Department of Environment and Agriculture

Regulation of vase life of waxflowers *(Chamelaucium* Desf.) with new ethylene antagonists

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Declaration

"To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university."

Signature: 

Date:...2/5/2017.....

Dedication

To:

My father (Moussa Abdalmaged)

My mother (Khadija Saad)

To souls of my brother Abdallah and my sister Randa

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Abstract

Waxflower (*Chamelaucium uncinatum* Schauer) is an Australian native flower that is available in different colours, size and flowers shape. Waxflower is one of the most popular cut flowers worldwide with a production of more than 300 million cut flower stems per year. Waxflower is one of the leading commercial Australian native flowers and is now in the top 20 types of flowers, in terms of volume sold in Europe. However, most of the waxflower genotypes are sensitive to ethylene, which accelerates flower/bud abscission caused by the accumulation of exogenous ethylene in the post-harvest atmosphere. This can be a major issue for the international trade of Australian waxflowers. 1-Methylcyclopropene (1-MCP) and silver thiosulfate (STS) are commercially being used by the floriculture industry but there are some difficulties, such as 1-MCP is a gas and can be applied only in tightly closed chambers. STS is a heavy metal and detrimental to the environment. Therefore, these difficulties offer an attractive opportunity to search new effective ethylene action inhibitors. The present investigations are aimed to evaluate the efficacy of various alternative ethylene antagonists in inhibiting ethylene action and reducing abscission of flowers/buds in different genotypes of cut waxflowers. Some of the chemicals were synthesised into either stable liquids, solids or gases to facilitate their application, such as isoprene oxide, allyl butyl ether, 1-octene oxide, 1*H*-cyclopropa[*b*]naphthalene (NC), 1*H*-cyclopropabenzene (BC) and 1-hexylcyclopropene (1-HCP) and others including 1-octyne, butyl acrylate, 1-octene oxide purchased from different companies. Meanwhile, others have been derived from naturally occurring products such as common natural terpene molecules *trans*-cinnamaldehyde (CA), (+)-carvone, eugenol and (*S*)-(-)-limonene and also, these chemicals were purchased from different companies. All these compounds were chosen as ethylene antagonists are /or are structurally similar to reported antagonists of ethylene in fruits and vegetables.

The effects of different concentrations (0.5, 1.0 and 2.0 µM) of active ethylene antagonists such as CA, NC, BC and 1-HCP on ethylene action in reducing flowers/buds abscission in different genotypes of waxflowers were also examined. The effects of active ethylene antagonists such as CA, NC, BC and 1-HCP in combination with vase solutions containing distilled water, 58.5 µM sucrose or

fructose and/or supplemented with 100 mg L^{-1} 8-hydroxyquinoline sulphate (8-HQS) on vase life of different waxflowers were evaluated. The general testing method used was to fumigate flowering stems of different genotypes of waxflowers with different ethylene antagonists alone in 60 L plastic drums for 18 h. Sprigs were fumigated with (1 µM) ethylene antagonist alone for 18 h, stems were fumigated with 10 µL L^{-1} ethylene alone for 24 h, with an ethylene antagonist for 18 h followed by their exposure to ethylene (10 µL L^{-1}) for 24 h, untreated flowering stems were kept as control. Effects of various treatments on flowers/buds abscission were recorded following the treatments. In the vase life experiment sprigs of different waxflower genotypes were fumigated with 1 µM of ethylene antagonists including CA, NC, BC or 1-HCP chemicals for 18 h and then the stems were kept in vase solutions containing distilled water and held in a vase life room to assess flower and leaf vase life in four different genotypes of cut waxflowers. Meanwhile, in another experiment the flower stems of 'WX14' and 'WX74' were fumigated with 1 µM *trans*-cinnamaldehyde, 1*H*-cyclopropa[*b*]naphthalene and 1-hexylcyclopropene alone as ethylene antagonist for 18 h in 60 L plastic drums followed by treatments in vase solutions containing 58.5 µM sucrose or fructose and 8-HQS (100 mg L^{-1}). Untreated sprigs were kept in plastic drums as a control.

Among different fumigation treatments tested, 1-octyne, isoprene oxide, (+)-carvone, eugenol, allyl butyl ether or 1-octene (1 µM) for 18 h to flower stems of 'White Spring', 'WX17', 'WX116', 'Muchae Mauve', 'WX110' and 'Jenny' waxflowers and followed by exposure to ethylene treatment (10 µL L^{-1}) for 24 h did not reduce flowers/buds abscission and the ethylene treated sprigs exhibited 100% flowers/buds abscission. Meanwhile, the fumigation treatment of CA, (*S*)-(-)-limonene, NC, BC or 1-HCP were the most effective in inhibiting ethylene action and reducing ethylene-induced flowers/buds abscission as recorded in the average of the total reduction of flowers/buds abscission in each five effective chemicals.

1. *trans*-cinnamaldehyde: The sprigs of 'WX73', 'WXFU', 'WX17', 'WX56', 'WX58', 'Hybrid1', 'Revelation' and 'Purple Pride' genotypes fumigated with *trans*-cinnamaldehyde followed by ethylene treatment exhibited 67.72% mean reduction in flowers/buds abscission as compared to those fumigated with ethylene alone.

2. (*S*)-(-)-limonene: The flowering stems of ‘WX73’, ‘WXFU’, ‘WX17’, ‘WX56’, ‘WX58’ and ‘Purple Pride’ genotypes treated with (*S*)-(-)-limonene followed by ethylene fumigation showed 54.19% mean reduction in flowers/buds abscission as compared to those fumigated with ethylene alone.
3. 1*H*-cyclopropa[*b*]naphthalene: ‘WX73’, ‘WX56’, ‘WX58’, ’WX107’, ‘Jenny’, ‘Hybrid 1’, ‘Revelation’ and ‘Purple Pride’ genotypes stems fumigated with 1*H*-cyclopropa[*b*]naphthalene followed by ethylene fumigation showed 63.49% average reduction over treatment of ethylene alone.
4. 1*H*-cyclopropabenzene: The sprigs of ‘WX73’, ‘WX56’, ‘WX58’, ’WX17’, ‘Revelation’ and ‘Purple Pride’ genotypes treated with 1*H*-cyclopropabenzene followed by ethylene application showed 68.39% mean reduction in flowers/buds abscission in comparison to those treated with ethylene alone.
5. 1-Hexylcyclopropene: The flowering stems of hybrid and non-hybrid ‘WX73’, ‘WX56’, ‘WX58’, ‘Hybrid1’ and ‘Purple Pride’ genotypes fumigated with 1-hexylcyclopropene followed by ethylene fumigation exhibited 84.81% mean reduction in flowers/buds abscission compared to those fumigated with ethylene alone. Additionally, in ‘Purple Pride’, ‘Revelation’ and ‘Hybrid1’ waxflowers fumigated with *trans*-cinnamaldehyde, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene and 1-hexylcyclopropene (0, 0.5, 1.0 and 2.0 µM) for 18 h followed by ethylene exposure for 24 h, 1 µM concentration fumigation was the more effective in reducing flowers/buds abscission as compared to the other two concentrations tested.

In vase life experiments, the sprigs of ‘Crystal Pearl’, ‘Lady Stephanie’, ‘Purple Pride’ and ‘WX74’ waxflower were fumigated with 1 µM CA, NC, BC or 1-HCP and kept in DW distilled water alone. 1- HCP (1 µM) fumigation for 18 h resulted in significantly longest flower vase life (20.71 days) when kept in DW alone as compared to all other fumigation treatments and control (18.29 days). Meanwhile, 1 µM fumigation of NC, 1-HCP or CA for 18 h resulted in longer leaf vase life in comparison to the control and BC fumigation treatment. Meanwhile, flowering stems of ‘WX74’ and ‘WX14’ fumigated with CA, NC or 1-HCP alone and as a

combination of fumigation treatments with vase solution containing 58.5 µM of sucrose or fructose and/or 100 mg L^{-1} 8-HQS and the fumigation of 1-hexylcyclopropene or *trans*-cinnamaldehyde followed by vase solution containing sucrose and 8-HQS were effective in extending vase life of flowers/leaves in 'WX74' and 'WX14' as compared to treatments and the control.

In conclusion, five compounds including (*S*)-(-)-limonene, *trans*-cinnamaldehyde, 1*H*-cyclopropabenzene, 1*H*-cyclopropa[*b*]naphthalene, and 1-hexylcyclopropene were effective in inhibiting ethylene action consequently reducing flowers/buds abscission in a range of Australian waxflower 'WX73', 'WXFU', 'WX17', 'WX56', 'WX58', 'Hybrid1', 'Revelation', 'WX107', 'Jenny' and 'Purple Pride' genotypes. Additionally, fumigation treatments of *trans*-cinnamaldehyde, 1-hexylcyclopropene and 1*H*-cyclopropa[*b*]naphthalene were efficient in inhibiting the ethylene action consequently delaying flower and leaf senescence as well as extending the vase life of flowers and leaf in different genotypes of waxflower. The reduction in both abscission of flowers /buds and senescence of leaf and flowers in waxflowers with the fumigation of (*S*)-(-)-limonene, *trans*-cinnamaldehyde, 1*H*-cyclopropabenzene, 1*H*-cyclopropa[*b*]naphthalene, or 1-hexylcyclopropene suggests that these compounds are potential ethylene antagonists.

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List of symbols and abbreviations

-	No information available
–	Hyphen
$	US dollar
%	Percentage
(*S*)- (-) -Lim	(*S*)-(-) – Limonene
(*S*)- (-) -Lim	(*S*)-(-) – Limonene fumigation followed by ethylene
/	Divide
<	Less than
±	Plus/minus
×	multiply/interaction
µ	Micro
1-DCP	1-Decylcyclopropene
1-HC	1-Hexylcyclopropene
1-HCP+E	1-Hexylcyclopropene fumigation followed by ethylene
1-MCP	1-Methylcyclopropene
1-OCP	1-Octylcyclopropene
2, 5-NBD	2,5-Norbornadiene
3,3-DMCP	3,3 -Dimethylcyclopropene
8-HQS	8-hydroxyquniline sulphate
ABA	Abscisic acid
ACC	1-Aminocyclopropane–1–carboxylate
ACO	1-Aminocyclopropane-1-carboxylic acid oxidase
ACS	1-Aminocyclopropane-1-carboxylic acid synthase
Ag^{+}	Silver ion
$AgNO_3$	Silver nitrate
$Al_2(SO_4)_3$	Aluminium sulfate
ANOVA	Analysis of variance
AOA	Aminooxyacetic acid
APX	Ascorbate peroxidase

ATA	Aminotriazol
AUD	Australian Dollar
AVG	Aminoethoxyvinylglycine
AZ	Abscission zone
BA	Benzyl adenine
BA	Benzyladenine
BC	1*H*-cyclopropabenzene
BC+E	1*H*-cyclopropabenzene fumigation followed by ethylene
C	Control
CA	*trans*-Cinnamaldehyde
CA+E	*trans*-Cinnamaldehyde fumigation followed by ethylene
$CaCl_2$	Calcium chloride
CAT	Catalase
ClO_2	Chlorine dioxide
cm	Centimetre
CO_2	Carbon dioxide
CP	Cyclopropenes
CRD	Completely randomized design
Cu	Copper
DACP	Diazocyclopentadiene
DAFWA	Department of Agriculture and Food Western Australia
DNA	Deoxyribonucleic acid
DW	Distilled water
E	Ethylene alone
C_2H_4	Ethylene
Fig	Figure
g	Gram
GA_3	Gibberellic acid
GRAS	Generally Recognized As Safe
h	Hour
Ha	Hectares

IAA	Indole-3-acetic acid
IO	Isoprene oxide
KCl	Potassium chloride
$KMnO_4$	Potassium permanganate
L	Litre
L^{-1}	Per litre
LSD	Least significant difference
m^{-2}	Per square meter
mg	Milligram
mmol	Millimolar
MVG	Methoxyvinylglycine
MW	Molecular weight
N+E	1*H*-cyclopropa[*b*]naphthalene fumigation followed by ethylene
NAA	Naphthalene acetic acid
NC	1*H*-cyclopropa[*b*]naphthalene
nL	Nanolitre
NO	Nitric oxide
NSW	New South Wales
^oC	Degree Celsius
ppb, nLL^{-1}	Parts-per-billion
ppm, μLL^{-1}	Parts-per-million
RH	Relative humidity
RNA	Ribonucleic acid
s^{-2}	Per second squared
SA	Salicylic acid
SAM	S-adenosylmethionine
SE	Standard error of the mean
SE	Standard error
SEM	Standard error of mean
SOD	Superoxide dismutase
STS	Silver thiosulphate

TCO	*trans*-Cyclooctene
UK	United Kingdom
USA	United States of America
USD	United States Dollar
VL	Vase life
µM	Micromolar

CHAPTER 1

General introduction

The floricultural industry can be divided into different garden plants, cut flowers, cut foliage, potted plants nursery stock (trees), flowering leafy, annual, perennials flowers, tubers and bulbs (van Uffelen and de Groot, 2005). The annual consumption of commercially grown flowers in the world is estimated to be worth USD 40 - 60 billion (Ghule and Menon, 2013). Global exports of flowers have expanded by more than 10% annually by 2012 and the world exports are estimated to reach USD 25 billion (Ghule and Menon, 2013). In 2013, the international exports of cut foliage, cut flowers, living plant and flower bulbs had an estimated worth of USD 20.6 billion as against USD 21.1 billion in 2011 and in 2001 nearly USD 8.5 billion (van Rijswick, 2015). Australia exported fresh cut flowers and flower buds (AUD 20.2 million) during 2010-2011 to Japan, United States, Netherlands, Germany, Canada and others countries which are a declining trend as compared to the export of fresh cut flowers and flower buds (AUD 37.2 million) in 2007-2008 (Anon, 2012). Recently, the total area of flowers and pot plant production in the world has been estimated to be 650,000 ha (Hubner, 2016). Meanwhile, cut flowers in the open or under protection were cultivated on 4470 ha in Australia during 2013-2014 (Hubner, 2016). The cut flowers and foliage in Australia included traditional flowers, exotic and Australian native flowers. Fresh and dried Australian native flowers are economically important commodities in the export of floricultural crops (Anon, 2002). Australian flowers are mostly exported to Europe, Japan and North America as reported by Yan (2001). Australia has a sizeable native cut flower industry with an estimated 20% of world production with a value of AUD 500 million (Aldous and Hegde, 2013).

Waxflower has become one of the most significant commercial native cut flower in Australia during the recent past (Anon, 2002; Beasley and Joyce 2002; Gollnow and Worral, 2010; Seaton and Poulish, 2010). Ratanasanobon and Seaton (2010) reported that the annual production of waxflower globally is estimated at approximately 600 million stems. Flower lifetime in various species is often terminated by flowers and leaves senescence and/ or abscission of floral organs such

as petals, sepals, stamens, styles, inflorescences and leaves from the plant as well as fruit which is mainly regulated by ethylene (Ascough *et al*., 2005, 2006; Scariot *et al*., 2014).

As reported by Ascought *et al*. (2006) that the flowers and buds abscission is one of the horticultural industry problem which lead to losses cut and potted flowers quality. Extensive flowers/buds abscission induced by ethylene during transportation of waxflowers to export markets is a major hurdle in expansion of Australian waxflower trade. Accumulation of endogenous ethylene within cartons of waxflowers or unintentional exposure of flower stems to exogenous sources of ethylene leads to flower abscission thereby deteriorating postharvest quality and marketability (Joyce 1988, 1993; Dinh *et al*., 2008; Faragher *et al*., 2010; Seaton and Poulish, 2010). Floral organs (open flowers and flower buds) of waxflower are extremely sensitive to ethylene. Unintentional exposure of flowers to small amounts of ethylene can lead to 85% flower drop from the stems consequently shortening vase life causing high economic losses and reducing the marketability of several Australian native cut flowers including *Chamelaucium* Desf. (Myrtaceae) and *Boronia heterophylla* (Macnish *et al*., 1999; Seaton, 2006; Gollnow and Worral, 2010; Seaton and Poulish, 2010).

It may be as a result of adversity of causes, including flower senescence that reduces the life time of cut flowers, petals wilting or abscission of flowers, buds, leaves and/or petals (van Doorn and Stead, 1997; Reid, 2002). Flower aging is prompted by several factors such as pollination which, can promote the production of ethylene, (Wu *et al*., 1991a, Stead, 1992: Holden *et al*., 2003; Satoh *et al*., 2005; Ebrahimzadeh *et al*., 2008), ethylene exposure is another reason (van Doorn, 2001; Hunter *et al*., 2004a; Tripathi and Tuteja, 2007; Shahri and Tahir, 2011; Sapbua *et al*.,2012; Scariot *et al*., 2014) and post-harvest oxidative stress (Prochazkova *et al*., 2001). Drought and abscisic acid (ABA) have also been reported to enhance ethylene production and hasten floral senescence which may increase prior to/ or during senescence in attached or detached organs and act as abscission-accelerating signals (Smart, 1994; Rubinstein, 2000; Tripathi and Tuteja, 2007; Ebrahimzadeh *et al*., 2008; Sawicki *et al*., 2015). In addition, ethylene and other factors such as light, temperature, water supply, water quality, flowers maturity, photosynthates supply,

disease and mechanical damage are also associated with abscission of leaves, petals, buds and entire flowers (shattering) but ethylene plays a key role in accelerating this process and adversely affects postharvest quality (Reid, 2002, 2009; Beasley and Joyce, 2002; Ascough *et al*., 2005; Ascough *et al*., 2006; Sawicki *et al*., 2015).

Ethylene plays essential role in promoting the senescence, undesirable floral organs abscission, accelerating petal wilting and leaf yellowing in a number of export flowering plants therefore, reducing the ornamental visual appeal, causing loss of chlorophyll and pigmentations, damaging the product quality and shortening the floral life span thus reducing the market value (Woltering and van Doorn, 1988; Joyce, 1993, Joyce and Poole, 1993; Serek *et al*., 1994a, b; Serek *et al*.,1995b; van Doorn and Stead, 1997; Cameron and Reid, 2001; van Doorn, 2001; Beasly and Joyce, 2002; Celikel *et al*., 2002; Macnish *et al*., 2005; Serek *et al*., 2006; Ebrahimzadeh *et al*., 2008; Macnish *et al*., 2010; Sapbua *et al*., 2012; Scariot *et al*., 2014). Serek *et al*. (2006b) have suggested that at very low concentrations (nl-µL L^{-1}) ethylene will be biologically active however, there are substantial differences in sensitivity to exposure to ethylene among the species and cultivars of plants (Scariot *et al*., 2014).

Several methods have been used successfully for mitigating the harmful effects of exogenous and/or endogenous ethylene exposure in the floral industry to control premature senescence, abscission and extend floral lifetime in ornamental plants, for example breeding cultivars that are less sensitive to ethylene, inhibiting ethylene biosynthesis and/or inhibiting ethylene action. The sensitivity to ethylene is variable among and within different species and hybrids of waxflower (Macnish *et al*., 2004a; Seaton *et al*., 2007; Gollnow and Worrall, 2010). A wider range of hybrid waxflowers have been developed by breeding programs at Department of Agriculture and Food Western Australia (DAFWA) which may be a preferable method to reduce flowers/buds abscission. The main purpose of this program is to offer growers over 100 hybrids and cultivars of waxflower to ensure lower susceptibility to pests and disease, to develop new colours, early or late flowering seasons, improve vase life quality and display for export (Yan, 2001; Seaton *et al*., 2007; Gollnow and Worrall, 2010; Seaton and Poulish, 2010).

High CO_2 levels, ethanol, compounds such as aminooxyacetic acid (AOA), aminoethoxyvinylglycine (AVG) and silver ions inhibit the activities of ACS and or ACC and consequently ethylene biosynthesis which delays senescence and flowers/buds abscission (Rattanwisalanona *et al*., 2003; Ascough *et al*., 2006; Ebrahimzadeh *et al*., 2008). Silver thiosulfate (STS) and 1-methylcyclopropene (1-MCP) are used commercially to prevent ethylene action which mitigates the adverse effects of ethylene exposure on a range of floriculture crops including waxflowers (Joyce, 1993; Macnish *et al*., 2000b; Ichimura *et al*., 2002; Blankenship and Dole, 2003; Chamani *et al*., 2005; Reid and Celikel, 2008; Zencirkiran, 2010; Liu *et al*., 2012; Hassan and Ali, 2014). 1-MCP fumigation (10 nL L^{-1}) treatment failed to improve the flower vase life in 14 different genotypes of flowers in the absence of exogenous ethylene (Macnish *et al*., 2000b). In addition, application of another ethylene antagonist 2,5-norbornadiene (500 μL L^{-1}) has been reported to prolong the vase life of carnation cut flowers (Serek *et al*., 2006).

However, the application of STS and 1-MCP is limited due to some of their limitations such as that STS is harmful to human health and the environment. As STS is a heavy metal and remains in the soil for a long time and contaminates drinking water (Halevy, 1994; Sisler and Serek, 1997; Serek *et al*., 2007; Seaton and Poulish, 2010). Meanwhile, 1-MCP can be applied as a fumigant only in the tightly closed chambers or as sachets in flower cartons (locally developed method). It is highly unstable gas is difficult to handle. 1-MCP effectiveness wears off with time, in general, it can be from (2-25 days) depending on the flowers types and the treatment condition typically in waxflowers just effective only four days it possible to repeat the treatments. 1-MCP is expensive and sold as a service, not as a chemical. Due to the very low solubility of 1-MCP in water, it cannot be used as dip loading of cut flowers or applied as a pre-harvest spray in the field. The response of fumigation with 1-MCP is dependent on, number of factors such as, the concentration used of 1-MCP, genotype, exposure duration, temperature during treatment, storage and maturity stage of the produce in horticultural commodities (Blankenship, 2001; Grichko, 2006; Goren *et al*., 2008; Seaton and Poulish, 2010).

Antagonising ethylene action is one of the best effective approaches to reduce abscission of flowers/buds, retard senescence, extend flower and leaf vase life and maintain the flower quality in a range of flower crops. Presently, STS and 1-MCP are used to alleviate the adverse effects of ethylene in the postharvest phase of various flower crops including waxflower but various limitations are coupled with their usage. Various limitations linked with the usage of STS and 1-MCP offers the opportunity to researchers and technologists to develop new robust inhibitors of ethylene action which can be used not only to manage the adverse effects of ethylene on flower crops including waxflowers but also in other horticultural crops. The compounds with the extension of chain length (five or more carbon atoms) in the 1-position of the cyclopropene will improve their efficacy in antagonising ethylene action, for example, 1-hexylcyclopropene (1-HCP) is more effective anti-ethylene than 1-MCP in extending the shelf life of banana fruit (Sisler *et al.,* 2003). Similarly, Kebenei *et al*. (2003a) also reported that 1-HCP treated Alexandra *Kalanchoe¨ blossfeldiana* flowers exhibited delayed rolling of flower petals and prolonged vase life. Recently, Khan, (2014) reported that 1*H*-cyclopropabenzene at (50-100 nLL^{-1}) fumigation resulted in a reduction of flowers/buds abscission and extension vase life in waxflower 'WX73' and 'WX17' genotypes and maintained waxflower quality. Grichko *et al*. (2003) have also reported that the inhibitory effect of monoterpenes as anti-ethylene and some other naturally occurring compounds evaluated using green banana fruit ripening and suggested that these compounds interact with ethylene for binding to the receptor but were less effective than cyclopropenes. Jing *et al*. (2011) reported that application of cinnamaldehyde in vase solution prolongs vase life of 'Pink Queen' rose cut flowers by 2.8 days.

No research work has been reported on the effects of fumigation with a series of potential ethylene antagonists with different functional groups in their structure such as (1-octyne, isoprene oxide, *trans*-cinnamaldehyde, eugenol, (+)-carvone, (*S*) -(−)-limonene, 1-octene, allyl butyl ether, butyl acrylate, 1-octene oxide, 1*H*-cyclopropa[*b*]naphthalene (NC), 1*H*-cyclopropabenzene (BC) and 1-hexylcyclopropene (1-HCP) on inhibiting ethylene action in various genotypes of Australian native waxflowers. It was hypothesised that some of these new potential ethylene antagonists will be more effective in inhibiting flowers/buds abscission in

waxflowers. Therefore, this research was conducted with the following general objectives:

1. To test the efficacy of various new potential ethylene antagonists such as 1-octyne, isoprene oxide, *trans*-cinnamaldehyde, eugenol, (+)-carvone, (*S*)-(−)-limonene, 1-octene, allyl butyl ether, butyl acrylate, 1-octene oxide, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene and 1-hexylcyclopropene to inhibit ethylene action, particularly modulation of abscission of flowers/buds in different genotypes of waxflowers.
2. To investigate the effectiveness of some selected efficient ethylene antagonists such as *trans*-cinnamaldehyde, (*S*)-(−)-limonene, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene and 1-hexylcyclopropene in inhibiting ethylene action consequently reducing flowers/buds abscission in different genotypes of waxflowers.
3. To evaluate the efficiency of different concentrations of promising ethylene antagonists i.e. *trans*-cinnamaldehyde, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene and 1-hexylcyclopropene on inhibiting flowers/buds abscission in different genotypes of waxflowers.
4. To underpin the effectiveness of *trans*-cinnamaldehyde, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene and 1-hexylcyclopropene alone and in combination with vase solutions containing distilled water, sucrose or fructose and/or 200 mg L^{-1} 8-hydroxyquinoline sulphate (8-HQS) on vase life extension of different waxflower genotypes.

CHAPTER 2

General Review of Literature

2.1. Introduction

The floriculture is one of the branches of horticulture and contains a widespread variety of various categories of plants and flowers (van Uffelen and de Groot, 2005). It has been reported that more than 120 developed countries are involved in the floriculture production on a large scale and more than 90% of demand for floriculture comes from Europe, America, and Asia. Meanwhile, Colombia and Ecuador are the major suppliers to European markets and lead the market in the USA. Asia-Pacific countries are the central providers to Japan and Hong Kong (Ghule and Menon, 2013). The major countries with the leading share in cut and potted plants production are the Netherlands (35%), Germany (11%), and Italy (18%) (van Uffelen and de Groot, 2005). The largest production of various ornamentals plants, cut flowers, bulbs (for annuals and perennials) and potted plants is in the Netherlands. France is also a major player in different kinds of cut flowers, while the UK, Belgium and Spain are small producers of flowers (van Uffelen and de Groot, 2005). In addition, approximately 80% of potted plants and flowers are grown in North America (Canada and USA), whereas Colombia and Mexico contribute 6% and 3% respectively of potted plant and flowers.

The trends in international exports of cut flowers, cut foliage, living plant and flowers bulbs during 2001-2013 in the world are presented in (Fig.2.1) International exports of cut flowers, cut foliage and living plant and flower bulbs were an estimated USD 8.5 billion in 2001, and increasing to USD 21.1 billion in 2011and declining in 2013 to USD 20.6 billion.

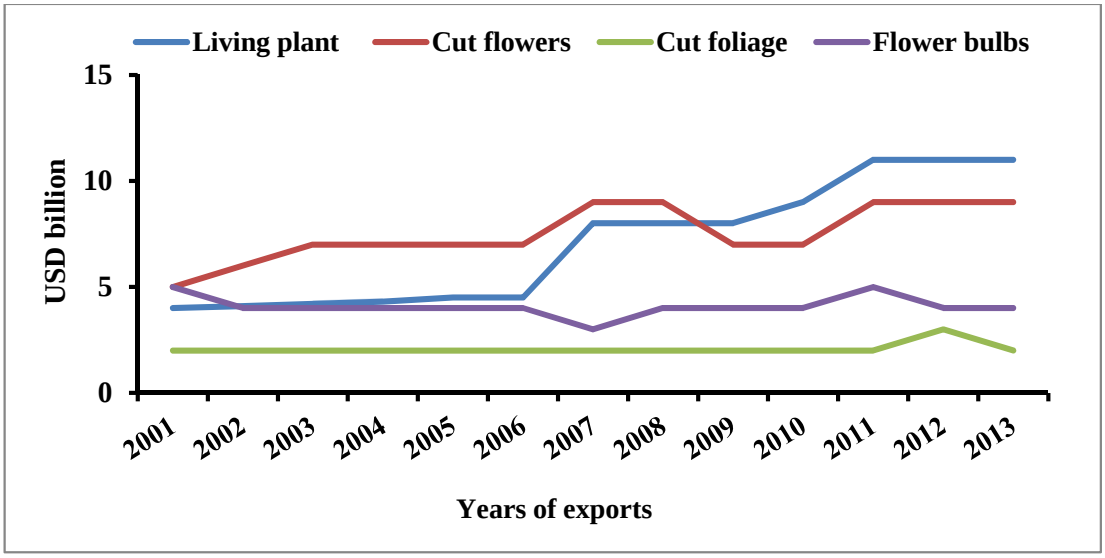


Figure. 2.1. Global trends in floriculture exports during 2001 to 2013 (van Rijswick, 2015)

Fresh and dried Australian native cut flowers and foliage, such as the banksia, kangaroo paw, thryptomene, boronia, waratah and Geraldton waxflower are economically important commodities of floricultural crops (Aldous and Hegde, 2013). Australian flowers are mainly exported to the United States, Western Europe and Japan (Ratanasanobon and Seaton, 2010; Aldous and Hegde, 2013). Australia has a sizeable native cut flower industry with an estimated 20% of world production with a value of A$500 million (Aldous and Hegde, 2013). Australian imports of fresh cut flowers and buds escalated from 52,103 flowers in 2007-08 to 71,145 flowers during 2010-11 Fig. 2.2 whilst the export of fresh cut flowers and buds declined from 37,223 flowers in 2007- 08 to 20,161 flowers in 2010-11 Fig. 2.3 (Anon, 2012). Waxflower and other *Chamelaucium* hybrids and species are one of the largest components of the fresh cut flower export industry from Australia (Lullfitz, 2001; Vitner *et al*., 2007; Gollnow and Worrall, 2010; Seaton and Poulish, 2010). The botanical name of the waxflower is (*Chamelaucium* spp. Desf.), while commonly

known as Geraldton waxflower. Waxflower is a native of Western Australia and is widely distributed in South Western Australia Fig.2.4. It has become one of the most desirable and valuable native cut flower exports from Western Australia which ranks first followed by Victoria and NSW. (Beasley and Joyce 2002; Vitner *et al*., 2007; Gollnow and Worrall, 2010; Ratanasanobon and Seaton, 2010; Seaton and Poulish, 2010; Aldous and Hegde, 2013). *Chamelaucium* are woody evergreen shrubs belongs to the family Myrtaceae. It is a short day cut flower. The distinguishing feature of the waxflower shrub is that it is covered with thousands of small waxy flowers during the blooming period with unique colours (Shillo *et al*., 1985; Gollnow and Worrall, 2010; Dinh *et al*., 2011). Earlier, Macnish *et al*. (2004a) reported that one of the most commonly cultivated species exported from Australia is *Chamelaucium uncinatum* estimated to be about 90% of the volume of all *Chamelaucium* spp. Waxflower is native to Australia has been used as a cut flower since the 1940s in California and was introduced into Israel in the 1970s. (Gollnow and Worrall, 2010). Yan (2001) and Dinh *et al*. (2008) reported that waxflower is a valuable perennial shrub and is grown as a large-scale floricultural crop used for cut flowers (Lamont, 1986). Seaton *et al*. (2007) have also reported that in Europe and the US, waxflowers are being successfully marketed as pot plants and amenity or landscape flowers. Vitner *et al*. (2007) and Dinh *et al*. (2008) suggested that waxflower sprigs are around 60-75 cm in length, straight and strong with attractive small white, pink, purple or red flowers and green leaves making the waxflower a good filler in floral arrangements or when displayed alone (Joyce, 1993; Olley *et al*., 1996; Dinh *et al*., 2011). Waxflowers contain 5 waxy petals, 5-minute sepals and 10 stamens, while the waxflower inflorescence forms a corymb (Slater and Beardsell, 1991; Beasley and Joyce, 2002). The flower has a pleasant aroma. It starts to produce nectar at anthesis stage and continues for 7-10 days (Olley *et al*., 1996). Some cultivars of *Chamelaucium uncinatum* are easy to cultivate and have higher productivity, while other species can offer a long flowering season (Seaton and Poulish, 2010). Flowering process in waxflowers depends on day length and air temperature and the flowering time of waxflower species/cultivars ranges from June to November in Mediterranean temperatures Table. 2.1 (Gollnow and Worrall, 2010; Seaton and Poulish, 2010). Vitner *et al*. (2007) reported that USA (California), Israel, Australia, Peru, Chile, and

South Africa are the main waxflower producing countries in the world (Ratanasanobon and Seaton, 2010).

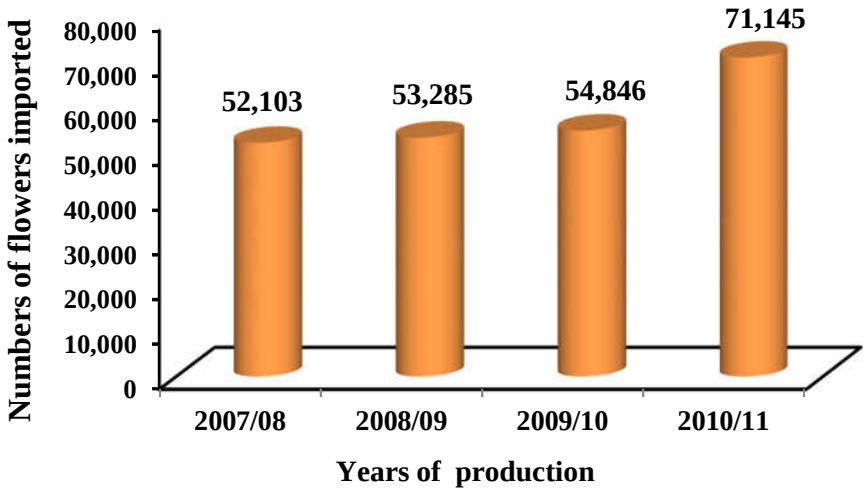


Figure. 2. 2. Total Australian imports (‘000 numbers) of fresh cut flower and flower buds during 2007 to 2011. (Anon, 2012)

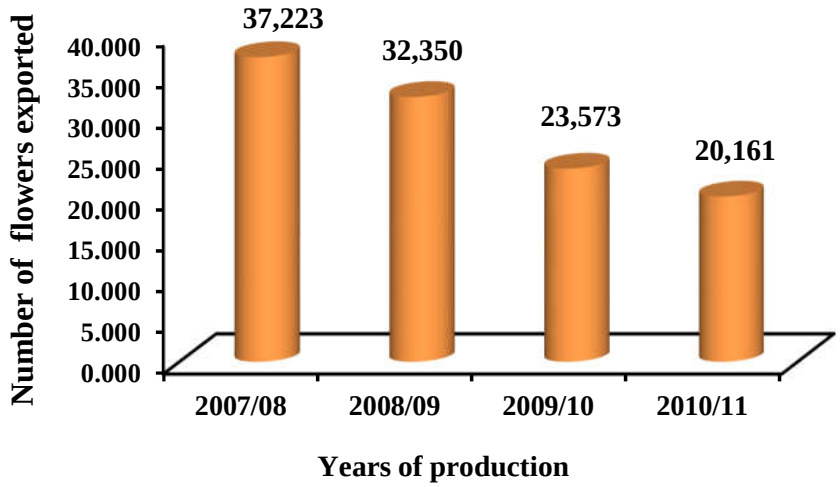


Figure. 2.3. Total Australia exports (‘000 numbers) of fresh cut flowers and flower buds during 2007 to 2011. (Anon, 2012).

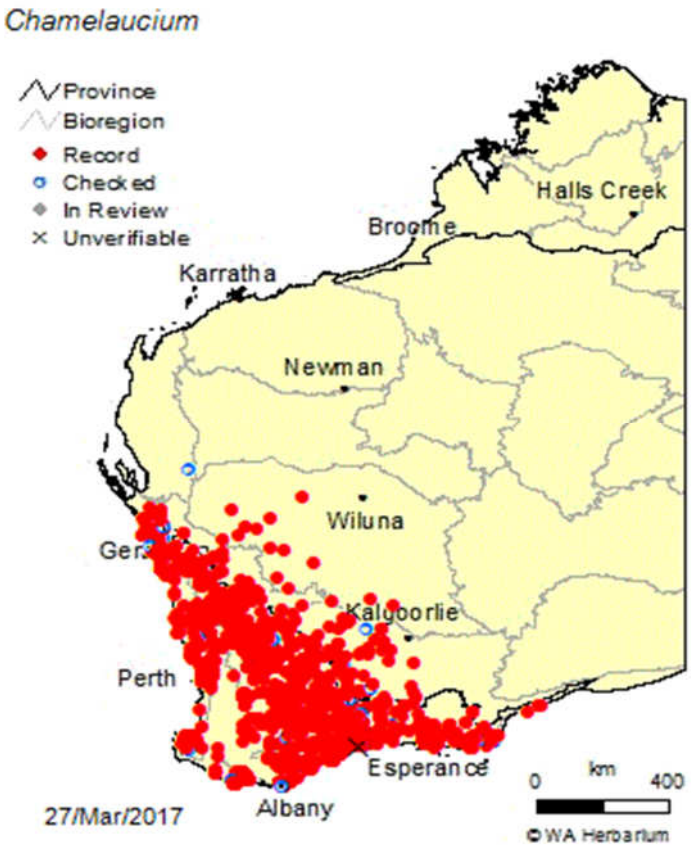


Figure.2.4. Distribution of *Chamelaucium* Desf. (red points) in Western Australia (WA) during 2017. https://florabase.dpaw.wa.gov.au/browse/profile/21818.

Table. 2.1. Some commercial varieties of waxflower available in Australia (Seaton and Poulish, 2010).

Variety	Flowering time
CWA Pink	Very early season June
Early NIR	Early season June to July
Revelation	Early season late June to July
Jurien Book	Early to mid-season July to early August
Purple Pride	Early to mid-season July to August
Eclipse	Early to mid-season July to August
Painted Lady	Early to mid-season July to August
Purple Giant	Early to mid-season July to August
Teina's Delight	Early to mid-season July to August
Lilac Spring	Mid- season July to October
Raspberry Ripple	Mid- season August
Crystal Pearl	Mid to late season August
WX74	Mid to late season mid to late August
Alba	Mid- season August to September
Ivory Pearl	Mid- season late August to early September
Mullering Brook	Mid to late season September
Chantilly Lace	Mid to late season September to October
Eric John	Late season mid -September to October
WX87	Late season September to October
Dancing Queen	Late season late-September to October
Lady Stephanie	Late season late-September to October
Paddy's Pink	Late season late-September to October
White Fire	Late season late-September to October
Lady Jennifer	Late season late-September to October
Cardinal Chris	Late season late-September to October
My Sweet Sixteen	Very late season October to November
Snowball	Very late season October to November
Mullering Brook White	Very late season October to November

Cut waxflowers are very sensitive to ethylene and their vase life is relatively short (Joyce, 1993). The cut waxflower industry in Australia has a significant problem with post-harvest flowers/buds abscission due to the accumulation of endogenous ethylene and/or exposure to exogenous ethylene in the post-harvest atmosphere (Joyce, 1988, 1992; Faragher, 1989). The sensitive flowers, such as waxflower genotypes and other flowers are damaged by exposure to ethylene as little as 0.01 μL L^{-1} for more than a day whilst, 1 μL L^{-1} for 12 - 24 h can cause substantial damage to both flower bud and open floral abscission and ageing after harvest (Dinh *et al*.,2008; Faragher *et al.*, 2010). Ethylene and *Botrytis cinerea* are also known to reduce the value of the stems during storage (Joyce 1988; Dinh *et al.*, 2008, 2011; Gollnow and Worrall, 2010; Seaton and Poulish, 2010). Anti-ethylene treatment during the post-harvest phase is one of the most effective methods for reducing the loss of flowers in waxflowers (Faragher *et al.*, 2010; Gollnow and Worrall, 2010; Seaton and Poulish, 2010).

2.2. Ethylene

Ethylene is a colourless gas and is produced by plants and acts as a plant hormone. Ethylene is the simplest unsaturated hydrocarbon with a molecular formula of C_2H_4 (Abeles *et al*., 1992; Saltveit, 1999, 2004; Gibson *et al*., 2000; Lurie, 2007; Martínez-Romero *et al*., 2007; Scariot *et al*., 2014). Ethylene is very active as a plant hormone even at low concentrations (nL - μL L^{-1}) but the response to ethylene is genotype dependent Table.2.2 (Saltveit, 1999; 2004; Gibson *et al*., 2000; Serek *et al*., 2006; Scariot *et al*., 2014). Abeles *et al*. (1992) reported that ethylene has a critical role in initiating and regulating the life cycle of plants from seed germination to organ senescence (Bleeker and Kende, 2000; Martínez-Romero *et al*., 2007). Blankenship (2001) illustrated that ethylene can be produced when plant tissues are injured either through disease or mechanical damage. There are several external sources of ethylene that can affect plants sources include heaters, smoke (from cigarettes, combustion engines and the welding), rotting vegetation and natural gas leaks (Gibson *et al.*, 2000). Ethylene has been shown to play a beneficial and detrimental role in a wide range of plant responses and developmental processes in horticultural crops. It promotes seed germination, the formation of the root, lateral bud development, initiation of flowering and flower opening but it also promotes

senescence, leaf, flower and fruit abscission, excessive softening of fruits, loss of chlorophyll and enhances discoloration. All the detrimental effects contribute to the hastening of the floral senescence pathway in a number of ornamental plants (Abeles *et al*., 1992; Joyce, 1993; Ten Have and Woltering, 1997; Saltveit, 1999; Bleeker and Kende, 2000; Blankenship, 2001; Saltveit, 2004; Binder *et al*., 2007; Lin *et al*., 2009; Golden *et al*., 2014). Ethylene is known as natural senescence hormone to cause petal senescence in *Dendrobium, Campanula, Trachelium, Gypsophila* (Newman *et al*.,1998; Serek *et al*., 2006) and carnation (*Dianthus caryophyllus*) (Borochov and Woodson, 1989; Serek *et al.,* 1995a, b), also plays a key role in shortening vase life of sensitive cut flowers and inducing undesirable flowers abscission (Serek *et al*., 2006) reduction in vase life of sweet pea (*Lathyrus odoratus* L.), (Kebenei *et al*., 2003b), enhanced petal senescence in (*Narcissus pseudonarcissus* L.) (Hunter *et al*., 2004a) and petal/flower/bud abscission in *Backhousia myrtifolia, Baeckea virgata, Boronia heterophylla, Ceratopetalum gummiferum, Chamelaucium uncinatum, Plectranthus, Pelargonium peltatum,* Zonal Geraniums and Oriental hybrid lilies (Joyce,1993; Cameron and Reid, 2001; Jones *et al*., 2001; Celikel *et al*., 2002 Ascough *et al*., 2006; Faragher *et al*., 2010).

Table.2.2. Sensitivity to ethylene exposure in different flowers (Reid, 2004).

Flower scientific name	Ethylene sensitivity
Alstroemeria	Sensitive to ethylene exposure
Anemone spp.	Cause petal drop and reduce vase life
Anthurium andraeanum	Insensitive to ethylene exposure
Asparagus spp.	Cause leaf drop
Aster spp.	Insensitive to ethylene exposure
Gypsophila paniculata	Cause sleepiness of open buds and wilting of flowers
Strelitzia reginae	Insensitive to ethylene exposure
Bouvardia spp.	Cause flowers abscission and wilting
Zantedeschia spp.	Insensitive to ethylene exposure
Dianthus caryophyllus	Sensitive to ethylene case petal wilting and sleepiness
Dendranthema grandiflorum	Insensitive to ethylene exposure
Narcissus cvs.	Sensitive to ethylene
Delphinium consolida spp.	Very sensitive to ethylene
Chamaedorea spp.	Insensitive to ethylene exposure
Eucalyptus spp.	Insensitive to ethylene exposure
Abies spp.	Insensitive to ethylene exposure
Freesia	Ethylene effect in young buds than open florets
Gerbera jamesonii	Insensitive to ethylene exposure
Alpinia zerumbet	Insensitive to ethylene exposure
Gladiolus cvs.	Insensitive to ethylene but ethylene can cause abortion to close buds
Heliconia humilis	Insensitive to ethylene exposure
Iris cvs.	Insensitive to ethylene exposure
Gaultheria shallon	Insensitive to ethylene exposure
Liatris pycnostachya	Insensitive to ethylene exposure
Liatris	Insensitive to ethylene exposure
Lilium spp.	Sensitive to ethylene can cause petal abscission

2.2.1. Biosynthesis of ethylene

Ethylene is produced from 1-aminocyclopropanecarboxylic acid (ACC) which in turn is made from methionine in a biosynthetic pathway known as the Yang's cycle. The two main enzymes which regulate this metabolic pathway are 1-aminocyclopropane-1-carboxylic acid ACC oxidase and ACC synthase (Adams and Yang, 1979). In the Yang's cycle, methionine is converted to S-adenosyl methionine (SAM) by SAM synthase. 1-aminocyclopropane-1-carboxylic acid (ACC) synthase then performs the cyclisation reaction to convert SAM to ACC Fig.2.5. The conversion of ACC into ethylene by ACC oxidase involves the loss of carbon dioxide and hydrogen cyanide (Yang and Hoffman, 1984; Bleeker and Kende, 2000; Srivasava, 2002; Muller and Stummann, 2003) The pathway of ethylene biosynthesis including activity of two main enzymes ACC synthase and ACC oxidase has been reviewed in detail (Adams and Yang, 1979; Abeles *et al*., 1992; Kende, 1993; Bleeker and Kende, 2000; Muller and Stummann, 2003).

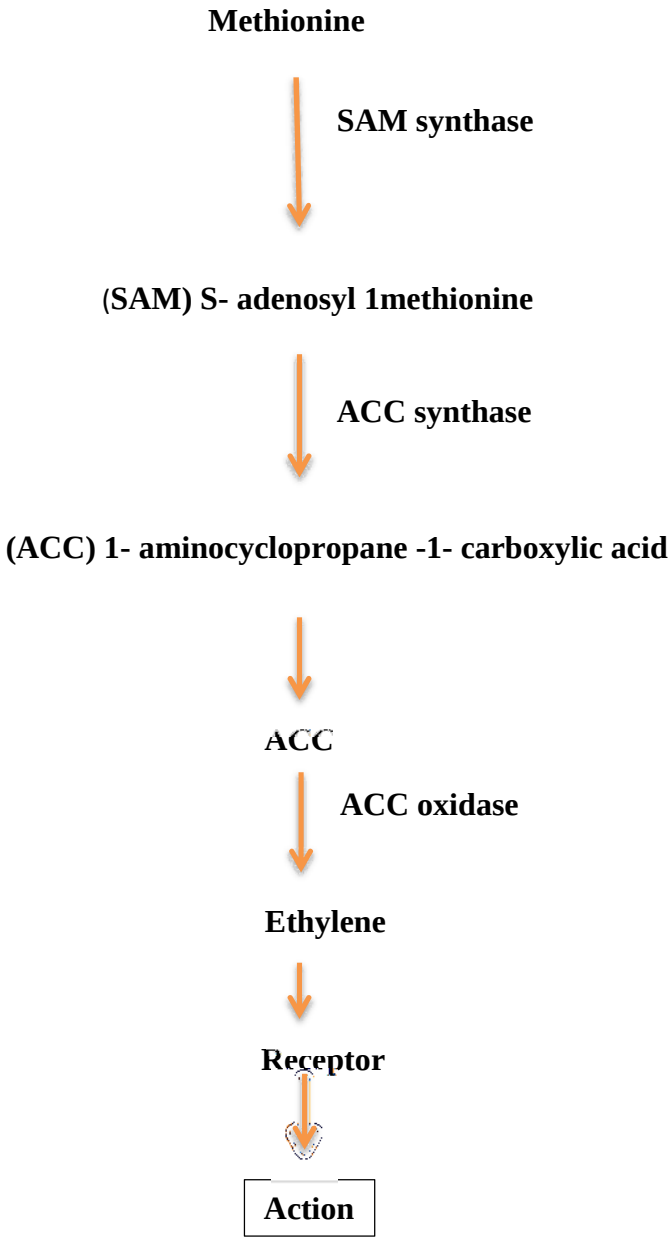


Figure. 2.5. Ethylene biosynthesis in plants and the different enzymes involved (Adams and Yang, 1979; Martinez-Romero *et al*., 2007)

2.3. Senescence

Senescence, aging and death are the main obstacles in the floricultural industry which leads to reduced lifetime and quality in a number of cut flowers (van Doorn and Wothering, 1991; Bowyer and Wills, 2003; Muller and Stummann, 2003; Ebrahimzadeh *et al*., 2008; Almasi *et al*., 2012; Rani and Singh, 2014). Flower senescence represents the ultimate event in the life cycle of many plant tissues. It is considered to be an integral part of the normal growth of plants, as reported in *Alstroemeria* flowers senescence characterised by wilting and in rolling, terminating by the flowers abscission. (Stead and Van Doorn, 1994; Wagstaff *et al*., 2003; Jones *et al*., 2005; Yamada *et al*., 2007; Tripathi and Tuteja, 2007; Ichimura *et al*., 2009; Shahri and Tahir, 2011). Senescence is known as a complex process which involves a series of highly coordinated changes in gene expression and other biochemical and physiological changes ultimately leading to cell death of an organ or an organism. It is usually observed in petals, leaves, stamens and style (Tripathi and Tuteja, 2007; van Doorn and Woltering, 2008; Shahri and Tahir, 2011; Desai *et al*., 2012).

A number of biochemical, physiological and genetic changes occur during floral senescence (Tripathi and Tuteja, 2007; Shahri and Tahir, 2011) such as, regulation of expression of genes, increased oxidative stress and decreased activities of protective enzymes, proteins degradation in cytoplasm loss of fatty acid, breakdown in pigmentation, loss of membrane permeability and loss of nucleic acids (DNA and RNA) at the last stages of senescence. Meanwhile, the ultra-structural changes during senescence, such as loss and collapse of tonoplast of organelles and loss of cytoplasmic content, has been reported in carnation and *Iris* flowers (Smith *et al*., 1992; van Doon *et al*., 2003; Van Doon and Woltering, 2004). Environmental factors can promote senescence processes such as biotic stresses, drought, light quality and other factors (Tripathi and Tuteja, 2007; van Doorn and Woltering, 2008; Shahri and Tahir, 2011). Flower senescence is induced by many factors such as water stress (Muller *et al*., 2000; Tripathi and Tuteja, 2007), ethylene (Cameron and Reid, 2001; Hunter *et al*., 2004a) , pollination (Nichols, 1977; Stead, 1992; van Doorn, 1997) and post-harvest oxidative stress (Prochazkova *et al*., 2001). Abscisic acid has been reported to implicated in floral senescence by increasing the ethylene production (Halevy and Mayak, 1981; Rubinstein, 2000; Tripathi and Tuteja, 2007).

Ethylene promotes leaf and floral organ senescence in many plant species (Woltering and van Doorn, 1988; Borochov and Woodson 1989; van Doorn, 2001; Müller and Stummann, 2003; Wagstaff *et al*., 2005; Tripathi and Tuteja, 2007; Scariot *et al*., 2014). Abscisic acid is another regulator in flower senescence in a number of flowers. In cut roses, abscisic acid reduces the level of carbohydrates and increases the rate of respiration (Borochov *et al*., 1976; Hunter *et al*., 2004b; Tripathi and Tuteja, 2007). However, cytokinins are anti-senescent phytohormones that delay floral senescence in various plant tissues by reducing ethylene production and sensitivity to ethylene (Eisinger, 1977; Chang *et al*., 2003; Tripathi and Tuteja, 2007; Shahri and Tahir, 2011). Shahri and Tahir. (2011) reported that polyamines and jasmonates have been found to retard and hasten senescence, respectively, in orchid species.

Anthocyanins, carotenoids and betalains are three main pigments contributing to the colour of flowers (Rani and Singh, 2014). Chlorophyll is known as the main pigment in plant tissues, oxygen radicals involve in decreasing the photosynthetic rate and pigment level as reported by Prochazkova *et al*., 2001. Remobilization of nitrogen from proteins occurs due to the breakdown of chlorophyll (Shahri and Tahir, 2011). According to Avila-Rostant *et al*. (2010) that one of the most important reasons for the termination of shelf life in several cut flowers and the factors involved in hampering to reduce flowers quality is colour fading and discoloration (Rani and Singh, 2014). Extensive studies have confirmed that the colour of flowers depends on the pigment present in the plant tissue and also on various factors which determine the colour of the flowers due to pH, light and temperature both important factors in anthocyanin accumulation (Shvart *et al*., 1997; Weiss, 2000; Dela *et al*., 2003; Meng and Wang, 2004). As such, exposure of flowers to low light and high temperature results in reduced pigment content in flower petals because of down-regulation of genes expression involved in the biosynthesis of anthocyanins (Rani and Singh, 2014). In addition, other changes occur during senescence. Membrane integrity is lost by lipid peroxidation. A range of reactive oxygen species such as singlet oxygen and hydroxyl radicals formed by lipid peroxidation which, break down the fatty acids ultimately influence membrane permeability (van Doorn and Woltering, 2008; Rogers, 2012). All of these reactive

oxygen species have the ability to oxidise a number of macromolecules with different specificities (Bailly *et al*., 2001).

During petal senescence, a change in protein synthesis and degradation is observed. Loss of proteins plays important role in most of the structural and functional properties of plant tissues. The senescence of petals and other parts of a flower causes a reduction in total protein content due to the increased protease activity (Shahri and Tahir, 2011; Rani and Singh, 2014). Rani and Singh. (2014) reported that in some species of flowers such as petunia and rose a significant reduction in protein levels have been reported before the symptoms of ageing appear. Similarly, Shahri and Tahir. (2011) also reported increased protease activity during sepal senescence in *Consolida ajacis* flowers. The breakdown of nucleic acids (DNA and RNA) during the senescence process is noted in many flowers species such as *Petunia inflata, Alstroemeria, Gladiolus* and *Actinidia deliciosa* (Orzaez and Granell, 1997; Xu and Hanson, 2000; Wagstaff *et al*., 2003; Yamada *et al*., 2003). Rani and Singh. (2014) also reported that activities of most enzymes such as catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) among others act in diverse ways during flower ageing.

2.4. Abscission of flowers/buds

The abscission process of flower parts (shattering) is referred to the natural processes where plant organs separate from the plant such as floral structures, fruit and leaves. Abscission is an integral characteristic of plant development and considered as a serious problem during postharvest handling of several flowering plants (Cameron and Reid, 1983; Joyce, 1993; Cameron and Reid, 2001; Macnish *et al*., 2005; Hvoslef-Eide, 2008). The various organs that are dropped in the abscission process include sepals, stamens, petals, styles, leaves, flowers, inflorescences and fruit (Ascough *et al*., 2006). Pollination-induced ethylene generation often provides the signal for petal or corolla abscission (Brown, 1997). Scientists have sought to elucidate abscission events that occur in response to developmental environmental and hormonal factors (Ascough *et al*. 2005). Stressful environmental factors trigger abscission in plant material which include low light, water stress, temperature, humidity, exposure to ethylene gas, improper packaging and transport. Endogenous

ethylene normally hastens abscission even though it is not the only factor regulating abscission (Sexton *et al*., 1985; Ascough *et al*. 2005). Abscisic acid (ABA) is a natural hormone which has also been implicated in playing an essential role in promoting senescence processes in various cut flowers (Hunter *et al*, 2004b). ABA accelerates senescence in rose cut flowers by increasing respiration rate and reducing carbohydrate levels (Borochov *et al*., 1976). ABA accumulation has also been reported to accelerate the senescence in daylily, carnation, narcissus and roses cut flowers. This is due to ABA-induced ethylene production and an increased sensitivity of flowers towards ethylene (Mayak and Halevy, 1972; Mayak and Dilley, 1976; Hunter *et al*., 2004b). Auxins have also been reported to reduce the sensitivity of the abscission zone to ethylene action (van Doorn and Stead, 1997). Macnish *et al*. (2005) reported that the abscission zone is known as a narrow constriction in tissue and loss in cell cohesion leading to the formation of separate layers in plant organs. For instance, in waxflowers genotypes, the abscission process occurred at a two to four cells wide separation layer within the abscission zone (Macnish *et al.*, 2005). In this process, there is a degradation of the middle lamella. The cell wall adjacent to the separation layer generally becomes more spherical and loosely packed with the deterioration of the protoplasm (Macnish *et al*., 2005). A number of studies have revealed that activation of the abscission zone refers to high secretory activity and high protein synthesis in cell walls especially hydrolytic enzymes (van Doorn and Stead, 1997). According to the authors, the activation of the abscission zone (AZ) is attributed to upregulation of protein synthesis in cell walls, especially cell wall hydrolysis enzymes such as cellulose and pectinase (Sexton *et al*., 1985).

Exogenous ethylene appears to cause most of the postharvest problems in flower and bud abscission, wilting and flower senescence during transport, handling, storage, and marketing of Australian native flowers including *Chamelaucium uncinatum, Leptospermum, Thryptomene calycina, Backhousia myrtifolia, Baeckea virgata, Boronia heterophylla, Ceratopetalum gummiferum* and *Grevillea* as well as cause short vase life of potted plants *Pelargonium peltatum and Plectranthus* cultivars (Joyce, 1993; Cameron and Reid, 2001; Ascough *et al*., 2006; Faragher *et al*., 2010). Waxflower is one of the most valuable economic cut flowers for export in the floriculture industry in Australia with up to 50 million sprigs sold to international markets. It has been estimated that 95% of total production of native flowers from

Australia is being exported (Beasley and Joyce 2002; Seaton and Poulish, 2010). However, as reported by Joyce (1993) waxflowers suffer from flower and bud abscission during export because of the ethylene accumulation in carton occuring during export. Macnish *et al*. (2004a) reported that most waxflowers are sensitive to ethylene and there are differences among waxflowers response to ethylene exposure such as that sensitive genotypes shed 10% of their flowers in response to exposure to ethylene including the extremely sensitive genotypes 'Purple Pride', 'Sweet Georgia' and C. *uncinatum cvv.* 'Early Nir', 'Paddy's Late', 'CWA Pink' and 'Early Hard'. Macnish *et al.* (2000a) reported that 1-MCP (10 nLL^{-1}) fumigation for 12 h at 20˚C prevented flower abscission in Grevillea '*Sylvia*' inflorescences and waxflower stems. Joyce (1993) proposed that in waxflower, ethylene production is mediated by various factors such as wounds, water deficit, pathogens, water stress and all these factors elevate ethylene biosynthesis. Moreover, abscission can be reduced with the treatment of various ethylene antagonists such as STS, 1-MCP, NAA with varying efficiencies in modulating ethylene action and biosynthesis and organ abscission.

2.5. Prevention of ethylene action

Numerous chemicals have been tested successfully for blocking the deleterious effects of ethylene in various plant and floriculture industries in order to control flowers and buds abscission and/or senescence in various ornamental plants, such as silver thiosulfate (STS) (Veen, 1979; Serek and Andersen 1993; Halevy, 1994; Macnish *et al*., 2000a; Tanase *et al*., 2009), 2,5-norbornadiene (2,5-NBD) (Sisler and Pian, 1973; Sisler *et al*., 1986; Peiser, 1989; Wang and Woodson 1989; Sisler *et al*., 1990; Sisler and Serek 2003; Ascough *et al*.,2006; Serek *et al*., 2007), Diazocyclopentadiene (DACP) (Sisler and Blankenship, 1993b; Serek *et al*., 1994a; Sisler and Serek, 1997; Sisler and Serek, 2003; Serek *et al*., 2006; Serek *et al*., 2007), *trans*-cyclooctene (TCO) (Sisler *et al.*, 1990; Sisler and Serek, 2003), cyclopropene (CP), (Sisler *et al*., 1996b; Sisler and Serek, 2003), 1-methylcyclopropene (1-MCP) and 3,3-dimethylcyclopropene (3,3-DMCP) (Sisler *et al*., 1996a; Sisler and Serek, 1997; Sisler *et al*., 2001; Sisler *et al*., 2003; Ascough *et al.*, 2006; Grichko, 2006; Reid and Staby, 2008; Chutichudet *et al*., 2010).

2.5.1. Inhibitors of ethylene biosynthesis

Senescence and abscission are significant postharvest problems induced by exogenous and/or endogenously produced ethylene (Joyce, 1993; Faragher *et al*., 2010; Scariot *et al*., 2014). In the past ten years, development of different strategies to inhibit ethylene production or its action led to substantial advances in postharvest science and postharvest technologies, which are used for blocking the deleterious effects of ethylene consequently reducing abscission of flowers and/or senescence in ornamental plants (Scariot *et al*., 2014). Exposure to exogenous or endogenously produced ethylene can be blocked in a number of methods including the use of ethylene biosynthesis inhibitors or ethylene action inhibitors (Serek *et al*., 2006; Ebrahimzadeh *et al*., 2008; Scariot *et al*., 2014). Various chemical substances have been used for blocking the damaging effects of ethylene alleviating abscission of flowers and/or senescence in different ornamental plants. Firstly, ethylene biosynthesis inhibitors have been used that are effective in reducing endogenous ethylene production in the plants, by inhibiting the activity of 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and/or 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) (Serek *et al*., 2006; Martinez-Romero *et al*., 2007; Scariot *et al*., 2014).

Ethylene biosynthesis inhibitors such as AVG, (1-aminoethoxyvinylglycine), (AOA) (amino oxyacetic acid) and methoxy vinyl glycine (MVG) have been proven to be effective tools in delaying senescence of flowers by inhibiting the activity of ACS (Fujino, *et al*., 1980; Dostal *et al*., 1991; Reid and Wu, 1992; Ascought *et al*., 2006; Martinez-Romero *et al*., 2007; Ebrahimzadeh *et al*., 2008; Tanase *et al*., 2009). Rattanwisalanona *et al*. (2003) reported that application of amino oxyacetic acid (AOA) extended vase life of *Dendrobium* flowers by inhibiting the activity of ACC synthase and was also effective in controlling the corolla abscission in Guinea impatiens *(Impatiens* × *hawkeri* 'Sunfire') (Dostal *et al*., 1991). Application of AVG was more effective in extending the vase life of cut *Eustoma* flowers (Shimizu-Yumoto and Ichimura, 2010). Martinez- Romero *et al*. (2007) reported that high rates of carbon dioxide CO_2 and low rates of oxygen have been found to be as an antagonist of ethylene biosynthesis and action consequently improving the shelf-life of fruit and vegetables (Serek *et al*., 2006; Ebrahimzadeh *et al*., 2008). However,

these chemicals have several weaknesses that have led to the reduction or the prohibition of its use such as high cost, the great difficulty in preparation of solutions and phytotoxicity (Scariot *et al*., 2014). Additionally, flowers treated with ethylene biosynthesis inhibitors will be prone to the deleterious effects of external ethylene during the postharvest phase.

2.5.2. Inhibition at the receptor level

Ethylene is known to interact with the ethylene receptor through a transition metal complex. The formation of this complex triggers the signal transduction pathway which is responsible for ethylene action (Sisler and Serek, 2003; Binder *et al*., 2007). Although many transition metals previously have been tested, only silver and copper can act as cofactors to promote ethylene binding activity. There are two types of compound that bind to the ethylene receptor through the copper (I) cofactor. (Thompson *et al*., 1983; Rodriguez *et al*., 1999; Binder *et al*., 2007; Pirrung *et al*., 2008; McDaniel and Binder, 2012). Compounds that bind to the receptor and trigger an ethylene response are called agonists and compounds that bind to the ethylene receptor but do not cause a response are called antagonists (Sisler and Serek; 1999, 2003) Ethylene antagonists promise to be commercially important in protecting different fruits, vegetables and ornamental plants against ethylene action.

2.5.2.1. Silver ions

Many inhibitors have been assessed for their effectiveness on inhibition of the signal transduction and gene activation by blocking the ethylene receptors in plants (Sisler and Serek, 1997; Serek *et al*., 2006; Ebrahimzadeh *et al*., 2008; Strader *et al*., 2009). Beyer (1976) suggested that the application of $AgNO_3$ as a spray solution on *Cattleya* orchid blooms delayed the flower senescence and prevented flowers from wilting and leaf abscission. Kofranek and Paul (1975) reported that the flower stems of Chrysanthemums, Gerbera, Carnation and Gladiolus dipped in 1000 and 1200 mg L^{-1} $AgNO_3$ solution for 10 minutes improved flower opening and shelf life of flowers. Halevy and Kofranek (1977) reported that the longevity of carnations cut flowers can be prolonged with pre-treatment of $AgNO_3$ salts. Ag^{+} is also known to exhibit anti-microbial properties when added to the vase solutions holding cut

carnation flower stems. Later on, Veen, (1983) also reported that (Ag^{+}) as a strong ethylene action inhibitor binding to ethylene receptors and inhibiting ethylene action (Veen, 1983; Serek *et al*., 2007) as silver ions. Nevertheless, this form of silver (silver nitrate) is a heavy metal often phytotoxic when applied as a spray, it was ineffective when used in vase solutions as moves slowly in cut flower stems also, cannot be applied to edible crops (Joyce, 1988; Staby *et al*., 1993; Da Silva, 2006; Sisler *et al*., 2006; Ebrahimzadeh *et al*., 2008; Reid and Staby, 2008). Silver thiosulfate (STS) is used commonly to protect sensitive ornamental plants against ethylene, for delaying senescence and extending postharvest shelf life of potted plants and cut flowers (Veen and van de Geijn, 1978; Veen, 1983; Mor *et al*., 1984; Rodriguez *et al*., 1999; Dole *et al*., 2004; Serek *et al*., 2006; Ebrahimzadeh *et al*., 2008; Seglie *et al*., 2010; Hassan and Ali, 2014). Staby *et al*. (1993) reported that more than 400 studies are available on the effectiveness of STS on different floral crops. The treatment of (STS) has been reported to be extremely effective by inhibiting ethylene action and in delaying the onset of senescence or preventing floral abscission (Ichimura *et al*., 2002).

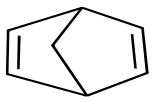
Joyce (1993) found that the application of STS (0.5 mM Ag^{+} L^{-1}) for 15- 22 h resulted in suppression of endogenous ethylene production and reduced flower fall in Geraldton waxflower in packaged flowers. Application of STS resulted in delaying in fresh weight loss in ethylene-treated flowers has also been reported for *Verticordia nitens* (Joyce and Poole, 1993). Macnish *et al.* (2000a) reported that the longest protection against ethylene effects was observed when the cut waxflowers sprigs treated with STS than the application of 1-MCP. Application of STS to carnation and delphinium flowers is also effective in delaying the onset of senescence or preventing floral abscission and retarding fresh weight loss after harvest (Ichimura *et al*., 2002) also, inhibited petal abscission (Cameron and Reid, 1983) and extended vase life in *Matthiola, Physostegia, Gypsophila*, several orchid species, *Delphinium*, sweet pea (Ichimura *et al*., 2002; Serek *et al*., 2006) and Victory Parade *Rosa hybrida* L. (Serek and Andersen, 1993).

According to Al-Humaid (2004) pulse treatment with STS is widely performed as an antimicrobial agent to reduce total microbial activity during vase life and significantly improved postharvest quality in ‘Supreme’ cut gladiolus spikes and

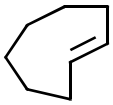
'Evel Tower' rose cultivars. Application of STS has been reported to extend the flowers vase life in Snapdragon (*Antirrhinum majus* L.) (Ichimura *et al.,* 2008). STS prolongs the life of florets and spikes in cut Freesia "Cordula" (Zencirkiran *et al.,* 2010). Adugna *et al*., (2012) claimed that Green- GO and Galy cultivars of carnation cut flowers treated with STS (0.6 mM) and 25g sucrose was effective to extend the days taken for flower opening in *Rosa hybrida* (Asghari *et al*., 2014).

STS is a highly toxic heavy metal and cannot be used in food and feed due to concerns for detrimental effects on human health and the environment (Serek and Reid 1993; Halevy, 1994; Mayers *et al*., 1997; Sisler and Serek, 1997; Ichimura and Suto, 1999; Cameron and Reid, 2001; Serek *et al*., 2007; Seaton and Poulish, 2010). STS has not been commonly adopted by growers because of the higher cost, requirement for specific time of application, proper temperatures and negative impact on human health which have led to the reduction or the prohibition of its use in the USA and in Holland, for example, its use on potted plants was banned (Halevy, 1994; Mayers *et al*., 1997). Later on, Seaton and Poulish (2010) reported that STS at higher concentrations can be phytotoxic and causes flower drop thus, shortening vase life in certain waxflowers.

2.5.2.2. 2,5-Norbornadiene (2,5-NBD) and *trans*-cyclooctene (TCO)



2,5-norbornadiene



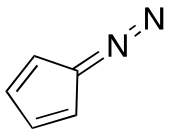
***trans*-cyclooctene**

Numbers of potent cyclic olefins are known to inhibit effect of ethylene and were found to compete with the ethylene receptor and very effective blocker of ethylene binding sites in plant tissue (Sisler and Pian 1973; Sisler *et al*., 1996b; Sisler and Serek, 1999; Serek *et al*., 2007; Ebrahimzadeh *et al.,* 2008). Sisler and Pian (1973) first described 2,5-norbornadiene as an inhibitor of ethylene action in carnation cut flower at anthesis stage as well as other flowers (Sisler *et al*., 1983; Peiser, 1989; Wang and Woodson, 1989). 2,5-norbornadiene is stable liquid at a boiling point of 89 °C. The use of 2,5-norbornadiene as an inhibitor of ethylene

action on flower senescence has been extensively investigated over 20 years (Sisler *et al*., 1983; Sisler *et al*.,1986; Sisler and Serek, 2003; Ascough *et al*., 2006). Sisler *et al*. (1985) reported that application of 2000 µL L^{-1} 2,5-norbornadiene was sufficient to reduce the activity of both enzymes ACO and ACS to inhibit the abscission rate of the citrus leaf. Serek *et al*. (2006) reported that application of 2,5-norbornadiene (500 µL L^{-1}) strongly delayed the onset of ethylene production and extended shelf life of carnation flowers. 2,5-norbornadiene was one of the effective ethylene antagonists until *trans*-cyclooctene was discovered (Sisler *et al*., 1990). Sisler *et al.* (2006) reported that *trans*-cyclooctene was not as highly strained as 2,5-norbornadiene, but the effective concentration to block ethylene action of *trans*-cyclooctene was nearly 100 times lower than 2,5-norbornadiene.

However, there are limitations on the practical use of both 2,5-norbornadiene and *trans*-cyclooctene. High concentrations of 2,5-norbornadiene will produce large amounts of endogenous ethylene. Moreover, 2,5-norbornadiene is toxic and has a noxious odour (Sisler and Serek, 1999; Sisler *et al.*, 2006). Additionally, both compounds can diffuse from the receptor site within a short time and *trans*-cyclooctene will only be effective if applied continuously. The antagonistic effects of both these compounds are temporary hence no attempts have been made to use these commercially (Sisler *et al*., 1985; Sisler and Serek, 2003; Serek *et al*., 2006; Sisler *et al*., 2006).

2.5.2.3 Diazocyclopentadiene (DACP)



Diazocyclopentadiene

Diazocyclopentadiene (DACP) was synthesised as a potential photo-activated antagonist which binds irreversibly to the ethylene receptor to decrease the ethylene responses (Sisler and Blankenship, 1993b; Sisler *et al.*, 1993; Serek *et al*., 1994a). Serek *et al*. (2006) reported that DACP is a highly reactive gaseous compound which competes with ethylene for the receptor particularly under fluorescent light (Sisler

and Blankenship, 1993a, b; Sisler and Serek, 2003; Serek *et al*., 2007; Reid and Staby, 2008). Irradiated DACP compound 1µL L^{-1} for 24 h was more effective in reducing the endogenous production of ethylene in carnation cut flowers (Sisler *et al.*, 1993) extended shelf life in 'Victory Parade' potted miniature roses (Serek *et al*., 1994a) and delayed wilting, flower and bud abscission in *Lathyrus odoratus* L. (Sexton *et al.*, 1995).

DACP suppresses ethylene production and also delays banana fruit ripening (Sisler and Blankenship, 1993; Reid and Staby, 2008). Sexton *et al.* (1995) showed that spikes of sweet pea flowers treated with DACP (170 µL L^{-1}) for 18 h under fluorescent lights exhibited reduced abscission of buds and promoted the opening of buds. It was also reported that tomato fruit treated with DACP were insensitive to ethylene exposure for 10 days (Sisler and Blankenship, 1993b). Although DACP is a very efficient inhibitor of ethylene action, it is not used in the horticulture industry because it is unstable, explosive and phytotoxic when used at higher concentration (Serek *et al*., 1994a; Sisler and Serek, 1999, 2003; Serek *et al*., 2006; Sisler, 2006; Serek *et al.*, 2007).

2.5.2.4.-Cyclopropenes

1-Methylcyclopropene

1-MCP

3,3 -dimethylcyclopropene

3,3-DMCP

Cyclopropene

CP

Cyclopropene is an interesting small molecule for study because it contains a highly-strained alkene situated within a three-membered ring (Stigliani *et al*., 1975). Cyclopropene (CP), 1-methylcyclopropene (1-MCP), and 3,3-dimethylcyclopropene (3,3-DMCP) were the first group of cyclopropenes tested as effective ethylene antagonists in several horticultural crops (Sisler *et al*., 1996a; Sisler and Serek, 1997). CP and 1-MCP extend shelf-life and delay ripening in the postharvest phase by preventing the physiological action of ethylene in a number of horticultural crops

(Sisler *et al*., 1996a; Sisler and Serek, 1997; Sisler and Serek, 2003; Blankenship and Dole, 2003; Sisler *et al.*, 2006). Walsh (2005) proposed that CP acts as an ethylene antagonist due to a highly strained ring which tends to bind to electron donor compounds such as low valent metals in the receptor (Sisler and Serek, 1997; Sisler *et al*., 2006; Rubin *et al*., 2007). As an example, application of CP (1 nL L^{-1}) to carnation cut flowers for 24 h was sufficient to protect them from exogenous ethylene exposure (Sisler *et al*., 1996a). Sisler and Serek (2000) also reported that the application of CP or 1-MCP to banana fruit reduced the sensitivity to ethylene and recovered sensitivity to ethylene in 12 days of treatment. CP has no odour at an optimum concentration and protects a number of plants from the deleterious effects of ethylene (Sisler and Serek, 1997). However, it is explosive and polymerises when exposed to high temperatures which limit its use (Sisler *et al*., 1996a; Sisler and Serek, 1997; Sisler and Serek, 2003). These ethylene action inhibitors have been commercially applied to extend the postharvest life of cut flowers and potted plants. Blankenship and Dole (2003) suggested that the main role of the anti-ethylene compound is to prevent ethylene action by blocking the receptor sites. Sisler and Serek (1999) reported that there are differences between the agonists and antagonists in the period of time these compounds remain bound longer to the receptor than ethylene. Some ethylene inhibitors require continuous exposure to be effective such as 2,5-NBD while with others such as 1-MCP a single exposure is sufficient to block ethylene receptors. Some ethylene antagonists bind to the receptor and the time of diffusion from the receptor is 3-6 h some active components block ethylene responses for 7-12 days (Sisler and Serek 1999; Buanong *et al*., 2005) and others appear to be not strong binds to the ethylene receptor (Sisler and Serek, 1997).

2.5.2.4.1. 1-Methylcyclopropene (1-MCP)

A number of cyclopropenes that appear to interact with the ethylene for receptor and inhibit the ethylene action for extended periods have been discovered (Sisler and Serek, 2003; Ebrahimzadeh *et al*., 2008). 1-Alkylcyclopropenes (1-MCP, 1-OCP, 1-DCP and 3-MCP) were more effective cyclopropenes in blocking ethylene responses in a range of ornamental crops by inactivating the receptor at very low concentrations for long periods thus, protecting the flowers from exogenous ethylene whereby, improving shelf life and reducing flowers, leaves and buds abscission

(Sisler and Serek 1997; Sisler *et al*., 1999; Cameron and Reid, 2001; Kebenei *et al*., 2003a; Sisler *et al*., 2003; Buanong *et al*., 2005; Lurie, 2007).

1-MCP is a tool which has been added to the list of ethylene binding inhibitors for overcoming the harmful effects of ethylene in ornamentals (Reid *et al*., 2001). 1-MCP is a gas at standard temperature and pressure (Blankenship and Dole 2003). 1-MCP is a well-known commercially available treatment to suppress or inhibit the adverse effects of ethylene by delaying postharvest senescence and maintaining the quality of horticultural and floricultural commodities by controlling ethylene signal transduction and ethylene action (Serek *et al*., 1995a; Sisler and Serek, 1997; Macnish *et al*., 2000a, b; Cameron and Reid, 2001; Serek and Sisler, 2001; Blankenship and Dole 2003; Lurie, 2005). It has been reported that 1-MCP has an excellent safety profile and is environmentally friendly (Ebrahimzadeh *et al*., 2008). 1-MCP is stable at room temperature, easily applied, odourless and has been successfully used on flowers, some vegetables, fruits, and plotted plants (Serek, *et al*., 2006). The formulation of 1-MCP has been developed as a powder for commercial use by two American companies under the name EthylBloc® especially for its application in ornamental crops and later as a commercial formulation under the name SmartFresh™, used for various horticultural commodities (Blankenship and Dole, 2003; Serek, *et al*., 2006; Reid and Staby, 2008). To ensure the effectiveness of 1-MCP, the treatment is commonly performed in an enclosed area such as shipping containers, greenhouses, or in trucks at higher concentrations (800 ppb) for more than 24 h. Some flowers only require treatment for 15 minutes to see beneficial effects of 1-MCP (Reid *et al*., 2001).

1-MCP is very beneficial in extending the vase life of both fresh cut flowers and potted plants (Reid *et al*., 2001; Grichko, 2006). Plants treated with 1-MCP at very low concentrations (0.5 nL L^{-1}) prevent the deleterious effects of ethylene on a range of cut flowers and potted plants and other horticultural commodities including flowers (Serek *et al*., 1994b; Serek *et al*., 1995a; Sisler *et al*., 2003). Treatment with 1-MCP provided protection against the adverse effects of exogenous ethylene and prevented ethylene-induced flower and bud abscission in *Begonia elatior* and *Rosa hybrid*a L. and extended display life in *Begonia tuberhybrida, Kalanchoe blossfeldiana* and *Rosa hybrida* (Serek *et al.*, 1994b). Positive and negative effects

were observed when 1-MCP was applied to various potted and cut flowers such as *Plectranthus* (Ascought *et al.*, 2006), *Cattleya alliances* (Yamane *et al*., 2004), *Dianthus caryophyllus* (Asil *et al*., 2013), tulip (Chutichudet *et al*., 2010), *Pelargonium peltatum* 'Pink Blizzard' (Cameron and Reid, 2001), *Campanula carpatica* and *Kalanchoë blossfeldiana* (Sisler *et al*., 1999), *Antirrhinum majus* (Serek *et al*., 1995a), waxflower and *Grevillea* (Macnish *et al*., 2000a), 'Wendy' Geraldton waxflower (Serek *et al*., 1995b), *Gypsophila paniculata* (Newman *et al*., 1998), *Hibiscus rosa* (Reid *et al*., 2002), seven cultivars of *Kalanchoe* (Serek and Reid, 2000), *Lilium* (Celikel *et al.,* 2002), *Asiatic hybrid* and *Lilium longiflorum* (Elgar *et al*., 1999), Zonal Geranium (Jones *et al*., 2001), *Lathyrus odoratus, Dianthus caryophyllus* and *Delphinium hybrid* (Ichimura *et al*., 2002), 14 different native Australian cut flowers (Macnish *et al*., 2000b, 2004b), *Rosa hybrida* L. 'Kardinal' (Liao *et al*., 2013), *Freesia* flowers (Zencirkiran, 2010), *Dendrobium* Aroon White, *Mokara* Jairak Gold and *Vascostylis sakura* (Obsuwan and Uthairatanakij, 2007), mini *Phalaenopsis* (Sun *et al*., 2009), *Boronia heterophylla* (Macnish *et al*., 2000c), *Lantana camara (*Rapaka *et al*., 2007), Tree peony (Jia *et al*., 2009) 38 cultivars of Tea rose (Macnish *et al*., 2010) and *Lathyrus odoratus* L. (Kebenei *et al.,* 2003b). The application of 1-MCP strongly inhibited the negative effects of exogenous ethylene such as by inhibiting ethylene-induced flowers and buds abscission, improving and maintaining life span and quality in some cultivars, delaying flower senescence and petal wilting, extending the longevity by keeping the water balance and accelerating flower opening (Serek *et al.,* 1994b; Ichimura *et al.,* 2002; Picchioi *et al*., 2002; Kebenei *et al*., 2003b; Ascough *et al.,* 2006; Obsuwan and Uthairatanakij, 2007).

In contrast, Elgar *et al*. (1999) showed that the application of 1-MCP for 6h at different concentrations on *Asiatic hybrid, Oriental hybrid* and *Lilium longiflora* provided a small effect on the extension of vase life. Celikel *et al*. (2002) suggested that 1-MCP did not prevent normal abscission or senescence in *Lilium* cut flowers. The application of 1-MCP (10 nL L^{-1}) over 12 h lead to delayed senescence in 14 various native Australian cut flowers, however, no significant protection was seen against exogenously applied ethylene (Macnish *et al.,* 2000b). *Plectranthus* cultivars treated with 1-MCP (100 ppb or 500 ppb) did not reduce abscission (Ascough *et al*., 2006). A similar observation was reported when 1-MCP was applied to *Kalanchoe*

blossfeldiana 'Oriba', as there were no significant differences in the numbers of 'Oriba'open flowers and buds as compared to control (Park *et al*., 2011).

The 1-mcp application appears to be efficient and has low toxicity. 1-MCP is one of the most promising antagonists for commercial use however, it is not exploited to its full potential in the ornamentals industry due to the highest cost, differential response to horticultural commodities in respect to concentration, exposure duration, temperature during treatment and storage, variety and maturity stage of the produce and interaction among response the genotype (Obsuwan and Uthairatanakij, 2007; Seaton and Poulish, 2010; Khan, 2014) As 1-MCP is a gas, it can only be used as a fumigant in closed containers. It cannot be applied as a spray in a large field of flowers and has very low solubility in water (Grichko, 2006; Goren *et al*., 2008). However, it is used in sachets in flower cartons effectively (Seaton and Poulish, 2010). The development of newer anti-ethylene compounds which will be effective in reducing flowers/buds abscission and extending the vase life of flowers is warranted to overcome major postharvest problems in waxflower and other floricultural crops.

2.6. Vase life (VL)

The postharvest losses in cut flowers have been estimated at about 20 - 25% of total global production (Bharathi and Barman, 2015). The vase life or longevity of cut flowers is known as the time from placement of the flowering stems in vase solution to the time flowers lose their quality and aesthetic value rapidly such as colour and appearance (Halevy and Mayak, 1981; Fanourakis *et al*., 2013; Saeed *et al*., 2014; Scariot *et al*., 2014). Various phenotypic attributes such as the opening of flowers, the length of florets and stems, changes in fresh weight, the colour of petals and senescence pattern can be important factors to determine the vase life of cut flowers (De and Bhattacharjee, 2000). In the most cut and potted flowers, the vase life parameter is the major factor to estimate the commercial value of cut flowers (Nukui *et al*., 2004; Seighalani *et al*., 2013). In different cut flowers, the lifespan of flowers is affected by a number of factors playing important roles in accelerating or shortening flowers vase life includes genetic differences among different species of flowers such as *Verticordia* sp. Desf. (Seaton, 2006), water balance, the presence of

endogenous or exogenous ethylene, pathogens and microorganisms that can cause stem vascular occlusion and modulation in levels of carbohydrates (Halevy and Mayak, 1981; Darras *et al*., 2004; Schroeder and Stimart, 2005; Slootweg, 2005; Zencirkiran, 2010). Environmental conditions such as temperature, light, and nutrition can be involved in reducing flower vase life (Celikel and Kraracaly, 1995).

Native waxflower is endemic to Australia. It is grown commercially as a filler flower in south-west Western Australia and comes in many colours, unique flower forms and in different seasons (Seaton *et al*., 2007; Gollnow and Worral, 2010; Seaton and Poulish, 2010). Waxflower is sensitive to ethylene and hence usually has short vase life compared to other flowers (Olley *et al.*, 1996). Due to its extreme sensitivity to exogenous ethylene, which is one of the major postharvest problems, floral organ (flowers, leaves and buds) drop consequently shortening vase life, leading to loss of sales (Joyce, 1988; Seaton, 2006; Gollnow and Worral, 2010; Seaton and Poulish, 2010). Seaton (2006) reported that the vase life of *Verticordia* flowers shortened with increased floral drop. Ethylene is responsible for premature floral senescence and flower and bud abscission as well as reduced the longevity in several cut flowers (Joyce, 1993; van Doorn and Stead, 1997; Ichimura *et al*., 2002; Scariot *et al*., 2014). The typical vase life of waxflower genotypes can range from 7 days to 32 days depending upon the species of a genus and varieties (Seaton *et al*., 2007). Recently, the floriculture industry searched to find an economical method to prolong the life span in different cut flowers (Khana *et al*., 2015). Vase life of various cut flowers can be extended by delaying the onset of flower senescence using different chemicals in vase solution. Effective chemicals can extend flower vase life by improving water uptake and reducing the transpiration process (Lu *et al*., 2010). Earlier, van Meeteren (1992) reported that the flowering stems of 'Cassa' *Chrysanthemum morifolium* placed in cold water for 2 h improved the water balance in flowers. When the stems were pulse treated (0.5 mM for 24 h) with STS, it was effective in preventing postharvest abscission in waxflowers but had no effect on extending waxflower vase life (Joyce, 1988, 1993).

In addition, STS plus sucrose was more effective than STS alone in prolonging vase life of snapdragon (Ichimura *et al*., 2008). Treatment of sucrose with 8-hydroxyquinoline sulphate 8-HQS maintained flower vase life in sweet pea

more than that in the 8-HQS only treatment, (Elhindi, 2012) while amino oxyacetic acid (AOA) in combination with sucrose prolonged vase life in *Dendrobium* (Rattanawisalanon *et al*., 2003). The spray or dipping treatment of benzyl adenine (BA) reported extending vase life in a number of tropical flowers such as *Anthurium andraeanum, Heliconia psittacorum* and *Alpinia purpurata* (Paull and Chantrachit, 2001). Gibberellic acid treatment enhanced the vase life quality in gladiolus cut flowers (Saeed *et al.*, 2014). Also, when the flowering sprigs of *Polianthes tuberosa* L. treated with 10 or 20 mg L^{-1} of GA_3 and 200 mg L^{-1} 8-HQS for 24 h followed by placement of the flowers in vase solution containing sucrose, improved *Polianthes tuberosa* vase life and flowers bud opening (Su *et al*., 2001). Vase solutions containing chlorine dioxide (2 or 10 µL L^{-1}) extended the vase life of different cut flowers such as *Alstroemeria peruviana, Antirrhinum majus, Dianthus caryophyllus, Gerbera jamesonii, Gypsophila paniculata, Lilium asiaticum, Matthiola incana* and *Rosa hybrida* flowers by 0.9 -13.4 days (Macnish *et al.,* 2008). Pulsing treatment of calcium chloride ($CaCl_2$) is an unsuccessful method in prolonging waxflowers shelf life or reducing the abscission of flowers and buds (Taylor *et al*., 2003). Application of ($CaCl_2$) and salicylic acid (SA) was effective in improving the vase life of rose cut flowers (Abdolmaleki *et al*., 2015). Seighalani *et al*. (2013) reported acetaldehyde (2%) in vase solution was effective in extending vase life and improving the opening of *Eustoma grandiflora* flowers. In addition, several metallic salts such as cobalt chloride, silver nitrate, zinc sulphate, calcium nitrate and aluminium sulphate have been used widely in extending the shelf life of different cut flowers (De and Singh, 2016).

Sugar in the vase solution generally acts as a substrate source for increasing the respiration process, keeps the water balance in several cut flowers and delays the petal and flower senescence (Halevy and Mayak, 1979; Pun and Ichimura, 2003). In *Ranunculus asiaticus* L., the sugar concentration increased during the flower opening period in the petal tissues more than during the senescence period (Shahri and Tahir, 2011).

The application of exogenous sugars played a significant role to delay the wilting process and suppress flower abscission and promote bud opening in *Dendrobium* 'Jew Yuay Tew' flowers (Rattanawisalanon *et al*., 2003). The treatment

of cultivars 'Delilah' *Rosa hybrida* L. with sucrose and (8-HQS) markedly extended vase life (Kazuo *et al*., 2005). Vase solutions containing sugars and/or the antimicrobial 8-Hydroxyquinoline sulphate (8-HQS) have become significantly effective treatments in extending the shelf life in a number of cut flowers such as *Antirrhinum majus,* various genotypes of waxflowers and rose cut flowers (Asrar, 2012; Dung, 2013; Elgimabi and Sliai, 2013). Asrar (2012) found that the application of 200 mg L^{-1} 8-HQS combined with 2% sucrose solution delayed snapdragon flower senescence, improved the quality and extended the flowers vase life. Dung (2013) investigated the effect of a number of sugars all at concentrations of 58.48 mM including maltose, glucose, fructose or galactose and sucrose supplemented with 200 mg L^{-1} 8-HQS on vase life of different genotypes of waxflowers and found that sucrose, fructose and glucose were more effective in extending waxflower vase life as compared to maltose and galactose. The vase life of cut spikes of *Cymbidium* orchid hybrid 'Pine Clash Moon Venus' was extended to 56 and 54.8 days when the spikes were pulsed in 5% and 8% sucrose compared to other treatments (Bharathi and Barman, 2015). Sugars at 4% and 100 mg L^{-1} of aluminium sulfate $Al_2 (SO_4)_3$ extended the vase life of *Dendrobium* 'Thongchai Gold' to 36 days (De and Singh, 2016). Khandaker *et al*. (2017) showed that 6% sucrose in vase solution was the most effective treatment to extend the vase life and keep flower quality of orchid cut flowers.

1-MCP is the most effective blocker of ethylene action in many cut flowers and is also beneficial in extending lifetime of both fresh cut flower and potted flowers (Celikel *et al*., 2002; Hassan and Gerzson, 2002; Ichimura *et al*.,2002; Kebenei *et al*., 2003b; Grichko, 2006; Zencirkiran, 2010; Asil *et al*., 2013). However, 1-MCP is limited in its application to enclosed areas to be effective and cannot be applied in fields or open areas. Some waxflower genotypes become more sensitive to ethylene after 2 to 4 days of treatment with 1-MCP and not enough soluble in water (Macnish *et al*., 2000b*;* Goren *et al*., 2008; Seaton and Poulish, 2010; Seglie *et al*., 2010). As reported previously in Section 2.5.2.5.**,** development of new compounds which will be more effective in inhibiting the ethylene action at the receptor level in flowers is warranted to extend the vase life of flowers and leaves.

CHAPTER 3

General materials and methods

Various experiments were conducted to determine the effectiveness of potential new ethylene antagonists in reducing flowers/buds abscission during 2014 and 2015. Efficacy of selected ethylene antagonists was also tested in extending the vase life of flowers/leaves of different genotypes of waxflowers in 2015. All the experiments were conducted at the Horticultural Research Laboratory located at Technology Park, Department of Environment and Agriculture, Curtin University, Bentley, Perth and the Department of Agriculture and Food Western Australia (DAFWA), South Perth, during June to October in 2014 and 2015.

Figure. 3.1. Waxflower bushes of different genotypes at the Department of Agriculture and Food Western Australia (DAFWA), South Perth, Western Australia used in various experiments.

3.1. Plant material

A range of waxflower genotypes differing in their sensitivity to ethylene was used in various experiments Table. 3.1 sourced from the Department of Agriculture and Food Western Australia (DAFWA), South Perth, Western Australia. Waxflower bushes were cultivated using irrigation and fertigation Fig. 3.1 best practice previously detailed by (Seaton and Poulish, 2010).

Table 3.1.Various genotypes of waxflower differing in their sensitivity to ethylene used in the experiments (Seaton and Poulish, 2010).

Genotypes	Sensitivity to ethylene
'WX107'	Medium
'WX17'	Highly sensitive
'WX73'	Medium
'WX56'	Medium
'WX58'	Medium
'WXFU'	Medium
'WX116'	Highly sensitive
'WX14'	Medium
'WX74'	Medium
'WX110'	Medium
'Purple Pride'	Highly sensitive
'Crystal Pearl'	Medium
'Lady Stephanie'	Medium
'White Spring'	Highly sensitive
'Southern Stars'	Low sensitive
'Revelation'	Medium
'Hybrid 1'	-
'Jenny'	Highly sensitive
'Muchae Mauve'	Highly sensitive

- No information available

3.2. Harvesting flowering stems

The leafy flowering sprigs (leaves, open flowers and flower buds) were picked using secateurs in the early morning exhibiting 57.5 to 98.7% open flowers (in different genotypes) from mature bushes (five - six years old). The harvested stems were 60 to 70 cm long and placed with their cut ends in pots of clean water following their harvest Fig. 3.2. The flowering stems were transported within 30 - 40 minutes after harvest to the Horticulture Research Laboratory at Technology Park,

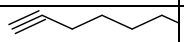
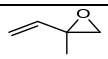
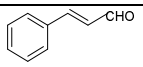
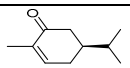
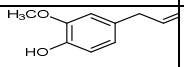
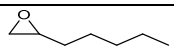
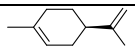
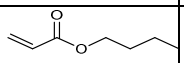
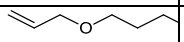
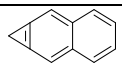
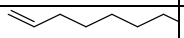
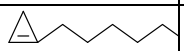
Department of Environment and Agriculture Curtin University, Bentley. At the laboratory, the stem ends were recut under water to remove air embolisms and 30% of the leaves were removed from the lower portion of each stem in each treatment. The flowering stems were selected randomly (Seaton, 2006).

Figure. 3.2. Flowering stems harvested from various genotypes used in different experiments: (A) 'WXFU' .(B) 'White Spring' .(C) 'Purple Pride' .(D) 'WX58' .(E) 'Revelation' .(F) 'WX56' .(G) 'WX74' and Pink 'WX14'.(H) 'Crystal Pearl' .(I) 'Muchae Mauve' waxflowers.

3.3. Sources of chemicals

Ethylene antagonists were synthesised by Dr Payne's group at the Department of Chemistry, Curtin University or purchased from different companies. A range of new compounds was synthesised that are structurally different compared to 1-methycyclopropene (1-MCP) and are either stable liquids, solids or gases to facilitate their potential application as a spray, dipping, coating and a fumigant. Various potential ethylene antagonists differing in chemical formula, structure and molecular weight were tested in different experiments are listed in Table. 3.2.

Table. 3.2. Chemical name, formula, structure, molecular weight (MW) and sources of potential antagonists tested in various experiments.

No	Chemical name	formula	Structure	MW (g)	Sources
1	1-Octyne	C_8H_{14}		110.2	Alfa Aesar, Lancashire, UK
2	Isoprene oxide	C_5H_8O		84.1	Department of Chemistry *
3	*trans*-Cinnamaldehyde (CA)	C_9H_8O		132.2	Acros Organics, New Jersey, US
4	(+)-Carvone	$C_{10}H_{16}O$		152.2	Sigma-Aldrich, Castle Hill, NSW, Australia
5	Eugenol	$C_{10}H_{12}O_2$		164.2	Sigma-Aldrich, Castle Hill, NSW, Australia
6	1-Octene oxide	$C_8H_{16}O$		128.2	Department of Chemistry*
7	(*S*)-(-)-Limonene	$C_{10}H_{16}$		136.2	Sigma Aldrich, Castle Hill, NSW, Australia
8	Butyl acrylate	$C_7H_{12}O_2$		128.2	Alfa Aesar, Lancashire, UK
9	Allyl butyl ether	$C_7H_{14}O$		114.2	Department of Chemistry *
10	1*H*-cyclopropa[*b*]naphthalene (NC)	$C_{11}H_8$		140.2	Department of Chemistry *
11	1-Octene	C_8H_{16}		112.2	Merck, Victoria, Australia
12	1*H*-cyclopropabenzene (BC)	C_7H_6		90.1	Department of Chemistry *
13	1-Hexylcyclopropene (1-HCP)	C_9H_{16}		124	Department of Chemistry *

* Ethylene antagonists were synthesised by Dr Payne's group at the Department of Chemistry, Curtin University.

3.3.1. Preparation of solution of different compounds.

Stock solutions of each chemical were freshly prepared prior to their use in experiments. Each compound was dissolved in sufficient ethanol to prepare a 100 µM of stock solution. The stock solution was diluted in ethanol to prepare 1 µM of concentration which was then applied to flower sprigs in plastic drums (60 L volume) as a fumigant as mentioned in Section 3.4.

3.4. Application of ethylene antagonists to cut waxflowers at 1 µM and different three concentrations (0.5, 1.0 and 2.0 µM).

All the experiments were conducted having four treatments per potential ethylene antagonist such as (i) control as untreated flowers and exposed to air (ii) control followed by exposure to (10 µL L^{-1}) ethylene 24 h prior to their exposure to the air (iii) antagonist fumigation treatment (1 µM) for 18 h then exposed to air only and (iv) antagonist fumigation treatment (1 µM) for 18 h followed by 24 h exposure to 10 µL L^{-1} of ethylene then exposed to the air. In total, 13 different ethylene inhibitors were tested. 3.Also the efficiency of different three (0.5, 1.0 and 2.0 µM) concentrations of promising ethylene antagonists i.e. *trans*-cinnamaldehyde, 1*H*-cyclopropa[b]naphthalene, 1*H*-cyclopropabenzene and 1-hexylcyclopropene in inhibiting ethylene action consequently reducing flowers/buds abscission in different genotypes of waxflowers have been tested. In all the experiments, the flower bunches were kept in small 100ml translucent plastic vases containing distilled water. Flowering sprigs of different genotypes were fumigated with different ethylene antagonists each ethylene antagonist solution was poured on a filter paper in a petri dish in 60 L plastic drums. A small plastic fan was left on inside the container to circulate the vapour of the chemical around the flower stems. The treatments were applied for 18 h at 20 ± 1ºC and 65 ± 5% RH. Also, 30 g of soda lime was placed in petri dishes in the drums during treatment of flowering stems to absorb any CO_2 that might have been produced by respiration process from the flowering stems (Seaton, 2006) and Fig. 3.3.

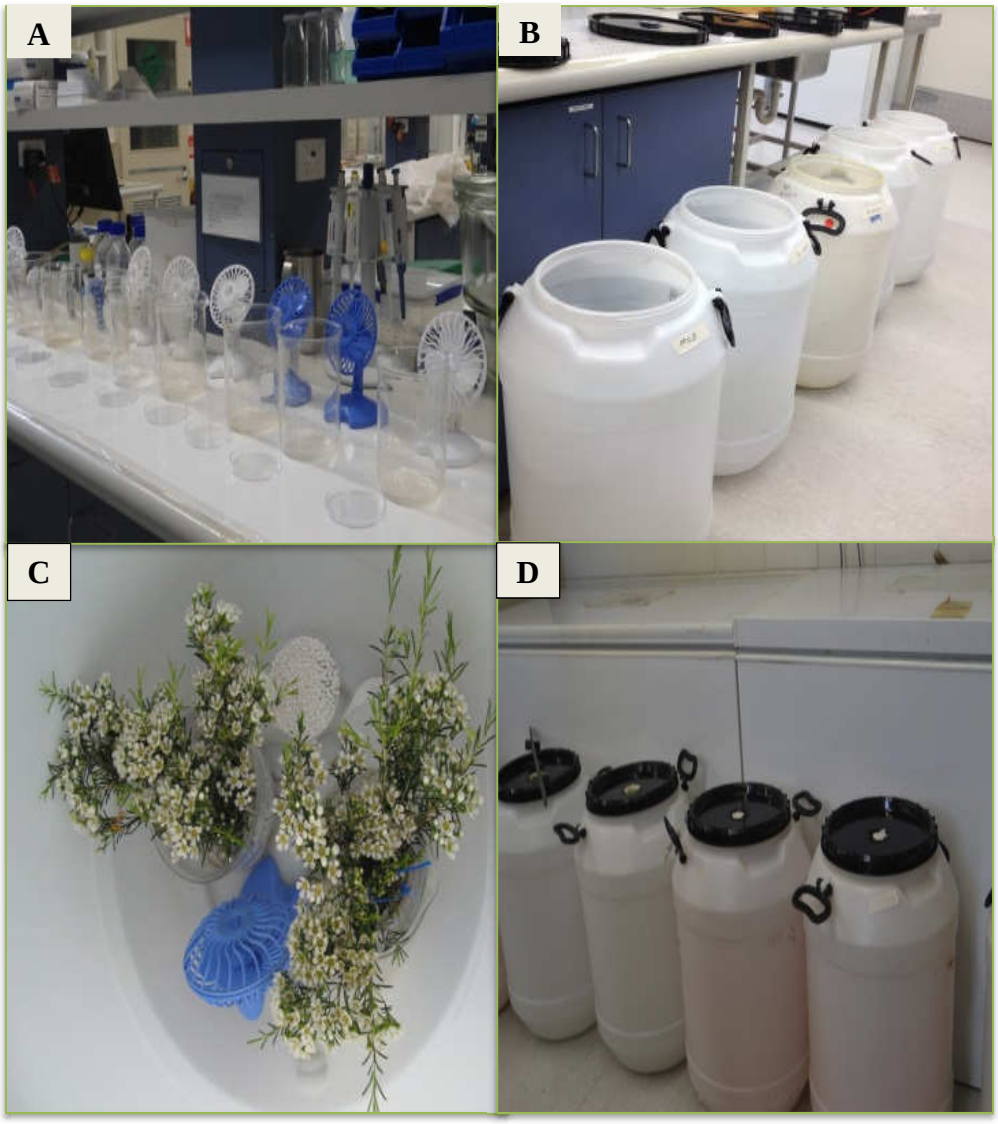


Figure. 3.3. Application of ethylene antagonist treatment as 18 h fumigation to waxflowers in a plastic drum. (A) Small plastic fan used inside the container to circulate the vapour of the chemical around the flower stems. (B) 60 L plastic drum used for 18 h fumigation treatment. (C) Plastic drum with flower stems, soda lime and battery operated the plastic fan. (D) Fumigation treatment of ethylene antagonist being applied to flowers stems in sealed plastic drums containing soda lime and battery operated the plastic fan.

3.5. Ethylene treatment

To test the protection provided by the ethylene antagonists flowers were subjected to exogenously applied ethylene. Following the completion of 18 h ethylene antagonist fumigation treatments the flowering bunches were taken out of

the drums and enclosed in plexiglass chambers (60 L volume) and then subjected to 10 µL L^{-1} of ethylene for 24 h according to (Seaton, 2006) Fig. 3.4.

Figure. 3.4. Application of ethylene treatment (10 µL L^{-1}) by injecting pure ethylene into glass chambers containing waxflower stems for 24 h. Flowering stems were placed in mesh cones inside the glass chambers prior to injecting the pure ethylene using a 1ml syringe inserted through a rubber port located at the top of each chamber (Seaton, 2006).

3.6. Flowers/buds abscission

The flowers/buds abscission was recorded following ethylene treatment. The sprigs were softly beaten against the bench for each replicate. The abscised flowers/buds were counted and the number of flowers/buds remaining on the stems also counted. The percentage of flowers/buds abscised was calculated daily for four consecutive days. In each experiment the efficacy of different concentrations of ethylene antagonists against flowers/buds abscission was recorded on one, two, three and four days after treatments and the percentage of flowers/buds abscission was calculated as:

$$(\%)\text{of flowers/buds abscission} = \frac{\text{the number of flowers/buds abscised}}{\text{Total (flowers/buds abscised} + \text{ remaining)}} \times 100$$

$$\text{Relative abscission} = \frac{\text{abscission (chemical} + C_2H_4)\% - \text{abscission } (C_2H_4)\%}{\text{abscission } (C_2H_4)\%} \times 100$$

3.7. Effects of ethylene antagonists and different types of vase solutions on vase life of different genotypes of waxflowers.

3.7.1. Plant material

Flowering bunches of ‘Crystal Pearl’, ‘Lady Stephanie’, ‘Purple Pride’ and ‘WX74’ were harvested from mature bushes (five years old) grown at the Department of Agriculture and Food Western Australia (DAFWA), South Perth, during 2016 Fig. 3.5. Immediately following harvest, the stems were kept upright in buckets filled with tap water and transported within half an hour to the Horticultural Research Laboratory located at Technology Park, Department of Environment and Agriculture, Curtin University, Bentley. For vase life treatments, the flower stems were also recut with secateurs in water to 30 cm in length (from the cut ends to the most extreme opened-flowers) and prior to placing the stems in vases the lower leaves were removed (Seaton, 2006)

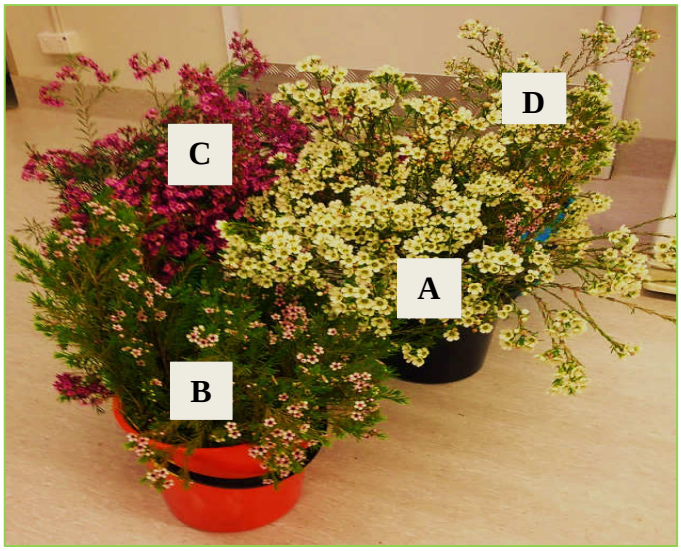


Figure. 3.5. Flower stems of (A) ‘Crystal Pearl’. (B) ‘Lady Stephanie’. (C) ‘Purple Pride’ and. (D) ‘WX74’ waxflowers harvested were kept upright in buckets filled with tap water and used in the experiments.

3.7.2. Application of ethylene antagonist fumigation treatments.

This experiment was carried out to investigate the effectiveness of four different ethylene antagonists at a concentration (1 µM) using four different

genotypes of waxflowers 'Crystal Pearl', 'Lady Stephanie', 'Purple Pride' and 'WX74'. The treatments included fumigation of randomly selected flower stems with 1*H*-cyclopropa[*b*]naphthalene alone, 1*H*-cyclopropabenzene alone, *trans*-cinnamaldehyde or 1-hexylcyclopropene alone. Untreated flower stems were treated as control. Flower stems of different genotypes were fumigated with (1 µM) for 18 h by using 60 L plastic drums at 20 ± 1ºC and 65 ± 5% RH as explained previously in section 3.4. Following the completion of ethylene antagonist treatments, the flowers stems were transferred within 35 minutes to (DAFWA) at South Perth. For vase life treatments, flower stems were recut with secateurs in water to 30 cm in length and placed in 250 ml individual plastic vases containing distilled water and kept in a vase room at DAFWA for observation of the lifetime of flowers and leaves (days). All vase life assessment experiments were held in an air-conditioned room at 20 ± 2ºC, 60 ± 10% RH with a 12 h photoperiod Fig. 3.6. The light flux densities were 8 µm m^{-2} s^{-2}. The experiment was laid out as a completely randomised two factors (treatment x genotypes) factorial design and each treatment was replicated six times consisting of a single stem in individual vases.

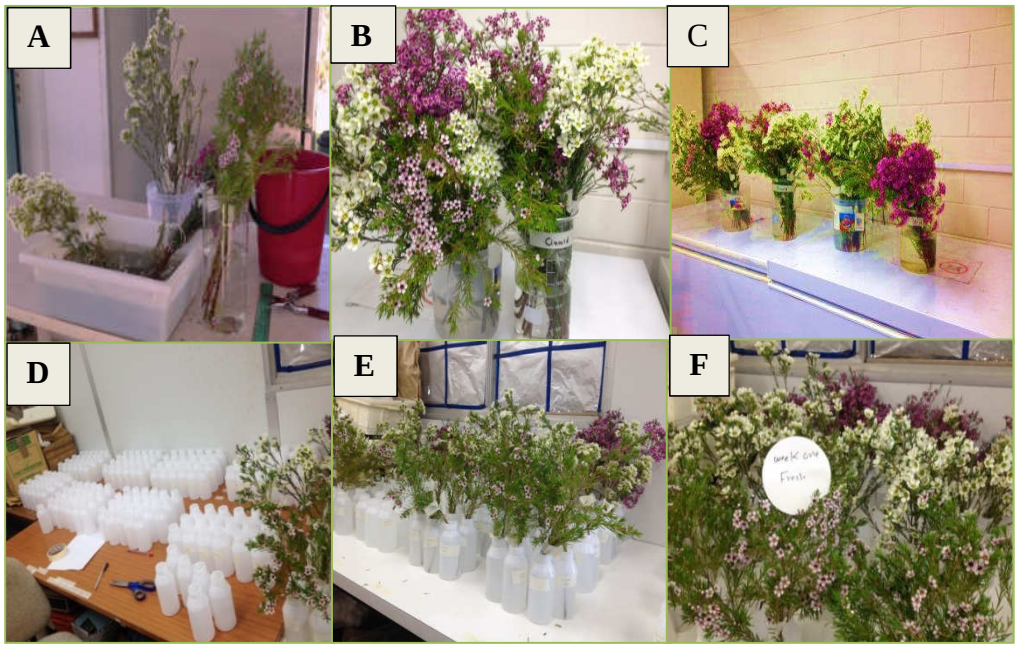


Figure. 3.6. Preparation of the flowers stems for placing in vase life room. (A) Flower stems were recut with secateurs in water to 30 cm in length. (B and C) four genotypes after fumigation with different ethylene antagonists. (D) 250 ml plastic vases containing distilled water. (E) Flowering stems placed in vases. (F) The flowering stems after one week of chemical treatment still fresh.

3.7.3. Assessment of vase life of flowers and leaves

Vase life (VL) of flowers and leaves was noted using a rating scale 1-5 as detailed Table. 3.3 and Fig. 3.7 and also as previously detailed by (Dung, 2013). Vase life of flowers was determined when more than 50% of opened flowers were more than 50% closed or the petals showed damage (Seaton and Joyce 1992 and 1993). For genotypes, where flowers dropped before closing, the end of vase life was assessed when more than 50% of opened flowers had dropped. Vase life of leaves was recorded when more than 50% of the leaves were fully desiccated or yellow for their full length following rating score (1 to 5) (Seaton and Joyce, 1992 and 1993).

Table. 3.3. Rating scale of (1-5) to assess the flowers and leaves vase life.

Score	Description
1	Fresh no closure or wilting
2	10-25% of flowers at least half closed or leaves with half of their length desiccated
3	25-50% flowers closed or leaves desiccated
4	50-75% flowers closed or leaves desiccated
5	75-100% of flowers closed or leaves desiccated

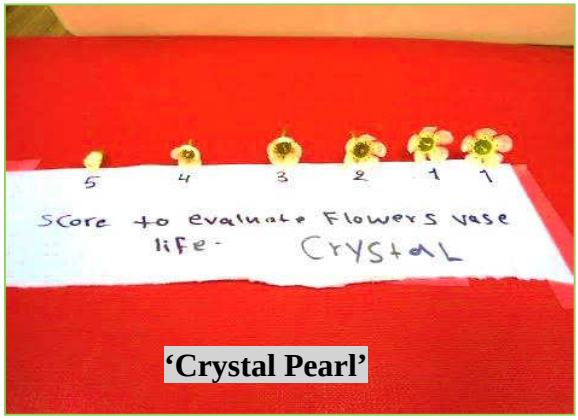


Figure. 3.7. Score for rating vase life of flowers.

3.8. Effect of fumigation of 1*H*-cyclopropa[*b*]naphthalene (NC), *trans*-cinnamaldehyde (CA) and 1-hexylcyclopropene (1-HCP) in combination with vase solution containing sucrose, fructose supplemented with 8-hydroxyquinoline sulphate on the extension of vase life of flowers in 'WX74' and 'WX14' waxflowers.

3.8.1. Plant material and harvesting flowering stems

Flower stems of 'WX74' and 'WX14' waxflowers were harvested from the field grown five-year-old bushes at DAFWA, South Perth. Flower stems exhibiting 75 - 96% open flowers were harvested in the early morning, and the cuts end of flowering stems were immediately held into buckets of tap water in the field.

3.8.2. Vase life solutions.

Vase solutions containing distilled water only, 58.5 µM concentrations of sucrose, or fructose or 100 mg L^{-1} 8-HQS were used.

3.8.3. Application of treatments of ethylene inhibitors on vase life of waxflowers.

The flowering stems of 'WX74' and 'WX14' genotypes were fumigated with three different inhibitors at 1 µM 1*H*-cyclopropa[*b*]naphthalene, *trans*-cinnamaldehyde and 1-hexylcyclopropene for 18 h by using 60 L plastic drums and the same procedure as detailed in section 3.4. Following the ethylene antagonists' treatments, the stems were kept in different types of vase solutions. The experiment was laid out following two-factor (treatment x genotype) factorial completely randomised design, and each treatment was replicated five times with one stem included in each replication. Flower and leaf vase life assessment were conducted in an air-conditioned room at 20 ± 2 °C, 60 ± 10% RH with a 12 h photoperiod. The light flux densities were 8 µmol m^{-2} s^{-2}.

3.8.4. Assessment of vase life and leaves

During this experiment, the vase life of flowers/leaves of the two treated genotypes was recorded as described previously in section 3.7.3.

3.8.5. Experimental design and statistical analysis of data

The data from various experiments was subjected to one-way or two-way analysis of variance (ANOVA) using the statistical package GenStat 14th edition (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). Least significant differences were calculated by following a significant F test ($P < 0.05$). Treatment means were compared by LSD at ($P < 0.05$) and means (± SE) were shown as appropriate. Differences among treatments were further analysed using Duncan's Multiple Range Test. To ensure the validity of the statistical analysis, all the assumptions of analysis of variance (ANOVA) were checked.

CHAPTER 4

Screening of potential antagonists of ethylene action through reducing abscission of flowers/buds in waxflowers.

Abstract

Ethylene is a natural plant hormone that promotes flower senescence and shortens the display life in many cut and potted flowers. 1-MCP is commercially used to extend the postharvest life of flowers but it is highly unstable is applied only as a fumigant and response is concentration and genotype specific. The aim of a series of experiments was to evaluate the efficacy of a range of potential ethylene antagonists with different functional groups in their structure such as 1-octyne, isoprene oxide, *trans*-cinnamaldehyde (CA), eugenol, (+)-carvone, (*S*)-(-)-limonene, 1-octene, allyl butyl ether, butyl acrylate, 1-octene oxide, 1*H*-cyclopropa[*b*]naphthalene (NC), 1*H*-cyclopropabenzene (BC) and 1-hexylcyclopropene (1-HCP) in inhibiting ethylene action in various genotypes of waxflowers investigated over 13 independent experiments in 2014. Varieties tested represented a range of waxflower types with differing ethylene sensitivity including 'White Spring', 'WXFU', 'WX116', 'Muchae Mauve', 'Southern Stars', 'WX73', 'WX110', 'WX107', 'Jenny' and 'WX17'. In different treatments, the flowers stem of waxflowers was treated with ethylene (10 µL L^{-1}) alone for 24 h, fumigated with one chemical (1 µM) alone for 18 h and flowering stems fumigated with this chemical followed by exposure to 10 µL L^{-1} ethylene. Untreated stems served as control. Percentage flower/bud abscission was recorded on completion of 24 h ethylene exposure in each treatment. Amongst tested compounds, fumigation of 1-octyne, isoprene oxide, (+)-carvone, eugenol, allyl butyl ether or 1-octene followed by ethylene exposure for 24 h did not show any reduction in flower/bud abscission compared to those exposed to the ethylene alone. Meanwhile, fumigation with 1-octene oxide, butyl acrylate, *trans*-cinnamaldehyde, (*S*)-(-)-limonene, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene or 1-hexylcyclopropene followed by ethylene exposure showed a reduction in the percentage of flower/bud abscission in different genotypes tested (46.4% 'Southern Stars', 56.8% 'Southern Stars', 47.2% 'WXFU', 66.4% 'WXFU', 81.1% 'WX107', 86.0% 'WX73' 1*H*-

cyclopropabenzene and 98.6% 'WX73' 1-hexylcyclopropene respectively) compared to those exposed to the ethylene alone.

These results suggested that 1-octene oxide, butyl acrylate, *trans*-cinnamaldehyde, (*S*)-(-)-limonene, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene or 1-hexylcyclopropene seem to be effective ethylene receptor blockers as evidenced by a reduction in flower/bud abscission of ethylene treated stems in different genotypes of waxflower. The efficacy of these compounds as ethylene antagonists was tested in detail on various genotypes of waxflowers in subsequent experiments.

4.1. Introduction

Ethylene is an ageing hormone in plants. It is a very simple hydrocarbon with chemical structure (C_2H_4) comprised of two carbon atoms linked with only one double bond and is a colourless gas (Abeles *et al*., 1992; Saltveit, 1999; Gibson *et al*., 2000; Blankenship, 2001; Martinez-Romero *et al*., 2007; Scariot *et al*., 2014). Ethylene is biologically very active at very low concentrations ranging from parts-per-million (ppm, $\mu L\ L^{-1}$) to parts-per-billion (ppb, $nL\ L^{-1}$) and effects on many process plant growth, development process and storage life of different fruits, vegetables and ornamental crops (Abeles *et al*., 1992; Saltveit, 1999; Blankenship, 2001; Muller and Stummann, 2003; Martinez-Romero *et al*., 2007; Faragher *et al*., 2010). Presence or absence of ethylene induced by various biotic and abiotic stresses has profound positive or negative effects during plant growth and development from seed germination to tissue senescence (Bleecker and Kende, 2000; Gibson *et al*., 2000; Blankenship, 2001; Binder *et al*., 2007). Ethylene production modulates various physiological processes in plant tissues including acceleration of abscission of leaves and flowers (Joyce, 1988; Blankenship, 2001; Cameron and Reid, 2001). This plant growth regulator plays a basic role in postharvest life of cut flowers by reducing their visual appearance and hastening shedding of leaves and flowers in several ornamental plants (Joyce and Poole, 1993; van Doorn and Stead, 1997; Celikel *et al*., 2002; Reid, 2002; Han and Miller., 2003). Ethylene causes 30 % postharvest losses in different floricultural crops (Faragher *et al.*, 2010). Green (1987) reported that generally, flowers are more sensitive when exposed to trace amounts of ethylene than are foliage plants. Ethylene is known to cause premature

abscission of flowers, other plant organs and accelerated senescence of cut flowers (Halevy and Mayak 1981; Green, 1987; Woltering and van Doorn 1988; Joyce and Pool, 1993; Elgar *et al*., 1999; Gibson *et al*., 2000; Cameron and Reid, 2001; Faragher *et al*., 2010; Scariot *et al*., 2014).

Beside the ethylene exposure effect, there are various factors such as environmental stress including light, temperature, water stress, humidity and disease accelerate the abscission process consequently leading to flower drop (Beasley and Joyce, 2002; Ascough *et al*., 2006; Sawicki *et al*., 2015). There are substantial differences in sensitivity to ethylene exposure among the cultivars and species of plants (Serek *et al*., 2006). The sensitivity to ethylene correlated with upregulation of genes expression involved in ethylene production and was accompanied by increased levels of ethylene biosynthesis that hastened cellular breakdown (Bartoli *et al*., 1996; Shahri and Tahir, 2011). Ethylene causes abscission of flowers in Australian native flowers such as, cut waxflower (*Chamelaucium uncinatum*) (Joyce,1993; Macnish *et al*., 2000a), *Backhousia myrtifolia*, *Baeckea virgata*, some *Leptospermum*, *Telopea speciosissima*, *Thryptomene calycina*, *Verticordia nitens*, *Verticordia cooloomia*, *Verticordia grandis* and *Verticordia serrata* and some *Grevillea* species (Faragher *et al*., 2010). Different species of *Verticordia* exhibit different degrees of ethylene sensitivity (Seaton, 2006). For instance, in the ethylene-sensitive flowers such as ornamentals Gibson *et al*. (2000) reported that ethylene caused a number of damaging effects on abscission of flowers and flower buds in, Achimene, Calecolaria, Crossandra, Cyclamen, Exacum, *Freesia*, Fuchsia, Hyacinth, Impatien and Saliva flowers, Begonia, *Kalanchoe blossfeldiana* Poelln. (Serek and Reid, 2000), *Pelargonium peltatum* (Cameron and Reid, 2001) and *Lilium* (Celikel *et al*., 2002). Also, ethylene can cause premature flower ageing in Aconitum, Alyssum, Campanula, Peperomia cut flowers and leaf drop in Azalea (Gibson *et al*., 2000). Exposure of Geraldton waxflower to ethylene causes many undesirable effects during transport and postharvest handling (Joyce 1993; Olley *et al*., 1996; Beasley and Joyce, 2002; Seaton and Poulish, 2010).

The floriculture industry seeks to supply attractive and long-lived flowers to consumers by using effective methods involving reduction of ethylene biosynthesis and/or by preventing ethylene action in ethylene sensitive cut flowers (Philosoph-

Hadas *et al*., 2003; Serek *et al*., 2006; Serek *et al*., 2007; Scariot *et al*., 2014). Serek *et al*. (2007) argued that the inhibition of ethylene production by different ethylene antagonists in various cut flowers was not much successful in controlling the response of flowers to exogenous ethylene exposure in extending shelf life. However, application of antagonists of ethylene action is a more effective method for extending the postharvest life of flowers (Scariot *et al*., 2014). 1-Methylcyclopropene (1-MCP) is a very effective antagonist of ethylene action and is used commercially in a large range of ornamentals crops (Sisler and Serek, 1997; Blankenship and Dole, 2003; Saleh-Lakha *et al*., 2004; Serek *et al*., 2006, 2007). Silver thiosulfate (STS) also acts as an antagonist to the adverse effects of ethylene action in a range of flowers crops but use is limited because of the adverse environmental effects (Veen, 1983; Serek and Reid, 1993; Mayers *et al*., 1997; Serek *et al*., 2007; Ebrahimzadeh *et al*., 2008; Seaton and Poulish, 2010).

1-MCP is a very powerful tool to extend the storage life and keeping the quality of both fresh cut flowers and potted plants by competing with ethylene for the ethylene receptor (Blankenship, 2001; Grichko, 2006). However, there are restrictions to using of 1-MCP it is high cost. In addition, 1-MCP is a highly unstable gas, can be used only as a fumigant and it requires enclosed rooms for preventing gas leakage as a powder in sachets in the middle of flower cartons that are activated by moist conditions (Grichko, 2006; Sisler, 2006; Goren *et al*., 2008; Reid and Celikel, 2008; Seaton and Poulish, 2010; Paul *et al*., 2010). In recent years, global awareness towards the reduction of postharvest losses in crops from farm to markets has widely increased. Thus, developing new user and environmentally friendly, non-phytotoxic active inhibitors of ethylene action to reduce postharvest losses has gained interest with several researchers. There is a need to develop compounds which are more stable than current antagonists and can be applied by fumigation, spray or dip treatments also, longer protection of plant material against ethylene exposure (Feng *et al*., 2004; Grichko, 2006; Sisler, 2006; Goren *et al*., 2008).

The various limitations of 1-MCP offer an attractive opportunity to develop other ethylene antagonists requiring testing of their efficacy in reducing the adverse effects of ethylene action. Preliminary, research work has been reported on ethylene antagonistic effects of 1*H*-cyclopropabenzene and 1-hexylcyclopropene on

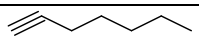
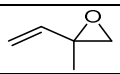
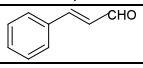
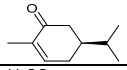
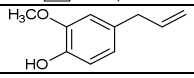
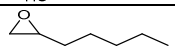
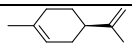
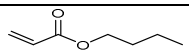
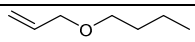
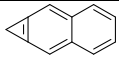
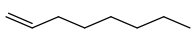
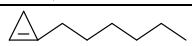
Kalanchoe blossfeldiana Poelln (Kebenei *et al.*, 2003a). Jing *et al*. (2011) found that cinnamaldehyde (0.1 - 0.3%) in vase solution extended the vase life of 'Pink Queen' rose cut flowers by 2.8 days. Additionally, Damunupola *et al*. (2010) reported that S-carvone enhanced lifetime parameters for the non-proteaceous species. In addition, played a role in antibacterial and antifungal activity in *Acacia holosericea* (Mimosaceae), *Baeckea frutescens* (Myrtaceae), *Chamelaucium uncinatum* cv. 'Mullering Brook' (Myrtaceae) and *Chrysanthemum* sp. cv. 'Dark Splendid Reagan' (Asteraceae). Currently, there is no published information on the effects of *trans*-cinnamaldehyde, eugenol, (+)-carvone, 1-octyne, (*S*)-(-)-limonene, 1-octene, allyl butyl ether, butyl acrylate compounds and new synthesis compounds such as, isoprene oxide, 1-octene oxide, allyl butyl ether, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene and 1-hexylcyclopropene (synthesised with different chemical structures) as ethylene antagonists to reduce abscission of flowers/buds in Geraldton waxflowers. It was hypothesised that some of these novel compounds will be more effective as ethylene antagonists. Therefore, the efficacy of various new potential ethylene antagonists (thirteen) to regulate abscission of flowers/buds in waxflowers was tested.

4.2. Materials and Methods

4.2.1. Sources of chemicals

Isoprene oxide (IO), 1-octene oxide, allyl butyl ether, 1*H*-cyclopropa[*b*]naphthalene (NC), 1*H*-cyclopropabenzene (BC) and 1-hexylcyclopropene (1-HCP) have been synthesised and provided by Muftah Musa and Dr, Alan Payne, Department of Chemistry, Curtin University and others chemicals were purchased from different companies such as, Alfa Aesar, Lancashire, United Kingdom. Acros Organics, New Jersey, US. Sigma-Aldrich, Castle Hill, NSW, Australia and Merck, Victoria, Australia as detailed in Chapter 3, Table. 3.2. Chemical name and structure of various chemicals tested are listed in Table. 4.1 below. The pressurised cylinder of pure ethylene (98%) gas was procured from BOC Gases, Australia Ltd., Perth, Australia.

Table. 4.1. Chemical name and structure of potential antagonists of ethylene action.

No	Chemical name	Chemical structure
1	1-Octyne	
2	Isoprene oxide	
3	*Trans*-Cinnamaldehyde	
4	(+)-Carvone	
5	Eugenol	
6	1-Octane oxide	
7	(*S*)-(-)-Limonene	
8	Butyl acrylate	
9	Allyl butyl ether	
10	1*H*-cyclopropa[*b*]naphthalene	
11	1-Octene	
12	1*H*-cyclopropabenzene	
13	1-Hexylcyclopropene	

4.2.2. Plant material

Thirteen independent experiments were conducted to investigate the efficacy of 13 different chemicals listed in Table. 4.1 as fumigants to protect against the adverse effects of ethylene and reducing abscission of flowers/buds in different genotypes of Australian native waxflowers. The experiments were carried out on ten different genotypes of Australian 'Geraldton waxflower varying in flower colour, size, flowering time and sensitivity to ethylene such as 'Southern Stars', 'Muchae Mauve', 'White Spring', 'WXFU', 'WX116', 'WX110', 'WX17', 'WX107', 'WX73' and 'Jenny' (Seaton and Poulish, 2010) during flowering season 2014. All the genotypes of waxflowers were sourced from the plant nursery Department of Agriculture and Food Western Australia (DAFWA) Fig. 4.1A.

4.2.3. Harvesting flowering stems

Depending upon the time of each experiment, the leafy flowering sprigs (with leaves plus flower buds and open flowers) of various genotypes were picked and used in different experiments. The stems were 60-75 cm long with 62% to 99% open flowers and were harvested in the early morning as mentioned in Table. 4. 2 from fully grown shrubs at DAFWA, South Perth, Western Australia. The sprigs were placed upright with their cut ends immersed in buckets of tap water, before commencing the application of different treatments. The sprigs were trimmed to 30 cm length by re-cutting the stem ends underwater to avoid air embolism. Also, the leaves from the lower portion of each flowering stems were removed.

Table. 4.2. Percentage (%) of flowers open on the stems of different genotypes of waxflowers when treated with different chemicals.

No	Chemical name	Genotype	Flowers open on the stems (%)
1	1-Octyne	'White Spring'	92.5 ± 8.4%
2	Isoprene oxide (IO)	'WX17'	80.5 ± 12.7%
3	*trans*-Cinnamaldehyde (CA)	'WXFU'	90.6 ± 7.1%
4	(+)-Carvone	'WX116'	72.7 ± 14.1%
5	Eugenol	'Muchae Mauve'	62.2 ± 6.6%
6	1-Octene oxide	'Southern Stars'	79.3 ± 16.4%
7	(*S*)-(-)-limonene	'WX73'	81.7 ± 11.2%
8	Butyl acrylate	'Southern Stars'	89.3 ± 10.1%
9	Allyl butyl ether	'WX110'	99.1 ± 1.3%
10	1*H*-Cyclopropa[*b*]naphthalene (NC)	'WX107'	99.8 ± 0.4 %
11	1-Octene	'Jenny'	89.6 ± 8.4%
12	1*H*-Cyclopropabenzene (BC)	'WX17'	65.3 ± 19.5%
13	1-Hexylcyclopropene (1-HCP)	'WX73'	78.7 ± 15.1%

(±) = Standard error of the mean (SE)

4.2.4. Experiments

4.2.4.1. Experiment 1: Effect of 1-octyne fumigation on abscission of flowers/buds in 'White Spring' waxflower.

In the first experiment, 'White Spring' flower stems without any treatment served as a control in distilled water and placed in a 60 L plastic drum. 'White

Spring' stems were fumigated with 1-octyne (1 µM) alone for 18 h and exposed to air and flower stems were treated with 1-octyne (1 µM) for 18 h followed by exposure to ethylene (10 µL L^{-1}) 24 h prior to their exposure to air. The stems were exposed to ethylene (10 µL L^{-1}) alone for 24 h prior to exposure to the air. The flower stems were kept in individual 250 ml small translucent plastic vases containing distilled water in all the treatments. The different treatments were applied in 60 L plastic drums Fig. 4.1C. The chemicals were dissolved in ethanol and pipetted on filter paper in a petri dish which was placed inside the plastic drum. A small fan was kept inside the container to distribute the vapour of the chemical around the flowers and stems Fig. 4.1D. All the treatments were applied at 20 ± 1ºC and 65 ± 5% RH. In addition, 30 g of soda lime was kept in each plastic drum with the sprigs to absorb any excessive accumulation of CO_2 possibly produced by respiration from the flower stems during the application of treatments Fig. 4.1E. The detailed procedure of treatment application has been described earlier in Chapter 3. All the treatments were replicated three times and three stems were included in each replication Fig. 4.1F. Following 24 h ethylene exposure, the vases containing flowering stems from all treatments were taken out from the chamber and softly beaten three times in a counting platter to collect abscised flowers/buds Fig. 4.1G and H. Flowers/buds abscission was recorded following 24 h ethylene exposure Fig. 4.1I. Percentage flower/bud abscission was calculated in each treatment. Reduction in flowers/buds abscission over ethylene alone treated stems on day four were calculated and expressed as a percentage and three stems were included in each replication.

4.2.4.2. Experiment 2: Effect of isoprene oxide fumigation on abscission of flowers/buds in 'WX17' waxflower.

The flower stems of 'WX17' with open flowers 80.5 ± 12.7 were fumigated with isoprene oxide (1 µM) alone for 18 h, followed by exposure to 10 µL L^{-1} of ethylene alone for 24 h, stems were also fumigated with 1 µM isoprene oxide alone. Untreated 'WX17' sprigs kept as control. All the details of treatment application and observations recorded on flowers/buds abscission have been explained previously in Experiment 1 above.

4.2.4.3. Experiment 3: Effect of *trans*-cinnamaldehyde fumigation on abscission of flowers/buds in 'WXFU' waxflower.

The same four treatments were also applied in this experiment to the flowering bunches of 'WXFU' 90.6 ± 7.13% with open flowers including control treatment, *trans*-cinnamaldehyde fumigation (1 µM) alone for 18 h, *trans*-cinnamaldehyde fumigated for 18 h followed by ethylene exposure and 'WXFU' stems treated with ethylene alone for 24 h. The same experimental methodology was followed for application of treatments and the observations recorded as explained previously in Experiment 1.

4.2.4.4. Experiment 4: Effect of (+)-carvone fumigation on abscission of flowers/buds in 'WX116' waxflower.

The flower stems of 'WX116' used in this experiment exhibited open flowers on the stems 72.7 ± 14.1%. Flower sprigs were fumigated with exogenous ethylene (10 µL L^{-1}) alone for 24h, stems fumigated with (+)-carvone (1 µM) alone for 18 h, and (+)-carvone (1 µM) for 18 h followed by exposure to 10 µL L^{-1} of ethylene for 24 h. Untreated stems were treated as a control. All the experimental treatments and observations on flowers/buds abscission recorded were similar to those previously detailed in Experiment 1.

4.2.4.5. Experiment 5: Effect of eugenol fumigation on abscission of flowers/buds in 'Muchae Mauve' waxflower.

The highly sensitive flowering stems of 'Muchae Mauve' waxflower with 62.2 ± 6.3% open flowers were treated with 10 µL L^{-1} of exogenous ethylene for 24 h, bunches were fumigated for 18 h with 1 µM eugenol followed by exposure to ethylene (10 µL L^{-1}) for 24 h, cut flowers stems treated with 1 µM eugenol alone and flowering stems without any treatments were kept as a control. All the treatments, their application and observations on flowers/buds abscission were similar to those explained previously in Experiment 1.

4.2.4.6. Experiment 6: Effect of 1-octene oxide fumigation on abscission of flowers/buds in 'Southern 'Stars' waxflower.

Stems of 'Southern Stars' waxflowers showing open flowers 79.3 ± 16.4% were used in this experiment. This genotype has low sensitivity to ethylene. Four treatments including stems underwent exposure to ethylene (10 µL L^{-1}) alone for 24 h, fumigation with 1 µM 1-octene oxide for 18 h, fumigation for 18 h with same concentration of 1-octene oxide followed by exposure to 10 µL L^{-1} of ethylene. Untreated sprigs were kept as a control. This trial was conducted following the same treatments as mentioned in Experiments 1. All the observations of flowers/buds abscission were recorded as explained in Experiment 1.

4.2.4.7. Experiment 7: Effect of (*S*)-(-)-limonene fumigation on abscission of flowers/buds in 'WX73' waxflower.

The flower stems with 89.3 ± 10.1% open flowers on the stems of 'WX73' waxflower were harvested and exposed to different treatments such as, fumigation with ethylene (10 µL L^{-1}) alone for 24 h, (*S*)-(-)-limonene (1 µM) fumigation alone for 18 h and (*S*)-(-)-limonene (1 µM) fumigation for 18 h followed by exposure to ethylene (10 µL L^{-1}) for 24 h. Untreated 'WX73'flower sprigs were treated as a control. All the treatments applied and method of recording observations on flowers/buds abscission is previously explained in Experiment 1.

4.2.4.8. Experiment 8: Effect of butyl acrylate fumigation on abscission of flowers/buds in 'Southern Stars' waxflower.

In this experiment 'Southern Stars' flower stems with 89.3 ± 10.1% open flowers were harvested. Stems without any treatment served as a control. In other treatments, the stems were exposed for 24 h to ethylene (10 µL L^{-1}) alone, fumigation with butyl acrylate (1 µM) alone for 18 h and butyl acrylate (1 µM) fumigation for 18 h followed by exposure to ethylene (10 µL L^{-1}) for 24 h in the plastic chamber. All the treatments and observations on flowers/buds abscission recorded in this experiment were similar to those previously mentioned in Experiment 1.

4.2.4.9. Experiment 9: Effect of allyl butyl ether fumigation on abscission of flowers/buds in 'WX110' waxflower.

Four different treatments were applied in plastic drums with 'WX110' waxflower stems showing 99.1 ± 1.3% open flowers on the stems and were treated for 24 h with ethylene (10 µL L^{-1}) alone, allyl butyl ether (1 µM) fumigation for 18 h followed by exposure to 10 µL L^{-1} of ethylene for 24 h, stems fumigated with allyl butyl ether (1 µM) for 18 h. Untreated stalks were kept as a control. All the experimental treatments and observations on flowers/buds abscission recorded in the experiment were similar to those previously stated in Experiment 1.

4.2.4.10. Experiment 10: Effect of 1H-cyclopropa[b]naphthalene fumigation on abscission of flowers/buds in 'WX107' waxflower.

Flower sprigs of 'WX107' waxflower showing 99.8 ± 0.36% open flowers constituted the experimental plant material. Untreated flower stems were kept as a control. The stems were treated with ethylene alone for 24 h, bunches fumigated with 1 µM 1*H*-cyclopropa[*b*]naphthalene for 18 h and in the last treatment, the stems were fumigated with 1*H*-cyclopropa[*b*]naphthalene for 18 h and then followed by exposure to ethylene (10 µL L^{-1}) for 24 h during this experiment. All the observations on flowers/buds abscission were recorded in a similar method as explained in Experiment 1.

4.2.4.11. Experiment 11: Effect of 1-octene fumigation on abscission of flowers/buds in 'Jenny' waxflower.

'Jenny' waxflowers are highly sensitive to ethylene. The flower stems were treated with four different treatments including a control, fumigation with 1-octene (1 µM) for 18 h in a plastic drum, stems exposed to exogenous ethylene alone for 24 h and bunches were fumigated for 18 h with 1-octene followed by exposure to ethylene similar to the procedure explained in Experiment 1. The observations on flowers/buds abscission were recorded as detailed in Experiment 1.

4.2.4.12. Experiment 12: Effect of 1*H*-cyclopropabenzene fumigation on abscission of flowers/buds in 'WX17' waxflower.

The stems of 'WX17' waxflower were fumigated with 1 µM 1*H*-cyclopropabenzene for 18 h, 10 µL L^{-1} ethylene alone for 24 h and 'WX17' bunches fumigated with 1*H*-cyclopropabenzene for 18 h followed by exposure to ethylene 24 h. All untreated stems were kept as a control. All the treatments were applied and observations on flowers/buds abscission were recorded as mentioned in Experiment 1.

4.2.4.13. Experiment 13: Effect of 1-hexylcyclopropene fumigation on abscission of flowers/buds in 'WX73' waxflower.

'WX73' waxflower was used as experimental plant material in this experiment. Four treatments were applied in 60 L plastic drum including, fumigation with 1-hexylcyclopropene for 18 h alone, 1-hexylcyclopropene fumigation followed by 10 µL L^{-1} exposure to exogenous ethylene for 24 h. 'WX73' stems were also treated for 24 h with 10 µL L^{-1} exogenous ethylene alone for 24 h. The untreated flowering stems were kept as a control. All the experimental observations on flowers/buds abscission were recorded as mentioned in Experiment 1.

4.2.5. Experimental design and statistical analysis of data

The experimental data of various 13 trials were arranged in completely randomised design (CRD) composed of two factors with three replicates. Analysis of variance (ANOVA) by employing GenStat 14th edition (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The effect of different anti-ethylene fumigation treatments on abscission of flowers/buds in various genotypes of waxflower, time and their interactions were evaluated within (ANOVA) by using least significant differences (LSD) at ($P \leq 0.05$).

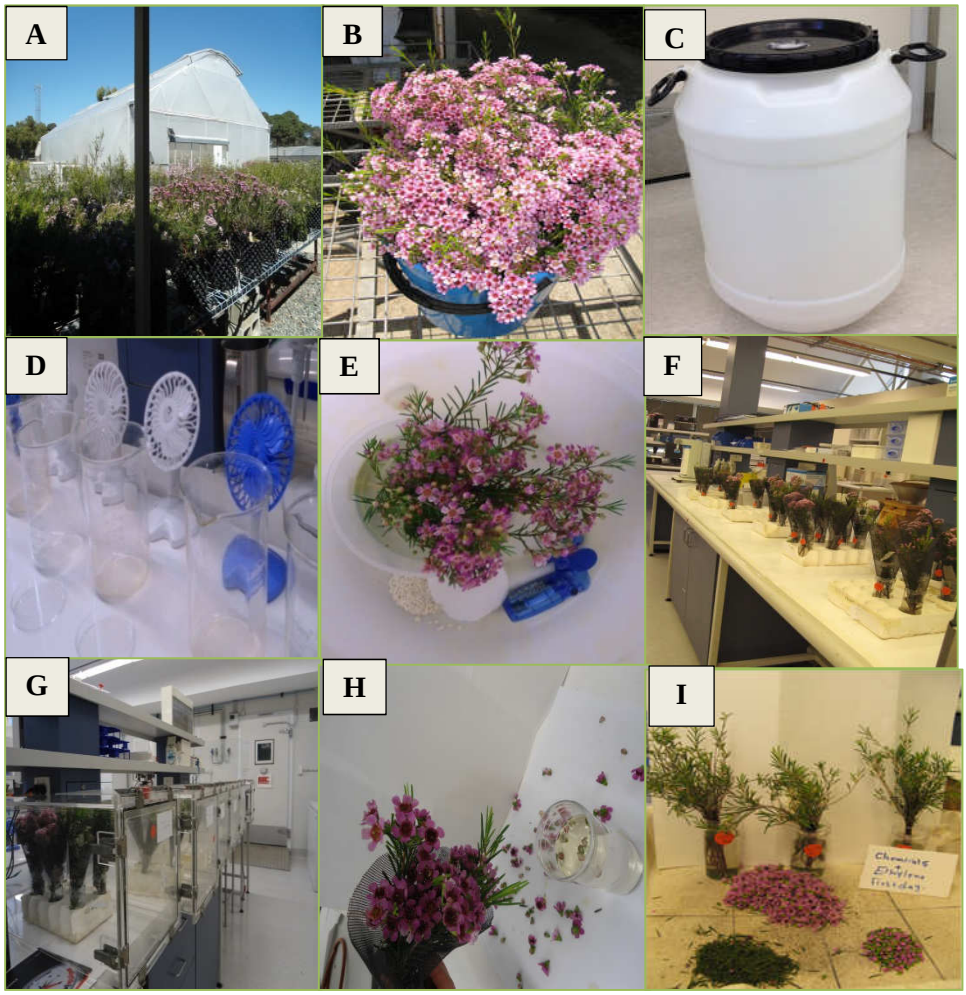


Figure. 4.1. Flower harvesting and method of application of the different chemicals and ethylene exposure. (A) Waxflower shrubs in field. (B) Flowers picked up and placed in bucket of tap water. (C) 60 L plastic drum to apply the chemicals for 18 h. (D) Small plastic fan used during chemical treatment. (E) Chemical treatment. (F) Individual flower stems in vases containing distilled water to place in the ethylene chamber. (G) flower stems placed in ethylene chamber. (H) Floral organs drop (flowers, buds and leaves) collected in a mesh cone. (I) The number of abscised and remaining flowers/buds were counted in a counting platter.

Figure. 4.2. The mesh cones were designed to retain any floral organs drop during and after 24 h of exposure to ethylene treatment (Seaton, 2006).

4.3. Results

4.3.1. Effect of different chemicals on mean flowers/buds abscission in different genotypes of 'Geraldton wax' in a flowering season of 2014.

Effects of 13 different potential ethylene antagonists on percentage reduction in flowers/buds abscission over ethylene treated stems in different genotypes of waxflowers were investigated in 13 different independent experiments during 2014 Table.4.3. The flower stems of 'White Spring', 'WX17', 'WX116', 'Muchae Mauve', 'WX110' and 'Jenny' fumigated with 1 µM 1-octyne, Isoprene, (+)-carvone, eugenol, allyl butyl ether or 1-octene respectively for 18 h followed by exposure to ethylene (10 µL L^{-1}) for 24 h did not show any reduction in flowers/buds abscission over ethylene treated stems Table.4.3. Meanwhile, fumigation of *trans*-cinnamaldehyde, (*S*)-(-)-limonene, 1*H*-cyclopropa[*b*]naphthalene (NC), 1*H*-cyclopropabenzene, 1-octene oxide, butyl acrylate or 1-hexylcyclopropene were effective in reducing flowers/buds abscission over ethylene treated stems (47.2%, 6.4%, 81.1%, 86.0%, 46.4%, 56.8% and 98.6% in 'WXFU', 'WXFU', 'WX107', 'WX73', 'Southern Stars', 'Southern Stars' and 'WX73' waxflowers respectively Table. 4.3.

Table. 4.3. Percentage reduction in flowers/buds abscission over ethylene treated stems in different genotypes of waxflower fumigated with different chemicals (1 µM) after day one following ethylene treatment in 2014.

	Chemical name	Genotype tested	Reduction in flowers/buds abscission over ethylene treated stems (%)
1	1-Octyne	'White Spring'	Nil
2	Isoprene oxide	'WX17'	Nil
3	*Trans*-cinnamaldehyde	'WXFU'	47.2
4	(+)-Carvone	'WX116'	Nil
5	Eugenol	'Muchae Mauve'	nil
6	1-Octene oxide	'Southern Stars'	46.4
7	(*S*)-(-)-limonene	'WXFU'	66.4
8	Butyl acrylate	'Southern Stars'	56.8
9	Allyl butyl ether	'WX110'	Nil
10	1*H*-cyclopropa[*b*]naphthalene	'WX107'	81.1
11	1-Octene	'Jenny'	Nil
12	1*H*-cyclopropabenzene	'WX73'	86.0%
13	1-Hexylcyclopropene	'WX73'	98.6%

4.4. Discussion

Ethylene during production, transport or handling and marketing causes premature floral senescence. Petal and flower abscission consequently shortens the storage/vase life of several ornamental crops (Joyce, 1988, 1993; Skog *et al*., 2001; Serek *et al*., 2007; Scariot *et al*., 2014). Various responses to ethylene exposure in many ornamental crops have been reported such as wilt and bud drop in *Phalaenopsis* spp. (Sun *et al*., 2009), abscission of flowers and buds in *Chamelaucium uncinatum* (Macnish *et al*., 2000b) and in potted plants such as

Pelargonium peltatum (Cameron and Reid, 2001). Controlling ethylene biosynthesis in the sensitive flowers is not an efficient method to prevent the postharvest losses whilst, antagonists of ethylene action are more effective (Goren *et al*., 2008). 1-MCP is used commercially to prevent these postharvest losses in a range of flower crops (Reid *et al*., 2001; Sisler and Serek, 2003). 1-MCP has several shortcomings that limit its use. The response of 1-MCP to horticultural commodities is dependent upon genotype, concentration applied of 1-MCP, exposure duration and temperature during treatment and variety, maturity stage of the produce and is very expensive (Sisler and Serek, 1997; Blankenship, 2001). Additionally, 1-MCP treatment protects the flowers from adverse effects of ethylene only for a short time (Macnish *et al*., 2000b; Seaton and Poulish, 2010).

Thirteen different chemicals with diverse structure and functional groups (one or more double bond (s) within the ring and some outside the ring, presence of an oxygen atom in their structure near the double bond, some have aldehyde or keto group containing carbonyl group (a carbon-oxygen double bond), or hydroxyl group, methyl group as electron, donating and/ or presence of a cyclopropene ring) were tested to assess their effectiveness as ethylene antagonists. Four common natural terpenes molecules *trans*-cinnamaldehyde, (+)-carvone, eugenol and (*S*)-(-)-limonene are active ethylene antagonists containing five to ten carbon atoms which may interact with ethylene receptors (Grichko *et al*., 2003). Three compounds from the cyclopropene group including, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene and 1-hexylcyclopropene and some other cyclopropene compounds containing the 1-alkene part in their structure (isoprene oxide, 1-octane oxide, 1-octyne, butyl acrylate and allyl butyl ether were also tested for their ability to inhibit of ethylene action and provide protection against ethylene-induced flowers/buds abscission.

Fumigation with 1-octyne, isoprene oxide, (+)-carvone, eugenol, allyl butyl ether or 1-octene followed by ethylene exposure to flowers stems of ‘White Spring’, ‘WX17’, ‘WX116’, ‘Muchae Mauve’, ‘WX110’ and ‘Jenny’ did not show any reduction in flowers/buds abscission over ethylene alone treated stems Table. 4.3. All these compounds did not protect different genotypes of waxflowers tested against ethylene action a possible reason for no activity of these compounds could be the

potency of these compounds tested was not sufficient to block the ethylene receptor irreversibly. Both, 1-octyne and 1-octene compounds have the same 1-alkene component in their structure and possibly the bond of these compounds to the ethylene receptor is not sufficient thus, these compounds were not effective in reducing the abscission of flowers/buds referring to the difference in double bond in their structure. Previously, Sisler (2008) tested 1-alkenes using pea growth and banana fruit ripening and claimed 1-octene to be an active competitive inhibitor of ethylene action in peas but was inactive as ethylene action compounds. Sisler (2008) also reported that competitive inhibitors of ethylene action reach to the receptor and binding site was not restricted. It was also attributed to the insufficient hydrophobic interactions with the ethylene receptor or compounds associated with the receptor. Carvone is a naturally occurring monoterpene in caraway (*Carum carvi*) (Damunupola *et al*., 2010) and in dill (*Anethum graveoleus*) seeds (De Carvalho *et al*., 2006) and exhibits antibacterial and antifungal activity (Oosterhaven *et al.*, 1995a, b). As reported earlier, by Grichko *et al*. (2003) carvone and eugenol contain the double bond inside the aromatic ring which could make the ring inactive and fail to bind to the receptor. Isoprene oxide was not an effective to block ethylene action in waxflowers. Similarly, Grichko *et al*. (2003) also reported earlier that isoprene was not an effective inhibitor of ethylene action when tested on the regulation of green banana fruit ripening. However, isoprene oxide contains oxygen atoms that can increase the ring strain. All these compounds tested do not inhibit the ethylene action as reflected by no reduction in the abscission of flowers/buds in different genotypes of waxflower.

Fumigation of 1-octene oxide, butyl acrylate, *trans*-cinnamaldehyde, (*S*)-(-)-limonene, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene or 1-hexylcyclopropene has significantly reduced flowers/buds abscission compared to the ethylene treated flower stems in different genotypes of waxflowers Table. 4.3 and demonstrates that these compounds act as inhibitors of ethylene action. It may be attributed to the presence of oxygen atoms adjacent to the double bond as the functional group in its structure that made the *trans*-cinnamaldehyde more effective in interacting with ethylene for the receptor (Gricko *et al*., 2003). Earlier, Sisler *et al*. (2001) also reported that any compound with a double bond at position one will be more active to block the receptor for a longer time. 1*H*-cyclopropa[*b*]naphthalene or

1*H*-cyclopropabenzene and 1-hexylcyclopropene inhibit ethylene action for certain periods because of a highly strained cyclopropene ring as a functional group which will be highly effective for inhibition of ethylene action as reported earlier (Sisler *et al*., 1996a, b, Sisler *et al*., 2001; Sisler, 2008). Moreover, the methyl group adjacent to the double bond in the chemical structure of the cyclopropene group also render them potent inhibitors for blocking ethylene action (Saleh-Lakha *et al*., 2004). 1-hexylcyclopropene has a six-carbon chain substituted in the 1-position with a longer side chain contributing to the effectiveness of this compound as ethylene antagonist (Kebenei *et al*., 2003a; Serek *et al*., 2007).

In conclusion, 1-octene oxide, butyl acrylate, *trans*-cinnamaldehyde, (*S*)-(-)-limonene, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene or 1-hexylcyclopropene were effective inhibitors of action of ethylene and consequently reduced flowers/buds abscission in different genotypes of waxflowers. The efficacy of these compounds as ethylene action antagonists has been investigated in detail and presented in subsequent different chapters.

CHAPTER 5

Trans-cinnamaldehyde fumigation protects different genotypes of waxflowers from detrimental effect of ethylene on abscission of flowers/buds.

Abstract

Waxflower (*Chamelaucium uncinatum* Schauer) is one of the most widely and commercially important flower crops available in Australia. Short postharvest life, excessive abscission of flowers/buds and leaf yellowing of waxflower, when exposed to trace amounts of ethylene during postharvest, can cause serious economic losses. Effects of fumigation of *trans*-cinnamaldehyde (1 µM) and ethylene (10 µLL^{-1}) alone, *trans*-cinnamaldehyde (1 µM) followed by exposure to 10 µL L^{-1} of ethylene on flowers/buds abscission in 'WX73', 'WXFU', 'WX17', 'WX58', and 'WX56' were investigated. Also, the effect of different concentrations 0.5, 1.0 and 2.0 µM of *trans*-cinnamaldehyde and ethylene (10µL L^{-1}) alone and for different concentrations of *trans*-cinnamaldehyde followed by exposure to exogenous ethylene on flowers/buds abscission was examined in 'Purple Pride', 'Revelation' and 'Hybrid1'. Flower stems were fumigated with *trans*-cinnamaldehyde (1 µM) in 60 L plastic drums sealed for 18 h followed by 24 h exposure to ethylene (10 µL L^{-1}) at 20 ± 1ºC and 65 ± 5% RH, in specially designed dosing chambers. In all the experiments, untreated flower stems were held as a control with stems standing in vases of distilled water (DW). Fumigation of flowering stems with *trans*-cinnamaldehyde (1 µM) followed by a single exposure to ethylene (10 µL L^{-1}) significantly ($P \leq 0.05$) reduced abscission of flowers/buds (13.7% 'WX58', 14.1% 'WX73', 25.2% 'WX56' and 36.7% 'WXFU') when compared to ethylene alone (68.8% 'WX58', 89.9% 'WX73', 85.9% 'WX56' and 66.2% 'WXFU'). The reduction in flowers/buds abscission was not noted in 'WX17' genotype when treated with *trans*-cinnamaldehyde (1 µM) followed by exposure to ethylene (10 µLL^{-1}). Amongst the three concentrations of *trans*-cinnamaldehyde tested (1 µM) fumigation for 18 h followed by exposure to ethylene (10 µL L^{-1}) for 24 h was the most effective concentration in reducing flowers/buds abscission. These results suggest that when *trans*-cinnamaldehyde (1 µM) was applied to waxflower stems for 18 h followed by exposure to 10 µL L^{-1} of ethylene for 24 h *trans*-cinnamaldehyde was effective in controlling ethylene-induced flowers/buds abscission by inhibiting

ethylene action. In conclusion, *trans*-cinnamaldehyde (1 µM) seemed to have some effective as an anti-ethylene inhibitor in the abscission process of waxflowers in all hybrid genotypes tested except C. *uncinatum* 'WX17' genotype.

5.1. Introduction

Abscission is a natural physiological process involving detachment of plant organs such as petals, sepals, leaves, entire flowers, inflorescences and fruit (Taylor and Whitelaw, 2001; Ascough, *et al*., 2005; Ascough *et al*., 2006). Abscission is an integral characteristic developmental event that occurs in a particular part of the organ known as the abscission zone (Reid, 1985; Macnish *et al*., 2005; Ascough *et al*., 2006). During the postharvest period, various environmental stresses including high temperature, low irradiance, water stresses, humidity, physical injury, diseases and poor nutrition, enhance ethylene biosynthesis that accelerate the abscission process consequently inducing the separation of floral organs (Joyce, 1992; Beasley and Joyce, 2002; Ascough *et al*., 2005; Da Silva, 2006).

Abscission of flowers, buds, leaves and premature floral senescence causes significant losses in floricultural crops (Joyce, 1993; Taylor and Whitelaw, 2001; Beasley and Joyce, 2002; Ascough *et al*., 2005; Ascough *et al*., 2006). Ethylene is known to mediate floral senescence and petal or flower by prompting changes prior to the abscission process that affects postharvest longevity of many species of economically important flowers (Burg, 1968; van Doorn and Stead, 1997; Blankenship, 2001; Cameron and Reid, 2001; Taylor and Whitelaw, 2001; van Doorn, 2001; Macnish *et al*., 2005; Scariot *et al*., 2014). Endogenous ethylene produced in vegetative and reproductive tissues of a plant as well as exogenous ethylene can act at very low concentrations ranging from part-per-million to part-per-billion (Saltveit, 1999; Gibson *et al*., 2000; Martinez-Romero *et al*., 2007; Gollnow and Worrall, 2010). Ethylene causes abscission of floral organs of a wide number of ornamental plants including *Hibiscus, Begonia, Clerodendron, Fuchsia* and *Agapanthus,* and can hasten petals senescence in *Aconitum, Delphinium, Rosa, Potentilla, Geranium, Antirrhinum, Veronica* (Woltering, 1987; Serek *et al*., 2006), *Chamelaucium* (Joyce, 1992), *Leptospermum* and *Thryptomene calycina* (Faragher *et al*., 2010) and *Verticordia* (Seaton, 2006).

Waxflower *(Chamelaucium* sp. and hybrids, Myrtaceae) commonly known as 'Geraldton wax' is one of the most desirable cut flowers in Australia and is economically important in the world because of wide range of colours, sizes and shapes of flowers making waxflower attractive for export (Beasley and Joyce, 2002; Terry *et al*., 2003; Gollnow and Worrall, 2010; Seaton and Poulish, 2010). Export of Australian flowers is worth an estimated A$14 million and 95 percent are native species (Seaton and Poulish, 2010). Postharvest senescence, short postharvest life, abscission of waxflowers and flower buds (up to 85%) as well as leaf yellowing when exposed to trace amounts of ethylene in the postharvest phase, are the main limitations in expanding export of waxflowers due to economic losses (Joyce, 1993; Faragher *et al*., 2010; Gollnow and Worrall, 2010; Seaton and Poulish, 2010).

In plant tissues, the ethylene molecule binds to specific receptors and activates downstream gene transcription and translation (Bleecker and Kende, 2000). Ethylene causes abscission of plant organs and induces a series of changes leading to an upsurge in respiration rates in plant tissue resulting in the synthesis of more RNA and protein which in turn increases the rough endoplasmic reticulum in cells and the quantities of polyribosomes. Following this, the vesicles of these organs synthesise more proteins that move out to the plasmalemma and excrete their contents finally into the cell wall which becomes swollen and the abscission process occurs (Reid, 1985). Thus, separation in the abscission layer is known to occur due to the breakdown of the main cell wall and active middle lamella solubilisation, thereby weakening most of the cell wall in waxflower (Macnish *et al*., 2005).

Various strategies have also been applied to avoid the detrimental effect of ethylene gas on cut flowers including removal of the ethylene from the atmosphere or from packaging by absorbing ethylene in potassium permanganate ($KMnO_4$) (Terry *et al*., 2007) and oxidizing agents such as ozone (Dickson *et al*., 1992), copper, cobalt, palladium and titanium (Maneerat *et al*., 2003). Some success has been reported for breeding new cultivars in Australian native waxflowers such as ethylene resistant varieties (*Chamelaucium* X *Verticordia plumosa* (Desf.) hybrids Druce), varieties with improved resistance to pests and diseases, providing different new colours and an extended flowering season (Yan, 2001; Seaton *et al*., 2007; Shan and Seaton, 2008; Seaton and Poulish, 2010).

In addition, several other strategies have been tested to curtail the harmful effects of ethylene on abscission of flowers and/or senescence in ornamental plants and flowers such as inhibiting ethylene biosynthesis using amino oxyacetic acid (AOA), aminoethoxy vinyl glycine (AVG) and methoxy vinyl glycine (MVG) (Fujino *et al*., 1980; Serek and Andersen. 1993; Serek and Reid,1993; Staby *et al*., 1993; Rattanwisalanona *et al*., 2003; Ascough *et al*, 2006; Ebrahimzadeh *et al*., 2008; Tanase *et al*., 2009) and aminotriazole (ATA) (Serrano *et al*., 1990; Ebrahimzadeh *et al*., 2008). Earlier, down-regulation of ethylene biosynthesis through antisense ACO gene in carnation (Savin *et al*., 1995) , antisense ACS and ACO genes in Petunia, Iris, Alstroemeria, Carnation, Sandersonia and Dendrobium orchid has also been reported (Rogers, 2013; Scariot *et al*., 2014; Sornchai *et al*., 2015).

Ethylene action inhibitors such as 2,5-norbornadiene (NBD) (Sisler and Pian, 1973; Sisler *et al*., 1983; Sisler *et al*., 1985; Wang and Woodson, 1989; Sisler and Serek, 2003; Serek *et al*., 2006; Sisler, 2006), diazocyclopentadiene (DACP) (Blankenship and Sisler, 1992; Sisler *et al*., 1993; Serek *et al*., 1994a; Sisler and Serek, 2003; Serek *et al*., 2006; Sisler, 2006), silver thiosulfate (STS) (Veen, 1979; Joyce, 1993; Ichimura *et al*., 2002; Serek *et al*., 2006; Asrar, 2012), nitric oxide (NO) (Ebrahimzadeh *et al*., 2008), 1-methylcyclopropene (1-MCP) (Serek *et al*., 1995a, 2006; Macnish *et al*., 2000a; Cameron and Reid, 2001; Zencirkiran, 2010) have also been tested to reduce the adverse effects of ethylene action in various flowers.

Application of 1-MCP at low concentration was more effective to prevent the biological action of ethylene by inactivating the ethylene receptors (Sisler *et al*., 1996a; Sisler and Serek, 1997). Therefore, flowers were protected for long period of time from the damaging effect of exogenous ethylene, whereby delaying flower senescence and maintaining vase life quality (Sisler and Serek 1997; Sisler and Serek, 1999; Sisler *et al*., 2003; Buanong *et al*., 2005; Lurie, 2005; Seglie *et al*., 2010). Macnish *et al*. (2000b) reported that the application of 1-MCP (10 nL L^{-1}) over 12 h led to a delay of senescence in 14 different native Australian cut flowers, however, it did not provide significant protection against exogenously applied ethylene. Further studies by Macnish *et al*. (2004b) suggested that the 1-MCP application to CWA Pink waxflowers prior to export and continual exposure of 1-

MCP during export was effective in reducing flower abscission induced by exposure to ethylene. Hunter *et al*. (2004a) reported that when the *Narcissus pseudonarcissus* flowers fumigated with 500 nL L^{-1} of 1-MCP for 6 h at 20 ºC resulted in only a modest extension in prolonged existence of attached flowers held in the air and no effects on the life span of the flowers held in water. In addition, Kim *et al*. (2007) indicated that Regal Pelargonium cultivars treated with 1 µL L^{-1} of 1-MCP prevents petal abscission of florets but, no effect on prolonging shelf life in any of the cultivars tested. 1-MCP has become a valuable tool for the horticulture industry, but it is a volatile gas at room temperature and is unstable often difficult to handle (Sisler and Serek, 1997; Blankenship, 2001; Reid and Seaton, 2001; Sisler and Serek, 2003; Grichko, 2006; Ebrahimzadeh *et al*., 2008; Paul *et al*., 2010). A major obstacle is that expensive for growers (Khan, 2014), not easy to apply because it is a gas (Reid and Seaton, 2001), there are practical difficulties in treatment, as the inhibitor is gaseous in nature, therefore, requiring appropriate treatment systems in trucks (Reid and Celikl, 2008; Macnish *et al*., 2010). Moreover, the response of 1-MCP to horticultural commodities is dependent upon genotype, concentration, exposure duration, the temperature during treatment and storage (Sisler and Serek, 1997; Blankenship, 2001).

Trans-cinnamaldehyde and cinnamyl alcohol and other compounds derived from the phenylpropanoid pathway have been reported to be effective ethylene antagonists in green bananas as a test material possibly due to the effect of oxygen atoms present being close to the double bond in its structure (Grichko *et al*., 2003). Jing *et al*. (2011) found that application of cinnamaldehyde (0.1 - 0.3%) in vase solution resulted in mitigation of oxidative stress in 'Pink Queen' rose cut flowers thus, prolong the flowers vase life to 2.8 days. However, no research work has been reported on the effect of *trans*-cinnamaldehyde on regulating abscission of flowers/buds in 'Geraldton wax'. It was hypothesised that *trans*-cinnamaldehyde will act as an antagonist to inhibit ethylene action and will reduce abscission of flowers/buds in waxflowers. Therefore, the effects of *trans*-cinnamaldehyde as an ethylene antagonist on flowers/buds abscission in different genotypes of waxflowers were investigated during 2014 -2015. The effects of different concentrations on flowers/buds abscission were also examined on three different genotypes of waxflower including 'Purple Pride', 'Revelation' and 'Hybrid1' during 2015.

5.2. Materials and Methods

5.2.1. Sources of chemicals

Trans-cinnamaldehyde Fig. 5.6 was purchased from Acros Organics™, New Jersey, USA. The pressurised cylinder of pure ethylene (98%) gas was procured from BOC Gases, Australia Ltd., Perth, Australia.

5.2.2. Plant material

To investigate the efficacy of *trans*-cinnamaldehyde as ethylene antagonist, eight different experiments were conducted using a range of fresh waxflower genotypes differing in sensitivity to ethylene. The experiments were conducted during June to October in 2014 and 2015. Sprigs of *C. uncinatum* cv. ('WX73', 'WXFU' and 'WX17') during 2014 and ('WX58', 'WX56', 'Hybrid1', 'Revelation' and 'Purple Pride') during 2015 were harvested with at least 50% flowers open from five-year old bushes which were grown under irrigation and fertigation (Seaton and Poulish, 2010) at the Department of Agriculture and Food Western Australia (DAFWA).

5.2.3. Harvesting flowering stems

The flowering sprigs (60 to 70 cm) of the genotypes were harvested in the early morning and placed with their cut ends in the containers containing clean water. Stems used in each treatment were randomly selected from harvested stems and placed in bunches. Prior to applying different treatments, the stems were recut with secateurs in water to 30 cm length from cuts ends to the extremities of the open flowers.

Table. 5.1. The percentage of open flowers on stems at the time of harvest.

Year	Genotype	Flowers open on stems (%)
2014	'WX73'	74.8±13.6%
2014	'WXFU'	90.6±7.1%
2014	'WX17'	69.3±13.4%
2015	'Purple Pride'	62.2±9.9%
2015	'WX56'	66.3±11.5%
2015	'WX58'	79.3±8.4%
2015	'Hybird1'	98.7±1.6%
2015	'Revelation'	84.8±16.7%
2015	'Purple Pride'	64.9±7.8%

± = Standard error of the mean (SE)

5.2.4. Treatments and methods

Untreated flower stems were kept as control. Flower stems were fumigated with ethylene (10µL L^{-1}) alone for 24 h, *trans*-cinnamaldehyde (1µM) alone for 18 h, and *trans*-cinnamaldehyde (1µM) for 18 h followed by exposure to ethylene (10µL L^{-1}) for 24h. The waxflower stems were fumigated with *trans*-cinnamaldehyde (1µM) by using 60L plastic drums. *trans*-cinnamaldehyde was loaded on a small filter paper and put in a petri dish in the plastic drum. A small plastic fan was kept on inside the container to distribute the vapour of the chemical around the flower stems. Untreated and *trans*-cinnamaldehyde-treated flower stems were fumigated with ethylene (10µL L^{-1}) for 24 h in 60L plastic drums. The flower bunches were kept in small translucent plastic vases containing distilled water during the application of treatments. All the treatments were applied at 20 ± 1ºC and 65 ± 5% Relative Humidity (RH). Also, 30g of soda lime was kept in the drums with the flowering stems to avoid excessive accumulation of CO_2. Stems from treatments were placed in 250ml vases containing distilled water with each placed in small mesh nylon cones installed at the base of the stalks to collect any loss of abscised flowers and flower buds during vase life assessment daily for four consecutive days (Seaton, 2006).

5.2.5. Experiments.

5.2.5.1. Experiment 1: Effect of fumigation of *trans*-cinnamaldehyde, ethylene alone and *trans*-cinnamaldehyde treated flowers followed by ethylene exposure on abscission flowers/buds of 'WX73' 'WXFU' and 'WX17' genotypes of waxflowers in 2014.

The antagonistic effect of *trans*-cinnamaldehyde to ethylene was tested in the first experiment, using the flower sprigs of 'WX73' waxflowers. The flower stems were treated with *trans*-cinnamaldehyde (1 µM) or ethylene (10 µL L^{-1}) alone and *trans*-cinnamaldehyde followed by exposure to 10 µL L^{-1} of ethylene. Untreated flower stems were kept as control. Following the ethylene treatment, the flower stems were taken out from the container and softly beaten to collect abscised flowers/buds. In each replicate, the abscised flowers/buds were counted and the number of flowers/buds remaining on the stems was also counted. The percentage of flowers/buds abscised was calculated daily for four consecutive days. Additionally, two independent experiments were also conducted on 'WXFU' and 'WX17' waxflowers keeping all the experimental treatments and design as mentioned in the first experiment.

5.2.5.2. Experiment 2: Effect of fumigation of *trans*-cinnamaldehyde, ethylene alone and trans–cinnamaldehyde treated flowers followed by ethylene exposure on abscission flowers/buds of 'WX58' and 'WX56' genotypes of waxflowers in 2015.

In 2015, the antagonistic effect of *trans*-cinnamaldehyde to ethylene was also evaluated by conducting two independent experiments using flower stems of two genotypes of waxflower including 'WX58' and 'WX56'. Both trials were conducted following the same treatments and design as mentioned in Section 5.2.6. The percentage of flowers/buds abscission in each replication was calculated daily for four days as detailed previously.

5.2.5.3. Experiment 3: Effects of different concentrations of *trans*-cinnamaldehyde, ethylene alone and *trans*-cinnamaldehyde followed by exposure to ethylene on abscission of flowers/buds of 'Purple Pride', 'Revelation' and 'Hybrid1' genotypes of waxflowers in 2015.

'Purple Pride' waxflower stems were fumigated with different concentrations 0.5, 1.0 and 2.0 µM of *trans*-cinnamaldehyde alone, ethylene (10 µL L^{-1}) alone and *trans*-cinnamaldehyde treatments followed by exposure to ethylene (10 µL L^{-1}). The flower stems without any treatment were kept as a control. The experiment was laid out by following one -factor factorial completely randomised design, with three replications and three stems per replication as an experimental unit. Effects of different treatments on percentage flowers/buds abscission as detailed in above experiments were recorded on the fourth day after the ethylene treatment. Two more separate experiments were conducted on cultivar 'Revelation' and 'Hybrid1' using the same treatments and experimental design as explained above. The flowers/buds abscission was recorded on day four after treatments and expressed as percentage flowers/buds abscission.

5.2.6. Experimental design and statistical analysis of data

The experiment 1 and 2 were laid out by following two-factor factorial and experiment 3 one -factor factorial completely randomised design with three stems were treated as an experimental unit and three replications. The experimental data obtained was analysed using one or two - way analysis of variance (ANOVA) using the statistical package Genstat 14th edition (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). Treatment means were compared by LSD at ($P < 0.05$) and means (± SE) were shown as appropriate. Differences among various treatments were further analysed using Duncan's Multiple Range Test.

5.3. Results

5.3.1. Experiment1.

5.3.1.1. Effect of *trans*-cinnamaldehyde (CA) fumigation on mean flowers/buds abscission in 'WX73' 'WXFU' and 'WX17' genotypes of waxflower in 2014 - 2015.

When averaged over four days, mean flowers/buds abscission was significantly ($P \leq 0.05$) highest (89.9% and 66.2%) on 'WX73' and 'WXFU' flowering stems treated with ethylene (10 $\mu L L^{-1}$) alone for 24 h as compared to the control (5.5% and 3.9%) and all other treatments respectively in 2014 Fig.5.1 A and B. The mean flowers/buds abscission was significantly ($P \leq 0.05$) increased (99.8% and 97.8%) on 'WX17' flowering stems fumigated with 1 µM *trans*-cinnamaldehyde for 18 h followed by exposure to ethylene (10 $\mu L\ L^{-1}$) and the ethylene treatment alone respectively for 24 h as compared to the control (78.2%) and all other treatments Fig.5.1 C. Flower stems of 'WX73' and 'WXFU' fumigated with *trans*-cinnamaldehyde (1 µM) for 18 h followed by exposure to 10 $\mu L\ L^{-1}$ of ethylene exhibited significantly reduced mean flowers/buds abscission (14.1% and 36.7%) as compared to those treated with ethylene alone (89.9% and 66.2%) respectively Fig.5.1A and B. Such a response to flowers/buds abscission to those treatments was lacking in 'WX17' waxflowers in 2014.

5.3.2. Experiment 2.

5.3.2.1. Effect of *trans*-cinnamaldehyde (CA) fumigation on mean flowers/buds abscission in 'WX56' and 'WX58' genotypes of waxflower in 2014-2015.

Flower stems treated with ethylene (10 $\mu L\ L^{-1}$) alone for 24 h exhibited significantly ($P \leq 0.05$) higher mean flowers/buds abscission (85.9% and 68.8%) as compared to the control (7.1% and 1.3%) in 'WX56' and 'WX58' waxflower respectively in 2015 Fig.5.2 D and E. Meanwhile, *trans*-cinnamaldehyde (1µM) fumigation for 18 h followed by exposure to 10 $\mu L\ L^{-1}$ of ethylene for 24 h significantly reduced mean flowers/buds abscission (25.2% and 13.7%) as compared to the treatment of ethylene alone (85.9% and 68.8%) in 'WX56' and 'WX58' respectively Fig.5.2 D and E.

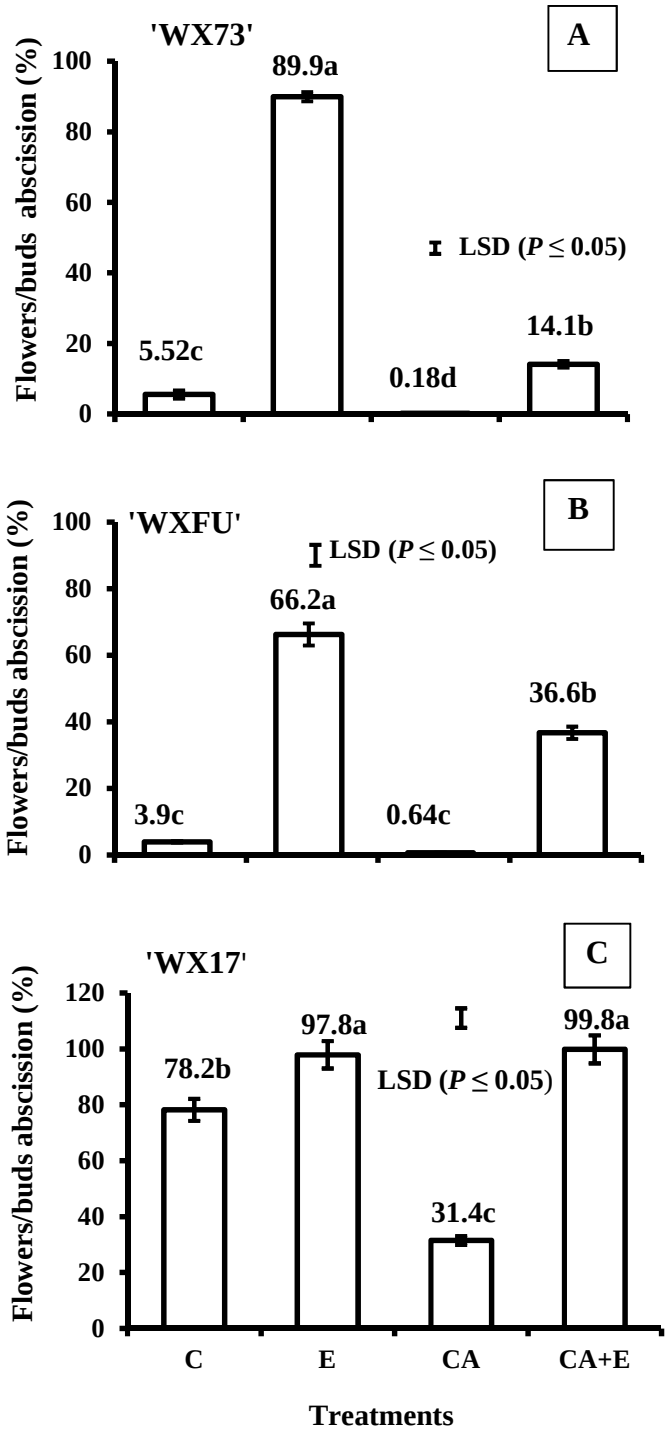


Figure. 5.1. Effects of fumigation of *trans*-cinnamaldehyde (1 µM), ethylene (10 µL L^{-1}) alone and *trans*-cinnamaldehyde followed by exposure to ethylene (10 µL L^{-1}) on mean flowers/buds abscission in (A) 'WX73', (B) 'WXFU' and (C) 'WX17' waxflower during 2014. Vertical bars represent (SE). C = control, E = ethylene alone (10 µL L^{-1}), CA = *trans*-cinnamaldehyde alone, CA + E = *trans*-cinnamaldehyde fumigation followed by ethylene.

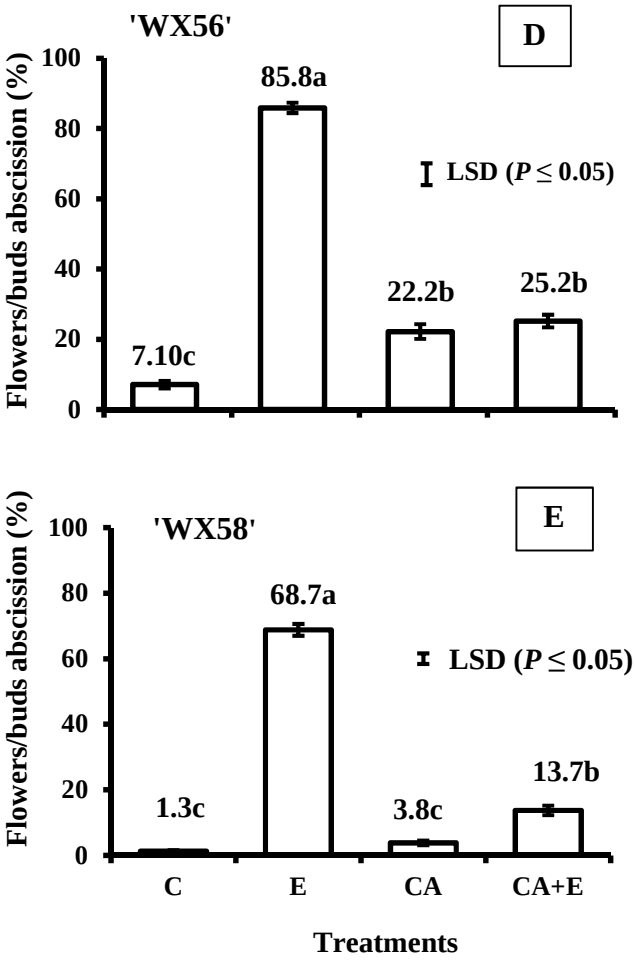


Figure.5.2. Effects of fumigation of *trans*-cinnamaldehyde (1 µM) or ethylene (10 µL L^{-1}) alone and *trans*-cinnamaldehyde followed by exposure to ethylene (10 µL L^{-1}) on mean flowers/buds abscission in (D) 'WX56' and (E) 'WX58' waxflower during 2015. Vertical bars represent (SE). C = control, E = ethylene alone (10 µL L^{-1}), CA = *trans*-cinnamaldehyde alone, CA+E = *trans*-cinnamaldehyde fumigation followed by ethylene.

5.3.2.2. Effect of *trans*-cinnamaldehyde (CA) fumigation on cumulative flowers/buds abscission in 'WX73', 'WXFU' and 'WX17' genotypes of waxflower.

Stems of 'WX73' waxflower fumigated with *trans*-cinnamaldehyde (1 µM) for 18 h followed by exposure to ethylene (10 µL L^{-1}) exhibited substantially reduced cumulative flowers/buds abscission (9.5% to 17.0%) as compared to those treated with ethylene alone (86.2% to 91.3%) from day one to four respectively in 2014 Fig. 5.3 A. As expected, fumigation of ethylene (10 µL L^{-1}) alone for 24 h promoted cumulative abscission of flowers/buds in 'WX73' waxflower as compared to the

control over a four day period Fig.5.3A. Sprigs of 'WX73' waxflower fumigated with *trans*-cinnamaldehyde (1 µM) alone exhibited lowest cumulative flowers/buds abscission (0.0% to 0.7%) over a four day period as compared to the control and all other treatments.

The flower sprigs of 'WXFU' waxflower fumigated with *trans*-cinnamaldehyde (1 µM) and followed by exposure to 10 µL L^{-1} of ethylene for 24 h showed significantly reduced cumulative flowers/buds abscission (27.7% to 40.3%) over a four day period during 2014 as compared to those treated with (10 µL L^{-1}) ethylene alone (65.3% to 67.2%). Meanwhile, *trans*-cinnamaldehyde treatment alone and untreated control waxflower stems showed lowest cumulative flowers/buds abscission (0.0% to 1.5% and 3.6% to 4.3% respectively) Fig.5.3B.

Contrarily, a significant increase of cumulative flowers/buds abscission over a four day period during 2014 was noted when the 'WX17' genotype stems were fumigated with *trans*-cinnamaldehyde (1 µM) followed by ethylene (10 µL L^{-1}) exposure or ethylene alone as compared to all other treatments. Meanwhile, the *trans*-cinnamaldehyde treatment (1 µM) alone for 18 h showed lowest cumulative flowers/buds abscission in 'WX17' waxflower (11.5% to 58.5%) over a four day period as compared to the control and all other treatments in 2014 Fig.5.3C.

5.3.2.3. Effect of *trans*-cinnamaldehyde (CA) fumigation on cumulative flowers/buds abscission in 'WX56' and 'WX58' genotypes of waxflower.

Stems of 'WX56' and 'WX58' waxflower fumigated with (1 µM) *trans*-cinnamaldehyde and followed by exposure to 10 µL L^{-1} of ethylene showed significantly ($P \leq 0.05$) reduced cumulative flowers/buds abscission (17.4% to 31.1% and 11.8% to 17.6% respectively) as compared to those treated with ethylene treatment alone from day one to day four (83.9% to 86.7% and 57.6 % to 74.6% respectively). As expected, ethylene (10 µL L^{-1}) applied alone for 24 h enhanced cumulative abscission of flowers/buds in 'WX56' and 'WX58' waxflowers as compared to the control over a four day period. The cumulative flowers/buds abscission was lowest in 'WX56' and 'WX58' in control (4.9% to 9.8% and 0.4% to 2.3% respectively) as compared to all other treatments in 2015 Fig.5.4 D and E.

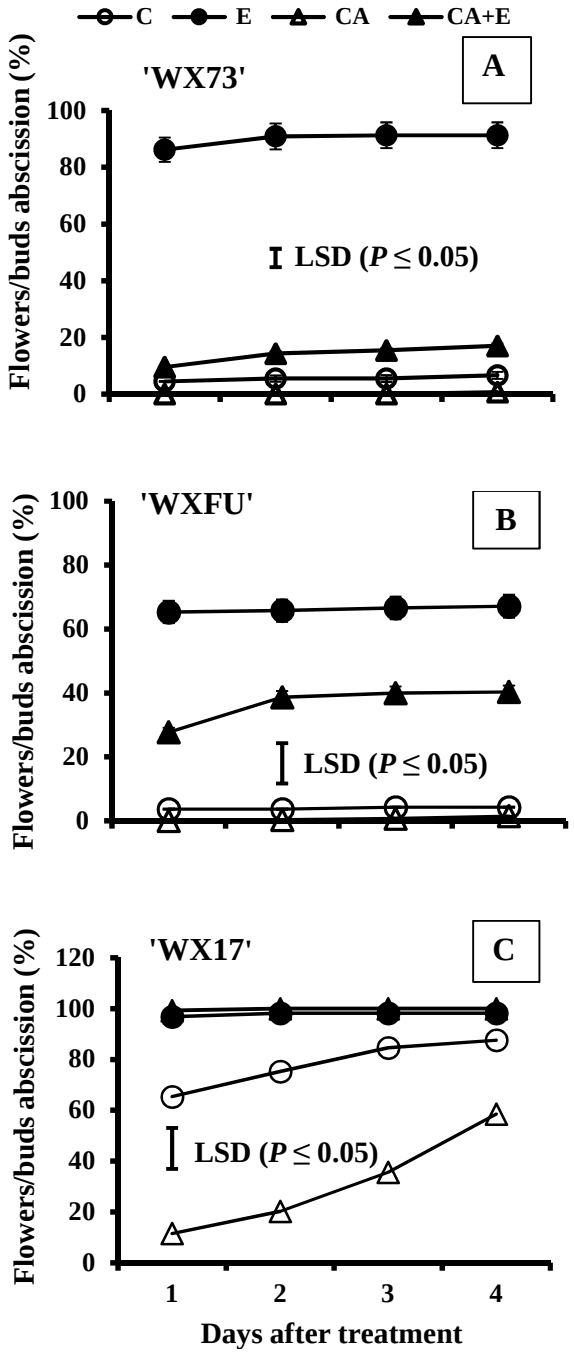


Figure. 5.3. Effects of fumigation of *trans*-cinnamaldehyde (1 µM) or ethylene (10 µLL^{-1}) alone and *trans*-cinnamaldehyde (1 µM) followed by ethylene (10 µL L^{-1}) on cumulative abscission of flowers/buds four days after treatment in (A) 'WX73', (B) 'WXFU' and (C) 'WX17' waxflower in 2014. n = three replications (three stems per replication), vertical bars represent SE, C = control, E = ethylene alone (10 µL L^{-1}), CA = *trans*-cinnamaldehyde alone, CA + E = *trans*-cinnamaldehyde fumigation followed by ethylene.

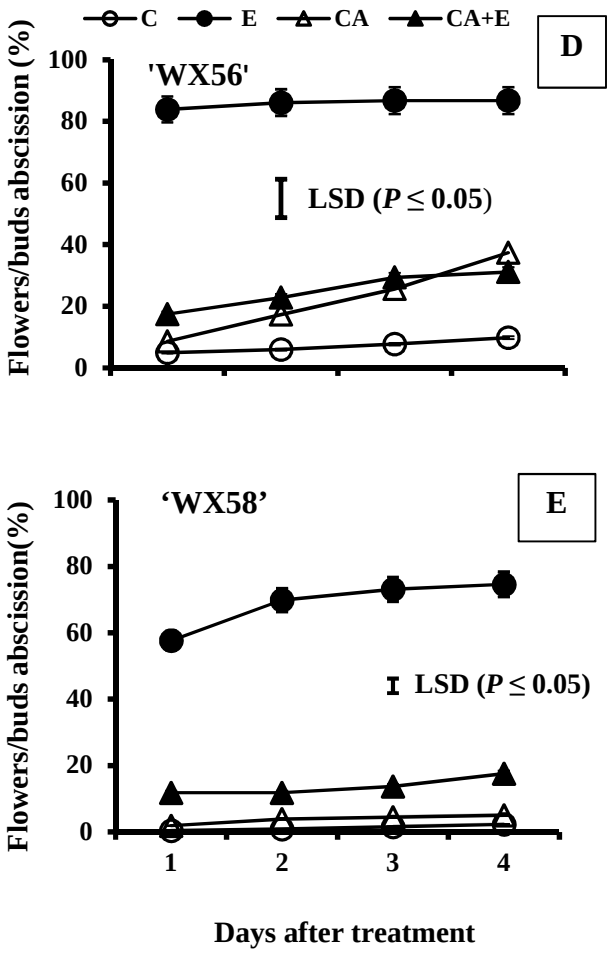


Figure. 5.4. Effects of fumigation of *trans*-cinnamaldehyde (1 µM) or ethylene (10 µL L^{-1}) alone and *trans*-cinnamaldehyde (1 µM) followed by ethylene (10 µL L^{-1}) on cumulative abscission of flowers/buds four days after treatment in (D) 'WX56' and (E) 'WX58' waxflower in 2015. n = three replications (three stems per replication), vertical bars represent SE, C = control, E = ethylene alone (10 µLL^{-1}), CA = *trans*-cinnamaldehyde alone, CA + E = *trans*-cinnamaldehyde fumigation followed by ethylene.

5.3.3. Experiment 3: Effects of different concentrations of *trans*-cinnamaldehyde fumigation alone and *trans*-cinnamaldehyde fumigation followed by exposure to ethylene on flowers/buds abscission of 'Purple Pride', 'Revelation' and 'Hybrid1' waxflowers in 2015.

As expected, ethylene (10 µL L^{-1}) treatment alone for 24h resulted in significantly ($P \leq 0.05$) highest flowers/buds abscission (84.7%, 47.1% and 83.9%) on day four as compared to the control (3.1%, 7.1% and 9.1%) in 'Purple Pride', 'Revelation' and 'Hybrid1' waxflowers respectively in 2015 Fig.5.5A, B and C. All the *trans*-cinnamaldehyde fumigation treatments irrespective of concentration applied followed by exposure to 10 µL L^{-1} of ethylene for 24 h resulted in significantly reduced flowers/buds abscission as compared to the ethylene treatment alone on day four in 'Revelation' and 'Hybrid1' waxflowers in 2015 Fig.5.5 B and C.

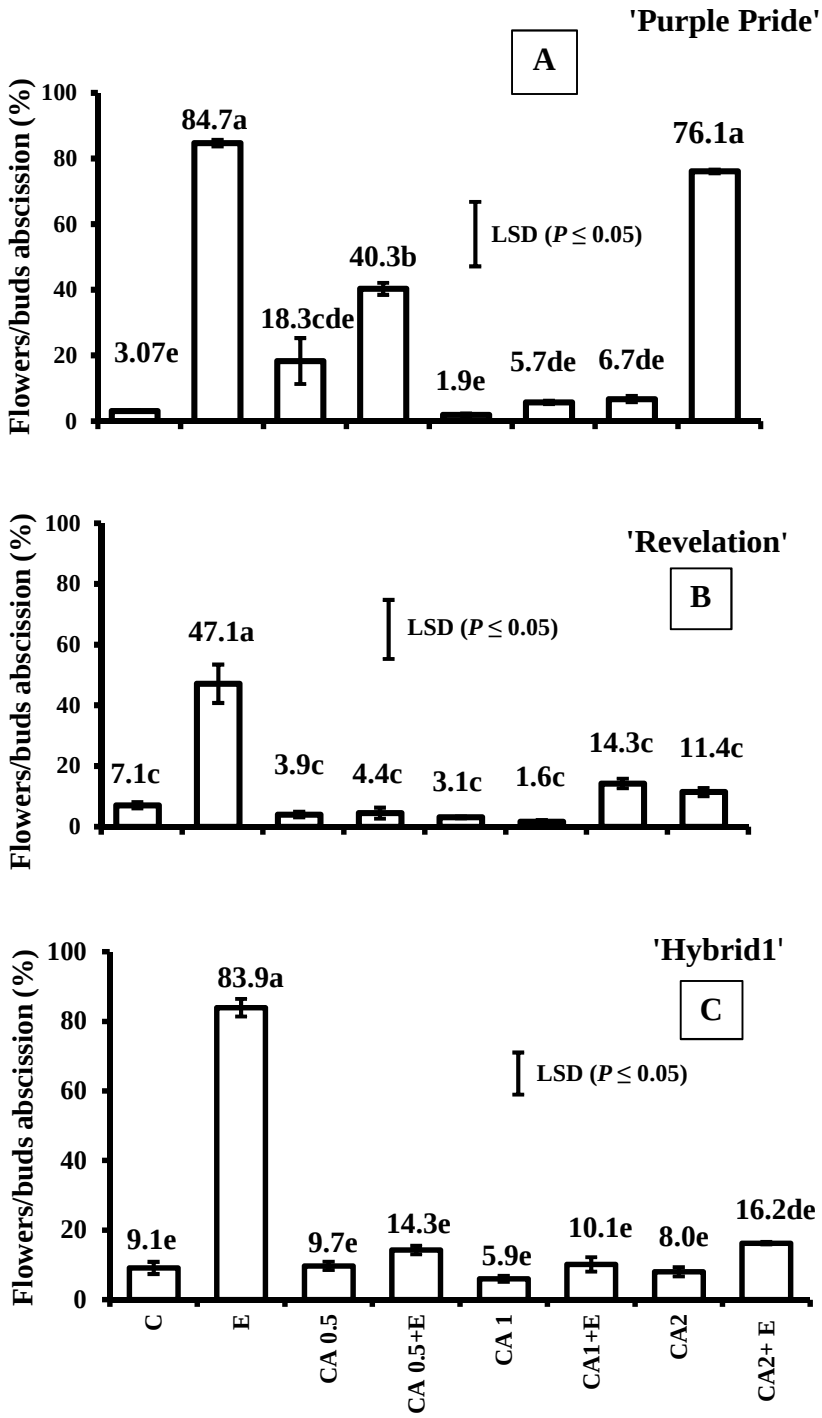


Figure. 5.5. Effects of different concentrations 0.5, 1.0 and 2.0 µM of fumigation of *trans*-cinnamaldehyde or ethylene (10 µL L^{-1}) alone and *trans*-cinnamaldehyde treatment followed by exposure to ethylene (10 µL L^{-1}) on flowers/buds abscission in (A) 'Purple Pride', (B)'Revelation' and (C) 'Hybrid1' waxflower on day four after treatments in 2015. Vertical bars represent SE, C = control, E = ethylene alone (10 µL L^{-1}), CA = *trans*-cinnamaldehyde alone, CA + E = *trans*-cinnamaldehyde fumigation followed by ethylene.

5.4. Discussion

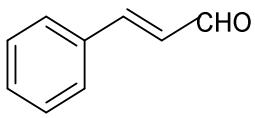
Abscission of flowers/buds is a major problem in Australian native waxflowers during the postharvest phase in transportation, handling, storage, and marketing which limits the potential of its international trade (Joyce, 1988; 1993; Faragher *et al*., 2010). Exposure of flower stems to ethylene induces flower petals abscission and causes premature wilting and yellowing of leaves consequently reducing display value in a range of flower crops including waxflower (Joyce 1988; Woltering and van Doorn, 1988; Cameron and Reid, 2001; Serek *et al*., 2006; Seaton and Poulish, 2010; Scariot *et al*., 2014). Flower stems fumigated with *trans*-cinnamaldehyde (1 µM) for 18 h followed by exposure to 10 µL L^{-1} of ethylene for 24 h has exhibited reduced flowers/buds abscission as compared to those exposed to ethylene (10 µL L^{-1}) alone in 'WX73' , 'WXFU','WX56' and 'WX58', waxflower during 2014 and 2015 Fig.5.7. Meanwhile, no such reduction in flowers/buds abscission was noted when 'WX17' waxflower stems fumigated with *trans*-cinnamaldehyde (1 µM) for 18 h followed by ethylene (10 µL L^{-1}) exposure for 24 h in 2014 Fig.5.1C. In 2015, amongst various concentrations 0.5, 1.0 and 2.0 µM of *trans*-cinnamaldehyde tested (1 µM) fumigation for 18 h followed by exposure to ethylene (10 µL L^{-1}) for 24 h was most effective in reducing flowers/buds abscission in 'Purple Pride', 'Revelation' and 'Hybrid1' waxflowers Fig.5.5A, B and C. The reduction of flowers/buds abscission with the fumigation of *trans*-cinnamaldehyde followed by exposure to ethylene (10 µL L^{-1}) for 24 h in 'WX73', 'WXFU', 'WX56', 'WX58', 'Purple Pride', 'Revelation' and 'Hybrid1' waxflowers in 2014 and 2015 may be ascribed to the inhibition of ethylene action by *trans*-cinnamaldehyde. Previously, the action of *trans*-cinnamaldehyde as ethylene antagonist has been attributed maybe to the presence of an oxygen functional group adjacent to the double bond in its structure that could make the *trans*-cinnamaldehyde more effectively interact with the ethylene for receptor than one not having an oxygen atom Fig.5.6. (Gricko *et al*., 2003). An alternative hypothesis is that the electron poor nature of the double bond in *trans*- cinnamaldehyde leads to stronger binding of ethylene to the ethylene receptor (Gricko *et al*., 2003). Earlier, Macnish *et al.* (1999) stated that the ethylene action antagonists bind to the ethylene receptor site thus turning off the ethylene signal transduction and gene expression pathway. Meanwhile, *trans*-cinnamaldehyde fumigation for 18 h followed by

exposure to ethylene (10 µL L^{-1}) for 24 h was not effective in reducing flowers/buds abscission in 'WX17' waxflower and can possibly be ascribed to the sensitivity of 'WX17' to ethylene. Similarly, Tieman and Klee, (1999) also showed that the differences among the *Chamelaucium* genotypes in the sensitivity to ethylene exposure may be due to genetic differences in the number of ethylene receptors or affinity of ethylene receptors and/or the result of the activity of the signal transduction pathway in plants tissue. Macnish *et al.* (2004a) reported that the sensitivity to ethylene exposure of cut Geraldton waxflowers seems to be an inherited trait. As an example, among the most highly sensitive genotypes to ethylene in waxflowers, *C. uncinatum* × *C. micranthum* cv. Sweet Georgia and *C. uncinatum* cv. 'Early Nir', 'Paddy's Late', 'Purple Pride', 'CWA Pink' and 'Early Hard' can shed 10% of their flowers when treated for 12 h with less than 0.01 µLL^{-1} of ethylene.

Trans-cinnamaldehyde (1 µM) treatment was more effective as compared to (0.5 and 2.0 µM) in reducing flowers/buds abscission in 'Purple Pride', 'Revelation' and 'Hybrid1' waxflowers when exposed to ethylene, suggesting its response is concentration dependent. Both *trans*-cinnamaldehyde and 1-MCP are similar as antagonising ethylene action (Serek and Sisler, 2001; Gricko *et al*., 2003). In Siam Tulip (*Curcuma aeruqinosa* Roxb.) flowers fumigated with different concentrations (0, 300, 600, 900 ppb) of 1-MCP, the treatment of 300 ppb for 8 h was most effective in reducing ethylene production and improving the keeping postharvest quality of cut tulip flowers by blocking the ethylene receptor site in flowers compared to other concentrations tested (Chutichudet *et al*., 2010). Furthermore, Serek *et al*. (1995b) found that application of 1-MCP (200 nL L^{-1}) was very effective in blocking ethylene responses in 'Wendy' waxflowers to reduce floral organs abscission and was even more useful to delay senescence. In *Cattleya alliances* flowers, 1-MCP during the first 2 days after treatment suppressed ACC oxidase activity and ethylene biosynthesis consequently extending flowers vase life (Yamane *et al*., 2004).

The results suggest that *trans*-cinnamaldehyde fumigation protects the waxflower organs from the adverse effects of ethylene because of its antagonist effects on ethylene action. Similarly, Cameron and Reid (2001) found that the treatment with 1-MCP (1 µL L^{-1}) has completely prevented ethylene-induced flowers and petal abscission in *Pelargonium peltatum.* In conclusion, *trans*-

cinnamaldehyde (1 µM) treatment seems to be effective in inhibiting the action of ethylene consequently reducing flowers/buds abscission in waxflower.



Trans-cinnamaldehyde C_9H_8O, Molecular weight: 132.2 g mol^{-1}

Figure. 5.6. Chemical structure of *trans*-cinnamaldehyde (C_9H_8O).

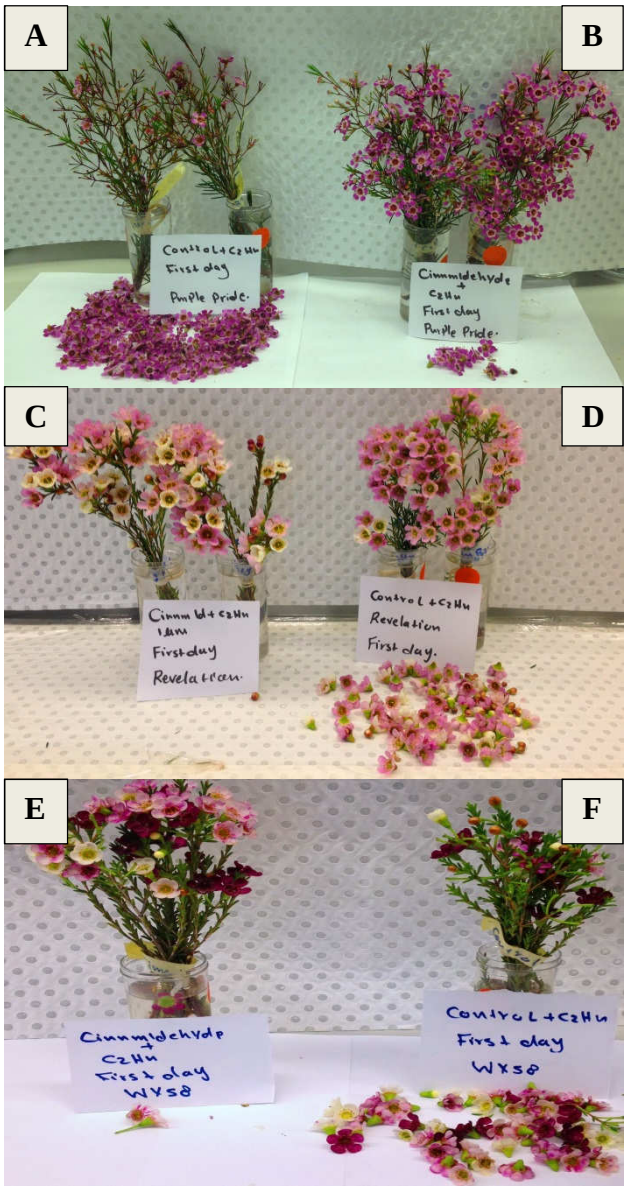


Figure. 5.7. Effects of fumigation of *trans*-cinnamaldehyde (1 µM) for 18 h and followed by exposure to ethylene (10 µL L^{-1}) for 24 h and the ethylene treatment alone on flower/buds abscission of 'Purple Pride', 'Revelation' and 'WX58' waxflowers. (A) 'Purple Pride' waxflower stems treated with ethylene alone (10 µLL^{-1}) for 24 h. (B) 'Purple Pride' waxflower stems treated with *trans*-cinnamaldehyde (1µM) for 18 h and followed by exposure to ethylene (10 µL L^{-1}) for 24 h. (C) 'Revelation' waxflower stems treated with *trans*-cinnamaldehyde (1 µM) for 18 h and followed by exposure to ethylene (10 µL L^{-1}) for 24 h. (D) 'Revelation' waxflower stems treated with ethylene alone (10µL L^{-1}) for 24 h. (E) 'WX58' waxflower stems treated with *trans*-cinnamaldehyde (1 µM) for 18 h and

followed by exposure to ethylene (10 µL L^{-1}) for 24 h. (F) 'WX58' waxflower stems treated with ethylene (10 µL L^{-1}) alone for 24 h.

CHAPTER 6

(*S*)-(-)-limonene fumigation protects waxflowers from detrimental effects of ethylene on abscission of flowers/buds.

Abstract

Postharvest flowers/buds abscission on stems of waxflowers causes serious economic losses to the Australian waxflower industry. This experiment aimed to investigate the effects of (*S*)-(-)-limonene in reducing the damaging effects of ethylene on abscission of flowers/buds in six genotypes of Geraldton waxflowers 'WX73', 'WXFU','WX17', 'WX58', 'WX56' and 'Purple Pride' in 2014 and 2015 respectively. The flowers stems were fumigated with ethylene (10 µL L^{-1}) for 24 h and (*S*)-(-)-limonene (1 µM) alone for 18 h and (*S*)-(-)-limonene fumigation followed by exposure to ethylene. Untreated sprigs served as control. The experimental design used in all the experiments was two-factor (treatments and time) factorial completely randomised design including three replications and three stems per replication. Cumulative abscission of flowers/buds was calculated for four consecutive days following 24 h of ethylene exposure. Fumigation with (*S*)-(-)-limonene (1 µM) for 18 h followed by exposure to 10 µL L^{-1} of ethylene significantly reduced flowers/buds abscission as compared to ethylene treatment alone in all genotypes except 'WX17'. When averaged over four days, mean flowers/buds abscission was significantly reduced when flower stems were fumigated with (*S*)-(-)-limonene followed by exposure to 10 µL L^{-1} of ethylene (26.7%, 30.9%, 62.4%, 13.6 and 6.4%) as compared to those exposed to ethylene treatment alone (89.9%, 82.0%, 77.4%, 85.9% and 68.8%) in 'WX73', 'WXFU', 'Purple Pride', 'WX56' and 'WX58' respectively. 'WX17' waxflower fumigated with (*S*)-(-)-limonene followed by exposure to 10 µL L^{-1} of ethylene did not show a significant reduction in flowers/buds abscission as compared to ethylene alone. The results reveal that (*S*)-(-)-limonene appears to be acting as an antagonist to ethylene action and consequent reduced flowers/buds abscission in waxflowers.

6.1. Introduction

Geraldton waxflower (*Chamelaucium uncinatum* Schauer), other *Chamelaucium* species and hybrids have become one of the attractive native plants and valuable cut flowers. *Chamelaucium* species are the major cut flowers exported from Australia in the recent past due to their relatively small pretty flowers and leaves (Beasley and Joyce, 2002; Growns, 2004; Seaton *et al*., 2007; Vitner *et al*., 2007; Gollnow and Worrall, 2010; Seaton and Poulish, 2010). Waxflower stems are strong enough and straight, and bear and support green leaves and attractive shiny small flowers (Yan, 2001; Gollnow and Worrall, 2010; Dinh et al., 2008, 2011). The Geraldton waxflower with possesses a distinct pleasant aroma. It starts to produce nectar at anthesis that continues for 7-10 days (Olley *et al*., 1996). Plummer *et al*. (2001) pointed out that the colours of petals range from white through pinks to mauve and purple, with the deep pinks and purple colours the most acceptable commercially. Dinh *et al*. (2011) estimated that the world production of waxflowers exceeds 300 million cut stems per year. Cut waxflowers are exported from Australia to European markets and are now ranked in the top 20 of sold volume of flowers (Yan, 2001; Gollnow and Worrall, 2010; Seaton and Poulish, 2010).

Extensive losses of floral organs (flowers/buds) in waxflower cut stems during transport, handling, storage and marketing can occur mainly as a result of unfavourable exposure to exogenous ethylene (Joyce, 1988; Faragher, 1989; Joyce, 1993). Faragher *et al*. (2010) stated that the abscission of floral organs of native Australian flowers such as *Boronia heterophylla, Backhousia myrtifolia, Baeckea virgata, Ceratopetalum gummiferum, Chamelaucium uncinatum,* some *Leptospermum, some Grevillea species, Telopea speciosissima, Thryptomene calycina, and Verticordia nitens, V. cooloomia, V. grandis* and *V. serrata*, is caused by the presence of ethylene around the flowers.

As a prelude, ethylene not only causes abscission of flowers/buds in the postharvest phase of waxflowers but also damage to both flower petals and leaves consequently reducing the value of the stems and resulting in a low price in export markets (Joyce, 1993). Several methods have been tested with different cultivars of waxflowers to downregulate ethylene production and ethylene action to overcome the adverse effects of ethylene on prolonging vase life. Joyce and Jones (1992)

suggested that the vase water containing 10 mg L^{-1} abscisic acid (ABA) alone or in combination with 10 mmol potassium chloride (KCl) was beneficial in promoting longevity of vase life of 'Purple Pride' and 'Alba' cultivars of waxflower. Studies carried out by Joyce *et al.* (1996) stated that addition of triadimenol fungicide (10 mg L^{-1}) into vase solution with accumulation of (ABA) regulated stomatal closure and improved water balance in waxflowers stems consequently extended vase life of flowers and leaves in 'Alba', 'Mullering Brook' and 'Purple Pride'. Damunupola *et al*. (2010) stated that the vase solutions containing an antibacterial compound such as (*S)*-carvone (0.318 to 0.636 mM) improved foliage and flower vase life in 'Mullering Brook' Geraldton waxflower. 'Mullering Brook', 'Alba' and 'Elegance' waxflowers treated with 0.5 mmol of silver thiosulphate (STS) for 15 - 22 h at 0°C resulted in inhibition of endogenous ethylene biosynthesis consequently reducing flower abscission (Joyce,1993). Beneficial effects of 1-MCP in protecting cut flowers from ethylene action have also been reported in 'Wendy' Geraldton waxflower, (Serek *et al*., 1995b), *Hibiscus rosa* (Reid *et al*., 2002), Zonal Geraniums (*Pelargonium x hortorum*) (Jones *et al*., 2001), *Rosa hybrida* (Liao *et al*., 2013), *Dianthus caryophyllus* and *Delphinium* (Ichimura *et al*. 2002). Moreover, a single application of 1-MCP (10 nLL^{-1}) for 12 h during the postharvest seems to be the most effective method for protecting and reducing waxflower losses (Macnish *et al*., 2000b; Gollnow and Worrall, 2010; Seaton and Poulish, 2010).

Application of different ethylene antagonists such as STS and 1-MCP are known to reduce damaging effects of ethylene in horticultural crops (Kader, 2003). Limonene is a natural monoterpene found in citrus and other fruit and is considered as environmentally friendly, when used as an adjuvant for agricultural chemicals as registered with the Environmental Protection Agency (Ibrahim *et al*., 2001; Hollingsworth, 2005). Previously, insecticidal, repellent and antimicrobial activity of limonene and its potential use in controlling insect pests as spray or dipping method for harvested commodities such as vegetables, fruits or cut and potted flowers have been reported by Ibrahim *et al*. (2001) and Hollingsworth (2005). In addition, limonene has been classified by the U.S. Food and Drug Administration as a Generally Recognised As Safe (GRAS) compound and can be used as an additive to food or flavouring (EPA, 1994). Hollingsworth (2005) also stated that (1%) of

limonene solution has no phytotoxic effects on thick and waxy leaves of ornamental plants such as orchids, palms and cycads.

In addition, some preliminary research on anti-ethylene properties of various monoterpenes in plants suggested that limonene with one double bond in the ring and one outside but, the mode of action as the antagonistic effect on ethylene is yet unclear (Grichko *et al.*, 2003). Currently, no information is available on the effect of (*S*)-(-)-limonene on inhibiting flowers/buds abscission in native waxflower or any other plant. It was hypothesised that an antagonistic effect of (*S*)-(-)-limonene on ethylene may regulate the postharvest abscission of flowers/buds in 'Geraldton wax'. Therefore, ethylene antagonistic effects of (*S*)-(-)-limonene were investigated in regulating abscission of flowers/buds on the stems of different genotypes of waxflowers by exposing to (*S*)-(-)-limonene and ethylene alone and (*S*)-(-)-limonene fumigation followed by ethylene exposure.

6.2. Materials and Methods

6.2.1. Sources of chemical

(*S*)-(-)-limonene Fig. 6.5. was purchased from Sigma-Aldrich, Castle Hill, NSW, Australia. Ethylene gas (98%) was procured from BOC Gases, Australia Ltd., Perth, Australia.

6.2.2. Plant material

The flower stems of *Chamelaucium* genotypes were harvested from five-year-old bushes which were grown under irrigation and fertigation (Seaton and Poulish, 2010) at the Department of Agriculture and Food Western Australia (DAFWA), South Perth. Six separate experiments were conducted during the winter-to-spring (June to October) in flowering season 2014 and 2015 to evaluate the effects of (*S*)-(-)-limonene as an ethylene antagonist on fresh cut waxflower stems of 'WX73', 'WXFU' and 'WX17' during 2014 and 'Purple Pride', 'WX56' and 'WX58' in 2015.

6.2.3. Harvesting flowering stems

The flowering stems of six genotypes 'WX73', 'WXFU', 'WX17', 'Purple Pride', 'WX56' and 'WX58' were picked (60 to 70 cm in length) in the early morning and immediately placed in the bucket containing clean tap water. At the laboratory, stems were completely randomised then recut under water to a length of 30cm to avoid air embolism prior to the application of different treatments. The table below presents the percentage of flowers open in six genotypes tested with a standard error of the mean (SE).

Table. 6.1. The percentage of open flowers on stems at the time of harvest.

Year	Genotype	Flowers open on stems (%)
2014	'WX73'	81.8±11.2%
2014	'WXFU'	95.7±3.4%
2014	'WX17'	67.7±14.2%
2015	'Purple Pride'	62.2±9.9%
2015	'WX56'	74.5±13.8%
2015	'WX58'	79.4±5.1%

± = Standard error of the mean (SE)

6.2.4. Treatments and methods

During the experimental period, in all the treatments the flower sprigs were kept in 250 ml small translucent plastic vases containing distilled water. Flower stems kept in distilled water with no fumigation treatment were assigned as a control. Flower branches were fumigated for 24 h with ethylene (10 µL L^{-1}) alone, (*S*)-(-)-limonene (1 µM) alone for 18 h and (*S*)-(-)-limonene (1 µM) for 18 h followed by exposure to ethylene (10 µL L^{-1}) 24 h. The (*S*)-(-)-limonene compound was applied (1µM) with the flower stems on filter papers in petri dishes inside the 60 L plastic drums. All other treatments were applied to flower stems in the plastic drums as detailed in Chapter 5, Section 5.2.4.

6.2.5. Experiments

6.2.5.1.Experiment 1: Effect of fumigation of (*S*)-(-)-limonene, ethylene alone and (*S*)-(-)-limonene treated flowers followed by ethylene exposure on abscission of flowers/buds of 'WX73' 'WXFU', 'WX17', 'Purple Pride', 'WX56' and 'WX58' genotypes of waxflowers in 2014 - 2015.

Antagonistic effect of (*S*)-(-)-limonene was tested in three independent experiments using 'WX73', 'WXFU' and 'WX17' in 2014 and three more independent experiments in 2015 on 'Purple Pride', 'WX56' and 'WX58'. After the termination of the 24h of ethylene treatment the flower bunches were taken out of the vases and softly beaten on the table to collect the abscised flowers/buds. Flowers/buds abscission was recorded daily following the treatments for four consecutive days. Cumulative flowers/buds abscission during the four days was calculated and expressed as a percentage of total abscised and intact flowers/buds as explained in Chapter 5, Section 5.2.4.

6.2.6. Experimental design and statistical analysis of data

All the six experiments were laid out by following two-factor factorial completely randomised design including the (treatment and time) with three replications and three stems were treated as an experimental unit. The data were subjected to a two-way analysis of variance (ANOVA) by employing GenStat 14th edition (Lawes Agricultural Trust, Rothamsted Experimental Station, Rothamsted, UK). Treatment means were compared by LSD at ($P < 0.05$) and means (± SE) were shown as appropriate. Differences among treatments were further analysed using Duncan's Multiple Range Test as explained in Chapter 5, Section 5.2.6.

6.3. Result

6.3.1. Experiment1. Effects of (*S*)-(-)-limonene fumigation on mean flowers/buds abscission in 'WX73', 'WXFU', 'WX17', 'Purple Pride', 'WX56' and 'WX58' genotypes of waxflower in 2014 -2015.

When averaged over four-day periods, mean abscission of flowers/buds was significantly ($P \leq 0.05$) higher (89.9 % and 82.1%) for 'WX73' and 'WXFU' waxflower stems when exposed for 24 h to exogenous ethylene (10 µL L^{-1}) alone as compared to the untreated control flowers (5.5% and 9.9%) and all other treatments respectively in 2014 Fig. 6.1A and B. In addition, 'WX17' genotype mean abscission of flowers/buds was significantly ($P \leq 0.05$) increased (99.6% and 97.8%) when 'WX17' stems were fumigated with (*S*)-(-)-limonene (1 µM) followed by ethylene (10 µL L^{-1}) and also in ethylene treatment alone Fig. 6.1C. Meanwhile, the flower sprigs of 'WX73' and 'WXFU' exhibited significantly lower mean abscission of flowers/buds when treated with (*S*)-(-)-limonene (1 µM) for 18 h followed by exposure to 10 µL L^{-1} of ethylene (26.7% and 30.9%) as compared to ethylene treatment alone (89.9 % and 82.1%) respectively Fig. 6. 1A and B. Such antagonistic effect of (*S*)-(−)-limonene to ethylene action in 'WX17' waxflower was lacking in 2014 Fig. 6.1C. Flower stems of 'Purple Pride', 'WX56' and 'WX58' exposed to ethylene (10 µL L^{-1}) alone showed significantly ($P \leq 0.05$) increased mean abscission of flowers/buds (77.4%, 85.9% and 68.8% respectively) as compared with the untreated control flowers (43.4%, 7.10% and 1.30%) and all other treatments in 2015 Fig. 6.2D, E and F. Meanwhile, (*S*)-(-)-limonene (1 µM) fumigation for 18 h followed by exposure to ethylene (10 µL L^{-1}) significantly ($P \leq 0.05$) reduced mean abscission of flowers/buds in 'Purple Pride', 'WX56' and 'WX58' (62.4%, 13.6% and 6.3%) respectively Fig. 6.2D, E and F.

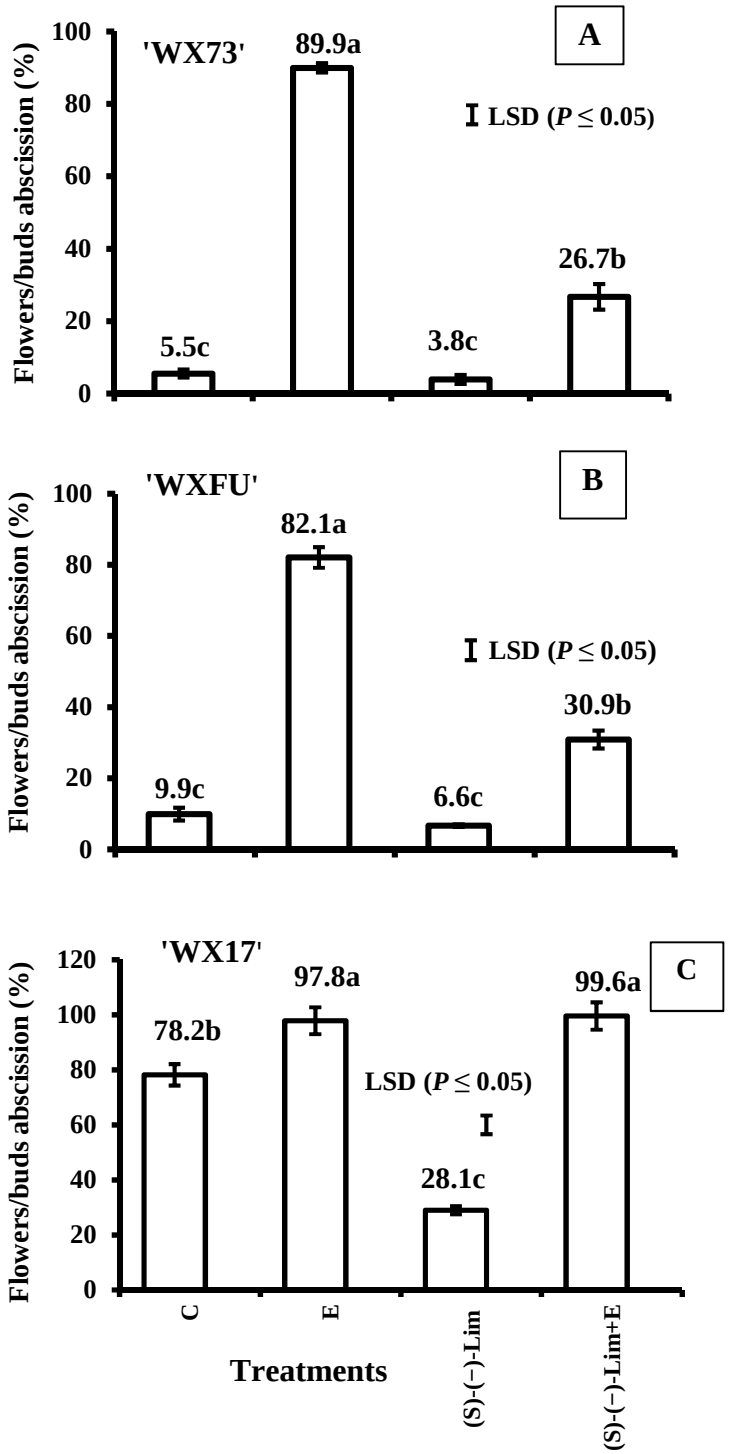


Figure. 6.1. Effects of fumigation of (*S*)-(-)-limonene (1 µM), ethylene (10 µL L^{-1}) alone and (*S*)-(−)-limonene followed by exposure to ethylene (10 µL L^{-1}) on mean flowers/buds abscission in (A) 'WX73', (B) 'WXFU' and (C) 'WX17' waxflower in 2014. Vertical bars represent SE. C = control, E = ethylene alone (10 µL L^{-1}), (*S*)-(-)-Lim = (*S*)-(-)-limonene alone, (*S*)-(−)-Lim + E = (*S*)-(-)-limonene followed by ethylene.

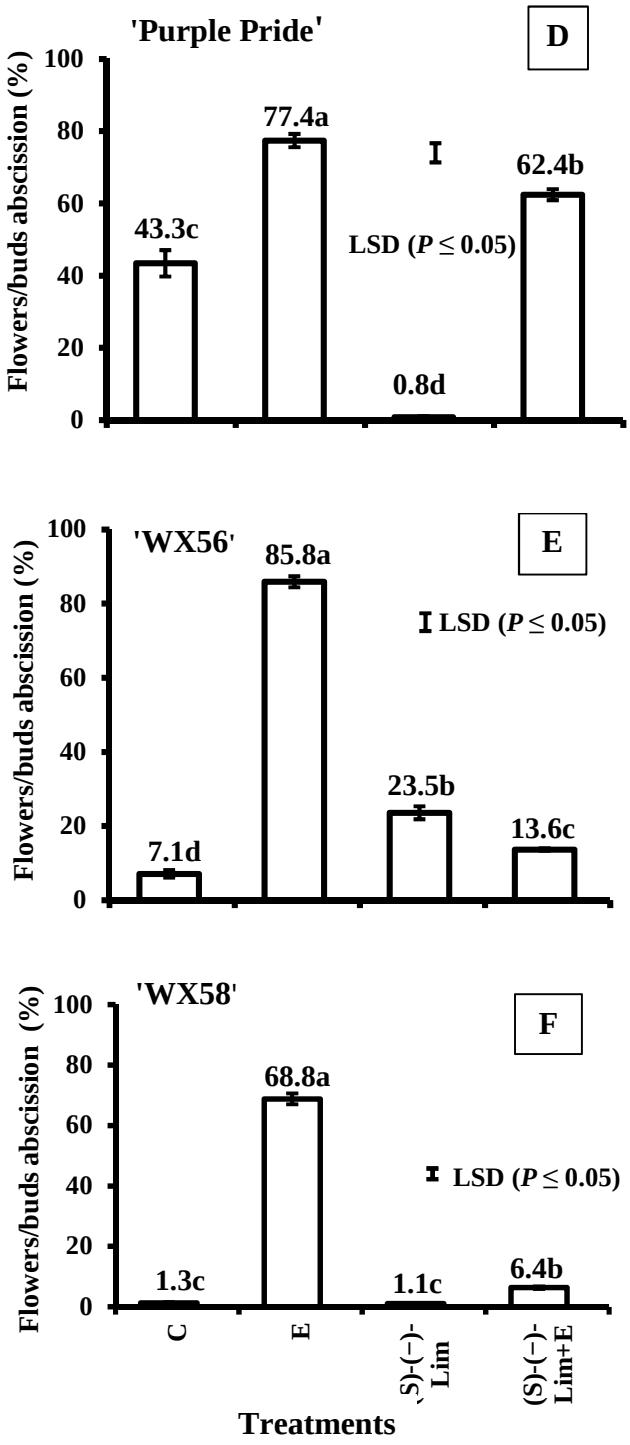


Figure. 6. 2. Effects of fumigation of (*S*)-(-)-limonene (1 µM), ethylene (10 µL L^{-1}) alone and (*S*)-(-)-limonene followed by exposure to ethylene (10 µL L^{-1}) on mean flowers/buds abscission in (D)'Purple Pride', (E) 'WX56' and (F)'WX58' waxflower in 2015. Vertical bars represent SE. C = control, E = ethylene alone (10 µL L^{-1}), (*S*)-(-)-Lim = (*S*)-(-)-limonene alone, (*S*)-(-)-Lim + E = (*S*)-(-)-limonene fumigation followed by ethylene.

6.3.2. Experiment 1. Effects of (*S*)-(-)-limonene fumigation on cumulative flowers/buds abscission in 'WX73', 'WXFU', 'WX17', 'Purple Pride', 'WX56' and 'WX58' genotypes of waxflower.

The antagonistic effects of (*S*)-(-)-limonene (1 µM) fumigation, ethylene (10 µL L^{-1}) alone and in combination on cumulative abscission of flowers/buds over a period of four days after treatment in 'WX73', 'WXFU', 'WX17', 'Purple Pride', 'WX56' and 'WX58' waxflowers were evaluated over four days during 2014 and 2015 Fig. 6.3 and 6.4. The flower sprigs of 'WX73' and 'WXFU' fumigated with 1 µM (*S*)-(-)-limonene and followed by exposure to ethylene (10 µL L^{-1}) for 24 h showed significantly reduced cumulative flowers/buds abscission from day one to four (19.3% to 31.1 and 26.8 to 33.8%) in 'WX73' and 'WXFU' respectively in 2014 as compared to those treated with 10 µL L^{-1} ethylene alone (86.2% to 91.3% and 79.1 to 83.9% respectively) Fig. 6.3A and B. Meanwhile, the lowest cumulative of flowers/buds abscission was shown on the 'WX73' and 'WXFU' stems treated with the (*S*)-(-)-limonene (1 µM) alone for 18 h (3.1% to 5.1% and 3.4% to 9.6% respectively) as compared to all other treatments and control Fig. 6.3A and B. However, cumulative flowers/buds abscission from day one to four in 'WX17' waxflower stems fumigated with (*S*)-(-)-limonene (1 µM) followed by ethylene exposure (10 µL L^{-1}) did not differ significantly from the flower stems treated with ethylene exposure (10 µL L^{-1}) alone Fig. 6. 3C.

The highest cumulative abscission of flowers/buds was observed on 'Purple Pride', 'WX56' and 'WX58' branches when fumigated with the ethylene treatment alone from day one to day four (75.5% to 79.1%, 83.9% to 86.7% and 57.6% to 74.6% respectively) as compared to the sprigs treated with 1 µM (*S*)-(-)-limonene and followed by exposure to ethylene (10 µL L^{-1}) for 24 h (52.7% to 66.3% 'Purple Pride', 11.6% to15.3% 'WX56' and 0.75 to 11.8% 'WX58') in 2015 Fig. 6.4D, E and F. Meanwhile, the (*S*)-(-)-limonene treatment alone showed the lowest cumulative flowers/buds abscission in 'Purple Pride' (0.0% to 1.5%) as compared to the untreated stems (39.8% to 46.5%). Untreated 'WX56' and 'WX58' waxflower stems exhibited the lowest cumulative abscission of flowers/buds as compared to all other treatments Fig. 6.4E and F.

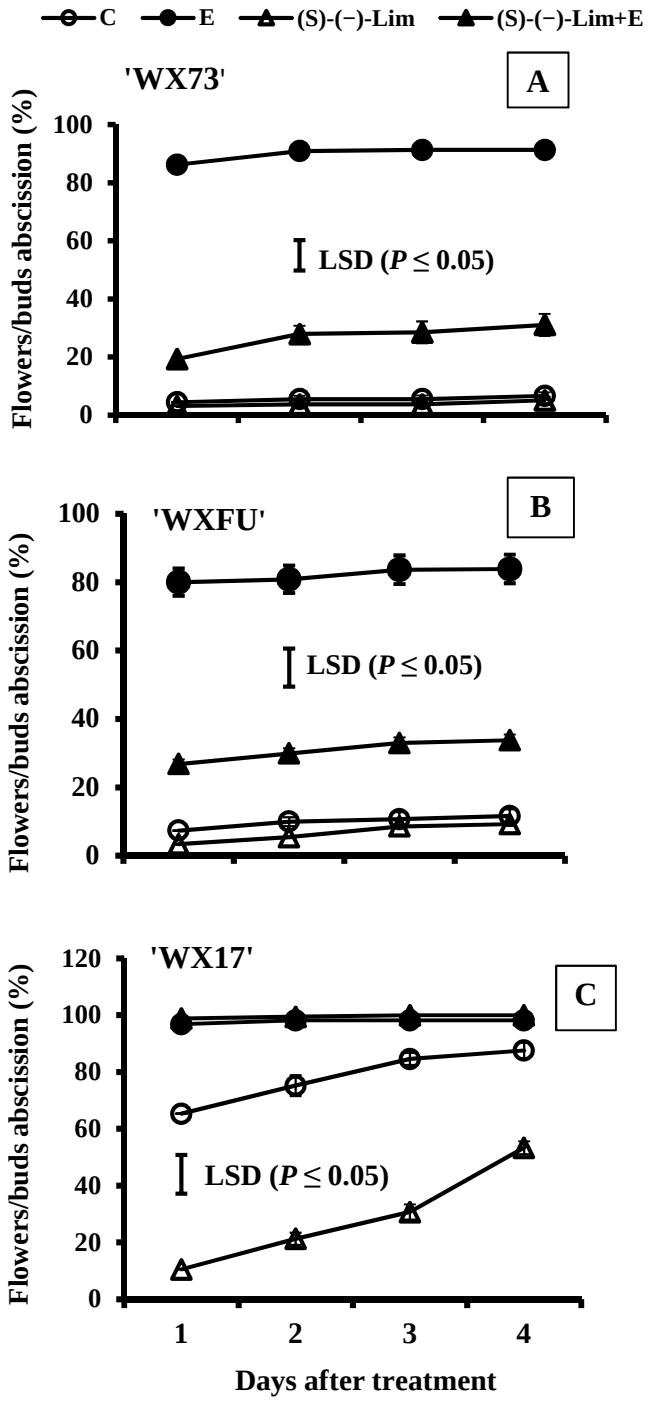


Figure. 6.3. Effects of fumigation of (*S*)-(-)-limonene (1 µM), ethylene (10 µL L^{-1}) alone and (*S*)-(-)-limonene followed by exposure to ethylene (10 µL L^{-1}) on cumulative abscission of flowers/buds over four days after treatment in (A) 'WX73', (B) 'WXFU' and (C)'WX17' waxflower in 2014. n = three replications (three stems per replication), vertical bars represent SE, C = control, E = ethylene alone (10 µL L^{-1}), (*S*)-(-)-Lim = (*S*)-(-)-limonene alone, (*S*)-(-)-Lim + E = (*S*)-(-)-fumigation followed by ethylene.

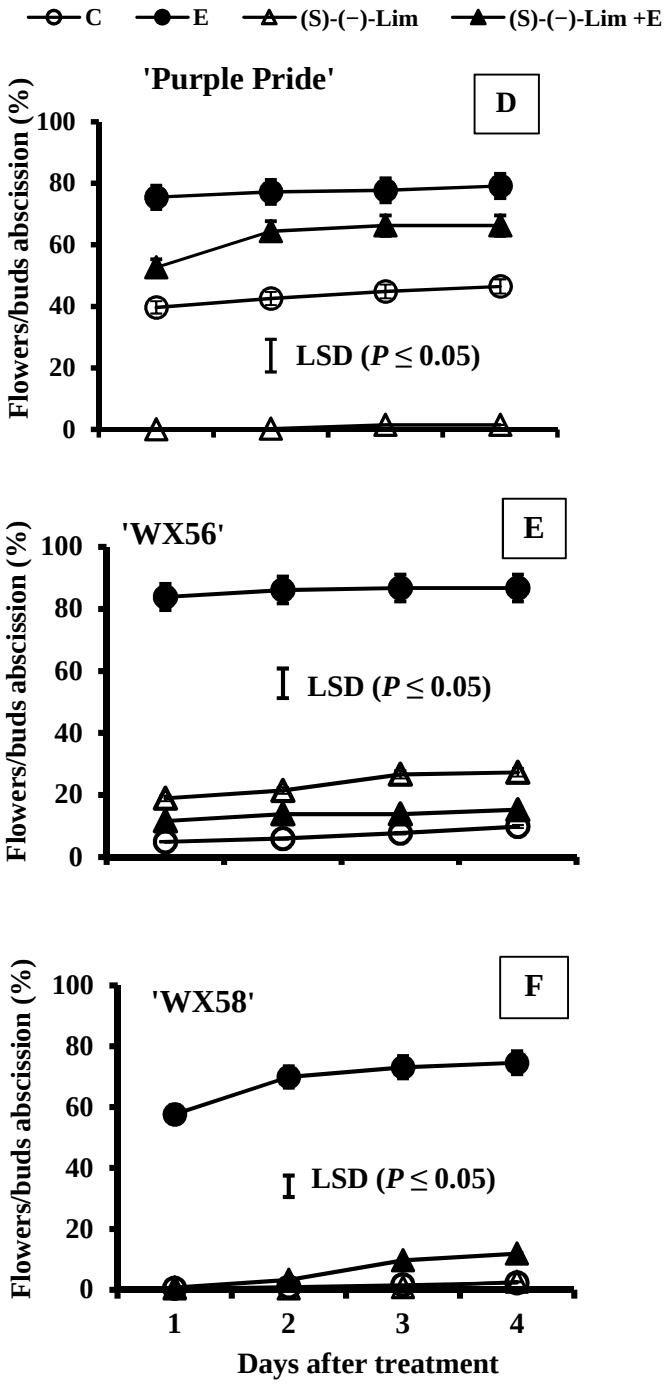


Figure. 6.4. Effects of fumigation of (*S*)-(-)-limonene (1 µM), ethylene (10 µL L^{-1}) alone and (*S*)-(-)-limonene followed by exposure to ethylene (10 µL L^{-1}) on cumulative abscission of flowers/buds over four days after treatment in (D) 'Purple Pride', (E) 'WX56' and (F)'WX58' waxflower in 2014. n = three replications (three stems per replication), vertical bars represent SE, C = control, E = ethylene alone (10 µL L^{-1}), (*S*)-(-)-Lim = (*S*)-(-)-limonene alone, (*S*)-(-)-Lim + E = (*S*)-(-)-limonene fumigation followed by ethylene.

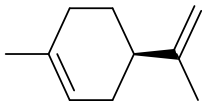
6.4. Discussion

Postharvest abscission of flowers/buds, petal and leaf senescence in waxflower causes serious economic losses to the Australian waxflower industry (Joyce, 1993; Dinh *et al*., 2008; Seaton and Poulish, 2010) as discussed in Chapter 5, Section 5.1. To avoid the harmful effects of exposure to unfavourable conditions that accelerate ethylene biosynthesis or render the flower more sensitive to ethylene may be an effective approach to reducing postharvest losses in horticulture produce including flowers (Kader, 2003; Ebrahimzadeh *et al*., 2008; Scariot *et al*., 2014). As a prelude, application of different ethylene antagonists such as STS, 1-MCP is effective in reducing damaging effects of ethylene in horticultural crops but, is coupled with some weaknesses (Blankenship, 2001; Blankenship and Dole, 2003; Kader, 2003; Seaton and Poulish, 2010) as also discussed in Chapter 5, Section 5.1.

Fumigation of (*S*)-(-)-limonene (1 µM) for18 h followed by exposure 10 µL L^{-1} of ethylene for 24 h has substantially lowered flowers/buds abscission on 'WX73', 'WXFU', 'Purple Pride', 'WX56' and 'WX58' waxflower during 2014 and 2015 Fig. 6.1A and B. Fig. 6.2D, E and F as compared to those treated with ethylene alone. Significant reduction in abscission of flowers/buds was shown in different genotypes of waxflower when stems were treated with (*S*)-(-)-limonene followed by exposure to ethylene Fig. 6.6. This can probably be ascribed to the inhibitory activity of applied (*S*)-(-)-limonene on ethylene action in waxflower. The experimental data suggest that (*S*)-(-)-limonene treatment seems to be very effective in blocking the ethylene action in waxflower consequently reducing the abscission of flowers/buds. Possibly, (*S*)-(-)-limonene seems to be binding to the ethylene receptor on 'WX73', 'WXFU', 'Purple Pride', 'WX56' and 'WX58' waxflower but, the exact mode of action of (*S*)-(-)-limonene as an ethylene antagonist warrants to be further investigated. Previously, Gricko *et al*. (2003) also reported the existence of one double bond as functional groups in the chemical structure one inside the ring and the other outside the ring Fig. 6.5. Possibly, the interaction of the double bonds in this chemical structure with the ethylene receptor site may make these compounds active in masking ethylene receptor sites in flowers.

Meanwhile, no such reduction in abscission of flowers/buds in 'WX17' was noted when the stems were treated with (*S*)-(-)-limonene followed by ethylene

exposure and with ethylene Fig. 6.1C. This shows genetic variation in the response of different genotypes of waxflowers. Similarly, the sensitivity of waxflower to ethylene exposure appeared to be a genetic trait particularly ascribed to the signal transduction pathways and/or the number as well as the affinity of ethylene receptors in the floral tissues (Tieman and Klee, 1999; Macnish *et al*., 2004a). A substantial reduction in abscission of flowers/buds in five different genotypes of waxflower treated with (*S*)-(-)-limonene prior to the exposure to ethylene suggests that it acts as an ethylene antagonist. (*S*)-(-)-limonene also has potential as a postharvest treatment to prevent flower/buds abscission in waxflower. Variation among waxflowers genotypes may require further investigation to ensure that it is effective for varieties of waxflower being treated. Also, the degree of ethylene control needs to be at a sufficient level to be considered as a replacement for other methods such as STS and 1-MCP.



(*S*)- (-)-Limonene ($C_{10}H_{16}$) Molecular Weight: 136.2 g mol^{-1}

Figure. 6.5. Chemical structure of (*S*)-(-)-limonene ($C_{10}H_{16}$).

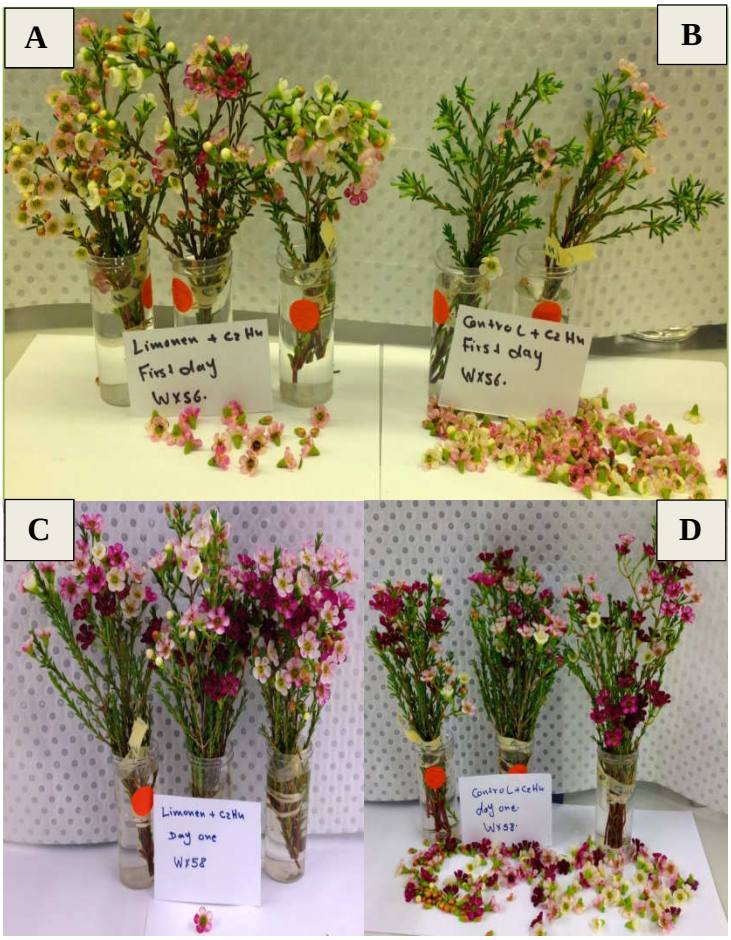


Figure. 6.6. Effects of fumigation of (*S*)-(-)-limonene (1 µM) for 18 h and followed by exposure to ethylene (10 µL L^{-1}) for 24 h and the ethylene treatment alone on flowers/buds abscission of 'WX58' and 'WX56' waxflower. (A) 'WX58' waxflower stems treated with (*S*)-(-)-limonene (1 µM) for 18 h and followed by exposure to ethylene (10 µL L^{-1}) for 24 h. (B) 'WX58' waxflower stems treated with ethylene (10 µL L^{-1}) alone for 24 h. (C) 'WX56' waxflower stems treated with (*S*)-(-)-limonene (1µM) for 18 h and followed by exposure to ethylene (10 µL L^{-1}) for 24 h. (D) 'WX56' waxflower stems treated with ethylene (10 µL L^{-1}) alone for 24 h.

CHAPTER 7

Influence of 1-Hexylcyclopropene fumigation on reducing detrimental effects of ethylene on abscission of flowers/ buds in different genotypes of cut waxflowers.

Abstract

Various methods have been reported for preventing the ethylene damaging effect on postharvest characteristics of ornamental waxflower by blocking ethylene production or inhibition of ethylene action. The objective of this study was to demonstrate the effectiveness of fumigation with 1-hexylcyclopropene (1-HCP) as anti-ethylene in five genotypes of waxflowers 'WX73', 'Purple Pride', 'WX56', 'WX58' during 2014 and 'Purple Pride' and 'Hybrid1' during 2015. The effects of fumigation 1-hexylcyclopropene at 1 µM followed by exposure to ethylene (10 µL L^{-1}) for 24 h and also, the effect of three different concentrations 0.5, 1.0 and 2.0 µM of 1-hexylcyclopropene followed by exposure to ethylene on flowers/ buds abscission were also evaluated in 'Purple Pride' and 'Hybrid1' during 2015. The flower sprigs were fumigated with 1-hexylcyclopropene using 60 L plastic drums for 18 h followed by 24 h exposure to ethylene with untreated flower stems kept as control. The design was a one or two- factor factorial completely randomised design with three replicates and three stems per replication. Cumulative abscission of flowers/buds was calculated for four consecutive days following 24 h of ethylene exposure. The percentage of flowers/buds abscission in each replicate was calculated daily for four days, while with different concentrations experiment the data was calculated in day one after treatment. Fumigation of 1-hexylcyclopropene (1 µM) followed by single exposure to ethylene (10 µL L^{-1}) has significantly ($P \leq 0.05$) reduced flowers/ buds abscission by (2.7% 'WX73', 4.8% 'Purple Pride', 14.6% 'WX58' and 23.5% 'WX56') when compared to ethylene treatment alone significantly where flowers/buds abscission increased by (95.1% 'WX73', 77.4% 'Purple Pride', 68.8% 'WX58' and 85.9% 'WX56'). 1-HCP concentration of 1 µM was the most effective amongst three concentrations tested in reducing flowers/ buds abscission when fumigated for 18 h followed by exposure to ethylene (10 µL L^{-1}) for 24 h. The results clearly indicate that application of 1 µM 1-hexylcyclopropene on several waxflowers genotypes substantially reduced flowers/ buds abscission.

7.1. Introduction

The maintenance of quality of ornamental plants through minimization of the adverse effects of ethylene during the supply chain is a prerequisite for commercial success (Ebrahimzadeh *et al*., 2008; Ferrante *et al*., 2015). The sensitivity to ethylene exposure has significant implications for transport and handling of 'Geraldton wax' cut flowers (Joyce, 1988, 1993). Flowers/buds abscission in waxflower during the postharvest phase causes serious economic losses (Faragher, 1988; Joyce 1988; Seaton and Poulish, 2010). Gollnow and Worral (2010) reported that the waxflower stems can lose up to 85% of their flowers if exposed to ethylene (Seaton and Poulish, 2010). Eyre *et al*. (2006) stated that the economic success in Australian 'Geraldton wax' depends on the protection of the flowers from the floral organ abscission and senescence process. Sutton (2004) reported that extension of waxflower lifespan of more than five days could achieve more than 20% higher profit.

Ethylene is known to modulate a range of physiological and developmental processes at various stages in plants commencing from seed germination to tissue senescence (Sisler *et al*., 2001; Martinez-Romero *et al*., 2007; Lin *et al*., 2009). Ethylene antagonists are considered as a more effective tool in inhibiting the response of the plant tissues to exogenous and endogenous ethylene and consequently, extend the vase life in a range of sensitive ornamental cut flowers (Philosoph-Hadas *et al*., 2003; Sisler and Serek, 2003; Serek *et al*., 2006; Apelbaum *et al*., 2008).

Several reports have indicated that cyclopropenes and some derivatives including 1-methylcyclopropene (1-MCP) irreversibly bind to the ethylene receptor and delay the senescence in ornamental and horticultural crops (Serek *et al*., 1995a, 1995b; Ichimura *et al*., 2002; Blankenship and Dole, 2003; Serek *et al*., 2007; Reid and Staby, 2008). 1-MCP has been found to be a very effective ethylene blocking agent and extends storage and shelf life of fruits, leafy vegetables, herbs and flower crops (Serek and Sisler, 2001; Sisler and Serek, 2003; Saleh-Lakha *et al*., 2004; Serek *et al*., 2006).

Exogenous application of 1-MCP has been reported to extend the shelf life of flowers including *Primula sinensis* L. (Ghassemzade *et al*., 2013), *Penstemon*

hartwegii Benth. (Serek *et al*., 1995a), *Freesia* (Zencirkiran, 2010) and waxflower (Serek *et al*., 1995b). Although, 1-MCP has been reported to be an effective blocker of ethylene responses (Serek *et al*., 1994b; Sisler and Serek, 1997; Blankenship, 2001). The practical usage of 1-MCP is coupled with a number of limitations such as it can be applied as a fumigant only or as sachets in flower cartons, not as a dip or loading and spray to cut flowers, typically providing protection to waxflowers genotypes for 4 days (Grichko, 2006; Goren *et al*., 2008; Seaton and Poulish, 2010). Grichko (2006) reported that newer suitable ethylene antagonists to control the harmful effects of ethylene such as through long lasting protection of plants upon a single exposure, water solubility, potency, non-phototoxicity, efficacy at minute concentrations, colourlessness and ease to the application. The use of such ethylene antagonists is essential to extend postharvest life and maintain the freshness of horticultural produce (Feng *et al*., 2004; Apelbaum *et al*., 2008; Goren *et al*., 2008).

Recently, novel compounds made of 1-substituted cyclopropene analogues of 1-MCP with a similar action and structure with a number of carbon chains at the 1-position in their chemical structure have been developed which are more effective in terms of concentration and timing of application required to inhibit the ethylene effects in plants (Sisler *et al*., 2001, 2003). 1-Hexylcyclopropene (1-HCP) is one of the series of substituted cyclopropenes with a molecular weight (124 g) which, has already been screened for its potency as an ethylene antagonist on *Kalanchoe blossfeldiana* Poelln. flower and achieved a high protection of display life of up to 5 - 6 days. This is comparable to 1-MCP in its capability to compete with ethylene for the receptor, by binding to metal in the ethylene receptor and blocking the receptor (Kebenei *et al.*, 2003a) and may be due to the structure or electronic effect of 1-hexylcyclopropene (Sisler *et al*., 2001; Kebenei *et al.*, 2003a).

Sisler *et al*. (2003) demonstrated that compounds with a longer side chain of more than 5 carbons in the 1-position have an enhanced efficacy and these compounds as anti-ethylene beside the low concentration required to inactivate the ethylene receptor and provide the longer time protection from ethylene. For example, 1-hexylcyclopropene (1-HCP) and 1-octylcyclopropene (1-OCP) (Buanong *et al*., 2005; Sisler *et al*., 2005; Serek *et al.*, 2007). In addition, green bananas (*Musa sapientum* L.) treated with 0.4 ± 0.01 nL L^{-1}1-HCP following exposure to 333 μL L^{-1}

ethylene for 18 h was effective in protecting banana fruit from ethylene action and delaying the ripening process for 20 days (Sisler *et al*., 2003). Kebenei *et al*. (2003b) reported that fumigation with 1-OCP or 1-MCP was (200 nL L^{-1}) effective in protecting the cut flowers of sweet pea (*Lathyrus odoratus* L.) against the adverse effects of ethylene.

Only limited research work has reported about the effective activity of 1-substituted cyclopropene (1-HCP) in extending the flower life of potted *Kalanchoe blossfeldiana* plant (Kebenei *et al*., 2003a). Apparently, no research work has been reported about the efficacy of 1-HCP in reducing the abscission of flowers/buds in Australian waxflowers genotypes. It was hypothesised that 1-HCP may block ethylene action and consequently reduce flower/bud abscission in different Australian waxflowers. The objective of this study was to investigate the effects of 1-HCP and different concentrations on flowers/ buds abscission in different waxflower genotypes.

7.2. Materials and Methods

7.2.1. Sources of chemicals

1-Hexylcyclopropene Fig.7.7 was synthesised at the Department of Chemistry, Curtin University by Dr Payne's group. Pure ethylene gas (98%) was purchased from BOC Gases, Australia Ltd., Perth, Australia.

7.2.2. Plant material

Five different genotypes of 'Geraldton wax' 'WX73', 'Purple Pride', 'WX56', 'WX58' and 'Hybrid1' have been used in the experiments to examine the efficacy of 1-HCP fumigation as ethylene inhibitor during two consecutive years 2014 and 2015. Also, the effect of different concentrations 0.5, 1.0 and 2.0 µM of 1-HCP was evaluated on 'Hybrid1' and 'Purple Pride' during 2015. The waxflower branches were sourced from six-year-old bushes from the Department of Agriculture and Food Western Australia (DAFWA).

7.2.3. Harvesting flowering stems

The flowering stalks of different genotypes (60 - 75 cm) were collected with flowers 57% to 98% fully opened in the early morning from shrubs located at (DAFWA) and placed in buckets containing tap water. The branches were trimmed to 30 cm length and placed immediately in distilled water prior to starting different treatments.

Table. 7.1.The percentage of open flowers on stems at the time of harvest.

Year	Genotype	Flowers open on stems (%)
2014	'WX73'	78.7±15.2%
2015	'WX56'	66.9±8.7%
2015	'WX58'	77.4±5.0%
2015	'Purple Pride'	57.5±11.1%
2015	'Purple Pride'	65.5±6.3%
2015	'Hybrid1'	98.3±1.6%

± = Standard error of the mean (SE)

7.2.4. Experiments

7.2.4.1. Experiment 1: Effect of fumigation of 1-hexylcyclopropene, ethylene alone and 1-hexylcyclopropene treated flowers followed by ethylene exposure on abscission of flowers/buds in 'WX73', 'Purple Pride', 'WX56' and 'WX58' genotypes of waxflowers in 2014 -2015.

In the first experiment, four different treatments were applied to the flower stems of waxflower including 1-hexylcyclopropene (1 µM) fumigation alone, 1-hexylcyclopropene (1 µM) fumigation alone for 18 h followed by exposure to 10 µL L^{-1} ethylene for 24 h,10 µL L^{-1} ethylene for 24 h alone and untreated. Fumigation of 1-hexylcyclopropene was applied in a 60 L plastic drum for 18 h as explained in Chapter 5, Section 5.2.4. The sprigs were treated with ethylene 10 µLL^{-1} for 24 h as explained in Chapter 5, Section 5.2.4. The efficacy of application of 1-hexylcyclopropene as ethylene antagonist was examined during season 2014 on

'WX73' genotype and four more separate independent experiments were executed by using different genotypes such as 'Purple Pride', 'WX56' and 'WX58' in 2015. Flowers/buds abscission was recorded daily following the treatments for four consecutive days. Flowers/buds abscission was expressed as a percentage as detailed in Chapter 5, Section 5.2.4.

7.2.4.2. Experiment 2: Effects of different concentrations of 1-hexylcyclopropene, ethylene alone and 1-hexylcyclopropene followed by exposure to ethylene on flowers/buds abscission in 'Hybrid1' and 'Purple Pride' genotypes of waxflowers in 2015.

Two independent experiments were conducted using 'Hybrid1' in the first experiment and 'Purple Pride' waxflowers in the second experiment. Three different concentrations (0.5, 1.0 and 2.0 µM) were tested on 'Hybrid1'genotype to evaluate the effective concentration to reduce the postharvest flowers/buds abscission in waxflower stems. The sprigs of 'Hybrid1' were fumigated with 1-hexylcyclopropene alone, ethylene alone and 1-hexylcyclopropene treatments followed by exposure to ethylene (10 µL L^{-1}) and the untreated flower sprigs were treated as a control. The flowers/buds abscission was recorded 24 h after the completion of treatments on day one. Flowers/buds abscission was expressed as a percentage as explained in Chapter 5, Section 5.2.5.3. Another experiment was repeated on 'Purple Pride' keeping all the treatments, an experimental design similar to those explained in the first experiment.

7.2.5. Experimental design and statistical analysis of data

All the trials were arranged in completely randomised design composed of one or two factors with three replicates. Three stems were used in each replication. Depending upon the experiment, the data were subjected to one or two - way analysis of variance (ANOVA) using GenStat 14th edition (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). Following significant F-test ($P < 0.05$), least significant differences (LSD) were calculated. The effects of different treatments and times were compared using least significance differences (LSD). Means standard error (± SE) was also calculated. Differences among treatments were further analysed using Duncan's Multiple Range Test.

7.3. Results

7.3.1. Experiment 1: Effect of 1-hexylcyclopropene (1-HCP) fumigation on mean flowers/buds abscission in 'WX73', 'Purple Pride', 'WX58' and 'WX56' genotypes of waxflower in 2014 -2015.

As expected, the flowers stems of 'WX73', 'Purple Pride', 'WX58' and 'WX56' genotypes fumigated with exogenous ethylene alone (10 µL L^{-1}) for 24 h resulted in significantly ($P \leq 0.05$) highest mean flowers/buds abscission (95.1%, 77.4%, 68.8% and 85.9% respectively) as compared to the untreated stems (2.2%, 43.4%, 1.3% and 7.1%) and all other treatments respectively Fig.7.1 A and Fig.7.2 B,C and D. Meanwhile, the mean flowers/buds abscission was found to be significantly ($P \leq 0.05$) reduced (2.7%, 4.8%, 14.6% and 23.5%) when the sprigs of 'WX73', 'Purple Pride', 'WX58' and 'WX56' waxflowers were treated with 1-hexylcyclopropene (1 µM) for 18 h followed by 24 h of exposure to 10 µL L^{-1} of ethylene Fig.7.1 A and Fig.7.2 B,C and D. Also, treatment of 1-1-HCP alone exhibited the lowest mean flowers/buds abscission (1.2%, 0.9%, 2.4% and 21.3% respectively) as compared to all the other treatments.

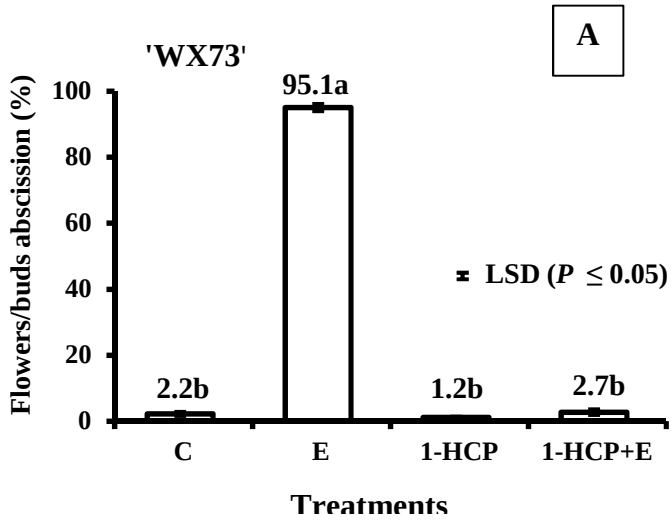


Figure.7.1. Effects of fumigation of 1-hexylcyclopropene (1 µM) or ethylene (10 µL L^{-1}) alone and 1-hexylcyclopropene followed by exposure to ethylene (10 µL L^{-1}) on mean flowers/buds abscission in (A) 'WX73' waxflowers during 2014. Vertical bars represent (SE). C = control, E = ethylene alone (10 µL L^{-1}), 1-HCP = 1-hexylcyclopropene alone, 1-HCP + E = 1-hexylcyclopropene fumigation followed by ethylene.

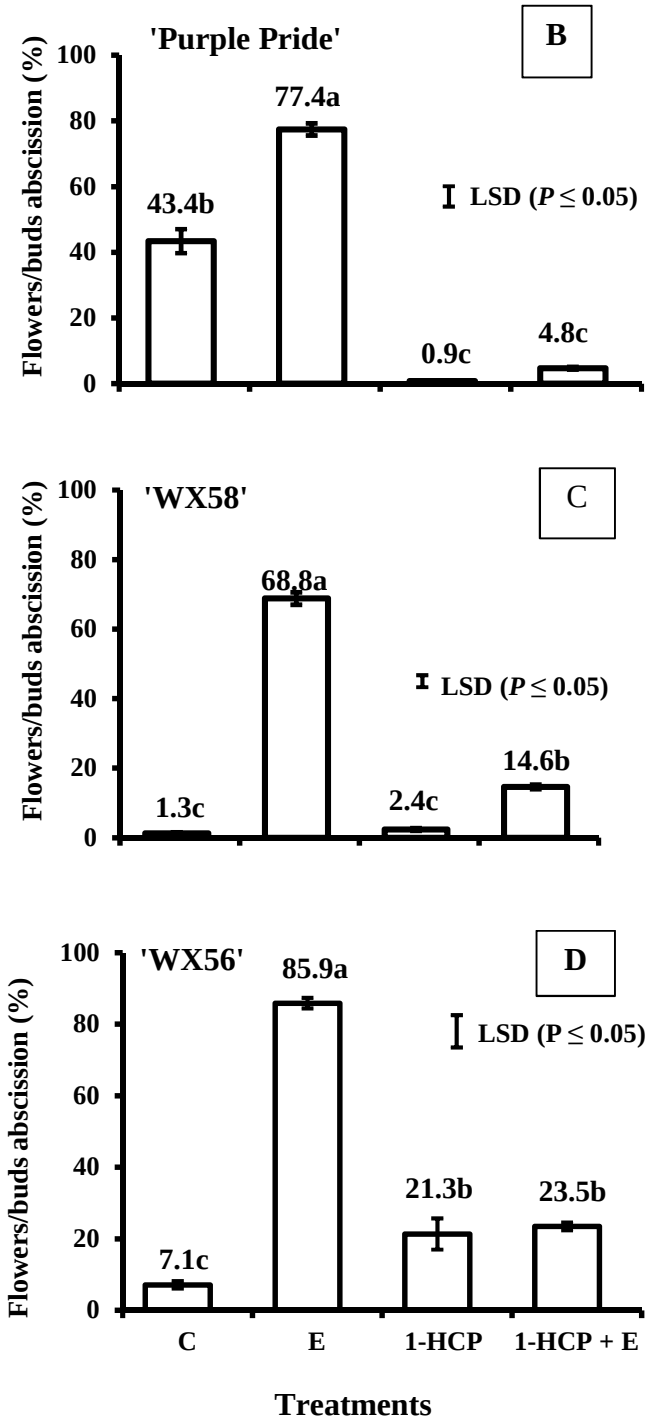


Figure.7.2. Effects of fumigation of 1-hexylcyclopropene (1 µM), or ethylene (10 µL L^{-1}) alone and 1-hexylcyclopropene followed by exposure to ethylene (10 µL L^{-1}) on mean flowers/buds abscission in (B)'Purple Pride', (C) 'WX58' and (D) 'WX56' waxflowers during 2015. Vertical bars represent (SE). C = control, E = ethylene alone (10 µL L^{-1}), 1-HCP = 1-hexylcyclopropene alone, 1-HCP + E = 1-hexylcyclopropene fumigation followed by ethylene.

7.3.2. Experiment 2: Effect of 1-hexylcyclopropene (1-HCP) fumigation on cumulative flowers/buds abscission in 'WX73', 'Purple Pride', 'WX58' and 'WX56' genotypes of waxflowers in 2014 - 2015.

The flower sprigs of 'WX73' genotype were fumigated with 1-hexylcyclopropene (1 µM) for 18 h and followed by exposure to 10 µL L^{-1} of ethylene for 24 h resulted in significantly ($P \leq 0.05$) lowest (1.3% to 3.2%) cumulative abscission of flowers/buds over four days as compared to those exposed to the ethylene treatment alone (94.7% to 95.2%) during 2014 Fig.7.3A. Meanwhile, the 1-hexylcyclopropene treatment alone resulted in the lowest cumulative abscission of flowers/buds over four days (0.7% to 1.3%) as compared to the control and all other treatments during 2014 Fig.7.3A.

Similarly, the flower bunches of 'Purple Pride', 'WX58' and 'WX56' fumigated with 1-hexylcyclopropene (1 µM) for 18 h and followed by ethylene exposure (10 µL L^{-1}) for 24 h resulted in the significantly ($P \leq 0.05$) lowest (2.7 to 5.8%, 5.10 to 24.30% and 15.1 to 30.9%) cumulative buds/flowers abscission as compared to the ethylene treatment (10 µL L^{-1}) alone (75.5 to 79.1%, 57.6 to 74.6% and 83.9% to 86.7% respectively) and all other treatments during 2015 (Fig. 7.4B,C and D).

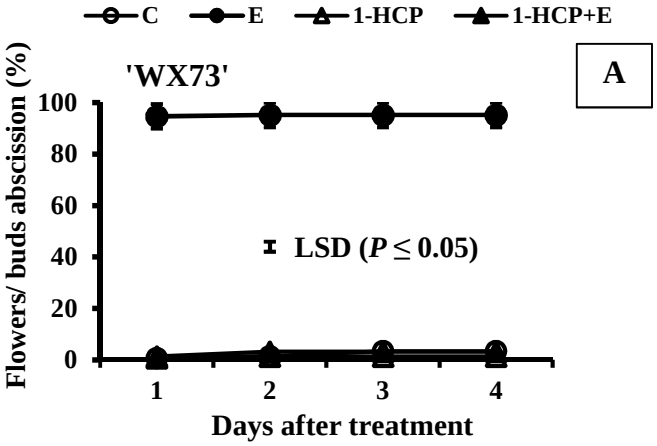


Figure.7.3. Effects of fumigation of 1-hexylcyclopropene (1 µM) or ethylene (10µL L^{-1}) alone and 1-hexylcyclopropene (1µM) followed by ethylene (10 µL L^{-1}) on cumulative abscission of flowers/buds four days after treatment in (A) 'WX73' waxflowers in 2014. n = three replications (three stems per replication), vertical bars represent SE, C = control, E = ethylene alone (10 µL L^{-1}), 1-HCP = 1-hexylcyclopropene alone, 1-HCP + E = 1-hexylcyclopropene fumigation followed by ethylene.

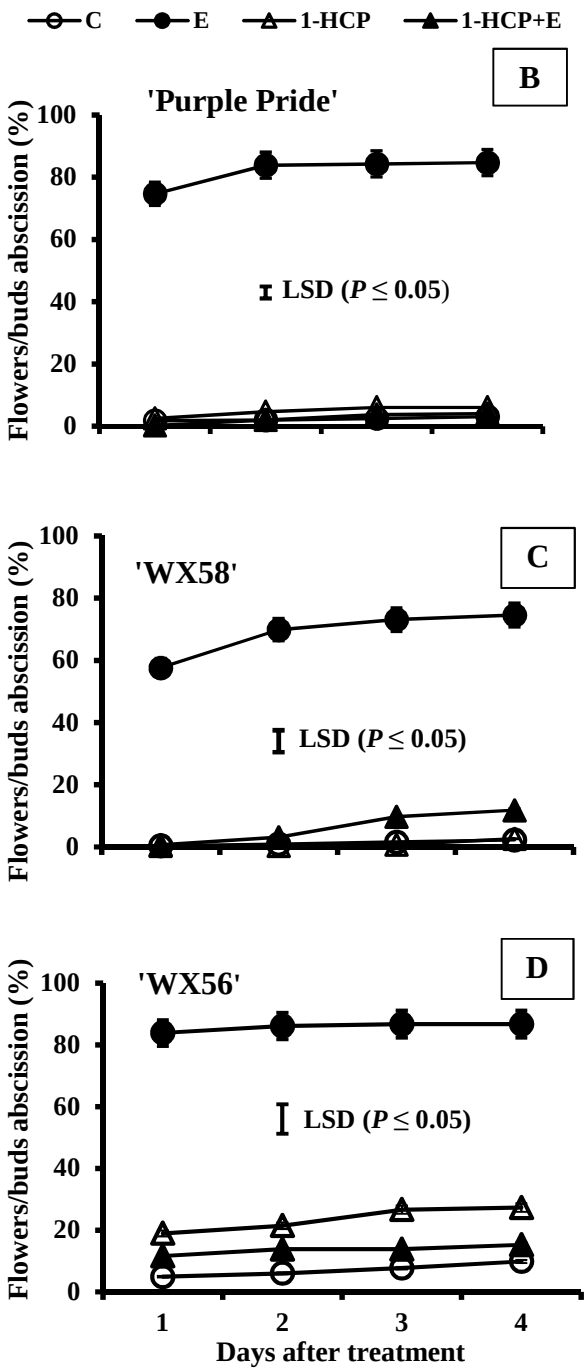


Figure.7.4. Effects of fumigation of 1-hexylcyclopropene (1 µM) or ethylene (10 µL L^{-1}) alone and 1-hexylcyclopropene (1 µM) followed by ethylene (10 µL L^{-1}) on cumulative abscission of flowers/buds four days after treatment in (B) 'Purple Pride', (C) 'WX58' and (D) 'WX56' waxflower in 2015. n = three replications (three stems per replication), vertical bars represent SE, C = control, E = ethylene alone (10 µL L^{-1}), 1-HCP = 1-hexylcyclopropene alone, 1-HCP + E = 1-hexylcyclopropene fumigation followed by ethylene.

7.3.3. Experiment 3: Effects of different concentrations of 1-hexylcyclopropene fumigation alone and 1-hexylcyclopropene fumigation followed by exposure to ethylene (10 µL L^{-1}) on flowers/buds abscission of 'Hybrid1' and 'Purple Pride' waxflowers in 2015.

The stems of 'Hybrid1' treated with 0.5, 1.0 and 2.0 µM 1-hexylcyclopropene for 18 h followed by exposure to ethylene for 24 h resulted in significantly ($P \leq 0.05$) reduced flowers/buds abscission as compared to stems treated with ethylene alone one day after the treatments Fig.7.5A. Meanwhile, flowers/buds abscission was significantly ($P \leq 0.05$) reduced when 'Purple Pride' stems were fumigated with all different concentrations of 1-HCP except 0.5 µM followed by exposure to the ethylene treatment as compared to all other treatments one day after the treatments Fig.7.5B. As expected, ethylene treatment alone 10 µL L^{-1} for 24 h significantly ($P \leq 0.05$) increased (83.4% and 74.7% respectively) flowers/buds abscission in both 'Hybrid1' and 'Purple Pride' genotypes one day after the treatments when compared to control treatment and all other treatments Fig.7.5A and B.

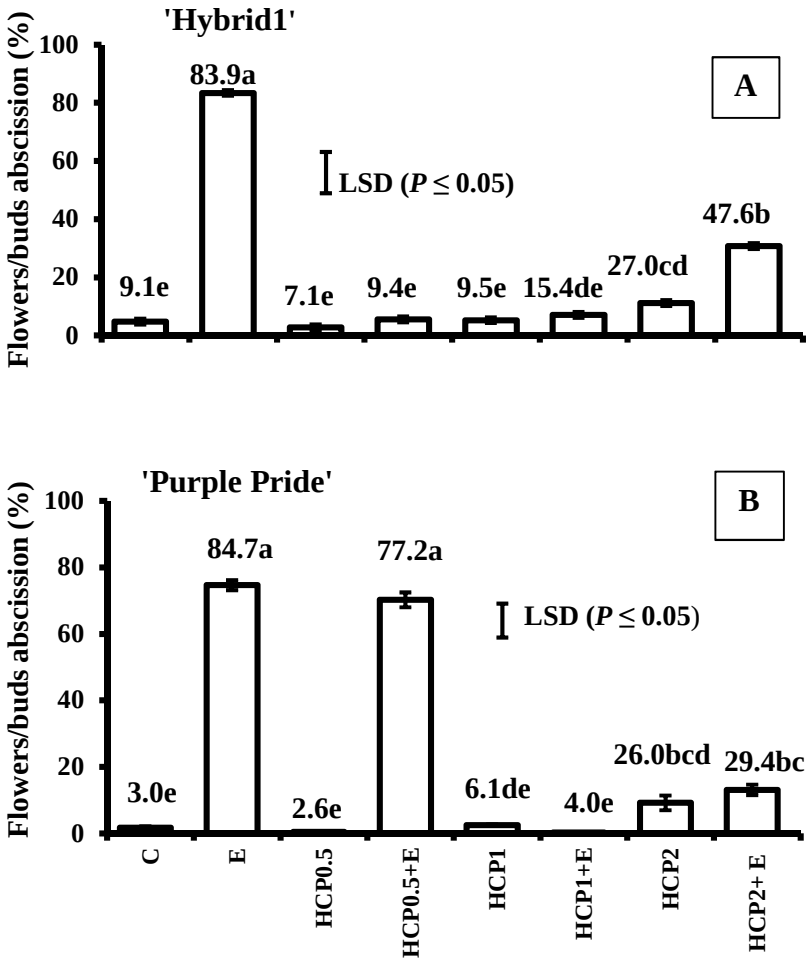


Figure.7.5. Effects of different concentrations 0.5, 1.0 and 2.0 µM of fumigation of 1-hexylcyclopropene or ethylene (10 µL L^{-1}) alone and 1-hexylcyclopropene treatment followed by exposure to ethylene (10 µL L^{-1}) on flowers/buds abscission in (A)'Hybrid1' and (B)'Purple Pride' waxflower on day 1 after treatments in 2015. Vertical bars represent SE, C = control, E = ethylene alone (10 µL L^{-1}), 1-HCP = 1-hexylcyclopropene alone, 1-HCP + E = 1-hexylcyclopropene fumigation followed by ethylene.

7.4. Discussion

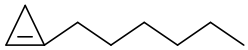
Small cyclopropenes are known to be highly effective in counteracting ethylene response at the receptor level in plants (Sisler *et al*., 2006, 2009) for long periods of time at very low concentration (Sisler *et al*., 1999; Sisler *et al*., 2005). For example, 1-MCP is promising as a post-harvest treatment for both fresh cut flowers and potted plants (Grichko, 2006; Mayers *et al.,* 1997). 1-MCP is coupled with various weaknesses as reported some by Seaton and Poulish, (2010) such as that 1-MCP is known to protect the waxflowers for a short time as low as four days, and 2 to 25 days depending on flowers type it may require repeating the treatment and can be used as a fumigation treatment only (Macnish *et al*., 2000b*;* Macnish *et al*., 2004b; Goren *et al*., 2008). In addition, it is expensive (AU$9000/100 g of 1-MCP), consequently reducing profit to growers. Agrofresh® does not directly sell 1-MCP therefore; it offers an attractive opportunity to develop new ethylene antagonists.

In this study, ethylene antagonistic effects of the application of 1-hexylcyclopropene on four different genotypes of waxflowers were assessed. Flower stems of different genotypes of waxflowers such as 'WX73', 'Purple Pride', 'WX58' and 'WX56' fumigated with 1-hexylcyclopropene (1 µM) for 18 h and followed by exposure to ethylene for 24 h exhibited significant reduction in abscission of flowers/buds (2.7%, 4.8%, 14.6% and 23.5% respectively) as compared to the ethylene treated stems (95.1%, 77.4%, 68.8% and 85.9% respectively) Fig.7.1A and Fig.7.2B, C and D. The experimental results suggested that possibly the 1-HCP treatment inhibited ethylene action by blocking the ethylene receptor and consequently inhibited abscission of flowers and buds in different genotypes of waxflowers Fig.7.7. Amongst various concentrations of 1-HCP tested 1 µM fumigation for 18 h followed by exposure to ethylene (10 µL L^{-1}) for 24 h was most effective in reducing flowers/buds abscission in 'Hybrid1' and 'Purple Pride' waxflowers. Therefore, the response of 1-HCP in inhibiting flowers/buds abscission in waxflower is concentration dependent. Similarly, as found by Chutichudet *et al*. (2010) *Siam Tulip* flowers fumigated with 1-MCP at four concentrations (0, 300, 600, 900ppb) and two fumigation periods (4 or 8 h) showed an optimum concentration of 300 ppb of 1-MCP for 8 h could prevent ethylene biosynthesis and maintain the cut *tulip* flower quality. In addition, Jones *et al*. (2001) found a

reduction in the petal abscission in all cultivars of Zonal Geraniums treated with different *concentrations* (0, 0.1 or 1.0 µLL^{-1}) of 1-MCP for 3, 6, 12 or 24 h followed by exposure to 1.0 µL L^{-1} of ethylene. In general, ethylene action on abscission of flowers/buds in waxflowers was significantly inhibited with the fumigation treatment of 1-HCP when flower stems were exposed to the ethylene treatment in 'WX73', 'Purple Pride', 'WX58', 'WX56' and 'Hybrid1' waxflowers in 2014 and 2015. Possibly, the action of 1-HCP as ethylene antagonist it could be due to the presence of the small three-membered ring as a functional group which is a structural analogue of 1-MCP and capable of binding to ethylene receptor sites (Khan, 2014). It may also be argued that ethylene antagonism of 1-HCP noted in different genotypes of waxflowers may be ascribed to molecular strain which is a major factor attributed to the effectiveness of the antagonist and the compound with high strain will be able to withdraw the electrons from the metal in the receptor consequently binding tightly to the ethylene receptor Fig. 7.6. (Sisler and Shang, 1984; Sisler *et al*., 1996a, b; Sisler and Serek, 1997; Sisler *et al*., 1999; Buanong *et al*., 2005; Sisler *et al*., 2009). It maybe the existence of a double bond in this chemical structure can also be another factor competing with ethylene for the receptor Fig. 7.6. (Sisler *et al.*, 2006, 2009). It is proposed that the action of 1-HCP to reduce the abscission of floral organs in waxflowers may be ascribed to the ability of 1-HCP to compete with ethylene for receptor and remain for a long time thus inhibiting ethylene action role as suggested earlier by (Sisler *et al*. 1996a, 1999). In addition, Sisler *et al*. (2001) have also mentioned that substitution in 1-position is more effective at low concentration due to their stabilising effect. Kebenei *et al.* (2003a) suggested that 1-HCP has a hexyl group, which is highly electron donating and it has a six carbon atom chain substituted in the 1-position which supports to prolong binding time. Moreover, the compound with more hydrophobic side chains seems to have longer activity and it is thought that the ethylene receptors located in a cell membrane and a hydrophobic attachment probably because low diffusion from the cell and the compound will remain bonded to the ethylene receptor (Serek *et al.*, 2007; Sisler *et al.*, 2009).

Similarly, Kebenei *et al*. (2003a) claimed that 1-HCP protected the (*Kalanchoe blossfeldiana*) longer than 1-MCP by delaying the petals in-rolling. Later on, Apelbaum *et al*., (2008) demonstrated that 1-HCP is one of the six effective ethylene inhibitors which have been tested on tomato and avocado fruit. Sisler *et al*.

(2003) reported that application of 0.4 nL L^{-1} 1-HCP with the chain longer than five carbon atoms was active to protect banana fruit from ethylene action and delay the fruit ripening to 20 d. As a conclusion, 1-HCP fumigation exhibits antagonism to ethylene action through a reduction in flowers/buds abscission in 'WX73', 'Purple Pride', 'WX58', 'WX56' and 'Hybrid1' waxflowers.



1-Hexylcyclopropene C_9H_{16}, Molecular weight: 124 g mol^{-1}

Figure. 7.6. Chemical structure of 1-hexylcyclopropene C_9H_{16}.

Figure.7.7. Effects of fumigation of 1-hexylcyclopropene (1 µM) for 18 h and followed by exposure to ethylene (10 µL L^{-1}) for 24 h and the ethylene treatment alone on flower/buds abscission of 'Purple Pride'. (A) 'Purple Pride' waxflower stems treated with ethylene alone (10 µL L^{-1}) for 24 h.(B) 'Purple Pride' waxflower stems treated with 1-hexylcyclopropene (1µM) for 18 h and followed by exposure to ethylene (10 µL L^{-1}) for 24 h.

CHAPTER 8

1*H*-cyclopropabenzene and 1*H*-cyclopropa[*b*]naphthalene fumigation protects different genotypes of cut waxflowers from the detrimental effects of ethylene on abscission of flowers/buds.

Abstract

Abscission of flowers/buds in different genotypes of waxflowers in postharvest phase causes serious economic losses in Australia. The aim was to investigate the antagonistic effects of 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) at one and different concentrations on ethylene action in reducing abscission of flowers/buds in different genotypes of waxflowers. Experiments tested inhibitory effects of BC and NC fumigation at 1 µM in reducing abscission of flowers/buds exposed to ethylene (10 µL L^{-1}) in different waxflower genotypes 'WX73', 'WX17', 'WX58', 'WX56', 'WX107', 'Jenny' and 'Purple Pride' over two flowering seasons in 2014 and 2015. Also tested were the effects of different concentrations 0.5, 1.0 and 2.0 µM of BC and NC followed by exposure to ethylene (10 µL L^{-1}) on flowers/buds abscission in 'Purple Pride', 'Revelation' and 'Hybrid1' in 2015. In all the experiments, the flower sprigs were fumigated separately with BC or NC (1 µM) using 60 L plastic drums for 18 h then 24 h exposure to ethylene (10 µL L^{-1}). These results were compared against controls bunches fumigated for 18 h of BC or NC (1 µM) alone and ethylene (10 µL L^{-1}) alone. As well as untreated flower sprigs were kept as control. The experiments were one or two-factor factorial completely randomised design, with three replications and three stems per replication. Fumigation of BC (1 µM) for 18 h followed by exposure to 10 µL L^{-1} of ethylene showed a significant ($P \leq 0.05$) overall (77%) reduction in the mean abscission of flowers/buds in different genotypes (16.0% 'WX73', 0.0% 'WX58', 13.8% 'WX56'% and 44.8% 'Purple Pride') as compared to those fumigated with ethylene alone (95.1%, 68.8%, 85.9% and 77.4% respectively). Similarly, mean flowers/buds abscission was significantly ($P \leq 0.05$) overall (94%) decreased when the stems were fumigated with NC (1 µM) for 18 h followed by exposure to ethylene (10 µL L^{-1}) for 24 h in different genotypes (38.1%'WX73', 25.5%'WX107, 8.9% WX58, 18.8%'WX56' and 27.0% 'Purple Pride' respectively) as compared with ethylene treatment alone (86.9%, 73.1%, 68.9%, 85.8% and 77.4% respectively). However, no response in reduction of mean flowers/buds abscission

was noted when 'WX17' and 'Jenny' when fumigated with BC or NC (1 µM) for 18 h followed by exposure to ethylene (10 µL L^{-1}) alone for 24 h respectively in 2014. Amongst different concentrations 0.5, 1.0 and 2.0 µM of BC tested on 'Revelation' waxflowers, all the concentrations of BC fumigation applied prior to the ethylene exposure were significantly effective in reducing mean flowers/buds abscission and the differences among the concentration were non-significant. Meanwhile, amongst various concentrations of NC fumigation evaluated, NC (0.5 - 1.0 µM) fumigation prior to the ethylene exposure significantly reduced mean flowers/buds abscission at 0.5 ('Hybrid1'7.9% and 'Revelation'12.5%) and at 1 µM (33.3%'Purple Pride', 28.4%'Hybrid1' and 2.9% 'Revelation')

In conclusion, the experimental results clearly demonstrated the BC and NC (0.5 -1.0 µM) are antagonists to ethylene action as apparent in a reduction in ethylene-induced flowers/buds abscission in different genotypes of waxflowers but to some degree also influenced by the sensitivity of genotype to ethylene. Thereby, BC and NC are highly effective at low concentration (1 µM) as an ethylene antagonist by preventing the ethylene action in cut waxflower. As both inhibitors were found to be non-toxic they might be considered as candidates for practical use.

8.1. Introduction

Cyclopropenes are very small organic molecules that appear to counteract with the ethylene receptor in plants and have been tested as an ethylene antagonist in different horticultural commodities (Sisler and Serek. 1997; Sisler *et al*., 1999; Walsh, 2005; Grichko, 2006; Sisler, 2006). Various cyclopropenes such as cyclopropene, 3-methylcyclopropene (3-MCP), 3,3-dimethylcyclopropene 3,3-(DMCP) and 1-methylcyclopropene (1-MCP) have been evaluated as ethylene antagonists (Sisler and Serek, 1997; Sisler and Serek, 2000; Sisler and Serek, 2003). Walsh (2005) reported that cyclopropenes are commonly proposed as an effective tool to compete with ethylene for binding to the ethylene receptor (Blankenship, 2001; Sisler and Serek, 2003). The stability is the main factor in determining the activity of ethylene antagonists (Sisler *et al*., 1996a, 1996b; Sisler and Serek, 2000). However, cyclopropenes are very unstable in the vapour state and can also be explosive at high temperatures (Schipperijn and Smael, 1973; Sisler, 2006) hence require a low temperature to store these cyclopropenes. 1-MCP and 3-MCP are more stable in the

gas phase than other cyclopropenes in the liquid phase. However, 3-MCP is less active than 1-MCP and also requires higher concentration probably due to the presence of the functional methyl group at position 3 and protects from ethylene effect only 7days (Sisler, 2006). 1-MCP seems to be relatively more stable at room temperature (Sisler 1996a, 1996b; Sisler *et al*., 1999; Sisler *et al*., 2001). 1-MCP and 3-MCP contain a double bond in the cyclopropene ring which can be involved inhibiting ethylene action by interaction with the receptor site (Sisler *et al*., 1999; Sisler and Serek, 2000). Sisler *et al*. (1996b) reported that CP (1nL/L) and 3,3-DMCP 0.5 -1.0 µL L^{-1} for 24 h seem to protect cut carnation flowers from the adverse effect of ethylene which is attributed to the presence of the strained alkene in this chemical structure.

1-MCP is one of cyclopropene group that apparently has highly strained its binds to a metal in the ethylene receptor than ethylene (Sisler and Serek, 1997; Rodriguez *et al*., 1999; Sisler and Serek, 2000) and used commercially as an effective blocker of ethylene action to delay senescence in ornamental and other horticultural crops (Sisler and Serek., 1997; Blankenship and Dole; 2003; Watkins, 2008). 1-MCP is the effective ethylene inhibitor which competes with ethylene for receptors thus, leading to an inhibition of ethylene responses (Sisler *et al*., 1996a; Sisler and Serek, 1997; Blankenship, 2001; Sisler and Serek, 2003; Saleh-Lakha, *et al*., 2004). Blankenship and Dole (2003) found that 1-MCP is 10 times more efficient in binding to the ethylene receptor than ethylene. It acts at very low concentrations to hinder ethylene responses in different plants at the receptor level even for very high amounts of ethylene (Sisler and Serek, 1997; Sisler and Serek, 2000; Blankenship and Dole, 2003).

Various reports have been published documenting the positive and negative effects of application of 1-MCP in ornamentals such as *Antirrhinum majus* and *Alstroemeria spp*. (Serek *et al*., 1995a), *Gypsophila paniculata* L. 'Perfecta', 'Gilboa', and 'Golan' (Newman *et al*., 1998) *Dianthus caryophyllus* L. (Asil *et al*., 2013), *Curcuma erauqinosa* Roxb. (Chutichudet *et al*., 2010), *Campanula carpatica* and *Kalanchoe blossfeldiana* Poelln. (Sisler *et al*., 1999), *Hibiscus rosa-sinensis* L. (Reid *et al*., 2002) and *Lilies* (Celikel *et al*., 2002). Macnish *et al*. (2010) reported that the Hybrid Tea rose treated with 1-MCP resulted in protecting rose flowers against exogenous ethylene on day 6 of dry transport. It also has various limitations that

prevent its widespread use in agriculture (Goren *et al*., 2008). 1-MCP is highly unstable the longevity of its action is affected by genotype, its short-term residual activity in some plants, is highly expensive and sold as a service not a chemical, and can be applied as a fumigation not as a dip or spray (Sisler and Serek, 1997; Macnish *et al*., 2004b; Sisler *et al*., 2006; Ebrahimzadeh *et al*., 2008; Goren *et al*., 2008; Seaton and Poulish, 2010) as also explained in Chapter 5, Section 5.1 and Chapter 7, Section 7.1.

Kader (2001) stated that there are several strategies in postharvest technology to reduce the damage in quantity and quality between the harvest and customers. Scariot *et al*. (2014) mentioned a number of approaches can be used to reduce the ethylene production or inhibit its action to prolong the postharvest life of flowers. As suggested by Ebrahimzadeh *et al*. (2008) the search for new analogues such as 1-MCP might lead to prolonging the vase life in carnation flowers yet must be easy to apply, economically viable and environmentally friendly. 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) are two of the most highly strained members of the cyclopropene group and represented more stable compounds (Ullman and Buncel, 1963; Halton, 1973). Also, there is no significant difference between BC and analogue NC in chemical structure as both contain the benzene and cyclopropene ring and a double bond.

Earlier, preliminary research on the beneficial effect of BC (50 -100 nL L^{-1}) fumigation on inhibiting flowers/buds abscission in 'WX73' and 'WX17' waxflower has been reported by Khan (2014). Waxflowers vary in their sensitivity to ethylene among genotypes as reported earlier by Macnish *et al*. (2004a) and *C. uncinatum* × *C. micranthum* cv. 'Sweet Georgia' and *C. uncinatum* cv. 'Early Nir', 'Paddy's Late', 'Purple Pride', 'CWA Pink' and 'Early Hard' were highly sensitive to ethylene. However, no research work has been reported on the effects of different concentrations of BC and NC fumigation on flowers/buds abscission in different genotypes of waxflowers which differ in their sensitivity to ethylene. It was hypothesised that BC and NC may act as an ethylene antagonist depending upon the concentration applied in inhibiting abscission of flowers/buds in different genotypes of waxflowers. Therefore, the effects of different concentrations of BC and NC on abscission of

flowers/buds in different genotypes of waxflowers were investigated during two consecutive years.

8.2. Materials and Methods

8.2.1. Sources of chemicals

1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) Fig.8.11 were synthesised by Dr Alan Payne and his research group at the Department of Chemistry, Curtin University.

8.2.2. Plant material

Fresh sprigs of different 'Geraldton wax' genotypes 'WX73', 'WX17', 'WX56', 'WX58', 'WX107', 'Purple Pride', 'Revelation', 'Jenny' and 'Hybrid1' were picked up from more than five mature bushes grown at the Department of Agriculture and Food Western Australia (DAFWA), South Perth, at Medina Research Station (31°59'18'S, 115°53'E) and used in different experiments.

8.2.3. Harvesting flowering stems

During the experimental period, the flowering stems (60 to 70 cm long) of different genotypes were harvested with flowers open, arranged in the early morning and held in buckets containing clean tap water. Stems were arranged randomly with ends re-cut to 30 cm in length (from cut ends to the extreme open flowers) under water with secateurs and stood in buckets of water and then placed in 600 ml glass beakers containing distilled water ready for treatments. Percentage of open flowers of genotypes fumigated with BC and NC compounds during 2014 and 2015 are indicated in Table. 8.1.

Table. 8.1. The percentage of open waxflowers on stems at the time of harvest different genotypes used in different experiments in 2014 and 2015.

Chemical	Year	Genotypes	Open flowers on the stem (%)
BC	2014	'WX73'	87.3±11.8%
	2014	'WX17'	65.3±19.5%
	2015	'WX56'	69.3±14.9%
	2015	'WX58'	78.2±11.4%
	2015	'Purple Pride'	67.8±6.60%
	2015	'Revelation'	83.4±15.8%
NC	2014	'WX73'	80.2±15.4%
	2014	'WX107'	99.9±0.36%
	2014	'Jenny'	90.2±9.38%
	2015	'WX56'	71.9±13.3%
	2015	'WX58'	81.8±8.7%
	2015	'Purple Pride'	67.3±7.6%
	2015	'Hybrid1'	97.1±4.4%
	2015	'Revelation'	81.9±15.6%
	2015	'Purple Pride'	68.4±8.8%

(±) = Standard error of the mean (SE)

8.2.4. Experiments

8.2.4.1. Experiment 1: Effect of 1*H*-cyclopropabenzene (BC) fumigation on flowers/buds abscission of 'WX73' and 'WX17' in 2014.

In 2014, the flower stems of 'WX73' were exposed to different treatments such as fumigation with ethylene (10 µL L^{-1}) alone for 24 h, BC (1 µM) fumigation alone for 18 h and BC (1 µM) fumigation for 18 h followed by exposure to ethylene (10 µL L^{-1}) 24 h. Untreated flower sprigs served as a control. BC and ethylene fumigation was applied to flower stems in 60 L plastic drums as explained in detail in Chapter 5, Section 5.2.4. The experiment was designed following two factors (treatment and time) factorial completely randomised design. Each treatment was replicated three times and three stems were included in each replicate. Flowers/buds abscission was recorded

daily for four consecutive days. Flowers/buds abscission was expressed as a percentage of total flowers/buds present. The second experiment in 2014 was conducted using the 'WX17' genotype keeping the same treatments and experimental design as in the first experiment. Flowers/buds abscission was recorded for four days at one-day intervals.

8.2.4.2. Experiment 2: Effect of 1*H*-cyclopropabenzene (BC) fumigation on flowers/buds abscission in 'WX58', 'WX56' and 'Purple Pride' in 2015.

The effectiveness of BC fumigation as an ethylene antagonist on inhibiting flowers/buds abscission was investigated in three independent experiments using 'WX58', 'WX56' and 'Purple Pride' waxflowers during 2015. All the treatments and experimental design for these three experiments were similar to Experiment 1. Flowers/buds abscission was recorded for four days at one-day intervals in all three experiments and expressed as a percentage.

8.2.4.3. Experiment 3: Effect of different concentrations of 1*H*-cyclopropabenzene (BC) fumigation on abscission of flowers/buds in 'Revelation' in 2015.

'Revelation' waxflower stems were fumigated with different concentrations 0.5, 1.0 and 2.0 µM of 1*H*-cyclopropabenzene alone for 18 h, all 1*H*-cyclopropabenzene treated flowers stems were exposed to ethylene (10 µL L^{-1}) for 24 h, and ethylene fumigation alone. Untreated flower stems were treated as a control. The application of BC and ethylene fumigation treatments has been explained in Chapter 5, Section 5.2.4. The experiment was conducted as a one-factor factorial completely randomised design, with three replications and three stems per replication. Flowers/buds abscission was recorded on day three after treatments and expressed as percentage flowers/buds abscission as explained in Chapter 5, Section 5.2.4.

8.2.4.4. Experiment 4: Effect of 1*H*-cyclopropa[*b*]naphthalene (NC) fumigation on flowers/buds abscission in 'WX73' and 'WX107' and 'Jenny' in 2014.

The 'Jenny' flower sprigs used in this experiment exhibited 90.2 ± 9.38% open flowers and sprigs were treated with 1 µM NC fumigation alone for 18 h, exposure to ethylene alone for 24 h and NC (1 µM) fumigation for 18 h followed by exposure to

10 µL L^{-1} of ethylene for 24 h. Flower stems were also kept as an untreated control. The flower sprigs were fumigated in separate 60 L volume plastic drums as explained in Chapter 5, Section 5.2.4. The experiment was arranged in two - factor (treatments and time) factorial completely randomised design (CRD). Three replications and three stems per replication were used for each treatment. Effects of treatments on flowers/buds abscission were noted in each treatment for four consecutive days. Flowers/buds abscission was expressed as a percentage. Two independent experiments were conducted using 'WX73' and 'WX107' genotypes in flowering season 2014 keeping all the experimental treatments and design as mentioned in Experiment 4. Flowers/buds abscission was recorded over four days.

8.2.4.5. Experiment 5: Effect of 1*H*-cyclopropa[*b*]naphthalene (NC) fumigation on flowers/buds abscission in 'WX58', 'WX56' and 'Purple Pride' in 2015.

'WX58' stems displaying 81.8 ± 8.7 % open flowers were used in this experiment to assess the inhibitory effect of fumigation with NC. The sprigs were treated with NC alone, ethylene alone and NC followed by exposure to ethylene in 2015 as explained in Experiment 4. Untreated flowering stems were kept as a control. Flowers/buds abscission was noted over four days at one-day intervals and expressed as a percentage. Two other independent experiments were repeated on genotype 'WX56' and 'Purple Pride' waxflowers open flowers on the shoots 71.9 ± 13.3% and 67.3 ± 7.6% respectively using the same treatments and experimental design as mentioned in Experiment 4. The percentage of flowers/buds abscission in each replication was calculated daily over four days as detailed earlier.

8.2.4.6. Experiment 6: Effect of different concentrations of 1*H*-cyclopropa[*b*]naphthalene (NC) fumigation on flowers/buds abscission in 'Purple Pride', 'Hybrid1' and 'Revelation' genotypes of waxflowers in 2015.

In 2015, the efficacy of different concentrations 0.5, 1.0 and 2.0 µM of NC fumigation alone for 18 h and all the NC treated flower sprigs were also fumigated with ethylene (10 µL L^{-1}) for 24 h, and ethylene fumigation alone. Untreated flower sprig was considered as a control. The detailed method of NC and ethylene fumigation has been explained earlier in Chapter 5, Section 5.2.4. The experimental layout was one-factor factorial completely randomised design. All the treatments and control

included three replications. Three stems were included in each replication. Flowers/buds abscission was noted on day two after treatments and expressed as a percentage also explained in Chapter 5, Section 5.2.5.3. Two additional independent experiments were conducted to test the efficacy of different concentrations of NC in protecting from the detrimental effects of ethylene on flowers/buds abscission in 'Hybrid1' and 'Revelation' waxflowers. The same experiment was repeated with the same treatments and experimental design on two genotypes. All the treatments and experimental designs were similar to those used in Experiment 6. The observation of flowers/buds abscission was recorded on day two in both experiments and expressed as percentage flowers/buds abscission.

8.2.5. Experimental design and statistical analysis of data

Depending upon the experiment, the data was analysed using one or two - way analysis of variance (ANOVA) using the statistical package GeneStat 14th edition (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The effects of different treatments, time and their interaction were assessed using LSD, which was calculated following significant F-test ($P < 0.05$). Duncan's Multiple Range Test was also used to distinguish the differences among treatments.

8.3. Results

8.3.1. Experiment 1 and 2: Effect of 1*H*-cyclopropabenzene (BC) fumigation on mean flowers/buds abscission in 'WX73', 'WX58', 'WX56', 'Purple Pride' and 'WX17' genotypes of waxflower in 2014 - 2015.

Mean flowers/buds abscission over four days was significantly ($P \leq 0.05$) higher (95.1%, 68.8%, 85.9% and 77.4%) when the flower stems of 'WX73', 'WX58', 'WX56' and 'Purple Pride' fumigated with exogenous ethylene (10 µL L^{-1}) alone for 24 h, as compared to untreated flowers (2.2%, 1.3%, 7.1% and 43.4% respectively) and all other treatments in 2014/2015 Fig. 8.1A and Fig. 8.2. While the sprigs fumigated with 1 µM BC for 18 h followed by 24 h of exposure to 10 µL L^{-1} exogenous ethylene significantly ($P \leq 0.05$) decreased mean flowers/buds abscission in all the genotypes tested (16.0%'WX73', 0.0%'WX58', 13.8% 'WX56' and 44.8%'Purple Pride') as compared to those fumigated with ethylene treatment alone (95.1%, 68.8%,

85.9% and 77.4% respectively) Fig. 8.1 and 8.2. However, in 'WX17', the BC (1 µM) fumigation for 18 h followed by 24 h of exposure to 10 µLL^{-1} of ethylene did not significantly reduce flowers/buds abscission (95.42%) compared to ethylene treatment alone (100.00%) Fig. 8.1.

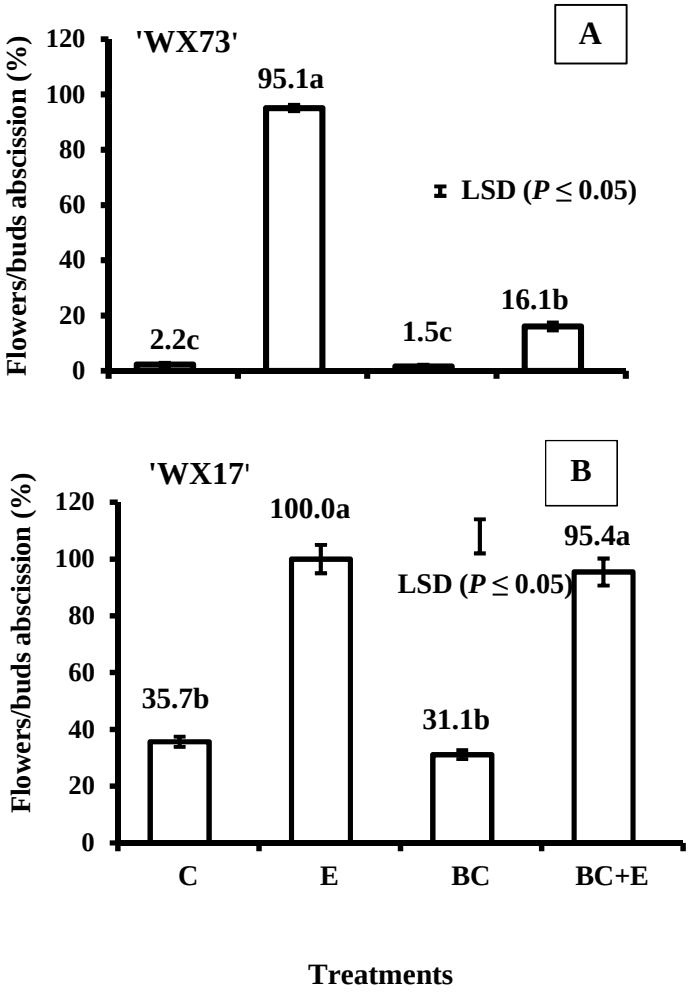


Figure. 8.1. Effects of fumigation of 1*H*-cyclopropabenzene (1 µM), ethylene (10 µL L^{-1}) alone and 1*H*-cyclopropabenzene followed by exposure to ethylene (10 µL L^{-1}) on mean flowers/buds abscission in (A)'WX73' and (B) 'WX17' waxflower during 2014. Vertical bars represent SE, C = control, E = ethylene alone (10 µL L^{-1}), BC = 1*H*-cyclopropabenzene alone, BC + E = 1*H*-cyclopropabenzene fumigation followed by ethylene.

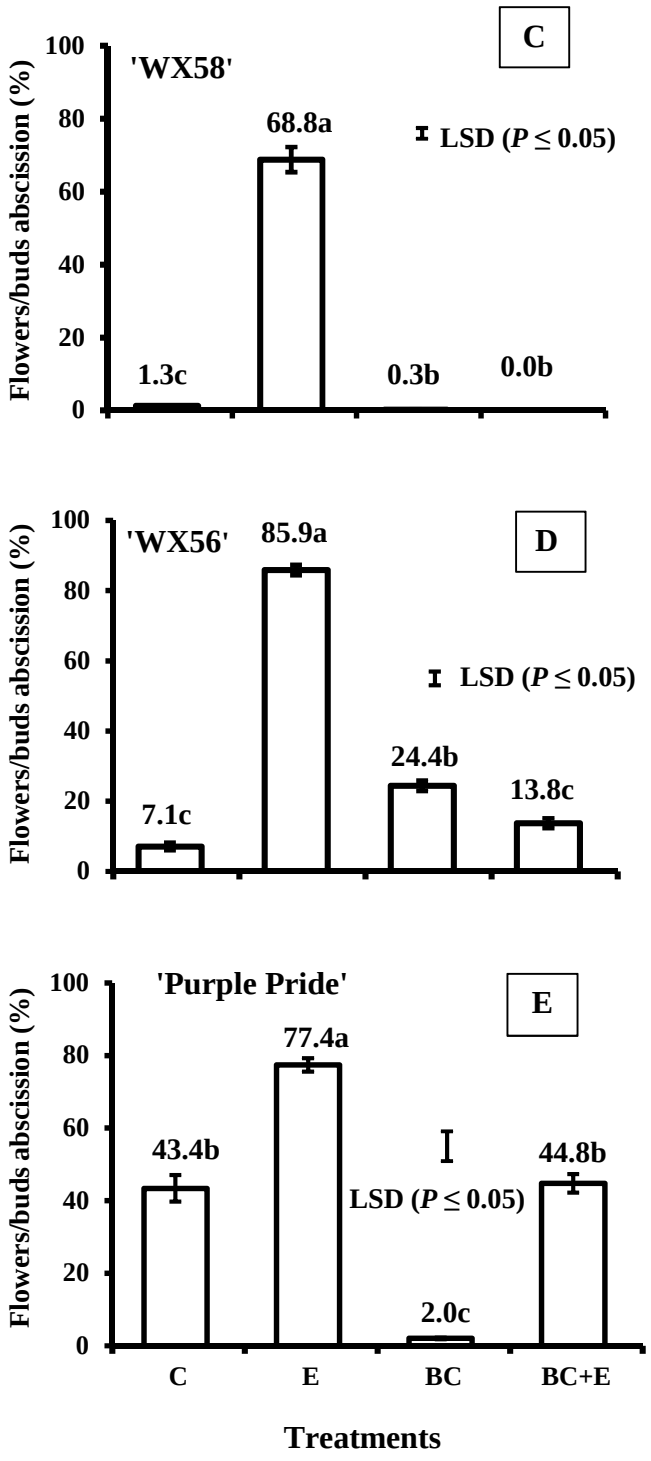


Figure. 8.2. Effects of fumigation of 1*H*-cyclopropabenzene (1 µM), ethylene (10 µL L^{-1}) alone and 1*H*-cyclopropabenzene followed by exposure to ethylene (10 µL L^{-1}) on mean flowers/buds abscission in (C) 'WX58', (D)'WX56' and (E) 'Purple Pride' waxflower during 2015. Vertical bars represent SE, C = control, E = ethylene alone (10 µL L^{-1}), BC = 1*H*-cyclopropabenzene alone BC + E = 1*H*-cyclopropabenzene fumigation followed by ethylene.

8.3.2. Experiment 1 and 2: Effect of 1*H*-cyclopropabenzene (BC) fumigation on cumulative flowers/buds abscission in 'WX73', 'WX17', 'WX58', 'WX56' and 'Purple Pride' genotypes of waxflowers in 2014 and 2015.

As expected, the flower sprigs of 'WX73', 'WX17', 'WX58', 'WX56' and 'Purple Pride' fumigated with ethylene (10 µL L^{-1}) alone promoted cumulative flowers/buds abscission from day one to day four (94.6% to 95.20%, 100% to 100%, 57.6% to 74.6%, 83.9% to 86.8% and 75.5% to 79.1% respectively) during the season 2014/2015 Fig. 8.3 and Fig. 8.4. Untreated flower stems exhibited the lowest cumulative flowers/buds abscission (0.72% to 3.5%, 19.6% to 56.4%, 0.4% to 2.9%, 4.91% to 9.8% and 39.8% to 46.5%) in 'WX73', 'WX17', 'WX58', 'WX56' and 'Purple Pride' respectively during four days. Meanwhile, cumulative flowers/buds abscission over four days was significantly reduced (13.3% to 16.9%, 0.00% to 0.00%, 10.5% to 17.8% and 29.6% to 50.9%) when the flower sprigs of 'WX73', 'WX58', 'WX56' and 'Purple Pride' respectively were fumigated with BC (1 µM) for 18 h followed by exposure to ethylene (10 µL L^{-1}) for 24 h as compared to the ethylene fumigation alone during 2014 and 2015 Fig. 8.3A .Fig. 8.4 C, D and E. In 'WX17' there was no significant difference between the cumulative flowers/buds abscission over four days in ethylene treatment alone and fumigation with BC (1 µM) followed by exposure to ethylene (10 µL L^{-1}) for 24 h during 2014 Fig. 8.3B.

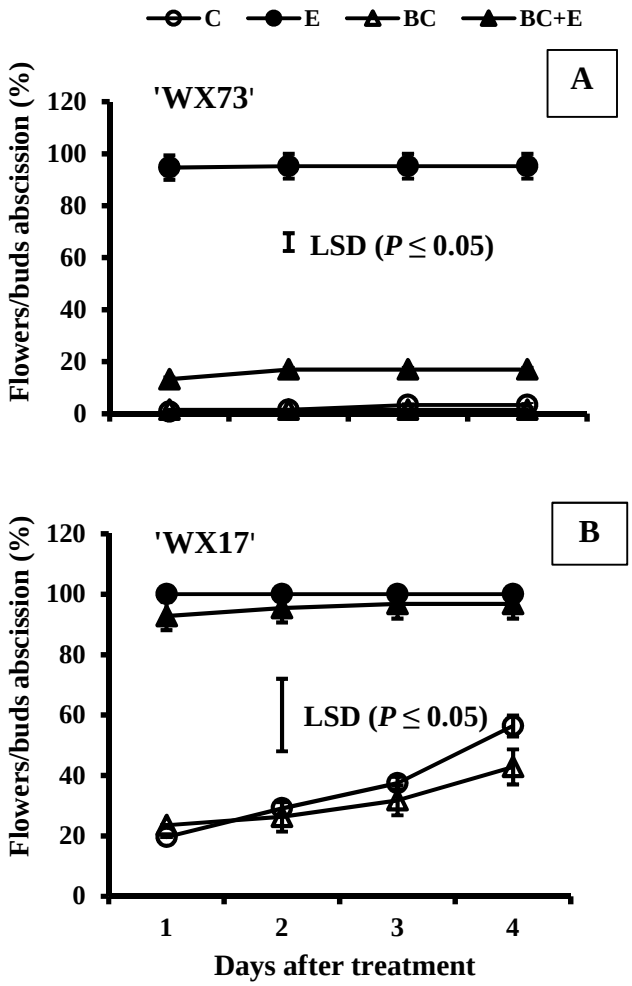


Figure. 8.3. Effects of fumigation of 1*H*-cyclopropabenzene (1 µM) or ethylene (10 µL L^{-1}) alone and 1*H*-cyclopropabenzene (1 µM) followed by ethylene (10 µL L^{-1}) on cumulative abscission of flowers/buds four days after treatments in (A) 'WX73' and (B) 'WX17' waxflower in 2014. n = three replications (three stems per replication), vertical bars represent SE. C = control, E = ethylene alone (10µL L^{-1}), BC = 1*H*-cyclopropabenzene alone, BC + E = 1*H*-cyclopropabenzene fumigation followed by ethylene.

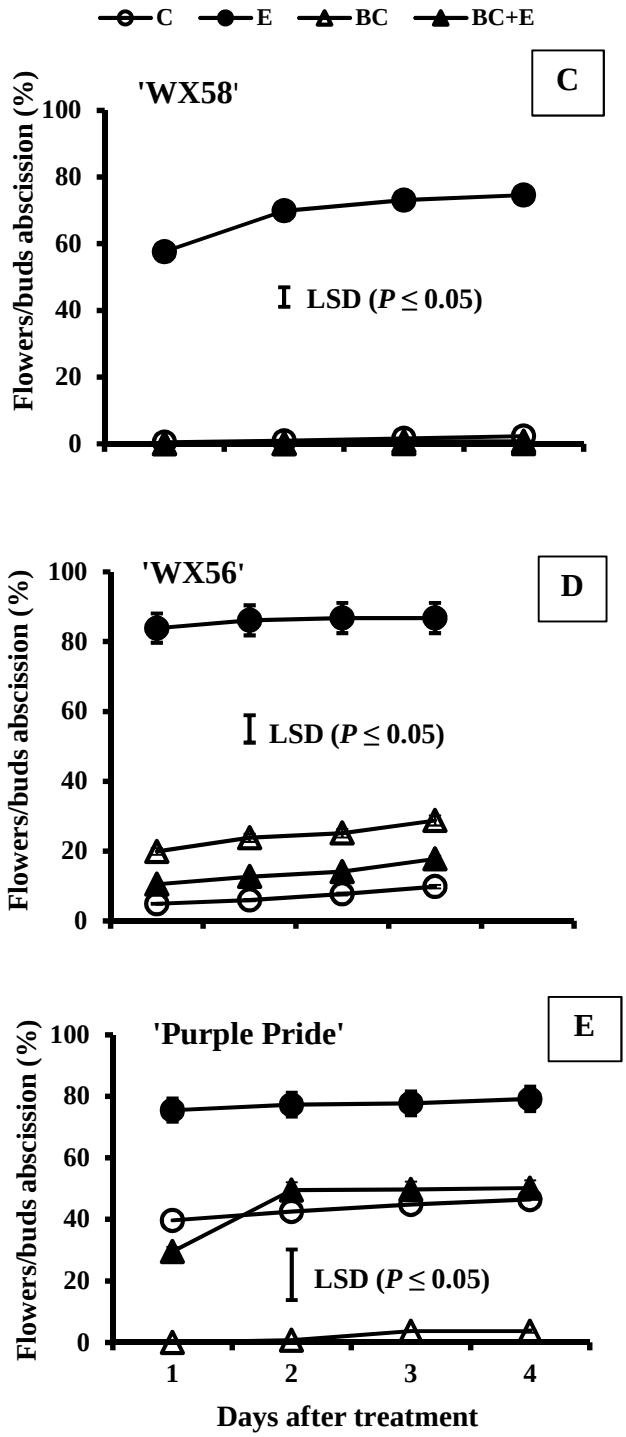


Figure. 8.4. Effects of fumigation of 1*H*-cyclopropabenzene (1 µM) or ethylene (10 µL L^{-1}) alone and 1*H*-cyclopropabenzene (1 µM) followed by ethylene (10 µL L^{-1}) on cumulative abscission of flowers/buds four days after treatment in (C)'WX58', (D) 'WX56' and (E) 'Purple Pride' waxflowers in 2015. n = three replications (three stems per replication), vertical bars represent SE. C = control, E = ethylene alone (10 µL L^{-1}), BC =1*H*-cyclopropabenzene alone, BC + E = 1*H*-cyclopropabenzene fumigation followed by ethylene.

8.3.3. Experiment 3: Effects of different concentrations of 1*H*-cyclopropabenzene fumigation alone and 1*H*-cyclopropabenzene fumigation followed by exposure to ethylene on flowers/buds abscission of 'Revelation' waxflowers in 2015.

Unsurprisingly, the ethylene fumigation alone resulted in the significantly ($P \leq 0.05$) highest flowers/buds abscission (47.1%) as compared to untreated control stems (5.9%) on day three after treatments in 'Revelation' waxflower in 2015 Fig. 8.5A. All the three 0.5, 1.0 and 2.0 µM concentrations of BC fumigation for 18 h and followed by exposure to 10 µL L^{-1} of ethylene for 24 h have significantly reduced flowers/buds abscission as compared to the ethylene alone on day three in 'Revelation' waxflower. The differences among different BC fumigation treatments followed by ethylene fumigation in reducing flowers/buds abscission were non-significant but BC (1 µM) fumigation was most effective Fig. 8.5A.

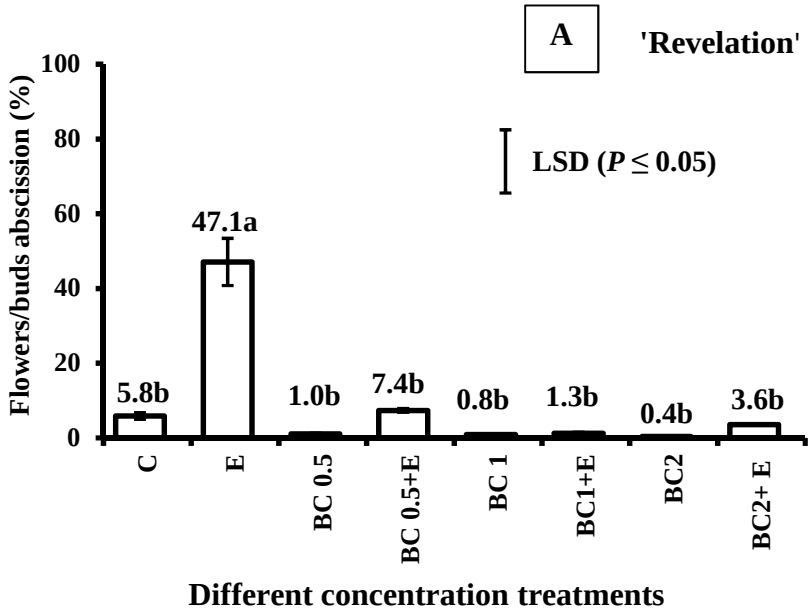


Figure. 8.5. Effects of different concentrations 0.5, 1.0 and 2.0 µM of fumigation of 1*H*-cyclopropabenzene or ethylene (10 µL L^{-1}) alone and 1*H*-cyclopropabenzene treatment followed by exposure to ethylene (10 µL L^{-1}) on flowers/buds abscission in (A) 'Revelation' waxflower on day three after treatments in 2015. Vertical bars represent SE, C = control, E = ethylene alone (10 µL L^{-1}), BC = 1*H*-cyclopropabenzene alone, BC + E = 1*H*-cyclopropabenzene fumigation followed by ethylene.

8.3.4. Experiment 4 and 5: Effect of 1*H*-cyclopropa[*b*]naphthalene (NC) fumigation on mean flowers/buds abscission in 'WX73', 'WX107', 'Jenny', 'WX58', 'WX56' and 'Purple Pride' genotypes of waxflower in 2014 - 2015.

The possibility of NC acting as ethylene antagonist was evaluated on six genotypes differing in their sensitivity to ethylene during two consecutive flowering seasons 2014 and 2015. The flowering sprigs of 'WX73', 'WX107', 'WX58', 'WX56' and 'Purple Pride' treated with ethylene (10 µL L^{-1}) alone for 24 h resulted in the significantly ($P \leq 0.05$) highest mean flowers/buds abscission over four days (86.9%, 73.14%, 68.8%, 85.9% and 77.4% respectively) in all the genotypes examined as compared with the untreated control stems (8.15%, 12.4%, 1.3%, 7.1% and 43.4% respectively) during 2014/2015 Fig. 8.6 A and B. Fig. 8.7 D, E and F. Meanwhile, the flower stems of 'WX73', 'WX107', 'WX58', 'WX56' and 'Purple Pride' fumigated with of NC (1 µM) for 18 h followed by exposure to 10 µL L^{-1} of ethylene alone for 24 h showed significantly ($P \leq 0.05$) reduced mean flowers/buds abscission (38.1% 'WX73', 25.5% 'WX107', 8.9% 'WX58', 18.8% 'WX56' and 27.0% 'Purple Pride') when compared with ethylene treatment alone for 24 h and all other treatments during four days Fig. 8.6 A and B. Fig. 8.7 D, E and F. Meanwhile, such a response in reduction of mean flowers/buds abscission with NC fumigation followed by exposure to ethylene in 'Jenny' waxflower was not noted during 2014 Fig. 8.6C.

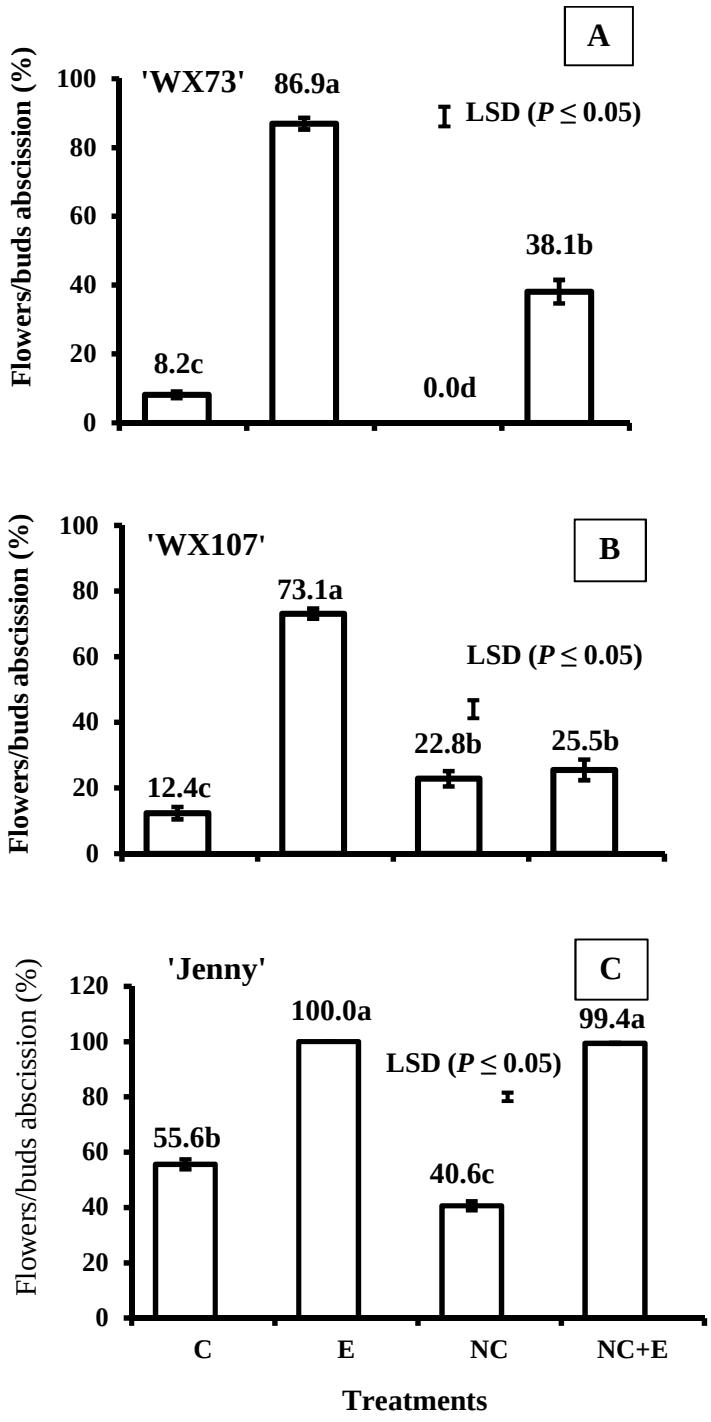


Figure. 8.6. Effects of fumigation of 1*H*-cyclopropa[*b*]naphthalene (1 µM), ethylene (10 µL L^{-1}) alone and 1*H*-cyclopropa[*b*]naphthalene followed by exposure to ethylene (10 µL L^{-1}) on mean flowers/buds abscission in (A)'WX73', (B)'WX107' and (C)'Jenny' waxflower during 2014. Vertical bars represent SE, C = control, E = ethylene alone (10 µL L^{-1}), NC = 1*H*-cyclopropa[*b*]naphthalene alone, NC + E = 1*H*-cyclopropa[*b*]naphthalene fumigation followed by ethylene.

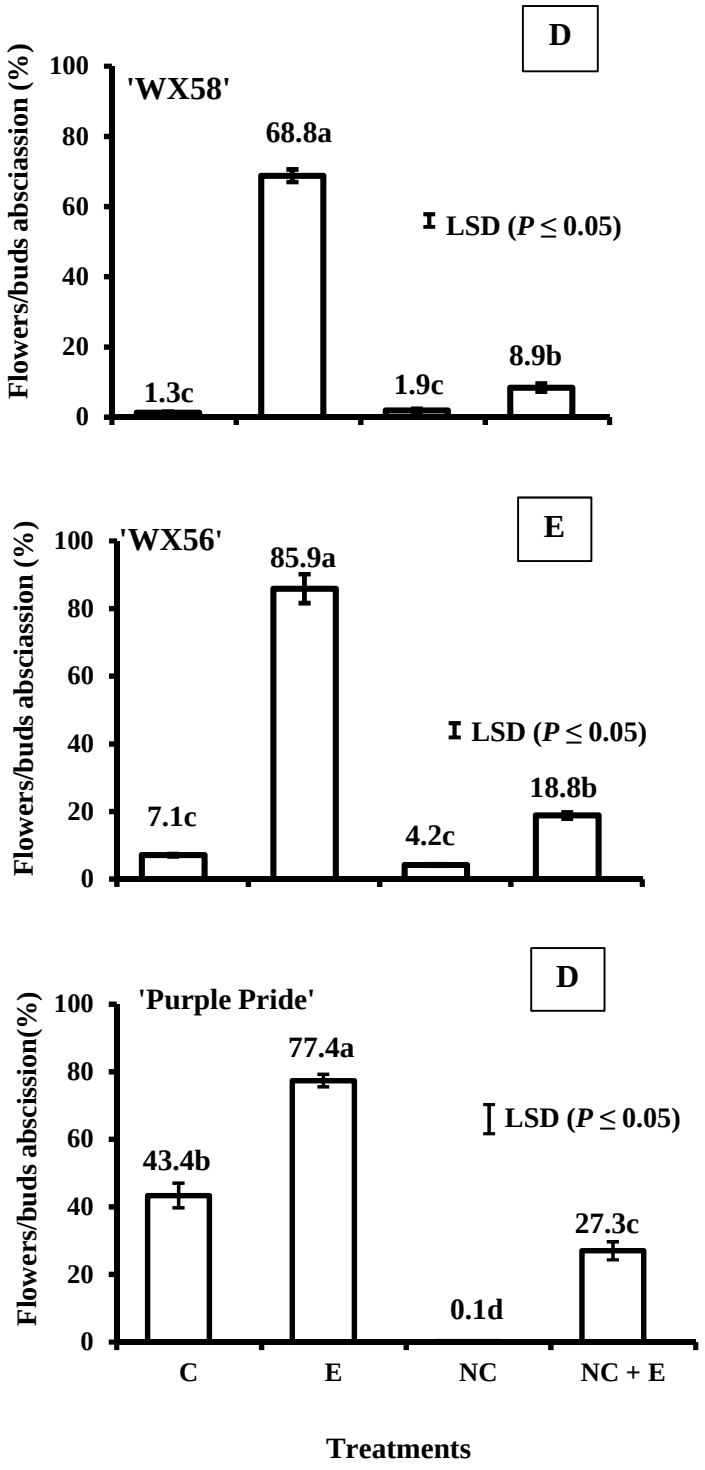


Figure. 8.7. Effects of fumigation of 1*H*-cyclopropa[*b*]naphthalene (1 µM), ethylene (10 µL L^{-1}) alone and 1*H*-cyclopropa[*b*]naphthalene followed by exposure to ethylene (10 µL L^{-1}) on mean flowers/buds abscission in (D)'WX58', (E) 'WX56' and (F) 'Purple Pride' waxflower during 2015. Vertical bars represent SE, C = control, E = ethylene alone (10 µL L^{-1}), NC = 1*H*-cyclopropa[*b*]naphthalene alone, NC + E = 1*H*-cyclopropa[*b*]naphthalene fumigation followed by ethylene.

8.3.5. Experiment 4 and 5: Effect of 1*H*-cyclopropa[*b*]naphthalene (NC) fumigation on cumulative flowers/buds abscission in 'WX73', 'WX107', 'Jenny', 'WX58', 'WX56', 'Purple Pride' genotypes of waxflower in 2014 -2015.

The effects of NC (1 µM), ethylene (10 µL L^{-1}) alone and combination of NC followed by exposure to ethylene (10 µL L^{-1}) on cumulative flowers/buds abscission during a four-day period was evaluated in 'WX73', 'WX107', 'Jenny', 'WX58', 'WX56' and 'Purple Pride' waxflowers during 2014 and 2015. As expected, the highest cumulative flowers/buds abscission (86.5% to 87.4% 'WX73', 67.7% to 77.7% 'WX107', 57.6% to 74.6% 'WX58', 83.9 to 86.7% 'WX56' and 75.5% to 79.1% 'Purple Pride') was observed in ethylene-treated flowers (10 µL L^{-1}) alone for 24 h as compared to the control (4.4% to 12.3%, 3.2% to 20.2%, 0.4% to 2.3%, 4.9% to 9.8%, and 39.7% to 46.5% respectively) Fig. 8.8 A and B. Fig. 8.9D, E and F. Meanwhile, the flowers sprigs of 'WX73', 'WX107', 'WX58', 'WX56', and 'Purple Pride' fumigated with 1 µM of NC and followed by exposure to 10 µL L^{-1} of exogenous ethylene for 24 h exhibited significantly decreased cumulative flowers/buds abscission over four day (29.5% to 44.3% 'WX73', 12.8% to 37.1 'WX107', 3.3% to 11.7% 'WX58', 22.8% to 17.6% 'WX56' and 23.1% to 28.6% 'Purple Pride') Fig. 8.8 A and B. Fig. 8.9D, E and F. Whilst 'Jenny' genotypes exhibited the highest (100% to 100% and 98.5% to 100%) cumulative flowers/buds abscission from day one to day four when the stems were exposed to ethylene treatment (10 µL L^{-1}) alone and when fumigated with 1 µM of NC and followed by exposure to 10 µL L^{-1} of ethylene for 24 h as compared to untreated control stems during 2014 Fig. 8.8C.

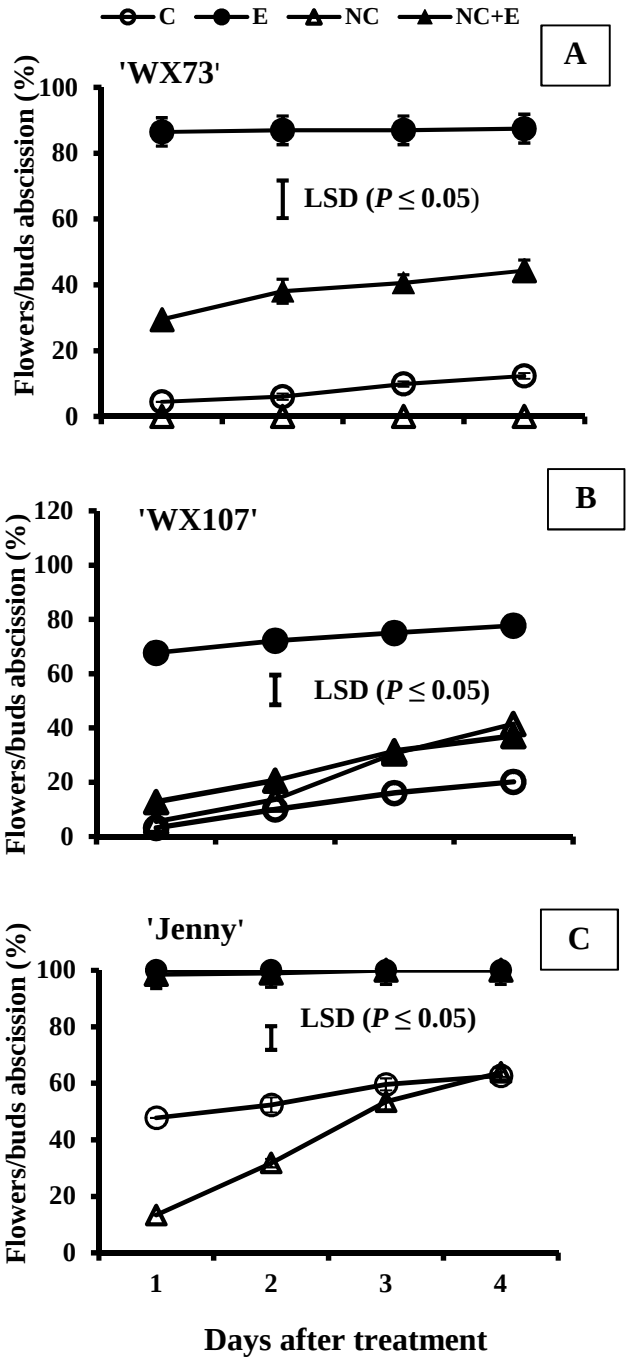


Figure. 8.8. Effects of fumigation of 1*H*-cyclopropa[*b*]naphthalene (1 µM) or ethylene (10 µL L^{-1}) alone and 1*H*-cyclopropa[*b*]naphthalene (1 µM) followed by ethylene (10µL L^{-1}) on cumulative abscission of flowers/buds four days after treatment in (A) 'WX73', (B) 'WX107' and (C) 'Jenny' waxflower in 2014. n = three replications (three stems per replication), vertical bars represent SE. C = control, E = ethylene alone (10 µL L^{-1}), NC = 1*H*-cyclopropa[*b*]naphthalene alone, NC + E = 1*H*-cyclopropa[*b*]naphthalene fumigation followed by ethylene.

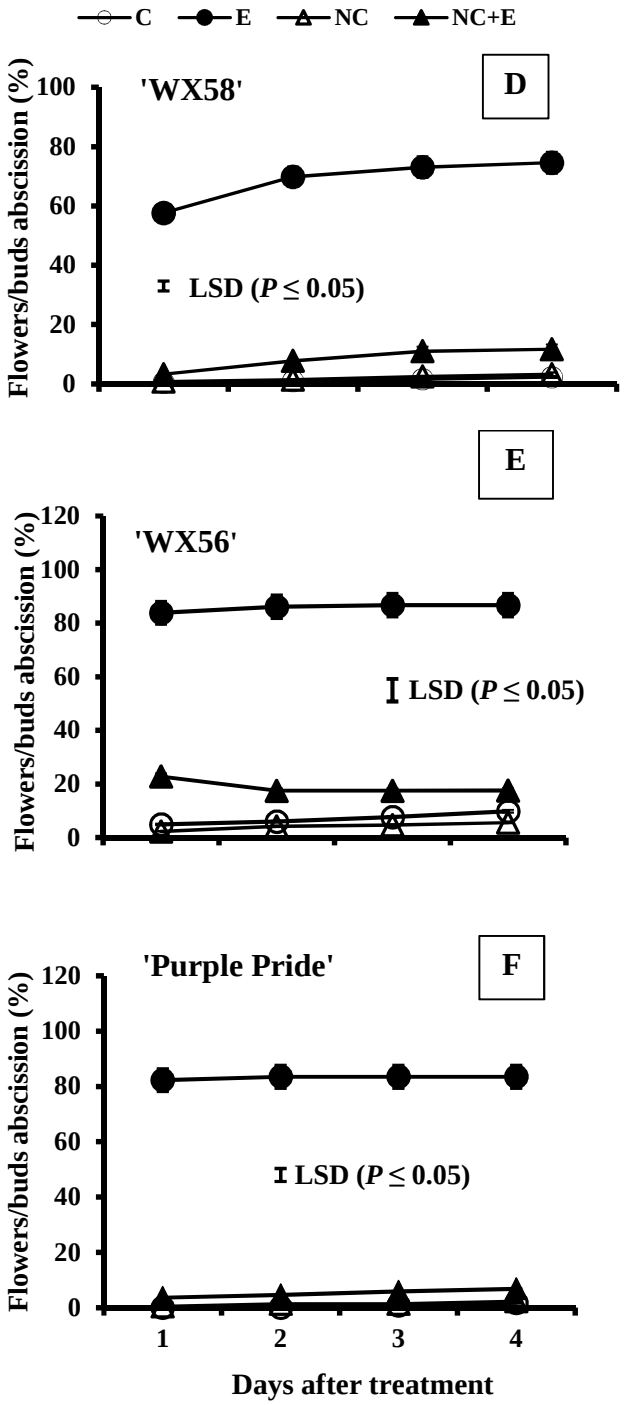


Figure. 8.9. Effects of fumigation of 1*H*-cyclopropa[*b*]naphthalene (1 µM) or ethylene (10 µL L^{-1}) alone and 1*H*-cyclopropa[*b*]naphthalene (1 µM) followed by ethylene (10 µL L^{-1}) on cumulative abscission of flowers/buds four days after treatment in (D) 'WX58', (E) 'WX56' and (F) 'Purple Pride' waxflower in 2015. n = three replications (three stems per replication), vertical bars represent SE. C = control, E = ethylene alone (10 µL L^{-1}), NC = 1*H*-cyclopropa[*b*]naphthalene alone, NC + E = 1*H*-cyclopropa[*b*]naphthalene fumigation followed by ethylene.

8.3.6. Experiment 6: Effects of different concentrations of 1*H*-cyclopropa[*b*]naphthalene fumigation alone and 1*H*-cyclopropa[*b*]naphthalene fumigation followed by exposure to ethylene on flowers/buds abscission of 'Purple Pride', 'Hybrid1' and 'Revelation' waxflowers in 2015.

The flowers bunches treated with ethylene treatment alone for 24 h showed significantly ($P \leq 0.05$) highest mean flowers/buds abscission (83.9%, 83.4% and 45.9% in 'Purple Pride', 'Hybrid1' and 'Revelation' respectively) as compared to untreated control flowers (2.0%, 8.0% and 2.5% respectively) Fig.8.10A, B and C. 'Purple Pride' and 'Revelation' flower stems fumigated with of 1*H*-cyclopropa[*b*]naphthalene (1.0 µM) followed by ethylene exposure (10 µL L^{-1}) resulted in significantly ($P \leq 0.05$) lowest flowers/buds abscission on the second day after the treatments as compared to other concentrations of 1*H*-cyclopropa[*b*]naphthalene applied Fig. 8.10A and C. Meanwhile, the flower stems of 'Hybrid1' fumigated with of 1*H*-cyclopropa[*b*]naphthalene (0.5 µM) followed by ethylene exposure for 24 h resulted in significantly ($P \leq 0.05$) reduced mean flowers/buds abscission on day two as compared to the other concentrations of 1*H*-cyclopropa[*b*]naphthalene fumigation applied Fig. 8.10 B. In general, of 1*H*-cyclopropa[*b*]naphthalene fumigation (0.5 and 1.0 µM) was the most effective in reducing adverse effects of ethylene in curtailing mean flowers/buds abscission as compared to all other treatments except control in 'Hybrid1', 'Revelation' and 'Purple Pride' during 2015.

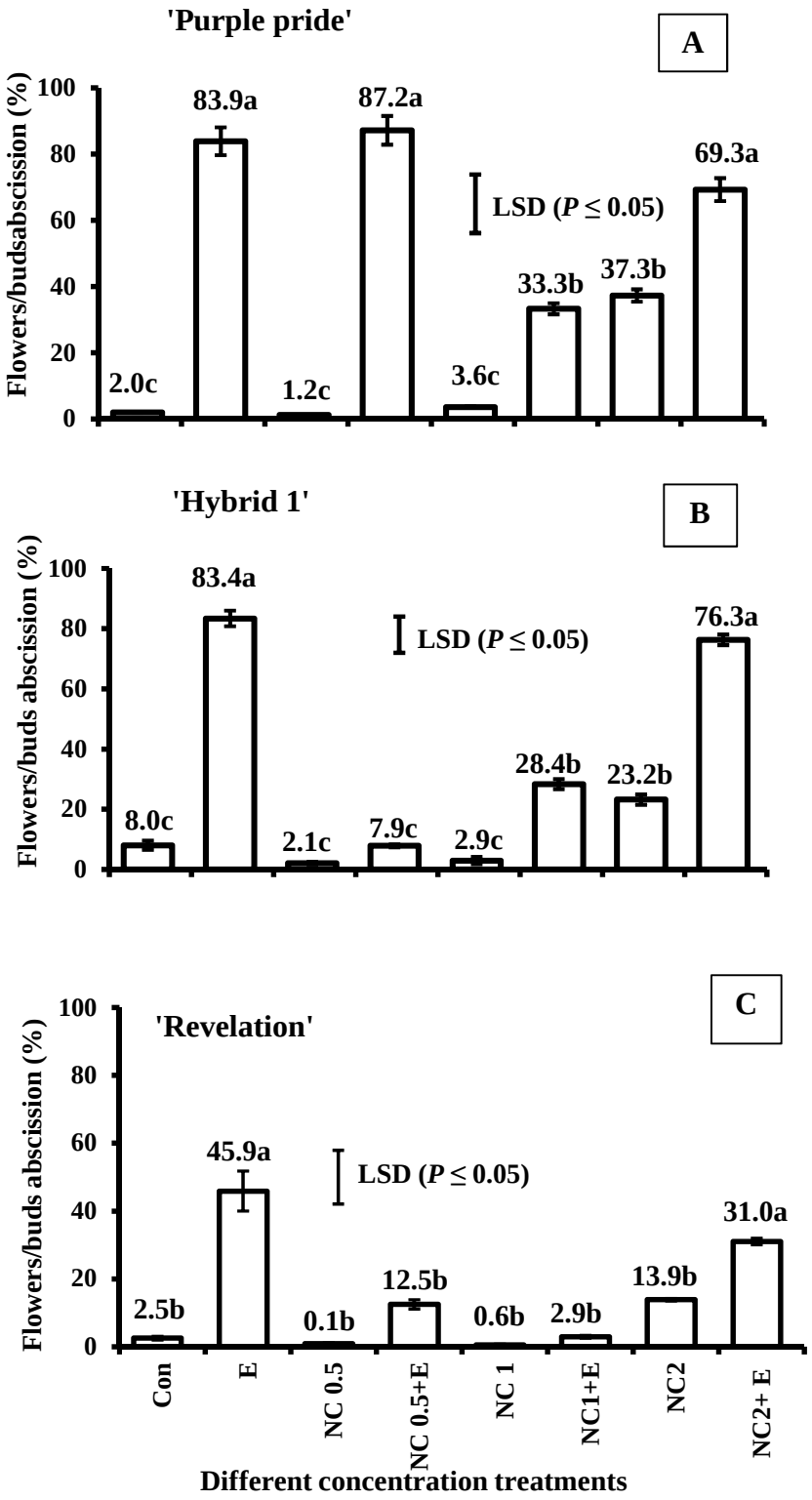


Figure. 8.10. Effects of different concentrations 0.5, 1.0 and 2.0 µM of fumigation NC or ethylene (10 µL L^{-1}) alone and NC treatment followed by exposure to ethylene (10 µL L^{-1}) on flowers/buds abscission in (A)'Purple Pride',(B) 'Hybrid1' and (C)'Revelation' waxflower on day 2 in 2015. Vertical bars represent SE. C = control, E = ethylene alone (10µ L L^{-1}), NC = 1*H*-cyclopropa[*b*]naphthalene alone, NC + E = 1*H*-cyclopropa[*b*]naphthalene fumigation followed by ethylene.

8.4. Discussion

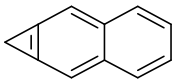
Cyclopropenes and their alkyl derivatives have recently been shown to interact with the ethylene for receptor and their ability to inhibit ethylene-induced different responses in plant tissues (Saleh-Lakha *et al.*,2004; Sisler *et al*., 2006; Apelbaum *et al*., 2008; Reid and Staby, 2008; Sisler *et al*., 2009). Earlier, Sisler (2008) reported that the molecular structure was an essential factor to affect the reactivity of the cyclopropene group. Previously, Sisler *et al*. (2001) claimed that molecule structure, size, shape, high ring strain, presence of the functional groups, level of saturation, hydrophobicity and number of double bonds location near to functional groups seem to play important role in the activity of a compound as an ethylene inhibitor (Sisler, 2008).

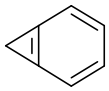
BC fumigation (1 µM) for 18 h prior to the 24 h of exposure to ethylene treatment was markedly effective in reducing flowers/buds abscission in 'WX73', 'WX58', 'WX56' and 'Purple Pride' genotypes of waxflowers as compared to those fumigated with ethylene (10 µL L^{-1}) alone in 2014 and 2015 Fig. 8.1 A and Fig. 8.2 C, D and E. Similarly, NC (1 µM) fumigation for 18 h followed by exposure to ethylene (10 µL L^{-1}) alone for 24 h decreased the mean flowers/buds abscission in 'WX73', 'WX107', 'WX58', 'WX56' and 'Purple Pride' genotypes as compared with ethylene treatment alone for 24 h during 2014 and 2015 Fig. 8.6 A and B . Fig. 8.7 D, E and F. There was a significant reduction in ethylene-induced flowers/buds abscission in waxflower genotypes using new potent ethylene antagonists BC or NC which, may be attributed to their structural molecular strain antagonising the effect of ethylene as reported about cyclopropene compounds earlier by (Sisler and Serek, 1997; Sisler, 2008; Sisler *et al*., 2009). Because both compounds contain the cyclopropene ring as the functional group Fig.8.11. Which, render them potent inhibitors for blocking ethylene action, BC has a much higher strain value than cyclopropene (52.6 kcal mol^{-1}) (Halton, 1973; Bach and Dmitrenko, 2004). Earlier, Sisler *et al*. (2006) reported that the energy strain seems to be the main associated factor in the activity of the antagonists in blocking the ethylene receptors and inhibits ethylene action beside some other factors (Sisler, 2008). According to Sisler and Serek, (1997) mode of action of these compounds containing the cyclopropene ring is similar to 1-MCP as they can bind to ethylene receptor sites irreversibly. It has been demonstrated by some other

studies that ring strain of these ethylene antagonists reacts with co- factor (Cu) present in the ethylene receptor ETR1 sites, which ultimately leads to very strong bonding (Rodrıguez, *et al*., 1999; Pirrung *et al*., 2008; Reid and Celikel, 2008). Sisler (2008) reported that the inhibitory effect of ethylene antagonists is due to the ring strain. Due to the ethylene, antagonistic potential of BC and NC comparable to 1-MCP, it is supposed these both these compounds made an irreversible bond with ethylene receptor sites and their double bond may cause an inactivation of ethylene receptors (Sisler, 2008). Contrarily, BC or NC (1 µM) fumigation for 18 h followed by 24 h of exposure to ethylene (10 µLL^{-1}) did not reduce the flowers/buds abscission in 'WX17' and 'Jenny' respectively Fig. 8.1B and Fig. 8.6 C. Probably these genotypes are much more sensitive to ethylene because of their inherited trait as reported earlier (Macnish *et al*., 2004a).

Amongst three different concentrations 0.5, 1.0 and 2.0 µM of 1*H*-cyclopropabenzene fumigation for 18 h followed by 24 h exposure to 10 µL L^{-1} ethylene tested, all the concentrations of BC treatments significantly reduced mean flowers/buds abscission in 'Revelation' waxflowers compared to the ethylene alone treatment but the differences among different concentrations of BC applied did not differ significantly Fig. 8.5A. Similarly, Khan (2014) reported that 'WX17' and 'WX73' genotypes fumigated with 50 nLL^{-1} to 100 nL L^{-1} of BC and followed by ethylene exposure (10 µL L^{-1}) exhibited significantly lower abscission of the flowers to the fourth day compared to those treated with ethylene alone. Among three different concentrations 0.5 and 1.0 µM of NC fumigation tested following by ethylene exposure for 24 h 0.5 and 1.0 µM concentrations were the most efficient in lessening mean flowers/buds abscission as compared to all other treatments except control in 'Hybrid1', 'Revelation' and 'Purple Pride' during 2015 Fig. 8.10 B,C and A.

In conclusion, the experimental results demonstrated that the 1*H*-cyclopropabenzene or 1*H*-cyclopropa[*b*]naphthalene are antagonists to ethylene action as evident by reduction in ethylene-induced flowers/buds abscission in different genotypes of waxflowers but dependent upon the genotype to some degree Fig. 8.12.

1. 

2. 

1. 1*H*-cyclopropa[*b*]naphthalene, ($C_{11}H_8$) Molecular weight: 140.2 g mol^{-1}
2. 1*H*-cyclopropabenzene, (C_7H_6) Molecular weight: 132.2 g mol^{-1}

Figure.8.11. Chemical structures 1*H*-cyclopropa[*b*]naphthalene and 1*H*-cyclopropabenzene.

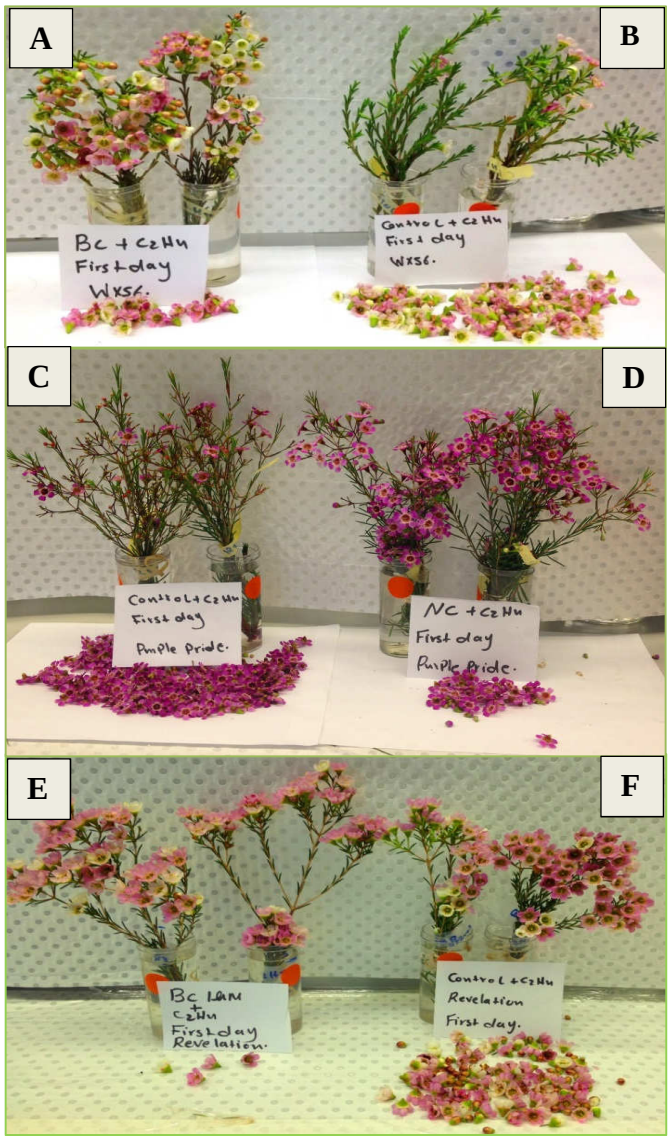


Figure. 8.12. Effects of fumigation of BC and NC (1 µM) for 18 h and followed by exposure to ethylene (10 µL L^{-1}) for 24 h and the ethylene treatment alone on flower/buds abscission 'WX56', 'Purple Pride' and 'Revelation' waxflowers. (A) 'WX56' waxflower stems treated with BC (1 µM) for 18 h and followed by exposure to ethylene (10 µL L^{-1}) for 24 h. (B) 'WX56'waxflower stems treated with ethylene alone (10 µL L^{-1}) for 24 h. (C) 'Purple Pride' waxflower stems treated with ethylene (10 µL L^{-1}) alone for 24 h. (D) 'Purple Pride' waxflower stems treated with NC (1 µM) for 18 h and followed by exposure to ethylene (10 µL L^{-1}) for 24 h. (E) 'Revelation' waxflower stems treated with BC (1 µM) for 18 h and followed by exposure to ethylene (10 µL L^{-1}) for 24 h. (F) 'Revelation' waxflower stems treated with ethylene (10 µL L^{-1}) alone for 24 h.

CHAPTER 9

Effect of fumigation of 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene *trans*-cinnamaldehyde and 1-hexylcyclopropene on flower/leaf vase life of 'Crystal Pearl', 'Lady Stephanie', 'Purple Pride' and 'WX74' waxflower.

Abstract

Flowers senescence is the main limiting factor in the supply chain of various species of potted and cut flowers. The aim of this study was to assess the effect of fumigation with new ethylene antagonists (NC, BC, CA and 1-HCP) on extending the vase life of 'Crystal Pearl', 'Lady Stephanie', 'Purple Pride' and 'WX74' waxflower. The study also aimed to evaluate the influence of fumigation with CA, NC and 1-HCP alone and in combination with vase solutions of distilled water (DW) and supplemented with 8-HQS, sucrose and fructose on vase life of flowers/leaves of waxflowers. In the first experiment, flower sprigs were fumigated with different NC, BC , CA and 1-HCP ethylene antagonists (1 µM for18 h) followed by placement in a vase solution of DW. In the second experiment, the flower sprigs of 'WX14' and 'WX74' were fumigated with CA, NC or 1-HCP for18 h followed by placement in vase solutions containing distilled water, 8-HQS (100 mg L^{-1}), sucrose and/or fructose (58.5 µM). The 1-HCP treatment was more effective in extending flower vase life in 'Crystal Pearl', 'Lady Stephanie', 'Purple Pride' and 'WX74' waxflowers when kept in DW alone compared to the other three chemicals (mentioned above). Meanwhile, NC and 1-HCP fumigation were more effective in extending leaf vase life of these four genotypes as compared to DW and other vase solution treatments. 'Crystal Pearl', 'Lady Stephanie' and 'WX74' genotypes were more responsive to ethylene antagonist fumigation treatments compared to 'Purple Pride' in prolonging flower and leaf vase life. In the second experiment, the flowering stems of 'WX74' and 'WX14' fumigated with 1-HCP or CA followed by placement in vase solutions containing both sucrose and 8-HQS or followed by 8-HQS or DW exhibited the longest mean flower and leaf vase life as compared to all other treatments and non-fumigated controls. In conclusion, 1-HCP or NC fumigation treatments in DW alone or 1-HCP or CA fumigation in combination in vase solutions containing sucrose and 8-HQS were the most effective treatments for extending flower/leaf vase life of cut waxflower genotypes.

9.1. Introduction

Geraldton waxflower (*Chamelaucium uncinatum* Schauer.) is one of the major native cut flowers in Australia due to its value as a cut flower and its availability in many colours for export throughout the world (Newell *et al*., 1999; Gollnow and Worrall, 2010; Seaton and Poulish, 2010; Seaton, 2012). However, a major cause of quality deterioration in waxflower is high flowers/buds abscission on cut flower sprigs during marketing consequently shortening the vase life and limiting trade (Joyce, 1993; Macnish *et al*., 2000b; Beasley and Joyce, 2002). Consumers prefer high-quality flowers with maximum vase life (Elhindi, 2012; Mahmood *et al*., 2015). Short postharvest vase life is one of the common problems of cut flower crops and waxflowers are sensitive and vulnerable to postharvest losses such as senescence, leaf yellowing, premature wilting, organs abscission and diseases caused by fungal pathogens (Seaton and Poulish, 2010; Abbasi and Asil, 2011; Kazemi *et al*., 2011; Scariot *et al*., 2014).

Flower vase life is influenced by genetic factors, such as vase life of different cultivars of native waxflowers varying from 7 to 30 days while for hybrid waxflowers variation is from 17 to 30 days (Seaton and Poulish, 2010). In *Verticordia,* vase life of flowers of different genotypes varies from 5 to 19 days and for leaves from 5 to 30 days (Seaton, 2006). While, vase life of hybrid tea rose varies from 4.5 to 18.8 days (Macnish *et al*., 2010) depending on water uptake ability and the internal carbohydrate levels (Halevy and Mayak, 1981; Kazuo *et al*., 2005). The vase life of cut flowers is also influenced by flower handling methods (Pizano, 2009), growing conditions such as temperature, light, nutrition (Celikel and Kraracaly, 1995) and the presence of blockage of xylem vessels (Edrisi *et al*., 2012; Hassan and Ali, 2014; Mahmood *et al*., 2015). However, ethylene plays a key role in promoting senescence in various flowers (Ichimura *et al*., 2002; Muller and Stummann, 2003; Serek *et al*., 2006; Asil and Karimi, 2010; Seglie *et al*., 2010; Scariot *et al.,* 2014) and so affects vase life. Senescence shortens vase life of various cut flowers and leaves and consequently limiting their marketability (van Doorn and Wothering, 1991; Bowyer and Wills, 2003). Ethylene has also been implicated in accelerating flower/leaf abscission, flower senescence and deterioration of visual

appearance and display life in cut waxflowers (Joyce, 1988; Abeles *et al.,* 1992; Gollnow and Worrall, 2010; Seaton and Poulish, 2010).

Vase life of different cut flowers was prolonged with the application of silver thiosulfate (STS) (Cameron and Reid, 1983; Joyce, 1988; Staby *et al*., 1993; Dole *et al*., 2004; Sexton *et al*., 2005; Ebrahimzadeh *et al*., 2008; Williamson and Joyce, 2013). Additionally, Serek *et al*. (2006) reported that application of 500 μLL^{-1} 2-5, norbornadiene was effective in extending the vase life of carnation cut flowers. Applying STS (4 mmol) for 20 min as a pulse was effective in reducing the floral drop in C. *uncinatum* cultivars (Joyce,1988; Seaton, 2005) and also, extended the lifetime of sensitive cut *Freesia* flowers (Zencirkiran, 2010). 1-MCP inhibited the effects of exogenously applied ethylene and improved the shelf life of plant products and quality depending upon genotype (Blankenship and Dole, 2003; Serek and Reid, 2000; Cameron and Reid, 2001; Zencirkiran, 2010). Macnish *et al*. (2000b) reported that 14 different genotypes of Australian native cut flowers fumigated with 10 nLL^{-1} of 1-MCP protected flowers from ethylene and did not increase the vase life of native flowers in the absence of exogenous ethylene.

The addition of 8-hydroxyquinoline sulphate (8-HQS) a well-known antimicrobial agent used by the floral industry to inhibit microbial growth results in an increase in water uptake of the cut flower stems as reported by (Nowak and Rudnicki, 1990; Asrar, 2012). Vase solutions containing 8-HQS were significantly effective in prolonging the vase life and quality of *Strelitzia reginae* Ait. (Ail and Hassan, 2014) and pulse treatment with 8-HQS alone effective treatment to extend the vase life of florets 'Diana' *Lathyrus odoratus* (Elhindi, 2012), 8-HQS with chlorine dioxide (ClO_2) extended vase life of *Gerbera jamesonii* 'Julia', 'Lorca' and 'Vilassar' flowers (Macnish *et al*., 2008) and 8-HQS in combination with ABA prolonged the vase life of flowers, but not of foliage in waxflowers (Joyce and Jones, 1992).

Previously, Ichimura and Suto (1999) and Elhindi (2012) reported the positive effects of sugars in plants which was ascribed to the supply of adequate substrates for respiration, materials for cell wall biosynthesis and decreased osmotic values for increased water balance in cut flowers. Sugars have been found to be more effective in slowing down flower senescence by reducing ethylene sensitivity in

ethylene sensitive flowers than ethylene insensitive flowers (Pun and Ichimura, 2003; van Doorn, 2004; van Doorn and Woltering, 2008).

Vase solutions with soluble sugars have been reported to improve vase life in different cut flowers (Halevy and Mayak, 1979; Pun and Ichimura, 2003; Arrom and Munne-Bosch 2012; Ikeura *et al*., 2012). Joyce and Jones (1992) found that addition of 58.5 mmol sucrose alone in vase solution reduced the foliage longevity but increased vase life of 'Purple Pride' waxflowers. However, this concentration of sucrose has damaging effects on the foliage of *C. uncinatum* 'Alba'. Dung (2013) also claimed addition of 58.48 mmol of sucrose caused desiccation of the leaves in *C. megapetalum* hybrids and leaf tip injury in 'Purple Pride' while lower levels were less damaging and extended vase life. Also, Dung (2013) found that application of fructose, sucrose and glucose was more effective than maltose and galactose in improving vase life in different cultivars of waxflowers.

Vase solutions containing two effective components such as germicides and sugars were found to be effective techniques for extending different flowers vase life (Asrar, 2012). Elgimabi and Sliai (2013) reported that the application of 8-HQS (200 mg L^{-1}) significantly improved Taif rose flower vase life when combined with 7% sucrose. In addition, Beura and Singh (2001) showed that the combination treatment of 8-HQS and sucrose in vase solution improved the gladiolus spikes flowers quality. The 8-HQS combined with 2% sucrose in vase water treatment maintained water uptake and prolonged vase life of *Antirrhinum majus* L. up to 18 days and delayed flower senescence (Asrar, 2012). Dung (2013) reported that 200 mg L^{-1} 8-HQS in combination with sucrose concentration up to 116.9 mmol increased the vase life in six genotypes of waxflowers.

Joyce and Jones (1992) reported the positive effect of applying sucrose alone in vase solutions to prolong the lifetime of cut 'Geraldton wax'. While Joyce (1988) reported that sucrose in combination with HQS significantly improved *C. uncinatum* vase life. In addition, Dung (2013) investigated the effect of different types of sugars alone as vase solutions such as maltose, glucose, fructose and galactose and sucrose at different concentration supplemented with 200 mg L^{-1} 8-HQS in extending the vase life of flowers/leaves of different cultivars of waxflowers. This is the first time that fumigation with new types of ethylene antagonists such as NC, BC, CA and 1-

HCP alone or in combination with vase solution containing 8-HQS and/or sugars on vase life of flowers/leaf in waxflowers has been investigated. It was hypothesised that waxflower sprigs fumigated with ethylene antagonist in combination with vase solution containing 8-HQS and/or sugars would be more effective in extending the vase life of waxflower and leaf compared to their application alone. The effects of NC, CA and 1-HCP fumigation alone and in combination with vase solution containing 8-HQS and/or supplemented with sucrose and fructose on extension of vase life of flowers and leaves in different genotypes of waxflowers was investigated.

9.2. Materials and Methods.

9.2.1. Sources of chemicals

Trans-cinnamaldehyde was purchased from Acros Organics™, New Jersey, USA. Meanwhile, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene and 1-hexylcyclopropene were synthesised by Dr Alan Payne and his research group at the Department of Chemistry, Curtin University. Ethylene gas was purchased from BOC Gases, Australia Ltd., Perth, Australia as mentioned in Chapter 5, Section 5.2.1.

9.2.2. Plant material

In both experiments, the fresh flowering stems of ‘Crystal Pearl’, ‘Lady Stephanie’, ‘Purple Pride’, ‘WX14’ and ‘WX74’ were harvested from DAFWA, South Perth, Western Australia.

9.2.3. Harvesting flowering stems

The leafy flowering shoots of ‘Crystal Pearl’, ‘Lady Stephanie’, ‘Purple Pride’, ‘WX14’ and ‘WX74’ genotypes were harvested when 60 to 99% flowers were open in the early morning as mentioned in Chapter 5, Section 5.2.3.

9.2.4. Experiments.

9.2.4.1. Experiment 1: Effect of CA, NC, BC and 1-HCP fumigation on flower/leaf vase life of 'Crystal Pearl', 'Lady Stephanie', 'Purple Pride' and 'WX74' in 2015.

The fresh cut flowers of 'Crystal Pearl', 'Lady Stephanie', 'Purple Pride' and 'WX74' genotype were prepared for the treatments as mentioned in Chapter 5, Section 5.2.3. Five treatments were applied in this experiment to each genotype including untreated flower stems. Flower stems were fumigated with CA, NC, BC and 1-HCP for 18 h as detailed in Chapter 5, Section 5.2.4. Following the completion of 18 h fumigation treatment, the stems were transferred to vase life room at (DAFWA) and the stems for each genotype trimmed to a length of 30 to 35 cm and placed in individual 250 ml translucent plastic vases containing distilled DW and kept in the vase room at DAFWA, South Perth. The vase life room was maintained at 20º ± 2°C, 60 ± 10% relative humidity with a 12 h photoperiod (Seaton, 2006). The experiment was conducted following two factors (treatments and genotypes) factorial completely randomised design with six replications and one stem per replication. The vase life of flowers and leaf was recorded on the control and treated sprigs over several weeks.

9.2.4.2. Experiment 2: Influence of fumigation of CA, NC and 1-HCP alone and in combination with vase solutions supplemented with 8-HQS, sucrose and fructose on vase life of flowers/leaf in 'WX14' and 'WX74' waxflowers.

In this experiment, the effectiveness was evaluated of fumigation of CA, NC, and 1-HCP alone as ethylene antagonist and in a combination of fumigation treatments with vase solution containing 58.5 µM of sugars or fructose and/or 100 mgL^{-1} 8-HQS in extending the flowers/leaf vase life in 'WX14' and 'WX74' waxflowers. Fumigation treatments 1 µM (CA, NC and 1-HCP) for 18 h were applied in 60 L plastic drums as explained in Chapter 5, Section 5.2.4. Untreated stems were treated as a control. After 18 h of chemical fumigation, the flowers were transported to the vase room at the DAFWA laboratory at South Perth for vase life treatments. Following the ethylene antagonists treatments, the stems were kept in different types of vase solutions containing 8-HQS and sugars with 16 treatments as

mentioned in Table. 9.1. The experimental design was completely randomised two factors (treatments and genotypes) factorials, and each treatment was replicated five times, with one stem in each replication. Vase life of flowers and leaf were noted in all treatments including the control in both genotypes.

Table. 9.1. Chemical fumigation alone and in combination with vase solutions supplemented with 8-HQS, and /or sucrose and fructose in 'WX14' and 'WX74'waxflowers.

Treatments
No fumigation, vase solution with DW (control)
No fumigation, vase solution with HQS only
No fumigation, vase solution with HQS+ sucrose
No fumigation, vase solution with HQS+ fructose
NC fumigation, vase solution with DW
NC fumigation, vase solution with HQS only
NC fumigation, vase solution with HQS+ sucrose
NC fumigation, vase solution with HQS+ fructose
CA fumigation, vase solution with DW
CA fumigation, vase solution with HQS only
CA fumigation, vase solution with HQS+ sucrose
CA fumigation, vase solution with HQS+ fructose
1-HCP fumigation, vase solution with DW
1-HCP fumigation, vase solution with HQS only
1-HCP fumigation, vase solution with HQS+ sucrose
1-HCP fumigation, vase solution with HQS+ fructose

9.2.5. Assessment of vase life of flowers and leaf.

In both experiments, the vase life of stems was determined as the time from placement of flowering stems in different vase solutions for evaluation until they lost their ornamental visual appeal (drop and wilting) using the rating scale ranging from 1 to 5 as detailed in Table. 3.3. Chapter 3, section 3.7.3. For flowers, the end of vase life was assessed when more than 50% of opened flowers had dropped or showed wilting. Vase life of leaf was determined when more than 50% of the leaves were fully desiccated or yellow for their full length (Seaton and Joyce, 1992 and 1993).

9.2.6. Experimental design and statistical analysis of data

The data on vase life of flowers and leaves from both experimental treatments were subjected to two-way ANOVA using the statistical package GenStat 14th edition (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The effect of treatments, genotypes and their interaction was assessed and means were compared by LSD at $P < 0.05$. The standard errors of the mean (±, SEM) were also shown as appropriate. Where possible, mean comparisons were made using Duncan's Multiple Range Test. All the assumptions of ANOVA were checked to ensure the validity of the statistical analysis.

9.3. Results

9.3.1. Effect of CA, NC, BC and 1-HCP fumigation on flowers/leaf vase life in 'Crystal Pearl', 'Lady Stephanie', 'Purple Pride' and 'WX74' waxflowers in 2015.

9.3.1.1. Flower vase life

When averaged over different genotypes, treated flower sprigs fumigated with 1-HCP (1 µM) for 18 h exhibited significantly ($P \leq 0.05$) the longest mean vase life of flowers (20.7 days) when kept in DW as compared to all other fumigation treatments including CA , BC or NC and control Table. 9.2. When averaged over different treatments, the flower vase life varied significantly ($P \leq 0.05$) among four genotypes of waxflowers Fig. 9.1 A. 'Lady Stephanie' flowers showed significantly longer flower vase life (23.7 days) compared to 'Crystal Pearl' (20.8days), 'Purple

Pride' (12.1days) and 'WX74' (18.1days). The interaction between different treatments and genotypes was found to be non-significant for extending flower vase life as shown in Fig.9.2B.

Table. 9.2. Effect of fumigation of 1*H*-cyclopropa[*b*]naphthalene (NC), 1*H*-cyclopropabenzene (BC), *trans*-cinnamaldehyde (CA) and 1-hexylcyclopropene (1-HCP) on mean flowers vase life in four genotypes of waxflowers. Vertical bars represent SE mean.

Treatments	Genotypes				
	'Crystal Pearl'	'Lady Stephanie'	'Purple Pride'	'WX74'	Mean (Treatments)
DW	19.00cd	23.7ab	11.0h	19.5c	18.3b
NC	20.7bc	23.5ab	12.3gh	18.5cdef	18.8b
BC	19.0cde	24.5ab	11.0h	15.3defg	17.5b
CA	21.5bc	21.3bc	11.0h	18.8cdef	18.2b
1-HCP	23.7ab	25.7a	15.2dfg	18.3cdef	20.7a
Mean (Genotypes)	20.8 b	23.7a	12.1d	18.1c	
LSD($P \leq 0.05$)	Treatment=1.67, genotypes=1.49, Treatment x genotypes=3.35				

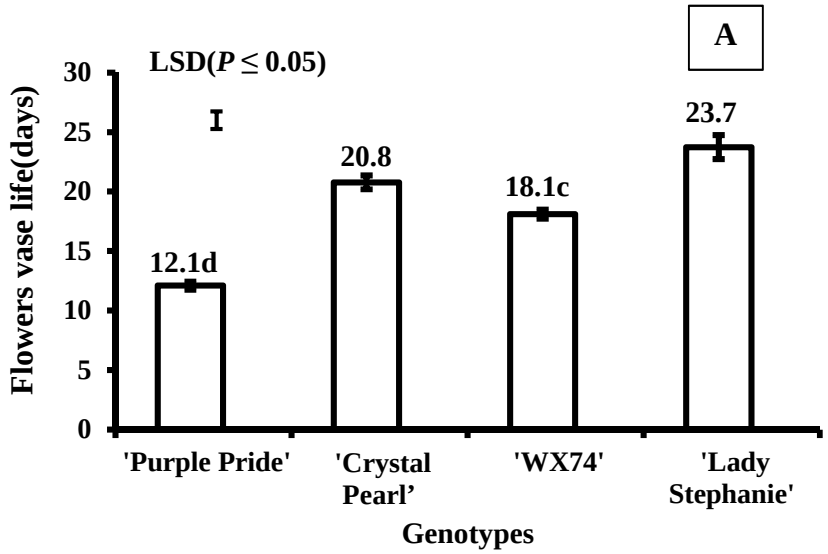


Figure. 9.1. Mean flower vase life in four genotypes following fumigation with 1*H*-cyclopropa[*b*]naphthalene (NC), 1*H*-cyclopropabenzene (BC), *trans*-cinnamaldehyde (CA) or 1-hexylcyclopropene (1-HCP). Vertical bars represent SE mean.

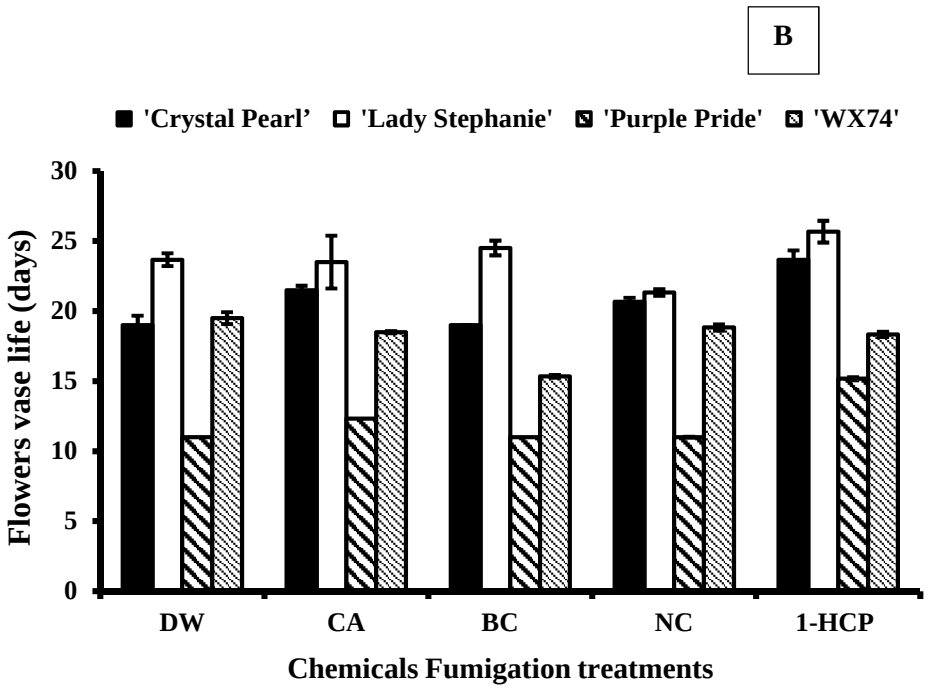


Figure. 9.2. Flower vase life (days) of 'Crystal Pearl', 'Lady Stephanie', 'Purple Pride' and 'WX74' waxflower fumigated with (1 µM) of *trans*-cinnamaldehyde (CA),1*H*-cyclopropa[*b*]naphthalene (NC), 1*H*-cyclopropabenzene (BC), 1-hexylcyclopropene (1-HCP) in vase solution of DW only. Vertical bars represent SE mean.

9.3.1.2. Leaf vase life

Different fumigation treatments of 1 µM CA, NC, BC and 1-HCP significantly ($P \leq 0.05$) influenced leaf vase life of waxflowers. Averaged over different genotypes, the flowering stems fumigated with 1 µM of NC, 1-HCP and CA, for 18 h exhibited significantly ($P \leq 0.05$) longer mean leaf vase life (28.8, 27.5, 26.6 days respectively) than when the flowering stems after fumigation were kept in DW as compared to untreated stems (26.1 days) and those fumigated with BC (24.5 days) Table. 9.3.

Meanwhile, when averaged over all treatments tested, mean leaf vase life was significantly ($P \leq 0.05$) different for each genotype of waxflowers Fig. 9.3A. The stems of 'Crystal Pearl' and 'Lady Stephanie' showed the significantly longest leaf vase life (30.2 and 29.4 days respectively) compared to 'Purple Pride' (20.9 days) and 'WX74' stems (26.3 days) Fig. 9.3A. The interaction between treatments and genotypes tested was found to be significant ($P \leq 0.05$) for leaf vase life of waxflowers. 1*H*-cyclopropabenzene fumigated sprigs of 'Lady Stephanie' resulted in the significantly ($P \leq 0.05$) longest leaf vase life (33.0 days) as compared to the control and all other fumigation treatments in different genotypes except CA, 1-HCP or NC treated 'Crystal Pearl' (32.3, 31.2, 31.0 days respectively), 1*H*-cyclopropa[*b*]naphthalene treated 'Lady Stephanie' (31.0 days), NC, 1-HCP or CA-treated 'WX74' (30.0, 29.0, 29.8 days respectively) and untreated 'Lady Stephanie' and 'Crystal Pearl' (30.0 and 29.0 days respectively) Fig. 9.4B.

Table.9.3. Effect of fumigation of 1*H*-cyclopropa[*b*]naphthalene (NC), 1*H*-cyclopropabenzene (BC), *trans*-cinnamaldehyde (CA) and 1-hexylcyclopropene (1-HCP) on mean leaf vase life in four genotypes of waxflowers . Vertical bars represent SE mean.

Treatments	Genotypes				
	'Crystal Pearl'	'Lady Stephanie'	'Purple Pride'	'WX74'	Mean (Treatments)
DW	29.0abcd	30.0abcd	21.3fg	24.0ef	26.1bc
NC	31.0abcd	31.0abcd	23.0efg	30.0g	28.8a
BC	27.5bcde	33.0a	19.0g	18.5abcd	24.5c
CA	32.3ab	26.0def	18.3g	29.8abcd	26.6abc
1-HCP	31.2abc	26.8cde	23.0efg	29.0abcd	27.5ab
Mean (Genotypes)	30.2a	29.4a	20.9c	26.3b	
LSD ($P \leq 0.05$)	Treatment=2.16, genotypes=1.93, Treatment x genotypes=4.33				

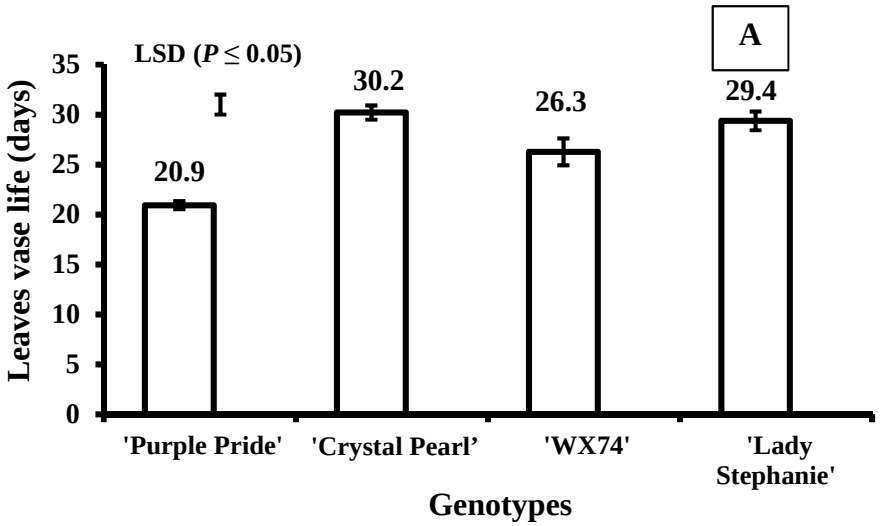


Figure.9.3. Mean leaf vase life in four genotypes following the fumigation with 1*H*-cyclopropa[*b*]naphthalene (NC), 1*H*-cyclopropabenzene (BC), *trans*-cinnamaldehyde (CA) or 1-hexylcyclopropene (1-HCP). Vertical bars represent SE mean.

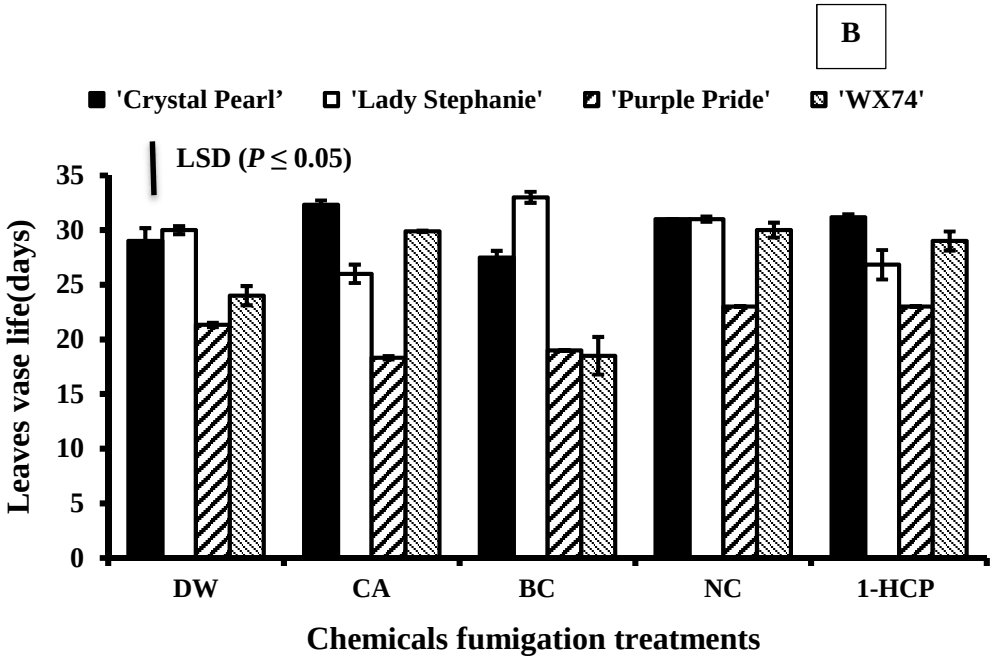


Figure. 9.4. Leaf vase life (days) of 'Crystal Pearl', 'Lady Stephanie', 'Purple Pride' and 'WX74' waxflower fumigated with (1 µM) of *trans*-cinnamaldehyde (CA), 1*H*-cyclopropa[*b*]naphthalene (NC), 1*H*-cyclopropabenzene (BC), 1-hexylcyclopropene(1-HCP) in vase solution of DW only. Vertical bars represent SE mean.

9.3.2. Influence of fumigation of *trans*-cinnamaldehyde, 1*H*-cyclopropa[*b*]naphthalene and 1-hexylcyclopropene alone and in combination with vase solutions supplemented with 8-HQS, sucrose and fructose on vase life of flower/leaf in 'WX14' and 'WX74' waxflowers.

9.3.2.1. Flower vase life.

The mean flowers vase life was significantly ($P \leq 0.05$) longer (22.8, 22.6, 23.4, 23.6 and 23.5 days) when sprigs of 'WX14' and 'WX74' waxflowers were fumigated with CA and kept in DW, 1-HCP or CA fumigated and kept in vase solution containing 8-HQS alone (100 mg L^{-1}), CA fumigated in vase solution containing fructose (58.5 µM) and 8-HQS, and 1-HCP or CA followed by vase solution containing sucrose (58.5 µM) and 8-HQS as compared to control and all other treatments Fig. 9.5A.

When averaged over different treatments, the mean flower vase life varied significantly ($P \leq 0.05$) between two tested genotypes of waxflowers 'WX74' exhibited longer flowers vase life (21.3 days) than 'WX14' (18.9 days) Fig. 9.6B.

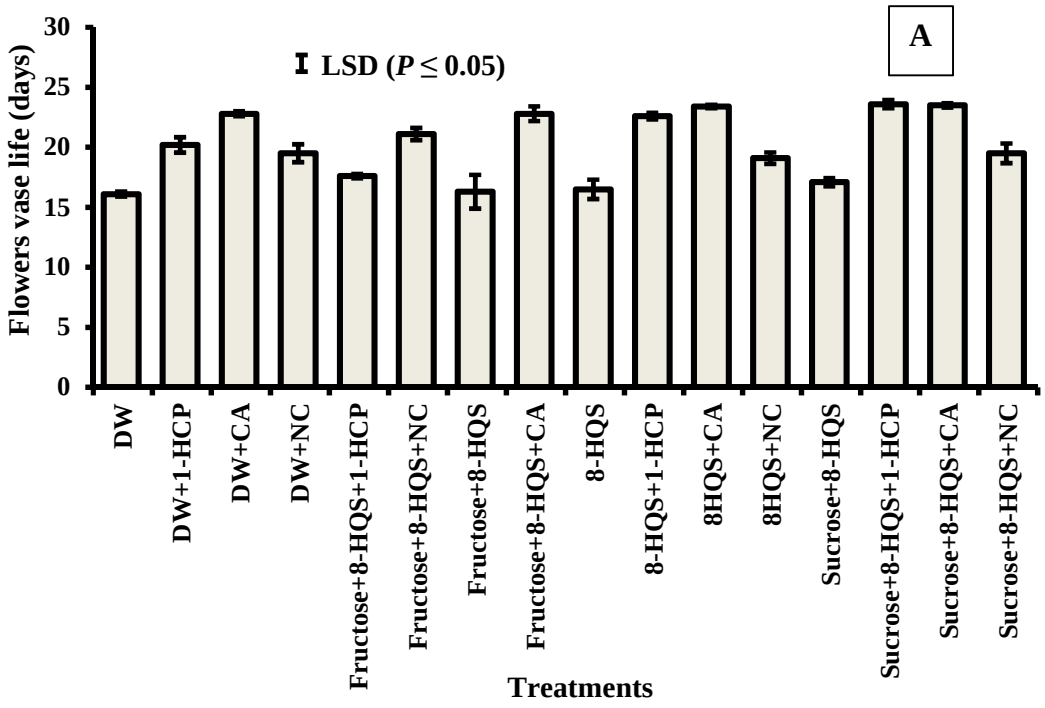


Figure. 9.5. Effect of different treatments on extending mean flower vase life of 'WX74' and 'WX14' waxflowers. Vertical bars represent SE mean.

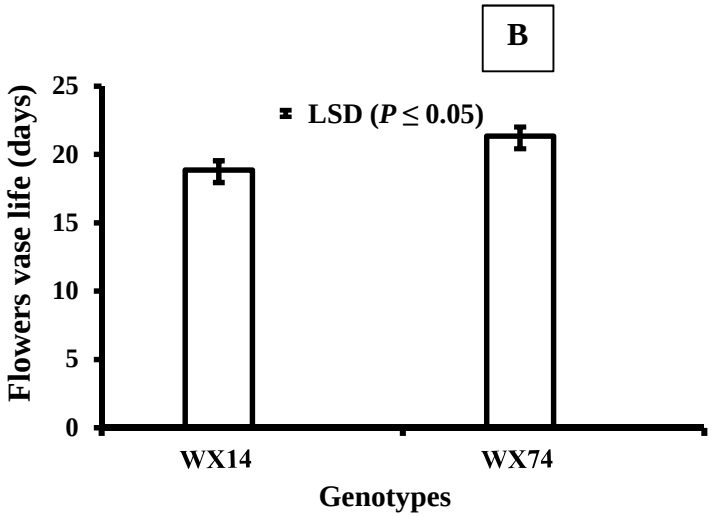


Figure. 9.6. Mean flowers vase life influenced by 1*H*-cyclopropa[*b*]naphthalene, *trans*-cinnamaldehyde and 1-hexylcyclopropene fumigation and various vase solutions containing DW, 8-HQS and /or sucrose and or fructose in 'WX74' and 'WX14' genotypes. Vertical bars represent SE mean.

The interaction between the various treatments and genotypes was found to be significant ($P \leq 0.05$) for flower vase life. All the CA fumigated 'WX14' sprigs followed by vase solution containing DW only, 8-HQS, or sucrose and 8-HQS, or when 'WX14' stems fumigated with 1-HCP followed by vase solution containing DW only or 8-HQS as a vase solution, resulted in the longest flower vase life (23.0 days) as compared to all other treatments and the DW water control (15.6 days) Fig. 9.7C. Meanwhile, in 'WX74' the sprigs fumigated with CA and kept in vase solution containing fructose along with 8-HQS, or when the stems fumigated with 1-HCP followed by vase solution containing sucrose and 8-HQS, CA fumigated and the 'WX74' stems kept in vase solution containing sucrose and 8-HQS, CA fumigated sprigs and kept in vase solution containing 8-HQS or when the stems fumigated with NC and kept in vase solution of sucrose and 8-HQS exhibited significantly longest flower vase life (25.0, 24.8, 24.0, 23.8 and 23.0 days respectively) as compared to the control (16.6 days) and all other treatments Fig. 9.7C.

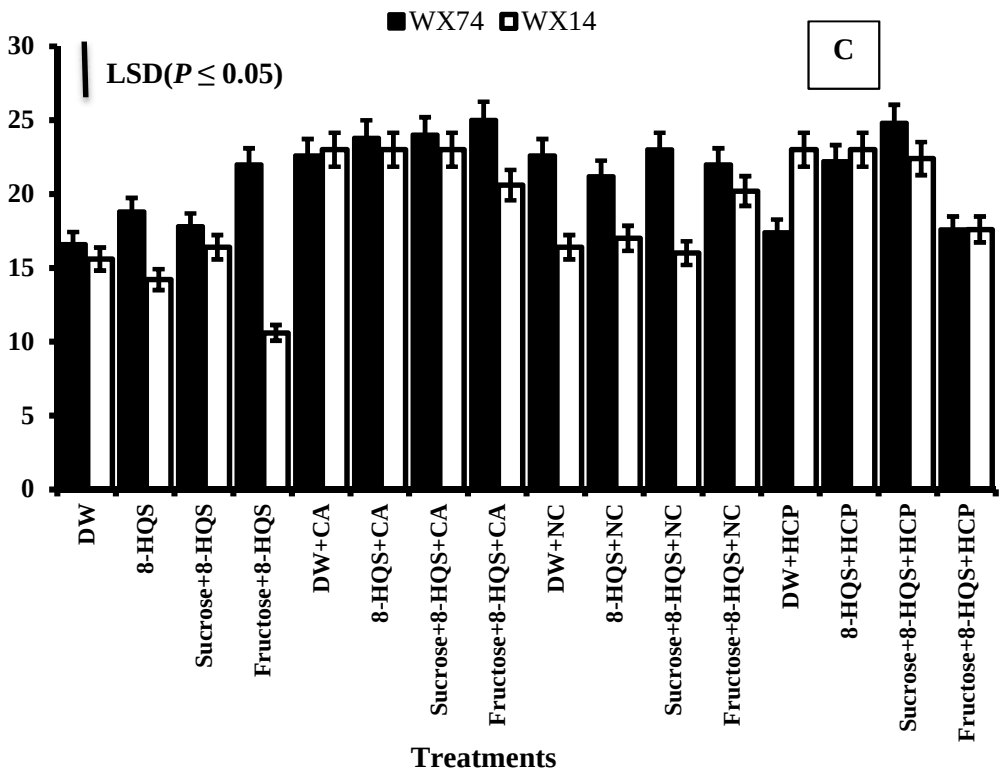


Figure. 9.7. Effects of 1*H*-cyclopropa[*b*]naphthalene (NC), *trans*-cinnamaldehyde (CA) and 1-hexylcyclopropene (1-HCP) fumigation and different vase solutions containing DW, 8-HQS and /or sucrose and/ or fructose on flowers vase life in 'WX74' and 'WX14' waxflower. n= 5 replications, one stem per replication. Vertical bars represent SE mean.

9.3.2.2. Leaf vase life

When averaged over both genotypes, mean leaf vase life was significantly ($P \leq 0.05$) longer (25.5, 24.6, 24.1 and 24.1 days) when 'WX14' and 'WX74' sprigs were fumigated with 1-HCP or CA and followed by vase solution containing sucrose and 8-HQS, CA fumigated stems kept in vase solution fortified with 8-HQS or distilled water only respectively, as compared to the control (17.4 days) and all other treatments Fig. 9.8A. When averaged over all treatments, mean leaf vase life was significantly ($P \leq 0.05$) longer in 'WX74' (22.7 days) than 'WX14' (21.5 days) Fig. 9.9B.

The interaction between different treatments and genotypes of waxflower was found to be significant ($P \leq 0.05$) for the leaf vase life. The sprigs of 'WX74' fumigated with 1-HCP or CA and kept in vase solution containing sucrose and 8-HQS, CA fumigated stems kept in vase solution containing DW only or 8-HQS or fructose and 8-HQS resulted in longer leaf vase life (27.0, 26.2, 25.2, 25.2 and 25.2 days respectively) as compared to all other treatments and the untreated sprigs (16.8 days) Fig. 9.10C. The leaf vase life of 'WX14' waxflower was significantly ($P \leq 0.05$) longest (24.0, 23.0, 23.0 23.0, 23.0 and 23.0 days) when the 'WX14' sprigs were fumigated with 1-HCP and kept in vase solution containing sucrose and 8-HQS, 1-HCP fumigated sprigs and kept in vase solution with fructose and 8-HQS or 8-HQS only or DW only, CA fumigated with sucrose and 8-HQS and CA fumigated with 8-HQS only or distilled water only respectively Fig. 9.10C, as compared to the control (18.0 days) and other treatments.

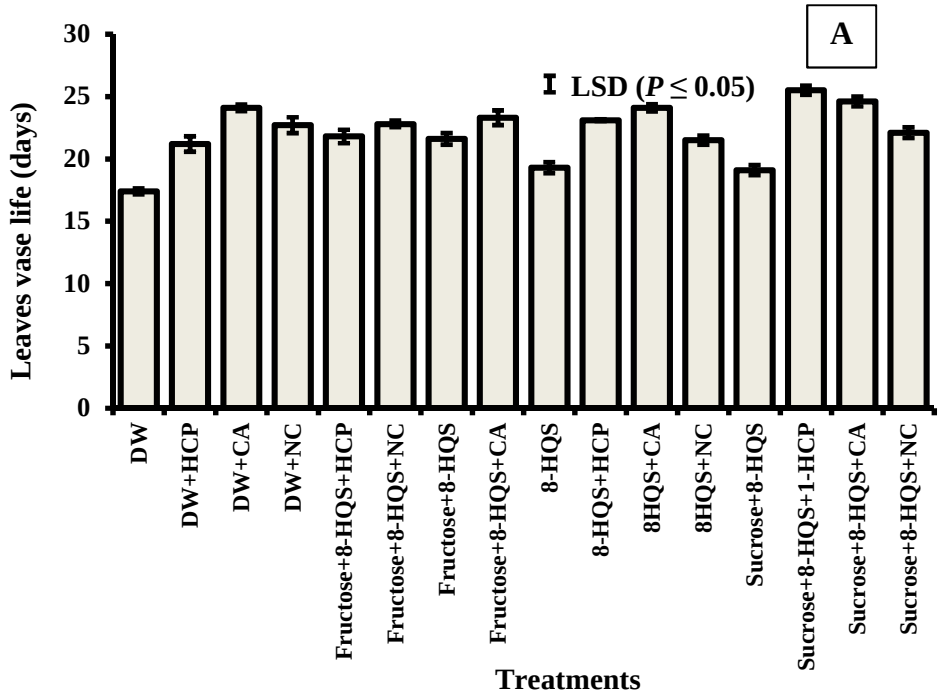


Figure 9.8. Effect of different treatments on extending mean leaf vase life of 'WX74' and 'WX14' waxflowers. Vertical bars represent SE mean.

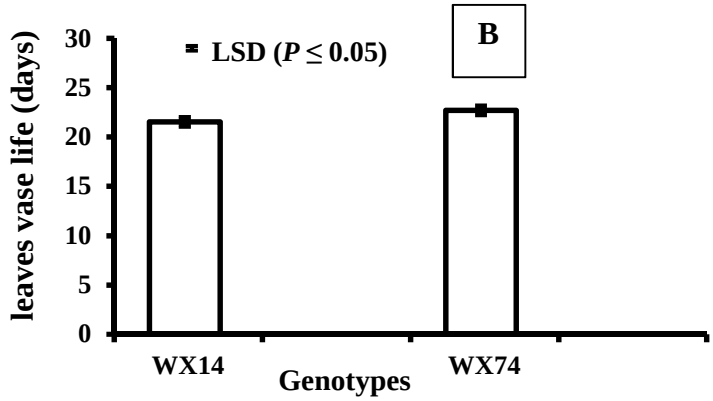


Figure.9.9. Mean leaf vase life influenced by 1*H* cyclopropa[*b*]naphthalene, *trans*-cinnamaldehyde and 1-hexylcyclopropene fumigation and various vase solutions containing DW, 8-HQS and /or sucrose and or fructose in 'WX74' and 'WX14' genotypes. Vertical bars represent SE mean.

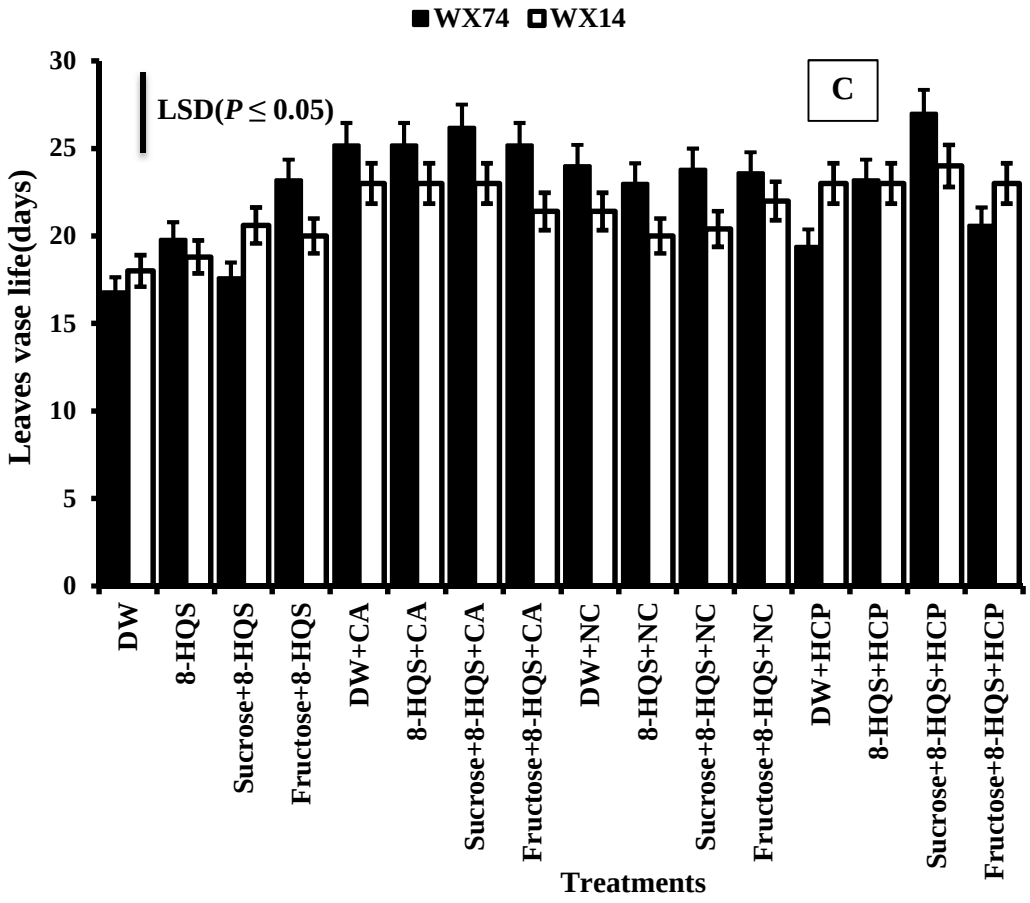


Figure. 9.10. Effects of 1*H* cyclopropa[*b*]naphthalene (NC), *trans*-cinnamaldehyde (CA) and 1-hexylcyclopropene (1-HCP) fumigation and different vase solutions containing DW, 8-HQS and /or sucrose and or fructose on leaf vase life in 'WX74' and 'WX14' waxflower. n= 5 replications, one stem per replication. Vertical bars represent SE.

9.4. Discussion

The flower and leaf vase lives in waxflowers are limited in many genotypes because of flowers/ buds abscission and leaf yellowing owing to their sensitivity to ethylene (Macnish *et al*., 2004a; Gollnow and Worrall, 2010; Seaton and Poulish, 2010). In general, different cultivars of waxflowers exhibit lifespans of 11 to 12 days as previously reported (Manning, 1996; Beasley and Joyce, 2002). Joyce and Jones (1992) and Seaton and Poulish, (2010) reported that the vase life of waxflowers varied among cultivars with shorter vase life in *C. uncinatum* cultivars of 'Alba' and

'Purple Pride' and longer vase life in *C. megalopectalum* hybrids of 'Albany Pearl' and 'Bridal Pearl'. Earlier, Joyce and Jones, (1992) also reported that the foliage of waxflower stems commenced desiccating before the flowers onset to close and/or begin to dry. In this study, the effects of ethylene antagonist fumigation such, CA, NC, BC and 1-HCP alone and in combination with vase solutions fortified with 8-HQS and/or sugars were effective in extending flower and leaf vase-lives in different genotypes of 'Geraldton waxflowers'.

In the first experiment, amongst CA, NC, BC and 1-HCP fumigation treatments applied to 'Crystal Pearl', 'Lady Stephanie', 'Purple Pride' and 'WX74' 1-hexylcyclopropene (1 µM) with stems kept in distilled water exhibited the longest mean flower vase life (20.7 days) compared to all other treatments and untreated control flowers (18.3days) Table . 9.2. Meanwhile, the stems fumigated with NC and kept in DW maintained leaf freshness with prolonged their vase life (28.8 days) as compared to control (26.1 days) and all other treatments except fumigation of 1-HCP and CA Table. 9.3. These positive effects of 1-HCP and NC fumigation on enhancing the postharvest quality and maintenance of flower and leaf vase life of cut waxflower may be ascribed to its antagonist effects on ethylene action attributed to the presence of small cyclopropene rings functional group as explained in Chapter 8, Section 8.4. Recently, Khan *et al*. (2016) proposed that the mode of action of 1-HCP in inhibiting ethylene action is due to the ring-opening reaction which appears to be responsible for the potency of cyclopropene ethylene antagonists. Khan *et al.* (2016) also claimed that 1-HCP is an effective anti-ethylene compound and its mode of action is similar to 1-methylcyclopropene (1-MCP). Earlier, it has been hypothesized that 1-MCP antagonist has more affinity to bind the receptor site than ethylene (Sisler and Serek, 1997) which acts as a competitor with ethylene and irreversible inhibitor of binding of ethylene to its receptor thereby acting to delay the senescence of flowers and reducing the effect of ethylene in promoting flower senescence (Sisler *et al*., 1996a; Sisler and Serek, 1997). Similarly, it has been reported by Macnish *et al*. (2000b) that the application of 1-MCP was effective in delaying flower senescence *C. uncinatum* and different native Australian cut flowers. It may also be argued that extension in waxflower vase life with the application of 1-HCP and NC may be due to the existence of a double bond that competes with ethylene for receptors thereby, blocking ethylene binding. Additionally, their structural molecular

strain also allows very tight bonding to electron donor compounds in the ethylene receptor (Kebenei *et al.*, 2003a; Buanong *et al*., 2005; Sisler, 2008).

Although each antagonistic chemical was effective to differing degrees in prolonging vase life this was dependent in many cases on the type of solution used to hold flowers after treatment with the 1- HCP or NC fumigation treatments in vase solution of distilled water alone. However, overall the most effective combination depended on the particular waxflowers tested 'WX14' and 'WX74' the most effective treatments for prolonging flowers/leaf vase life of cut waxflowers when stems of both genotypes fumigated with 1 µM of 1-HCP or CA in combination in vase solution containing sucrose and 8-HQS.

Flower stems fumigated with 1-HCP or CA and kept in vase solutions containing both sucrose (58.5 µM) and 8-HQS (100 mg L^{-1}) exhibited the longest mean leaf vase life (25.5 and 24.6 days) and mean flower vase life (23.6 and 23.5 days) as compared to all other treatments and control. The incremental improvements in the leaf and flower vase life with 1-HCP or CA fumigated flower stems in combination with vase solutions containing sucrose and 8-HQS may be ascribed to their synergistic effects. The extension in leaf and flower vase lives with the fumigation of 1-HCP or CA has been explained earlier in this section.

Possibly, the major effect of sucrose on extending the flower and leaf waxflower vase lives is through improving water balance when added to vase solution as reported earlier (Halevy and Mayak, 1974; Kuiper *et al.*1995). This effect may also possibly be attributed to the sucrose increasing demand for carbohydrates thereby increasing leaf and flower vase life and higher osmotic concentration of the flowers consequently improving water uptake (Ichimura *et al*., 2003; Asrar, 2012; Dung, 2013). Similarly, sucrose has been reported to extend flowers vase life of Geraldton waxflower (Joyce, 1988). Joyce and Jones, (1992) reported an extension of vase life 'Purple Pride' and 'Alba' waxflower when the flower stems were treated with sucrose and 8-HQS. Later on, Dung (2013) also reported that sucrose was more effective in improving vase life in different genotypes of waxflowers compared to maltose and galactose. Possibly, the extension of leaf and flowers vase lives with addition of 8-HQS to the vase solution may be ascribed to its antimicrobial activities thereby extending flower longevity (Dung, 2013). Similarly, vase solutions

containing 8-HQS and sucrose were a most effective treatment for enhancing quality and prolonging the vase life of *Lathyrus odoratus* L. (Elhindi, 2012).

The extension of flower and leaf vase lives in 'WX14' and 'WX74' waxflower stems fumigated with 1-HCP or CA and addition of sucrose and 8-HQS in the vase solution may be attributed collectively due to the inhibitory effect of 1-HCP or CA fumigation on ethylene action as explained previously and/or also attributed to the critical role of sucrose and 8-HQS in supporting water absorption and metabolic processes within flowers as explained earlier by Asrar (2012). However, Joyce (1988) also indicated that increased sucrose concentrations up to 146.2 mmol combined with 200 mg L^{-1} 8-HQS reduced vase life of flowers and leaves of cultivars of *C. uncinatum*.

In conclusion, flower stems fumigated with 1-hexylcyclopropene or 1*H* cyclopropa[*b*]naphthalene fumigation were more effective as compared to the control and all other treatments when kept in vase solutions containing distilled water only. 1-hexylcyclopropene or *trans*-cinnamaldehyde fumigation in combination with vase solutions containing sucrose and 8-HQS is more effective in extending flower and leaf vase lives as compared to the 1-hexylcyclopropene or *trans*-cinnamaldehyde fumigation alone and all other treatments.

CHAPTER 10

General discussion, conclusion and future research

10.1. Introduction

Ethylene is a known natural plant hormone which regulates various plant growth and developmental process, including acceleration of senescence and abscission of leaf, flowers/buds and fruits (Abeles *et al*., 1992; Bleeker and Kende, 2000; Lurie, 2007; Scariot *et al*., 2014). Also, ethylene adversely affects postharvest quality of many cut flowers (Joyce and Poole, 1993; Elgar *et al*. 1999; Gibson *et al*., 2000; Ichimura *et al*., 2002). Previously, ethylene has been reported to be associated with abscission of flowers/buds, yellowing and abscission of leaves in *Chamelaucium uncinatum*, *Verticordia spp., Leptospermum and Thryptomene calycina, Backhousia myrtifolia*, *Baeckea virgata*, *Boronia heterophylla, Ceratopetalum gummiferum*, some *Grevillea* species, *Telopea speciosissima*, *Thryptomene calycina* and *Pelargonium peltatum*, *Oriental* lilies and *Leucocoryne coquimbensis* (Joyce, 1993; Joyce and Poole, 1993; Elgar *et al*.,1999; Cameron and Reid, 2001; Celikel *et al*., 2002; Han and Miller, 2003; Faragher *et al*., 2010). In addition, ethylene has also been reported to cause flower wilting in *Boronia heterophylla* and *Telopea speciosissima* (Faragher *et al*., 2010). Earlier, Bleecker and Kende (2000) reported that the response of ethylene in plant cells refers to ethylene binding to membrane-located protein receptors and activates a phosphorylation signal transduction pathway that initiates downstream ethylene responses. Native Australian waxflower (*Chamelaucium* Desf.) is an attractive important cut flower in Australia (Anon, 2002; Beasley and Joyce 2002; Gollnow and Worrall, 2010; Seaton and Poulish, 2010). Most waxflower genotypes are sensitive to ethylene exposure (1 mgL^{-1}) which causes up to 85% abscission of flowers and buds in *C. uncinatum* cultivars (Joyce, 1988; Macnish *et al*., 2000a, b; Gollnow and Worrall, 2010; Seaton and Poulish, 2010). Accumulation of ethylene in the post-harvest atmosphere during distribution to long distance transport often without water, usually in darkness, causes extensive abscission of floral organs in both flowers and petals that reduces the value of the stems of waxflowers (Joyce, 1988, 1993). The application of ethylene action inhibitors such as silver thiosulfate (STS) and 1-methylcyclopropene (1-MCP) is more effective to reduce postharvest

losses in flowers than ethylene biosynthesis inhibitors such as AOA and AVG because their effects are negated by exogenous ethylene presence in the storage environment (Joyce, 1993; Macnish *et al*., 2000a; Ascough *et al*., 2006). Application of STS or 1-MCP is also effective in reducing adverse effects of ethylene on the quality of flowers (Staby *et al*., 1993; Zencirkiran, 2010). Additionally, the application of STS on flower crops cannot be exploited to its full potential due to its toxic and harmful effects as it is a heavy metal. The effectiveness of STS application also depends on variety, stems size and temperature and in some case can cause flower drop (Staby *et al*., 1993; Mayers *et al*., 1997; Seaton and Poulish, 2010). Sisler *et al*. (2006) claimed that 1-MCP acts as an ethylene action inhibitor by competitively binding to ethylene receptors thereby preventing flower senescence (Sisler *et al*., 1996a; Sisler and Serek, 1997; Yamane *et al*., 2004). However, 1-MCP has many shortcomings that limit its use (Grichko, 2006; Goren *et al*., 2008). 1-MCP is a highly unstable gas, costly, can be used as fumigation only and requires a reasonably sealed room for application. Also, the response of 1-MCP to horticultural commodities is concentration, exposure duration and temperature dependent (Sisler and Serek, 1997; Blankenship, 2001; Blankenship and Dole, 2003; Grichko, 2006; Reid and Celikl, 2008; Seaton and Poulish, 2010; Acuna *et al*., 2011). Meanwhile, the flowers of waxflowers may only be protected for a short period of four days and require repeated application to extend postharvest life (Seaton and Poulish, 2010). Used in sachets added to flowers carton has been found may be an effective way to use 1-MCP (Seaton and Poulish, 2010). Shortcomings of 1-MCP offer an attractive opportunity to develop alternative ethylene antagonists which may be used to antagonise ethylene action and consequently extend postharvest life of horticultural produce (Goren *et al*., 2008). Based on the predicted chemical properties of ethylene receptors in plants, various compounds were synthesised by Dr Alan Payne and his group in the Department of Chemistry Curtin University which either suppress or enhance ethylene production as well as its action. It was hypothesised that the chemicals with a cyclopropene ring as a functional group in their structure allow them to bind to active ethylene sites and allow these compounds to compete with ethylene. Therefore, a large number of potential ethylene antagonists such as 1-octyne, *trans*-cinnamaldehyde, eugenol, (+)-carvone, (*S*)-(-)-limonene, 1-octene, butyl acrylate, isoprene oxide, 1-octene oxide, allyl butyl ether, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene and 1-hexylcyclopropene were

tested to determine their ability to inhibit ethylene action particularly in regulation of abscission of flowers/buds in different genotypes of waxflowers. The second aim of my research was to evaluate the efficacy of effective ethylene antagonists in inhibiting ethylene action consequently reducing flowers/buds abscission in different genotypes of waxflowers. The effects of different concentrations 0, 0.5, 1.0 and 2.0 µM of active ethylene antagonists such as NC, BC, 1-HCP and CA on flowers/buds abscission were also tested using different genotypes of waxflowers. The efficacy of different effective ethylene antagonists such as NC, BC, 1-HCP and CA alone and in combination with two types of sugars (sucrose and fructose) supplemented with 200 mg L^{-1} 8-hydroxyquinoline sulphate (8-HQS) on vase life of different waxflower genotypes was also investigated.

10.2. Screening of potential antagonists 1-octyne, isoprene oxide, *trans*-cinnamaldehyde, eugenol, (+)-carvone, (*S*)-(-)-limonene, 1-octene, allyl butyl ether, butyl acrylate, 1-octene oxide, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene and 1-hexylcyclopropene of ethylene action through reducing abscission of flowers/buds in waxflowers.

A number of different compounds were tested for their ability to prevent ethylene-induced abscission in floral organs of Australian native waxflowers. Fumigation of 1-octyne, isoprene oxide, (+)-carvone, eugenol, allyl butyl ether or 1-octene (1 µM) for 18 h to flower stems of 'White Spring', 'WX17', 'WX116', 'Muchae Mauve', 'WX110' and 'Jenny' waxflowers followed by exposure to ethylene treatment (10 µL L^{-1}) for 24 h did not reduce flowers/buds abscission. All these compounds were very weak in inhibiting ethylene action and the mean flowers/buds abscission was 100% in the flowers treated with all six compounds followed by exposure to ethylene and thereby did not protect waxflower genotypes 'White Spring', 'WX17', 'WX116', 'Muchae Mauve', 'WX110' and 'Jenny' from ethylene-induced abscission of flowers/buds. In general, these compounds did not act as ethylene antagonists. Similarly, Grichko *et al*., (2003) also reported that isoprene was not effective as an ethylene action blocker when applied on green banana fruit. Meanwhile, (+)-carvone in vase solutions played a role in antibacterial and antifungal activity in a number of cut flowers, such as *Chamelaucium uncinatum* cv. 'Mullering Brook' (Myrtaceae) (Damunupola *et al*., 2010).

Amongst thirteen potential ethylene antagonists tested, NC, BC, 1-HCP, CA and (*S*)-(-)-limonene were effective inhibitors of ethylene action as evidenced by substantial reduction in ethylene-induced flowers/buds abscission in waxflower genotypes. The stems of different waxflower genotypes fumigated for 18 h with CA or (*S*)-(-)-limonene or NC or BC or 1-HCP, followed by ethylene (10 µL L^{-1}) exposure for 24 h significantly reduced flowers/buds abscission (47.2% 'WXFU' CA, 66.4% 'WXFU' (*S*)-(-)-limonene, 81.1% 'WX107' NC, 86.0% 'WX73' BC and 98.6% 'WX73' 1-HCP) compared to the ethylene treatment alone. Meanwhile, 1-octene oxide and butyl acrylate showed a reduction in abscission of flowers/buds in 'Southern Stars' but not on other genotypes tested. The effectiveness of ethylene antagonists to inhibit ethylene action in waxflowers can possibly be ascribed to their steric and inductive effects as reported earlier (Sisler *et al*., 1996b, 1999, 2001). Presently, limited information is available on the effect of these chemicals, such as 1*H*-cyclopropabenzene as ethylene action inhibitors by suppressing climacteric ethylene production during fruit ripening in 'Black Amber' plum (Khan, 2014). The application of 1-HCP has been reported to extend the vase life of *Kalanchoe blossfeldiana* Poelln. flowers, retard fruit ripening in tomato, avocado and banana suggesting it is an ethylene antagonist (Kebenei *et al*., 2003a; Sisler *et al*., 2003; Apelbaum *et al*., 2008). CA has also been reported to be an active ethylene action inhibitor retarding ripening of green banana fruit and playing a role in reducing oxidative stress in 'Pink Queen' rose cut flowers (Gricko *et al*., 2003; Jing *et al*., 2011). Grichko *et al*. (2003) suggested that the double bond in limonene is important, however, the mode of (*S*)-(-)-limonene action was still unclear. No research works have been reported on the efficacy of 1-octene oxide and butyl acrylate as ethylene action inhibitors.

10.3. **Effect of *trans*-cinnamaldehyde fumigation on flowers/buds abscission in 'WX73', 'WXFU', 'WX17', 'WX56' and 'WX58' genotypes and the effects of different concentrations on flowers/buds abscission in 'Purple Pride', 'Revelation' and 'Hybrid1' waxflowers.**

The fumigation of waxflower stems with CA (1 µM) for 18 h followed by exposure to 10 µL L^{-1} of ethylene for 24 h resulted in significantly reduced flowers/buds abscission ranging from 17.3 to 36.7% as compared to those exposed to

ethylene. CA fumigation treatment significantly reduced flowers/buds abscission in 'WX73', 'WXFU', 'WX56' and 'WX58' waxflower genotypes as compared to those treated with ethylene alone. Also, different concentrations 0.5, 1.0 and 2.0 µM of CA fumigation for 18 h followed by exposure to ethylene (10 µL L^{-1}) for 24 h have been tested on modulating flowers/buds abscission in 'Purple Pride', 'Revelation' and 'Hybrid 1' genotypes. The experimental results showed that (1 µM) of CA was more effective in reducing abscission of flowers/buds (5.7% in 'Purple Pride', 1.6% in 'Revelation' and 10.1% in 'Hybrid 1') as compared to 0.5 and 2.0 µM concentrations and the ethylene treatment alone. It seems that CA protects waxflower flowers/buds from ethylene action, consequently reducing the abscission of flowers/buds.

Possibly, CA prevents ethylene binding to the receptor in waxflowers and blocks the ethylene receptor for some time consequently preventing ethylene action. It may also be argued that the inhibitory action of CA application may be due to the aldehyde by its ability to withdraw electrons from the alkene to improve binding (Grichko *et al*., 2003). No findings have been reported previously on the effect of CA fumigation as ethylene antagonist on cut waxflower crops or in general ornamental plants to inhibit ethylene action. However, Gricko *et al*. (2003) reported an inhibitory effect of CA on ripening of green bananas and CA fumigation enhanced flowers/buds abscission in 'WX17' waxflower which is an ethylene sensitive genotype and may be due to the genetic differences in the number of ethylene receptors (Tieman and Klee, 1999).

10.4. Effect of fumigation of (*S*)-(-)-limonene on flowers/buds abscission in 'WX73', 'WXFU', 'WX17', 'Purple Pride', 'WX56' and 'WX58' genotypes of waxflowers.

The sprigs of 'WX73', 'WXFU', 'Purple Pride', 'WX56' and 'WX58' fumigated with (*S*)-(-)-limonene (1 µM) for18 h and followed by exposure to ethylene (10 µL L^{-1}) for 24 h have shown significant protection from ethylene by reducing abscission of flowers/buds (26.7%, 30.9%, 62.4%, 13.6% and 6.4% respectively) than the stems exposed only to ethylene treatment. The antagonistic effect of fumigation with (*S*)-(-)-limonene in blocking the ethylene action is unclear but monoterpenes containing a six-membered ring are more active than acyclic terpenes as reported previously by (Grichko *et al*., 2003). It may also be argued that

the effectiveness of this monoterpene is due to the interaction of the double bond with the receptor site which may block the ethylene receptor sites in flowers and delay or prevent the abscission of flowers/buds in different waxflowers genotypes tested thus, (*S*)-(-)-limonene, appears to be one of the natural useful active ethylene antagonists (Grichko *et al*., 2003). No research work has been reported on the effect of (*S*)-(-)-limonene fumigation on reducing flowers/buds abscission in native waxflowers or other plants except one study reported by Grichko *et al*. (2003) on inhibition of ripening in banana fruit. Meanwhile, no reduction in abscission of flowers/buds in 'WX17' when the stems were treated with (*S*)-(-)-limonene followed by ethylene exposure may be attributed to the sensitivity of this C.*uncinatum* genotype to the ethylene as discussed in the previous section.

10.5. Effect of 1-hexylcyclopropene fumigation on flowers/buds abscission in 'WX73', 'Purple Pride', 'WX56' and 'WX58' genotypes of waxflowers and the effects of its different concentrations on flowers/buds abscission in 'Hybrid 1' and 'Purple Pride' waxflowers.

1-Hexylcyclopropene (1-HCP) is a potent ethylene inhibitor due to an ability to compete with ethylene for the receptor (Kebenei *et al*., 2003a). The stems of 'WX73', 'Purple Pride', 'WX56' and 'WX58' waxflower genotypes fumigated with 1 µM of 1-HCP for 18 h and followed by exposure to exogenous ethylene for 24 h reduced flowers/buds abscission (2.7%, 4.8%, 14.6% and 23.5% respectively) as compared to ethylene exposure treatment alone. It is apparent from these results that 1-HCP application significantly maintains the flowering stem quality by reducing the abscission of flowers/buds in the genotypes for some time. Amongst different concentrations of 1-HCP (0.5, 1.0 and 2.0 µM) tested on the stems of 'Hybrid 1' and 'Purple Pride' followed by exogenous ethylene exposure, 1.0 and 2.0 µM concentrations were very effective in reducing the flowers/buds abscission in both genotypes. The activity of this compound in preventing the damaging effect of ethylene on waxflowers may possibly be ascribed to steric effects and high molecular strain which is the more important factor involved in inactivating the ethylene receptor by binding to it for some time (Sisler *et al*., 1996 a,b 1999; Sisler, 2008; Sisler *et al*., 2009). 1-HCP treatment for 2 h was sufficient to extend the shelf life of kalanchoe pot plant from 2 to 7.3 days (Kebenei *et al*., 2003a). 1-Alkylcyclopropenes

with chain lengths greater than 5 carbons, like 1-HCP, are more efficient antagonists as reported earlier by Kebenei *et al*., (2003a). In addition, the mode of action of 1-HCP is similar to that of 1-methylcyclopropene (1-MCP) as both contain the high strain cyclopropene ring.

10.6. Effect of fumigation of 1*H*-cyclopropabenzene and 1*H*-cyclopropa[*b*]naphthalene on flowers/buds abscission in 'WX73', 'WX17', 'WX56', 'WX58', 'WX107' and 'Jenny' genotypes of waxflowers and the effects of their different concentrations on flowers/buds abscission in 'Purple Pride', 'Revelation' and 'Hybrid 1' waxflowers.

In this study, the results of different experiments showed that waxflower stems of different genotypes fumigated with BC for 18 h followed by exposure to ethylene for 24 h displayed significantly reduced mean flower/buds abscission ranging from 0.00 to 44.8% in 'WX73', 'WX58', 'WX56' and 'Purple Pride' waxflower genotypes over four days as compared to ethylene treatment alone that promoted flowers/buds abscission. All the three (0.5, 1.0 and 2.0 µM) concentrations tested were significantly effective in reducing flowers/buds abscission as compared to the ethylene alone on day three in 'Revelation' waxflower. Similarly, waxflower sprigs of different genotypes ('WX73', 'WX107', 'WX58', 'WX56' and 'Purple Pride') fumigated with 1 µM NC for 18 h followed by exposure to ethylene (10 µL L^{-1}) alone for 24 h showed decreased mean flowers/buds abscission varying from 8.9 to 38.1% thus implying that ethylene treatment did not induce ethylene action in different genotypes of waxflowers when the stems were fumigated with NC compound. In addition, when the stems of 'Purple Pride', 'Hybrid 1' and 'Revelation' were treated with NC at three concentrations 0.5, 1.0 and 2.0 µM, NC fumigation (0.5 and 1.0 µM) both were the most effective concentration in reducing flowers/buds abscission on the second day after the treatments as compared to all other treatments. However, fumigation with BC and NC (1µ M) for 18 h followed by exposure to ethylene (10 µL L^{-1}) alone for 24 h did not prevent ethylene-induced abscission of flowers/buds in C. *uncinatum* such as 'WX17' and 'Jenny' genotypes.

Significant reduction in the abscission of flowers/buds of waxflowers with BC and NC is not fully understood but could be attributed to their high strain energy and reactivity in the presence of copper (I). Pirrung and co-workers attempted to

form copper complexes of 1-HCP but only obtained ring-opened dimerised products, which are formed through a copper carbenoid species. This suggested that the high potency of 1-HCP is not solely due to complexation but is due to its reactivity (Pirrung *et al*. 2008). They suggested that the reaction intermediate, the copper carbenoid, could covalently react with the ethylene receptor and inactivate it. NC undergoes the same ring-opening reactions with copper (I) as 1-HCP (Khan, 2014) and could indicate a similar mode of action.

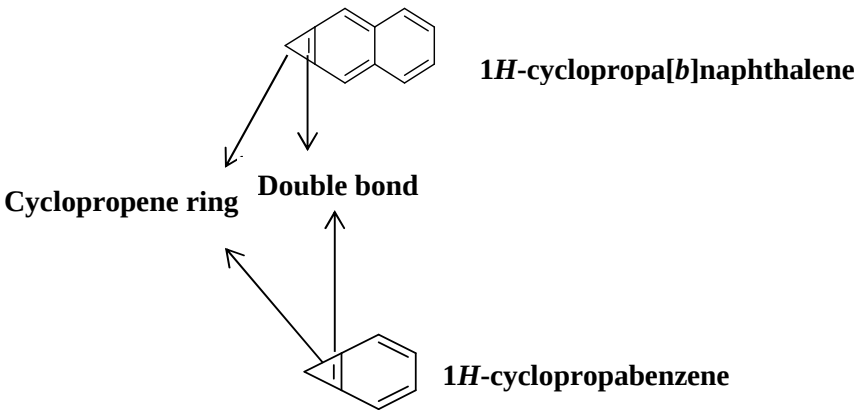


Figure 10.1. Chemical structure of 1*H*-cyclopropa[*b*]naphthalene and 1*H*-cyclopropabenzene

Previous work with the group has shown BC to reduce flowers/buds abscission in waxflowers. Earlier, Khan, (2014) reported that 1*H*-cyclopropabenzene fumigation (50 or 100 nL L^{-1}) was more effective in extending the vase life time of 'WX17' and 'WX73' waxflowers. Probably the mode of action of both NC and BC is similar to the mode of action of 1-MCP. Fumigation with 1-MCP proved a very powerful inhibitor of ethylene action in various types of flowers such as Zonal Geraniums, *Pelargonium peltatum,* cut carnation, *Delphinium* and sweet pea, *Cattleya alliance, Dianthus caryophyllus* L. waxflower, *Grevillea* 'Sylvia', *Hibiscus rosa and Lilies* (Macnish *et al*., 2000a; Cameron and Reid, 2001; Ichimura *et al*., 2002; Reid *et al*., 2002; Yamane *et al*., 2004; Abadi *et al*., 2009). These experimental results show that both NC and BC inhibit ethylene action and reduce flowers/buds abscission in different genotypes of waxflowers.

10.7. Effect of fumigation of 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene, *trans*-cinnamaldehyde and 1-hexylcyclopropene on flowers/leaf vase life of 'Crystal Pearl', 'Lady Stephanie', 'Purple Pride' and 'WX74' waxflower.

The sprigs of different genotypes of waxflower ('Crystal Pearl', 'Lady Stephanie', 'Purple Pride' and 'WX74') fumigated with 1 µM of NC , BC , CA or 1-HCP for 18 h followed by placement of flowers in vase solution of distilled water showed that only 1-HCP treatment was more effective in extending flowers vase life and maintaining flowers quality in 'Crystal Pearl', 'Lady Stephanie', 'Purple Pride' and 'WX74' waxflowers by 20.7 days compared to control only 18.2 days and other ethylene antagonists treatments. Meanwhile, the fumigation of NC or 1-HCP fumigation was effective in prolonging leaf vase life of the aforementioned four genotypes (28.8 and 27.5 days respectively) in comparison to the control (26.0 days) and other treatments. NC and 1-HCP were more effective than other compounds tested in extending the vase life of flowers and leaf in four genotypes. Their activity is probably due to the cyclopropene substructure present in both compounds (Sisler *et al*., 1996b, 1999, 2001) as explained previously in Section, 9.5 and 9.6.

10.8. Influence of fumigation of *trans*-cinnamaldehyde, 1*H*-cyclopropa[*b*]naphthalene and 1-hexylcyclopropene alone and in combination with vase solutions supplemented with 8-HQS, sucrose and fructose on vase life of flowers/leaves in 'WX14' and 'WX74' waxflowers.

The waxflower genotypes ('WX14' and 'WX74') fumigated with CA or 1-HCP and kept in vase solution containing 250 mmol sucrose and 100 mg L^{-1} 8-HQS or followed by DW alone or followed by vase solution of 1-HQS extended vase life of flowers and leaf as compared to control and all other treatments. This positive effect of fumigation with CA or 1-HCP followed by different vase solution may be ascribed to the antagonistic role of these two ethylene antagonists as previously described in Sections 9.3 and 9.5. Additionally, increased water uptake with the application of the sugars and the antimicrobial activity due to the application of 8-HQS may reduce the stem plugging consequently contributing to the incremental improvement of vase life of flowers and leaf (Ichimura *et al*., 2003; Asrar, 2012; Dung, 2013; Elgimabi and Sliai, 2013). Earlier, Dung (2013) also similarly reported

that 200 mg L^{-1} of 8-HQS in combination with sucrose was a more effective treatment in improving the vase life in six genotypes of waxflowers.

In conclusion, fumigation with CA and 1-HCP in combination with vase solution containing 250 mmol sucrose and 100 mg L^{-1} of 8-HQS were effective in extending the vase life of flowers (23.50 and 23.60 days) compared to control (16.1 days) and leaf (24.60 and 25.50 days) compared to untreated (17.4 days) of 'WX14' and 'WX74' hybrid waxflowers.

10.9. General Conclusions

1. Out of thirteen different potential ethylene antagonists tested to inhibit the ethylene action and abscission of flowers/buds in waxflower genotypes, five compounds *trans*-cinnamaldehyde, (*S*)-(-)-limonene, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene and 1-hexylcyclopropene were the most effective inhibitors of ethylene action.
2. The effectiveness of five active ethylene antagonists depends on the variety of Geraldton waxflower. For example, the sprigs of six hybrids 'WX73', 'WX56', 'WX58', 'WXFU', 'Hybrid 1', 'Revelation' and non-hybrid waxflower treated with *trans*-cinnamaldehyde followed by ethylene exposure showed 76.79% mean flowers/buds abscission reduction than those exposed to ethylene alone. The flowering stems of four hybrids ('WX73', 'WX56', 'WX58' and 'WXFU') waxflower treated with (*S*)-(-)-limonene followed by ethylene exposure for 24 h exhibited 76.90% mean flowers/buds abscission reduction as compared to those exposed to ethylene alone. The sprigs of six hybrids 'WX73', 'WX56', 'WX58', 'WX107', 'Hybrid1' and 'Revelation' fumigated with 1*H*-cyclopropa[*b*]naphthalene followed by exposure to ethylene, or four hybrid 'WX73', 'WX56', 'WX58' and 'Revelation' treated with 1*H*-cyclopropabenzene followed by ethylene exposure and four hybrid 'WX73', 'WX56', 'WX58' and 'Hybrid1' fumigated with 1-hexylcyclopropene followed by ethylene exposure showed 73.8%, 90.9% and 82.5% flowers/buds abscission reduction respectively as compared to ethylene treatment alone. The sprigs of non-hybrid waxflower 'WX17' exposed to *trans*-cinnamaldehyde, (*S*)-(-)-limonene or 1*H*-cyclopropabenzene followed by ethylene exposure showed flowers/buds abscission -2.01, -1.78 and 4.58% respectively. Meanwhile, the

stems of 'Purple Pride' fumigated with *trans*-cinnamaldehyde, (*S*)-(-)-limonene, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene or 1-hexylcyclopropene followed by ethylene exposure showed 83.05%, 19.35%, 65.08%, 42.17% and 93.86% reduction in flowers/buds abscission respectively as compared to ethylene treatment alone.

3. The efficacy of fumigation with different ethylene antagonists including *trans*-cinnamaldehyde, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene or 1-hexylcyclopropene in extending flowers/leaf vase life depends upon the type of vase solution and genotypes. The waxflower stems ('Crystal Pearl', 'Lady Stephanie', 'Purple Pride' and 'WX74') were fumigated with 1-hexylcyclopropene, followed by keeping in DW showed longest mean flower vase life (20.7 days) compared to all other treatments and control (18.2 days). Meanwhile, the mean leaf vase life was longest (28.7 and 27.5 days) when the flower stems of these genotypes were fumigated with 1*H*-cyclopropa[*b*]naphthalene and 1-hexylcyclopropene respectively compared to the control (26.08 days). The flower stems of 'WX14' and 'WX 74' fumigated with 1-hexylcyclopropene or *trans*-cinnamaldehyde and kept in vase solution containing 8-HQS and sucrose exhibited 7.5, 7.4, 8.1 and 7.2 days longer mean flower and leaf vase life respectively compared to the untreated flower stems which were kept in DW water only.

10.10. Future research

Trans-cinnamaldehyde, (*S*)-(-)-limonene, 1*H*-cyclopropa[*b*]naphthalene, 1*H*-cyclopropabenzene and 1-hexylcyclopropene are effective in reducing flowers/buds abscission even when flower stems were exposed to ethylene suggesting that these compounds are effective ethylene antagonists. The future research may focus on:

1. Efficacy of these ethylene antagonists in extending vase life and quality of flower may be investigated in other commercial flower crops.
2. Physiological and biochemical mechanisms of these ethylene antagonists in modulating abscission zone of different genotypes of cut waxflowers warrants to be investigated in detail.

3. Effect of these antagonists on the up or down regulation of expression of genes involved in abscission of flowers/buds and petal senescence in waxflower and other ornamental flowers is yet to be investigated.
4. Different types of formulations may be developed so that these compounds can be applied as fumigation, spray, coating and dipping for a range of horticultural produce.
5. It is yet to be investigated that these five ethylene antagonists are safe to use by the industry and their impacts on the environment when applied to the different horticultural commodities warrants investigation.

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