This is to certify that the

dissertation entitled

ALTERNATIVE STRATEGIES TO CONTROL SCALD OF APPLES AND SOME BIOCHEMICAL BASES

presented by

ZHENYONG WANG

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in **HORTICULTURE**

David R. Dilley
Major professor

Date Dec 14, 1998

ALTERNATIVE STRATEGIES TO CONTROL SCALD OF APPLES AND SOME BIOCHEMICAL BASES

By

Zhenyong Wang

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1998

ABSTRACT

ALTERNATIVE STRATEGIES TO CONTROL SCALD OF APPLES
AND SOME BIOCHEMICAL BASES

By

Zhenyong Wang

Scald is a pervasive physiological disorder of apples and pears induced by storage at refrigerated temperatures in air or long term controlled atmosphere (CA). Scald has the potential to destroy the market value and utility of millions of tons of apples and pears annually unless the fruits are treated with a postharvest drench with diphenylamine (DPA) or ethoxyquin along with a fungicide. Numerous countries have banned the use of DPA and prohibited importation of fruits so treated. The objectives of these studies were to develop alternative strategies to control scald of apples to avoid applying postharvest treatment with the scald inhibitor DPA and to ensure fruit quality and food safety. The physiological and biochemical bases of apple scald were also investigated.

Granny Smith and Law Rome apples were placed under hypobaric storage immediately after harvest or after 0.5, 1, 2, 3, 3.5, 4, 4.5, 5 or 6 months storage in air at 1°C to determine the effects of delaying imposition of hypobaric storage on ripening and scald development and production of α-farnesene and its oxidation product 6-methyl-5-heptene-2-one (MHO). Fruits did not scald during hypobaric storage or afterwards when transferred to static air at one atmosphere continuously for 4 months if they were placed under hypobaric conditions within one month after

harvest while held in air at 1°C; after 3 months delay, scald development was similar to that for fruits stored in air. MHO accumulated in the epicuticular wax when fruits were placed under hypobaric storage after one month delay in air. MHO in the epicuticular wax of fruits stored hypobarically after 2 or more months delay was released upon transfer of fruits to 20°C; MHO accumulated in direct proportion to the duration of the delay to hypobaric storage. Hypobaric ventilation apparently removes scald-related volatile substances including α-farnesene and MHO that otherwise accumulates and partitions into the epicuticular wax of fruits stored in air at atmospheric pressure.

Scald susceptible and not susceptible cvs. fruits were treated with different concentrations of ethanol vapor and different durations. Fruits were then stored in 3% O_2 with 0% CO_2 in flow-through CA and in air at 1°C. The treatments with 6000 $\mu L \cdot L^{-1}$ ethanol vapor for 2 weeks were more effective for scald control than the other treatments; higher levels of ethanol for over 2 months caused fruit injury and off-flavor. The ethanol vapor treatment diminished the level of several low molecular weight volatiles produced by the fruit. The rate and pattern of α-farnesene production was similar for fruits treated or not treated with ethanol vapor. Ethanol vapor treatments reduced the rate of MHO production. This indicates that the α-farnesene is not as closely related to scald development as its oxidation product MHO.

Fruits were treated with initial low O_2 stress (ILOS) at different levels of low O_2 and various durations and then stored in different CA storage conditions and air at 0.5-1°C. Superficial scald was markedly reduced by ILOS at 0.5% O_2 for up to two weeks followed by air storage. With CA storage at 3% O_2 with 0% CO_2, following 0.5% and 0.25% ILOS for 2 weeks reduced scald; and with CA at 1.5% O_2 with 3% CO_2, scald was prevented. ILOS at 0.25% O_2 for two weeks or also when followed with an additional two weeks of low O_2 stress after 2 months of the storage were the most effective treatments regimens for scald control. The production of α-farnesene and MHO was inhibited by ILOS and CA at 1.5% O_2. The 0.25% O_2 ILOS caused stronger inhibition on α-farnesene and MHO production than 0.5% O_2 ILOS. Collectively, this results suggest that the accumulation of MHO is highly related to scald development of apples. A commercial test of initial low O_2 stress confirmed the efficacy of ILOS for controlling scald.

The 'scald-like' disorder of Empire apples is a CO_2-linked physiological disorder. The factors of acclimatization of preclimacteric fruits at 3°C in air or low O_2 levels prior to elevating the CO_2 concentration for subsequent long-term controlled atmosphere were investigated. The results indicate that the Empire 'scald-like' disorder can be effectively controlled by holding the fruits at 3°C for 3-4 weeks at 1.5 to 3% O_2 without CO_2 prior to CA storage at 1.5% O_2 + 3% CO_2. Acclimatization of fruits against the disorder was also achieved by storage in air but this resulted in excessive ripening development with flesh firmness loss during subsequent CA storage.

Dedicated to my eternal companion Xiaozhu Pan,

whose faith in me encouraged me to achieve excellence in all I attempted to do,

and to our children Shuyu and Brice Libai,

whose successful future I have strived to ensure.

ACKNOWLEDGMENTS

The timely completion of this dissertation would not have been possible if not for the support, mentoring, patience, and confidence shown me by Dr. David R. Dilley, my major professor and chair of my advisory committee. I sincerely appreciate his advice for my personal as well as professional development.

I also appreciate the service of Drs. Randolph M. Beaudry, Stephen A. Boyd, Jerry N. Cash as members of my advisory committee for their advice and encouragement.

I 'm grateful to Drs. Robert C. Herner, Muraleedharan G. Nair, Jun Song, Douglas Bermeister, John Everard, Dina Kadyrzhanova, Nazir Mir, and Manit Kosttrakun for their assistance and encouragement.

I gratefully acknowledge the assistance of Weimin Deng, Tom J. McCully, Konstantinos Vlachonasios, Hongying Jiang, Toni M. Warner and many other colleagues in the lab for assistance in data collection and moral support to make it possible for me to pursue this goal.

I am also grateful to Lorri K. Busick, Joyce Jackson, Sherry Mulvaney, and Gabrielle Seyka in the Department Office for their assistance.

I wish to express special thanks to my friend Xiaoyu Lin for his genuine friendship and assistance, moral and spiritual support.

I owe special thanks to my mother and brothers for their love, kindness, sincerity, encouragement, moral and financial support.

Finally, I express deep love and appreciation to my dear wife Xiaozhu and our children Shuyu and Brice Libai for their tireless support, understanding and sacrifice, confidence, encouragement, and enthusiasm.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER V

LIST OF FIGURES

CHAPTER II

CHAPTER III

CHAPTER V

INTRODUCTION

Overview of the scald problem:

Apples and pears are major US horticultural crops and are consumed as fresh fruits, minimally processed sliced fruits and after processing as canned or frozen, sauce, bakery and juice items. The domestic and export market is large and growing for these fresh fruits. The large size of the crops requires that more than 50 percent of the fruits grown be stored for extended periods of up to one year to provide orderly marketing of fresh fruits and crop utilization for processing. Refrigerated air storage and controlled atmosphere storage technologies are used for this purpose. Scald is a devastating physiological disorder induced by storage of apples and pears at refrigerated temperatures required to delay ripening and extend the storage period of these fruits. Scald has the potential to destroy the market value and utility of millions of tons of apples and pears annually unless the fruits are treated with a postharvest drench with diphenylamine (DPA) or ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline). The concentration of these chemicals can be as high as 2,000 $mg \cdot L^{-1}$ (w/v) for DPA and 2,700 $mg \cdot L^{-1}$ for ethoxyquin which leaves a considerable and detectable residue both on and within the fruit at near maximal FDA tolerance levels. The postharvest drench solution must contain a fungicide along with a wetting agent to minimize decay development which may arise from a long list of fruit pathogens (Hardenburg and Spalding, 1972). These pathogens are carried on the fruit surface as spores or decay lesions within the fruits. The drench solutions become heavily loaded with inoculum unless a fungicide is included. The only fungicide approved for postharvest application is the thiabendazole (MerTect) and it is used to kill the pathogens and prevent infection through wounds in the fruit surface derived from mechanical damage in harvesting and handling and through natural openings in the fruit surface. Captan has been used for this purpose. Numerous countries have banned the use of DPA and numerous fungicides including Captan and prohibit importation of fruits so treated. If the US is to market apples and pears world-wide and year-around, alternative strategies must be developed to provide safe and economical control of this important disorder. Since the use of DPA is diminishing globally because of environmental and health concerns and government mandates, this means that apples and pears will generally not have any fungicides applied postharvest to protect them against fungal pathogens during storage and marketing. Many of the fungicides once commonly used have been or may be withdrawn. The costs for developing and registering new fungicides are prohibitive and contra-indicated by market size and environmental concerns, respectively. Alternative strategies to control pathological and physiological disorders of apples and pears are urgently needed to ensure that these important fruits which can be available from storage will continue to be marketed on a year-around basis. Moreover, there is growing concern among consumers, growers and processors of apples and pears that many of the pesticide chemicals employed to produce the crop and control postharvest pathological and physiological disorders may be potentially harmful to humans.

Rationale for new research:

The possibility of losing the use of DPA for controlling apple scald in the future has prompted renewed interest in developing alternative strategies to control this potentially devastating disorder. A special symposium held in Yakima, Washington in 1994 was dedicated to apple scald research needs. It was sponsored mainly by the Washington State Tree Fruits Research Committee (WSTFRC). Researchers from all over the world gathered to summarize the current situation and to address what must be done to control scald in the event that DPA was not re-registered for use on apples and pears to control scald. At that time there was still a question as to whether or not DPA would be approved. It was. In spite of this reprieve, the development of alternative strategies to control scald has been a high priority research objective for many laboratories since 1994.

Recent strategies to control scald:

Over the past several years, we and other researchers have found several non-chemical treatments that reduce or control scald incidence and severity. These include: initial low O_2 stress; initial high carbon dioxide stress; heat-treatment; intermittent warming and initial ethanol vapor treatment. However, none of these treatments are yet practical to employ commercially for long-term controlled atmosphere (CA) storage without placing the fruits and the industry at risk without first confirming the validity and practicality of the most promising procedures to employ. Decreasing the O_2 level to ca. 0.7% during CA can effectively control scald of Red Delicious apples in some growing regions and seasons but not in others

(Lau, 1990; Yearsley et al., 1996). Moreover, some cvs. are susceptible to low O_2 injury and all cvs. do not reach optimum dessert quality subsequent to long-term CA storage at O_2 levels below 1% (Gran and Beaudry, 1993; Little, 1985; Truter et al., 1994). This may have a negative effect on consumer acceptance of apples in the US as was found in Europe using ultra-low O_2 CA. And this is now proving to be the case in the US as well.

Current CA storage technology to control scald:

Our laboratory studies with CA atmosphere purging at 1.5% O_2 have continued to show good control of apple scald. This has been demonstrated at the commercial level at the Michigan State University Clarksville Horticulture Experiment Station (MSU CHES) CA facility since 1987 using 1.5% O_2 with purging to scrub CO_2 (Dilley, 1990). Numerous Michigan CA operators have verified this procedure with good results for at least 6 months of CA storage. Success using this procedure is predicated on harvesting apples at the onset of the ethylene climacteric, having the fruits under CA within a week after harvest and employing a hollow fiber membrane air separator such as the Permea system for CO_2 scrubbing. These parameters are not always readily achieved. For CA storage beyond 6 months, fruits generally must be harvested at preclimacteric ethylene levels. Our laboratory studies since 1960 employing continuous purging with low O_2 atmospheres have shown good control of scald for at least 9 months. This suggests that atmosphere purging may remove a fruit-produced volatile substance that otherwise accumulates and results in scald. There is much precedence for this scenario dating back to the seminal studies of Brooks et al. (1919) more than 70 years ago showing that using mineral oil coated wraps can sometimes be effective to control scald. Fortunately, as a direct result of the recently expanded research activities the world-over, much information has been generated on fundamental aspects of the scald disorder. This is pointing the way towards understanding the basis of the disorder and offers promise that new strategies to control the disorder may be developed to control scald.

New strategies to control scald:

Two areas of our research have been especially fruitful in this regard; ethanol vapor treatments and initial low O_2 stress followed by CA storage at 1.5% O_2. We find that the initial low O_2 stress induces the fruits to produce their own ethanol and this may explain the efficacy of the exogenously applied ethanol vapor treatments in reducing scald. The biochemical mechanism responsible for the beneficial effects on scald control by low O_2 stress and the ensuing metabolism of ethanol remain to be elucidated. Extensive analyses of the volatiles produced by apples receiving low O_2 stress or ethanol vapor treatments support the premise that metabolism of α-farnesene in the pigment bearing cells is closely associated with development of the scald disorder.

Role of volatiles in scald:

We have found that an oxidation product of α-farnesene (Song and Beaudry, 1996; Wang and Dilley, 1997), namely 6-methyl-5-heptene-2-one abbreviated as MHO, is highly correlated with scald development. Moreover, DPA was found to inhibit production of MHO but not α-farnesene from which it is derived. Our studies with fruits stored with continuous hypobaric (subatmospheric pressure) ventilation at O_2 partial pressures equivalent to low O_2 CA indicate that apples (even the Granny Smith cv.) do not scald even after a year of such storage or after subsequent removal to air at ambient temperature. Hypobaric storage favors the removal of all volatiles including ethylene produced by the fruits; although the fruits produce normal levels of α-farnesene and its oxidation product MHO following hypobaric storage. This suggests that some metabolic pathway responsible for scald may be affected. We suspect that the fruits capacity to metabolize α-farnesene to produce the MHO ketone in the scald development pathway has been down-regulated by hypobaric ventilation. The same down-regulation effect may occur under ultra-low O_2 CA, e.g., 0.7% O_2, but this remains to be proven.

Summary current knowledge about scald:

The mechanism whereby conventional CA storage at 1.5 to 2% O_2 retards and ultra-low O_2 CA, e.g., 0.7% O_2 prevents scald is not known. The results from a vast body of CA storage and physiological research spanning the past 60 years can be succinctly summarized as follows.

Scald:

- is a manifestation of membrane-based chilling injury,
- is a dysfunction of the pigment bearing cells of the hypodermis, especially,

but not limited to, the non-red portion of the fruit surface,

- becomes more prevalent as the fruits senescence or age during storage and upon subsequent warming,
- is caused by accumulation of a volatile by-product of fruit metabolism in the affected cells,
- is a consequence of oxidative metabolism involving reduced species of O_2, e.g., active O_2 species (AOS) such as O_2^-, H_2O_2 and free radicals derived from them such as the hydroxyl radical (·OH), peroxyl radical (·OOH) and perhaps singlet ($^1O_2$),
- may be controlled *in vivo* by naturally occurring antioxidants such as ascorbic acid, carotenoids, α-tocopherol and glutathione among others which act to scavenge free-radicals,
- is largely controlled by exogenously applied anti-oxidants such as diphenylamine (DPA), ethoxyquin, ascorbate-6-palmitate, butylated hydroxy toluene (BHT), butylated hydroxyanisole (BHA), squalene and others which scavenge free-radicals if applied shortly after harvest,
- is controlled by hypobaric storage if fruits are under hypobaric conditions within one month,
- is less prevalent with fruits harvested after exposure to cool temperatures late in the growing season,
- is a metabolic dysfunction likely caused as a consequence of oxidation of α-farnesene to 6-methyl-5-heptene-2-one and its subsequent metabolism via free-radical intermediates,

- affects most but not all cvs. of apples which suggests a genetic basis for the disorder,
- results in the oxidation of essential amino acid functional groups of enzymes and of membrane lipids to carbonyl derivatives disabling them from functioning or targeting the proteins for proteolysis leading to cell death.

Role of active oxygen species:

All plant cells produce active O_2 species (AOS) such as O_2^- and H_2O_2 as part of their normal aerobic metabolism and this is augmented by biotic and abiotic stresses. These AOS are normally dissipated in the cells by enzymatic reactions and by naturally occurring antioxidants. Failure of these defense systems to keep the titer of AOS at low levels can result in AOS interacting non-enzymatically to produce more highly reactive species such as the hydroxyl radical ($\cdot$OH), peroxyl radical ($\cdot$OOH) and singlet O_2 ($^1O_2$). Ubiquitious enzymes such as catalase, peroxidase, ascorbic acid peroxidase and glutathione peroxidase metabolize H_2O_2 to water. These enzymes exist in most of the subcellular compartments even in the cell wall (apoplast). Superoxide dismutase is likewise distributed and dismutates O_2^- to water. The combined action of these enzymes with naturally occurring antioxidants maintains non-damaging levels of $^{\cdot}$OH radicals which otherwise may non-discriminantly destroy membrane-bound lipids and proteins and cytosolic enzymes essential for cellular homeostasis.

Cellular senescence and scald:

The normal progression of plant cell senescence as occurs in ripening results in lowering the cells ability to cope with biotic and abiotic stresses. Consequently, as the reserves of naturally occurring antioxidants decline, the defense systems decline. This is one reason plants lose the ability to counter microbial invasion. As enzymes and the lipo-protein membrane matrix become oxidatively and irreversibly damaged, catastrophic events occur resulting in death. Cells of the fruit hypodermis affected by scald succumb in this manner and then display the well recognized symptoms. However, the potential for the progression of cellular catabolism leading to the eventual symptoms of scald develops within the first few weeks in storage but symptoms remain unseen for many months by constrainment at low temperature and low O_2 levels during storage.

CHAPTER I

LITERATURE REVIEW

Scald of apples:

Superficial scald - a physiological disorder: Superficial scald is a pervasive physiological disorder of apples and pears that results in the browning of the skin during or after low temperature storage in air or after controlled atmosphere storage which markedly extends the useful life span of these fruits. The affected cells of the hypodermis die and dehydrate (Bain, 1956, 1963). Most cvs. of apples and pears are susceptible to scald (Ingle and D'Souza, 1989). The disorder destroys the appearance and therefore the fresh market value of the fruits. Affected fruits may be processed as juice, sauce or sliced for bakery products but with product yield reduction attending losses due to trimming. Product quality may be adversely affected and costs are increased due to a higher labor input. No evidence was found to involve flavanols, condensation products between flavonoid glycosides and gallic acid or polymerisation of flavan-3,4-diols in the disorder of scald and the oxidative coupling of o-dihydroxyphenols in damaged tissue seems highly related to the browning (Piretti et al., 1994). The physiology and control of scald has been periodically reviewed (Smock 1961; Meigh, 1970; Ingle and D'Souza, 1989; Emongor et al., 1994).

Empire and Braeburn disorders: Scald-like disorders on Empire and Braeburn apples have symptoms similar to superficial scald and are also controlled by DPA treatment (Burmeister and Dilley, 1995; Burmeister and Rowan, 1997). These disorders could occur only a few weeks after harvest while in storage and is highly related to the CO_2 level in the storage (Burmeister and Dilley, 1995) while superficial scald only occurs after a few months of storage and is more severe when stored in air. Johnson et al. (1998) concluded that fruits susceptible to CO_2-linked disorder were known to have higher resistance to gas diffusion than cvs. not affected. Wang et al. (1997b) found that Empire 'scald-like' disorder could be controlled by acclimatizating fruits in low O_2 level and without CO_2 for a few weeks at the beginning of storage. Braeburn apples also have internal browning symptom (Burmeister and Rowan, 1997) and an ultra-low CO_2 CA and a slow or 14-day delay in establishing the CA conditions have been found to reduce the risk of Braeburn browning disorder (Elgar et al., 1998; Lau, 1998)

Factors related to susceptibility of superficial scald of apples:

Varieties: Some cvs. of apples are particulary susceptible to develop scald eg. Law Rome, Granny Smith, Red Delicious, McIntosh and Cortland among others, while other cvs. show some natural resistance eg. Empire and Golden Delicious (Ingle and D'Souza, 1989; Emongor et al., 1994).

Preharvest factors: Environment during the growing season also influences scald susceptibility as does mineral nutrition (Emongor et et., 1994; Fidler, 1957). Apples with low calcium levels often develop more scald than those with high levels (Bramlage et al., 1974). Scald susceptibility was reported to be increased by high application of nitrogenous fertilizers in Delicious apples (Weeks et al., 1958). High nitrogen may increase α-farnesene (2,6,10-trimethyl-2,6,9,11-dodecatetraene) and/or conjugated trienes indirectly in relation to fruit maturity and/or the development of wax on the fruit during storage (Emongor et et., 1994).

Maturity: Fruit maturity at harvest is a major factor; less mature fruits are prone to scald while the more mature fruits are not when sampled from the same trees in the same season (Anet, 1972b; Christophen, 1941; Ingle and D'Souza, 1989). The fruit industry has been driven to harvest apples at a relatively immature stage of development when attempting to extend the storage season beyond six months in CA storage. Delayed harvest increased the concentrations of α-tocopherol and carotenoids but not ascorbic acid and greatly reduced scald development (Barden and Bramlage, 1994c). Early initiation of ripening by preharvest applications of 400 $mg \cdot L^{-1}$ ethephon 3-5 weeks before harvest reduced superficial scald and the content of lipid soluble antioxidants in the fruit peel of ethephon-treated apples after eight months in storage was increased (Curry, 1994). Similar results with ethephon found by other researchers (Couey and Williams, 1973; Greene et al., 1977; Hammett, 1976; Lurie et al., 1989b; Windus and Shutak, 1977).

Antioxidants: Anet (1972b) proposed that the high scald susceptibility of immature apples was due to an inefficient antioxidant system in their peel. Eleven antioxidants from apple peel were isolated by using thin-layer chromatography, but only three tocopherols were identified (Anet, 1974). He concluded that scald did not occur if the concentrations of the antioxidants remained adequate to limit α-farnesene oxidation sufficiently. Lurie, et al. (1989a) concluded that DPA prevents scald by its general antioxidant effect and not specifically by preventing the oxidation of α-farnesene. An estimate of lipid-soluble antioxidant activity in apple peel at harvest was negatively correlated with scald development (Meir and Bramlage, 1988). Scalded tissue contained less α-tocopherol than non-scalded tissue in the same fruit (Gallerani et al., 1990). Antioxidant concentrations at harvest were inversely related to maximum conjugated triene concentrations at the end of storage and to scald development. However, no individual antioxidant was associated consistently with conjugated triene accumulation or scald development. And as conjugated triene concentrations increased, total lipid-soluble antioxidant activity also increased but water-soluble antioxidants generally decreased during storage (Barden and Bramlage, 1994b).

Scald is a chilling injury-related disorder: Superficial scald is considered to be a chilling injury-related disorder (Watkins et al., 1995). Melville and Hardisty (1953) found good control of scald of Granny Smith apples by storing them at ca. 5°C in air for several weeks followed by storage at 0°C. This supports the premise that scald is a chilling injury-related disorder. Apple scald does not occur on fruits stored above 10°C. However, a disorder similar to scald can be induced to develop at 20°C by an anaerobic atmosphere followed by returning the fruits to air (Dilley et al., 1963). It too was prevented by DPA. Fruits gain potential to scald by storing them in air at 0 to 5°C while actual symptom development may appear many months later. Evidence for this comes from studies with timing of application of scald inhibitors. DPA must be applied shortly after harvest to be effective in controlling scald (Shutak and Christopher, 1960). It becomes ineffectual once the fruits have been in air storage for more than 3-4 weeks. This suggests that low temperature storage in air potentiates scald development. This is consistent with scald being a manifestation of chilling injury.

Intermittant warming treatments are sometimes effective in preventing scald. Fruits warmed to 20°C after 2 weeks in air at 0°C reduced scald while warming after 4 weeks was not effective (personal communication, Dr. C.B. Watkins, Cornell University, Ithaca, NY).

Role of Volatiles: Early investigations by Brooks et al. (1919) and studies by Fidler (1950) suggested that volatile substances produced by the fruits were the cause of the scald disorder. This was suggested since wrapping the fruits with mineral oil impregnated paper was partially successful in controlling scald so they hypothesized that some volatile organic substance produced by the fruit was absorbed in the oil and thus prevented its accumulation in the cuticle. Many researchers believed that natural volatiles produced by apple fruits including α-farnesene and it oxidation products such as conjugated trienes, conjugated trienols and 6-methyl-5-heptene-2-one were involved in apple scald development (Du and Bramlage, 1993; Filmer and Meigh, 1971; Hall et al., 1953; Huelin and Coggiola, 1970a; Meigh and Filmer, 1969; Murray et al., 1964; Murray, 1969; Spicer et al., 1993; Song and Beaudry, 1996; Watkins et al., 1993; Whitaker et al., 1997). Fidler (1950) concluded that scald involves a volatile as well as a non-volatile substance. A volatile sesquiterpene, α-farnesene, was implicated in scald development more than four decades ago (Meigh, 1969). α-Farnesene accumulates soon after harvest (Meigh and Filmer, 1969) and it gradually autoxidizes at 1 °C due to being attacked by a free radicals and the primary monomeric products are conjugated triene hydroperoxides (Anet, 1969). The conjugated trienes accumulate during subsequent storage (Anet, 1972a). Song and Beaudry (1996) found that MHO applied to apple fruits caused a scald-like disorder and this is a product of α-farnesene oxidation. Scald was induced by the products of α-farnesene oxidation, and the concentration and time of appearance of these products determined the severity of the disorder (Anet, 1972b). Scald development in Granny Smith, a cv. very susceptible to scald, was correlated to the oxidation of α-farnesene to conjugated triene hydroperoxides (Huelin and Coggiola, 1970a; Filmer and Meigh, 1971). Extensive investigations in Australia (Huelin and Murray, 1966; Huelin and Coggiola, 1968, 1970b, 1970c; Anet, 1972a; Anet and Coggiola, 1974) suggest that scald results from the autooxidation of a volatile sesquiterpene hydrocarbon α-farnesene in the fruit skin (Meigh and Filmer,1969). The oxidation of α-farnesene yields conjugated triene hydroperoxide free-radicals and conjugated trienols (Whitaker et al., 1997) which injure the cells and give rise to the symptoms of scald (Rowan et al.,1995). New techniques have been developed for rapid analysis of volatile compounds (Matich et al., 1996; Song et al., 1997). Recently, Rupasinghe et al. (1998) found that incorporation of radiolabel into α-farnesene from trans,

trans-[1-3H]-farnesenepyrophosphate (FPP) was nearly 3-fold lower in scald-developing skin tissue than in scald-free skin tissue of the same apple. Ventilation increases loss of α-farnesene and reduces scald (Anet, 1972b). Whereas at the same time Matich et al. (1998) suggested that reduction of apple scald by ventilation may not be explicable merely by enhanced evaporative depletion of α-farnesene since the rate of loss of α-farnesene from the surface of the fruit depends more on storage time than on its concentration in the wax (Matich et al., 1998). Scrubbing CO_2 with low O_2 atmosphere during CA storage at 1.5% O_2 controls scald (Dilley, 1990); this also removes other volatiles. Collectively, research strongly supports the premise that some volatile(s) is closely related to scald development.

6-methyl-5-heptene-2-one: Among the products of the oxidation of α-farnesene are low-molecular-weight carbonyl compounds, some of which were identified to no effect on scald, however, 6-methyl-5-heptene-2-one, pyruvaldehyde, and methylvinyl ketone require more study (Filmer and Meigh, 1971). The primary volatile product during early stages of α-farnesene oxidation is 6-methyl-5-heptene-2-one (Anet, 1972a). Exogenous application of 6-methyl-5-heptene-2-one vapor can induced scald symptom on 9 cultivars of apple; scald -susceptible cultivar were more sensitive than scald-resistant cultivars (Song and Beaudry, 1996).

Active oxygen species: All plant cells produce active oxygen species (AOS) such as O_2^- and H_2O_2 as part of their normal aerobic metabolism and this is augmented by biotic and abiotic stresses. These AOS are normally dissipated in the cells by enzymatic reactions and by naturally occurring antioxidants. Failure of these defense systems to keep the titer of AOS at low levels can result in AOS interacting non-enzymatically to produce more highly reactive species (Halliwell and Gutteridge, 1989) such as the hydroxyl radical (·OH), peroxyl radical (·OOH) and singlet oxygen ($^1O_2$). Ubiquitious enzymes such as catalase, peroxidase, ascorbic acid peroxidase and glutathione peroxidase metabolize H_2O_2 to water. These enzymes exist in most of the subcellular compartments and even in the cell wall (apoplast). Superoxide dismutase is likewise distributed and dismutates O_2^- to water. The combined action of these enzymes with naturally occurring antioxidants maintains non-damaging levels of ·OH radicals which otherwise may non-discriminantly destroy membrane-bound lipids and proteins and cytosolic enzymes essential for cellular homeostasis (Halliwell and Guttridge, 1989). The normal progression of plant cell senescence as occurs in ripening results in lowering the cells ability to cope with biotic and abiotic stresses. Consequently, as the reserves of naturally occurring antioxidants declines, the defense systems decline. This is one reason plants lose the ability to counter physiological disorders such as superficial scald of apples.

Technologies to Control superficial scald of apples:

Storage conditions: High O_2 levels, low carbon dioxide levels, ethylene accumulation and poor ventilation conditions during storage increase the scald incidence and severity (Lau, 1985a, 1985b; Little et al., 1985; Little and Peggie, 1987; Manseka and Vasilakakis, 1993; Porritt, 1966; Roberts et al., 1963).

Low oxygen CA: Ultra-low O_2 levels in CA storage can also reduce scald (Little and Peggie, 1987). Storage under ultra low CA conditions led to markedly lower scald levels on the post-mature fruit, but did not greatly reduce scald on the pre-mature and mature fruit (Truter et al., 1994). CA storage at 0.7 % O_2 can be effective in control of scald in Red Delicious apples in the Northwest (Lau, 1990). For other regions the results have been less promising and some cvs. show intolerance to the low O_2 levels needed to control scald (Gran and Beaudry, 1993). Low O_2 or ultra low O_2 also showed similar results in reducing or controlling apple scald in other studies (Patterson and Workman, 1962; Roberts et al., 1963; Porritt, 1966; Sharples and Johnson, 1981; Chen et al., 1985; Little, 1985; Truter et al., 1994; Yearsley et al., 1996). CA storage at 1.5 % O_2 appears to maintain flesh firmness and other quality attributes such as flavor for most of the cvs. we have examined over many years and largely control scald whereas long-term CA storage at higher O_2 levels does not (Dilley, 1990).

Low ethylene CA: Low ethylene concentration in the storage atmosphere was partially effective in reducing scald of apples (Little et al., 1985; Liu, 1985; Skrzynski et al., 1985). It was suggested that ethylene played a fundamental role in changes associated with scald development based on Du and Bramlage (1994). Ethylene was included because the delay in ripening by storage in a controlled atmosphere and by modified atmosphere created by coatings are partially due to alternation in ethylene production and action (Kader, 1986). Removing ethylene from the storage atmosphere can reduce scald (Knee and Hatfield, 1981). Our preliminary studies (unpublished) with ethylene action inhibitors suggests that some aspect of ethylene action as a plant hormone is implicated in scald development. Our observation was recently confirmed by researchers in Washington State (Postharvest Physiology Review by the Washington State Tree Fruits Research Committee, July 20-21, 1998 in Wenatchee, WA). Low ethylene atmosphere during storage can significantly reduce scald incidence (Little et al., 1985; Liu, 1985; Skrzynski et al., 1985; Du and Bramlage, 1994).

Low oxygen and low ethylene CA: Low O_2 and low ethylene during storage have been shown to reduce superficial scald development (Lau, 1989, 1990, 1993; Fica, 1991;). Ventilation of apples with low O_2 atmospheres can reduce scald (Dilley, 1990). Hypobaric storage can completely control scald (Dilley, 1982). Low O_2 and low ethylene storage conditions can reduce or control scald development (Lau, 1983, 1985a, 1985b; Little, 1985; Johnson et al., 1989; Fica, 1991). Many of these studies suggest, as proposed by Brooks et al.(1919) more than 70 years ago and extended by Fidler (1950), that scald development is caused by a volatile substance produced naturally by the fruit.

Hypobaric storage: Refrigerated storage of apples at reduced atmospheric pressures of 0.1 to 0.2 atmospheres prevents scald (Dilley, 1982). At 0.1 atmosphere of air the partial pressure of O_2 is 76 mm-Hg which is equivalent to about 2% O_2 at atmospheric pressure. Storage at 2% O_2 can delay but not prevent scald. Since hypobaric ventilation favors the removal of volatiles produced by the fruits including ethylene this may explain how it can prevent scald.

Heat-treatment: Early studies by Hardenburg and Anderson (1965) showed that prestorage heat-treatment could reduce the incidence of superficial scald of apples. Prestorage heat-treatments of apples for 4 days at 38°C provided control of scald on Granny Smith apples, but this affected the flavor due to a large reduction in organic acids (Lurie et al., 1990, 1991). Moreover, heat-treatments are problematic in commercial due to large volumes of fruits.

Chemical control: Partial control of scald was achieved by wrapping fruits in mineral oil impregnated paper. This labor intensive practice was in common use until the 1960s when the antioxidant diphenylamine was introduced and commercially developed as a postharvest control procedure (Smock, 1957). In this procedure, fruits are drenched in an emulsified solution of 2,000 $mg \cdot L^{-1}$ (w/v) DPA (Hall et al., 1961). DPA is known to prevent the autoxidation of α-farnesene *in vivo* and *in vitro* (Anet and Coggiola, 1974) and control scald *in vivo*. Baker (1963) reported that diphenylamine inhibited electron transport in plant mitochondria. DPA also caused other physiological changes on stored apples in controlling scald (Lurie et al., 1989a). Chen et al. (1990) reported that superficial scald of 'd'Anjou' pears was effectively controlled by ethoxyquin. Squalene is also known to reduce scald (Curry, 1998). Some amine-type antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisle (BHA) are less effective and some others are potential carcinogenic compounds (Wills and Scott, 1977). Phorone (2,6-dimethyl-2,5-heptadien-4-one) is known to control scald and limit the accumulation of α-farnesene on fruits during storage and the amount of conjugated triene oxidation products derived from α-farnesene. It was more effective than monoterpenes in controlling scald (Scott et al., 1980) but taints the flavor of the apples and no toxicology data is available. Some monoterpenes are effective in controlling scald (Wills et al., 1977). The antioxidant ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethyl quinoline) has also been used to control scald of apples (Johnson et al., 1980). It is an additive commonly used to control oxidative rancidity in poultry feeds. Ascorbate-6-palmitate has shown some control of scald (Bauchot and John, 1996). However, there is great interest in the public sector and among research scientists as well as in the fruit industry, to avoid using postharvest applied antioxidant chemicals to apples and pears.

Coating with food compatible antioxidant treatment: Use of chemicals such as Semperfresh with food-compatible antioxidants can reduce superficial scald for a few months but not in long term storage (Little and Barrand, 1989; Bauchot et al., 1995b). Kallay (1994) found that Semperfresh and antioxidant combinations were effective to control scald of Granny Smith apples. Bauchot et al. (1995a) concluded that the limited control of scald by ascorbyl palmitate plus Semperfresh is partially related to the observed modification of the internal atmosphere of the apple. A sucrose ester coating has a potential to improve the storage quality of apples (Smith and Stow, 1994). Use of ascorbyl palmitate mixed with oil resulted in marked reduction in scald (Dodd and Bester, 1993). Ascorbic acid reduced scald incidence and severity when apples were stored in CA for 43 weeks but did not reduce it consistently when apples were stored in air (Chellew and Little, 1995). Vegetable oils reduced superficial scald probably because of a physical effect but not related to chain length of fatty acids or degree of unsaturation (Scott et al., 1995b; Little and Barrand, 1989; Bauchot et al., 1995b; Kallay, 1994; Bauchot et al., 1995a; Smith and Stow, 1994; Dodd and Bester, 1993; Chellew and Little, 1995).

Prediction of superficial scald: There has been some success in predicting scald susceptibility of Cortland and Red Delicious apples in the Northeast (Barden and Bramlage, 1994a) based upon the accumulation of days or hours at temperature below 10°C during the late maturation stage of fruit development. For other regions and cvs. this has given mixed results but they are still promising (Curry, 1998).

Alternative strategies to control scald are needed:

The issues of quality maintenance and control of fruit and vegetable physiological and pathological disorders have been addressed for over the past several decades by postharvest researchers (Hall, 1957; Harvey, 1978). The storage technologies which have been developed and implemented in commercial application by postharvest researchers have addressed prolonging the useful shelf-life of these produce items (Blanpied, 1990; Curry, 1990). This research has largely addressed the issues of quality maintenance and control of physiological disorders of fruits and vegetables (Hintlian and Hotchkiss, 1986). The over-all goal has been to develop and extend improved fruit storage technologies to provide high quality, wholesome and safe foods to the consuming public at an affordable price while at the same time assuring the producers and handlers of the produce a sufficient profit margin to encourage them to adopt these technologies.

DPA and ethoxyquin have been used as described above for nearly forty years to control superficial scald. Early research with DPA as a dip treatment of fruits in bulk bins was conducted in 1963 (Dilley and Dewey, 1963). This was quickly followed by development of over-head drench treatment facilities for treating entire truck loads of apples before they were put in CA storage (Dewey and Dilley, 1964); a system still widely used today throughout the US and Canada. In Michigan alone at least 5 million bushels (1x 10^5 tons) of apples are treated each year with a postharvest drench treatment with DPA and the thiabendazole fungicide to control scald and decay of fruits during storage. Nationally, more than half of the apples and pears grown are treated in a similar manner with scald inhibitors and fungicide prior to storage.

Disposal of the waste treating solutions poses a potential problem of contamination of surface, stream and/or ground water. At least one instance of fish kill has occurred from DPA contamination from improperly disposed solutions. Disposal of DPA testing solutions to maintain the correct titer of DPA in the treating solutions also poses a serious problem because the chemical reaction to analyze for DPA forms a highly carcinogenic nitroso-derivative (personal communication with Scott

Ager of Olympia, WA who developed the analytical procedure widely used throughout the world while he was with Shieldbrite Corporation the major supplier of DPA formulated for use for scald control).

DPA has been recently found to control some 'scald-like' CO_2-induced disorders (Burmeister and Dilley, 1995) and internal breakdown disorders in apple fruits (Burmeister and Rowan, 1997) and concern has been raised by researchers that its use may be expanded for this purpose as well as for scald control. Scald affects nearly all of the important apple and pear cvs.

A major goal of the MSU postharvest research program for the past 6 years has been to develop alternative strategies to control scald of apples to avoid using any postharvest-applied chemicals. These studies (Burmeister et al. 1994; Wang et al., 1995, 1997a, 1997b; Wang and Dilley, 1996, 1997; Dilley and Beaudry, 1998) indicate that this may be achieved in the not-to-distant future. These investigations encompass fundamental studies of the etiology and biochemistry of the scald disorder, controlled atmosphere (CA) storage experiments employing various O_2 and carbon dioxide atmosphere regimens, initial low O_2 stress regimens and semi-commercial testing of CA regimens found from laboratory studies to be potentially applicable on commercial scale for the fruit industry.

Many researchers are searching alternatives to DPA for controlling superficial scald of apples and searching effective methods to predict scald development. Two areas of our research include ethanol vapor treatments and initial low O_2 stress followed by CA storage at 1.5% O_2 (Wang and Dilley, 1996,1997; Wang et al.,1997a) have been especially fruitful in this regard.

Ethanol vapor treatment: Our studies (Wang and Dilley 1995,1997; Wang et al., 1997a) on the efficacy of ethanol vapor treatments to control scald were apparently initiated at about the same time as those of Scott's group in Australia (Scott et al., 1995a) who have extended their investigations (Ghahramani and Scott, 1998a) on ethanol vapor treatments.

Initial low oxygen stress: We (Wang and Dilley, 1996,1997; Wang et al., 1997a) and Ghahramani and Scott (1998b) find that the initial low O_2 stress induces the fruits to produce their own ethanol and this may explain the efficacy of the exogenously applied ethanol vapor treatments in reducing scald even when the fruits are subsequently stored in air. The biochemical mechanisms responsible for the beneficial effects on scald control by low O_2 stress and the ensuing metabolism of ethanol remain to be elucidated. Extensive analyses of the volatiles produced by apples receiving low O_2 stress or ethanol vapor treatments support the premise that metabolism of α-farnesene in the pigment bearing cells is closely associated with development of the scald disorder (Dilley and Beaudry, 1998; Wang et al., 1997a; Wang and Dilley 1996; 1997; Ghahramani and Scott, 1998b). Truter et al., (1994) reported that storage under ultra low CA conditions led to markedly lower scald levels on the post-mature fruit, but did not greatly reduce scald on the pre-mature and mature fruit. Storage under the initial low O_2 stress + ultra low O_2 CA regime, however, conferred low levels of superficial scald on pre-mature, mature and post-mature fruit (Little et al., 1982). Recent studies by Van der Merwe et al. (1997) indicate good control of scald by initial low O_2 stress followed by CA storage at 1% O_2 with 3% CO_2 at 1°C.

This dissertation focuses on investigations of alternative strategies such as ethanol vapor, initial low O_2 stress, temperature and atmosphere, CA acclimatization and hypobaric storage to control apple scald and their physiological and biochemical bases.

HYPOTHESES:

1. The scald disorder is a manifestation of chilling injury affecting the pigment bearing cells of the fruit hypodermis. Fruits may acquire after harvest resistance to scald. Symptoms of the disorder are consequences of autooxidative processes in cell membranes brought about by low temperature storage beyond the ability of naturally occurring antioxidants to prevent the dysfunction.

2. Hypobaric storage controls scald by effectively decreasing the accumulatiom of scald-related volatile compounds in fruits which may affect fruit metabolism such as ethylene, α-farnesene and its oxidation product MHO.

3. Initial low O_2 stress prior to CA storage controls scald by affecting the metabolism including ethanol, α-farnesene and its oxidation product MHO.

4. The Empire 'scald-like' disorder may be related to superficial scald since the disorders of both Empire and Braeburn apples can be effectively controlled by drenching with DPA before storage.

OBJECTIVES:

1. Determine and optimize the level and duration of ethanol vapor treatment to control scald of apples and discuss the possible mechanism of its action.

2. Determine and optimize the level and duration of initial low O_2 stress and subsequent CA conditions to safely and effectively control scald without using postharvest- applied chemicals and without compromising fruit quality.

3. Determine the effective treatment regimens and gain insight into the mechanism of scald control by identifying and quantifying scald related volatile compounds in fruits or in their epicuticular wax substance in CA or air storage and atmosphere purging through hypobaric storage through GC/MS/SPME profile analysis.

4. Determine the pathway of formation of 6-methyl-5-heptene-2-one which has been implicated in scald development and the molecular mechanism of

its action.

5. Determine the effects of acclimatizing fruits in various gas atmosphere regimens prior to CA storage to control Empire disorder.

LITERATURE CITED

Anet, E.F.L.J. 1969. Auto oxidation of α-farnesene. Austral. J. Chem. 22: 2403-2410.

Anet, E.F.L.J. 1972a. Superficial scald, a functional disorder of stored apples: VIII. Volatile products from the autoxidation of α-farnesene. J. Sci. Food Agr. 23: 605-608.

Anet, E.F.L.J. 1972b. Superficial scald, a functional disorder of stored apples:IX. Effect of maturity and ventilation. J. Sci. Food Agric. 23: 763-769.

Anet, E.F.L.J. and Coggiola, I.M. 1974. Superficial scald, a functional disorder of stored apples: X. Control of α-farnesene autoxidation. J. Sci. Food Agr. 25: 293-298.

Anet, E.F.L.J. 1974. Superficial scald, a functional disorder of stored apples: XI. Apple antioxidants. J. Sci. Food Agric. 25: 299-304.

Bain, J.M. 1956. A histological study of the development of superficial scald in 'Granny Smith' apples. J. Hort. Sci. 31: 234-238.

Bain, J.M. and Ercer, F.V. 1963. The submicroscopic cytology of superficial scald, a physiological disease of apples. Aust.J. Biol. Sci. 16: 442-449.

Baker, J.E. 1963. Diphenylamine inhibition of electron transport in plant mitochondria. Arch. Biochem. Biophys. 103: 148-155.

Barden, C.L. and Bramlage, W.J. 1994a. Separating the effects of low temperature, ripening and light on loss of scald susceptibility in apples before harvest. J. Amer. Soc. Hort. Sci. 119: 54-58.

Barden, C.L. and Bramlage, W.J. 1994b. Relationships of antioxidants in apple peel to changes in α-farnesene and conjugated trienes during storage, and to superficial scald development after storage. Postharvest Biol. Technology 4: 23-

33.

Barden, C.L. and Bramlage, W.J. 1994c. Accumulation of antioxidants in apple peel as related to preharvest factors and superficial scald susceptibility of the fruit. J. Amer. Soc. Hort. Sci. 119: 264-269.

Bauchot, A.D., John, P., Soria, Y. and Recasens, I. 1995a. Carbon dioxide, oxygen, and ethylene changes in relation to the development of scald in Granny Smith apples after cold storage. J. Agric. Food Chem. 43: 3007-3011.

Bauchot, A.D., John, P., Soria, Y. and Recasens, I. 1995b. Sucrose ester-based coatings formulated with food-compatible antioxidants in the prevention of superficial scald in stored apples. J. Amer. Soc. Hort. Sci. 120: 491-496.

Bauchot, A.D. and John, P. 1996. Scald development and the levels of α-farnesene and conjugated triene hydroperoxides in apple peel after treatment with sucrose ester-based coatings in combination with food-approved antioxidants. Postharvest Bio. and Technol. 7: 41-49.

Blanpied, G.D. 1990. A review of the biology of storage scald and the technology of its control. Washington State Univ. Tree Fruit Postharvest J. 1: 14-16.

Bramlage, W.J., Drake, M. and Baker, J.H. 1974. Relationships of calcium content to respiration and postharvest condition of apples. J. Amer. Soc. Hort. Sci. 99: 376-378.

Brooks, C., Cooley, I.S. and Fisher, D.F. 1919. Nature and control of apple scald. J. Agric. Res. 18: 211-240.

Burmeister, D.M., Wang, Z. and Dilley, D.R. 1994. The Empire 'scald-like' disorder-1994 studies. Proc. Mich. State Hort. Soc. 124: 207-209.

Burmeister, D.M. and Dilley, D.R. 1995. A 'scald-like' controlled atmosphere storage disorder of Empire apples- a chilling injury induced by CO_2. Postharvest Biol. Technol. 6: 1-7.

Burmeister, D.M. and Rowan, S.1997. Physiological and biochemical basis for the Braeburne browning disorder (BBD). In: E.J. Mitchman (ed.) Apples and Pears. Proc. 7[th] Int'l Nat. CA Res. Conf., 2: 126-131.

Chellew, J.P. and Little C.R. 1995. Alternative methods of scald control in 'Granny Smith' apples. J. Hort. Sci. 70: 109-115.

Chen, P.M., Varga, D.M., Mielke, E.A., Facteau, T.J. and Drake, S.R. 1990. Control of superficial scald on 'd'Anjou' pears by ethoxyquin: Oxidation of α-farnesene and its inhibition. J. Food Sci. 55: 171-174.

Chen, P.M., Olsen, K.L. and Meheriuk, M. 1985. Effect of low-oxygen atmosphere on storage scald and quality preservation of 'Delicious' apples. J. Amer. Soc. Hort. Sci. 110: 16-20.

Christopher, E.P. 1941. Influence of time of harvest on storage scald development of Rhode Island Greening and Cortland apples. Proc. Amer. Soc. Hort. Sci. 39:58-.

Couey, H.M. and Williams, M.W. 1973. Preharvest application of ethephon on scald and quality of stored 'Delicious' apples. HortScience 8: 56-57.

Curry, E.A. 1990. Alternatives to DPA and ethoxyquin for controlling superficial scald of apples. Washington State Univ. Tree Fruit Postharvest J. 1: 17-20.

Curry, E.A. 1994. Preharvest applications of ethephon reduce superficial scald of 'Fuji' and 'Granny Smith' apples in storage. J. Hort. Sci. 69: 1111-1116.

Curry, E.A. 1998. Farnesene and squalene reduce scald in apples and pears. Proc. 25th International Horticulture Congress. Brussels, BE (in press).

Dewey, D.H. and Dilley, D.R. 1964. Control of storage scald. Mich. State Univ. Ext. Bul. 470.

Dilley, D.R. 1982. Principles and effects of hypobaric storage of fruits and vegetables. Am. Soc. Heating, Refrigeration and Air Conditioning Engineering Transactions, 88: 1461-1478.

Dilley, D.R. 1990. Application of air separator technology for the control of superficial scald of apples not treated with scald inhibiting chemicals. Proc. 23 Int'l. Hort. Congress, Firenze, Italy, August 27-September 1, 1990. pp. 656.

Dilley, D.R. and Beaudry, R.M. 1998. NE-103 Postharvest Physiology of Fruits. Annual Report Michigan Agric. Expt. Stat., Michigan State Univ., East Lansing, MI.

Dilley, D.R., and Dewey, D.H. 1963. Dip treatment of apples in bulk boxes with diphenylamine for control of storage scald. Mich. Agr. Expt. Sta. Quart. Bul. 46: 73-79.

Dilley, D.R., Dedolph, R.R., MacLean, D.C. and Dewey, D.H. 1963. Apple scald induction by anaerobiosis. Nature. 200:1229-1230.

Dodd, M.C. and Bester, R. 1993. Alternative antioxidants for control of superficial scald. Washington State Univ. Tree Fruit Postharvest J. 4: 33-34.

Du, Z. and Bramlage, W.J. 1993. A modified hypothesis on the role of conjugated trienes in superficial scald development on stored apples. J. Amer. Soc. Hort. Sci. 118: 807-813.

Du, Z. and Bramlage, W.J. 1994. Roles of ethylene in the development of superficial scald in 'Cordland' apples. J. Amer. Soc. Hort. Sci. 119: 516-523.

Elgar, H.J., Burmeister, D.M., Watkins, C.B. 1998. Storage and handling effects on a CO2-related internal browning disorder of 'Braeburn' apples. HortScience 33: 719-722.

Emongor, V.E., Murr, D.P. and Lougheed, E.C. 1994. Preharvest factors that predispose apples to superficial scald. Postharvest Biol. and Technol. 4: 289-300.

Fica, J. 1991. The response of Cortland apples to low oxygen and low ethylene CA storage. Fruit Sci. Reports 18: 63-67.

Fidler, J.C. 1950. Studies of the physiologically-active volatile organic compounds produced by fruits: The rate of production of carbon dioxide and volatile organic compounds by King Edward VII apples in gas storage, and the effect of the removal of the volatiles from the atmosphere of the store in the incidence of superficial scald. J. Hort. Sci. 25: 81.

Fidler,J.C. 1957. Scald and weather. Food Science Abstracts, 28: 545-554.

Filmer, A.A.E. and Meigh, D.F. 1971. Natural skin coating of the apple and its influence on scald in storage. IV.-Oxidation products of α-farnesene. J.Sci.Fd Agric. 22: 188-190.

Gallerani, G., Pratella, G.C. and Budini, R. A. 1990. The distribution and role of natural antioxidant substances in apple fruit affected by superficial scald . Adv. Hort. Sci. 4: 144-146.

Ghahramani, F. and Scott, K.J. 1998a. The action of ethanol in controlling superficial scald of apples. Aust. J. Agric. Res. 49: 199-205.

Ghahramani, F. and Scott, K.J. 1998b. Oxygen stress of Granny Smith apples in relation to superficial scald, ethanol, α-farnesene and conjugated trienes. Aust. J. Agric. Res. 49: 207-210.

Gran, C.D. and Beaudry, R.M. 1993. Determination of the low oxygen limit for several commercial apple cultivars by respiratory quotient breakpoint. Postharvest. Biol. and Technol. 3: 259-267.

Greene, D.W., Lord, W.J. and Bramlage, W.J. 1977. Mid-summer applications of ethephon and daminozide on apples. II. Effects on 'Delicious'. J. Amer. Soc. Hort. Sci. 102: 494-497.

Hall, E.G., Sykes, S.M. and Trout, S.A. 1953. Effects of skin coatings on the behaviour of apples in storage, III. Cool storage investigations. Aust. J. Agric. Res. 4: 365-383.

Hall, E.G. 1957. Control of apple scald. Food Preservation Quarterly 17: 2-6.

Hall, E.G., Scott, K.J. and Coote, G.G. 1961. Control of superficial scald on Granny Smith apples with diphenylamine. Aust. J. Agric. Res. 12: 834-853.

Halliwell, B. and Gutteridge,I.C. 1989. Free radicals in biology and medicine. Clarendon Press, Oxford. pp543.

Hammett, L.K. 1976. Ethephon influence on storage quality of 'Starkrimson Delicious' and 'Golden Delicious' apples. HortScience 11: 57-59.

Hardenburg, R.E. and Anderson, R.E. 1965. Postharvest chemical, hot water, and packaging treatments to control apple scald. Proc. Amer. Soc. Hort. Sci. 87: 93-99.

Hardenburg, R.E. and Spalding, D.H. 1972. Postharvest benomyl and thiabendazole treatments, alone and with scald inhibitors, to control blue and grey mold in wounded apples. J. Amer. Soc. Hort. Sci. 97: 154-158.

Harvey, J.M. 1978. Reduction of losses in fresh market fruits and vegetables. Annu. Rev. Phytopathol. 16: 321-341.

Hintlian,C.B. and and Hotchkiss, J.H. 1986. The safety of modified atmosphere packaging: A review. Food Technol. 40: 70-76.

Huelin, F.E. and K.E. Murray. 1966. α-Farnesene in the natural coating of apples. Nature (London) 210: 1260-1261.

Huelin, F.E. and Coggiola, I.M. 1968. Superficial scald, a functional disorder of stored apples: IV. Effect of variety, maturity, oiled wraps and diphenylamine on the concentration of α-farnesene in the fruit. J. Sci. Food Agr. 19: 297-301.

Huelin, F.E. and Coggiola, I.M. 1970a. Superficial scald, a functional disorder of stored apples: V. Oxidation of α-farnesene and its inhibition by diphenylamine. J. Sci. Food. Agr. 21: 44-48.

Huelin, F.E. and Coggiola, I.M. 1970b. Superficial scald, a functional disorder of stored apples. VI.-Evaporation of α-farnesene from the fruit. J. Sci. Fd Agric. 21: 82-86

Huelin, F.E. and Coggiola, I.M. 1970c. Superficial scald, a functional disorder of stored apples. VII.-Effect of applied α-farnesene, temperature and diphenylamine on scald and the concentration and oxidation of α-farnesene in the fruit. J. Sci. Fd Agric. 21: 584-589.

Ingle, M. and D'Souza, M. 1989. Physiology and control of superficial scald of apples: A review. HortScience 24: 28-31.

Johnson, D.S., Allen, J.G. and Warman, T.M. 1980. Postharvest application of diphenylamine and ethoxyquin for the control of superficial scald on 'Bramley's Seedling' apples. J. Sci. Food Agr. 31: 1189-1194.

Johnson, D.S., Dover, C.J. and Colgan, R.J. 1998. Effect of rate of establishment of CA conditions on the development of 'CO_2 injury' in Bramley's Seedling apples. Acta Hort. 464: 351-356.

Johnson, D.S., Prinja, J. and Smith, S.M. 1989. The use of controlled atmosphere (CA) conditions for the control of bitter pit and superficial scald in Bramley's Seedling apples. Proc. 5th International CA Conference 1: 157-168.

Kader, A.A. 1986. Biological and physiological basis for effects of controlled and modified atmosphere on fruits and vegetables. Food Technology 40: 99-103.

Knee, M. and Hatfield, S.G.S. 1981. Benefits of ethylene removal during apple storage. Ann. Applied Biol. 98: 157-165.

Lau, O.L. 1983. Effects of storage procedures and low oxygen and high carbon dioxide atmospheres and storage quality of 'Spartan' apples. J. Amer. Soc. Hort. Sci. 108: 953-957.

Lau, O.L. 1985a. Storage procedures, low oxygen and low carbon dioxide atmospheres on storage quality of 'Golden Delicious' and 'Delicious' apples. J. Amer. Soc. Hort. Sci. 110: 541-547.

Lau, O.L. 1985b. Storage responses of four apple cultivars to low oxygen atmosphere. In: S.M. Blankemship (ed.), Controlled Atmospheres for Storage and Transport of Perishable Agricultural Commodities. Proc. 4th Natl. CA Res. Conf., North Carolina State Univ., Hortic. Rep., 126: 43-56.

Lau, O.L. 1989. Control of storage scald in 'Delicious' apples by diphenylamine, low oxygen atmosphere and ethylene scrubbling. Proc. 5th International CA Conference 1: 177-184.

Lau, O.L. 1990. Efficacy of diphenylamine, ultra-low oxygen, and ethylene scrubbing on scald control in 'Delicious' apples. J. Amer. Soc. Hort. Sci. 115: 959-961.

Lau, O.L. 1993. Scald and its control: The north American situation. Acta Hortic. 326: 225-230.

Lau, O.L. 1998. Effect of growing season, harvest maturity, waxing, low O_2 and elevated CO_2 on flesh browning disorders in 'Braeburn' apples. Postharvest Biol. and Technol. 14: 131-141.

Little, C.R., Faragher, J.D. and Taylor, H.S. 1982. Effects of initial low oxygen stress treatments in low oxygen modified atmosphere storage of Granny Smith apples. J. Amer. Soc. Hort. Sci. 107: 320-323.

Little, C.R. 1985. The advantages of ultra low oxygen storage. In:Sharkey, P.J. (ed.) Proc. Postharvest Hort. Workshop. Melbourne Australia. CSIRO. Sydney, Australia. pp. 80-90.

Little, C.R. Taylor, H.J. and McFarlane, F. 1985. Postharvest and storage factors affecting superficial scald and core flush of Granny Smith apples. HortScience 20: 1080-1082.

Little, C.R. and Peggie, I.A. 1987. Storage injury of pome fruits caused by stress levels of O_2, CO_2, temperature and ethylene. HortScience 22: 783-790.

Little, C.R. and Barrand, L. 1989. Seasonal orchard and storage conditions affecting storage scald in pome fruit. Proc. 5th International CA Conference, Idaho, USA, 177-184.

Liu, F.W. 1985. Conditions for low ethylene CA storage of apples: A review. In: S.M. Blankemship (ed.), Controlled Atmospheres for Storage and Transport of Perishable Agricultural Commodities. Proc. 4th Natl. CA Res. Conf., North Carolina State Univ., Hortic. Rep., 126: 127-134.

Lurie, S., Klein, J. and Ben-Arie, R. 1989a. Physiological changes in diphenylamine-related 'Granny Smith' apples. Israel J. Botany 38: 199-207.

Lurie, S., Meir, S. and Ben-Arie, R. 1989b. Preharvest ethephon sprays reduce superficial scald of 'Granny Smith' apples. HortScience 24: 104-106.

Lurie, S., Klein, J.D. and Ben-Arie, R. 1990. Postharvest heat treatment as a possible means of reducing superficial scald of apples. J. Hort. Sci. 65: 503-509.

Lurie, S., Klein, J.D. and Ben-Arie, R. 1991. Prestorage heat treatment delays development of superficial scald on 'Granny Smith' apples. HortScience 26: 166-167.

Manseka, V.S. and Vasilakakis, M. 1993. Effect of storage maturity, postharvest treatments and storage conditions on superficial scald and quality of apples. Acta Hortic. 326: 213-224.

Matich, A.J., Rowan, D.D. and Banks, N.H. 1996. Solid phase microextraction for quantitative headspace sampling of apple volatiles. Anal. Chem. 68: 4114-4118.

Matich, A.J., Banks, N.H. and Rowan, D.D. 1998. Modification of α-farnesene levels in cool-stored 'Granny Smith' apples by ventilation. Postharvest Biol. and Technol. 14: 159-170.

Meigh, D.F. 1969. Production of farnesene and incidence of scald in stored apples. Qual. Plant. Mater. Veg. 19:243-254.

Meigh, D.F. and Filmer, A.A.E. 1969. The natural skin coating of the apple and its influence on scald in storage: III. α-farnesene. J. Sci. Food Agr. 20: 139-143.

Meigh, D.F. 1970. Apple scald. In: A.C. Hulme (Ed.), The Biochemistry of Fruits and Their Products. Vol. 1. Academic Press, London, pp. 555-569.

Meir, S. and Bramlage, W.J. 1988. Antioxidant activity in 'Cortland' apple peel and susceptibility to superficial scald after storage. J. Amer. Soc. Hort. Sci. 113: 412-418.

Melville, F. and Hardisty, S.E. 1953. Scald in Granny Smith apples. Practical aspects of control. J. Agric. W. Australia 2: 101-107.

Murray, K.E., Huelin, F.E. and Davenport, J.B. 1964. Occurrence of α-farnesene in the natural coating of apples. Nature(London) 204: 80.

Murray, K.E. 1969. α-Farnesene: Isolation from the natural coating of apples. Aust. J. Chem. 22: 197-204.

Patterson, M.E. and Workman, M. 1962. The influence of oxygen and carbon dioxide on the development of apple scald . Proc. Amer. Soc. Hort. Sci. 80: 130-136.

Piretti, M.V., Gallerani, G. and Pratella G.C. 1994. Polyphenol fate and superficial scald in apple. Postharvest Biol. and Technol. 4: 213-224.

Porritt, S.W. 1966. The effect of low oxygen and low concentrations of carbon dioxide on the quality of apples stored in controlled atmospheres. Can. J. Plant Sci. 46: 317-321.

Roberts, E.A., Hall, E.G. and Scott, K.J. 1963. The effect of carbon dioxide and oxygen concentration on superficial scald on Granny Smith apples. Australian J. Agric. Res. 14: 765-777.

Rowan, D.D., Allen, J.M., Fielder, S., Spicer, J.A. and Brimble, M.A. 1995. Identification of conjugated triene oxidation products of α-farnesene in apple skin. J. Ag. Food Chem. 43: 2040-2045.

Rupasinghe, H.P.V., Paliyath, G. and Murr, D.P. 1998. Biosynthesis of α-farnesene and its relation to superficial scald development in 'Delicious apples. J. Amer. Soc. Hort. Sci. 123: 882-886.

Scott, K.J., Wills, R.B.H. and McBailey, W. 1980. The action of phorone and other compounds in controlling superficial scald of apples. Scientia Hort. 13: 9-14.

Scott, K.J., Yuen, C.M.C. and Ghahramani, F. 1995a. Ethanol vapor- a new anti-scald treatment for apples. Postharvest Biol. Technol. 6: 201-208.

Scott, K.J., Yuen, C.M.C. and Kim, G.H. 1995b. Reduction of superficial scald of apples with vegetable oils. Postharvest Biol. and Technol. 6: 219-223.

Sharples, R.O. and Johnson, D.S. 1981. Storage of cox in ultra low oxygen - Sensitivity to low temperature injury. Rpt. E. Malling Res. Sta. pp. 133.

Shutak, V. and Christopher, E.P. 1960. Role of the cuticle in development of storage scald on Corland apples. Pro. Amer. Soc. Hort. Sci. 76: 106-111.

Skrzynski, J., Fica, J. and Dilley, D.R. 1985. The effect of ethylene removal during controlled atmosphere storage of Red Delicious, Empire, Jonathan, Idared, and Law Rome apples. In: S.M. Blankemship (ed.), Controlled Atmospheres for Storage and Transport of Perishable Agricultural Commodities. Proc. 4th Natl. CA Res. Conf., North Carolina State Univ., Hortic. Rep., 126: 115-126.

Smith, S.M. and Stow, J.R. 1994. The potential of a sucrose ester coating material for improving the storage and shelf-life qualities of Cox's Orange Pippin apples. Ann. Appl. Biol. 104: 383-391.

Smock, R.M. 1957. A comparison of treatments for control of the apple scald disease. Proc. Amer. Soc. Hort. Sci. 69: 91-100.

Smock, R.M. 1961. Methods of scald control on the apple. Cornell Univ. Agric. Exp. Stn., Bull. No. 970, pp. 55.

Song, J. and Beaudry, R.M. 1996. Rethinking apple scald: new hypothesis on the causal reason for development of scald in apples. HortScience 31: 605.

Song, J., Gardner, B.D., Holland, J.F. and Beaudry, R.M. 1997. Rapid analysis of volatile flavor compounds in apple fruit using SPME and GC/Time-of-Flight Mass Spectrometry. J. Agri. Food Chem. 45: 1801-1807.

Spicer, J.A., Brimble, M.A. and Rowan, D.D. 1993. Oxidation of α-farnesene. Aust. J. Chem. 46: 1929-1939.

Truter, A.B., Combrink, J.C. and Burger, S.A. 1994. Control of superficial scald in 'Granny Smith' apples by ultra-low and stress levels of oxygen as an alternative to diphenylamine. J. Hort. Sci. 69: 581-587.

Van der Merwe, J.A., Combrink, J.C., Truter, A.B. and Calitz, F.J. 1997. Effect of initial low oxygen stress treatment and CA storage at increased carbon dioxide levels on post-storage quality of South African-grown 'Granny Smith' and 'Topred' apples. In: E.J. Mitchman (ed.) Apples and Pears. Proc. 7^{th} Int'l Nat. CA Res. Conf., 2: 79-84.

Wang, Z., Kosittrakun, M. and Dilley, D.R. 1995. Developments to control the Empire 'scald-like' disorder and superficial scald of apples. Proc. Mich. State Hort. Soc. 125: 220-221.

Wang, Z. and Dilley, D.R. 1996. Ethanol vapor treatment: A new alternative approach to control superficial scald of apples. Proc. Mich. State Hort. Soc. 126: 35-40.

Wang, Z. and Dilley, D.R. 1997. The relationship of α-farnesene production and its oxidation product 6-methyl-5-heptene-2-one to superficial scald of Granny Smith, Law Rome, Red Delicious and Idared apples during controlled atmosphere and air storage. In: E.J. Mitchman (ed.) Apples and Pears. Proc. 7^{th} Int'l Nat. CA Res. Conf., 2: 98-104.

Wang, Z., McCully, T.J. and Dilley, D.R. 1997a. The effect of ultra low oxygen storage, initial oxygen stress and ethanol vapor treatments on controlling superficial scald of Granny Smith, Law Rome, Red Delicious and Idared apples. In: E.J. Mitchman (ed.) Apples and Pears. Proc. 7^{th} Int'l Nat. CA Res. Conf., 2: 105-111.

Wang, Z., Kosittrakun, M. and Dilley, D.R. 1997b. The effect of acclimatization of fruits on the control of a CO_2-linked disorder of Empire apples. In: E.J. Mitchman (ed.) Apples and Pears. Proc. 7^{th} Int'l Nat. CA Res. Conf., 2: 193-197.

Watkins, C.B., Barden, C.L. and Bramlage, W.J. 1993. Relationships among α-farnesene, conjugated trienes and ethylene production with superficial scald development of apples. Acta Hort. 343: 155-160.

Watkins, C.B., Bramlage, W.J and Cregoe, B.A. 1995. Superficial scald of granny Smith apples is expressed as a typical chilling injury. J. Am. Soc. Hort. Sci. 120: 88-94.

Weeks, W.D., Southwick, F.W., Drake, M. and Steckel, J.E. 1958. The effect of varying rates of nitrogen and potassium on the mineral composition of 'McIntosh' foliage and fruit color. Prpc. Am. Soc. Hort. Sci. 71:11-19.

Whitaker, B.D., Solomos, T. and Harrison, D.J. 1997. Quantification of α-farnesene and its conjugated trienol oxidation products from apple peel by C_{18}-HPLC with UV detection. J. Agric. Food Chem. 45: 760-765.

Wills, R.B.H. and Scott, K.J. 1977. Evaluation of the use of butylated hydroxytoluene to reduce superficial scald of apples. Scientia Hort. 6: 125-127.

Wills, R.B.H., Scott, K.J. and McBailey, W. 1977. Reduction of superficial scald in apples with monoterpenes. Austral. J. Agr. Res. 28: 445-448.

Windus, N.D. and Shutak, V.G. 1977. Effect of ethephon, diphenylamine and daminozide on the incidence of scald development on 'Cortland' apples. J. Amer. Soc. Hort. Sci. 102: 715-718.

Yearsley, C.W., Banks, N.H., Ganesh, S. and Cleland, D.J. 1996. Determination of lower oxygen limits for apple fruit. Postharvest Biol. and Technol. 8: 95-109.

CHAPTER II

EFFECTS OF HYPOBARIC STORAGE ON THE CONTROL OF APPLE SCALD

ABSTRACT

Law Rome and Granny Smith fruits were stored hypobarically at 0.05 atmosphere pressure of air with a ventilation rate of one vessel void volume change per hour at 97% RH and also in air and in CA storage at 1.5 and 3% O_2 with 0 and 3% CO_2 for 8 months at 1°C. Fruits were placed under hypobaric storage immediately after harvest or after 0.5, 1, 2, 3, 3.5, 4, 4.5, 5 or 6 months storage in air at 1°C to determine the effects of delaying imposition of hypobaric storage on ripening and scald development and production of α-farnesene and its oxidation product 6-methyl-5-heptene-2-one (MHO). Granny Smith and Law Rome apples did not scald during hypobaric storage or afterwards when transfered to static air at one atmosphere continuously for 4 months if they were placed under hypobaric conditions within one month after harvest while held in air at 1°C. After 3 months delay, scald development was similar to that for fruits stored in air. Fruits of both cvs. produced MHO and it accumulated in the epicuticular wax when placed under hypobaric storage after one month delay in air at one atmosphere at 1°C. MHO which had partitioned in the epicuticular wax of fruits stored hypobarically after 2 or more months delay was released upon transfer of fruits to 20°C; MHO accumulated in direct proportion to the duration of the delay to hypobaric storage. In another experiment with 5 apple cvs., the production rates of α-farnesene and MHO ketone

were low during hypobaric storage but increased upon removal from storage after 7 months over a 7 day period in air at 20°C and then sharply decreased afterward. After storage, α-farnesene and MHO production rates were similar and high for Law Rome, Mutsu, Red Delicious and Golden Delicious apples and were the lowest for Granny Smith. Scald did not develop on any hypobarically stored fruits whereas it did on all cvs. except Golden Delicious stored in air at one atmosphere. Hypobaric ventilation apparently removes a scald-related volatile substance that otherwise accumulates and partitions into the epicuticular wax of fruits stored in air at atmospheric pressure.

INTRODUCTION

Hypobaric storage is a system of storing commodities while ventilating with air at less than atmospheric pressure. The commodity is placed in a vacuum-tight container in which the absolute pressure, air temperature and humidity are precisely controlled and the rate of air exchange is closely regulated. A vacuum pump operating continuously evacuates the chamber to the desired absolute pressure (commonly to 0.05 to 0.1 atmosphere) at which time a vacuum regulator opens and admits air to the vessel. The partial pressure of each of the component gases comprising the air mixture is reduced in direct proportion to the total pressure as consequence of Dalton's Law. Thus at 0.1 atmosphere absolute total pressure, the O_2 partial pressure is equivalent to 2.1% (v/v) O_2 at atmospheric pressure. And the same is true for the other gases. Therefore, after pressure reduction, the partial pressure of water vapor in the ambient air is likewise reduced 10-fold. This precisely why the air entering the vacuum vessel must be rehumidified and this is done by passing the incoming air (after it comes through the vacuum regulator) through a container of water which is at the same pressure as the vacuum vessel. This humidifies the rarified air which is then flushed through the vacuum vessel containing the commodity. The temperature of the water through which the air is admitted determines its vapor pressure and thus the relative humidity of the storage vessel. No other gas other than air is required.

The concept of hypobaric storage of fruits was introduced by Burg and Burg (1966). Since then it has been widely investigated (Dilley, 1972, 1977, 1982; Salunkhe and Wu, 1973; Spalding and Reeder, 1976, 1977) and reviewed (Jamieson,1980; Salunkhe and Wu,1975; Tolle,1972). Hypobaric storage extends the useful life of perishable commodities much beyond that achievable by CA storage at the equivalent O_2 levels. Preclimacteric apples at harvest remain preclimacteric during hypobaric storage (Dilley,1972). This phenomenon is related to another basic principle of hypobaric storage related to the diffusivity of gases; 'the rate of gas diffusion varies inversely with the absolute pressure of the gaseous medium through which it diffuses'. Therefore at 0.1 atmosphere the rate of gas diffusion is 10-fold greater than at 1 atmosphere. This has a direct bearing on the effective concentration of ethylene within plant tissues producing ethylene at a steady-state rate. For example, the preclimacteric ethylene production rate of apples is fairly constant at about 0.05 $\mu L \cdot L^{-1} \cdot kg^{-1} \cdot hr^{-1}$ while the ethylene concentration in the intercellular air space is about 0.1 $\mu L \cdot L^{-1}$ at normal atmospheric pressure. If the absolute pressure is decreased to 0.1 atmosphere, the internal ethylene concentration is decreased 10-fold which is much below the level to induce ripening because it diffuses away from the fruits 10 times as fast. When this is coupled to a reduction in the rate at which ethylene is produced at the reduced O_2 level equivalent to 2.1%, an even further reduction in ethylene concentration may exist within the fruit tissue. Moreover, the concentration of CO_2 produced in fruit metabolism is likewise reduced. Additionally, the concentration of metabolic products such as ethanol, acetaldehyde and other compounds (eg. α-farnesene) which have significant vapor pressures at fruit storage tempertures would likewise be reduced under hypobaric ventilation compared to the concentration at atmospheric pressure. α-Farnesene and its oxidation products have been implicated as factors in the superficial scald disorder (Anet, 1972; Song and Beaudry, 1996). Apple fruits do not develop scald when stored hypobarically (Dilley, 1972) while fruits stored at the equivalent partial pressure of O_2 in CA storage develop scald. The combined effects of lower production rates of oxidatively produced metabolites with significant vapor pressures at reduced O_2 levels and reduced levels in the tissue under hypobaric storage conditions may at least partially explain prevention of scald. This indicates that fruits produce some metabolite(s) or factor(s) related to scald that can be attenuated by hypobaric storage conditions. Hypobaric storage thus offers an experimental system to elucidate the nature of these substances.

We hypothesized that hypobaric storage controls scald by effectively decreasing the accumulatiom of scald-related volatile compounds in fruits which may affect fruit metabolism such as ethylene, α-farnesene and its oxidation product MHO. Hypobaric storage was investigated to determine effective treatment regimens and to gain insight into the mechanism of scald control.

MATERIALS AND METHODS

Plant materials:

Law Rome, Granny Smith, Red Delicious, Mutsu and Golden Delicious apples were harvested at the preclimacteric stage of maturity from the MSU CHES.

Hypobaric studies in 1995/96:

Apples were placed in two 1270-L vacuum vessels in a 1°C storage room. The vessels were maintained hypobarically at 0.05 atmosphere pressure of air with a ventilation rate of one vessel void volume change per hour at 97 RH (Dilley, 1982). These conditions have proven to prevent apples from developing scald in more than 25 years of experimentation. After two months of hypobaric storage at 1°C, fruits were transferred to CA at 1.5% O_2 plus 3% CO_2 and in air. After CA and air storage for 6 months, scald index was measured based on the percentage of fruit surface area affected. Scald index was measured again after an additional 10 months of storage in air.

Hypobaric studies 1996/97:

Fruits were stored hypobarically and also stored in air and in CA storage at 1.5 and

3% O_2 with 0 and 3% CO_2 for 8 months at 1°C. The hypobaric fruits were then placed in air for an additional 4 or 10 months. Some fruits stored in CA for 2 months were transferred to air. Scald index was measured as before at various intervals of storage. α-Farnesene and MHO of 5 cultivars were measured as described below.

Hypobaric studies 1997/98:

Law Rome and Granny Smith fruits were stored hypobarically at 1°C and also stored in air at the same temperature. Fruits were placed under hypobaric storage immediately after harvest or after 0.5, 1, 2, 3, 3.5, 4, 4.5, 5 or 6 months storage in air at 1°C to determine the effects of delaying imposition of hypobaric storage on ripening and scald development and production of α-farnesene and its oxidation product 6-methyl-5-heptene-2-one (MHO). At appropriate intervals, fruits were removed from hypobaric storage and transferred to 20°C in air in glass jars. Subsamples were used for fruit firmness measurements and ripening and again after 7 days. The headspace in the jars was sampled by SPME/GC/MS as described by Song et al., (1997) to determine α-farnesene and MHO production rates at daily or bi-daily intervals. Samples of epicuticular wax were removed from the fruits at each time interval and placed in glass vials to determine the amount of α-farnesene and MHO partioned in the cuticle by measuring the vial headspace.

RESULTS

Hypobaric studies in 1995-97:

Fruits stored hypobarically for 2 months then under CA at 1.5% O_2 plus 3% CO_2 for 6 months did not develop scald during storage (Table 1). A slight amount of scald developed on the Granny Smith and Law Rome cvs. for two months hypobarically plus 6 months in CA and then in air continuous for 10 months. Fruits stored hypobarically and then in air for 6 or 16 months had scald indices of 30 and 85, respectively (Table 1). Fruits stored hypobarically for 8 months plus 4 months in air did not develop scald (Table 2), whereas, scald developed after an additional 6 months of storage in air. Granny Smith and Law Rome fruits stored under CA with 1.5% O_2 for 8 months developed slight scald with indices of 8.3 and 10.3, respectively; those stored for 2 months in CA then moved to air or those under CA at 3% or more O_2 scalded severely (Table 2). Collectively, the data indicate that apples stored hypobarically do not scald during storage but may do so after subsequent and extended periods of time in a static air atmosphere (Table 2). Moreover, CA storage is much less of a deterrent to scald development than hypobaric storage. Since hypobarically stored fruits eventually scald when transferred to air at one atmosphere, this indicates that hypobaric storage does not irreversibly inhibit scald development. And that only as little as 2 months of hypobaric storage largely prevent scald suggesting that its effect is primarily manifested during the first few months of storage at low temperature by ameliorating the effect of a volatile substance produced by the fruits. This data is consistent with our observation that fruits do not scald when flow-through CA is employed at 1.5%

O_2 with 3% CO_2. Moreover, scald is largely controlled in commercial storages at 1.5% O_2 with 3% CO_2 employing air separators to remove CO_2 by flushing. Flushing also removes other volatile substances.

Table 1. Effect of pretreatment with hypobaric storage (0.05 atmosphere of air) followed by CA or air storage on scald development of Granny Smith and Law Rome apples at 1°C.

Treatments (1995)	Scald index[z]	
	Granny Smith	Law Rome
Hypobaric 2 mo. + CA (1.5% O_2 + 3% CO_2) 6 mo.	0 c	0 c
Hypobaric 2 mo. + CA (1.5% O_2 + 3% CO_2) 6 mo. + 10 mo. in air	4.0 c	9.2 b
Hypobaric 2 mo. + 6 mo. in air	31.7 b	29.6 a
Hypobaric 2 mo. + 16 mo. in air	85.3 a	-

[z] Scald index (0=none; 1=slight; 2=moderate; 3=severe) was normalized to 100. Means within columns followed by different letters differ at $p \leq .05$.

Table 2. Effect of hypobaric storage (0.05 atmosphere of air) and CA storage on scald development of Granny Smith and Law Rome apples at 1°C.

Treatments (1996)	Scald index[z]	
	Granny Smith	Law Rome
Hypobaric 8 mo.	0 e	0 f
Hypobaric 8 mo. + 4 mo. in air	0 e	0 f
Hypobaric 8 mo. + 10 mo. in air	38.3 d	31.9 d
CA (1.5% O_2 + 3% CO_2) 8 mo.	8.3 e	10.3 e
CA (3% O_2 + 0% CO_2) 8 mo.	87.6 b	77.3 b
CA (1.5% O_2 + 3% CO_2) 2 mo. + 6 mo. in air	61.7 c	49.9 c
Control (Air for 8 mo.)	100.0 a	100.0 a

[z] Scald index (0=none; 1=slight; 2=moderate; 3=severe) was normalized to 100. Means within columns followed by different letters differ at $p \leq .05$.

Hypobaric studies 1997/98:

Hypobaric storage at 0.05 atmosphere prevents scald of Granny Smith and Law Rome apples only if they are placed under hypobaric conditions within one month after harvest and held in air at 1°C (Figure 1). After 3 months delay, scald development is similar to that for fruits stored in air.

Progressively longer periods of storage in air at 1°C before Granny Smith apples were placed in hypobaric storage resulted in greater capacity of the fruits to produce a burst of α-farnesene production (Figure 2A) but not for Law Rome fruits for which a nearly opposite trend was found (Figure 2B). α-Farnesene production rates were higher with Law Rome apples stored hypobarically within one month after harvest but lower with Granny Smith apples treated similarly. The α-farnesene production capacity of Granny Smith fruits placed in hypobaric storage within one month of harvest steadily increased after transferring the fruits to air at 20°C eventually reaching or exceeding that of fruits stored after 2 months delay (Figure 2A). The amount of α-farnesene partioned in the cuticle largely followed the pattern of its production capacity for both cvs. (Figure 2C,2D). Upon transfer to 20°C, the α-farnesene evolution rate from cuticle of Law Rome apples was higher from apples which were under hypobaric condition within two months than those more than two months (Figure 2B,2D). This again mimicked the pattern of the production capacity.

MHO production rates were progressively greater as the delay to hypobaric storage increased with both cvs. (Figure 3A,3B). And, following a burst observed one day

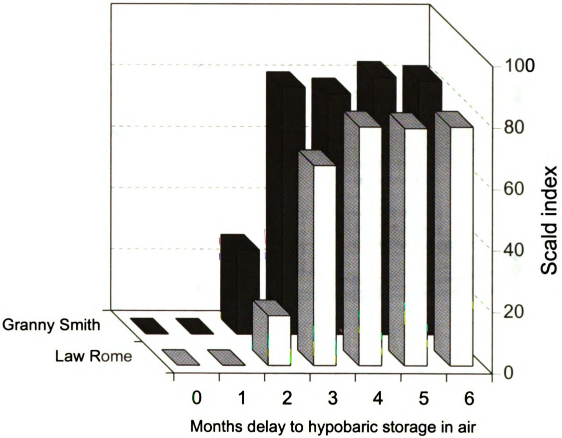

Figure 1. Effect of delaying hypobaric storage on control of scald of Granny Smith and Law Rome apples. Fruits were stored in air at 1°C prior to storing them hypobarically at 0.05 atmosphere in air. Scald index (0=none; 1=slight; 2=moderate; 3=severe) was normalized to 100.

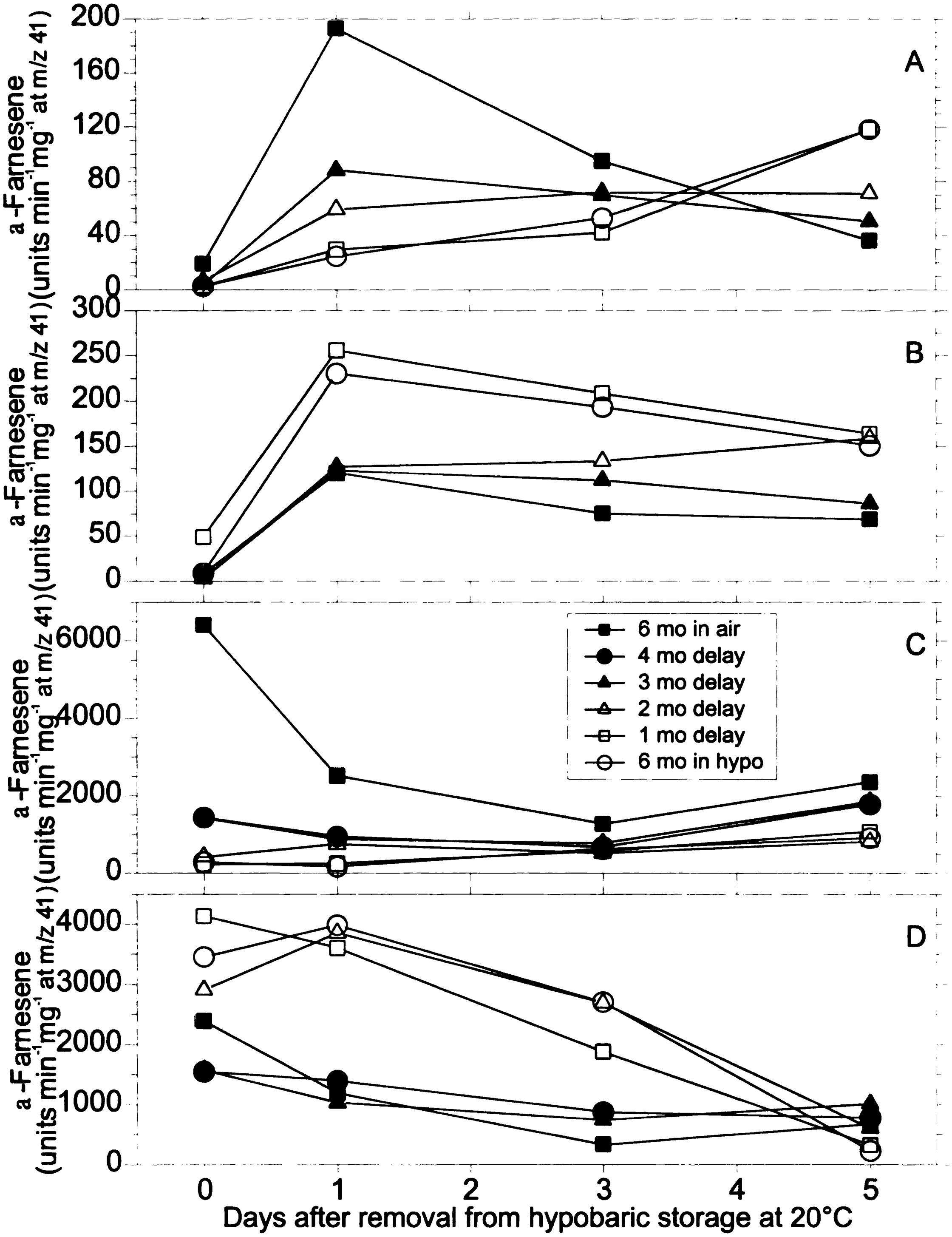

Figure 2. Change in α-farnesene levels in headspace (A, B) and in the epicuticular wax (C, D) of Granny Smith (A, C) and Law Rome (B, D) apples stored in air for 0 to 6 months before hypobaric storage at 0.05 atmosphere in air.

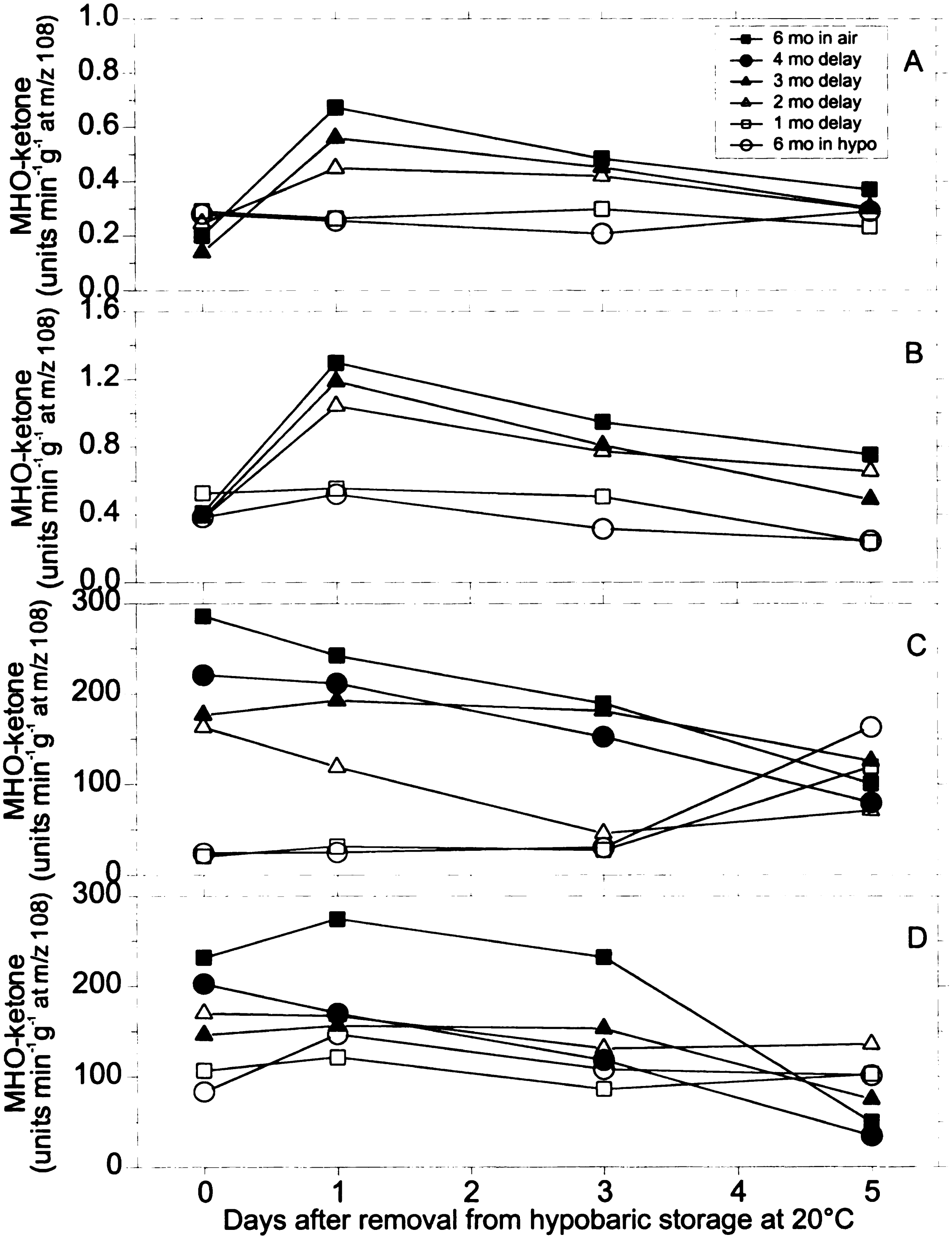

Figure 3. Change in 6-methyl-5-heptene-2-one (MHO) levels in headspace (A, B) and in the epicuticular wax (C, D) of Granny Smith (A, C) and Law Rome (B, D) apples stored in air for 0 to 6 months before hypobaric storage at 0.05 atmosphere in air.

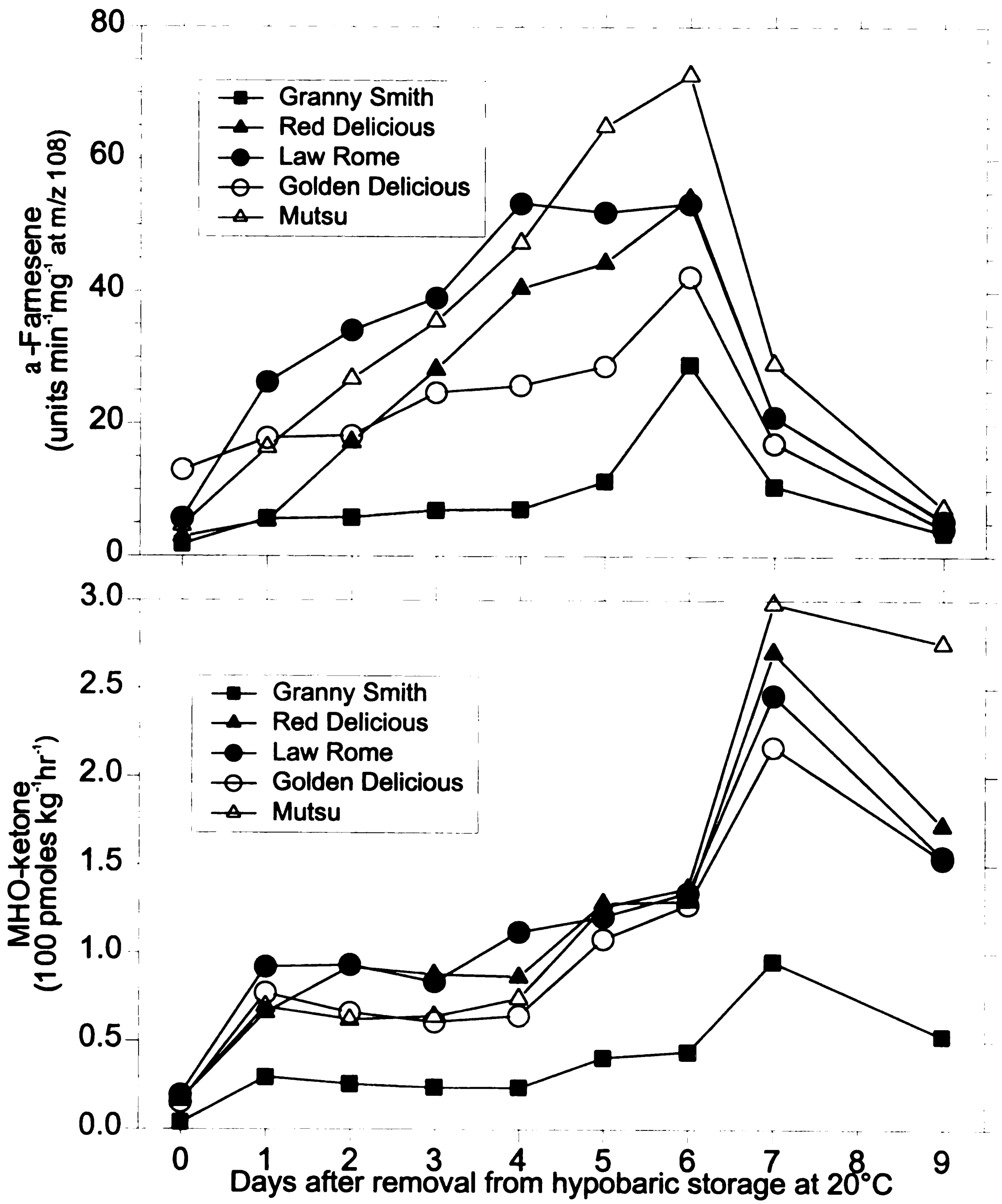

Figure 4. Change in α-farnesene (A) and its oxidation product 6-methyl-5-heptene-2-one (B) levels in headspace for different cultivars of apples after 7 months of hypobaric storage at 1°C and then at 20°C in air. (Scald did not develop on any fruits stored hypobarically but developed on all others except Golden Delicious stored at atmospheric pressure.)

after transfer to 20°C, the rates gradually diminished. Fruits that were under hypobaric storage within one month produced a nearly constant, low level of MHO. MHO which accumulated in the cuticle of fruits delayed to hypobaric storage gradually dissipated upon transfer of fruits to 20°C. Again, these fruits had the lowest levels of MHO production if the fruits were under hypobaric condition within one month (Figure 3C,3D).

Based on the measurement of 5 different cultivars including scald susceptible and not susceptible cultivars, the production rates of α-farnesene and MHO ketone were low during hypobaric storage but quickly increased upon removal from storage after 7 months of storage and held in air at 20°C; rates then, unexplicably, sharply decreased after about a week (Figure 4). The Granny Smith cv. had the lowest rates among the 5 cvs. examined. All cvs. stored hypobarically did not scald but they did when stored in air except Golden Delicious.

DISCUSSION

Removing volatiles controls scald:

Apple fruits stored hypobarically at O_2 partial pressures equivalent to CA storage do not scald during or after storage unless stored for prolonged periods of air storage (Table 2). Hypobaric storage favors the removal of ethylene and other volatile substances produced by the fruits (Dilley, 1982). A low rate of synthesis coupled with continuous removal of these substances by hypobaric ventilation results in complete control of scald during storage and even after the fruits are returned to a static air atmosphere for a few months. Fruits so stored will eventually scald. This indicates that the fruits produce some metabolites or factor(s) related to scald development that are greatly attenuated by hypobaric storage.

Our hypobaric storage studies in the 1997/98 season have again confirmed that hypobaric storage controls scald very effectively. But, to do so the fruits must be under hypobaric storage within about one months of being harvested (Figure 1). Fruits stored in air at 1°C for 2 months or longer prior to placing them under hypobaric storage at 0.05 atmosphere developed scald following storage while those kept in air for progressively shorter times before hypobaric storage did not. This is significant because it indicates that the metabolism involved in scald development becomes irreversible after 2 months storage in air. Moreover, although hypobaric storage favors the removal of all volatiles produced by the fruits, none of these volatiles including α-farnesene and its breakdown products can be implicated as factors in scald beyond the initial 2 months of storage. A trienol oxidation product of α-farnesene has been implicated in scald (Whitaker, et al., 1997) They may however be involved during this early period during which the potential for scald development is gained by storage in air (Meigh, 1970; Shutak and Christoffer, 1960; Watkins et al., 1995). These results are consistent with the observation that for DPA to be effective in controlling scald it must be applied within a few weeks of placing the fruits in air storage (Dilley and Dewey, 1963). In addition, these studies reinforce the concept that scald is a chilling injury phenomenon (Watkins et al., 1995).

It is apparent that some volatiles that can be removed by effective ventilation may be involved in scald development (Meigh and Filmer, 1969). DPA has been shown to attenuate conversion of α-farnesene to triene hydroperoxides (Anet and Coggiola, 1974). Apple fruits stored hypobarically at O_2 partial pressures equivalent to CA storage do not scald during storage but do after extended periods in air storage upon removal (Tables 1,2). Since hypobaric storage favors the removal of ethylene and other volatile substances produced by the fruits, this suggests some involvement of a volatile substance (Dilley, 1977, 1982; Jamieson, 1980). But if fruits were stored hypobarically only a couple of months, scald still developed after many months of subsequent storage in air (Table 1). This indicates that short term hypobaric storage does not irreversibly inhibit scald development. Continuous removal of those volatile substances by hypobaric ventilation for longer time results in complete control of scald even after the fruits are returned to a static air atmosphere for up to 4 months (Table 2). This suggests that the ability of fruits to produce some metabolite(s) or factor(s) related to scald development has been altered by hypobaric ventilation.

Involvement of α-farnesene and MHO:

We also assessed the role of α-farnesene and 6-methyl-5-heptene-2-one in scald development. The pattern of α-farnesene production capacity following hypobaric storage was similar for five cvs. examined in 1996 (Figure 4A). Moreover, the production levels are similar to those of fruits stored in CA and scald develops during CA storage. The ability of the fruits to produce the α-farnesene oxidation product 6-methyl-5-heptene-2-one was also demonstrated. Fruits stored hypobarically for 7 months did not develop scald; the production rates of α-farnesene and MHO ketone were low but quickly increased upon removal from storage and held in air at 20°C and then sharply decreased afterward (Figure 4). Hypobaric ventilation apparently removes a volatile substance that otherwise accumulates perhaps by partitioning into the epicuticular wax. The high level of the ketone production and absence of scald strongly suggests that the sensitivity of response in cvs. to MHO ketone is much different or that the ketone may not be the factor or only factor causing scald development. These data also indicate that the ketone is not produced as a consequence of scald development. The dramatic increase then decrease in α-farnesene production at 20°C was seen in all cvs. and virtually all treatments (Figure 4). Very interestingly, α-farnesene production abruptly decreased after 7 days while the MHO production rate was still increasing. Changes in the MHO followed the same trend except for a couple of days' delay (Figure 4). This suggests that the trends are not due to abberancies of analysis. This data clearly supports the notion that the 6-methyl-5-heptene-2-one is derived from α-farnesene.

Alternatively, the hypobarically stored fruit may lack the mechanism for the ketone to cause scald. There is ample precedence for this. Some portions of the fruit surface scald while adjoining portions do not scald. Yet, all cells would be expected to produce α-farnesene and MHO. Fruits lose susceptibility to scald as they mature (Couey and Williams, 1973;Hammett, 1976) but they don't lose ability to produce

α-farnesene and MHO. It is conceivable that the levels of natural antioxidants may mitigate scald development (Anet, 1972,1974; Meir and Bramlage, 1988; Gallerani et al., 1990). The levels of the natural antioxidant ascorbic acid increase as apple fruits mature whereas water-soluble antioxidants generally decrease during storage (Barden and Bramlage, 1994a, 1994b); ascorbic acid and other natural antioxidants may be protected from degradation when fruits are stored hypobarically. The red portion of the red apple cvs. has higher levels of ascorbic acid than the green portions. and it is the green portion of the fruit surface that is prone to scald (Chapter V, Table 3). Moreover, this correlation of the disorder with the pigment bearing cells of the hypodermis implicates pathways of pigment biosynthesis. It is apparent that susceptibility of fruits to the scald disorder is developmentally regulated by some aspect of ethylene action since inhibition of ethylene synthesis or action can control scald (Personal communication with Meiseng Tian, HortResearch Institute, New Zealand) but it is unlikely that this affects α-farnesene or MHO production.

LITERATURE CITED

Anet, E.F.L.J. 1972. Superficial scald, a functional disorder of stored apples: VIII. Volatile products from the autoxidation of α-farnesene. J. Sci. Food Agr. 23: 605-608.

Anet, E.F.L.J. 1974. Superficial scald, a functional disorder of stored apples: XI. Apple antioxidants. J. Sci. Food Agric. 25: 299-304.

Anet, E.F.L.J. and Coggiola, I.M. 1974. Superficial scald, a functional disorder of stored apples: X. Control of α-farnesene autoxidation. J. Sci. Food Agr. 25: 293-298.

Barden, C.L. and Bramlage, W.J. 1994a. Relationships of antioxidants in apple peel to changes in α-farnesene and conjugated trienes during storage, and to superficial scald development after storage. Postharvest Biol. Technology 4: 23-33.

Barden, C.L. and Bramlage, W.J. 1994b. Accumulation of antioxidants in apple peel as related to preharvest factors and superficial scald susceptibility of the fruit. J. Amer. Soc. Hort. Sci. 119: 264-269.

Burg, S.P. and Burg, E.A. 1966. Fruit storage at subatmospheric pressures. Science 153: 314-315.

Couey, H.M. and Williams, M.W. 1973. Preharvest application of ethephon on scald and quality of stored 'Delicious' apples. HortScience 8: 56-57.

Dilley, D.R. 1972. Hypobaric storage - a new concept for preservation of perishables. 102 Ann. Rpt., State Hort. Soc. Mich. 1972:82-89.

Dilley, D.R. 1977. The hypobaric concept for controlled atmosphere storage. In: D.H.Dewey (ed.) Horticultural Report. Proc. 2nd Int'l Nat. CA Res. Conf., pp 29-37.

Dilley, D.R. 1982. Principles and effects of hypobaric storage of fruits and vegetables. Am. Soc. Heating, Refrigeration and Air Conditioning Engineering Transactions, 88: 1461-1478.

Dilley, D.R., Carpenter, W.J. and Burg, S.P. 1975. Principles and application of hypobaric storage of cut flowers. Acta Hort. 41: 249-268.

Dilley, D.R. and Dewey, D.H. 1963. Dip treatment of apples in bulk boxes with diphenylamine for control of storage scald. Mich. Agr. Expt. Sta. Quart. Bul. 46: 73-79.

Dilley, D.R. and Dewey, D.H. 1973. Hypobaric storage of apples. HortScience 8: 273.

Gallerani, G., Pratella, G.C. and Budini, R. A. 1990. The distribution and role of natural antioxidant substances in apple fruit affected by superficial scald . Adv. Hort. Sci. 4: 144-146.

Ghahramani, F. and Scott, K.J. 1998a. The action of ethanol in controlling superficial scald of apples. Aust. J. Agric. Res. 49: 199-205.

Jamieson, W. 1980. Use of hypobaric conditions for refrigerated storage of meats, fruits, and vegetables. Food Technology. 1980: 64-71.

Meigh, D.F. 1970. Apple scald. In: A.C. Hulme (Ed.), The Biochemistry of Fruits and Their Products. Vol. 1. Academic Press, London, pp. 555-569.

Meir, S. and Bramlage, W.J. 1988. Antioxidant activity in 'Cortland' apple peel and susceptibility to superficial scald after storage. J. Amer. Soc. Hort. Sci. 113: 412-418.

Salunkhe, D.K. and Wu, M.T. 1973. Effects of subatmospheric pressure storage on ripening and associated chemical changes of certaindeciduous fruits. J. Amer. Soc. Hort. Sci. 98: 113-116.

Salunkhe, D.K. and Wu, M.T. 1975. Subatmospheric storage of fruits and vegetables. In N.F.Haard and D.K. Salunkhe (ed.) Postharvest Biology and Handling of Fruits and Vegetables. AVI.

Shutak, V. and Christopher, E.P. 1960. Role of the cuticle in development of storage scald on Corland apples. Pro. Amer. Soc. Hort. Sci. 76: 106-111.

Song, J. and Beaudry, R.M. 1996. Rethinking apple scald: new hypothesis on the causal reason for development of scald in apples. HortScience 31: 605.

Spalding, D.H. and Reeder, W.F. 1976. Low pressure (hypobaric) storage of limes. J. amer. Soc. Hort. Sci. 10:367-370.

Spalding, D.H. 1977. Current recommendations of atmospheres for transport and storage of tropical fruits. In: D.H.Dewey (ed.) Horticultural Report. Proc. 2nd Int'l Nat. CA Res. Conf., pp 242-249.

Tolle, W.E. 1969. Hypobaric storage of fresh produce. Year Book. United Fruit and Veg. Assoc. Pp. 27, 28, 30, 33, 34, 36, 38, 43.

Watkins, C.B., Bramlage, W.J and Cregoe, B.A. 1995. Superficial scald of granny Smith apples is expressed as a typical chilling injury. J. Am. Soc. Hort. Sci. 120: 88-94.

Whitaker, B.D., Solomos, T. and Harrison, D.J. 1997. Quantification of α-farnesene and its conjugated trienol oxidation products from apple peel by C_{18}-HPLC with UV detection. J. Agric. Food Chem. 45: 760-765.

CHAPTER III

ETHANOL VAPOR CONTROLS SUPERFICIAL SCALD OF APPLES DURING STORAGE

ABSTRACT

The objective was to develop a new alternative strategy to control superficial scald of apple to avoid applying postharvest treatment with the scald inhibitor diphenylamine (DPA).

Granny Smith, Law Rome, Red Delicious (scald susceptible) and Idared (not susceptible) were harvested at the preclimacteric stage of physiological development. Fruits were treated at 1 °C with a) different concentrations of ethanol vapor, b) different durations of pretreatment with ethanol vapor, or c) without ethanol treatment as control. Fruits were then stored in 3% O_2 with 0% CO_2 in flow-through CA and in air at 1 °C. Scald was controlled when ethanol vapor was applied to Law Rome and Red Delicious apples in the storage chambers ventilated air with enriched ethanol vapor and also in modified atmosphere packages by initially adding ethanol. In CA at 3% O_2 with 0% CO_2, fruits treated with 6000 $\mu L \cdot L^{-1}$ of ethanol vapor for one week showed significantly less superficial scald. The effectiveness of ethanol vapor treatments decreased when the concentration or exposure time to the ethanol vapor was decreased. All cultivars showed similar responses to treatments. Even though apple fruits scalded when stored in air and scald developed upon warming on the fruits from CA storage, the effects of ethanol

vapor pretreatments on reducing scald were evident in all treatments. The treatments with 6000 $\mu L \cdot L^{-1}$ ethanol vapor were more effective for scald control than the other treatments.

The ethanol vapor treatment diminished the level of several low molecular weight volatiles produced by the fruit. The rate and pattern of α-farnesene production were similar for fruits treated or not treated with ethanol vapor. The rate of MHO production was much higher in control than for the ethanol vapor treatments. This indicates that the α-farnesene is not as closely related to scald development as its oxidation product MHO.

INTRODUCTION

Superficial scald is a pervasive physiological disorder of apples and pears that results in the browning of the skin during or after low temperature storage in air or after controlled atmosphere storage which markedly extends the useful life span of these fruits. The affected cells of the hypodermis die and dehydrate (Bain, 1956). Most cvs. of apples and pears are susceptible to scald. The disorder destroys the appearance and therefore the fresh market value of the fruits. The physiology and control of scald has been periodically reviewed (Smock 1961; Meigh, 1970; Ingle and D'Souza, 1989; Emongor et al., 1994). The disorder is prevented commercially by postharvest drench treatment with diphenylamine (DPA). Numerous countries have banned the use of DPA or importation of DPA-treated fruits which mandates development of alternative methods to control scald. Fruits may possess at harvest or may acquire after harvest resistance to scald (Ingle and D'Souza, 1989). The skin browning symptoms of the disorder result from autooxidative processes in cell membranes brought about by low temperature storage beyond the ability of endogenous antioxidants such as ascorbic acid or α-tocopherol to prevent the dysfunction (Gallerani et al., 1990). Free-radicals such as hydroxyl (·OH), hydroperoxyl (HO_2·) and superoxide (O_2^-) may attack and destroy lipid and protein macro-molecular structures essential for cellular function (Stadtman, 1993). The scald inhibitor chemicals DPA and ethoxyquin are known to be effective inhibitors of lipid peroxidation so this may be their mechanism of action in controlling scald (Huellin and Coggiola, 1970a). Scott et al.(1995) found that ethanol vapor treatment controlled scald of Granny Smith apples and we have confirmed this (Wang and Dilley, 1996). Ethanol is known to be a free-radical scavenger (Halliwell and Gutteridge, 1989) and this may be a mechanism of action in scald control. Little et al.(1982) reported that scald of Granny Smith apples was largely controlled by an initial low O_2 stress followed by CA storage. Ghahramani and Scott (1998a) found that the initial low O_2 stress induces the fruits to produce their own ethanol and this may be related to the efficacy of the exogenously applied ethanol vapor treatments in reducing scald even when the fruits are subsequently stored in air. The biochemical mechanism responsible for the beneficial effects on scald control by low O_2 stress and the ensuing metabolism of ethanol remain to be elucidated. Extensive analyses of the volatiles produced by apples receiving low O_2 stress or ethanol vapor treatments support the premise that metabolism of α-farnesene in the pigment bearing cells is closely associated with development of the scald disorder

(Dilley and Beaudry, 1998; Wang et al., 1997; Wang and Dilley 1996; 1997; Ghahramani and Scott, 1998b).

We hypothesize that the scald disorder is a manifestation of chilling injury affecting the pigment bearing cells of the fruit hypodermis. Symptoms of the disorder are consequences of autooxidative processes in cell membranes brought about by low temperature storage beyond the ability of naturally occurring antioxidants to prevent the dysfunction. We investigated initial ethanol vapor treatments to determine effective treatment regimens and to gain insight into the mechanism of scald control.

MATERIALS AND METHODS

Preliminary studies 1995:

Fruits: The apples used for these experiments came from the MSU CHES. They were harvested at the preclimacteric stage of maturity approximately one week from the onset of the endogenous ethylene climactacteric. Fruits free of defects were randomized into treatment replicates comprised of 40 to 60 fruits each.

Ethanol vapor treatments: Ethanol vapors were applied to Law Rome apples in 20-L storage chambers at 3°C by circulating air at a flow rate of 80 mL min^{-1} through aqueous solutions of ethanol in flasks in series with the chambers. The initial ethanol (ETOH) concentrations were 0, 1, 2, 4 and 8% (v/v). Law Rome and Red Delicious apples were also treated at static ethanol vapor concentrations developed by equilibration with 0, 5, 10, 20 and 40% (v/v) in the chambers. Law Rome apples in modified atmosphere packages (MAP) with 2 mil low density polyethylene (LDPE) were also treated by evaporating 0.25 g ethanol per fruit within the package as was done by Scott et al.(1995). The oxygen and carbon dioxide levels in the MAP equilibrated to 5% and 8%, respectively. Fruits from all treatments were stored at 1°C.

The ethanol concentration in the storage chamber or MAP headspaces and in fruits was analyzed during storage. Ethanol was measured by gas chromatography employing a FID and column of Porapakt GTT 80/100. Volatile compounds including α-farnesene and 6-methyl-5-heptene-2-one were measured by SPME combined with gas chromatography/time of flight mass spectrometer (SPME/GC/TOF MS) as described by Song et al. (1997). Fruits were examined for scald after storage and the data is expressed as a scald index based on the percentage of fruit surface area affected where no scald = 0, <25%=1, 25-50%=2 and >50%=3.

An experiment was set up to test the response for different cultivars of apples to produce ethanol after exposed to low levels of O_2. O_2 concentrations of 0.25, 0.5, 1.0, 2.0, and 21%, were employed in equal volume MAP with 5 mil LDPE at 1°C by mixing N_2 produced with a Permea air separator with air and distributing the gas mixtures to the packages at an equal flow rate. Four cultivars, Granny Smith, Law Rome, Red Delicious and Idared, five fruits each, were contained in each MAP for 2 days then the internal ethanol was measured on GC equipped with Porapak GTT

80/100 column and FID detector.

1996 studies:

Granny Smith, Law Rome, Red Delicious and Idared fruits were harvested from trees at the MSU CHES at the preclimacteric stage of physiological development. The maturity stage at harvest was about one week before the onset of the endogenous ethylene climacteric. Fruits were treated with a) different concentrations of ethanol vapor, b) different durations of pretreatment with ethanol vapor, or c) without ethanol vapor pretreatment as control. Fruits were then stored in 3% O_2 with 0% CO_2 (dry lime added) in flow-through CA (0.12 atmosphere turnovers per hr) for 4 to 7 months and in air at 1°C. Scald data is expressed as a scald index based on the percentage of fruit surface area affected described above and scald index was normalized to 100. Scald index was measured after 4 and 7 months of CA storage and 5 months of air storage and after 7 days in air at 20°C. Three replicates of 40-60 fruits each for each cv. were used to measure the scald index for each treatment at each storage period.

To determine the effects on ripening and scald development and production of α-farnesene and its oxidation product 6-methyl-5-heptene-2-one (MHO) fruits were removed from CA and air storage, at appropriate intervals, and transferred to 20C in air in 4-L glass jars. Headspace analysis of 1.2 kilograms of fruits in a jar was done after a one hr enclosure period. α-Farnesene and MHO in the headspace were sampled by SPME and analyzed by SPME combined with GC/TOF MS. α-

Farnesene production rate is expressed as relative units min^{-1} $mg^{-1}fw$ at 108 m/z while the production rate of MHO-ketone was expressed as nmoles or 100 pmoles kg^{-1} hr^{-1} based on external standard.

RESULTS

The ethanol concentration in the headspace of the fruit storage chambers decreased with time in storage from the initial levels established as shown in Figure 1. The decrease in ethanol concentration is a consequence of the decrease in ethanol in the solution because the solution was purged with air in a flow-through system. Additionally, some of the ethanol would be expected to be metabolized by the fruit. An experiment was conducted to test this at 0 and 20°C. In chambers without fruit (Figure 2A), the headspace ethanol concentration remained at near initial levels for at least 7 days at 0°C and for 4 days at 20°C after which the level declined. The higher ethanol vapor concentration at 20°C compared to 0°C is a direct consequence of the vapor pressure/temperature relationship. In chambers with fruits at 0°C (Figure 2B), the ethanol vapor concentration decreased slowly to about one/half its initial level in 7 days. At 20°C ETOH decreased dramatically within 2 days and then declined slowly to a minimum at 4 days and then slowly increased. This pattern suggests that a considerable amount of the applied ETOH was dissolved in and metabolized by the fruit. The slow increase in ETOH after day 4 at 20°C may be a consequence of ethanol toxicity.

A similar experiment was conducted with the McIntosh cv (Figure 3). Fruits were

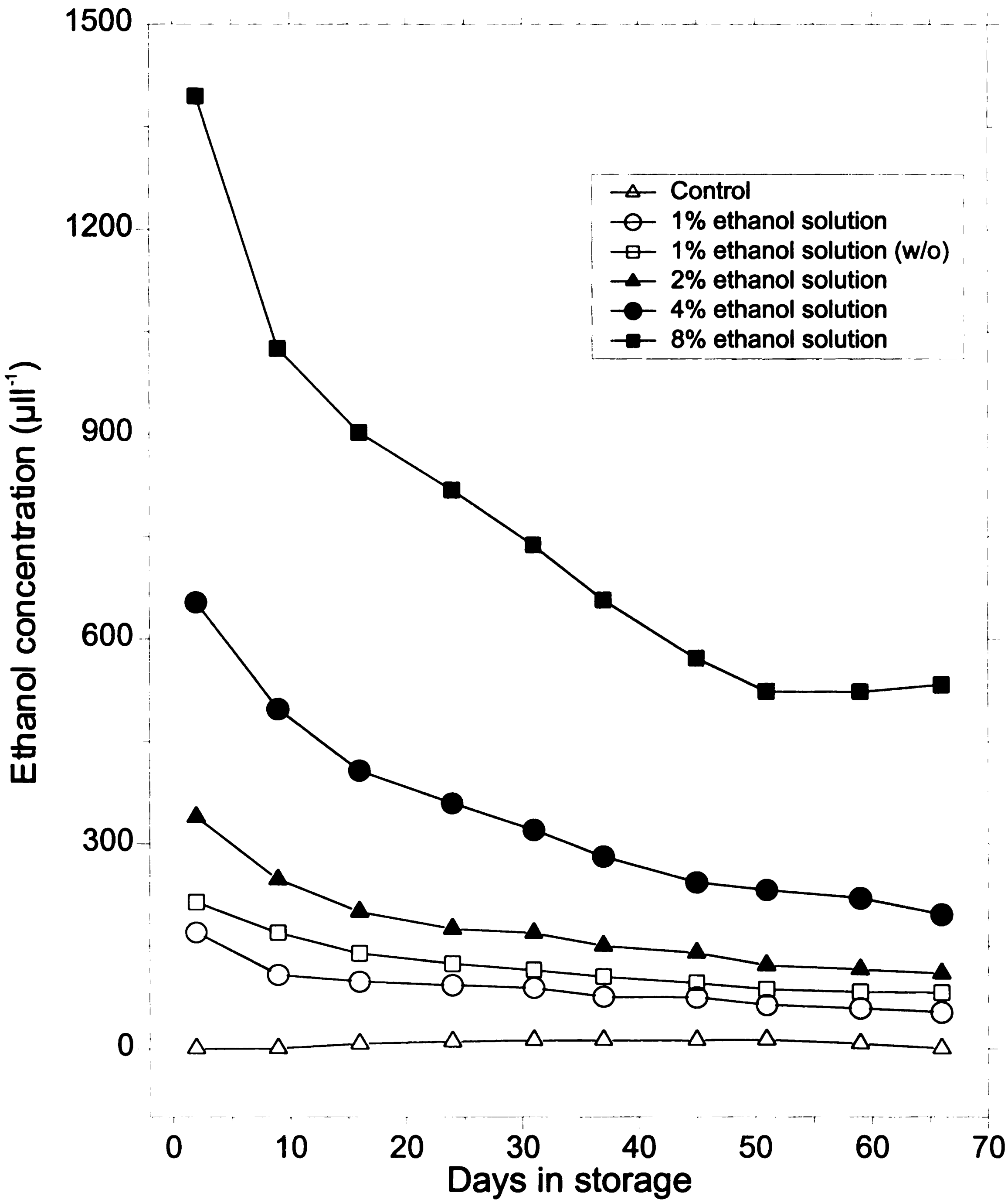

Figure 1. Changes in headspace ethanol vapor concentrations as a function of time. Ethanol vapors were applied to Law Rome apples in the experimental storage chambers at 3°C by circulating air through aqueous solutions of ethanol at 0, 1, 2, 4 and 8% (v/v). Designation 1% w/o is for a chamber without fruit.

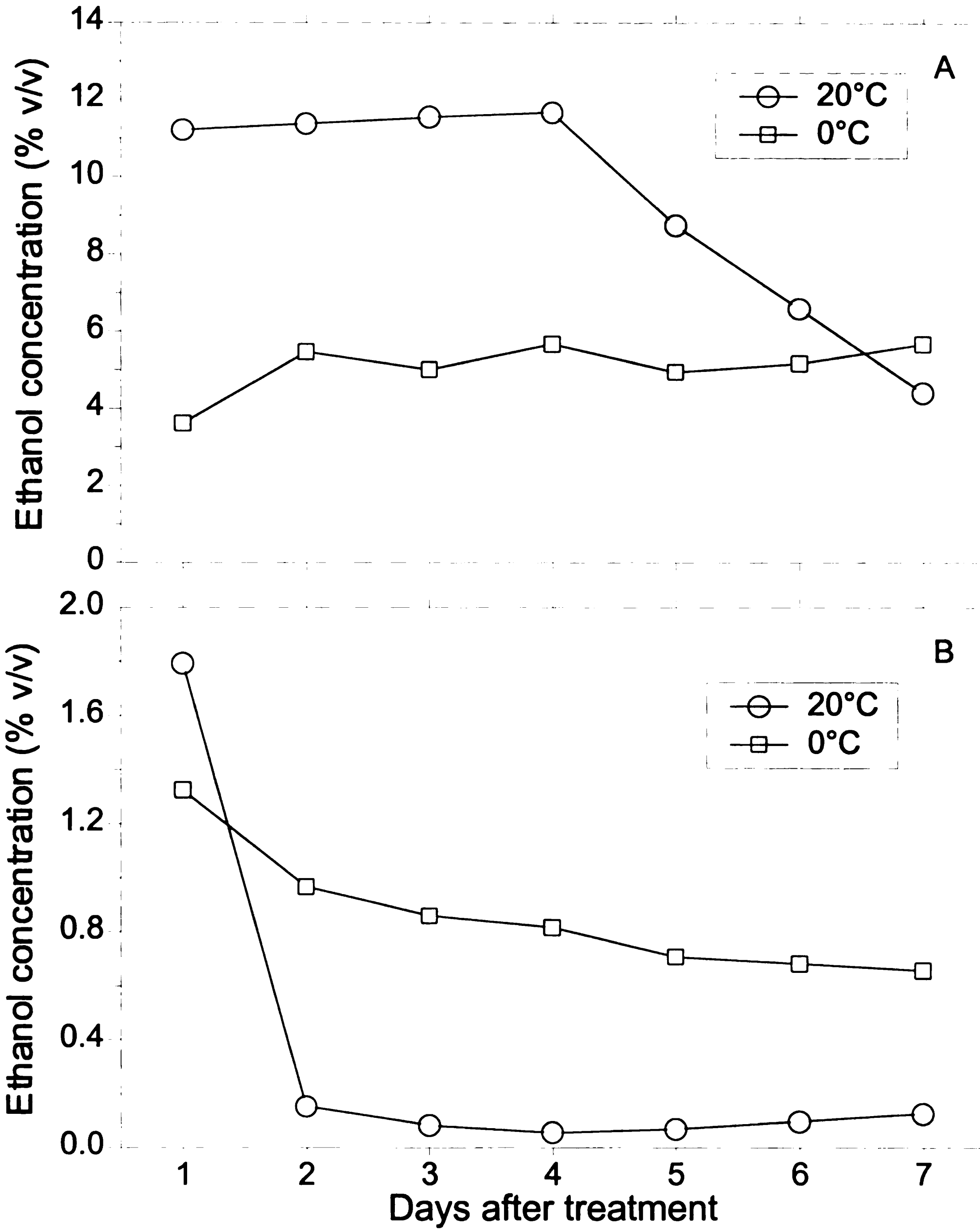

Figure 2. Changes in headspace ethanol vapor concentrations without (A) or with (B) 20 fruits at 0 and 20°C. 5g of pure ethanol was added to 10 g of vermiculite in the experimental storage chamber.

enclosed in chambers to which ETOH (0.2 to 12.8 g) was added. The ETOH concentration in the headspace was measured from 6 to 72 hours. The headspace ethanol concentration remained at near initial level within 72 hrs in no fruit chamber while in the chamber with fruits the ETOH concentration declined very fast (Figure 3A). The headspace ethanol concentration gradually decreased for the treatments with initial amounts of ethanol at 1.6 g or below in a 20-L chamber while headspace ethanol concentration of others increased continuously up to 24h then started to decline (Figure 3B). This indicates that it requires some time for the ethanol to vaporize at the low temperature condition when provided up to 3.2 g of liquid per chamber. The data in Figure 3 clearly shows that fruits readily metabolize ETOH but as the concentration increased beyond 1.6 g administered the fruits' ability to utilize it diminished. Again, ETOH toxicity may explain the decrease in the rate of ETOH consumption.

Direct evidence that fruit metabolism can be altered as a consequence of metabolizing exogenously applied ETOH was obtained. Law Rome and Red Delicious apples were ventilated with 0 to 8,000 $\mu L \cdot L^{-1}$ ETOH vapor continuously for 2 months at 1°C. As the ETOH administered increased, the internal ethanol level increased and the internal ethylene level decreased (Figure 4) indicating a reduction in the ethylene production rate. The correlation between internal ethanol and ethylene is given in Figure 5. This pertubation in ethylene production can be interpreted as a metabolic response to ETOH stress.

In addition, the ETOH concentration in the fruit increased in direct proportion to the

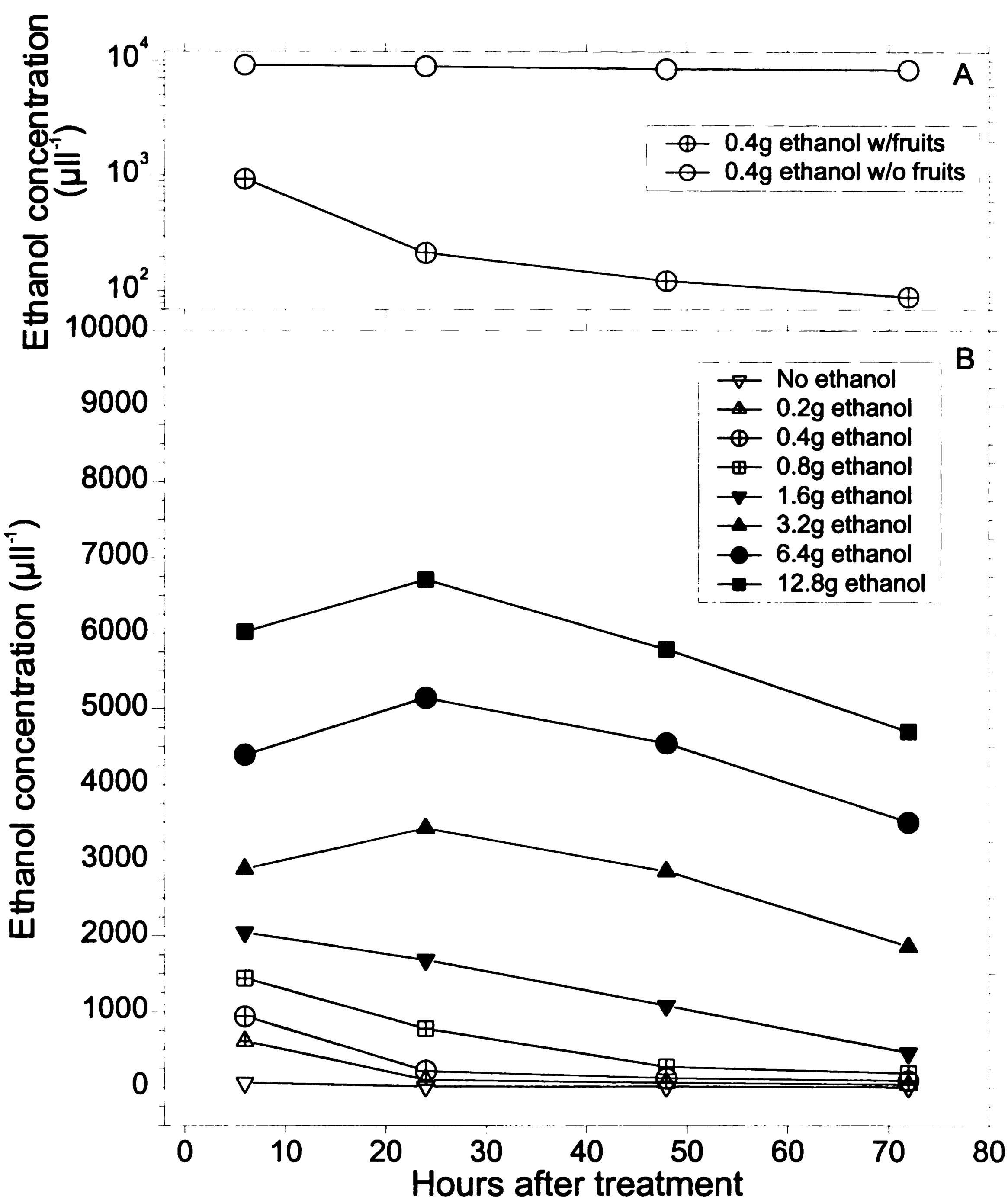

Figure 3. Changes in headspace ethanol vapor concentrations. Ethanol was added to 10 g of vermiculite in the experimental storage chamber containing McIntosh apples at 3°C.

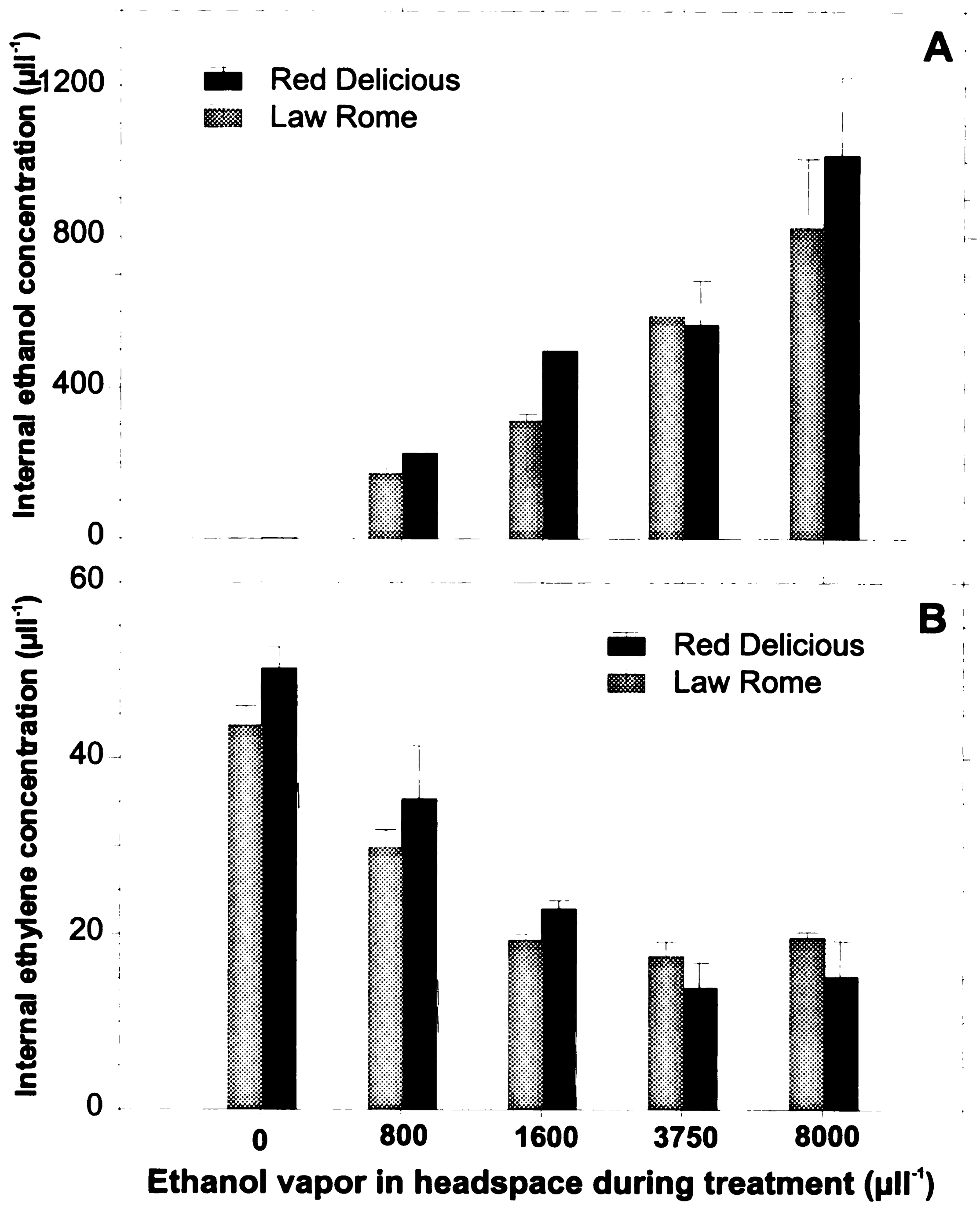

Figure 4. Internal ethanol vapor (A) and ethylene (B) concentrations in Law Rome and Red Delicious apple fruits as a function of ethanol concentration (% v/v) in the vapor generator. Ethanol concentrations were maintained for 2 months by replenishing weekly with ethanol as necessary.

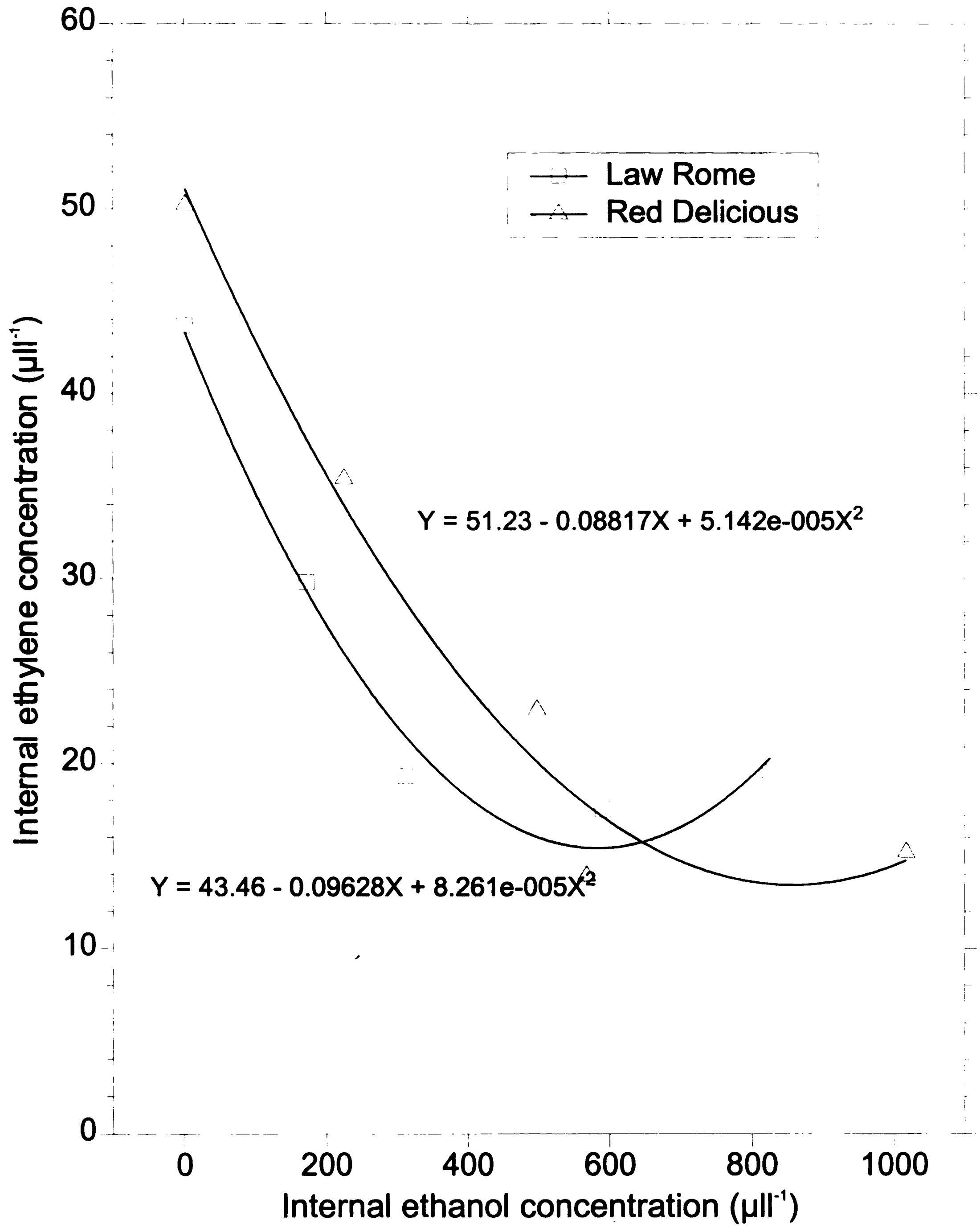

Figure 5. The correlation between internal ethanol and ethylene from the fruits exposed to the maintained ethanol concentrations for 2 months by replenishing weekly with ethanol as necessary.

ETOH vapor concentration (Figure 4A). In contrast, the ethylene concentration decreased as the internal ETOH level increased up to 200 $\mu L \cdot L^{-1}$ and 250 $\mu L \cdot L^{-1}$ for Law Rome and Red Delicious fruits, respectively. (Figure 4B). This indicates that ETOH directly affected ethylene production but the mechanism is not known. It is clear that apple fruits readily absorb ETOH vapors and can metabolize a considerable amount of applied ETOH without damage to the tissue.

Treatment with ETOH vapor generated by 5 and 10% ETOH (v/v) decreased the production of volatile compounds such as hexylacetate, hexylbutanoate/ butylhexanoate, hexyl-2-methylbutanoate and hexylhexanoate by Law Rome (Figure 6 A2,A3) and Red Delicious (Figure 6 B2,B3) apples when applied continuously for 2 months compared to ventilation with air only. Whereas treatment with ETOH vapor generated by initial 2 and 4% ETOH (v/v) was less effective (Figure 6 C2,C3). The level of α-farnesene was not markedly affected by ETOH treatment. These results indicate that ETOH vapor treatment still significantly alters fruit metabolism even though not affecting α-farnesene production.

Apple cvs. vary in their response to low O_2 levels. This is evident by ethanol accumulation in the fruits as a function of O_2 level (Figure 7). The internal ethanol levels were higher for Red Delicious and Idared apples at 1% O_2 level than for Granny Smith and Law Rome apples. Whereas, when stored at 0.25% O_2, Granny Smith and Law Rome apples accumulated more ethanol than the others.

As a consequence of ETOH in the tissue or as a result of its metabolism or

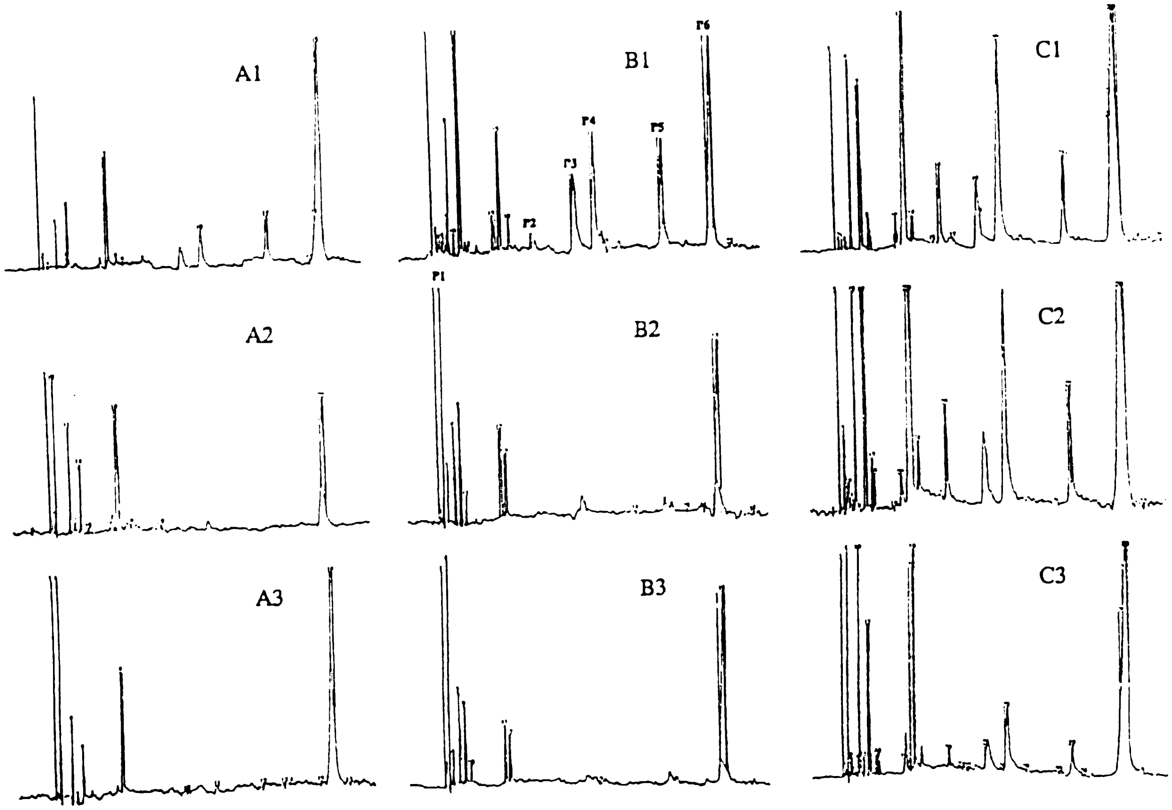

Figure 6. Changes in relative amounts of some volatile compounds in apple fruits treated with ethanol vapors which were generated by circulating air through various concentrations (% v/v) of ethanol in water: A1, 0%; A2, 5% and A3, 10% were maintained for 2 months on Law Rome apples; B1, 0%; B2, 5% and B3, 10% were maintained for 2 months on Red Delicious apples; C1, 0%; C2, 2% and C3, 4% were added initially to Law Rome apples. All fruits were stored in air for 3.5 months at 3°C. P1-P6 stand for the peaks of ethanol, hexylacetate, hexylbutanoate/ butylhexanoate, hexyl-2-methylbutanoate, hexylhexantoate, and α-farnesene, respectively.

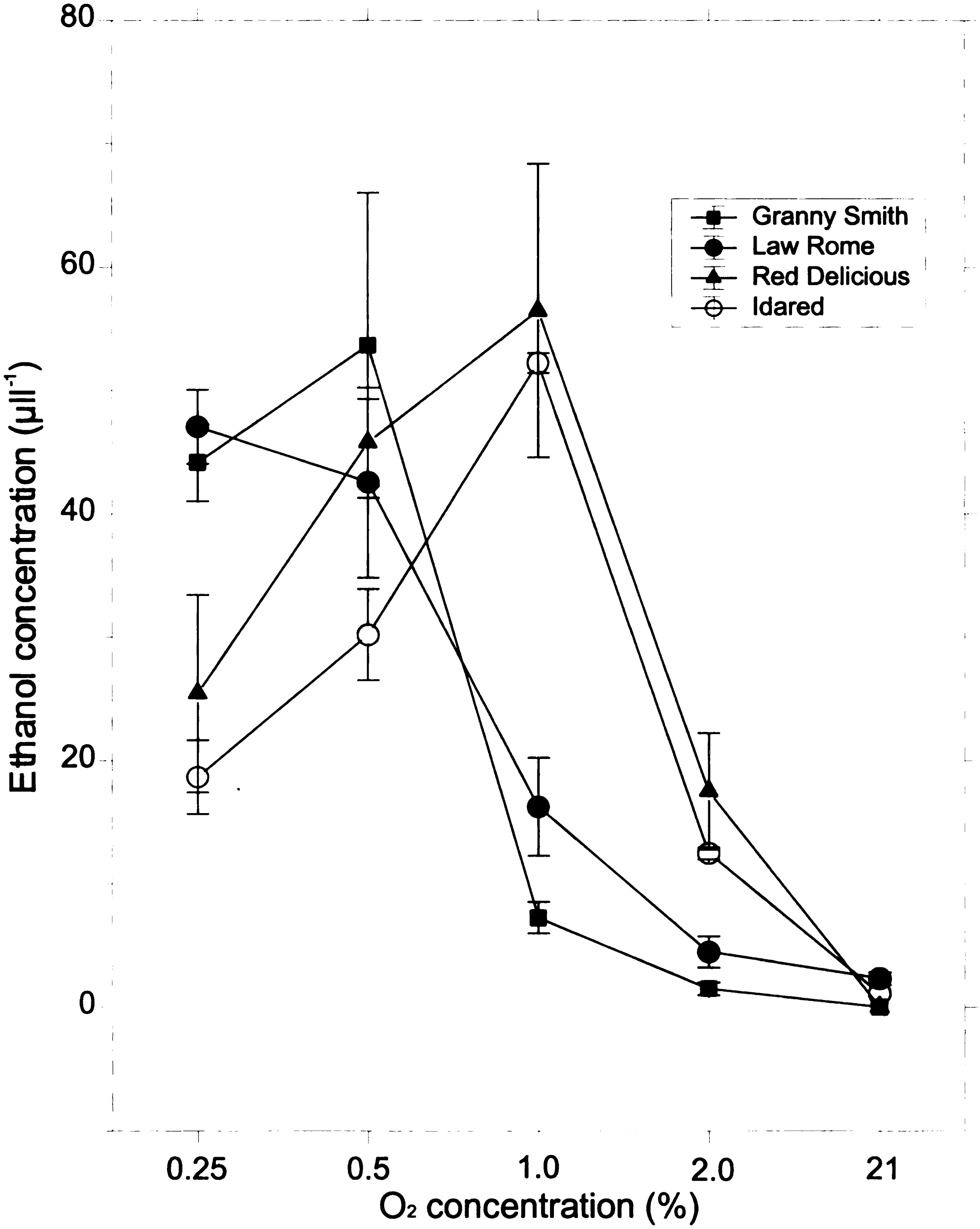

Figure 7. Effect of oxygen levels on internal ethanol concentration for Granny Smith, Law Rome, Red Delicious and Idared apples. Atmospheres were equilibrated at the respective oxygen level for 2 days prior to sampling.

pertubation of normal tissue metabolism, the incidence and severity of scald is reduced as shown in Tables 1 and 2. ETOH vapor treatment was found to diminish or control scald of Law Rome apples stored in air (Tables 1, 2). As the ETOH vapor concentration increased, the scald index for Law Rome apples stored in air was reduced (Tables1 and 2). Fruits treated with ETOH vapor generated by an 8% (v/v) ETOH:water solution received an initial ETOH vapor level of about 1400 $\mu L \cdot L^{-1}$ ETOH and by 66 days the generator was delivering only 533 $\mu L \cdot L^{-1}$. These fruits had the lowest amount of scald while those initially receiving 653 $\mu L \cdot L^{-1}$ ETOH had a higher scald index. Fruits treated with ETOH vapor at 800 and 1600 $\mu L \cdot L^{-1}$ continuously showed good scald control (Table 1). These data suggest that the primary benefit of ETOH vapor treatment is derived early in the storage period.

MAP studies:

Ethanol vapor treatment in MAP also controlled scald (Table 3) when fruits received an initial treatment with ETOH in MAP at about 9,000 $\mu L \cdot L^{-1}$ (Table 4). After 13 days the ETOH concentration declined to about 200 $\mu L \cdot L^{-1}$ (Table 4). During this time the O_2 and CO_2 levels equilibrated to about 5 and 8%, respectively. The decrease in the headspace ETOH level is a consequence of it becoming dissolved in the water of the tissue and metabolized with adequate O_2 being available. Scott et al., (1995) found that ethanol vapor treatment was effective in controlling scald of Granny Smith apples in MAP when ETOH was added initially.

Table 1. Effect of maintaining a constant ethanol vapor concentration for 2 months on control of scald of Law Rome apples stored in air at 3°C for 3.5 months.

Ethanol conc. (%(v/v))	Ethanol vapor ($\mu l\ l^{-1}$ (v/v))	Scald index[z]	
		0d	+7d at 20°C
0	0	1.0	2.33
5	800	0	0.25
10	1600	0	0
20	3750	Slight ETOH injury	Slight ETOH injury
40	8000	Severe ETOH injury	Severe ETOH injury

[z]Scald index: 0=0%, 1=<25%, 2=25 to 50%, 3=>50% of surface scalded.

Table 2. Effect of initial ethanol vapor treatment on control of scald of Law Rome apples stored in air for 3.5 months at 3°C.

Ethanol conc. (%(v/v))	Ethanol vapor ($\mu l\ l^{-1}$ (v/v))			Scald index[z]	
	0d	31d	66d	0d	+7d at 20°C
0	0	12	0	2.63	2.93
1	170	89	53	2.67	2.83
2	339	169	110	2.40	2.79
4	653	320	196	1.67	2.24
8	1394	737	533	0.83	1.53

[z]Scald index: 0=0%, 1=<25%, 2=25 to 50%, 3=>50% of surface scalded.

Table 3. Effect of initial ethanol vapor treatment on control of scald of Law Rome apples stored in MAP with 2 mil LDPE for 3 months at 1°C.

Time exposed to ethanol (weeks)	Ethanol added (g/fruit)	Scald index[z]	
		0d	+7d at 20°C
0 (control)	0	0.98	1.66
1	0.25	0.38	0.58
2	0.25	0.27	0.54
4	0.25	0.40	0.62

[z]Scald index: 0=0%, 1=<25%, 2=25 to 50%, 3=>50% of surface scalded.

Table 4. Ethanol vapor concentration change in MAP with 2 mil LDPE at 1°C. Ethanol at 0.25 g per fruit was added initially.

Time (days)	Ethanol ($\mu l\ l^{-1}$±SD)	O_2 (%±SD)	CO_2 (%±SD)
1	9068±462	16.9±0.45	1.8±0.22
3	5927±388	11.2±1.8	4.1±0.55
5	3292±311	6.6±1.9	6.1±0.48
7	1538±179	3.4±2.5	7.7±0.66
9	645±71	2.8±3.3	8.3±1.03
11	346±54	4.9±2.6	8.2±0.88
13	220±23	5.2±2.0	8.4±0.58

1996 studies:

Our experiments in 1996-97 confirmed our earlier studies that ethanol vapor treatment at the beginning of the storage period can prevent or largely diminish superficial scald of apples as first reported by K. Scott et al. (1995) in Australia. We observed that ethanol vapor treatment induced apples to accumulate ethanol or to produce ethanol aerobically. Since apples produce ethanol when the O_2 level is below the extinction point of alcohol fermentation (ca. 1%) suggests that a storage regimen of initial low O_2 stress sufficient to induce the fruits to produce ethanol may be employed to control scald.

We conducted extensive studies in 1996-97 to ascertain the effects of ethanol vapor treatments on control of scald of Granny Smith, Red Delicious, Law Rome and Idared apples. Fruits were stored in CA subsequent to receiving treatments.

Ethanol vapor treatment reduced scald incidence for all scald susceptible cvs. (Granny Smith, Red Delicious, Law Rome) examined in a dose dependent manner (Figure 8). The most effective treatment was one week exposure to 6000 $\mu L \cdot L^{-1}$ ethanol vapor. Reducing the ethanol concentration and duration of exposure at a given concentration reduced the effectiveness for scald control. Continuous exposure of fruits to ethanol vapor at 800 to 1600 $\mu L \cdot L^{-1}$ for 8 weeks at 3% O_2 with subsequent storage at 3% O_2 reduced but did not prevent scald. Likewise, exposure to 2700 $\mu L \cdot L^{-1}$ ethanol vapor for up to two weeks gave only marginal control of scald. This suggests that it is the initial period in storage that ethanol vapor is

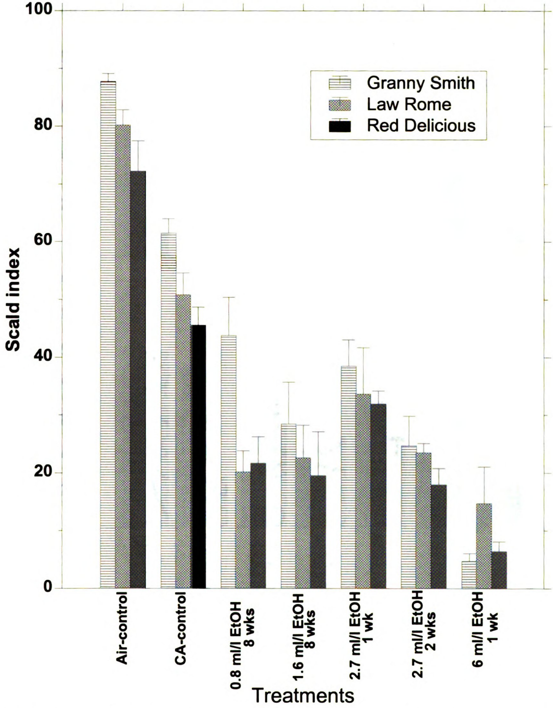

Figure 8. Effect of ethanol vapor on controlling apple scald for Granny Smith, Law Rome and Red Delicious after 4 months of CA storage (3% O_2 + 0% CO_2) at 1°C plus 7 days at 20°C in air. Scald index (0=none; 1=slight; 2=moderate; 3=severe) was normalized to 100.

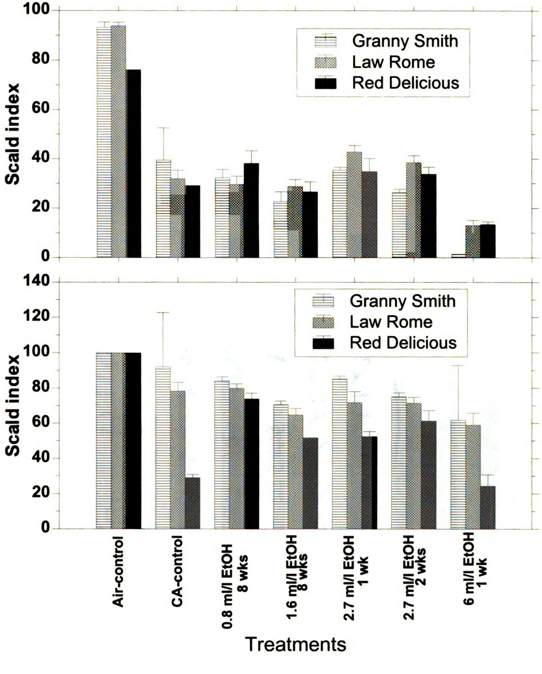

Figure 9. Effect of ethanol vapor on controlling apple scald for Granny Smith, Law Rome and Red Delicious after 7 months of CA storage (3% O_2 + 0% CO_2) at 1°C after storage (A) and plus 7 days at 20°C in air (B). Scald index (0=none; 1=slight; 2=moderate; 3=severe) was normalized to 100.

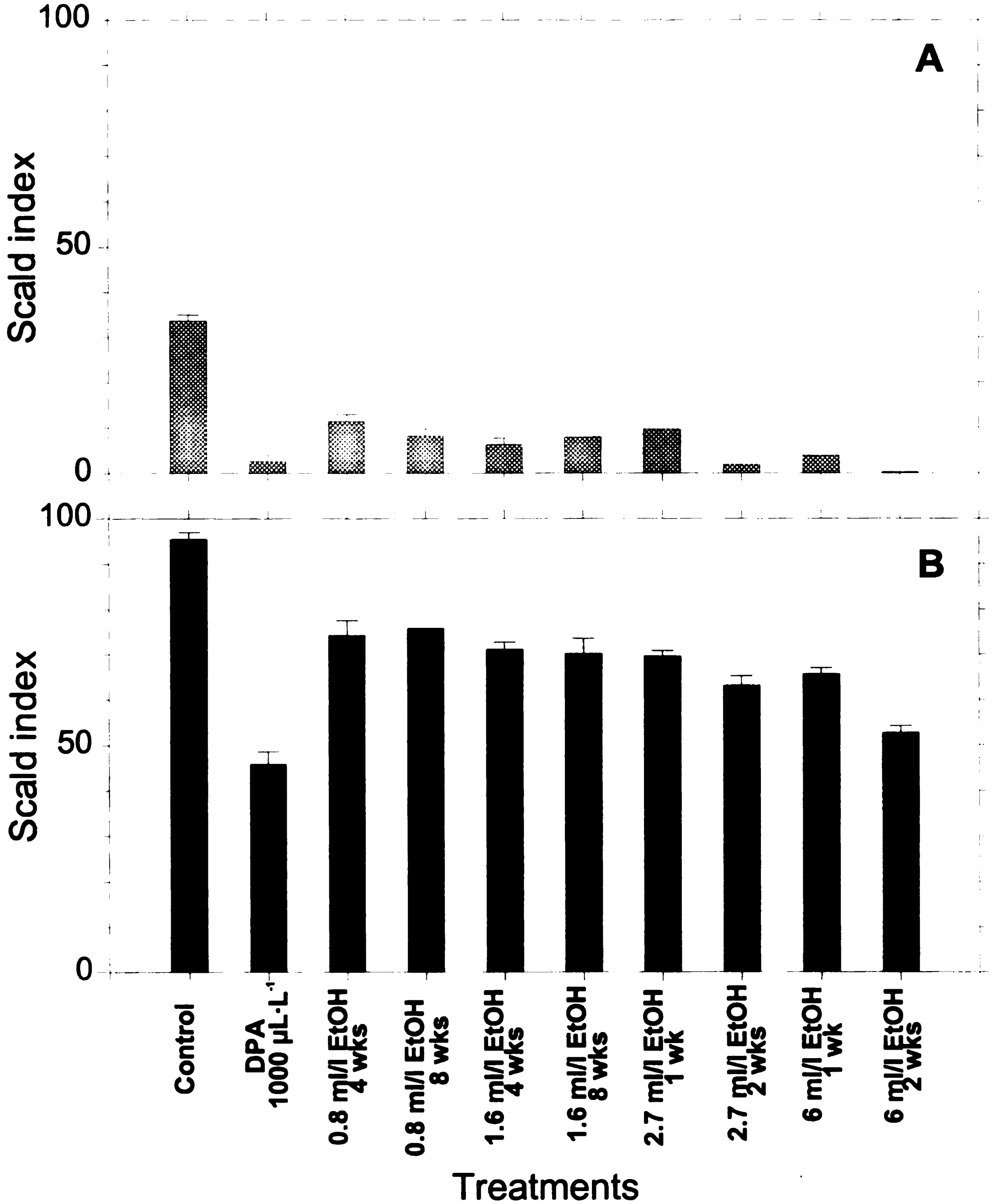

Figure 10. Effect of ethanol vapor or diphenylamine (DPA) on controlling scald of Granny Smith apples stored in air for 5 months at 1°C after storage (A) and plus 7 days at 20°C (B). Scald index (0=none; 1=slight; 2=moderate; 3=severe) was normalized to 100.

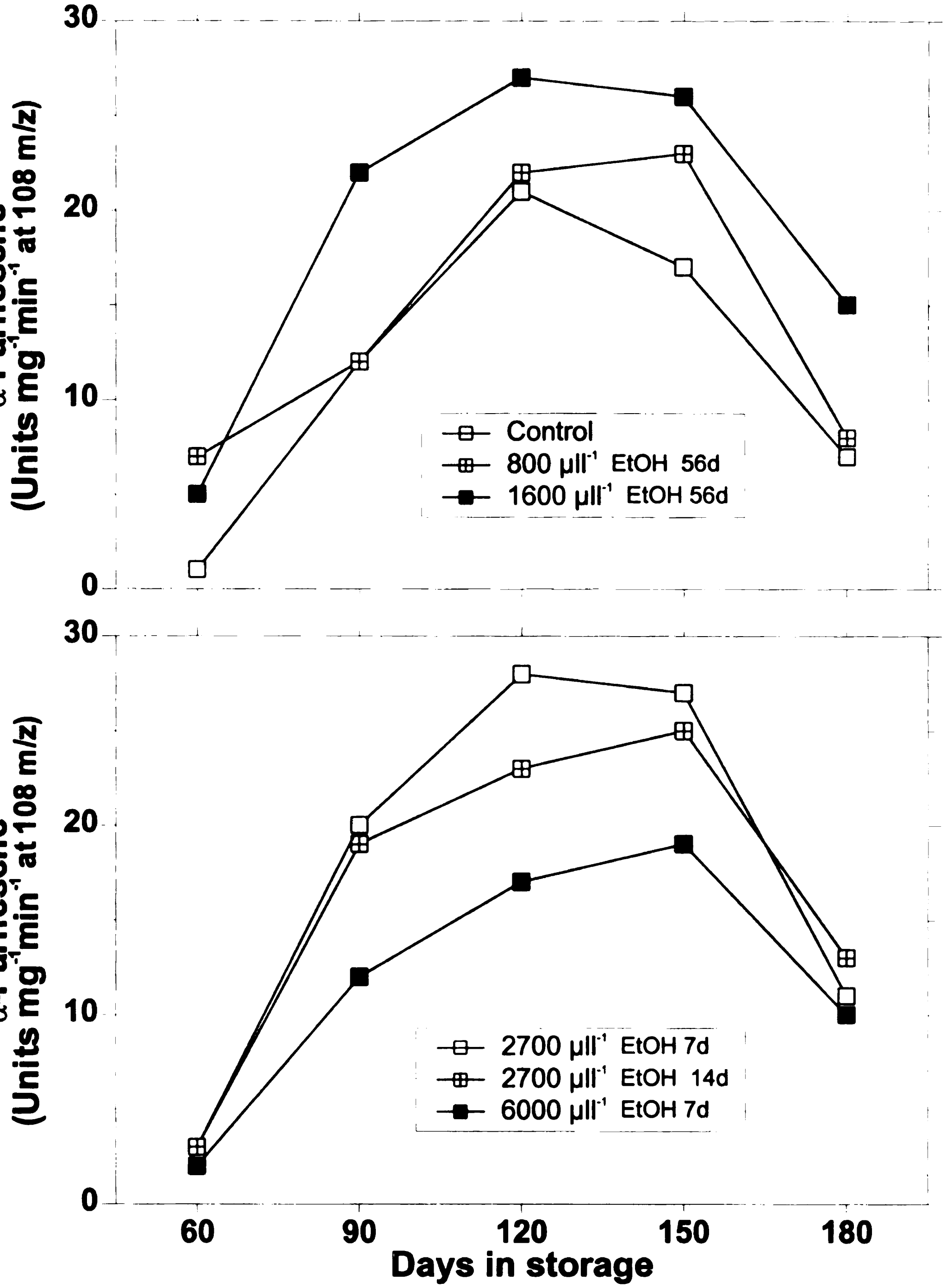

Figure 11. Effect of ethanol vapor on the production rates of α-farnesene for Granny Smith apples during CA storage (3% O_2 + 0% CO_2) at 1°C.

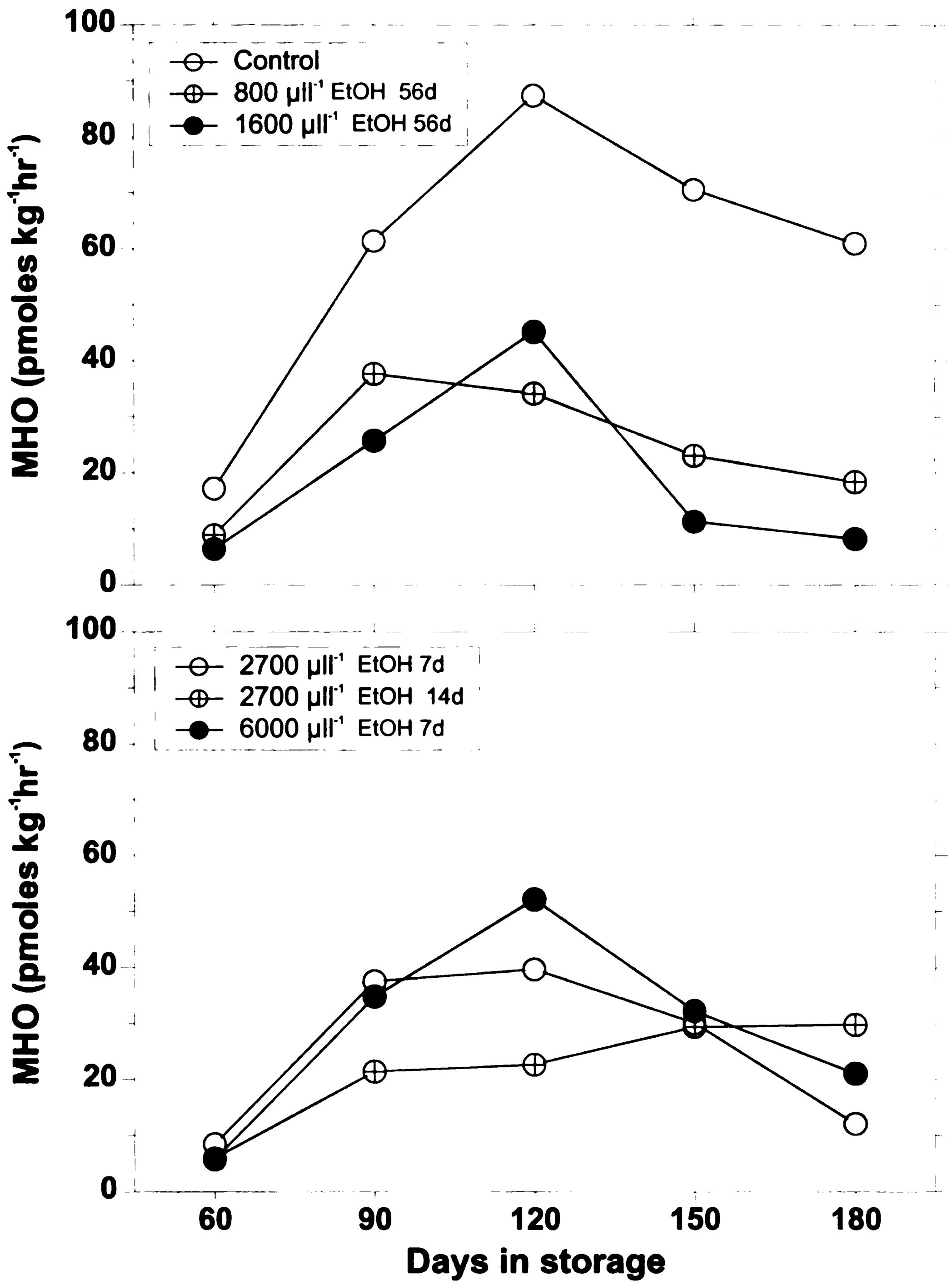

Figure 12. Effect of ethanol vapor on the production rates of 6-methyl-5-heptene-2-one for Granny Smith apples during CA storage (3% O_2 + 0% CO_2) at 1 °C.

effective. The high initial ethanol vapor treatment of Red Delicious was quite effective in controlling scald.

Whereas good control of scald was achieved during the first 4 months in CA by ethanol vapor treatments, these treatments were not effective for longer CA storage periods (Figure 9A, 9B). Moreover, DPA treatment at 1000 mg·L^{-1} was also ineffective for scald control for Granny Smith apples in this season (Figure 10A, 10B). The fruits for these experiments were harvested quite immature to provide high potential to scald.

The rate and pattern of α-farnesene production were similar for fruits treated or not treated with ETOH vapor (Figure 11A, 11B). The rate of MHO production was much higher in control than for the ethanol vapor treatments (Figure 12A, 12B). This indicates that the α-farnesene is not as closely related to scald development as its oxidation product MHO.

DISCUSSION

DPA and α-farnesene metabolism:

After 5 months of air storage, DPA was found to promote α-farnesene production but inhibit production of the MHO ketone and this was associated with low incidence of scald of Granny Smith apples (Figure 10; Chapter IV, Figure 5). In general, treatments which resulted in low MHO production following storage were those giving the best control of scald. The potential for scald to develop following many months of storage is largely determined during the first 2 months of storage (Meigh,1970; Shutak and Christopher, 1960; Watkins et al., 1995). If the mechanism of DPA action is to inhibit the oxidation of α-farnesene to the MHO ketone, this must be demonstrated during the first few months of storage. Production rate of MHO was lower for DPA-treated fruits than other treatments while production rate of α-farnesene was higher when measured after 5.5 months of storage at 20°C (Chapter IV, Figure 5A,5B). Therefore, we conclude that the mechanism of DPA in reducing scald may be related to inhibiting the oxidation of α-farnesene. Moreover, the conversion of α-farnesene to the MHO appears to be correlated to scald development but the role of MHO as a factor in causing scald may be indirect; since a dramatic increase occurs in MHO production upon warming the fruit of all cvs. including those which do not develop scald (Chapter I).

Ethanol vapor controls scald:

We have determined that ethanol supplied to fruits as a vapor is readily absorbed and metabolized by the tissue; moreover, one of the consequences is reduced rate of ethylene production (Wang and Dilley,1996). In addition, the production of low molecular weight volatile compounds including hexylacetate, hexylbutanoate/ butylhexanoate, hexyl-2-methylbutanoate and hexylhexanoate is reduced by ethanol vapor treatment (Figure 6). These data suggest that ethanol reduce the effects of ethylene or ameliorate other metabolism to control scald.

Ethanol vapors controlled apple scald when applied to Law Rome and Red

Delicious apples in the storage chambers by ventilating air through aqueous solutions of ethanol at different concentrations and in modified atmosphere package by adding various initial concentrations of ethanol vapor. This confirms the observation of Scott et al. (1995). Fruits in storage chambers treated with ethanol vapor at 1,600 $\mu L \cdot L^{-1}$ for about 2 months showed no scald when stored for an additional period in air storage whereas the scald index for control fruits was up to 2.33 (Table 1). The similar results in the modified atmosphere experiments confirmed that ethanol vapor could prevent apple scald (Scott et al., 1995). But, when the concentrations of ethanol were up to 3750 or 8000 $\mu L \cdot L^{-1}$ and were applied to fruits continuously for 2 months, the fruits were injured as evidenced by darkening of the skin color and the fruits showed some fermentation damage (Table 1). Ethanol vapor treatments were not correlated with a reduction of α-farnesene production by the fruits. α-Farnesene is an isoprenoid metabolite in the pathway to carotenoid synthesis that has been implicated indirectly as a factor in scald development (Meigh, 1970). Evidence for this is based on DPA reducing the level of a conjugated terpene product of α-farnesene oxidation (Anet and Coggiola, 1974). Our results suggest that the control of scald by ethanol vapor treatment may be related to a reduction of α-farnesene conversion to its oxidation product MHO. Ethanol vapor treatment resulted in accumulation of ethanol in the fruits in direct proportion to the ethanol vapor concentration administered and reduced the rate of ethylene production. The internal ethanol levels dropped rapidly when fruits were returned to air without ethanol vapor except the fruits with high ethanol vapor 3750 or 8000 $\mu L \cdot L^{-1}$ for 2 months continuously (Table 1). Those fruits were irreversibly injured by the alcohol treatments and remained with off-flavors when returned to air.

Ethanol vapor treatment reduced apple scald, exposure to 6000 $\mu L \cdot L^{-1}$ ethanol vapor for one or two weeks was the most effective. The effectiveness of ethanol vapor treatments diminished after long term CA storage at 3% O_2 (Figure 9A,9B). Perhaps better effectiveness of controlling scald may have been achieved if the CA treatment had been at 1.2 to 1.5% O_2 with 3% CO_2.

Fruits did not show off-flavor after exposing to 6000 $\mu L \cdot L^{-1}$ ethanol vapor for two weeks before storage but fruits did have an off-flavor after exposing to 3750 $\mu L \cdot L^{-1}$ or higher levels of ethanol vapor for 2 months. Newly harvested apples may have a higher resistance to ethanol while after some time of storage the resistance may decreased. Prolonged exposure to ethanol vapor concentrations at 3700 $\mu L \cdot L$ -1 apparently causes irreversible damage to cellular metabolism.

Low oxygen induces ethanol production:

Fruits held under ultra low levels of O_2 resulted in ethanol production (Figure 7). The internal ethanol levels were different since apple cvs. vary in their response to low O_2 levels. Ethanol accumulation at 0.5% O_2 was the highest for Granny Smith > Red Delicious > Law Rome > Idared; at 0.25% O_2, Granny Smith and Law Rome apples accumulated more ethanol than others. This suggest that apples may become resistant to scald through producing ethanol. To achieve the maximum resistance to scald each cv. of apples may require a specific and low O_2 level for a specific period of time. This remains to be elucidated.

Subjecting apple fruits to low O_2 levels which induce ethanol production for a period of two weeks at the beginning of the storage period was found to reduce the incidence of scald on fruits subsequently stored at higher O_2 levels. This suggests that some aspect of ethanol metabolism may reduce scald. This may involve its action as a free-radical scavenger, esterification of some intermediate, increasing membrane fluidity or other means. The mechanism of ethanol action in reducing scald needs further study.

LITERATURE CITED

Anet, E.F.L.J. and Coggiola, I.M. 1974. Superficial scald, a functional disorder of stored apples: X. Control of α-farnesene autoxidation. J. Sci. Food Agr. 25: 293-298.

Bain, J.M. 1956. A histological study of the development of superficial scald in 'Granny Smith' apples. J. Hort. Sci. 31: 234-238.

Dilley, D.R. and Beaudry, R.M. 1998. NE-103 Postharvest Physiology of Fruits. Annual Report Michigan Agric. Expt. Stat., Michigan State Univ., East Lansing, MI.

Emongor, V.E., Murr, D.P. and Lougheed, E.C. 1994. Preharvest factors that predispose apples to superficial scald. Postharvest Biol. and Technol. 4: 289-300.

Gallerani, G., Pratella, G.C. and Budini, R. A. 1990. The distribution and role of natural antioxidant substances in apple fruit affected by superficial scald . Adv. Hort. Sci. 4: 144-146.

Ghahramani, F. and Scott, K.J. 1998a. The action of ethanol in controlling superficial scald of apples. Aust. J. Agric. Res. 49: 199-205.

Ghahramani, F. and Scott, K.J. 1998b. Oxygen stress of Granny Smith apples in relation to superficial scald, ethanol, α-farnesene and conjugated trienes. Aust. J. Agric. Res. 49: 207-210.

Halliwell, B. and Gutteridge,I.C. 1989. Free radicals in biology and medicine. Clarendon Press, Oxford. pp543.

Huelin, F.E. and Coggiola, I.M. 1970a. Superficial scald, a functional disorder of stored apples: V. Oxidation of α-farnesene and its inhibition by diphenylamine. J. Sci. Food. Agr. 21: 44-48.

Ingle, M. and D'Souza, M. 1989. Physiology and control of superficial scald of apples: A review. HortScience 24: 28-31.

Little, C.R., Faragher, J.D. and Taylor, H.S. 1982. Effects of initial low oxygen stress treatments in low oxygen modified atmosphere storage of Granny Smith apples. J. Amer. Soc. Hort. Sci. 107: 320-323.

Meigh, D.F. 1970. Apple scald. In: A.C. Hulme (Ed.), The Biochemistry of Fruits and Their Products. Vol. 1. Academic Press, London, pp. 555-569.

Scott, K.J., Yuen, C.M.C. and Ghahramani, F. 1995. Ethanol vapor- a new anti-scald treatment for apples. Postharvest Biol. Technol. 6: 201-208.

Shutak, V. and Christopher, E.P. 1960. Role of the cuticle in development of storage scald on Corland apples. Pro. Amer. Soc. Hort. Sci. 76: 106-111.

Smock, R.M. 1961. Methods of scald control on the apple. Cornell Univ. Agric. Exp. Stn., Bull. No. 970, pp. 55.

Song, J., Gardner, B.D., Holland, J.F. and Beaudry, R.M. 1997. Rapid analysis of volatile flavor compounds in apple fruit using SPME and GC/Time-of-Flight Mass Spectrometry. J. Agri. Food Chem. 45: 1801-1807.

Stadtman, E.R. 1993*. Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalyzed reactions. Annu. Rev. Biochem. 62: 797-821.

Wang, Z. and Dilley, D.R. 1996. Ethanol vapor treatment: A new alternative approach to control superficial scald of apples. Proc. Mich. State Hort. Soc. 126: 35-40.

Wang, Z. and Dilley, D.R. 1997. The relationship of α-farnesene production and its oxidation product 6-methyl-5-heptene-2-one to superficial scald of Granny Smith, Law Rome, Red Delicious and Idared apples during controlled atmosphere and air storage. In: E.J. Mitchman (ed.) Apples and Pears. Proc.

7th Int'l Nat. CA Res. Conf., 2: 98-104.

Wang, Z., McCully, T.J. and Dilley, D.R. 1997a. The effect of ultra low oxygen storage, initial oxygen stress and ethanol vapor treatments on controlling superficial scald of Granny Smith, Law Rome, Red Delicious and Idared apples. In: E.J. Mitchman (ed.) Apples and Pears. Proc. 7th Int'l Nat. CA Res. Conf., 2: 105-111.

Watkins, C.B., Bramlage, W.J and Cregoe, B.A. 1995. Superficial scald of granny Smith apples is expressed as a typical chilling injury. J. Am. Soc. Hort. Sci. 120: 88-94.

CHAPTER IV

INITIAL LOW OXYGEN STRESS CONTROLS SUPERFICIAL SCALD OF APPLES

ABSTRACT

Alternative strategies to control apple scald to avoid the postharvest application of scald inhibitors and fungicides were investigated. Granny Smith, Law Rome, Red Delicious (scald susceptible) and Idared (not susceptible) were harvested at the preclimacteric stage of physiological development. Fruits were treated at 1 °C with a) initial low O_2 stress (ILOS) at different levels of low O_2 and various durations of pretreatment, b) different CA storage conditions, c) ultra low O_2 storage, d) without any pretreatment as control. Fruits were then stored in 3% O_2 with 0% CO_2, 0.7-0.8% O_2 with 3% CO_2 and 1.5% O_2 with 3% CO_2 in flow-through CA and in air at 0.5-1 °C. Superficial scald was markedly reduced by initial low O_2 conditioning at 0.5% O_2 for up to two weeks followed by CA storage and with 0.8% O_2 and 3% CO_2. With CA storage at 3% O_2 with 0% CO_2, 0.5% and 0.25% initial low O_2 pretreatment for 2 weeks resulted in excellent control of scald, especially at 0.25% O_2 for 2 weeks. All cultivars showed similar responses to treatments. Even though apple fruits scalded when stored in air and scald developed quickly upon warming after storage, the effects on reducing scald by initial low O_2 stress pretreatment were evident in all treatments. The treatment with 0.25% O_2 for two weeks or when this treatment was followed with an additional two weeks of low O_2 stress after 2 months of storage, were more effective treatment regimens for scald control than the other

treatments. The production of α-farnesene and MHO was inhibited by initial low O_2 stress treatments and 1.5% O_2 CA condition. The 0.25% O_2 initial stress treatment caused stronger inhibition on α-Farnesene and MHO production than 0.5% O_2 initial stress treament. This suggests that the accumulation of MHO is highly related to scald development of apples.

A commercial test of initial low O_2 stress confirmed the efficacy of ILOS for controlling scald.

INTRODUCTION

Superficial scald of apples is a devastating physiological disorder which has the potential to destroy the market value and utility of millions of tons of fresh apples annually during long-term storage. The physiology and control of scald has been periodically reviewed (Smock 1961; Meigh, 1970; Ingle and D'Souza, 1989; Emongor et al., 1994). The disorder is prevented commercially by postharvest drench treatment with diphenylamine (DPA) (Smock, 1957). The drench treatment must include a fungicide to prevent decay development. Numerous countries have banned the use of DPA or importation of DPA-treated fruits which mandates development of alternative methods to control scald (Ke and Kadar, 1992). Researchers (Barden and Bramlage, 1994a, 1994b; Gallerani et al., 1990) proposed that fruits may possess at harvest or may acquire after harvest resistance to scald; it is speculated that the skin browning symptoms of the disorder result from autooxidative processes affecting cell membranes brought about by low temperature storage beyond the ability of endogenous antioxidants such as ascorbic acid or α-tocopherol to prevent the dysfunction. Free-radicals such as hydroxyl ($OH^{\cdot}$), hydroperoxyl ($HO_2^{\cdot}$) and superoxide (O_2^{-}) may attack and destroy lipid and protein macro-molecular structures essential for cellular function (Anet, 1969; Halliwell and Gutteridge, 1989; Stadtman, 1993). Scald development in Granny Smith, a cv. very susceptible to scald, was correlated to the oxidation of α-farnesene to conjugated triene hydroperoxides (Huelin and Coggiola, 1970a; Filmer and Meigh, 1971). Extensive investigations in Australia (Huelin and Murray, 1966; Huelin and Coggiola, 1968, 1970a, 1970b; Anet, 1972a, 1972b; Anet and Coggiola, 1974) suggest that scald results from the autooxidation of a volatile sesquiterpene hydrocarbon α-farnesene (2,6,10-trimethyl-2,6,9,11-dodecatetraene) in the fruit skin (Meigh and Filmer,1969). The oxidation of α-farnesene is hypothesized to yield conjugated triene hydroperoxide free-radicals which injure the cells and give rise to the symptoms of scald (Rowan et al.,1995). Song and Beaudry (1996) found that MHO applied to apple fruits caused a scald-like disorder and this is a product of α-farnesene oxidation. Scald can be induced by the products of α-farnesene oxidation, and the concentration and time of appearance of these products affects the severity of the disorder (Anet, 1972b). The scald inhibitor chemicals DPA and ethoxyquin are known to be effective inhibitors of lipid peroxidation so this may be the mechanism of action in controlling scald. DPA is also an inhibitor of electron transport in plant mitochondria (Baker, 1963). Scott et al.(1995) found that ethanol vapor treatment controlled scald of Granny Smith apples and we have confirmed this (Wang and Dilley, 1996, 1997; Wang et al., 1997) as has Ghahramani and

Scott (1998a). Ethanol is known to be a free-radical scavenger (Halliwell and Gutteridge, 1989) and this may be a mechanism of action in scald control. Little et al.(1982) reported that scald of Granny Smith apples was largely controlled by an initial low O_2 stress followed by CA storage. We hypothesized that initial low O_2 stress prior to CA storage controls scald by affecting the metabolism including ethanol, α-farnesene and its oxidation product MHO. Initial low O_2 stress treatments were investigated to determine effective treatment regimens and to gain insight into the mechanism of scald control.

MATERIALS AND METHODS

Preliminary investigations (1996):

Granny Smith, Law Rome, Red Delicious and Idared fruits were harvested from trees at the MSU CHES at the preclimacteric stage of physiological development. The maturity stage at harvest was about one week before the onset of the endogenous ethylene climacteric. Three replicates of 40-60 fruits each for each storage period evaluation for each cv. were treated with initial low O_2 stress at 0.5% O_2 for one or two weeks. Nitrogen gas prepared with a Permea air separator adjusted to provide 0.5% O_2 was employed to provide the low O_2 atmosphere which was distributed through a capillary flow board system for maintaining controlled atmospheres in a flow-through system. Fruits were then stored in 3% O_2 with 0% CO_2 (dry lime added) and 0.8% O_2 with 3% CO_2 in the flow-through CA (0.12 atmosphere turnovers per hr) and in air for up to 7 months at 1°C. To bring out the effect of treatments, CA at 3% O_2 was employed since it is only partially effective in controlling scald. Some fruits were treated with DPA 1000 mg$\cdot$ L^{-1} and also placed in air at 1°C. Scald data is expressed as a scald index based on the percentage of fruit surface area affected where no scald = 0, <25%=1, 25-50%=2 and >50%=3 and scald index was normalized to 100 by mutiplying 33.33. Scald index was measured after 4 and 7 months of CA storage and 5 months of air storage and after 7 days in air at 20°C.

To determine the effects on ripening and scald development and production of α-farnesene and its oxidation product 6-methyl-5-heptene-2-one (MHO), fruits were removed from CA and air storage at appropriate intervals, and transferred to 20°C in air in glass jars. Subsamples were used for fruit firmness measurements and ripening and again after 7 days. The headspace in the jars was sampled by SPME/GC/MS as described by Song et al. (1997) to determine the α-farnesene and MHO production at daily or every other day intervals. Samples of epicuticular wax were removed from the fruits at each time interval and placed in glass vials to determine the amount of α-farnesene and MHO partitioned in the epicuticular wax.

Initial low oxygen stress studies(1997):

Preclimacteric Law Rome apples were obtained from the MSU CHES and Granny Smith apples were obtained from a grower in Southwest Michigan. Maturity of both cvs. was about two weeks prior to the onset of the endogenous ethylene climacteric to assure high potential for scald development. Fruits were placed in 20-L CA chambers employing about 40-60 fruits in each of three replicates for each storage period evaluation. Two levels of initial low O_2 stress were employed; 0.5 and 0.25% O_2 for two weeks at 1°C. The control fruits did not receive low O_2 stress. The low O_2 stress atmospheres were generated using a Permea air separator adjusted to produce nitrogen which was blended with O_2 in a gas mixing apparatus and distributed to the chambers using a capillary flow board. After the low O_2 stress, the CA chambers were stored in dynamic (slowly ventilated) CA at 3 or 1.5% O_2 at 1°C. A slow flow rate of 0.18-chamber atmospheres per hour was employed. Some fruits so treated were again subjected to the same low O_2 stress treatments after two months in CA. Fruits were also stored in air and in CA without prior low O_2 stress.

Examination of fruits for scald and quality parameters was made after 5 and 8 months of storage plus one week in air at 20°C. Scald data is expressed as a scald index based on the percentage of fruit surface area affected as mentioned above.

Analysis of volatiles: SPME GC Mass spectrometry was employed to identify and quantify the volatiles including α-farnesene and 6-methyl-5-heptene-2-one (MHO). Five to 8 fruits from each treatment were enclosed in 4-L glass jars at 20°C for 1.5 hrs. and the headspace was sampled by SPME and analyzed employing a GC/MS according to the procedure of Song et al.(1997). At the same time intervals, a sample of the epicuticular wax scraped from the fruit surface was placed in small glass vial at 20°C and the headspace gas was sampled by SPME and analyzed by GC/MS to determine the nature of the compounds that had partitioned into the epicuticular wax or cuticle.

Continuous low O_2 stress studies:

Granny Smith apples as used for the initial low O_2 stress studies were employed. Fruits were placed in 10-L glass desiccators and ventilated with 0.13, 0.25, 0.4, 0.5, 0.75, 1.0, 1.25 and 1.5% O_2 or with air at 1°C. The low O_2 atmospheres were made by mixing nitrogen generated by a Permea air separator with air employing a gas mixing apparatus and a capillary flow board. The ventilation rate of the gas mixture through the chambers was 54 mL·min^{-1} (approximately 0.6 void volume exchanges per hr). At weekly intervals throughout the 8 month storage period, the volatiles produced by the fruits while under the storage atmospheres were sampled by SPME directly from the headspaces of the storage chambers and analyzed by GC/MS. The volatile profile produced by intact fruits of more than 60 compounds was obtained. The volatile profile of fruits after transfer to 20°C was obtained in a similar manner. Fruits were examined for scald and other physiolgical disorders after removal from storage and again after 7 days at 20C.

Initial low O_2 stress to control scald; a commercial test at the MSU CHES CA Storage Facility:

Apples from a hail-damaged area of Sparta, Michigan were employed. Fruits came from five different orchards plus CHES. Red Delicious strains were Red Chief, Starkrimson, NuRed, Ace and Super Spur. Romes were Law Rome and Galia Beauty. Winesaps were Stayman and Turley. All fruits were of preclimacteric maturity stage and were harvested and loaded into the rooms between October 6 and 13. Each room contained 126 bins (about 18 bu capacity per bin) and these were loaded with an identical stacking pattern. CA rooms were sealed on October 13 and brought to 2.3% O_2 by October 18. Dry lime was employed to scrub carbon dioxide. A Permea nitrogen generator was used for initial O_2 pull-down and as needed to maintain O_2 levels. CA room #1 was maintained at 0.7% O_2 with 0.5% carbon dioxide throughout the storage period at 0.1°C. CA room #2 was maintained at 1.5% O_2 with 0.5% carbon dioxide throughout the storage period at 0.1°C. Fruits in CA room # 3 had initial low O_2 stress at 0.55% +/- 0.19 for 18 days from October 20-November 6 and between Oct. 24-Nov. 4 (12 days) the O_2 level was 0.48% +/- 0.11. Fruits in CA room # 4 had initial low O_2 stress at 0.61% +/- .21 for 18 days beginning on October 20th and between Oct. 27-Nov. 7 (12 days) the O_2 level was 0.51% +/- 0.13. For CA rooms 3 and 4 the carbon dioxide level was 0.5%. At the end of the initial low O_2 stress, the temperature was raised in CA room 3 and 4 from 0.1°C to 2°C for three days then returned to 0.1°C and the O_2 level was raised to 1.5% for the duration of the storage period. The rooms were opened on June 27th after about 8 months in CA. Three forty-fruit samples were obtained from each lot and examined for scald, internal disorders and flavor and again after a week at 20°C.

RESULTS

1996-97 studies:

Initial low oxygen stress followed by CA storage:

Granny Smith, Law Rome and Red Delicious apples without low O_2 stress and stored in air at 1°C were severely affected by scald and CA storage at 3% O_2 gave only partially control (Figure 1). Initial low O_2 stress at 0.5% O_2 for 1 or 2 weeks with subsequent storage in CA at 3% O_2 largely attenuated scald. Initial low O_2 stress reduced scald incidence and severity and 2 weeks at 0.5% O_2 was more effective than 1 week (Figure 1).

Whereas good control of scald was achieved during the first 4 months in CA by initial low O_2 stress treatments, these treatments were not effective for longer CA storage periods (Figure 2A, 2B). Continuous CA storage at 0.8% O_2 prevented scald development but this treatment resulted in some damage from fermentation resulting in low flavor development and off-flavors.

During 3% O_2 CA storage, α-farnesene production by fruits without initial low O_2 stress treatment was similar to that from initial low O_2 stress except the peak was at 120 days of CA storage while α-farnesene peaked at 150 days of storage in the fruits with initial low O_2 stress treatment (Figure 3A). Control fruits produced more MHO than those fruits with 0.5% O_2 initial treatment for 1 or 2 weeks (Figure 3B).

Initial low oxygen stress followed by air storage:

For Granny Smith apples stored in air, all of the treatments including DPA gave good apparent reduction in scald during storage at 1 °C but not after fruits were held for 7 days at 20°C (Figure 4). Moreover, DPA treatment at 1000 $mg \cdot L^{-1}$ was also

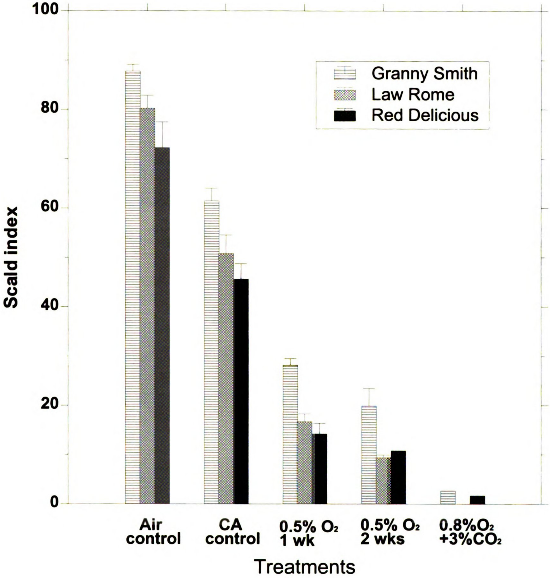

Figure 1. Effect of initial low oxygen stress and ultra low oxygen level continuously on controlling apple scald of Granny Smith, Law Rome and Red Delicious apples after 4 months of CA storage (3% O_2 + 0% CO_2) at 1°C plus 7 days at 20°C in air. Scald index (0=none; 1=slight; 2=moderate; 3=severe) was normalized to 100.

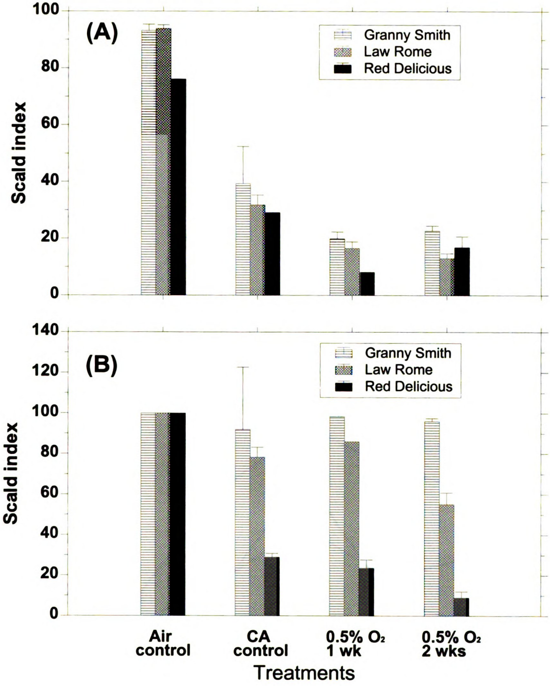

Figure 2. Effect of initial low oxygen stress on controlling apple scald for Granny Smith, Law Rome and Red Delicious after 7 months of CA storage (3% O_2 + 0% CO_2) at 1°C on removal from storage (A) and plus 7 days at 20°C in air (B). Scald index (0=none; 1=slight; 2=moderate; 3=severe) was normalized to 100.

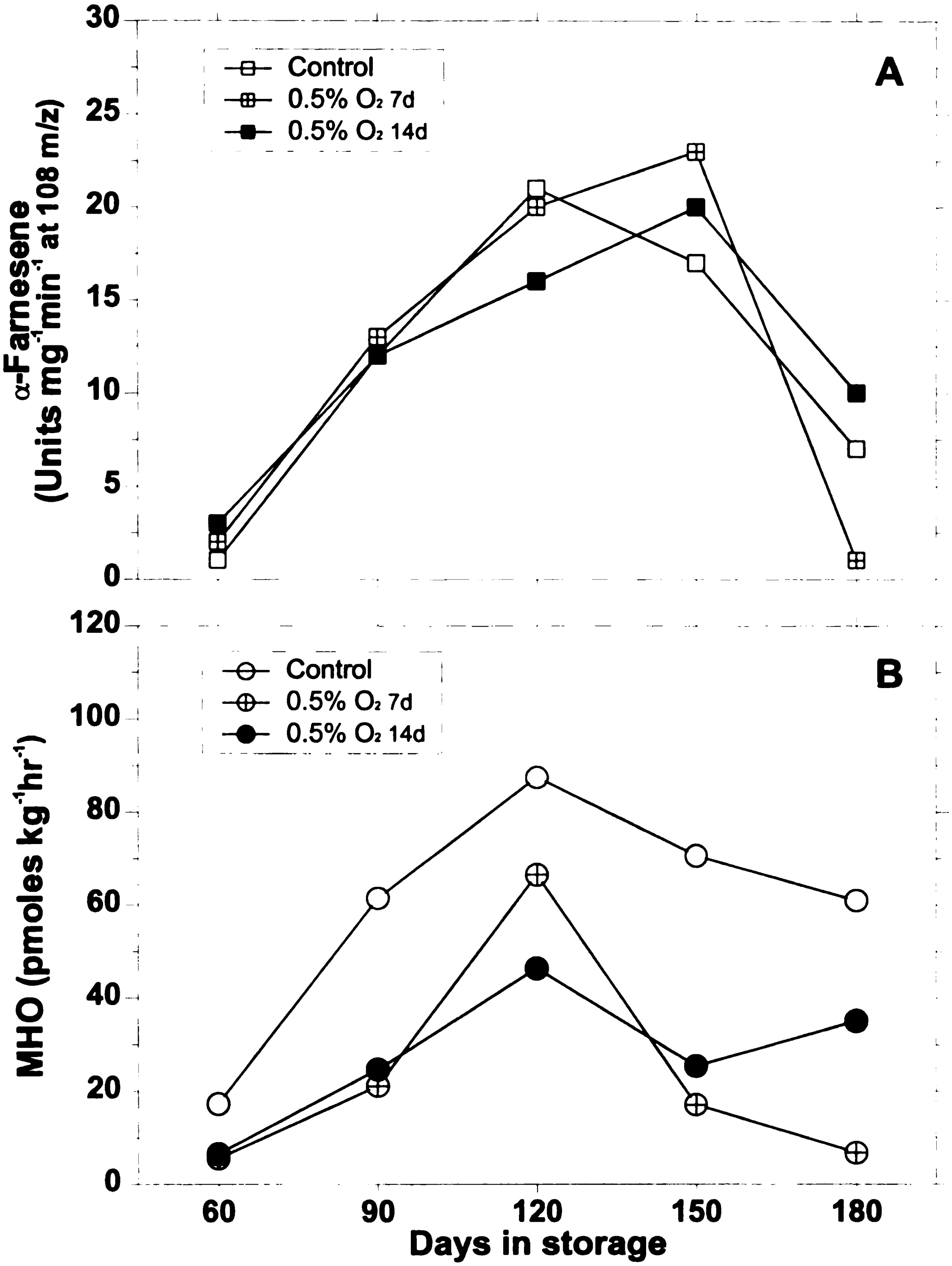

Figure 3. Effect of initial low oxygen stress on the production rates of α-farnesene (A) and 6-methyl-5-heptene-2-one (B) for Granny Smith apples during CA storage (3% O_2 + 0% CO_2) at 1°C.

ineffective for scald control for Granny Smith apples. The fruits for these experiments were harvested quite immature to provide high potential to scald. More mature fruits with naturally lower scald potential would likely have shown good scald control using similar treatments and storing them in CA at 1.5% O_2.

After 5- and -a-half months of air storage without pretreatment at 1°C, α-farnesene production by Granny Smith fruits at 20°C was lower than for the other treatments. Fruits treated with 1000 $mg \cdot L^{-1}$ DPA produced a higher level of α-farnesene but a lower level of MHO while control fruits produced less α-farnesene but more MHO (Figure 5A, 5B). α-Farnesene levels from fruits with initial low O_2 stress or ethanol vapor treatment were between those of the control and DPA treatments but MHO levels were even higher than that in control (Figure 5). This indicates that the production of α-farnesene and MHO for control apples has peaked out before removal from storage.

1997/98 Studies:

Initial low oxygen stress studies:

Increasing the intensity of low O_2 stress progressively diminished the incidence and severity of scald of Granny Smith and Law Rome fruits subsequently stored in CA at 3% O_2 whereas fruits stored at 1.5% O_2 did not develop scald significantly (Figure 6A, 6B). CA storage at 1.5% O_2 of O_2-stressed fruits completely controlled internal browning in both cvs. whereas CA storage at 3% O_2 did not (Figure 7A, 7B). The nature of internal browning was largely of minor intensity and was judged to be

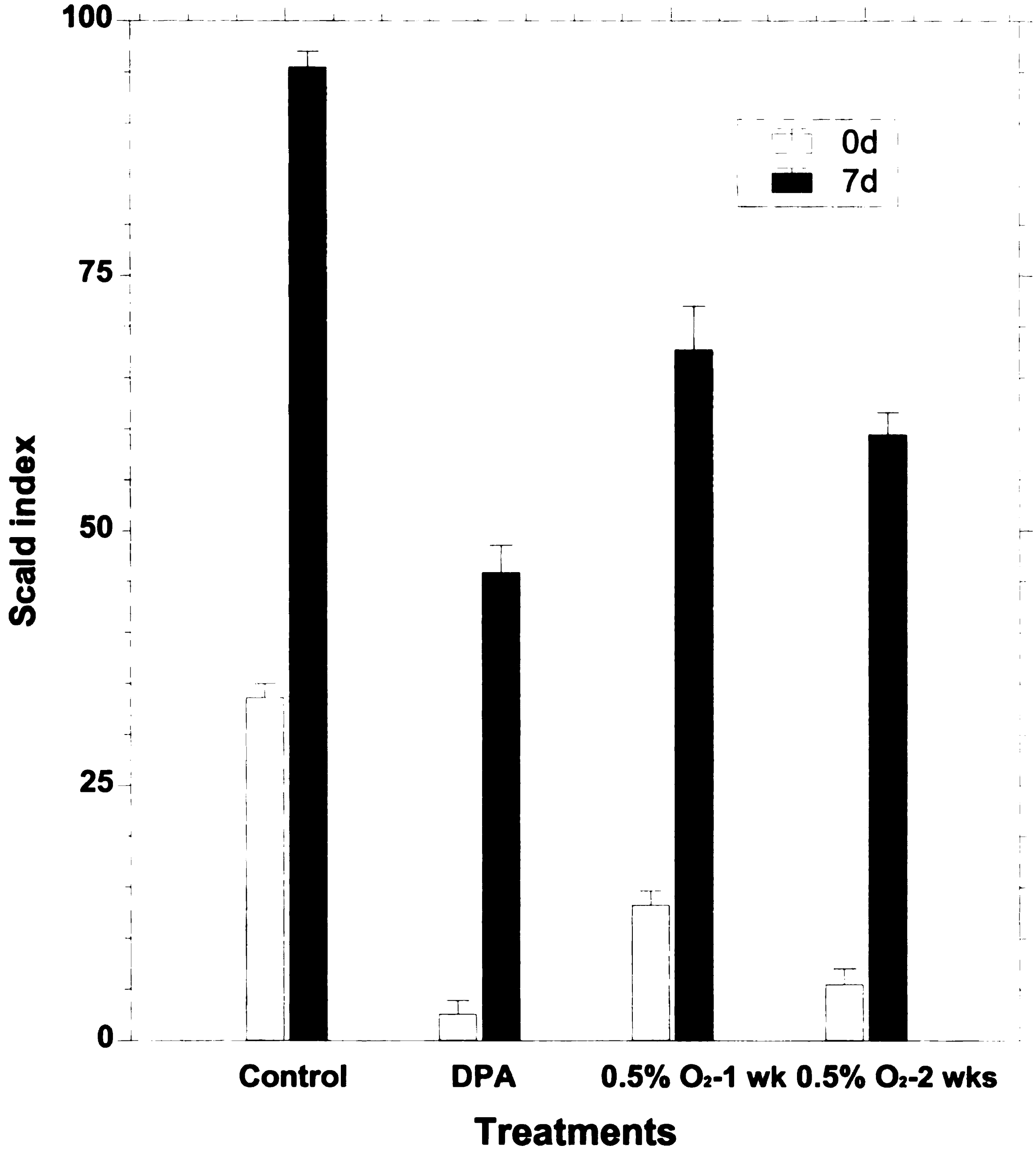

Figure 4. Initial low oxygen stress attenuates scald of Granny Smith apples stored in air for 5 months at 1°C. Scald index (0=none; 1=slight; 2=moderate; 3=severe) was normalized to 100. (□ after storage; ■ plus 7 days in air at 20°C).

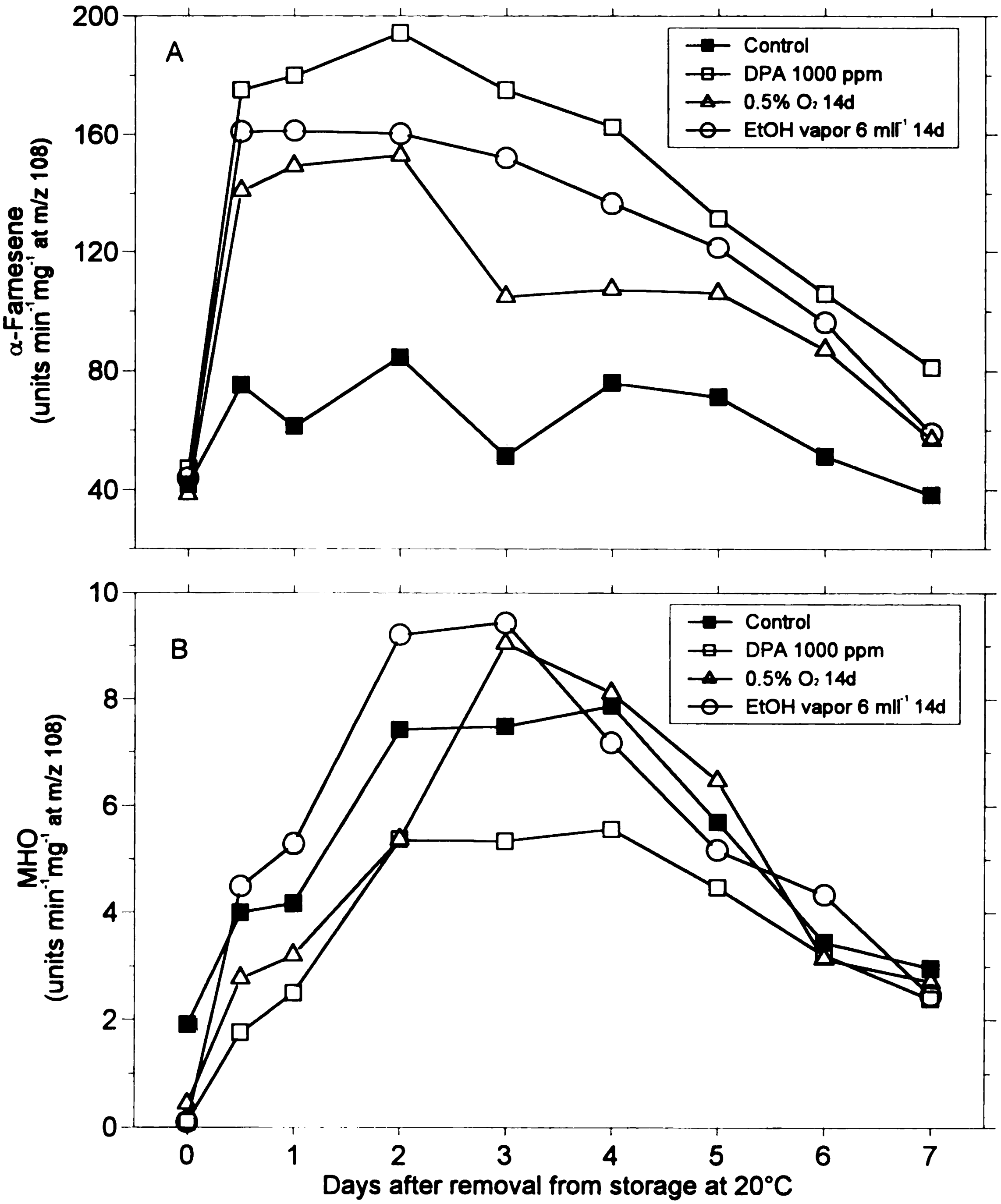

Figure 5. Effects of initial low oxygen stress, DPA and EtOH treatments on the production rates of α-farnesene (A) and 6-methyl-5-heptene-2-one (B) for Granny Smith apples after 5.5 months of air storage at 1°C.

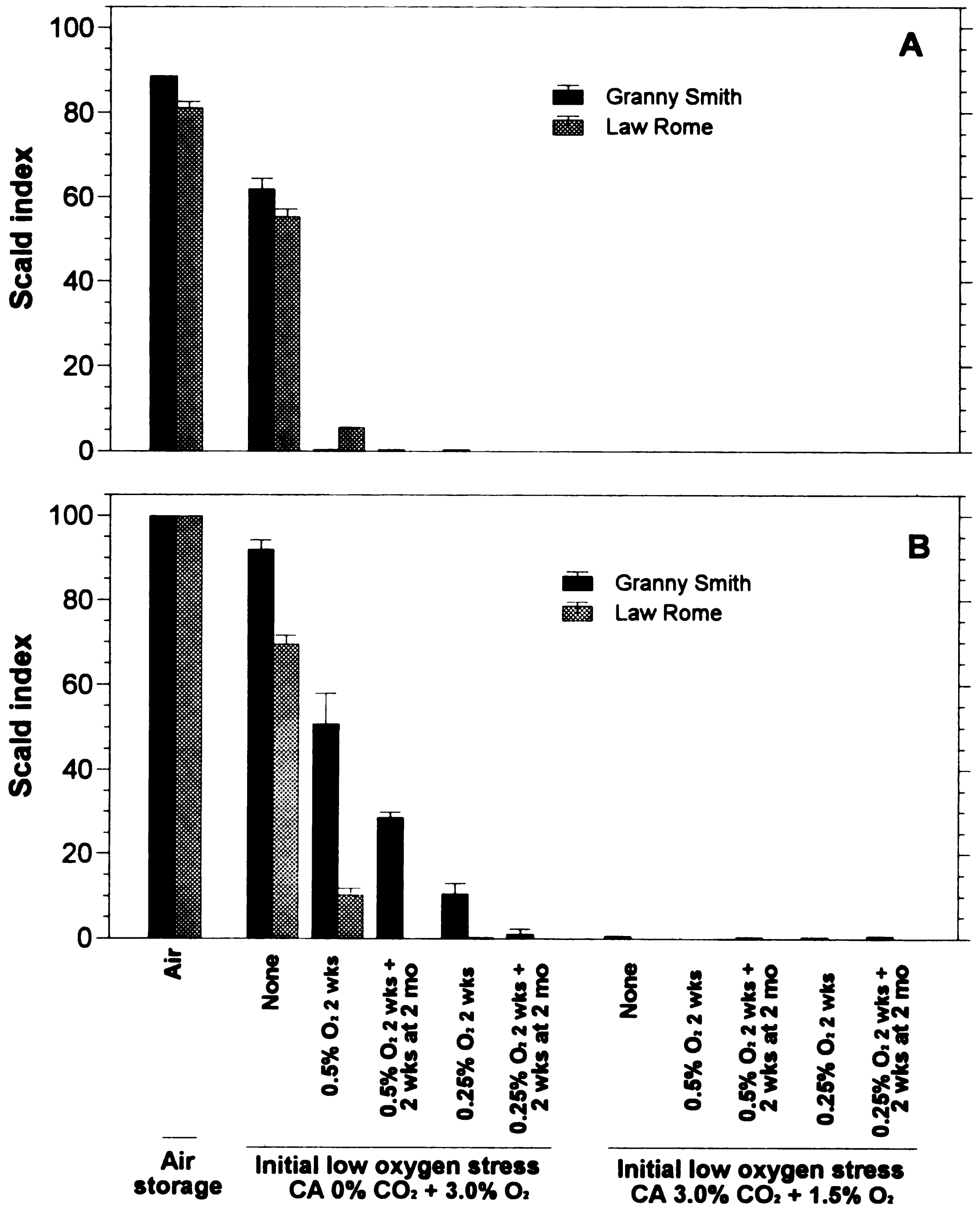

Figure 6. Initial low oxygen stress attenuates scald of Granny Smith and Law Rome apples stored in CA (3% O_2 + 0% CO_2 or 1.5% O_2 + 3% CO_2) for 5 months(A) and 8 months (B) at 0.5°C plus 7 days at 20°C in air. Scald index (0=none; 1=slight; 2=moderate; 3=severe) was normalized to 100.

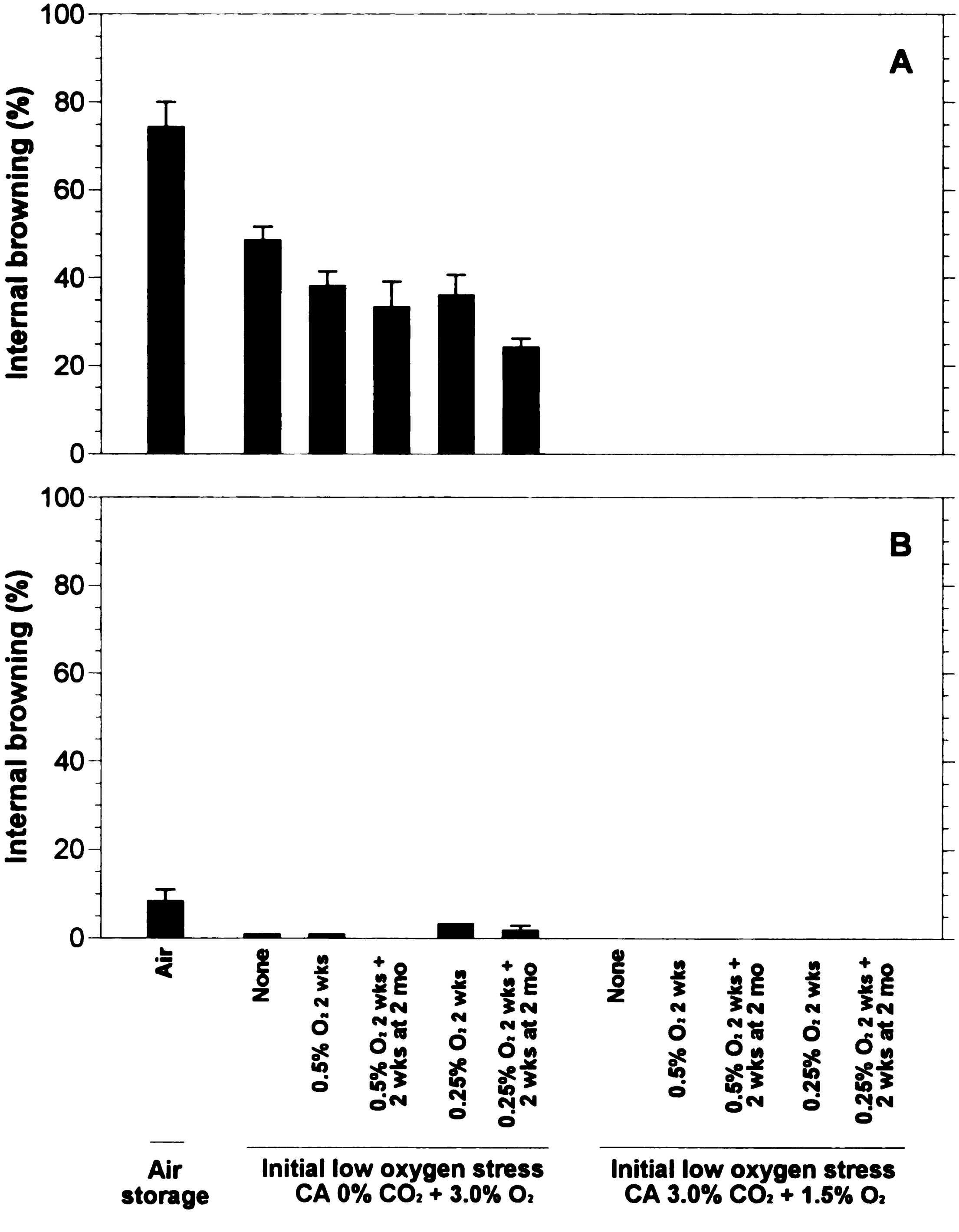

Figure 7. Effects of initial low oxygen stress and oxygen levels in CA storage (3% O_2 + 0% CO_2 or 1.5% O_2 + 3% CO_2) on internal browning of Law Rome (A) and Granny Smith (B) apples stored for 8 months at 0.5°C plus 7 days at 20°C in air.

senescence-related in that it was most evident in the air stored fruits and in 3% O_2 CA. This is supported by the observation that core browning (a slight discoloration of tissue immediately adjacent to the seed cavity related to immaturity at harvest) was most prevalent in Law Rome subjected to the most low O_2 stress and only when stored at 1.5% O_2 CA (Figure 8A). This was less evident for Granny Smith apples (Figure 8B) from all other CA atmosphere regimens which exhibited varying degrees of core browning with no clear association with CA treatments. The incidence of decay was generally inversely related to the degree of low O_2 stress; with more low O_2 stress decay decreased. Decay was most prevalent in the air control fruits for both cvs. (Figure 9). The lowest incidence of decay was generally found for fruits subjected to two weeks of 0.25% O_2.

Flesh firmness was retained better for both Granny Smith and Law Rome cvs after receiving initial low O_2 stress treatment than those without (Figures 10, 11). Compared to the initial flesh firmness (82.5N for Granny Smith and 89.2N for Law Rome) fruits of both cvs. had higher firmness when stored in 1.5% O_2 CA than those stored in 3% O_2 CA (Figure 10, 11) as would be expected.

Apples without initial low O_2 stress produced more α-farnesene after 5 months of storage in 1.5% O_2 CA than those with initial low O_2 stress treatments (Figure 12 A, 12C) whereas fruits produced lower amounts of α-farnesene when removed from 3% O_2 CA (Figure 12B, 12D). A similar pattern was seen for fruits held at the same conditions for 8 months (Figure 13). Also, similar to the data in Figure 12, apples

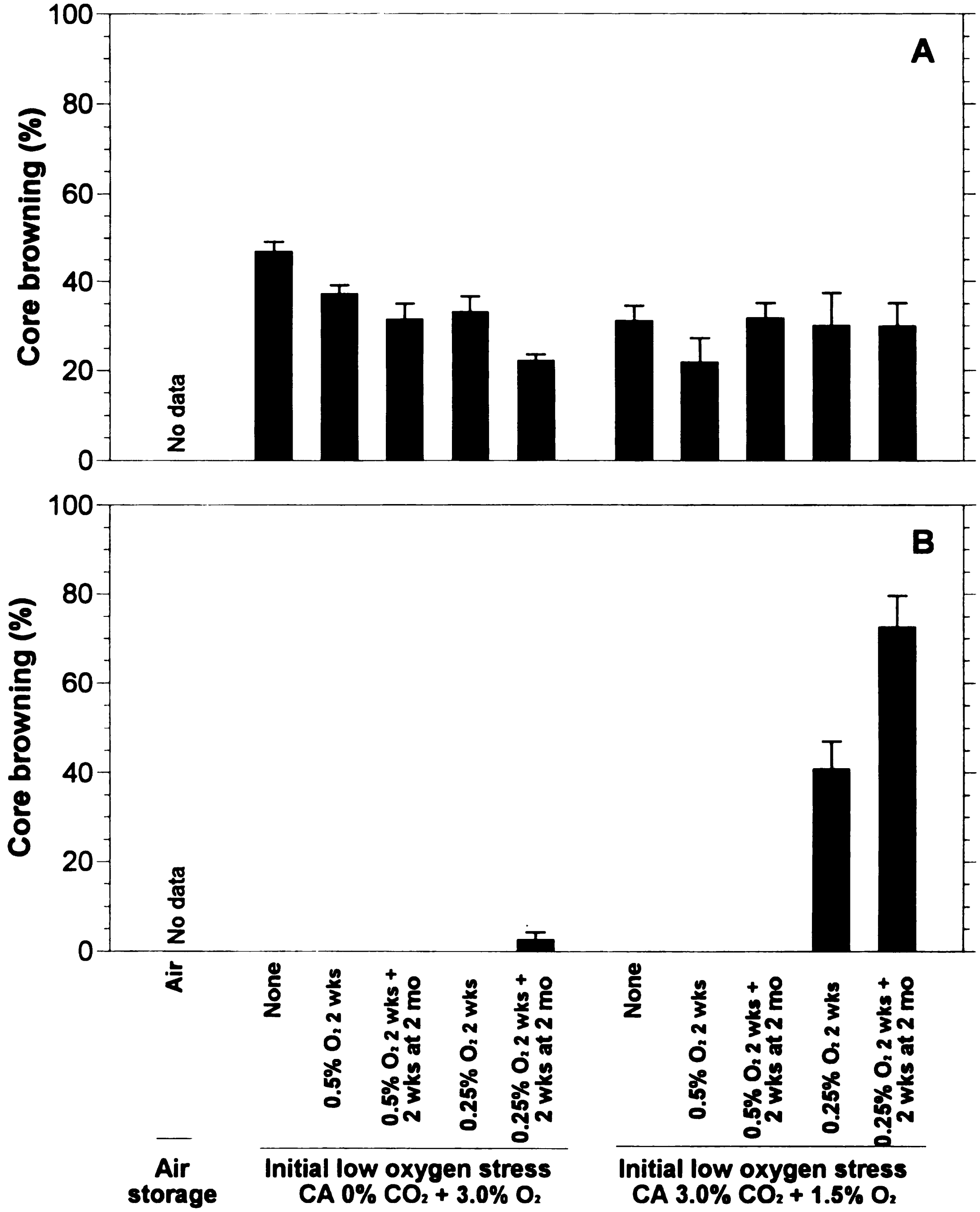

Figure 8. Effects of initial low oxygen stress and oxygen levels in CA storage (3% O_2 + 0% CO_2 or 1.5% O_2 + 3% CO_2) on core browning of Law Rome (A) and Granny Smith (B) apples stored for 8 months at 0.5°C plus 7 days at 20°C in air.

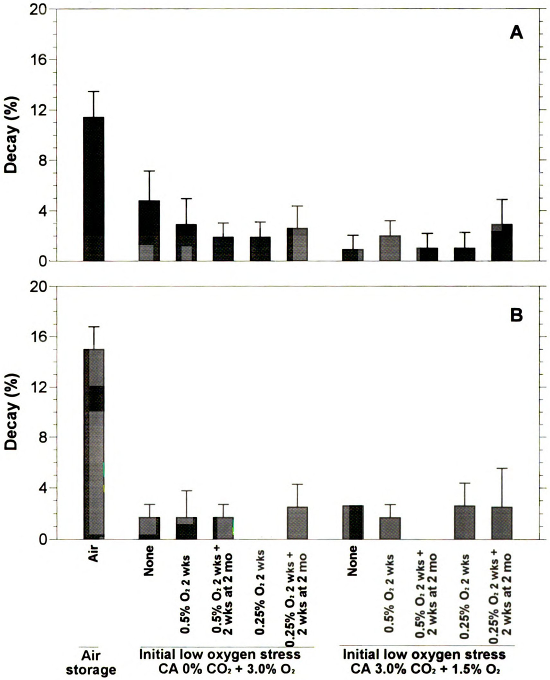

Figure 9. Effects of initial low oxygen stress and oxygen levels in CA storage (3% O_2 + 0% CO_2 or 1.5% O_2 + 3% CO_2) on decay of Granny Smith (A) and Law Rome (B) apples stored for 8 months at 0.5°C plus 7 days at 20°C in air.

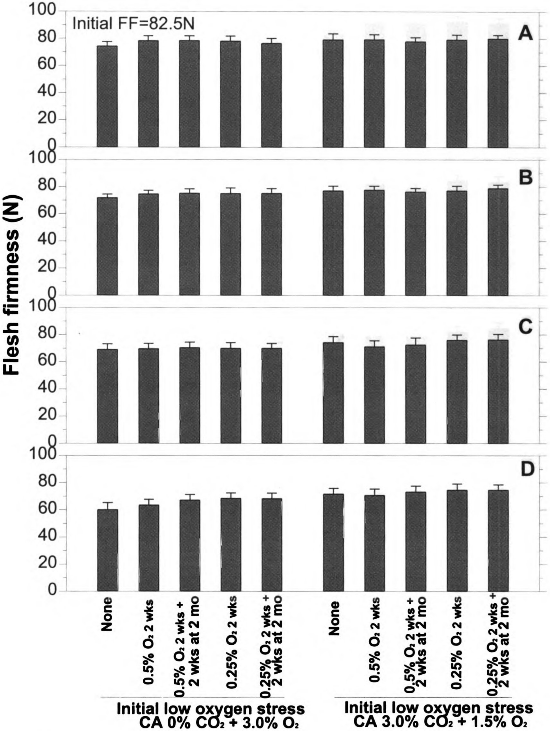

Figure 10. Effect of initial low oxygen stress and CA conditions (3% O_2 + 0% CO_2 or 1.5% O_2 + 3% CO_2) on flesh firmness retention of Granny Smith apples stored 5 (A,B) and 8 (C,D) months at 0.5°C. Flesh firmness on removal from storage (A,C) and after 7 days at 20°C (B,D).

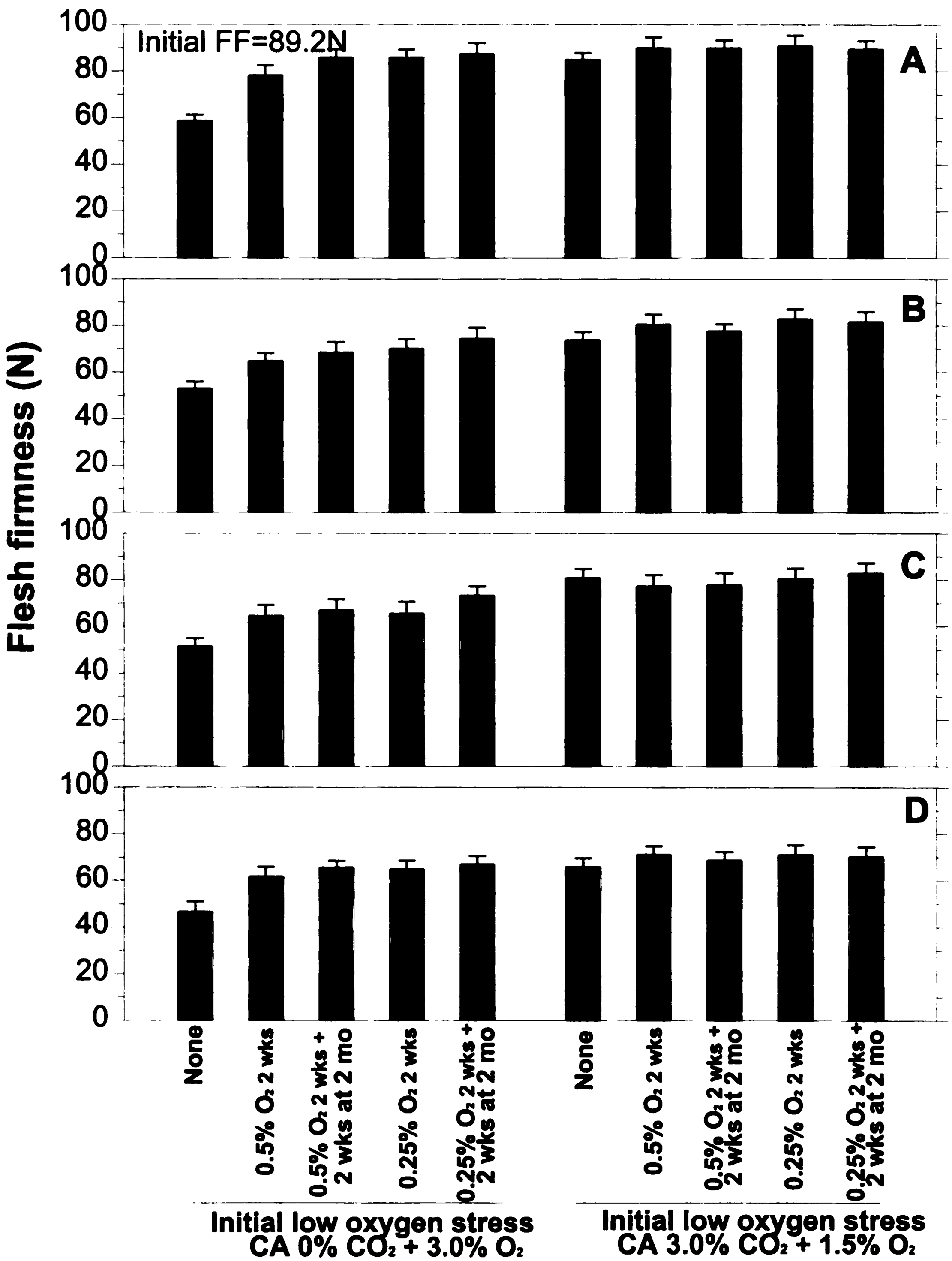

Figure 11. Effect of initial low oxygen stress and CA conditions (3% O_2 + 0% CO_2 or 1.5% O_2 + 3% CO_2) on flesh firmness retention of Law Rome apples stored 5 (A,B) and 8 (C,D) months at 0.5°C. Flesh firmness on removal from storage (A,C) and after 7 days at 20°C (B,D).

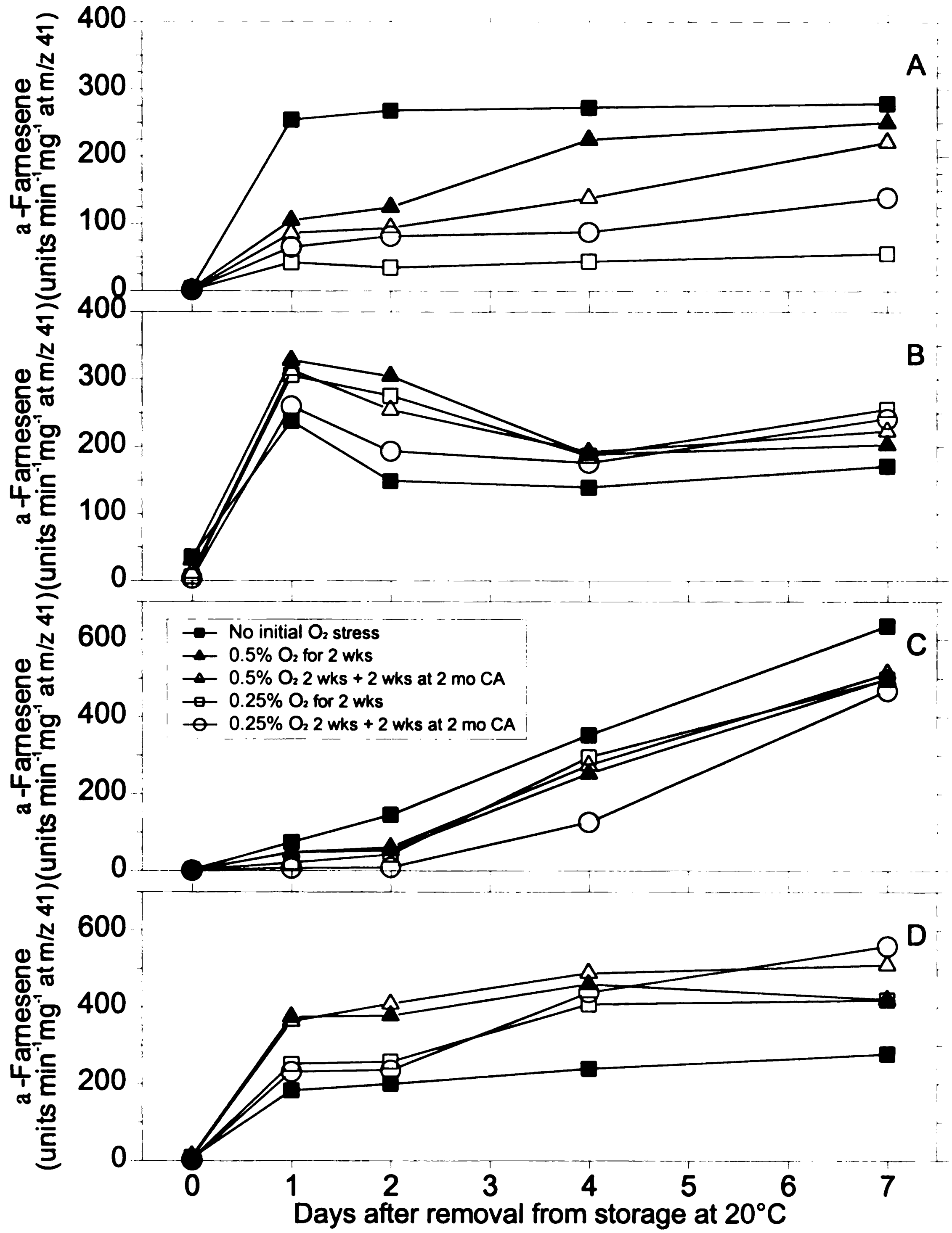

Figure 12. Effect of initial low oxygen stress on α-farnesene levels in the headspace of Granny Smith (A, B) and Law Rome (C, D) apples stored in 1.5% (A, C) and 3% (B, D) O_2 CA for 5 months at 0.5°C.

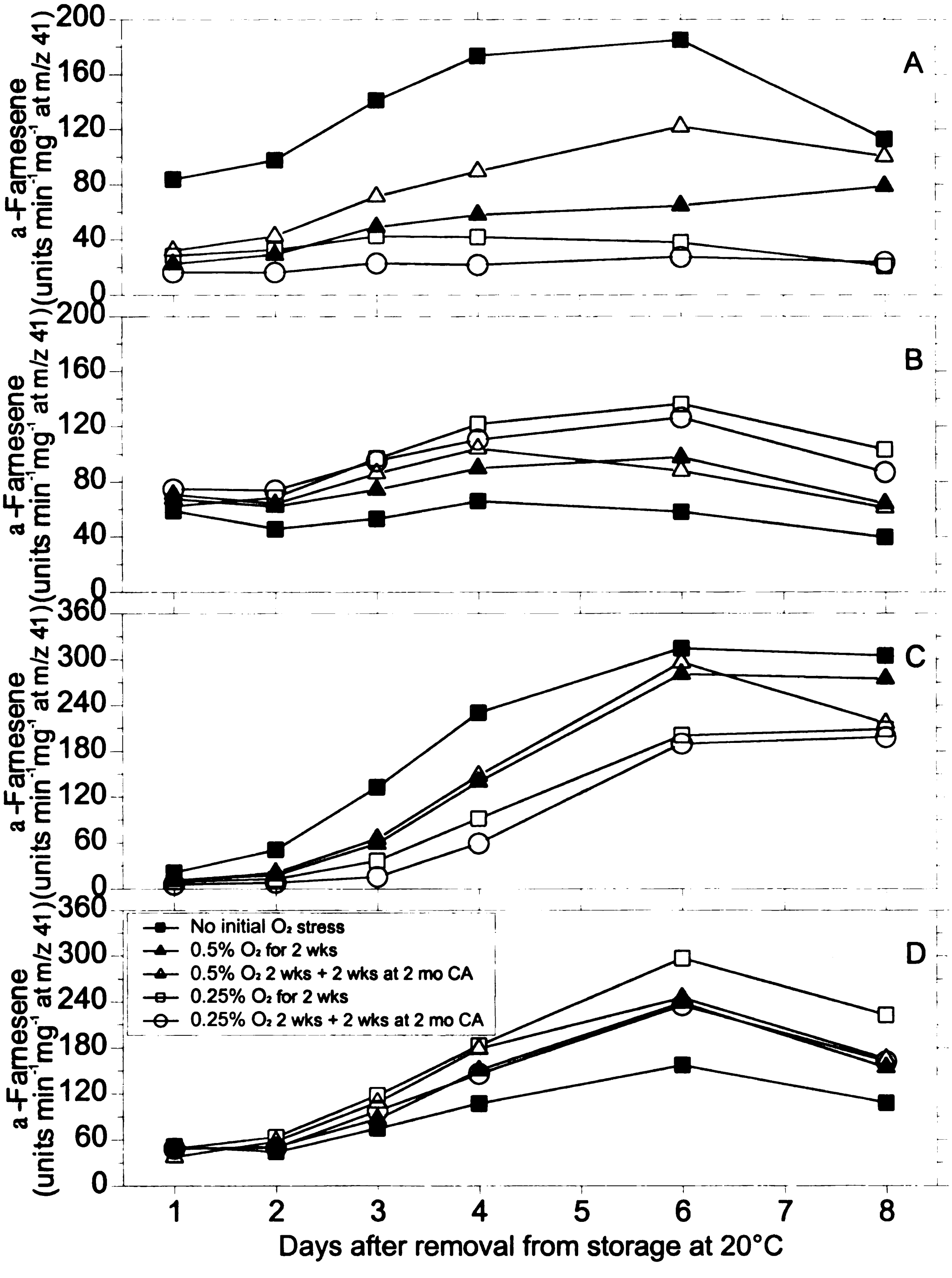

Figure 13. Effect of initial low oxygen stress on α-farnesene levels in the headspace of Granny Smith (A, B) and Law Rome (C, D) apples stored in 1.5% (A, C) and 3% (B, D) O_2 CA for 8 months at 0.5°C.

with initial low O_2 stress at 0.25% O_2 level produced less α-farnesene when fruits were held at 1.5% O_2 storage than those with 0.5% initial O_2 stress treatment at 5 and 8 months (Figure 12A,C;13A,C), respectively. This indicates that the α-farnesene production of apples was inhibited by initial low O_2 stress treatments and also indicates initial O_2 stress at the 0.25% O_2 level and/or CA at the 1.5% O_2 level more strongly inhibits α-farnesene production than was observed for fruits without initial low O_2 stress and/or CA at the 3% O_2 level.

After transfer from 1.5% O_2 CA to air at 20°C more α-farnesene accumulated in the epicuticular wax of Granny Smith apple fruits without initial low O_2 stress treatment than those transferred from 3% O_2 storage (Figure 14A, 14B). Law Rome fruits with initial low O_2 stress had a higher potential to produce α-farnesene than Granny Smith fruits after 8 months of storage at both 1.5% and 3% O_2 (Figure 14C, 14D).

Fruits receiving 0.25% O_2 initial stress treatments produced less MHO than those with 0.5% O_2 stress at all storage conditions for both Granny Smith and Law Rome cvs. after 5 months of storage (Figure 15). Whereas, after an additional 3 months of storage, apples moved from 3% O_2 produced more MHO than those held at 1.5% O_2 (Figure 16). A similar pattern of MHO levels was seen under the various O_2 levels of CA (Figure 17). In contrast, after 8 months of storage, some of the potential to produce less MHO with 0.25% O_2 initial stress treatments have been decreased based on the analyses of headspace (Figures 16) and epicuticular wax (Figure 17). This data indicate that both 0.25% O_2 initial stress treatment and CA

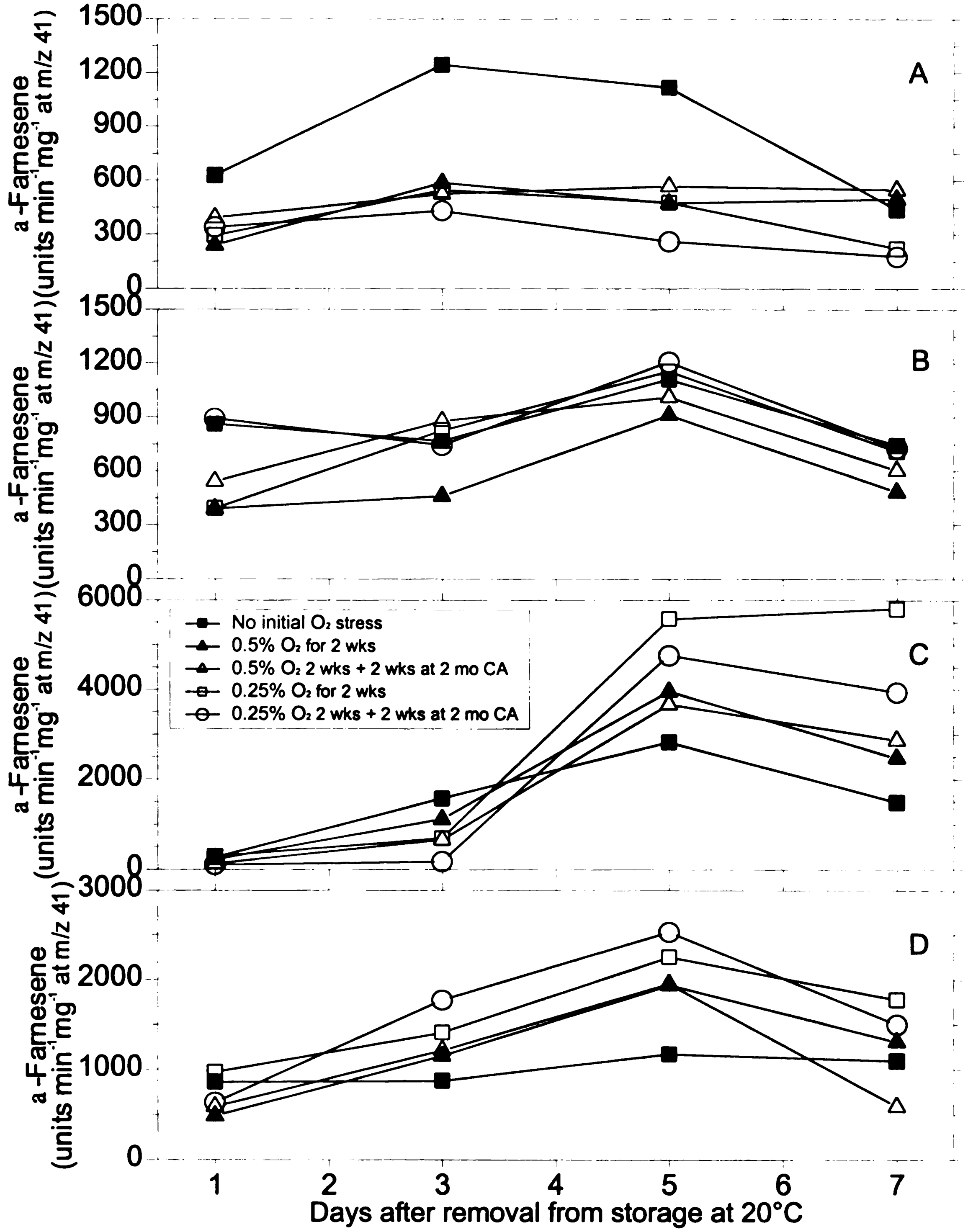

Figure 14. Effect of initial low oxygen stress on α-farnesene levels in the epicuticular wax of Granny Smith (A, B) and Law Rome (C, D) apples stored in 1.5% (A, C) and 3% (B, D) O_2 CA for 8 months at 0.5°C.(Note different scale for C and D).

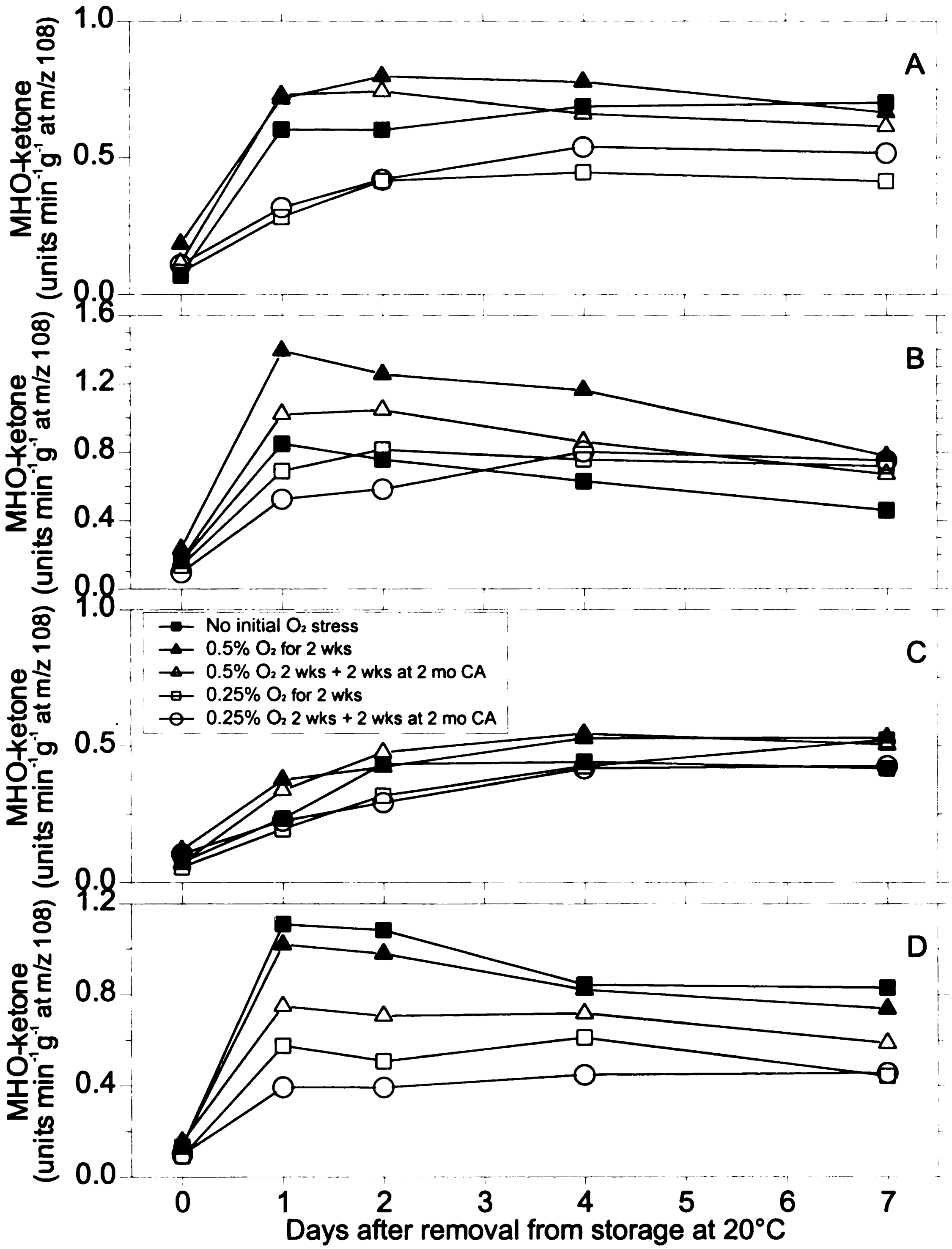

Figure 15. Effect of initial low oxygen stress on 6-methyl-5-heptene-2-one (MHO) levels in the headspace of Granny Smith (A, B) and Law Rome (C, D) apples stored in 1.5% (A, C) and 3% (B, D) O_2 CA for 5 months at 0.5°C.

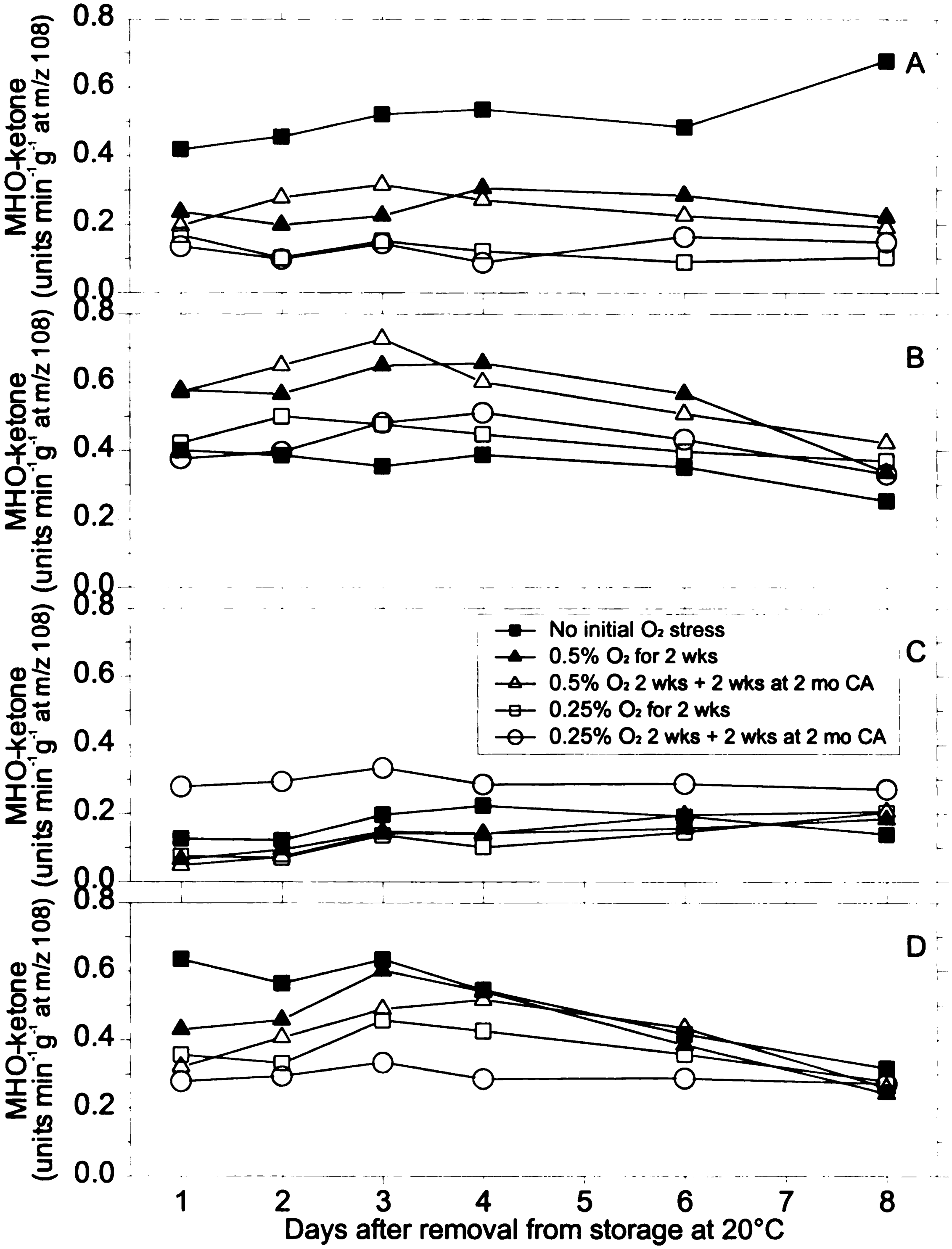

Figure 16. Effect of initial low oxygen stress on 6-methyl-5-heptene-2-one (MHO) levels in the headspace of Granny Smith (A, B) and Law Rome (C, D) apples stored in 1.5% (A, C) and 3% (B, D) O_2 CA for 8 months at 0.5°C.

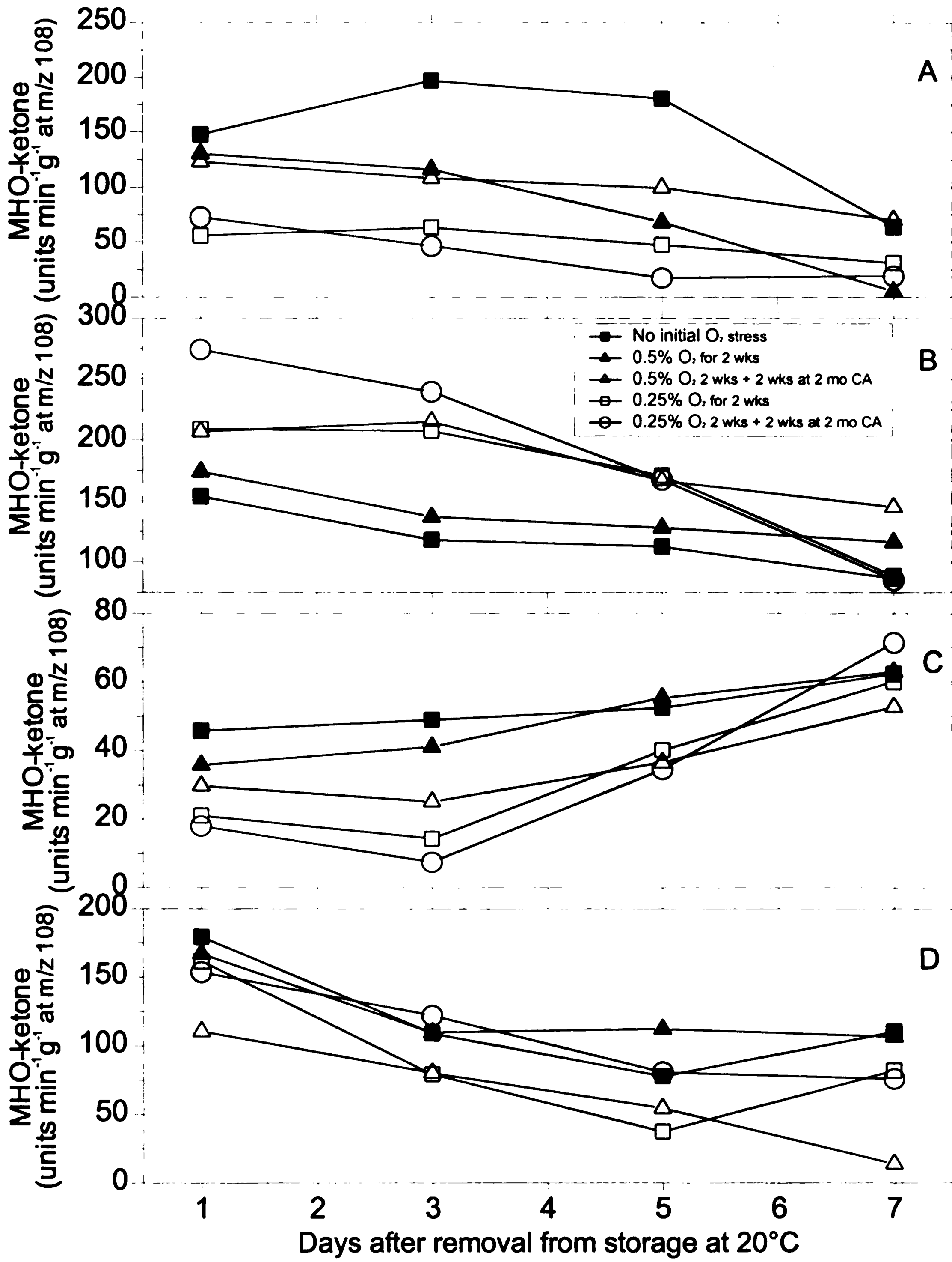

Figure 17. Effect of initial low oxygen stress on 6-methyl-5-heptene-2-one (MHO) levels in the epicuticular wax of Granny Smith (A, B) and Law Rome (C, D) apples stored in 1.5% (A, C) and 3% (B, D) O_2 CA for 8 months at 0.5°C.

at1.5% O_2 level has a stronger effect on inhibition of MHO production than does 0.5% initial O_2 stress and CA at 3% O_2 level.

Continuous low or ultra low oxygen stress studies:

GC/MS analyses of more than 60 volatiles produced by intact fruits and those which accumulated in the cuticle were conducted throughout the 8-month storage period at 0.5°C. Volatiles analyses of Granny Smith apples subjected to continuous low O_2 stress levels ranging from 0.13 to 1.5% O_2 and in air commenced at weekly intervals beginning one week after the low O_2 levels were established. The results for acetaldehyde, ethanol and ethylacetate are shown in Figure 18 . The results for fruits held at 0.75, 1.0, 1.25 and 1.5% O_2 were similar to values for fruits held in air and are ommitted from the Figure 18). Ethanol, ethylacetate and acetaldehyde were detected during the storage only on O_2 levels below 0.4% O_2 stored fruits (Figure 18A, 18B, 18C). Very small amounts of ethanol and ethylacetate were detected within the first 2 or 3 weeks of storage. Ethanol production was observed by fruits held below 0.5% O_2 beginning one week after commencing the low O_2 regimens. At 0.13% O_2, ethanol production rose to a peak after two weeks and then declined to a steady-state value followed by an increase after 150 days. Fruits held at 0.25% O_2 produced ethanol at a lower level than those at 0.13% and rose to a peak at 21 days followed by a gradual decline for the remainder of the storage period (Figure 18A). A similar pattern was seen for fruits held at 0.4% O_2 but the level of ethanol production was much lower and declined to non-detectable levels after a month of storage. The pattern of ethylacetate production was similar to that

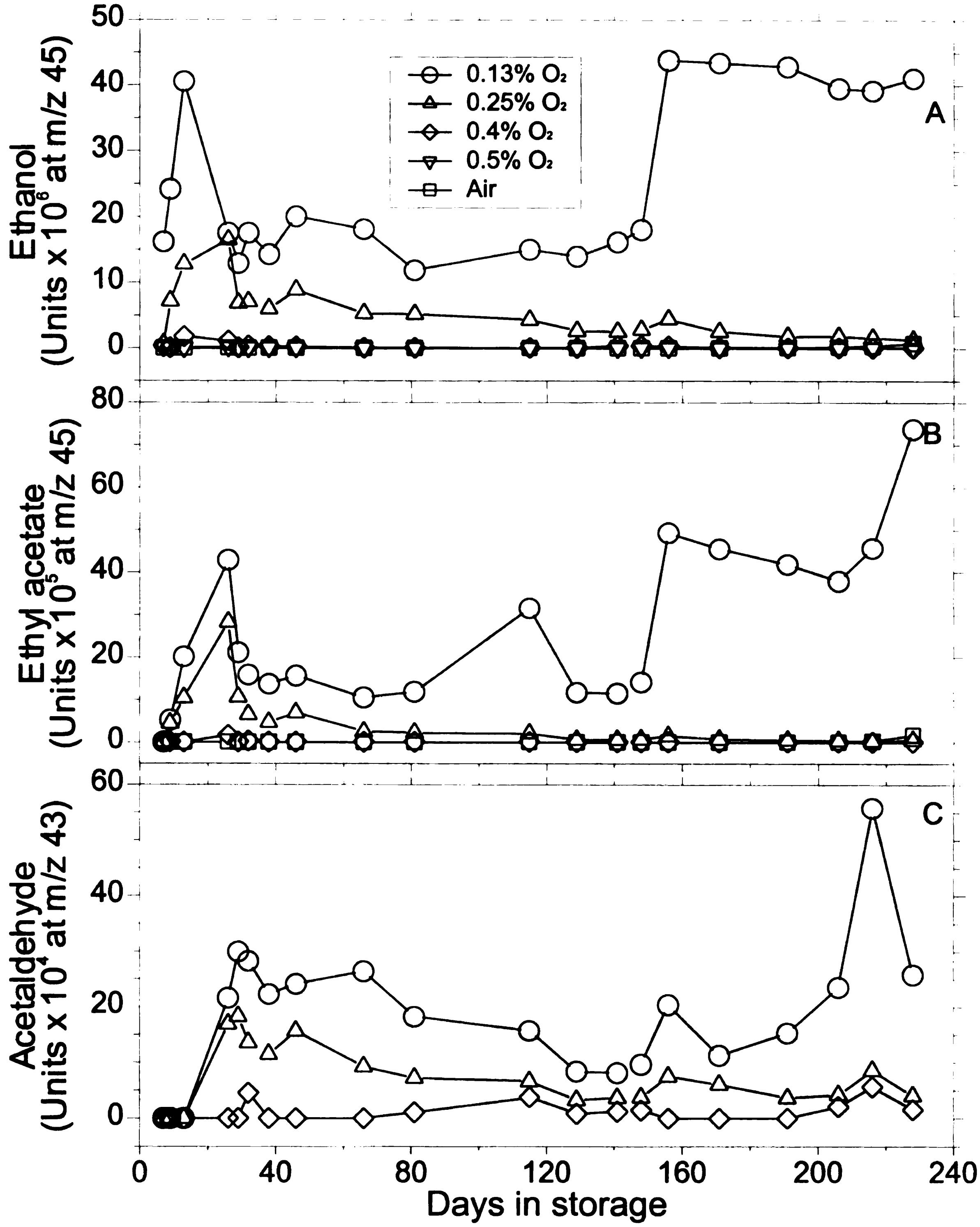

Figure 18. Change in production rates of ethanol (A), ethyl acetate (B) and acetaldehyde (C) of Granny Smith at low levels of oxygen and air storage at 0.5°C.

of ethanol (Figure 18B) but commenced slightly later. Acetaldehyde began to progressively accumulate after 4 weeks at 0.4% O_2 and below but was not measurable until production of ethanol and ethylacetate reached their maximum values (Figure 18C). Actaldehyde production at 0.13 and 0.25% O_2 remained at high values for several weeks before gradually declining. Near the end of the storage period, fruits held at 0.13% O_2 again increased in acetaldehyde production and this again followed an increase in ethanol and ethylacetate production. This may be related to irreversible damage to metabolism caused by O_2 deprivation.

α-Farnesene was produced in copious amounts by fruits in air after 3 weeks and was generally progressively lower as the O_2 level decreased (Figure 19A,19B). MHO was first detected from fruits held in air after about 45 days of storage and its production peaked at 3 months then declined to half-peak values for the remainder of the storage period (Figure 19C). MHO was not detected at O_2 levels below 1.25% and was generally detectable only at 1.5% O_2 or above. The strong relationship between the levels of O_2 favoring ethanol production and reduced levels of MHO suggest that ethanol metabolism may be directly involved in keeping MHO at low levels. Alternatively, MHO production may involve an oxidative enzyme with a fairly high affinity for molecular O_2. The mechanism for scald control by initial low O_2 stress remains to be resolved. Scald developed on the air-stored fruits after 4 months of storage and on the 1.5% O_2 stored fruits after 10 months of storage. (data not shown.)

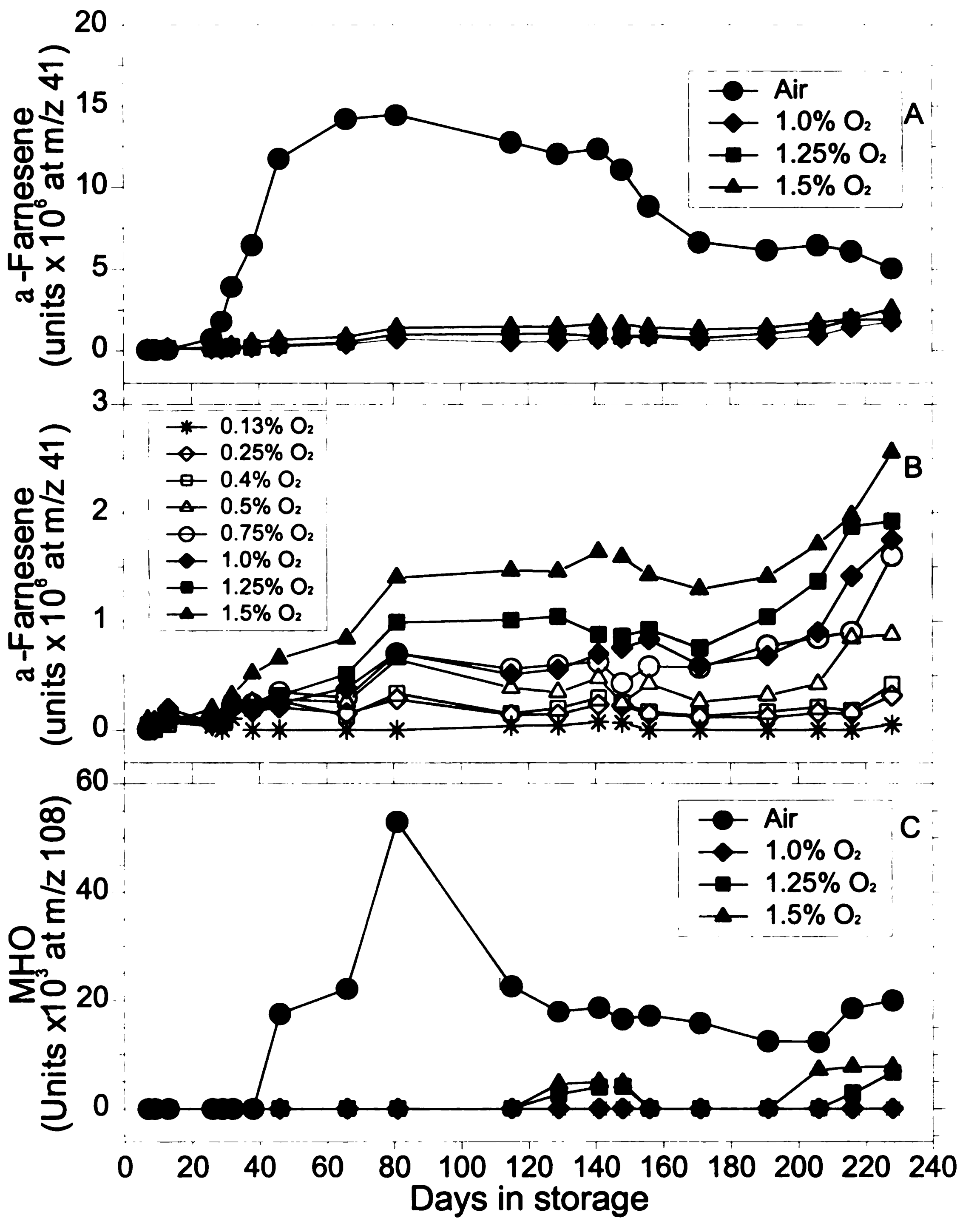

Figure 19. Change in production rates of α-farnesene (A,B) and its oxidation product MHO (C) of Granny Smith at low levels of oxygen and air storage at 0.5°C. (Fruits did not produce MHO when O_2 lower than 1.25%)

Initial low O_2 stress studies at the MSU CHES CA Storage Facility, a commercial test:

No internal disorders or off-flavors were detected in fruits from any of the storage regimens; scald was evident on fruits from the continuous 1.5% O_2 CA upon removal from storage but not for the other storage regimens and this intensified after a week at 20°C. Fruits from ultra low CA (0.7% O_2 throughout) were scald-free (Table 1 and Figure 20). Some of the Red Delicious and Law Rome fruits exhibited pigment purpling on removal from 0.7% O_2 storage but this largely cleared after a few days in air at 20°C. All cultivars from CA room #2 (1.5% O_2 throughout) exhibited scald of varying degrees with an over-all scald index average of 0.52. Fruits from CA room 3 and 4 (initial low O_2 stress for 18 days at 0.55 and 0.61% O_2) had very low scald index values of 0.09 and 0.03, respectively. Galia Beauty Rome was the only cv. with appreciable scald (last two entries in Table 1). These fruits were very immature at harvest.

Table 1. Effect of initial low oxygen stress on control of scald of apples in the CHES CA storage trial [z].

	Scald index							
	No initial low oxygen stress				Initial low oxygen stress			
Grower[y] - Cultivar	0.7% O_2 ± 0.1		1.5% O_2 ± 0.1		0.55% O_2 ± 0.2 18 days		0.61% O_2 ± 0.2 18 days	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
I- Starkrimson	0.00	0.00	0.04	0.05	0.00	0.00	0.00	0.00
IV- Starkrimson	0.00	0.00	0.04	0.03	0.01	0.01	0.00	0.00
V- Starkrimson	0.00	0.00	0.05	0.04	0.06	0.04	0.00	0.00
I- Nu Red	0.00	0.00	0.09	0.06	0.00	0.00	0.00	0.00

Table 1 continued ...

III- Super Spur	0.00	0.00	-	-	0.06	0.04	0.00	0.00
VI- Red Chief	0.00	0.00	-	-	-	-	0.00	0.00
I- Red Chief	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00
V- Red Chief	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.00
II- Red Chief	0.00	0.00	0.15	0.13	-	-	0.00	0.00
V-Winesap, Turley	0.00	0.00	0.15	0.04	0.07	0.08	0.00	0.00
I-Winesap, Stayman	0.00	0.00	0.18	0.10	0.04	0.05	0.00	0.00
III- Rome, Law	0.00	0.00	0.06	0.04	0.00	0.00	0.00	0.00
IV- Rome, Law	0.00	0.00	0.34	0.10	0.01	0.01	0.00	0.00
V- Rome, Law	0.00	0.00	0.46	0.25	0.01	0.02	0.00	0.00
VI- Rome, Law	0.00	0.00	1.10	0.25	0.07	0.04	0.00	0.00
II- Rome, Law	0.00	0.00	1.22	0.11	0.12	0.09	0.00	0.00
I- Rome, Law	0.00	0.00	1.51	0.34	0.04	0.03	0.00	0.00
II- Rome, Galia	0.00	0.00	1.16	0.17	0.25	0.13	0.12	0.08
I- Rome, Galia	0.00	0.00	2.22	0.37	0.71	0.23	0.37	0.16
Average	0.00		0.52		0.08		0.02	

[z] Harvest date October 7-13, 1997;Removal date of fruits from CA rooms June 27-30, 1998. Analysis date July 4-6, 1998;

Scald index expressed as 0-none, 1-slight, 2-moderate, 3-severe;

Storage CA condition: Room #1: 0.7% O_2 + 0.5% CO_2 continuous; Room #2: 1.5% O_2 + 0.5% CO_2 continuous; Room #3,#4: 1.5% O_2 + 0.5% CO_2 after initial low oxygen stress.

[y] 6 growers were designated as I, II, III, IV, V and VI.

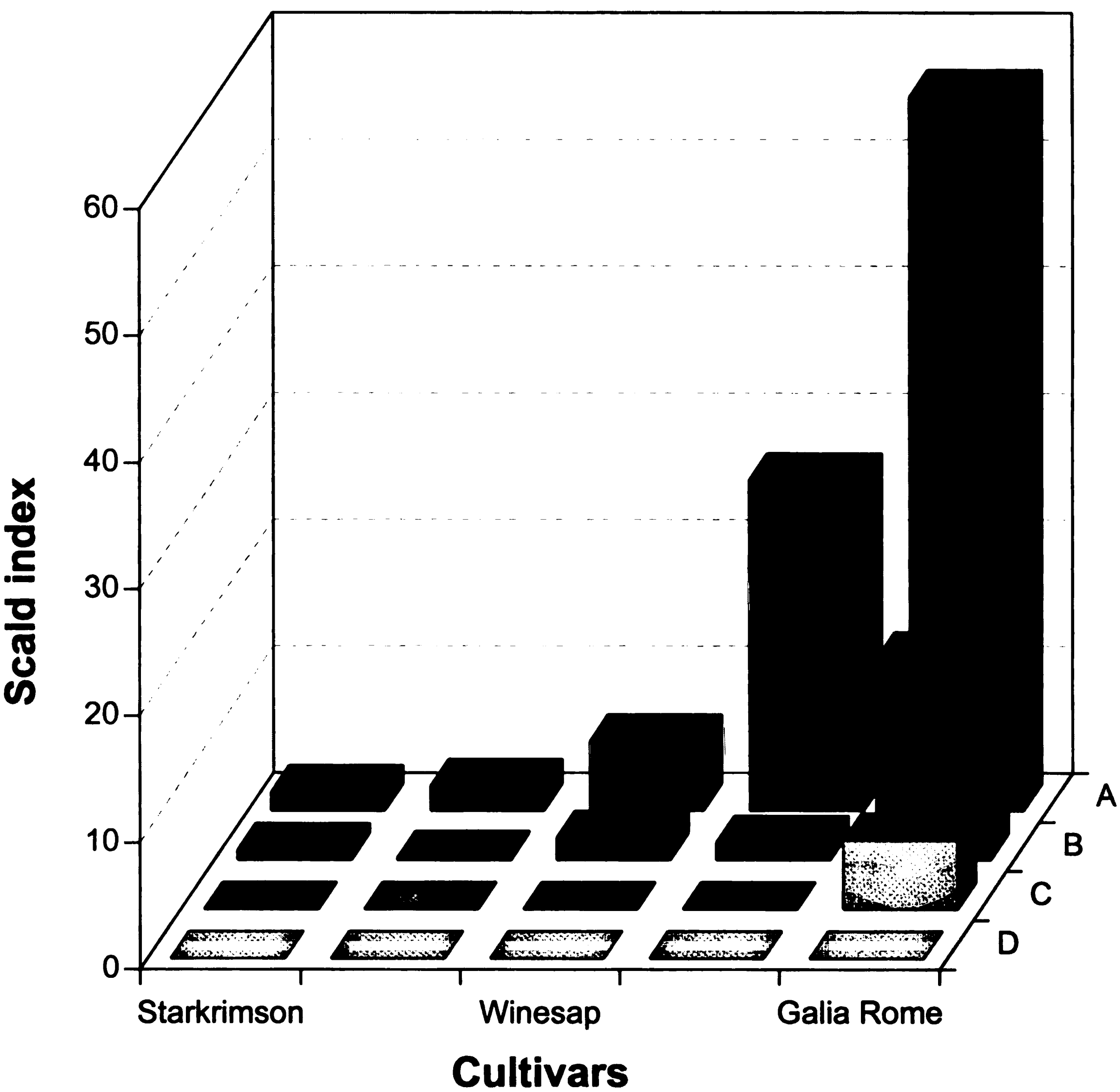

Figure 20. Effects of initial low oxygen stress and oxygen levels in CA storage (1.5% O_2 + 0.5% CO_2) at the MSU CHES facility on controlling scald of apples stored for 8 months at 0.1°C plus 7 days at 20°C in air. Treatment designation: A is for 1.5% O_2 CA as control; B is for 0.55% O_2 initial stress for 18 days then 1.5% O_2 CA; C is for 0.61% O_2 initial stress for 18 days then 1.5% O_2 CA; D is for 0.7% ultra-low O_2 CA continuously. Scald index (0=none; 1=slight; 2=moderate; 3=severe) was normalized to 100.

DISCUSSION

Our experiments in 1996-97 confirmed our earlier studies that initial low O_2 treatment at the beginning of the storage period can largely diminish superficial scald of apples as first reported by Little et al.(1982) in Australia and extended by Ghahramari and Scott (1998b). We observed that initial low O_2 stress treatment induced apples to produce ethanol aerobically (Chapter III). Moreover, ethanol vapor treatment reduced scald (Chapter III). That apples produce ethanol when the O_2 level is below the extinction point of alcohol fermentation (ca. 1%) suggests that a storage regimen of initial low O_2 stress may induce apples to produce ethanol and be an effective means to control scald. Little et al.(1982) reported that scald of Granny Smith apples was largely controlled by initial low O_2 stress followed by low O_2 CA storage. While some studies of initial low O_2 stress indicate reduction of scald others do not (Truter et al., 1994). Recent studies by Van der Merwe et al. (1997) indicate good control of scald by initial low O_2 stress followed by CA storage at 1% O_2 with 3% CO_2 at 1°C.

Apples receiving the 0.5% O_2 initial stress produced ethanol during the low O_2 stress as would be expected and has been reported (Ghahramari and Scott, 1998b; Wang et al., 1997). This suggests that the reduction of scald incidence by the initial low O_2 stress treatment may be a consequence of the ethanol produced and/or metabolism induced to reduce concentration of some metabolites or ameliorate their action as related to scald development.

Initial low oxygen stress controls scald:

Initial low O_2 stress of 0.5% O_2 for two weeks administered prior to CA storage at 3% O_2 reduced the incidence and severity of apple scald; 0.5% O_2 for 2 weeks gave better control than for 1 week (Figure 1, 2). The effectiveness of initial O_2 stress treatments diminished after long term CA storage at 3% O_2 (Figure 4). Initial low O_2 stress at 0.5 and 0.25% O_2 for two weeks at 1°C followed by CA storage at 1.5% O_2 at 1°C very effectively controlled scald of preclimacteric Law Rome and Granny Smith apples not treated with DPA (Figure 6). These results confirm the observation of Van der Merwe et al. (1997). This treatment regimen resulted in excellent retention of fruit firmness and over-all quality while CA storage at 3% O_2 following low O_2 stress did not (Figure 10, 11); the incidence of scald was however progressively less as the degree of low O_2 stress increased (Figure 6).

α-Farnesene and MHO production:

Our results support the concept that α-farnesene and its breakdown product MHO are related to the scald disorder (Anet, 1972b; Filmer and Meigh, 1971; Huelin and Coggiola, 1970a; Rowan et al.,1995; Song and Beaudry, 1996). We find that preclimacteric Law Rome and Granny Smith apples are remarkably tolerant of low O_2 stress. O_2 levels below 1.25% completely arrests MHO production (Figure 19) and α-farnesene production decreased as O_2 levels declined from 1.5 down to 0.13%. The data indicates that α-farnesene is not the limiting factor but rather its metabolism leading to MHO production and or metabolism may be.

MHO production by intact fruits and that which accumulated in the cuticle of both Law Rome and Granny Smith apples as determined after 8 months in CA storage revealed that initial low stress at 0.25% O_2 for 2 weeks markedly lowered its production/accumulation values (Figure 6). High MHO production/accumulation values were strongly and directly proportional to the scald index. This supports a putative role of MHO in the scald disorder but when it is a factor in subsequent scald development must be early vs late during storage. This is because fruits stored hypobarically within 2 months of being harvested do not scald whereas those stored with longer delays do scald (Chapter II). Capacity for MHO production begins within the first 2 months when fruits are stored in air.

Conclusions and implications:

We conclude that initial low O_2 stress of ca. 0.25-0.5% O_2 for 14 days at 0°C followed by warming to 2°C for 3 days can effectively control scald of apples not treated with DPA and subsequently stored at 1.5% O_2 at 0°C employing a static CA regimen. This confirms the results reported by Little and his colleagues (1982) and others (Van der Merwe et al., 1997). Storage at 0.7% O_2 throughout CA storage completely controlled scald (Figure 20) confirming the results of Lau (1990). However, some these fruits exhibited reversible evidence of anaerobic stress and prolonging storage beyond 8 months at 0.7% O_2 may have resulted in irreversible damage and flavor quality impairment. Since the initial low O_2 stress followed by CA at 1.5% O_2 controlled scald effectively and at commercially acceptable levels this CA storage regimen may provide a safe and effective means to store apples not treated with DPA. This also avoids the use of postharvest fungicides which is an issue of growing concern. We previously found that an initial ethanol vapor treatment of apples subsequently stored in air or CA at 3% O_2 was partially effective in controlling scald (Chapter III, Figure 8; Wang et al., 1997). Our present results support the concept that the beneficial effects of initial low O_2 stress may be related to ethanol metabolism in some manner. The residual effect of ethanol vapor treatment in reducing scald of apples subsequently stored in air (Wang and Dilley, 1996) suggests that ethanol may act apart from the effect of low CO_2 alone. A similar conclusion was recently reported by Ghahramani and Scott (1998b) who suggested that control of scald by initial low O_2 stress may be due to increased production of ethanol and consequent reduction in α-farnesene and conjugated trienes accumulation.

In our flow-through CA experiments we use gas mixtures at a flow rate that is just sufficient to remove CO_2 produced in respiration and provide a constant CO_2 and O_2 level. This amount of fresh atmosphere flushing is sufficient to remove the trace amounts of volatile substances that may partition into the epicuticular wax (Matich et al., 1998). Such substances may include α-farnesene and 6-methyl-5-heptene-2-one derived from the biological oxidation of α-farnesene (Filmer and Meigh, 1971; Spicer et al., 1993) or perhaps a trienol derived from α-farnesene (Whitaker et al., 1997). This has not been proven definitively. In theory removal of this volatile by use of a scrubber is feasible. However, the efficacy of mineral oil impreguated wraps (Brooks et al., 1919) and vegetable oil coatings (Scott, 1995b) to reduce scald is thought to be as a consequence of volatiles being partioned into them.

Our data is consistent with our observation that fruits do not scald during 8-month storage when flow-through CA is employed at 1.5% O_2 with 3% CO_2. Moreover, scald is largely controlled in commercial storages at 1.5% O_2 with 3% CO_2 employing air separators to remove CO_2 by flushing (Dilley, 1990). Flushing the atmosphere removes volatile substances.

Alternative strategies:

Several different strategies may be useful to control scald (Chellew and Little, 1995; Kader, 1986; Kallay, 1994). These may depend on cv. and the duration of the storage period. For fruits which are to be marketed after only 6 months in CA, using an air separator to maintain 1.5% O_2 with 3% CO_2 has provided satisfactory control of scald. Fruits for long-term CA for 7 or more months may require a more demanding strategy. This may include initial low O_2 stress at 0.25-0.5% O_2 levels for two weeks prior to CA at 1.5% O_2 and 3% CO_2. CA at 1 to 1.5% O_2 using an air separator to control CO_2 at 1 to 2% may provide adequate scald control for mature fruits not yet producing significant levels of ethylene. The commercial scale trial at the MSU CHES CA Facility of initial low O_2 stress demonstrated excellent control of scald by employing this treatment regimen.

LITERATURE CITED

Anet, E.F.L.J. 1969. Auto oxidation of α-farnesene. Austral. J. Chem. 22: 2403-2410.

Anet, E.F.L.J. 1972a. Superficial scald, a functional disorder of stored apples: VIII. Volatile products from the autoxidation of α-farnesene. J. Sci. Food Agr. 23: 605-608.

Anet, E.F.L.J. 1972b. Superficial scald, a functional disorder of stored apples:IX. Effect of maturity and ventilation. J. Sci. Food Agric. 23: 763-769.

Anet, E.F.L.J. and Coggiola, I.M. 1974. Superficial scald, a functional disorder of stored apples: X. Control of α-farnesene autoxidation. J. Sci. Food Agr. 25: 293-298.

Baker, J.E. 1963. Diphenylamine inhibition of electron transport in plant mitochondria. Arch. Biochem. Biophys. 103: 148-155.

Barden, C.L. and Bramlage, W.J. 1994a. Relationships of antioxidants in apple peel to changes in α-farnesene and conjugated trienes during storage, and to superficial scald development after storage. Postharvest Biol. Technology 4: 23-33.

Barden, C.L. and Bramlage, W.J. 1994b. Accumulation of antioxidants in apple peel as related to preharvest factors and superficial scald susceptibility of the fruit. J. Amer. Soc. Hort. Sci. 119: 264-269.

Brooks, C., Cooley, I.S. and Fisher, D.F. 1919. Nature and control of apple scald. J. Agric. Res. 18: 211-240.

Chellew, J.P. and Little C.R. 1995. Alternative methods of scald control in 'Granny Smith' apples. J. Hort. Sci. 70: 109-115.

Dilley, D.R. 1990. Application of air separator technology for the control of superficial scald of apples not treated with scald inhibiting chemicals. Proc. 23 Int'l. Hort. Congress, Firenze, Italy, August 27-September 1, 1990. pp. 656.

Emonger, V.E., Murr, D.P. and Lougheed, E.C. 1994. Preharvest factors that predispose apples to superficial scald. Postharvest Biol. and Technol. 4: 289-300.

Filmer, A.A.E. and Meigh, D.F. 1971. Natural skin coating of the apple and its influence on scald in storage. IV.-Oxidation products of α-farnesene. J.Sci.Fd Agric. 22: 188-190.

Gallerani, G., Pratella, G.C. and Budini, R. A. 1990. The distribution and role of natural antioxidant substances in apple fruit affected by superficial scald . Adv. Hort. Sci. 4: 144-146.

Ghahramani, F. and Scott, K.J. 1998a. The action of ethanol in controlling superficial scald of apples. Aust. J. Agric. Res. 49: 199-205.

Ghahramani, F. and Scott, K.J. 1998b. Oxygen stress of Granny Smith apples in relation to superfical sacld, ethanol, a-farnesene and conjugated trienes. Aust. J. Agric. Res. 49: 207-210.

Halliwell, B. and Gutteridge,I.C. 1989. Free radicals in biology and medicine. Clarendon Press, Oxford. pp543.

Huelin, F.E. and K.E. Murray. 1966. α-Farnesene in the natural coating of apples. Nature (London) 210: 1260-1261.

Huelin, F.E. and Coggiola, I.M. 1968. Superficial scald, a functional disorder of stored apples: IV. Effect of variety, maturity, oiled wraps and diphenylamine on the concentration of α-farnesene in the fruit. J. Sci. Food Agr. 19: 297-301.

Huelin, F.E. and Coggiola, I.M. 1970a. Superficial scald, a functional disorder of stored apples: V. Oxidation of α-farnesene and its inhibition by diphenylamine. J. Sci. Food. Agr. 21: 44-48.

Huelin, F.E. and Coggiola, I.M. 1970b. Superficial scald, a functional disorder of stored apples. VI.-Evaporation of α-farnesene from the fruit. J.Sci.Fd Agric. 21: 82-86

Ingle, M. and D'Souza, M. 1989. Physiology and control of superficial scald of apples: A review. HortScience 24: 28-31.

Kader, A.A. 1986. Biological and physiological basis for effects of controlled and modified atmosphere on fruits and vegetables. Food Technology 40: 99-103.

Kallay, T. 1994. New measures against superficial scald in apples. Acta Hortic. 368: 220-224.

Ke, D. and Kadar, A.A. 1992. Potential of controlled atmosphere for postharvest insect disinfestation of fruits and vegetables. Postharvest News and Information. 3: 31-37.

Lau, O.L. 1990. Efficacy of diphenylamine, ultra-low oxygen, and ethylene scrubbing on scald control in 'Delicious' apples. J. Amer. Soc. Hort. Sci. 115: 959-961.

Little, C.R., Faragher, J.D. and Taylor, H.S. 1982. Effects of initial low oxygen stress treatments in low oxygen modified atmosphere storage of Granny Smith apples. J. Amer. Soc. Hort. Sci. 107: 320-323.

Matich, A.J., Banks, N.H. and Rowan, D.D. 1998. Modification of α-farnesene levels in cool-stored 'Granny Smith' apples by ventilation. Postharvest Biol. and Technol. 14: 159-170.

Meigh, D.F. and Filmer, A.A.E. 1969. The natural skin coating of the apple and its influence on scald in storage: III. α-farnesene. J. Sci. Food Agr. 20: 139-143.

Meigh, D.F. 1970. Apple scald. In: A.C. Hulme (Ed.), The Biochemistry of Fruits and Their Products. Vol. 1. Academic Press, London, pp. 555-569.

Rowan, D.D., Allen, J.M., Fielder, S., Spicer, J.A. and Brimble, M.A. 1995. Identification of conjugated triene oxidation products of α-farnesene in apple skin. J. Ag. Food Chem. 43: 2040-2045.

Scott, K.J., Yuen, C.M.C. and Ghahramani, F. 1995a. Ethanol vapor- a new anti-scald treatment for apples. Postharvest Biol. Technol. 6: 201-208.

Scott, K.J., Yuen, C.M.C. and Kim, G.H. 1995b. Reduction of superficial scald of apples with vegetable oils. Postharvest Biol. and Technol. 6: 219-223.

Smock, R.M. 1957. A comparison of treatments for control of the apple scald disease. Proc. Amer. Soc. Hort. Sci. 69: 91-100.

Smock, R.M. 1961. Methods of scald control on the apple. Cornell Univ. Agric. Exp. Stn., Bull. No. 970, pp. 55.

Song, J. and Beaudry, R.M. 1996. Rethinking apple scald: new hypothesis on the causal reason for development of scald in apples. HortScience 31: 605.

Song, J., Gardner, B.D., Holland, J.F. and Beaudry, R.M. 1997. Rapid analysis of volatile flavor compounds in apple fruit using SPME and GC/Time-of-Flight Mass Spectrometry. J. Agri. Food Chem. 45: 1801-1807.

Spicer, J.A., Brimble, M.A. and Rowan, D.D. 1993. Oxidation of α-farnesene. Aust. J. Chem. 46: 1929-1939.

Stadtman, E.R. 1993. Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalyzed reactions. Annu. Rev. Biochem. 62: 797-821.

Truter, A.B., Combrink, J.C. and Burger, S.A. 1994. Control of superficial scald in

'Granny Smith' apples by ultra-low and stress levels of oxygen as an alternative to diphenylamine. J. Hort. Sci. 69: 581-587.

Van der Merwe, J.A., Combrink, J.C., Truter, A.B. and Calitz, F.J. 1997. Effect of initial low oxygen stress treatment and CA storage at increased carbon dioxide levels on post-storage quality of South African-grown 'Granny Smith' and 'Topred' apples. In: E.J. Mitchman (ed.) Apples and Pears. Proc. 7th Int'l Nat. CA Res. Conf., 2: 79-84.

Wang, Z. and Dilley, D.R. 1996. Ethanol vapor treatment: A new alternative approach to control superficial scald of apples. Proc. Mich. State Hort. Soc. 126: 35-40.

Wang, Z. and Dilley, D.R. 1997. The relationship of α-farnesene production and its oxidation product 6-methyl-5-heptene-2-one to superficial scald of Granny Smith, Law Rome, Red Delicious and Idared apples during controlled atmosphere and air storage. In: E.J. Mitchman (ed.) Apples and Pears. Proc. 7th Int'l Nat. CA Res. Conf., 2: 98-104.

Wang, Z., McCully, T.J. and Dilley, D.R. 1997. The effect of ultra low oxygen storage, initial oxygen stress and ethanol vapor treatments on controlling superficial scald of Granny Smith, Law Rome, Red Delicious and Idared apples. In: E.J. Mitchman (ed.) Apples and Pears. Proc. 7th Int'l Nat. CA Res. Conf., 2: 105-111.

Whitaker, B.D., Solomos, T. and Harrison, D.J. 1997. Quantification of α-farnesene and its conjugated trienol oxidation products from apple peel by C_{18}-HPLC with UV detection. J. Agric. Food Chem. 45: 760-765.

CHAPTER V

THE EFFECT OF ACCLIMATIZATION OF FRUITS ON THE CONTROL OF A CO_2-LINKED DISORDER OF EMPIRE APPLES

ABSTRACT

The 'scald-like' disorder of Empire apples is a CO_2-linked physiological disorder. In this study the factors of acclimatization of preclimacteric fruits at 3°C in air or low O_2 levels prior to elevating the CO_2 concentration for subsequent long-term controlled atmosphere were investigated. The results indicate that the Empire 'scald-like' disorder can be effectively controlled by holding the fruits at 3°C for 3-4 weeks at 1.5 to 3% O_2 without CO_2 prior to CA storage at 1.5% O_2 + 3% CO_2. Acclimatization of fruits against the disorder was also achieved by storage in air but this resulted in excessive ripening development with flesh firmness loss during subsequent CA storage.

INTRODUCTION

The Empire 'scald-like' physiological disorder can occur during controlled atmosphere (CA) storage (Burmeister and Dilley, 1995). It was concluded that it is a CO_2-linked disorder that is (1) exacerbated by storage at 0°C at O_2 levels below 1.5% with CO_2 at or over 3%, (2) controlled by adding dry lime to the CA storage chambers (0% CO_2), and (3) controlled by maintaining CO_2 at or below 2%. The disorder has many but not all of the attributes of superficial scald (Ingle and D'Souza, 1989). The disorder is more prevalent with immature fruits; affects the non-red surface of the fruits; is induced during the first few weeks of storage; and is controlled by diphenylamine (DPA), an antioxidant employed to prevent scald. This disorder has resulted in as much as a 20% reduction in packout by shippers to meet U.S. and export standards. A similar physiological disorder has been observed on immature Northern Spy (Burmeister and Dilley, 1995) and Braeburn apples (Burmeister and Rowan, 1997; Elgar et al., 1998). This prompted us to investigate alternative methods to control the disorder. A recent report by Lau (1998) suggests that the Braeburn browning disorder is more prevalent with late-harvested fruits. The Braeburn browning can be controlled by DPA (Burmeister and Rowan, 1997). We hypothesized that the Empire 'scald-like' disorder may be related to superficial scald since the disorders of both Empire and Braeburn apples can be effectively controlled by drenching with DPA before storage. The two disorders may not be the same since the Empire disorder does not happen in air storage and was related to high CO_2 levels especially at the beginning of the storage period. The Empire disorder can occur after only a few weeks in storage while superficial scald occurs after several months. In this study, we investigated the effects of acclimatizing fruits in various gas atmosphere regimens prior to CA storage to control the disorder.

MATERIALS AND METHODS

Preliminary experiments (1994):

Preclimacteric Empire fruits with or without DPA at 1000 mg$\cdot$L^{-1} were placed in 20-L chambers ventilated with 0 or 5.5% CO_2 in humidified air at 0.1 and 3°C. Ethanol vapors were applied to apples in some storage chambers by circulating 0 or 5.5% CO_2 through 1% aqueous solutions of ethanol in flasks in series with the chambers to generate about 115 $\mu L \cdot L^{-1}$ ethanol vapor into the chambers. The ventilation rate was 0.4 atmosphere turnover per hr. Three replicates of 50 fruits were employed each. Fruits were examined for the scald-like disorder incidence at weekly intervals for 3 and half weeks. (See Table 1 for results).

Preclimacteric Empire apples were placed under CA at 1.5% O_2 with 0, 3 and 5% CO_2 and in air at 3°C. Half the fruits for CA at 1.5% O_2 +3% CO_2 were added a 500g dry lime-packet to each chamber to scrub CO_2, while half the fruits for CA at 1.5% O_2 + 5% CO_2 were treated with DPA 1000 $mg \cdot L^{-1}$. The Empire disorder index was measured after 1 and 5 months of storage and plus 7 days at 20°C in air. The index was based on the percentage of fruit surface area affected where no disorder = 0, <25%=1, 25-50%=2 and >50%=3 and disorder index was normalized to 100. (See Table 2 for results).

Carbonyl content determination:

The carbonyl content of apple peel tissue was determined by the methods of Levine et al. (1994) and Stadtman (1993).

Main experiment (1995):

Fruits from the MSU CHES were harvested at the preclimacteric stage of maturity about 10 days before the onset of the endogenous ethylene climacteric. This was done so the fruits would have a high potential to develop the 'scald-like' disorder (Burmeister and Dilley, 1995). Fruits were randomized to fill 132 20-L chambers with 45-65 fruits each. Atmospheres of 1.5 or 3% O_2 were established within 1 day of harvest and the CO_2 level was brought up to 3 or 5% after 0, 0.5, 1, 2, 4, or 8 weeks in storage at 3C. The gas atmospheres were established by mixing nitrogen produced by a Permea air separator with CO_2 and air in a gas mixing apparatus. The gas mixtures were humidified and distributed to the CA chambers using a capillary flow-board system at a flow rate sufficient to provide 0.4 chamber void volume changes per hour. Some fruits were kept at 1.5 or 3.0% O_2 without CO_2, some were held in air for 1 or 2 weeks prior to adjusting to the CA conditions while others were held in air throughout the storage period of 6 months. A David Bishop Oxystat II Analyzer was employed to monitor the O_2 and carbon dioxide levels on a daily basis. Three replicates were used for each treatment. After 6 months of storage, the disorder index was determined: no disorder = 0, <25%=1, 25-50%=2 and >50%=3 based on the percentage of the fruit surface area affected by the disorder. Disorder index was normalized to 100 by multiplying 33.33.

RESULTS

Preliminary experiments:

Supplementing the CA atmosphere with ethanol vapor at 115 $\mu L \cdot L^{-1}$ (v/v) reduced the disorder incidence during a 2 week period at 5% CO_2 + 1.5% O_2 but became ineffective with longer storage durations (Table 1). Fruits at 3°C without CO_2 in the atmosphere were not affected with the disorder but with 3 or 5% CO_2 had serious

Table 1. Effect of ethanol vapor treatment on the incidence (%) of the `scald like' disorder of Empire apples stored at 0 or 5.5% CO_2 + 1.5% O_2 at 0.1 and 3°C with and without pretreatment with diphenylamine (1000 $mg \cdot L^{-1}$).

Storage temperature (°C)	Treatments			Disorder incidence (%)			
	Ethanol vapor ($\mu l \cdot L^{-1}$)	CO_2 (%)	DPA ($mg \cdot L^{-1}$)	14d	20d	24d	24d + 2d at 20°C
0.1	0	5.5	0	62	71	65	79
0.1	115	5.5	0	15	37	43	83
0.1	115	5.5	1000	0	0	0	0
0.1	0	0	0	0	0	0	0
0.1	115	0	0	0	0	0	0
0.1	115	0	1000	0	0	0	0
3	0	5.5	0	58	60	69	82
3	115	5.5	0	23	40	50	80
3	115	5.5	1000	0	0	0	0
3	0	0	0	0	0	0	0
3	115	0	0	0	0	0	0
3	115	0	1000	0	0	0	0

disorder incidence (Table 2). Fruits at 3°C with 500g of dry lime-packets to absorb CO_2 in the chambers with 3% CO_2 + 1.5% O_2 significantly reduced the disorder index. 1000 mg·L^{-1} DPA completely controlled the Empire disorder. Fruits stored at 1.5% O_2 without CO_2 for 30d prior to CA with 3% CO_2 + 1.5% O_2 or 5% CO_2 + 1.5% O_2 did not show the disorder (Table 2).

Table 2. The effect of carbon dioxide and diphenylamine (DPA) on the development of Empire disorder at 3°C. Disorder index (0=none; 1=slight; 2=moderate; 3=severe) was normalized to 100.

Treatments	Disorder index			
	30d+7d at 20°C		50d+7d at 20°C	
	Mean	SE	Mean	SE
Control-air	0	0	0	0
$0\%CO_2+1.5\%O_2$	0	0	0	0
$3\%CO_2+1.5\%O_2$	47.7	3.7	49.7	5.4
$5\%CO_2+1.5\%O_2$	71.3	6.8	73.0	9.2
$0\%CO_2+1.5\%O_2$ for 30d prior to $3\%CO_2+1.5\%O_2$	-	-	0	0
$0\%CO_2+1.5\%O_2$ for 30d prior to $5\%CO_2+1.5\%O_2$	-	-	0	0
$3\%CO_2+1.5\%O_2$+Lime-Package	11.7	2.1	12.3	3.3
$5\%CO_2+1.5\%O_2$+DPA (1000 mg·L^{-1})	0	0	0	0

Main experiment:

Establishing CA conditions of 5% CO_2 + 1.5% O_2 at 3°C within 1 day of harvest resulted in the highest incidence and severity of the Empire disorder followed by 3% CO_2 + 1.5% O_2. The incidence of the disorder was decreased when the level of O_2 was increased from 1.5% O_2 to 3% O_2 (Figure 1). After acclimatizating the fruit for two weeks, the scald-like disorder index for fruits stored at 5% CO_2 + 1.5% O_2 reduced 86% while disorder index for fruits stored at 3% CO_2 + 1.5% O_2 reduced 94% comparing to the control (Figure 1). The incidence of the disorder did not increase after removal from storage at 3°C to 20°C for 7 days (Figure 1A,1B). Acclimatizating the fruit in air at 3°C before CA storage attenuated or prevented the disorder. The fruits had to be acclimated at 3°C for 3-4 weeks at 1.5 or 3% O_2 to become insensitive to 5 or 3% CO_2. Holding the fruits in air for 1 week prior to administering 3 or 5% CO_2 with 1.5% O_2 during storage was insufficient to control the disorder whereas the fruits tolerated 3 or 5% CO_2 after holding the fruits in air for 2 weeks(Figure 1A,1B). But the 2 week delay to CA resulted in accelerating flesh softening (Figure 2). Supplemental carbon dioxide was not necessary for at least 1 month to maintain flesh firmness at initial firmness values when fruits were kept at 1.5% O_2. At 3% O_2 the presence of 3 or 5% CO_2 from the beginning of the storage period was required to retard flesh firmness decrease. Fruits stored for the entire storage duration without CO_2 softened markedly (Figure 2A,2B).

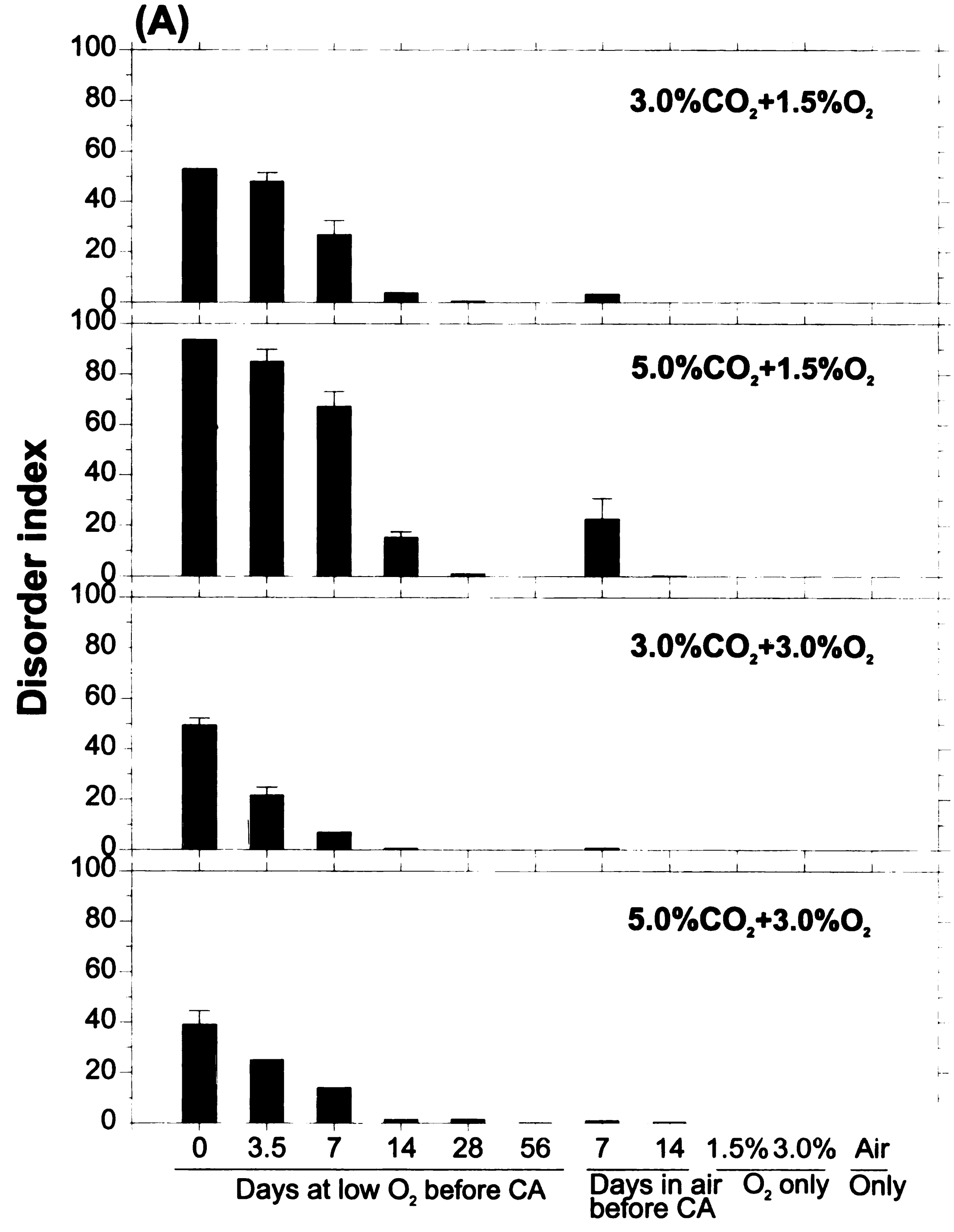

Figure 1. (A) Effect of acclimatization atmosphere on the incidence and severity of the scald-like disorder of Empire apples stored 6 months in CA at 3°C. Disorder index on removal from storage. Disorder index (0=none; 1=slight; 2=moderate; 3=severe) was normalized to 100.

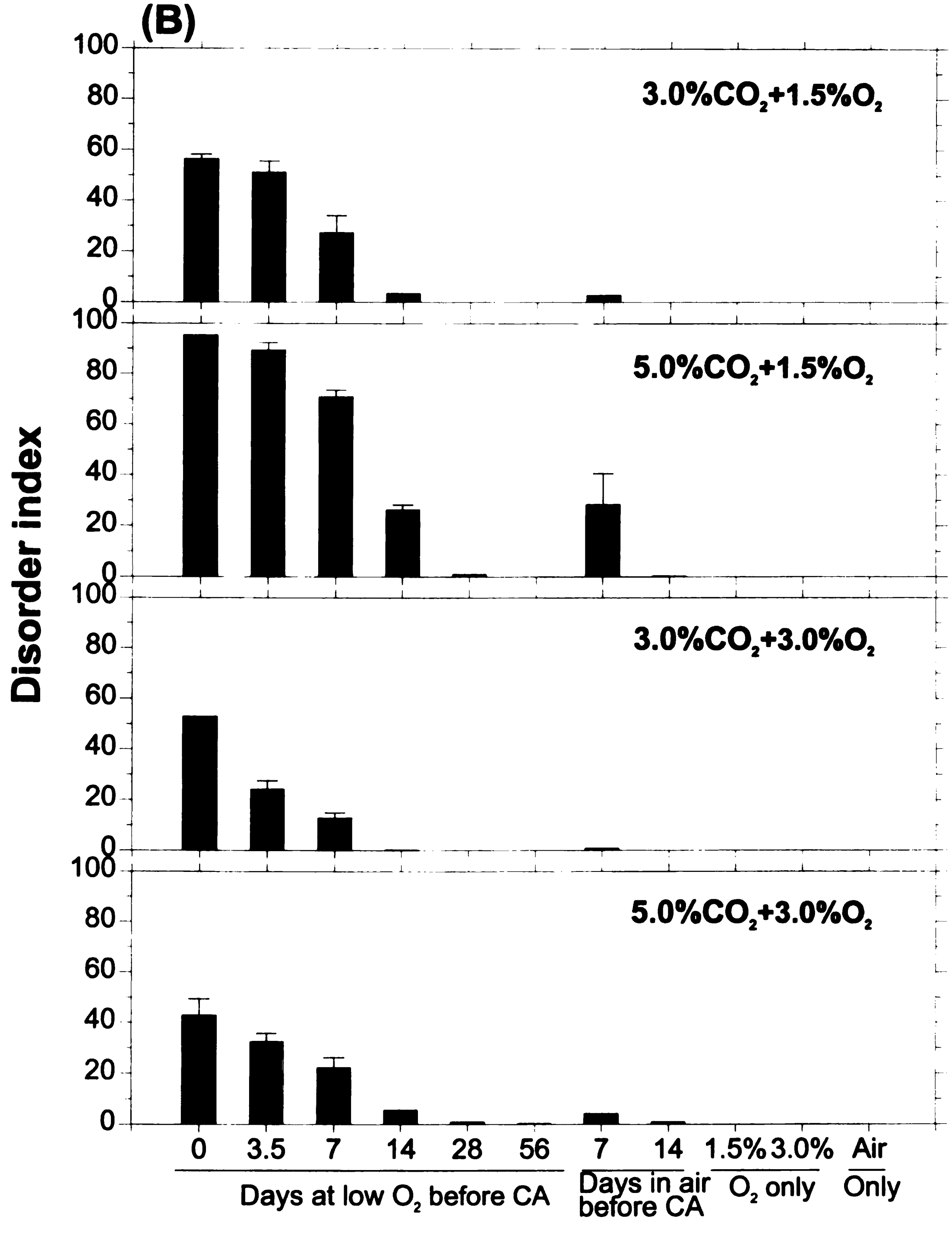

Figure 1. (B) Effect of acclimatization atmosphere on the incidence and severity of the scald-like disorder of Empire apples stored 6 months in CA at 3°C plus 7 days at 20°C in air. Disorder index (0=none; 1=slight; 2=moderate; 3=severe) was normalized to 100.

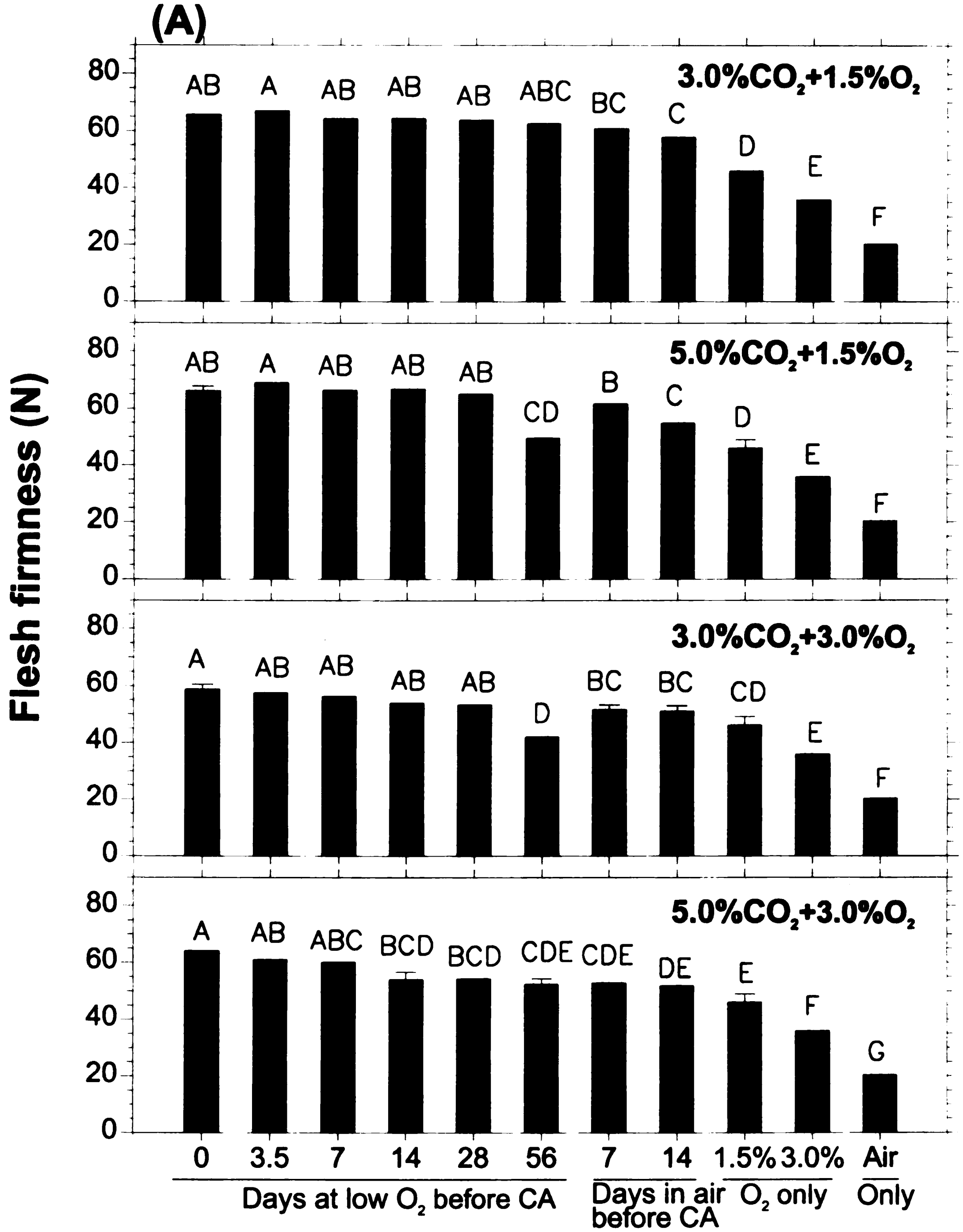

Figure 2. (A) Effect of acclimatization atmosphere on flesh firmness retention of Empire apples stored 6 months in CA at 3°C. Flesh firmness on removal from storage. Disorder index (0=none; 1=slight; 2=moderate; 3=severe) was normalized to 100. The different letters at the top of the bars designate the difference at $P \leq 0.01$.

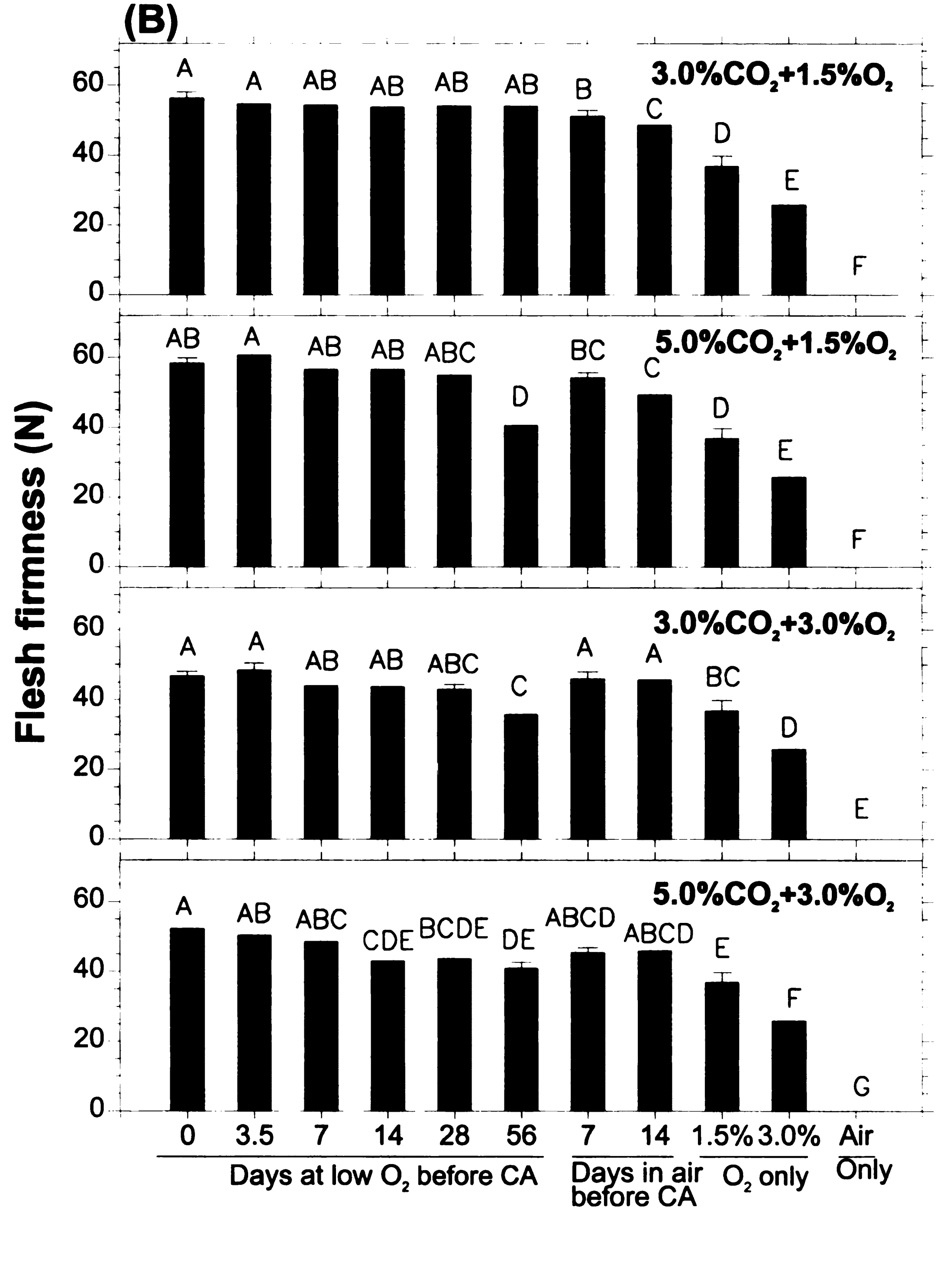

Figure 2. (B) Effect of acclimatization atmosphere on flesh firmness retention of Empire apples stored 6 months in CA at 3°C plus 7 days at 20°C in air. Disorder index (0=none; 1=slight; 2=moderate; 3=severe) was normalized to 100. The different letters at the top of the bars designate the difference at P≤ 0.01.

DISCUSSION

Fruits for long-term CA storage and those destined for export must be harvested at the preclimacteric stage of maturity to ensure delay of ripening and excessive firmness loss to meet market requirements. Moreover, the presence of CO_2 during CA storage helps to delay ripening. However, the presence of CO_2 during the first few weeks of CA storage at the low O_2 level of 1.5% required to ensure good delay of ripening during storage while the fruits are at the optimum temperature of 3°C can lead to high incidence of the Empire 'scald-like' disorder (Burmeister et al., 1994; Burmeister and Dilley, 1995). Storage at 0-1°C, is not advisable for Empire apples since the disorder is exacerbated and can cause another disorder described as internal flesh browning which is known to be a chilling-related and CO_2-linked physiological disorder. This poses a restriction on the use of CA atmospheres and low temperature to extend the storage period of Empire apples for certain markets and/or potentially profitable marketing windows. While establishing CA conditions of 3 to 5% CO_2 with 1.5 or 3% O_2 at 3°C within one week of harvest results in good control of ripening with high retention of flesh firmness this storage regimen can lead to serious incidence of the Empire disorder with preclimacteric fruits.

The nature of the disorder:

We confirmed that: (1) the disorder is caused by high CO_2 levels; (2) this is exacerbated at O_2 levels below 1.5%; (3) the disorder is more prevalent with immature fruits; and (4) this affects primarily the non-red fruit surface. Diphenylamine (DPA) at 1000 $mg{\cdot}L^{-1}$ has been found to control the Empire disorder in laboratory experiments (Tables 1,2) and in commercial CA storage experience. DAP also controls the Braeburm browning disorder (Burmeister and Rowan, 1997).

We do not recommend that Empires be treated with DPA. The fact that diphenylamine applied as a postharvest drench treatment controlled the disorder suggests the involvement of free-radicals as the mechanism responsible for the dysfunction (Burmeister et al., 1994; Burmeister and Dilley, 1995; Burmeister and Rowan, 1997).

The role of active oxygen species:

Internal CO_2 may be high enough in the hypodermal cells to cause the disorder in conjunction with low O_2 in these fruits. Ethanol is a free-radical scavenger and its effectiveness in diminishing the disorder supports our hypothesis that high CO_2 levels at low O_2 levels foster the accumulation of free-radicals through a Fenton reaction involving hydrogen peroxide (H_2O_2) and consequential oxidative modification of protein amino groups (Halliwell and Gutteridge, 1989; Stadkman, 1993). The Fenton reaction is: $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + HO^\bullet$. Ascorbic acid, which can serve as an antioxidant in preventing the accumulation and reactions of free-radicals thought to be involved in scald and the scald-like disorder, is much lower in the non-red than in the red fruit skin colored with the anthocyanidin pigment cyanidin (Table 3). Apple cultivars with high ascorbic acid levels are generally less prone to scald than those with lower levels (Anet, 1972, 1974; Lurie

Table 3. Ascorbate and dehydroascorbate content of scalded and non-scalded peel tissue of Jonathan, Golden Delicious and Stayman apples.

Cultivar	Scald	Ascorbate	Dehydroascorbate	Total
			(mg/100g FW)[z]	
Jonathan	-	8.9a	11.9a	20.8a
	+	4.5b	6.3b	10.8b
Golden Delicious	-	29.2a	9.9a	39.1a
	+	17.6b	10.6a	28.4b
Stayman (Green)	-	13.4a	1.1a	14.5a
(Green)	+	6.9b	3.7b	10.6b

Means within columns for the same cv. followed by different letters differ at $p \leq .05$.

Table 4. Carbonyl content of Law Rome apples skin in relation to scald development of fruits stored for 6 months in air or CA at 3% CO_2 + 3% O_2 at 1 or 3°C.

Storage Treatments	Scald incidence (%)		Carbonyl content (out of storage)
	0	7d	nmols/mg protein
1) 1°C 3:3 control	0 c	20 b	7.80 ± 0.38 abc
2) 1°C 3:3 + DPA	0 c	0 c	7.33 ± 0.07 bc
3) 1°C air control	13 b	53 a	7.40 ± 0.42 abc
4) 1°C air control + DPA	0 c	0 c	6.91 ± 0.56 c
5) 3°C 3:3 control	0 c	27 b	8.05 ± 1.01 ab
6) 3°C 3:3 + DPA	0 c	0 c	7.25 ± 0.52 bc
7) 3°C air control	20 a	47 a	8.50 ± 0.21 a
8) 3°C air control + DPA	0 c	7 c	7.08 ± 0.45 b

Means within columns followed by different letters differ at $p \leq .05$.

et al., 1989; Meir and Bramlage, 1988; Gallerani et al., 1990). However, ascorbate levels generally do not change substantially as apple fruits mature and although fruits become less prone to scald as they mature on the tree. The Empire 'scald-like' disorder affects the non-red portion of the fruit and hardly ever the portion of the surface that is highly pigmented with anthocyanins; the ascorbic acid levels in the red portion are higher than in the green portion of the skin (Table 3). This is consistent with the effect of ascorbic acid as an antioxidant. It is also consistent with the observation that the antioxidant DPA can control the Empire disorder (Table 4) and the Braeburn browning disorder (Burmeister and Rowan, 1997).

Protein oxidation may be related to scald development and also to the Empire 'scald-like' disorder. The carbonyl content in a protein is a measure of the extent of protein oxidation that has occurred. Protein oxidation can disable proteins from functioning as active enzymes (Chen et al., 1993; Halliwell and Gutteridge, 1989). The carbonyl content of Law Rome apples was 4 to 4.9 nmol mg^{-1} protein at harvest and increased to 7 to 8 nmol mg^{-1} protein during 6 months in storage (Table 4). DPA-treated apples had a lower carbonyl content than nontreated fruits. Fruits which developed scald had a higher carbonyl content than the non-scalded fruits (Table 4). Fruits stored in air at 3°C had a higher carbonyl content than those stored at 1°C and the incidence of scald was about 50% at each temperature.

The carbonyl content of air stored fruits without DPA averaged over 1° and 3°C was 7.95 nmol mg^{-1} protein while that for DPA-treated fruits was 7.0 nmol mg^{-1} protein

(Table 4). This represents an actual increase of about 1% in carbonyl content per mg of protein. This is a significant increase in actual carbonyl content as expressed in relation to mg of protein. Collectively, the data indicate that: (1) scald is related to the increase in carbonyl content and (2) DPA marginally lowers the carbonyl content while it strongly inhibits scald development. This may indicate that if only certain proteins are prevented from becoming oxidized by DPA scald may be controlled.

The acclimatization phenomenon:

The results of the current studies indicate that Empire apples can be acclimatized during the first few weeks of storage at 3°C to attenuate the incidence and severity of the 'scald-like' disorder. Storage in air or at 1.5 or 3% O_2 without CO_2 for several weeks before CA storage at 1.5 or 3% O_2 with 3 or 5% CO_2 reduces or prevents the disorder. Fruits susceptible to CO_2-linked disorders are known to have higher resistance to gas diffusion than cvs. not affected (Rajapakse et al., 1990; Park et al., 1993; Johnson et al., 1998). This favors the accumulation of CO_2 in the internal atmosphere and this may contribute to disorder incidence (Lau, 1998). Moreover, fruits acclimate better in air than at low O_2 levels with respect to tolerating CO_2 during subsequent CA storage. Elgar et al. (1998) found that rapid establishment of CA increases the incidence of the Braeburn browning disorder. This suggests a requirement of oxidative metabolism in acclimatization for CO_2 tolerance. This may involve some aspect of ethylene action. Storage of apples of all cvs. in air beyond about 7 days induces preclimacteric fruits to produce ethylene autocatalytically. This is associated with induction of the ripening processes and CA storage is much less effective in delaying subsquent ripening development during storage. Consequently, the fruits soften and marketing of such fruits becomes problematic. Acclimatization in air at 3°C beyond 7 days before CA is established results in excessive flesh softening even though it does prevent the disorder incidence. However, acclimatization at 3°C at 1.5 or 3% O_2 without CO_2 for up to 4 weeks and then raising the CO_2 to 3 or 5% to gain the ripening retardation effects of CO_2 largely eliminates the Empire 'scald-like' disorder while providing good control of ripening. This storage regimen fits well with current CA technology employing the use of air separators for establishing and maintaining O_2 and CO_2 levels during CA storage.

LITERATURE CITED

Anet, E.F.L.J. 1972. Superficial scald, a functional disorder of stored apples: VIII. Volatile products from the autoxidation of α-farnesene. J. Sci. Food Agr. 23: 605-608.

Anet, E.F.L.J. 1974. Superficial scald, a functional disorder of stored apples: XI. Apple antioxidants. J. Sci. Food Agric. 25: 299-304.

Burmeister, D.M., Wang, Z. and Dilley, D.R. 1994. The Empire 'scald-like' disorder-1994 studies. Proc. Mich. State Hort. Soc. 124: 207-209.

Burmeister, D.M. and Dilley, D.R. 1995. A 'scald-like' controlled atmosphere storage disorder of Empire apples- a chilling injury induced by CO_2. Postharvest Biol. Technol. 6: 1-7.

Burmeister, D.M. and Rowan, S.1997. Physiological and biochemical basis for the Braeburn browning disorder (BBD). In: E.J. Mitchman (ed.) Apples and Pears. Proc. 7th Int'l Nat. CA Res. Conf., 2: 126-131.

Chen, Z., Silva, H. and Klessig, D.F. 1993. Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science 262: 1883-1886.

Elgar, H.J., Burmeister, D.M., Watkins, C.B. 1998. Storage and handling effects on a CO2-related internal browning disorder of 'Braeburn' apples. HortScience 33: 719-722.

Gallerani, G., Pratella, G.C. and Budini, R. A. 1990. The distribution and role of natural antioxidant substances in apple fruit affected by superficial scald . Adv. Hort. Sci. 4: 144-146.

Halliwell, B. and Gutteridge, I.C. 1989. Free radicals in biology and medicine. Clarendon Press, Oxford. pp543.

Ingle, M. and D'Souza, M. 1989. Physiology and control of superficial scald of apples: A review. HortScience 24: 28-31.

Johnson, D.S., Dover, C.J. and Colgan, R.J. 1998. Effect of rate of establishment of CA conditions on the development of 'CO_2 injury' in Bramley's Seedling apples. Acta Hort. 464: 351-356.

Lau, O.L. 1998. Effect of growing season, harvest maturity, waxing, low O_2 and elevated CO_2 on flesh browning disorders in 'Braeburn' apples. Postharvest Biol. and Technol. 14: 131-141.

vine, R.L., Williams, J.A. Stadtman, E.R. and Shacter, E. 1994. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol. 233, Part C: 346-363.

Lurie, S., Klein, J. and Ben-Arie, R. 1989. Physiological changes in diphenylamine-related 'Granny Smith' apples. Israel J. Botany 38: 199-207.

Meir, S. and Bramlage, W.J. 1988. Antioxidant activity in 'Cortland' apple peel and susceptibility to superficial scald after storage. J. Amer. Soc. Hort. Sci. 113: 412-418.

Park, Y.M., Blanpied, G.D., Jozwiak, Z. and Liu, F.W. 1993. Postharvest studies of resistance to gas diffusion in McIntosh apples. Postharvest Biol. Technol. 2: 329-339.

Rajapakse, N.C., Banks, N.H., Hewett, E.W. and Cleland, D.J. 1990. Development of oxygen concentration gradients in flesh tissues of bulky plant organs. J. Amer. Soc. Hort. Sci. 115: 793-797.

Stadtman, E.R. 1993. Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalyzed reactions. Annu. Rev. Biochem. 62: 797-821.

SUMMARY

Scald is a pervasive physiological disorder of apples and pears induced by storage at refrigerated temperatures in air or long term CA. Scald has the potential to destroy the market value and utility of millions of tons of apples and pears annually unless the fruits are treated with a postharvest drench with diphenylamine (DPA) or ethoxyquin along with a fungicide. Numerous countries have banned the use of DPA and prohibit importation of fruits so treated.

Hypobaric storage at 0.05 atmosphere prevents scald of Granny Smith and Law Rome apples only if they are placed under hypobaric conditions within one month after harvest and held in air at 1°C; after 3 months delay, scald development is similar to that for fruits stored in air. MHO accumulated in direct proportion to the duration of the delay in placing fruits in to hypobaric storage. Only as little as 2 months of hypobaric storage largely prevents scald. This suggests that the effect of hypobaric storage is primarily manifested during the first few months of storage at low temperature by ameliorating the effect of a volatile substance produced by the fruits. Fruits stored hypobarically for 8 months plus 4 months in air did not develop scald, whereas, scald developed after an additional 6 months of storage in air. This results indicate that apples stored hypobarically do not scald during

storage but may do so after subsequent and extended periods of time in a static air atmosphere. This is consistent with the observation that fruits remain preclimacteric while in hypobaric storage. Fruits from scald non-susceptible cvs. stored hypobarically for 7 months also produced similar amounts of α-farnesene and MHO upon removal to 20°C for a few days. The levels of the natural antioxidant ascorbic acid increase as apple fruits mature whereas water-soluble antioxidants generally decrease during storage. It is conceivable that the levels of natural antioxidants may mitigate scald development. Ascorbic acid and other natural antioxidants may be protected from degradation when fruits are stored hypobarically.

Ethanol vapor treatment reduced scald incidence for all scald susceptible cvs. (Granny Smith, Red Delicious, Law Rome) examined in a dose-dependent manner; the most effective treatment was one week exposure to 6000 $\mu L \cdot L^{-1}$ ethanol vapor. Reducing the ethanol concentration and duration of exposure at a given concentration reduced the effectiveness for scald control. Ethanol supplied to fruits as a vapor is readily absorbed and metabolized by the tissue and affected fruit metabolism. The consequences are reduced rate of ethylene production, MHO accumulation and the production of low molecular weight volatile compounds including hexylacetate, hexylbutanoate/ butylhexanoate, hexyl-2-methylbutanoate and hexylhexanoate but not α-farnesene since the rate and pattern of α-farnesene production was similar for fruits treated or not treated with ETOH vapor. When the concentrations of ethanol vapor were up to 3750 or 8000 $\mu L \cdot L^{-1}$ and were applied to fruits continuously for 2 months, the fruits were injured as evidenced by darkening of the skin color and the fruits showed some fermentation damage. The rate of MHO production was much higher in control than for the ethanol vapor treatments indicating that α-farnesene is not as closely related to scald development as its oxidation product MHO. Or α-farnesene is not the limiting factor but rather its metabolism leading to MHO production and or metabolism may be.

Increasing the intensity of low O_2 stress progressively diminished the incidence and severity of scald of Granny Smith and Law Rome fruits subsequently stored in CA at 3% O_2 whereas fruits stored at 1.5% O_2 did not develop scald significantly. Fruits held under ultra low levels of O_2 resulted in ethanol production. The internal ethanol levels were different since apple cvs. vary in their response to low O_2 levels. Ethanol accumulation at 0.5% O_2 was the highest for Granny Smith > Red Delicious > Law Rome > Idared; at 0.25% O_2, Granny Smith and Law Rome apples accumulated more ethanol than others. This suggest that apples may become resistant to scald through producing ethanol. Or that the reduction of scald incidence by the initial low O_2 stress treatment may be a consequence of the ethanol produced and/or metabolism induced. It may reduce the concentration of some metabolites or ameliorate their action as related to scald development. To achieve the maximum resistance to scald each cv. of apples may require a specific and low O_2 level for a specific period of time. This remains to be elucidated.

Subjecting apple fruits to low O_2 levels which induce ethanol production for a period of several weeks at the beginning of the storage period was found to reduce the incidence of scald on fruits subsequently stored at higher O_2 levels. This suggests that some aspect of ethanol metabolism may reduce scald and this needs further study as an alternative strategy to control scald on apples.

α-Farnesene production of apples was inhibited by initial low O_2 stress treatments and initial O_2 stress at the 0.25% O_2 level and/or CA at the 1.5% O_2 level more strongly inhibits α-farnesene production than was observed for fruits without initial low O_2 stress and/or CA at the 3% O_2 level. Fruits receiving 0.25% O_2 initial stress treatments produced less MHO than those with 0.5% O_2 stress at all storage conditions for both Granny Smith and Law Rome cvs. after 5 months of storage. This data indicate that the 0.25% O_2 initial stress treatment followed by CA at 1.5% O_2 level has a stronger effect on inhibition of MHO production than does 0.5% initial O_2 stress and CA at 3% O_2 level. The strong relationship between the levels of O_2 favoring ethanol production and reduced levels of MHO suggest that ethanol metabolism may be directly involved in keeping MHO at low levels. Alternatively, MHO production may involve an oxidative enzyme with a fairly high affinity for molecular O_2. The mechanism for scald control by initial low O_2 stress remains to be resolved.

High MHO production/accumulation values were strongly associated with scald development. Capacity for MHO production begins within the first 2 months when fruits are stored in air. This supports a putative role of MHO in the scald disorder but when it is a factor in subsequent scald development must be early vs late during storage. This is because fruits stored hypobarically within 1 months of being harvested do not scald whereas those stored with longer delays do scald.

We conclude that initial low O_2 stress of ca. 0.25-0.5% O_2 for 14 days at 0°C followed by warming to 2°C for 3 days can effectively control scald of apples not treated with DPA when subsequently stored at 1.5% O_2 at 0°C employing a static CA regimen. Storage at 0.7% O_2 throughout CA storage completely controlled scald. However, some these fruits exhibited reversible evidence of anaerobic stress and prolonging storage beyond 8 months at 0.7% O_2 may have resulted in irreversible damage and flavor quality impairment. Since the initial low O_2 stress followed by CA at 1.5% O_2 controlled scald effectively and at commercially acceptable levels this CA storage regimen may provide a safe and effective means to store apples not treated with DPA.

Several different strategies may be useful to control scald. These may depend on cv. and the duration of the storage period. For fruits which are to be marketed after only 6 months in CA, using an air separator to maintain 1.5% O_2 with 3% CO_2 has provided satisfactory control of scald. Fruits for long-term CA for 7 or more months may require a more demanding strategy. This may include initial low O_2 stress at 0.25-0.5% O_2 levels for two weeks prior to CA at 1.5% O_2 and 3% CO_2. CA at 1 to 1.5% O_2 using an air separator to control CO_2 at 1 to 2% may provide adequate scald control for mature fruits not yet producing significant levels of ethylene. The commercial scale trial at the MSU CHES CA Facility of initial low O_2 stress demonstrated excellent control of scald by employing this treatment regimen.

Establishing CA conditions of 5% CO_2 + 1.5% O_2 at 3°C within 1 day of harvest resulted in the highest incidence and severity of the Empire disorder of the treatments tested followed by 3% CO_2 + 1.5% O_2. The incidence of the disorder was decreased when the level of O_2 was increased from 1.5% O_2 to 3% O_2. The incidence of the disorder did not increase after removal from storage at 3°C to 20°C for 7 days. Holding the fruits in air for 1 week prior to administering 3 or 5% CO_2 with 1.5% O_2 during storage was insufficient to control the disorder whereas the fruits tolerated 3 or 5% CO_2 after holding the fruits in air for 2 weeks. But the 2 week delay to CA resulted in accelerating flesh softening. This suggests a requirement of oxidative metabolism in acclimatization for CO_2 tolerance.

The results of the current studies confirmed that: (1) the Empire scald-like disorder is caused by high CO_2 levels; (2) this is exacerbated at O_2 levels below 1.5%; (3) the disorder is more prevalent with immature fruits;(4) this affects primarily the non-red fruit surface; and (5) DPA at 1000 $mg \cdot L^{-1}$ controls the Empire disorder. Our results also indicate that Empire apples can be acclimatized during the first few weeks of storage at 3°C to attenuate the incidence and severity of the 'scald-like' disorder to avoid using DPA. Acclimatization at 3°C at 1.5 or 3% O_2 without CO_2 for up to 4 weeks and then raising the CO_2 to 3 or 5% to gain the ripening retardation effects of CO_2 largely eliminates the Empire 'scald-like' disorder while providing good control of ripening. The susceptibility of fruits to CO_2-linked disorders may be related to differences in resistance to gas diffusion among cvs. This storage regimen fits well with current CA technology employing the use of air separators for establishing and maintaining O_2 and CO_2 levels during CA storage.