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Crops at the Receptor Level**

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THE CONTROLLING ETHYLENE RESPONSES IN HORTICULTURAL CROPS AT
RECEPTOR LEVEL

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ABSTRACT

Cyclopropenes: 1-heptylcyclopropene, 1-nonylcyclopropene, 1-decylcyclopropene, cyclopropenylmethyl butyl ether, cyclopropenylmethyl hexyl ether, N-(1-Cyclopropenylmethyl)ethyl-amine, 3-methylcyclopropene, 3-pentylcyclopropene, 3-heptylcyclopropene, 1-(3-hydroxypropyl)-cyclopropene, 3-cycloprop-1-enyl-propanoic acid ethyl ester, 3-cycloprop-1-enyl-propanoic acid and 3-cycloprop-1-enyl-propanoic acid isopropylamine salt were prepared and checked for the anti-ethylene activity using mature banana fruits and carnation flowers. Changes in both a banana peel color and flesh softness were observed during the experiments. Degreening of a peel and softening of the flesh tissue were significantly but unequally delayed by treatment of banana fruits with substituted cyclopropenes. Anti-ethylene potency of cyclopropenes was found to depend on the ability of compounds to retain intact ring upon interaction with the receptor, their molecular weight and hydrophobicity.

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1. INTRODUCTION

This study was undertaken to further extend a time of insensitivity to ethylene in cyclopropene-treated banana fruits, and to research the effect of molecular structure of substituted cyclopropenes on their antagonistic properties. It was reported previously that cyclopropene, 1-methylcyclopropene (1-MCP), 3-methylcyclopropene (3-MCP), 1,2-dimethylcyclopropene, 1,3-dimethylcyclopropene, 3,3-dimethylcyclopropene, 1,3,3-trimethylcyclopropene, 3-methyl-3-vinylcyclopropene, and 3-methyl-3-ethynylcyclopropene irreversibly inactivated ethylene receptor in banana fruit (Sisler *et al.*, 2001). Light cyclopropenes bound to ethylene receptor both *in vivo* and *in vitro* preventing ethylene perception for up to 12 d (Sisler *et al.*, 1996; Sisler *et al.*, 2001). Minimum concentrations of methyl, vinyl- and ethynyl-substituted cyclopropenes were within a broad range from $0.7 \text{ nl}\cdot\text{L}^{-1}$ to $20 \text{ }\mu\text{l}\cdot\text{L}^{-1}$ (Sisler *et al.*, 1996; Sisler *et al.*, 2001). Here, manipulation of substitution patterns in cyclopropenes was further exploited to control anti-ethylene properties of cyclopropenes.

At present time, the structure of ethylene-binding center in ethylene receptor and the mechanism of interaction of both ethylene and cyclopropenes with the receptor are not known. As a consequence, it is not possible to predict accurately the effect of molecular structure of cyclopropene on its antagonistic properties. With notwithstanding, the hydrophobic cyclopropenes that contain the long, straight, saturated chain substituents on cyclopropene ring might be expected to be the excellent membrane-targeting agents. Hydrophilic substituents might allow discrimination between different fruit tissues. In addition, electron-withdrawing and electron-donating substituents may affect biological activity of cyclopropenes allowing time-resolved control of ripening. Esters seem to be the promising soft-acting anti-ethylene agents allowing preparation of water-soluble inhibitors that may be applied to cut flowers and ornamental flowers as well.

Both a difference in a time of induced insensitivity of banana fruits to ethylene and the substantial difference in a concentration of cyclopropenes required to block ethylene receptors have demonstrated a great potential of cyclopropene derivatives for a flexible control of fruit ripening.

II. DESCRIPTION OF THE RESEARCH ACTIVITY

1. Preparation of the compounds

Substituted cyclopropenes can be prepared by a number of different methods (Halton and Banwell, 1987). The 1- and 3-substituted cyclopropenes were synthesized by the reaction of a corresponding bromo-olefins with dibromocarbene followed by the conversion of a tribromocyclopropane to the cyclopropene with methyllithium as shown (Fig 1 A&B). The raw compounds were purified and characterized by GC, IR, NMR and MS, when applicable. All newly prepared cyclopropenes (Table 1) were preliminary screened for the anti-ethylene activity in the US. The activity in banana ripening test when applied as a gas was used as representative of the activity of these compounds.

Fig. 1A. General method of synthesis of 1-substituted cyclopropenes.

Fig. 1B. General method of synthesis of 3-substituted cyclopropenes.

Table 1. Newly synthesized 1- and 3-substituted cyclopropenes

	Compound	Structure
1	3-Methylcyclopropene	
2	3-Pentylcyclopropene	
3	3-Heptylcyclopropene	
4	1-Heptylcyclopropene	
5	1-Nonylcyclopropene	
6	1-Decylcyclopropene	
7	1-Cyclopropenylmethyl butyl ether	
8	1-Cyclopropenylmethyl hexyl ether	
9	1-(3-Hydroxypropyl)-cyclopropene	
10	3-Cycloprop-1-enyl-propanoic acid	
11	3-Cycloprop-1-enyl-propanoic acid ethyl ester	
12	3-Cycloprop-1-enyl-propanoic acid isopropylamine salt	
13	N-(1-Cyclopropenylmethyl)ethyl-amine	

1.1 Synthesis of 1-heptylcyclopropene, 1-nonylcyclopropene and 1-decylcyclopropene

1-Heptylcyclopropene was prepared from 2-bromo-1-nonene by addition of dibromocarbene under phase transfer conditions to produce 1,1,2-tribromo-2-heptylcyclopropane, followed by reaction with methyl lithium in diethyl ether under nitrogen atmosphere and then with a water (Al Dulayymi *et al.*, 1996a). 2-Bromo-1-nonene was obtained by direct hydrobromination of 1-nonyne with tetraethylammonium hydrogen dibromide in dichloromethane (Cousseau, 1980). 1-Nonylcyclopropene was prepared in an analogous way starting with the synthesis of 2-bromo-1-undecene. 1-Decylcyclopropene was synthesized starting from the preparation of 2-bromo-1-dodecene.

1.2 Synthesis of 1-cyclopropenylmethyl butyl ether and 1-cyclopropenylmethyl hexyl ether

1-Cyclopropenylmethyl butyl ether was prepared from 2-bromo-2-propenyl butyl ether by addition of dibromocarbene under phase transfer conditions to produce 1-(1,2,2-tribromocyclopropyl)methyl butyl ether followed by reaction with methyl lithium and after that with a water (Al Dulayymi *et al.*, 1996a). 2-Bromo-2-propenyl butyl ether was synthesized by direct hydrobromination of propargyl butyl ether with tetraethylammonium hydrogen dibromide in dichloromethane (Cousseau, 1980). Propargyl butyl ether was prepared by the direct alkylation of propargyl alcohol with 1-bromobutane in the presence of powdered potassium hydroxide in cyclohexanone (Vartanian *et al.*, 1974). 1-Cyclopropenylmethyl hexyl ether was prepared in an analogous way starting with the direct alkylation of propargyl alcohol with 1-bromohexane in the presence of powdered potassium hydroxide in cyclohexanone (Vartanian *et al.*, 1974).

1.3 Synthesis of 3-methylcyclopropene

Freshly prepared aqueous sodium hydroxide (50%, 84 g in 84 ml of water) was added slowly via a funnel to a rapidly stirred mixture of 1-bromo-1-propene (25 g, 0.21 mole), bromoform (106.6 g, 0.42 mole) and cetrimide (7 g) keeping an internal temperature below 40°C (Al Dulayymi *et al.*, 1996a). The resulting mixture was stirred

rapidly for 24 h at room temperature, added 750 ml of dichloromethane and then 300 ml of brine. The resulting mixture was transferred to a separatory funnel and the organic layer was separated. The aqueous phase was extracted with dichloromethane (3 x 200). The organic layers were combined and dried, and dichloromethane was distilled off to give brown oil. The excess of bromoform was distilled off at 10 mm Hg. The residue was added 200 ml of ether and the precipitate and the solvent were removed from the filtrate *in vacuo* to give brown oil, which was distilled to yield colorless 1,1,2-tribromo-3-methylcyclopropane (b.p. 69.5°C and 2 mm Hg) which showed ^1H NMR (CDCl_3) 1.23-1.25 (d, 3H), 1.80-1.90 (m, 1H), 3.74-3.77 (d, 1H, J 3.7 Hz); ν_{max} (KCl) 3046, 2955, 2926, 2870, 2857, 1460, 690 cm^{-1} .

To prepare 3-methylcyclopropene (3-MCP) a solution of 0.2 ml of neat 1,1,2-tribromo-3-methylcyclopropane in 5 ml of diethyl ether was placed under a nitrogen atmosphere into a test tube. While cooling in an ice water bath, 2 ml of 1.5 M methyl lithium in diethyl ether was added slowly by syringe (Al Dulayymi *et al.*, 1996a). After 30 min, 3 ml of water was added via syringe. The test tube was removed out of ice water bath, allowed to stay under ambient conditions for 30 min and was placed in a freezer at -20°C to freeze up the water phase. The solution of 3-MCP in ether was separated by decantation and kept in a freezer prior to further use.

1.4 Synthesis of 3-pentylcyclopropene and 3-heptylcyclopropene

The *trans*-2-octenoic acid (30 g, 0.21 mole) was used to prepare (2R*, 3S*)-2,3-dibromooctanoic acid, which was used to synthesize (*Z*)-1-bromo-1-heptene (Brevet and Mori, 1992). 1,1,2-Tribromo-3-pentylcyclopropane was prepared from (*Z*)-1-bromo-1-heptene in a way analogous to that described for 1,1,2-tribromo-3-methylcyclopropane. Crude preparation of 1,1,2-tribromo-3-pentylcyclopropane was distilled to yield neat colorless oil (90°C and 2 mm Hg), which showed MW 348.90; ^1H NMR (CDCl_3) 0.85-0.95 (t, 3H), 1.20-1.45 (m, 8H), 1.60-1.70 (m, 1H), 3.65-3.70 (d, 1H, J 3.7 Hz); ν_{max} (KCl) 3046, 2986, 2970, 2928, 2866, 1450, 690 cm^{-1} . Molecular weight was determined using the JEOL HX-110 high resolution, double focusing mass spectrometer.

To prepare 3-pentylcyclopropene a solution of 0.2 ml of neat 1,1,2-tribromo-3-pentylcyclopropane in 5 ml of diethyl ether was placed into a test tube under a nitrogen

atmosphere. While cooling in a dry ice acetone bath, 2 ml of 1.5 M methyl lithium in diethyl ether was added slowly by syringe (Al Dulayymi *et al.*, 1996a). After 30 min, 3 ml of water was added via syringe. The test tube was removed out of dry ice bath, allowed to stay under ambient conditions for 30 min and was placed in a dry ice acetone bath to freeze up the water phase. The solution of 3-pentylcyclopropene in ether was separated by decantation and kept in a freezer prior to further use.

The *trans*-2-decenoic acid (25 mL) was purchased from Acros Organics (New Jersey, USA) and was used to prepare 2,3-dibromodecenoic acid that was used to synthesize (Z)-1-bromo-1-nonene (Brevet and Mori, 1992). 3-Heptylcyclopropene was prepared in a way analogous to that described for 3-pentylcyclopropene.

1.5 Preparation of N-(1-cyclopropenylmethyl)ethylamine

1-(1,2,2-tribromocyclopropyl)methylethylamine was obtained from N-(2-bromoallyl)ethylamine by addition of dibromocarbene under phase transfer conditions (Al Dulayymi *et al.*, 1996a). N-(2-bromoallyl)ethylamine was prepared from 2,3-dibromopropene (Baumgarten, 1973).

1.6 Preparation of 1-(3-hydroxypropyl)-cyclopropene, 3-cycloprop-1-enyl-propanoic acid, 3-cycloprop-1-enyl-propanoic acid ethyl ester and 3-cycloprop-1-enyl-propanoic acid isopropylamine salt

4-Pentyl-1-ol was used to prepare 4-bromopent-4-en-1-ol, which was reacted with 2-methoxypropene in the presence of catalyst pyridinium toluene-4-sulphonate to give 4-bromo-1-((methoxy-1-methyl)ethoxy)pent-4-ene (Al Dulayymi *et al.*, 1996a). 1,1,2-tribromo-2-((1-methoxy-1-methyl)ethoxypropyl)cyclopropane was obtained from 4-bromo-1-((methoxy-1-methyl)ethoxy)pent-4-ene accordingly to the procedure described elsewhere (Al Dulayymi *et al.*, 1996a). 1,1,2-tribromo-2-((1-methoxy-1-methyl)ethoxypropyl)cyclopropane was converted to 1,1,2-tribromo-2-(3-hydroxyl)-cyclopropane, which then was used to prepare 1-(3-hydroxypropyl)-cyclopropene by a standard procedure (Al Dulayymi *et al.*, 1996a). 1,1,2-Tribromo-2-(3-hydroxyl)-cyclopropane was oxidized to 3-(1,1,2-tribromo-cyclopropyl)-propanoic acid with chromium trioxide in glacial acetic acid, and 3-(1,1,2-tribromo-cyclopropyl)-propanoic

acid was used for the preparation of 4-cycloprop-1-enyl-propanoic acid by a standard procedure (Al Dulayymi *et al.*, 1996b). To prepare 3-cycloprop-1-enyl-propanoic acid ethyl ester 1-(3-carboxypropyl)-cyclopropene was treated with triethylamine and methylchloroformate. The product was purified and treated with 21% sodium ethoxide in ethanol solution. The ether phase was washed, dried over MgSO_4 and filtered to give 3.9 mM 3-cycloprop-1-enyl-propanoic acid ethyl ester in ether. To prepare 3-cycloprop-1-enyl-propanoic acid isopropylamine salt a solution of 3-cycloprop-1-enyl-propanoic acid ethyl ester in 10 ml of ether was treated with 0.1 g of isopropyl amine at room temperature to give about 100 mg of 3-cycloprop-1-enyl-propanoic acid isopropylamine salt.

1.7 GC, IR, and NMR

Gas chromatograph Varian 2700 with FID and a 5-ft, 2-mm packed glass column was used for the analysis. The column was packed with 10% SE-30, 80/100 packing obtained from Supelco (Supelco Park Bellefonte, PA 16823-0048). The flow rate of nitrogen carrier gas was 2-10 mL/min. To ascertain a separation of the obtained products the oven was operated in a programmed temperature range to elute the low boilers in a well-spaced pattern and the high boilers in a reasonable period of time.

IR spectra were recorded on a FT-IR spectrometer MB100 (Bomem, Quebec, Canada) equipped with a cell with KCl windows with a resolution of 1 cm^{-1} . NMR spectra were recorded on GE 300 MHz spectrometer. The chemical shifts of the impurities were not analyzed, and the integrals were adjusted to reflect only the contribution of the target compound.

2. Preliminary scanning of the new cyclopropenes for activity

2.1 Plant material

Mature green bananas (*Musa sapientum* L.), which have not been exposed to ethylene, were purchased from a commercial source. Bananas were kept at 20°C and usually were used within two days. White carnations (*Dianthus caryophyllus*), which have not been treated with silver salts and not been exposed to ethylene, were purchased

from J.A. Balistreri Farms, Inc. (Denver, CO). Flowers were kept at 4°C and usually were used within two weeks.

2.2 Determination of minimum active concentration and maximum time of protection

In order to determine the minimum active concentration prior to volatile gas treatment banana fruit was placed into 3-L glass jar. The required amount of cyclopropene solution in ether was pipetted onto the paper filter, which was allowed to air for 5-10 s. The filter was then placed on the bottom of a jar and jar was immediately sealed. Twenty-four hours later the jars were opened in an exhaust hood, paper filters were taken out and jars were vented. Ethylene gas equal to $333 \mu\text{L}\cdot\text{L}^{-1}$ final concentration was injected into each jar and the jar was immediately sealed. Twenty-four hours later the jars were opened and banana fruits were taken out and placed on a laboratory bench. Color and softness readings were taken a few days later allowing the control banana fruit to turn yellow. 3-Methylcyclopropene was applied as a concentrated solution in diethyl ether, which was stored at -20°C.

In order to determine the time of induced insensitivity of banana fruits to ethylene, banana fruits were treated with sufficient amount of concentrated solution of cyclopropene in ether for 24 h. The solution was pipetted onto paper filters and jars were tightly closed. The jars were opened in an exhaust hood, paper filters were taken out and jars were thoroughly vented. Banana fruits were then taken out and placed on a laboratory bench allowing ripening in an air. Periodically one banana fruit was treated with $333 \mu\text{L}\cdot\text{L}^{-1}$ ethylene for 24 h. In general, observations on a fruit color and softness were recorded on a daily basis.

2.3 Chlorophyll measurements

Relative chlorophyll content (index) in banana fruits was measured with CM1000 Chlorophyll Meter (Spectrum Technologies, Inc., Plainfield, IL) accordingly to the instructions provided by manufacturer.

3. Determination of minimum active concentration and maximum time

of protection

3.1 Effect of new cyclopropenes on ripening of banana fruit

Minimum concentration of newly synthesized cyclopropenes necessary to protect banana fruits against ethylene and a maximum time of induced insensitivity to ethylene are summarized in the Table 2.

Table 2. Minimum concentration and time of insensitivity to ethylene induced in banana fruits by cyclopropenes applied as a gas for 24 h (Data of Varvara Grichko)

	Compound	Molecular formula	FW	Concentration nl·L ⁻¹		Time d
				Peel	Flesh	
1	3-Methylcyclopropene	C ₄ H ₆	54.1	53.0	53.0	18
2	3-Pentylcyclopropene	C ₈ H ₁₄	110.2	4.3	11.5	7
3	3-Heptylcyclopropene	C ₁₀ H ₁₈	138.3	15.9	158.9	15
4	1-Heptylcyclopropene	C ₁₀ H ₁₈	138.3	4.0	7.9	21
5	1-Nonylcyclopropene	C ₁₂ H ₂₂	166.3	2.4	13.2	37
6	1-Decylcyclopropene	C ₁₃ H ₂₄	180.3	7.5	43.0	33
7	1-Cyclopropenylmethyl butyl ether	C ₈ H ₁₄ O	126.2	277.1	277.1	15
8	1-Cyclopropenylmethyl hexyl ether	C ₁₀ H ₁₈ O	154.3	12.3	12.3	29
9	1-(3-Hydroxypropyl)-cyclopropene	C ₆ H ₁₀ O	98.1	554.2	554.2	24
10	3-Cycloprop-1-enyl-propanoic acid	C ₆ H ₈ O ₂	112.1	410.2	493.9	18
11	3-Cycloprop-1-enyl-propanoic acid ethyl ester	C ₈ H ₁₂ O ₂	140.2	158.9	635.7	22
12	3-Cycloprop-1-enyl-propanoic acid isopropylamine salt	C ₉ H ₁₇ NO ₂	171.1	214.0	1711.5	23
13	N-(1-Cyclopropenylmethyl)ethyl-amine	C ₆ H ₁₀ N	98.1	497.2	497.2	26

The data in Table 2 show that the 3-substituted cyclopropenes protect bananas against ethylene for the shorter period of time when compared to 1-substituted compounds. 3-Pentylcyclopropene was found to be more efficient ethylene antagonist

when compared to 3-methylcyclopropene. However, a time of protection against ethylene imposed by 3-pentylcyclopropene was found to be essentially shorter in comparison with a time of protection found in a case of 3-methylcyclopropene. 1-Nonylcyclopropene was able to protect fruits for a longer period of time compared to 1-heptylcyclopropene by sixteen days. Further increase in a number of carbon atoms present in a side chain of 1-substituted cyclopropene has resulted in both the decrease of the protection period and an increase in the minimal concentration of a compound necessary to protect banana fruits against ethylene. Though 1-decylcyclopropene was shown to induce insensitivity to ethylene in banana fruits for thirty-three days a high concentration of the compound per a treatment has been required. For all alkyl-substituted compounds, except for 3-MCP, the substantial difference was observed between the concentrations that were necessary to protect a fruit peel against ethylene and a concentrations needed to protect flesh tissue. The difference increases as a molecular weight of a compound increases.

Both 1-cyclopropenylmethyl hexyl ether and 1-cyclopropenylmethyl butyl ether were found to be the potent ethylene antagonists (Table 2). Quite unexpectedly, no difference has been observed between the concentration of a compound required to protect fruit peel and the concentration needed to protect flesh tissue. 1-Cyclopropenylmethyl hexyl ether was found to be at least twenty-two times more effective compared to 1-cyclopropenylmethyl butyl ether. Moreover, 1-cyclopropenylmethyl hexyl ether allowed banana fruits staying green for a period of time twice as long as 1-cyclopropenylmethyl butyl ether did. Application of both 1-cyclopropenylmethyl butyl ether and 1-cyclopropenylmethyl hexyl ether delayed fruit ripening in a dose dependent manner when solutions of relatively low concentrations were applied (Fig. 2). Application of the solutions with higher concentrations of cyclopropenyl ethers has resulted in the saturation of the response. 1-(3-Hydroxypropyl)-cyclopropene, 3-cycloprop-1-enyl-propanoic acid, 3-cycloprop-1-enyl-propanoic acid ethyl ester and 3-cycloprop-1-enyl-propanoic acid isopropylamine salt were able to protect banana fruits for up to 24 days though very high concentration have been required for the protection (Table 2). Significant difference has been observed between the concentration of a compound required to protect fruit peel and the concentration needed to protect flesh tissue. N-(1-Cyclopropenylmethyl)ethyl-amine protected banana fruits

against ethylene for a long period of time while it required an application of highly concentrated solutions (Table 2).

Fig. 2. Effect of ethers on delay in fruit ripening. Banana fruits were treated with the indicated amount of an ether for 24 h and next day were exposed to $300 \mu\text{l}\cdot\text{L}^{-1}$ of ethylene for 24 h. Fruits were then left to ripen in an air. Legend: squares, 1-cyclopropenylmethyl butyl ether; circles, 1-cyclopropenylmethyl hexyl ether.

3.2 Effect of new cyclopropenes on flower senescence

It was preliminary demonstrated that the way of treatment had an important effect on the antagonistic properties of cyclopropene derivatives. In that study, carnation flowers were treated for 24 h with 3-cycloprop-1-enyl-propanoic acid salt, which was applied either as a gas or as a 5 μ M solution, followed by 24-h ethylene treatment. At 48 h an average diameter of carnation flowers treated with ethylene only was 57.1% of the control value. Average diameter of carnation flowers treated with 3-cycloprop-1-enyl-propanoic acid isopropylamine salt, which was applied as a gas, and then treated with ethylene was 98.9% of control value. Average diameter of flowers treated with 3-cycloprop-1-enyl-propanoic acid salt, which was applied as a solution, and then treated with ethylene was 73.3% of control value.

III. DISCUSSION AND SUGGESTIONS FOR FURTHER RESEARCH

1. Possible mechanism of inhibition (Proposal of Varvara Grichko)

It is a challenge to explain how cyclopropenes inhibit ethylene receptor. The receptor is thought to use a dimeric mechanism of signal transduction with the feature of the disulfide bonds (cysteine 4 and cysteine 6) between monomers (Schaller *et al.*, 1995; Pirrung, 1999) and is active in its dimeric form with copper ion bonded to cysteines 65 and histidines 69 (Rodriguez *et al.*, 1999). Dissociation of transmembrane region induced by ethylene binding would turn on the response (Pirrung, 1999). Cyclopropenes that would form a long-lived complex with ring retention with the copper cofactor are likely to promote dissociation of the dimeric receptor also. With notwithstanding, in contrast to ethylene cyclopropenes inhibit the receptor.

Substituted cyclopropenes readily undergo ring-opening in the presence of Cu^+ and Ag^+ (McKenna *et al.*, 1995; Isaka and Nakamura, 1990; Gettwert *et al.*, 1999). because π -bonding is the dominant interaction in cyclopropene complexes (Halton and Banwell, 1987) compared to σ -bonding in olefin complexes (Thompson *et al.*, 1983; Muhs and Weiss, 1962). Neither metal-complexed or free vinyl carbenes that are the products of ring-opening would account for the inhibition.

Because inhibition by cyclopropenes is irreversible (Sisler *et al.*, 1996) it might be suggested that inhibition occurs via the covalent attachment of cyclopropene moiety to the active side of the receptor. Cyclopropenes react with thiols with the formation of alkylthiocyclopropanes, and cyclopropene fatty acid irreversibly inhibit fatty acyl CoA desaturases by the reaction of cyclopropene ring with a cysteine residue of the active site of the enzyme (Reiser and Raju, 1964; Quintana *et al.*, 1998; Triola *et al.*, 2001). It is possible that the similar mechanism takes place in a case of ethylene receptor. Reaction of alkylcyclopropene with cysteine 65 would irreversibly upset the active center in the receptor. The effect would be similar to the mutation of the cysteine 65 resulting into plant insensitivity to ethylene (Bleecker *et al.*, 1988).

A suggested mechanism for cyclopropene inhibiting of ethylene receptor is depicted in Fig. 3. Complexation to copper would prevent formation of disulfide bond while ethylene binding would promote the linkage. Formation of disulfide bond would result into a non-functional conformation of the receptor followed by the activation of response pathways. Upon ethylene binding, the copper ion would become coordinated to ethylene molecule and to a nitrogen atoms from each of two histidines. Three-coordinate trigonal-planar geometry is the dominant structural type for Cu^+ -monololefin complexes [27] and 18-electron configuration can be attained via copper bonding to the forth ligand. Sulfur-ligated Cu^+ -ethylene complex exhibits very weak metal-ligand bonding interactions (Hirsh *et al.*, 2001) that may serve as an indirect evidence for the suggested mechanism.

Fig. 3. Suggested mechanism for cyclopropene inhibiting of ethylene receptor.

2. Evaluation of the research achievements (Comments of Varvara Grichko)

1-Alkyl-substituted cyclopropenes were found to be the most competent ethylene antagonists (Table 2). 3-Alkyl-substituted cyclopropenes protected banana fruits for somewhat shorter period of time and were active at elevated levels that can be attributed to the escalation in ring-opening. Indeed, 3-alkyl-substituted cyclopropenes are generally less stable than 1-substituted cyclopropenes. For example, 3-methylcyclopropene is 3 kcal/mol less stable than 1-methylcyclopropene (Han *et al.*, 2000). Somewhat low antagonistic competence of relatively hydrophilic cyclopropenes 1-cyclopropenylmethyl butyl ether, 1-cyclopropenylmethyl hexyl ether, 1-(3-hydroxypropyl)-cyclopropene, 3-cycloprop-1-enyl-propanoic acid, 3-cycloprop-1-enyl-propanoic acid ethyl ester and 3-cycloprop-1-enyl-propanoic acid isopropylamine salt is in a good agreement with the data reported earlier by Quintana *et al.* (1998), who found that in carboxycyclopropene the addition of the thiol compound to the cyclopropene double bond was prevented by the presence of the carboxylic group as substituent in the ring. Instability of cyclopropenes in acidic medium (Halton and Banwell, 1987) may account for the significant difference in concentration of a compound required to protect a fruit peel and the concentration needed to protect flesh tissue that were observed for the acid and, especially, the salt (Table 2). No such difference was found in a case of amine (Table 2).

3-Cycloprop-1-enyl-propanoic acid resembles naturally occurring cyclopropenoid fatty acid (CPFA), the sterculic and malvalic acids that are widespread in the seed oil of plants (Salaun and Baird, 1995). Sterculic acid inhibits desaturases (Reiser and Raju, 1964) and unsaturated fatty acid biosynthesis in plants (James *et al.*, 1968), and exhibit moderate anti-ethylene activity (Sisler, 2001). CPFA contained in seeds may account for the stimulatory effect of exogenous ethylene on seed germination. Interestingly, CPFA content reduces drastically during the initial stages of germination (Pandey and Subrahmanyam, 1986).

There is a close resemblance in the inhibition of ethylene receptor and desaturases by cyclopropenes that can be useful in designing new compounds. For instance, cyclopropenes with exocyclic double bond do not block effectively ethylene receptor, and the structural analogue of sterculic acid with an exocyclic double bond does not inhibit desaturases (Sisler, 2001; Triola *et al.*, 2001). Substituted cyclopropene with branched saturated side chain is likely to be a less effective ethylene antagonist because of the steric hindrance affecting the yield of cyclopropene addition to the S-H bond.

It appears that time of protection and, in some cases, minimum active concentration were both dependent on alkyl chain length (Table 2). The effect faded when compounds turned too heavy to volatile. Another indication that the effect could be a diffusion-controlled one came from the observation that time of protection decreased as temperature increased. These lead to the following suggestions. During 24-h treatment, cell membranes became saturated with a free cyclopropene. After withdrawal of cyclopropene from the atmosphere heavy cyclopropene was expected to stay longer in a lipid membrane than gaseous 1-MCP or light cyclopropene. For example, sterculate has a higher preference for incorporation into membranes than malvalate (Pawlowski *et al.*, 1985). Cyclopropene incorporated into membrane would continue to block the newly synthesized receptors postponing tissue recovery. Because some cyclopropenes are co-carcinogenic (Pawlowski *et al.*, 1985) thorough testing of cyclopropenes that are able to incorporate into membranes is required prior the commercial usage.

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BARD - קמח

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CONTROLLING ETHYLENE RESPONSES IN HORTICULTURAL CROPS AT THE RECEPTOR LEVEL

Final Report (1999/2000) BARD Project No.: US-2786-96R

Submitted by

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Signature:
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Signature:
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Master theses	---	---	---	---
Ph.D. theses	---	---	---	---
Abstracts	5	---	---	5
Not refereed (proceedings, reports, etc.)	5	---	---	5

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted	---	---	---	---
Issued (allowed)	---	---	---	---
Licensed	---	---	---	---

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Visits/Meetings	1	4	---	5
Sabbaticals	---	---	---	---
Postdoctorates	---	---	---	---

Cooperation, briefly explain whether synergistic, complementary or supportive.

Synergistic cooperation:

US scientist synthesized 1-MCP and its analogues that were sent to the Israeli group which did the postharvest, physiology and handling studies. Constant contacts via e-mail, telephone and fax exchanges, mutual consultations were carried routinely.

ABSTRACT

Ethylene is a plant hormone that controls many plant responses, such as growth, senescence, ripening, abscission and seed germination. Recently, 1-methylcyclopropene (1-MCP), was shown to bind to ethylene receptor for a certain period of time and prevent ethylene action. The objectives of this research were to synthesize analogues of 1-MCP and test their potency to block the ethylene receptor and inhibit ethylene action.

During the course of this project, procedures for synthesis and shipment of the cyclopropene compounds were developed as well as assay procedures for each compound were worked out. Thirteen new compounds were synthesized. All of them are structural analogues of 1-MCP, with substitution in the 1-position and a side chain containing 2 to 10 carbons. After preliminary studies, nine promising compounds were selected for in-depth study. The potency of the compounds to inhibit ethylene action was tested on a wide scope of systems like: climacteric fruits (banana, avocado and tomato), the triple response (etiolated peas), and leaf abscission (citrus). As the putative inhibitors are suspected to compete for the site of binding and a competitive type of inhibition could be considered, a high concentration of ethylene ($300 \mu\text{l}\cdot\text{L}^{-1}$) was used to induce ripening and other physiological processes. The tests were conducted under extreme conditions which hasten ripening like treatment and storage at 22 to 25°C. There were fluctuations in the responses as related to the concentrations of the inhibitors. Some required much higher concentration to exert the same effect, while some, when applied at the same concentration, blocked the receptor for a longer period of time than the others. Some fruits and other plant organs responded differently to the same inhibitor, indicating differences in characteristics and availability of the ethylene receptors in the various tissues. The potency of the putative inhibitors was found to be greatly affected by their molecular structural and size. In addition, it was found that treatment with the inhibitor should be given before the onset of ethylene action. In the case of fruit, treatment should be carried out before the pre-climacteric stage. Simultaneous treatment with ethylene and the inhibitors reduced the inhibitors' effect. The relationship between ethylene and the inhibitors is of a non-competitive nature. All the fruits treated with the putative inhibitors resumed normal ripening after recovery from the inhibition. This fact is of great importance when considering the inhibitors for practical use.

The advantage of using inhibitors of ethylene action over inhibitors of ethylene production lies in the ability of the inhibitors of ethylene action to protect the tissue against both endogenous and exogenous ethylene, thus providing better overall protection. Our findings indicate that 1-MCP and its structural analogues are potent inhibitors of ethylene action capable of providing good protection against endogenous and exogenous ethylene. The fact that the compounds are in a gas phase and are non-phytotoxic, odorless and effective at minute concentrations, renders them promising candidates for commercial use. However, the development of water-soluble inhibitors will expand the potential use of the inhibitors in agriculture.

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I. INTRODUCTION

Ethylene is a plant hormone that controls many plant responses, such as growth, senescence, ripening, abscission and seed germination. Ethylene is a natural product of metabolism and plants respond to both endogenous and exogenous ethylene (Abeles *et al.*, 1992). Ethylene responses can be controlled by regulating its production or action, or by selecting mutants which do not respond to ethylene. Inhibitors of ethylene action are considered very potent for basic research and agriculture use. The use of heavy metal silver (silver thiosulfate) is an excellent means of controlling ethylene responses, but its toxicity limits its usefulness. It has been known for many years that certain strained olefins will compete with ethylene for the receptor and control ethylene responses even when the tissue is continuously exposed to ethylene. The compound 2,5-norbornadiene (NBD) is an effective inhibitor of ethylene responses, however, its strong odor and corrosive nature renders it not useful for agricultural purposes. Recently, it has become apparent that compounds exist which will bind to the receptor for long periods of time and prevent ethylene from acting; thus, they are effective inhibitors of ethylene responses. These compounds are very highly strained olefins which bind in an apparent irreversible manner to the physiological receptor; however, after a certain period the tissue becomes again sensitive to ethylene. Depending on the compound, the time of insensitivity differs. The aim of the present research project was to widen our knowledge regarding the inhibitor 1-methylcyclopropene (1-MCP) and assess the potency of newly synthesized highly strained olefins as putative inhibitors of ethylene action.

The result of this research project was an achievement of both the excellent cooperation of the American and Israeli groups. The American investigator was responsible for preparing the new ethylene receptor blockers and ship them over to the Israeli co-investigators for studying the efficiency at the physiological and practical levels, using the relevant plant experimental models outlined below.

The objective of the American group (objective 1 in the proposal) was to develop analogues of 1-MCP. Compounds were selected to extend the useful parameters of compounds already available. The major factors that were considered during selection of the compounds were: (1) The activity of the compound. (2) The stability of the compound. (3) Compounds that are more water-soluble and easier to handle under agricultural circumstances. (4) Compounds that are less volatile.

The objective of the Israeli group (objective 2 in the proposal) was to select different plant models in a wide spectrum of growth, development, senescence and fruit ripening processes. The models were used in order to evaluate the ethylene blocking action in controlling ethylene action in different practical and physiological processes in which ethylene is involved as a regulator. The plant model systems that have been chosen include: counteracting the action of ethylene on abscission and the involvement of ethylene in the autocatalysis and in autoinhibition of ethylene biosynthesis. Another set of plant models has been selected in order to elucidate the effectiveness of the blockers to inhibit the ethylene-induced senescence process like chlorophyll degradation, as demonstrated by degreening of orange fruit peel or by preventing degreening of the peel of Sweetie (Oroblanco) fruits. The climacteric fruit model was used for testing prolong storage and shelf life. Tomato, apple and avocado

fruits were chosen as representatives of climacteric fruits. The etiolated pea seedling model represented the effectiveness of the blockers in controlling cell elongation.

Table 1. The structure of the newly synthesized compounds tested during the research of the project

Compound	Structure
1-MCP (1-methylecyclopropene)	CH_3
H1-MCP (cyclopropene-1-methanol)	$\text{CH}_2 \text{OH}$
1-ECP (1-ethylcyclopropene)	$\text{CH}_2 \text{CH}_3$
1-PCP (1-propylcyclopropene)	$\text{CH}_2 \text{CH}_2 \text{CH}_3$
1-BCP (1-butylcyclopropene)	$(\text{CH}_2)_3 \text{CH}_3$
1-PentCP (1-pentylcyclopropene)	$(\text{CH}_2)_4 \text{CH}_3$
1-HCP (1-Hexylcyclopropene)	$(\text{CH}_2)_5 \text{CH}_3$
1-HeptCP (1-heptylcyclopropene)	$(\text{CH}_2)_6 \text{CH}_3$
1-OCP (1-octylcyclopropene)	$(\text{CH}_2)_7 \text{CH}_3$
1-DCP (1-decatylcyclopropene)	$(\text{CH}_2)_9 \text{CH}_3$

During the three years of this research project, we tested the potency of the following inhibitors of ethylene action: 1-MCP and 9 newly synthesized putative inhibitors termed: H1-MCP, 1-ECP, 1-PCP, 1-BCP, 1-PentCP, 1-HCP, 1-HeptCP, 1-OCP and 1-DCP (Table 1). Our test model systems were designated to assess the potency of the newly synthesized inhibitors which counteract ethylene action. These test systems included climacteric fruits, like banana, tomato and avocado, and non-climacteric fruits like 'Shamouti' oranges and 'Sweetie' (Oroblanco) fruits. Typical ripening parameters for each climacteric fruit, like color change, softening, ethylene production, and the activity of the hydrolytic enzymes cellulase and Polygalacturonase, were followed during storage to determine the effect of the inhibitors on ripening. In non-climacteric fruits, ethylene-related processes like peel color change and calyx abscission were followed. The putative inhibitors were also tested on vegetative tissue. Pea seedlings and leaf explants were included in our test model systems. In order to assess the potency of the putative inhibitors to counteract ethylene action, the effect of the inhibitors were tested on ethylene-induced ripening rather than spontaneously ripening. As the putative inhibitors are suspected to compete for the site of binding and a competitive type of inhibition was suggested (Sisler et al., 1997), a physiological high concentration of ethylene ($300 \mu\text{l}\cdot\text{L}^{-1}$) was used to induce ripening. The tests were conducted under extreme conditions which hasten ripening like treatment and storage at 22-25°C.

The result of preliminary experiments with 1-MCP showed maximal inhibitory effect of 1-MCP was obtained only when the compound was applied at the preclimacteric stage immediately after harvest. Any delay in application of the inhibitor reduced the efficiency of the treatment or annihilated it. In addition, our preliminary

experiments have also shown that treatment with the inhibitor 1-MCP was effective only if applied prior to exposure to ethylene. Simultaneous exposure of the fruit to the inhibitor and ethylene reduced the potency of the inhibitor. In light of the above, in all the studies with the newly synthesized putative inhibitors of ethylene action presented herein, the fruit or vegetative tissue were exposed to the inhibitor immediately after harvest prior to ethylene treatment.

Within the framework of this project, we also conducted some large-scale studies to explore possible commercial use of 1-MCP. In addition, the influence of the temperature during the treatment and storage on the potency of 1-MCP was investigated. We also studied the effect of 1-MCP on the growth of callus originated from the albedo of citrus fruit peel and the influence of 1-ECP and 1-PCP on color change in citrus fruit. Studies were conducted as well to elucidate the mode of action of the inhibitors.

II. DESCRIPTION OF THE RESEARCH ACTIVITY

1. Developing, preparing and supplying compounds to the Israeli group

The putative inhibitors of ethylene action were prepared in the US and shipped to the Israeli group for testing. Each compound poses somewhat different problems and these problems were solved as the work progressed.

1.1 Preparation of the compounds

The compounds (Table 2, 3) were prepared by literature methods (Rappoport, 1987; Schipperijin and Smael, 1973; Closs, 1966). None of the compounds is commercially available and in some cases many intermediates needed to be prepared for the synthesis. A problem that developed was to assay the amount of the compound that was present. Gas chromatography (GC) is the method of choice. The compounds are prepared in solvents and these frequently interfere with the assay methods. For this reason it was necessary to use more than a single GC column to assay the compounds. Another problem inherited in the study is the stability of the compounds. Most of the compounds are gasses at room temperature and can be readily handled in the gas phase. They normally are stable for weeks in the gas phase, provided no organic solvents are present. It was found that a saturated ammonium sulfate solution seal was suitable for containing most of them because they readily penetrate rubber seals. If the

Fig. 1. Exposure time and concentration of 1-MCP, 1-ECP and 1-PCP on preservation of banana fruit

presence of water causes instability, low temperatures (-80°C) is about the only way the free compound can be handled.

In order to find the full potential of compounds blocking the ethylene receptor, it is necessary to prepare analogues of the compounds and to test them for activity. In previous studies some cyclopropenes were tested, but there are hundreds of cyclopropenes.

They fit into different classes, and these classes have divergent activities and protection times to the fruits (Sisler and Serek 1997,1999). Also the compounds have widely different stabilities.

Fig. 2. Exposure time and concentration of 1-BCP, 1-HCP and 1-OCP on preservation of banana fruit

Cyclopropenes are usually very unstable compounds, they are not commercially available. They must therefore be prepared by synthesis. All of the compounds listed in Tables 2 and 3 are the products of our synthetic efforts.

1.1.1 Cyclopropenes substituted in the 1 position.

1.1.1.1 Synthesis. Although each has some specific synthetic requirements, only general procedures are described. These compounds can be prepared as in the

case of 1-MCP by treating 3-chloro-2-methyl-cyclopropene with phenyllithium or lithium diisopropylamide (Magid *et al.*, 1971). Other compounds of varying chain length could be prepared this way by extending the chain length of the 2-methyl position. The intermediates are not available to do this and their synthesis would be difficult. The method used was the preparation of the-bromo-compound from the 1-Alkyne (Cousseau, 1980). HBr adds to the triple bond to give a double bond with bromine in the 2 position. Treatment of this with 50% NaOH and bromoform gives a tribromocyclopropane. When these compounds are treated with excess methylithium, the cyclopropenes are obtained (Al Dulayymi *et al* 1996, Al Dulayymi *et al* 1997, Baird *et al*, 1986). This method is long and tedious, and sometimes several intermediates must be prepared. Many 1-Alkynes are available and the method seems to be the method of choice for most 1-substituted cyclopropenes.

Table 2. Minimum concentration of 1-cyclopropenes needed for protection of bananas

Compound	Amount required for 24 hr exposure (n l·L ⁻¹)	Protection time (days)
1-MCP (1-methylecyclopropene)	0.7	12
1-ECP (1-ethylcyclopropene)	4.0	12
1-PCP (1-propylcyclopropene)	6.0	12
1-PentCP (1-pentylcyclopropene)	0.5	14
1-BCP (1-butylcyclopropene)	3.0	12
1-HCP (1-hexylcyclopropene)	0.4	20
1-OCp (1-octylcyclopropene)	0.3	25

1.1.1.2 Testing. Banana was used as the primary test method (Sisler *et al*, 1999) to ascertain activity and to test for stability and method of application and

duration of inactivation. Some preliminary data had been previously obtained. Amounts of compound required are in Tables 2 and 3 and Figures 1 and 2. The time protection by a single saturating exposure to 1-MCP is also listed in Table 2. Figures 1 and 2 give the minimum concentration of compound needed to protect the fruit against ethylene after exposure for a given amount of time up to 48 h.

Table 3. Minimum concentration required to inhibit ethylene-induced ripening of banana for the indicated duration

Compound	Concentration (nl·L ⁻¹)	Time (days)
Cyclopropene	0.7	12
1-methylcyclopropene	0.7	12
3-methylcyclopropene	2	12
3,3-dimethylcyclopropene	500	7
3-methyl-3-vinylcyclopropene	120	5
1,3-dimethylcyclopropene	250	12
1,2-dimethylcyclopropene	3000	3
1,3,3-trimethylcyclopropene	20,000	12
3-methyl-3-ethynylcyclopropene	240	5
Cyclopropene-1-methanol	30	10*

* Peel only

The results we obtained with these compounds were quite surprising. Data obtained by Burg and Burg (1967), in pea growth, indicate ethylene to have a 1/2 maximum concentration of 0.1 $\mu\text{l}\cdot\text{L}^{-1}$ and 1-butene to have a 1/2 maximum of 25,000 $\mu\text{l}\cdot\text{L}^{-1}$. Data obtained by Sisler and Yang (1984) gave a value of 25,000 $\mu\text{l}\cdot\text{L}^{-1}$ for cis-butene for blocking the ethylene receptor. These results show that as the chain is lengthened on cyclopropenes, the minimum concentration does increase some, but not

nearly as much as might have been expected. It also shows that as the chain length goes to C-6 and C-8, the amount required for effective inhibition appears to be even lower than 1-MCP.

Table 4. Structures of Compounds

cyclopropene
1-methylcyclopropene
3-methylcyclopropene
3,3-dimethylcyclopropene
3-methyl-3-vinylcyclopropene
1,3-dimethylcyclopropene
1,2-dimethylcyclopropene
1,3,3-trimethylcyclopropene
3-methyl-3-ethynylcyclopropene
cyclopropene-1-methanol

It is not known why this occurs. But the estimated boiling points for C-6 and C-8 would be around 150°C and 200°C. Perhaps as low molecular weight compounds enter, some of it comes back out before binding while the high molecular weight compounds remain in the tissue (membrane?) and binds.

Another interesting observation was that with the C-9 (the compound of C-6 chain) both the pulp and peel of the fruits were not protected against ethylene at low concentrations but as the concentration increased, both parts of the fruits were protected at the same concentration, but with the C-11 and C-12 compound at low concentrations, the peel was protected, but the pulp was not. At higher concentrations, both were protected. Although the pulp in the C-11 and C12 compounds was not protected at very low concentrations, the concentration needed was low. The reason for this is unknown but may be due to the slow diffusion of the high molecular weight compounds in bulky tissue such as banana. Evaluation of these compounds is still being made.

1.1.2 Cyclopropenes substituted in the 3 position.

1.1.2.1 Di-substitution in the 3 position. A number of compounds were reported and described in the first annual report and thus not refereed on in this report.

1.1.2.2 Mono-substitution in the 3 position. Preparing these compounds is limited by stability. The methods used in preparing di-substitution would destroy the compounds with a single substituent. Compounds containing a hydroxy group can be converted to a chloride by fairly easy procedures and some of these should be converted to mono-cyclopropenes by lithium diisopropylamide. This method has been previously used to prepare 3-methylcyclopropene. Banana was used as the primary test method to ascertain activity of these compounds and to test for stability and method of

application and duration of inactivation. This synthetic method did not appear to work, and a different method has been devised which should allow for preparation of some of these compounds. This is currently under investigation.

1.2 Consideration for long distances shipping of the compounds and storage

The problem of long distance shipping of these unstable compounds was explored. Long distance shipping of the compounds could possibly be done on dry ice, but a more reliable method was needed. It is known that the lithium salts of the compounds are more stable than the free compounds. It was found that the solvents could be removed under vacuum without much loss of activity. As a solid they can be shipped at ambient temperature and then stored in the cold upon arrival. It is necessary to add back a suitable solvent and chill to -80°C before adding water which gives the free compound. The free compound then can be stored at -80°C . The use of this method has proved a success in furthering the research, and this method should prove useful in storage of the synthetic compounds.

2. Preliminary scanning of the new compounds for activity

Every compound undergoes a preliminary screening for activity before sending it to Israel for further physiological studies. Banana has been used as a model system as they are readily available throughout the year, and give consistently good results. Although some problems are noted during the hottest days of summer, the bananas appear to be sensitized to ethylene on some occasions.

The compounds prepared to date and some properties of these compounds are given in Tables 2 and 3 and the structure presented in Table 4. This lists the cyclopropenes prepared earlier as well as those prepared on the BARD grant so that comparison of all of the compounds can be made in a single table. The preliminary banana assay gives a good indication of a compound's inhibitory potential.

3. Materials and Methods for Testing the Putative Inhibitors

3.1 Plant materials

Avocado cv. 'Ettinger', 'Hass', 'Reed' and 'Fuerte', banana fruit cv, 'Grandnine', 'Ziv' and 'Ailon', citrus cv. 'Shamouti' and 'Sweetie' fruit were harvested in local orchards and tomato fruit, cv. 'Faculty 189' and 'Faculty 191', were picked from plants growing in a local greenhouse. All fruit were harvested during the commercial harvesting season. The fruit were treated with the inhibitors within 6 h after harvest.

3.2 Synthesis of 1-MCP

1-MCP was synthesized according to the procedure described by Sisler and Serek (1997). We have noticed that in this publication the authors indicated that "the preparation contains some impurities, but as it contains so much activity the impurities usually can be ignored". In light of that, attention was paid to check possible presence of physiological active impurities in the 1-MCP preparation. It was found that employing iodine, which reacts with the double bond in 1-MCP to form the physiological inactive diiodine compound, renders the preparation physiologically

inactive. Thus, 1-MCP was the only physiological active compound in the preparation capable of inhibiting ethylene action.

3.3 Extract of the putative inhibitors from materials synthesized by USA group

Most of the compounds synthesized by USA group are unstable in their active form. Since the lithium salt of these compounds is very reactive. It was found that under nitrogen, the lithium salt is stable and can be easily handled transported to the Israeli group without loss of activity. Upon material the salt was dissolved in water and the compound extracted in ether. The ether solution was parted from the water by freezing. The compound was applied as an ether solution and kept in the freezer.

3.4 Determination of the inhibitors concentration

A 1 ml gas sample of the inhibitor was injected into a Varian 3300 gas chromatograph equipped with a 23% SP-1700 on 80/100 chromosorb P AW column and a flame ionization detector. Ethylene or 1-butene was used as a calibration standard for 1-MCP. Cyclohexane was used as a calibration standard for 1-ECP, 1-PCP and 1-BCP. Octane was used for both 1-PentCP and 1-HCP, decane for both 1-HeptCP and 1-OCP and tridecane for 1-DCP.

3.5 Testing model system

A test system has been established for testing all the newly synthesized putative ethylene inhibitors. This test system includes some climacteric fruit (banana, tomato and avocado), citrus leaf explants and pea seedlings. In addition, some physiological or biochemical processes in nonclimacteric fruit which are related to ethylene action, such

as the peel color change of citrus fruit, were also used as indicators in the test system on some of the putative inhibitors.

3.5.1 Testing the putative inhibitors of ethylene action using climacteric fruit. The potency of the inhibitors was first tested with the climacteric fruit avocado, banana and tomato. In these experiments, a single fruit was sealed in a 3-l glass jar and the proper amount of the inhibitor was injected into the jar. Each treatment was composed of 5 replicates (5 fruits per treatment) and all the experiments were repeated 3 times. The fruits were first treated with the inhibitors for 24 h and then exposed to ethylene ($300 \mu\text{L}^{-1}$) for 24 h. After the ethylene treatment, the fruit were stored in air at 25°C for ripening assessment. Fruit treated with the inhibitors were compared with untreated fruit and with those treated with ethylene ($300 \mu\text{L}^{-1}$).

3.5.2 Testing the inhibitors using citrus leaf explants. Six to 10 months old leaves from 50 years old 'Shamouti' orange trees, were picked early in the morning, carried to the laboratory in plastic bags and treated immediately. Twenty mm long explants composed of 10 mm tissue from the petiole and 10 mm tissue from the leaf blade were excised. Thirty explants were placed in a 3 liter glass jar and treated for 6 h with the desired concentration of the inhibitors and subsequently treated with ethylene ($20 \mu\text{L}^{-1}$) for 24 h. The treated explants were then placed in 3-l glass jars with the lids open, at room temperature, and the rate of abscission was examined daily.

3.5.3 Testing the inhibitors using pea seedlings. Pea seeds cv. 'Alaska' were soaked for 24 h in aerated water, rinsed, and planted in plastic pots filled with vermiculite. The pots were placed in 4-l jars. Three days old seedlings were treated for 24 h with various concentrations of the inhibitors and afterwards kept continuously in ethylene ($100 \mu\text{L}^{-1}$) until the end of the experiments (approximately 96 h). At the end

of the incubation morphological parameters were measured. All experiments were conducted in the dark at 20°C. Treatments and observations were made under deem green safe light.

3.6 Large scale experiments with 1-MCP

Experiments were conducted with large quantities of fruit at various temperatures. The effects of 1-MCP on ripening of avocado fruit and on color change of 'Sweetie' fruit were investigated. Twenty-five to 35 fruits were enclosed in a 30 liter plastic jars and treated with proper concentrations of 1-MCP for 24 h at 4°C, 8°C, 12°C, 16°C or 20°C and then stored in air for one month at those temperatures. Afterwards, the fruit were held for one week to simulate shelf life at 20°C. During the whole experimental period, ripening was assessed at one week interval.

3.7 Fruit quality assessment and biochemical analysis

3.7.1 Cellulase and PG analysis. Extraction and measurement of cellulase (endo-1,4-b-glucanase; EC 3.2.1.4) and polygalacturonase (PG; EC 3.2.1.15) activity were performed following the methods of Goren and Huberman (1979) and Pesis et al. (1978) with slight modifications. All extractions were performed at 4°C. Three samples of mesocarp tissue (2 g) were taken from the equatorial region of three fruits from each treatment. Each sample was homogenized for 2 min at 24,000 r/min (SEV homogenizer) in 20 ml of 0.2 M K-phosphate buffer pH 5.5 containing 1 M NaCl and 0.05% L-cysteine. The homogenate was stirred for 30 min in an ice bath, filtered through four layers of cheese cloth and centrifuged for 10 min at 20,000g (Sorvall RC 5C Plus centrifuge, rotor SS-34). The supernatant was dialyzed overnight against the

above indicated buffer, diluted 100 times, and recentrifuged for 10 min at 12,000 r/min. The supernatant was checked for cellulase and PG activity.

Cellulase activity was assayed viscosimetrically at 37°C using 1.3% Na-carboxymethylcellulose dissolved in 0.02 M K-phosphate buffer pH 6 as substrate. Activity was expressed as percentage change in viscosity. PG activity was determined spectrophotometrically by measuring reducing groups liberated from Na-polypectate with the dinitrosalicylic acid reagent. D-galacturonic acid was used as a standard and 1 mM Na-hydrosulfite was added as an inhibitor of uronic acid oxidase (Riov, 1974). The release of 1 mg of D-galacturonic acid in 6 h was defined as one unit of PG activity. The activity of both enzymes was calculated and expressed per fresh weight.

3.7.2 Chlorophyll and lycopene analysis. Chlorophyll was determined by the method of Arnon (1949). Lycopene was estimated by measuring the density at 504 nm (Gross, 1991). One g of the tissue was taken and ground with 80% acetone. The extraction was filtered and fixed to 5 ml. For measuring chlorophyll, the density at 663 nm and 645 nm (D_{663} and D_{645}) were measured and the chlorophyll content (C_{a+b}) was calculated according to the following formula:

$$C_{a+b} \text{ (mg/L)} = 20.2 D_{645} + 8.02 D_{663}$$

Lycopene was measured at 504 nm (D_{504}).

3.7.3 Ethylene measurement. Jars containing the plant materials were left opened between measurements for ventilation and sealed for 1 h prior to ethylene sampling. A 1 ml gas sample was withdrawn by syringe from each jar through a septum fitted in the jar lid. The sample was injected into a Varian 3300 gas chromatograph equipped with a flame ionization detector (FID) and a alumina column.

3.7.4 Firmness measurement. Fruit firmness was determined using a Chatillon digital force gauge model DFG-50 fitted with a conical probe, 6 mm diameter and expressed in *kg* or *N*.

3.7.5 Color assessment. Peel color in avocado, banana, tomato was assessed visually and recorded following a 0-10 scale (0 indicating no color change and 10 complete change). Peel color in 'Sweetie' hybrid was assessed and recorded following a 1-4 scale. For the large scale experiments with 1-MCP, a Minolta Chromo Meter, model CR-200 was used for color measurements. The color index of the 'Sweetie' hybrid fruit, was expressed as the ratio between the 'a' and 'b' parameters.

3.7.6 CO₂ measurement in nectarine and plum. Five nectarine or plum fruits from each treatment were weighed and then placed into a 1.7-l jar at 20°C. The jars were sealed for 1 h. A 5 ml gas sample was withdrawn by syringes from each jar through a septum fitted in the jar lid. The 5 ml gas sample was injected into a gas chromatograph with a thermal conductivity detector (TCD) and a Poropak column for CO₂ determination. The jars were left open for ventilation between measurements.

3.7.7 Soluble solids concentration (SSC) and titratable acidity (TA) in apple fruit. SSC and TA were measured in 4 replicates on juice extracted from 5 sections, each one was taken from a different apple. SSC was determined with an Atago digital refractometer (Atago, PR-100, Tokyo) and TA by titrating the juice with 0.1 N NaOH to pH 8.2 using a pH meter (665 Dosimat, Metrohm Ltd, Swiss). The results were expressed as percent malic acid.

3.7.8 α -Farnesene and conjugated trienes in apple peel. For the measurement of α -farnesene and conjugated trienes, 6 discs of 1 cm diameter were taken with a cork borer from a peel strip of the fruit equator part. Five apples were

used for each treatment. Ten discs (2 from each apple) were put in 150 ml hexane for 15 min. The hexane extraction was used for determination of α -farnesene and conjugated trienes in apple peel. The absorption was measured at 232 nm for α -farnesene and at 258, 281 and 290 nm for conjugated trienes. A spectrophotometer (UV / VIS 911, GBS, Australia) was used. Three replicates were in each treatment. The result was calculated using the following formula:

$$\text{Content of } \alpha\text{-farnesene } (\mu\text{mol}/\text{cm}^2) = \text{OD}_{232\text{nm}} \times 36 \times 7.5 / \pi R^2$$

$$\text{Content of conjugated triene } (\mu\text{mol}/\text{cm}^2) = (\text{OD}_{258\text{nm}} - \text{OD}_{290\text{nm}}) \times 40 \times 7.5 / \pi R^2$$

$$\text{or: } = (\text{OD}_{281\text{nm}} - \text{OD}_{290\text{nm}}) \times 40 \times 7.5 / \pi R^2$$

R -- disc radius (nm)

3.7.9 Assessment of fruit physiological disorders

3.7.9.1 Woolliness in nectarine fruit. To assess woolliness in nectarine fruit, 20 nectarines for each treatment were halved along the calyx and base. Woolliness was examined on the cut surface and rated as light, medium or severe if it occupied up to 1/3, 1/3 to 2/3 or more than 2/3 of the cut surface, respectively. Woolliness was determined using the following formula:

$$\text{Woolliness index} = (1 \times \text{percent of light} + 2 \times \text{percent of medium} + 4 \times \text{percent of severe}) / 4.$$

3.7.9.2 Flesh reddening in nectarine fruit. To assess the flesh reddening in nectarine fruit, at least 20 nectarines for each treatment were halved along the calyx and base. Flesh reddening was examined on the cutting surface and rated as light, medium or severe if it covered up to 1/3, 1/3 to 2/3 or more than 2/3 of the cutting surface, respectively. Flesh reddening was determined following an index calculated by using the formula:

Index of flesh reddening

$$= (1 * \text{percent of light} + 2 * \text{percent of medium} + 4 * \text{percent of severe}) / 4.$$

3.7.9.3 Superficial scald in apple fruit. Twenty apples were used for each treatment. Superficial scald was rated as light, medium or severe if it covered up to 25%, 50% or more than 50% of the fruit surface, respectively, and the damage was expressed following a scald index using the formula:

$$\text{Scald index} = (1 * \text{percent of light} + 2 * \text{percent of medium} + 4 * \text{percent of severe}) / 4.$$

3.7.9.4 Internal grey pulp in avocado fruit. Twenty avocados were used for each treatment. Internal grey pulp in avocado fruit was assessed visually following a 0-10 scale (0 indicating no grey pulp and 10 complete grey pulp). Values of 1-2 were considered as mild, 3-5 as visible and greater than 5 as serious. The result was calculated using the following formula:

$$\text{Grey pulp index (\%)} = \text{No. of fruit with scale greater than 3} / \text{No. of fruit checked}$$

3.8 Molecular biology research

3.8.1 RNA extraction. Total RNA extraction was based on the phenol/SDS method by Sambrook et al. (1989). mRNA was prepared from total RNA using PolyATtract mRNA Isolation System III (Promega) according to the manufacturer's instructions.

3.8.2 Northern Blot Analyses. For northern blot analysis, 10 µg of total RNA were separated by formaldehyde-formamide agarose gel electrophoresis and blotted onto a nylon membrane (GeneScreen Plus). Hybridization was performed according to the instructions of the membrane supplier.

4. Effect of new inhibitors of ethylene action on ripening of banana fruit

Ethylene is synthesized by ripening bananas, and unripe fruit can normally be initiated to ripen by exposure to an exogenous source of the gas at $100 \mu\text{l}\cdot\text{L}^{-1}$ for 24 h (Hall, 1967). Banana is one of the models in our test system for assessment of the potency of the newly synthesized putative inhibitors of ethylene action. Increased ethylene production, peel color change from green to yellow and fruit pulp softening are the most obvious phenomena observed in the ripening process of banana fruit. These phenomena were used as indicators for banana fruit ripening in our research to determine the potency of the putative inhibitors. Fruit peel color change during ripening was determined according to an eleven grade-scale with -0- indicating complete green and -10- complete yellow. Fruit softening during ripening was determined following a five grade-scale with -5- indicating very firm, -4- firm, -3- relatively firm, -2- soft and -1- very soft. Chlorophyll content in fruit peel was determined 10 days after treatment. In our studies, banana fruit were defined ripe when color index was more than 8, softening index less than 2 and when the fruit displayed a climacteric peak of ethylene production. According to this ripening definition, banana fruit treated with $300 \mu\text{l}\cdot\text{L}^{-1}$ ethylene ripened 2-4 days after the ethylene treatment.

4.1 Effect of H1-MCP on ripening processes in banana fruit

Banana fruit treated with 25, 50, 100, 250 or $500 \text{ nl}\cdot\text{L}^{-1}$ H1-MCP for 24 h and then exposed to $300 \mu\text{l}\cdot\text{L}^{-1}$ ethylene for 24 h retained their green peel color for at least

10 days at room temperature whereas the ethylene treated control fruit turned yellow 2-3 days after treatment. However, pulp softening in the H1-MCP treated fruit was delayed by only 1-2 days compared with ethylene treated fruit. The ethylene climacteric peak in the H1-MCP treated fruit was delayed by 3-5 days compared with those treated with $300 \mu\text{L}^{-1}$ ethylene.

Our studies with 1-MCP on banana fruit (refer to 10.1.1 of this section) showed that 1-MCP at low concentration (1 nL^{-1}) delayed all ripening processes in banana fruit by 13 days. In contrast the putative inhibitor H1-MCP applied at high concentration up to 500 nL^{-1} did not delay all ripening processes to the same extent; color change was efficiently delayed (by 10 days) but softening and the climacteric ethylene peak were delayed by only 1 and 3 days, respectively. This differential effect of H1-MCP on the various ripening processes of banana fruit warrant further studies aiming at exploring possible different ethylene receptors for each of the ripening processes in this fruit.

4.2 Effect of 1-ECP on ripening of banana fruit

Treatment with 1-ECP (100 nL^{-1}) 4-6 h after harvest, prior to exposure to ethylene, delayed ethylene-induced ripening by 12 days in banana fruit stored at room temperature. This was determined by peel color change, climacteric ethylene production and fruit softening. Treatments with 500 nL^{-1} 1-ECP delayed ethylene-induced ripening by 16 days. Increasing the concentration of 1-ECP to 1 or $2 \mu\text{L}^{-1}$ did not further delay ripening. The results indicate that 500 nL^{-1} 1-ECP was sufficient to exert the maximal response.

4.3 Effect of 1-PCP on ripening of banana fruit

The concentration required to inhibit ripening by 1-PCP was similar to that for 1-ECP. The effect of 1-PCP intensified as the concentration increased. Treatment with 12.5, 25, 250 and 500 $\text{nl}\cdot\text{L}^{-1}$ 1-PCP delayed ethylene-induced banana fruit ripening by 2, 4, 11 and 13 days respectively. Treatment with lower concentration (5 or 7.5 $\text{nl}\cdot\text{L}^{-1}$ 1-PCP) delayed fruit ripening by only 1 day. The results show that there was no significant difference between 250 and 500 $\text{nl}\cdot\text{L}^{-1}$ 1-PCP treatments in terms of ethylene peak, fruit softening and color change. Therefore, treatments with 1-PCP at concentrations within the range between 250 to 500 $\text{nl}\cdot\text{L}^{-1}$ is sufficient to exert a maximal inhibitory effect.

4.4 Effect of 1-BCP on ripening of banana fruit

Our results show that treatment with 10, 25, 50 and 100 $\text{nl}\cdot\text{L}^{-1}$ 1-BCP before exposure to ethylene (300 $\mu\text{l}\cdot\text{L}^{-1}$) delayed ethylene-induced banana fruit ripening by 3, 5, 12 and 13 days respectively. Treatment with 100 $\text{nl}\cdot\text{L}^{-1}$ did not significantly increase the effect. Thus, treatment with 50 $\text{nl}\cdot\text{L}^{-1}$ 1-BCP was sufficient to achieve maximal effect.

4.5 Effect of 1-PentCP on ripening of banana fruit

In this study, the inhibitory effects of treatments with 1, 5, 50 or 500 $\text{nl}\cdot\text{L}^{-1}$ 1-PentCP on ethylene-induced ripening in banana fruit were investigated. The results show that treatment with 1 $\text{nl}\cdot\text{L}^{-1}$ 1-PentCP delayed the ethylene-induced fruit ripening by 2 days (Fig. 3). Increasing the concentration resulted in further delay in

Fig. 3. Effect of 1-PentCP on color change (A), fruit softening (B) and ethylene production (C) in banana fruits. The fruits were treated for 24 h with 0 (▲), 1 (Δ), 5 (□), 50 (○) or 500 (●) nl L^{-1} 1-PentCP, ventilated, and then exposed to ethylene ($300 \mu\text{l L}^{-1}$) for 24 h. Untreated fruits (■) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

Fig. 4. Effect of 1-PentCP on chlorophyll content of banana fruits 10 days after treatment. The fruits were treated for 24 h with 0 (A), 1 (C), 5 (D), 50 (E) or 500 (F) nl L^{-1} 1-PentCP, ventilated, and then exposed to ethylene ($300 \mu\text{l L}^{-1}$) for 24 h. Untreated fruits (B) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

ethylene-induced fruit ripening. In fruit treated with higher concentrations of 1-PentCP (5 , 50 or $500 \text{ nl}\cdot\text{L}^{-1}$), ripening was delayed by 9, 13 and 15 days respectively (Fig. 4). Chlorophyll measurement on the 10th day after treatment revealed that chlorophyll content in the peel of fruit treated with 1-PentCP at concentrations high than $5 \text{ nl}\cdot\text{L}^{-1}$ was maintained at a high level (Fig. 4). Of all the four concentrations tested, treatment with $500 \text{ nl}\cdot\text{L}^{-1}$ 1-PentCP was found the most effective concentration to inhibit ethylene-induced ripening in banana fruit. Increasing the concentration of 1-PentCP from $50 \text{ nl}\cdot\text{L}^{-1}$ to $500 \text{ nl}\cdot\text{L}^{-1}$ delayed ripening by just additional 2 days (Fig. 3), thus, increasing the concentration over $500 \text{ nl}\cdot\text{L}^{-1}$ would not increase markedly the inhibitory effect.

4.6 Effect of 1-HCP on ripening of banana fruit

The effect of 1-HCP on ethylene-induced ripening of banana fruit was investigated. The results show that untreated fruit ripened at 22°C 11 days after

Fig. 5. Effect of 1-HCP on color change (A), fruit softening (B) and ethylene production (C) in banana fruits. The fruits were treated for 24 h with 0 (▲), 1 (△), 5 (□), 50 (○) or 500 (●) nl L^{-1} 1-HCP, ventilated, and then exposed to ethylene ($300 \mu\text{l L}^{-1}$) for 24 h. Untreated fruits (■) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

harvest, fruit treated with $300 \mu\text{L}^{-1}$ ethylene for 24 h ripened at 22°C 5 days after harvest (Fig. 5). Treatment with 1, 5, 50 or 500 nL^{-1} 1-HCP for 24 h immediately after harvest prior to ethylene exposure (24 h to $300 \mu\text{L}^{-1}$) delayed the ethylene-induced banana fruit ripening by 2, 7, 11 and 13 days respectively (Fig. 5). Chlorophyll content in the fruit peel on the 10th day after treatment with various

Fig. 6. Effect of 1-HCP on peel chlorophyll content of banana fruits 10 days after treatment. The fruits were treated for 24 h with 0 (A), 1 (C), 5 (D), 50 (E) or 500 nL^{-1} 1-HCP, ventilated, and then exposed to ethylene ($300 \mu\text{L}^{-1}$) for 24 h. Untreated fruits (B) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

concentrations of 1-HCP was much higher than that in ethylene treated fruit. In fruit treated with concentrations of 1-HCP higher than 5 nL^{-1} , chlorophyll content was 3.4-4.6 fold that in the peel of untreated fruit (Fig. 6). Of all the concentrations tested, treatment with 500 nL^{-1} 1-HCP was the most effective in delaying ethylene-induced ripening in banana fruit. As there was no marked difference in delaying ethylene-induced fruit ripening between treatments with 50 nL^{-1} and 500 nL^{-1}

1-HCP (Fig. 5), further increasing the concentration of 1-HCP over $500 \text{ nl}\cdot\text{L}^{-1}$ may not result in further inhibitory effect.

4.7 Effect of 1-HeptCP on ripening of banana fruit

In the present study the effect of 1-HeptCP on ethylene-induced ripening of banana fruit was investigated. The results show that the untreated fruit ripened 11 days after harvest while fruit treated with ethylene ($300 \mu\text{l}\cdot\text{L}^{-1}$) for 24 h ripened 6 days after

Fig. 7. Effect of 1-HeptCP on chlorophyll content in banana fruits 10 days after treatment. The fruits were treated for 24 h with 0 (A), 50 (C), 150 (D), 250 (E) or 2000 (F) nl L^{-1} 1-HeptCP, ventilated, and then exposed to ethylene ($300 \mu\text{l L}^{-1}$) for 24 h. Untreated fruits (B), were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

harvest at 22°C . Treatment for 24 h with 50, 150, 250 or $2000 \text{ nl}\cdot\text{L}^{-1}$ 1-HeptCP immediately after harvest prior to exposure to ethylene ($300 \mu\text{l}\cdot\text{L}^{-1}$, 24 h) delayed the ethylene-induced ripening of banana fruit by 9, 11, 12 and 15 days respectively.

Analysis of chlorophyll content in the fruit peel on the 10th day after treatment showed that treatment with various concentrations of 1-HeptCP delayed chlorophyll

decomposition in the peel of banana fruit (Fig. 7). Chlorophyll contents in the peel of fruits treated with the various concentrations of 1-HeptCP tested were 3-7 fold higher than that of ethylene-treated fruit. It should be noted that as the fruits treated with the various concentrations of 1-HeptCP ripened 4-10 days later than the untreated fruit, concomitantly their chlorophyll contents were 77%-255% higher than that of the untreated fruit (Fig. 7).

Among the four concentrations of 1-HeptCP tested, treatment with 2000 $\text{nl}\cdot\text{L}^{-1}$ 1-HeptCP is the most effective to delay ethylene-induced ripening of banana fruit. Increasing the concentration of 1-HeptCP may further intensify its effect.

4.8 Effect of 1-OCP on ripening of banana fruit

It appears that 1-OCP is a very potent inhibitor of ethylene action. Treatment with 5 $\text{nl}\cdot\text{L}^{-1}$ 1-OCP delayed ethylene-induced banana fruit ripening by 16 days. Increasing the concentration to 10, 30 or 100 $\text{nl}\cdot\text{L}^{-1}$ did not further delay ripening. Chlorophyll measurements on the 12th day after treatment revealed that the chlorophyll content in fruit peel, treated with all of the 1-OCP concentrations tested, was retained at a high level, and there was no significant difference between the treatments. As chlorophyll breakdown is inhibited to a greater extent than other ripening processes in banana, different ethylene receptors may be presented in the flesh and in the chloroplasts of the peel. This observation is a subject for further exploration.

4.9 Effect of 1-DCP on ripening of banana fruit

The inhibitory effect of 1-DCP on ethylene-induced ripening in banana fruit was found to be concentration-dependent. The results show that banana fruit treated

Fig. 8. Effect of 1-DCP on chlorophyll content in banana fruits 10 days after treatment. The fruits were treated for 24 h with 0 (A), 50 (C), 150 (D), 250 (E) or 2000 (F) $\text{nl}\cdot\text{L}^{-1}$ 1-DCP, ventilated, and then exposed to ethylene ($300\ \mu\text{l}\cdot\text{L}^{-1}$) for 24 h. Untreated fruits (B), were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

with ethylene ($300\ \mu\text{l}\cdot\text{L}^{-1}$) for 24 h and stored at 22°C ripened 4 days after treatment and the untreated fruit ripened 11 days after harvest. Treatment with 50, 150 or 250 $\text{nl}\cdot\text{L}^{-1}$ 1-DCP delayed the ethylene-induced ripening of banana fruit by 6, 7 and 9 days respectively. The appearances of climacteric, fruit color change as well as fruit softening were concomitantly delayed. The inhibitory effect of 1-DCP on ethylene-induced ripening of banana fruit was markedly intensified by increasing the concentration of 1-DCP to $2000\ \text{nl}\cdot\text{L}^{-1}$. As compared with the fruit treated with 250 $\text{nl}\cdot\text{L}^{-1}$ 1-DCP, treatment with $2000\ \text{nl}\cdot\text{L}^{-1}$ 1-DCP further delayed the ethylene-induced fruit ripening by 3 days. Further increasing the concentration of 1-DCP may result in a further delay of ripening.

Analysis of chlorophyll content on the 10th day after treatment revealed that concomitant with the fruit ripening, chlorophyll content in the peel of fruit treated with

Fig. 9. Chlorophyll content in banana fruit peel treated with eight new putative inhibitors of ethylene action. The fruits were treated for 24 h with the indicated inhibitor, ventilated, and then exposed to ethylene (300 µl.L⁻¹) for 24 h. Untreated fruits (A) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

the various concentration of 1-DCP tested was 1.5-4.5 fold higher than that of ethylene treated fruit, and 6%-146% higher than that of the untreated fruit (Fig. 8).

4.10 Comparison of the effects of the inhibitors on ripening of banana fruit

In all the studies presented above, we have tested the effects of nine newly synthesized putative inhibitors of ethylene action on ethylene-induced ripening of banana fruit. As the chemical structure of H1-MCP is different from the other eight compounds, it is possible that the potency might be different as well. The potency of the effective concentrations of all the other new inhibitors of ethylene action on banana fruit ripening is presented in Fig. 9 and Table 5.

Table 5 shows that the concentrations of all the new inhibitors required to delay ethylene-induced ripening of banana fruit are much higher than that of 1-MCP. The inhibitor 1-OCP was the most potent one of all the new inhibitors since only 5 nl.L⁻¹ of this compound delayed ripening by 16 days (Fig. 9 and Table

Table 5. Days after harvest to reach the ethylene climacteric peak, fruit softening firmness (index 2) and complete skin color change in banana fruit. Fruit were treated for 24 h with the indicated inhibitor and then exposed for 24 h to ethylene (300 $\mu\text{l}\cdot\text{L}^{-1}$). Thereafter the fruits were stored at 22°C for ripening assessment

Inhibitor	Concentration	Ethylene	Softening	Color	Delay of ripening (days)
Untreated	--	10.3 d	11.5 d	12.0 d	--
C ₂ H ₄	300 $\mu\text{l}\cdot\text{L}^{-1}$	4.3 e	5.0 e	7.3 e	--
1-ECP	500 $\text{nl}\cdot\text{L}^{-1}$	20.0 a	21.5 a	23.0 a	16 a
1-PCP	500 $\text{nl}\cdot\text{L}^{-1}$	18.0 b	16.0 c	18.3 b	13 b
1-BCP	50 $\text{nl}\cdot\text{L}^{-1}$	17.0 c	17.3 b	16.5 c	12 b
1-PentCP	500 $\text{nl}\cdot\text{L}^{-1}$	19.8 ab	19.2 a	18.8 b	15 a
1-HCP	500 $\text{nl}\cdot\text{L}^{-1}$	18.0 b	17.8 b	17.2 cb	13 b
1-HeptCP	2000 $\text{nl}\cdot\text{L}^{-1}$	21.5 a	20.8 a	20.6 b	15 a
1-OCP	5 $\text{nl}\cdot\text{L}^{-1}$	20.0 a	21.3 a	19.5 b	16 a
1-DCP	2000 $\text{nl}\cdot\text{L}^{-1}$	18.6 ab	18.5 ab	18.2 b	12 b

Different letters indicate statistical differences at $P = 0.05$.

5). This was determined by ethylene climacteric peak, fruit softening and color change as well as chlorophyll content in the fruit peel. This concentration is very low compared with the effective concentrations of all the other seven inhibitors (Table 5). The less potent compound was 1-ECP, which delayed ethylene-induced ripening of banana fruit by 16 days at the concentration of 500 $\text{nl}\cdot\text{L}^{-1}$ (Table 5). The order of decrease in potency of the tested inhibitors is: 1-OCP > 1-ECP > 1-PentCP > 1-HeptCP > 1-PCP > 1-HCP > 1-BCP > 1-DCP.

5. Effect of new inhibitors of ethylene action on ripening of avocado fruit

Avocado is a climacteric fruit and its ripening can be stimulated by using ethylene (Hardenburg *et al.*, 1986). Thus, avocado was chosen as one of the fruit in our test system set up for assessing the potency of all the newly synthesized putative inhibitors of ethylene action. The ripening indicators used to assess the potency of the inhibitors to delay ethylene-induced ripening in avocado fruit included: ethylene production, softening, activity of the hydrolytic enzymes cellulase and polygalacturonase (PG) and color change in 'Hass' cultivar. Color change in 'Hass' fruit was determined on an eleven grade-scale with -0- indicating complete green and -10- complete color change (egg-plant). PG and cellulase activity were analyzed on the 8th day after harvest following the method described in (Huberman and Goren, 1979). In these studies, we have used the avocado cultivars 'Fuerte', 'Hass', 'Ettinger' and 'Reed', which were harvested in a local plantation. We observed that depending on the date of harvest during the season, the untreated fruit of the 'Fuerte', 'Hass', 'Ettinger' and 'Reed' fruit stored at room temperature ripened within 5-8 days after harvest. Treatment with ethylene ($300 \mu\text{l}\cdot\text{L}^{-1}$) significantly shortened the period to ripening by 3-4 days.

5.1 Effect of H1-MCP on avocado fruit ripening

Results of the studies with the putative inhibitor of ethylene action H1-MCP conducted on 'Reed' avocado fruit show that treatment with 50, 100, 250 or $500 \text{ nl}\cdot\text{L}^{-1}$,

prior to 24 h exposure to ethylene ($300 \mu\text{L}^{-1}$), had no effect on the rate of ethylene production, the onset of the climacteric peak and on fruit softening.

5.2 Effect of 1-ECP on ripening of avocado fruit

When 'Hass' fruit were treated with 100, 500, 1000 or 2000 nL^{-1} 1-ECP, prior to the ethylene treatment, the onset of ethylene climacteric peak was delayed by 7, 8, 10 and 10 days respectively compared with fruit treated with $300 \mu\text{L}^{-1}$ ethylene. Treatments with 1000 nL^{-1} 1-ECP also reduced the height of the ethylene climacteric peak by about 40% in both 'Hass' and 'Fuerte' fruit. The time needed to complete color change was delayed by 6, 8, 14 and 12 days respectively; and fruit softening (to 15-20 N) was delayed by 6, 7, 12 and 11 days respectively. Similar results were obtained with 'Fuerte' fruit treated with the same concentrations of 1-ECP.

Analysis of PG and cellulase activity on the 8th day after treatment (Fig. 10) showed that ethylene treatment increased the activity of both these enzymes in 'Hass' and 'Fuerte' avocado fruit as compared with untreated fruit. Treatment with 1-ECP prior to ethylene exposure reduced the activity of PG by 26%-64% and of cellulase by 30%-61%. Maximal inhibition of both PG and cellulase activity in 'Hass' and 'Fuerte' avocado fruit was achieved with 1-ECP at the concentration of 1000 nL^{-1} . Increasing the concentration to 2000 nL^{-1} did not intensify its inhibitory effect, but rather slightly decreased it.

5.3 Effect of 1-PCP on ripening of avocado fruit

Treatment with 150 or 300 nL^{-1} 1-PCP did not affect ethylene-induced ripening in both 'Hass' and 'Fuerte' avocado fruit; 'Hass' fruit ripened 2-4 days after

Fig. 10. Activity of PG and cellulase, in 'Fuerte' and 'Hass' avocado fruits, treated with 1-ECP or 1-PCP. The fruits were treated for 24 h with the indicated inhibitor and then exposed for 24 h to ethylene (300 $\mu\text{l}\cdot\text{L}^{-1}$) and thereafter stored in air at 25°C for ripening assessment. A: Untreated, B: C_2H_4 (300 $\mu\text{l}\cdot\text{L}^{-1}$)

treatment and 'Fuerte' 4-5 days. In 'Fuerte' fruit, increasing the concentration of 1-PCP to 1000 and 1500 $\text{nl}\cdot\text{L}^{-1}$ delayed the climacteric peak of ethylene production by 7 and 10 days respectively and the fruit softening (15-20 *N*) by 9 and 10 days respectively. Unlike the effect on 'Fuerte', the effect of treatment with 1500 $\text{nl}\cdot\text{L}^{-1}$ 1-PCP 'Hass' was more pronounced than that of treatment with 1000 $\text{nl}\cdot\text{L}^{-1}$ 1-PCP. The onset of the ethylene climacteric peak was delayed by 11 days in fruit treated with 1500 $\text{nl}\cdot\text{L}^{-1}$ 1-PCP and only by 8 days in those treated with 1000 $\text{nl}\cdot\text{L}^{-1}$ 1-PCP.

The analysis of PG and cellulase activity on the 8th day after harvest (Fig. 10) showed that treatment with 1-PCP, compared with ethylene treated fruit, reduced the activity of PG by 14%-63% and of cellulase by 9%-59%. Treatment with 1500 $\text{nl}\cdot\text{L}^{-1}$ 1-PCP had the maximal inhibitory effect on both PG and cellulase activity in 'Hass' and 'Fuerte' avocado fruit.

Table 6. Days after harvest to reach the climacteric peak of ethylene production, fruit softening (firmness 15-20 *N*) and complete skin color change in 'Hass' and 'Fuerte' avocado fruit. The fruit were treated for 24 h with the indicated inhibitor and then exposed for 24 h to ethylene (300 $\mu\text{l}\cdot\text{L}^{-1}$) and thereafter stored in air at 22°C for ripening assessment.

Cultivars	Treatment	Ethylene	Softening	Color	Delayed ripening by (days)
'Fuerte'	C_2H_4 (300 $\mu\text{l}\cdot\text{L}^{-1}$)	4 c	5 b	3 c	--
	1000 $\text{nl}\cdot\text{L}^{-1}$ 1-ECP	13 b	14 a	12 b	10 a
	1500 $\text{nl}\cdot\text{L}^{-1}$ 1-PCP	14 a	15 a	14 a	9 b
'Hass'	C_2H_4 (300 $\mu\text{l}\cdot\text{L}^{-1}$)	4 c	4 b	3 c	--
	1000 $\text{nl}\cdot\text{L}^{-1}$ 1-ECP	13 b	15 a	14 a	10 a
	1500 $\text{nl}\cdot\text{L}^{-1}$ 1-PCP	15 a	14 a	12 b	10 a

*Different letters indicate statistical differences at $P = 0.05$. **Fruit not treated with ethylene nor with inhibitors ripened 5 to 7 days after harvest, depending on the harvest date during the season.

5.4 Effect of 1-BCP on ripening of avocado fruit

In the present study, the effect of 1-BCP on ethylene-induced ripening of 'Reed' avocado fruit was investigated. 'Reed' fruit were treated for 24 h with 50, 250, 500 or 2000 $\text{nl}\cdot\text{L}^{-1}$ 1-BCP prior to exposure to ethylene ($300\ \mu\text{l}\cdot\text{L}^{-1}$) for 24 h and then put at

Fig. 11. Effect of 1-BCP on fruit softening (left) and ethylene production (right) of 'Reed' avocado fruits. The fruits were treated for 24 h with 0 (▲), 50 (△), 250 (□), 500 (○) or 2000 (●) $\text{nl}\cdot\text{L}^{-1}$ 1-BCP, ventilated, and then exposed to ethylene ($300\ \mu\text{l}\cdot\text{L}^{-1}$) for 24 h. Untreated fruits (■) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

Fig. 12. Effect of 1-BCP on activity of PG (left) and cellulase (right) in 'Reed' avocado fruits 10 days after treatment. The fruits were treated for 24 h with 0 (B), 50 (C), 250 (D), 500 (E) or 2000 (F) $\text{nl}\cdot\text{L}^{-1}$ 1-BCP, ventilated, and then exposed to ethylene ($300\ \mu\text{l}\cdot\text{L}^{-1}$) for 24 h. Untreated fruits (A) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

22°C in air for ripening assessment.

The results show that treatment with 50 nl·L⁻¹ 1-BCP had only a little inhibitory effect on ethylene-induced ripening of 'Reed' avocado fruit. Raising the concentration of 1-BCP to 250 nl·L⁻¹ or 500 nl·L⁻¹ greatly increased the inhibitory effect; ethylene-induced ripening was delayed by 8 and 12 days respectively, and the onset of climacteric ethylene peak and fruit softening were also delayed. Further increase in concentration to 2000 nl·L⁻¹ did not increase the inhibitory effect, but rather slightly decreased it (Fig. 11).

Measurements of PG and cellulase activity on the 8th day after harvest showed that compared with ethylene treated fruit,

treatment with 1-BCP reduced the activity of PG by 17.9%-78.9% and of cellulase by 14.2%-77.3%. There were no significant differences between treatments with 500 or 2000 nl·L⁻¹ 1-BCP on the activity of both PG and cellulase (Fig. 11). Treatment with

Fig. 13. Effect of 1-PentCP on softening (A), color change (B) and ethylene production (C) in 'Hass' avocado fruits. The fruits were treated for 24 h with 0 (▲), 50 (Δ), 100 (□), 500 (○) or 1000 (●) nl·L⁻¹ 1-PentCP, ventilated, and then exposed to ethylene (300 μl·L⁻¹) for 24 h. Untreated fruits (■) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

500 $\text{nl}\cdot\text{L}^{-1}$ 1-BCP had the maximal inhibitory effect on ethylene-induced ripening of 'Reed' avocado fruit (Fig. 11).

5.5 Effect of 1-PentCP on ripening of avocado fruit

Our results show that the inhibitory effect of 1-PentCP on ethylene-induced ripening in 'Hass' avocado fruit was concentration-dependent. Untreated 'Hass' fruit ripened 7 days after harvest. Fruit treated with ethylene ($300\ \mu\text{l}\cdot\text{L}^{-1}$) for 24 h ripened 3 days after treatment at 22°C . However, treatment with 50, 100 or $500\ \text{nl}\cdot\text{L}^{-1}$ 1-PentCP

Fig. 14. Effect of 1-PentCP on activity of PG (left) and cellulase (right) in "Hass" avocado fruits 10 days after treatment. The fruits were treated for 24 h with 0 (B), 50 (C), 100 (D), 500 (E) or $1000\ \text{nl}\cdot\text{L}^{-1}$ 1-PentCP, ventilated, and then exposed to ethylene ($300\ \mu\text{l}\cdot\text{L}^{-1}$) for 24 h. Untreated fruits (A) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

delayed the ethylene-induced ripening by 2, 4 and 11 days respectively (Fig. 13). Increasing the concentration to $1000\ \text{nl}\cdot\text{L}^{-1}$ did not result in further inhibition of the ethylene-induced fruit ripening. Activity of PG and cellulase measurement on the 8th day after harvest revealed that treatment with 500 or $1000\ \text{nl}\cdot\text{L}^{-1}$ 1-PentCP greatly decreased the activity of these two enzymes. There were no significant differences in activity of PG and cellulase between fruit treated with 500 or $1000\ \text{nl}\cdot\text{L}^{-1}$ 1-PentCP

(Fig. 14). Of all the four concentrations tested, treatment with $500 \text{ nl}\cdot\text{L}^{-1}$ 1-PentCP was sufficient to cause maximal inhibitory effect on ethylene-induced ripening in 'Hass' avocado fruit.

5.6 Effect of 1-HCP on ripening of avocado fruit

The effect of 1-HCP on ethylene-induced ripening of 'Reed' avocado fruit was investigated in this study. The results show that the untreated 'Reed' fruits ripened at

Fig. 15. Effect of 1-HCP on fruit softening PG (left) and ethylene production (right) in 'Reed' avocado fruits. The fruits were treated for 24 h with 0 (▲), 50 (△), 100 (□), 250 (○) or 1000 (●) $\text{nl}\cdot\text{L}^{-1}$ 1-HCP, ventilated, and then exposed to ethylene ($300 \mu\text{l}\cdot\text{L}^{-1}$) for 24 h. Untreated fruits (■) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

22°C 9 days after harvest while fruit treated with ethylene ($300 \mu\text{l}\cdot\text{L}^{-1}$) for 24 h ripened 5 days after harvest at room temperature (Fig. 15). Treatment with 50 or $100 \text{ nl}\cdot\text{L}^{-1}$ 1-HCP for 24 h immediately after harvest prior to ethylene exposure (24 h to $300 \mu\text{l}\cdot\text{L}^{-1}$) delayed the ethylene-induced ripening of 'Reed' avocado fruit by only 1 and 2 days respectively (Fig. 15). Increasing the concentrations of 1-HCP to 250 or 1000

nl·L⁻¹ intensified the inhibitory effect; Ethylene-induced ripening of 'Reed' fruit was delayed by 6 and 12 days respectively (Fig. 15).

Table 7. Effect of 1-HeptCP on ethylene-induced ripening in 'Hass' and 'Reed' avocado fruit on the 4th day after treatment. The fruit were treated for 24 h with the indicated compound and then exposed for 24 h to ethylene (300 µl·L⁻¹) and thereafter stored in air at 22°C for ripening assessment.

Cultivars	Treatment	Firmness (N)	Color index
'Hass'	untreated	56.8 d	2 a
	C ₂ H ₄ (300 µl·L ⁻¹)	12.6 ab	10 b
	50 nl·L ⁻¹ 1-HeptCP	13.6 ab	10 b
	150 nl·L ⁻¹ 1-HeptCP	12.9 ab	10 b
	250 nl·L ⁻¹ 1-HeptCP	14.8 b	10 b
	1000 nl·L ⁻¹ 1-HeptCP	13.5 ab	10 b
	0.1% 1-HeptCP dip	12.7 ab	10 b
'Reed'	untreated	48.6 c	--
	C ₂ H ₄ (300 µl·L ⁻¹)	8.5 a	--
	50 nl·L ⁻¹ 1-HeptCP	11.6 a	--
	150 nl·L ⁻¹ 1-HeptCP	9.7 a	--
	250 nl·L ⁻¹ 1-HeptCP	10.6 a	--
	1000 nl·L ⁻¹ 1-HeptCP	9.5 ab	--
	0.1% 1-HeptCP dip	11.9 a	--

Different letters indicate statistical differences at $P = 0.05$.

Analysis of the activity of PG and cellulase in 'Reed' fruit mesocarp on the 8th day after treatment showed that treatment with various concentrations of 1-HCP decreased the activity of both PG and cellulase. Maximal inhibition was observed with 1000 nl·L⁻¹ 1-HCP where PG activity was decreased by 79.3% and 69.6% compared with ethylene treated fruit or with the untreated control fruit respectively. Cellulase activity was reduced by 73.6% and 59.7% respectively (Fig. 16).

Fig. 16. Effect of 1-HCP on activity of PG (left) and cellulase (right) in 'Reed' avocado fruits 8 days after treatment. The fruits were treated for 24 h with 0 (B), 50 (C), 100 (D), 250 (E) or 1000 (F) nl L^{-1} 1-HCP, ventilated, and then exposed to ethylene ($300 \mu\text{l L}^{-1}$) for 24 h. Untreated fruits (A) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

Of all the four concentrations tested, treatment with $1000 \text{ nl}\cdot\text{L}^{-1}$ 1-HCP was found to be the most effective to delay ethylene-induced ripening of 'Reed' avocado fruit. In light of the results obtained from the experiment, it was suggested that ethylene-induced ripening of 'Reed' avocado fruit can possibly be further delayed by further increasing the treatment concentration of 1-HCP over $1000 \text{ nl}\cdot\text{L}^{-1}$.

5.7 Effect of 1-HeptCP on ripening of avocado fruit

Treatment of 'Hass' and 'Reed' avocado fruit for 24 h with 50, 150, 250 or $1000 \text{ nl}\cdot\text{L}^{-1}$ 1-HeptCP, prior to ethylene exposure ($300 \mu\text{l}\cdot\text{L}^{-1}$), did not significantly delay the ethylene-induced fruit ripening. Firmness of untreated 'Hass' and 'Reed' fruits 4 days after treatment was 56.8 N and 48.6 N respectively. Treatment with the various concentration of 1-HeptCP did not delay fruit softening of both 'Hass' and 'Reed' fruit (Table 7). Ethylene-induced color change of 'Hass' fruit was also not affected by treatment with any of the four 1-HeptCP concentrations tested (Table 7).

To explore the possibility of using the newly synthesized putative inhibitor in liquid form, the effect of aqueous solution of 1-HeptCP on ethylene-induced ripening of 'Hass' and 'Reed' avocado fruit was investigated. 'Hass' and 'Reed' fruits were dipped in 0.1% aqueous solution of 1-HeptCP for 2 min and placed in air, 24 h later they were treated with ethylene ($300 \mu\text{L}\cdot\text{L}^{-1}$) for 24 h and then stored in air at 22°C for ripening assessment. Results of this experiment show that the treatment had no significant effect on the ripening of 'Hass' or 'Reed' fruit, compared with the ethylene treated fruits (Table 7).

5.8 Effect of 1-OCP on ripening of avocado fruit

The results show that 1-OCP had no effect on ethylene-induced ripening of 'Ettinger' fruit when treated with 50, 100 or $250 \text{ nl}\cdot\text{L}^{-1}$ 1-OCP, prior to the ethylene

Table 8. Days after harvest to reach the climacteric peak of ethylene production and fruit softening (firmness 15-20 *N*) in 'Ettinger' avocado fruit. The fruit were treated for 24 h with the indicated concentrations of 1-OCP and then exposed for 24 h to ethylene ($300 \mu\text{L}\cdot\text{L}^{-1}$) and thereafter stored in air at 22°C for ripening assessment.

Treatment	Ethylene	Softening
C_2H_4 ($300 \mu\text{L}\cdot\text{L}^{-1}$)	5.2 a	5.4 a
Untreated	9.3 b	9.5 b
$50 \text{ nl}\cdot\text{L}^{-1}$ 1-OCP	5.3 a	5.1 a
$100 \text{ nl}\cdot\text{L}^{-1}$ 1-OCP	6.1 a	6.4 a
$250 \text{ nl}\cdot\text{L}^{-1}$ 1-OCP	6.8 ab	7.2 ab
$1000 \text{ nl}\cdot\text{L}^{-1}$ 1-OCP	8.2 b	8.4 b

Different letters indicate statistical differences at $P = 0.05$.

treatment. No significant delay in the onset of ethylene climacteric peak and fruit softening were observed compared with fruit treated with ethylene ($300 \mu\text{l}\cdot\text{L}^{-1}$) (Table 8). Increasing the concentration to $1000 \text{ nl}\cdot\text{L}^{-1}$ showed some inhibitory effect on ethylene-induced fruit ripening: the onset of ethylene climacteric peak and fruit softening were delayed concomitantly by 3 days (Table 8). Analysis of PG and cellulase activity on the 8th day after treatment (Fig. 17) showed that ethylene treatment increased the activity of both these enzymes in 'Ettinger' avocado fruit as compared with the untreated fruit. However, treatment with $1000 \text{ nl}\cdot\text{L}^{-1}$ 1-OCP prior

Fig. 17. Effect of 1-OCP on activity of PG (left) and cellulase (right) in "Ettinger" avocado fruits 8 days after treatment. The fruits were treated for 24 h with 0 (B), 50 (C), 100 (D), 250 (E) or 1000 (F) $\text{nl}\cdot\text{L}^{-1}$ 1-OCP, ventilated, and then exposed to ethylene ($300 \mu\text{l}\cdot\text{L}^{-1}$) for 24 h. Untreated fruits (A) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

to exposure to ethylene significantly reduced the activity of PG and cellulase (Figure 5-8). The effective concentration of 1-OCP found in this study ($1000 \text{ nl}\cdot\text{L}^{-1}$) was very high as compared with that of 1-MCP ($50 \text{ nl}\cdot\text{L}^{-1}$). Even at this high concentration the ethylene-induced ripening was delayed by only 3 days.

5.9 Effect of 1-DCP on ripening of avocado fruit

In the present study, the effect of 1-DCP on ethylene-induced ripening of 'Hass' and 'Reed' avocado fruit, was investigated. The fruit were treated for 24 h with 50, 150, 250 or 1000 nl·L⁻¹ 1-DCP prior to 24 h exposure to ethylene (300 µl·L⁻¹) and then stored at 22°C in air for ripening assessment. The results show that treatment with 1-DCP did not significantly delay the ethylene-induced ripening of 'Hass' and

Table 9. Effect of 1-DCP on ethylene-induced ripening in 'Hass' and 'Reed' avocado fruit on the 4th day after treatment. The fruit were treated for 24 h with the indicated compound and then exposed for 24 h to ethylene (300 µl·L⁻¹) and thereafter stored in air at 22°C for ripening assessment.

Cultivars	Treatment	Firmness (N)	Color index
'Hass'	untreated	56.8 d	2 a
	C ₂ H ₄ (300 µl·L ⁻¹)	12.6 ab	10 b
	50 nl·L ⁻¹ 1-DCP	12.9 ab	10 b
	150 nl·L ⁻¹ 1-DCP	15.8 b	10 b
	250 nl·L ⁻¹ 1-DCP	12.7 ab	10 b
	1000 nl·L ⁻¹ 1-DCP	11.5 ab	10 b
	0.1% 1-DCP dip	14.7 b	10 b
'Reed'	untreated	48.6 c	--
	C ₂ H ₄ (300 µl·L ⁻¹)	8.5 a	--
	50 nl·L ⁻¹ 1-DCP	9.6 a	--
	150 nl·L ⁻¹ 1-DCP	10.2 a	--
	250 nl·L ⁻¹ 1-DCP	8.8 a	--
	1000 nl·L ⁻¹ 1-DCP	12.5 ab	--
	0.1% 1-DCP dip	10.9 a	--

Different letters indicate statistical differences at $P = 0.05$.

'Reed' fruits. On the 4th day after treatment, no significant difference in fruit firmness were found between ethylene treated control and fruits treated with four different concentrations of 1-DCP prior to exposure to ethylene (Table 9). 1-DCP was also found to have no effect on ethylene-induced LA-AZ abscission in citrus leaf explants (refer to 7.9 of this section), but was found to have effect on ethylene-induced growth modification in pea seedlings (refer to 8.8 of this section). This raises the question why 1-DCP inhibits ethylene action in some plant materials and in some not.

Results of study on the effect of 1-DCP on ethylene-induced ripening of 'Hass' and 'Reed' avocado fruit following postharvest dip in the inhibitor show that dipping the fruits in 0.1% aqueous solution of 1-HeptCP for 2 min did not delay significantly ethylene-induced ripening in both 'Hass' and 'Reed' fruit (Table 9).

5.10 Comparison of the effects of the various putative inhibitors

on ripening of avocado fruit

We have tested the effects of nine newly synthesized putative inhibitors of ethylene action on ethylene-induced ripening of avocado fruit. One or two of the cultivars 'Hass', 'Reed', 'Fuerte' or 'Ettinger' avocado fruit were used for testing each putative inhibitor. The effects of all the putative inhibitors tested are compared in Table 10. The results show that if rated by the concentration of the inhibitor used and the period of protection from ethylene action resulted from the treatment, the most potent compound 1-BCP. Treatment with 500 nl·L⁻¹ 1-BCP delayed ethylene-induced ripening in 'Reed' fruit by 12 days (Table 10). This was determined by assessment of ethylene climacteric peak, fruit softening and color change as well as PG and cellulase activity. Less potent was 1-PentCP which delayed ethylene-induced ripening in 'Hass'

Table 10. Comparison between the effects of tested putative ethylene inhibitors on ethylene-induced ripening of avocado fruit: Days after harvest to reach the climacteric peak of ethylene production, fruit softening (firmness 15-20 *N*) and complete skin color change in 'Hass' fruit. The fruit were treated for 24 h with the indicated inhibitor and then exposed for 24 h to ethylene (300 $\mu\text{L}\cdot\text{L}^{-1}$) and thereafter stored in air at 22°C for ripening assessment.

Inhibitors	Cultivars	Ethylene	Softening	Color	Delayed ripening by (days)
C ₂ H ₄ , 300 $\mu\text{L}\cdot\text{L}^{-1}$	'Hass'	5 a	5 a	6 a	--
H1-MCP, 500 $\text{nl}\cdot\text{L}^{-1}$	'Reed'	5 a	5 a	5 a	--
1-ECP, 1000 $\text{nl}\cdot\text{L}^{-1}$	'Hass'	13 c	15 c	14 c	10 b
1-PCP, 1500 $\text{nl}\cdot\text{L}^{-1}$	'Hass'	15 d	14 c	12 b	10 b
1-BCP, 500 $\text{nl}\cdot\text{L}^{-1}$	'Reed'	17 e	18 d	--	12 c
1-PentCP, 500 $\text{nl}\cdot\text{L}^{-1}$	'Hass'	16 de	17 d	19 d	11 bc
1-HCP, 1000 $\text{nl}\cdot\text{L}^{-1}$	'Reed'	17 e	17 d	--	12 c
1-HeptCP, 1000 $\text{nl}\cdot\text{L}^{-1}$	'Hass'	5 a	5 a	5 a	--
1-OCp, 1000 $\text{nl}\cdot\text{L}^{-1}$	'Ettinger'	8 b	8 b	--	3 a
1-DCP, 1000 $\text{nl}\cdot\text{L}^{-1}$	'Hass'	5 a	5 a	5 a	--

*Different letters indicate statistical differences at $P = 0.05$. **Fruit not treated with ethylene nor with inhibitors ripened 5 to 7 days after harvest, depending on the harvest date during the season.

fruit by 11 days at the concentration of 500 $\text{nl}\cdot\text{L}^{-1}$ (Table 10). The order of decrease in potency of the tested inhibitors is: 1-BCP>1-PentCP>1-HCP>1-ECP>1-PCP>1-OCp, whereas H1-MCP, 1-HeptCP and 1-DCP had no effect.

6. Effect of new inhibitors of ethylene action on ripening

of tomato fruit

Tomato is a climacteric fruit, in which ripening processes are strongly influenced by endogenously produced or exogenously applied ethylene. In our system,

set up for testing all the newly synthesized putative inhibitors of ethylene action, tomato fruits were chosen as one of the test models. In the following investigations, the evaluation of ripening of tomato fruit was based on the parameters: peel color change, determination of lycopene, ethylene production and fruit softening. Visual color change was determined using a eleven grade-scale with -0- indicating complete green and -10- complete red. Fruit softening during ripening was measured using a five grade-scale with -5- indicating very firm, -4- firm, -3- relatively firm, -2- soft and -1- very soft. Lycopene content in fruit pulp of all treatments was analyzed on the 8th day after treatment. The fruits were considered to be ripe when color index was 7, softening index reached 2-2.5 and the climacteric ethylene peak appeared. As determined by these ripening criteria, untreated mature green tomato fruits ripened at 22°C, 7-9 days after harvest and fruits treated with ethylene (300 $\mu\text{L}\cdot\text{L}^{-1}$) and kept at 22°C ripened 2-5 days after the treatment.

6.1 Effect of H1-MCP on ripening of tomato fruit

Mature green tomato fruit treated with ethylene turned red 2 days after treatment. Fruit treated with 50, 100, 250 or 500 $\text{nl}\cdot\text{L}^{-1}$ H1-MCP for 24 h immediately after harvest prior to the ethylene treatment (24 h, 300 $\mu\text{L}\cdot\text{L}^{-1}$) retained their green color for just 2 or 3 days at 22°C. The ethylene climacteric peak in the H1-MCP treated fruits appeared on the 3rd day after treatment, same as in ethylene treatment. Thus, treatment with H1-MCP at the indicated concentration (up to 500 $\text{nl}\cdot\text{L}^{-1}$) had very little effect on ethylene-induced ripening of tomato fruits.

6.2 Effect of 1-ECP on ripening of tomato fruit

Treatment with 100, 500, 1000 or 2000 $\text{nl}\cdot\text{L}^{-1}$ 1-ECP 4-6 h after harvest prior to ethylene ($300\ \mu\text{l}\cdot\text{L}^{-1}$) treatment delayed ripening of mature green tomato fruit by 11, 13, 12 and 12 days respectively. No significant difference was found between

Fig. 18. Effect of 1-ECP and 1-PCP on lycopene content in mature green tomato fruits 8 days after treatment. The fruits were treated for 24 h with the indicated inhibitor, ventilated, and then exposed to ethylene ($300\ \mu\text{l}\cdot\text{L}^{-1}$) for 24 h. Untreated fruits (A) were kept in air containing jars during treatment. For ethylene treatment (B), fruits were closed in the jars for 24 h with air instead of with the inhibitor before exposure to ethylene for additional 24 h.

treatments with 500, 1000 or 2000 $\text{nl}\cdot\text{L}^{-1}$ 1-ECP regarding color change, fruit softening and ethylene climacteric peak. Lycopene analysis in the fruit pulp on the 8th day after treatment (Fig. 18) showed that in fruits treated with 500, 1000 or 2000 $\text{nl}\cdot\text{L}^{-1}$ 1-ECP, lycopene content was 5.6%-9.6% that of control (100%) and no significant difference was found between them. Treatment with 500 $\text{nl}\cdot\text{L}^{-1}$ 1-ECP was sufficient to exert maximal inhibitory effect on ethylene action, and treatment with higher concentrations did not show further significant increase in the inhibitory effect.

6.3 Effect of 1-PCP on ripening

of tomato fruit

The results show that mature green tomato fruit treated with ethylene ($300 \mu\text{L}\cdot\text{L}^{-1}$) ripened 3-5 days after harvest. Treatment with $1000 \text{ nl}\cdot\text{L}^{-1}$ 1-PCP delayed ripening by 5 days. In fruit treated with $500 \text{ nl}\cdot\text{L}^{-1}$ 1-PCP, the climacteric ethylene peak appeared on the 4th day after treatment, just 1 day after that in the ethylene treated fruit. Thus, treatment with $500 \text{ nl}\cdot\text{L}^{-1}$ 1-PCP had little effect on ethylene-induced ripening of tomato fruit. Treatments with higher concentrations of 1-PCP (1500 or $2000 \text{ nl}\cdot\text{L}^{-1}$) showed a reduction in the ability to inhibit ethylene action rather than an increase. On the 8th day after treatment lycopene content (Fig.

Fig. 19. Effect of 1-PentCP on color change (A), fruit softening (B) and ethylene production (C) in mature green tomato fruits. The fruits were treated for 24 h with 0 ($\blacktriangle$), 1 ($\triangle$), 10 ($\square$), 100 ($\circ$) or 1000 ($\bullet$) $\text{nl}\cdot\text{L}^{-1}$ 1-PentCP, ventilated, and then exposed to ethylene ($300 \mu\text{L}\cdot\text{L}^{-1}$) for 24 h. Untreated fruits ($\blacksquare$) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

18) in fruit treated with $1000 \text{ nl}\cdot\text{L}^{-1}$ 1-PCP was 28.3% of that in the ethylene treated

fruit. This lycopene content was significantly lower than that in fruit treated with 500, 1500 or 2000 $\text{nl}\cdot\text{L}^{-1}$ (68.8%, 48.4% and 64.5% respectively). Thus results indicate that 1000 $\text{nl}\cdot\text{L}^{-1}$ 1-PCP was the most effective concentration to inhibit ethylene-induced ripening in mature green tomato fruit.

6.4 Effect of 1-BCP on ripening of tomato fruit

In this study, the inhibitory effects of treatments with 50, 100, 250 or 500 $\text{nl}\cdot\text{L}^{-1}$ 1-BCP on ethylene-induced ripening in tomato fruit was investigated. Fruit ripening

Fig. 20. Effect of 1-PentCP on lycopene content in mature green tomato fruits 8 days after treatment. The fruits were treated for 24 h with 0 (A), 50 (C), 100 (D), 250 (E) or 1000 (F) $\text{nl}\cdot\text{L}^{-1}$ 1-PentCP, ventilated, and then exposed to ethylene ($300\ \mu\text{l}\cdot\text{L}^{-1}$) for 24 h. Untreated fruits (B) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

was defined by determination of ethylene climacteric peak, fruit softening and color change. The results show that treatment with 50 $\text{nl}\cdot\text{L}^{-1}$ 1-BCP delayed ethylene-induced ripening in tomato fruit by 2 days. Treatment with 100 $\text{nl}\cdot\text{L}^{-1}$ 1-BCP extended the period until fruit ripening by 5 days whereas treatments with 250 and 500 $\text{nl}\cdot\text{L}^{-1}$ 1-BCP prolonged it by 6 days. Measurement of fruit softening, color change, lycopene content and timing of ethylene climacteric peak revealed no significant

difference between 250 and 500 $\text{nl}\cdot\text{L}^{-1}$ 1-BCP treatments. This indicates that treatment with 250 $\text{nl}\cdot\text{L}^{-1}$ 1-BCP had the maximal inhibitory effect on ripening of mature green tomato fruit.

6.5 Effect of 1-PentCP on ripening of tomato fruit

In the present study, the effect of 1-PentCP on ethylene-induced ripening of mature green tomato fruit was investigated. Mature green tomato fruit treated with 300 $\mu\text{l}\cdot\text{L}^{-1}$ ethylene for 24 h ripened 5 days after treatment at 22°C. The untreated fruit ripened 4 days later than those treated with only 300 $\mu\text{l}\cdot\text{L}^{-1}$ ethylene. Treatment with various concentrations of 1-PentCP delayed the ethylene-induced ripening and was found to be concentration dependent. Treatment with 1, 10, 100 or 1000 $\text{nl}\cdot\text{L}^{-1}$ 1-PentCP for 24 h immediately after harvest prior to exposure to ethylene (24 h to 300 $\mu\text{l}\cdot\text{L}^{-1}$) delayed the ethylene-induced fruit ripening by 2, 5, 9 and 12 days respectively (Fig. 19). Analysis of lycopene content in the fruit pulp on the 8th day after treatment showed that lycopene content in fruit treated with various concentrations of 1-PentCP was 20.3%-87.8% lower than that of the ethylene treated control (100%) (Fig. 20). In the fruit treated with 1 $\text{nl}\cdot\text{L}^{-1}$ 1-PentCP, lycopene content was 7.8% lower than that of the untreated fruit, lycopene content in all the fruit treated with higher concentrations of 1-PentCP (10, 100 or 1000 $\text{nl}\cdot\text{L}^{-1}$) was 21.8%-84.7% lower than that in the untreated fruit (Fig. 20). Of all the concentrations of 1-PentCP tested, treatment with 1000 $\text{nl}\cdot\text{L}^{-1}$ was most effective in delaying ethylene-induced ripening in mature green tomato fruit. Raising the concentration of 1-PentCP over 1000 $\text{nl}\cdot\text{L}^{-1}$ may have a more pronounced effect.

Fig. 21. Effect of 1-HCP on color change (A), fruit softening (B) and ethylene production (C) in "Hass" avocado fruits. The fruits were treated for 24 h with 0 ($\blacktriangle$), 1 ($\triangle$), 10 ($\square$), 100 ($\circ$) or 1000 ($\bullet$) $\text{nl}\cdot\text{L}^{-1}$ 1-HCP, ventilated, and then exposed to ethylene ($300\ \mu\text{l}\cdot\text{L}^{-1}$) for 24 h. Untreated fruits ($\blacksquare$) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

6.6 Effect of 1-HCP on ripening of tomato fruit

In this study, the inhibitory effects of treatments with 1, 10, 100 or $1000\ \text{nl}\cdot\text{L}^{-1}$ 1-HCP on ethylene-induced ripening in mature green tomato fruit were investigated. The results show that treatment with 100 or $1000\ \text{nl}\cdot\text{L}^{-1}$ 1-HCP delayed the ethylene-induced fruit ripening by 7 and 9 days respectively. In fruit treated with lower concentrations of 1-HCP (1 or $10\ \text{nl}\cdot\text{L}^{-1}$), the climacteric ethylene peak appeared on the 6th to 8th day after treatment, which were 1-3 days after the ethylene treated fruit and 1-3 days before the untreated fruit (Fig. 21). Lycopene content on 8th day after treatment in the pulp of fruit treated with the four concentrations of 1-HCP tested was 8.9%-71.5% lower than that of ethylene treated fruit (Fig. 22). As compared with the

untreated fruit, lycopene content in fruit treated with higher concentrations of 1-HCP (100 or 1000 $\text{nl}\cdot\text{L}^{-1}$) was 51.5%-64.2% lower than the untreated fruit, whereas that in

Fig. 22. Effect of 1-HCP on lycopene content in mature green tomato fruits 8 days after treatment. The fruits were treated for 24 h with 0 (A), 50 (C), 100 (D), 250 (E) or 1000 (F) $\text{nl}\cdot\text{L}^{-1}$ 1-HCP, ventilated, and then exposed to ethylene (300 $\mu\text{l}\cdot\text{L}^{-1}$) for 24 h. Untreated fruits (B) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

fruit treated with lower concentrations of 1-HCP (1 or 10 $\text{nl}\cdot\text{L}^{-1}$) was 0.8%-14.3% higher than the untreated fruit (Fig. 22). Thus our results indicate that 1000 $\text{nl}\cdot\text{L}^{-1}$ 1-HCP was the most effective concentration among the four concentrations tested to inhibit ethylene-induced ripening in mature green tomato fruit. As fruit ripening was just further delayed by 3 days when treatment concentration was increased from 100 $\text{nl}\cdot\text{L}^{-1}$ to 1000 $\text{nl}\cdot\text{L}^{-1}$ (Fig. 21), it seems that the effect could not be greatly increased by further raising the treatment concentration over 1000 $\text{nl}\cdot\text{L}^{-1}$.

6.7 Effect of 1-HeptCP on ripening of tomato fruit

The effect of 1-HeptCP on ethylene-induced ripening of mature green tomato fruit was investigated in this study. The results show that the untreated fruit ripened 9

days after harvest while fruit treated with $300 \mu\text{L}^{-1}$ ethylene for 24 h ripened at 22°C 5 days after harvest (Fig. 23). Treatment with 50, 150 or 250 nL^{-1} 1-HeptCP for 24 h immediately after harvest prior to exposure to ethylene (24 h to $300 \mu\text{L}^{-1}$) delayed the ethylene-induced ripening of mature green tomato fruit by 5, 7 and 8 days respectively (Fig. 23). Increasing the concentration of 1-HeptCP to 2000 nL^{-1} did not greatly intensify its inhibitory effect: Ethylene-induced fruit ripening was further delayed by only 1 day as compare with fruits treated with 250 nL^{-1} 1-HeptCP (Fig. 23).

Analysis of lycopene content in the fruit pulp on the 8th day after treatment showed that treatment with 1-HeptCP delayed lycopene synthesis in mature green termite

Fig. 23. Effect of 1-HeptCP on color change (A), fruit softening (B) and ethylene production (C) in mature green tomato fruits. The fruits were treated for 24 h with 0 ($\blacktriangle$), 50 ($\triangle$), 150 ($\square$), 250 ($\circ$) or 2000 ($\bullet$) nL^{-1} 1-HeptCP, ventilated, and then exposed to ethylene ($300 \mu\text{L}^{-1}$) for 24 h. Untreated fruits ($\blacksquare$) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

fruit (Fig. 24). Lycopene contents in fruits treated with 50, 150, 250 or 2000 nL^{-1}

were 27.4%, 49.5%, 61.6% and 70.2% lower than that of ethylene-treated fruit (100%) respectively. As the fruits treated with the various concentrations of 1-HeptCP ripened 1-5 days later than the untreated fruit (Fig. 23), concomitantly their lycopene contents were 12.1%-63.9% lower than that of the untreated fruit (Fig. 24).

Of all concentrations of 1-HeptCP $2000 \text{ nl}\cdot\text{L}^{-1}$ was found to be the most effective in delaying ethylene-induced ripening. However, only a 1 day difference in fruit ripening was observed between fruits treated with $250 \text{ nl}\cdot\text{L}^{-1}$ and $2000 \text{ nl}\cdot\text{L}^{-1}$ 1-HeptCP. In light of this finding, it is suggested that ethylene-induced ripening of mature green tomato fruit can not be further delayed by increasing the concentration of 1-HeptCP over $2000 \text{ nl}\cdot\text{L}^{-1}$.

Fig. 24. Effect of 1-HeptCP on lycopene content in mature green tomato fruits 8 days after treatment. The fruits were treated for 24 h with 0 (A), 50 (C), 150 (D), 250 (E) or 2000 (F) $\text{nl}\cdot\text{L}^{-1}$ 1-HeptCP, ventilated, and then exposed to ethylene ($300 \mu\text{l}\cdot\text{L}^{-1}$) for 24 h. Untreated fruits (B) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

6.8 Effect of 1-OCP on ripening of tomato fruit

All the treatments with 30, 75, 150 or $300 \text{ nl}\cdot\text{L}^{-1}$ 1-OCP delayed ethylene-induced ripening in mature green tomato fruits by 6 days. No significant

difference was found between these four concentrations regarding fruit softening, color change, lycopene content and ethylene climacteric peak appearance. The results indicate that of all the concentrations tested, 30 $\text{nl}\cdot\text{L}^{-1}$ 1-DCP was sufficient to exert maximal inhibition of tomato fruit ripening induced by 300 $\mu\text{l}\cdot\text{L}^{-1}$ ethylene.

6.9 Effect of 1-DCP on ripening of tomato fruit

The inhibitory effect of 1-DCP on ethylene-induced ripening in mature green tomato fruit was found to be concentration-dependent. The results show that the tomato fruit treated with 300 $\mu\text{l}\cdot\text{L}^{-1}$ ethylene for 24 h ripened 3 days after treatment and the untreated fruit ripened 9 days after harvest at 22°C (Fig. 25). Treatment with 50, 150 or 250 $\text{nl}\cdot\text{L}^{-1}$ 1-DCP prior to

Fig. 25. Effect of 1-DCP on color change (A), fruit softening (B) and ethylene production (C) in mature green tomato fruits. The fruits were treated for 24 h with 0 ($\blacktriangle$), 50 ($\triangle$), 150 ($\square$), 250 ($\circ$) or 2000 ($\bullet$) $\text{nl}\cdot\text{L}^{-1}$ 1-DCP, ventilated, and then exposed to ethylene (300 $\mu\text{l}\cdot\text{L}^{-1}$) for 24 h. Untreated fruits ($\blacksquare$) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

ethylene ($300 \mu\text{L}^{-1}$) treatment delayed the ethylene-induced ripening of mature green tomato fruit by 5, 6 and 7 days respectively. The appearances of ethylene climacteric

Fig. 26. Effect of 1-DCP on lycopene content in mature green tomato fruits 8 days after treatment. The fruits were treated for 24 h with 0 (A), 50 (C), 150 (D), 250 (E) or 2000 (F) $\text{nl}\cdot\text{L}^{-1}$ 1-DCP, ventilated, and then exposed to ethylene ($300 \mu\text{L}\cdot\text{L}^{-1}$) for 24 h. Untreated fruits (B) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

peak, fruit color change as well as fruit softening were concomitantly delayed (Fig. 25). The inhibitory effect of 1-DCP on ethylene-induced ripening in mature green tomato fruit was not greatly intensified by increasing the concentration to $2000 \text{ nl}\cdot\text{L}^{-1}$. It delayed the ethylene-induced fruit ripening by only 1 day compare with the treatment with $250 \text{ nl}\cdot\text{L}^{-1}$ 1-DCP (Fig. 25). As the concentration difference between these two treatments was 8 times, a period of 7-8 days was the maximal by using 1-DCP to delay ethylene-induced ripening of mature green tomato fruit.

Analysis of lycopene content in the fruit pulp on the 8th day after treatment revealed that lycopene content in the pulp of fruit treated with the four concentrations of 1-DCP tested was 29.4%-57.1% lower than that of ethylene treated fruit (Fig. 26).

As compared with the untreated fruit, lycopene content in fruit treated with 1-DCP was 14.6%-48.1% lower (Fig. 26).

6.10 Comparison of the effects of the inhibitors on ripening of tomato fruit

In all the studies presented above, we have tested the effects of nine newly synthesized putative inhibitors of ethylene action on ethylene-induced ripening of mature green tomato fruit. As the chemical structure of H1-MCP is quite different from all the other eight compounds, the eight putative inhibitors of ethylene action with similar chemical structure other than H1-MCP are compared in Table 11 and Table 12. The potency of the inhibitor at its most effective concentration tested is

Table 11. Lycopene content in mature green tomato fruits 8 days after treatment with the indicated inhibitor prior to ethylene treatment. Tomato fruit were treated for 24 h with the indicated inhibitor and then exposed for 24 h to ethylene (300 $\mu\text{L}\cdot\text{L}^{-1}$) and thereafter stored in air at 22°C for ripening assessment

Inhibitor	Treatment concentration ($\text{nL}\cdot\text{L}^{-1}$)	Lycopene (% of control)
1-ECP	500	5.6 c
1-PCP	1000	31.1 b
1-BCP	250	38.3 a
1-PentCP	1000	12.2 b
1-HCP	1000	28.5 a
1-HeptCP	2000	29.8 b
1-OCP	30	38.3 a
1-DCP	2000	42.9 a

Different letters indicate statistical differences at $P = 0.05$.

showed in Table 11 and Table 12. Of all the eight new ethylene inhibitors tested, 1-ECP was the most potent one. This was determined by ethylene climacteric peak, fruit softening and color change and lycopene content in the fruit pulp. Treatment with the most effective concentration of 1-ECP ($500 \text{ nl}\cdot\text{L}^{-1}$) delayed ethylene-induced ripening of mature green tomato fruit by 13 days, which was double that of fruits treated with three of the other inhibitors: 1-BCP, 1-PCP and 1-OCP (Table 12). In fruits treated with $500 \text{ nl}\cdot\text{L}^{-1}$ 1-ECP a 1-8 days delay of fruit softening, color change and climacteric ethylene peak was recorded as compared with all the other seven

Table 12. Days to reach the climacteric peak of ethylene production, fruit softening (firmness 2-2.5 kg) and color change (index > 7) in mature green tomato fruits treated with the indicated inhibitor prior to ethylene treatment. Tomato fruits were treated for 24 h with the indicated inhibitor and then exposed for 24 h to ethylene ($300 \mu\text{l}\cdot\text{L}^{-1}$) and thereafter stored in air at 22°C for ripening assessment

Inhibitor	Ethylene	Softening	Color	Delayed ripening by (days)
1-ECP ($500 \text{ nl}\cdot\text{L}^{-1}$)	19.0 a	18.3 a	17.8 a	13 a
1-BCP ($250 \text{ nl}\cdot\text{L}^{-1}$)	12.6 b	12.3 bc	12.0 b	6 b
1-PCP ($1000 \text{ nl}\cdot\text{L}^{-1}$)	11.6 b	11.8 c	10.6 c	5 c
1-PentCP ($1000 \text{ nl}\cdot\text{L}^{-1}$)	19.5 b	19.7 bc	18.2 b	12 b
1-HCP ($1000 \text{ nl}\cdot\text{L}^{-1}$)	17.1 b	16.3 bc	15.6 b	9 b
1-HeptCP ($2000 \text{ nl}\cdot\text{L}^{-1}$)	14.2 b	14.5 c	13.6 c	9 c
1-OCP ($30 \text{ nl}\cdot\text{L}^{-1}$)	11.8 b	12.6 b	12.3 b	6 b
1-DCP ($2000 \text{ nl}\cdot\text{L}^{-1}$)	13.3 b	13.7 b	12.8 b	8 b

Different letters indicate statistical differences at $P = 0.05$.

inhibitor treatments. In addition, 8 days after treatment, lycopene content in 1-ECP treated fruit was about 13%-46% of those in fruits treated with any of the other seven inhibitors (Table 11). However, the concentration of 1-ECP ($500 \text{ nl}\cdot\text{L}^{-1}$) required to exert its effect is much higher than that of 1-BCP ($250 \text{ nl}\cdot\text{L}^{-1}$) or 1-OCP ($30 \text{ nl}\cdot\text{L}^{-1}$), but is much lower than that of 1-HeptCP ($2000 \text{ nl}\cdot\text{L}^{-1}$) or 1-DCP ($2000 \text{ nl}\cdot\text{L}^{-1}$).

Of all the treatments, 1-OCP exerted its inhibitory effect on the ethylene-induced ripening of tomato fruit at the lowest concentration ($30 \text{ nl}\cdot\text{L}^{-1}$) compared with the other seven new inhibitors of ethylene action (Table 12). Nevertheless, treatment with $30 \text{ nl}\cdot\text{L}^{-1}$ 1-OCP delayed ethylene-induced ripening of mature green tomato fruit by only 6 days, which was less than 50% of the delay by treatment with $500 \text{ nl}\cdot\text{L}^{-1}$ 1-ECP. In addition, increasing the concentration of 1-OCP to $300 \text{ nl}\cdot\text{L}^{-1}$ did not further prolong the protection period of fruit from ethylene (refer to 6.8 of this section). This means that a delay of 6 days in ethylene-induced ripening of mature green tomato fruit was the longest period which can be obtained by treatment with 1-OCP.

The results show that if rated by the concentration and the period of protection from ethylene action, the most potent compound among the eight inhibitors tested was 1-ECP, which delayed fruit ripening by 13 days at the concentration of $500 \text{ nl}\cdot\text{L}^{-1}$. Less potent was 1-PentCP, which delayed ethylene-induced fruit ripening by 12 days at the concentration of $1000 \text{ nl}\cdot\text{L}^{-1}$ (Table 12). The order of decrease in potency of the tested inhibitors is: 1-ECP>1-PentCP>1-HCP>1-HeptCP>1-DCP>1-OCP>1-BCP>1-PCP.

7. Effect of new inhibitors of ethylene action on ethylene-induced abscission in citrus leaf explants

It has been well established that ethylene is involved in organ abscission in many plants (Abeles *et al.*, 1992; Sagee *et al.*, 1980). During the abscission process, the activity of the enzymes polygalacturonase (PG) and cellulase in the abscission zone increase, which induces breakdown of the cell walls (Greenberg *et al.*, 1975). In this research project ethylene-induced abscission in citrus leaf explants has been employed as a test model in our test system set up for assessing the potency of all the putative inhibitors of ethylene action synthesized by USA group.

Preliminary studies in our lab revealed that treatment for 24 h with 20 $\mu\text{l}\cdot\text{L}^{-1}$ ethylene was the most effective for induction of abscission in 'Shamouti' [*Citrus sinensis* (L.) Osbeck] orange leaf explants. Treatment with 100 $\text{nl}\cdot\text{L}^{-1}$ 1-MCP for 6 h prior to exposure to ethylene (20 $\mu\text{l}\cdot\text{L}^{-1}$) effectively delayed the ethylene-induced abscission and greatly reduced exo- and endo-cellular cellulase and PG activity in the petiole / blade abscission zone (LA-AZ) (refer to 10.1.4 of this section). In the present study, we investigated the effects of all the newly synthesized putative inhibitors on ethylene-induced LA-AZ abscission in citrus leaf explants. Based on the results of our studies with 1-MCP, the treatment period in the current experiments with all the new putative inhibitors was shortened from 24 h to 6 h. However, the exposure of the leaf explants to ethylene (20 $\mu\text{l}\cdot\text{L}^{-1}$) was kept for 24 h, so that maximal induction of abscission was achieved and the effect of the new putative inhibitors were assessed under extreme conditions.

7.1 Effect of H1-MCP on ethylene-induced abscission in citrus leaf explants

Treatment with H1-MCP (50, 250 or 500 $\text{nl}\cdot\text{L}^{-1}$) did not affect the ethylene (20 $\mu\text{l}\cdot\text{L}^{-1}$) induced abscission in citrus leaf explants. The results of experiment with H1-MCP on banana fruit show that ethylene-induced fruit color breakdown was delayed by treatment with this compound but ethylene-induced fruit pulp softening was not (refer to 4.1 of this section). These results may indicate that different ethylene receptors exist in plant tissue and that the same compound can not bind to all types of the receptors. Thus, H1-MCP probably binds to ethylene receptor related to color breakdown but not to the ethylene receptors related to abscission and banana pulp softening.

7.2 Effect of 1-ECP on ethylene-induced abscission in citrus leaf explants

‘Shamouti’ citrus leaf explants treated with ethylene (20 $\mu\text{l}\cdot\text{L}^{-1}$) completely abscised immediately after treatment. In leaves treated with 100, 400 or 700 $\text{nl}\cdot\text{L}^{-1}$ 1-ECP for 6 h prior to the ethylene treatment (20 $\mu\text{l}\cdot\text{L}^{-1}$), abscission recorded immediately after ethylene treatment was 100%, 67% and 6% respectively. However, 48 h after ethylene treatment, 100% abscission was found in leaves treated with 400 $\text{nl}\cdot\text{L}^{-1}$ 1-ECP and 72 h after ethylene treatment in leaves treated with 700 $\text{nl}\cdot\text{L}^{-1}$ 1-ECP. When the concentration of 1-ECP was lower below 100 $\text{nl}\cdot\text{L}^{-1}$, no effect on ethylene-induced LA-AZ abscission in leaf explants was found.

Of the different concentrations of 1-ECP tested, application of 700 $\text{nl}\cdot\text{L}^{-1}$ 1-ECP for 6 h was found to be most effective in delaying ethylene-induced abscission in leaf explants. Similar to the findings with 1-MCP application, ethylene production by

the explants 24 h after treatment with $700 \text{ nl}\cdot\text{L}^{-1}$ 1-ECP increased 3.6 fold over the untreated control (Fig. 27). The results indicate that treatment with $700 \text{ nl}\cdot\text{L}^{-1}$ 1-ECP counteracted the “ethylene autoinhibition” (Riov and Yang, 1982) and resulted in over production of ethylene by the leaves. It seems that although 1-ECP induced high rate of ethylene production, as it blocks the site of ethylene binding, its ability to delay abscission is not affected.

The results of the studies with cellulase and PG at LA-AZ, showed that compared with untreated fruits ethylene treatment ($20 \mu\text{l}\cdot\text{L}^{-1}$) increased the activity of exo-, endo-cellular cellulase and PG by 50.2%, 52.8% and 88.5% respectively. Treatment with $700 \text{ nl}\cdot\text{L}^{-1}$ 1-ECP reduced the activity of exo-cellulase by 49.8%, endo-cellular cellulase by 47.2% and PG by 71.7% at 6 h after treatment (Fig. 28). Similar results were obtained with 1-MCP treatment (refer to 10.1.4 of this section).

7.3 Effect of 1-PCP on ethylene-induced abscission in citrus leaf explants

Treatment for 6 h with $1000 \text{ nl}\cdot\text{L}^{-1}$ 1-PCP had a minor inhibitory effect on ethylene-induced abscission in citrus leaf explants; 93% abscission was recorded after treatment. However, in explants treated with 1500 or 2000 $\text{nl}\cdot\text{L}^{-1}$ 1-PCP, only 17% and 8% abscission was recorded respectively after treatment. Nevertheless, 92% of the explants treated with $1500 \text{ nl}\cdot\text{L}^{-1}$ 1-PCP abscised 48 h after ethylene treatment. In explants treated with $2000 \text{ nl}\cdot\text{L}^{-1}$ 1-PCP, 98% abscission was recorded 72 h after treatment. Of all the concentrations tested, treatment with $2000 \text{ nl}\cdot\text{L}^{-1}$ 1-PCP for 6 h was found to be most effective in inhibiting ethylene-induced abscission in leaf explants. It seems that the inhibitory effect could be intensified by prolonging the treatment period or by increasing 1-PCP concentration.

Fig. 27. Effect of putative inhibitors of ethylene action on ethylene production in citrus leaf explants. Explants were treated for 6 h with the indicated inhibitors and then exposed to ethylene (20 $\mu\text{l}\cdot\text{L}^{-1}$) for 24 h and thereafter placed in air at 22°C for abscission assessment. For ethylene treatment, explants were closed in the jars for 6 h with air instead of with the inhibitors before exposure to ethylene for 24 h. Untreated explants were kept in air containing jars during treatment.

The results also showed that 24 h after 6 h treatment with 2000 $\text{nl}\cdot\text{L}^{-1}$ 1-PCP and 24 h exposure to ethylene (20 $\mu\text{l}\cdot\text{L}^{-1}$), the production of ethylene by the explants increased 4.3 fold over the untreated control (Fig. 27). This treatment also greatly reduced the activity of exo-, endo-cellular cellulase and PG by 41.4%, 29.2% and 66.7% respectively at 6 h after 24 h exposure to ethylene (Fig. 28).

7.4 Effect of 1-BCP on ethylene-induced abscission in citrus leaf explants

Treatment with 250 or 500 $\text{nl}\cdot\text{L}^{-1}$ 1-BCP for 6 h and then 24 h exposure to ethylene had little effect on ethylene-induced abscission in leaf explants: 97% and 73% of the explants abscised respectively after treatment. Explants treated with 800 $\text{nl}\cdot\text{L}^{-1}$ 1-BCP showed only 13%, 19% and 31% abscission at 0, 24 and 48 h after treatment respectively. However, 100% abscission was recorded in these explants 72 h after the

Fig. 28. Effect of putative inhibitors of ethylene action on the activity of endo- (A) and exo- (B) cellulase and PG (C) at LA-AZ of 'Shamouti' orange citrus leaf explants 6 h after treatment. Explants were treated for 6 h with the indicated inhibitors and then exposed for 24 h to ethylene ($20 \mu\text{l}\cdot\text{L}^{-1}$) and thereafter stored in air at 25°C for abscission assessment. Untreated explants were kept in air containing jars during treatment.

ethylene treatment. Treatment with $800 \text{ nl}\cdot\text{L}^{-1}$ 1-BCP also increased ethylene production by 4 fold over the untreated control (Fig. 27) and reduced the activity of exo-, endo-cellular cellulase and PG by 33.9%, 41% and 64% respectively at 6 h after treatment (Fig. 28).

7.5 Effect of 1-PentCP on ethylene-induced abscission in citrus leaf explants

The effect of 1-PentCP on ethylene-induced LA-AZ abscission in citrus leaf explants was investigated in this study. Explants treated for 6 h with 50 $\text{nl}\cdot\text{L}^{-1}$ 1-PentCP and then exposed to ethylene (24 h, 20 $\mu\text{l}\cdot\text{L}^{-1}$) showed 60%, 80% and 100% abscission at 48, 60 and 72 h after ethylene exposure respectively. Increasing the concentration resulted in elevating the effect of this compound. In explants treated with 250 $\text{nl}\cdot\text{L}^{-1}$ 1-PentCP, 40%, 70% and 100% leaf abscission were recorded 72, 84 and 96 h after ethylene exposure respectively. A further increase in the concentration of 1-PentCP to 500 or 1000 $\text{nl}\cdot\text{L}^{-1}$ did not cause any further significant inhibition of ethylene-induced leaf abscission compared with that treated with 50 $\text{nl}\cdot\text{L}^{-1}$ 1-PentCP. Therefore, of all the concentrations tested, treatment with 50 $\text{nl}\cdot\text{L}^{-1}$ 1-PentCP for 6 h was most effective in inhibiting of ethylene-induced abscission in leaf explants. The results also showed that 24 h after treatment, ethylene production by the explants treated with 50 $\text{nl}\cdot\text{L}^{-1}$ 1-PentCP increased 3.6 fold over the untreated control (Fig. 27). This treatment also reduced the activity of exo-, endo-cellular cellulase and PG in the abscission zone by 57%, 50.6% and 76% respectively (Fig. 28).

7.6 Effect of 1-HCP on ethylene-induced abscission in citrus leaf explants

In this study, the inhibitory effect of treatments with 75, 150, 300 or 600 $\text{nl}\cdot\text{L}^{-1}$ 1-HCP on ethylene-induced abscission in citrus leaf explants was investigated. The results show that treatment with 75 or 150 $\text{nl}\cdot\text{L}^{-1}$ 1-HCP for 6 h and then 24 h exposure to ethylene (20 $\mu\text{l}\cdot\text{L}^{-1}$) had little effect on ethylene-induced abscission in citrus leaf explants. 97% and 87% of the explants abscised respectively after treatment.

Explants treated with 300 and 600 $\text{nl}\cdot\text{L}^{-1}$ showed 14% and 16%, 19% and 21%, 30% and 34% abscission at 0, 24 and 48 h after treatment respectively. However, 100% abscission was recorded in explants treated with these two concentrations of 1-HCP 72 h after the treatment. Ethylene production in the explants treated with 300 or 600 $\text{nl}\cdot\text{L}^{-1}$ 1-HCP increased 4.2 and 4.1 fold respectively over the untreated control (Fig. 27). No significant difference in abscission of the explants between treatments with 300 and 600 $\text{nl}\cdot\text{L}^{-1}$ 1-HCP. Thus, treatment with 300 $\text{nl}\cdot\text{L}^{-1}$ 1-HCP was sufficient to cause maximal inhibition in ethylene-induced abscission of citrus leaf explants. Treatment with 300 $\text{nl}\cdot\text{L}^{-1}$ 1-HCP also reduced the activity of exo-cellulase by 37.9%, endo-cellular cellulase by 34.3% and PG by 66.9% (Fig. 28).

7.7 Effect of 1-HeptCP on ethylene-induced abscission in citrus leaf explants

Treatment for 6 h with 50, 100, 500 or 1000 $\text{nl}\cdot\text{L}^{-1}$ 1-HeptCP and prior to a 24 h exposure to ethylene ($20\ \mu\text{l}\cdot\text{L}^{-1}$) had a similar effect on ethylene-induced abscission in citrus leaf explants. Abscission in all treatments was delayed about 3 days. No significant difference in leaf abscission was found between the treatments. Of the four concentrations tested, treatment with 50 $\text{nl}\cdot\text{L}^{-1}$ 1-HeptCP was sufficient to cause maximal inhibitory effect. Thus, the optimal concentration may be lower than 50 $\text{nl}\cdot\text{L}^{-1}$. To verify this, another experiment with lower concentrations of 1-HeptCP 5, 20, 35 or 50 $\text{nl}\cdot\text{L}^{-1}$ was carried out. The results show that treatment with 5 or 20 $\text{nl}\cdot\text{L}^{-1}$ had little effect on ethylene-induced abscission in leaf explants: 90% and 80 % of the explants abscised after treatment respectively. Explants treated with 35 $\text{nl}\cdot\text{L}^{-1}$ 1-HeptCP showed 90% and complete abscission at 48 h and 60 h after ethylene exposure respectively. However, in explants treated with 50 $\text{nl}\cdot\text{L}^{-1}$ 1-HeptCP, just as the result obtained in the

former experiment, ethylene-induced leaf abscission was delayed by 3 days. So, the results of both of experiments showed that treatment with $50 \text{ nl}\cdot\text{L}^{-1}$ 1-HeptCP for 6 h is the most effective treatment to inhibit ethylene-induced abscission in citrus leaf explants. The results also show that ethylene production by the explants 24 h after treatment with $50 \text{ nl}\cdot\text{L}^{-1}$ 1-HeptCP increased 3.4 fold over the untreated control (Fig. 27).

7.8 Effect of 1-OCP on ethylene-induced abscission in citrus leaf explants

In explants treated with 75, 150 or $300 \text{ nl}\cdot\text{L}^{-1}$ 1-OCP prior to a 24 h exposure to ethylene, 100%, 71% and 11% abscission were recorded after treatment respectively. Ethylene production in the explants treated with $300 \text{ nl}\cdot\text{L}^{-1}$ 1-OCP increased 4 fold over the untreated explants 24 h after treatment (Fig. 27), the activity of exo-cellulase, endo-cellular cellulase and PG at 6 h after treatment were reduced by 26.4%, 22.5%, 56.3% respectively (Fig. 28).

7.9 Effect of 1-DCP on ethylene-induced abscission in citrus leaf explants

In the present study, the inhibitory effect of treatments with 50, 100, 500 or $1000 \text{ nl}\cdot\text{L}^{-1}$ 1-HCP on ethylene-induced abscission in citrus leaf explants was investigated. The results show that all the treatments with the above mentioned concentrations of 1-DCP for 6 h prior to exposure to ethylene ($20 \mu\text{l}\cdot\text{L}^{-1}$), did not have any effect on ethylene-induced abscission in citrus leaf explants in spite of the high concentration ($1000 \text{ nl}\cdot\text{L}^{-1}$).

7.10 Comparison between the effects of the various inhibitors tested on ethylene induced abscission in citrus leaf explants

Our results dealing with all the newly synthesized putative inhibitors of ethylene action showed that except for 1-DCP, treatments with all the other inhibitors tested at the proper concentrations, prior to exposure to ethylene ($20 \mu\text{L}^{-1}$) for 24 h inhibited ethylene-induced abscission in LA-AZ of citrus leaf explants. The most effective concentrations tested were: 700 nL^{-1} for 1-ECP, 2000 nL^{-1} for 1-PCP, 800 nL^{-1} for 1-BCP, 50 nL^{-1} for 1-PentCP, 300 nL^{-1} for 1-HCP, 50 nL^{-1} for 1-HeptCP and 300 nL^{-1} for 1-OCP. It should be noted that the difference in concentrations required to cause maximal effect for each of the putative inhibitors was large. For example, a 40-fold higher concentration of 1-DCP is required to achieve the same effect as 1-PentCP. As the difference between these compounds, except for H1-MCP, is in the length of side-chain (the number of carbon), this might be the reason for the difference in potency.

All the treatments with these new putative inhibitors at the above mentioned concentrations increased ethylene production by the explants 24 h after treatment (Fig. 27). Treatment with $20 \mu\text{L}^{-1}$ ethylene reduced ethylene production in the leaf explants (Fig. 27), due to "ethylene autoinhibition" (Riov and Yang, 1982). Therefore, the increase of ethylene production in the explants induced by treatments with these new putative ethylene inhibitors was possibly due to counteracting the ethylene autoinhibition phenomenon. However, as the ethylene receptors were blocked by the inhibitor, this increase in ethylene production could not induce abscission.

Of all the putative inhibitors tested, 1-HeptCP was found to be the most effective compound to inhibit ethylene-induced abscission in citrus leaf explants, since it

required the lowest concentration to exert its effect and provided longer inhibitory period. The order of decreasing potency of the other inhibitors is: 1-PentCP>1-HCP>1-OCP>1-ECP>1-BCP>1-PCP, and 1-DCP had no effect at all.

8. Effect of new inhibitors of ethylene action on ethylene-induced growth modification in pea seedlings

Exposure of etiolated pea seedlings to exogenous ethylene inhibits stem elongation, induces swelling of the sub apex of the epicotyle and root and apical hook closure. Endogenous ethylene has to some extent the same effects. These phenomena of etiolated pea seedlings were used widely as a model for research regarding ethylene action (Abeles *et al.*, 1992; Apelbaum, 1990; Apelbaum *et al.*, 1972). In our preliminary experiments, this model was first used to study the effect of 1-MCP on ethylene-induced growth modification of pea seedlings. The results show that treatment with 50 nl·L⁻¹ 1-MCP was sufficient to cause maximal inhibition of ethylene action of both exogenously applied and endogenously produced ethylene (refer to 10.1.6 of this section).

As the 1-MCP was found to inhibit ethylene-induced growth modification of pea seedlings, in the present study, we investigated the effect of the newly synthesized putative inhibitors of ethylene action using this pea seedling model.

Two days old etiolated pea seedlings grown in pots in the dark were placed in glass containers and treated for 24 h with various concentrations of the inhibitors. The inhibitors were applied by injecting them through a septum into the sealed glass jars containing the seedlings. After the treatment the containers were aerated and 100

μL^{-1} ethylene was injected into the jars to induce ethylene growth modification. The seedlings were continuously exposed to ethylene for 3 to 4 days. Measurements of epicotyle and root elongation, and the swelling as well as degree of hook opening were conducted at the end of the examination.

8.1 Effect of 1-ECP on ethylene-induced growth modification in pea seedlings

The results (Table 13 and Fig. 29) showed that when pea seedlings were exposed for 4 days to $100 \mu\text{L}^{-1}$ ethylene, epicotyle growth was suppressed by 30%

Fig. 29. Effect of 1-ECP on ethylene-induced growth modification in pea seedlings. Pea seeds cv. Alaska were soaked for 24 h in aerated water, rinsed, and planted in plastic pots. Three days old seedlings were treated for 24 h with the indicated concentrations of the inhibitor and afterwards kept continuously in ethylene ($100 \mu\text{L}^{-1}$) until measured. Untreated pea seedlings were kept in air containing jars during experiment.

and root growth reduced by 18%. Epicotyle and root swelling induced by ethylene was 379% and 136% of the control respectively. Treatment with $500 \text{ nl}\cdot\text{L}^{-1}$ 1-ECP reversed the ethylene-induced inhibition of elongation by 91% and 89% in epicotyle and root

respectively. Ethylene-induced epicotyle and root swelling was completely reversed by treatment with the higher concentration of the inhibitor. Consequently, no significant difference was observed between the plants treated with 1000 $\text{nl}\cdot\text{L}^{-1}$ 1-ECP and

Table 13. Effect of treatment with 1-ECP on ethylene induced growth inhibition and swelling in etiolated pea seedlings. Pea seeds cv. 'Alaska' were soaked for 24 h in aerated water, rinsed, and planted in plastic pots. Three days old seedlings were treated for 24 h with various concentrations of the inhibitors and afterwards kept continuously in ethylene ($100\ \mu\text{l}\cdot\text{L}^{-1}$) until measured.

Treatment	Length (cm)		Swelling (g/cm)	
	<u>Epicotyle</u>	<u>Root</u>	<u>Epicotyle</u>	<u>Root</u>
Untreated	9.04 b	6.49 ab	0.038 b	0.022 b
C_2H_4 ($100\ \mu\text{l}\cdot\text{L}^{-1}$)	2.28 d	5.35 c	0.144 a	0.030 a
1-ECP ($500\ \text{nl}\cdot\text{L}^{-1}$)+ C_2H_4	8.45 c	6.36 b	0.041 d	0.024 b
1-ECP ($1000\ \text{nl}\cdot\text{L}^{-1}$)+ C_2H_4	9.02 b	6.38 b	0.038 b	0.024 b
1-ECP ($1500\ \text{nl}\cdot\text{L}^{-1}$)+ C_2H_4	9.60 a	6.53 a	0.035 c	0.023 b
1-ECP ($2000\ \text{nl}\cdot\text{L}^{-1}$)+ C_2H_4	10.05 a	6.65 a	0.033 c	0.022 b

Different letters indicate statistical differences at $P = 0.05$.

untreated plants. This means that treatment with $1000\ \text{nl}\cdot\text{L}^{-1}$ 1-ECP was sufficient to completely reverse the growth modification induced by $100\ \mu\text{l}\cdot\text{L}^{-1}$ exogenous ethylene. Treatments with 1.5 or $2000\ \text{nl}\cdot\text{L}^{-1}$ 1-ECP reversed the ethylene-induced growth modification to such an extent that epicotyle length was 8.5% longer than the untreated plants and root length was 1.5% longer. The epicotyle was 10% thinner than the untreated plants. The fact that plants treated with 1500 or $2000\ \text{nl}\cdot\text{L}^{-1}$ 1-ECP, prior to ethylene treatment, were longer and thinner than control, indicates that inhibition of not only exogenous but also endogenous ethylene action occurred. No significant difference between treatments with 1500 and $2000\ \text{nl}\cdot\text{L}^{-1}$ 1-ECP on ethylene-induced

growth modification was found. Treatment with ethylene caused closure of the plumular hook, in all the plants treated with 1-ECP prior to ethylene treatment a plumular hook opening was observed compared with the ethylene treated ones. In summery, treatment with $1500 \text{ nl}\cdot\text{L}^{-1}$ 1-ECP was found to be sufficient to overcome the growth modification induced by the ethylene treatment.

8.2 Effect of 1-PCP on ethylene-induced growth modification in pea seedlings

Treatment with 50, 100, 200 or 500 $\text{nl}\cdot\text{L}^{-1}$ 1-PCP prior to ethylene treatment ($100 \mu\text{l}\cdot\text{L}^{-1}$) resulted in recovery from ethylene-induced growth inhibition and swelling

Fig. 30. Effect of 1-PCP on ethylene-induced growth inhibition (up) and swelling (down) in pea seedlings. Pea seeds cv. Alaska were soaked for 24 h in aerated water, rinsed, and planted in plastic pots. Three days old seedlings were treated for 24 h with the indicated concentrations of 1-PCP and afterwards kept continuously in ethylene ($100 \mu\text{l}\cdot\text{L}^{-1}$) until measured. Untreated pea seedlings were kept in air containing jars during experiment.

(Fig. 30). The recovery recorded was 2%, 7%, 48%, 55% in epicotyle elongation and 10%, 21%, 58%, 67% in root growth respectively. Epicotyle swelling was reduced by 11%, 60%, 92%, 99% and root swelling decreased by 26%, 45%, 71%, 86% respectively. Compare with the ethylene-treated plants, in the plants treated with 1-PCP prior to ethylene treatment, plumular hook opening was observed. Of all the concentrations of 1-PCP tested, treatment with $500 \text{ nl}\cdot\text{L}^{-1}$ exerted the most complete recovery from ethylene growth modification.

8.3 Effect of 1-BCP on ethylene-induced growth modification in pea seedlings

Treatment high concentration of 1-BCP ($2000 \text{ nl}\cdot\text{L}^{-1}$) only slightly reversed the ethylene-induced growth inhibition in pea seedlings. Plumular hook opening was not observed in the 1-BCP treated plants compared with the ethylene treated ones. Of all the concentrations tested (50, 500, 1000 and $2000 \text{ nl}\cdot\text{L}^{-1}$), treatment with $2000 \text{ nl}\cdot\text{L}^{-1}$ 1-BCP was found to be more effective than the others.

8.4 Effect of 1-PentCP on ethylene-induced growth modification in pea seedlings

Treatment with 50, 100, 500 or $1000 \text{ nl}\cdot\text{L}^{-1}$ 1-PentCP prior to ethylene treatment ($100 \mu\text{l}\cdot\text{L}^{-1}$) had different effect on ethylene-induced growth inhibition and swelling (Fig. 31). Treatment with low concentration ($50 \text{ nl}\cdot\text{L}^{-1}$) had little effect; recovery in epicotyle elongation was only 31% and that in root growth was 43%. When treatment concentration increased to $100 \text{ nl}\cdot\text{L}^{-1}$, the inhibitory effect was greatly intensified: recovery in epicotyle elongation and in root growth was 80% and 89% respectively; epicotyle swelling was reduced by 99% and root swelling by 92%. Further increase in treatment concentration to $500 \text{ nl}\cdot\text{L}^{-1}$ or $1000 \text{ nl}\cdot\text{L}^{-1}$ did not show

Fig. 31. Effect of 1-PentCP on ethylene-induced growth inhibition (up) and swelling (down) in pea seedlings. Pea seeds cv. Alaska were soaked for 24 h in aerated water, rinsed, and planted in plastic pots. Three days old seedlings were treated for 24 h with the indicated concentrations of 1-PentCP and afterwards kept continuously in ethylene (100 $\mu\text{l}\cdot\text{L}^{-1}$) until measured. Untreated pea seedlings were kept in air containing jars during experiment.

further significant recovery of ethylene-induced growth modification (Fig. 31). In all cases, plumular hook opening was observed in the 1-PentCP treated plants compared with the ethylene treated ones. Of all the concentrations tested, treatment with 100 $\text{nl}\cdot\text{L}^{-1}$ 1-PentCP was sufficient to exert the maximal recovery from ethylene growth modification in pea seedlings.

8.5 Effect of 1-HCP on ethylene-induced growth modification in pea seedlings

Treatment with 50, 250 or 500 $\text{nl}\cdot\text{L}^{-1}$ 1-HCP prior to ethylene treatment (100 $\mu\text{l}\cdot\text{L}^{-1}$) caused recovery from ethylene-induced growth inhibition and swelling in pea

seedlings (Fig. 32). The recovery recorded was 29%, 48%, 72% in epicotyle elongation and 26%, 48%, 78% in root growth respectively. Epicotyle swelling was decreased 75%, 89%, 93% and root swelling reduced 40%, 56%, 68% respectively. It

Fig. 32. Effect of 1-HCP on ethylene-induced growth inhibition (up) and swelling (down) in pea seedlings. Pea seeds cv. Alaska were soaked for 24 h in aerated water, rinsed, and planted in plastic pots. Three days old seedlings were treated for 24 h with the indicated concentrations of 1-HCP and afterwards kept continuously in ethylene (100 $\mu\text{L}\cdot\text{L}^{-1}$) until measured. Untreated pea seedlings were kept in air containing jars during experiment

appears that the recovery effect resulted from treatment with 1-HCP intensified as the treatment concentration increased within the range indicated above. Increasing the concentration of 1-HCP to 2000 $\text{nl}\cdot\text{L}^{-1}$ did not show further recovery effect. Thus, of all the four concentrations tested, treatment with 500 $\text{nl}\cdot\text{L}^{-1}$ 1-HCP exerted the maximal recovery from ethylene growth modification in pea seedlings. In all cases plumular hook opening was observed in the 1-HCP treated plants compared with the ethylene treated ones.

8.6 Effect of 1-HeptCP on ethylene-induced growth modification in pea seedlings

Treatment with various concentrations of 1-HeptCP prior to ethylene treatment ($100 \mu\text{l}\cdot\text{L}^{-1}$) resulted in a recovery from the ethylene-induced growth inhibition and swelling (Fig. 33). The extent of the recovery was concentration-dependent. The

Fig. 33. Effect of 1-HeptCP on ethylene-induced growth inhibition (up) and swelling (down) in pea seedlings. Pea seeds cv. Alaska were soaked for 24 h in aerated water, rinsed, and planted in plastic pots. Three days old seedlings were treated for 24 h with the indicated concentrations of 1-HeptCP and afterwards kept continuously in ethylene ($100 \mu\text{l}\cdot\text{L}^{-1}$) until measured. Untreated pea seedlings were kept in air containing jars during experiment.

recovery in epicotyle elongation caused by treatment with 50, 150, 250 or $1000 \text{ nl}\cdot\text{L}^{-1}$ 1-HeptCP was 50%, 76%, 91% and 87% respectively, and the recovery from root growth inhibition was 55%, 89%, 91% and 86% respectively. Ethylene-induced epicotyle swelling was reduced by 85% in plants treated with $50 \text{ nl}\cdot\text{L}^{-1}$ 1-HeptCP. The other three higher concentrations caused complete recovery from the ethylene-induced

epicotyle swelling. Ethylene-induced root swelling was decreased by 80%-82% by treatment with the four concentrations of 1-HeptCP tested (Fig. 33). Of all the concentrations tested, treatment with 250 $\text{nl}\cdot\text{L}^{-1}$ 1-HeptCP was sufficient to exert maximal recovery from ethylene growth modification in pea seedlings. Increasing concentration to 1000 $\text{nl}\cdot\text{L}^{-1}$ did not show further recovery effect (Fig. 33). Plumular hook opening was observed in plants treated with all concentrations of 1-HeptCP compared with the ethylene treated ones.

8.7 Effect of 1-OCP on ethylene-induced growth modification in pea seedlings

The results show that treatment with 50, 100, 150 or 500 $\text{nl}\cdot\text{L}^{-1}$ 1-OCP prior to ethylene exposure (100 $\mu\text{L}\cdot\text{L}^{-1}$), caused a recovery from the ethylene-induced growth

Fig. 34. Effect of 1-OCP on ethylene-induced growth inhibition (up) and swelling (down) in pea seedlings. Pea seeds cv. Alaska were soaked for 24 h in aerated water, rinsed, and planted in plastic pots. Three days old seedlings were treated for 24 h with the indicated concentrations of 1-OCP and afterwards kept continuously in ethylene (100 $\mu\text{L}\cdot\text{L}^{-1}$) until measured. Untreated pea seedlings were kept in air containing jars during experiment.

inhibition of the epicotyle and root (Fig. 34) Recovery of the epicotyle growth inhibition ranged by 6%-33% and that of the root by 7%-35% compared with ethylene treated plants. Epicotyle swelling was reduced between 26% and 66% and root swelling was decreased between 2% and 9%. In all the treatments, plumular hook opening was also observed. As no significant differences in recovery from the ethylene action was found between 150 and 500 $\text{nl}\cdot\text{L}^{-1}$ 1-OCP treatments, it seems that the recovery effect of 1-OCP would not be increased much by increasing the concentration of 1-OCP.

Fig. 35. Effect of 1-DCP on ethylene-induced growth inhibition (up) and swelling (down) in pea seedlings. Pea seeds cv. Alaska were soaked for 24 h in aerated water, rinsed, and planted in plastic pots. Three days old seedlings were treated for 24 h with the indicated concentrations of 1-DCP and afterwards kept continuously in ethylene ($100 \mu\text{l}\cdot\text{L}^{-1}$) until measured. Untreated pea seedlings were kept in air containing jars during experiment.

8.8 Effect of 1-DCP on ethylene-induced growth modification in pea seedlings

Treatment with various concentrations of 1-DCP prior to exposure to ethylene ($100 \mu\text{l}\cdot\text{L}^{-1}$) reversed the ethylene-induced growth inhibition in epicotyle and root elongation and swellings. The effect was concentration-dependent (Fig. 35). Ethylene-induced epicotyle and root elongations were reversed by 57%-101% and 47%-98% respectively, the ethylene-induced epicotyle and root swellings were reversed by 88%-99% and 50%-71% respectively (Fig. 35). Of the four concentrations tested, treatment with $250 \text{ nl}\cdot\text{L}^{-1}$ 1-DCP caused the maximal effect. Decreasing the concentration to $150 \text{ nl}\cdot\text{L}^{-1}$ or $50 \text{ nl}\cdot\text{L}^{-1}$ or increasing it to $1000 \text{ nl}\cdot\text{L}^{-1}$ did not improve the recovery from ethylene-induced growth modification (Fig. 35). In all the plants treated with the various concentrations of 1-DCP plumular hook opening was observed compared with the ethylene treated plants.

8.9 Comparison between the effects of the various inhibitors tested on ethylene-induced growth modification in pea seedlings

A comparison between the effect of 1-ECP, 1-PCP, 1-BCP and 1-OCP, at their optimal concentration, on ethylene-induced growth modification is shown in Table 14. Of all the inhibitors tested, treatment with $1500 \text{ nl}\cdot\text{L}^{-1}$ 1-ECP was the most effective. The inhibitor 1-DCP is less effective, and then come 1-HeptCP, 1-PentCP, 1-HCP, 1-PCP, 1-BCP and 1-OCP which is the least effective.

It appears that except for 1-OCP, compounds with a longer side chain containing more than six carbons were more effective to reverse ethylene-induced growth modification in pea seedlings (Table 14). Although 1-ECP was found to be the

most effective one from the experiments, the concentration required to cause the maximal effect was very high ($1500 \text{ nl}\cdot\text{L}^{-1}$) if compared with other compounds (less than $500 \text{ nl}\cdot\text{L}^{-1}$ except for 1-BCP that was $2000 \text{ nl}\cdot\text{L}^{-1}$). As the number of carbons in the side chain increases from 2 to 4, the effect of the compounds decreased. However, any relationship between the molecular size of the putative ethylene inhibitors and its potency can not be found from the experimental results reported herein.

Table 14. Effects of the new inhibitors of ethylene action on ethylene-induced growth modification in pea seedlings. Pea seeds cv. 'Alaska' were soaked for 24 h in aerated water, rinsed, and planted in plastic pots. Three days old seedlings were treated for 24 h with various concentrations of the inhibitors and afterwards kept continuously in ethylene ($100 \mu\text{l}\cdot\text{L}^{-1}$) until measured.

Inhibitors*	Recovery from ethylene-induced growth modification (%)			
	<u>Epicotyle growth</u>	<u>Root growth</u>	<u>Epicotyle swelling</u>	<u>Root swelling</u>
$1.5 \mu\text{l}\cdot\text{L}^{-1}$ 1-ECP	112	109	104	100
$500 \text{ nl}\cdot\text{L}^{-1}$ 1-PCP	54	67	99	86
$2000 \text{ nl}\cdot\text{L}^{-1}$ 1-BCP	27	49	47	29
$100 \text{ nl}\cdot\text{L}^{-1}$ 1-PentCP	80	89	99	92
$500 \text{ nl}\cdot\text{L}^{-1}$ 1-HCP	72	78	93	68
$250 \text{ nl}\cdot\text{L}^{-1}$ 1-HeptCP	91	91	100	82
$500 \text{ nl}\cdot\text{L}^{-1}$ 1-OCP	33	35	66	9
$250 \text{ nl}\cdot\text{L}^{-1}$ 1-DCP	101	98	99	71

* The optimal concentration for each inhibitor is presented.

9. Influence of new inhibitors of ethylene action on color change

of citrus fruit

It has been shown that ethylene is involved in the process of color change in citrus fruits (Apelbaum *et al.*, 1976; Stewart and Wheaton, 1972). In the experiments

during the first year it was found that treatment with 50 nl·L⁻¹ 1-MCP effectively inhibited the ethylene-induced degreening of 'Shamouti' [*Citrus sinensis* (L.) Osbeck] orange fruits (First Annual Report, page 15; Porat *et. al*, 1999). 'Sweetie' (grapefruit x pomelo) is an economically important fruit and is preferably marketed green. Therefore, the possibility to delay color change of 'Sweetie' by treating with the new putative ethylene action inhibitors was investigated.

'Sweetie' fruits were treated with various concentrations of 1-ECP or 1-PCP for 24 h and subsequently exposed to ethylene (20 µl·L⁻¹) for 24 h. After treatment the fruits were stored at room temperature for color change observation. Fruit peel color was measured visually and determined following a four grade-scale; -1- indicating yellow, -2- green-yellow, -3- yellow-green and -4- indicating green. The results showed that the color of fruits treated with ethylene (20 µl·L⁻¹) turned completely

Table 15. Effect of 1-ECP and 1-PCP on calyx abscission in 'Sweetie'.
Fruits were treated for 24 h with the indicated concentration of the inhibitors and then exposed for 24 h to ethylene (20 µl·L⁻¹).

Treatment	Calyx abscised in 10 fruits on days after harvest					
	5	6	7	8	9	10
Ethylene (20 µl·L ⁻¹)	10 a	--	--	--	--	--
0.7 µl·L ⁻¹ 1-ECP	0 c	0 b	0 a	0 a	2 a	3 a
1.5 µl·L ⁻¹ 1-ECP	0 c	0 b	1 a	1 a	3 a	4 a
3.0 µl·L ⁻¹ 1-ECP	0 c	0 b	0 a	1 a	2 a	4 a
0.5 µl·L ⁻¹ 1-PCP	10 a	--	--	--	--	--
1.0 µl·L ⁻¹ 1-PCP	10 a	--	--	--	--	--
2.0 µl·L ⁻¹ 1-PCP	7 b	10 a	--	--	--	--

Different letters indicate statistical differences at $P = 0.05$.

Table 16. Effect of 1-ECP and 1-PCP on rust-like spots on the peel of 'Sweetie'. Fruits were treated for 24 h with the indicated concentration of the inhibitors and then exposed for 24 h to ethylene (20 $\mu\text{l}\cdot\text{L}^{-1}$).

Treatment	Area of rust-like spots appearance (%) on days after harvest					
	5	6	7	8	9	10
Ethylene (20 $\mu\text{l}\cdot\text{L}^{-1}$)	0 d	0 e	0 e	0 e	0 e	0 f
0.7 $\mu\text{l}\cdot\text{L}^{-1}$ 1-ECP	10 c	12 c	16 c	18 c	20 c	25 c
1.5 $\mu\text{l}\cdot\text{L}^{-1}$ 1-ECP	20 b	25 b	30 b	40 b	65 b	80 b
3.0 $\mu\text{l}\cdot\text{L}^{-1}$ 1-ECP	40 a	50 a	65 a	75 a	90 a	100 a
0.5 $\mu\text{l}\cdot\text{L}^{-1}$ 1-PCP	0 d	2 d	2 d	3 d	4 d	5 e
1.0 $\mu\text{l}\cdot\text{L}^{-1}$ 1-PCP	0 d	1 d	2 d	3 d	4 d	5 e
2.0 $\mu\text{l}\cdot\text{L}^{-1}$ 1-PCP	0 d	1 e	2 d	4 d	6 d	10 d

Different letters indicate statistical differences at $P = 0.05$.

yellow within 7 days after ethylene treatment and in all of them the calyx abscised immediately after treatment. Treatment with 0.7, 1.5 or 3 $\mu\text{l}\cdot\text{L}^{-1}$ 1-ECP delayed ethylene-induced calyx abscission by 6-7 days (Table 15), but no significant delay of color change was observed. It was also found that treatment with 1-ECP caused some rust-like spots on the fruit surface and this adverse effect intensified as 1-ECP concentration increased (Table 16). Like the results of banana treated with 1-OCP, again it seems that the inhibition has a differential effect on color breakdown and calyx abscission.

Treatment with 0.5, 1 or 2 $\mu\text{l}\cdot\text{L}^{-1}$ 1-PCP did not inhibit calyx abscission (Table 15). This treatment had no effect on color change as well. The results that both 1-ECP and 1-PCP treatment did not inhibit peel color change are consistent with those of the study with 1-MCP on 'Sweetie' fruits intended to delay color change (page 38 of this report). In light of the results presented, it is suggested that, in contrast to other citrus

cultivars, ethylene might not be involved in the process of color change in 'Sweetie' fruit peel. This could be further supported by our finding that ethylene treatment did not significantly accelerate the color change of 'Sweetie' fruits.

10. Research on 1-MCP

10.1 Effect of 1-MCP on ethylene action in plant

10.1.1 Effect of 1-MCP on ripening of banana fruit. Several processes occur during ripening of banana fruits, the most obvious are climacteric ethylene peak, peel color change and pulp softening. These phenomena were used in our studies as indicators to assess banana fruit ripening and to determine the potency of the inhibitor to delay ripening.

Fig. 36. Effect of 1-MCP on ethylene production in banana fruits. Bananas were treated for 24 h with the indicated concentrations of 1-MCP, ventilated, and then exposed to ethylene ($300 \mu\text{l} \cdot \text{L}^{-1}$) for 24 h and thereafter stored in air at 22°C for ripening assessment. For ethylene treatment, fruits were closed in the jars for 24 h with air instead of with 1-MCP before exposure to ethylene for additional 24 h. Untreated fruits were kept in air containing jars during experiment.

10.1.1.1 Delaying ripening of banana fruit. Bananas treated with ethylene ($300 \mu\text{L}^{-1}$) ripened at 22°C 3 days after the treatment as determined by peel color change, climacteric ethylene production (Fig. 36) and fruit softening. Treatment with 1, 5, 10, 15 and $30 \text{ nl}\cdot\text{L}^{-1}$ 1-MCP immediately after harvest prior to exposure to ethylene ($300 \mu\text{L}^{-1}$, 24 h) delayed ethylene-induced ripening by 13 days in banana fruit stored at 22°C . The results show that as little as $1 \text{ nl}\cdot\text{L}^{-1}$ 1-MCP was sufficient to exert a maximal response.

10.1.1.2 Effect of repetitive treatment with 1-MCP. It appears that the duration of the inhibitory effect of 1-MCP on ethylene-induced fruit ripening is limited. In case of banana fruit, 13-14 days after the treatment the fruit resumes sensitivity to ethylene. This could be due to either (1) Dissociation of 1-MCP from the binding sites at the ethylene receptor or (2) Formation of new ethylene binding sites. (3) Metabolism of 1-MCP. In an additional study we treated banana fruit with $1 \text{ nl}\cdot\text{L}^{-1}$ 1-MCP for 24 h and then repeated this treatment after 10 days in storage at room temperature. It was found that this repetitive treatment with 1-MCP doubled the storage life of banana fruit compared with a single treatment. These results indicate that 10 days after the treatment with 1-MCP, active ethylene receptor binding sites not occupied with 1-MCP were present in the fruit. These sites could be blocked with 1-MCP 10 days after the first 1-MCP treatment. To determine whether the free ethylene-binding sites are newly formed sites or old sites dissociated from 1-MCP, studies with radioactive 1-MCP should be conducted.

Our preliminary studies show that the effect of 1-MCP in delaying ripening is maximal only if the treatment is applied immediately after harvest. Any delay in 1-MCP application reduces the efficiency of the treatment. We also found that 1-MCP

treatment should be given prior to ethylene treatment. Application of 1-MCP together with ethylene renders the 1-MCP not effective.

10.1.2 Effect of 1-MCP on ripening of avocado fruit. The most pronounced ripening processes in avocado fruit are: increased ethylene production, softening, increased activity of hydrolytic enzymes like cellulase and polygalacturonase (PG) and color change in some varieties like 'Hass'. In this study we have followed these ripening process in fruit treated with 1-MCP and assessed the potency of this inhibitor to delay ethylene-induced ripening in avocado fruit.

The fruit were harvested in a local plantation, placed in glass jars and treated with different concentrations of the 1-MCP for 24 h and then treated with ethylene ($300 \mu\text{L}\cdot\text{L}^{-1}$) for 24 h. All the experiments were conducted at 22°C . In these studies, we have used the avocado varieties 'Fuerte', 'Reed' and 'Hass'. We observed that in all the varieties, depending on the time of harvest during the season, the untreated fruit ripened within 5-7 days after harvest and treatment with ethylene shortened the period to ripening by 4 days.

10.1.2.1 Effect of 1-MCP on ethylene production by the fruit. When 'Fuerte' fruit were treated with low concentrations of 1-MCP ($1, 5$ or $15 \text{ nl}\cdot\text{L}^{-1}$), prior to the ethylene treatment, ethylene production increased 9-11 fold over the untreated fruit during the first 24 h. Thereafter, ethylene production decreased significantly, but still remained 3-5 fold of control. Compared with ethylene-treated fruits the onset of the ethylene climacteric peak was not affected by these treatments; the peak appeared 2-3 days after the treatment. Similar results were obtained with 'Hass' fruit. Increasing the concentration of 1-MCP to $30, 50$ or $70 \text{ nl}\cdot\text{L}^{-1}$ caused some further increase in ethylene production during the first 24 h, but ethylene production declined thereafter

to control level. The onset of the ethylene climacteric peak was delayed by 14 days in 'Hass' fruit and 13 days in 'Reed' fruit. Treatment with these concentrations of 1-MCP also reduced the height of the ethylene climacteric peak by 20%-30% compared with ethylene-treated fruits in both 'Hass' and 'Reed' fruit.

10.1.2.2 Effect of 1-MCP on color change in 'Hass' avocado fruit. Fruit treated with ethylene ($300 \mu\text{l}\cdot\text{L}^{-1}$) for 24 h displayed color change within 4 days. However, when the fruits were treated with 30, 50 or $70 \text{ nl}\cdot\text{L}^{-1}$ 1-MCP prior to the ethylene treatment color change was delayed by 12-14 days. Treatment with $70 \text{ nl}\cdot\text{L}^{-1}$ 1-MCP was the most effective in delaying color change.

10.1.2.3 Effect of 1-MCP on softening in avocado fruit. At harvest time, fruit firmness was about 13 kg (Fig. 37). Firmness in untreated fruit after 8 days after harvest was 3 kg. In the ethylene treated fruit firmness was 2 kg 4 days after harvest. However, fruit treated with 30, 50 or $70 \text{ nl}\cdot\text{L}^{-1}$ 1-MCP prior to ethylene treatment

Fig. 37. Effect of 1-MCP on softening of "Reed" avocado fruits. The fruits were treated for 24 h with 0 (▲), 30 (△), 50 (□) or $70 \text{ nl}\cdot\text{L}^{-1}$ 1-MCP, ventilated, and then exposed to ethylene ($300 \mu\text{l}\cdot\text{L}^{-1}$) for 24 h. Untreated fruits (■) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

retained their firmness (9 kg) for 16 days after harvest. Of the various concentrations of 1-MCP tested, 50 nl·L⁻¹ was sufficient to cause maximal inhibition of softening.

10.1.2.4 Effect of 1-MCP on cellulase activity. In ethylene treated fruit cellulase activity reached its highest value (2.5 fold over untreated fruits) 4 days after harvest. In untreated control fruit or fruit treated with 1-MCP (30, 50 or 70 nl·L⁻¹) prior to ethylene treatment, cellulase activity on day 4 was maintained at a level similar to that at harvest time. On the 8th day after harvest the activity in untreated control fruit doubled whereas in the 1-MCP treated fruit it was unchanged. On day 12, activity in control fruit reached its maximal value and among the 1-MCP treated fruit, in those treated with 30 nl·L⁻¹ the activity started to increase while in fruit treated with 50 or 70 nl·L⁻¹ 1-MCP the activity remained at a level similar to that at harvest. On the 16th day after harvest, cellulase activity in fruit

treated with 30 or 50 nl·L⁻¹ 1-MCP increased significantly whereas in fruit treated with 70 nl·L⁻¹ 1-MCP the activity of cellulase remained at the same value as at harvest. These results indicate that 70 nl·L⁻¹ is the most potent concentration of 1-MCP for the inhibition of cellulase activity in avocado fruit.

Fig. 38. Effect of 1-MCP on PG activity of "Hass" avocado fruits. The fruits were treated for 24 h with 0 (▲), 30 (Δ), 50 (□) or 70 (○) nl·L⁻¹ 1-MCP, ventilated, and then exposed to ethylene (300 μl·L⁻¹) for 24 h. Untreated fruits (■) were kept in air containing jars during treatment. After treatment, the fruits were stored at 22°C for ripening assessment.

10.1.2.5 Effect of 1-MCP on polygalacturonase (PG) activity. Ethylene treatment increased the activity of PG in 'Reed' and 'Hass' avocado fruit (Fig. 38). Treatment with 1-MCP prior to ethylene treatment greatly reduced the activity of this enzyme. On the 4th day after harvest, PG activity in ethylene treated fruit increase by 6 fold and reached its maximal value, in the untreated control fruit it increased by 2 fold whereas in 1-MCP treated fruit the activity increased only slightly. On the 8th day of harvest, PG activity in untreated control fruit continued to increase linearly reaching 5 fold over that at harvest time and among the fruit treated with 1-MCP, only in those treated with 30 $\text{nl}\cdot\text{L}^{-1}$ activity started to increase. On the 12th day after harvest the activity in untreated fruits reached maximal value while fruit treated with 30 $\text{nl}\cdot\text{L}^{-1}$ showed a continuous low rate increase in PG activity. On the 16th day after harvest, PG activity in fruit treated with 30 $\text{nl}\cdot\text{L}^{-1}$ 1-MCP increased slightly while in those treated with 50 or 70 $\text{nl}\cdot\text{L}^{-1}$ 1-MCP activity remained relatively low. It appears that the treatments with 70 $\text{nl}\cdot\text{L}^{-1}$ 1-MCP had the most inhibitory effect on PG activity in 'Hass' fruit while in 'Reed' 50 $\text{nl}\cdot\text{L}^{-1}$ 1-MCP was sufficient as well.

10.1.2.6 Effect of 1-MCP on pathogen infection in avocado fruit. During prolonged storage, avocado fruit treated with 30, 50 or 70 $\text{nl}\cdot\text{L}^{-1}$ 1-MCP showed less pathogen infections than the untreated or ethylene treated fruit. This effect could be either due to direct effect of 1-MCP on the pathogen or due to increased fruit resistance to pathogen infection via delaying ripening and fruit senescence.

10.1.2.7 Effect of 1-MCP on pathogen growth in avocado fruit peel. The purpose of this study is to investigate the effect of 1-MCP treatment on pathogen growth in avocado fruit. Sections of 'Fuerte' avocado peel 2 by 4 cm were placed in 0.5-l jars (2 in each). Each peel section was inoculated with 2 drops of *Colletotrichum*

or *Alternaria* suspension. Ten jars were used for each pathogen, the jars were sealed and 1-MCP ($300 \text{ nl}\cdot\text{L}^{-1}$) was injected through a septum stopper into half of the jars inoculated with each of the pathogen. Thus, each treatment contained 5 replicates. The treatment lasted for 24 h. The rate of germination of the pathogens on the fruit peels was examined. The results show no significant differences in the rate of pathogen germination between peel sections treated with 1-MCP and the untreated sections. Therefore, it was concluded that 1-MCP does not have a direct effect on pathogen

Fig. 39. Effect of 1-MCP on internal grey pulp in 'Fuerte' and 'Hass' avocado fruits after storage at 3°C (left) or 5°C (right) for 5 weeks. 'Fuerte' and 'Hass' were treated with 100 (B) or 300 (C) $\text{nl}\cdot\text{L}^{-1}$ 1-MCP for 24 h at 3°C or 5°C respectively and then stored in air at the same temperature for 5 weeks before the internal grey pulp was examined. Untreated fruits (A) were exposed to air during treatment.

infection in avocado fruit and the reduced pathogen infection in avocado fruit treated with 1-MCP was rather due to fruit resistance via delaying fruit ripening and senescence.

10.1.2.8 Effect of 1-MCP on physiological disorders in 'Fuerte' and 'Hass' avocado fruit. One of the physiological disorders in avocado fruit after harvest is the gradual appearance of internal grey pulp. In previous studies it was shown that ethylene treatment promoted the appearance of internal grey pulp in avocado fruit.

Treatment with 1-MCP inhibit this physiological disorder (refer to 10.2.1 of this section). The present study was carried out to further characterize effect of 1-MCP on the appearance of the disorder -- internal grey pulp.

'Hass' and 'Fuerte' avocados, harvested from the same local orchard during the commercial harvest time, were placed in 30-l plastic container (25 fruits in each); The fruits were treated for 24 h with 1-MCP (100 or 300 $\text{nl}\cdot\text{L}^{-1}$) at 3°C or 5°C and

Fig. 40. Effect of repetitive treatment of 1-MCP on ethylene production (left) and fruit softening (right) in 'Ettinger' avocados. After harvest, the fruits were treated with 50 $\text{nl}\cdot\text{L}^{-1}$ 1-MCP (○ and ●) or closed in air (■) for 24 h and then stored at 22°C for ripening assessment. Ten days later, half of the 1-MCP treated fruits were again treated with 50 $\text{nl}\cdot\text{L}^{-1}$ 1-MCP (●) for additional 24 h and then continued to store at 22°C for ripening assessment.

then stored at the same temperature for 5 weeks. The internal grey pulp was examined at the end of the storage period. Although both of the two cultivars were harvested from the same orchard the results show (Fig. 39) that 'Fuerte' fruits were more prone to develop internal grey pulp at both 3°C and 5°C than 'Hass' fruit. Treatment with 100 or 300 $\text{nl}\cdot\text{L}^{-1}$ 1-MCP markedly reduced the rate of internal grey pulp in both 'Fuerte' and 'Hass' fruit stored at 3°C and 5°C. In addition, storage temperature had a distinct influence on the occurrence of internal grey pulp. Both 'Fuerte' and 'Hass' fruit

stored at 5°C had much higher degree of internal grey pulp than those stored at 3°C. In conclusion, postharvest internal grey pulp can be completely inhibited by a combined treatment with 1-MCP (100-300 nl·L⁻¹) and storage at 3°C.

10.1.2.9 Effect of repetitive treatment of 1-MCP on ripening of avocado fruit. Results of experiments with 1-MCP on banana fruit show that two treatments with 1-MCP doubled the storage life of banana fruit compared with a single treatment (refer to 10.1.1.2 of this section). To investigate possible prolonging storage life of avocado fruit by repetitive treatment with 1-MCP, the following study was undertaken.

There were three treatments in this experiment: single treatment, repetitive treatment and untreated fruits. 'Ettinger' avocado fruit were placed 15-l jars (25 in each) and sealed. The fruit in 2 jars were treated with 1-MCP (50 nl·L⁻¹) for 24 h and one jar was left untreated. Ten days later, fruit in one of the jars treated again with 1-MCP (50 nl·L⁻¹) for 24 h (double treatment). Treatment and storage were conducted at 22°C.

The results show that compared with the untreated control fruit, the climacteric ethylene peak in fruit that received a single was delayed by 9 and double treatment by 16 days. Firmness of about 20 N was recorded 10 and 16 days later than in the ethylene treatment respectively (Fig. 40). The results indicate that as in the case of banana fruit, repetitive treatments with 1-MCP prolonged the storage life of 'Ettinger' avocado fruit as compared with a single treatment. These findings provide a method which enable to further prolong storage life of avocado fruit.

10.1.3 Effect of 1-MCP on ripening of tomato fruit. When mature green tomato fruit are exposed to external they ripen. Among the most obvious ripening processes are: peel color change, climacteric ethylene peak and fruit softening. These

phenomena were used as indicators in our study to determine ripening and the potency of the inhibitors to delay ripening of tomato fruit.

Mature green tomato fruit treated with ethylene ($300 \mu\text{L}\cdot\text{L}^{-1}$) for 24 h and kept at room temperature ripened 4-6 days after the treatment, as determined by peel color change, onset of the climacteric ethylene peak and fruit softening. Treatment with 5, 25, 100 or $250 \text{ nl}\cdot\text{L}^{-1}$ 1-MCP immediately after harvest prior to ethylene treatment ($300 \mu\text{L}\cdot\text{L}^{-1}$, 24 h) delayed ethylene-induced ripening by 6, 8, 10 and 11 days respectively in fruit stored at 22°C . Analysis of lycopene content in the fruit pulp on the 8th day after treatment revealed that lycopene content in fruits treated with 5, 25, 100 or $250 \text{ nl}\cdot\text{L}^{-1}$ 1-MCP was 31.5%, 49.4%, 67.3% and 71.6% respectively lower than that in ethylene-treated fruits.

10.1.4 Effect of 1-MCP on abscission of citrus leaves. One of the effects of ethylene on plants is related to abscission. During the abscission process, the activity of the enzymes cellulase and polygalacturonase (PG) in the abscission zone increase (Greenberg *et al.*, 1975), and breakdown of the cell walls occurs. The involvement of ethylene in enhancing abscission has been proven unequivocally by experiments conducted in many plants (Abeles *et al.*, 1992). In citrus leaf explants grown in culture, supplying ACC or ethephon accelerates abscission, while AVG (Sagee *et al.*, 1980) and hypobaric atmosphere delays abscission. The inhibitor of ethylene action, 2,5-norbornadiene, blocked abscission and the induction of cellulase and PG at the abscission zone (AZ) (Sisler *et al.*, 1985).

The aim of this study was to elucidate the effect of 1-MCP on ethylene induced abscission in citrus leaf explants. Our studies show that in 'Shamouti' orange explants treated with $20 \mu\text{L}\cdot\text{L}^{-1}$ ethylene for 6, 9, 18 and 24 h, abscission was recorded in 0%,

1%, 6% and 100% present of the explants respectively. This result indicates that the ethylene treatment duration between 18 and 24 h is the most effective for induction of abscission since it increased during this period of time by 94%. When citrus leaf explants were treated with 50 $\text{nl}\cdot\text{L}^{-1}$ 1-MCP for 3, 6, 9, 12 and 18 h prior to the ethylene treatment (20 $\mu\text{l}\cdot\text{L}^{-1}$ ethylene for 24 h), the abscission induced by ethylene was completely prevented during the first 36 h in all of 1-MCP treatments. However, after 48 h, 10-23% abscission was recorded in all 1-MCP treatments whereas in the ethylene treatment 100% of the explants abscised. Of all the different concentrations of 1-MCP, application of 50 $\text{nl}\cdot\text{L}^{-1}$ 1-MCP for 6 h was found to be sufficient to cause a maximal delay in ethylene-induced citrus leaf explants abscission. However, 96 h after the 1-MCP treatment, 100% of the explants abscised. When the 1-MCP concentration was increased to 100 $\text{nl}\cdot\text{L}^{-1}$, explant abscission was totally inhibited for 36 h after treatment (same as with 50 $\text{nl}\cdot\text{L}^{-1}$). But the treatment duration required for maximal effect with 100 $\text{nl}\cdot\text{L}^{-1}$ 1-MCP was shortened to 3 h. This indicates that increasing the 1-MCP concentration did not prolong the time that the explants were protected against abscission but rather shortened the treatment duration required to reach maximal efficiency.

10.1.4.1 Effect of 1-MCP on ethylene production and ethylene autoinhibition in citrus leaf explants. When citrus leaf explants were prepared, the leaves suffered dissection injuries. These injuries induce an increase in ethylene production (Abeles *et al.*, 1992). We found that the ethylene production in the leaves of untreated explants (not treated with ethylene or 1-MCP) was 202 $\text{nl}\cdot\text{gFW}^{-1}\cdot\text{h}^{-1}$. Treatment with ethylene (20 $\mu\text{l}\cdot\text{L}^{-1}$) reduced the ethylene production in the leaf explants by 40% 1 h after treatment. This phenomenon is known as "ethylene

autoinhibition" (Riov and Yang, 1982). Treatment with 1-MCP caused an increase in ethylene production over the control. Although treatment with 1-MCP delayed citrus leaf explant abscission, it induced an increase in ethylene production by the leaves.

Fig. 41. Effect of 1-MCP on the activity of endo- (A), endo- (B) cellulase and PG in the LA-ZA, the blade and the petiole parts near the abscission zone of 'Shamouti' orange citrus leaf explants. Explants were treated for 6 h with 20 µl·L⁻¹ or the indicated concentration of 1-MCP and then exposed to ethylene (20 µl·L⁻¹) for 18 h and thereafter stored in air at 22°C for abscission assessment. Untreated explants were kept in air containing jars during treatment. Activity of the enzymes was measured 6 h after treatment.

Ethylene production increased 5 fold 24 h after treatment with 1-MCP $50 \text{ nl}\cdot\text{L}^{-1}$ for 6 h (not related to cutting-injury-induced ethylene production). It appeared that since 1-MCP blocks the site of ethylene binding the induction of high rate of ethylene production by this inhibitor did not affect its ability to delay abscission.

10.1.4.2 Activities of *exo-cellulase*, *endo-cellulase* and *PG* in 1-MCP treated leaf explants. To investigate the relationship between abscission of citrus leaf explants and the changes in the activity of cellulase and PG under the influence of 1-MCP, we measured the activity of these enzymes in the blade part, LA-AZ (petiole and blade abscission zone) and in the petiole of citrus leaf explants treated with either $20 \mu\text{l}\cdot\text{L}^{-1}$ ethylene, 250 or $500 \text{ nl}\cdot\text{L}^{-1}$ 1-MCP or air (untreated control) for 6 h and then exposed to ethylene ($20 \mu\text{l}\cdot\text{L}^{-1}$) for 18 h. Six h after treatment, activity of *exo*- and *endo*-cellulase as well as of PG was measured. Our result show that while ethylene treatment significantly increased the activities of these enzymes, about 60%, 6 h after treatment, treatment with 1-MCP greatly reduced (between 50%-75%) the activity of *exo*- and *endo*-cellulase and PG in the abscission zone LA-ZA (Fig. 41). A similar effect was found in the blade and petiole parts near the abscission zone as well, but the changes in these regions were much less pronounced than in the abscission zone (Fig. 41).

10.1.5 Effect of 1-MCP on abscission in avocado leaf explants. Leaf explants prepared from 'Reed' avocado leaves behaved differently from those of citrus leaves; untreated control explants (not treated with ethylene) did not show abscission throughout the entire experiment which lasted 4 days. Treatment with ethylene ($1000 \mu\text{l}\cdot\text{L}^{-1}$) induced 82% abscission after 4 days. Treatment with 1-MCP (5, 15 and $100 \text{ nl}\cdot\text{L}^{-1}$) did not reverse the ethylene induced abscission in the avocado leaf explants.

10.1.6 Effect of 1-MCP on ethylene-induced growth modification in pea seedlings. Etiolated pea seedlings are widely used as a model for ethylene studies (Abeles *et al.*, 1992). When etiolated pea seedlings are exposed to ethylene, elongation is inhibited and swelling occurs in the epicotyle and root. Previous studies showed that under low pressure conditions, where the endogenous ethylene concentration is lowered (Apelbaum *et al.*, 1972) or as response to 2,5-NBD (Apelbaum, 1990), elongation is enhanced and the plumular hook opens to a degree which is correlated with the intensity of the treatment.

In the present study 2 days old etiolated pea seedlings grown in pots were exposed for 24 h to 1-MCP (5, 20, 30, 50, 70, 80, 100, 300 or 500 nl·L⁻¹) by injecting the inhibitor into the sealed glass containers, into which the plants were introduced. At the end of the 1-MCP treatment the containers were aerated and ethylene (50, 75 or 100 µl·L⁻¹) was injected into the containers. The seedlings were continuously exposed to ethylene for 5 days and then checked for epicotyle and root elongation, swelling and degree of hook opening.

Table 17. Effect of treatment with 1-MCP on ethylene induced growth inhibition and swelling in etiolated pea seedlings

Treatment	Length (cm)		Weight (g)		Swelling (g/cm)	
	<u>Epicotyle</u>	<u>Root</u>	<u>Epicotyle</u>	<u>Root</u>	<u>Epicotyle</u>	<u>Root</u>
Untreated control	9.08	9.78	0.34	0.14	0.037	0.014
1-MCP (50 nl·L ⁻¹)	9.61	10.56	0.36	0.17	0.038	0.016
C ₂ H ₄ (100 µl·L ⁻¹)	2.35	5.96	0.13	0.11	0.056	0.018
1-MCP(30 nl·L ⁻¹)+C ₂ H ₄	4.52	7.62	0.23	0.15	0.051	0.019
1-MCP(50 nl·L ⁻¹)+C ₂ H ₄	4.49	7.69	0.22	0.15	0.049	0.020
1-MCP(70 nl·L ⁻¹)+C ₂ H ₄	4.76	7.86	0.23	0.14	0.048	0.017
1-MCP(100 nl·L ⁻¹)+C ₂ H ₄	4.48	7.96	0.21	0.14	0.047	0.017

The results show (Table 17) that when the pea seedlings were exposed for 5 days to ethylene ($100 \mu\text{l}\cdot\text{L}^{-1}$) epicotyle growth was suppressed by 4 fold and root growth reduced by 40%, epicotyle weight was reduced 2.6 fold and root weight by 22%. Ethylene caused swelling; in the ethylene treated plants the epicotyle was 1.5 fold thicker and the root 28% thicker than the untreated control. Treatment with $50 \text{ nl}\cdot\text{L}^{-1}$ 1-MCP alone slightly increased growth as compared with untreated seedlings due to inhibition of the action of endogenous ethylene. Compared with the ethylene treatment, exposure to 1-MCP partially reversed the growth inhibition induced by exogenously ethylene. In plants treated with 1-MCP ($50, 70$ and $100 \text{ nl}\cdot\text{L}^{-1}$) prior to ethylene ($100 \mu\text{l}\cdot\text{L}^{-1}$), epicotyle growth increased 3.2 fold and root growth by 25%. In all cases plumular hook opening was observed in the 1-MCP treated plants compared with the ethylene treated ones. Treatment with $50 \text{ nl}\cdot\text{L}^{-1}$ 1-MCP was found sufficient to cause maximal inhibitory effect of ethylene action.

10.1.7 Influence of 1-MCP on ethylene-induced adverse effects in citrus fruit¹. Citrus fruit are nonclimacteric and produce only small amount of ethylene. However, endogenous ethylene and exogenously applied ethylene are involved in the regulation of citrus fruit maturation and senescence, and thereby have some adverse effects on keeping quality of the fruit (Kader, 1992; Stewart and Wheaton, 1972; Apelbaum *et al.*, 1976). In the present study, we examined the effects of treatments with ethylene and the inhibitor of the ethylene action 1-MCP, on the postharvest quality of 'Shamouti' [*Citrus sinensis* (L.) Osbeck] oranges. The results show that neither ethylene nor 1-MCP had any effect on fruit weight loss and firmness. However,

¹ Experiments carried out at the laboratory of Dr. Ron Porat, Dept. of Postharvest science of Fresh Produce, ARO, The Volcani Center, Bet-Dagan, 50250, Israel

ethylene increased the appearance of chilling injury symptoms, increased stem-end rot, increased the content of off flavor volatiles in the juice head space and in the fruit internal atmosphere, but reduced mold rots. Treatment with 1-MCP ($50 \text{ nl}\cdot\text{L}^{-1}$) effectively inhibited the ethylene induced degreening of 'Shamouti' orange, but was ineffective in preventing the adverse effects of ethylene during storage; it rather accelerated peel aging and increased chilling symptoms as well as increased decay and accumulation of off flavor volatile. In the light of these findings it is suggested that while high concentrations of exogenously applied ethylene are undesirable during storage due to their enhancement of fruit deterioration, the low level of ethylene produced by the fruit is required to maintain the natural fruit resistance to various environmental and pathological stresses. Therefore, 1-MCP may be applied as a postharvest treatment to inhibit the degreening process in citrus fruit varieties which are preferably marketed green. However special care must be taken to avoid chilling injury symptoms and decay development in these fruit.

10.1.8 Effect of 1-MCP on superficial scald of 'Granny Smith' apple².

Superficial scald is one of the physiological disorders in 'Granny Smith' apple (*Malus domestica* Borkh.) fruit. In the present study, the effect of 1-MCP on the development of superficial scald on 'Granny Smith' apple fruit was investigated. Granny-Smith apples of uniform size and color were harvested at Kfar Etzion, Israel, during the commercial harvest period. At harvest time, 20 apples were measured for firmness, SSC (soluble solids concentration) and TA (titratable acidity) content. Immediately after harvest, the fruit were divided into four lots, each one of them containing 40 apples. All the four lots were closed in four 15-l jars respectively, two of them were

² Experiments carried out with the cooperation of Dr. Susan Lurie at her laboratory at the Dept. of Postharvest Science of Fresh Produce, ARO, The Volcani Center, Bet-Dagan, 50250

treated with 1000 nl·L⁻¹ 1-MCP and two of them with air (as control) for 24 h at room temperature. At the end of treatment, O₂ in the jars was found to be 15%, CO₂ 2.3% and C₂H₄ 0.2 µl·L⁻¹. After treatment the fruit were placed in storage at 0°C for 3 or 5 months. At each removal from the storage, 10 apples from each treatment were assayed for α-farnesene and conjugated trienes in the peel. The rest were held at 20°C for a period of 7 days of shelf-life simulation. During the simulation period, ethylene production and respiration were measured daily on 10 apples from each treatment. At the end of simulation, superficial scald, firmness, SSC and TA were determined on 20 apples from each treatment.

The results show that treatment with 1000 nl·L⁻¹ 1-MCP completely inhibited superficial scald on 'Granny Smith' apples. During shelf life following storage at 0°C

Table 18. Effect of 1-MCP on ripening parameters and superficial scald of 'Granny Smith' apple fruit. The fruit were treated for 24 h with 1000 nl·L⁻¹ 1-MCP followed by storage at 0°C for 3 and 5 months plus a 7 day simulation of shelf life at 20°C. Data were collected at harvest as well as at removal from storage and at the end of shelf life.

Treatment	Scald index (%)	Firmness (N)		SSC (% TA (%))	
Harvest		62.3 fg		11.0 a	0.80 c
3 months + 7 days		At removal from storage		End of shelf life	
1000 nl·L ⁻¹ 1-MCP	0	62.3 fg	39.2 c	12.0 b	0.80 c
Control	88	53.8 e	31.1 ab	11.4 a	0.62 b
5 months + 7 days		At removal from storage		End of shelf life	
1000 nl·L ⁻¹ 1-MCP	0	58.3 f	32.5 b	11.7 a	0.68 b
Control	93	45.4 d	25.8 a	11.7 a	0.55 a

Different letters indicate statistical differences at $P = 0.05$.

Table 19. Effect of 1-MCP on ripening parameters and superficial scald of 'Granny Smith' apple fruit. The fruit were treated for 24 h with 1000 nl·L⁻¹ 1-MCP followed by storage at 0°C for 3 and 5 months plus a 7 day simulation of shelf life at 20°C. Data were collected at harvest as well as at removal from storage and at the end of shelf life.

Treatment	α -Farnesene (nm/cm ²)	Conjugated trienes (nm/cm ²)
3 month storage		
1000 nl·L ⁻¹ 1-MCP	12	1
Control	61	15
5 month storage		
1000 nl·L ⁻¹ 1-MCP	23	5
Control	78	21

*Different letters indicate statistical differences at $P = 0.05$.

Fig. 42. Effect of 1-MCP on ethylene production (A, B) and respiration (C, D) of 'Granny Smith' apples during shelf-life simulation. After harvest, the fruits were treated with 1000 nl·L⁻¹ 1-MCP (●) or exposed to air (○) for 24 h at 0°C and then stored at the same temperature for 3 (A, C) or 5 (B, D) months and thereafter held at 20°C for shelf-life simulation.

for 3 or 5 months, the apples lost 37%-49% of their firmness. However, 1-MCP treated fruit were firmer than control fruit and retained higher TA (Table 18). After 3 and 5 months of storage at 0°C plus a 7 day simulation of shelf life at 20°C, the 1-MCP treated fruit were completely healthy while superficial scald on control fruit was serious and the scald

index of control fruit reached 88%-93% (Table 18). The levels of α -farnesene and conjugated trienes in the peel of 1-MCP treated fruit were concomitantly lower than that of control fruit (Table 19). In addition, 1-MCP depressed respiration and totally inhibited ethylene production by the fruit (Fig. 42). It was suggested that 1-MCP inhibited superficial scald by inhibiting volatile development. This inhibition may be due to the effect of

1-MCP on ethylene production and hence its inhibitory action on fruit ripening and senescence.

Fig. 43. Effect of 1-MCP on fruit softening of 'Flavortop' nectarines during shelf life at 20°C after treatment (up) or after storage at 0°C for 4 weeks (down). After harvest, the fruits were treated with the indicated concentrations of 1-MCP for 24 h at 20°C, and then immediately or after storage at 0°C for 4 weeks, held at 20°C for 7 days of shelf-life simulation. Fruit firmness was measured at the end of shelf life.

10.1.9 Effect of 1-MCP on Ripening and Storage of 'Flavortop' Nectarines³.

Fruit of 'Flavortop' nectarine (*Prunus persica*) were harvested on July 14, 1999 from Gush Etzion. Sixty

nectarines were placed in each of four 15-l glass jars and sealed.

Three concentrations

of 1-MCP, 10, 100 or

1000 $\text{nl}\cdot\text{L}^{-1}$ were

injected respectively

into 3 jars, and the last

held sealed as a

control. Incubation

was for 24 h at 20°C

and upon opening 20

fruits were removed

to shelf life at 20°C,

and 40 placed for 4

weeks storage at 0°C and then removed to 20 °C shelf life.

CO_2 , O_2 , C_2H_4 gas concentration accumulated during 24 h of 1-MCP incubation in the sealed containers was measured before opening the jars. Ethylene and CO_2 production by the fruit were followed with 5 fruits from each treatment during

Fig. 44. Effect of 1-MCP on pulp woolliness (up) and reddening (down) of 'Flavortop' nectarines during shelf life at 20°C. After harvest, the fruits were treated with the indicated concentrations of 1-MCP for 24 h at 20°C and then stored at 0°C for 4 weeks, before being held at 20°C for 7 days of shelf-life simulation. Fruit pulp woolliness and reddening were measured at the end of shelf life.

³ Experiments carried out with the cooperation of Dr. Susan Lurie at her laboratory at the Dept. of Postharvest Science of Fresh Produce, ARO, The Volcani Center, Bet-Dagan, 50250

20°C shelf life. Fruit firmness was measured on 15 fruits from each treatment after 7 days at 20°C shelf life. Physiological disorders (woolliness and flesh reddening) were determined in the nectarine flesh at the end of shelf life after storage.

During 24 h 1-MCP incubation CO₂ concentration increased to 30%, O₂ dropped to 1.4% and ethylene accumulation to 2 µl·L⁻¹ inside the jars. There were no significant differences between the 3 concentrations of 1-MCP used.

10.1.9.1 Effect of 1-MCP during 20 °C shelf life after treatment. Ethylene production was low for the first 6 days and then began to increase. Only 1000 nl·L⁻¹ 1-MCP affected ethylene production by decreasing it. Respiration was not affected by 1-MCP. Fruit firmness measured at the end of 7 day shelf life at 20°C showed that control fruit were the softest, while increasing 1-MCP concentrations led to increased firmness (Fig. 43).

10.1.9.2 Effect of 1-MCP during 20 °C shelf life after 0 °C storage. At the end of storage at 0°C for 4 weeks, 5 fruits from each treatment were again monitored for ethylene and CO₂ production. Respiration was similar in fruit from all treatments. Control fruit had higher ethylene production than 1-MCP treated fruit for the first 4 days of shelf life and then 1-MCP treated fruit increased their ethylene production.

Fruit firmness at the end of 7 day shelf life at 20°C following storage showed the same pattern as without storage. Control fruit were softest while increasing concentrations of 1-MCP led to increased firmness (Fig. 10-8).

Physiological disorders were determined in the nectarine flesh at the end of shelf life after storage. The 1-MCP treated fruit had a higher woolliness index (Fig. 44) and more flesh reddening (Fig. 44) than the control fruit. Woolliness in nectarines is caused due to water absorption by the pectin in the tissue. PG activity which degrade

Fig. 45. Effect of 1-MCP on fruit softening of 'Red Rosa' plums during shelf life at 20°C after treatment (left) or after storage at 0°C for 5 weeks (right). Upon harvest, the fruits were treated with the indicated concentrations of 1-MCP for 24 h at 20°C, and then immediately or after storage at 0°C for 5 weeks, held at 20°C for 14 days of shelf-life simulation. Fruit firmness was measured 7 and 14 days of shelf life.

the pectin can prevent the woolliness. It is suggested that since ethylene induces PG activity, inhibition of ethylene action by 1-MCP which prevents the induction of increase in PG activity is, at least in part, the cause of woolliness in 1-MCP treated fruit.

10.1.10 Effect of 1-MCP on Ripening and Storage of 'Red Rosa' Plums⁴.

Fruit of 'Red Rosa' plum (*Prunus saliciana*) were harvested on August 1, 1999 from Kvitzat Shiller. One hundred fruits were placed in each of four 15-l glass jars and sealed. 1-MCP of 10, 100 and 1000 nl·L⁻¹ was injected into 3 jars and the fourth left as a control. Incubation was for 24 h at 20°C. After opening 30 fruits were removed to 20°C shelf life, and the rest placed in 0°C storage for 5 weeks.

CO₂, O₂, C₂H₄ gas concentration accumulated during 24 h of 1-MCP incubation in the sealed containers was measured before opening the jars. Ethylene and

⁴ Experiments carried out with the cooperation of Dr. Susan Lurie at her laboratory at the Dept. of Postharvest Science of Fresh Produce, ARO, The Volcani Center, Bet-Dagan, 50250

CO₂ production by the fruit were followed with 5 fruits from each treatment during 20°C shelf life. Fruit firmness was measured on 15 fruits from each treatment after 7 and 14 days at 20°C shelf life. Physiological disorders (woolliness and flesh reddening) were determined in the plum flesh at the end of shelf life after storage.

At the end of 24 h 1-MCP incubation CO₂ concentration was 9%, O₂ was 10% and ethylene 300 nl·L⁻¹ inside the jars. There were no significant differences between the 1-MCP treatments.

10.1.10.1 Effect of 1-MCP during 20°C shelf life after treatment. Ethylene production was very low for 11 days, and then control fruits began to increase in ethylene while 1-MCP treated fruit remained low. There was no difference in respiration among the treatments. Firmness of 1-MCP treated fruit was higher after both 7 and 14 days of shelf life, although relationship between 1-MCP concentration and reduction in firmness was not seen here (Fig. 45).

10.1.10.2 Effect of 1-MCP during 20°C shelf life after 0°C storage. After removal from storage at 0°C for 5 weeks, ethylene production was very low for 10 days, at which point all began to increase in ethylene, although production from control fruit was higher than from 1-MCP treated fruit. Respiration was not affected by 1-MCP. Fruit firmness retention was observed in 1-MCP treated fruit during ripening after storage, but not as dramatically as before storage (Fig. 45).

10.1.11 Effect of 1-MCP on citrus albedo callus growth. Callus, formed from the albedo of Valencia or Star Ruby grapefruit grown in solid media, produces ethylene, which accumulate in the culture tube and may cause inhibition of callus growth. To overcome this possible growth inhibition caused by ethylene, the callus was exposed to 1-MCP.

The tubes containing the albedo callus were placed in 3-l jars with the covers of the tubes loose to allow penetrating of 1-MCP into the tubes. The jars were sealed and the appropriate concentration of 1-MCP injected. The treatment lasted 24 h.

The results show that treatment with 1 or 50 $\text{nl}\cdot\text{L}^{-1}$ 1-MCP slightly stimulated callus growth compared with the control. The little effect of 1-MCP on callus growth in this system may be attributed to three factors: 1) The concentration was insufficient. 2) 1-MCP did not reach the callus as the cover of the tube containing the callus was not completely open during treatment. 3) Possible absorption of 1-MCP by the culture agar media. Therefore we designed a second experiment.

In the second experiment, the tubes containing the albedo callus were placed in 3-l jars with their covers opened to allow better exposure of the callus to 1-MCP. Higher concentrations of 1-MCP ($100 \text{ nl}\cdot\text{L}^{-1}$ and $1000 \text{ nl}\cdot\text{L}^{-1}$) were used to treat the callus. However, in this second experiment, the results obtained were similar to those of the first one. This suggests that the ethylene accumulated in the culture tubes does not inhibit the growth of the citrus albedo callus and other factors inhibit the growth of the citrus albedo callus.

10.2 Preliminary studies for the evaluation of possible commercial use of 1-MCP in postharvest handling of fruit

The action of ethylene, whether it is endogenously produced or exogenously applied, is a key factor in the induction and stimulation of ripening in climacteric fruit (Abeles *et al.*, 1992). Thus, it is of outmost importance to control ethylene action during the process of postharvest handling of climacteric fruit. We have examined the potency of 1-MCP to inhibit ethylene action in the climacteric fruit: banana, avocado

and tomato as well as orange which is non-climacteric fruit (refer to 10.1.1-3 and 10.1.7 of this section). In laboratory experiments an individual fruit in a 3 liter glass jar was treated with 1-MCP. In the following studies, we investigated the effect of 1-MCP on ethylene action on large quantities of fruit: 25-40 fruit were placed in a 30-l plastic container and treated with 1-MCP. In addition, we checked the influence of storage temperature on the efficiency of 1-MCP treatment. These experiments we found necessary to assess possible commercial use of 1-MCP. In all the experiments designated to evaluate the potency of 1-MCP for use in a commercial scale, the fruit were not treated with ethylene, only the effect of 1-MCP on spontaneous ripening was tested.

10.2.1 Preliminary studies with 1-MCP for the use in avocado industry ⁵.

We found in laboratory experiments that treatments with 30, 50 or 70 nl·L⁻¹ 1-MCP effectively delayed ethylene-induced ripening in avocado fruit (refer to 10.1.2 of this section). However, all these experiments were carried out on one fruit in a 3-l jar. In the present experiments, we investigated the effect of 1-MCP on spontaneous ripening in large quantities of avocado fruit at three different temperatures. Twenty to 30 avocados (cultivars: 'Ettinger', 'Fuerte' and 'Hass') were placed in a 30 liter plastic container and treated for 24 h with 100 or 300 nl·L⁻¹ 1-MCP. The temperature during the treatment and storage was at 4°C, 8°C or 20°C. The fruit were stored at each temperature for one month and then transferred to shelf life at 20°C for one week.

The results show that after storage for one month and transferred to shelf life at 20°C, spontaneous fruit ripening in all the cultivars tested was delayed by only 3-5

⁵ Experiments carried out with the cooperation of Prof. Dov Prusky at his laboratory at the Dept. of Postharvest Science of Fresh Produce, ARO, Volcani Center, Bet-Dagan, 50250

days by treatment with 1-MCP compared with the untreated fruit stored at the same temperature. Although from the commercial point of view prolonging shelf life by 3-5 days is of great significance for the avocado industry, these results are quite different from those of the laboratory experiment carried out in 3 liter glass jars, where avocado ripening was delayed by 8-14 days (refer to 10.1.2 of this section). The reason for this reduced potency of 1-MCP in the present experiment could be explained by partial absorption of the compound by the plastic container. Nevertheless, the results indicated that the efficiency of the 1-MCP treatment increased with the increase in 1-MCP concentration and decreased with lowering the temperature from 20°C to 4°C. In another, similar, experiment carried out with 25 fruit in a 25 liter glass container the results were similar. It should be noted that we found in these experiments that treatment with 1-MCP decreased the development of internal grey pulp in avocado fruit and this effect intensified as the treatment prolonged (Table 20).

10.2.2 Preliminary studies with 1-MCP for the use in 'Sweetie' industry ⁶.

'Sweetie' is an early-maturing seedless pummelo-grapefruit hybrid. The color of 'Sweetie' fruit turns from green to yellow within several days after harvest. In Israel, the fruit is picked early in the season and is marketed to Japan and European countries in its green color. However, in some cases the fruit degreens during postharvest shipping and storage and, then, loses its commercial value, since the consumers prefer green 'Sweetie' fruit. Endogenous ethylene and exogenously applied ethylene are involved in the regulation of citrus fruit maturation and senescence (Kader, 1992; Apelbaum *et al.*, 1976; Stewart and Wheaton, 1972). In some citrus cultivars,

⁶ Experiments carried out with the cooperation of Dr. Ron Porat at his laboratory at the Dept. of Postharvest Science of Fresh Produce, ARO, The Volcani Center, Bet-Dagan, 50250

treatment with ethylene accelerates color change (Porat *et al.*, 1998). At the beginning of this project, the effect of treatment with 1-MCP on the postharvest quality of 'Shamouti' [*Citrus sinensis* (L.) Osbeck] orange fruit was investigated (refer to 10.1.7

Table 20. Effect of 1-MCP on the development of internal grey pulp in 'Fuerte' avocado fruit. The fruit were treated with 100 nl·L⁻¹ 1-MCP for 24 h or 48 h and then stored in air at 20°C before internal grey pulp measurement.

Treatment	Internal grey pulp (%)
untreated control	50.1 d
C ₂ H ₄ , 5 µl·L ⁻¹	100.0 c
1-MCP, 100 nl·L ⁻¹ , treated for 24 h	29.8 b
1-MCP, 100 nl·L ⁻¹ , treated for 48 h	11.6 a

*Different letters indicate statistical differences at $P = 0.05$.

of this section; Porat *et. al*, 1999). The results showed that treatment with 1-MCP (50 nl·L⁻¹) effectively inhibited the ethylene induced degreening of 'Shamouti' orange. In the light of this finding it was assumed that 1-MCP could be applied as a postharvest treatment to inhibit the degreening of 'Sweetie'.

To investigate possible delay in color change of 'Sweetie' fruit by inhibition of ethylene action, the effect of 1-MCP, ethylene and gibberellic acid at various temperatures on the postharvest degreening of 'Sweetie' fruit was assessed. For assessment the effect of 1-MCP, 25 fruits per treatment were exposed to 100 or 200 nl·L⁻¹ 1-MCP for 24 h at 2, 6 12 and 20°C and stored for four weeks at these temperatures respectively. After storage the fruit were transferred for one week to 20°C to simulate shelf life. During the storage and shelf life period, color change of the fruit was measured weekly using a Minolta Colorimeter. We found that the storage

temperature was critical for retaining fruit color: at low temperature (2°C) the fruit retained its green color for a period of up to 5 weeks, whereas at higher temperatures (6, 12 and 20°C) there was a gradual increase in the rate of degreening respectively. Also 50 nl·L⁻¹ gibberellic acid application, either given as preharvest sprays in the field or as postharvest dipping treatment, effectively retained the fruit green color and reduced the rate of degreening. Exposure to ethylene at concentration up to 100 µl·L⁻¹ for 3 days had little effect on fruit degreening and treatment with 1-MCP at concentration up to 200 nl·L⁻¹ for 24 h had no effect at all on the peel color change in 'Sweetie'. This raised the question whether the rate of color change in this fruit after harvest is influenced by ethylene.

The fact that 1-MCP did not influence color change in 'Sweetie' while it did in 'Shamouti' oranges, and that color change in 'Shamouti' oranges can be induced by ethylene, but not in 'Sweetie' fruit, may suggest that the mode of induction of color change may be different in these two citrus cultivars. This is further demonstrated by our finding that newly synthesized putative inhibitors of ethylene action, such as 1-ECP and 1-PCP, also have no effect on color change in 'Sweetie' (refer to 9 of this section).

10.2.3 Preliminary study with 1-MCP for the use in tomato industry.

Mature green tomato fruit ripen within 2-4 days after exposure to ethylene. In previous experiments we found that treatment with 5 nl·L⁻¹ 1-MCP effectively delayed ethylene-induced (300 µl·L⁻¹) ripening of mature green tomato fruit (refer to 10.1.3 of this section). The most obvious phenomena among the ripening processes of mature green tomato fruit are peel color change from green to yellow, climacteric ethylene peak and fruit softening. Using these phenomena as indicators for ripening, 25 mature green tomatoes were enclosed in a 15 liter glass jar and treated with 5 nl·L⁻¹ 1-MCP

for 24 h (no treatment with ethylene). The results showed that these fruit were maintained in an unripening stage for 14-16 days at room temperature. This is 6-8 days longer than those fruit not treated with 1-MCP under the same condition. The result of this experiment provides another tool to prolong the storage period in postharvest handling of tomato fruit.

III. DESCRIPTION OF THE COOPERATION

During the three years of this research project, there was a constant contact by e-mail, fax and telephone calls between the Israeli and American groups. Dr. Prof. E. C. Sisler routinely supplied, by air delivery, the inhibitors that he developed and synthesized. In August 1997, just before this project began, Dr. Prof. R. Goren visited the USA laboratory for a period of two weeks in order to get familiar with the methods of 1-MCP synthesis and handling techniques for the inhibitor. This visit was very successful, it allowed the Israeli group to adopt the method of synthesis used and treatment procedures in a relatively short time. The research plans for the first year by both groups were discussed and determined. In August 1998 and in July 1999 and in July 2000, Dr. Prof. Goren visited again the USA group laboratory for the second and third and fourth time, respectively. In April 2000, Dr. Prof. Sisler visited the Israeli laboratory for a period of one week. Each meeting of such kinds was an excellent occasion to discuss and evaluate the results obtained thus far by the Israeli and by the American groups. During the visiting, the research results of the two groups were summarized, the annual reports of the two groups combined and future studies discussed. This kind of visiting also provided good chances for both the Israeli and American groups to exchange opinions and to communicate directly. The close cooperation and the constant exchange of ideas and evaluation of each step of the research, developed a very fruitful collaboration between the two groups and laid an excellent basis for satisfactorily finishing the project.

IV. EVALUATION OF THE RESEARCH

ACHIEVEMENTS

The objectives outlined for this research were: (1) To synthesis new compounds that interact with and block the ethylene receptor. (2) To test the new and recently synthesized blockers of the ethylene receptor, in various plant systems, with special emphasis on inhibition of ethylene action in agricultural important plants.

The following chapter will describe how the objectives and goals set forward in the research proposal were met during the course of this research project.

Normal start up problems were encountered by the Israeli group at the beginning of this project. However, these were quickly solved. Procedures for preparing the analogue compounds of 1-MCP were successfully developed and assay procedures for each compound worked out. A major problem with the newly synthesized compounds was to develop methods for transporting and handling them. The compounds are unstable in their active form. The lithium salt of these compounds is stable but very reactive. It was found that under nitrogen, the lithium salt can be easily handled and the compounds can be transferred from the USA to Israel without loss of activity. This method developed for the transportation of the compounds will, certainly, be useful for future utilization of the compounds in practice.

Thirteen new putative inhibitors of ethylene action were synthesized during the course of this project. After some preliminary studies, nine promising inhibitors were selected for in-depth studies to assess their potency to inhibit ethylene action. The

following eight compounds are structural analogues of 1-MCP with substitution in the 1-position with a side chain containing 2 to 10 carbons: 1-ECP (1-ethylcyclopropene), 1-PCP (1-propylcyclopropene), 1-BCP (1-butylcyclopropene), 1-PentCP (1-pentylcyclopropene), 1-HCP (1-Hexylcyclopropene), 1-HeptCP (1-heptylcyclopropene), 1-OCP (1-octylcyclopropene) and 1-DCP (1-decylcyclopropene). The compound H1-MCP (cyclopropene-1-methanol) is an exception, it contains a methanol group in the 1-position.

The Israeli group developed procedures to evaluate the potency of the compounds to block the receptor and inhibit ethylene action and thereby assess their scientific and practical potential. A wide scope of systems like climacteric fruits (banana, avocado and tomato), non-climacteric fruits (citrus), the triple response (etiolated peas), and leaf abscission (citrus) as well as effect on ethylene-induced disorders or adverse effect like: woolliness in nectarine, grey pulp in avocados, softening in plums, superficial scald in apples and degreening of Sweetie (Oroblanco) fruit. Procedures were developed to elucidate the generality of the response of plants to the compounds synthesized. In physiological and hormonal studies, the generality of the effects is to be established (Jacobs 1959, Hua *et al.* 1998). Our results indicate that in principle the general inhibitory effect of all the synthesized compounds, 1-MCP and its analogues, was well recognized in the above systems.

As with 1-MCP, the compounds synthesized were expected to be putative inhibitors of ethylene action exerting their effect by binding to the ethylene receptor sites (Sisler and Serek, 1997). In the present study, several measures were undertaken and extreme conditions were employed to determine the potency of these compounds in delaying fruit ripening: (1) The effect of the compounds on ethylene-induced

ripening rather than on spontaneous ripening was studied. (2) High concentration of ethylene ($300 \mu\text{l}\cdot\text{L}^{-1}$) was used to induce ripening. This concentration was up to several thousand fold of the concentration of the inhibitors used. This measure was taken in case the inhibition of ethylene binding by 1-MCP is competitive rather than non-competitive in nature. (3) During the treatment and the follow-up of ripening the fruits were held at 22-25°C, which is considered close to the optimal temperature for acceleration of fruit ripening.

The results of preliminary experiments with 1-MCP showed that maximal inhibitory effect of 1-MCP was obtained only when the compound was applied at the pre-climacteric stage immediately after harvest. Any delay in application of the inhibitor reduced the efficiency of the treatment or annihilated it. In addition, we found that treatment with the inhibitors was effective only if applied prior to exposure to ethylene. Therefore simultaneous exposure of the fruit to the inhibitor and ethylene reduced the potency of the inhibitor. In light of the above, in all the studies with the newly synthesized putative inhibitors presented herein, the fruit or vegetative tissue were exposed to the inhibitor immediately after harvest prior to ethylene treatment.

The results of the first year's research indicate that in principle the general inhibitory effect of 1-MCP was well recognized in all of the above systems. In contrary to 1-MCP, the analogue H1-MCP has differential responses in banana as far as the color breakdown and pulp softening are concerned. Although it is known that these are two different physiological events, the finding that H1-MCP is capable to inhibit ethylene-induced chlorophyll breakdown while failing to delay the ethylene induced softening may serve as an experimental tool to further understand the mechanism by which ethylene controls the two systems. Like 2,5-norbornadiene (Sisler *et al.*, 1985),

1-MCP inhibited the activity of cellulase and polygalacturonase which was causing a delay in citrus leaf abscission, but it did not reverse ethylene-induced abscission of avocado leaves. It is noteworthy that citrus and avocado, both belonging to subtropical evergreen trees, responded differently to 1-MCP and ethylene in relation to their abscission behavior. These results indicate that a more specific insight to the availability of ethylene receptors should be investigated.

The results showed that potency of the new compounds to inhibit fruit ripening and other ethylene-induced phenomena in plants, is well recognized in all of our test systems. Most of the inhibitors tested are capable of inhibiting ethylene action in all or some of our test systems, probably by competing for the site of binding in the ethylene receptor. Treatments with six of the nine inhibitors: 1-ECP, 1-PCP, 1-BCP, 1-PentCP, 1-HCP or 1-OCP delayed ethylene-induced ripening of banana, avocado and tomato fruits. They delayed ethylene-induced abscission in citrus leaf and reversed ethylene-induced growth inhibition and swelling in pea seedlings. However, the compounds H1-MCP, 1-HeptCP and 1-DCP were not effective on some of the plant materials tested. H1-MCP was revealed to have only little effect on ethylene-induced ripening in mature green tomato fruit. The compound 1-HeptCP did not have any effect on ethylene-induced ripening in avocado fruit and 1-DCP did not show any effect on ethylene-induced avocado fruit ripening and abscission in citrus leaf explants, but both of them inhibited ethylene action in all of the other plant materials tested.

There are also fluctuations in the responses as related to the concentrations of the various putative inhibitors of ethylene action used as a pre-treatment application. However, it is noteworthy that a competition exists between the analogues and ethylene when applied simultaneously. The concentrations of the inhibitors required to

exert a maximal effect in vegetative tissues, like leaves explants and pea seedlings, are higher than those required for fruits. The differences in these responses may originate from: (1) differences in cell sensitivity to the inhibitor, (2) availability of free receptor sites and (3) differences in catabolic breakdown of the inhibitor. In some cases delay in treatment time may lead to ethylene production and as a result higher concentration of the inhibitor is needed. Maximal inhibition of the various ethylene-induced phenomena studied was achieved within a range of concentrations between $5 \text{ nl}\cdot\text{L}^{-1}$ and $2000 \text{ nl}\cdot\text{L}^{-1}$. Some of the new inhibitors tested require much higher concentration than others to exert the same effect and some when applied at the same concentration, block the receptor for a longer time than others like in the case of banana fruits (Table 5 in section 4.10, II). The data may indicate that the characteristics and availability of the ethylene receptors in the various organs and plants are different, and the potency of the inhibitors is greatly affected by their molecular structure and size.

The results obtained in this study are quite surprising and important. Burg and Burg (1967) determined the $1/2$ maximum value for ethylene activity of ethylene, propylene and 1-butene in pea growth. The amount required was $0.1 \mu\text{l}\cdot\text{L}^{-1}$ for ethylene, $10 \mu\text{l}\cdot\text{L}^{-1}$ for propylene and $25,000 \mu\text{l}\cdot\text{L}^{-1}$ for 1-butene. The action of these unsaturated hydrocarbones reflects their interaction with the receptor. It might be expected that inhibitors blocking the receptor would similarly follow this pattern. This did not happen. Although the amount of inhibitor required did increase with 1-methyl, 1-ethyl and 1-propyl cyclopropene, the magnitude was much smaller, and with butyl, hexyl and octyl propenes, it declined in some cases to a level lower than 1-MCP. Why these larger compounds are so very active is not known, but the fact that they would be very lipid soluble probably is involved.

Most of the nine new compounds were shown to be potent inhibitors since they counteracted ethylene action under extreme conditions. As a result, fruits were kept in an unripe stage even though they were treated for 24 h with high concentration of ethylene ($300 \mu\text{l}\cdot\text{L}^{-1}$) and were stored at the range of 22-25°C which is optional for hastening fruit ripening. The duration of the period that the fruit were kept unripe after treatment with the inhibitors, varied from 2 to 16 days. The factors affecting this period were: (1) The molecular structure of the inhibitor; (2) The concentration of the inhibitor; (3) The plant materials tested.

All the fruits treated with the various inhibitors resumed normal ripening after recovery from the inhibition. The renewed sensitivity to ethylene suggests that at this point, free binding sites on the ethylene receptor are present in the tissue. Whether these are newly formed binding sites or sites that have become dissociated from the inhibitor or possible metabolism of the inhibitor in the tissue is not yet clear. At any event, the fact that the fruits recover from the inhibition of ripening after a certain period of time is of great importance when considering the inhibitors for practical use.

It appears that the newly synthesized analogues of 1-MCP are less potent inhibitors of ethylene action than 1-MCP. At lower concentrations, some of the analogues are more effective than others in certain plant system or certain ripening processes. Thus, presenting new inhibitors of ethylene action which have differential effects on various plant responses to ethylene, provide a broad spectrum of means and tools for controlling ethylene action in scientific studies and / or in practical use. The determination of analogue thus should depend on the plant organ to be used and the duration of delay of ethylene effect that is required. Each organ and the required delay will determine which analogue to be used.

The advantage of using inhibitors of ethylene action over inhibitors of ethylene production lies in the ability of the inhibitors of ethylene action to protect the tissue against both endogenous and exogenous ethylene, thus providing better overall protection.

Our findings indicate that 1-MCP and its analogues are potent inhibitors, capable of providing good protection against ethylene. The fact that the compounds are in a gas phase and are non-phytotoxic, odorless, and effective at low concentrations, renders them promising candidates for commercial use.

V. CONCLUSION AND SUGGESTIONS FOR FUTURE RESEARCH

The goal of the current project was to broaden our knowledge of the effectiveness of cyclopropene compounds as blockers of the ethylene receptors and inhibitors of ethylene responses. Thirteen new ethylene receptor blockers were synthesized. Nine of these appear to be very effective. All of them are structural analogues of 1-MCP, with substitution in the 1-position with a side chain containing from 2-10 carbons. The potency of the compounds to block the receptor and inhibit ethylene action was found to be effective on a wide scope of systems like: climacteric fruits (banana, avocado and tomato), non-climacteric fruits (citrus), the triple response (etiolated peas), and leaf abscission (citrus) as well as effect on ethylene-induced disorders or adverse effect like: woolliness in nectarine, grey pulp in avocados, softening in plums, superficial scald in apples and degreening of Sweetie (Oroblanco) fruit. Treatment with the inhibitors was effective only if applied prior to exposure to ethylene. Simultaneous exposure of the fruit to the inhibitor and ethylene reduced the potency of the inhibitors as a result of their competition relationship with ethylene under this condition. In fact this method is used to demonstrate that the inhibitor is acting at the receptor site. This does not work if the inhibitor is allowed to bind first. Then there is no competition. In principle the general inhibitory effect of 1-MCP is well recognized in all the systems tested. However, there are fluctuations in the responses as related from the concentration and the plant system used.

It can be concluded that most of the new inhibitors tested are capable of inhibiting ethylene action in all or some of our test systems, probably by competing for the site of binding in the ethylene receptor.

Every compound used in this study needed to be prepared by synthesis (1-MCP is now available from "Biotechnologies for Horticulture Inc". 751 Thunderbolt Dr. Waltersboro, SC 29488, USA). The synthesis of these compounds was generally done according to literature methods, and usually involved several steps and the preparation of several intermediates. Some of the compounds have not previously reported. Synthetic methods have developed slowly but recently methods have been reported making many more compounds available. In some areas, methods are yet lacking to develop the desired analogue. During this research, some analogues were not prepared because suitable methods were not available. Future research likely will involve developing new synthetic methods. In conclusion, now a number of compounds are available from our work. We conclude that more types of derivatives in the 1-position should lead to some more useful compounds. In particular, it should be possible to make and test compounds that are non-volatile, of limited volatility for use as sprays. Also there is a need to check some additional types of substitution in the three position. It is possible that a strong electron-withdrawing group such as a carboxy-ester would be active at low concentrations and have unusual properties. Consideration should be given to prepare water-soluble compounds both volatile and non-volatile. These compounds obviously can be useful in agriculture. Although experimental quantities can be produced, the production and commercial availability will depend on making wide tests and getting appropriate clearance of governmental agencies. Every indication is that this will be done soon, as commercial companies are

now starting to do these things. This is the first time most of these compounds were conceived of and synthesized for the purpose of controlling ethylene responses. The use of 1-MCP has received worldwide recognition in plant ethylene research, in general, and in postharvest physiology, in particular. These new compounds should make a useful addition to those areas. They have obvious scientific value as well as practical significance. Although some of the analogues were not very effective, scientifically the results are very important. They show that a series of compounds with finite binding times is possible. They also show that substitution in the 1-position leads to longer binding compounds and concentration wise are more effective. It was the generation of this information that led to the preparation of the 1-substituted compounds which are very effective. These in turn show that long chain compounds are very effective and thus point to other possibilities, many of which have not yet been tested. The long chain can support other types of chemical groups and may be the path to water-soluble compounds and non-volatile compounds which can be very useful. This avenue is now being explored.

The problem of preparing and shipping compounds was an important part of the experience gained. Some of the compounds can be handled as a gas (low boiling). Those can be shipped in capped bottles in the gaseous state with a saturated ammonium sulfate solution seal. Samples that have a high boiling point are better applied in a solution of a low boiling solvent. To be shipped the solvent needs to be removed, and the compounds shipped under inert gas as they are highly reactive with water and oxygen. Before application, the compound needs to be hydrolyzed at -40°C to -60°C and extracted into the desired solvent for application.

According to the achievement in our research, we suggest that future studies should be directed to the following aspects:

(1) Substitution in the 1 position seems to be promising. Molecular size although important does not seem to make as much difference as might be expected as long as the boiling point of the compound is not too high. There are groups such as methyl esters and ethers that can be incorporated into the molecule and may give good inhibitory effect. A free acid group in the position 1 should be tested. It is noted that all active compounds, both as ethylene agonists and ethylene antagonists, are volatile compounds. It may only be a coincidence, but the idea needs to be further explored. A free acid may give this information. The compound cyclopropene-1-methanol, has a selective activity in banana, being more active in the peel than the pulp. Hydroxy groups can be placed in different positions giving secondary alcohol and tertiary alcohol and may give different results. These compounds can be tested for specificity and other desirable properties in a large number of fruits and vegetables.

(2) Substitution in the 3 position needs to be further studied. Although a number of hydrophobic group have been tested, all are relatively short hydrophobic groups. The effect of hydrophylic group such as methylhydroxy- and ethylhydroxy-groups needs further work. Also the ester group may give good results.

(3) On the basis of the current available methods for the identification and isolation of the ethylene receptor(s), and by using ^{14}C -ethylene, and if available also ^{14}C -1-MCP, it will be possible to study the persistence of 1-MCP binding at the receptor site. Furthermore, the competitive nature of the two chemicals, at the receptor(s) site(s), could be studied. It is suggested that 1-MCP exert its effect of protecting plant tissue against ethylene action by competing for the site of binding at

the ethylene receptor (Sisler *et. al*, 1997). It has been demonstrated that *ETR1* gene encodes an ethylene receptor in *Arabidopsis* and expression of truncated forms of ETR1 in yeast provided evidence that the amino-terminal hydrophobic domain of the protein is the site of ethylene binding (Schaller and Bleecker, 1995). Recently the *ERS* gene homologue of *ETR1* has been identified and characterized from 'Valencia' orange in our laboratory (Li *et. al*, 1998). Therefore, it is now possible to investigate whether 1-MCP could bind directly to the ethylene receptor in citrus.

VI. PUBLICATIONS ORIGINATED FROM THE BARD PROJECT

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3. Porat, R., Weiss, B., Cohen, L., Daus, A., Goren, R. and Droby, S. 1999. Effects of ethylene and 1-methylcyclopropene on the postharvest quality of 'Shamouti' oranges. *Postharvest Biology and Technology*. 15: 155-163

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