RELATIONSHIPS BETWEEN PREHARVEST SOYBEAN OIL APPLICATION AND POSTHARVEST BEHAVIOUR OF APPLES

By

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A dissertation submitted in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

WASHINGTON STATE UNIVERSITY
Department of Horticulture and Landscape Architecture

May 2005

To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of INES MÜLLER find it satisfactory and recommend that it be accepted.

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ACKNOWLEDGMENTS

First, I thank Mark Hanrahan, who never stopped reminding me that it is possible. He recommended cooperators, organized and repaired equipment, collected samples, stored and hauled fruit. Thanks for the incredible patience and most importantly, for unconditional love.

Secondly, thanks to my committee members John Fellman, Jim Mattheis, Kathleen Willemsen, and Mechthild Tegeder for their sound advice. John, I am grateful you had the courage to put up with me, regardless. Jim, your prompt and concise advice was always greatly appreciated. Thanks to Kathleen for encouraging me and for editing my papers. Mechthild, thanks for being my German connection, it was much needed.

Over the years I had tremendous support from a multitude of people. Thanks to everybody in the postharvest lab. Scott Mattinson provided technical support and endured my seemingly never-ending generation of data. Margo Haines made me laugh and cheered me on. Thanks for always lending a hand to Greg Hoffman, Guy Lévesque and Sam Fuchs. I am also thankful for the lab assistance from Crystal Nelson, Travis Belisle, Naomi Keilwitz, Mary Fellman, Kate Watson, Tristan Boyce, Holly Robinette, and Marie Railing.

My dear friend and accomplished scientist Claudia Stoefer was brave enough to visit two years in a row to assist me during harvest, even if it meant illuminating trees with the help of car headlights in order to spray them after sunset or spending countless hours chopping apple pieces and calling it biochemistry. Harvest would not have been possible without the ability to use the home of Peter and Patricia Hanrahan as base camp. The taco dinners will be remembered forever. Special thanks to my fellow graduate student and friend Gregory Peck. I learned a lot from you about professional conduct and integrity.

Valerie Lynch-Holm and Nathan Tarlyn went out of their way to help process all my samples for electron microscopy, no matter what. Chris Skidmore always found a time slot to squease in my samples for fatty acid analysis. Statistical advice was provided by Mark Evans. Molly Stock, you are my inspiration.

Thanks to everybody who provided orchard space for my field experiments: Auvil Fruit Company, Green Acres Farms, Hanrahan Orchards and to Wayne Shull and Deb Pehrson who guarded my fruit from beeing picked. I am grateful to Dana Faubion and Ron Britt for letting me use their sprayers. The Washington State Tree Fruit Research Commission provided financial support for this research and for my graduate education.

RELATIONSHIPS BETWEEN PREHARVEST SOYBEAN OIL APPLICATION AND POSTHARVEST BEHAVIOUR OF APPLES

Abstract

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May 2005

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The present study characterized the effects of growing-season applied soybean oil emulsions on at-harvest and postharvest behaviour of 'Golden Delicious' and 'Gala' apples. Three single treatments (midseason = soy1, 21 days before harvest = soy2, three days before harvest = soy3) of soybean oil emulsion (1% food grade oil, emuslified with 0.1% Latron®; v/v) were administered to apple trees grown in two different locations within Washington State, USA, to test the following variables: time of application, duration of storage, and influence of storage atmospheres. Fruit measurements included maturity indices (firmness, acidity, soluble solids, starch conversion), respiration rate, ethylene evolution, internal ethylene concentration, volatile aroma emission, flavour regeneration capacity, peel fatty acid distribution, fruit colour development, weight loss in storage, development of cuticular cracks and epicuticular wax crystallization patterns. The fruit was harvested at commercial maturity and stored for up to 6 months at 0.5°C in refrigerated air (RA) or under 2% O_2 and 0.2% CO_2 controlled atmosphere (CA) conditions.

Fruit firmness, titratable acidity, soluble solids content, and fatty acid distribution in the peel tissue were unaffected by the soybean oil treatment. At harvest and after storage 'Golden Delicious' apples treated with soy2 emitted more aldehydes (mainly hexanal), and oil

applications closer to harvest (soy2, soy3) consistently yielded fruit with improved ester regeneration capacity after CA storage. 'Gala' apples treated with soy1 had significantly higher alcohol and ester levels when compared to control fruit. Delayed degreening was observed on 'Golden Delicious' apples after soy1 and soy2 treatment in 2003, but no treatment effect was noted in 2004. As observed by scanning electron microscopy (SEM), oil application decreased the occurrence and severity of cuticular cracks in susceptible varieties such as 'Golden Delicious'. The rate of weight loss during storage was slowed down for all soybean oil treated apples and was directly related to the development of cracks. All apples showed altered wax crystallization patterns after soybean oil application.

In conclusion, field-applied soybean oil emulsions have demonstrated potential to improve postharvest quality of apples by stimulating volatile aroma emission of fruit, delaying weight loss in storage and the improvement of cuticular structures.

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For grandma.

CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

Washington is the leading apple (*Malus domestica* Borkh.) producing state in the USA with over two million tonnes of apples harvested from nearly 66,000 hectares in 2003 (Washington State Department of Agriculture, 2004). Increased consumer demand for products grown without the use of synthetic chemicals coupled with increased production from sustainable farming enterprises, and tighter regulations around agrochemicals due to environmental concerns are compelling reasons to search for alternative crop protection agents. Additionally, consumers are increasingly demanding that stored apples more closely match the appearance, taste, and texture of freshly harvested fruit. This represents a particular challenge, since most of the apples produced in Washington State have to be transported long distances to principal markets within North America or elsewhere in the world. To maintain apple fruit quality, controlled atmosphere (CA) storage is used extensively. However, both long-term air and CA storage have also been associated with the loss of acceptable apple flavour and aroma (Bangerth and Streif, 1987; Fellman et al., 2003).

During the growing season horticultural oils (also called petroleum, mineral, white, or narrow range spray oils) function as insecticides, when applied to apples (Cranshaw and Baxendale, 2004). Oil washes have been used for over a century to suppress insect and mite pests in orchards (Willett and Westigard, 1988) and this method

of controlling mites is becoming increasingly important as mites are rapidly developing resistance to chemical acaricides (Cremlyn, 1990). Oils are unique because no target pest species has developed resistance to them. This is mainly due to the fact that oils kill by suffocating the insect or simply act as a repellant. Oils can be further used as fungicides and spray adjuvants (Cranshaw and Baxendale, 2004) and can also be mixed with other insecticides or fungicides to provide a broader spectrum and greater persistence of control. For example, Davis et al. (1992) found hydrolysed cellulose together with a plant-oil based spreader-sticker enhanced the foliar tenacity and control of fungal apple diseases by the antagonist *Chaetomium globosum*.

Traditionally, the apple industry in Washington State has relied on petroleum-based horticultural oils. However, using a plant-based oil would have the added advantage to contribute to more sustainable farming practices, since it is derived from renewable resources. Moreover, plant oils are regarded safe for human consumption as food and thus will not adversely affect consumer preferences. In addition, plant based oils improve fruit quality.

Recently, new methods have allowed researchers to examine biosynthetic pathways and control mechanisms in the synthesis and subsequent accumulation and release of volatiles from apples in more detail (Dixon and Hewett, 2000b). This is resulting in a better understanding on how biochemical and environmental factors influence aroma and flavour of apple fruit (Yahia, 1994). Increased interest in non-chemical pre-treatments to preserve or improve apple fruit quality, and as disinfestation treatments, has highlighted deficiencies in the knowledge of factors that affect postharvest apple flavour development (Dixon and Hewett, 2000b).

IMPORTANT QUALITY PARAMETERS AND PHYSIOLOGICAL INDICATORS DURING THE MATURATION OF APPLES

Ripening of apple fruit involves many physiological and biochemical changes. The most important of these, from an applied perspective, are softening, the change of background colour from green to yellow, loss of acidity, conversion of starch to sugars, formation of cuticular waxes and synthesis of aromatic compounds. The metabolism involved in these changes has been described by Knee (1993). In the natural world, these changes result in a desirable product for seed dispersal by the activity of animals and/or breakdown and decay of the fruit. Many of these changes are at least partly desirable for human consumption. The goal of the apple industry is to harvest fruit at the appropriate maturity and apply postharvest technologies to control the rates of these changes in order to provide the consumer with an acceptable product.

The harvest date within the maturation and ripening period has a profound effect on the storage quality of fruit. As quality factors such as flavour and aroma of the fruit increase, the storage potential of the fruit decreases. More precisely, the length of storage of apples can usually be increased by harvesting fruit before they fully mature, but quality characteristics such as colour and varietal flavour develop less in these fruit. Additionally, early harvested fruit can be more susceptible to physiological disorders like bitter pit and superficial scald. Fruit harvested over-mature tend to be softer and more easily damaged and may have water core. Further on they might be more susceptible to fungal diseases and physiological disorders, such as senescent breakdown. Therefore,

harvest decisions are a compromise between the quality and the storabilty of fruit (Watkins, 2003).

Respiration rate. Apples are considered climacteric fruit (Kidd and West, 1945). When they reach physiological maturity and ripening processes are initiated, there is a marked increase in respiratory activity resulting in the increased evolution of carbon dioxide. Respiration is a major metabolic process of any living plant product. During respiration more complex organic material such as starch, sugars and organic acids are oxidatively broken down into simple molecules, such as carbon dioxide and water. Concurrently, energy is produced. The respiration rate of produce is a useful guide to the potential storage life of produce, because it is an indicator of metabolic activity (Wills et al., 1989). The respiration rate of a fruit is measured as either oxygen consumed or carbon dioxide produced. At harvest respiration rate and ethylene evolution within one apple cultivar can be influenced by factors such as growing region, cultivar strain, growing-season conditions and maturity (Blanpied and Silsby, 1992).

Ethylene evolution. During the climacteric, ethylene production follows a similar pattern to carbon dioxide evolution. Ethylene concentration of produce may increase between 10- and 1000- fold during the climacteric period (Reid et al., 1973). In contrast, ethylene production may not be relevant for determining the harvest of some cultivars, such as 'Golden Delicious', because it does not increase during the harvest period (Watkins et al., 1989). As summarized by Watkins (2003), ethylene advances the timing of the climacteric, autocatalytic production continues after removal of ethylene and, in contrast to non-climacteric fruit, the magnitude of the respiratory rise is independent of

the concentration of the applied ethylene. Thus, timing of the climacteric and ripening of apple fruit is advanced by exposure to ethylene.

Internal ethylene concentration (IEC) might be a better indicator for measuring the onset of ripening processes, since ethylene evolution starts in the core region of the fruit (Rudell et al., 2000). Ethylene concentration can be measured in several ways. The most common methods are extracting a gas sample from the internal core space of fruit, or extracting it from a sealed container in which fruit have been kept for a length of time and analyzing it using gas chromatography. Apples grown in Washington state are considered ready for commercial harvest when internal ethylene emission reaches one μl l^{-1} (Fellman et al., 2003).

Fruit firmness. As pome fruits, such as apples, ripen, the cementing material between the cells, the middle lamella, dissolves. This softening can be measured using a penetrometer, which records the resistance of peeled fruit flesh to the insertion of a plunger of known diameter (Kingston, 1992). Decreasing firmness as maturity approaches is well-documented in apples (Harker et al., 1997). However, firmness is being used as an internal quality criterion by fruit storage facilities and wholesalers rather than as a maturity index.

Soluble solids. As apples mature, starches are converted to sugars. This increase in sugars renders the fruit much sweeter, and therefore more acceptable to consumers (Kingston, 1992). Sugar content can be measured directly by chemical means. However, as sugar is the major component of soluble solids it is easier to measure soluble solids in extracted juice using a refractometer (Wills et al., 1989). Soluble solids concentration tends to increase as apples ripen (Blankenship and Unrath, 1988). As with firmness,

soluble-solids concentrations are increasingly being used as a quality criterion by wholesalers.

Titratable acidity (TA). Malic acid is the predominant acid contributing to the TA of apples. During the greater metabolic activity that occurs during ripening organic acids decline since they are respired. Acids are generally considered a reserve source of energy to the fruit (Wills et al., 1989). Consumer acceptability of apples in European countries is closely correlated to acid content (Blanpied and Blak, 1977). TA at harvest cannot be used to predict TA after storage (Kingston, 1992).

Fruit colour. Colour is the most obvious change that occurs during ripening in many fruit, including most apple cultivars. Most consumers use colour change as the major criterion to determine the ripeness of fruit.

The colour of apple fruit is determined primarily by the relative amounts of pigments in the fruit skin, namely anthocyanins, chlorophylls, and carotenoids (Saure, 1990). Fruit pigments undergo considerable changes during fruit development. As chlorophyll is degraded or disappears in fruit that turn yellow or red as they mature, chloroplasts become chromoplasts. For 'Golden Delicious' apples, the change in skin colour from green to yellow is primarily associated with a decline in chlorophyll concentration and an increase in xanthophylls (Workman, 1963). In fruit that remains green when mature, chlorophyll persists, although its concentration decreases with maturity.

Prior to harvest, the rate of chlorophyll degradation can be hastened by treatment with ethylene (Purvis and Barmore, 1981) or ethylene generating compounds as well as fungicide applications. Ethylene application is used commercially to promote degreening

of bananas (*Musa* sp.) and tomatoes (*Lycopersicon esculentum* L.) before sale in retail markets. Chlorophyll breakdown however, can also be influenced in an ethylene independent manner. Fan et al. (1998) and Fan and Mattheis (1999) have suggested this possibility in conclusion of their work on the involvement of methyl jasmonate in fruit ripening. In their experiments they noticed that 'Fuji' and 'Golden Delicious' apples degreened faster after treatment with methyl jasmonate without an increase in ethylene emission. Additionally, 'Golden Delicious' apples treated with aminoethoxyvinylglycine (AVG), an inhibitor of autocatalytic ethylene production, showed a non-appreciable effect on the chlorophyll degradation of epidermal tissue during cold storage (Halder-Doll and Bangerth, 1987). The existence of distinct subsets within the overall ripening program, as proposed by Srivastava (2002), might explain these seemingly contradictory results. As shown on tomato mutants such as *yellowflesh*, *greenflesh*, and *tangerine*, while the overall regulation of ripening might be regulated by ethylene, subsets like chlorophyll degradation or carotenoid synthesis may be controlled independently.

Furthermore, the amount of nitrogen within the fruit is negatively correlated with chlorophyll degradation (Fallahi et al., 1985; Reay et al., 1998). The rate of citrus fruit degreening was slowed down if fruit was harvested 7 or 14 days after application of 2% of petroleum-based horticultural oil (Lee et al., 1994). Degreening took 72 hours when fruit was harvested 14 days after spraying. De Lee et al. (1994) attributed this delayed effect to a requirement of the oil to penetrate into the fruit over time.

The chloroplast to chromoplast conversion in broccoli (*Brassica oleracea* L. var. Italica) floret sepals was inhibited by ethanol vapour (Suzuki et al., 2005).

Weight loss. Fresh fruits commonly release water vapour into the surrounding atmosphere by transpiration. Transpiration by fruit involves the diffusion of water vapour from the fruit into the surrounding environment. Fruits also exchange CO_2 for oxygen as a result of respiration. The weight loss of fruit is principally due to the loss of water in transpiration and to a lesser extent to the loss of carbon in the respiration process. Total weight loss from fruit is most often expressed as a percentage of the original weight.

Excessive weight loss in fruit can result in a shriveled appearance caused by decreased turgidity and can render it unsaleable. Only a 5% loss of apple weight may cause a shriveled appearance (Hatfield and Knee, 1988). Wilcke (1992) described weight loss being cultivar dependent with 'Golden Delicious', one of the more susceptible varieties, losing 5% on a fresh weight basis after 100 days of RA storage. He attributed the high loss in cold storage to ventilation. In CA, the loss of weight was lower, about 2% in 100 days. Additionally, losses can vary between years. Carnauba or shellac based wax coatings, commonly applied to apples to improve appearance, have also been linked to extended shelf-life by reducing water loss, respiration rates and fruit ripening (Saftner, 1999; Bai et al., 2002).

Fruit that demonstrate high water vapour permeance lose water and shrivel faster (Maguire et al., 2001). Maguire et al. (1998) established a link between cuticular micro-cracking and fruit water vapour permeance. As cracking seems to be caused by stress in the fruit skin (Meyer, 1944), it seems likely that cuticular elasticity would affect incidence and severity of cuticular cracking. Both of these may depend upon production practices such as irrigation management and timing of harvest. The influences of

production practices on fruit permeance are largely unexplored at this point. However, cracks in the cuticle should be minimized to enhance the barrier properties of the cuticle.

Volatile aroma synthesis. Over 300 volatile compounds have been measured in the aroma profile of apples. These compounds include alcohols, aldehydes, carboxylic esters, ketones, and ethers (Dimick and Hoskin, 1983). Most volatiles are thought to be synthesized in the peel and outer flesh of the apple (Guadagni et al., 1971; Bundschuh, 1987) and enzyme activities related to the volatile synthesis are highest in the peel also (Feys et al., 1980). Although in some cases the typical fruit aroma can be ascribed to a specific compound, in general the overall aroma quality is the sum of a multitude of components. About 20 of these chemicals are 'character impact' compounds, contributing to the typical apple aroma, aroma intensity, or are related to aroma quality (Dixon and Hewett, 2000b). The most abundant esters contain even numbered carbon chains and include combinations of acetic, butanoic, and hexanoic acids with ethyl, butyl, and hexyl alcohols (Paillard, 1979). Most aroma compounds, in variable portions, are present in volatile emissions from most apple cultivars and there appear to be no key characteristic compounds for any given cultivar (Yahia, 1994).

Biogenesis of volatiles. As volatiles are comprised of at least five chemical classes, there are several pathways involved in volatile synthesis. Although these pathways have not been fully described yet, it seems clear that most volatiles are derived from the enzymatically controlled amino acid, lipid, and carbohydrate metabolism (Schreier, 1984; Yahia, 1994). Generally, aroma volatiles in intact fruit are formed via the β-oxidation catabolic pathway, whereas when fruit tissue is disrupted, volatiles are

formed via the lipoxygenase pathway both of which use fatty acids as substrates (Schreier, 1984).

Fatty acids. The main precursors of straight chain ester-, alcohol-, and aldehyde-volatiles produced by apple fruit during development and maturation are free fatty acids (FA) or those liberated by lipase activity and further metabolized by beta-oxidative enzymes and/or lipoxygenase (Schreier, 1984). Numerous volatile aroma compounds are derived from fatty acids (Bundschuh, 1987), most notably the unsaturated linoleic (C18:2) and linolenic (C18:3) acids, which comprise precursors for the synthesis of volatile aroma compounds. Supplying additional FA to fruit leads to incorporation into volatile aroma components, as has been shown by several authors (Bartley et al., 1985; Brackmann et al., 1993; Harb et al., 1994). Song and Bangerth (2003) described a rapid increase of linoleic (C18:2) and oleic (C18:1) acid during the climacteric rise of 'Golden Delicious' apples or after ethylene treatment of pre-climacteric fruit. They also described a strong positive correlation between IEC and these fatty acids. Polar lipids make up the largest portion of membrane lipids, and little change in FA concentrations during fruit development occurs in this fraction, whereas changes in the concentration of the free fatty acid fraction are highly dynamic. However, the free fatty acid pool in the epidermis of the apple fruit has the lowest share of the total fatty acid pool. Overall, lower storage temperatures retard fatty acid degradation and therefore influence lipid metabolism. Brackmann et al. (1993) observed an increased synthesis of unsaturated fatty acids in membranes of 'Golden Delicious' apples during long-term storage at low temperatures. Alternately, under low oxygen conditions, such as commonly observed during CA storage, Mazliak (1970) reported a significant decrease in unsaturated fatty acid

synthesis. This causes an increase in membrane fluidity and consequently accelerates senescence, as observed by the premature breakdown of fruit tissue (Mazliak, 1983; Kimura et al., 1986).

β-oxidation. β-oxidation of fatty acids is the primary pathway providing alcohols and acyl-CoA for ester formation (Paillard, 1979; Bartley et al., 1985). Substrate feeding experiments with 'Golden Delicious' apples using C1-C6 aldehydes, or C2-C6 carboxylic acid vapours induced increases in esters typical of those expected for β-oxidation of the added compounds (De Pooter et al., 1987). Perdeuterated linoleic acid fed to 'Delicious' apples produced only C6 metabolites, implying that saturated ester volatiles arise via β-oxidation, rather than peroxidation of fatty acid precursors (Rowan et al., 1996). Fatty acid acyl-CoA derivatives are converted to shorter chain acyl-CoAs by losing two carbons in every round of the β-oxidation cycle, requiring flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD), and free CoA. Acyl-CoAs are reduced by acyl-CoA reductase to aldehydes that in turn are reduced by alcohol dehydrogenase (ADH) to alcohols for the use of alcohol acyl-CoA transferase (AAT) to produce esters (Bartley et al., 1985). Bartley et al. (1985) proposed varietal differences in volatile composition of apples to depend on the specific activities of β-oxidation enzymes. While AAT has an ethylene dependent pattern of regulation, ADH seems to act independently of endogenous ethylene levels (Defilippi et al., 2005).

Lipoxygenase (LOX). When apple fruit tissue is disrupted, volatiles are formed via the LOX pathway (Schreier, 1984). Lipoxygenases are nonheme iron containing dioxygenases widely distributed in plants and animals. The enzyme LOX catalyses the hydroperoxidation of polyunsaturated fatty acids, the preferred substrates in plants being

linoleic (C18:2) and linolenic (C18:3) acids (Porta and Rocha-Sosa, 2002). Only short exposure times to air (10-30 min) after crushing are necessary to induce the formation of aldehydes like hexanal and 2-hexenal via LOX, especially when substates are available (Drawert et al., 1973; Feys et al., 1980). These aldehydes are also the dominant detectable volatile compounds in intact immature fruit contributing to 'green' odour notes and are known to decrease in concentration as fruit mature (De Pooter et al., 1987; Mattheis et al., 1991). Furthermore, aldehydes can act as antifungal agents. For example, hexanal is known to inhibit the hyphal growth of *Penicillium expansum, Alternaria alternata* and *Botrytis cinerea* on various fruit species (Hamilton-Kemp, 1996; Song and Bangerth, 1996). Aldehydes are also important for the formation of C_6 alcohols and C_6 esters, which are among the most abundant volatile components in ripe apples. Supplying aldehydes can increase the overall aroma production of apples by stimulating alcohol and ester synthesis (Song and Bangerth, 1994).

In intact fruit, enzymes and their substrates in the LOX pathway have different subcellular locations, preventing the formation of volatile compounds (Sanz et al., 1997). During ripening, cell walls and membranes may become more permeable, allowing the LOX pathway to become more active without tissue disruption (Sanz et al., 1997). There is still controversy in the research community regarding the actual involvement of the LOX pathway in volatile aroma emission during apple ripening. On the one hand, LOX activity increased during ripening of apples and/or was greatest during the climacteric peak, coinciding with peak volatile production (Kim and Grosch, 1979; Feys et al., 1980; Echeverria et al., 2004). Hence, LOX could have the potential to provide substrates for ester production by acting as an alternative to β-oxidation of fatty acids (De Pooter et al.,

1983; Dixon and Hewett, 2000b; Echeverria et al., 2004). Contrary, LOX activity increases slower when compared to volatile aroma production according to Song and Bangerth (2003). This would suggest the fatty acids for the volatile aroma synthesis are derived by means other than the LOX pathway. Song and Bangerth (2003) hypothesize, the FA could be derived from *de novo* biosynthesis. Another reason for these varying results could be the existence of more than one LOX in apples. In tomato three different LOX mRNAs, corresponding to the nuclear genes encoding TomloxA, TomloxB, and TomloxC, are active during fruit ripening. These genes are differentially regulated during fruit ripening and their expression is affected by ethylene and unknown developmental factors (Griffiths et al., 1999). Griffiths et al. (1999) further suggested, in addition to the involvement in the flavour synthesis, LOXs have a possible plant defense function and might be involved in the degradation of the thylakoid membranes during the transition from chloroplast to chromoplast.

Amino acids. Although information on the biogenesis of plant volatiles from amino acids is rather scarce, work done with banana and tomato fruit demonstrated the importance of amino acids as precursors of plant volatiles (Schreier, 1984). Branched-chain alcohols and esters are derived from the amino acid metabolism (Schreier, 1984; Wyllie and Fellman, 2000). The conversion into branched-chain alcohols proceeds through the production of a α-keto acid decarboxylase and alcohol dehydrogenase (Echeverria et al., 2004). Isoleucine is considered to be the biosynthetic precursor of 2-methyl butanoic acid and its esters in apples (Paillard, 1990). Deuterated isoleucine was metabolized by ‘Delicious’ apples to 2-methyl butanol and to 2-methyl butyl or 2-methyl butenyl esters, whereas ‘Granny Smith’ apples produced ethyl-2-methyl butanoate almost

exclusively (Rowan et al., 1996). Rowan et al. (1996) suggested that different enzyme activity and selectivity, rather than substrate availability of the amino acid degradation pathway determines the concentration of branched chain esters and is cultivar specific in apples. Ackermann et al. (1992) showed that, although concentrations of amino acids decreased with increased ripening of 'Glockenapfel' apples, they were generally stable in storage. The supply of precursors arising from amino acid metabolism is apparently less variable than that derived from fatty acids via β-oxidation. If amino acid concentrations determine the type of volatile compounds produced by apples during ripening remains a topic for further research.

Esters. Esters are the most significant contributors to aroma in apples, accounting for up to 80% of odour active volatiles in 'Golden Delicious' (López et al., 1998). Among the most prevalent esters described for 'Golden Delicious' are butyl acetate, 2-methyl butyl acetate and hexyl acetate (Brackmann et al., 1993). Ester production in fruit tissue is the result of esterification of alcohols, carboxylic acids, and acyl-CoAs in an oxygen dependent reaction (Knee and Hatfield, 1981; Bartley et al., 1985). Alcohol acyltransferase (AAT) enzymes catalyze the last step in the ester formation (Bartley et al., 1985) and combinations between different alcohols and acyl-CoAs will result in the formation of a range of esters in different fruit species (Beekwilder et al., 2004). Since the most likely precursors for esters are lipids and amino acids, their metabolism during ripening will play an important role in determining both the levels and types of esters formed (Beekwilder et al., 2004). Alcohols serve as direct precursors of esters in apples (Dimick and Hoskin, 1983). Thus, any reduction in concentration of alcohols will adversely affect the most significant group of flavour molecules. Increased acetate ester

production is directly related to the availability of related alcohol precursors (Echeverria et al., 2004). However, the substrate specificity of AAT differs from fruit to fruit and esterification of straight-chain alcohols is preferred over branched-chain alcohols (Rowan et al., 1996). Such differences in preference for acyl-CoAs and alcohols may determine variety specific concentration of esters in apple aroma profiles. On the other hand, changes in alcohol concentration may result from increased esterase activity during the climacteric (Goodenough, 1983). Esterase functions by converting esters back to alcohols and carboxylic acids. Thus ester and alcohol levels in apple tissue may result from an equilibrium between synthesis, hydrolysis, and diffusion from tissue (Knee and Hatfield, 1981).

Factors influencing volatile aroma synthesis. The synthesis of aroma volatile compounds is associated with fruit ripening and therefore will depend on the developmental stage of the fruit at harvest (Fellman et al., 2003). An early harvest may have some advantages with regard to transportation and shelf-life, but has a strong negative influence on aroma volatile production (Mattheis et al., 1991; Song and Bangerth, 1996). Various storage procedures, particularily those reducing/eliminating ethylene biosynthesis and/or action, or reducing oxygen availability in the storage atmosphere have a negative impact on the production of aroma volatiles of apples (Halder-Doll and Bangerth, 1987; Bangerth and Streif, 1987; Mattheis et al., 1998; Fellman et al., 2003; Defilippi et al., 2005).

The limiting factor in aroma production is thought to be the substrate availability rather than the enzyme activity (Bartley et al., 1985; Bangerth et al., 1998). For example, it is generally accepted that the enzymes required to catabolize FA (LOX, and/or beta

oxidative enzymes) as well as downstream enzymes are not the limiting factors in aroma production. Song and Bangerth (2003) concluded: "…it seems that *de novo* biosynthesis of FA rather than their release from membranes or storage pools represents the limiting step in the volatile aroma production of apple fruit".

PREVIOUS STUDIES RELATING PLANT OIL USE AND FRUIT QUALITY PARAMETERS

Horticultural oils have been used extensively as insecticides and acaricides during the dormant season on fruit trees. Traditionally, their use during the growing season has been somewhat curbed by the fear of causing phytotoxicity. Though used extensively as oil paper wrap in the earlier parts of the 20th century, postharvest oil application has only recently received renewed interest by the research community. There are a growing number of publications about the replacement of the traditionally petroleum-based oils with plant-based oils.

Dormancy. Vegetable oils derived from storage seeds have been previously reported to delay bloom in tree fruit (Myers et al., 1996) and grapevines (*Vitis vinifera* L.) (Dami and Beam, 2004). The application of 10% crude or degummed soybean oil delayed bloom of peach trees (*Prunus persica* L.) for up to six days. Repeated sprays of oils with 5% concentration delayed bloom an additional four days (Myers et al., 1996). When used on dormant grapevines, the effectiveness of the oils depended on application timing and oil concentration. Optimum rates were between 8 and 10%. These rates were effective in delaying budbreak but not fruit ripening and did not affect fruit set, yield components, or fruit composition (Dami and Beam, 2004). However, the success of the

treatment also depended on the oil itself. From the two commercially available soybean oil emulsions (Prime Oil®, Amigo®) tested, only Amigo® did not have adverse effects.

In another study by Pless et al. (1995) 5% soybean oil emulsions effectively killed European Red mites and San Jose scale on apples and terrapin scale on peaches. The authors concluded that soybean oil showed promise as a substitute for petroleum oil during winter control of these very destructive fruit tree pests.

Growing season. Although plant derived oils, like soybean, corn and canola oil have been used sucessfully as an insecticide (Liu and Stansly, 1995; Csizinszky et al., 1997), a fungicide (Young, 1994; Finger et al., 2002), and as a spray adjuvant (Davis et al., 1992; Schmitz-Eiberger et al., 2002) within the growing season on various crops, the effect of such applications on overall fruit quality remains largely elusive.

One of the few studies directly relating the use of horticultural oils (including plant-based oils) to fruit quality has been published by Finger et al. (2002). The authors related the effects of horticultural oils to photosynthesis, fruit maturity, and crop yield of wine grapes. Sugar accumulation is an important quality parameter for wine grapes, yet reduced photosynthetic rate caused by reduced vegetative growth, as often observed after multiple oil applications, impairs appropriate sugar accumulation. Horticultural oils were applied three times as a 1.5% emulsion (v/v). Photosynthetic rate, transpiration, and stomatal conductance were reduced, with high volumes of oil causing greater decreases. Furthermore, high volume oil application delayed soluble solid accumulation and reduced fruit crop yield.

Plant based oils have also shown to be an effective bloom thinner for various fruit crops including apples, cherries and peaches (Myers et al., 1996; Ju et al., 2001).

Thinning success on cherries (*Prunus avium* L.), peaches and apples in China was both concentration and application time dependent. When applied at the same time, the higher concentration was more effective (1%, 3%). At the same concentration (3%), oil emulsion was more effective when applied earlier in the bloom stage (bud break or 20% full bloom) (Ju et al., 2001). Although oil emulsion sprays at 3 and 5% decreased the overall yield of cherries, cherry fruit size and soluble solids were increased. No foliage damage was observed and bee activity was not affected. Oil treatment improved return bloom in apples, but did not affect peaches and cherries (Ju et al., 2001).

Plant oil emulsion applied to growing cherries significantly reduced cracking, while firmness and fruit finish were not affected (Granger and Träger, 2002). When a 0.3% vegetable oil emulsion (Synertrol®) was applied to cherry trees, during the three weeks preceding harvest, before rainfall, reduced cracking was observed. While fruit firmness, fruit finish and soluble solids were not affected, fruit size was increased, especially during dry seasons.

The biological efficacy and rainfastness of foliar applied calcium chloride solutions was enhanced on 'Braeburn' fruit surfaces by addition of rapeseed oil surfactants (Schmitz-Eiberger et al., 2002). Hence, the calcium content of the fruit could be increased and a reduced incidence of bitter pit was observed.

Biological control agents are increasingly used in organic and intergrated pest management systems to suppress pests in apple orchards. A problem commonly encountered under field conditions is the question on how to provide nutrients for the foliar applied antagonists that selectively enhance the antagonist's survival and growth, thereby achieving biological disease control. In 1992, Davis et al., utilized a formulation

composed of colloidal cellulose and a vegetable oil-based spreader sticker (Soy-Dex) that exhibited long-term foliar tenacity. This cellulose suspension plus *Chaetomium globosum* ascospores suppressed flyspeck and sooty blotch on apple trees as effectively as the fungicide control.

Postharvest. Most studies relating fruit quality and oil applications have been done after the plant material had been harvested. The predominant reason for the postharvest use of oils has been to prevent the occurrence of physiological disorders.

As a postharvest treatment vegetable oils reduced superficial scald (Ju and Curry, 2000a+b; Ju et al., 2000b; Scott et al., 1995). An Australian study (Scott et al., 1995) reported that wiping 'Granny Smith' apples with either commercial vegetable oils (canola, castor, palm, peanut, sunflower) or a petroleum-based mineral oil was effective in reducing scald after four months of regular storage. Since all oils were effective, the authors concluded that a physical effect may be responsible for the control of superficial scald by oils. Ju and Curry (2000b) found a 5 or 10% oil emulsion to be as effective as DPA (Diphenylamine) in controlling scald in fruit stored for six months. However, no treatment was as effective as DPA during storage periods beyond the six months. Wills et al. (1977) described the potential of using a methylated fatty acid (methyl linoleate) to reduce soft scald in 'Jonathan' apples. In d'Anjou pears, corn oil emulsions (5 or 10%) reduced or completely inhibited decay after eight months in storage (Ju and Curry, 2000b). In the same study an elimination of core flush caused by chilling injury in 'Granny Smith' apples was reported. Consequently the authors concluded that oil treatment may have great potential in reducing chilling injury related disorders.

Generally, no adverse effects on fruit quality have been reported if horticultural oils are applied during the postharvest period. Ju et al. (2000b) concluded that plant oil applications maintained apple fruit quality. Some of the studies found improvements in certain quality traits, mainly when oils were applied at rates between 5 and 10%. For example, Ju and Curry (2000a) described a 2.5% stripped corn oil emulsion to have no effect on ethylene emission rates in 'Granny Smith' apples, while treatment with higher oil concentrations (5 or 10%) supressed ethylene emission for the first three months of storage and later exceeded control levels. After storage, oil treated fruit was greener, firmer and contained higher levels of titratable acidity mainly due to partially inhibited ethylene production (Ju and Curry, 2000a+b; Ju et al., 2000b). Treatment of 'Golden Supreme' apples with 10% corn oil emulsion reduced production of ethylene, α-farnesene, and major volatile esters in the first three months of RA storage, but this trend was reversed after five months. The response to the oil treatment was more pronounced if applied to preclimacteric fruit (Ju et al., 2000b).

SYSTEMS CHOSEN FOR THE STUDY

Horticultural oils to be applied to apples. Traditionally, the apple industry in Washington State has relied on petroleum-based horticultural oils.

However, plant derived oils, like soybean, corn and canola oil have been used sucessfully as insecticides (Csizinszky et al., 1997; Liu and Stansly, 1995), fungicides (Young, 1994; Finger et al., 2002), and as spray adjuvants (Davis et al., 1992; Schmitz-Eiberger et al., 2002) during the growing season on various crops. Plant-based oils also

improve fruit quality. Plant oil emulsions applied to growing cherries (*Prunus avium* L.) significantly reduced cracking, but did not affect firmness and fruit finish (Granger and Träger, 2002). When applied as a postharvest treatment to apples, plant oils reduced superficial scald (Scott et al., 1995; Ju and Curry, 2000) and physiological disorders like core flush in 'Granny Smith' apples (Ju and Curry, 2000), while maintaining fruit quality. After storage, oil treated fruit was greener, firmer and contained higher levels of titratable acidity (Ju and Curry, 2000; Ju et al., 2000a).

These effects can be explained by the chemical structure of the plant oil itself. The main oil constituents are acyl esters of glycerol (mainly triacylglycerides). Plant oils are usually characterized by their fatty acid composition (Hamilton, 1993). Soybean oil is derived from the seeds of the soybean plant (*Glycine max* (L.) Merr.). According to Souci et al. (1994) the predominant fatty acids present in soybean oil are linoleic (18:2), oleic (18:1) and linolenic acids (18:3), contributing 45%, 20%, and 14% respectively to the overall fatty acid profile of the oil. In plants, these fatty acids serve important roles as structural and metabolic constituents of cells. Besides being an essential component of membranes, fatty acids act as precursors of signalling (e.g. jasmonates) and volatile aroma components in most fruit including apple (Bundschuh, 1987). Supplying additional fatty acids to apples can increase the production of related volatile aroma compounds (Bartley et al., 1985; Harb et al., 1994). Most natural acids, whether saturated or unsaturated, are straight chain compounds with an even number of carbon atoms in each molecule. The most common chain lengths are C_{16}, C_{18}, C_{20}, and C_{22} (Gunstone and Norris, 1983). In contrast, petroleum-based oils show no such even chain-length preponderance (Hamilton, 1993).

A common problem associated with the use of spray oils is the risk of causing phytotoxic plant responses. Phytotoxic reactions can lead to a decrease in net photosynthesis, compromising yield potential (Furness and Maelzer, 1981), or cosmetically mark fruit resulting in loss of marketability (Willett and Westigard, 1988). In the field, emulsion-concentrations containing up to 2% of oil are used to avoid phytotoxic plant responses, while still being effective as pest control agents (Cranshaw and Baxendale, 2004). Bondada et. al (2000) successfully applied a 1% soybean oil emulsion to peach (*Prunus persica* L.) and apple trees without compromising the photosynthetic efficiency of the trees. Nonetheless, successful postharvest oil treatment to reduce scald on apples required concentrations of 5% or greater (Ju and Curry, 2000; Ju et al., 2000a). Administering oil treatments after harvesting the fruit is a challenge when adapting to current production systems. Furthermore, the effectiveness of a postharvest plant oil treatment was higher when administered to preclimacteric fruit i.e. before the onset of the ripening related surge in respiration (Ju et al., 2000a), leading to the speculation that administering oil treatments to unripe fruit in the orchard might be most beneficial.

Apple varieties used. Yellow apple cultivars such as 'Golden Delicious' have a thin cuticle and some of the common problems related to this genetic predisposition are russeting, shriveling and bruising (Meyer, 1944). The distinct differences of cuticular lipid and wax layers between the apple varieties have been linked to fruit storage life. 'Golden Delicious' apples that lost the most weight during storage had tube-like wax crystals on their surface, while the wax on fruit showing low weight loss in storage

crystallized into wax plates Rittweger (1986). Neubeller (1963) observed a direct correlation between weight loss and lipid/wax degradation during storage.

New apple varieties, like 'Gala' are being produced in increasing numbers in Washington state and elsewhere because of consumer preference. Both varieties have shown increased potential to physiological disorders like lenticel breakdown.

Epicuticular wax of apples. A waxy cuticle covers all primary aerial organs of land plants and is essential for their protection and interaction with the environment. For example, it waterproofs the plant surface and hinders attacks by pathogens and insects (Jenks and Ashworth, 1999). The cuticle consists of amorphous intracuticular wax embedded in a cutin polymer. In most plant species, including apples, the cuticle is superimposed by a thin wax layer containing very-long-chained fatty acids and their derivatives with carbon chain lengths ranging from C_{20} to C_{35} (Kunst and Samuels, 2003). Ursolic acid, a triterpenoid, is also widespread on the surface of apples (Mazliak, 1970). The outer surface of cuticular wax often crystallizes in an intricate pattern of rods, tubes, or plates (Barthlott et al., 1998). Generally, the development of cuticular lipids and waxes on the surface of fruit is a genetically controlled characteristic (Morice and Shorland, 1973; Belding et al., 1998).

The wax structure differs between apple varieties. Most cultivars have small platelets on their surface, except for 'Golden Delicious' apples, whose wax appears amorphous (Roy et al., 1999). When fruit with the amorphous wax type enlarge, the cuticular wax layer often cracks (Faust and Shear, 1972). As the fruit matures, the cracks extend and widen (Meyer, 1944; Roy et al., 1999). In contrast, a fruit surface with a platelet type of wax does not crack. The wax platelets simply move apart and the spaces

are filled with new platelets (Faust and Shear, 1972). Further, the development of cracks depends on climatic conditions, as fruit grown in an arid climate (i.e. Wenatchee, WA) was free of cracks (Faust and Shear, 1972). On the other hand, deeply indented or uneven cutin that penetrates between cells might render fruit of susceptible cultivars prone to cracking (Tetley, 1930; Shutak and Schrader, 1948).

The amount and composition of epicuticular wax on apples can be influenced by preharvest crop management techniques such as light intensity, rootstock, fungicides and surfactants (Wolter et al., 1988). Postharvest storage temperature and oxygen concentration in storage are also reported to affect cutin and wax characteristics (Mazliak, 1970; Brackmann et al., 1993). Research has focused on the epidermal lipids of apples because their occurance has been correlated with fruit storage life and the development of abiotic postharvest disorders like superficial scald. Studies as early as 1944 (Pieniazek, 1944) indicated that lipids on fruit surfaces are important transpiration barriers and thus, directly influence water loss during storage.

Hydrophobic materials such as carnauba and shellac based fruit waxes, have been shown to partially fill cracks on the surface of apple fruit when applied after harvest (Glenn et al., 1990). Prior to harvest, hydrophobic materials like horticultural oils are used extensivley as insecticides, fungicides, and spray adjuvants (Cranshaw and Baxendale, 2004). There is contradictory evidence as to how the use of horticultural oil emulsions influences epicuticular wax development and no correlation to weight loss in storage has yet been made. Thus far, Bondada et al. (2000) did not observe an effect on apple and peach leaf morphology after a 1% soybean oil emulsion application. However,

an application of pure soybean oil at 9 l ha^{-1} dissolved wax plates on the leaf surface of Johnsongrass (*Sorghum halepense* (L.) Pers.) (McWhorter and Barrentine, 1988).

Storage treatments. Depending on growing region and cultivar, some apples are stored for up to one year, just before the following year's crop becomes available. In order to store fruit for longer periods of time, metabolic rates need to be decreased. The principal mechanisms available to achieve this are, control of temperatures and atmospheres. The tradeoff of longer storage periods is usually a considerable loss of variety-specific volatile aroma components. Some of the reasons for such losses include harvesting fruit too early, the negative effects of controlled atomosphere (CA) storage and the development of off-flavors when lowering the oxygen concentration below tolerable levels. Furthermore, the elevated amount of carbon dioxide in the CA atmosphere not only slows down respiration but acts as an antagonist of aroma synthesis. Carbon dioxide inhibits alcohol dehydrogenase acitivity and thus acts on carboxylic acid metabolism (De Pooter et al., 1987).

Major apple aroma and flavour compounds have been shown to be reduced after exposure to CA conditions (Brackmann et al., 1993; Saquet et al., 2003). Suppressed aroma production of apples after CA storage is related to free linoleic acid availability (Saquet et al., 2003). Fatty acids serve as main precursors for volatile aroma components in apples, and CA storage decreases fatty acid synthesis (Bangerth et al., 1998) . The loss of aroma compounds in CA storage has been of great concern, and practical methods to alleviate these effects have been considered. Among those, dynamic atmosphere storage (Mattheis et al., 1998) and hypoxic treatments (Dixon and Hewett, 2001) seem to be promising. Supplying additional FA to fruit leads to incorporation into volatile aroma

components, as has been shown by several authors (Bartley et al., 1985; Brackmann et al., 1993; Harb et al., 1994).

RESEARCH FOCUS

'Golden Delicious' and 'Gala' apples are important commercial apple varieties worldwide. Skin and peel related disorders of these varieties are thought to be related to epidermal structure, especially when compared to other cultivars. A better understanding of epicuticular wax structures, their seasonal changes in apples and the influence of hydrophobic surfactants on their development and function are essential for sucessful postharvest apple management. Every treatment that strengthens the fruit skin will diminish susceptibility for pathogen infection, as well as bruising, russeting and water loss. However, the effect of a preharvest spray application of soybean oil on fruit quality of apples is unknown. In addition, only the free fatty acids in the epidermis of apples are highly dynamic during maturation (Song and Bangerth, 2003). Since fatty acids are known precursors for apple volatile aroma components, the application of soybean oils, rich in fatty acid aroma precursors, during the growing season could potentially improve the retention and regeneration capacity of important volatile compounds contributing to the aroma of apples. Hence, the use of non-traditional, plant-based horticultural oils has the potential to benefit the growth, development, and storage longevity of apples.

Vegetable oils, such as soybean oil, could provide a new tool for organic growers since mineral oil is only allowed as a dormant spray for disease and insect pest control. Additionally, oil application could be beneficial as an alternative in integrated pest management programs. Approved chemicals for summer applications are becoming more

limited every year due to development of resistance and loss of regulatory agency approval. Discovery research like this may result in improved, non-toxic products for producer use. In response to increased consumer pressure for a more wholesome product, use of materials, such as soybean oil, could provide increased marketing opportunities to a growing sector of fruit buyers while contributing to the long-term sustainability of the fruit-growing enterprise. Finally, soybean oil is nontoxic for humans, environment and animals. It is a renewable resource. No species of insects or mites are known to have developed resistance against oils.

OBJECTIVES

The study presented here had two objectives:

1. Ascertain and quantify the effect of growing season-applied oil sprays on at-harvest and storage quality of ‘Golden Delicious’ and ‘Gala’ apples.

2. Determine the effect of the oil application on the cuticular structure of growing and mature apples.

Field and storage experiments were performed to test the following variables: cultivar, orchard location, time of application, and storage regimen. The effects of soybean oil were compared to the industry standard Orchex 796®, a petroleum-based horticultural oil.

Experimental methods employed in this work are outlined as follows:

1. Study plots were established at the research orchard in Pullman (Tukey farm) and in the Yakima valley using criteria based on the variables of interest.

2. Apples were treated at different times during their growth period with a soybean oil emulsion and samples of immature and mature apples were collected for the study.

3. Apple quality, chemistry, and skin structure were evaluated at harvest and after storage.

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CHAPTER 2

PREHARVEST SOYBEAN OIL APPLICATION ALTERS EPICUTICULAR WAX CRYSTALLIZATION PATTERNS AND RESISTANCE TO WEIGHT LOSS OF APPLES AT HARVEST AND DURING STORAGE

ABSTRACT

The effect of field-applied soybean oil emulsions on weight loss in storage, development of cuticular cracks and epicuticular wax crystallization patterns of apples was studied utilizing three orchard locations within Washington State, USA. Three single treatments (midseason = soy1, 21 days before harvest = soy2, three days before harvest = soy3) of soybean oil emulsion (1% food grade oil, 0.1% Latron®, v/v) were administered to 'Golden Delicious' and 'Gala' apples between 2002 and 2004. Apples were harvested at commercial maturity and stored for up to 6 months at 0.5°C in refrigerated air (RA) or under 1.5% O_2 and 0.2% CO_2 controlled atmosphere (CA) conditions. The rate of weight loss was decreased in all soybean oil treated apples. Fruit grown in a cooler climate and/or stored under sub-standard relative humidity conditions benefitted the most from preharvest soybean oil treatments. As observed by scanning electron microscopy (SEM), 'Golden Delicious' apples developed cracks that intensified with maturity, while 'Gala' apples retained a smooth surface. Under arid growing conditions, fewer cracks developed. The preharvest application of soybean oil emulsions decreased the occurrence and severity of cuticular cracks in susceptible varieties such as 'Golden Delicious'. Oil was less influencial on varieties with an innate smooth topography of the cuticle. The rate

of weight loss during storage was directly related to the development of cracks. All apples showed altered wax crystallization patterns after soybean oil application.

INTRODUCTION

A waxy cuticle covers all primary aerial organs of land plants and is essential for their protection and interaction with the environment. For example, it renders the plant surface waterproof and hinders attacks by pathogens and insects (Jenks and Ashworth, 1999). The cuticle consists of amorphous intracuticular wax embedded in a cutin polymer. In most plant species, including apples, the cuticle is superimposed by a thin wax layer containing very-long-chained fatty acids and their derivatives with carbon chain lengths ranging from C_{20} to C_{35} (Kunst and Samuels, 2003). Ursolic acid, a triterpenoid, is also widespread on the surface of apples (*Malus domestica* Borkh.) (Mazliak, 1970). The outer surface of cuticular wax often crystallizes in an intricate pattern of rods, tubes, or plates (Barthlott et al., 1998). Generally, the development of cuticular lipids and waxes on the surface of fruit is a genetically controlled characteristic (Morice and Shorland, 1973; Belding et al., 1998).

The wax structure differs between apple varieties. Most cultivars have small platelets on their surface, except for 'Golden Delicious' apples, whose wax appears amorphous (Roy et al., 1999). When fruit with the amorphous wax type enlarge, the cuticular wax layer often cracks (Faust and Shear, 1972). As the fruit matures, the cracks extend and widen (Meyer, 1944; Roy et al., 1999). In contrast, a fruit surface with a platelet type of wax does not crack. The wax platelets simply move apart and the spaces are filled with new platelets (Faust and Shear, 1972). Additionally, deeply indented or

uneven cutin that penetrates between cells might render fruit of susceptible cultivars prone to cracking (Tetley, 1930; Shutak and Schrader 1948).

The amount and composition of epicuticular wax on apples can be influenced by preharvest crop management factors including light intensity, rootstock, fungicides and surfactants (Wolter et al., 1998), as well as temperature and oxygen concentration in storage after harvest (Mazliak, 1970; Brackmann et al., 1993). Research has focused on the epidermal lipids of apples because their occurrence has been correlated with fruit storage life and the development of abiotic postharvest disorders like superficial scald. Studies as early as 1944 (Pieniazek) indicated that lipids on fruit surfaces are important transpiration barriers and thus, directly influence water loss during storage.

The weight loss of fruit is principally due to the loss of water in transpiration and to a much lesser extent, the loss of carbon in the respiration process. A 5% decrease in apple weight can make the fruit appear shriveled and hence unsaleable (Hatfield and Knee, 1988). However, apples with a low water vapour permeance do not lose water or shrivel as quickly as those with high permeance (Maguire et al., 2001).

Maguire et al. (1998) established a link between cuticular micro-cracking and fruit water vapour permeance. As cracking seems to be caused by stress in the fruit skin (Meyer, 1944), it is likely that cuticular elasticity would affect the incidence and severity of cuticular cracking. Both of these may depend upon production practices such as irrigation management and timing of harvest. The influences of production practices on fruit permeance are largely unexplored at this point. However, cracks in the cuticle should be minimized to enhance the barrier properties of the cuticle. Further, the

development of cracks depends on climatic conditions, as fruit grown in an arid climate (i.e. Wenatchee, Wa.) was free of cracks (Faust and Shear, 1972).

Hydrophobic materials such as carnauba and shellac based fruit waxes, have been shown to partially smother cracks on the surface of apple fruit when applied after harvest. (Glenn et al., 1990). Prior to harvest, hydrophobic materials like horticultural oils are used extensively as insecticides, fungicides, and spray adjuvants (Cranshaw and Baxendale, 2004). There is contradictory evidence as to how the use of horticultural oil emulsions influences epicuticular wax development and no correlation to weight loss in storage has yet been made. Thus far, Bondada et al. (2000) did not observe an effect on apple and peach leaf morphology after a 1% soybean oil emulsion application. However, an application of pure soybean oil at 9 l ha^{-1} dissolved wax plates on the leaf surface of Johnsongrass (*Sorghum halepense* (L.) Pers.) (McWhorter and Barrentine, 1988). When a 0.3% vegetable oil emulsion (Synertrol®) was applied to cherry trees (*Prunus avium* L.) during the three weeks preceding harvest, before rainfall, reduced cracking of the fruit was observed (Granger and Träger, 2002). Additionally, Pinolene, a liquid polyterpene plastic film, treatment of 'Valencia' oranges (*Citrus sinensis* L.) two months prior to harvest resulted in oranges that were greener at harvest, lost less weight in storage and showed altered peel topography (Albrigo and Brown, 1970).

The objectives of this study were to describe and compare the development of epicuticular wax during maturation and storage of 'Golden Delicious' and 'Gala' apples, determine the impact of a preharvest application of soybean oil emulsions on fruit morphology, as well as compare the rate of weight loss during storage and subsequent shelf-life between untreated and oil-treated fruit.

MATERIALS AND METHODS

Experimental design. The study was carried out between 2002 and 2004 and included three separate experiments testing two varieties and two orchard locations. General orchard management procedures were performed according to industry standards. 'Golden Delicious' (MM106, 17 years old) and 'Gala' (M7, 15 years old) apple trees grown in a commercial orchard near Harrah (latitude 46°18´N, longitude 123° 8´W) in the Yakima Valley, WA, USA, were used in a randomized complete block design with four replications in 2003. Each replication consisted of 8 to 10 trees/ treatment of uniform size and fruit density. The 20.6 cm of annual average precipitation were supplemented with a sub-surface drip irrigation system. No overhead cooling was supplied.

'Golden Delicious' (M7, 22 years old) from the Tukey research orchard (latitude 46°73´N, longitude 117° 19´W) at Washington State University in Pullman, WA, USA were used in a completely randomized design with four replications and four trees/treatment in 2002 and 2003. At this location an overhead sprinkler system complements the 50.8 cm annual precipitation and functions as hydrocooling system if needed.

In 2004 'Golden Delicious' (M7, 25 years old) apple trees grown in a commercial orchard near Buena (latitude 46°43´N, longitude 122° 21´W) in the Yakima Valley, Wa., USA, were used in a randomized complete block design with four replications. Each replication consisted of 5 trees/ treatment of uniform size and fruit density. No overhead cooling was supplied.

Soybean oil treatments. The treatments for all locations and varieties consisted of three one-time applications of 1% food-grade soybean oil (Safeway Inc., Pleasanton, CA, USA) emulsified with Latron B-1956®, a nonionic surfactant (0.1%) (Rohm and Haas Co., Philadelphia, PA, USA). Treatments were applied to fruit and foliage, using a hand-held sprayer consisting of a single hollow cone nozzle, 5-horsepower petroleum driven diaphragm pump. In an effort to completely coat every fruit, each treatment was applied to the point of runoff, which resulted in a rate of one to two gallons/tree (3.8-7.6 liters) depending on tree size.

The oil emulsion was sprayed at midseason (soy1), 21 days before harvest (soy2), and 3 days before harvest (soy3). The experiment in 2002 included soy1 and soy2. In 2004 only the soy2 application was repeated. To avoid phytotoxic reactions, trees were sprayed in the evenings after temperatures had dropped below 90 °F (32 °C). Since all orchard locations receive very little natural percipitation during the summer, we did not have to consider possible rain events when timing the oil applications. However, the orchard in Pullman had an overhead irrigation system. Irrigation was scheduled one day before spraying maximizing the time between oil application and irrigation sets (average six days between oil application and next irrigation set) to keep the spray solution from washing off.

For comparative purposes, four randomly selected ‘Golden Delicious’ trees in Pullman received a treatment with 1% Orchex 796® (Exxon), a petroleum based horticultural oil, utilizing the same emulsifier in 2003. In 2004, five trees received a 0.1% emulsifier application.

Storage treatments. Storage treatments consisted of 0.5 °C in refrigerated air (RA) or 0.5 °C at 1.5 kPa oxygen and 0.2 kPa carbon dioxide (controlled atomosphere: CA) for 90 and 180 days in 2003 only. 'Gala' apples were sampled after 180 days storage. Fruit from CA was moved into regular atmosphere (RA) one week prior to sampling.

Sample preparation. Apples were harvested at commercial maturity as determined by the orchard management in 2003. In 2004, fruit was harvested 1, 10, 18 and 27 days following the oil applications. Fruit was picked exclusively from lateral branches between one and two meters from the orchard floor. Only fruit of uniform size, without visible defects and not directly exposed to the sun was used. Special care was taken to avoid touching the fruit surface area intended for observation while picking, transporting and preparing the apples for scanning electron microscopy (SEM). Fruit were transported to the laboratory in boxes containing a single layer of fruit.

Microscopy samples (3x3x5 mm) were excised from the equatorial area of the fruit with a razor blade from the upward facing cheek of the apple and immediately dropped into screw top glass vials containing distilled water to clean the surface. After removal of the water, samples were frozen in liquid nitrogen and immediately freeze dried for either 24 hours (Virtis, The Virtis company, Inc., Gardiner, NY, USA) or stored at –80 °C before freeze drying. Preliminary studies verified the absence of artifacts when comparing this procedure to common fixation and dehydration procedures (data not shown).

In each experiment four individual fruit per block were sampled and stored. A minimum of four samples of each cultivar, obtained from different apples, were

examined at a magnification range of 100-2500X to verify consistency of the surface wax. Pictures were taken of representative samples.

The rate of weight loss was determined in 2003 at harvest and in storage, as well as during a 15-day shelf-life period. All fruit was allowed to stabilize at ambient laboratory temperature (22 °C) for 12 hours before measurements were taken. Fruit for the shelf-life study were kept under those conditions after removal from storage. All weight loss measurements were performed on four replications per treatment consisting of five fruit each. The individual weight of each apple was determined at harvest, after completion of storage treatments, and after 3, 7, 11, 15 days at 22 °C. Weight loss was calculated as percent weight lost from initial fruit weight at harvest. After 180 days in CA storage the occurance and severity of shrivel was determined for 'Golden Delicious' from Pullman in 2003 using a code (1-4) developed in our lab: 1 = no shrivel, 2 = shrivel at calyx end, 3 = shrivel as unconnected ridges on entire fruit, 4 = ridges connected in net-like structure.

Scanning electron microscopy. Freeze dried pieces, with the exterior side exposed, were affixed to aluminium stubs (Specimen mount, Ted Pella, Inc., Redding, CA, USA) using Pelco tabs (Ted Pella, Inc., Redding, CA, USA) self-adhesive paper tracks. The stubs were gold-coated on a Hummer sputter coater (Anatech, San Jose, CA) and observed with a Scanning electron microscope (Hitachi, Tokyo, Japan) using an accelerating voltage of 10 KV.

Statistical analyses. Data for each variety and location were analyzed separately as a randomized complete block design or completely randomized design respectively. An analysis of variance (PROC GLM) was carried out using SAS statistical software

(SAS Institute, Cary, NC). The separation of means was accomplished using the protected least significant difference (LSD) test at the five percent level.

RESULTS

Epicuticular wax structure. *'Golden Delicious' from Pullman.* In 2002, untreated fruit had an intact wax layer in mid-season (approx. 60 dafb) (Fig. 2.1). Epicuticular waxes appeared relatively smooth and homogenous. The upper layer of the wax crystallized into platelets (5μm). When approaching maturity, surface cracks started to develop and the platelets were less apparent and smaller in size (1-2 μm). The cracks became more pronounced during storage and formed an interconnected network after 90 days in RA storage. The progression of crack development was retarded in fruit stored under CA conditions. The development of the cracks was caused by a thinning of the cutin (Fig. 2.1). The stretching of the cutin caused the epicuticular wax to collapse into the so-formed ridges, giving the fruit a cracked appearance.

Overall, midseason soybean oil application (soy1) in 2002 diminished the severity of surface cracks, while a preharvest application (soy2) prevented the occurrence of cracks (Fig. 2.1). Less wax crystals developed on the surface of soybean oil treated fruit. The wax bloom appeared to have been replaced by an amorphous wax layer.

In comparison, at-harvest control fruit in 2003 showed cracks on the surface, most of which were connected in a net-like structural pattern (Fig. 2.2). Midseason application (soy1) apples appeared very similar to the untreated fruit. About half of the observed samples from soy2 treated fruit showed no cracks while the remainder had cracks of varying sizes. The soybean oil application three days before harvest (soy3) made some

samples look ‘smoothed out’ with less pronounced wax crystals, while others looked like the control. Prior to storage, only soy2 treated fruit showed noticeable differences in the surface wax development.

‘Golden Delicious’ from Yakima. In 2003, untreated fruit had a smooth appearance with small wax crystals distributed across the surface and some small cracks at harvest (Fig. 2.2, 2.3). Midseason soybean oil application (soy1) did not significantly alter wax crystallization patterns, while soy2 treated fruit lacked the occurrence of cracks and had less crystals on the surface (Fig. 2.3). Apples from the last oil application before harvest had no cracks at all. Two structural types were prevalent; Type 1 had many crystals, while type 2 was smooth with structural remnants remaining. After 90 days of RA storage, untreated fruit had no cracks. The surface was either smooth with small crystals or covered by a net of ‘ladder-like’ structures, possibly healed over cracks. Fruit treated with soy1 had less crystals than the control but overall the same appearance, while fruit treated with soy2 was smooth with some areas of less pronounced ‘ladders’. Soy3 treated apples showed small cracks on about half of the samples. After storage of fruit for an additional 90 days under RA conditions, control fruit became smoother with less crystals, some ladders were still visible, but less pronounced. Occasional cracks were observed. The cuticular structure of all soybean oil treated fruit was similar to the control. There was no apparent difference between fruit of any treatment after 90 and 180 days of CA storage. Surface wax appeared smooth with little cracks and fine crystals on the surface.

'Gala'. In 'Gala' apples no difference in wax crystallization patterns between treatments was observed at harvest. Generally the fruit was covered with a smooth epicuticular wax layer that crystallized into small plates on the surface (Fig. 2.2).

Weight loss. *'Golden Delicious' from Yakima.* The rate of weight loss for untreated fruit was above 1% of the initial weight after 90 days of storage, regardless of storage treatment (RA or CA) (Table 2.1). Further storage for 90 days resulted in higher weight loss rates for RA treated fruit (2.7% vs. 2.0% for CA). No significant treatment effects were noted. The following are trends. After 180 days in RA and/or CA storage all soybean oil treated fruit lost less weight than the control (15-35%). There was one exception; after 180 days in CA the soy3 treated fruit lost 10% more weight compared to untreated fruit.

'Golden Delicious' from Pullman: Control fruit designated for CA storage were stored in a low relative humidity CA room, prone to producing shriveled apples. After 90 days under CA conditions, apples had lost weight at double the rate compared to RA stored fruit (4.45% vs. 2.31%) (Table 2.1). Further storage for 90 days in CA resulted in accelerated weight loss (8.12%).

All fruit subjected to soybean oil applications lost weight at a slower rate (significant overall treatment effect) (Table 2.1). When stored under RA conditions, all soybean oil treated fruit lost 10-20% less weight compared to control fruit. The effect of the soybean oil treatment on the rate of weight loss in apples was most pronounced after CA storage. Oil treated fruit lost 20-30% less weight than control fruit, regardless of the time of oil application. Overall, soy2 treated fruit lost the least amount of weight.

After 180 days in CA, the occurrence and severity of shrivel was assessed. Untreated fruit had a higher incidence of shrivel. After 8 days under shelf-life conditions all soybean oil treatments had not reached the severity of shrivel of control fruit directly after storage removal (Figure 2.4).

'Gala' from Yakima: Untreated fruit lost weight faster under CA storage conditions than during RA storage (20%). No differences between the treatments were observed (Table 2.1).

Comparison between orchard location. Untreated apples from Pullman lost weight in RA storage at higher rates than control apples from Yakima (double for 90 days RA, 50% more for 180 days in RA) (Table 2.1). After 90 days under CA conditions apples from Pullman had lost weight at double the rate than RA stored fruit (4.45% vs. 2.31%). Further storage for 90 days in CA resulted in accelerated weight loss (8.12%). In contrast, fruit from Yakima stored for 90 days in CA lost weight at about the same rate than fruit stored for the same amount of time in RA storage. The rate of weight loss slowed down in untreated apples from Yakima after further CA storage, but remained consistent in RA.

Only Pullman grown fruit treated with soybean oil had decreased weight loss rates in storage compared to control fruit after 90 days. Oil treated apples from both locations showed weight loss rates below control levels during the 180 day storage period.

DISCUSSION

Soybean oil treated 'Golden Delicious' from Pullman lost significantly less weight in storage and during subsequent shelf-life period. Wilcke (1992) described weight loss being cultivar dependent with 'Golden Delicious', one of the more

susceptible varieties, losing 5% after 100 days of RA storage. He attributed the high loss in cold storage to ventilation. In CA, the loss of weight was lower, about 2% in 100 days. Additionally, losses can vary between years. Carnauba or shellac based wax coatings, commonly applied to apples to improve appearance after storage, have also been linked to extended shelf-life by reducing water loss, respiration rates and fruit ripening (Saftner et al., 1999; Bai et al., 2002).

Oil seems to function differently, since in our experiments we found soybean oil to decrease the rate of weight loss, without affecting respiration rates. This might be due to a waterproofing effect of the oil application, without restriction of air movement. Thus, soybean oil treatments could potentially slow down weight loss of apples in storage, especially when fruit is stored for longer periods under CA conditions. In our study, an application three weeks before harvest seemed to be the most effective. Fruit grown in cooler climates and/or stored in less than optimal relative humidity benefitted the most from preharvest oil treatments.

Since the surface of 'Golden Delicious' fruit from Pullman had deep cracks compared to a smooth appearance after oil treatment, a direct relation between cuticular cracking and water loss is likely. The cuticle of apples serves as the main barrier to water loss (Schönherr, 1976; Kolattukudy, 1980). Meyer (1944) suggested cracks and breaks in the cuticle to be the overriding factor of overall weight loss and Maguire et al. (1998) established a link between cuticular micro-cracking and fruit water vapour permeance. These authors noted the absence of studies linking those two phemonena. Soybean oil application did influence microcracking of apples while simultaneously reducing water loss in storage. One possible mode of action of the oil might be the restriction of water

movement by physically smothering these cracks. However, not all effects can be explained with this theory, since 'Gala' and 'Fuji' (data not shown) apples did not develop cracks during their maturation. Changes in water vapour permeance might be the main factor in determining rates of weight loss in those varieties.

Cracks are usually described as ruptures in the cuticle without a separation between the involvement of epicuticular wax and/or the cutin fraction. While observing cracks on the surface of 'Golden Delicious' it occurred to us that cracking might be due to changes in the thickness of the cutin, rather than the wax itself. The stretching of the cutin caused the wax to collapse into the so-formed ridges, giving the fruit the cracked appearance. Previously, Shutak and Schrader (1948) found badly cracked 'York Imperial' apples had deeply indented cutin and Tetley (1930) described cultivars with uneven cutin were more susceptible to cracking than others. As summarized by Neinhuis et al. (2001), while the cuticle and intracuticular waxes mainly function as a barrier against water loss, epicuticular waxes serve different purposes such as light reflection and waterproofing. Additionally, the development of cracks has been associated with rapid fruit growth (Roy et al., 1999). However, this phenomenon does not explain why cracks continue to develop after six months of storage. Based on our study we propose the cause of cuticular cracking to be a stretching and/or weakening of the cutin layer. A thorough investigation of this issue seems pertinent as causes of uneven cutin development have not been investigated to date.

The reason for altered wax crystallization patterns due to soybean oil application could be the ability of wax crystals to move through the oil layer, thereby forming a characteristic three-dimensional structure of altered crystallization patterns. A similar

phenomenon has been observed after dewaxing *Quercus robur* leaves (Neinhuis et al., 2001). Neinhuis et al. (2001) further showed the molecules involved in the formation of epicuticular wax crystals are moved within the cuticular water current in a process resembling steam distillation. Epicuticular wax crystallization affects a variety of processes such as light reflection, water repellancy, cuticular sorption of agrochemicals and plant insect interactions (Eigenbrode and Espelie, 1995; Neinhuis and Barthlott, 1998; Bally, 1999; Wisniewski et al., 2002). Whether soybean oil applications changed any of those parameters remains unknown. Further studies are needed to establish appropriate cause-and-effect interactions.

In conclusion, the application of soybean oil emulsions during the second part of the growing season decreased the occurance and severity of cuticular cracks in 'Golden Delicious' apples, while being less influencial on varieties with an innate smooth topography of the cuticle. The rate of weight loss during storage was directly related to the development or absence of cracks. Soybean oil emulsions benefitted apple quality retention during storage by reducing dehydration.

Table 2.1. Influence of soybean oil treatments on weight loss (in % of total weight) after 90 and 180 days (d) in regular (RA) and controlled atmosphere (CA) storage.

Treatment		Weight loss in storage as % loss			
		90d RA	180d RA	90d CA	180d CA
		'Golden Delicious' from Yakima			
Control		1.16	2.70	1.23	2.00
Soy1, m.s.[a]		1.21	2.51	1.26	1.69
Soy2, 21d.b.h.[b]		1.13	2.21	1.15	1.50
Soy3, 3d.b.h.		1.20	2.24	1.40	2.20
Significance[c] *:*					
Oil treatment		NS	NS	NS	NS
Storage		***	***	***	***
Oil treatment (storage)		NS	NS	NS	NS
		'Golden Delicious' from Pullman			
Control	(A)[d]	2.32 a	3.88	4.43	8.11 a
Soy1, m.s.	(B)	2.05 b	3.72	3.50	6.25 b
Soy2,21 d.b.h.	(B)	1.80 c	3.48	2.99	5.77 b
Soy3,3 d.b.h.	(B)	1.91bc	3.34	3.31	6.60 b
Significance:					
Oil treatment		**	**	**	**
Storage		***	***	***	***
Oil treatment (storage)		***	NS	NS	***
		'Gala'			
Control		-	2.37	-	2.84
Soy1, m.s.		-	2.22	-	2.63
Soy2, 21d.b.h.		-	2.34	-	2.55
Soy3, 3d.b.h.		-	2.36	-	2.60
Significance:					
Oil treatment		-	NS	-	NS
Storage		-	***	-	***
Oil treatment (storage)		-	NS	-	NS

Mean separation by protected LSD ($P \leq 0.05$).
Means (n = 20) followed by different letters within the same column and variety are significantly different.

[a]m.s.= midseason, based on prediction model of harvest date for each apple variety using days after full bloom.

[b]d.b.h. = days before harvest.

[c]NS,*,**,*** = Not significant or significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively.

[d]Capital letters in parenthesis indicate significant overall treatment effect.

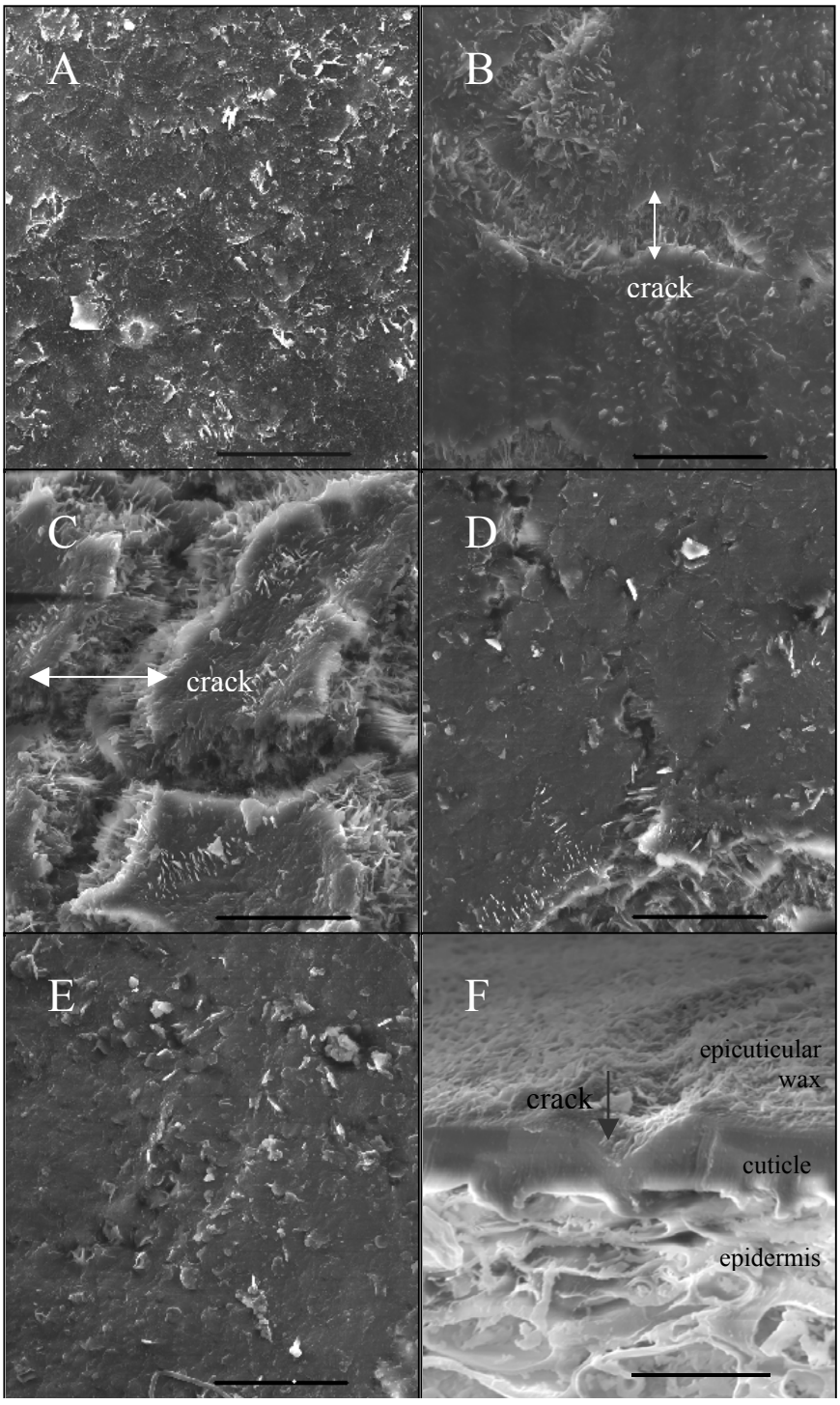

Figure 2.1. SEM micrographs of epicuticular wax of 'Golden Delicious' apples from Pullman. (A-C) untreated fruit during maturation, no cracks visible midseason (A), cracks are developing at harvest (B), after 90 days in RA storage cracks have deepened and widened (C). Fruit treated with soybean oil emulsions midseason (D) and three weeks before harvest (E) has reduced appearance of cracks after 90 days in RA. Cracks are formed by stretching of the cuticle (F). Bar = 43 μm

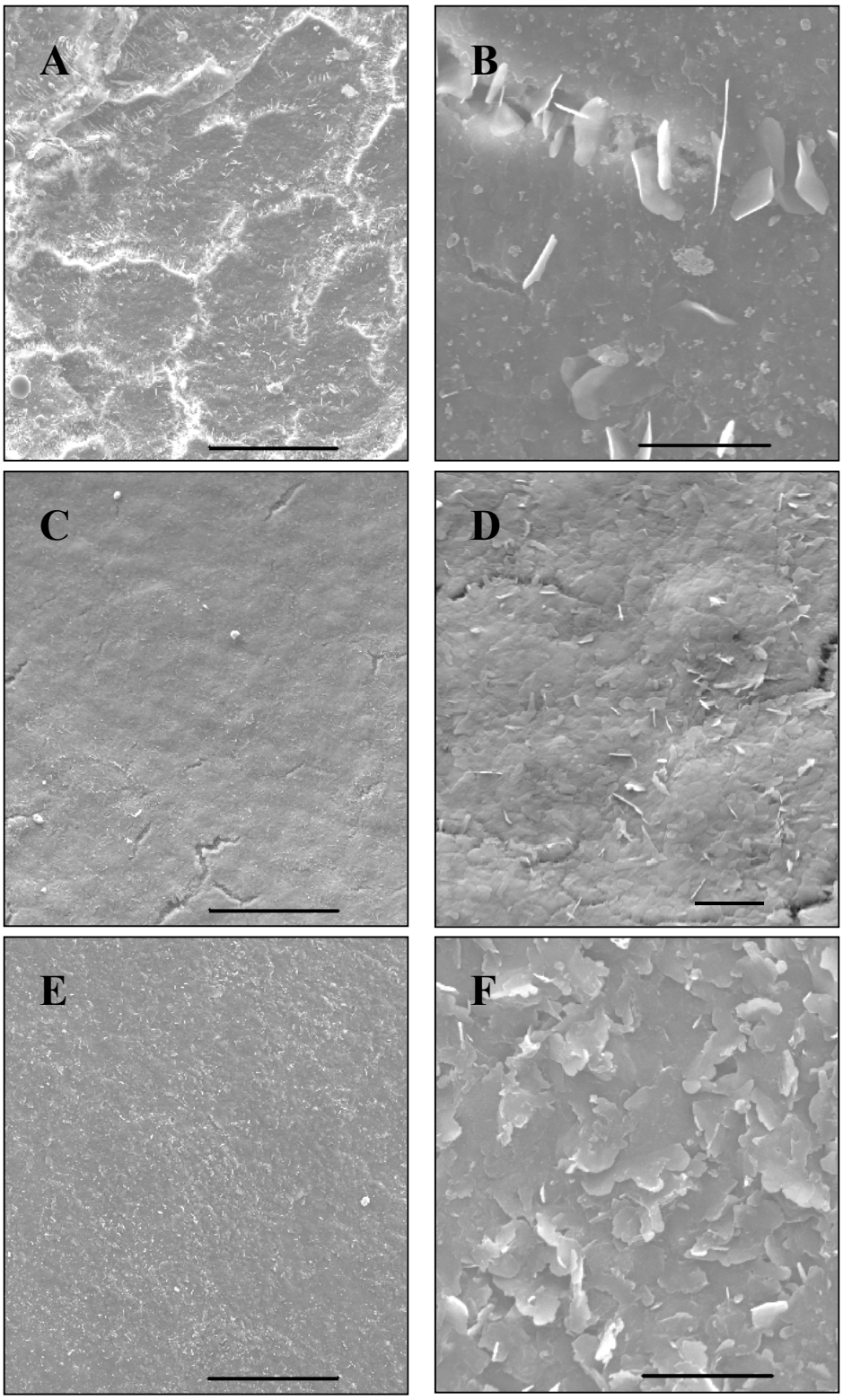

Figure 2.2. SEM micrograph of epicuticular wax of 'Golden Delicious' apples from Pullman (A+B) and Yakima (C+D) and 'Gala' (E+F) apples from Yakima at harvest. Bar = 150 µm (A, C, E); Bar = 15µm (B, D, F).

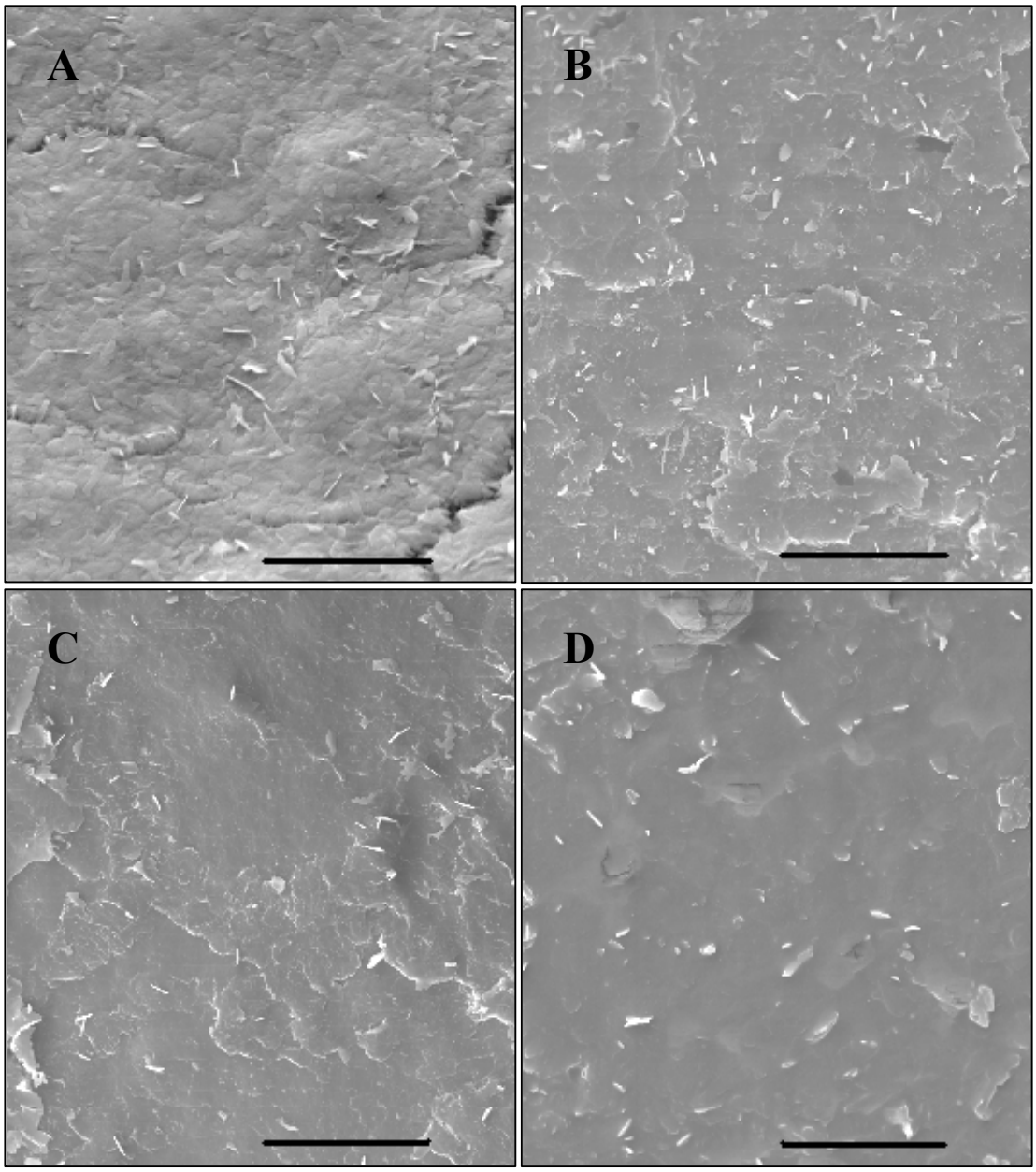

Figure 2.3. SEM micrographs of epicuticular wax of 'Golden Delicious' apples from Yakima at harvest. (A) control, (B-D) soybean oil treatments: (B) midseason, (C) three weeks before harvest, (D) three days before harvest. Bar = 30 μm

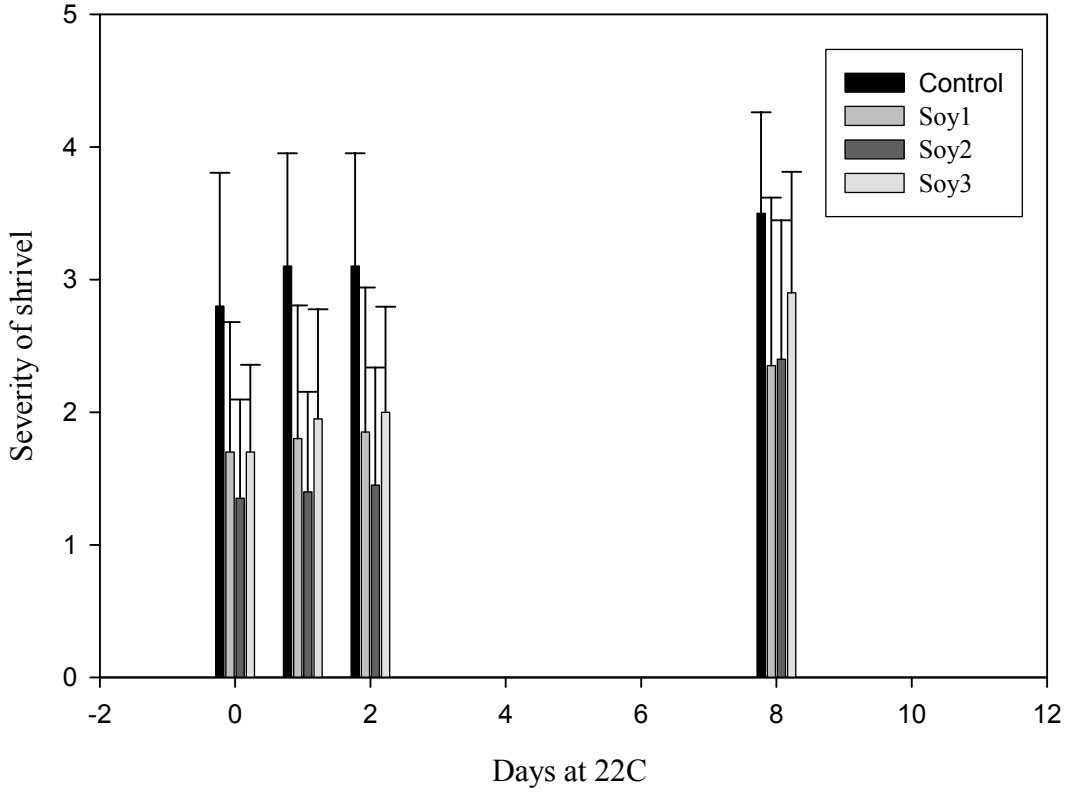

Figure 2.4. Severity of shrivel of 'Golden Delicious' apples after 180 days in controlled atmosphere (CA) storage followed by eight days in 22°C.

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CHAPTER 3

APPLE POSTHARVEST PERFORMANCE AS INFLUENCED BY PREHARVEST SOYBEAN OIL APPLICATIONS

ABSTRACT

The present study characterized the effects of growing-season applied soybean oil on at-harvest and postharvest behaviour of 'Golden Delicious' and 'Gala' apples. Three single treatments (midseason = soy1, 21 days before harvest = soy2, three days before harvest = soy3) of soybean oil emulsion (1% food grade oil, emuslified with 0.1% Latron®; v/v) were administered to apple trees grown in two different locations within Washington state, USA, to test the following variables: time of application, duration of storage, and influence of storage atmospheres. Fruit measurements included maturity indices (firmness, acidity, soluble solids, starch conversion), respiration rate, ethylene evolution, internal ethylene concentration, volatile aroma emission, flavour regeneration capacity and peel fatty acid distribution. 'Golden Delicious' and 'Gala' apples were harvested at commercial maturity and stored for up to 6 months at 0.5 °C in refrigerated air (RA) or under 1.5% O_2 and 0.2% CO_2 controlled atmosphere (CA) conditions. The oil application did not cause phytotoxicity and/or reduced fruit growth. No adverse effects on fruit finish were noticed. Fruit firmness, titratable acidity, soluble solids content, and fatty acid distribution in the peel tissue and flavour regeneration capacity were unaffected by the soybean oil treatment. 'Golden Delicious' apples treated with soy2 emitted more aldehydes (mainly hexanal), while soy3 treated fruit produced more esters. Fruit grown in warmer conditions reacted with more pronounced changes within the volatile profile. The

overall amount of volatiles emitted by fruit at harvest was similar between growing regions. Directly after CA storage, fruit from Pullman had higher alcohol and ester emission rates. Yakima grown fruit had higher alcohol and ester regeneration capacities after fruit was kept at 22 °C for seven days following CA storage. 'Gala' apples treated with soy1 had significantly higher alcohol and ester levels when compared to control fruit. Delayed loss of green colour was observed on 'Golden Delicious' apples from both locations after soy1 and soy2 treatment.

INTRODUCTION

Washington is the leading apple producing state in the USA with over two million tonnes of apples were harvested from nearly 66,000 hectares in 2003 (Washington State Department of Agriculture, 2004). Increased consumer demand for products grown without the use of synthetic chemicals coupled with increased production from sustainable farming enterprises, and tighter regulations around agrochemicals due to environmental concerns are compelling reasons to search for alternative crop protection agents.

Horticultural oils (also called petroleum, mineral, white, or narrow range spray oils) function as insecticides, fungicides, and spray adjuvants (Cranshaw and Baxendale, 2004) when applied to apples during the growing season. Oil washes have been used for over a century to suppress insect and mite pests in orchards (Willett and Westigard, 1988). This method of controlling mites is becoming increasingly important as mites are rapidly developing resistance to chemical acaricides (Cremlyn, 1990). Oils are unique because no target pest species has developed resistance to them. This is mainly due to the

fact that oils kill by suffocating the insect or simply act as a repellant. Oils also can be mixed with other insecticides or fungicides, providing a broader spectrum and greater persistence of control. For example, Davis et al. (1992) found hydrolysed cellulose together with a plant-oil based spreader-sticker enhanced the foliar tenacity and control of fungal apple diseases by the antagonist *Chaetomium globosum*.

Suitable oils for use as spray material on plants are distilled from petroleum or plant materials. In order to be suitable for plant application during the growing season, impurities are removed via filtration, distillation and dewaxing to avoid phytotoxic plant responses that can be caused by oil impurities. Final formulations of horticultural oils are commonly combined with an emulsifying agent that allows the oil to mix with water.

Traditionally, the apple industry in Washington State has relied on petroleum-based horticultural oils. However, using a plant-based oil would have the added advantage to contribute to more sustainable farming practices, since it is derived from renewable resources. Moreover, plant oils are regarded safe for human consumption as food and thus will not adversely affect consumer preferences. In fact, plant oils have demonstrable health benefits including reduction of serum LDL cholesterol levels, decreased risk of heart disease, stabilization of blood pressure and blood sugar levels, and increase in fat soluble vitamin absorption (Dept. of Agriculture, Dept. of Health and Human Services, 2005). These effects are mainly due to the high degree of unsaturated fatty acids, a major constituent of the oil itself.

Plant derived oils, like soybean, corn and canola oil have already been used sucessfully as an insecticide (Csizinszky et al., 1997; Liu and Stansly, 2000), a fungicide (Young, 1994; Finger et al., 2002), and as a spray adjuvant (Davis et al., 1992; Schmitz-

Eiberger et al., 2002) during the growing season on various crops. Plant-based oils also improve fruit quality. Plant oil emulsions applied to growing cherries (*Prunus avium* L.) significantly reduced cracking, but did not affect firmness and fruit finish (Granger and Träger, 2002). When applied as a postharvest treatment to apples, plant oils reduced superficial scald (Scott et al., 1995; Ju and Curry, 2000) and physiological disorders like core flush in 'Granny Smith' apples (Ju and Curry, 2000), while maintaining fruit quality. After storage, oil treated fruit was greener, firmer and contained higher levels of titratable acidity (Ju and Curry, 2000; Ju et al., 2000a).

These effects can be explained by the chemical structure of the plant oil itself. The main oil constituents are acyl esters of glycerol (mainly triacylglycerides). Plant oils are usually characterised by their fatty acid composition (Hamilton, 1993). Soybean oil is derived from the seeds of the soybean plant (*Glycine max* (L.) Merr.). According to Souci et al. (1994) the predominant fatty acids present in soybean oil are linoleic (18:2), oleic (18:1) and linolenic acids (18:3), contributing 45%, 20%, and 14% respectively to the overall fatty acid profile of the oil. In plants, these fatty acids serve important roles as structural and metabolic constituents of cells. Besides being an essential component of membranes, fatty acids act as precursors of signalling (e.g. jasmonates) and volatile aroma components in most fruit including apple (Bundschuh, 1987). Supplying additional fatty acids to apples can increase the production of related volatile aroma compounds (Bartley et al., 1985; Harb et al., 1994). Most natural acids, whether saturated or unsaturated, are straight chain compounds with an even number of carbon atoms in each molecule. The most common chain lengths are C_{16}, C_{18}, C_{20}, and C_{22} (Gunstone and

Norris, 1983). In contrast, petroleum-based oils show no such even chain-length preponderance (Hamilton, 1993).

A common problem associated with the use of spray oils is the risk of causing phytotoxic plant responses. Phytotoxic reactions can lead to a decrease in net photosynthesis, compromising yield potential (Furness and Maelzer, 1981), or cosmetically mark fruit resulting in loss of marketability (Willett and Westigard, 1988). In the field, emulsion-concentrations containing up to 2% of oil are used to avoid phytotoxic plant responses, while still being effective as pest control agent (Cranshaw and Baxendale, 2004). Bondada et al. (2000) successfully applied a 1% soybean oil emulsion to peach (*Prunus persica* L.) and apple trees without compromising the photosynthetic efficiency of the trees. Nonetheless, successful postharvest oil treatment to reduce scald on apples required concentrations of 5% or greater (Ju and Curry, 2000; Ju et al., 2000a). Administering oil treatments after harvesting the fruit is a challenge when adapting to current production systems. Furthermore, the effectiveness of a postharvest plant oil treatment was higher when administered to preclimacteric fruit i.e. before the onset of the ripening related surge in respiration (Ju et al., 2000a), leading to the speculation that administering oil treatments to unripe fruit in the orchard might be most beneficial.

The influence of preharvest plant oil applications on fruit quality and storage behaviour of apples is not well understood. This is the first attempt to identify physiological effects caused by the use of a plant-based oil on growing apples. The purpose of this experiment was to determine the influence of a growing season-applied soybean oil spray on harvest quality and postharvest behaviour of apples. It ascertained

the effects of a one-time soybean oil application on harvest quality, ripening patterns, storability, and volatile aroma production capacity of 'Golden Delicious' and 'Gala' apples. In addition, the influences of application timing and the orchard location were determined.

MATERIALS AND METHODS

Experimental design. Following a pilot study in 2002, the main study was carried out in 2003 and included three separate experiments testing two varieties and two orchard locations. Fertilization, pest and disease control, irrigation and other orchard management procedures were performed according to industry standards and were the same between all treatments at a given site. 'Golden Delicious' (MM106, 17 years old) and 'Gala' (M7, 15 years old) apple trees grown in a commercial orchard near Harrah (latitude 46° 18´N, longitude 123° 8´W) in the Yakima Valley, WA, USA, were used in a randomized complete block design with four replications. Each replication consisted of 8 to 10 trees/ treatment of uniform size and fruit density. The 20.6 cm of annual average precipitation were supplemented with a sub-surface drip irrigation system. No overhead cooling was supplied. 'Golden Delicious' (M7, 22 years old) from the 'Tukey' research orchard (latitude 46° 73´N, longitude 117° 19´W) at Washington State University in Pullman, WA, USA were used in a completely randomized design with four replications and four trees/treatment within replication. At this location an overhead sprinkler system complements the 50.8 cm annual precipitation and functions as hydrocooling system if needed.

Soybean oil treatments. The treatments for all locations and varieties consisted of three one-time applications of 1% food-grade soybean oil (Safeway Inc., Pleasanton, CA, USA) emulsified with Latron B-1956®, a nonionic surfactant (0.1%) (Rohm and Haas Co., Philadelphia, PA, USA). Treatments were applied to fruit and foliage, using a hand-held sprayer consisting of a single hollow cone nozzle, 5-horsepower, petrol driven diaphragm pump. In an effort to completely coat every fruit, each treatment was applied to the point of runoff, which resulted in a rate of one and two gallons/tree (3.8-7.6 liters) depending on tree size. The oil emulsion was sprayed at midseason (soy1), 21 days before harvest (soy2), and three days before harvest (soy3). To avoid phytotoxic reactions, trees were sprayed in the evenings after temperatures had dropped below 90 °F (32 °C). As both orchard locations receive almost no natural precipitation during the summer, possible rain events were disregarded when timing the oil applications. However, the orchard in Pullman had an overhead irrigation system. Irrigation was scheduled one day before spraying to prevent washing off the spray solution by maximizing the time between oil application and irrigation sets (average six days between oil application and next irrigation set). For comparative purposes, four randomly selected 'Golden Delicious' trees in Pullman received a treatment with 1% Orchex® 796 (Exxon, Houston, TX, USA), a petroleum based horticultural oil, utilizing the same emulsifier.

Fruit quality analyses. Apples were harvested at commercial maturity as determined by the orchard management. Fruit was picked exclusively from lateral branches between one and two meters from the orchard floor. Only fruit of uniform size and without visible defects were used. Harvest maturity and quality indices as well as

internal ethylene concentration, evolved ethylene, respiration, volatile aroma compound emission, weight loss in storage, and peel fatty acid distribution were analyzed at each harvest and after storage. All fruit was allowed to stabilize at ambient laboratory temperature (22 °C) for 12 hours before measurements were taken. Unless otherwise stated, all measurements were performed on four individual replications per treatment based on field replications.

Storage and shelf-life treatments. Storage treatments consisted of 0.5 °C in air (RA) or 0.5 °C at 1.5 kPa oxygen and 0.2 kPa carbon dioxide (controlled atmosphere: CA) for 90 and 180 days. 'Gala' apples were sampled after 180 days storage only. Using volatile aroma compound emission, flavour regeneration capacity for fruit stored in CA was determined after 7 days at 20 °C shelf life conditions for 'Golden Delicious' apples from both locations. Fruit from CA was moved into regular atmosphere (RA) one week prior to sampling.

Respiration rates, ethylene evolution, and internal ethylene emission rates. Evolved ethylene and CO_2 production were measured by placing quadruplicate samples of preweighed groups of five apples/treatment for two weeks in air tight flow chambers using an automated sampling system (Patterson and Apel, 1984). These plexiglass chambers (18 l) were supplied with ethylene-free air at approximately 100 ml min^{-1}. The carbon dioxide (CO_2) and ethylene (C_2H_4) concentrations from each chamber were automatically measured every 8 h for 24 h using a HP 5890A gas chromatograph (Hewlett-Packard Co., Palo Alto, California, USA) equipped with a thermal conductivity detector connected each to a GS-Q PLOT column (0.53 mm x 30 m) (Agilent Technologies, Avondale, PA, USA), and an electronic switching valve. Oven, injector,

and detector temperatures were held at 30 ˚C, 90 ˚C, and 200 ˚C, respectively. The helium carrier gas flow rate was 8 and 10 ml min^{-1} for CO_2 and C_2H_4.

Internal ethylene concentration (IEC) was assayed by withdrawing a 0.5cm^3 gas sample from the core space of a whole apple fruit through the calyx end of the fruit into the core with a 50-gauge hypodermic needle fitted with a serum stopper (n = 20). The gas sample was injected into a gas chromatograph (HP 5830A, Hewlett Packard, Palo Alto, CA) with a J&W CARBONPLOT column (30m x 0.53mm I.D.) with 3µm film. The injector was packed type with a flame ionization detector. The pre- purified nitrogen gas flow was adjusted to 8 mL/min. Injector and detector temperature was set at 200 °C and the oven temperature was set at 100 °C.

Measurements of fruit quality. All quality measurements were taken on eight fruit from each of four replications. Flesh firmness was analyzed using a Topping penetrometer (Topping, 1981). Starch content was assayed by staining transverse cross-sections of the fruit with iodine (I-KI) and visually rating the color change using a 1-5 scale (1 = 100% of the area stained; 5 = 0%). Soluble solids content was estimated using a Reichert ABBE Mark II refractometer (AO Scientific Instruments, Keene, NH, USA). Titratable acidity (malic acid) was measured using a Metrohm 672 autotitrator (Herisau, Switzerland) and expressed as malic acid equivalents.

Volatile aroma compound emission. Four replications containing juice of eight fruit/replication were stored at –20 °C until analysis. Solid phase microextraction (SPME) was employed to determine the concentration of major volatile compounds in apple flesh. One sample consisted of 2.0 ml of apple juice with 0.65 g NaCl in a 4.0 ml vial with a Teflon lined cap. The SPME device (Supelco, Co., Bellefonte, PA, USA)

consisted of a fused silica fiber coated with 65 μm poly(dimethylsiloxane)/ divinylbenzene phase. The SPME fiber was exposed to the headspace of the sample for exactly 1 hour before GC injection. SPME injection was achieved by splitless injection for 2 min. at 200 ^{o}C into a Hewlett-Packard 5890II/5970 GC/MSD equipped with a DB-1 column (60m x 0.32 mm, 0.25 μm film). Chromatographic conditons were as described by Mattheis et al., (1991) except transfer line temperature and ion source was held at 250 ^{o}C. The GC inlet contained a 0.75 mm SPME injection sleeve which assures peak sharpness, especially for early eluting peaks (Yang and Peppard, 1994). The compound identification was made by comparison of spectra from sample compounds with those contained in the Wiley-NIST library and by comparing retention indices of sample compounds and authentic standards. Quantification was accomplished by using selected ion monitoring for base peaks. Values were calculated using response factors generated from injection of authentic standard compounds.

Peel fatty acid content. Tissue disks (1.2 cm diameter, 3 mm thick) of fruit peel tissue (including epidermis, hypodermis, and several cell layers of cortical tissue) were obtained from each of 5 apples per replication and immediately frozen in liquid nitrogen and stored at –40 °C until further analysis. To determine the fatty acid content of fruit peel tissue eight disks per replication were randomly chosen and triplicate samples of tissue (5x5 mm) were placed into glass screw-capped centrifuge tubes and immediately topped with 1ml of 2.5% H_2SO_4 in methanol to extract the fatty acids from the tissues and transmethylate them. The samples were capped and incubated at 80 °C for 1h. After the addition of 0.4 ml of hexane and 1.5 ml H_2O, the fatty acid methyl esters were extracted into the hexane layer by vigorously shaking and centrifuging the tubes at low

speed until the hexane layer was clear. Samples (2 µl) of the organic phase were analyzed by GC using an Agilent 6890 series gas chromatograph (Agilent Technologies, Avondale, PA, USA) equipped with an Alltech AT-WAX column having a film thickness of 0.25µm, helium as the carrier gas at 1.4 ml/min, and a flame ionization detector. The GC was programmed for an initial temperature of 150 °C . The temperature was ramped to 200 °C at a rate of 5 °C/min followed by an increase to 204 °C at 1.5 °C/min, and reaching the final temperature of 260 °C at a rate of 30 °C/min after a total runtime of 16.53 min. The carrier gas flow rate was 1ml/min. The peaks were identified based on co-chromatography with authentic standards (Nu Check Prep, Elysian, MN, USA) and quantification achieved by generation of detector response factors for individual constituents. Previous work (Song and Bangerth, 2003) determined dynamic fluctuations during fruit growth and development to occur mainly among five fatty acids. These fatty acids are: palmitic (C16), stearic (C18), oleic (18:1), linoleic (18:2), and linolenic (C18:3) acids and these are the predominant fatty acids in soybean oil (Hamilton, 1993). Individual fatty acid content is reported as percent of the five detected fatty acid total.

Statistical analyses. Data for each variety and location were analyzed separately as a randomized complete block design or completely randomized design respectively. An analysis of variance (PROC GLM) was carried out using SAS statistical software (SAS Institute, Cary, NC). The separation of means was accomplished using the protected least significant difference (LSD) test at the five percent level.

RESULTS

Phytotoxicity was not observed as indicated by leaf damage, growth retardation and reduced fruit growth rate (Appendix 3.1). No adverse effects on fruit finish such as extensive russeting were noted.

Respiration rates, ethylene evolution, and internal ethylene emission rates

'Golden Delicious' from Yakima. Respiration rates in fruit from the soy2 treatment were on average 20% higher than untreated fruit at harvest (Figure 3.1). After 90 days in RA, fruit from all soybean oil treatments had higher respiration rates than control (between 10-24%), but all fruit respired at rates similar to untreated fruit after 180 days in RA. Apples stored in CA did not show any differences in respiration rates. The ethylene evolution rate at-harvest was similar to the respiration pattern. Fruit from the soy2 treatment had the highest ethylene emission rates (Figure 3.1). Compared to controls, storage did not influence the ethylene emission pattern of fruit from all soybean oil treatments. Internal ethylene concentration (IEC) was not significantly different between treatments and controls for all storage conditions (Table 3.1).

'Golden Delicious' from Pullman. Compared to untreated fruit, apples from the soy1 treatment had 20% and 74% lower respiration and ethylene emission rates at harvest respectively (Figure 3.2). During storage fruit from the soy3 treatment had lower respiration and ethylene emission rates, while fruit from the soy1 and soy2 treatments remained within 10% of control levels. The exception was the 90 day RA storage period were control fruit respired 20% below soybean oil treated fruit. IEC was not detectable at harvest (Table 3.2). No significant treatment effects were noted during storage. The fruit

colour (visual observation) did not match the perceived stage of ripeness. For 'Golden Delicious' from both locations soy3 appeared as yellow as the control, soy1 and soy2 remained much greener, despite respiration and ethylene trends (Appendix 3.2).

'Gala' from Yakima. Untreated apples had an average IEC of 1 ppm at harvest, 2ppm after 180 days of CA storage and above 50 ppm after 180 days in RA. No differences in IEC between treated and untreated fruit were observed (Table 3.3).

Measurements of fruit quality

Fruit firmness and titratable acidity (TA) decreased during prolonged storage in untreated apples for all varieties and storage treatments, with RA storage having the most pronounced responses (Tables 3.1, 3.2, 3.3). Generally, untreated fruit entered storage with slightly higher firmness. These differences persisted throughout the storage period. The type of storage itself had the most pronounced influence on these fruit quality parameters. For example, while 'Golden Delicious' fruit from Pullman lost approximately 4 N of firmness during the first 90 days of CA storage, firmness levels remained consistent for the next 90 days. The effect was even more pronounced for RA. Firmness loss was 20 N after 90 days, with less than 4 N additional firmness loss after 180 days (Figure 3.3). Similar trends for fruit firmness were observed for the 'Golden Delicious' and 'Gala' apples from the Yakima Valley. Generally, fruit TA levels of all fruit tested were unchanged during CA storage and did not show any significant treatment effects. Only Yakima grown soy1 treated 'Golden Delicious' fruit had significantly higher TA levels after 90 days in CA storage (Table 3.1).

Soluble solids content (SSC) increased during storage for untreated fruit within all storage treatments and varieties tested (Tables 3.1, 3.2, 3.3). After 180 days of CA storage untreated 'Golden Delicious' apples from Pullman had significantly higher SSC levels. Soybean oil treatments did not significantly affect SSC of fruit tested across all varieties and storage treatments.

Volatile aroma compound emission

'Golden Delicious'-Yakima. The major volatile concentration for 'Golden Delicious' apples from Yakima comprised a total of 11 compounds (3 aldehydes, 4 alcohols, 4 esters). The mean fruit volatile concentration measured 12 hours after warming from 0 °C to 22 °C increased with increasing storage time in RA stored control fruit: 41% increase at 90 and 71% increase at 180 days of storage (Table 3.4). Soybean oil-treated fruit showed increased mean volatile emission similar to controls after 180 days in storage. Shorter storage periods (90 days) resulted in significantly higher mean volatile levels for the soy2 treated fruit (approx. 20%). Total volatiles in untreated fruit increased faster in 90 days of CA storage (61% higher compared to at harvest) than during the longer storage period (180 days, 43%). Soybean oil-treated fruit showed similar trends. Overall, storage treatments significantly influenced the ability to produce aroma volatiles. Long-term (180 days) RA and short term (90 days) CA lead to the highest overall volatile aroma compound emission. However, RA treated fruit achieved high volatile levels due to enhanced ester and alcohol emission, while CA had the highest rates of aldehyde production. After 7 days at 22 °C, CA-stored untreated fruit produced more total volatiles when compared to at-harvest concentrations (130% in 90 days CA

and 86% 180 days CA) (data not shown). Soybean oil-treated fruit emitted volatiles at or above control levels (exception: soy3 after 90 days CA + shelf-life period) (Table 3.4).

Aldehydes. Total aldehyde (principally hexanal) production paralleled the pattern of the mean volatile production (Table 3.4). Untreated apples held under RA conditions had decreased hexanal concentrations after 180 days of storage. Control fruit from 90 days of CA storage exhibited about 50% higher hexanal levels compared to at-harvest fruit (Table 3.5). During further storage the hexanal decreased less (30% above at harvest). Regardless of storage treatment, fruit from soy2 had significantly higher hexanal concentrations when compared to untreated fruit. 2-Hexenal emission ceased from RA-stored untreated fruit, but remained above at-harvest levels in fruit from CA. Fruit from the soy2 treatment consistently emitted 2-hexenal at or above control levels regardless of storage treatment. The effect was most pronounced for CA storage, yet no significant treatment effects were observed.

Alcohols. Mean alcohol concentrations in untreated apples increased regardless of storage regimen (Table 3.4). The steepest increase was observed for fruit stored 180 days in RA, followed by 90 days in RA. Alcohol levels in CA storage were below RA and independent of the storage length. No overall treatment effects on fruit alcohol levels were observed. Apples treated with soy1 had significantly lower methyl butanol and butanol levels after 90 and 180 days in CA respectively (Table 3.5).

Esters. Regular atmosphere storage resulted in untreated apples with up to 6 times higher overall ester concentration after 180 days in storage, with fruit from soy2 and soy3 treatments emitting esters above control levels (Table 3.4). Simultaneously, fruit from the soy1 treatment had significantly lower mean ester concentrations than the other soybean

oil treated fruit. Untreated fruit stored under CA conditions had the highest ester levels (five times above at harvest) after 90 days in respective storage. After 180 days in CA, untreated fruit emitted three times the amount of esters compared to at harvest levels. Controlled atmosphere storage caused fruit from the soy2 treatment to emit esters below control levels (similar to fruit from soy1 treatment), and only apples from the soy3 treatment had higher ester levels when compared to untreated fruit. Regardless of storage treatment, soy2 and soy3 treated fruit exhibited a total ester emission greater than fruit from control treatments (Table 3.4). Soy1 treated fruit had ester levels significantly below fruit from the other soybean oil treatments. Butyl acetate was the ester present in the highest concentration, followed by 2-methyl butyl acetate and hexyl acetate (Table 3.5). Apples treated with soy3 had significantly higher butyl acetate concentrations compared to untreated fruit, and apples from the soy 1 treatment emitted butyl acetate significantly below control fruit levels. Hexyl acetate emission of soy3 treated fruit was significantly lower than control fruit. No overall treatment effects were noted for 2-methyl butyl acetate concentrations, but after 180 days in RA soy3 treated fruit had significantly lower levels compared to untreated apples.

Flavour regeneration. All untreated fruit were able to regenerate volatiles after a 7 day shelf-life period, with fruit held longer in CA storage being less effective (Figure 3.4). No significant treatment effects on flavour regeneration were noted. The following are trends only. Higher hexanal concentrations were largely responsible for fruit from soy1 and soy2 treatments emitting the most overall aldehydes (10-20% above control). The alcohol regeneration capacity remained consistent with storage length. Fruit from soy2 and soy3 treatments regenerated 20% more alcohols after 180 days in CA than

untreated fruit. When untreated apples were removed from CA, ester emission doubled within 7 days of shelf-life and was not influenced by time held in storage. Fruit from soy3 treatment consistently emitted more esters (10-40%) than untreated fruit, mainly butyl acetate and 2-methyl-butyl acetate.

'Golden Delicious'-Pullman. The mean volatile concentration in untreated fruit nearly doubled during the first 90 days of RA and CA storage and decreased in further storage (Table 3.6). Soybean oil-treated fruit behaved like control fruit and no significant differences were observed. After seven days at 22 °C, CA-stored, untreated fruit produced more total volatiles when compared to fruit immediately removed from storage (data not shown). Fruit from the soy2 treatment had the highest mean volatile concentration after 90 days of CA and seven days shelf-life, yet differences were statistically comparable to controls. Fruit treated with Orchex had the lowest mean volatile concentration after 90 days of RA storage and no differences were observed during CA storage (Appendix 3.3).

Aldehydes. The total aldehyde (principally hexanal) production of untreated fruit paralleled the mean volatile production pattern (Table 3.6). Maximum aldehyde levels in fruit were reached after CA storage and subsequent shelf-life. The highest aldehyde emissions were observed for soy2 treated apples under all storage conditions. Apples from the soy3 treatment had the lowest mean aldehyde production (exception: 90 days of CA storage). Hexanal levels in fruit increased during storage within all treatments (exception: 180 days of CA stoarge) (Table 3.7). Hexanal in fruit from soy1 and soy2 treatments remained at or above control levels, while fruit receiving the last soybean oil

application (soy3) had lower hexanal levels after the 180 day storage treatments. The emission of 2-hexenal ceased from fruit as the storage period increased. Apples from soy3 treatments had the lowest levels throughout storage, except after 90 days of CA.

Alcohols. The alcohol levels for untreated fruit increased during the first 90 days of storage and slightly decreased with additional 90 days, regardless of RA or CA storage conditions (Table 3.6). Maximum alcohol concentration in fruit was measured after 90 days CA storage. Overall, soybean oil treatments had no influence on alcohol levels of fruit tested. The most abundant single alcohol component was 1-butanol.

Esters. Ester emission rates for untreated fruit increased rapidly during storage (approximately ten-fold) regardless if RA or CA conditions were applied (Table 3.6). The maximum release of esters was observed after 90 days of RA storage. All soybean oil treated fruit behaved similarily to untreated apples. Butyl acetate, 2-methyl butyl acetate, and hexyl acetate were the most abundant ester components (Table 3.7).

Flavour regeneration. All untreated fruit were able to regenerate volatiles after a seven day shelf-life period, with fruit held in CA storage longer being less responsive (data not shown). The mean volatile emission increased mainly due to increasing aldehyde levels. The ability to regenerate alcohols decreased after 90 days of CA, and the ester regeneration ability decreased regardless of time in CA storage. Similar overall effects were noted for all soybean oil treatments except soy3. Esters regenerated remained constant during the shelf-life period after 90 days in CA. Due to higher overall aldehyde emission rates, fruit from the soy2 treatment emitted more volatiles (mean volatile concentration) when compared to untreated control fruit after 90 days in CA storage and the subsequent shelf-life period (data not shown).

'Gala'-Yakima. The mean volatile emission for untreated fruit increased approximately 30% during 180 days of RA storage and decreased by about 20% if stored under CA conditions (Table 3.8). Drastically lower alcohol levels in fruit can be considered the cause. No treatment effect was noted. However, fruit from the soy1 treatment had a 75% increase of total volatiles in RA storage.

Aldehydes. While RA storage maintained total aldehyde concentrations in fruit, the maximum aldehyde levels in fruit from all treatments were produced after CA storage (Table 3.8). Even though apples from the soy1 treatment had the lowest aldehyde levels at harvest, after storage soy1 treated fruit emitted the most aldehydes regardless of storage conditions. Interestingly, 2-hexenal emission did not cease from fruit during storage. While RA storage lowered 2-hexenal levels of all fruit (except soy1), CA storage increased emissions respectively.

Alcohols. The total alcohol levels for untreated fruit doubled during RA storage, but decreased almost ten-fold during CA. The fruit from later oil treatments (soy2 and 3) showed no differences compared to control fruit, while CA-stored apples treated with soy1 had increased total alcohol levels (Table 3.8). The most prevalent single alcohol component was 1-butanol and most of the effects described earlier can be attributed to the absence of this compound after CA storage (Table 3.9). Fruit from the midseason soybean oil application (soy1) had the highest total alcohol levels going into storage. After storage, all oil-treated fruit had higher alcohol concentrations, with soy1 and soy2 treated fruit being the most pronounced (Table 3.8).

Esters. Ester emission rates for untreated fruit increased rapidly during RA storage, and declined 10-fold during CA storage. All soybean oil-treated fruit behaved similarily to untreated apples during storage, but soy1 treated fruit entered storage with higher overall ester concentrations (Table 3.8). Butyl acetate was the ester present in the highest concentration after RA storage and 2-methyl butyl acetate dominated after CA storage (Table 3.9).

Peel fatty acid content

The total fatty acid (FA) content of the fruit skin was measured for 'Golden Delicious' fruit from Pullman only and expressed as % of total FA content based on the five FA included in the analysis (see material/methods section). The most predominant FA in the apple fruit peel was linoleic acid (C18:2, around 50% of total) (Table 3.10). Untreated fruit showed a declining proportion of palmitic (C16:0) and linolenic (C18:3) acid, constant levels of stearic (C18:0) and oleic (C18:1) acid, and increasing proportions of linoleic (C18:2) acid during storage (Figure 3.5). The proportion of linoleic acid increased 30% during RA storage. The proportional decline of palmitic and linolenic acid in apples occured faster during RA storage conditions. No significant treatment effects were determined (Table 3.10), but untreated fruit had consistently higher linoleic acid contents compared to any oil-treated fruit, while soy3 treated apples had more linolenic acid.

Comparison of orchard locations

Respiration rates. Apple respiration rates at harvest were at similar levels (16-18 ml CO_2/kg/hr) and reached a plateau after 17 and 12 days, regardless of growing location (Fig. 3.1, 3.2). While soy2 treated fruit from Yakima respired at rates 20% above control fruit levels, soy1 treated fruit from Pullman respired at levels 20% below that of untreated fruit. Regardless of storage duration, fruit held under RA conditions showed flat respiration rates when removed from storage. Apples from Pullman respired at rates above those obtained from Yakima fruit (7.5μg CO_2/kg/hr) after removal from RA storage. All soybean oil treated fruit from Yakima respired above control fruit levels (10-24%), while no differences were observed for oil-treated fruit from Pullman. After 90 days of CA storage fruit from Yakima reached a respiration plateau after 14 days. Fruit from Pullman leveled out after 3 days. A flat respiration curve was observed for fruit from both locations when removed from 180 days of CA storage. Fruit from Pullman respired at higher rates regardless of time in storage (5.6-7.5 μg CO_2/kg/hr).

Ethylene evolution. The ethylene evolution showed a continuous increase for apples from both locations at harvest and after 90 days in RA or CA storage. No differences in the amount of ethylene released were noted in fruit from different growing regions. Storage of fruit for 180 days resulted in a flattening out of the ethylene emission curve after 5 days (RA) or 7 days (CA). Apples from the Pullman location emitted ethylene above rates from Yakima grown fruit (20%). Fruit treated with soy2 in Yakima emitted ethylene at higher rates than control fruit at harvest. The same treatment in Pullman had no effect on the fruit ethylene production, while soy1 treated fruit emitted less ethylene (74%) at harvest. Except for 90 days in RA, all other storage treatments of

Pullman grown fruit resulted in soy3 fruit having lower ethylene production rates (Fig. 3.1, 3.2).

Internal ethylene concentration. The IEC for fruit from Pullman was generally well above the IEC for fruit from Yakima. The IEC for fruit from both locations varied in the response to the soybean oil treatment. Yakima-grown apples showed higher IEC in response to the soy1 treatment (90 days RA, 180 days CA). Apples from Pullman had lower IEC after storage following soy3 treatment (Table 3.2).

Standard quality parameters. Fruit from Yakima had higher firmness levels (4-5N) compared to apples from Pullman at harvest. These firmness differences disappeared with storage time. No differences between growing regions and SSC content were noted. The TA was consistently higher (+/- 0.1% of malic acid equivalent) for fruit from Pullman (Table 3.2).

Aroma volatile production. a) General trends for untreated fruit: The overall amount of volatiles emitted from fruit was similar between the growing regions evaluated in the present study. The mean volatile emission for fruit from Pullman doubled during the first 90 days of storage (RA and CA) and decreased with further storage (Figure 3.6). Fruit from Yakima emitted volatiles above at harvest levels for all storage treatments (RA: +41/71%, CA: +61/43% for 90/180 days of storage). The aldehyde emission from Pullman grown fruit paralleled Yakima fruit emission rates at harvest and after 90 days in RA. Longer time in RA (180 days) caused fruit from Pullman to produce aldehydes at four times the rate of fruit grown in Yakima. During CA storage the mean aldehyde emission rates from Pullman grown fruit were about half of those from Yakima. Generally, aldehyde emission rates followed the mean volatile production trends and

hexanal was the main aldehyde produced for both locations. The mean alcohol emission from Pullman grown fruit was four times higher at harvest and after subsequent CA storage when compared to Yakima grown fruit. After RA storage the mean alcohol production measured in fruit from Pullman was half the amount of fruit from Yakima. Apples grown in Pullman had maximum alcohol emission rates after 90 days of CA storage. In contrast, apples grown in Yakima increased alcohol production with time in storage and RA storage resulted in overall higher alcohol levels than fruit stored in CA. Overall, Pullman-grown fruit emitted more esters than fruit from Yakima (exception: 180 days in RA, -50%). The effect was most pronounced after CA storage (Figure 3.7). While fruit from Pullman had four times the amount of esters at harvest (compared to Yakima fruit), 90 days in CA resulted in seven times and 180 days in CA resulted in ten times the amount of esters compared to fruit grown in Yakima. The ester emission increased up to ten-fold in apples grown in Pullman regardless of storage treatment. The maximum ester emission was observed after 90 days in RA. Fruit from Yakima reached maximum ester emission after 180 days in RA (Table 3.4). Less volatiles were regenerated as fruit from both locations was stored under CA conditions for longer periods of time. While aldehyde levels increased following CA storage and seven days at ambient temperatures for apples grown in Pullman, aldehyde levels form Yakima grown fruit remained unchanged (data not shown). The reverse effect was observed for alcohol and ester regeneration capacities. Fruit from Yakima was able to regenerate these compounds, fruit from Pullman had up to 50% reduced emission rates during the shelf-life period.

b) Comparison of soybean oil treatment effects: Generally, ‘Golden Delicious’ grown in Yakima had a more pronounced reaction to the oil treatment. An overall treatment effect

for hexanal emission of soy2 treated Yakima grown fruit was noted as a non significant trend in Pullman grown apples. Additionally, all soy3 treated fruit from Pullman had a lower aldehyde emission rate. Fruit from neither location reacted to any of the soybean oil applications with altered alcohol emission rates. Only fruit from Yakima exhibited responses in ester production after storage due to the soybean oil application. The soy2 and soy3 treatments had significantly higher ester emission rates than soy1 treated apples.

DISCUSSION

These results document that a one time preharvest soybean oil application may transiently interfere with fruit ripening, while maintaining other quality attributes in an apple variety and orchard location specific manner. Oil treatments altered aroma related-volatile compounds without affecting fatty acid metabolism in the fruit peel.

Our results are in agreement with previously determined values for respiration rates, ethylene evolution, and IEC for 'Golden Delicious' and 'Gala' apples grown in Washington state (Ju and Curry, 2000) and elsewhere (Song and Bangerth, 1996). The oil treatment affected respiration rates at harvest only. In comparison, Ju and Curry (2000) found a 2.5% stripped corn oil emulsion to have no effect on ethylene emission rates in 'Granny Smith' apples, while higher oil concentrations (5 or 10%) supressed ethylene emission for the first 3 months of storage and later exceeded control levels. Even though we did not observe a change in internal CO_2 concentration after storage, it is possible that soybean oil application caused a temporary change in the internal atmosphere of the fruit at harvest. At harvest respiration rate and ethylene evolution within one apple cultivar can be influenced by factors such as growing region, cultivar strain, growing-season

conditions and maturity. Alternately, ethylene production may not be relevant for determining the harvest of some cultivars, such as 'Golden Delicious' because it does not increase during the harvest period (Watkins et al., 1989). The orchard location influenced the rate of respiration and the shelf-life in the present study. Generally, 'Golden Delicious' apples from Pullman respired at higher rates and reached the climacteric high earlier, suggesting faster maturation and senescence. We cannot explain why 'Golden Delicious' fruit from Yakima developed senescent breakdown after storage (Appendix 3.4), with control and soy1 treated fruit being the most severely affected.

After storage, the fruit colour did not match the perceived stage of ripeness. For 'Golden Delicious' from both locations soy3 treated fruit appeared as yellow as the control, soy1 and soy2 treated fruit remained much greener, despite respiration and ethylene trends showing no differences among treatments. This suggests, that chlorophyll breakdown was delayed in soy1 and soy2 treated fruit in an ethylene independent manner. Fan et al. (1998) and Fan and Mattheis (1999) have suggested this possibility in conclusion of their work on the involvement of methyl jasmonate in fruit ripening. In their experiments they noticed that 'Fuji' and 'Golden Delicious' apples degreened faster after treatment with methyl jasmonate without an increase in ethylene emission. However, respiration rates were elevated. Respiration rates were not different for soybean oil treated fruit in our experiments after storage. Ju and Curry (2000) and Ju et al. (2000a) reported reduced green colour loss of apples was also influenced by applications of higher oil concentrations. The responses to the oil treatment were more pronounced when applied to preclimacteric fruit. Our results might be explained with the existence of distinct subsets within the overall ripening program, as proposed by Srivastava (2002).

As shown on tomato mutants such as *yellowflesh*, *greenflesh*, and *tangerine*, while the overall regulation of ripening might be regulated by ethylene, subsets like chlorophyll degradation or carotenoid synthesis may be controlled independently. In 'Golden Delicious' apples treated with aminoethoxyvinylglycine (AVG), an inhibitor of autocatalytic ethylene production, Halder-Doll and Bangerth (1987) found a non-appreciable effect on the chlorophyll degradation of epidermal tissue during cold storage. As summarized by Halder-Doll and Bangerth (1987), ethylene is not particulary important in regulating chlorophyll breakdown during storage of fruits including banana, tomato, and apples.

Fruit firmness, TA, and SSC were unaffected by the soybean oil treatments. This is in agreement with Ju and Curry (2000). Only higher concentrations of oil reduced changes in these maturity indices (5 or 10%). We noticed that most of the fruit firmness lost in storage occurred during the first 90 days in storage. Additional storage caused only minute losses. Thus, storing fruit under CA first and then switching to RA could be of practical importance. As previously reported in the literature, it is possible to aerate CA storages. The main methods tested to date are: a) dynamic CA conditions (Lidster, et al., 1987; Mattheis et al., 1998), and b) so-called broken storage (Wilcke, 1992). However, the success of such storage is dependent on the physiological development of the fruit. Typically some kind of atmospheric switch to higher oxygen concentration needs to occur within three to five months of storage to be effective (Schulz, 2000). The fruit metabolism is activated considerably by aerating the CA storage without raising temperatures (Mattheis et al., 1998). Fruit can ripen evenly and develop the variety specific aroma and a good colour. In taste panels, fruit stored in this fashion were

considered better than fruit stored in CA the entire time (Wilcke, 1992) This is an easy method to improve sensory quality of fruit, i.e. to round off the green notes of early harvest CA fruit with flavour components of ripe apples. Additionally, since the costs to run a RA storage are lower ($2/bin), it might be feasable to turn off certain CA storage systems (specifically continuous purge systems) earlier than the anticipated storage period. This could make warehouse operations more cost effective, and save energy while not compromising fruit quality. Informal taste panels conducted in our lab indicated a preference for fruit stored in such a way. In fact, the volatile aroma regeneration capacity is known to decrease with time in CA storage (Fellman et al., 2003). Flavour of fruit could be improved by the application of shorter storage periods under CA conditions.

Fruit grown in Pullman had lower storage potential as manifested by overall higher respiration and ethylene emission rates, as well as IEC. Consequently, fruit from Pullman lost weight in storage faster (see Chapter 2). But other quality parameters like firmness and SSC were unaffected. Although the overall amount of volatiles emitted from fruit was similar between growing regions, certain volatile classes, namely alcohol and ester compounds, showed regional differences. The effect was most pronounced in CA storage, were apples from Pullman had higher emission rates. In concert with overall higher TA of apples from Pullman this could amount to a better eating experience. However, Yakima grown fruit had better alcohol and ester regeneration capacities.

The elevated amount of carbon dioxide in the CA atmosphere inhibits not only respiration but acts as an antagonist of aroma synthesis. Carbon dioxide inhibits alcohol dehydrogenase acitivity and thus acts on carboxylic acid metabolism (De Pooter et al., 1987). Aroma volatiles in intact fruit are formed via the β-oxidation biosynthetic

pathway, whereas when fruit tissue is disrupted, volatiles are formed via the lipoxygenase pathway (Schreier, 1984). Major apple aroma and flavour compounds have been shown to be reduced after exposure to CA conditions (Brackmann et al., 1993; Saquet et al., 2003). Mean volatile production was not impaired by CA in this study. However, this was mainly due to consistent aldehyde levels. Juicing the samples might have contributed greatly by stimulating lipoxygenase-activated lipid oxidation. Only short exposure times to air after crushing are necessary to induce the formation of hexanal and 2-hexenal especially when substates are available (Feys et al., 1980). These compounds are formed by the enzymatic oxidation of linoleic and linolenic acids after the fruit is crushed and exposed to oxygen (Drawert et al., 1973) and usually these aldehydes are the dominant detectable volatile compounds in intact immature fruit (De Pooter et al., 1987; Mattheis et al., 1991). When soybean oil treatments were applied to immature fruit, they had not only higher aldehyde levels, but remained greener throughout storage.

Aldehydes contribute to the green notes of apples and are known to decrease in concentration as apples mature (Mattheis et al., 1991). Hexanal and 2-hexenal, the most abundant aldehydes observed in our study, are important flavour impact compounds in 'Golden Delicious' apples (De Pooter et al., 1987). Furthermore, flavour volatiles can act as antifungal agents, especially six carbon (C_6) aldehydes like hexanal, Cis-3- hexenal and others. Six carbon aldehydes like hexanal are known to inhibit the hyphal growth of *Penicillium expansum, Alternaria alternata* and *Botrytis cinerea* on various fruit species (Hamilton-Kemp, 1996; Song et al., 1996). Aldehydes are are also important for the formation of C_6 alcohols and C_6 esters, which are among the most abundant volatile components in ripe apples. Supplying aldehydes can increase the overall aroma

production of apples by stimulating alcohol and ester synthesis (Song and Bangerth, 1994). Our study showed no significant differences between total aldehyde levels of fruit among treatments and between apple varieties or location. However, cis-3-hexenal emission ceased in 'Golden Delicious' apples from both locations after long-term storage, while the same compound increased in 'Gala' apples after CA storage. Further studies are needed to relate cis-3-hexanal emission of apple fruit to antifungal activity.

Esters are the most significant contributors to aroma in apples, accounting for up to 80% of odour active volatiles in 'Golden Delicious' (López et al., 1998). Among the most prevalent esters described for 'Golden Delicious' are butyl acetate, 2-methyl butyl acetate and hexyl acetate (Brackmann et al., 1993). Alcohols serve as important precursors of esters in apples (Dimick and Hoskin, 1983). Thus, any reduction in concentration of alcohols will adversely affect the most significant group of flavour molecules. Increased acetate ester production is directly related to the availability of related alcohol precursors (Echeverria et al., 2004). Higher alcohol emission rates in 'Gala' at harvest for fruit treated with soy1 (midseason) were most notable. Possibly, soybean oil applications in the middle of the growing season have functioned like an antitranspirant. Hence, less carbohydrates were lost due to respiration and made available for other processes. Another possibility could be a conditioning effect ('positive stress'). On the other hand, changes in alcohol concentration may result from increased esterase activity during the climacteric (Goodenough, 1983). Esterase functions by converting esters back to alcohols and carboxylic acids. Thus ester and alcohol levels in apple tissue may result from an equilibrium between synthesis, hydrolysis, and diffusion from tissue (Knee and Hatfield, 1981). Additionally, supplying aldehydes can increase the overall

aroma production of apples, since alcohol and ester synthesis can be stimulated (Song and Bangerth, 1994). Higher aldehyde production in fruit after soybean oil treatment, as observed in our study for soy2 treated fruit, has therefore the potential to improve aroma development.

The main precursors of ester-, alcohol-, and aldehyde-volatiles produced by apple fruit during development and maturation are free FA or those liberated by lipase activity and further metabolization by beta-oxidative enzymes and/or lipoxygenase. Our results are in agreement with Song and Bangerth (2003), who described a rapid increase of linoleic acid (C18:2) during the climacteric rise of 'Golden Delicious' apples or after ethylene treatment of pre-climacteric fruit. They also described a strong positive correlation between IEC and the concentration of C18:2. However, we did not find a considerable increase in the C18:1 fraction of the FA profile as previously observed by Song and Bangerth (2003). Polar lipids make up the largest portion of membrane lipids, and little change in FA concentrations during fruit development occur in this fraction, whereas changes in the concentration of the free fatty acid fraction were highly dynamic. However, the free fatty acid pool in the epidermis of the apple fruit had the lowest share of the total fatty acid pool (Song and Bangerth, 2003). In our study we determined FA concentration based on the total FA present in all lipid fractions of the epidermis. Since C18:1 contributed only 5-6.5% to the total FA in our study, changes in this FA might have been unnoticed.

Our results are also in agreement with Gaillard (1968) who found the proportion of linolenic acid (C18:3) in lipids of post-climacteric apples to be lower than in pre-climacteric apples. Gaillard (1968) explains the lower linolenic acid concentrations are

due to lower concentrations of certain lipid fractions rather than to an overall change to the fatty acid distribution of individual lipids. Additionally, Gaillard (1968) observed decreases in chlorophyll concentration in conjunction with decreasing lipid concentrations, no doubt concurring with ripening-related chloroplast breakdown. Dixon and Hewett (2000) suggested chloroplast breakdown could provide the major source of linoleic and linolenic fatty acids for volatile biosynthesis in fruit. If that were true, the relatively higher concentrations of linolenic acid in soy3 treated fruit in our experiment could be explained as coming from the oil-application itself, since no skin colour difference, indicating altered chloroplast degradation, of apples between this treatment and the control was observed. Also, soy1 and soy2 treated fruit remained greener after storage without discernable differences in maturation patterns or FA proportions, when compared to untreated fruit. This suggests the fatty acids needed for volatile synthesis in those treatments may originate from a source other than chloroplast breakdown, possibly soybean oil applications.

The limiting factor in aroma production is thought to be substrate availability rather than enzyme activity (Bartley et al., 1985; Bangerth et al., 1998). It is generally accepted that the enzymes required to catabolize FA (lipoxygenase, and/or beta oxidative enzymes) as well as downstream enzymes are not the limiting factors in aroma production. Song and Bangerth (2003) concluded: "…it seems that de novo biosynthesis of FA rather than their release from membranes or storage pools represents the limiting step in the volatile aroma production of apple fruit". Supplying additional FA to fruit leads to incorporation into volatile aroma components, as has been shown by several authors (Bartley, 1985; Brackmann et al., 1993; Harb et al., 1994).

In conclusion, spraying a soybean oil emulsion one time onto apple trees seems to have no adverse effects on fruit growth and finish. Fruit quality and volatile aroma emission were largely unchanged by the oil treatment in storage. Multiple applications and/or higher oil concentrations could potentially improve fruit aroma after harvest.

Table 3.1. Influence of soybean oil treatments on firmness, soluble solids content (SSC), titratable acidity (TA), and internal ethylene concentration (IEC) of 'Golden Delicious' apples from Yakima in the 2003 season. Measurements were taken at harvest, and after 90 or 180 days in regular (RA) or controlled atmosphere (CA) storage.

Quality index	Treatment	At harvest	After Storage			
			RA		CA	
			Time in Storage (months)			
			90	180	90	180
Firmness (N)	Control	82.29 a	53.02	46.99	77.36	73.81
	Soy1, m.s.[a]	76.51 b	52.02	46.12	74.59	70.10
	Soy2, 21d.b.h.[b]	77.34 b	51.80	46.02	74.89	69.73
	Soy3, 3d.b.h.	78.29 b	53.02	47.46	75.20	73.02
Significance[c] *:*						
Oil treatment		NS	NS	NS	NS	NS
Storage		***	***	***	***	***
Oil treatment (storage)		**	NS	NS	NS	NS
SSC (%)	Control	12.3	13.6	12.9	13.5	13.5
	Soy1, m.s.	11.7	12.9	12.8	12.6	12.6
	Soy2,21 d.b.h.	12.3	13.5	12.7	13.1	13.1
	Soy3,3 d.b.h.	12.3	13.8	13.2	12.9	13.9
Significance:						
Oil treatment		NS	NS	NS	NS	NS
Storage		**	**	**	**	**
Oil treatment (storage)		NS	NS	NS	NS	NS
TA (%)	Control	0.620	0.445	0.271	0.588 b	0.537
	Soy1, m.s.	0.630	0.466	0.273	0.664 a	0.536
	Soy2, 21d.b.h.	0.610	0.394	0.234	0.580 b	0.511
	Soy3, 3d.b.h.	0.630	0.469	0.244	0.592 b	0.557
Significance:						
Oil treatment		NS	NS	NS	NS	NS
Storage		***	***	***	***	***
Oil treatment (storage)		NS	NS	NS	*	NS
IEC ($\mu l\ l^{-1}$)	Control	nd[d]	221.5	202.1	5.7	88.1
	Soy1, m.s.	nd	271.1	184.5	15.9	97.6
	Soy2, 21d.b.h.	nd	209.1	179.5	8.8	78.2
	Soy3, 3d.b.h.	nd	219.9	191.3	14.2	64.4
Significance:						
Oil treatment		NS	NS	NS	NS	NS
Storage		***	***	***	***	***
Oil treatment (storage)		NS	NS	NS	NS	NS

Mean separation by protected LSD ($P \leq 0.05$). Means (n = 4) followed by different letters within the same column and quality index are significantly different.

[a]m.s.= midseason, based on prediction model of harvest date for each apple variety using days after full bloom.

[b]d.b.h. = days before harvest.

[c]NS,*,**,*** = Not significant or significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively.

[d]nd = not detectable.

Table 3.2. Influence of soybean oil treatments on firmness, soluble solids content (SSC), titratable acidity (TA), and internal ethylene concentration (IEC) of 'Golden Delicious' apples from Pullman in the 2003 season. Measurements were taken at harvest, and after 90 and 180 days in regular (RA) or controlled atmosphere (CA) storage.

Quality index	Treatment	At harvest	After Storage			
			RA		CA	
			Time in Storage (months)			
			90	180	90	180
Firmness (N)	Control	75.8	53.7	47.5	69.7	72.6 a
	Soy1, m.s.[a]	72.3	52.5	47.3	68.3	67.8 b
	Soy2, 21d.b.h.[b]	72.5	51.6	47.6	66.7	66.6 b
	Soy3, 3d.b.h.	73.0	50.9	45.6	68.4	69.8ab
Significance[c] *:*						
Oil treatment		NS	NS	NS	NS	NS
Storage		***	***	***	***	***
Oil treatment (storage)		NS	NS	NS	NS	*
SSC (%)	Control	12.9	13.9	13.3	13.7	14.3 a
	Soy1, m.s.	12.6	13.3	13.0	13.2	13.4 b
	Soy2,21 d.b.h.	12.6	13.4	13.1	13.4	13.1 b
	Soy3,3 d.b.h.	12.6	13.2	12.7	13.6	13.6 b
Significance:						
Oil treatment		NS	NS	NS	NS	NS
Storage		NS	NS	NS	NS	NS
Oil treatment (storage)		NS	NS	NS	NS	**
TA (%)	Control	0.76	0.63	0.41	0.71	0.64
	Soy1, m.s.	0.75	0.62	0.41	0.70	0.66
	Soy2, 21d.b.h.	0.72	0.62	0.39	0.70	0.59
	Soy3, 3d.b.h.	0.76	0.63	0.40	0.71	0.64
Significance:						
Oil treatment		NS	NS	NS	NS	NS
Storage		***	***	***	***	***
Oil treatment (storage)		NS	NS	NS	NS	NS
IEC ($\mu l\ l^{-1}$)	Control	nd[d]	229.4	453.6	118.1	316.5
	Soy1, m.s.	nd	265.1	421.3	118.0	402.3
	Soy2, 21d.b.h.	nd	234.4	407.4	116.2	371.9
	Soy3, 3d.b.h.	nd	219.5	394.9	78.3	341.4
Significance:						
Oil treatment		NS	NS	NS	NS	NS
Storage		***	***	***	***	***
Oil treatment (storage)		NS	NS	NS	NS	NS

Mean separation by protected LSD ($P \leq 0.05$).
Means (n = 4) followed by different letters within the same column and quality index are significantly different.

[a]m.s.= midseason, based on prediction model of harvest date for each apple variety using days after full bloom.

[b]d.b.h. = days before harvest.

[c]NS,*,**,*** = Not significant or significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively.

[d]nd = not detectable.

Table 3.3. Influence of soybean oil treatments on firmness, soluble solids content (SSC), titratable acidity (TA), starch staining and internal ethylene concentration (IEC) of 'Gala' apples from the Yakima valley in the 2003 season. Measurements were taken at harvest and after 180 days in regular (RA) or controlled atmosphere (CA) storage.

Quality index	Treatment	At harvest	After storage	
			180 days RA	180 days CA
Firmness (N)	Control	81.9 ± 3.4	56.8 ± 1.3	71.7 ± 1.3
	Soy1, midseason[a]	79.7 ± 1.5	54.5 ± 0.6	69.2 ± 2.0
	Soy2, 21 d.b.h.[b]	80.5 ± 1.4	56.4 ± 0.7	66.7 ± 3.1
	Soy3, 3 d.b.h.	80.4 ± 1.0	54.4 ± 1.3	70.3 ± 2.3
Significance[c] *:*				
Oil treatment		NS	NS	NS
Storage		***	***	***
Oil treatment (storage)		NS	NS	NS
SSC (%)	Control	12.1 ± 0.3	12.1 ± 0.2	12.7 ± 0.2
	Soy1, midseason	12.2 ± 0.1	12.0 ± 0.1	12.6 ± 0.2
	Soy2, 21 d.b.h.	11.8 ± 0.1	12.3 ± 0.3	12.2 ± 0.1
	Soy3, 3 d.b.h.	12.1 ± 0.1	12.0 ± 0.1	12.4 ± 0.2
Significance:				
Oil treatment		NS	NS	NS
Storage		*	*	*
Oil treatment (storage)		NS	NS	NS
TA (%)	Control	0.47 ± 0.00	0.25 ± 0.01	0.42 ± 0.01
	Soy1, midseason	0.47 ± 0.03	0.26 ± 0.02	0.41 ± 0.01
	Soy2, 21 d.b.h.	0.47 ± 0.03	0.24 ± 0.01	0.39 ± 0.01
	Soy3, 3 d.b.h.	0.48 ± 0.04	0.27 ± 0.01	0.43 ± 0.01
Significance:				
Oil treatment		NS	NS	NS
Storage		***	***	***
Oil treatment (storage)		NS	NS	NS
Starch (1-6)	Control	3.2 ± 0.3	nd[d]	nd
	Soy1, midseason	3.8 ± 0.2	nd	nd
	Soy2, 21 d.b.h.	3.3 ± 0.4	nd	nd
	Soy3, 3 d.b.h.	3.1 ± 0.3	nd	nd
Significance:				
Oil treatment		NS	-	-
Storage		-	-	-
Oil treatment (storage)		NS	-	-
IEC ($\mu l\ l^{-1}$)	Control	0.9 ± 0.9	73.7 ± 29.4	2.1 ± 1.3
	Soy1, midseason	1.1 ± 1.0	55.9 ± 39.2	2.0 ± 0.9
	Soy2, 21 d.b.h.	1.0 ± 1.0	87.3 ± 40.6	1.8 ± 0.9
	Soy3, 3 d.b.h.	1.3 ± 1.1	81.5 ± 26.7	2.3 ± 1.3
Significance:				
Oil treatment		NS	NS	NS
Storage		***	***	***
Oil treatment (storage)		NS	NS	NS

Mean separation by protected LSD ($P \leq 0.05$). Values are means ± SD (n = 4).

[a] midseason = based on prediction model of harvest date for each apple variety using days after full bloom.

[b] d.b.h. = days before harvest.

[c] NS,*,**,*** = Not significant or significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively.

[d] nd = not detectable.

Table 3.4. Influence of soybean oil treatments on predominant volatile groups of 'Golden Delicious' apples from Yakima in the 2003 season. Measurements were taken at harvest, and after 90 or 180 days in regular (RA) or controlled atmosphere (CA) storage.

Volatile group (μg ml^{-1})	Treatment		At harvest	After Storage				Pooled SD
				RA		CA		
				Time in Storage (months)				
				90	180	90	180	
Overall means	Control		1.691	2.385 b	2.894	2.725	2.416	
	Soy1, m.s.[a]		1.795	2.575ab	2.707	2.836	2.112	
	Soy2, 21d.b.h.[b]		1.834	2.914 a	3.099	2.959	2.596	
	Soy3, 3d.b.h.		1.634	2.487 b	3.141	2.860	2.107	0.320
Significance[c] *:*								
Oil treatment			NS	NS	NS	NS	NS	
Storage			***	***	***	***	***	
Oil treatment (storage)			NS	*	NS	NS	NS	
Aldehydes	Control	(B)[d]	1.627	1.475 b	0.433	2.368	2.163	
	Soy1, m.s.	(AB)	1.718	1.748ab	0.456	2.598	1.913	
	Soy2,21 d.b.h.	(A)	1.768	1.985 a	0.450	2.683	2.351	
	Soy3,3 d.b.h.	(B)	1.560	1.511 b	0.411	2.499	1.802	0.249
Significance:								
Oil treatment			*	*	*	*	*	
Storage			***	***	***	***	***	
Oil treatment (storage)			NS	*	NS	NS	NS	
Alcohols	Control		0.050	1.391	1.276	0.273	0.203	
	Soy1, m.s.		0.061	1.479	1.129	0.186	0.161	
	Soy2, 21d.b.h.		0.051	1.140	1.314	0.215	0.196	
	Soy3, 3d.b.h.		0.058	0.856	1.453	0.283	0.236	0.260
Significance:								
Oil treatment			NS	NS	NS	NS	NS	
Storage			***	***	***	***	***	
Oil treatment (storage)			NS	NS	NS	NS	NS	
Esters	Control	(AB)	0.014	0.309	1.184bc	0.084	0.050	
	Soy1, m.s.	(B)	0.016	0.267	1.122 c	0.052	0.038	
	Soy2, 21d.b.h.	(A)	0.015	0.324	1.335 a	0.061	0.049	
	Soy3, 3d.b.h.	(A)	0.017	0.342	1.277ab	0.078	0.069	0.085
Significance:								
Oil treatment			**	**	**	**	**	
Storage			***	***	***	***	***	
Oil treatment (storage)			NS	NS	*	NS	NS	

Mean separation by protected LSD ($P \leq 0.05$).
Means (n = 4) followed by different letters within the same column and volatile group are significantly different.

[a]m.s.= midseason, based on prediction model of harvest date for each apple variety using days after full bloom.

[b]d.b.h. = days before harvest.

[c]NS,*,**,*** = Not significant or significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively.

[d]Capital letters in parenthesis indicate overall treatment effect.

Table 3.5. Influence of soybean oil treatments on single volatile compounds of 'Golden Delicious' apples from Yakima in the 2003 season. Measurements were taken at harvest, and after 90 or 180 days in regular (RA) or controlled atmosphere (CA) storage.

Volatile compound (μg ml^{-1})	Treatment		At harvest	After Storage				Pooled SD
				RA		CA		
				Time in Storage (months)				
				90	180	90	180	
Aldehydes								
Cis-3-Hexenal	Control		0.172	0.008	nd[d]	0.273	0.149	
	Soy1, m.s.[a]		0.188	0.022	nd	0.268	0.077	
	Soy2, 21 d.b.h.[b]		0.203	0.000	nd	0.158	0.080	
	Soy3, 3 d.b.h.		0.153	0.028	nd	0.167	0.122	0.061
Hexanal	Control	(B)[c]	1.057	1.152 b	0.392	1.579	1.578	
	Soy1, m.s.	(AB)	1.155	1.365ab	0.408	1.817	1.415	
	Soy2, 21 d.b.h.	(A)	1.168	1.613 a	0.405	1.968	1.796	
	Soy3, 3 d.b.h.	(B)	1.019	1.143 b	0.367	1.810	1.289	0.216
2-Hexenal	Control		0.397	0.314	0.041	0.516	0.436	
	Soy1, m.s.		0.376	0.361	0.049	0.512	0.421	
	Soy2, 21 d.b.h.		0.397	0.372	0.044	0.558	0.476	
	Soy3, 3 d.b.h.		0.388	0.340	0.044	0.522	0.391	0.044
Alcohols								
1-Butanol	Control		0.022	0.441	1.086	0.164	0.081ab	
	Soy1, m.s.		0.030	0.397	0.934	0.087	0.057 b	
	Soy2, 21 d.b.h.		0.021	0.447	1.122	0.118	0.075ab	
	Soy3, 3 d.b.h.		0.028	0.428	1.264	0.170	0.108 a	0.113
2-Methyl-1-butanol	Control		0.008	0.049	0.037	0.035 b	0.057	
	Soy1, m.s.		0.009	0.046	0.043	0.044 a	0.053	
	Soy2, 21 d.b.h.		0.008	0.044	0.034	0.035 b	0.056	
	Soy3, 3 d.b.h.		0.008	0.053	0.034	0.036 b	0.052	0.012
1-Pentanol	Control		nd	0.006	nd	0.003	nd	
	Soy1, m.s.		nd	0.010	nd	0.003	nd	
	Soy2, 21 d.b.h.		nd	0.005	nd	0.003	nd	
	Soy3, 3 d.b.h.		nd	0.006	nd	0.003	nd	0.002
1-Hexanol	Control		0.020	0.106	0.153	0.071	0.064	
	Soy1, m.s.		0.023	0.105	0.152	0.052	0.052	
	Soy2, 21 d.b.h.		0.022	0.109	0.158	0.060	0.065	
	Soy3, 3 d.b.h.		0.022	0.146	0.155	0.073	0.076	0.034
Esters								
Butyl Acetate	Control	(B)	0.005	0.243	1.043bc	0.059	0.031	
	Soy1, m.s.	(C)	0.006	0.209	0.968 c	0.027	0.015	
	Soy2, 21 d.b.h.	(AB)	0.005	0.265	1.182 a	0.037	0.021	
	Soy3, 3 d.b.h.	(A)	0.008	0.277	1.164ab	0.050	0.036	0.070
2-Methyl-butyl acetate	Control		0.007	0.051	0.073 a	0.020	0.014	
	Soy1, m.s.		0.007	0.045	0.085 a	0.020	0.020	
	Soy2, 21 d.b.h.		0.007	0.048	0.078 a	0.020	0.024	
	Soy3, 3 d.b.h.		0.007	0.055	0.057 b	0.022	0.027	0.012
Pentyl acetate	Control		0.001	0.002	0.008	0.001	0.000	
	Soy1, m.s.		0.001	0.002	0.009	0.001	0.000	
	Soy2, 21 d.b.h.		0.001	0.002	0.010	0.001	0.000	
	Soy3, 3 d.b.h.		0.001	0.002	0.007	0.001	0.000	0.001
Hexyl acetate	Control	(A)	0.002	0.012	0.061 a	0.005	0.005	
	Soy1, m.s.	(A)	0.002	0.011	0.061 a	0.004	0.004	
	Soy2, 21 d.b.h.	(A)	0.002	0.009	0.065 a	0.004	0.004	
	Soy3, 3 d.b.h.	(B)	0.002	0.009	0.049 b	0.005	0.005	0.005

Mean separation by protected LSD ($P \leq 0.05$).

Means (n = 4) followed by different letters within the same column and volatile compound are significantly different.

[a]m.s. = midseason, based on prediction model of harvest date for each apple variety using days after full bloom.

[b]d.b.h. = days before harvest.

[c]Capital letters in parenthesis indicate overall treatment effect.

[d]nd = not detectable.

Table 3.6. Influence of soybean oil treatments on predominant volatile groups of 'Golden Delicious' apples from Pullman in the 2003 season. Measurements were taken at harvest, and after 90 or 180 days in regular (RA) or controlled atmosphere (CA) storage.

Volatile group (µg ml^{-1})	Treatment		At harvest	After Storage				Pooled SD
				RA		CA		
				Time in Storage (months)				
				90	180	90	180	
Overall means	Control		1.937	3.572	2.912	3.465	2.359	
	Soy1, m.s.[a]		1.747	3.720	2.902	3.430	2.403	
	Soy2, 21d.b.h.[b]		1.881	3.654	3.015	3.273	2.480	
	Soy3, 3d.b.h.		1.683	3.537	2.557	3.234	2.107	0.115
Significance[c]*:*								
Oil treatment			NS	NS	NS	NS	NS	
Storage			***	***	***	***	***	
Oil treatment (storage)			NS	NS	NS	NS	NS	
Aldehydes	Control		1.679	2.045	1.645	1.751	1.144ab	
	Soy1, m.s.		1.524	2.136	1.612	1.758	1.208 a	
	Soy2,21 d.b.h.		1.636	2.283	1.712	1.843	1.346 a	
	Soy3,3 d.b.h.		1.467	2.089	1.298	1.852	0.927 b	0.075
Significance:								
Oil treatment			NS	NS	NS	NS	NS	
Storage			***	***	***	***	***	
Oil treatment (storage)			NS	NS	NS	NS	*	
Alcohols	Control		0.193	0.845	0.733	1.132	0.727	
	Soy1, m.s.		0.168	0.840	0.710	1.088	0.662	
	Soy2, 21d.b.h.		0.191	0.781	0.795	0.966	0.676	
	Soy3, 3d.b.h.		0.167	0.768	0.704	0.983	0.710	0.084
Significance:								
Oil treatment			NS	NS	NS	NS	NS	
Storage			***	***	***	***	***	
Oil treatment (storage)			NS	NS	NS	NS	NS	
Esters	Control	(AB)[d]	0.064	0.682	0.533	0.581	0.488	
	Soy1, m.s.	(A)	0.055	0.744	0.580	0.584	0.533	
	Soy2, 21d.b.h.	(B)	0.054	0.590	0.508	0.464	0.458	
	Soy3, 3d.b.h.	(B)	0.049	0.680	0.555	0.399	0.470	0.056
Significance:								
Oil treatment			*	*	*	*	*	
Storage			***	***	***	***	***	
Oil treatment (storage)			NS	NS	NS	NS	NS	

Mean separation by protected LSD ($P \leq 0.05$).
Means (n = 4) followed by different letters within the same column and volatile group are significantly different.

[a] m.s.= midseason, based on prediction model of harvest date for each apple variety using days after full bloom.

[b] d.b.h. = days before harvest.

[c] NS,*,**,*** = Not significant or significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively.

[d] Capital letters in parenthesis indicate significant overall treatment effect.

Table 3.7. Influence of soybean oil treatments on single volatile compounds of 'Golden Delicious' apples from Pullman in the 2003 season. Measurements were taken at harvest, and after 90 or 180 days in regular (RA) or controlled atmosphere (CA) storage.

Volatile compound (µg ml^{-1})	Treatment		At harvest	After Storage				Pooled SD
				RA		CA		
				Time in Storage (months)				
				90	180	90	180	
Aldehydes								
Cis-3-Hexenal	Control		0.077	0.008	0.000	0.055 b	0.005	
	Soy1, m.s.[a]		0.082	0.000	0.000	0.037 b	0.005	
	Soy2, 21 d.b.h.[b]		0.072	0.000	0.000	0.050 b	0.012	
	Soy3, 3 d.b.h.		0.092	0.012	0.000	0.101 a	0.009	0.012
Hexanal	Control		1.150	1.664	1.480	1.373	0.972ab	
	Soy1, m.s.		1.028	1.791	1.449	1.396	1.061 a	
	Soy2, 21 d.b.h.		1.138	1.917	1.535	1.459	1.170 a	
	Soy3, 3 d.b.h.		0.984	1.754	1.168	1.411	0.786 b	0.066
2-Hexenal	Control	(A)[c]	0.452	0.374	0.165 a	0.322	0.167	
	Soy1, m.s.	(AB)	0.415	0.345	0.163 a	0.324	0.142	
	Soy2, 21 d.b.h.	(A)	0.426	0.366	0.177 a	0.333	0.164	
	Soy3, 3 d.b.h.	(B)	0.391	0.323	0.130 b	0.341	0.132	0.011
Alcohols								
1-Butanol	Control		0.113	0.662	0.599	0.790	0.405	
	Soy1, m.s.		0.094	0.669	0.584	0.770	0.375	
	Soy2, 21 d.b.h.		0.109	0.611	0.662	0.668	0.404	
	Soy3, 3 d.b.h.		0.095	0.608	0.581	0.668	0.416	0.069
2-Methyl-1-butanol	Control		0.040	0.050	0.025	0.085	0.177 a	
	Soy1, m.s.		0.030	0.039	0.022	0.076	0.146 b	
	Soy2, 21 d.b.h.		0.031	0.035	0.023	0.066	0.149 b	
	Soy3, 3 d.b.h.		0.032	0.031	0.021	0.087	0.162ab	0.008
1-Pentanol	Control		0.006	0.005	0.005	0.008	0.004	
	Soy1, m.s.		0.005	0.005	0.004	0.008	0.004	
	Soy2, 21 d.b.h.		0.006	0.005	0.005	0.010	0.004	
	Soy3, 3 d.b.h.		0.004	0.004	0.005	0.008	0.004	0.001
1-Hexanol	Control		0.035	0.129	0.105	0.249	0.141	
	Soy1, m.s.		0.040	0.127	0.100	0.235	0.137	
	Soy2, 21 d.b.h.		0.045	0.131	0.105	0.222	0.120	
	Soy3, 3 d.b.h.		0.037	0.125	0.097	0.219	0.128	0.012
Esters								
Butyl Acetate	Control	(AB)	0.028	0.580	0.475	0.456	0.259	
	Soy1, m.s.	(A)	0.024	0.644	0.520	0.459	0.317	
	Soy2, 21 d.b.h.	(B)	0.026	0.517	0.458	0.371	0.263	
	Soy3, 3 d.b.h.	(B)	0.020	0.598	0.499	0.301	0.268	0.048
2-Methyl-butyl acetate	Control		0.030	0.080	0.038 a	0.093	0.203	
	Soy1, m.s.		0.025	0.076	0.039 a	0.093	0.187	
	Soy2, 21 d.b.h.		0.023	0.053	0.030 b	0.069	0.172	
	Soy3, 3 d.b.h.		0.022	0.059	0.036 a	0.077	0.178	0.010
Pentyl acetate	Control		0.001	0.003	0.003	0.003	0.002	
	Soy1, m.s.		0.001	0.003	0.003	0.003	0.002	
	Soy2, 21 d.b.h.		0.001	0.003	0.003	0.003	0.002	
	Soy3, 3 d.b.h.		0.003	0.003	0.003	0.002	0.002	0.001
Hexyl acetate	Control		0.004	0.018	0.017	0.030	0.024	
	Soy1, m.s.		0.004	0.020	0.018	0.028	0.028	
	Soy2, 21 d.b.h.		0.003	0.017	0.018	0.023	0.021	
	Soy3, 3 d.b.h.		0.004	0.020	0.017	0.019	0.022	0.003

Mean separation by protected LSD ($P \leq 0.05$).

Means (n = 4) followed by different letters within the same column and volatile compound are significantly different.

[a]m.s. = midseason, based on prediction model of harvest date for each apple variety using days after full bloom.

[b]d.b.h. = days before harvest.

[c]Capital letters in parenthesis indicate significant overall treatment effect.

Table 3.8. Influence of soybean oil treatments on predominant volatile groups of 'Gala' apples from the Yakima valley in the 2003 season. Measurements were taken at harvest, and after 180 days in regular (RA) or controlled atmosphere (CA) storage.

Volatile group (µg ml^{-1})	Treatment		At harvest	After storage		Pooled SD
				180 days RA	180 days CA	
Overall means	Control		2.040	2.943	1.573	
	Soy1, m.s.[a]		2.173	3.520	1.681	
	Soy2, 21 d.b.h.[b]		1.951	3.212	1.595	
	Soy3, 3 d.b.h.		1.940	3.078	1.583	0.318
Significance[c]*:*						
Oil treatment			NS	NS	NS	
Storage			***	***	***	
Oil treatment (storage)			NS	NS	NS	
Aldehydes	Control		1.097	0.929	1.472	
	Soy1, m.s.		0.823	1.288	1.555	
	Soy2, 21 d.b.h.		1.031	1.071	1.475	
	Soy3, 3 d.b.h.		1.076	1.031	1.472	0.204
Significance:						
Oil treatment			NS	NS	NS	
Storage			***	***	***	
Oil treatment (storage)			NS	NS	NS	
Alcohols	Control	(B)[d]	0.639 b	1.133	0.078	
	Soy1, m.s.	(A)	0.861 a	1.396	0.100	
	Soy2, 21 d.b.h.	(AB)	0.607 b	1.357	0.097	
	Soy3, 3 d.b.h.	(B)	0.568 b	1.150	0.084	0.139
Significance:						
Oil treatment			*	*	*	
Storage			***	***	***	
Oil treatment (storage)			*	NS	NS	
Esters	Control		0.304 b	0.880	0.024	
	Soy1, m.s.		0.489 a	0.835	0.026	
	Soy2, 21 d.b.h.		0.312 b	0.783	0.024	
	Soy3, 3 d.b.h.		0.296 b	0.896	0.026	0.07
Significance:						
Oil treatment			NS	NS	NS	
Storage			***	***	***	
Oil treatment (storage)			*	NS	NS	

Mean separation by protected LSD ($P \leq 0.05$).
Means (n = 4) followed by different letters within the same column and volatile group are significantly different.

[a]m.s. = mid-season, based on prediction model of harvest date for each apple variety using days after full bloom.

[b]d.b.h. = days before harvest.

[c]NS,*,**,*** = Not significant or significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively.

[d]Capital letters in parenthesis indicate overall significant treatment effect.

Table 3.9. Influence of soybean oil treatments on single volatile compounds of 'Gala' apples from the Yakima valley in the 2003 season. Measurements were taken at harvest, and after 180 days in regular (RA) or controlled atmosphere (CA) storage.

Volatile compound (µg ml^{-1})	Treatment		At harvest	After storage		Pooled SD
				180 days RA	180 days CA	
Aldehydes						
Cis-3-Hexenal	Control		0.0225[c]	nd[d]	0.0949	
	Soy1, m.s.[a]		0.0160	nd	0.2055	
	Soy2, 21 d.b.h.[b]		0.0323	nd	0.0880	
	Soy3, 3 d.b.h.		0.0191	nd	0.1231	0.055
Hexanal	Control		0.8427	0.7988	1.0174	
	Soy1, m.s.		0.6456	1.1157	1.0010	
	Soy2, 21 d.b.h.		0.7961	0.9198	1.0171	
	Soy3, 3 d.b.h.		0.8385	0.8952	0.9818	0.191
2-Hexenal	Control		0.2316 a	0.1305	0.3600	
	Soy1, m.s.		0.1610 b	0.1726	0.3490	
	Soy2, 21 d.b.h.		0.2027ab	0.1516	0.3695	
	Soy3, 3 d.b.h.		0.2183 a	0.1362	0.3674	0.036
Alcohols						
1-Butanol	Control		0.4607	1.0084	nd	
	Soy1, m.s.		0.6160	1.2391	0.0021	
	Soy2, 21 d.b.h.		0.4320	1.2052	nd	
	Soy3, 3 d.b.h.		0.4151	1.0132	nd	0.154
2-Methyl-1-butanol	Control	(B)[e]	0.0697 b	0.0245	0.0663	
	Soy1, m.s.	(A)	0.1142 a	0.0360	0.0831	
	Soy2, 21 d.b.h.	(AB)	0.0717 b	0.0361	0.0823	
	Soy3, 3 d.b.h.	(B)	0.0623 b	0.0251	0.0711	0.016
1-Pentanol	Control	(BC)	0.0091	0.0065	nd	
	Soy1, m.s.	(A)	0.0099	0.0084	nd	
	Soy2, 21 d.b.h.	(AB)	0.0093	0.0077	nd	
	Soy3, 3 d.b.h.	(BC)	0.0067	0.0069	nd	0.002
1-Hexanol	Control	(B)	0.0996 b	0.0935	0.0113	
	Soy1, m.s.	(A)	0.1209 a	0.1123	0.0147	
	Soy2, 21 d.b.h.	(B)	0.0945 b	0.1083	0.0147	
	Soy3, 3 d.b.h.	(B)	0.0837 b	0.1048	0.0134	0.011
Esters						
Butyl Acetate	Control		0.2059 b	0.7939	0.0008	
	Soy1, m.s.		0.2966 a	0.7622	0.0011	
	Soy2, 21 d.b.h.		0.1933 b	0.7050	0.0006	
	Soy3, 3 d.b.h.		0.1941 b	0.8092	0.0009	0.053
2-Methyl-butyl acetate	Control		0.0805 b	0.0529	0.0220	
	Soy1, m.s.		0.1658 a	0.0458	0.0239	
	Soy2, 21 d.b.h.		0.0964 b	0.0516	0.0223	
	Soy3, 3 d.b.h.		0.0872 b	0.0530	0.0246	0.019
Pentyl acetate	Control		0.0032	0.0057	nd	
	Soy1, m.s.		0.0045	0.0041	nd	
	Soy2, 21 d.b.h.		0.0084	0.0043	nd	
	Soy3, 3 d.b.h.		0.0026	0.0053	nd	0.002
Hexyl acetate	Control		0.0147 b	0.0279	0.0007	
	Soy1, m.s.		0.0219 a	0.0233	0.0010	
	Soy2, 21 d.b.h.		0.0140 b	0.0222	0.0009	
	Soy3, 3 d.b.h.		0.0121 b	0.0288	0.0007	0.003

Mean separation by protected LSD ($P \leq 0.05$).

Means (n = 4) followed by different letters within the same column and volatile compound are significantly different.

[a]m.s. = mid-season, based on prediction model of harvest date for each apple variety using days after full bloom.

[b]d.b.h. = days before harvest.

[c]NS,*,**,*** = Not significant or significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively.

[d]nd = not detectable.

[e]Capital letters in parenthesis indicate overall significant treatment effect.

Table 3.10. Influence of soybean oil treatments on fatty acid (FA) profiles (relative % by weight) of 'Golden Delicious' apples from Pullman in the 2003 season. Measurements were taken at harvest, and after 90 or 180 days in regular (RA) or controlled atmosphere (CA) storage.

FA	Treatment	At harvest	After Storage			
			RA		CA	
			Time in Storage (days)			
			90	180	90	180
16:0[a]	Control	21.7 ± 0.8	17.8 ± 1.7	14.5 ± 0.7	18.7 ± 0.6	16.6 ± 0.8
	Soy1, m.s.[b]	21.4 ± 0.8	18.1 ± 1.6	14.7 ± 0.7	19.2 ± 0.3	17.0 ± 0.9
	Soy2, 21 d.b.h.[c]	22.1 ± 1.0	18.2 ± 1.5	15.4 ± 0.7	18.7 ± 0.3	17.7 ± 0.7
	Soy3, 3 d.b.h.	21.6 ± 1.2	17.3 ± 1.2	15.0 ± 0.3	18.9 ± 0.6	16.6 ± 0.3
18:0	Control	6.7 ± 0.7	6.8 ± 0.3	7.6 ± 0.3	7.0 ± 0.4	8.0 ± 0.3
	Soy1, m.s.	6.9 ± 0.4	7.1 ± 0.3	8.2 ± 0.2	6.9 ± 0.2	8.1 ± 0.2
	Soy2, 21 d.b.h.	6.4 ± 0.8	7.4 ± 0.4	7.8 ± 0.5	7.5 ± 0.3	8.3 ± 0.5
	Soy3, 3 d.b.h.	6.2 ± 0.8	7.5 ± 0.5	8.0 ± 0.7	7.0 ± 0.8	8.1 ± 0.2
18:1	Control	5.4 ± 1.7	6.5 ± 1.6	6.5 ± 0.7	5.6 ± 0.5	6.5 ± 0.8
	Soy1, m.s.	5.4 ± 1.6	5.9 ± 1.1	6.5 ± 0.6	5.4 ± 0.7	6.1 ± 1.0
	Soy2, 21 d.b.h.	5.5 ± 1.3	5.7 ± 1.0	6.4 ± 1.0	6.0 ± 0.3	5.9 ± 1.3
	Soy3, 3 d.b.h.	5.1 ± 1.3	6.7 ± 1.2	6.5 ± 0.5	5.5 ± 0.6	6.3 ± 0.4
18:2	Control	48.7 ± 2.7	59.5 ± 1.4	64.0 ± 0.9	58.3 ± 1.5	60.4 ± 0.9
	Soy1, m.s.	48.6 ± 0.9	58.8 ± 1.5	62.7 ± 0.8	56.8 ± 1.3	61.0 ± 0.3
	Soy2, 21 d.b.h.	48.0 ± 1.1	59.2 ± 1.0	62.8 ± 0.4	56.7 ± 2.2	59.6 ± 1.2
	Soy3, 3 d.b.h.	48.8 ± 1.1	58.3 ± 0.7	62.3 ± 1.5	57.5 ± 0.9	59.7 ± 1.4
18:3	Control	17.5 ± 1.1	9.4 ± 1.2	7.4 ± 0.9	10.5 ± 1.4	8.5 ± 1.2
	Soy1, m.s.	17.7 ± 0.8	10.1 ± 1.1	7.9 ± 0.7	11.7 ± 1.0	7.8 ± 0.5
	Soy2, 21 d.b.h.	18.0 ± 1.2	9.5 ± 0.6	7.6 ± 0.6	11.2 ± 1.8	8.5 ± 0.7
	Soy3, 3 d.b.h.	18.4 ± 1.0	10.3 ± 0.4	8.2 ± 1.7	11.1 ± 0.9	9.3 ± 1.7

Significance:[d]	16:0	18:0	18:1	18:2	18:3
Oil treatment	NS	NS	NS	NS	NS
Storage	***	***	**	***	***
Oil treatment (storage)	NS	NS	NS	NS	NS

Values are means ± SD (n = 12). Mean separation by protected LSD (P≤0.05).

[a]16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid.

[b] m.s.= midseason, based on prediction model of harvest date for each apple variety using days after full bloom.

[c]d.b.h. = days before harvest.

[d]NS,*,**,*** = Not significant or significant at P≤0.05, P≤0.01, P≤0.001 respectively.

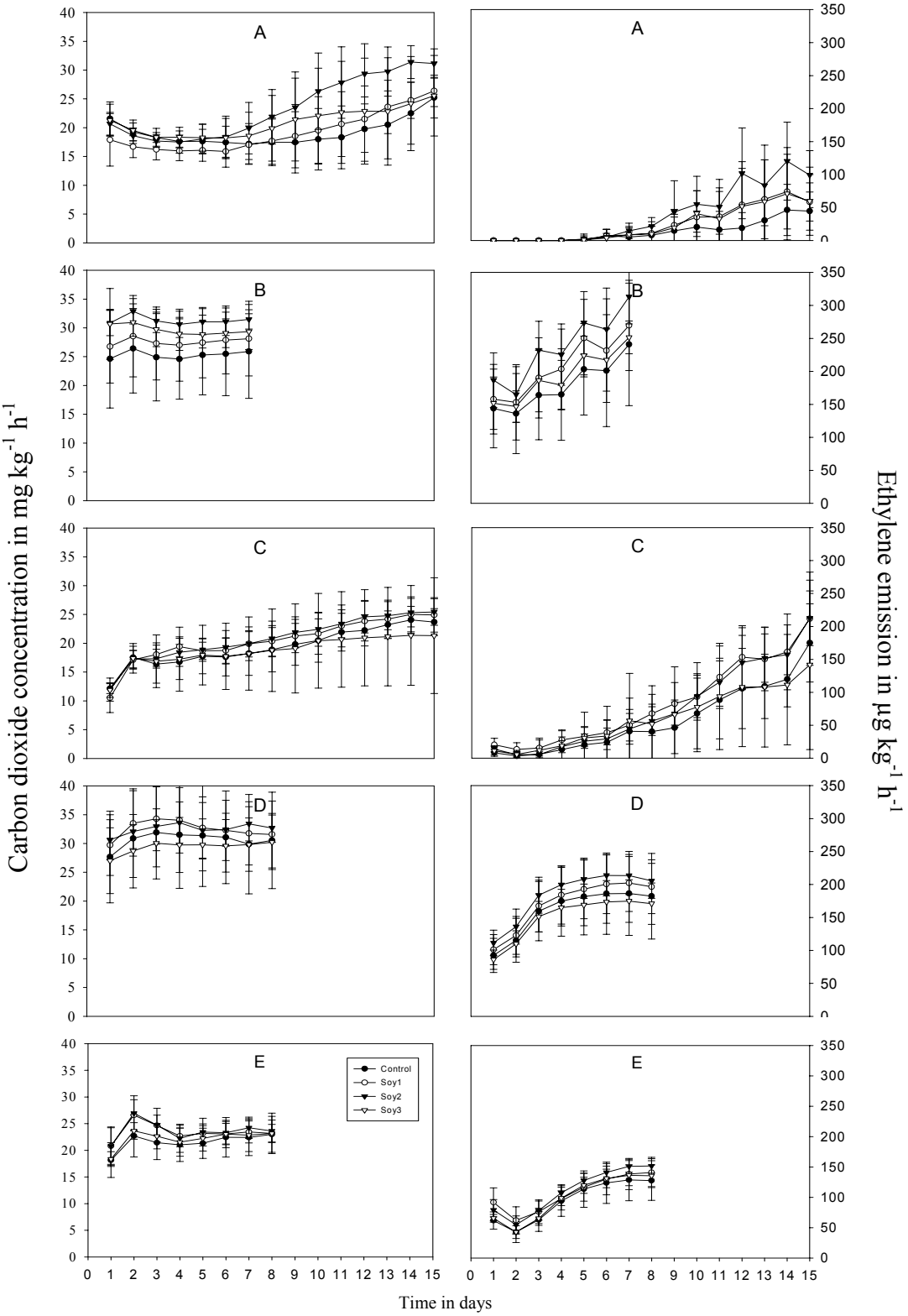

Figure 3.1. Progression of respiration and ethylene production for 'Golden Delicious' from Yakima. Vertical bars represent SE of means (n=3).
A = at harvest, B = 90 days in RA, C = 90 days in CA, D = 180 days in RA, E = 180 days in CA (RA = regular atmosphere, CA = controlled atmosphere).

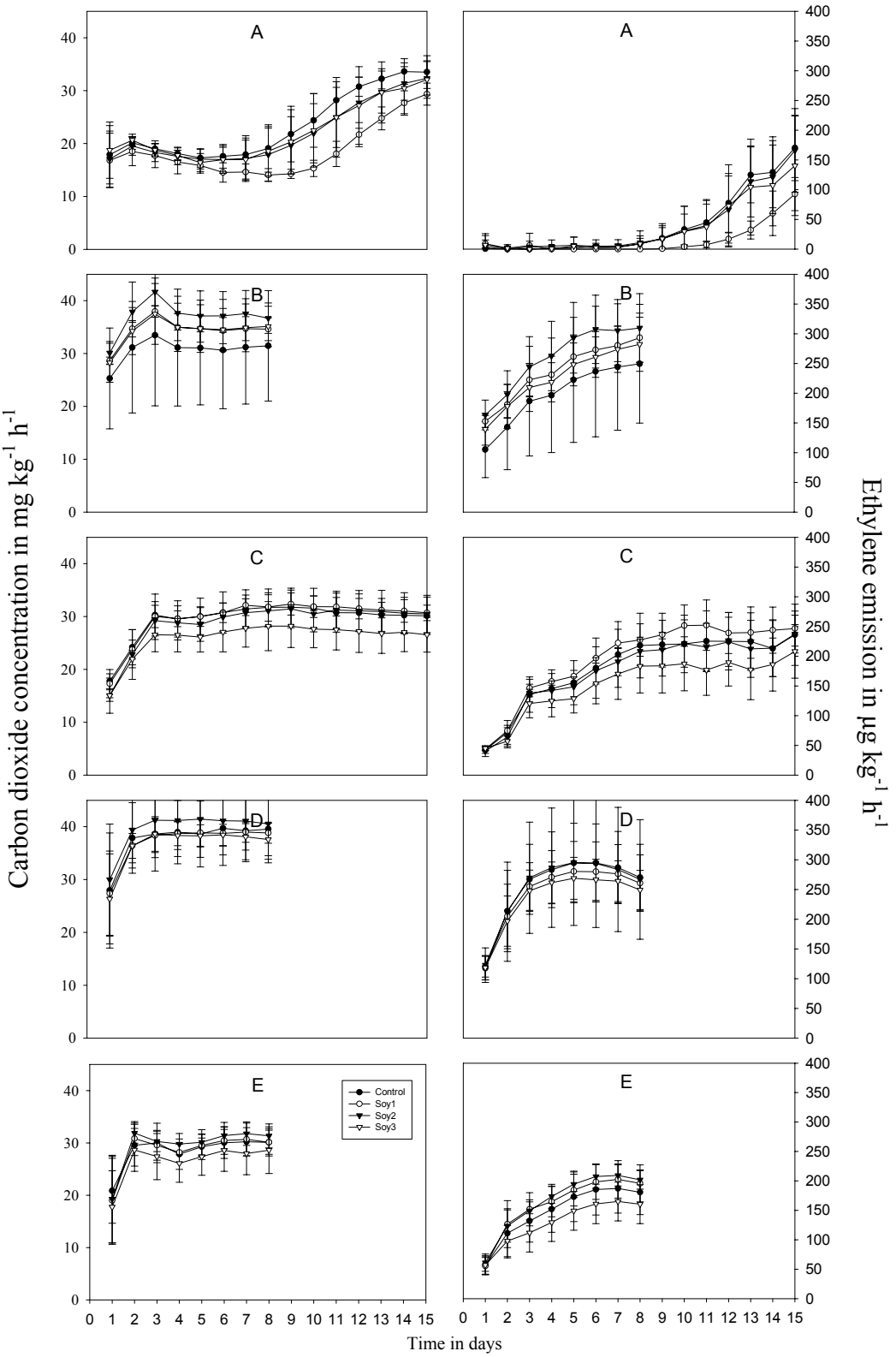

Figure 3.2. Progression of respiration and ethylene production for 'Golden Delicious' from Pullman. Vertical bars represent SE of means (n=3).
A = at harvest, B = 90 days in RA, C = 90 days in CA, D = 180 days in RA, E = 180 days in CA (RA = regular atmosphere, CA = controlled atmosphere).

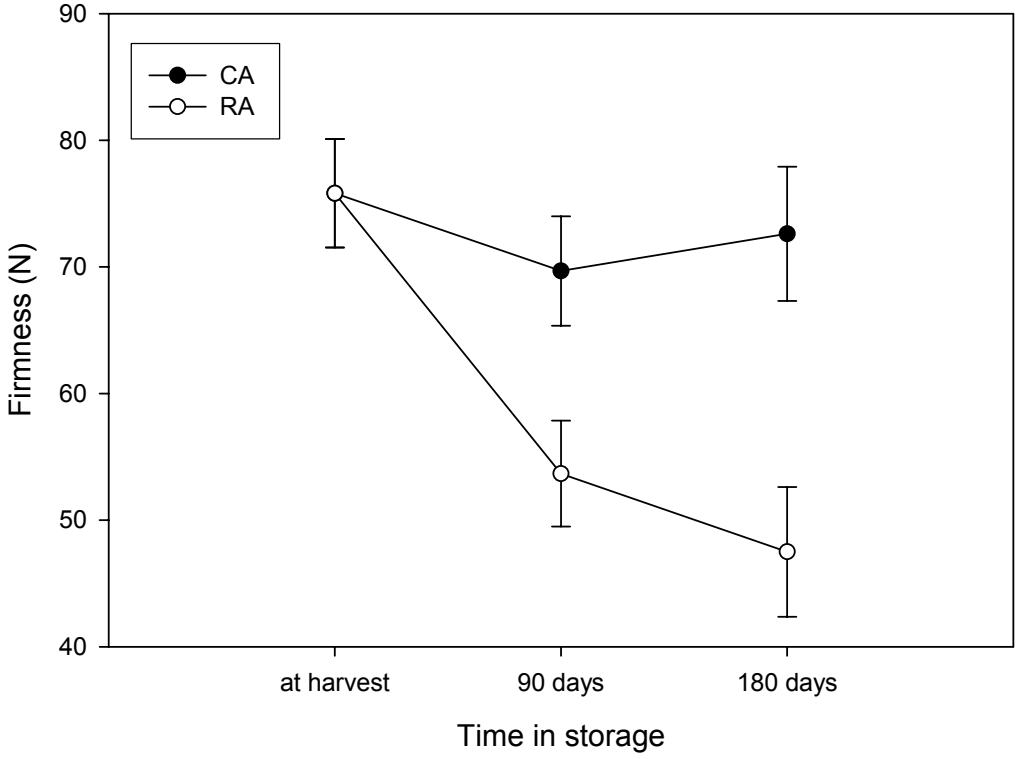

Figure 3.3. Changes in firmness (N) during regular (RA) and controlled atmosphere (CA) storage of untreated 'Golden Delicious' apples grown in Pullman. Vertical bars represent SE of means (n = 4).

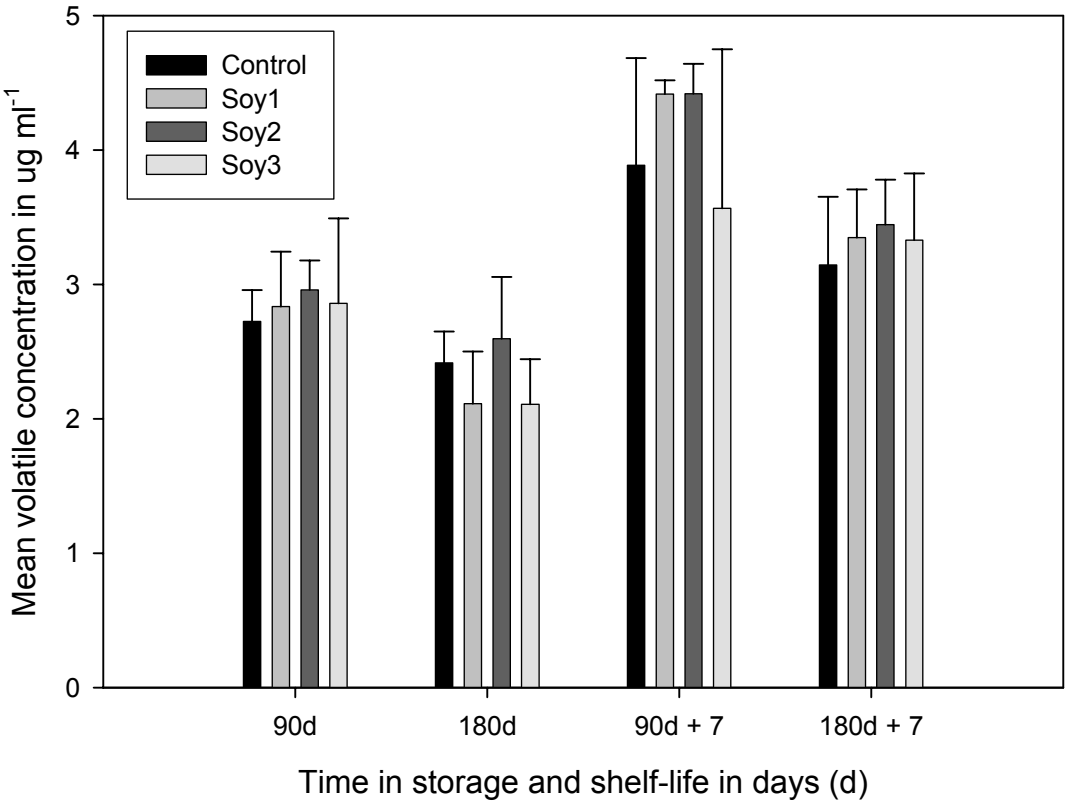

Figure 3.4. Flavour regeneration of Yakima grown 'Golden Delicious' apples after controlled atmosphere storage (CA) and 7 days at 22 °C. Vertical bars represent SE of means (n = 4).

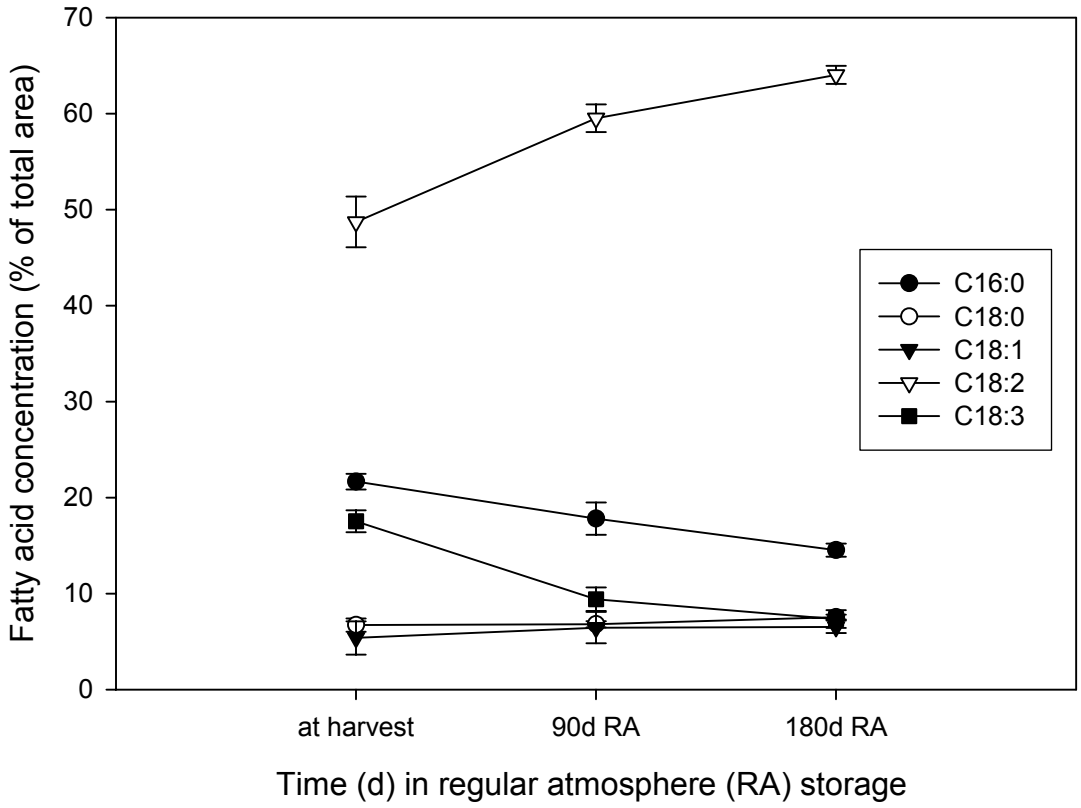

Figure 3.5. Production of five fatty acids in the peel of untreated 'Golden Delicious' apples during 180 days of regular atmosphere (RA) storage. Vertical bars represent SE of means (n = 4).

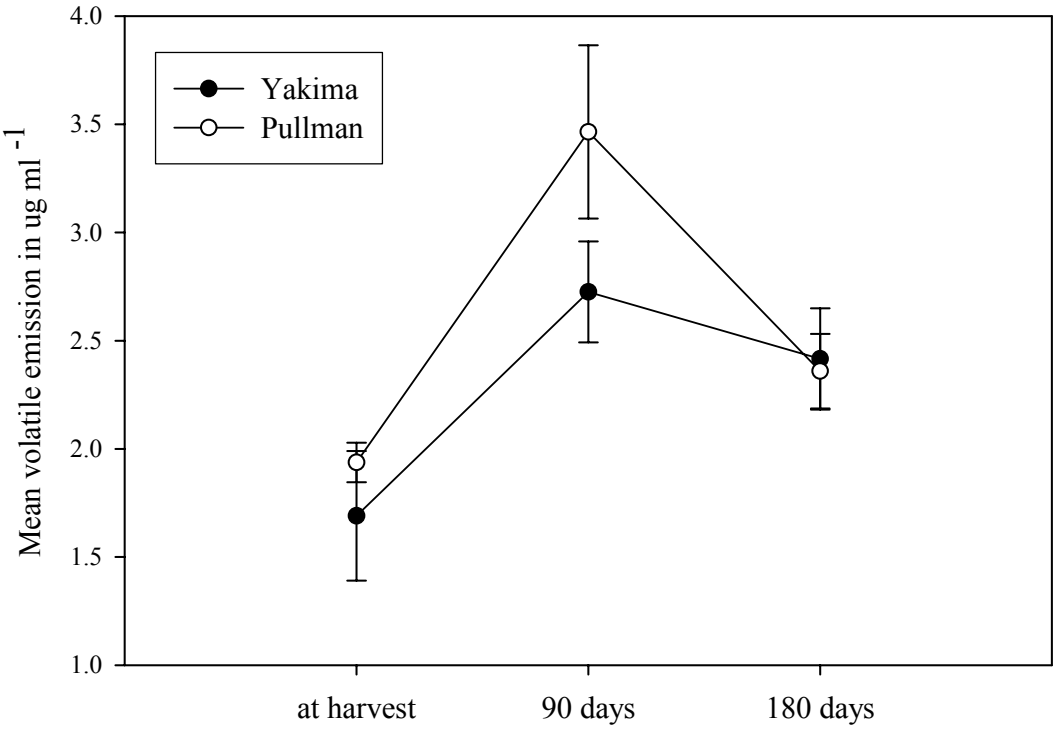

Figure 3.6. Changes in mean volatile emission of untreated 'Golden Delicious' apples grown in Yakima or Pullman during controlled atmosphere (CA) storage. Vertical bars represent SE of means (n = 4).

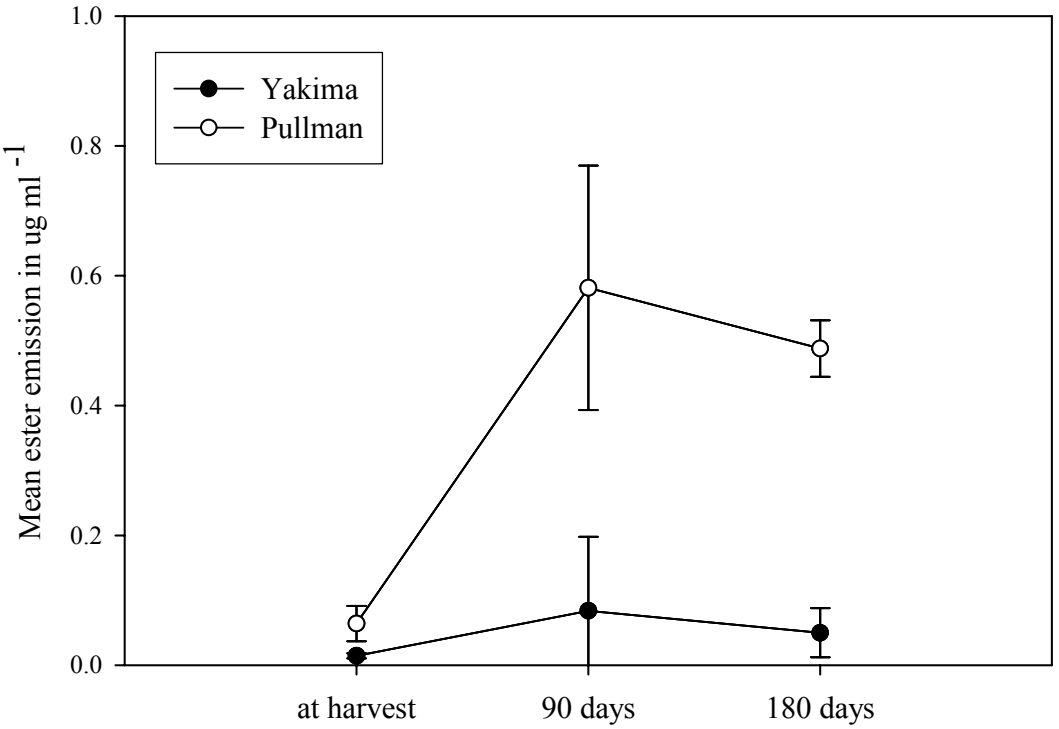

Figure 3.7. Changes in mean ester emission of untreated 'Golden Delicious' apples grown in Yakima or Pullman during controlled atmosphere (CA) storage.
Vertical bars represent SE of means (n = 4).

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CHAPTER 4

PREHARVEST SOYBEAN OIL AND POSTHARVEST 1-METHYLCYCLOPROPENE (1-MCP) APPLICATION TO 'GOLDEN DELICIOUS' APPLES AFFECTS VOLATILE AROMA PRODUCTION AFTER CONTROLLED ATMOSPHERE STORAGE

ABSTRACT

To study the effect of growing season applied soybean oil on pre- and post-storage respiration rates, volatile aroma regeneration capacities, colour development, and fatty acid metabolism of apples, one single treatment (21 days before harvest) of soybean oil emulsion (1% food grade oil, 0.1% Latron®) was administered to 'Golden Delicious' apple trees. Apples were harvested at commercial maturity. At the time of storage a second treatment was applied to either control or soybean treated pre-climacteric fruit, consisting of 1-methylcyclopropene (1-MCP). The fruit were then stored for up to 140 days at 0.5 °C under 1% O_2 and 0.2% CO_2 controlled atmosphere (CA) storage conditions and analyzed after holding at 22 °C for 0, 3, 7, 11, and 15 days. Additionally, fruit firmness, soluble solids content (SSC), titratable acidity (TA), and internal ethylene concentration (IEC) were determined at day 0.

No significant effects due to soybean oil treatment were noted for internal ethylene concentrations, fruit firmness, TA, SSC, fatty acid distribution, and yellowing of fruit colour. Soybean oil application significantly increased the mean volatile and aldehyde emission, as well as hexanal and 2-hexenal levels of fruit directly after CA storage (day 0). No significant treatment effects were observed for mean alcohol

concentrations of fruit after storage and/or at-harvest shelf-life period. Soybean oil application did improve regeneration capacities of straight chain esters and significantly increased the emission of the branched chain ester 2-methyl-butyl acetate compared to untreated fruit. 1-MCP treated fruit lost linolenic acid (C18:3) at a slower rate and linoleic acid (C18:2) did not increase as fast compared to untreated fruit stored under CA conditions.

INTRODUCTION

The interest in the use of alternative crop protection agents such as plant-based oils has increased due to heightened consumer demand for products grown without the use of synthetic chemicals, coupled with increased production from sustainable farming enterprises, and tighter regulations for agrochemicals due to environmental concerns. Horticultural oils, including plant-based oils, are used extensivley as insecticides, fungicides, and spray adjuvants (Cranshaw and Baxendale, 2004). Additionally, plant-based oils potentially improve fruit quality when applied before the harvest (Granger and Träger, 2002) or postharvest (Scott et al., 1995; Ju and Curry, 2000).

Our laboratory has studied the influence of preharvest soybean oil applications on fruit quality and storage behaviour of apples (*Malus domestica* Borkh.) (see chapter 3). We found that maturity indices such as fruit firmness, titratable acidity, soluble solids content of 'Golden Delicious' and 'Gala' apples were unaffected by a treatment with an emulsion containing 1% soybean oil. However, the apple volatile aroma emission was altered due to soybean oil application, for example 'Golden Delicious' apples treated with soybean oil three weeks before harvest emitted more aldehydes (mainly hexanal), while fruit treated with the oil three days before harvest produced more esters after

harvest and/or storage. Fruit grown in warmer conditions reacted with more pronounced changes within the volatile profile. Furthermore, fruit from soybean oil applications close to the anticipated harvest date had higher alcohol and ester regeneration capacities after fruit were kept at 22 °C for seven days following CA storage. Nothing is known about the persistence of those effects during longer shelf-life periods.

The main precursors of ester-, alcohol-, and aldehyde-volatiles produced by apple fruit during development and maturation are free fatty acids (FA) or those liberated by lipase activity and further metabolism by beta-oxidative enzymes and/or lipoxygenase. The limiting factor in aroma production is thought to be substrate availability rather than enzyme activity (Bartley et al., 1985; Bangerth et al., 1998). It is generally accepted that the enzymes required to catabolize FA (lipoxygenase, and/or beta oxidative enzymes) as well as downstream enzymes are not the limiting factors in aroma production. Supplying additional FA to fruit leads to incorporation into volatile aroma components, as has been shown by several authors (Bartley et al., 1985; Brackmann et al., 1993; Harb et al., 1994).

Plant oils are rich in fatty acids. There is indirect evidence that applying soybean oil to growing apples can provide FA for volatile aroma synthesis. First, soybean oil treatments altered aroma related volatile compounds without affecting fatty acid metabolism in the fruit peel (see chapter 3). Secondly, apples treated with soybean oil midseason or three weeks before harvest remained greener after storage without discernable differences in maturation patterns or FA proportions, when compared to untreated fruit (see chapter 3). Clearly, chloroplast breakdown, as suggested by Dixon and Hewett (2000b), did not provide FA, namely linoleic and linolenic acids, for volatile aroma biosynthesis in this case.

Ethylene can promote chlorophyll degradation in fruit (Purvis and Barmore, 1981) and is used commercially to promote degreening of bananas (*Musa* sp.) and tomatoes (*Lycopersicon esculentum* L.) before sale in retail markets. According to previous results (see chapter 3) chlorophyll breakdown was delayed in soybean oil treated fruit in an ethylene independent manner. Fan et al. (1998) and Fan and Mattheis (1999) have suggested this possibility in conclusion of their work on the involvement of methyl jasmonate in fruit ripening. In their experiments they noticed that 'Fuji' and 'Golden Delicious' apples degreened faster after treatment with methyl jasmonate without an increase in ethylene emission. In 'Golden Delicious' apples treated with aminoethoxyvinylglycine (AVG), an inhibitor of autocatalytic ethylene production, Halder-Doll and Bangerth (1987) found a non-appreciable effect on the chlorophyll degradation of epidermal tissue during cold storage. As summarized by Halder-Doll and Bangerth (1987), ethylene does not seem particulary important in regulating chlorophyll breakdown during storage of fruits such as bananas, tomatoes, and apples.

Whether the delay in degreening of apples after soybean oil application is truly ethylene independent could be investigated by utilizing a known ethylene action inhibitor (1-methylcyclopropene, 1-MCP) in plants, including apples (Fan et al., 1998).

The objective of this study was to determine the effects of a preharvest soybean oil application on colour development, fatty acid metabolism, and flavour regeneration capacity after controlled atmosphere storage and under shelf-life conditions. Furthermore, we tested whether soybean oil applications act independently of ethylene action on these parameters by inhibiting ethylene responses of 'Golden Delicious' apples with 1-MCP.

MATERIALS AND METHODS

Experimental design. In 2004 'Golden Delicious' (M7, 25 years old) apple trees grown in a commercial orchard near Buena (latitude 46°43´N, longitude 122° 21´W) in the Yakima Valley, WA, USA, were used in a randomized complete block design with four replications. Each replication consisted of 8 trees/ treatment of uniform size and fruit density. No overhead cooling was supplied. Fertilization, pest and disease control, irrigation and other orchard management procedures were performed according to industry standards and were the same for all treatments at the site.

Soybean oil treatment. The treatment consisted of a one-time application of an emulsion containing 1% food-grade soybean oil (Safeway Inc., Pleasanton, CA, USA) and 0.1% Latron B-1956, a nonionic surfactant (Rohm and Haas Co., Philadelphia, PA, USA). Treatments were applied to fruit and foliage, using a hand-held sprayer consisting of a single hollow cone nozzle, 5-horsepower, petroleum driven diaphragm pump. In an effort to completely coat every fruit, each treatment was applied to the point of runoff, which resulted in a rate of two gallons/tree (7.6 liters). The oil emulsion was sprayed three weeks before harvest. To avoid phytotoxic reactions, trees were sprayed in the evening after temperatures had dropped below 90 °F (32 °C). As the orchard location receives almost no natural precipitation during the summer, possible rain events were disregarded when timing the oil application.

Fruit quality analyses. Apples were harvested at commercial maturity as determined by the orchard management. Fruit was picked exclusively from lateral branches between one and two meters from the orchard floor. Only fruit of uniform size and without visible defects were used. Harvest maturity and quality indices as well as internal ethylene concentration, evolved ethylene, respiration, volatile aroma compound

emission, fruit colour development, and peel fatty acid distribution were analyzed at each harvest and after storage. All fruit was allowed to stabilize at ambient laboratory temperature (22 °C) for 12 hours before measurements were taken. Unless otherwise stated, all measurements were performed on four individual replications per treatment based on field replications.

1-MCP treatment. Immediately after harvest and transport to the storage facilities apples were treated with 1-methylcyclopropene (1-MCP) by placing fruit in a 49.21 l (13 gal) autoclave bag with a 50 ml beaker containing 1-MCP from EthylBloc® (BioTechnologies for Horticulture, Inc., Walterboro, SC, USA) according to label recommendations to obtain a concentration of 0.70 $\mu l\ l^{-1}$. The bag was quickly sealed and the apples were pulsed for 12 hours at 22 °C.

Storage and shelf-life treatments. After treatment with 1-MCP all fruit was cooled to 32 °F (0.5 °C) and placed into 180 l plexiglass chambers for 140 days of storage at 0.5 °C in a 1.0 kPa oxygen and 0.2 kPa carbon dioxide controlled atmosphere. Fruit was moved into regular atmosphere (RA, 0.5 °C) storage one week prior to sampling. Using volatile aroma compound emission, flavour regeneration capacity for fruit as well as fruit colour change, and fatty acid concentrations were determined after 0, 3, 7, 11, 15 days at 22 °C shelf-life conditions directly after harvest and following CA storage.

Internal ethylene, ethylene evolution, and fruit respiration. Evolved ethylene and CO_2 production were measured by placing quadruplicate samples of preweighed groups of five apples/treatment for two weeks in air tight flow chambers using an automated sampling system (Patterson and Apel, 1984). These plexiglass chambers (18 l)

were supplied with ethylene-free air at approximately 100 ml min^{-1}. The carbon dioxide (CO_2) and ethylene (C_2H_4) concentrations from each chamber were automatically measured every 8 h for 24 h using a HP 5890A gas chromatograph (Hewlett-Packard Co., Palo Alto, California, USA) equipped with a thermal conductivity detector connected each to a GS-Q PLOT column (0.53 mm x 30 m) (Agilent Technologies, Avondale, PA, USA), and an electronic switching valve. Oven, injector, and detector temperatures were held at 30 ˚C, 90 ˚C, and 200 ˚C, respectively. The helium carrier gas flow rate was 8 and 10 ml min^{-1} for CO_2 and C_2H_4.

Internal ethylene concentration (IEC) was assayed by withdrawing a 0.5 cm^3 gas sample from the core space of a whole apple fruit through the calyx end of the fruit into the core with a 50-gauge hypodermic needle fitted with a serum stopper (n = 20). The gas sample was injected into a gas chromatograph (HP 5830A, Hewlett Packard, Palo Alto, CA, USA) with a J&W CARBONPLOT column (30 m x 0.53 mm I.D.) with 3 µm film. The injector was a packed type with a flame ionization detector. The pre-purified nitrogen gas flow was adjusted to 8 mL/min. Injector and detector temperature was set at 200 °C and the oven temperature was set at 100 °C.

Measurements of fruit quality. All quality measurements were taken from eight fruit from each of four replications. Flesh firmness was analyzed using a Topping penetrometer (Topping, 1981). Starch content was assayed by staining transverse cross-sections of the fruit with iodine (I-KI) and visually rating the color change using a 1-5 scale (1 = 100% of the area stained; 5 = 0%). Soluble solids content was estimated using a Reichert ABBE Mark II refractometer (AO Scientific Instruments, Keene, NH, USA).

Titratable acidity (malic acid) was measured using a Metrohm 672 autotitrator (Herisau, Switzerland) and expressed as malic acid equivalents.

Volatile aroma compound emission. Four replications containing juice from eight fruit/replication were stored at –20 °C until analysis. Solid phase microextraction (SPME) was employed to determine the concentration of major volatile compounds in apple flesh. One sample consisted of 2.0 ml of apple juice with 0.65 g NaCl in a 4.0 ml vial with a Teflon lined cap. The SPME device (Supelco, Co., Bellefonte, PA, USA) consisted of a fused silica fiber coated with 65 μm poly(dimethylsiloxane)/ divinylbenzene phase. The SPME fiber was exposed to the headspace of the sample for exactly 1 hour before GC injection. SPME injection was achieved by splitless injection for 2 min. at 200^{o}C into a Hewlett-Packard 5890II/5970 GC/MSD equipped with a DB-1 column (60 m x 0.32 mm, 0.25 μm film). Chromatographic conditons were as described by Mattheis et al., (1991) except transfer line temperature and ion source was held at 250 °C. The GC inlet contained a 0.75mm SPME injection sleeve which assures peak sharpness, especially for early eluting peaks (Yang and Peppard, 1994). The compound identification was made by comparing the spectra from sample compounds to those contained in the Wiley-NIST library and by comparing retention indices of sample compounds and authentic standards. Quantification was accomplished by using selected ion monitoring for base peaks. Values were calculated using response factors generated from injection of authentic standard compounds.

Peel fatty acid content. Tissue disks (1.2 cm diameter, 3 mm thick) of fruit peel tissue (including epidermis, hypodermis, and several cell layers of cortical tissue) were obtained from each of 5 apples per replication and immediately frozen in liquid nitrogen

and stored at –40 °C until further analysis. To determine the fatty acid content of fruit peel tissue, eight disks per replication were randomly chosen. Triplicate samples of tissue (5 x 5 mm) were placed into glass screw-capped centrifuge tubes and immediately topped with 1ml of 2.5% H_2SO_4 in methanol to extract the fatty acids from the tissues and transmethylate them. The samples were capped and incubated at 80 °C for 1h. After the addition of 0.4ml of hexane and 1.5 ml H_2O, the fatty acid methyl esters were extracted into the hexane layer by vigorously shaking and centrifuging the tubes at low speed until the hexane layer was clear. Samples (2 µl) of the organic phase were analyzed by GC using an Agilent 6890 series gas chromatograph (Agilent Technologies, Avondale, PA, USA) equipped with an Alltech AT-WAX column having a film thickness of 0.25µm, helium as the carrier gas at 1.4 ml/min, and a flame ionization detector. The GC was programmed for an initial temperature of 150 °C. The temperature was ramped to 200 °C at a rate of 5 °C/min followed by an increase to 204 °C at 1.5 °C/min, and reaching the final temperature of 260 °C at a rate of 30 °C/min after a total runtime of 16.53 min. The carrier gas flow rate was 1 ml/min. The peaks were identified based on co-chromatography with authentic standards (Nu Check Prep, Elysian, MN, USA) and quantification achieved by generation of detector response factors for individual constituents. Previous work (Song and Bangerth, 2003) determined dynamic fluctuations during fruit growth and development to occur mainly among five fatty acids. These fatty acids are: palmitic (C16), stearic (C18), oleic (18:1), linoleic (18:2), and linolenic (C18:3) acids and are also the predominant fatty acids in soybean oil (Hamilton, 1993). Individual fatty acid content is reported as percent of five detected fatty acid total.

Fruit colour. Fruit colour was measured on five fruit/replication with a Minolta CR-200 chroma meter (Minolta Co., Ramsey, NJ, USA) using the CIE L*a*b coordinate system. The meter was calibrated before use with a white standard (Minolta model CR 200/300). At each sampling time, peel ground colour was measured three times around the fruit's equator. Care was taken to avoid marked or discoloured areas of the skin. Chroma and hue angle were calculated according to McGuire (1992).

Statistical analyses. Data for each variety and location were analyzed separately as a randomized complete block design or completely randomized design respectively. An analysis of variance (PROC GLM) was carried out using SAS statistical software (SAS Institute, Cary, NC, USA). The separation of means was accomplished using the protected least significant difference (LSD) test at the five percent level.

RESULTS

Internal ethylene, ethylene evolution, and fruit respiration. At-harvest fruit was in preclimacteric condition as indicated by respiration rate measurements (Fig. 4.1.). After 140 days in CA storage apples had entered the respiratory climacteric. The ascending respiration rates of fruit at harvest were between 15 and 25 mg kg^{-1} h^{-1} and between 12 and 20 mg kg^{-1} h^{-1} after CA storage (Fig. 4.1). 1-MCP treated fruit remained in the preclimacteric state for 33 days after CA storage (data not shown) The trend of the ethylene emission rates followed the pattern of respiration and reached 50 µg kg^{-1} h^{-1} at the end of the at-harvest recording period. Fruit emitted ethylene at rates up to 120µg kg^{-1} h^{-1} after CA storage (Fig. 4.1). Apples treated with 1-MCP did not start to emit ethylene until 33 days after CA storage. Although respiration and ethylene emission rates of fruit

treated with soybean oil emulsions were always below control fruit levels, no significant treatment effects were noted. IEC concentrations of apples were below detectable levels at harvest and after 1-MCP-treated fruit had been stored for 140 days under CA conditions (Table 4.1). Fruit stored in CA without prior 1-MCP treatment had between 23.5 and 28.9 $\mu l\ l^{-1}$ of internal ethylene. No significant effect on IEC due to soybean oil treatment was observed.

Measurements of fruit quality. Fruit firmness decreased by 4-7 N and TA by 0.09-0.16% while SSC increased by 1 to 1.5 % during CA storage (Table 4.1). No significant treatment effects were noted.

Volatile aroma compound emission. The mean volatile and aldehyde emission rates from apples nearly doubled when fruit was stored for 140 days under CA conditions, regardless of whether it was treated with 1-MCP before storage (Table 4.2, Fig. 4.2). Soybean oil application significantly increased the mean volatile and aldehyde emission, as well as hexanal and 2-hexenal levels of fruit after CA storage (Tables 4.2, 4.3, Fig. 4.3). During the 15 day shelf-life period immediately following harvest, the mean volatile emission of apples increased (10-50 %), with control fruit emitting significantly more volatiles after 7 and 15 days (Fig. 4.2). The mean volatile emission of fruit also increased during the shelf-life period following CA storage as well (25-100 %) without significant treatment effects. The mean aldehyde emission of fruit decreased approximately 20% during the at-harvest shelf-life period (Table 4.2). Untreated fruit emitted aldehydes significantly above soybean oil treated fruit after 7 days. In contrast, after CA storage, regardless of 1-MCP treatment, the mean aldehyde emission of fruit increased over time (25-50 %) when fruit was kept at 22 °C. No significant treatment

effect on mean aldehyde emission rates was observed during the shelf-life study. However, 2-hexenal emission of untreated fruit was significantly higher compared to soybean oil- treated apples after 7 and 11 days of shelflife. 2-Hexenal emission of 1-MCP treated control fruit was significantly above soybean oil treated fruit after 7 days at ambient temperature.

The mean alcohol and ester emission rates from fruit increased during 140 days of CA storage compared to at-harvest emission rates (3 to 4-fold during CA, 2-fold during CA + 1-MCP) (Table 4.2, Fig. 4.2). Fruit treated with 1-MCP had declining rates of alcohol and ester emission (-50 %), while fruit stored under CA conditions without prior 1-MCP treatment exhibited increased emission rates of alcohols and esters when kept at ambient temperatures for 15 days following storage (3-4 fold). During the shelf-life period following CA storage, mean ester emission rates of soybean oil treated apples were significantly above emission rates from untreated fruit after 7 and 11 days (Table 4.2). Butanol emission was significantly higher in 1-MCP treated fruit without prior soybean oil application (control + 1-MCP), and 1-pentanol emission rates of untreated fruit exhibited a treatment effect significantly above the soybean oil treatment at harvest (Table 4.3). Soybean oil application had no statistically significant effect on mean alcohol and ester emission rates of fruit during the at-harvest shelf-life period (Table 4.2, Fig. 4.2). After seven days at ambient temperature untreated fruit emitted 2-methyl butanol and pentyl acetate at significantly higher rates when compared to soybean oil treated apples (Table 4.3). However, soybean oil application did significantly increase 2-methyl-butyl acetate emission af apples compared to untreated fruit, regardless of storage regimen (overall treatment effect) (Fig. 4.4). After CA storage, fruit treated with soybean

oil had significantly higher 2-methyl-butyl acetate emission rates beginning after day 7 at 22 °C (Table 4.3).

Peel fatty acid content. The fatty acid (FA) content of the fruit skin was expressed as percent (%) of total FA content based on the five FA included in the analysis (see material/methods section). The most predominant FA in the apple fruit peel was linoleic acid (C18:2, around 50% of total) (Table 4.4). Untreated fruit showed a declining proportion of palmitic (C16:0) and linolenic (C18:3) acid, constant levels of stearic (C18:0) and oleic (C18:1) acid, and increasing proportions of linoleic (C18:2) acid during storage (Table 4.4, Fig. 4.5). Overall, the proportion of linoleic acid increased between 6 and 9 % during CA storage, which was almost the exact rate at which linolenic acid decreased at the same time. Similar trends were observed for fruit treated with 1-MCP, however linolenic acid was lost at a slower rate, thus linoleic acid did not increase as fast.

No significant treatment effects were determined (Table 4.4) when peel fatty acids were measured directly at harvest or after CA storage. During the at-harvest shelf-life period, untreated fruit contained significantly higher levels of C18:0 (day 0) and C18:3 (day 7) fatty acids compared to soybean oil-treated apples. After 140 days of CA storage untreated fruit had significantly higher C18:2 fatty acid levels after three days at 22 °C. 1-MCP treated fruit with preharvest soybean oil application had significantly higher C18:1 fatty acid concentrations (7 and 11 days) during the shelf-life period, while C18:2 concentrations were significantly lower compared to control levels after 11 days on the shelf.

Fruit colour. During 'Golden Delicious' apple fruit ripening, as observed during the at-harvest shelf-life study and after CA storage, L*, a*, and b* values increased, resulting in decreased hue values, increased chroma values, and a visual colour change from green to yellow (Table 4.5). Fruit treated with 1-MCP did not show changes in chroma and had a reduced loss of hue values after storage and during the subsequent shelf-life period. No significant treatment effects due to soybean oil application were noted.

DISCUSSION

Respiration rates and ethylene emission of apples were not affected by the soybean oil treatment at harvest or after storage according to the present study. Previous studies in our laboratory had found soybean oil to alter those parameters in apples at harvest only, depending on the time of application. In comparison, Ju and Curry (2000) found a 2.5% stripped corn oil emulsion to have no effect on ethylene emission rates in 'Granny Smith' apples, while oil concentrations of 5 or 10% supressed ethylene emission for the first 3 months of storage, which later exceeded control levels. Other maturity indices such as IEC, firmness, SSC, and TA were unchanged after oil application according to our study. Thus we conclude, a 1% emulsion of soybean oil applied within three weeks of harvest has no influence on the rate of maturation of 'Golden Delicious' apples as well as IEC, firmness, SSC, and TA. We speculate that oil concentration might have been the determining factor for the observed response.

Soybean oil application as observed in the present study did not visibly change the rate of loss of green colour compared to untreated fruit as observed in earlier studies in

our lab (see chapter 3) and elsewhere (Ju and Curry, 2000; Ju et al., 2000a). The degreening of apples is influenced by a variety of preharvest cultural and environmental factors such as fruit nutrition, mineral content, and climatic conditions (Fallahi, 1985; Reay et al., 1998). Most notably nitrogen content can have a marked influence on chlorophyll degradation. Fruit mineral content was not assessed in the current study, hence altered nutritional status remains a possible explanation for retention/loss of green colour. Year to year variation in climatic conditions may also explain inconsistent degreening responses. The colour of apple fruit is determined primarily by the relative amounts of pigments in the fruit skin, namely chlorophylls, carotenoids, and anthocyanins (Saure, 1990). Fruit pigments undergo considerable changes during fruit development. For 'Golden Delicious' apples, the change in skin colour from green to yellow is primarily associated with a decline in chlorophyll concentration and an increase in xanthophylls (Workman, 1963). According to Gross and Lenz (1979) xanthophylls, particularily violaxanthin, show a steep increase during maturation, while the loss of chlorophyll is relatively slow. Hence, the importance of carotenoid metabolism for colour development of 'Golden Delicious' apples is probably underestimated when evaluating methods that alter the degreening rate. Finally, as Ju and Curry (2000) and Ju et al. (2000a) reported, green colour loss was also influenced by application of higher oil concentrations. Further studies using higher oil concentrations to evaluate the effect on degreening are needed.

Generally, the most pronounced change in the FA profile of 'Golden Delicious' observed in this and previous studies is the loss of linolenic acid (C18:3) and the subsequent increase in the less unsaturated linoleic acid (C18:2). Linolenic acid was lost

at a slower rate in fruit treated with 1-MCP, thus linoleic acid did not increase as fast. Our results are in agreement with Song and Bangerth (2003), who described a rapid increase of linoleic acid (C18:2) during the climacteric rise of 'Golden Delicious' apples or after ethylene treatment of pre-climacteric fruit. They also described a strong positive correlation between IEC and the concentration of C18:2. However, we did not find a considerable increase in the C18:1 fraction of the FA profile, as previously observed by Song and Bangerth (2003). We analyzed FA based on concentrations in all lipid fractions, yet changes during ripening are known to occur predominantly in the smallest lipid fraction (free FA portion) (Song and Bangerth, 2003). As a result, changes in a less dominant FA, such as C18:1, could remain undetected in our work.

No significant treatment effects due to soybean oil application were determined when fatty acids were measured on fruit directly at harvest or after CA storage, validating results from previous work. Though not significant, soybean oil-treated fruit seemed to retain more fatty acid unsaturation during the shelf-life study following CA storage. Alternately, 1-MCP treated fruit with preharvest soybean oil application had higher oleic acid (C18:1) concentrations during the shelf-life period while C18:2 concentrations were lower compared to control fruit. This may indicate slightly increased metabolic activity due to soybean oil application while partially overcoming inhibitory effects due to 1-MCP treatment. To the best of our knowledge no studies have investigated the relationship between blocking ethylene reception and fatty acid metabolism.

At harvest, all major volatile groups of soybean oil treated fruit were lower than controls. However, this effect appeared reversed after CA storage. One explanation would be the breakdown of the oil during storage, thus supplying FA precursors for

volatile aroma metabolism. Another possible scenario would be the interaction of cold temperatures, low oxygen concentration and the oil under CA storage conditions. As suggested by Ju and Curry (2000b), plant oils may alter the susceptibility of apples to chilling injury caused by sub-optimal temperatures. Aldehyde emission rates of 1-MCP treated fruit were comparable to control fruit, yet the post-stoarge emission rate increased slower compared to that of controls after removal from CA storage. Soybean oil application did not significantly influence the reaction of the fruit to 1-MCP. Apparently, fruit treated with 1-MCP did not cease to produce aldehydes after CA storage. But the initial high rate might have been due to temperature-induced outgassing of accumulated compounds (Fellman et al., 2003).

Aldehydes are the dominant detectable volatile compounds in intact immature fruit contributing to the green notes of apples and are known to decrease in concentration as apples mature (De Pooter et al., 1987; Mattheis et al., 1991). The overall increase in aldehyde emission of fruit after CA storage observed in our study might be related to sample preparation (see chapter 3). Hexanal and 2-hexenal, the most abundant aldehydes observed in our study, are important flavour impact compounds in 'Golden Delicious' apples (De Pooter et al., 1987). After CA storage soybean oil treated fruit emitted more aldehydes compared to untreated fruit. However, during the shelf-life period this effect disappeared. It seems likely that aldehydes were converted to respective alcohols and esters, since supplying aldehydes can increase the overall aroma production of apples, by stimulating alcohol and ester synthesis (Song and Bangerth, 1994). Higher aldehyde production in fruit after soybean oil treatment, as observed in our study has the potential to improve aroma development.

Esters are the most significant contributors to aroma in apples, accounting for up to 80% of odour active volatiles in 'Golden Delicious' (López et al., 1998). Among the most prevalent esters described for 'Golden Delicious' are butyl acetate, 2-methyl-butyl acetate and hexyl acetate (Brackmann et al., 1993). Alcohols serve as important precursors of esters in apples (Dimick and Hoskin, 1983). Thus any reduction in concentration of alcohols will adversely affect the most significant group of flavour molecules. Increased acetate ester production is directly related to the availability of related alcohol precursors (Echeverria et al., 2004). The elevated amount of carbon dioxide in CA atmospheres inhibits not only respiration but acts as an antagonist of aroma synthesis. Carbon dioxide inhibits alcohol hydrogenase acitivity and thus acts on carboxylic acid metabolism (De Pooter et al., 1987). All ester regeneration capacity was improved for straight chain compounds due to soybean oil application. The branched chain ester 2-methyl-butyl acetate showed a significant treatment effect due to soybean oil application, regardless of storage treatment with the most pronounced response observed during the shelf-life period after CA storage.

The increase in ester regeneration capacity was facilitated by the availability of necessary alcohol precursors, the concentration of which also increased during the shelf-life. However, the increased capability to regenerate esters, particularily the branched chain ester 2-methyl-butyl acetate cannot be easily explained because alcohol emission rates did not show treatment effects due to soybean oil application. Generally, it is thought that branched-chain esters are derived from amino acid metabolism (Drawert et al., 1973). Thereby, alcohol acyl-CoA transferase (AAT) combines alcohols and CoA derivatives of fatty acids to form esters. Echeverria et al. (2004) reported that the rise in

acetate esters in 'Fuji' apples was caused by increased substrate availability, rather than from increased enzyme activity. On the other hand, Rowan et al. (1996) suggested that different enzyme activity and selectivity, rather than substrate availability of the amino acid degradation pathway determines the concentration of branched chain esters, and is cultivar specific in apples. It seems possible that soybean oil application induced changes in the concentration of the acyl-CoAs by supplying fatty acids to the β-oxidation catabolic pathway. However, as noted by Dixon and Hewett (2000a), little is known of the factors that affect acyl-CoA concentrations and synthesis in fruits; this would be a most interesting and valuable topic for further research.

Apparently, the primary mode of action for soybean oil treatment is an increase in the supply of fatty acid precursors for volatile aroma synthesis. This would explain why no major changes in the FA profiles were observed, since additional substrate was metabolised immediately. The limiting factor in aroma production is thought to be substrate availability rather than enzyme activity (Bartley et al., 1985; Bangerth et al., 1998; Song and Bangerth, 2003). It is generally accepted that the enzymes required to catabolize FA (lipoxygenase, and/or beta oxidative enzymes) as well as downstream enzymes are not the limiting factors in aroma production. Supplying additional FA to fruit leads to incorporation into volatile aroma components, as has been shown by several authors (Bartley et al., 1985; Brackmann et al., 1993; Harb et al., 1994).

Table 4.1. Influence of soybean oil treatments on firmness, soluble solids content (SSC), titratable acidity (TA), and internal ethylene concentration (IEC) of 'Golden Delicious' apples from Yakima in the 2004 season. Measurements were taken at harvest, and after 140 days in controlled atmosphere (CA) storage.

Quality index	At harvest		After Storage			
			CA		1-MCP[b] + CA	
	Control	Soy[a]	Control	Soy	Control	Soy
Firmness (N)	73.967	74.930	67.122	68.784	70.401	68.139
SSC (%)	12.303	12.147	13.276	13.513	13.613	13.600
TA (%)	0.716	0.698	0.599	0.592	0.575	0.612
IEC ($\mu l \cdot l^{-1}$)	nd[c]	nd	28.872	23.491	nd	nd

Mean separation by protected LSD ($P \leq 0.05$).
Means (n = 4) within the same column and quality index are not significantly different.

[a] Soybean oil was applied as a 1% emulsion three weeks before harvest.

[b] 1-Methylcyclopropene was applied after harvest at 0.7ul l^{-1} for 12 hours .

[c] nd = not detectable.

Table 4.2. Influence of soybean oil treatments on major volatile groups of 'Golden Delicious' apples from Yakima in the 2004 season. Measurements were taken at harvest, and after 140 days in controlled atmosphere (CA) storage.

Volatile group ($\mu g\ ml^{-1}$)	Days at 22°C	At harvest		After Storage			
				CA		1-MCP[b] + CA	
		Control	Soy[a]	Control	Soy	Control	Soy
Means	0	2.240	2.267	3.678 b	4.733 a	4.156	4.170
	3	1.820	1.935	5.224	5.098	3.994	4.154
	7	2.440 a	1.958 b	7.102	7.173	4.856	4.804
	11	2.549	2.534	7.574	7.476	4.921	5.096
	15	3.069 a	2.561 b				
Aldehydes	0	2.123	2.180	3.299 b	4.416 a	3.981	3.963
	3	1.702	1.853	5.033	4.945	3.843	3.992
	7	2.204 a	1.858 b	6.403	6.281	4.740	4.682
	11	2.002	2.158	6.122	5.693	4.826	4.992
	15	1.724	1.609				
Alcohols	0	0.047	0.035	0.219	0.160	0.076	0.095
	3	0.078	0.048	0.099	0.090	0.077	0.071
	7	0.129	0.061	0.353	0.397	0.056	0.056
	11	0.272	0.211	0.713	0.765	0.052	0.056
	15	0.691	0.470				
Esters	0	0.069	0.053	0.160	0.157	0.099	0.112
	3	0.040	0.034	0.093	0.064	0.074	0.091
	7	0.106	0.039	0.346 b	0.494 a	0.060	0.066
	11	0.275	0.165	0.739 b	1.018 a	0.044	0.048
	15	0.654	0.482				

Mean separation by protected LSD ($P \leq 0.05$).
Means (n = 4) followed by different letters within the same time at 22 °C and storage treatment are significantly different.

[a] Soybean oil was applied as a 1% emulsion three weeks before harvest.

[b] 1-Methylcyclopropene was applied after harvest at $0.7ul\ l^{-1}$ for 12 hours .

Table 4.3. Influence of soybean oil treatments on single volatile compounds of 'Golden Delicious' apples from Yakima in the 2004 season. Measurements were taken at harvest, and after 140 days in controlled atmosphere (CA) storage.

Volatile compound ($\mu g\ ml^{-1}$)	Days at 22°C	At harvest		After Storage				Treatment effect
				CA		1-MCP[c] + CA		
		Control	Soy[b]	Control	Soy	Control	Soy	
Aldehydes								
Cis-3-Hexenal	0	0.451	0.580	nd	nd	nd	nd	not significant
	3	0.065	0.187	nd	nd	nd	nd	
	7	nd[a]	0.018	nd	nd	nd	nd	
	11	0.021	0.023	nd	nd	nd	nd	
	15	nd	nd					
Hexanal	0	1.372	1.320	2.662 b	3.601 a	3.101	3.079	not significant
	3	1.244	1.286	4.137	4.001	2.979	3.166	
	7	1.799	1.752	5.546	5.429	3.842	3.826	
	11	1.635	1.751	5.342	4.993	3.996	4.155	
	15	1.469	1.413					
2-Hexenal	0	0.300	0.280	0.637 b	0.815 a	0.880	0.884	not significant
	3	0.393	0.380	0.895	0.943	0.865	0.826	
	7	0.405 a	0.088 b	0.857	0.852	0.898 a	0.856 b	
	11	0.346 b	0.384 a	0.780 a	0.700 b	0.830	0.837	
	15	0.255	0.197					
Alcohols								
1-Butanol	0	0.026	0.016	0.105	0.059	0.012 a	0.005 b	not significant
	3	0.037	0.025	0.014	0.008	nd	nd	
	7	0.079	0.026	0.107	0.136	nd	nd	
	11	0.172	0.139	0.287	0.354	nd	nd	
	15	0.534	0.363					
2-Methyl-1-butanol	0	0.003	0.002	0.029	0.031	0.036	0.043	not significant
	3	0.007	0.004	0.030	0.028	0.031	0.034	
	7	0.013 a	0.007 b	0.099	0.109	0.014	0.014	
	11	0.028	0.018	0.199	0.196	0.006	0.007	
	15	0.055	0.033					
1-Pentanol	0	0.001 a	0.001 b	0.002	0.002	0.001	0.001	not significant
	3	0.002	0.001	0.001	0.000	0.000	0.000	
	7	0.001	0.002	0.004	0.005	0.000	nd	
	11	0.048	0.004	0.008	0.008	nd	nd	
	15	0.006	0.004					
1-Hexanol	0	0.017	0.016	0.083	0.069	0.028	0.046	not significant
	3	0.032	0.019	0.054	0.053	0.046 a	0.037 b	
	7	0.037	0.025	0.143	0.147	0.042	0.042	
	11	0.068	0.050	0.218	0.206	0.045	0.049	
	15	0.096	0.070					
Esters								
Butyl acetate	0	0.041	0.032	0.089	0.062	0.011	0.009	not significant
	3	0.021	0.017	0.016	0.007	0.000	0.000	
	7	0.067	0.017	0.110	0.157	0.001	0.001	
	11	0.192	0.109	0.274	0.408	0.001	0.001	
	15	0.464	0.338					
2-Methyl-butyl acetate	0	0.023	0.017	0.059	0.081	0.083	0.098	significant
	3	0.014	0.014	0.068	0.051	0.072	0.090	(P=0.02)
	7	0.032	0.018	0.218 b	0.314 a	0.059	0.064	
	11	0.070	0.047	0.433 b	0.567 a	0.042	0.046	
	15	0.160	0.119					
Pentyl acetate	0	0.001	0.001	0.001	0.001	0.001	0.000	not significant
	3	0.001	0.001	0.001	0.000	0.000	0.000	
	7	0.002 a	0.001 b	0.003	0.004	0.000	0.000	
	11	0.003	0.002	0.006	0.008	0.000	0.000	
	15	0.006	0.005					
Hexyl acetate	0	0.004	0.003	0.010	0.013	0.004	0.005	not significant
	3	0.004	0.002	0.007	0.005	0.001	0.001	
	7	0.005	0.003	0.016	0.020	0.001	0.001	
	11	0.010	0.007	0.026	0.034	0.001	0.001	
	15	0.024	0.020					

Mean separation by protected LSD ($P \leq 0.05$).

Means (n = 4) followed by different letters within the same time at 22 °C and storage treatment are significantly different.

[a] nd = not detectable.

[b] Soybean oil was applied as a 1% emulsion three weeks before harvest.

[c] 1-Methylcyclopropene was applied after harvest at $0.7ul\ l^{-1}$ for 12 hours .

Table 4.4. Influence of soybean oil treatments on fatty acid (FA) profiles (relative % by weight) of 'Golden Delicious' apples from Yakima in the 2004 season. Measurements were taken at harvest, and after 140 days in controlled atmosphere (CA) storage.

FA	Days at 22 °C	At harvest		After Storage			
				CA		1-MCP[c] + CA	
		Control	Soy[b]	Control	Soy	Control	Soy
16:0 [a]	0	23.71	24.15	22.85	22.83	22.75	22.72
	3	24.53	24.65	22.83	24.04	23.01	22.60
	7	25.52	25.05	23.58	23.42	22.37	24.06
	11	23.55	23.82	23.94	23.40	23.58	23.82
	15	20.83	20.75	21.89	22.28	24.16	24.71
18:0	0	6.37 a	5.78 b	6.94	7.42	5.98	6.26
	3	6.64	6.70	7.45	7.66	6.60	6.75
	7	7.28	7.97	7.70	7.82	6.66	7.07
	11	8.04	8.20	6.61	6.59	6.39	6.32
	15	7.42	8.03	6.66	6.32	6.29	6.47
18:1	0	4.62	4.63	4.06	4.50	3.63	3.75
	3	3.03	3.15	4.22	4.69	3.47	4.12
	7	3.61	4.09	5.64	6.03	4.09 b	4.68 a
	11	8.77	7.96	8.52	8.28	3.57 b	4.30 a
	15	13.53	13.14	11.06	10.51	4.27	4.63
18:2	0	45.24	44.81	54.33	52.96	51.18	51.21
	3	47.35	46.94	54.11 a	52.31 b	50.16	50.43
	7	46.41	47.01	53.67	53.41	50.64	48.86
	11	48.85	47.94	54.24	55.15	50.56 a	49.78 b
	15	51.85	52.17	55.35	55.40	50.44	49.54
18:3	0	19.65	20.64	12.04	12.28	16.47	16.06
	3	18.44	18.56	11.39	11.31	16.75	16.10
	7	17.24 a	15.89 b	9.38	9.31	16.22	15.33
	11	10.79	12.08	6.68	6.58	15.91	15.77
	15	6.37	5.92	5.04	5.48	14.84	14.65

Mean separation by protected LSD ($P \leq 0.05$).
Means (n = 12) follwed by different letters, within the same time at 22 °C and storage treatment, are significantly different.

[a] 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid.

[b] Soybean oil was applied as a 1% emulsion three weeks before harvest.

[c] 1-Methylcyclopropene was applied after harvest at 0.7ul l^{-1} for 12 hours .

Table 4.5. Effect of soybean oil treatments on hue and chroma values of 'Golden Delicious' apples from Yakima in the 2004 season. Measurements were taken at harvest, and after 140 days in controlled atmosphere (CA) storage.

	Days at 22 °C	At harvest		After Storage			
				CA		1-MCP[b] + CA	
		Control	Soy[a]	Control	Soy	Control	Soy
Hue	0	113.31 [c]	113.69	108.61	108.87	109.14	108.07
	3	112.87	113.21	107.24	107.87	108.66	107.36
	7	112.17	112.51	104.78	104.94	108.48	107.11
	11	110.73	111.28	101.45	101.57	108.11	106.62
	15	108.98	109.89	99.11	99.17	107.67	105.94
Chroma	0	46.70	47.31	46.12	46.56	46.29	45.74
	3	46.83	47.50	47.31	47.70	46.15	45.51
	7	46.96	47.76	49.54	49.84	45.57	45.14
	11	47.47	47.91	51.18	51.61	45.83	45.58
	15	48.64	49.16	52.63	52.98	46.20	45.67

Mean separation by protected LSD ($P \leq 0.05$).
Means (n = 4), within the same time at 22 °C and storage treatment, are not significantly different at $P \leq 0.05$.

[a] Soybean oil was applied as a 1% emulsion three weeks before harvest.

[b] 1-Methylcyclopropene was applied after harvest at 0.7ul l^{-1} for 12 hours .

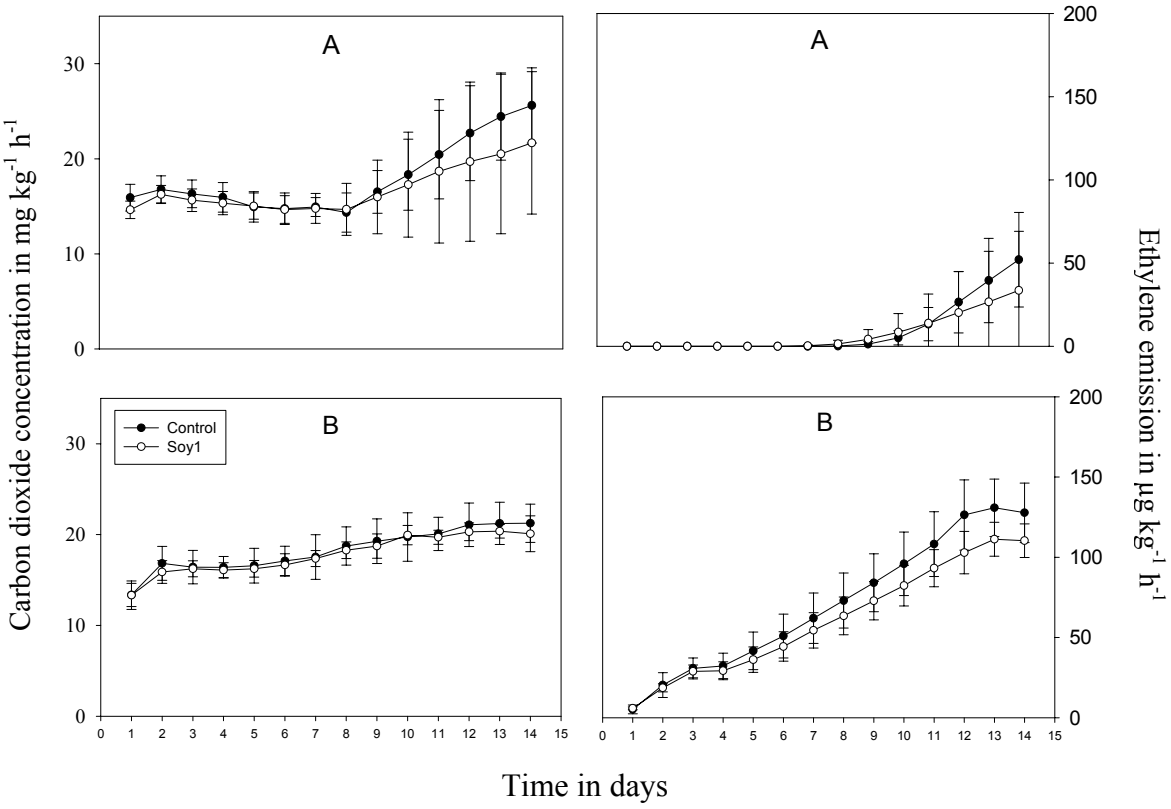

Figure 4.1. Progression of respiration and ethylene production of 'Golden Delicious' apples from Yakima. Vertical bars represent SE of means (n = 3).
A = at harvest, B = 140 days in CA (CA = controlled atmosphere).

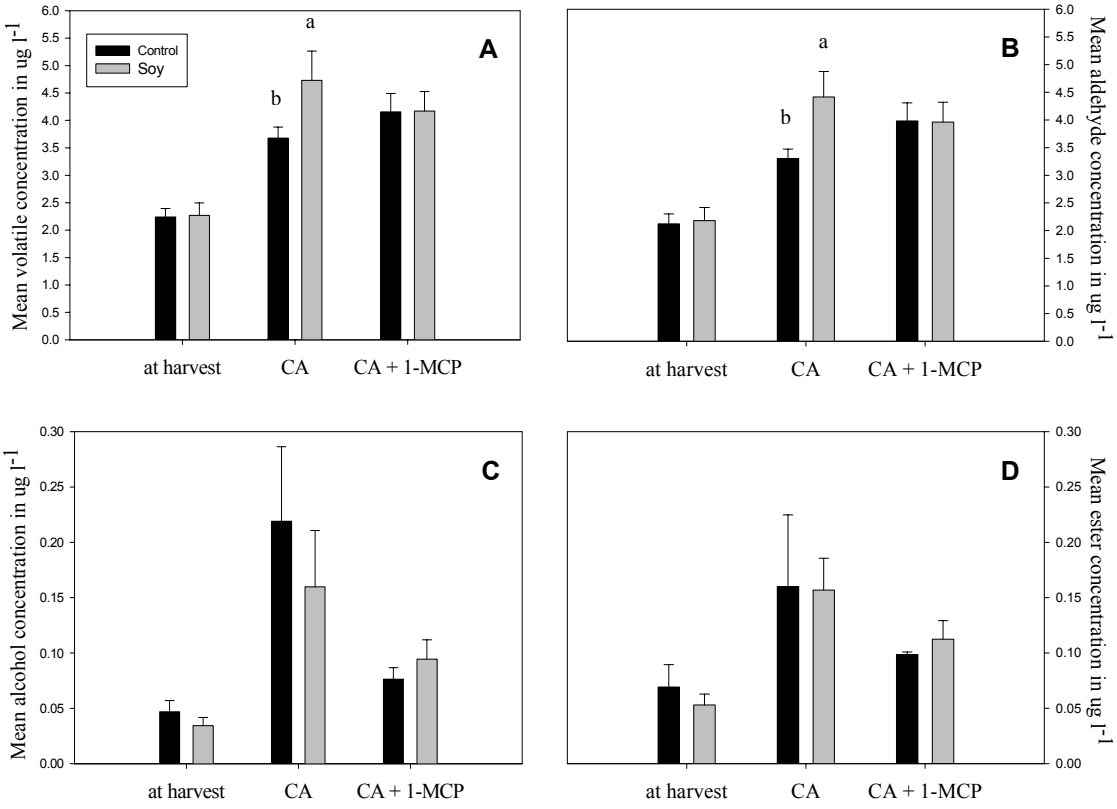

Figure 4.2. Influence of pre-harvest soybean oil application on mean volatile (A), aldehyde (B), alcohol (C), and ester (D) emission of 'Golden Delicious' apples at harvest and after controlled atmosphere (CA) storage with or without pre-storage 1-MCP treatment. Vertical bars represent SE of means (n = 4). Different letters above treatment columns indicate significant treatment effects at $P < 0.05$.

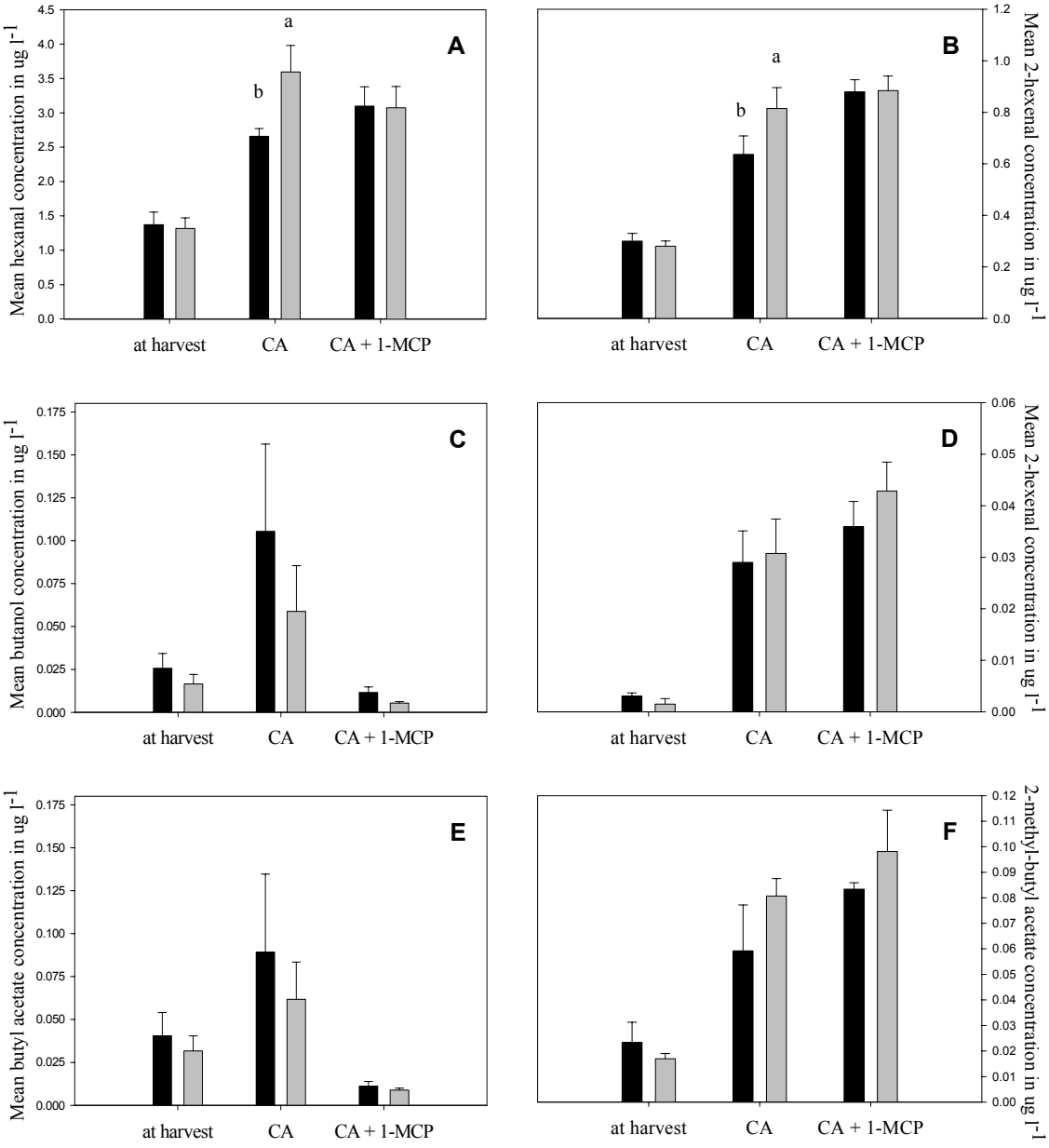

Figure 4.3. Influence of pre-harvest soybean oil application on hexanal (A), 2-hexenal (B), butanol (C), methyl butanol (D), butyl acetate (E), and 2-methyl butyl acetate (F) emission of 'Golden Delicious' apples at harvest and after controlled atmosphere (CA) storage with or without pre-storage 1-MCP treatment. Vertical bars represent SE of means (n = 4). Different letters above treatment columns indicate significant treatment effects at $P < 0.05$.

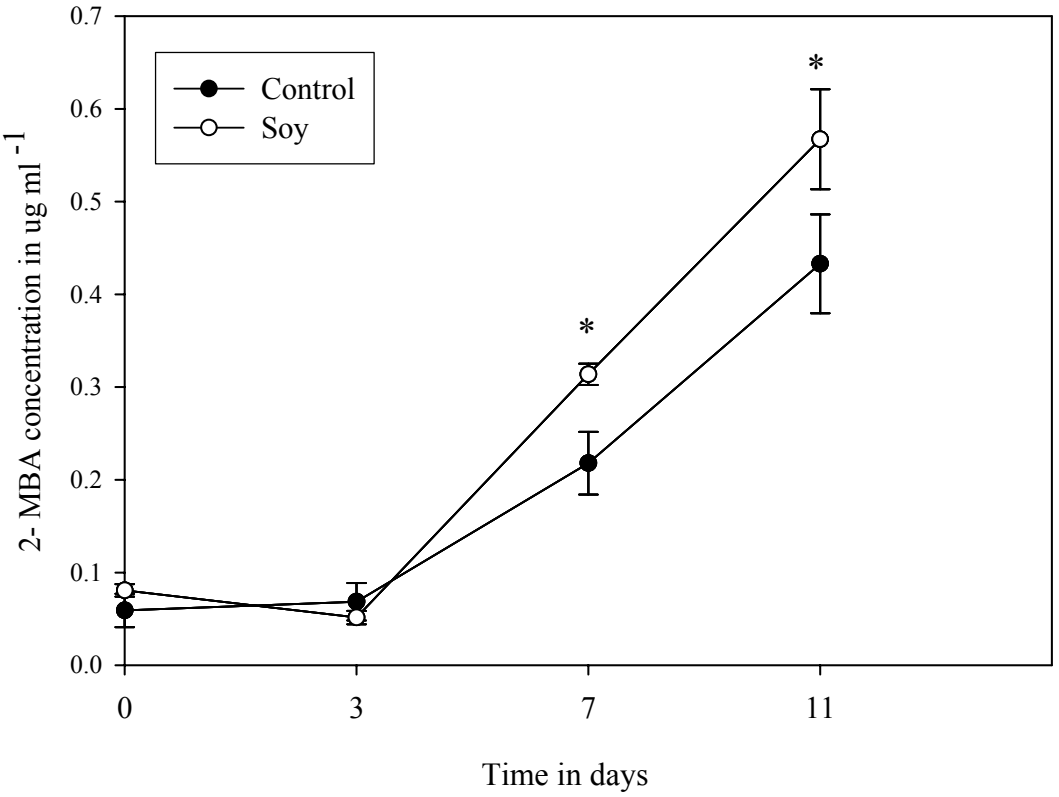

Figure 4.4. Influence of pre-harvest soybean oil application on 2-MBA (2-methyl-butyl acetate) emission of 'Golden Delicious' apples during a shelf-life period at 22 ^{o}C following 140 days of controlled atmosphere storage (CA). Vertical bars represent SE of means (n = 4).
* indicates significant treatment effects (LSD, $P < 0.05$).

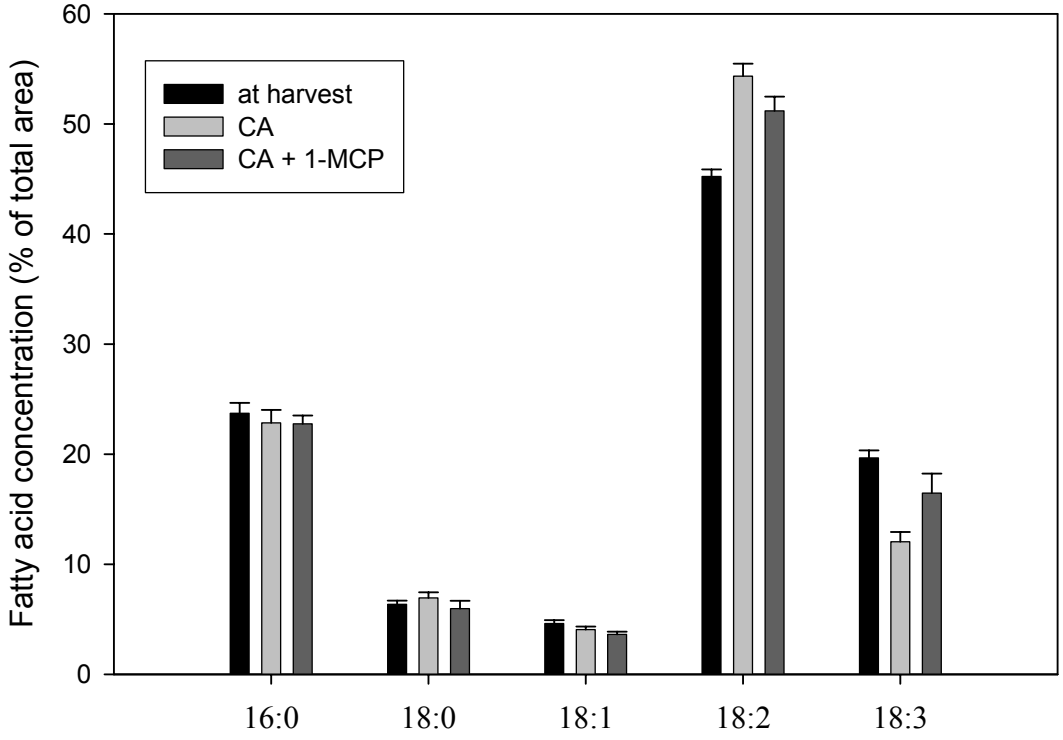

Figure 4.5. Production of five fatty acids in the peel of 'Golden Delicious' apples at harvest and after 140 days of controlled atmosphere (CA) storage with or without pre-storage 1-MCP (1-methylcyclopropene) treatment. Vertical bars represent SE of means (n = 4).
16:0 = palmitic, 18:0 = stearic, 18:1 = oleic, 18:2 = linoleic, 18:3 = linolenic acid.

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CHAPTER 5

GENERAL DISCUSSION

The application of soybean oil during the growing season has shown potential to improve the retention and regeneration capacity of important volatile compounds contributing to the aroma of 'Golden Delicious' and 'Gala' apples. However, the 1% oil concentration appears too low to sustain any effects. Consequently, a combination of two oil treatments within three weeks of harvest might result in even higher and more pronounced volatile aroma retention capacities. Additionally, the 1% concentration used in the field might not have been high enough to cause major physiological changes, as other groups have observed when using higher concentrations (5 or 10%) in a postharvest application. Higher concentrations should be field-tested, especially to determine threshold levels for phytotoxicity, with the goal to amplify any underlying physiological phenomena.

Additionally, since the current study has measured volatiles on the base of juice samples, results should be compared to those obtained by headspace analysis of intact apple fruit in order to account for volatiles produced by the crushing of the tissue. Given the low odour threshold of most of the esters responsible for the typical ripe apple aroma and the improvement of flavour regeneration capacities of fruit treated with soybean oil, it seems necessary to relate all volatile data to olfactory measurements. The use of taste panels is recommended to determine if measurable increases in volatile aroma emission translate into perceivable differences when eating the fruit.

The effect of oil application on the free fatty acid content needs to be re-evaluated since the current study only observed changes in overall fatty acid patterns including membrane bound FA. Further, if one particular fatty acid were to fluctuate strongly, an application of just this particular acid to the trees could be evaluated. By spraying of a plant oil emulsion containing high amounts of fatty acids required for aroma production just days before harvest, the loss of alcohols and associated esters could be somewhat decreased. There seems to be a relationship but, in order to confirm this, higher concentrations or multiple applications are probably necessary. The application of the oil seems to have supplied fatty acids for physiological processes associated with ripening, like volatile production, while chlorophyll degradation was slowed down. If and how these processes are interconnected it not clear yet, since the response varied from year to year.

One of the most pronounced effects of soybean oil application was the reduction of weight loss during storage and subsequent shelf-life periods. The exact mechanism of this waterproofing is unknown to date. It seems clear though that the mode of action of soybean oil applications is unique, since weight loss was reduced without affecting other quality parameters as observed elsewhere after application of traditional antitranspirants.

Few studies to date have linked cuticular micro-cracking and fruit water vapour permeance. Soybean oil application did influence micro-cracking of apples while simultaneously reducing water loss in storage. Apples with more severe crack development benefitted the most from oil treatment. Further studies including the measurement of water vapour permeance and relating it to the size and amount of cracks observed would be appropriate.

One of the still largely unexplored aspects of the development of cuticular cracks is the lack of a clear description of the involvement of the different epicuticular layers like epicuticular wax and/or cutin. In results reported here, cracks appeared to be due to changes in the thickness of the cutin without changes in the epicuticular wax fraction. This is plausible, as cutin functions as the principal barrier against water loss. Furthermore, the development of cracks has been attributed to rapid fruit growth, which does not explain why cracks continue to enlarge during storage of apples. In light of the present study it seems possible to improve cuticular characteristics through application of materials rich in fatty acids. If and how these compounds are incorporated remains unknown.

Soybean oil application also altered epicuticular wax crystallization patterns. It would be intriguing to study relationships between soybean oil application and light reflection, water repellancy, cuticular sorption of agrochemicals, and plant insect interactions, since all these processes are tightly linked to epicuticular wax crystallization patterns.

SUGGESTIONS FOR FUTURE RESEARCH

1. Apply soybean oil emulsions several times during the growing season.

2. Evaluate the effects of application of higher oil concentrations on quality parameters.

3. Determine changes in the free fatty acids of the cuticle after oil application.

4. Investigate fungicidal activities of volatile components of apples.

5. Determine the molecular mechanism for the colour change from green to yellow in apples.

6. Could horticultural oils be used as vectors for biological control agents in postharvest treatments?

7. What are the potential implications when using horticultural oils as vectors for biological control agents in the orchard?

8. Determine all 1-aminocyclopropane-1-carboxylate synthase (ACS) genes associated with ethylene responses in apple fruit growth and maturation and investigate upregulation and environmental control.

9. Evaluate soybean oil as a season-long tool to avoid cuticular microcracking.

10. Investigate the mechanism of the development of cuticular microcracking. For example, could it prevent early season russett?

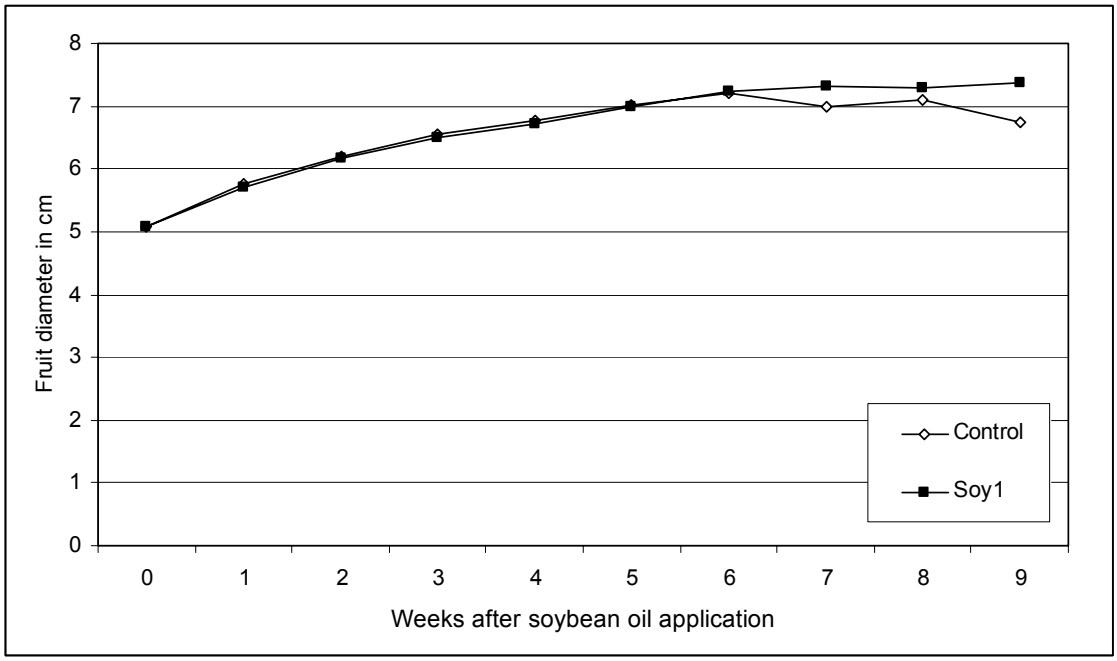
11. What are the factors involved in cutin development?

12. Determine how soybean oil application affects physical properties like light reflection, water repellency, cuticular sorption of agrochemicals, plant insect interactions of the epicuticular wax.

13. Conduct taste panels and determine if soybean oil application improves eating experience of apples.

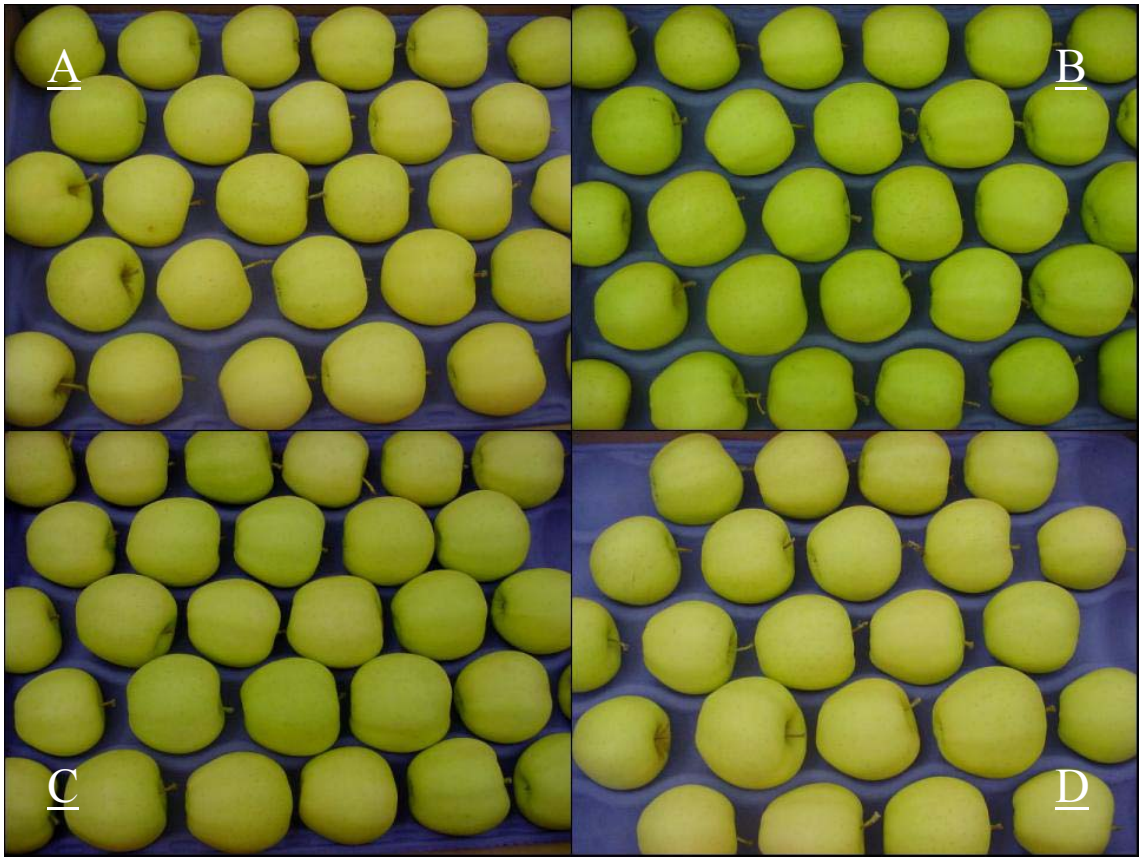
APPENDIX

Appendix 3.1. Fruit growth rate of 'Golden Delicious' apples from Pullman during the 2003 season.



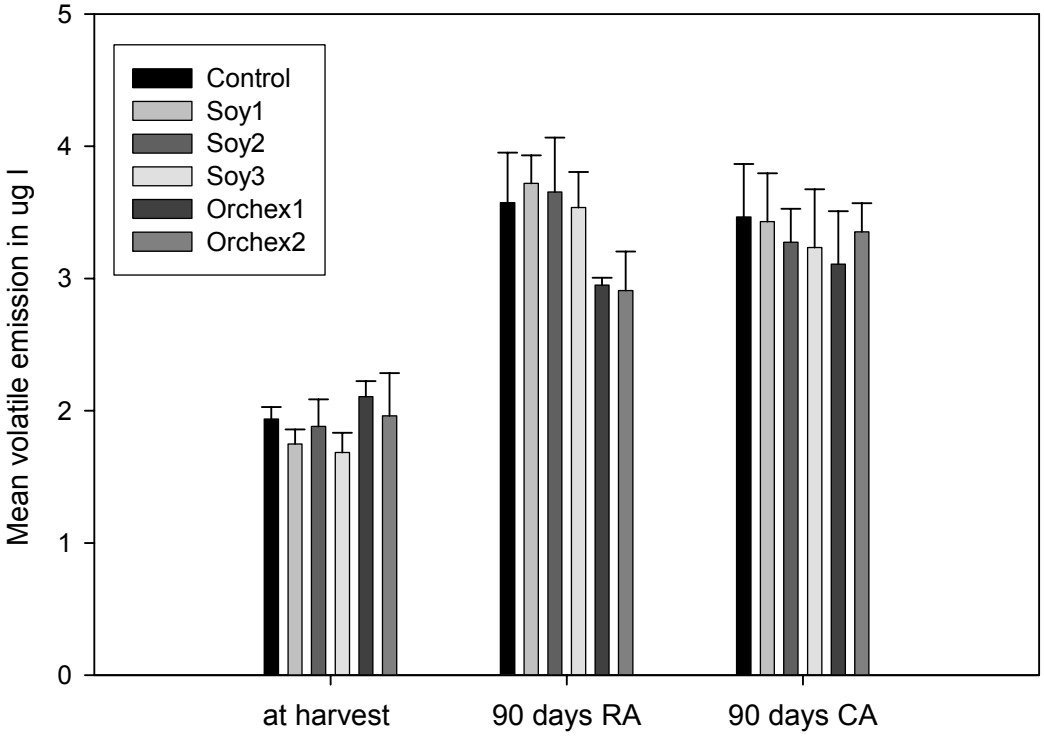
The fruit growth rate was not affected by soybean oil application (Soy1, 'Golden Delicious', Pullman). Due to high codling moth pressure in Pullman, about half the untreated fruit was lost due to insect damage. However, only half the amount of insect damage was observed on the oil-treated fruit.

Appendix 3.2. Effect of soybean oil application on rate of colour change of 'Golden Delicious' apples from Pullman after 90 days in regular atmosphere (RA) storage.



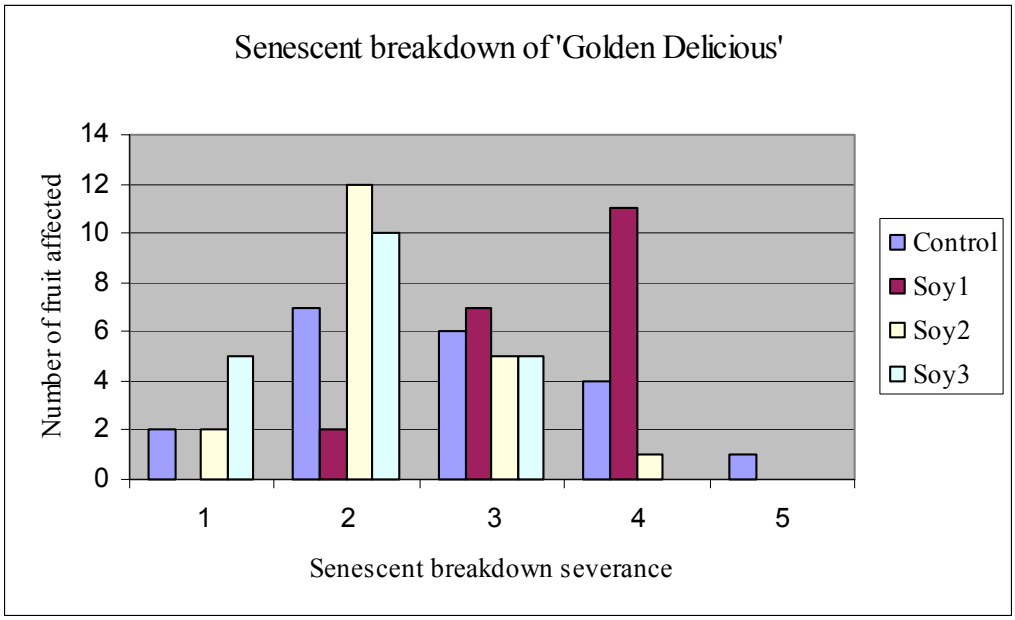
A = control, B-D= soybean oil treatments: B = midseason (soy1), C = three weeks before harvest (soy2), D = three days before harvest (soy3).

Appendix 3.3. Influence of Orchex treatments on predominant volatile groups of 'Golden Delicious' apples from Pullman in the 2003 season. Measurements were taken at harvest, and after 90 days in regular (RA) or controlled atmosphere (CA) storage.



Soy 1: midseason oil application.
Soy 2 / Orchex 1: application three weeks before harvest.
Soy 3 / Orchex 2: application three days before harvest.

Appendix 3.4. Severity of senescent breakdown of 'Golden Delicious' apples from Yakima in the 2003 season after 180 days in regular atmosphere (RA) storage.



After 180 days of RA storage, 'Golden Delicious' from Yakima had a high incidence of senescent breakdown. Fruit (n = 20) were scored for disorder incidence and severity and it was found that soy1 treated fruit and control fruit was most affected.