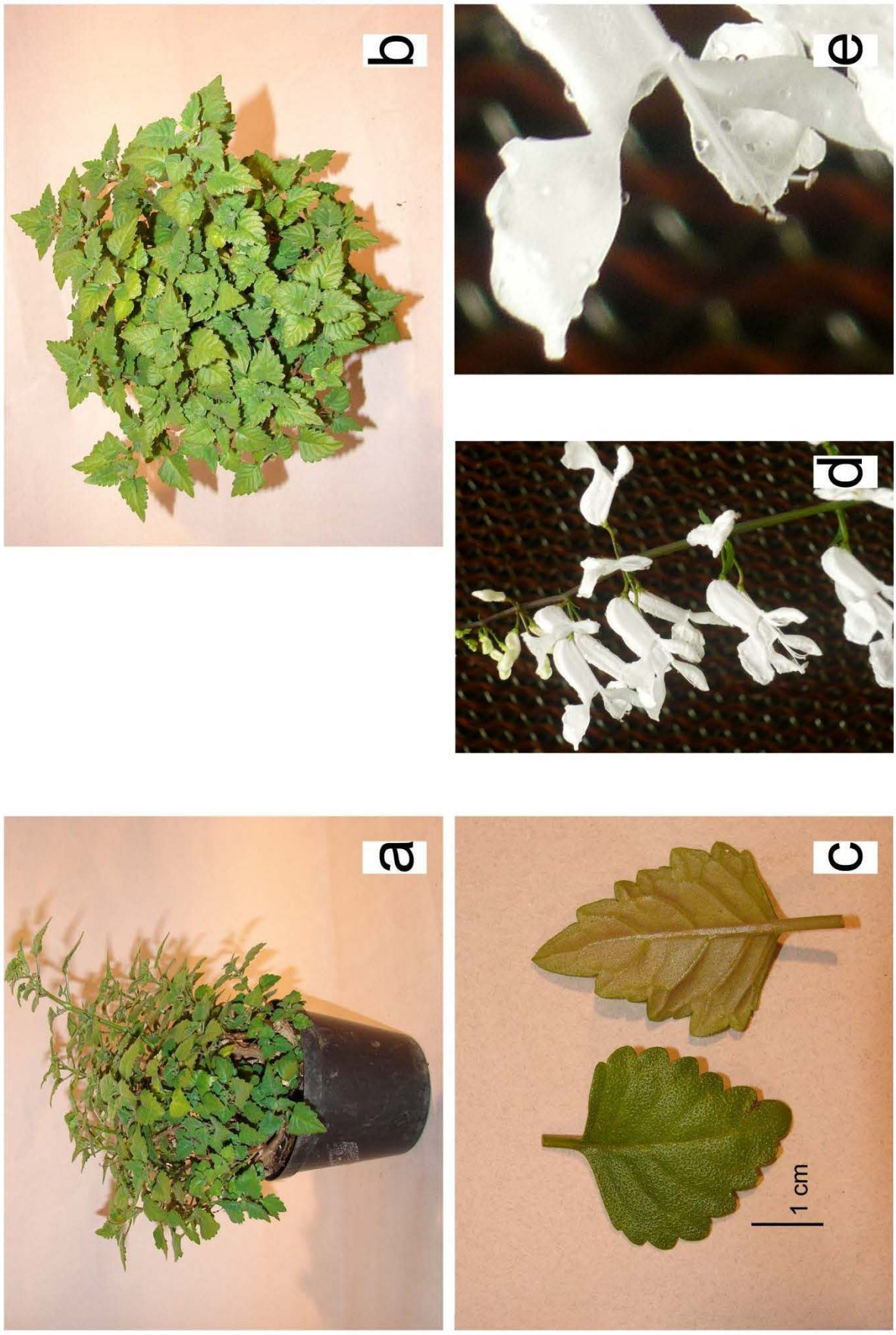

Figure 2.12. P 96 04 06, side view of potted plant (a), top view of potted plant (b), abaxial and adaxial leaf surface (c), inflorescence (d), petals (e).

2.4.3 Flow cytometry

2.4.3.1 Introduction

The determination of the ploidy of a plant can be very important when examining the effect of different conditions on the plant and the internal processes of the plant. The plants used in this project have all been bred from wild species. In some cases the breeder attempted to create polyploids using colchicine. Once a new variety was established the breeder could not be certain of the ploidy of the new variety nor if the polyploidization was successful.

When examining the reaction of different plant varieties to certain environmental conditions, it may be important to know the ploidy of each variety. Varieties with different ploidy levels may react differently under certain conditions.

In order to determine the ploidy level of each variety, flow cytometry was used. Flow cytometry estimates the DNA quantity of nuclei in plant cells (JOHNSTON *et al.*, 1999) (DOLEŽEL & BARTOŠ, 2005). The DNA in an aqueous solution containing intact nuclei is stained, and the fluorescence intensity of the solution is measured and used to determine the DNA content of the nuclei (DOLEŽEL *et al.*, 1989). Sample preparation is quick and this method may be used to detect mixoploidy and aneuploidy (DOLEŽEL *et al.*, 1989; GILISSEN *et al.*, 1993; SMULDERS *et al.*, 1994; SMULDERS *et al.*, 1995; DOLEŽEL *et al.*, 1998; MISHIBA & MII, 2000; DOLEŽEL & BARTOŠ, 2005).

Plectranthus species have 14 sets of chromosomes (BRITS & LI, 2008), diploid plants have 28 chromosomes (2n=28), triploid plants have 42 chromosomes (3n=42) and tetraploid plants have 56 chromosomes (4n=56). The goal of the breeder is often to create triploid plants as these are sterile and have restored vigour that is often lost in tetraploid plants (BRITS & LI, 2008).

2.4.3.2 Materials and methods

Young leaves were collected from plants from each of the eight varieties growing in the greenhouse. Leaves were collected from more than one plant so as to get a sample representative of the variety. Collected leaf material was immediately put onto ice.

Each sample, 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 (10 µl/ml) and insoluble PVPP 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 with a known ploidy level.

2.4.3.3 Results

Three of the eight varieties (P 00 06 03, P 01 05 09 and P 96 04 06) used in this study showed peaks in channel 200, the same position as the diploid control (Figure 2.13). P 00 06 07 was the only variety to display a peak in channel 300. P 05 04 08, P 04 05 18 and P 08 05 06 all showed peaks in channel 400. Variety P 00 06 03A displayed two peaks, one in channel 200 and one in channel 400.

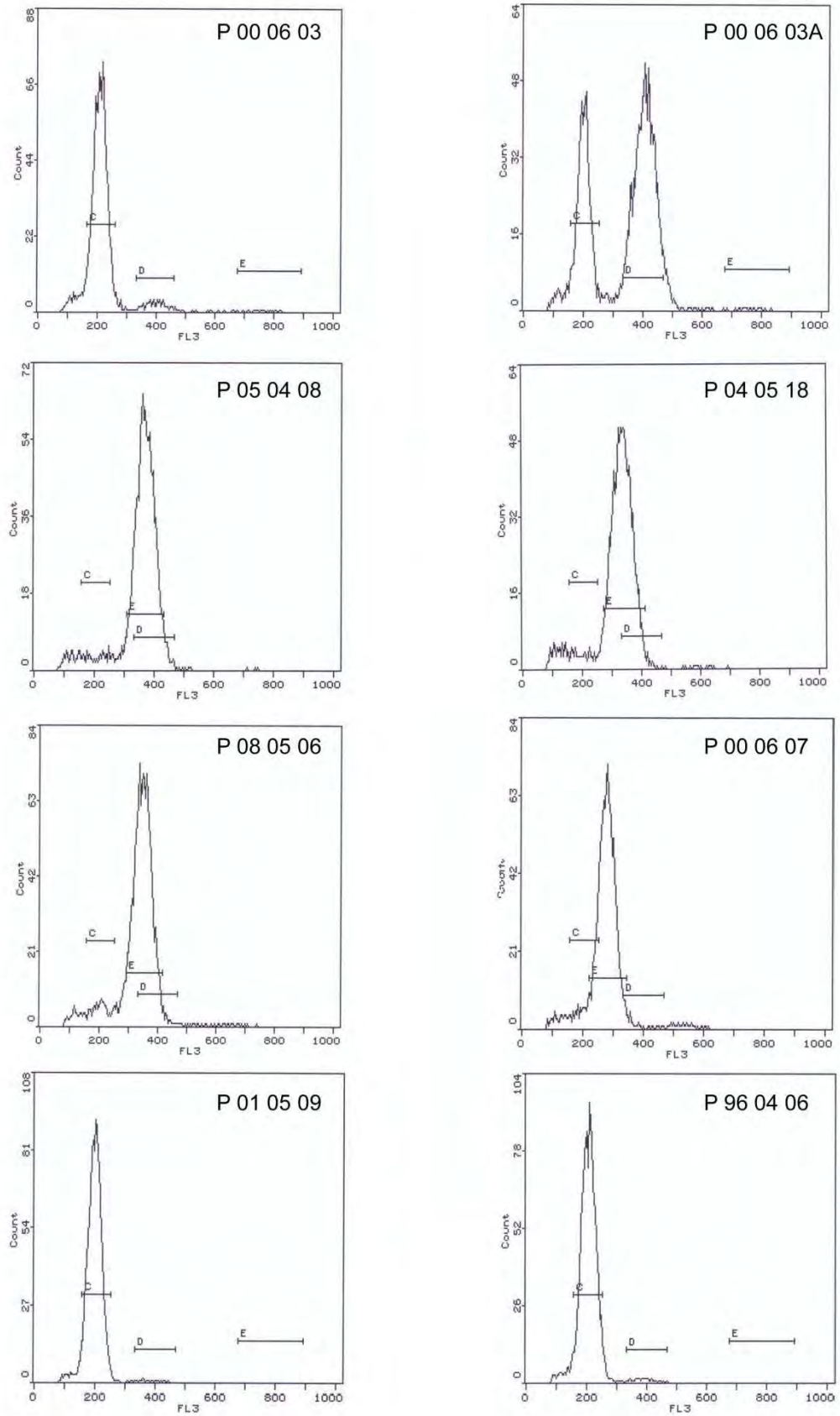

Figure 2.13. Flow cytometry histograms for *Plectranthus* varieties. The diploid peak is set to channel 200.

2.4.3.4 Discussion

Flow cytometry revealed that varieties P 00 06 03, P 01 05 09 and P 96 04 06 were all diploid (2n) as they yielded peaks in channel 200 (Figure 2.13). These three varieties have 28 chromosomes, 2n=28. Two of these varieties, P 00 06 03 and P 96 04 06, were the result of a cross between diploid parents and so it was expected that the resulting progeny would also be diploid. The third, P 01 05 09, was a natural sport from a diploid variety. It is possible that this individual may have been polyploid but it is not surprising that it is in fact diploid.

P 00 06 07 produced a peak in channel 300, indicating that this is a triploid (3n=42) variety. During correspondence with the breeder, it was established that this variety does not set seed. This sterility further confirms that this individual is triploid. Triploids are typically sterile (BRITS & LI, 2008). This variety is the result of a cross between a diploid (2n) individual and a tetraploid (4n) individual in order to obtain a triploid (3n).

P 05 04 08, P 04 05 18 and P 08 05 06 are tetraploid varieties, indicated by the peaks they yielded in channel 400 (Figure 2.13). In the histograms these peaks are not precisely in channel 400 (Figure 2.13). This is as a result of random drift during the use of the instrument. Although instrument settings are not changed while running samples, slight deviations from the channel can occur due to random instrument drift and variation in sample preparation and staining (DOLEŽEL & BARTOŠ, 2005). However, when these samples were mixed with the standard sample and re-run, as suggested by JOHNSTON *et al* (1999) it was clear that they had double the DNA content of the diploid standard, confirming that they are tetraploid. P 05 04 08 is suspected to be the result of polyploidization, as the parents of this variety were both diploid, polyploidization must have occurred to produce a tetraploid offspring. P 04 05 18 is the result of a cross between two tetraploid individuals and so was expected to be tetraploid. P 08 05 06 is the offspring of a cross between a natural tetraploid and P 00 06 03A, which was also used in this study.

Figure 2.13 shows two peaks for P 00 06 03A, one of which is in channel 200 and the other in channel 400. This is an indication of two different types of nuclei present in

this variety. There are some nuclei with diploid chromosome number and others with tetraploid chromosome number. This is a phenomenon known as polysomaty (SMULDERS *et al.*, 1994), or mixoploidy (DOLEŽEL & BARTOŠ, 2005). Polysomaty occurs when there are repeated cycles of DNA synthesis but cell division does not take place (GILISSEN *et al.*, 1993; SMULDERS *et al.*, 1994). This results in polysomatic tissues where cells at different levels of endoreduplication are present (SMULDERS *et al.*, 1994). This variety stems from a single and unique plant that resulted from a cross between diploid parents. In this individual, a problem occurred during the replication of cells for growth. The DNA in the cell was duplicated but cell division did not occur. This resulted in a cell with double the number of chromosomes as its parent cell. This tetraploid cell then continued to undergo mitosis which formed more tetraploid cells. There was continued mitosis by the original diploid cells and so both diploid and tetraploid cells are present in this variety. As mentioned earlier P 00 06 03A was a parent of the tetraploid variety P 08 05 06. In this cross the gamete contributed by the P 00 06 03A parent must have been the result of meiosis by a tetraploid cell. Polysomaty has also been observed in a number of other plant species, particularly those subjected to breeding practices. The same diploid and tetraploid state was found in tomato (*Lycopersicon esculentum* cv. Moneymaker) (SMULDERS *et al.*, 1994; SMULDERS *et al.*, 1995), cucumber (*Cucumis satiivus* L. cv. Hokus) (GILISSEN *et al.*, 1993), potato (*Solanum tuberosum*) (SREE RAMULU & DIJKHUIS, 1986), sugar-beet (*Beta vulgaris* L.) (SLIWINSKA & LUKASZEWSKA, 2005) and tobacco (*Nicotiana attenuate* Tarry ex. Watson) (BUBNER *et al.*, 2006). It has been observed in other important horticultural species such as *Portulaca grandiflora* Hook. (MISHIBA & MII, 2000), and *Cymbidium* hybrids (Flower Dance 'Christmas Kiss' and Twilight Moon 'Day Light') (FUKAI *et al.*, 2002).

Establishing the DNA content of each variety will play an important role in understanding the results in the rest of this study. The expression of abscission related genes will be examined and it is possible that the expression of these genes will be higher in tetraploid varieties when compared with the diploid and triploid varieties.

Chapter 3

The effects of simulated transport conditions on flower abscission

In both potted plants and cut flowers the majority of flower abscission occurs during postharvest handling (VAN MEETEREN & VAN GELDER, 1995) and prolonged transport (ASCOUGH *et al.*, 2005). Moving plants and cuttings from nursery to retailer can take several days. Transport conditions are stressful for the plants due to a number of environmental factors, such as temperature extremes, high humidity, exposure to ethylene and darkness (MÜLLER *et al.*, 1998; SEREK *et al.*, 1998). The loss of flowers and flower buds during this time is a problem in many potted plants including *Hibiscus rosa-sinensis* L. (VAN MEETEREN & VAN GELDER, 1995; VAN MEETEREN & VAN GELDER, 2000) and *Rosa hybrid* L. (MÜLLER *et al.*, 1998). Packing plants into confined spaces during transport puts plants under stress (ASCOUGH *et al.*, 2005) and allows stress ethylene produced by the plants and cuttings to build up (HØYER, 1995). This has an adverse effect on the plants and cuttings. Dark conditions during transport cause a number of physiological changes in the plants (WRIGHT, 1981; MAO *et al.*, 1989; TURNER & WIEN, 1994b). Problems such as flower and flower bud abscission in pot plants during transport cause problems when developing and marketing a variety (MÜLLER *et al.*, 1998). An understanding of the unfavourable conditions during transport, and how each of these affects flower abscission, will aid in developing a protocol to prevent transport-induced flower abscission.

3.1 Flower abscission under greenhouse conditions

3.1.1 Introduction

Flower abscission under natural conditions usually occurs after pollination (ASCOUGH *et al.*, 2005). The flower's purpose of attracting pollinators to the plant has been accomplished and as the flower cannot be pollinated a second time the corolla is no longer of any use to the plant. Flowers are energetically expensive organs

for a plant to produce and maintain and so as soon as the flower has served its purpose it is 'discarded' by the plant. Unopened flowers have not yet played their role in pollination and so under optimum conditions only open flowers are abscised from the plant. In some cases senescence occurs following pollination (VAN DOORN, 1997). Senescence is the controlled death of the flower (ASCOUGH *et al.*, 2005), which may be observed as wilting. If a flower is not pollinated, it will eventually abscise. In this case abscised flowers fall from the plant while still turgid (VAN DOORN & STEAD, 1997).

The plants used in this study were kept in a greenhouse. Here plants were under optimum light and temperature conditions. In order to understand the effects that transport conditions had on flower longevity and abscission, it was important to examine flower longevity and abscission whilst plants were under both favourable and simulated transport conditions. Flower longevity is the time for which a flower is open and functional (TEIXIDO *et al.*, 2011).

3.1.2 Materials and methods

Five inflorescences from each variety were tagged, before any flowers on the inflorescence had opened. Each inflorescence was checked daily and the days on which flowers opened and abscised were recorded. Flowers on the lowest ranks (Figure 3.1) open first and then sequentially up the inflorescence. The number of days for which an opened flower remained on the plant was calculated. An analysis of variance (ANOVA) was carried out on all data. Data were analysed using a Duncan's test at the 5% level in GenStat, 14th edition.

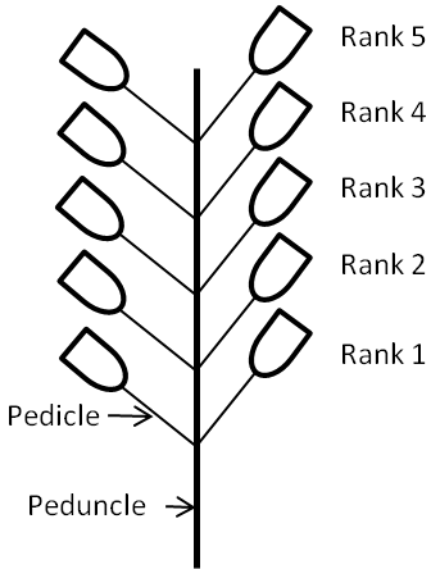

Figure 3.1: Inflorescence showing rank labelling.

3.1.3 Results

Under favourable (greenhouse) conditions only open flowers abscised. No unopened flowers abscised during this experiment for all eight varieties.

There was no significant difference in the longevity of flowers on different ranks of P 0 06 03 plants. Flowers on ranks 6 and 7 were the longest lasting flowers as they remained on the plant for 9.3 d (Figure 3.2). Flowers on rank 5 had the shortest open life, only being open and on the plant for 7.3 d. Flowers on ranks 1 to 4 remained on the plant as open flowers for between 7.4 d and 8.8 d.

On P 00 06 03A plants, flowers on rank 4 were open for the longest period of time (10.6 d) while flowers on rank 7 were only open for 5 d, the shortest period of time (Figure 3.2). Flowers on ranks 1 to 3, 5 and 6, and 8 were open for between 8.0 d and 10.1 d.

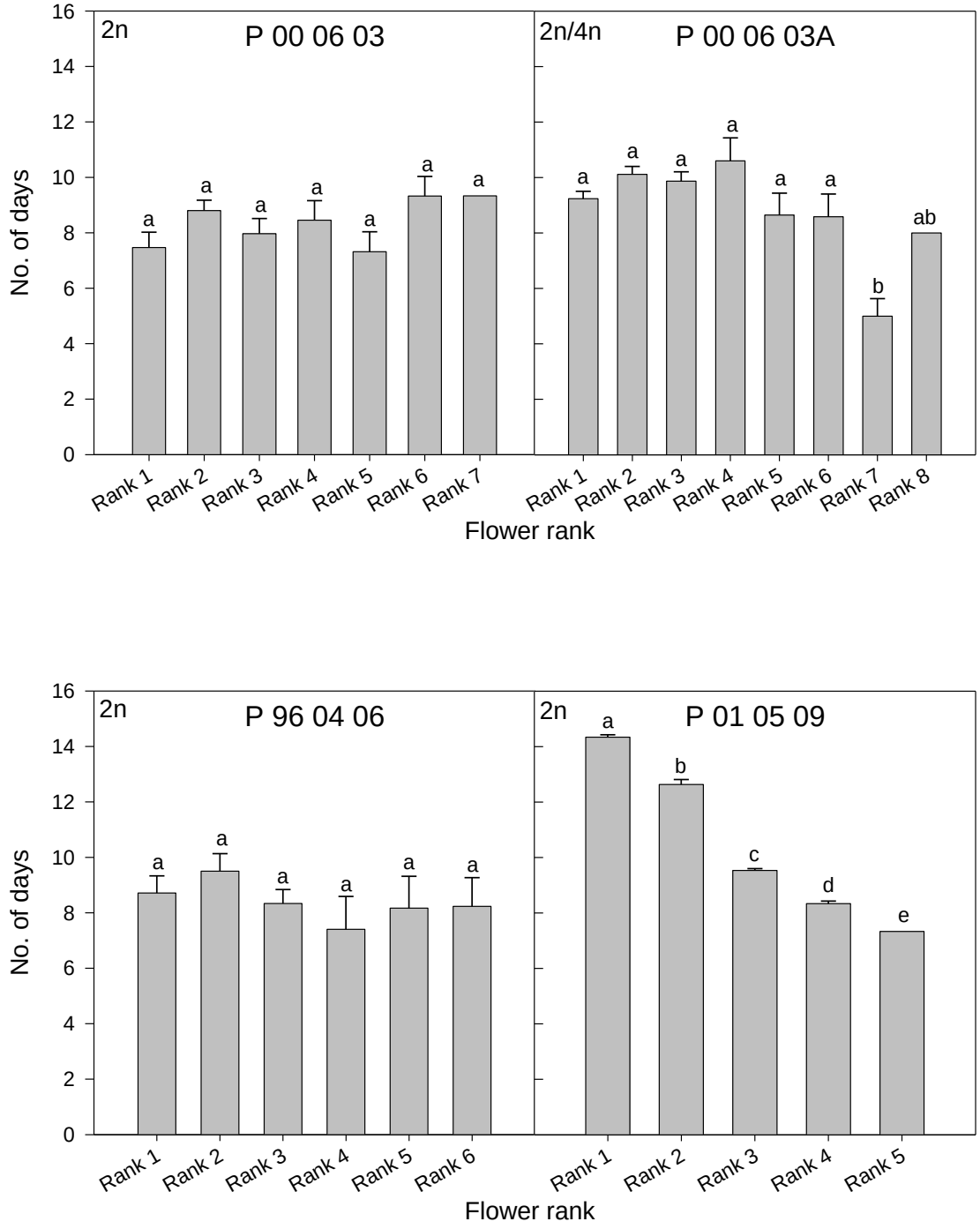

Figure 3.2: The number of days open flowers remained on plants under greenhouse conditions. Different letters indicate significant differences between treatments at a 5% level (ANOVA).

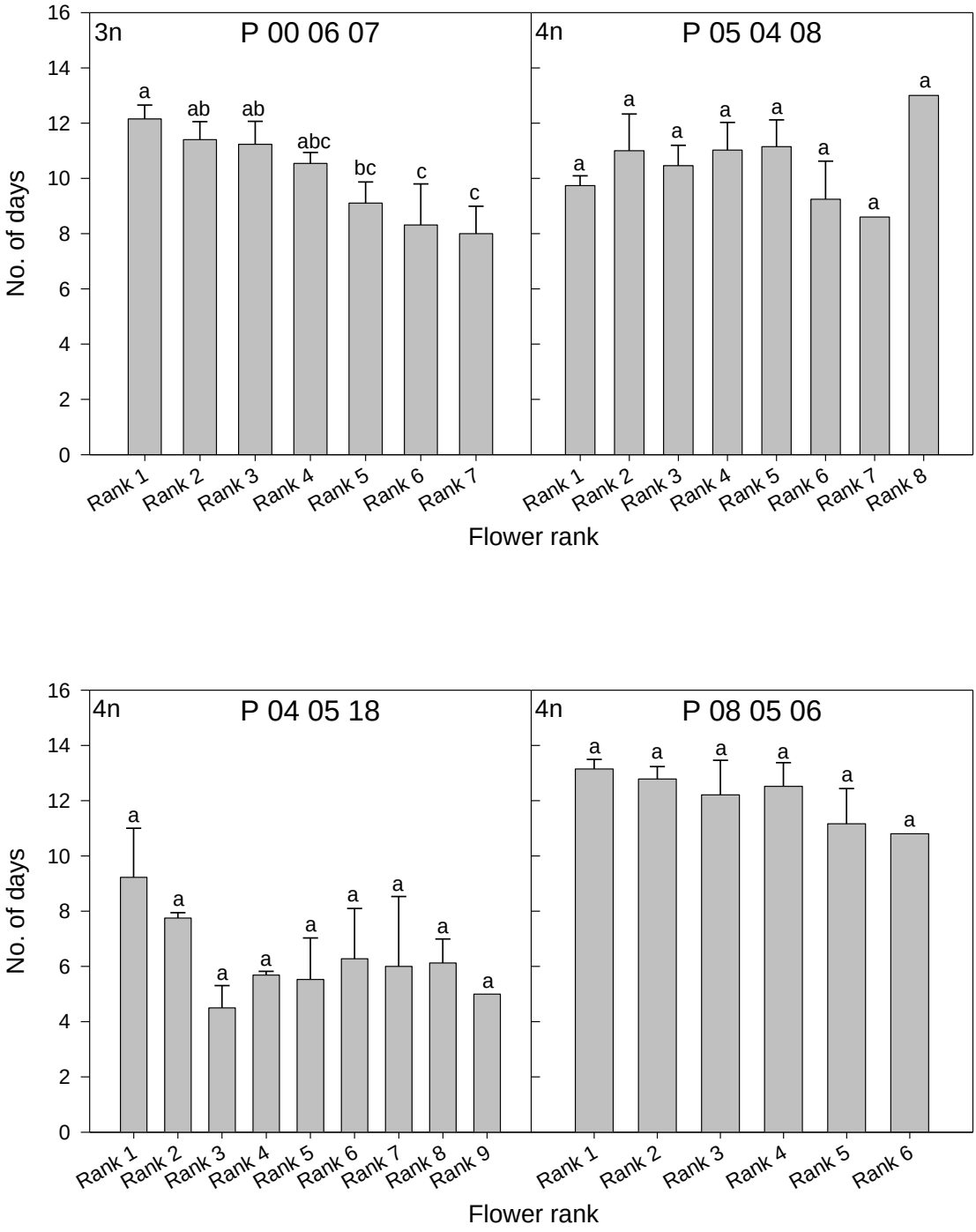

Figure 3.3: The number of days open flowers remained on plants under greenhouse conditions. Different letters indicate significant differences between treatments at a 5% level (ANOVA).

There was no significant difference in flower longevity of flowers on different ranks of P 96 04 06. Flowers on rank 2 were open for the longest time, 9.5 d (Figure 3.2) while flowers with the shortest longevity were those on rank 4 (7.4 d). Flowers on ranks 1, 3, 4 and 5 were open for between 8.2 d and 8.7 d.

Flowers on rank 1 remained open on plants of P 01 05 09 for 14.3 d, these were the flowers with the greatest longevity (Figure 3.2). Flowers on rank 5 remained open on plants for the shortest time (7.3 d). Flower longevity decreased the higher up the on the inflorescence the flower was produced. Flowers on ranks 2 to 4 were open on plants for between 8.3 d and 12.6 d.

Flowers on rank 1 remained open on the plants of P 00 06 07 for 12.2 d (Figure 3.3). The higher the rank on the inflorescence the shorter the lifespan of the flower. Flowers on rank 7 were open on the plant for the shortest time, 8.0 d. Flowers on ranks 2 to 6 remained open on the plant for between 8.3 d and 11.4 d.

There was no significant difference in the longevity of flowers on different ranks of P 05 04 08 plants. Flowers on rank 8 were open for 13 d, longer than the flowers on the other ranks in this variety (Figure 3.3). Flowers with the shortest open life on the plants are those on rank 7 (8.60 d). Flowers on ranks 1 to 6 were open for between 9.2 d and 11.2 d.

There was no significant difference in flower longevity of flowers on different ranks of P 04 05 18 plants. Flowers on rank 1 remained open on P 04 05 18 plants for 9.2 d, the longest period of time for this variety (Figure 3.3). Flowers on rank 3 were open for the shortest time in this variety (4.5 d). Flowers on rank 9 were also open for a short period of time, 5.0 d. Flowers on rank 2 and ranks 4 to 8 were open and remained attached to plants for between 5.5 d and 7.8 d.

There was no significant difference in flower longevity of flowers on different ranks of P 08 05 06 plants. Flowers on rank 1 remained open on the plant for the longest period of time (13.2 d) while flowers on rank 6 were open and on the plant for the

shortest time (10.8 d) (Figure 3.3). Flowers on ranks 2 to 5 remained on the plant as open flowers for between 11.2 d and 12.8 d.

3.1.4 Discussion

Under greenhouse conditions only open flowers were abscised from the plants. Unopened flowers are still of use to the plant as they are yet to open and provide the opportunity for pollination if they remain on the plant and open (VAN DOORN, 1997).

In varieties P00 06 03, P 96 04 06, P 01 05 09 (Figure 3.2), P 00 06 07, P 05 04 08 and P 08 05 06 (Figure 3.3), all flowers on all ranks remained on the plants for more than 7 d after opening. This was the case for flowers on most of the ranks in variety P 00 06 03A (Figure 3.2). Flowers on rank 7 of this variety remained open on the plant for 5 d before they abscised.

In P 04 05 18 only flowers on ranks 1 and 2 were open for longer than 7 d before they abscised (Figure 3.3). The rest of the flowers of this variety were open and on the plant for between 4.5 d and 6.3 d. The longevity of flowers in this variety is the shortest of all eight varieties studied. P 04 05 18 has large flowers and it is possible that the plant cannot maintain them for a very long time. Maintaining large showy flowers may limit the potential for future flower production (ASHMAN, 2004) and may demand more carbohydrates than the plant can supply (ASCOUGH *et al.*, 2005). TEIXIDO *et al*. (2011) suggested that larger flowers attract more visits by pollinators. Perhaps there is a trade-off between longevity and attractiveness. The optimum lifespan of a flower is a balance between reproductive benefits and the cost of maintenance (ASHMAN, 2004).

Certain varieties showed a definite trend when flower longevity is compared to which rank the flower is on. In P 00 06 07 (Figure 3.3) and P 01 05 09 (Figure 3.2) flowers on lower ranks are open for longer than those on higher ranks. Flowers open sequentially as the flowers on lower ranks open first followed by flowers on higher ranks. It is possible that flowers on higher ranks do not receive sufficient

carbohydrates to remain open for as long as the flowers lower down on the inflorescence. Carbohydrates are important for flower longevity (VAN DOORN & STEAD, 1997; MONTEIRO *et al.*, 2002), and those with a decreased carbohydrate supply will have a shorter lifespan than flowers that have a greater carbohydrate supply.

3.2 The effect of packaging on flower abscission

3.2.1 Introduction

In order to transport pot plants from the nursery/breeder to the retailer they must be put into a confined space. They may be packaged into boxes or put into the back of a truck. A lack of ventilation allows for ethylene to build up around the plants (HØYER, 1995). Ethylene is produced by the plants themselves as the transport conditions are stressful (HØYER, 1995; MÜLLER *et al.*, 1998). Ethylene is autocatalytic (BLEECKER & KENDE, 2000; DAVIS, 2004) meaning that ethylene in the environment will trigger production of more ethylene by the plant. When the plants produce ethylene during transport, this ethylene induces the production of further ethylene. If there is any ethylene in the atmosphere of the truck from a previous journey (HØYER, 1995) this will have the same effect.

Ethylene produced during transport can induce flower abscission (MUTUI *et al.*, 2007), as abscission is controlled by ethylene (VAN DOORN & STEAD, 1997). Transportation of pot plants and cuttings can take several days (SEREK *et al.*, 1998). *Plectranthus* plants are transported for a maximum of four days. During this time the truck is not opened and there is no ventilation. This would allow for ethylene to build up to concentrations harmful to the plants. Ethylene is active at very low concentrations, less than $1 \mu l l^{-1}$ (PECH *et al.*, 2004). It would not take long for the atmospheric ethylene in a truck full of plants producing ethylene to reach this concentration.

In this experiment the effect of packaging plants into a confined space (perspex chambers) on flower abscission was investigated by comparing to plants left in the

greenhouse. Plants were kept under artificial lights and a small amount of silica gel was placed inside the chambers. Silica gel was used to absorb the excess moisture from transpiration. This experiment aimed to investigate the effect of packaging plants (one component of transport conditions) alone on flower abscission.

3.2.2 Materials and methods

Individual plants having at least three inflorescences were transferred from the greenhouse to the laboratory and the number of inflorescences and flowers were recorded. At the start of experimentation, approximately 20-25% of flowers on a plant were open, the rest were unopened. They were placed inside sealed (gas-tight) impermeable Perspex chambers (small: 288 x 438 x 250 mm (ca. 31.5 L), medium: 138 x 538 x 300 mm (ca. 70.7 L); large: 488 x 636 x 350 mm (ca. 108.6 L)) at 21 ± 2°C and kept under a 16-h photoperiod illuminated by overhead fluorescent tubes providing 12.6 μmol m^{-2} s^{-1} radiant flux density. A small amount of silica gel was also placed in the chambers to absorb excess moisture produced by transpiration. Plants were observed every 24 h for 4 d and the number of flowers that had abscised were recorded. These abscised flowers were further categorized as being open at the time of abscission, or unopened (i.e., buds). When flower abscission was recorded, plants were removed from the chambers and the chambers were cleaned and vented to remove the excess moisture in the chambers. Plants were then replaced and the chambers resealed. Control plants were at the same stage of flowering as the plants used for the experiment. Control plants were kept in the greenhouse and observed every day.

The percentage flower abscission over time was recorded. Data were arcsine transformed and an analysis of variance (ANOVA) was carried out on all data. Data were analysed using a Duncan's test at the 5 % level in GenStat, 14th edition.

3.2.3 Results

Flower abscission of open and unopened flowers of P 00 06 03 plants was greater in sealed Perspex chambers than under greenhouse conditions (Figure 3.4). Abscission

of open flowers was considerably higher (17.73 %) than that of unopened flowers (1.28 %) over 96 h. Abscission of unopened flowers in the chambers was very low. There was no abscission of open or unopened flowers over 96 h by plants kept under greenhouse conditions (Table 3.1).

Table 3.1: Summary table of flower abscission of open and unopened flowers (±SD) after 96 h in the greenhouse and boxed in Perspex chambers in a 16 h photoperiod.

		Abscission after 96 h (%)				
		Greenhouse		Boxed		
Variety	Ploidy	Open	Unopened	Open	Unopened	Preferential shedding when boxed
P 00 06 03	2n	0	0	17.73±3.8	1.28±0.6	open
P 00 06 03A	2n/4n	0	0	4.67±2.5	5.9±5.0	open
P 96 04 06	2n	0	0	21.76±9.2	70.92±7.6	unopened
P 01 05 09	2n	0	0	4.43±1.1	6.38±4.4	unopened
P 00 06 07	3n	0	0	32.94±6.6	4.68±3.5	open
P 05 04 08	4n	0	0	4.97±2.5	35.06±12.1	unopened
P 04 05 18	4n	0	0	7.61±2.4	10.03±3.9	unopened
P 08 05 06	4n	0	0	7.42±4.4	24.79±9.3	unopened

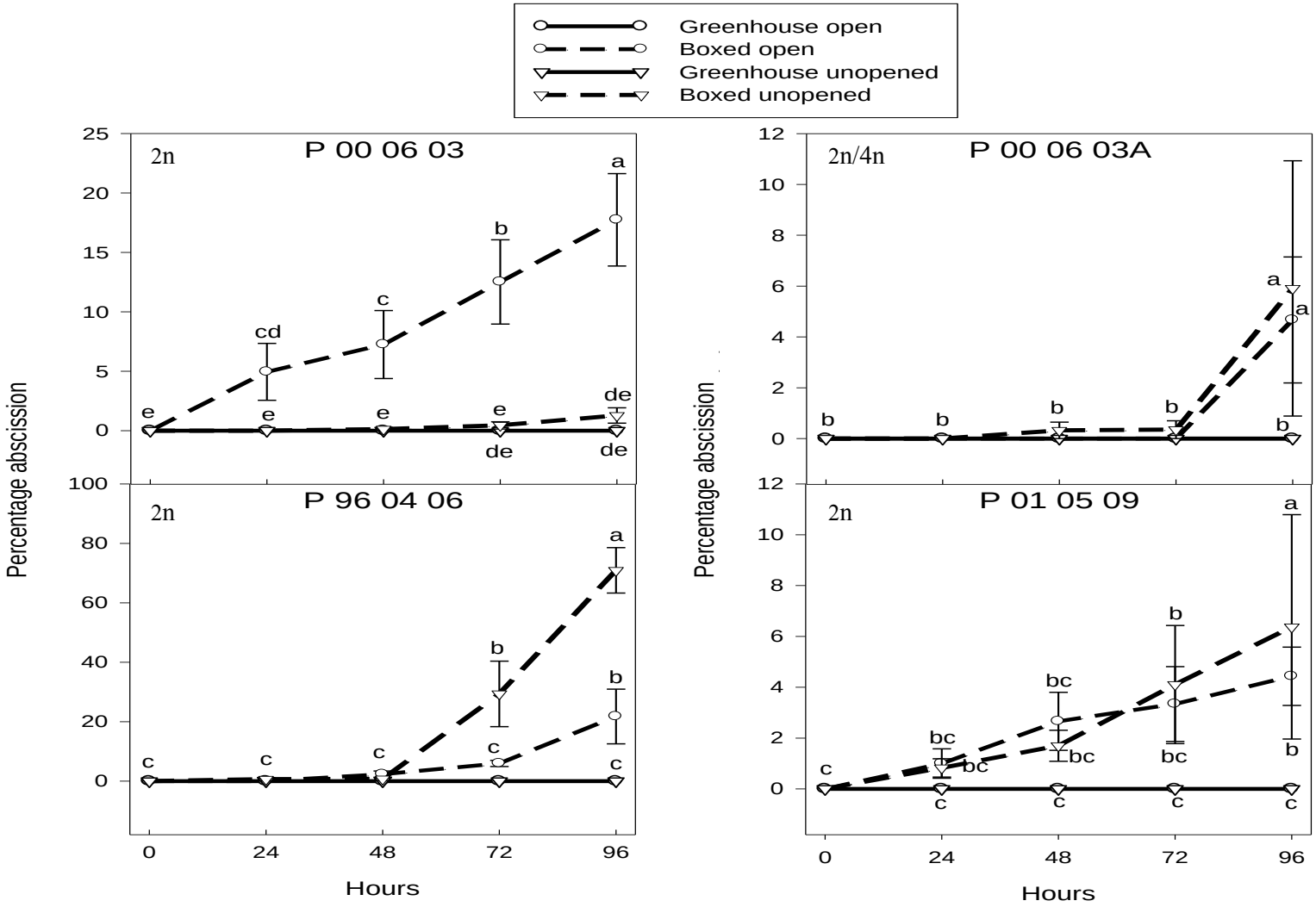

Figure 3.4: The effect of packing plants during simulated transport on abscission of open and unopened flowers. Different letters indicate significant differences between treatments at a 5% level (ANOVA). Note there are differences in the scale of the y-axis.

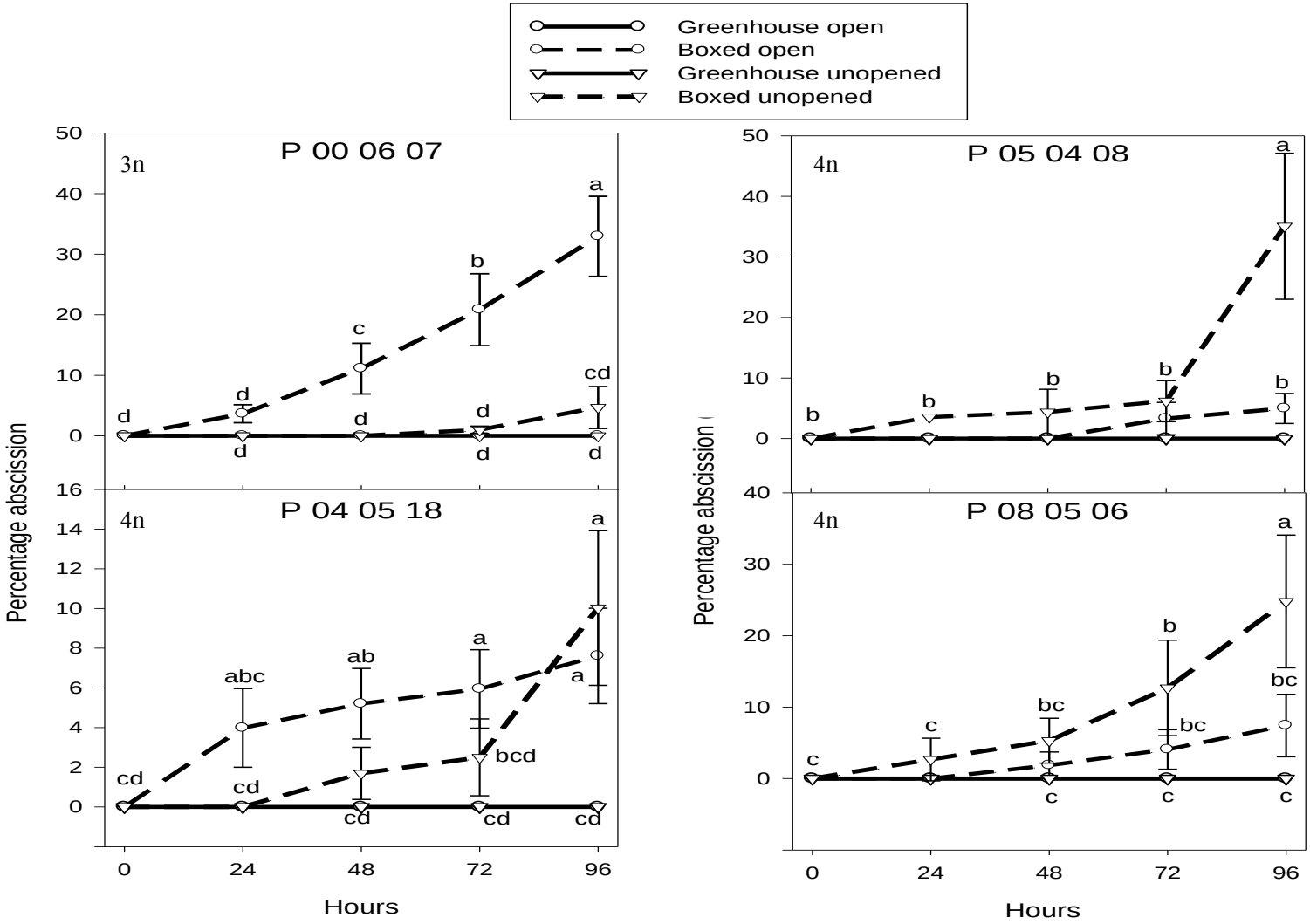

Figure 3.5: The effect of packing plants during simulated transport on abscission of open and unopened flowers. Different letters indicate significant differences between treatments at a 5% level (ANOVA). Note there are differences in the scale of the y-axis.

There was no flower abscission by P 00 06 03A plants in the greenhouse (Figure 3.4). Abscission of open flowers in the chambers was negligible before 72 h. After 72 h abscission of open and unopened flowers increased slightly. By the end of the experiment 5.9 % of unopened flowers and 4.7 % of open flowers had abscised from the plants.

There was no flower abscission by P 96 04 06 plants kept in the greenhouse (Figure 3.4). Abscission of both open and unopened flowers by plants in the chambers was exceptionally low for the first 48 h of the experiment. After this, abscission of unopened flowers increased rapidly until 96 h when 70.9 % of these flowers had been lost. Abscission of open flowers also increased after 48 h, with 21.7 % of open flowers abscised after 96 h in the chambers (Table 3.1).

There was no flower abscission by variety P 01 05 09 plants in the greenhouse (Figure 3.4). Both open and unopened flower abscised by plants in the chambers for the 96 h duration of the experiment (Table 3.1). For the first 48 h of the experiment abscission of open flowers was higher, however after 48 h abscission of unopened flowers was greater. 6.4% of unopened and 4.4% of open flowers abscised over 96 h.

There was no flower abscission by P 00 06 07 plants in the greenhouse over the 96 h experimental period (Figure 3.5). Abscission of open flowers by plants in the chambers increased steadily with time, 32.9% of open flowers abscised by the end of the experiment. Unopened flowers on plants in the chambers started to abscise after 48 h. However, only 4.7% of unopened flowers abscised at 96 h (Table 3.1).

Figure 3.5 shows the abscission of open and unopened flowers over 96 h by P05 04 08 plants kept in chambers and kept in the greenhouse. There was no flower abscission by greenhouse plants. The greatest abscission during the experiment was of unopened flowers on plants in the chambers. There was a sharp increase in abscission of unopened flowers in the chambers after 72 h. 35.1% of unopened flowers were abscised over 96 h. For the first 48 h there was no abscission of open flowers by plants in the chambers. Between 48 h and 96 h very few (4.9%) open flowers were abscised by the plants kept in chambers (Table 3.1).

There was no flower abscission by P 04 05 18 plants kept in the greenhouse (Figure 3.5). The greatest flower abscission for the first 72 h was of open flowers by plants in the chambers. 7.6% of open flowers were lost during the 96 h experiment. After 24 h there was no abscission of unopened flowers by plants in the chambers, but after 72 h there was an increase in abscission of unopened flowers by plants in the chambers resulting in 10.0% abscission.

There was no flower abscission by P 08 05 06 plants kept in the greenhouse over 96 h (Figure 3.5). Abscission of unopened flowers (24.8%) was greater than abscission of open flowers (7.4%) by plants in the chambers for the duration of the 96 h experiment. For the first 24 h there was no abscission of open flowers by plants in the chambers. As time progressed abscission of both open and unopened flowers by plants in the chambers increased.

3.2.4 Discussion

Packaging *Plectranthus* plants closed in containers to simulate transport conditions increased flower abscission in all eight of the varieties tested in this study. It is possible that this increased flower abscission is as a result of ethylene build up in the perspex containers during the experiment. HØYER (1995) found that there was a build-up of ethylene in trucks during the transport of pot plants. This could be an explanation for the increase in flower abscission when *Plectranthus* plants were put into the perspex chambers.

Varieties differed markedly in their abscission response to simulated transport. P 96 04 06 was most sensitive abscising 92% of flowers (open and unopened) after 96 h. Other varieties (P 08 05 06, P 05 04 08 and P 00 06 07) were moderately sensitive abscising 30-40% of flowers after 96 h. Still other varieties (P 00 06 03A, P 01 05 09 and P 04 05 18) were only slightly affected by boxing in chambers, abscising 10-17% of flowers after 96 h.

There was variation between varieties as to the extent that open or unopened flowers abscised when maintained in closed chambers. There was no significant difference in the abscission of open and unopened flowers in P 00 06 03A plants that were kept in chambers (Figure 3.4). Abscission of open flowers was significantly higher than abscission of unopened flowers in varieties P 00 06 03 (Figure 3.4) and P 00 06 07 (Figure 3.5). The same can be said for P 04 05 18 (Figure 3.5) for the first 72 h of the experiment, after which there was no significant difference in the abscission of open and unopened flowers. This same trend was observed by ASCOUGH *et al*. (2008) when *Plectranthus* plants were put into the dark. This supports the hypothesis that flowers are shed from the plant when they become energetically unprofitable in comparison to opening a new flower (ASHMAN & SCHOEN, 1994). Unopened flowers have the potential to be open for a longer time than flowers that have already opened. Thus they are of greater value to the plant.

This trend was not however seen in all the varieties. P 05 04 08, P 08 05 06 (Figure 3.5), and P 96 04 06 (Figure 3.4) abscised more unopened flowers than open flowers. P 01 05 09 plants showed no significant difference in abscission of open and unopened flowers when plants kept in chambers for the first 48 h of the experiment (Figure 3.4). After 48 h, abscission of unopened flowers was greater than abscission of open flowers. A similar pattern of flowers abscission was observed by *Vigna unguiculata* plants (DHANALAKSHMI *et al.*, 2003).

DHANALAKSHMI *et al*. (2003) suggests that even under natural environmental conditions different organs have dominance over others. In an inflorescence, flowers at a certain stage of development may be dominant over flowers at another stage of development. In cowpea (*Vigna unguiculata*), flowers which open first (lower down on the inflorescence), are dominant over the flowers which open after them (higher up on the inflorescence). This will cause the flowers higher up on the inflorescence to abscise. As a result all resources are put into developing a few pods and not into opening and maintaining new flowers that may have reproductive potential. (DHANALAKSHMI *et al.*, 2003).

When plants are in stressful environments they may have to compromise and may not be able to sustain as many flowers as they would under optimal conditions. It seems in *Plectranthus* that some varieties shed open flowers under stressful conditions whilst others shed unopened flowers. There are advantages and disadvantages to both strategies. If older, open flowers are shed then there is the potential for unopened flowers to open and be available for pollination for a longer time than the flowers which are already open. However, in this situation the resources already used to produce and maintain the open flowers are lost to the plant. If younger, unopened flowers are abscised then all resources can be concentrated into maintaining flowers that have already used substantial resources and where pollination may already have occurred. However, when the unopened flowers abscise their potential to attract pollinators and be of reproductive potential is also lost.

It should be noted that in this experiment the chambers were opened every 24 h. This was done to put new silica gel into the chambers. This allowed ethylene in the chambers to escape and fresh air to enter the chamber. If chambers had been kept sealed for the full96 h, abscission may have been even higher.

3.3 The effect of dark on flower abscission

3.3.1 Introduction

Another factor which is thought to contribute towards flower abscission during transport is darkness. The trucks which are used to transport pot plants do not have lights fitted in them. Darkness has been shown to induce flowers abscission in pepper cultivars (SHIFRISS *et al.*, 1994; TURNER & WIEN, 1994a), *Lilium* 'Enchantment' (VAN MEETEREN, 1981), *Hibiscus rosa-sinensis* L. (VAN MEETEREN & VAN GELDER, 1995; VAN MEETEREN & VAN GELDER, 2000), soybean (HEINDL & BRUN, 1983) and snapdragons (CRAKER & DECOTEAU, 1984). *Hibiscus rosa-sinensis* sheds a high percentage of flowers and flower buds after 4-6 d in the dark (VAN MEETEREN & VAN GELDER, 1995).

Abscission is controlled or partly controlled by light (VAN MEETEREN & VAN GELDER, 2000). Far-red light wavelengths indicate seasonal changes for plants and abscission is a red light regulated response (ASCOUGH *et al.*, 2005). Research has been done on preventing dark-induced abscission using red light (MAO *et al.*, 1989; VAN MEETEREN & VAN GELDER, 2000). Both continuous exposure and short pulses of red light, during darkness, reduced dark-induced flower abscission (VAN LIEBURG *et al.*, 1990). The aim of the following experiments was to determine the effect of darkness on flower abscission and compare this to the increased flower abscission observed when plants were placed in perspex containers.

3.3.2 Materials and methods

Individual plants having at least three inflorescences were transferred from the greenhouse to the laboratory and the number of inflorescences and flowers were recorded. At the start of experimentation, approximately 20-25% of flowers on a plant were open, the rest were unopened. They were placed inside sealed (gas-tight) impermeable Perspex chambers (small: 288 x 438 x 250 mm (ca. 31.5 L), medium: 138 x 538 x 300 mm (ca. 70.7 L); large: 488 x 636 x 350 mm (ca. 108.6 L)) at 21 ± 2°C and kept in darkness. Plants were observed every 24 h for 4 d and the number of flowers that had abscised was recorded. These abscised flowers were further categorized as being open at the time of abscission, or unopened (i.e., buds). When flower abscission was recorded, plants were removed from the chambers and the chambers were cleaned and vented to remove excess moisture. Plants were then replaced and the chambers resealed.

The percentage flower abscission over time was recorded. Data were arcsine transformed and an analysis of variance (ANOVA) was carried out on all data. Data were analysed using a Duncan's test at the 5% level in GenStat, 14th edition.

3.3.3 Results

Flower abscission of unopened flowers was not significantly different in variety P 00 06 03 plants under dark conditions compared with plants kept in the light (Figure 3.6).

Flower abscission of open flowers was significantly higher in plants kept in the dark compared to plants kept in the light. There was far greater abscission of open flowers than of unopened flowers in this variety.

There was no significant difference in the abscission patterns of opened and unopened flowers by plants of variety P 00 06 03A in the 16 h photoperiod (Figure 3.6). There was no significant difference in flower abscission by plants kept in the dark and those kept in the light for the first 72 h, after which flower abscission of both opened and unopened flowers was higher under dark conditions than under light conditions.

For the first 24 h of the experiment there was no significant difference in flower abscission when P 96 04 06 plants were put into the dark compared to those kept in the light (Figure 3.6). Between 24 h and 72 h abscission of unopened flowers was significantly higher in the dark than in the light. At 96 h abscission of unopened flowers was significantly higher than abscission of open flowers in the dark and in the light.

For the first 72 h of the experiment there was no significant difference in abscission of open or unopened flowers by variety P 01 05 09 plants in the light and in the dark (Figure 3.6). After 72 h the abscission of unopened flowers in the dark was higher than open and unopened flowers in the light and open flowers in the dark.

From 72 h abscission of open flowers was significantly higher by P 00 06 07 plants in the light compared to in the dark (Figure 3.7). For the duration of the 96 h experiment abscission of open flowers was significantly higher by plants in the light than plants in the dark. This is the only variety dark conditions reduced abscission which is interesting as it is the only triploid (3n) variety.

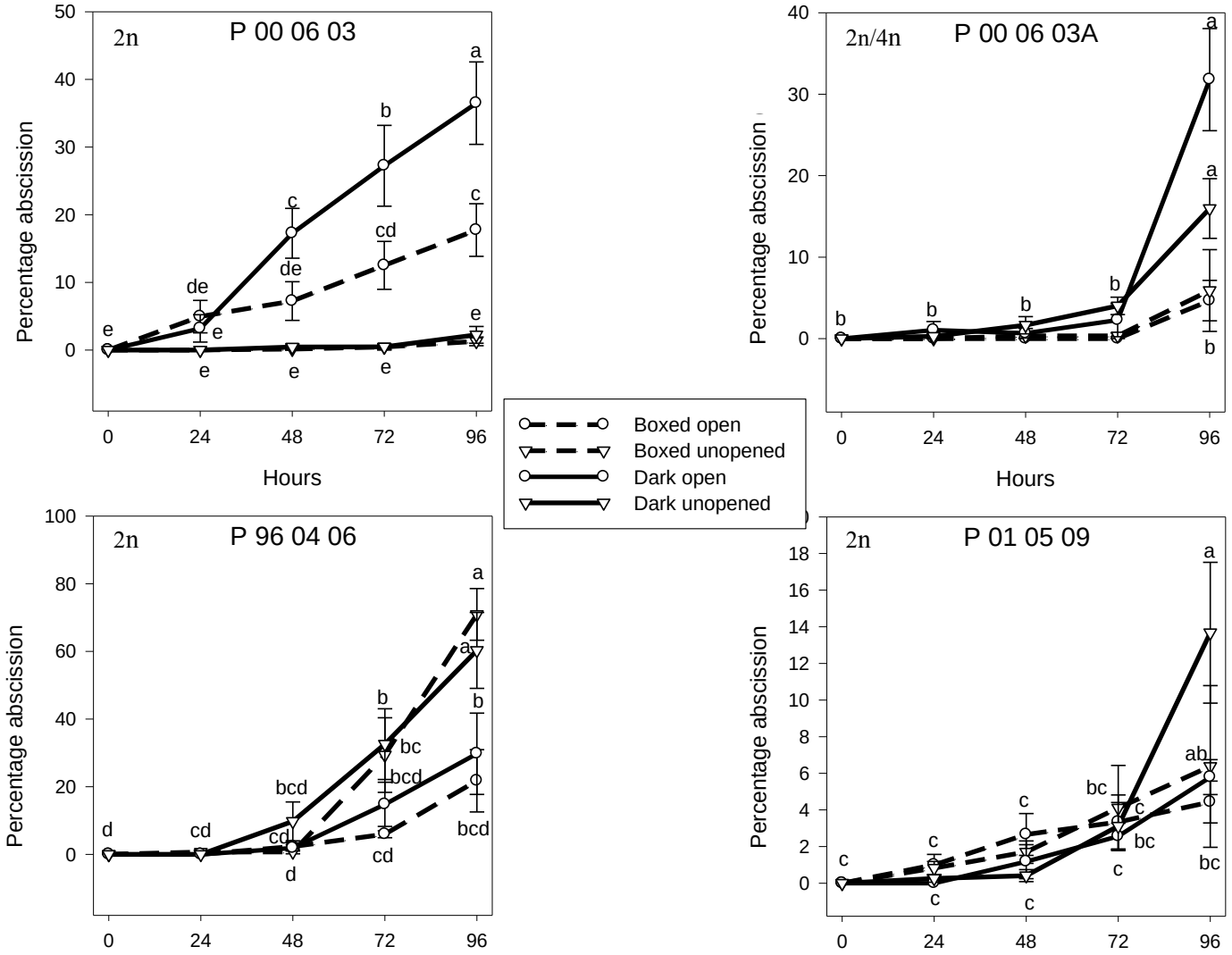

Figure 3.6: The effect of prolonged dark periods (96 h) on abscission of open and unopened flowers. Different letters indicate significant differences between treatments at a 5% level (ANOVA).

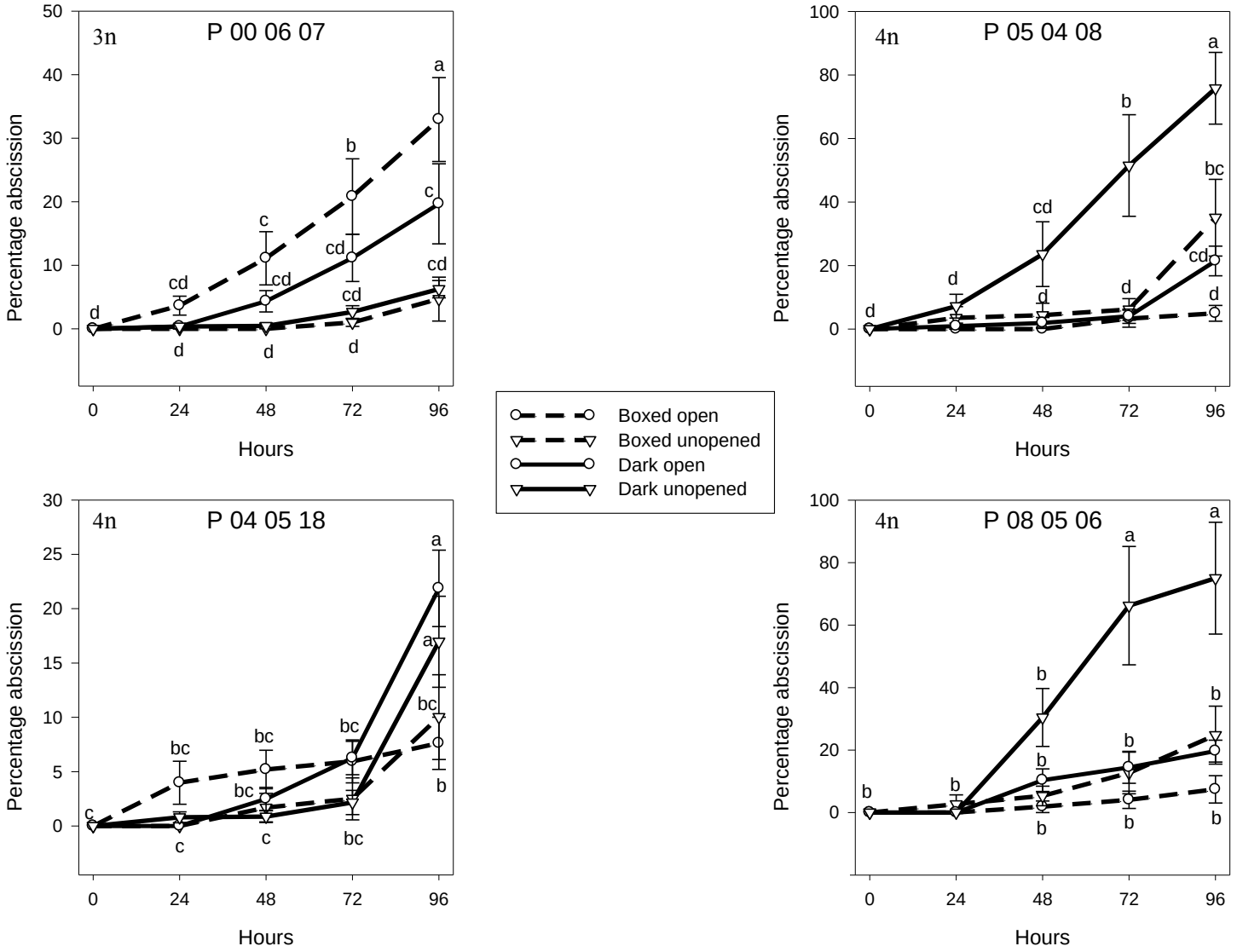

Figure 3.7: The effect of prolonged dark periods (96 h) on abscission of open and unopened flowers. Different letters indicate significant differences between treatments at a 5% level (ANOVA).

After 48 h duration of the experiment abscission of unopened flowers was significantly higher for P 05 04 08 plants in the dark than for plants in the light (Figure 3.7). There was no significant difference in abscission of open flowers by plants in the light compared to plants in the dark for the first 72 h of the experiment. However, after 72 h abscission of open flowers was higher in the dark than in the light.

For the first 48 h, flower abscission was higher for open flowers of P 05 04 18 plants in the 16 h photoperiod and there was no significant difference in flower abscission of open and unopened flowers in the light and unopened flowers in the dark (Fig. 3.7). Between 48 h and 96 h abscission of both open and unopened flowers in the dark increased to be significantly higher than abscission of open or unopened flowers in the light (Table 3.2).

Between 24 h and 96 h abscission of unopened flowers by P 08 05 06 plants in the dark was far higher than abscission of open or unopened flowers by plants in the 16 h photoperiod and open flowers by plants kept in the dark (Figure. 3.7). After 72 h abscission of unopened flowers in the light and open flowers in the dark was higher than abscission of open flowers by plants in the light.

For ease of comparison of abscission between varieties, results are summarised in Table 3.2. Exposure to darkness increased abscission in open flowers of all varieties except for P 00 06 07 where darkness decreased flower abscission of open flowers. Flower abscission of unopened flowers was greater when plants were exposed to darkness in all varieties except for P 96 04 06. However some of these changes were not significant.

Table 3.2: Summary of results showing percentage abscission of open and unopened flowers (±SD) after 96 h boxed in perspex chambers in a 16 h photoperiod or in the dark and preferential shedding of open or unopened flowers.

		Abscission after 96 h (%)				
		Boxed		Dark		
Variety	Ploidy	Open	Unopened	Open	Unopened	Preferential shedding in dark
P 00 06 03	2n	17.73±3.8	1.28±0.6	36.49±6.1	2.24±1.2	open
P 00 06 03A	2n/4n	4.67±2.5	5.9±5.0	31.79±6.2	15.97±3.6	open
P 96 04 06	2n	21.76±9.2	70.92±7.6	29.74±12.0	60.29±11.2	unopened
P 01 05 09	2n	4.43±1.1	6.38±4.4	5.79±0.9	13.68±3.8	unopened
P 00 06 07	3n	32.94±6.6	4.68±3.5	19.67±6.3	6.25±1.4	open
P 05 04 08	4n	4.97±2.5	35.06±12.1	21.44±4.7	75.79±11.3	unopened
P 04 05 18	4n	7.61±2.4	10.03±3.9	21.87±3.5	16.6±4.2	open
P 08 05 06	4n	7.42±4.4	24.79±9.3	19.67±3.5	75.03±17.8	unopened

3.3.4 Discussion

Darkness increased flower abscission in all but one of the eight varieties. Abscission of open flowers in variety P 00 06 07 was decreased by dark conditions (Figure 3.8 & Table 3.2). It is interesting to note that this is the only triploid variety. Darkness increased abscission of open flowers only in variety P 00 06 03 (Figure 3.7) and unopened flowers only in variety P 01 05 09 (Figure 3.6). In all other varieties abscission of both open and unopened flowers was increased in the dark. This confirms the results reported by ASCOUGH *et al*. (2008).

The effects of darkness on flower abscission have been attributed to a number of different factors. One group believe that darkness increases ethylene production by the plants (VAN MEETEREN, 1981; SEREK, 1993; SHIFRISS *et al.*, 1994; MÜLLER *et al.*, 1998) and that this in turn increases flower abscission. Others are of the opinion that reduced photosynthesis in the dark and so reduced assimilate (carbohydrate) supply induces flower abscission (HEINDL & BRUN, 1983; TURNER & WIEN, 1994b; LEBON *et al.*, 2004). A third group ascribe the effects of darkness on flower abscission to hormonal changes in the plant (MAO *et al.*, 1989)

Ethylene production was increased almost four fold in the dark in *Cucumis sativis* L. cv Ashy (ABDALLAH *et al.*, 1992). Light inhibited the production of ethylene by wheat leaves (WRIGHT, 1981) and SHIFRISS *et al* (1994) reported that darkness induced ethylene evolution by flowers. The same pattern was observed for flower buds of *Lilium* (VAN MEETEREN, 1982). Flower abscission induced by darkness is controlled through ethylene production by the flowers and flower buds (VAN MEETEREN & VAN GELDER, 2000). Ethylene biosynthesis in *Pelargonium* increased when cuttings were stored in the dark (MUTUI *et al.*, 2007). MUTUI *et al*. (2007) suggest that this increased ethylene production is due to increased activity by the enzymes involved in the ethylene biosynthetic pathway.

Subjecting plants to darkness limits assimilate accumulation which brings about flower and flower bud abscission (TURNER & WIEN, 1994a). When plants are put into the dark they cannot produce carbohydrates, a plant's source of energy, through photosynthesis. In the dark carbohydrate reserves are used up and not replenished (TURNER & WIEN, 1994b). In these conditions carbohydrate supply is concentrated in vegetative parts of the plants (TURNER & WIEN, 1994a). The supply to young buds and flowers may be reduced to a point where flowers and flower buds cannot be maintained (TURNER & WIEN, 1994a; ASCOUGH *et al.*, 2005). Higher carbohydrate reserves in the inflorescence favour flower development (LEBON *et al.*, 2004). Developing flowers need carbohydrates from floral initiation to maturation (YU *et al.*, 2000).

These other possible factors influencing flower abscission during transport conditions need to be investigated to completely understand the changes occurring within the plants when they are packaged and put into the dark.

3.4 Conclusions

Both packaging plants into closed container and darkness increased flower abscission of open and unopened flowers in all but one of the eight *Plectranthus* varieties compared to when plants were kept in the greenhouse. Flower abscission increased

when plants were put into the chambers and kept in a 16 h photoperiod. The effect of darkness on flower abscission was greater than the effect of packaging alone. Flower abscission increased considerably when plants were placed in the chambers and kept in the dark. From these experiments flower abscission during transport of *Plectranthus* can be attributed to a combination of packaging the plants and dark conditions.

Chapter Four

Ethylene sensitivity

4.1 Introduction

Ethylene is a major contributor to flower abscission during transportation of potted plants and cut flowers (REID *et al.*, 1989; BROWN, 1997). The sensitivity of plants and cuttings to ethylene has important implications during their transportation and handling (MÜLLER *et al.*, 1998). Knowing the ethylene sensitivity of a species, cultivar or variety, will allow for improved postharvest management of the plants (MACNISH *et al.*, 2004).

WOLTERING (1987) tested the ethylene sensitivity of 52 different species and based on the results classified the plants into 3 groups. Group 1 showed rapid wilting of flowers shortly after a treatment with exogenous ethylene and an increase in ethylene production. In this group wilting is mediated by ethylene. The flowers of group 2 wilted slowly after ethylene treatment and there was no significant increase in ethylene production by the plants. This suggests that wilting in this group is not mediated by ethylene. In group 3, flowers abscised without wilting. Here flower abscission is mediated by ethylene (WOLTERING, 1987). Additionally, showed that different species show varied ethylene sensitivity. However, differences in ethylene sensitivity have been recorded within different varieties and cultivars (MÜLLER *et al.*, 1998; MACNISH *et al.*, 2004; ONOZAKI *et al.*, 2008). The aim of this experiment was to establish the ethylene sensitivity of the eight different *Plectranthus* varieties provided by Dr Brits.

4.2 Materials and Methods

Sensitivity of plants to ethylene was determined by exposing plants to 0, 0.1, 0.25, 0.5, 1.0, or 2.0 $\mu l\ l^{-1}$ ethylene (Air Products, Johannesburg) for 4 d. A small amount of silica gel was placed inside the chambers to absorb excess moisture due to transpiration. This was replaced each day. Ethylene was injected using a syringe into the perspex chambers daily. Chambers of three different sizes were used small: 288 x

438 x 250 mm (ca. 31.5 L), medium: 138 x 538 x 300 mm (ca. 70.7 L); large: 488 x 636 x 350 mm (ca. 108.6 L). Flower abscission of open and unopened flowers was recorded every 24 h, during which time chambers were vented, re-sealed and re-injected with ethylene. Two or three plants were placed in each chamber, depending on the size of the plants.

4.3 Results

All varieties were sensitive to ethylene, with complete abscission occurring within 24 h when exposed to 1.0 or 2.0 $\mu l l^{-1}$ (Figures 4.1 and 4.2). Video 4.1 shows a time-lapsed video over 24 h of flower abscission by P 00 06 03 plants when chambers were injected with 2.0 $\mu l l^{-1}$. The video shows that 100% of flowers abscise in 24 h. Final abscission and rate of abscission decreased in a concentration-dependent manner.

In diploid varieties, unopened flowers were more sensitive to exogenous ethylene than open flowers as there was greater abscission of unopened flowers when plants were exposed to ethylene (Figure 4.1). In general, there was no significant difference in sensitivity of open and unopened flowers of tetraploid plants. Between 72 and 96 h there was higher abscission of unopened flowers at lower concentrations of ethylene (Figure 4.2). In P 00 06 03A (Figure 4.1) there was greater abscission of open flowers than unopened flowers.

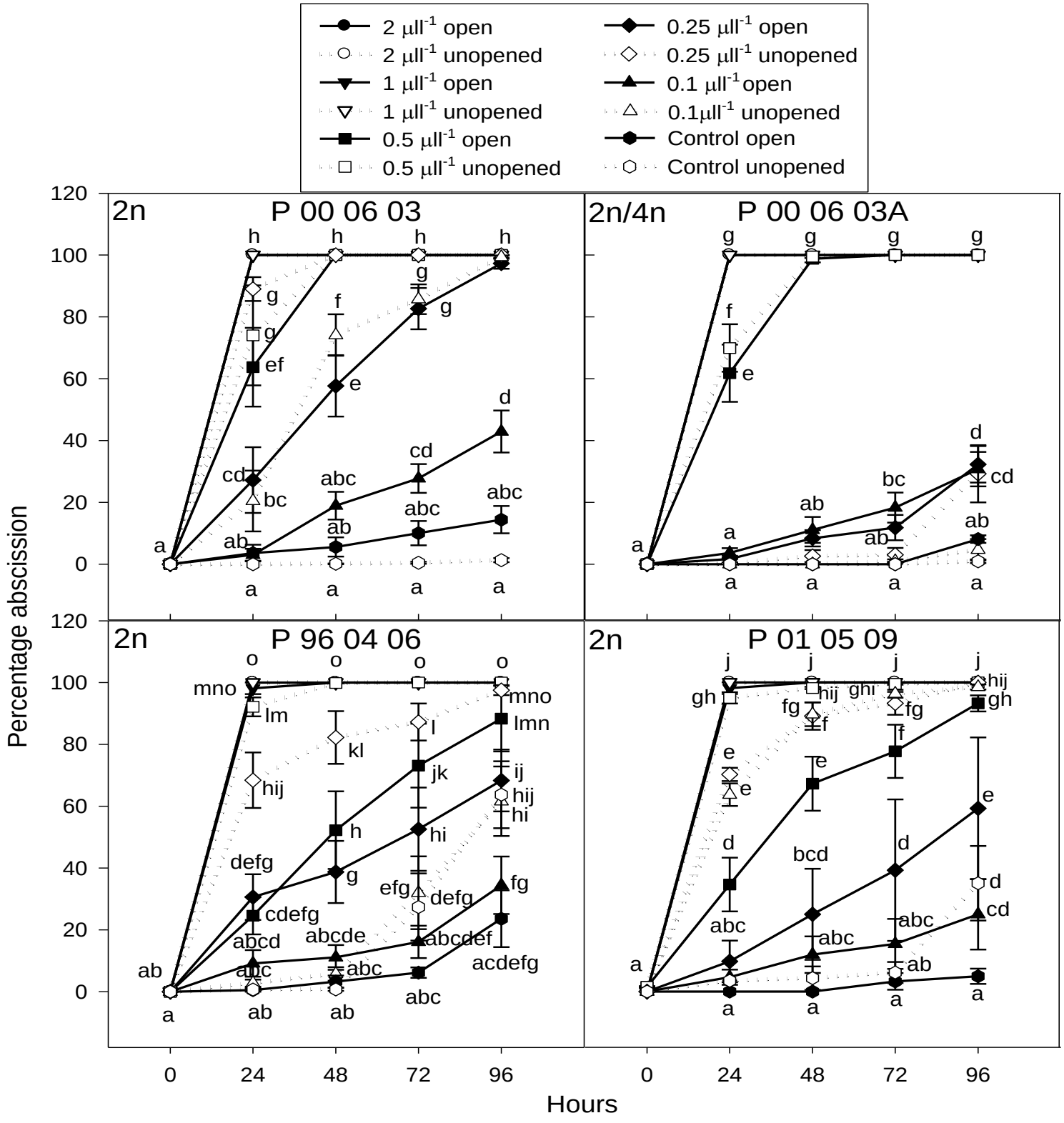

Figure 4.1. Flower abscission over time when plants were exposed to different concentrations of ethylene. Different letters indicate significant differences between treatments at a 5% level (ANOVA).

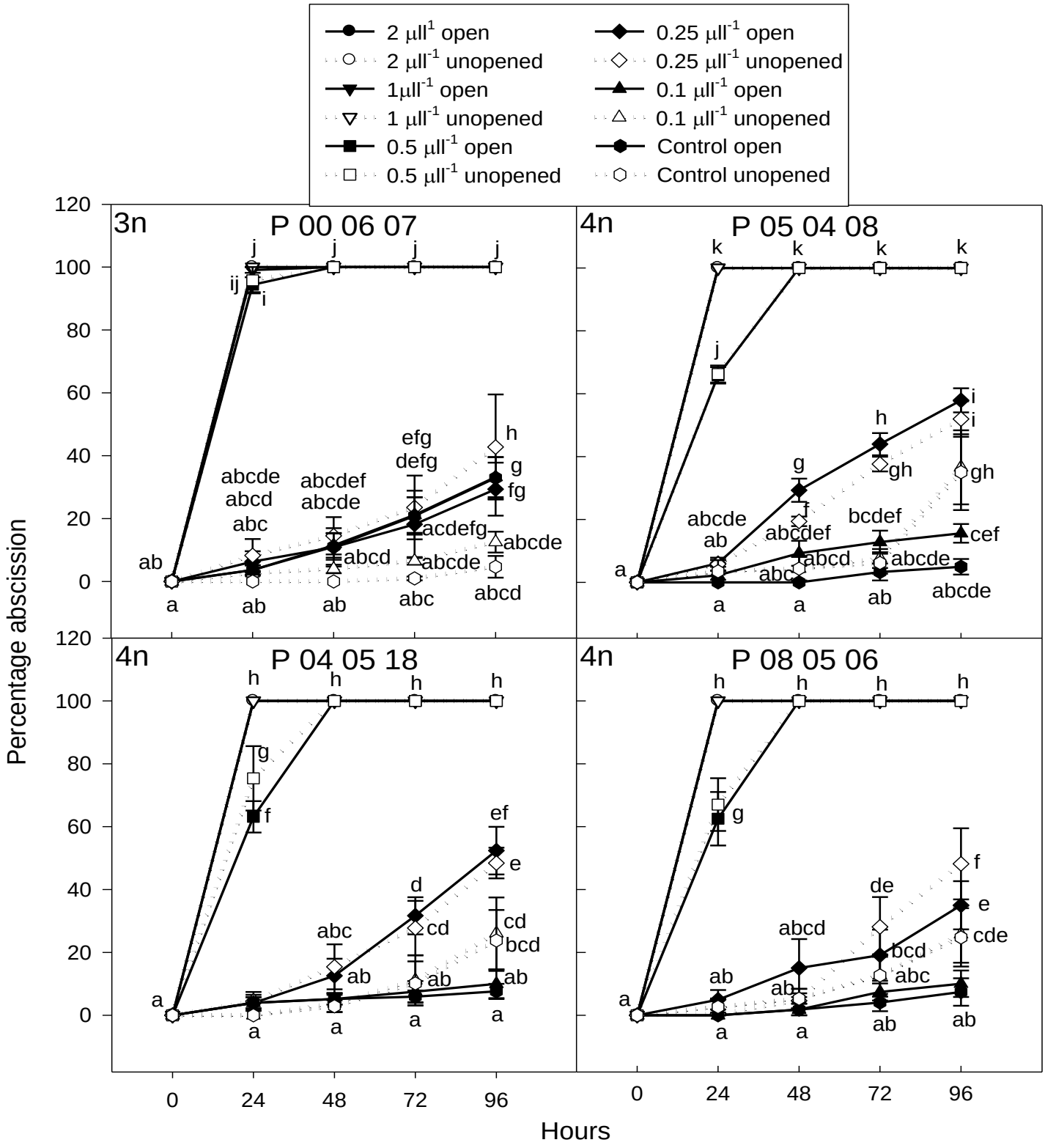

Figure 4.2. Flower abscission over time when plants were exposed to different concentrations of ethylene. Different letters indicate significant differences between treatments at a 5% level (ANOVA).

Diploid varieties (Figure 4.1) have different ethylene sensitivity patterns than the polyploid varieties (Figure 4.2). P 00 06 03A shows similar ethylene sensitivity to the other polyploid varieties as opposed to the diploid varieties. Polyploid varieties showed 100% abscission of open flowers within 48 h when chambers were injected with 0.5 $\mu l l^{-1}$ ethylene. In diploid varieties (P 96 04 06 and P 01 05 09) total abscission of open flowers was high when chambers were injected with 0.5 $\mu l l^{-1}$ ethylene, but it did not reach 100 %. Polyploid varieties (P 00 06 07, P 05 04 08, P 04 05 18 and P 08 05 06) were more sensitive to ethylene than diploid varieties.

4.4 Discussion

Flower abscission in *Plectranthus* is promoted by applied ethylene (Figures 4.1 and 4.2). In all varieties, complete abscission of open and unopened flowers occurred within 24 h when chambers were injected with 1.0 or 2.0 $\mu l l^{-1}$ ethylene. This places *Plectranthus* in the most sensitive category of pot plants (group 3) with *Clerodendron thomsonii* and *Browallia speciosa* (WOLTERING, 1987). Potted plants that exhibit petal abscission are very sensitive to ethylene (WOLTERING & VAN DOORN, 1988). At the lowest concentration tested, (0.1 $\mu l l^{-1}$) abscission in all varieties was significantly lower during the experiment compared to higher levels of ethylene treatment. When treated with 0.1 $\mu l l^{-1}$, flower abscission by varieties P 00 06 03A, P 04 05 18 and P 08 05 06 was not significantly different to flower abscission by control plants. Without ethylene, plants abscise very few flowers over a 96 h period. It appears that polyploid varieties are more sensitive to ethylene than diploid varieties at ethylene concentrations of 0.5 $\mu l l^{-1}$. However, at lower concentrations (0.1 and 0.25 $\mu l l^{-1}$) this was reversed and diploid varieties were generally more sensitive. In other species considerable variation has been observed with respect to cultivar sensitivity (MACNISH *et al.*, 2004).

In *Chamelaucium,* an ethylene concentration of 100 $\mu l\ l^{-1}$caused 100% flower abscission in some varieties while others were almost non-responsive to the treatment, losing only 10% of their flowers (MACNISH *et al.*, 2004). This not only highlights the extreme differences in ethylene sensitivity within cultivars, but also just how sensitive *Plectranthus* is to ethylene. In all varieties 100% abscission was observed

when chambers were injected with 1 $\mu l l^{-1}$ ethylene, 1/100 the concentration used by MACNISH (2004).

P 00 06 03A shows a polyploid pattern of flower abscission when exposed to ethylene rather than following the same patterns as the diploid varieties. This is interesting as this variety has both diploid and tetraploid cells but seemingly behaves as a tetraploid in this aspect. However, open flowers were more sensitive to ethylene when compared to unopened flowers. This pattern was different to that observed in both diploid and tetraploid varieties. If there was a significant difference in the abscission of opened and unopened flowers in the other varieties, there was greater abscission of unopened flowers. It seems that we currently cannot predict how mixoploid varieties will behave. Other examples of ethylene sensitivity in mixoploid plants have not been reported.

Polyploid varieties had different abscission patterns when exposed to ethylene compared to diploid varieties. Differences in ethylene sensitivity among varieties has been observed in miniature potted roses (MÜLLER *et al.*, 1998). This may be a reason for breeders of *Plectranthus* to attempt to achieve a triploid or tetraploid state in new varieties. These were less sensitive to ethylene at low concentrations, similar to those which they encounter during transport. Breeding efforts should be concentrated on cultivars which are less sensitive to ethylene (MÜLLER *et al.*, 1998). Insensitivity to ethylene is a heritable trait (WOLTERING *et al.*, 1993) and should be selected for when breeding for the flower industry (WOLTERING & VAN DOORN, 1988). Genes coding for ethylene insensitivity from one cultivar or variety can be transformed into another cultivar of variety.

Generally, unopened flowers were more sensitive to exogenous ethylene than open flowers, as there was greater abscission of buds. This is similar to the patterns observed for *Dendrobium* cv Miss Teen. Exogenous ethylene induced abscission of unopened buds but not of open flowers (BUNYA-ATICHART *et al.*, 2011). The opposite was found in tomato plants, where exogenous ethylene caused abscission of open flowers as opposed to unopened flowers (LASHBROOK *et al.*, 1998). It seems that there are also differences in sensitivity of flowers at different stages of maturation between species as in the case of *Plectranthus* varieties. P 00 06 03A is different to

the other varieties in this study as the open flowers on these plants are more sensitive to ethylene than the unopened flowers.

Plectranthus is extremely sensitive to ethylene and this is of foremost importance when considering transportation and handling of these plants. If there is a build-up of ethylene during transportation of *Plectranthus*, concentrations would only have to reach around 0.25 $\mu l l^{-1}$ to significantly increase flower abscission over a 96 h period.

Chapter 5

Effects of darkness on ACC oxidase and ACC synthase during flower abscission.

5.1 Introduction

Ethylene (C_2H_2) is a simple-structured hydrocarbon (YANG & HOFFMAN, 1984; SAKAI *et al.*, 1998; JOHNSON & ECKER, 1998; BLEECKER & KENDE, 2000) that acts as a plant hormone (CHANG *et al.*, 1993) responsible for regulating many plant processes and responses to environmental stimuli (JOHNSON & ECKER, 1998; PIERIK *et al.*, 2006). Ethylene is necessary for normal plant growth and development (DEIKMAN, 1997; CZARNY *et al.*, 2006) in a regulatory capacity (BLEECKER *et al.*, 1988), but is not needed for survival (JOHNSON & ECKER, 1998). This phytohormone is involved in regulating seed germination (JOHNSON & ECKER, 1998; BLEECKER & KENDE, 2000; KURODA *et al.*, 2004; MA *et al.*, 2006; CZARNY *et al.*, 2006), breaking seed dormancy (CHANG *et al.*, 1993), seedling growth rate (KURODA *et al.*, 2004), flower initiation (CHANG *et al.*, 1993) and development (CZARNY *et al.*, 2006), sex determination (TIAN & LU, 2006), fruit ripening (CHANG *et al.*, 1993; DEIKMAN, 1997; JOHNSON & ECKER, 1998; BLEECKER & KENDE, 2000; KURODA *et al.*, 2004; MA *et al.*, 2006), organ senescence (CHANG *et al.*, 1993; DEIKMAN, 1997; JOHNSON & ECKER, 1998; BLEECKER & KENDE, 2000; MA *et al.*, 2006), leaf and flower abscission (CHANG *et al.*, 1993; DEIKMAN, 1997; KURODA *et al.*, 2004; MA *et al.*, 2006) and the initiation of plant defense responses (JOHNSON & ECKER, 1998; CZARNY *et al.*, 2006). The increase in ethylene production under these circumstances is referred to as 'stress ethylene' (ABELES *et al.*, 1992).

In general ethylene synthesis by plant tissues is low but increases at certain developmental stages (JONES & WOODSON, 1999). Ethylene is active at very low concentrations, less than 1 $\mu l l^{-1}$ (PECH *et al.*, 2004) and apart from during 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) and drought (DEIKMAN, 1997). As a result processes such as senescence and abscission are accelerated (CHANG *et al.*, 1993)

The ethylene biosynthetic pathway (Figure 5.1) follows a series of processes that occur from the reception and recognition 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 & YANG, 1979). The ethylene biosynthetic pathway is well documented. However, the molecular action behind the pathway was only uncovered in the late 1990's (CHAO *et al.*, 1997). Two enzymes in the pathway ACC synthase (ACS) and ACC oxidase (ACO) are involved in regulating the synthesis of ethylene (KENDE & ZEEVAART, 1997; PECH *et al.*, 2004; TIAN & LU, 2006). 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 & ZEEVAART, 1997; BARRY *et al.*, 2000; WANG *et al.*, 2002; TIAN & LU, 2006; MA *et al.*, 2006), the members of which are expressed differentially in response to developmental, environmental and hormonal cues (KENDE & ZEEVAART, 1997). This enzyme is responsible for the conversion of SAM to ACC (PECH *et al.*, 2004) and therefore, expression and transcription of the ACS genes are important in regulating ethylene biosynthesis (VOGEL *et al.*, 1998; JOHNSON & ECKER, 1998). Initially it was thought that this was the only rate limiting step in the pathway, however evidence now shows that ACO is also involved in regulation (KENDE & ZEEVAART, 1997).

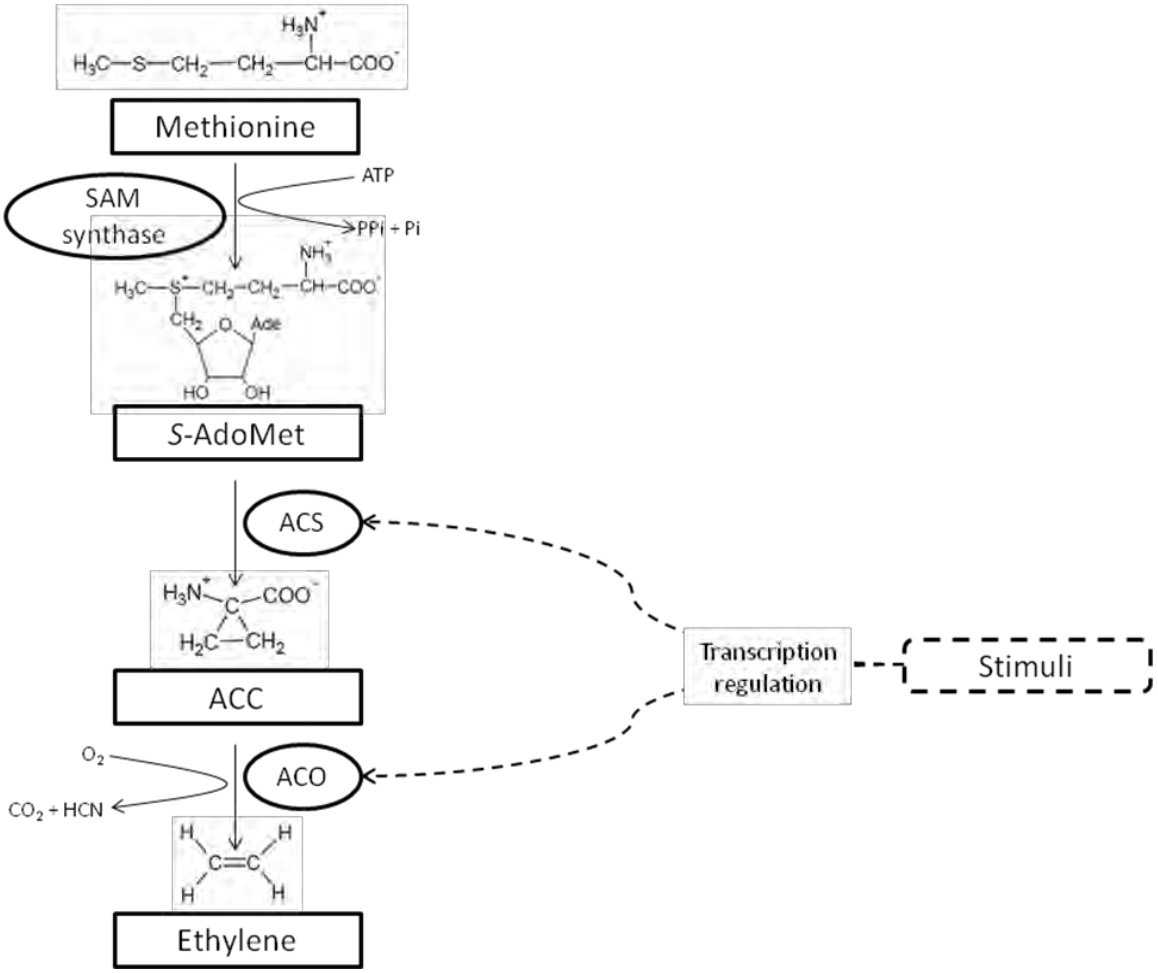

Figure 5.1. The ethylene biosynthetic pathway. Adapted from (BLEECKER & KENDE, 2000; WANG *et al.*, 2002; TIAN & LU, 2006).

Prior to the isolation of ACO in 1990 (HAMILTON *et al.*, 1990) it was referred to as Ethylene-forming enzyme (EFE) (WOODSON *et al.*, 1992; PECH *et al.*, 2004). ACO is encoded by a small multigene family (KENDE & 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 & ZEEVAART, 1997; JOHNSON & ECKER, 1998). Ethylene biosynthesis is also regulated during this step (KENDE & ZEEVAART, 1997).

Plants abscise their flowers due to environmental stresses such as frost, drought, pathogen attack and reduced sunlight (KALAITZIS *et al.*, 1997; TAYLOR & WHITELAW, 2001).

These are similar stresses that increase ethylene biosynthesis. Here we investigate the effect of darkness on the ethylene biosynthetic pathway and subsequently on flower abscission.

Studies have shown the effects of pollination (TANG & WOODSON, 1996) and exogenous ethylene treatments on ACS and ACO expression levels. Both pollination and exogenous ethylene upregulate the expression of ACS and ACO (TANG & WOODSON, 1996). The effect of darkness however does not seem to have been investigated. As ACS and ACO are involved in the regulation of ethylene biosynthesis we chose to examine the expression levels of these two enzymes. We investigated the effect of long periods of darkness (up to 96 h) on ACS and ACO levels in the pedicles (including the abscission zone) of open and closed flowers in four varieties (P 00 06 03A, P 00 06 03, P 04 05 18 and P 08 05 06) of *Plectranthus*. These four varieties were selected so that the experiment was conducted for both diploid and tetraploid varieties.

5.2 Materials and Methods

5.2.1 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 were used to design primer pairs manually. The following degenerate primers were used for cloning the corresponding sequences:
ACOF GDATTDCKSCHGAGTTTATGG and
ACOR TCGAAGTARGTCAGHAGRTTHTG;
ACSF GCDMTVTTTCAAGAYTATCATG and
ACSR GARAYBARVCCRAARCTCGACAT;
ACTINF GAAATYGTDAGGGACATCAAAGAGAAG and
ACTINR GCCACSACCTTRATCTTCATGCTGC.

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 ^{TM} 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 high fidelity enzyme (Clontech) and the degenerate primers. Samples were held at 72 °C for 3 min over 7 cycles. Step 2 was 30 sec 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 these were 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). Three vectors (clones) were sent for sequencing, and the inserts were sequenced from each side to reduce the risk of mismatch and sequencing errors. From the sequences specific primers for the genes of interest were designed using Primer3. The results of the ligation and cloning were checked by performing a restriction digest on the product of the miniprep. PstI and NcoI restriction enzymes were used. 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 found, 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).

5.2.2 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 P 00 06 03A, P 00 06 03, P 04 05 18 and P 08 05 06 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.

5.3 Results

Open flowers of P 00 06 03A and P 00 06 03 had increased levels of ACS mRNA when put into the dark (Figure 5.2). 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 (Figure 5.2), 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 (Figure 5.2).

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 (Figure 5.2).

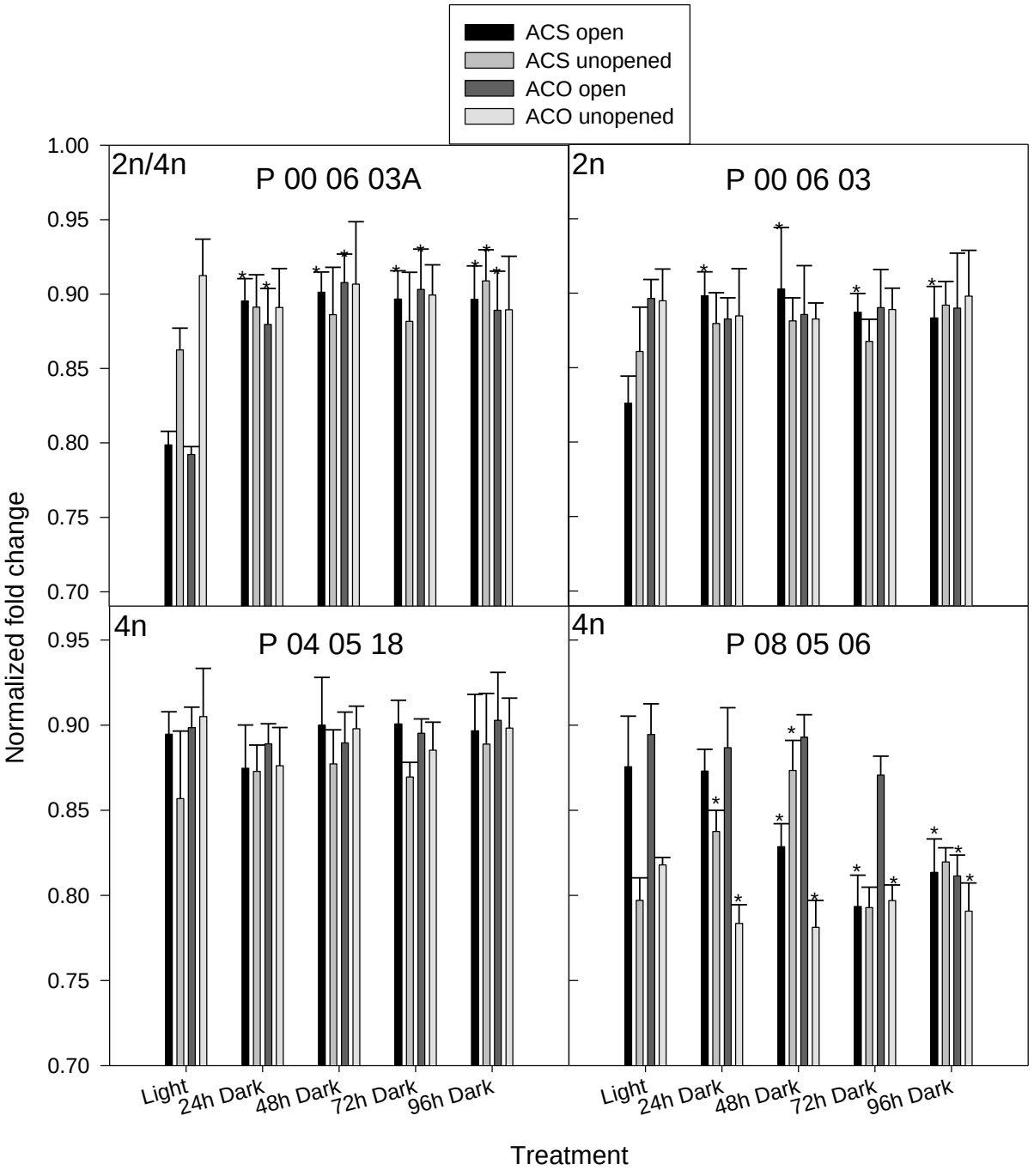

Figure 5.2. ACS and ACO mRNA levels in pedicels of open and unopened *Plectranthus* flowers kept in 16 h light: 8 h dark, 24, 48, 72 and 96 h dark.* marks treatments which differ significantly from the light treatment.

Open flowers on P 08 05 06 did not initially show any change in ACS mRNA levels when put into the dark (Figure 5.2). 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 variety 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.

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 (Figure 5.2). 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 (Figure 5.2)

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 (Figure 5.2). There was, however, a notable decrease in ACO mRNA levels once plants had been exposed to darkness for 96 h. There was a decrease in ACO mRNA levels in unopened flowers on P 08 05 06 when plants were put into the dark compared to those kept in the light (Figure 5.2). ACO mRNA levels did not differ significantly at different time periods during dark treatments.

5.4 Discussion

The two diploid (2n) varieties (P 00 06 03A and P 00 06 03) showed similar trends in ACS mRNA levels when placed in the dark (Figure 5.2). 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. Ethylene production is usually up-regulated when plants are stressed. 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 & HOFFMAN, 1984). Accumulation of ACS protein leads to elevated levels of cellular ACS activity and thus increased ethylene production (TIAN & LU, 2006).

The trends shown by P 00 06 03A and P 00 06 03 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 signaling. Flowers are energetically "expensive" organs to produce and maintain. In the case of *Plectranthus* they are necessary for reproduction, but are not vital for plant survival as the plants grow readily from cuttings. It may be beneficial to abscise flowers under stressed conditions ahead of other organs (for example leaves) which are necessary for the plant to survive.

Unopened flowers of P 00 06 03A and P 00 06 03 do not show a significant change in ACS mRNA levels and thus no notable change in abscission of unopened flowers occurs under dark conditions (Figure 5.2). This may indicate an attempt to retain unopened flowers as opposed to open flowers. ASCOUGH *et al*. (2008) showed that when P 00 06 03 plants were put in the dark there was preferential abscission of open flowers compared to unopened flowers. As P 00 06 03A show similar trends, this may also explain the unchanged ACS mRNA levels in unopened flowers in this variety. This supports the hypothesis that flowers are shed from the plant when they become energetically unprofitable in comparison to opening a new flower (ASHMAN & SCHOEN, 1994).

Changes in ACO mRNA levels in P 00 06 03A (Figure 5.2) are consistent with increased flower abscission of open flowers and maintaining unopened flowers on the plant. The increase in ACS and ACO mRNA levels (Figure 5.2) indicate that the whole ethylene biosynthetic pathway has been up regulated when plants of this variety are placed in the dark. Increases in ACS and ACO mRNAs are also observed in senescing flowers. An 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. TANG and WOODSON (1996) found that pollination in mature *Petunia hybrida* flowers was followed swiftly by an increase in ethylene production, but in immature flowers ethylene production was delayed. Perhaps the SAM and ACC needed to produce ethylene are not present in high enough concentrations in the tissues of unopened flowers, preventing ethylene synthesis from increasing.

No change in ACO mRNA levels were detected in either open or unopened flowers of P 00 06 03 kept in the dark (Figure 5.2). As there was no change in the levels of ACS mRNA in unopened flowers of this variety it was not expected that there would be a change in ACO mRNA levels. Unopened flowers did however show a change in ACS mRNA levels when placed in the dark. It is therefore strange that there is not a change in ACO mRNA levels in open flowers in the dark. This suggests that there is not an increase in ethylene production by open flowers in this variety.

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 (Figure 5.2). A change in ACO mRNA levels would depend on a change in ACS mRNA levels. 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 variety when kept in the dark. These tetraploid plants are larger than the other diploid varieties used in this study and they had much larger leaves, suggesting greater photosynthetic potential and so greater carbohydrate production potential. Perhaps this allows this variety to sustain their flowers for longer under stress before having to abscise them.

The trends shown by both open and unopened flowers of P 08 05 06 (Figure 5.2) are unusual when compared to the other varieties used in this study. Open flowers show a decrease in ACS mRNA and ACO mRNA when plants are placed in the dark suggesting that ethylene production is down-regulated in the dark. Unopened flowers show an initial increase in ACS mRNA suggesting that ethylene production increases in unopened flowers when initially in the dark but then decreases. However, ACO mRNA levels decrease in unopened flowers of this variety in the dark. This suggests a decrease in ethylene synthesis. Both ACS and ACO action are needed to synthesize ethylene and so ethylene production is not increased in these flowers. This may be an attempt to maintain the more valuable unopened flowers as opposed to open flowers in which senescence has already been partly initiated (ASHMAN & SCHOEN, 1994).

Although the changes in ACS and ACO expression were not as great as had been expected it is likely that these changes contribute to the increased flower abscission observed during transport in *Plectranthus*

Chapter 6

Dark induced changes in cytokinins

6.1 Introduction

There are numerous claims that organ abscission is primarily regulated by plant growth hormones (MARCELIS *et al.*, 2004). When plant growth hormones are discussed in the context of organ abscission regulation, ABA, ethylene and auxins dominate the research (VOGEL *et al.*, 1998; CHANG *et al.*, 2003; DEL POZO *et al.*, 2005; ABEBIE *et al.*, 2008). Many authors have investigated the roles and interactions of ABA, ethylene and auxins during the abscission process (YU *et al.*, 2000). The role of ethylene in inducing flower abscission is a recurring theme throughout this research project.

However, very little has been published regarding the role of cytokinins and the changes in endogenous cytokinins during the abscission process (VAN DOORN & STEAD, 1997; DAL CIN *et al.*, 2007). Extensive research has been conducted on the use of exogenous cytokinins in preventing organ abscission. Soybean has been the subject of much of this work (CROSBY *et al.*, 1981; CARLSON *et al.*, 1987; NAGEL *et al.*, 2001). The low fruiting rates due to high flower and pod abscission have driven the need for preventative measures. Cytokinins applied to the transpiration stream (NAGEL *et al.*, 2001) or as an aerosol spray to the terminal inflorescence (CROSBY *et al.*, 1981; CARLSON *et al.*, 1987) prevented both flower and pod abscission.

Cytokinins stimulate ethylene production to some extent in a number of plant tissues (YU *et al.*, 1979) and in young seedlings (FUCHS & LIEBERMAN, 1968). It is thought that many of the well-known effects of cytokinins are in fact due to an increase in ethylene synthesis caused by cytokinins as opposed to the cytokinins themselves. NOODÉN *et al*. (1990) and FANG *et al*. (2012) suggest that cytokinin concentrations change when environmental conditions change. This leads to the question of how cytokinins change when plants are put into the dark.

It has been established that when *Plectranthus* plants are kept under simulated transport conditions, flower abscission increases (Chapter 3). In an attempt to understand and explain the cause of this transport-induced abscission, the changes in cytokinin concentration in the flower pedicle during simulated transport condition were investigated.

6.2 Materials and methods

Due to cost and time constraints cytokinin analysis was only carried out for two of the *Plectranthus* varieties. The results showed similar trends in both of the chosen varieties, accordingly it was assumed that these same trends would be seen in the remaining six varieties.

Plants grown in the greenhouse were transferred to the dark at the beginning of the experiment. Plants were kept in the dark and were watered every second day in accordance with the watering regime maintained in the greenhouse. Pedicle samples were collected from plants which had been kept in the green house (0 hours dark) and from plants kept in the dark for 24, 48, 72 and 96 h. 500 mg of pedicle was collected for each treatment and three replicates of each treatment were collected. Replicates contained samples from at least three different individual plants. Data for light conditions are represented as 0 h and data for dark conditions are represented as 24, 48, 72 and 96 h on graphs.

Pedicle tissue was freeze-dried and ground in liquid nitrogen in a mortar and pestle. Ground samples were placed in airtight Eppendorf tubes and kept in a freezer at -20 °C until they were used for cytokinin purification and analysis. Cytokinin content of 43 different cytokinins was measured. Cytokinins measured were: tZ group (tZ, tZR, tZOG, ZROG, tZ9G and tZR5'MP), cZ group (cZ, cZR, cZOG, cZROG, cZ9G and cZR5'MP), DHZ group (DHZ. DHZR, DHZOG, DHZROG, DHZ9G and DHZR5'MP), iP group (iP, iPR, iP9G and iPR5'MP), BAP group (BAP, BAPR, BAP9G and BAPR5'MP), mT group (mT, mTR, mTOG, mTROG, mT9G and mTR5'MP), oT group (oT, oTR, oTOG, oTROG, oT9G and oTR5'MP) and pT group (pT, pTR, pTOG, pTROG, pT9G and pTR5'MP).

For cytokinin purification, a modified method described by FAISS *et al*. (1997) was used. Deuterium-labelled CK internal standards (Olchemim Ltd, Czech Republic) were added,1 pmol of each per sample, to check recovery during purification and to validate the determination (NOVÁK *et al.*, 2008). The samples were purified using a combination of cation (SCX-cartridge), anion [DEAE-Sephadex-C18-cartridge] exchangers and immunoaffinity chromatography (IAC) based on wide-range specific monoclonal antibodies against cytokinins (NOVÁK *et al.*, 2003). The eluates from the IAC columns were evaporated to dryness and dissolved in 20 µl of the mobile phase used for quantitative analysis. The samples were analysed by ultra performance liquid chromatography (UPLC) (Acquity UPLC^{TM}; Waters, Milford, MA, USA) coupled to a Quatro *micro*^{TM} API (Waters, Milford, MA, USA) triple quadrupole mass spectrometer equipped with an electrospray interface. The purified samples were injected onto a C18 reversed-phase column (BEH C18; 1.7 µm; 2.1 x 50 mm; Waters). The column was eluted with a linear gradient (0 min, 10% B; 0–8 min, 50% B; flow-rate of 0.25 ml/min; column temperature of 40°C) of 15 mM ammonium formate (pH 4.0, A) and methanol (B). Quantification was obtained by multiple reaction monitoring (MRM) of $[M + H]^+$ and the appropriate product ion. For selective MRM experiments, optimal conditions, dwell time, cone voltage, and collision energy in the collision cell corresponding to exact diagnostic transition were optimized for each cytokinin (NOVÁK *et al.*, 2008). Quantification was performed by Masslynx software using a standard isotope dilution method. The ratio of endogenous cytokinin to appropriate labelled standard was determined and used to quantify the level of endogenous compounds in the original extract, according to the known quantity of added internal standard (NOVÁK *et al.*, 2003).

6.3 Results

Cytokinin concentrations of BAP, mT, oT and pT groups were lower that the level of detection. To make trends in the data clearer, data for the remaining cytokinin groups was pooled and the mean content of cytokinin groups (i.e. tZ, cZ, DHZ and iP) was plotted against time. Results of individual cytokinin content during the experiment is presented and explained in Appendix 1.

In both varieties, cytokinins in open and unopened flowers show some similar trends. Concentrations of tZ and DHZ cytokinin groups are significantly lower than concentrations of cZ and iP cytokinin groups (Figure 6.1). In all, except unopened flowers of P 01 05 09, tZ and DHZ groups follow similar trends throughout the experiment. In unopened flowers of P 01 05 09, content of the tZ group spikes at 24 h and then again quite dramatically at 96 h while DHZ remains fairly stable.

Concentrations of DHZ and tZ groups peaked at 48 h and DHZ peaked a second time at 96 h in open flowers of both varieties (Figure 6.1a & 6.1c). In unopened flowers these groups peaked at 24 h in both varieties and then again at 96 h in P 01 05 09 (Figure 6.1b & 6.1d). These trends were only significant for tZ-type cytokinins in unopened P 01 05 09 flowers at 96 h.

Most of the cytokinin groups are present in pedicles in greater concentrations in the dark than in the light. Exceptions to this are the iP group in open flowers of P 00 06 03 and cZ and iP groups in open flowers of P 01 05 09 (Figure 6.1a & 6.1c). Concentrations of iP dropped below concentrations observed in the light at 72 and 96 h in P 00 06 03 and at 24 and 96 h in P 01 05 09. The cZ group showed concentration lower than those measured in the light at 96 h in P 00 06 03.

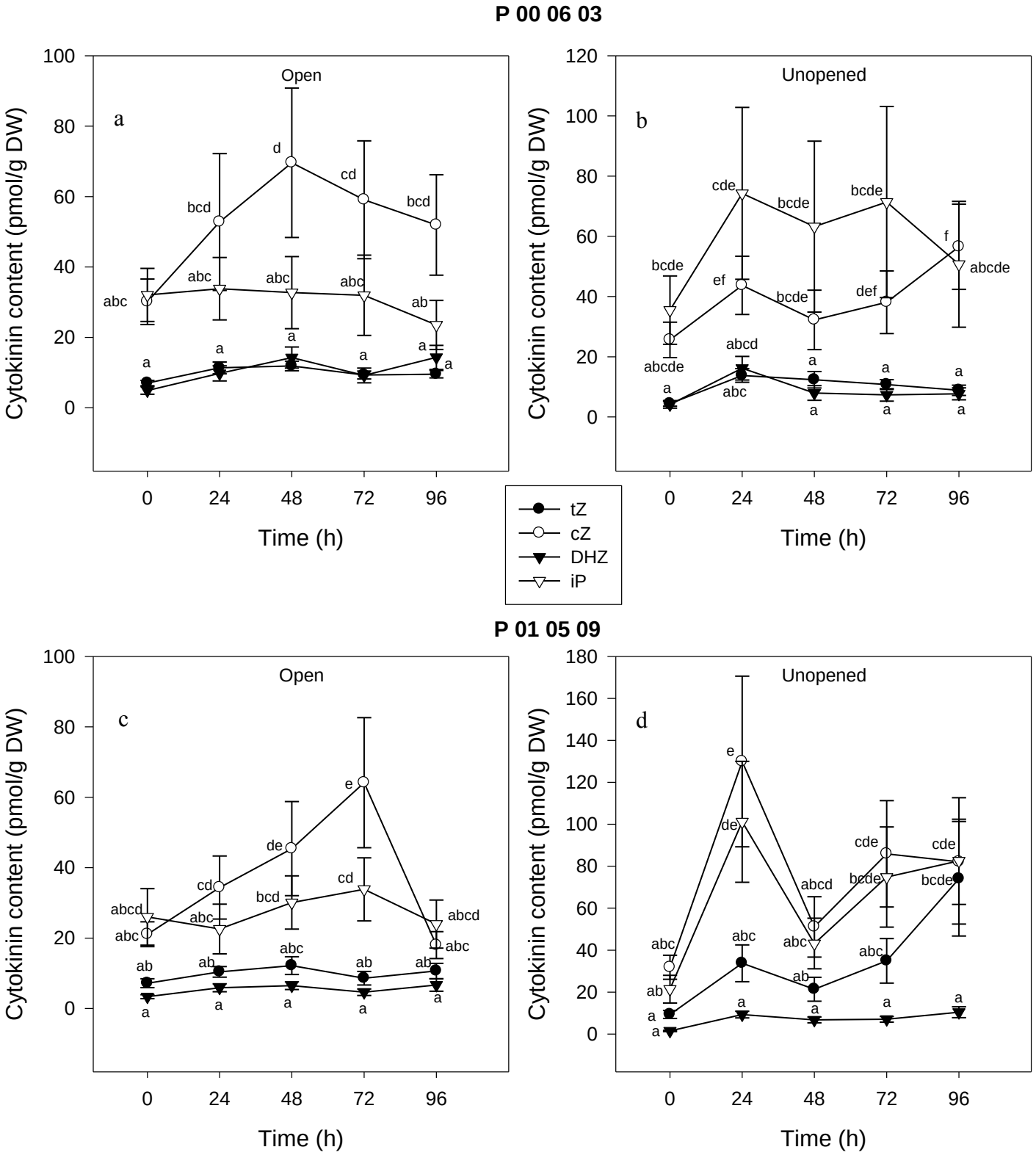

Figure 6.1: Changes in cytokinin concentrations (pmol/g dry weight) of cytokinin groups in pedicles of P 00 06 03 and P 01 05 09 plants taken from control light conditions (0 h) and placed under continuous darkness (24, 48, 72 and 96 h). Different letters indicate significant differences between treatments at a 5 % level (ANOVA).

In open flowers of P 00 06 03, iP concentrations were greatest at 24 h, however the change was very small and the concentration of this group was fairly stable (Figure 6.1a). In contrast the cZ group showed significant changes during the experiment with concentrations peaking at 48 h. In unopened flowers of P 00 06 03 concentrations of the iP group peaked at 24 h, while concentrations of the cZ group increased at 24 h but peaked at 96 h (Figure 6.1b). Concentrations of iP and cZ were greatest at 72 h in open flowers of P 01 05 09 (Figure 6.1c) while concentrations of both iP and cZ groups peaked at 24 h in unopened flowers (Figure 6.1d).

6.4 Discussion

Cytokinin groups behaved differently when plants were put into the dark. This was also observed by NOODÉN *et al*. (1990) in soybean and in *Chenopodium* species (MACHÁČKOVÁ *et al.*, 1993). FANG *et al*. (2012) suggested that cytokinins play a regulatory role in photosynthetic acclimation to canopy light gradients and senescence in wheat plants when they observed that wheat leave exposed to different light conditions showed differences in cytokinin concentrations. Generally, cytokinin concentrations increased when plants were put into the dark (Figure 6.1).

It is interesting to note where the peaks in cytokinins occur in relation to flower abscission. The increase in abscission of open flowers of P 00 06 03 after 48 h in the dark may be the effect of increased concentrations of tZ, DHZ and cZ cytokinins in pedicle tissue at 48 h in the dark.

An increase in flower abscission of open flowers in P 01 05 09 plants from 72 h in the dark follows a peak in DHZ and tZ cytokinins at 48 h and a peak in cZ and iP cytokinins at 72 h in the dark.

Similarly in unopened flowers of P 01 05 09, an increase in flower abscission occurred after 48 h in the dark. This was preceded by a peak in tZ, DHZ, cZ and iP cytokinin concentrations after 24 h in the dark.

Both increases and no changes in cytokinin levels as a result of dark exposure have been reported. In tobacco the levels of cytokinins increased when plants were kept in

the dark (BENKOVÁ *et al.*, 1999), whilst in wheat species, cytokinin levels remained relatively unchanged when plants were placed in the dark (MACHÁČKOVÁ *et al.*, 1993).

The aim of this experiment was to determine if changes in cytokinin levels in open and unopened flowers of *Plectranthus* could be responsible for the increased flower abscission under dark conditions. VAN STADEN (1973) suggested that an increase in cytokinins may induce abscission. There are reports that increased cytokinin levels result in increased ethylene production by stimulating the ethylene biosynthetic pathway (FUCHS & LIEBERMAN, 1968; VOGEL *et al.*, 1998), especially in stressed plant tissues (CARY *et al.*, 1995). It is possible that changes in cytokinin concentrations, when plants are put into the dark, are causing an increase in flower abscission. In this experiment, the changes in a number of cytokinins were observed and it is possible that it may be the change in only one of these or a combined effect which causes an increase in flower abscission in *Plectranthus*.

6.5 Conclusions

Changes in cytokinin concentrations, which occur in open and unopened flowers when *Plectranthus* plants are put into the dark, may induce ethylene production which in turn induces flower abscission. Although different groups of cytokinins behaved differently in dark conditions, it is possible that a change in only one of these can trigger an increase in the ethylene biosynthetic pathway. If this is true, then a change in only one group of cytokinins could induce flower abscission.

Chapter 7

Carbohydrate changes during simulated transport conditions

7.1 Introduction

In some species, carbohydrate supply plays an important role in regulating flower abscission (VAN DOORN & STEAD, 1997; VAN DOORN, 2004; BURGE *et al.*, 2010). Low carbohydrate levels in reproductive organs is thought to induce abscission in some species (MAO & CRAKER, 1990). In pepper, shading, high plant density and leaf removal resulted in flower and fruit abscission due to decreased carbohydrate accumulation (ALONI *et al.*, 1996; MARCELIS *et al.*, 2004), while petal abscission in rose was unrelated to petal carbohydrate levels (VAN DOORN & VOJINOVIC, 1996).

Photosynthetic rates decrease under low light conditions or in darkness, as light is necessary for photosynthesis. Without photosynthesis, plants cannot produce carbohydrates, their food source. During transportation poor light conditions cause a decrease in photosynthesis by plants. Photosynthetic rates decreased and flower senescence increased in potted *Hibiscus* plants that were kept indoors (REID *et al.*, 2002). Also, fewer flowers were produced indoors, and the dry matter content of these and the senesced flowers was significantly lower than those kept under greenhouse conditions.

A limited carbohydrate supply caused flower abscission in a number of plant species including pepper (TURNER & WIEN, 1994a) when plants were exposed to shading (MARCELIS *et al.*, 2004). A lack of stored carbohydrates or the inability to re-distribute reserve carbohydrates may be the cause of flower abscission (HWANG *et al.*, 2012). When there is a lack of carbohydrates in a plant, partitioning of these supplies to flowers and flower buds is limited as there is competition from the leaves and other vegetative parts of the plant (TURNER & WIEN, 1994a). Vegetative structures are more important for plant survival than flowers. Leaves are the site of energy production and without them the plant cannot survive. Flowers are reproductive structures and attract pollinators to the plant, without them the plant will

not reproduce but it will still survive to reproduce in the next season. In some species, flower retention depends on carbohydrate supply to the flowers and developing buds (ALONI *et al.*, 1996; MONERRI *et al.*, 2011). The aim of this experiment was to determine what carbohydrate related changes are occurring in the inflorescence as this may be influencing transport induce flower abscission.

7.2 Materials and Methods

Due to the availability of plant material and cost constraints, carbohydrate experiments were carried out on variety P 00 03 06 only. The effect of continuous dark and control treatments on soluble carbohydrate levels was examined by gas chromatography. Peduncle sections below the lowest rank of flowers were harvested at 0, 24, 48, 72 and 96 h from 3 different plants and combined. The peduncle was chosen since all assimilates pass through that point as they enter the inflorescence. Plants were held under control conditions (fluorescent lights), continuous darkness, or defoliated in the light. Additionally, inflorescences were classified into seven different categories based on their maturity as follows: 1) immature inflorescence, 2) inflorescence just prior to first flower opening, 3) 25% flowers open, 4) 50% flowers open, 5) 75% flowers open, 6) 100% flowers open, and 7) 100% flowers abscised under natural conditions. Some plants were maintained under greenhouse conditions, and samples taken every 3 h over a 24 h period to observe carbohydrate changes in untreated plants. Open flowers, unopened buds, and pedicels from open flowers and unopened buds were also harvested separately to establish their soluble carbohydrate status. Approximately 150 mg of stem sections were freeze dried and ground to a fine powder using liquid nitrogen. Soluble carbohydrates were extracted overnight on a rotary shaker (150 rpm) in a cold room with 80% ethanol. Samples were centrifuged at 2,000 *g* and supernatant transferred to a new 20 ml pill vial. Samples were dried under nitrogen at 40 °C. Sugar oximes were obtained by adding 500 µl hydroxylamine hydrochloride in pyridine (25 mg ml^{-1}) and heating at 40 °C for 40 min. Sugar oximes (100 µl) were transferred to new Eppendorf tubes and dried under nitrogen. The residue was re-dissolved in 100 µl Sil-BTZ (Supelco, USA) and incubated for 15 min at room temperature (SWEELEY *et al.*, 1963). Samples were analysed within 8 h of preparation by injecting 1 µl into a Varian model 3700 gas chromatograph. The sugars were detected by flame ionization on a 1.8 m x 3 mm

(i.d.) glass column packed with OV-17 Chromosorb HP 80/1000. The column was held at 125 °C for 3 min, followed by ramping at 4 °C min^{-1} to 270 °C. The final temperature was held for 10 min. Standard sugars (Merck, Germany) were treated similarly as ethanolic extracts of inflorescence stem sections at 1 mg ml^{-1} in 80% ethanol and used for co-chromatography (SWEELEY *et al.*, 1963).

7.3 Results

Only glucose and fructose were detected in peduncle sections from *Plectranthus* inflorescences. Over a 24 h period, slight variations were observed in fructose levels, while glucose levels fluctuated more (Figure 7.1). Glucose levels increased in the late afternoon and evening, but declined in the early morning before sunrise. Very young inflorescences (Stage 1) contained the lowest levels of glucose and fructose in peduncle sections, while inflorescences in stages 2-6 showed little variation in fructose levels (Figure 7.2). Glucose levels decreased from stages 2-6 as the inflorescence development stage increased. Glucose levels were lower in peduncle sections of mature inflorescences where abscission had occurred.

Glucose and fructose levels in control plants under fluorescent lights decreased after 24 h and remained low for the remaining 72 h of observation (Figures 7.3a & b). In contrast, under continuous dark conditions, glucose and fructose levels increased after 24 h, but then gradually declined until after 96 h, carbohydrate levels were marginally below the level of plants under fluorescent lights (Figures 7.3a & b). Defoliated plants kept under fluorescent lights showed a similar trend to non-defoliated plants in the light – a decrease in glucose and fructose levels after 24 h, followed by a levelling off for the remaining 72 h (Figure 7.2c).

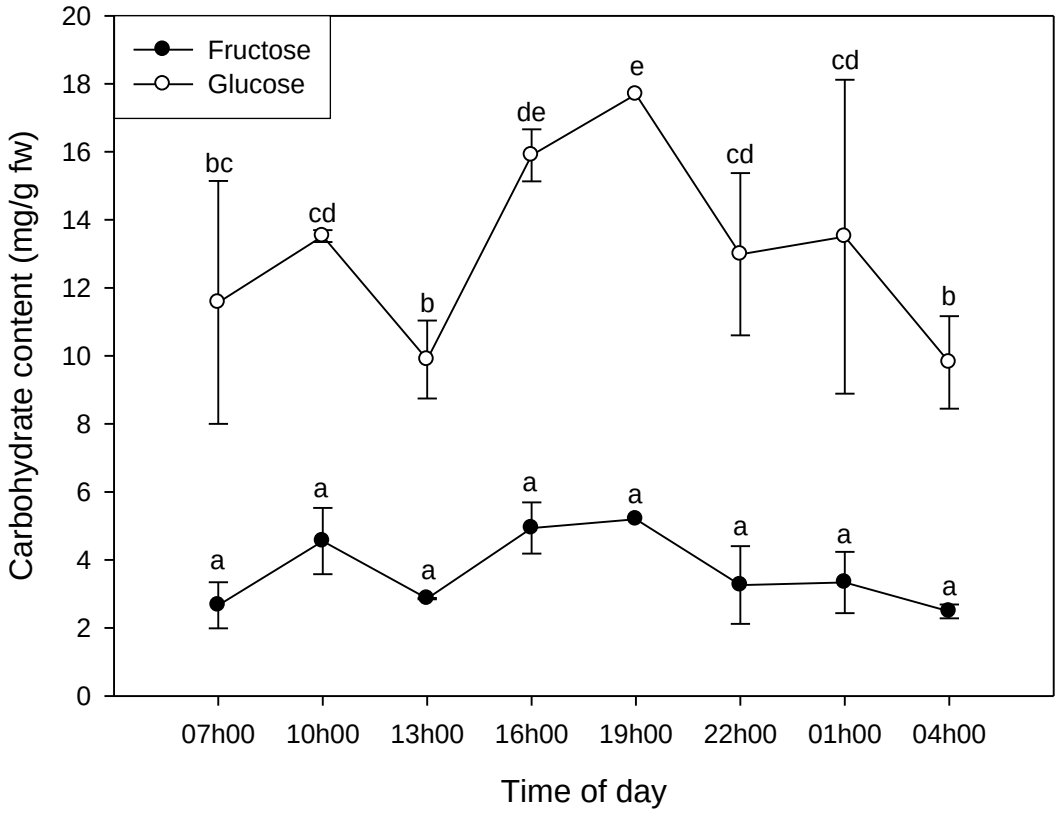

Figure 7.1. Soluble carbohydrate content in *Plectranthus* P 00 06 03 inflorescences during a 24 h cycle. Different letters indicate significant differences between treatments at a 5% level (ANOVA).

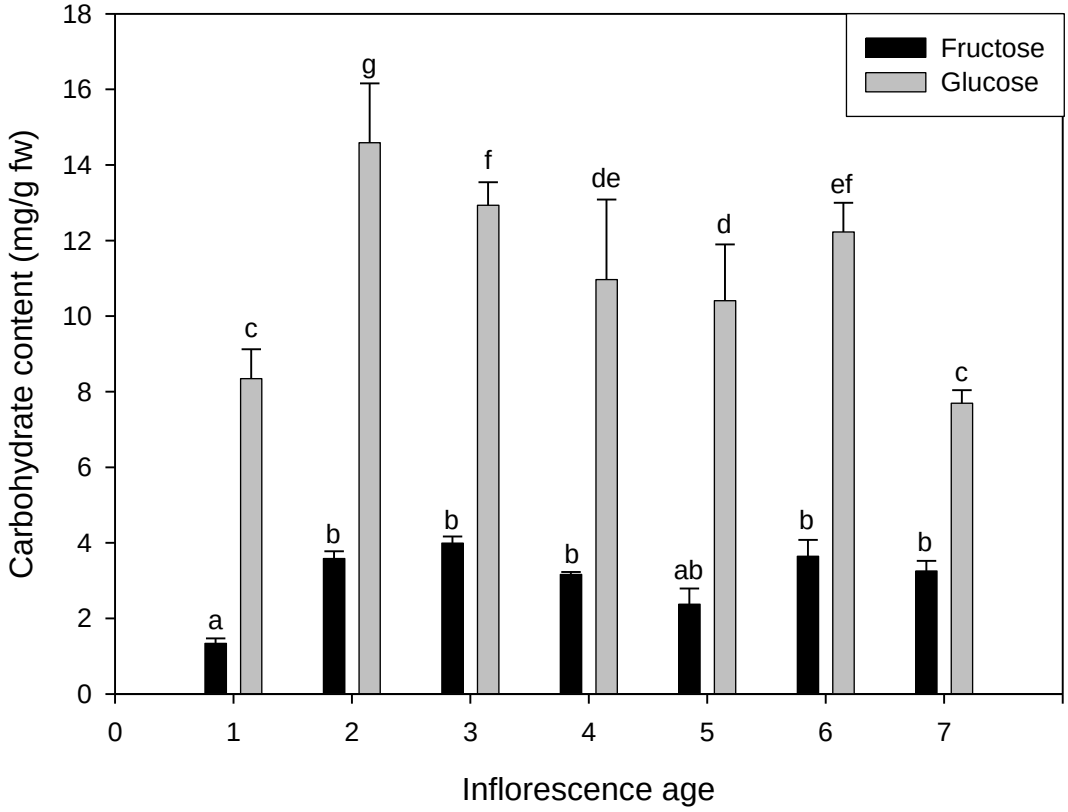

Figure 7.2. Soluble carbohydrate content in *Plectranthus* P 00 06 03 inflorescences of varying maturity. Categories are: 1) immature inflorescence, 2) inflorescence just prior to first flower opening, 3) 25% flowers open, 4) 50% flowers open, 5) 75% flowers open, 6) 100% flowers open, and 7) 100% flowers abscised under natural conditions. Different letters indicate significant differences between treatments at a 5% level (ANOVA).

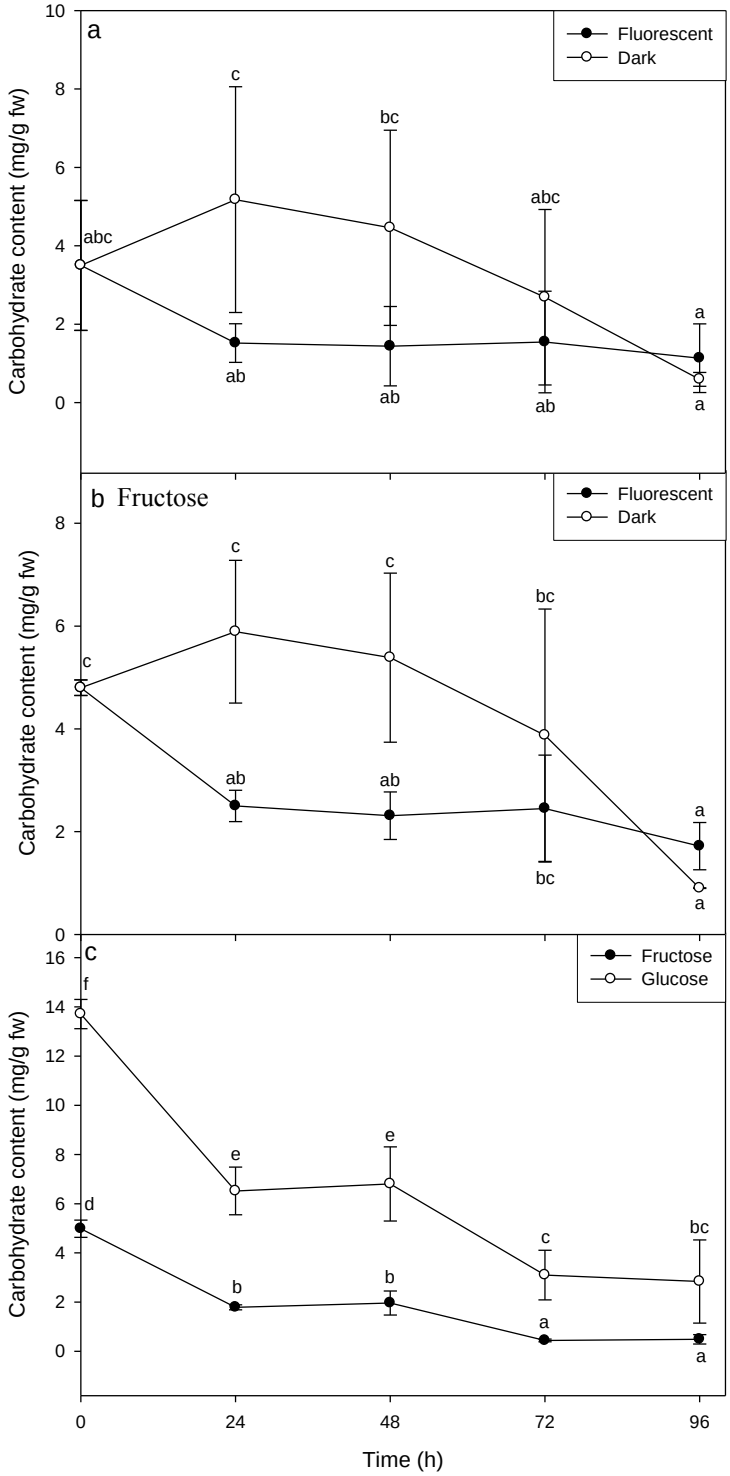

Figure 7.3 Glucose (a) and fructose (b) content of *Plectranthus* P 00 06 03 inflorescences under fluorescent lights (control) and continuous dark conditions. Levels of both carbohydrates increased initially under dark conditions before returning to levels similar to those of the controls. Soluble carbohydrate levels in defoliated plants kept under fluorescent lights (c) showed a similar trend to control plants. Different letters indicate significant differences between treatments at a 5% level (ANOVA)

7.4 Discussion

Under greenhouse conditions over a 24 h period, glucose levels increased in the late afternoon and early evening, but then declined in the early morning before sunrise (Figure 7.1). This is consistent with observations that elongation growth occurs in the hours preceding dawn, and hence energy is required for this growth (ERWIN *et al.*, 1989). Glucose levels in immature (Stage 1) inflorescences were substantially lower than in more mature (Stages 2-6) inflorescences, indicating these are weaker sinks. Fructose levels did not vary significantly across inflorescences at different development stages (Figure 7.2), or during a 24 h period (Figure 7.1) and were always lower than glucose levels, implying that glucose is the preferred/dominant respiratory substrate as no other sugars were detected.

Glucose and fructose levels of control plants under fluorescent lights decreased after 24 h, and remained low for the remaining 72 h of the experiment (Figures 7.3a & b). This is consistent with results in pepper where reduced light intensity decreased photosynthesis and the accumulation of total non-structural carbohydrates (ALONI *et al.*, 1996). Interestingly, glucose and fructose levels increased slightly after 24 h in the dark, but then had decreased to similar levels in plants kept in the light after 96 h. Glucose and fructose levels in defoliated plants showed similar trends to control plants held in the dark (Figure 7.3c). This suggests that sufficient carbohydrates are present in plants, even without leaves, to maintain and sustain flowering for at least 96 h. In cut petunia flowers, starch and soluble sugar levels dropped to a minimum within three hours after harvest, but that glucose and fructose levels subsequently increased as a result of gluconeogenesis (WHITEHEAD *et al.*, 2002). However, during gluconeogenesis lipids are converted to sugars, which indicate an early degradation of cellular membranes. Slight changes in membrane fluidity can affect binding of ethylene to its receptor, while accelerated senescence can also stimulate ethylene synthesis. It may also be possible that prolonged periods of darkness (or the interruption of the usual light-dark cycle) temporarily (24-48 h) alters soluble carbohydrate fluxes, thereby promoting ethylene synthesis and flower abscission.

It is generally accepted that sugar starvation or depletion leads to the initiation of senescence and mobilization of stored reserves out of leaves and flowers (VAN DOORN, 2004). In some cases, senescence occurs even though high concentrations of sugars are present in petals (EASON *et al.*, 1997), and application of sugars hastens senescence in stem sections of some species (VAN DOORN, 2004). These cases should not be taken as evidence that sugar accumulation promotes senescence, as the data may be confounded by unequal subcellular localization of sugars (VAN DOORN, 2004). In *Plectranthus*, we measured carbohydrate levels at the point of entry into the inflorescence were measured, and not for every open flower or bud, and thus results are speculative. However, it may be plausible that transient fluxes in carbohydrate levels could trigger ethylene production and promote abscission. Dark treatment caused increased ethylene production and upregulated the expression of two ACC synthase genes in *Pelargonium zonale* (MUTUI *et al.*, 2007), but carbohydrate levels were not recorded in that study. It is possible that similar mechanisms operate in *Plectranthus*, and may be linked to observed changes in cytokinin levels in the dark.

Chapter 8

The use of 1-MCP to prevent flower abscission

8.1 Introduction

Ethylene is well documented to cause major post-harvest problems in fruit, vegetables, cut flowers and potted plants (JONES *et al.*, 2001). The most relevant of which, to this study, is the induction of flower abscission. Potted plants which are bred, grown and sold for their floral displays are quickly rendered valueless when they lose their flowers during transportation due to ethylene exposure.

A number of 'anti-ethylene' compounds have been developed for use in agriculture, horticulture and floriculture over the years. Some ethylene antagonists prevent ethylene biosynthesis, while others prevent ethylene action. The latter is considered to be more successful in combating ethylene associated post-harvest problems, as preventing ethylene action protects plants from both endogenous and exogenous ethylene. Compounds which prevent ethylene action were also found to extend the vase/shelf-life of the flowers (ICHIMURA *et al.*, 2002).

For years the most widely used ethylene action inhibitor was silver thiosulfate (STS) (KIM *et al.*, 2007), however a number of negative characteristics began to emerge and it became unpopular. Firstly STS contains silver, a heavy metal and phytotoxin. Heavy metals sprayed onto plants and introduced into the soil are difficult to get rid of and can be harmful to humans and animals. Due to its phytotoxicity STS could not be used on food crops (KIM *et al.*, 2007) and in a number of cases, for example Geraniums, it caused root rot (JONES *et al.*, 2001). The short comings of STS led to the development of 1-methylcyclopropene (1-MCP) in the early 1990's (SISLER & SEREK, 1999).

1-MCP is a non-toxic, volatile olefin which binds in an apparently irreversible manner to the ethylene receptor (APELBAUM *et al.*, 2008), removing the need for continuous treatment (SISLER & SEREK, 1999). Other olefins, for example 2,5-norbornadiene (NBD), have a strong odor and corrosive nature which make them undesirable for

agricultural uses (APELBAUM *et al.*, 2008), but 1-MCP does not have these characteristics. Furthermore 1-MCP is active at relatively low concentrations in the parts per billion (ppb) range (SEREK & SISLER, 2001; KIM *et al.*, 2007). 1-MCP competes with ethylene for the active site on the receptor.

Since its release for commercial use, 1-MCP has been shown to successfully prevent fruit abscission in cherry tomatoes (BENO-MOUALEM *et al.*, 2004; LICHTER *et al.*, 2006), to prevent petal drop in roses (CHAMANI *et al.*, 2005), *Leonotis leonurus* (MEIR *et al.*, 2009), and, importantly, prevented flower abscission in potted plants such as *Pelargonium peltatum* (CAMERON & REID, 1983), *Campanula carptica* (SEREK & SISLER, 2001), and *Schlumbergera truncate* (SEREK & SISLER, 2001).

There is some doubt about the efficiency of 1-MCP, as some have found that it is not as effective as STS (SEREK & SISLER, 2001). However when the two are compared the negatives of using STS outweigh the slight inferiorities of the results obtained with 1-MCP.

Reports advise that plants must be treated with 1-MCP before they are exposed to increased levels of ethylene. This is to allow the 1-MCP to bind to the ethylene receptor sites and so successfully block ethylene itself from binding to the receptor sites. Common practice it to pre-treat plants with 1-MCP in its gaseous state for between 1 h and 24 h (JONES *et al.*, 2001) at concentrations ranging from 0.5 nll^{-1} (SISLER & SEREK, 1999) to 1 μll^{-1} (KIM *et al.*, 2007) prior to exposure to ethylene.

Numerous reports of 1-MCP successfully preventing flower abscission in potted plants do not mean that it is guaranteed to work for *Plectranthus*. As different plant species and varieties differ in their sensitivity to ethylene (JONES *et al.*, 2001), so it is true for the effectiveness of 1-MCP, on different plant species and varieties, in preventing flower abscission. In this study the effect of 1-MCP in preventing flower abscission in all eight varieties of potted *Plectranthus* was investigated.

8.2 Materials and Methods

Potted *Plectranthus* plants were placed in perspex chambers and then subjected to one of three different 1-MCP treatments: 1. (1-MCP-eth 1) plants were pre-treated with 100 nll^{-1} (SEREK *et al.*, 1995b) 1-MCP for 6 h after which chambers were vented, resealed and injected with 2 μll^{-1} ethylene. Ethylene (2 μll^{-1}) was injected into the chambers every 24 h; 2. (1-MCP-eth 2) plants were pre-treated with 100 nll^{-1} 1-MCP for 6 h after which chambers were vented, resealed and injected with 2 μll^{-1} ethylene. Plants were treated with 1-MCP (100 nll^{-1}) and chambers were injected with ethylene (2 μll^{-1}) every 24 h; 3. (1-MCP Dark) plants were pre-treated with 100 nll^{-1} 1-MCP for 6 h after which chambers were vented, resealed and then placed in the dark. Plants were treated with 1-MCP (100 nll^{-1}) every 24 h and were kept in the dark for the duration of the experiment. The number of open and unopened flowers abscised and those still remaining on the plants was recorded every 24 h. Five plants with three or more inflorescences were used for each experiment.

Plants (five plants per treatment) were treated with 1-MCP by dissolving the desired amount of carrier 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. Data were arcsine transformed prior to statistical evaluation (SCOTT *et al.*, 1984). An analysis of variance (ANOVA) was carried out on all data. Data were analysed using a Duncan's test at the 5% level in GenStat, 14th edition.

8.3 Results

For comparative reasons the percentage flower abscission recorded for plants treated with 2 μll^{-1} ethylene and for those kept in the light and in the dark untreated are presented along with the results of the 1-MCP treatments.

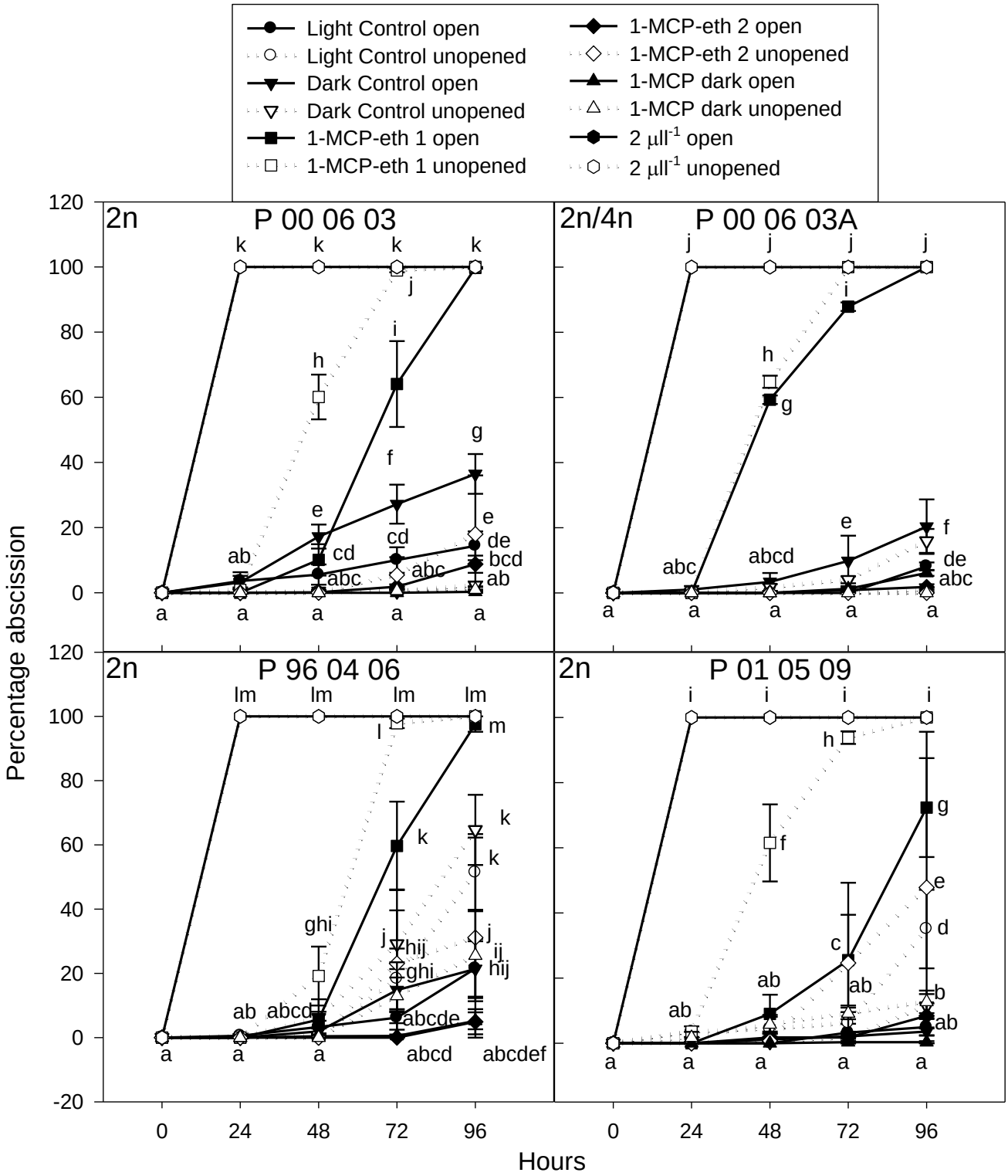

Figure 8.1: The effect of 1-MCP treatments on *Plectranthus* flower abscission in the light and in the dark. Different letters indicate significant differences between treatments at a 5% level (ANOVA).

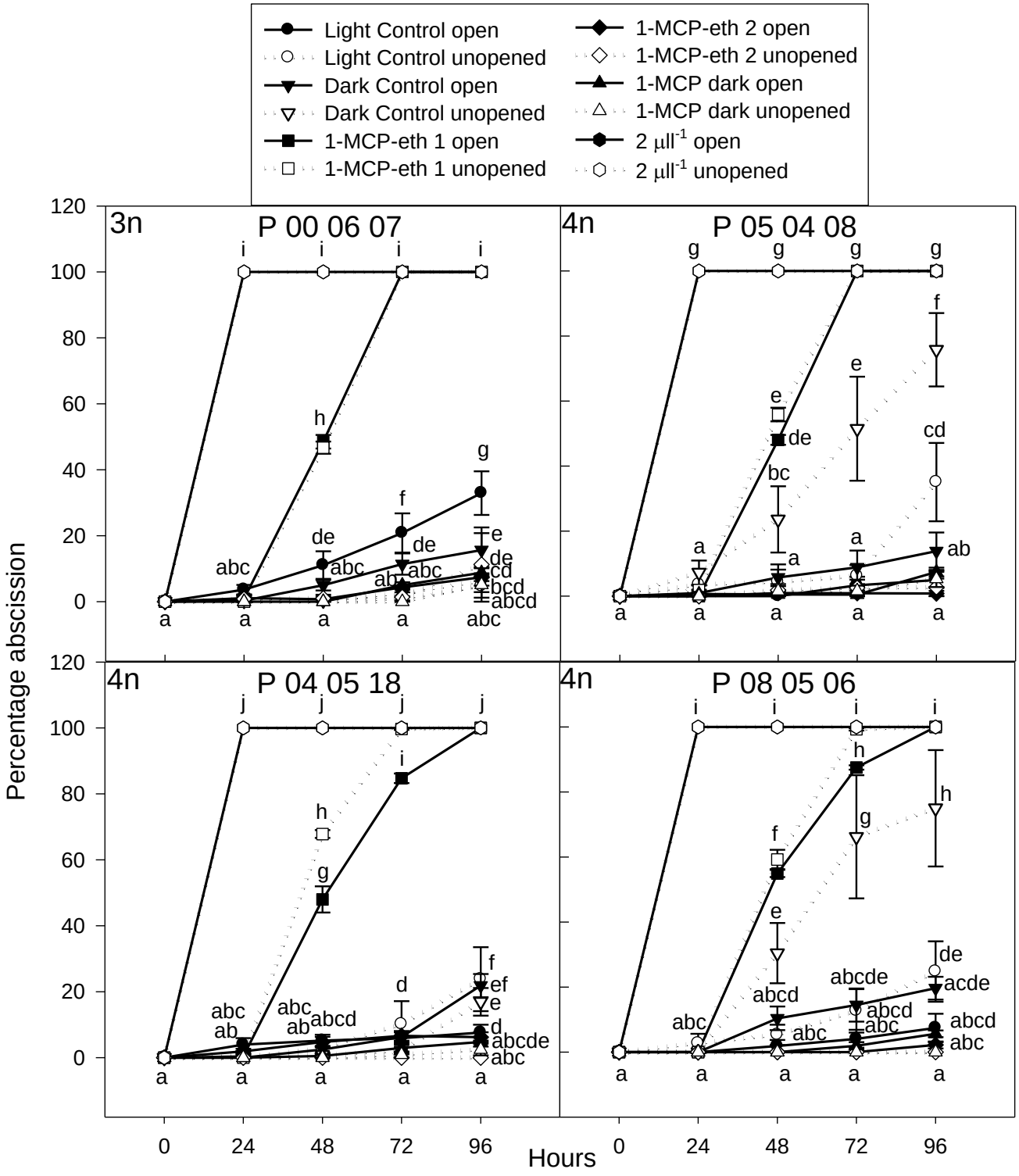

Figure 8.2: The effect of 1-MCP treatments on *Plectranthus* flower abscission in the light and in the dark. Different letters indicate significant differences between treatments at a 5% level (ANOVA).

When pre-treated with 1-MCP and subsequently exposed to 2 $\mu l l^{-1}$ (1-MCP-eth1), 100% of flowers in all eight varieties abscised within 96 h (Figures 8.1 & 8.2). This 6 h pre-treatment prevented flower abscission for the first 24 h and slowed the rate of flower abscission during this time in all eight varieties.

Continuous treatment with 1-MCP (1-MCP-eth2) reduced ethylene-induced and dark-induced flower abscission considerably compared with plants only exposed to 2 $\mu l l^{-1}$ ethylene only and those kept in the dark only. The lowest flower abscission when plant were treated with 1-MCP was observed in P 00 06 03A plants.

More unopened flowers were abscised than open flowers in response to ethylene treatments. This same trend was also observed in plants treated with 1-MCP and subsequently exposed to ethylene.

8.4 Discussion

In all eight *Plectranthus* varieties a single 6 h pre-treatment with 100 $n l l^{-1}$ 1-MCP was not sufficient to prevent flower abscission of open and unopened flowers for the duration of the 96 h experiment (Figures 8.1 & 8.2). Flower abscission of both open and unopened flowers increased dramatically after 24 h. This is consistent with results obtained for flower abscission in potted *Cansolida ajacis*, where a 1-MCP pre-treatment did not block all of the ethylene receptor sites for the duration of the experiment (DOS SANTOS *et al.*, 2005). A 1-MCP pre-treatment was not sufficient in preventing ethylene-induced flower abscission in *Pelargonium* x *domesticum* over a longer period of time (KIM *et al.*, 2007). Conversely a 1-MCP pre-treatment of potted *Campanula carpatica* and *Schlumbergera truncate* inhibited ethylene–induced flower and bud abscission for up to 12 d. However after this, flower and bud abscission increased (SEREK & SISLER, 2001). JONES *et al* (2001) state that the length of exposure to 1-MCP needed to protect the plant is dependent on the sensitivity of the plant.

When the plants were pre-treated with 1-MCP, the 1-MCP binds to the ethylene receptors within the plant. However the *Plectranthus* plants under investigation are potted plants and so they continued to grow throughout the experiment. During this

time the plant produces new ethylene receptors (DERVINIS *et al.*, 2000) which are then available for ethylene to bind to (MÜLLER *et al.*, 2000). Ethylene binds to the new ethylene receptors and flower abscission is induced. This explains why a single pre-treatment only prevents flower abscission for the first 24 h of the experiment and why continuous 1-MCP treatments were more successful in preventing flower abscission for the entire 96 h time period.

1-MCP pre-treatment has been reported successful for a number of cut flower species including *Rosa hybrid* 'Victory Parade', *Begonia elatior, Kalanchoe blossfeldiana* (SEREK *et al.*, 1995b), Geranium cultivars 'Fox', 'Kim', 'CottonCandy' and 'Veronica' (JONES *et al.*, 2001), *Dianthus caryophyllus*, *Delphinium* hybrid cv. Bellamosum (ICHIMURA *et al.*, 2002), *Dendrobium* 'Karen' (UTHAICHAY *et al.*, 2007), *Lathyrus odoratus* (ICHIMURA *et al.*, 2002; KEBENI *et al.*, 2003), *Leonotus leonurus* (MEIR *et al.*, 2009) and *Pelargonium peltatum* (CAMERON & REID, 2001). However, most cut flowers stop growing when they are harvested and so they, most probably, do not produce more ethylene receptors after this time. Pre-treatment with the correct concentration of 1-MCP at harvest will block all ethylene receptor sites which are present in the cut flowers at that time and so ethylene will not be able to bind to any sites after the flowers have been harvested.

When plants were treated every 24 h with 1-MCP, flower abscission of both open and unopened flowers was prevented for the duration of the 96 h experiment (Figures 8.1 & 8.2). With this 1-MCP treatment regime the new receptors which were formed while the plant grew were blocked every 24 h by the fresh application of 1-MCP, never allowing ethylene to bind to the receptor sites and successfully preventing ethylene induced flower abscission. This also applies to the plants kept in the dark for the duration of the experiment. KIM *et al*. (2007) stress the importance of the duration of 1-MCP treatments when working with potted plants.

Similar to the results of the dark experiments (Chapter 3) and the sensitivity experiments (Chapter 4), unopened flowers were more sensitive to ethylene and showed higher flower abscission when exposed to ethylene than opened flowers.

There were no significant differences in the effects of 1-MCP on varieties with different ploidy levels. Although different varieties with different ploidy levels of some plant species have been reported to react differently to 1-MCP and ethylene (KIM *et al.*, 2007), this has not been shown in any of the experiments with potted *Plectranthus* plants.

In all eight *Plectranthus* varieties, treatment with 1-MCP reduced the levels of flower abscission in the presence of ethylene to either the same as or below the level of flower abscission displayed by control plants in both the light and in the dark. These results suggest that 1-MCP may be used to prevent ethylene-induced flower abscission during the transport of potted *Plectranthus* plants.

Furthermore, the fact that 1-MCP reduced dark-induced flower abscission shows that the cause of the elevated abscission observed when plants were put into the dark, is indeed ethylene. Although this may be triggered by cytokinin fluxes, carbohydrate or gene expression changes under continuous dark conditions.

Chapter 9

Conclusions

This project set out to understand and, if possible, explain the processes involved in transport-induced flower abscission in potted *Plectranthus* with the view to preventing this problem. There were a number of factors which we suspected may influence flower abscission during transport.

Flower abscission is one of the most limiting factors when producing potted plants. Plants lose their value during transport, preventing them from being sold far from the nursery and preventing export. Combating this problem will allow for expansion in the potted plant industry into both local and international markets.

Dr Gert Brits, a plant breeder in Stellenbosch observed that his potted *Plectranthus* plants shed their flowers when they were transported across the country or internationally. Generous supplies of cuttings from eight different varieties of *Plectranthus*, some diploid and others tetraploid (Chapter 2), from Dr Brits allowed for *Plectranthus* to be used as a model plant for studying transport-induced flower abscission in potted plants.

When potted plants are put into transport conditions they are put into a dark and sealed environment for up to 4 d. During this time, potted *Plectranthus* plants abscised on average around 60% of their flowers, and up to 90% in the most sensitive varieties, compared to when plants were in the greenhouse where no abscission occurred (Chapter 3). In some varieties there was preferential shedding of open or unopened flowers. This is likely to be due to the dominance of flowers in a certain stage of development over flowers at another stage (DHANALAKSHMI *et al.*, 2003) and competition for assimilate supply, especially under stressful environmental conditions.

In order to ascertain the role that darkness plays in transport-induced abscission, plants were placed in the same sealed environment and kept under a 16 h photoperiod

to simulate natural light conditions. Under these light conditions fewer flowers were abscised (Chapter 3). It was thus concluded that although darkness was not the only factor impacting transport-induced flower abscission, it was a major contributor to the problem. Secondly, a sealed environment would allow for the build-up of any gasses that were produced during the transport period (HØYER, 1995). This may compound the problem of abscission further. Sensitivity to packaging and darkness varied among varieties. At this stage of the study it was determined that packaging plants increased flower abscission which was further increased when plants were put into the dark.

It was intriguing as to what internal changes occurred in the plant as a result of the transport conditions. It was important, however, to first establish the sensitivity of each of the varieties to ethylene, believed to be the primary control mechanism in flower abscission (WOLTERING, 1987; MÜLLER *et al.*, 1998; REID *et al.*, 2002).

Plants were placed in sealed airtight containers and exposed to ethylene concentrations ranging from 0.1 $\mu l l^{-1}$ to 2 $\mu l l^{-1}$ for up to 4 d. Every 24 h the number of abscised open and unopened flowers was recorded. All of the eight *Plectranthus* varieties were sensitive to ethylene, with 100% of flowers abscising within 4 d in all but two varieties in the presence of ethylene concentrations of 0.5 $\mu l l^{-1}$ and higher (Chapter 4). The two varieties that did not abscise 100% of flowers at 0.5 $\mu l l^{-1,}$ abscised between 88% and 93% of their flowers after 4 d. The different varieties showed roughly two patterns of sensitivity. All of the diploid plants followed a similar pattern and a different trend was observed in all of the tetraploid plants. Diploid varieties (P 00 06 03, P 96 04 06 and P 01 05 09) showed greater flower abscission when chambers were injected with 0.25 $\mu l l^{-1}$ ethylene compared to polyploidy varieties.

The view was formed that ethylene was contributing to the increased abscission during transport and that when plants were put into the simulated transport conditions, the generation of ethylene was increased. In other words, dark conditions may be causing an increase in ethylene synthesis. From this hypothesis, a number of experiments were designed relating to the ethylene-biosynthetic pathway and ethylene production by flowers.

The ethylene biosynthesis pathway was examined and two key enzymes were identified, namely ACC synthase (ACS) and ACC oxidase (ACO), which are responsible for regulating ethylene biosynthesis (VOGEL *et al.*, 1998; JOHNSON & ECKER, 1998). The expression of the genes which code for each of these enzymes changes under different environmental conditions (CLARK *et al.*, 1997; KENDE & ZEEVAART, 1997).

Variations in expression of ACS and ACO was investigated using real-time PCR to determine the levels of ACS and ACO transcripts (mRNA) in pedicel tissue from open and unopened flowers kept in the light and the dark for 24, 48, 72 and 96 h (Chapter 5). In general, there was an up-regulation of the ethylene biosynthetic pathway when plants are put into the dark. In turn, this resulted in an increase in ethylene production by the plant under simulated transport conditions. Increased ethylene production has been shown to accompany flower abscission (WOODSON *et al.*, 1992). However, the changes were not as dramatic as had been expected.

These results were surprising as it was expected that the changes in ACS and ACO expression would be the main cause of transport-induced flower abscission. However, we had not yet looked at all possible factors. Changes in cytokinins and carbohydrates during transport may influence flower abscission.

Discussions with collaborators of the Research Centre for Plant Growth and Development, and experts in measuring cytokinins, resulted in an interest as to what changes, if any, in cytokinin levels were occurring when plants were placed in transport conditions.

Pedicle samples were collected from plants kept in the dark for 24, 48, 72 and 96 h and from those kept in the light. These were prepared for extraction of cytokinins and the concentrations of 43 different cytokinins were measured (Chapter 6). Twenty one of these were below the level of detection and concentrations of 22 different cytokinins were measured.

In continuous dark conditions, levels of individual cytokinins varied substantially (Appendix 1). Some increased in concentration, some decreased in concentration

while others remained unchanged. The changes observed were also variety dependent, a trend that had been observed before in soybean (NOODÉN *et al.*, 1990).

When the changes in cytokinin groups were examined clearer trends emerged. Generally, cytokinin concentrations increased when plants were put into the dark. Links between the timing of peaks in cytokinin concentrations correlated with an increase in flower abscission in the dark. Increases in flower abscission were often preceded by a spike in cytokinin concentrations. It was concluded that an increase in cytokinin concentrations in the pedicles of flowers is likely to be one of the factors influencing transport-induced flower abscission

When plants are put into the dark they cannot photosynthesize nor produce carbohydrates. One of our theories was that this may be impacting on the increased flower abscission under simulated transport conditions. Carbohydrates were measured in the peduncle tissue of plants kept in the light and in the dark for 24, 48, 72 and 96 h (Chapter 7). There was no change in the carbohydrate flux into the inflorescence during this time. This indicates that the plants have sufficient stored carbohydrates to maintain flowers for the 96 h transportation period.

The primary cause of the observed transport-induced abscission was determined to be an increase in ethylene production when plants were put into simulated transport conditions. This was as a result of an up-regulation of the ethylene biosynthetic pathway and an increase in cytokinin concentrations in darkness. It is unlikely that carbohydrate changes play a role in increased flower abscission observed in *Plectranthus.*

Understanding the causes behind the increased flower abscission meant that in order to prevent abscission, ethylene production and reception could be targeted. Numerous authors reported on the success of ethylene antagonists in preventing flower abscission and specifically ethylene-induced flower abscission (KIM *et al.*, 2007). However, the majority of the reported work had been conducted on cut flowers and it was not definite that the same effects would be seen in potted *Plectranthus* plants.

After considering the array of ethylene antagonists available, 1-MCP was considered to be the safest and most 'user friendly' (JONES *et al.*, 2001; KIM *et al.*, 2007) and so it was decided that 1-MCP would be the subject of experimentation. Single treatments with 1-MCP, as used for cut flowers, prevented flower abscission for the first two days of the experiment after which flowers abscised (Chapter 8). The treatment regime was altered so that plants were treated every 24 h and flower abscission was prevented. It is thought that potted plants are continuously growing and in doing so, more ethylene receptors are produced. 1-MCP works by blocking these receptor sites making them no longer available for ethylene to bind to.

In conclusion, if potted *Plectranthus* plants are considered a model for other plants then the following assumptions can be made. Flower abscission increases during transport as a result of increased ethylene production under dark conditions. The sealed transport conditions allow for ethylene build up and plants abscise both open and unopened flowers. The high flower abscission means that the plants are of reduced value to the retailer after they have been transported. One possible solution is to use 1-MCP to prevent flower abscission. Plants should be treated with 100 nll^{-1} as soon as the plants are put into the transport conditions. Plants should be re-treated every 24 h for the duration of the transportation period.

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Appendix 1

Detailed results of cytokinin experiment (Chapter 6)

Table A.1: Changes in cytokinin concentrations (pmol/g dry weight) in open flowers when P 00 03 06 plants were put in the dark for 24, 48, 72 and 96 h. Different letters indicate significant differences between treatments at a 5 % level (ANOVA).

	Open				
Time (h)	**0**	**24**	**48**	**72**	**96**
Cytokinin					
tZ	7.53 ± 1.26 abc	6.66 ± 1.25 abc	4.77 ± 1.15 a	6.11 ± 0.59 ab	5.09 ± 1.35 ab
tZR	10.40 ± 2.52 bc	25.29 ± 4.55 f	18.16 ± 3.58 de	11.02 ± 0.83 bc	13.77 ± 3.83 cd
tZOG	10.29 ± 2.41 bcd	13.41 ± 1.77 d	18.50 ± 2.44 e	12.59 ± 1.88 cd	10.84 ± 1.28 bcd
tZROG	3.22 ± 0.73 a	7.29 ± 2.16 bc	9.64 ± 2.46 d	8.10 ± 1.41 bcd	9.48 ± 1.25 cd
tZ9G	6.53 ± 1.30 a	8.66 ± 1.31 ab	12.79 ± 3.56 ab	15.69 ± 3.70 bc	14.40 ± 2.01 ab
tZR5'MP	4.15 ± 0.68 bc	6.77 ± 0.41 d	7.41 ± 0.34 d	2.33 ± 0.73 ab	3.73 ± 1.01 abc
cZ	8.47 ± 1.68 bc	5.35 ± 1.23 ab	4.71 ± 1.37 ab	6.10 ± 1.81 ab	4.95 ± 1.28 a
cZR	40.94 ± 8.46 a	29.39 ± 4.20 a	65.91 ± 5.88 b	60.55 ± 0.85 b	60.82 ± 16.46 b
cZOG	5.45 ± 1.31 ab	5.34 ± 0.32 ab	7.55 ± 0.74 bc	8.87 ± 2.50 c	4.43 ± 0.47 a
cZROG	5.58 ± 1.61 bc	4.96 ± 0.82 b	8.14 ± 0.57 ef	9.33 ± 0.66 f	5.99 ± 0.56 bcd
cZ9G	46.62 ± 12.44 a	50.23 ± 16.80 a	78.36 ± 20.87 ab	69.26 ± 2.44 ab	70.08 ± 19.92 ab
cZR5'MP	73.58 ± 14.87 bc	221.34 ± 61.55 fg	252.81 ± 15.32 g	200.32 ± 32.96 ef	165.33 ± 27.24 de
DHZ	0.67 ± 0.23 ab	0.49 ± 0.15 a	0.76 ± 0.16 ab	0.78 ± 0.11 ab	0.78 ± 0.17 ab
DHZR	2.97 ± 1.01 ab	6.38 ± 0.38 de	7.70 ± 2.08 e	4.01 ± 0.95 bc	6.48 ± 0.67 de
DHZOG	3.16 ± 0.51 a	7.54 ± 1.96 bc	12.37 ± 4.06 d	7.65 ± 2.21 bc	10.57 ± 3.06 cd
DHZROG	9.02 ± 1.90 a	26.03 ± 4.40 b	31.12 ± 9.04 bc	23.73 ± 6.57 b	37.65 ± 4.98 c
DHZ9G	12.64 ± 1.80 a	15.43 ± 2.88 ab	28.36 ± 8.75 cd	16.37 ± 2.65 abc	27.44 ± 8.96 bcd
DHZR5'MP	1.07 ± 0.13 a	2.68 ± 0.79 c	5.30 ± 1.43 d	2.56 ± 0.45 c	2.97 ± 0.50 c
iP	7.92 ± 1.00 d	4.24 ± 1.20 abc	2.72 ± 0.74 a	3.44 ± 1.07 a	3.16 ± 0.66 a
iPR	55.14 ± 12.12 c	50.79 ± 12.86 c	29.59 ± 9.57 ab	18.75 ± 2.61 a	22.28 ± 3.67 a
iP9G	56.53 ± 14.04 a	71.09 ± 16.20 a	88.37 ± 7.96 ab	96.60 ± 3.87 ab	61.00 ± 9.78 a
iPR5'MP	8.55 ± 1.21 bc	9.10 ± 1.10 bc	10.15 ± 0.78 c	8.99 ± 3.05 bc	7.70 ± 0.56 abc

Table A.2: Changes in cytokinin concentrations (pmol/g dry weight) in unopened flowers when P 00 03 06 plants were put in the dark for 24, 48, 72 and 96 h. Different letters indicate significant differences between treatments at a 5 % level (ANOVA).

	Unopened				
Time (h)	**0**	**24**	**48**	**72**	**96**
Cytokinin					
tZ	7.72 ± 2.63 bc	4.66 ± 0.68 a	13.56 ± 1.64 d	9.41 ± 2.24 c	4.84 ± 1.15 ab
tZR	1.88 ± 0.32 a	19.21 ± 2.90 e	7.76 ± 0.38 b	11.74 ± 3.08 bc	11.68 ± 3.76 bc
tZOG	4.62 ± 1.24 a	10.44 ± 1.36 bcd	7.64 ± 1.73 ab	9.69 ± 2.41 bc	5.70 ± 1.51 a
tZROG	1.25 ± 0.11 a	6.13 ± 0.60 b	1.98 ± 0.10 a	2.81 ± 0.27 a	1.92 ± 0.52 a
tZ9G	9.45 ± 2.24 ab	30.85 ± 6.39 de	35.10 ± 9.40 e	23.06 ± 6.41 cd	22.72 ± 1.34 cd
tZR5'MP	1.48 ± 0.25 a	11.42 ± 2.44 e	8.12 ± 2.25 d	7.88 ± 2.04 d	6.27 ± 1.69 cd
cZ	19.91 ± 1.23 d	4.63 ± 1.43 a	10.05 ± 3.33 c	5.35 ± 1.52 ab	5.88 ± 1.31 ab
cZR	35.17 ± 7.45 a	55.71 ± 13.08 b	27.03 ± 3.60 a	27.24 ± 6.55 a	63.74 ± 8.11 b
cZOG	4.94 ± 0.54 a	8.14 ± 2.37 c	4.05 ± 0.35 a	5.13 ± 1.01 a	4.78 ± 0.26 a
cZROG	2.72 ± 0.87 a	7.29 ± 0.50 cde	2.52 ± 0.65 a	4.16 ± 1.11 ab	7.59 ± 1.99 def
cZ9G	72.59 ± 15.29 ab	96.42 ± 17.16 bc	119.35 ± 13.04 cd	116.36 ± 6.41 cd	135.37 ± 45.11 d
cZR5'MP	18.31 ± 3.19 a	90.10 ± 6.33 c	30.40 ± 8.96 ab	70.35 ± 23.93 bc	121.78 ± 35.72 cd
DHZ	0.69 ± 0.18 ab	0.84 ± 0.03 b	0.69 ± 0.16 ab	0.50 ± 0.08 a	0.68 ± 0.20 ab
DHZR	1.80 ± 0.41 ab	9.78 ± 1.78 f	2.10 ± 0.45 ab	2.86 ± 0.90 ab	5.28 ± 1.40 cd
DHZOG	2.28 ± 0.75 a	9.60 ± 1.52 cd	4.04 ± 0.70 ab	4.51 ± 1.14 ab	5.06 ± 1.45 ab
DHZROG	4.87 ± 1.66 a	26.34 ± 6.90 b	10.73 ± 1.32 a	9.79 ± 0.94 a	8.41 ± 2.27 a
DHZ9G	14.62 ± 4.52 a	46.13 ± 11.50 e	28.88 ± 4.31 d	24.45 ± 6.25 abcd	24.21 ± 6.24 abcd
DHZR5'MP	0.77 ± 0.12 a	4.42 ± 0.79 d	1.30 ± 0.25 ab	1.91 ± 0.58 abc	2.45 ± 0.67 bc
iP	15.42 ± 5.03 e	4.18 ± 1.41 ab	7.48 ± 1.83 bd	3.38 ± 0.82 a	3.31 ± 0.50 a
iPR	26.47 ± 7.50 ab	43.31 ± 11.22 bc	22.72 ± 7.90 a	31.87 ± 10.57 ab	30.48 ± 9.83 ab
iP9G	94.83 ± 32.49 ab	231.64 ± 54.08 cd	218.67 ± 67.03 cd	244.00 ± 77.81 d	162.45 ± 55.27 bc
iPR5'MP	5.22 ± 0.29 ab	17.98 ± 5.38 d	4.01 ± 1.13 a	6.32 ± 1.69 abc	6.61 ± 0.79 Abc

Table A.3: Changes in cytokinin concentrations (pmol/g dry weight) in open flowers when P 01 05 09 plants were put in the dark for 24, 48, 72 and 96 h. Different letters indicate significant differences between treatments at a 5 % level (ANOVA).

	Open				
Time (h)	**0**	**24**	**48**	**72**	**96**
Cytokinin					
tZ	5.27 ± 1.55 bc	2.37 ± 0.42 a	2.12 ± 0.63 a	1.02 ± 0.29 a	2.76 ± 0.50 Ab
tZR	8.80 ± 0.86 ab	14.63 ± 2.89 bc	10.09 ± 2.64 ab	6.25 ± 0.88 ab	8.53 ± 2.75 Ab
tZOG	17.77 ± 2.21 ab	20.47 ± 3.63 bc	33.76 ± 5.00 de	24.46 ± 6.90 bcd	28.51 ± 6.94 Cde
tZROG	5.41 ± 1.20 bc	9.50 ± 2.67 de	12.03 ± 3.25 e	7.50 ± 1.81 bcd	11.68 ± 2.05 E
tZ9G	3.25 ± 1.11 a	9.55 ± 2.12 a	9.33 ± 2.03 a	8.23 ± 1.81 a	8.67 ± 1.19 A
tZR5'MP	2.59 ± 0.71 a	5.87 ± 1.49 ab	5.74 ± 1.60 ab	4.15 ± 0.98 ab	3.84 ± 0.51 a
cZ	6.36 ± 1.96 ab	2.60 ± 0.71 a	3.64 ± 0.76 a	7.11 ± 1.54 ab	2.95 ± 0.91 A
cZR	33.78 ± 9.23 a	50.91 ± 13.53 a	63.25 ± 20.47 a	204.68 ± 62.48 bc	20.38 ± 2.60 A
cZOG	11.06 ± 2.25 d	4.76 ± 1.19 ab	5.08 ± 0.40 ab	5.23 ± 1.35 ab	4.38 ± 1.05 Ab
cZROG	5.49 ± 0.81 ab	6.90 ± 1.37 abc	9.76 ± 1.08 bc	17.06 ± 3.03 d	4.33 ± 1.08 a
cZ9G	37.52 ± 2.44 ab	38.77 ± 3.02 ab	35.85 ± 9.68 ab	36.59 ± 10.83 ab	34.13 ± 3.16 a
cZR5'MP	32.33 ± 10.32 a	102.21 ± 27.21 ab	154.86 ± 35.89 bc	114.10 ± 15.68 abc	41.82 ± 8.70 A
DHZ	0.43 ± 0.08 cd	0.30 ± 0.02 ab	0.33 ± 0.12 bc	0.20 ± 0.05 ab	0.32 ± 0.05 Abc
DHZR	4.31 ± 1.23 abc	5.21 ± 1.65 abc	4.84 ± 0.68 abc	4.54 ± 1.27 abc	2.87 ± 0.45 ab
DHZOG	3.41 ± 0.85 bc	4.30 ± 1.37 bc	6.95 ± 1.54 d	3.04 ± 0.99 bc	4.64 ± 1.52 Bc
DHZROG	7.35 ± 1.60 b	13.17 ± 4.21 bc	12.47 ± 3.61 bc	10.05 ± 2.68 b	21.34 ± 2.30 D
DHZ9G	3.06 ± 1.07 a	9.64 ± 2.23 bcd	11.14 ± 1.29 cde	8.57 ± 2.29 abc	9.31 ± 0.52 Bcd
DHZR5'MP	1.52 ± 0.44 ab	2.70 ± 0.87 bc	3.16 ± 1.00 cd	1.35 ± 0.46 ab	1.48 ± 0.47 Ab
iP	3.10 ± 0.66 a	0.76 ± 0.25 a	1.95 ± 0.66 a	1.84 ± 0.61 a	1.10 ± 0.24 A
iPR	34.14 ± 11.14 abcd	21.82 ± 5.36 ab	47.57 ± 8.95 bcde	50.60 ± 11.64 cde	28.11 ± 8.63 Abc
iP9G	63.20 ± 20.71 a	58.86 ± 16.72 a	58.88 ± 17.22 a	71.59 ± 17.53 a	58.14 ± 9.17 a
iPR5'MP	3.64 ± 0.95 a	8.89 ± 1.41 ab	11.98 ± 2.47 bc	11.31 ± 3.66 bc	8.58 ± 1.88 Ab

Table A.4: Changes in cytokinin concentrations (pmol/g dry weight) in unopened flowers when P 01 05 09 plants were put in the dark for 24, 48, 72 and 96 h. Different letters indicate significant differences between treatments at a 5 % level (ANOVA).

	Unopened				
Time (h)	**0**	**24**	**48**	**72**	**96**
Cytokinin					
tZ	6.72 ± 1.67 c	10.45 ± 2.56 b	3.40 ± 1.12 ab	5.39 ± 0.79 bc	23.27 ± 2.93 e
tZR	9.81 ± 2.04 ab	26.67 ± 7.52 de	17.97 ± 3.92 c	21.15 ± 4.84 cd	30.20 ± 4.34 e
tZOG	9.59 ± 2.31 a	38.46 ± 8.88 e	23.31 ± 4.85 bc	33.72 ± 5.08 de	49.72 ± 5.25 f
tZROG	1.04 ± 0.36 a	6.63 ± 1.54 bcd	4.06 ± 1.42 ab	4.53 ± 0.90 bc	7.92 ± 1.49 cd
tZ9G	24.52 ± 6.81 a	107.09 ± 28.91 bc	69.56 ± 16.59 b	129.81 ± 13.28 c	319.28 ± 62.99 d
tZR5'MP	4.51 ± 1.33 a	13.24 ± 3.26 cd	9.93 ± 3.07 bc	14.94 ± 4.43 d	13.84 ± 3.84 cd
cZ	34.73 ± 7.86 d	11.55 ± 3.15 b	5.20 ± 1.20 ab	6.89 ± 1.97 ab	20.14 ± 6.04 c
cZR	74.22 ± 10.69 a	247.61 ± 75.07 c	67.40 ± 11.46 a	161.00 ± 43.39 b	183.54 ± 32.62 bc
cZOG	5.83 ± 1.64 b	4.32 ± 1.39 ab	3.11 ± 0.43 a	4.44 ± 0.41 ab	8.18 ± 1.31 c
cZROG	6.66 ± 1.68 abc	19.81 ± 5.36 d	11.23 ± 1.76 c	10.58 ± 3.32 c	11.29 ± 3.09 c
cZ9G	34.29 ± 9.42 a	53.66 ± 8.32 c	50.57 ± 7.34 bc	60.92 ± 11.48 cd	69.09 ± 9.23 d
cZR5'MP	35.26 ± 6.43 a	442.58 ± 91.24 e	169.04 ± 35.88 bc	271.72 ± 84.46 d	200.41 ± 62.13 cd
DHZ	0.24 ± 0.04 ab	0.57 ± 0.10 e	0.29 ± 0.08 ab	0.48 ± 0.03 de	1.19 ± 0.06 f
DHZR	1.99 ± 0.52 a	13.31 ± 3.60 d	5.65 ± 1.30 bc	5.43 ± 1.70 bc	6.44 ± 2.17 c
DHZOG	0.63 ± 0.20 a	5.18 ± 1.13 cd	2.81 ± 0.93 b	3.65 ± 0.36 bc	5.12 ± 1.38 cd
DHZROG	1.47 ± 0.46 a	11.30 ± 3.66 bc	13.00 ± 3.73 bc	12.55 ± 1.61 bc	16.34 ± 4.35 cd
DHZ9G	3.79 ± 0.80 ab	19.21 ± 3.51 f	14.89 ± 3.69 def	15.83 ± 4.73 ef	30.67 ± 6.57 g
DHZR5'MP	0.87 ± 0.26 ab	6.16 ± 0.33 e	3.88 ± 1.26 cd	4.49 ± 1.53 d	2.87 ± 0.95 bc
iP	10.49 ± 3.41 bc	12.21 ± 3.97 cd	7.88 ± 1.70 b	8.10 ± 2.66 bc	14.95 ± 3.87 d
iPR	15.69 ± 5.22 a	116.86 ± 30.74 f	38.94 ± 6.95 abcde	65.24 ± 21.83 e	61.57 ± 15.04 de
iP9G	57.42 ± 9.22 a	244.41 ± 59.25 b	107.43 ± 21.98 a	202.14 ± 46.77 b	244.71 ± 67.56 B
iPR5'MP	2.05 ± 0.62 ab	31.26 ± 7.58 e	18.33 ± 5.94 cd	23.96 ± 5.62 d	8.99 ± 2.04 Ab

In open flowers of P 00 06 03 concentrations of tZR, tZOG and tZR'5MP increased when plants were initially put into the dark but then decreased again to similar concentrations found in plants in the light (Table A.1). tZROG and tZ9G were found in significantly higher concentrations when plants were kept in the dark compared to those in the light. No significant change in the concentration of tZ was detected when plants were put into the dark.

Similar trends in tZ group concentrations were observed in unopened flowers of P 00 06 03 (Table A.2). Concentrations of tZ, tZR, tZOG, tZROG and tZ9G increased initially when plants were placed in the dark and then dropped back down to similar concentrations as the plants kept in the light. tZR5'MP concentrations were significantly higher when plants were kept in the dark, throughout the experiment, compared to plants that were kept in the light.

Three CKs from the tZ group, tZR, tZOG and tZROG, were present in higher concentrations in open flowers of P 00 06 03 (Table A.1) when compared to unopened flowers (Table A.2) in both light and dark conditions. tZR5'MP was present in higher concentrations in open flowers in the light than in unopened flowers in the dark. Concentrations of tZ and tZ9G were relatively similar in opened and unopened flowers in the light, while in the dark this was only true for tZ. tz9G was present in much higher concentrations (2 x) in unopened flowers than in open flowers.

cZ concentrations in open flowers (Table A.1) showed a slight decrease when plants of P 00 03 06 were put into the dark but this change was not statistically significant. Concentrations of cZR and cZ9G did not change significantly when plants were put into the dark. Concentrations of cZOG, cZROG and cZR5'MP increased when plants were placed in the dark. In unopened flowers of P 00 06 03 (Table A.2) concentrations of cZ decreased, cZR showed no significant change (after 48 h) and cZR5'MP was

significantly higher (at all time points) when plants were put into the dark. cZOG and zZROG concentrations increased initially when plants were placed in the dark but then dropped to similar concentrations shown by plants kept in the light. Concentrations of cZ9G were generally higher in dark conditions than in light conditions.

Concentrations of cZR, cZOG and cZ9G were not significantly different in open (Table A.1) and unopened (Table A.2) flowers of P 00 06 03 in the dark and in the light. cZR5'MP concentrations were higher in open flowers both in the light and in the dark when compared to unopened flowers. Under dark conditions, concentrations of cZ and cZROG were the same in both open and unopened flowers. While in the light cZ concentrations were higher in unopened flowers than in open flowers and cZROG concentrations were greater in open flowers than in unopened flowers.

Concentrations of the DHZ group of CKs were all significantly higher in the dark in both opened (Table A.1) and unopened (Table A.2) flowers of P 00 06 03; except for DHZ, where there were no changes in concentrations when opened and unopened flowers were put into the dark.

DHZ concentrations were similar in open (Table A.1) and unopened (Table A2) flowers of P 00 06 03 in the light and in the dark. Concentrations of DHZR and DHZROG were greater in open flowers compared to unopened flowers in both light and dark conditions. In the light DHZOG, DHZ9G and DHZR5'MP concentrations were not significantly different in open and unopened flowers. Under dark conditions DHZOG and DHZR5'MP concentrations were higher in open flowers when compared to unopened flowers, while in unopened flowers DHZ9G concentrations were higher than in opened flowers.

iP and iPR concentrations decreased in open (Table A.1) flowers of P 00 06 03 when plants were put into the dark. iP9G concentrations increased initially and then decreased again and iPR5'MP concentrations remained stable when plants were placed in the dark.

In unopened (Table A.2) flowers, iP concentrations decreased while iP9G concentrations increased under dark conditions. Concentrations of iPR5'MP increased initially when plants were placed in the dark and then dropped back down to levels consistent with plants in the light. iPR concentrations did not change significantly when plants were put into the dark.

iP9G concentrations were greater in unopened (Table A.2) flowers than in opened (Table A.1) flowers in the light and the dark. Conversely iPR5'MP concentrations were greater in opened flowers than in unopened flowers in both the light and the dark. Under dark conditions iP and iPR concentrations were the same in open flowers as they were in unopened flowers. Unopened flowers had higher concentrations of iP in the light than open flowers and iPR concentrations were greater in open flowers than in unopened flowers.

In open flowers of P 01 05 09 (Table A.3) tZR and tZR5'MP, concentrations increased initially when plants were exposed to darkness but then decreased to levels similar to those found in the light. tZOG concentrations were significantly higher in open flowers when plants had been in the dark for 48 and 96 h compared to plants kept in the light. tZ concentrations decreased when plants were placed in the dark. tZ9G and tZROG concentrations were higher in dark conditions than in the light, although these results for tZROG were not significant.

In unopened flowers of P 01 05 09 (Table A.4) the concentrations of the tZ group of CKs increased when plants were put into the dark, except for tZ which decreased when plants were put into the dark and then increased after 72 h of dark exposure. tZ9G concentrations in the dark were more than two-fold those in the light.

The concentrations of tZROG were greater in opened flowers (Table A.3) of P 01 05 09 than in unopened flowers (Table A.4) in both the light and dark. Conversely, the concentrations of tZ9G were higher in unopened flowers than in opened flowers in both the light and the dark. In the light tZ, tZR and tZR5'MP concentrations were the same in open flowers as they were in unopened flowers, and tZOG concentrations were greater in opened flowers than in unopened flowers. In the dark tZ, tZR and tZR5'MP concentrations were higher in unopened flowers when compared to closed flowers, while there was no significant difference between concentrations of tZOG in opened and unopened flowers.

cZR and cZR5'MP concentrations increased initially in open flowers (Table A.3) when P 01 05 09 plants were put into the dark; however, after 24 h in the dark the concentrations of these CKs decreased back to levels which were the same as those measured in the light. cZ concentrations showed the opposite trend with an initial decrease when plants were put into the dark and a subsequent increase to levels which were consistent with plants kept in the light. Levels of cZROG increased when plants had been in the dark for 48 and 72 h. cZOG concentrations were significantly lower in plants in the dark compared to plants in the dark, while there were no significant changes in cZ9G concentrations when plants were put into the dark.

In unopened flowers (Table 6.4) of P 01 05 09 cZR, cZROG, cZ9G and cZR5'MP concentrations increased when P 01 05 09 were put into the dark. Concentrations of cZ

decreased significantly in the dark and there was no significant change in cZOG concentrations when plants were placed in the dark.

cZ9G concentrations were not significantly different in opened (Table A.3) and unopened (Table A.4) flowers of P 01 05 09 both in the light and in the dark. cZ and cZR concentrations were greater in unopened flowers than in open flowers in the light and in the dark. cZOG concentrations were higher in open flowers than in unopened flowers when plants were in the light, while there was no significant difference between the concentrations of cZROG and cZR5'MP in opened and unopened flowers in the light. Concentrations of cZROG and cZR5'MP were greater in unopened flowers in the dark when compared with open flowers. cZOG concentrations in the dark were the same in open flowers as they were in unopened flowers.

There was no significant change in FHZ and DHZR concentrations in open flowers (Table A.3) when P 01 05 09 plants were placed in the dark. DHZROG and DHZ9G concentrations increased when plants were put into the dark, while DHZOG and DHZR5'MP concentrations increased initially when plants went into the dark but then decreased back down to similar concentrations as were seen in plants in the light.

All DHZ group concentrations increased in unopened flowers (Table A.4) when P 01 05 09 plants were place in the dark.

In the light all of the DHZ group of CKs were present in greater concentrations in open flowers (Table A.3) of P 01 05 09 when compared with unopened flowers (Table A.4), except DHZ9G concentrations which were the same in open and unopened flowers. In the dark DHZ, DHZR, DHZ9G and DHZR5'MP concentrations were higher in unopened

flowers than in open flowers and there was no significant difference in the concentrations of DHZOG and DHZROG in opened and unopened flowers.

iP concentrations decreased in open flowers (Table A.3) when P 01 05 09 plants were put into the dark. There was no significant change in the concentrations of iPR and iP9G when plants were kept in the dark. iPR5'MP concentrations increased during dark treatments.

Concentrations of iPR, iP9G and iPR5'MP increased in unopened flowers (Table A.4) of P 01 05 09 plants when they were put into the dark. However, there was no significant change in the concentration of iP during dark treatments.

The concentrations of iPR and iPR5'MP were greater in open flowers (Table A.3) of P 01 05 09 than in unopened flowers (Table A.4) in the light and the dark. Conversely, concentrations of iP were greater in unopened flowers than in open flower in the light and the dark. iP9G concentrations were greater in unopened flowers than in open flowers in the dark, but were even in open and unopened flowers when plants were in the light.