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Förbättrad kvalitet och lagringsduglighet hos ekologiska äpplen

Ibrahim Tahir
Växtförädling och bioteknik

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Projektet utfördes under 2008-2010 i Kivik och Balsgård. Projektet har finansierats av SLU Ekoforsk.

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Abstract

Improvements in yield and quality by increasing the light distribution and carbohydrate uptake (summer pruning and ground covering), optimizing the physiological state of harvested fruit (cultivar-specific harvesting indices), postharvest fruit protection (treatment with hot water and ethanol, respectively) and optimizing storage conditions (cultivar-specific CA and ULO storage procedures) were investigated in a set of eight organically grown apple cultivars.

A combination of summer pruning and covering the orchard ground with white textile increased tree productivity, improved coloration at harvest, and increased contents of anthocyanin, ascorbic acid and total phenolic compounds in at least some of the cultivars. It also developed fruit resistance to *Neofabraea spp.* and *Colletotrichum gloeosporioides* which were the most common pathogens, causing fungal storage deseases.

Analyses of various fruit ripening parameters showed that starch hydrolysis point and Streif index (firmness/(starch hydrolysis point * soluble solid concentration)) were corrlated with internal ethylene concentration (IEC) at harvest. Thus, the optimal harvesting time can be deduced from the starch index in some cultivars (Agra, Santana and Sultanat) while the Streif index is more accurate for other cultivars (Eir, Delorina and Zarya alatau). In yet others, titratable acidity and flesh firmness also produced important information and have to use as an additional index beside starch hydrolysis for Ella and Dayton respectively which showed only close correlation between starch hydrolysis and IEC. By contrast, soluble solids concentration and skin color are not useful due to their sensitivity to weather conditions and light intensity.

Post-harvest hot water treatment (46º C for 120 seconds) decreased fungal decay during storage in only two cultivars (Dayton and Eir), whereas spraying the fruit with 10% ethanol decreased fungal decay in all six cultivars.

Optimization of CA and ULO storage conditions maintained fruit quality and reduced amount of fungal decay. Storage of eight organic cultivars in a wide range of pCO_2 achieved slight additional improvement in some cultivars, while flesh browning, causing by high CO_2 can be expected. CA (2.0 kPa O_2 and 2.0 kPa CO_2) can be recommended for Agra, Eir, Ella and Delorina; ULO (1.0 kPa O_2 and 1.0 kPa CO_2) for Zarya alatau and (1.0 kPa O_2 and 2.0 kPa CO_2) for Dayton, Santana and Sultanat.

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Förbättrad kvalitet och lagringsduglighet hos ekologiska äpplen

Sammanfattning

En ökning av arealen ekologiskt odlad frukt i Sverige kräver inte bara nya resistenta äpplesorter och lämplig odlingsteknik, utan också utveckling av de kvalitetsparametrar som gör frukten attraktiv hos konsumenterna samt transporttåliga och lagringsdugliga. Vårt projekt syftar till att uppnå detta mål genom undersökning av en modern odlingsmetod som ökar kolhydratackumuleringen och justerar ljusfördelningen i kronan, bestämning av optimal skördetidpunkt och optimala betingelser för kontrolled atmosfär (CA) respektive ultra låg syre (ULO) lagring, och minskning av svampangrepp med icke-kemiska efterskördebehandlingar.

Moderna odlingsmetoden som inkluderar sommarbeskärning och marktäckning med vita textilier ökar trädproduktiviteten, förbättrar fruktfärgen vid skörd och minskar kvalitetsnedgången under lagring. Denna metod ökar också antioxidanter, dvs. antocynininnehållet hos alla sorter, och askorbinsyra och totala fenoler hos några sorter. Odlingsmetoden har även förbättrat fruktmotstånd mot *Neofabraea sp.* och *Colletotrichum gloeosporioides*, som har varit de vanligaste patogena i området.

Optimal skördetidpunkt infaller i en period under fruktutvecklingen, den s.k. preklimakteriefasen, då förändringarna i etylenproduktionen och kvalitets´-parametrarna är mycket små. Frukt bör skördas när 25% av frukten börjar producera etylen för att minimera lagringsröta och förbättra kvalitén. En mycket stark korrelation mellan etylenproduktionen och stärkelsenedbrytningen (SNB) visar att SNB säkert kan användas som mognadsindex för vissa sorter (Agra, Santana och Sultanat) medan Streif index (fasthet / (SNB * lösliga torrsubstanser) som har visat sig ha en sådan korrelation med etylenproduktionen är en mer korrekt index för de andra sorterna (EIR, Delorina och Zarya Alatau). Syrligheten kan användas som ett ytterligare index till SNB för de sorter som visar en nära korrelation endast mellan etylenproduktionen och SNB (Ella och Dayton). Fasthet, lösliga torrsubstanser och skalfärg kan inte accepteras som korrekta index, eftersom de påverkas mycket av instabilt väder och svag ljustillgänglighet under skördeperioden.

Dopping av frukter i varmt vatten (46º C under 120 sekunder) minskar svampangrepp under lagring hos två sorter (Dayton och Eir) och kan inte rekommenderas för Agra och Sultanat. Efterskördebesprutning med 10% etanol minskade också svampangrepp i alla sorter. Våra resultat visar att CA lagring är mycket effektivt för att förbättra kvalitet och lagringsduglighet hos ekologiska äpplen. Lagring av åtta sorter i ett brett spektrum av pCO_2 innebär några ytterligare förbättringar hos vissa sorter, men höga koldioxidnivåer orsakar även vissa skalsjukdomar. CA (2,0 kPa O_2 och 2,0 CO_2 kPa) kan rekommenderas för Agra, Eir, Ella och Delorina, ULO (1,0 kPa O_2 och 1,0 kPa CO_2) för Zarya Alatau och (1,0 kPa O_2 och 2,0 kPa CO_2) för Dayton, Santana och Sultanat.

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Photos: Ibrahim Tahir.

The photo on the first page of this rapport represents organic cultivars: *1. Zarya alatau, 2. Delorina, 3. Eir, 4. Ella, 5. Sultanat, 6. Santana.*

Tack

Jag vill rikta ett tack till alla som har bidragit till att projektet kunnat genomföras. Ett speciellt tack riktas till SLU Ekoforsk, som har finansierat projektet, till Karl Wisen (KivikÅs) och Paul Ilg, som genomfört de praktiska momenten i försöket, samt Karl-Erik Gustavsson för tekniska support.

Foton är taget av Ibrahim Tahir.

Bilden på framsidan av rapporten visar olika sorter; 1. Zarya alatau, 2. Delorina, 3. Eir, 4. Ella, 5. Sultanat, 6. Santana.

1. Introduction

Due to the high market value and increasing interest from consumers, efforts have been made to promote organic apple production and solve some of the problems facing this system such as application of integrated pest and groundcover management (Weibel, 2002; Lima *et al.,* 2003) and fertilization regimes (Herencia *et al.,* 2007).

Commercial apple production in Sweden covers about 1440 ha and the total annual yield represents 8-9% of the national consumption (FAO, 2005). Organic orchards cover only 10% of this acreage. Because apple scab is the most detrimental disease in cool and moist production areas, a few of the growers have as yet taken the big step to organic apple growing. Therefore, cultivars with monogenic (vertical) or polygenic (horizontal) resistance to this disease have received considerable attention. Unfortunately, many of these scab-resistant cultivars however suffer from poor skin color at harvest, weak resistance to fungal decay and physiological disorders, and rapid deterioration in fruit quality during storage and post-storage handling (i.e. poor shelf-life) (Jönsson and Tahir, 2005; Tahir and Jönsson, 2005; Jönsson and Nybom, 2007).

The purpose of this work was to investigate potential improvements in yield and quality in a set of eight organically grown apple cultivars by increasing light distribution and carbohydrate uptake (summer pruning and ground covering), optimizing the physiological state of harvested fruit (cultivar-specific harvesting indices), application of postharvest fruit protection (heat treatment, ethanol) and optimizing storage conditions (cultivar-specific CA and ULO storage procedures).

2. Research hypotheses

Numerous measures have been suggested for the improvement of apple yield and quality, some of which could be very valuable also in organic apple production. Thus, fruit color and storage potential can be improved by summer pruning of the trees and covering the orchard ground with reflective materials (Lang *et al.,* 2001, Tahir *et al*., 2005, Tahir and Gustavsson, 2010). These procedures interact to provide a better light distribution in the canopy and thus enhance the red skin color, while a reduction in the competition between fruit and vegetative growth improves carbohydrate accumulation and thus internal quality and storability.

The stage of fruit maturation at the time of picking influences the overall dessert quality of apples, both at harvest and after storage (Kader, 1999) as well as the ability to withstand fungal attacks (Lafer, 2006). Accurate determination of an optimum harvesting date is therefore important for maintaining fruit quality and minimizing losses during storage. More information is, however, needed on the correlation between simple but often also cultivar- and environment-specific parameters, like starch conversion to sugar, fruit softening and color development (Willats *et al.,* 2001; Peirs *et al.,* 2004) and the more accurate but also more elaborate harvesting indices based on changes in physical and biochemical properties like internal ethylene concentration (IEC) and respiration rate (RR) (Echeverria *et al.,* 2002, Peirs *et al.,* 2004, Delong *et al.,* 2009).

In apple, various post-harvest treatments are applied to control fungal decay and maintain fruit quality during storage. For organically produced fruit, non-chemical methods have been used like post-harvest heating (Paull and Chen, 2000, Tahir *et al.*, 2009), which may enhance the wound repair process, inhibit the synthesis of cell wall hydrolytic enzymes (Ben-Shalom *et al*., 1996) and change apple skin structure (Veraverbeke *et al*., 2001). Dipping the fruit in warm water instead of applying hot air may be technically easier but the effectiveness of this method also depends on other factors such as cultivar and exposure period (Conway *et al.,* 2004, Maxin and Waber, 2011). Another alternative is to reduce postharvest decay by disinfecting the fruit with ethanol immediately after harvest. The major characteristic of ethanol is its antimicrobial effect, e.g. through denaturation of proteins in fungal cells, but it can also delaying fruit ripening (Karabulut *et al*., 2004; Zhang *et al*., 2007).

Storage in controlled atmosphere (CA) including ultra-low oxygen (ULO) helps to maintain fruit quality and, especially in the case of organic fruit, protects against fungal diseases. These storage methods are essentially based on delaying the natural ripening processes by slowing down the oxidative processes (respiration) due to lower concentration of oxygen around fresh apples. They can also reduce or eliminate the detrimental effects of ethylene as a ripening hormone, and cause a slowed decomposition of polysaccharides into fructose, and thereby retains fruit firmness (Kader, 2003). The well-recognized gas with antimicrobial properties is CO_2. High CO_2 with low temperature may increase the solubility of CO_2 in water, thereby allowing penetration of more bicarbonate ions into microbial cells and changes the cell permeability and metabolic processes. Unfavorable CA conditions can, however, induce physiological disorders (e.g. flesh browning), enhance susceptibility to decay, and shorten shelf life (de Castro *et al.,* 2007, Fawbush *et al.,* 2008). Thus, optimum CA conditions must be investigated for cultivars used for organic apple production (Vicente *et al.,* 2003).

3. Material and Method

Eight recently released cultivars, with a potential to become commercially important for organic apple production in Sweden, were studied: **Agra, Dayton, Delorina, Eir, Ella, Santana, Sultanat and Zarya Alatau.**

Trees on rootstock M26 had been planted at 1.25 x 4 m (in total 2000 trees per ha) in 1999 and trained according to the super spindle system in an organic orchard in Kivik, South Sweden. Pruning was performed each year in early April to maintain this shape. The orchard was fertilized (Bina blue 6:1:12, Garta, Sweden) with 300 g per tree in May, and with 116 g per tree in June. Monthly soil cultivation was conducted with a rotary hoe rototiller during April–July. Plant protection measures were applied according to the regulations for commercial organic apple production defined by the Swedish Board of Agriculture. Neither irrigation nor bloom or fruit thinning was undertaken. For the present analyses, trees were harvested during three years: 2008, 2009 and 2010.

3.1. Storage and shelf life

In all experiments, fruits were stored either in air (rooms 3 x 4 m, 2 ºC and 90% RH) or in controlled atmosphere (computer-controlled 350 L chambers, Nino-lab, Sweden; appendix 1). Gas composition was established three days before placing the apples in the chambers. After storage, fruits were held in a plastic chamber at 18 ± 2 ºC and 80% RH for one week (shelf life) before evaluation (appendix 2).

3.2. Quality estimation

In all experiments, the following fruit quality parameters were estimated at harvest in 10 fruits/tree, and after storage and shelf life in 15 fruits/replicate/treatment:

1. Fruit coloration was measured as the hue angle using a colorimeter (Chromameter, model CR200 with 8 mm diameter window; Minolta Camera Co, Ltd. Osaka, Japan). Measurements were conducted on three spots per fruit, and expressed as a* and b*; a* is negative for green and positive for red color, and b* is negative for blue and positive for yellow color (McGuire, 1992).
2. Firmness was measured on opposite, peeled sides of each fruit using a penetrometer (model FT 327, Effigi, Italy, plunger diameter of 11.1 mm, depth of 7.9 mm) and results expressed as kg per cm^2.
3. Starch hydrolysis was evaluated by dipping a slice of the fruit, cut at the equator, in an iodine solution (8.8 g potassium iodide and 2.2 g iodine per liter) for 1 minute, and graded from 1= full color to 10 = free of starch (no color) (appendix 3).
4. Titratable acidity (TA) was measured on juice extracted from composite samples of segments of 10 fruits/tree, using a titration unit (Titroline Easy, SCHOTT Instr. GmbH, Germany), and 0.1M NaOH to an end point of pH 8.2, results expressed as (%).
5. The extracted juice was also used for measurement of soluble solids concentration (SSC) using a refractometer (Atago PR-100, Atago Co. Ltd., Tokyo, Japan), results expressed as (%).

The following analyses were conducted only in experiment 1 and at harvest in 10 fruits/tree:

1. Total anthocyanin was determined by pH-differential spectrophotometry (Cary 50 Bio spectrophotometer, Varian Inc, Australian) (Wrolstad, 1993). Distilled water was added to 100 mg of powdered lyophilised apple flesh to a final amount of 25 g,

and the solution was sonicated for 45 min at room temp**erature.** Two samples of this extract, each with 1000 µl, were placed into two different 10 ml volumetric flasks and diluted to volume with buffers at pH 1.0 and pH 4.5, respectively. The absorbance was measured at 510 nm, using cyanidin-3-glycoside molar absorptivity 26900. Content of total anthocyanins was calculated according to Wrolstad (1993) and results expressed as $mg.kg^{-1}$ dry weight.

2. The total phenol content was determined with the Folin–Ciocalteau method as modified by Dewanto *et al.* (2002). One gram of lyophilized and milled apple flesh was extracted in 20 ml 70% ethanol over night on an orbital shaker (Forma Sci Canada) at 4 °C. 50 µl of this extract, 100 µl ddH_2O and 100 µl Folin-Ciocalteau phenol reagent (BDH, England) was added direct into a cuvette, mixed and held for 6 min at room temperature. 1 ml of 0.66 M Na_2CO_3 was added and the mixture held for 75 min at room temperature. The absorbance was measured at 765 nm using a Cary 50 Bio spectrophotometer. Quantification was achieved by comparing with external standard gallic acid, 3,4,5-trihydroxybenzoic acid (Sigma-Aldrich, Germany) and results expressed as gallic acids equivalents on a dry weight basis, $g.kg^{-1}$.
3. Contents of vitamin C, ascorbic acid, and dehydroascorbic acid were determined in apple flesh. Analyses were performed under dim green light. A total of 4 g of apple flesh were placed in a plastic bottle together with 20 mL of meta-phosphoric acid (1.5%) and homogenized with an Ultra Turrax apparatus (Model TP1810, IKA Werke GmbH, Staufen, Germany) for 1 min. Samples were stored in the freezer at –80 °C prior to high-performance liquid chromatography (HPLC) analysis. Before HPLC analysis, the samples were thawed in lukewarm water and centrifuged for 8 min at 12900 g, after which, 5 mL of each supernatant was filtered through a Sep-pak Plus C18 cartridge (Waters, Milford, MA), previously conditioned with 5 mL of methanol and 10 mL of water. The first 4 mL of each sample were discarded, and the remaining 1 mL was collected in a microcentrifuge. An aliquot of the collected extract was used for HPLC analysis, as described below. The dehydroascorbic acid concentration was calculated by subtracting the ascorbic acid concentration from the total vitamin C concentration, obtained after a reduction procedure. For this procedure, an aliquot of the extract was added to a 1% dithiothreitol solution and allowed to react for 30 min at pH 7.0, where the pH was adjusted with K_2HPO_4, prior to HPLC analysis. Samples were analyzed on an Agilent 1100 series (Agilent Technologies, Waldbronn, Germany) HPLC, using an isocratic method with a flow rate of 1.2 mL/min. The mobile phase consisted of 25% $NH_4H_2PO_4$ (15 mM) and 75% acetonitrile, with the pH adjusted to 3.9. A 20 µL portion was injected from each sample. A Waters carbohydrate analysis column, 300 × 3.9 mm with a particle size of 10 µm, was used. Absorbance was measured at 248 nm. Samples were quantified using an external ascorbic acid standard (Merck, Darmstadt, Germany). The concentrations were expressed as vitamin C on a dry weight basis, $mg.kg^{-1}$.

Ethylene production was estimated at harvest in experiment 2 using 10 fruits/tree for each picking date/cultivar. The fruits were placed in 0.5 L jars at 20 ºC. The jars were sealed for 3–4 h before measurements. Ethylene was quantified by gas chromatography (Agilent GC 6850, USA) equipped with a flame ionization detector, automatic gas sampling valve 1000 µl, 150 °C, and fitted with a capillary column HP plot Q 30 x 0.53 mm x 40 µm (Agilent). Analyses were run isothermally with an oven temperature of 70 °C and a detector temperature of 250 °C. Flow rates for nitrogen, hydrogen and compressed air were 20, 45 and 225 $ml.min^{-1}$. Ethylene was quantified by peak area, and external standards were used for calibration. Evaluation was achieved with chemstation version 9.03 (Agilent Corp).

3.3. Experiment 1. Orchard management: summer pruning and ground cover

Twenty-four trees of each of six cultivars (**Dayton, Delorina, Ella, Santana, Sultanat and Zarya Alatau**) were randomly divided into three blocks. In each block, four trees were treated according to standard practice (only early April pruning, no ground coverage). The other four trees were summer pruned at 10–15 July each year, and the ground under the trees was covered with a reflective textile (white sheet, 0.6 mm thick and 2 m wide, Lotico, Sweden) during 15 July–15 September (appendix 4). Light penetration within the canopy was estimated at 15:00 local time on a sunny day once a week during July–August in 2009 and 2010. These measurements were conducted at the center of the trunk, from four directions along the crown and 70 cm above the ground, using a light meter (L1-188; L1-COR, Frederickson, Copenhagen, Denmark). Full sunlight was measured (at the same time and height) in a free area (between rows). Sunlight penetration (%) was calculated as the amount of sunlight that reached the orchard floor and mid-canopy compared to full sunlight. In each of the three years, fruit was harvested according to common practice in commercial orchards (starch staining 4/10), counted and weighed. Fruit quality parameters were investigated in 10 newly picked fruits per tree as described above. A sample of 25 fruits was picked from the inside and outside of each tree (total of 100 fruits/replicate/cultivar), stored in air (2 ºC and 90% RH) for four months, followed by one week of shelf life as described above, and screened for physiological disorders (soft scald, flesh browning and internal breakdown) and storage rots (bull's eye rot, *Neofabraea spp.*). In addition, flesh firmness, TA and SSC was analyzed on 15 fruits from each sample as described above.

3.4. Experiment 2. Determination of optimal harvest date

Optimal harvesting date of the above eight cultivars was determined by fruit analysis for several parameters commonly employed for the development of a harvest index; internal ethylene concentration (IEC), starch hydrolysis, flesh firmness, skin color, TA and SSC, at three times a week in a total of four weeks/cultivar, starting at 95 days (for Agra), 100 days (for Eir), 105 days (for Ella), 120 days (for Dayton, Santana, and Zarya alatau), and 125 (for Delorina and Sultanat) days after full bloom (DAFB). The starting days were chosen from experiences in previous years in the same orchard.

Four similar fruits from both outside and inside of fifteen trees of each cultivar (arranged in three replicates, five trees each) were picked and analyzed early in the morning as explained above.

Each year, a large sample with 150 fruits/replicate/cultivar was picked early in the morning (to obtain cold fruit) at weekly intervals for a total of four times a season and stored in air (2 ºC and 90% RH) for four months. After storage and one week of shelf life (see above), flesh firmness, TA and SSC were evaluated on 15 fruits/replicate/cultivar/harvest date as described above. In addition, amount of physiological disorders and fungal decay was also estimated.

3.5. Experiment 3. Postharvest treatment with heating and ethanol

In 2009 and 2010, twenty fruits were picked from the outside and inside, respectively, of fifteen trees, arranged in three replicates with five trees each, and treated according to standard practice, resulting in a total of 300 apples. Four cultivars (**Agra, Dayton, Eir and Sultanat**) were used. The fruits were harvested at a pre-climacteric stage, with ethylene production at 0.1–0.3 $\mu l.L^{-1}.kg^{-1}.h^{-1}$, firmness approx. 8.0 $kg.cm^{-2}$ and starch hydrolysis approx. 4/10. Each sample was divided into four subsamples; the first subsample was sprayed with distilled water and the second with an ethanol solution, 100 ml.L-1. The third and fourth subsamples were immersed in water for 120 seconds, at 46 ºC and 20 ºC, respectively. All four subsamples were stored in air (2 ºC and 90% RH) for 16 weeks and one week of shelf life (see above) before estimation of physiological disorders and fungal decay.

3.6. Experiment 4. Controlled atmosphere storage

Each year, a lot of 420 fruit/ cultivar were picked from fifteen trees (randomly arranged in three replicates with five trees each, and treated according to standard practice) at a pre-climacteric stage. Maturity indices and fruit quality parameters were estimated in 30 fruits/ cultivar as explained above. The samples were transported to Balsgård, SLU, sorted for uniform size and absence of blemishes including bitter pit, and randomized to provide experimental units (replicates) of 30 fruits each. Three replicates per cultivar were placed in air, three in each of two ultra-low oxygen storage chambers, ULO 1 (1.0 kPa O_2 and 1.0 kPa CO_2) and ULO 2 (1.0 kPa O_2 and 2.0 kPa CO_2) and one in a CA storage chamber (2.0 kPa O_2 and 2.0 kPa CO_2) and 2 ºC as described above. After five months in storage and one week of shelf life (see above), flesh firmness, TA and SSC were evaluated as in experiments 1 and 2. The percentage of incidence of some physiological disorders (soft scald, flesh browning, and internal breakdown) and storage rots (*Colletotrichum gloeosporioides* and *Neofabrea spp.)* were also determined by visual examination.

3.7. Statistical analysis:

The experiment was carried out over three seasons. Three variables were controlled: four harvesting dates, three storage methods, two postharvest treatments, eight cultivars and three seasons. Data were subjected to arcsine square root transformation before analysis. Data were subjected to analyses of variance using the general linear model to determine main effects and interactions (SAS Inc., Cary, NC). Least significant difference values (0.05) were calculated for comparison of means. Regression analyses and Pearson correlation were carried out (using SAS) to quantify the relationships among harvesting date and changes in maturity indices.

4. Results and Discussion

4.1. Experiment 1. Effect of new orchard management method

The new management model, i.e., covering the orchard floor and adjusting vegetative growth in the summer, increased light transmission in the trees (*P≤0.001*) of all six cultivars (79% in Zarya Alatau, 62% in Delorina, 51% in Sultanat, 32% in Dayton and 40% in both Ella and Santana; Fig. 1). There was no significant interaction between the effects of season and management in any of the cultivars.

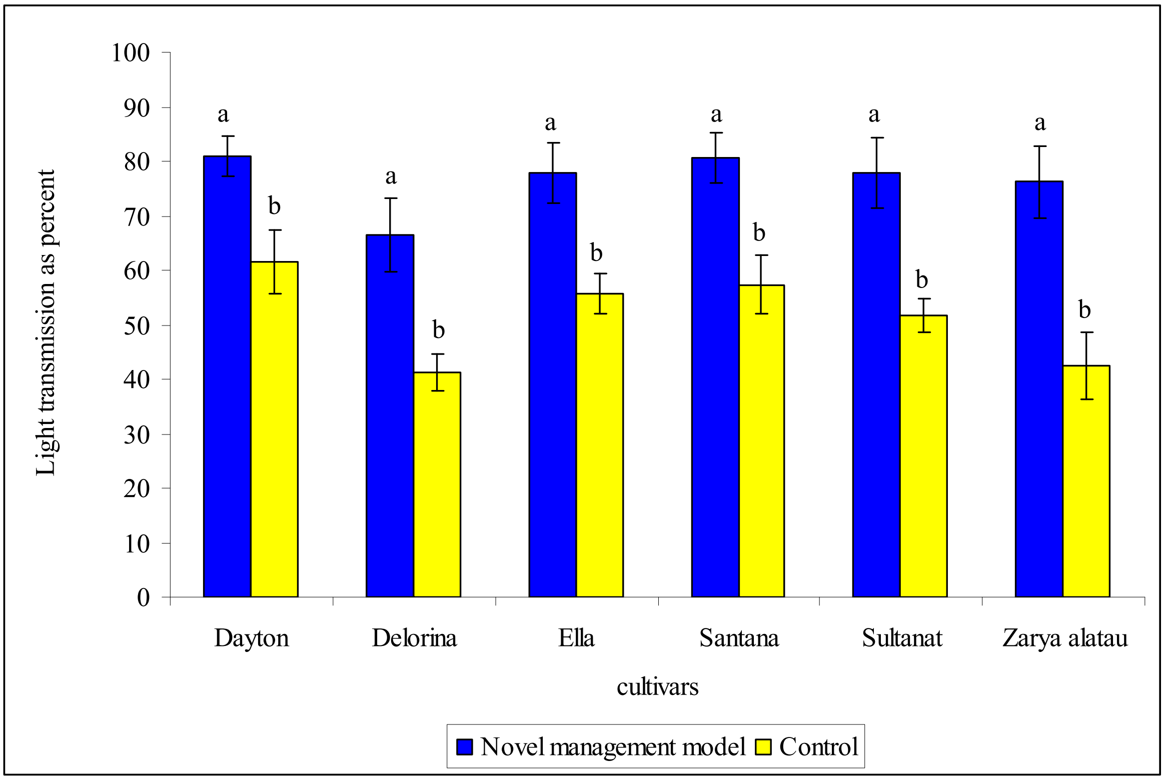

Fig. 1. Effect of novel management model (covering orchard floor by white textiles and shoot thinning by pruning in July) on the light transmission. Values are means of two years, three replicates of six measurements each. Bars for each cultivar with the same letter are not statistically different at P ≤ 0.001.

The new management model increased the cumulative yield over the three seasons in five of the cultivars (*P≤0.001*) (43% in Ella, 32% in Sultanat, 24% in Dayton, 20% in Delorina and 18% in Zarya Alatau; Fig. 2).

By contrast, fruit weight was not significantly affected (Table 1). Skin coloration was strongly improved (67% in Ella, 60% in Dayton, 45% in Delorina, 29% in Santana, 28% in Zarya Alatau and 26% in Sultanat) (Table 1 and Fig. 3; *P≤0.001*) but no significant interaction was found between season or management model in any cultivar at *P≤0.05* (Table 1). No effect on the other quality parameters at harvest such as firmness, SSC and TA was registered (Table 1).

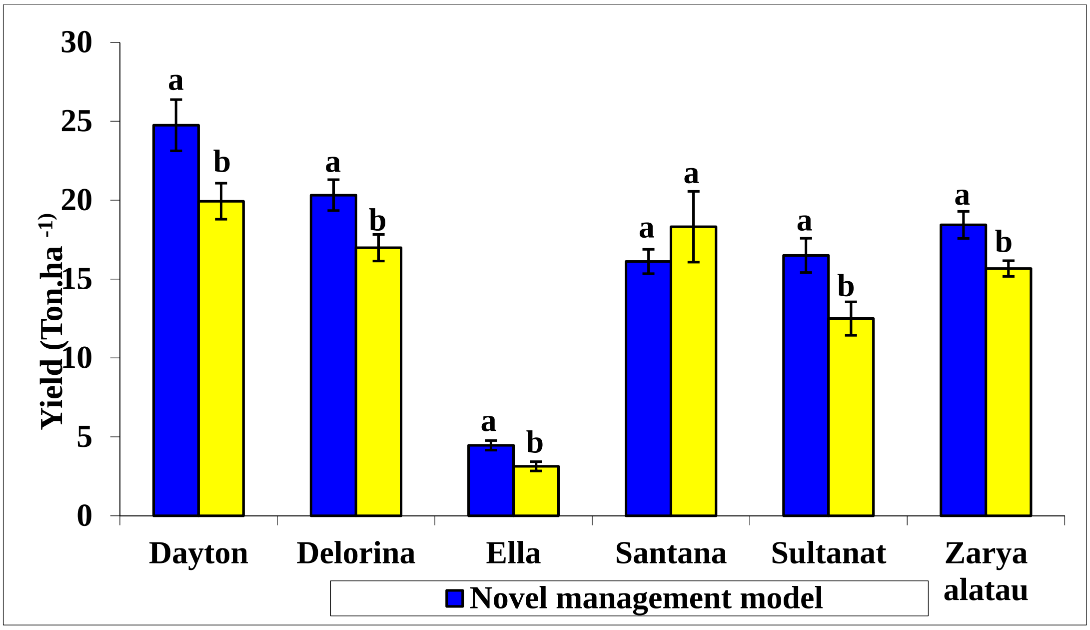

Fig. 2. Effect of novel management model (covering orchard floor by white textiles and shoot thinning by pruning in July) on the tree yield. Values are means of three years, three replicates of twelve trees each. Bars for each cultivar with the same letter are not statistically at $P \leq 0.001$.

Table 1. Effect of a novel orchard management (summer pruning in July and covering the orchard floor with a reflective textile) on fruit size and quality at harvest (1) and after cold storage in air (2), evaluated as firmness, SSC (solubles solids content), TA (titratable acid), skin color (a + b*only at harvest), and decay due mainly to fungal diseases (only after storage) of six apple cultivars grown in an organic orchard. Values are means of three years with three replicates of 30 fruits each at harvest and of 15 fruits each after storage.*

Cultivar	Management	Fruit weight (g)	Firmness ($kg.cm^{-1}$)		SSC (°Brix)		TA ($g.mL^{-1}$)		Color (a*+b*)	Decay (%)
			1	2	1	2	1	2		
Dayton	Novel	152.7 a[z]	7.8 a	5.5 a	12.2 a	12.2 a	0.8 a	0.7 a	45.6 a	0.0 b
	Standard	151.5 a	7.3 a	4.6 b	11.7 a	11.2 b	0.7 a	0.7 a	28.5 b	3.0 a
Delorina	Novel	117.3 a	9.8 a	6.8 a	9.8 a	13.6 a	0.9 a	0.6 a	53.4 a	0.8 b
	Standard	116.5 a	9.7 a	6.6 a	9.7 a	11.7 b	0.8 a	0.6 a	36.9 b	6.3 a
Ella	Novel	157.7 a	8.1 a	7.6 a	11.3 a	13.1 a	1.1 a	0.5 a	41.9 a	5.6 a
	Standard	156.3 a	7.7 a	7.1 a	10.6 a	11.6 b	1.0 a	0.3 b	25.1 b	6.4 a
Santana	Novel	165.1 a	8.7 a	7.3 a	11.4 a	12.8 a	1.1 a	0.8 a	55.1 a	0.5 b
	Standard	157.3 a	8.4 b	7.2 a	11.4 a	12.3 a	1.2 a	0.7 a	42.8 b	2.8 a
Sultanat	Novel	159.8 a	9.9 a	6.5 a	11.6 a	12.7 a	0.9 a	0.6 a	59.5 a	2.5 b
	Standard	158.7 a	9.0 a	5.3 b	11.3 a	11.6 b	0.8 a	0.5 a	47.1 b	3.2 a
Zarya Alatau	Novel	124.5 a	8.9 a	6.8 a	11.4 a	11.5 a	1.0 a	0.8 a	39.2 a	0.3 b
	Standard	126.8 a	8.6 a	5.3 b	11.3 a	10.8 a	1.0 a	0.8 a	30.6 b	2.7 a

a value = red color, b* value = yellow color. [z]within each cultivar, values with the same letter are not statistically different at $P \leq 0.001$.*

When analyzed after storage and shelf life, the application of summer pruning and ground cover produced less deterioration in fruit firmness in Dayton, Sultanat and Zarya Alatau, and in TA in all cultivars except Zarya Alatau, and a higher SSC in three cultivars, Delorina, Sultanat and Zarya Alatau, when compared to fruits grown with the standard management (Table 1, *P≤0.001*). *Neofabraea spp.* and *Colletotrichum gloeosporioides* (Fig. 3) were the most common pathogens, causing 3.0–7.0% damages (as bull's eye rot and bitter rot respectively). Fruit from the novel management model showed higher resistance (*P≤0.001*) to these pathogens in all cultivars except Ella (Table 1).

These fruit also had a higher anthocyanin content in all cultivars (Table 2, *P≤0.001*), a higher total phenolic content (25%, 28%, 29% and 39% in Dayton, Sultanat, Zarya Alatau and Ella, respectively) and a 13–15% higher ascorbic acid content in Delorina, Ella and Santana (Table 2, *P≤0.001*). Significant interactions between season and management were not found in any of the cultivars for anthocyanin or ascorbic acid, and only in Delorina (*P≤0.001*) and Sultanat (*P≤0.05*) for total phenolics (Table 2).

1 2

Fig. 3. Fungal storage diseases caused by 1. Neofabraea spp. And 2. Colletotrichum gloeosporicides in organic apples.

Table 2. Effects of a novel orchard management on antioxidant contents at harvest in six apple cultivars grown in an organic orchard. Values are means of three years.

Cultivars	Management	Anthocyanin mg.kg $^{-1}$ dw	Total phenolics g.kg $^{-1}$ dw	Ascorbic acid mg.kg $^{-1}$ dw
Dayton	Novel	56.4 a[z]	7.88 a	298.2 a
	Standard	14.1 b	6.31 b	311.1 a
Delorina	Novel	40.7 a	4.96 a	263.7 a
	Standard	18.0 b	4.21 a	231.1 b
Ella	Novel	37.8 a	6.74 a	227.1 a
	Standard	19.5 b	4.84 b	198.2 b
Santana	Novel	116.4 a	3.71 a	242.9 a
	Standard	53.6 b	3.20 a	215.1 b
Sultanat	Novel	115.3 a	6.52 a	410.9 a
	Standard	83.1 b	5.09 b	417.0 a
Zarya Alatau	Novel	47.6 a	4.76 a	362.7 a
	Standard	5.4 b	3.70 b	376.7 a

[z]*within each cultivar, values with the same letter are not statistically different at P≤0.001.*

The novel management model, with summer pruning and covering the ground with a reflective textile, increased light availability in July and August in all six studied cultivars. This management also resulted in a significantly higher yield in five of the cultivars compared with the standard practice, but did not affect fruit size.

Reducing crop load and removal of weak shoots and leaves (with low photosynthesis and high respiration rate) by summer pruning adjust the leaf/fruit ratio and carbohydrate partitioning, resulting in a positive influence on fruit quality (Yamaki, 1995; Barritt *et al*., 1997; Awad *et al*., 2001). We did, however, not see any significant differences between the two management models in fruit firmness, SSC and TA at harvest but there was a significantly lower decline for these parameters in fruit grown with this management model when analyzed after storage.

Application of summer pruning and reflective mulch has also been shown to increase fruit coloration (Tahir *et al*., 2005; Glenn and Puterka 2007), which in its turn is linked to an increased anthocyanin content (Sadilova *et al*., 2006). In our study, both anthocyanin and total phenolic content was higher in fruit produced with the new management model. In a previous study, the sun-exposed side of the fruit contained more wax than the shaded side (Tahir *et al*., 2009). Possibly both flavonoid content and wax accumulation as well as the retaining of firmness during storage may provide some physical protection or improved defense against pathogens, as evidenced by the lower rate of fungal damage in fruit grown with this management model (appendix 5).

4.2. Experiment 2. Determination of optimal harvest date

During the selected four weeks around the optimal harvesting time and in all three years, ethylene production increased and the cultivars were well differentiated. Early-ripening cultivars showed a faster increasing ethylene production in comparison with autumn- or late-ripening cultivars (Table 3). A significant increase in IEC occurred in the third harvesting week in Agra, Eir, Ella and Delorina (starting 109, 114, 119 and 139 DAFB, respectively) and in the fourth harvesting week in Dayton, Santana, Sultanat and Zarya Alatau (starting 141, 141, 146, 141 DAFB, respectively) (Table 3, $P\leq 0.001$). This rapid change took place towards the end of the critical week in 2008 and 2010, but five days earlier in 2009. Interaction between the effects of season and harvesting week for IEC was found only in two cultivars ($P\leq 0.05$), one early-ripening (Eir) and one late-ripening (Sultanat) (Table 3). However, the autocatalytic ethylene production remained at a threshold of 0.1 $\mu.L^{-1}$ for one week in Agra, Eir and Ella, and for two weeks in the other five cultivars (Table 3, $P\leq 0.001$).

A significant change in starch hydrolysis, calculated as weekly averages (three picking times a week), occurred in the last week of the harvesting period for Dayton, Santana and Sultanat (141, 141 and 146 DAFB respectively) (Table 3, $P\leq 0.001$). In Zarya Alatau and the more early-ripening cultivars (Agra, Eir and Ella), starch hydrolysis showed two peaks, one in the second week and the other in the last week of the harvesting period (Table 3, $P\leq 0.001$). Interaction between season and harvesting week for starch hydrolysis was found in Eir ($P\leq 0.001$), Delorina ($P\leq 0.05$) and Sultanat ($P\leq 0.001$); in 2009 the major change in starch index occurred five days earlier than in 2008 and 2010.

Fruit firmness decreased during the harvesting period. The maximum reduction in fruit firmness occurred at the last week, in conjunction with or particularly after the significant rise in IEC and starch hydrolysis (Table 4, $P\leq 0.001$). An interaction between harvesting date and season was found only in Eir ($P\leq 0.01$) and Sultanat ($P\leq 0.001$).

Soluble solid concentration (SSC) increased significantly during the harvesting period in all cultivars except Zarya Alatau. This change occurred during one week for Agra, Ella, Dayton, Delorina and Sultanat, and during two weeks for Eir and Santana, regardless of the season (Table 4, $P \leq 0.001$). Only for Eir and Sultanat a significant ($P \leq 0.01$) interaction between harvesting date and SSC was noted.

The Streif index decreased at the first and last week of the harvesting period and remained steady in the two intermediate weeks (Agra 102-109, Eir 107-114, Ella 112-119, Dayton 127-134, Delorina 132-139, Santana 127-134, Sultanat 132-139 and Zarya alatau 127-134 DAFB) (Table 3, $P \leq 0.001$). Significant interaction between harvesting date and season for Streif index was registered in Eir ($P \leq 0.01$), Delorina and Sultanat (both $P \leq 0.001$).

Table 3. Changes in three harvesting parameters during maturation for eight apple cultivars grown in an organic orchard. Values are means of three years, three picking a week, and three replicates of five trees each, and two fruit for starch conversion score and Streif index, and two for internal ethylene concentration.

Cultivar	Harvest week (DAFB)[x]	Internal ethylene concentration ($\mu l.L^{-1}.kg^{-1}.h^{-1}$)	Starch (1-10)	Streif index [Y]
Agra	95 - 101	0.1 c[z]	4.7 c	0.19 a
	102 - 108	0.5 c	6.1 b	0.11 b
	109 - 115	7.8 b	6.9 b	0.08 bc
	116 - 122	18.4 a	9.6 a	0.05 c
Eir	100 - 106	0.1 c	3.3 c	0.27 a
	107 - 113	0.8 c	4.5 b	0.15 b
	114 - 120	3.4 b	6.0 a	0.13 b
	121 - 127	5.3 a	6.7 a	0.09 c
Ella	105 - 111	0.1 c	3.2 c	0.28 a
	112 - 118	0.4 c	4.4 b	0.20 b
	119 - 125	0.8 b	5.0 b	0.16 b
	126 - 132	3.5 a	7.0 a	0.09 c
Dayton	120 - 126	0.02 b	3.1 b	0.27 a
	127 - 133	0.03 b	3.3 b	0.22 ab
	134 - 140	0.3 b	3.6 b	0.19 b
	141 - 147	2.5 a	5.8 a	0.10 c
Delorina	125 - 131	0.1 c	3.2 c	0.32 a
	132 - 138	0.1 c	4.1 c	0.25 b
	139 - 145	0.9 b	5.0 b	0.19 b
	146 - 152	2.1 a	6.4 a	0.11 c
Santana	120 - 126	0.06 b	2.4 b	0.34 a
	127 - 133	0.1 b	3.6 b	0.22 b
	134 - 140	0.6 b	4.0 b	0.18 b
	141 - 147	3.5 a	5.4 a	0.11 c
Sultanat	125 - 131	0.02 b	2.9 b	0.37 a
	132 - 138	0.1 b	3.7 b	0.28 b
	139 - 145	0.6 b	4.7 b	0.20 b
	146 - 152	3.4 a	6.9 a	0.11 c
Zarya Alatau	120 - 126	0.1 b	3.1 b	0.35 a
	127 - 133	0.1 b	4.2 b	0.20 b
	134 - 140	1.5 b	5.0 b	0.15 b
	141 - 147	4.4 a	7.0 a	0.09 c

*X. Days after full blooming. Y. Streif index= Firmness/ (SSC * starch hydrolysis point). Z. Values for each cultivar/column with the same letter are not statistically different at $P \leq 0.001$.*

Deterioration in fruit titratable acidity was higher at the third week in Eir, Dayton and Delorina and at the second week in the other five cultivars (Table 4, $P \leq 0.001$), again with interaction effects for Eir ($P \leq 0.05$) and Sultanat ($P < 0.001$).

Table 4. Changes in fruit quality evaluated as firmness, SSC (soluble solids content), TA (titratable acid) and skin color of eight apple cultivars grown in an organic orchard, during maturation and after four months of cold storage in air. Values are means of three years, three replicates of thirty fruits each at harvest and of fifteen fruits each after storage.

Cultivar	Harvest date (DAFB)[x]	Firmness ($kg.cm^{-1}$)		SSC (°Brix)		TA ($g.mL^{-1}$)		Red color (a* value)	
		At harvest	After storage	At harvest	After storage	At harvest	After storage	At harvest	After storage
Agra	98	8.8 a[z]	4.8 a	10.3 b	11.7 a	0.74 a	0.46 a	4.1 b	9.6 b
	105	7.3 b	3.4 b	11.2 a	11.2 ab	0.58 b	0.35 b	15.6 a	26.9 a
	112	6.6 b	2.5 c	11.9 a	10.9 ab	0.38 c	0.24 c	17.7 a	23.9 a
	119	5.5 c	2.0 c	11.9 a	10.5 b	0.27 c	0.18 d	16.9 a	21.1 a
Eir	103	8.4 a	4.4 a	10.2 c	12.0 a	0.79 a	0.46 a	3.6 c	10.1 b
	110	7.5 b	4.5 a	10.3 c	11.9 ab	0.68 a	0.40 a	8.6 b	29.3 a
	117	7.2 b	3.4 b	10.9 b	11.1 bc	0.51 b	0.19 b	14.5 a	23.9 ab
	124	6.2 c	2.2 c	11.4 a	10.9 c	0.29 c	0.15 b	14.9 a	27.7 a
Ella	108	9.1 a	4.2 a	10.0 c	11.4 a	1.04 a	0.75 a	-12.8 c	-4.1 b
	115	8.9 a	4.3 a	10.4 b	11.3 a	0.82 b	0.65 b	-7.3 b	-4.2 b
	112	8.1 b	3.1 b	10.8 a	11.1 a	0.64 c	0.54 c	0.9 a	3.2 a
	129	7.0 c	2.9 b	10.6 ab	10.9 a	0.34 d	0.06 d	3.3 a	5.1 a
Dayton	123	8.4 a	6.2 a	11.1 c	12.2 a	0.90 a	0.73 a	-3.4 b	-4.3 b
	130	8.1 a	5.4 b	11.8 ab	11.7 ab	0.79 ab	0.69 ab	1.3 ab	3.5 a
	137	7.8 a	4.9 b	11.6 bc	11.4 ab	0.66 b	0.59 b	3.1 a	5.7 a
	144	6.8 b	4.2 c	12.3 a	11.1 b	0.39 c	0.36 c	3.3 a	3.0 a
Delorina	128	10.3 a	7.6 a	10.0 b	12.8 a	0.86 a	0.27 a	-2.3 b	-5.5 c
	135	9.6 b	7.5 a	10.5 a	12.7 a	0.80 a	0.20 ab	-2.2 b	-0.6 bc
	142	9.2 b	6.5 b	10.6 a	12.4 a	0.62 b	0.12 b	2.7 ab	5.9 ab
	149	7.8 c	5.9 c	10.9 a	12.0 a	0.33 c	0.07 b	6.1 a	12.4 a
Santana	123	9.2 a	5.7 a	11.4 b	11.9 a	1.06 a	0.44 a	-0.2 b	-0.1 b
	130	9.2 a	4.8 a	11.7 b	11.7 a	0.92 b	0.25 b	3.2 b	5.5 b
	137	8.2 b	3.9 b	11.9 a	11.7 a	0.79 c	0.14 c	12.4 a	22.8 a
	144	7.4 c	3.6 b	12.4 a	11.6 a	0.51 d	0.06 d	18.1 a	17.1 a
Sultanat	128	10.2 a	6.5 a	10.5 b	12.4 a	0.98 a	0.63 a	-0.2 b	16.3 a
	135	10.1 a	6.2 a	11.5 a	11.8 ab	0.82 b	0.57 ab	3.2 b	22.6 a
	142	8.8 b	5.9 a	11.7 a	11.4 b	0.71 b	0.50 bc	12.4 a	19.2 a
	149	8.5 b	4.8 b	11.9 a	10.9 b	0.58 c	0.33 c	18.1 a	19.6 a
Zarya Alatau	123	11.2 a	7.6 a	11.4 a	12.1 a	0.92 a	0.73 a	-10.9 b	-4.2 b
	130	9.2 b	6.8 a	11.3 a	12.5 a	0.74 b	0.55 b	-7.8 b	-2.7 b
	137	8.3 c	6.4 a	11.2 a	11.5 b	0.50 c	0.40 c	1.0 a	1.9 a
	144	7.2 d	4.0 b	11.8 a	11.1 b	0.33 d	0.17 d	2.7 a	4.4 a

X. Days after full blooming. Z. Bars for each cultivar/column with the same letter are not statistically different at $P \leq 0.001$.

Decay occurrence during storage in air is associated with ethylene production. Fruit picked in all three years after significant raise in IEC showed higher decay in comparison with fruit picked before this raise ($P \leq 0.001$) except for the cultivar Dayton (Fig. 4).

Harvesting fruits just before the increase also helped to maintain their quality during storage and shelf life. Thus, firmness, SSC and TA remained more stable compared to in fruits picked two weeks before or after the increase (Table 5, *P≤0.001*). An interaction between the effects of season and harvesting date was found in Eir and Sultanat for decline in quality parameters (firmness, SSC and TA) and in Agra, Ella, Delorina and Zarya Alatau for storage decay (*P≤0.05*).

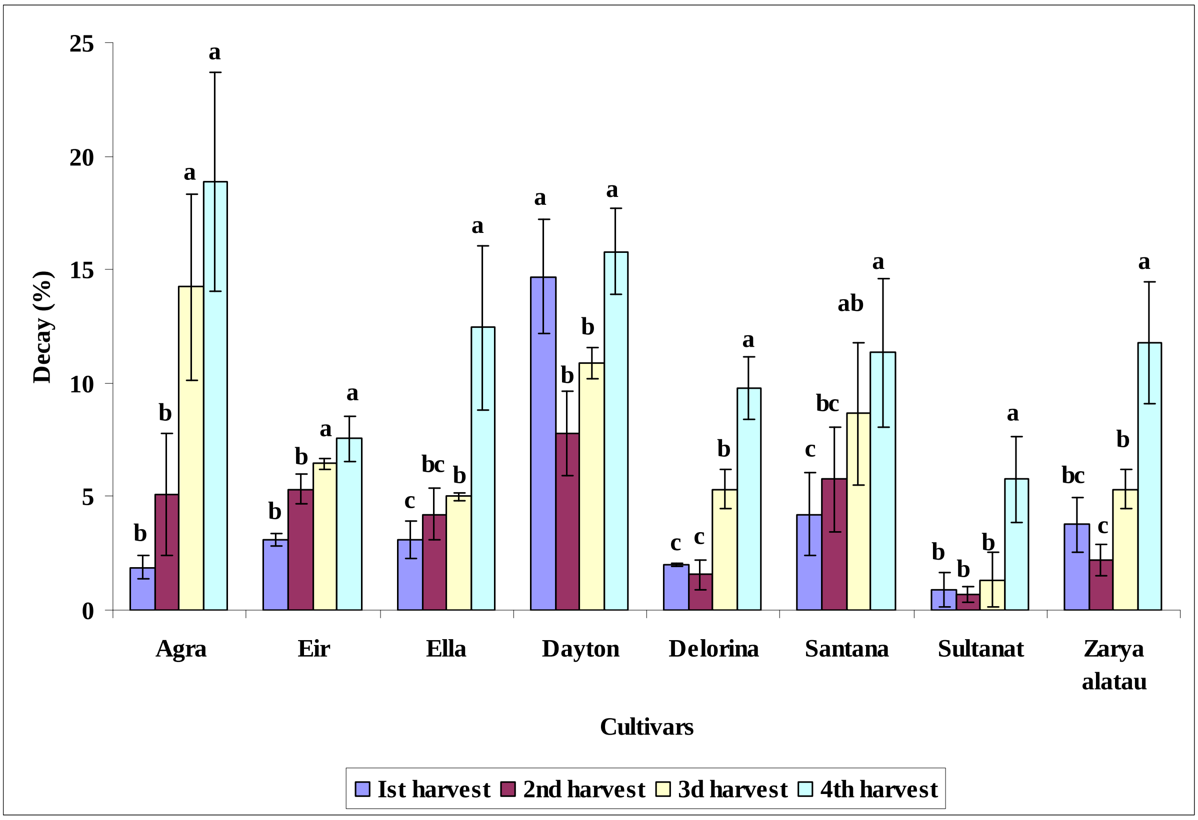

Fig. 4. The storability of different cultivars grown in an organic orchard and picked at different dates. Percentage fruit that had decayed due to fungi during cold storage in air for four months is indicated on the y-axis. Values are means of three year and three replicates of 150 fruits each. Within each cultivar, bars with the same letter are not statistically different at P≤ 0.001.

Highly significant positive correlations between ethylene production and starch hydrolysis during ripening were found in Agra, Santana and Sultanat (*P*≤0.001), medium significant correlations in Ella, Dayton and Zarya Alatau (*P*≤0.01) and only barely significant correlations in Delorina and Eir (*P*≤0.05) (Table 6). Ethylene production also showed highly significant negative correlations with fruit firmness in Agra, Dayton, Santana and Sultanat, medium significant in Eir, and only barely significant in Ella, Delorina and Zarya Alatau (Table 6; *P*≤0.001). Correlations between ethylene production and SSC were highly significant only in Eir, medium significant in Agra, Santana and Zarya Alatau, barely significant in Dayton, and non-significant in the remainder (Table 6).

Highly significant negative correlations were found between IEC and TA in all cultivars except Dayton and Santana which showed medium correlations. Highly significant correlations were found between ethylene production and Streif index in Eir, Delorina and Zarya Alatau, medium in Agra and barely significant in the remainder (Table 6). All these correlations were found in each of the three seasons.

Table 5. Quality decline during storage due to different harvesting dates (presented as percent, average of three years).

Cultivar	Harvest date (DAFB) [x]	Decline in firmness (%)	Decline in SSC[Y] (%)	Decline in TA[Y] (%)
Agra	98	45.5 c [z]	12.0 a	37.8 ab
	105	53.4 b	0.0 b	39.7 a
	112	62.1 a	-9.2 c	36.8 ab
	119	63.6 a	-13.3 c	33.3 b
Eir	103	47.6 c	15.0 a	29.1 d
	110	42.3 c	13.4 a	41.1 c
	117	52.8 b	1.8 b	62.7 a
	124	64.5 a	- 4.6 c	48.3 b
Ella	108	53.8 b	14.0 a	27.9 b
	115	51.7 b	8.6 b	20.7 c
	112	61.7 a	2.8 c	15.6 c
	129	58.6 a	2.8 c	82.4 a
Dayton	123	26.2 c	9.0 a	18.9 a
	130	33.3 b	-0.9 b	12.7 b
	137	37.2 a	-1.8 b	10.6 b
	144	38.2 a	-10.8 c	7.7 b
Delorina	128	26.2 b	21.9 a	68.6 c
	135	23.4 b	17.3 b	75.0 b
	142	29.3 a	14.5 b	80.6 a
	149	24.4 b	9.2 c	78.8 ab
Santana	123	38.0 c	4.2 a	58.5 d
	130	47.8 b	-1.7 b	72.8 c
	137	52.4 a	0.0 b	82.3 b
	144	51.4 a	-6.9 c	88.2 a
Sultanat	128	36.2 bc	15.3 a	35.7 b
	135	39,0 b	2.5 b	30,4 c
	142	32,9 c	-2.6 b	30,0 c
	149	43,5 a	-9.2 c	43,1 a
Zarya Alatau	123	32.1 a	6.1 b	20.7 b
	130	26.1 b	10.6 a	25.7 b
	137	22.9 b	2.6 c	20.0 b
	144	33.3 a	-6.3 d	48.4 a

X. Days after full blooming. Y. SSC= Solid soluble concentration, TA= titratable acidity. Z. Values for each cultivar with the same letter are not statistically different at $P \leq 0.001$.

Table 6. Pearson correlation between ethylene production ($\mu l.L^{-1}.kg^{-1}.h^{-1}$) and starch index (scale 1-10 points), firmness ($kg.cm^{-2}$), mean fruit soluble solids concentration (SSC. in gram per 100 ml) and titratable acidity (TA. as a percent) in the fruits of eight apple cultivars grown in an organic orchard.

Cultivar	IEC and starch	IEC and firmness	IEC and soluble solids concentration	IEC and titratable acidity	IEC and Streif index
Agra	0.8321 ***[z]	-0.7381 ***	0.6027 **	-0.8089 ***	-0.69 **
Eir	0.6962 *	-0.7027 **	0.7564 ***	-0.7739 ***	-0.71 ***
Ella	0.7067 **	-0.5705 *	-0.0206 ns	-0.7847 ***	-0.57 *
Dayton	0.7523 **	-0.7442 ***	0.5347 *	-0.7228 **	-0.62 *
Santana	0.8570 ***	-0.7801 ***	0.5757 **	-0.7202 **	-0.63 *
Zarya Alatau	0.7537 **	-0.5654 *	0.6175 **	-0.7750 ***	-0.70 ***
Delorina	0.6726 *	-0.6121 *	0.4146 ns	-0.7790 ***	-0.77 ***
Sultanat	0.8447 ***	-0.7233 ***	0.3267 ns	-0.7693 ***	-0.56 *

** Significant at $p \leq 0.05$, ** Significant at $p \leq 0.01$, *** Significant at $p \leq 0.001$ and ns. not significant at $p \leq 0.05$.*

Choice of an optimum harvesting date can help to maximize fruit quality and minimize storage disorders in organic apple production (Echeverria *et al*., 2002). Fruit storability is closely associated with the climacteric patterns of ethylene production (Elgar *et al*., 1998). Since the accelerated production of ethylene is one of the most important changes in apples as they begin to ripen, it can be used as an accurate indicator for determination of the optimum harvesting date (Sanders, 2003; Genard and Gouble, 2005). Harvesting should be conducted in the pre-climacteric period, when no significant quantities of ethylene have yet been produced or, at least, before half of the fruits have reached the threshold level of autocatalytic ethylene production (0.1 $\mu l.L^{-1}.kg^{-1}.h^{-1}$) (Bulens *et al*., 2010).

Our results showed that fruits of all of the investigated cultivars should be picked before the significant raise in IEC (when less than 50% of them started to produce ethylene) in order to avoid storage decay and maintain quality. However, early-ripening cultivars which showed rapid acceleration in IEC had short harvesting period and limited storage life. The IEC in Agra, Eir and Ella thus remained at a threshold of (0.1 $\mu l.L^{-1}.kg^{-1}.h^{-1}$) for only one week while it continued unaffected during two weeks in the other five cultivars.

To investigate a more commonly maturity index, easier than estimation of IEC, the starch conversion into sugar was controlled. The degradation of starch per interval of time was similar for each cultivar during the seasons. Fruits picked before a significant change in starch index showed overall very good storability.

According to the very close agreement between IEC and starch hydrolysis (for Agra, Santana and Sultanat) and close agreement (for Dayton, Ella and Zarya Alatau), starch index can be safety used as a maturity index (Peirs et al., 2004) for these cultivars, while due to the barely significant correlation between IEC and starch conversion score, the latter cannot be used as a maturity index for Eir and Delorina.

The Streif index, which makes use of three parameters: fruit firmness, SSC and starch hydrolysis (Streif, 1996; Baumann, 1998) is a commonly used method for determination of harvesting date. In our study, especially Eir, Delorina and Zarya Alatau, and, to a somewhat lesser degree Agra, showed a strong correlation between the Streif index and IEC, indicating that this index is very useful for determination of optimum harvest date in these cultivars.

The rise in ethylene production was closely associated also with fruit firmness and TA (for Ella and Dayton), and to a lesser extent with SSC. Using these parameters for determination of optimal harvesting date will be less accurate since they are strongly cultivar- and environment-dependent (Willats et al., 2001).

Finally, it should be mentioned that parameters involved in maturation must be determined on fruits collected from similar positions in the trees. Due to the widely varying weather conditions, it was still not possible to obtain estimates of color development that could be used as a maturity index.

Optimal harvesting date of eight apple cultivars grown in an organic orchard

Our results showed that the investigated cultivars should be picked according to the following information in order to decrease fungal decay and to achieve better maintain of firmness, SSC and TA, during storage in air:

- **Agra** apples: during one week (95 – 102 DAFB), when IEC is 0.1-0.5 $\mu l.L^{-1}.kg^{-1}.h^{-1}$ and/or starch hydrolysis point is 4.5 - 6.0.
- **Santana** apples: during two weeks (120-134 DAFB), when IEC is 0.06-0.6 $\mu l.L^{-1}.kg^{-1}.h^{-1}$ and/or starch hydrolysis point is 3.0-4.0.
- **Sultanat** apples: during two weeks (125-139 DAFB), when IEC is 0.02-0.6 $\mu l.L^{-1}.kg^{-1}.h^{-1}$ and/or starch hydrolysis point is 3.0-4.7.
- **Eir** apples: during one week, 100-107 DAFB, when IEC is 0.1-0.8 $\mu l.L^{-1}.kg^{-1}.h^{-1}$ and/or Streif index is 0.25-0.15.
- **Delorina** apples: during two weeks (125-139 DAFB), when IEC is 0.1-0.9 $\mu l.L^{-1}.kg^{-1}.h^{-1}$ and/or Streif index is not less than 0.19.
- **Zarya Alatau:** during two weeks (120-134 DAFB), when IEC is 0.1-1.5 $\mu l.L^{-1}.kg^{-1}.h^{-1}$ and/or Streif index is 0.20-0.15.
- **Ella** apples: during one week (105-112 DAFB), when IEC is 0.1-0.4 $\mu l.L^{-1}.kg^{-1}.h^{-1}$. Streif index is not an acceptable method to estimate optimum harvest date. Therefore, we recommended acidity which must be not less than 0.6% in additional to starch index 3-4.
- **Dayton** apples: during one week (127-134 DAFB), when IEC is 0.1-0.5 $\mu l.L^{-1}.kg^{-1}.h^{-1}$. Again Streif index is not an acceptable method to estimate optimum harvest date. Therefore, we recommended acidity which must be not less than 0.7% in additional to starch index 3-4.

4.3. Experiment 3. Postharvest treatment with heating and ethanol:

Dopping fruits in hot water (46º C for 120 seconds) decreased decay caused by *Neofabraea* during storage ($P \le 0.001$) in two cultivars, Dayton by 29% and Eir by 22%, while no effect was detected on fruit of Agra and Sultanat (Fig 5).

Post-harvest spraying fruits with 10% ethanol decreased the incidence of *Neofabraea* decay ($P \le 0.001$) in Dayton (by 83%), in Sultanat (by 72%), in Eir (by 51%) and in Agra (by 45%) (Fig 5). This positive effect was found in both years, and there was no significant interaction between year and treatment for any of the cultivars.

Enhancing the wound repair process, inhibition of the synthesis of cell wall hydrolytic enzymes (Ben-Shalom *et al*., 1996) and changes in apple skin structure may explain heating effectiveness (Veraverbeke *et al*., 2001). However, post-harvest heating by hot air hinders fungal decay. It changes epicuticular wax, stimulates wax synthesis and recrystallization (Tahir *et al*., 2009) and melts wax to fill micro-wounds and lenticels and cover germinated spores, conidia and hyphae (Tahir *et al*., 2009). Hot water may also melt skin wax and change its structure, causing better defense on the fruit surface (Roy *et al*., 1994; Díaz-Pérez *et al*., 2001; Fallik, 2006; Tahir *et al*., 2009). The effectiveness of hot water depended on cultivar confirming previous studies (Spadaro *et al*., 2004; Tahir *et al*., 2009). It can not be recommendered for Agra and Sultanat.

Ethanol application is considered a good substitute for controlling postharvest pathogens in in mango (Gutiérrez-Alonso *et al*., 2004; Plotto *et al.,* 2006; Gutiérrez-Martínez *et al*., 2012), grape (Chervin *et al.,* 2005), and peaches and nectarines (Margosan *et al*., 1997). In additional to its antimicrobial effect, it may be delay ripening, causing better defence against fungous attack (Chervin *et al*., 2005; Zhang *et al*., 2007). Our results showed that ethanol can be a good natural fungicides in organic apple production because the incidence of decay due to *Neofabraea spp.* was decreased in the treated fruit in comparison with untreated.

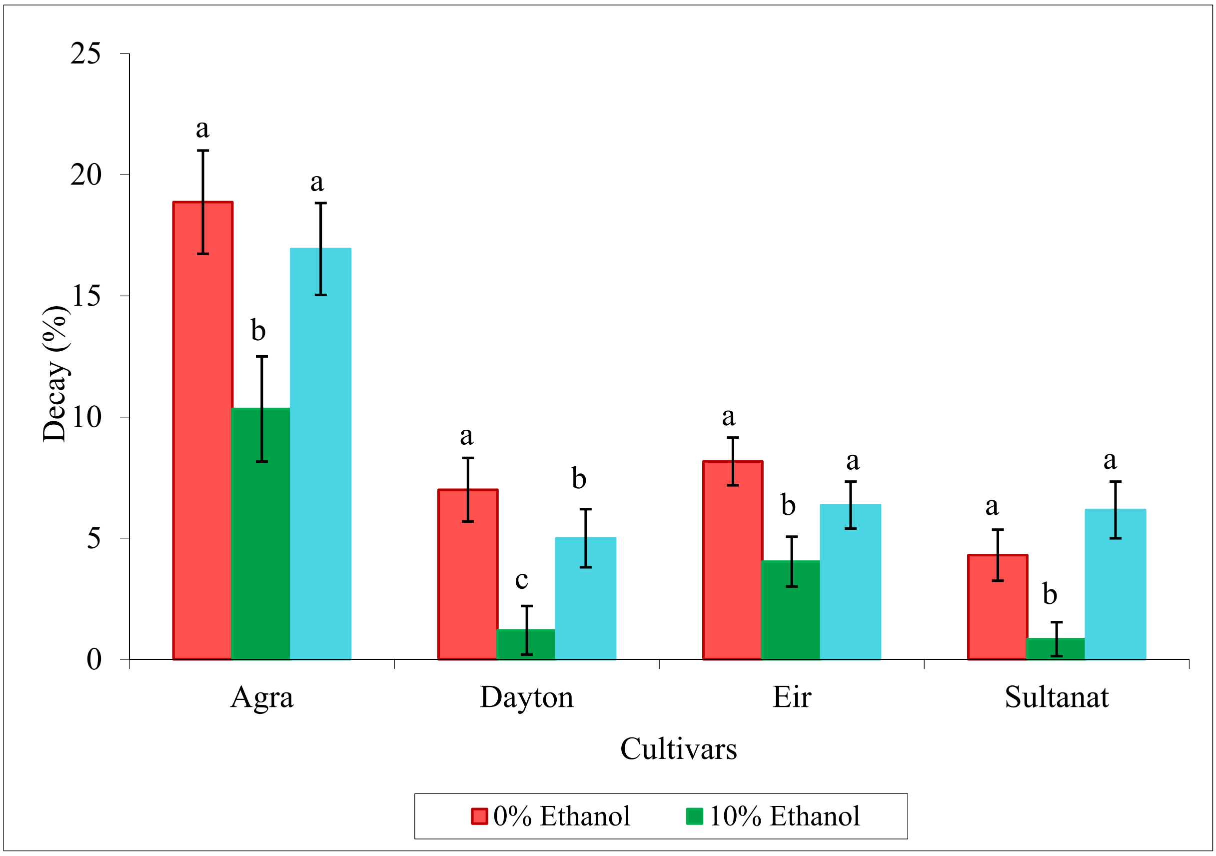

Fig. 5. Effect of postharvest treatments on the incidence of fungal decay. Values are means of three years, three treatments, three replicates of twenty five fruits each. Bars for each cultivar with the same letter are not statistically different at P ≤ 0.001.

4.4. Experiment 4. Controlled atmosphere storage of organic apple cultivars

Fruit appearance was positive affected by controlled atmosphere storage (2.0 O_2 kPa and 2.0 kPa CO_2) in all of the studied cultivars except Zarya alatau (Table 7; *P≤0.001*). CA storage also had a positive effect on fruit firmness and TA in all of the studied cultivars (*P≤0.001*) except Eir (Table 7). By contrast, SSC showed a positive response to CA only in Agra and Dayton (Table 7, *P≤0.001*). Since no storage disorders (except flesh browning in ULO 2 for Zarya Alatau) were noted during the three seasons, storage decay was restricted to fungal decay caused by *Neofabraea*. Amount of *Neofabraea* decay decreased due to CA storage by about 50% in Agra, Dayton, and Santana, and by 60–70% in the other cultivars in comparison with storage in air (Table 7, *P≤0.001*). The positive effect of CA on fruit storability was found in all three years (except in 2009 for Dayton) in spite of significant interactions between storage method and year in Eir and Dayton (*P≤ 0.01*) and in Delorina and Sultanat (*P≤ 0.001*).

Firmness, SSC and TA in Agra, Delorina, Santana and Sultanat apples were improved by CA storage. Eir apples showed better SSC, Ella apples showed better TA, and Dayton and Zarya alatau showed better firmness and TA due to CA storage in comparison with storage in air (Table 7). All fruits from controlled atmosphere storage had higher flavor quality (TA:SSC ratio). The incidence of fungal decay, soft scald and flesh browning decreased due to CA storage, by 44% in Agra, 53% in Dayton, 68% in Eir, Ella and Santana and 72% in Delorina, Sultanat and Zarya alatau, in comparison with storage in air (Table 7).

Table 7. Storability of eight apple cultivars grown in an organic orchard evaluated as percentage of decayed fruit and deterioration in firmness, SSC (soluble solids content) and TA (titratable acid) using two different storage methods. Values are means of three years with three replicates of thirty fruit each.

Cultivars	Storage methods	Appearance (1-10)	Firmness ($kg.cm^{-1}$)	SSC[Y] (°Brix)	TA[Y] ($g.mL^{-1}$)	Decay (%)
Agra	In air	3.5 b[z]	3.6 b	10.5 b	0.2 b	20.1 a
	CA[x]	5.5 a	4.6 a	10.9 a	0.6 a	11.3 b
Eir	In air	5.0 b	3.9 a	10.6 b	0.5 a	7.8 a
	CA	6.2 a	4.2 a	12.0 a	0.8 a	2.4 b
	In air	3.9 b	5.3 a	12.7 a	0.6 b	7.2 a
Ella	CA	5.9 a	9.3 a	12.4 a	0.9 a	2.3 b
	In air	4.7 b	5.1 b	12.2 a	0.5 b	6.6 a
Dayton	CA	7.5 a	7.4 a	12.5 a	0.8 a	3.1 b
	In air	4.7 b	4.8 b	10.7 b	0.4 b	10.1 a
Santana	CA	7.9 a	7.4 a	12.0 a	0.9 a	5.3 b
Zarya Alatau	In air	6.4 a	6.3 b	11.6 a	0.4 b	4.5 a
	CA	7.0 a	7.3 a	11.8 a	0.7 a	1.2 b
Delorina	In air	5.3 b	5.6 b	12.2 b	0.3 b	9.8 a
	CA	6.7 a	7.7 a	12.8 a	0.7 a	2.7 b
Sultanat	In air	5.4 b	4.8 b	11.6 b	0.5 b	3.6 a
	CA	8.0 a	7.0 a	12.9 a	0.8 a	1.0 b

X. Controlled atmosphere storage (CA): 2.0 kPa O_2 and 2.0 kPa CO_2. Y. SSC= Solid soluble concentration, TA= titratable acidity. Z. Values for each cultivar with the same letter are not statistically different at P≤ 0.001. Values are means of three years, two storage methods and three replicates of thirty fruits each.

Ultra-low oxygen (ULO), whether 1.0 kPa or 2.0 kPa CO_2, helped to stabilize SSC in Agra, Dayton and Zarya Alatau, and TA in Agra, Eir, Ella, Dayton and Santana (Table 8, *P≤0.001*). Agra, Delorina and Sultanat apples stored in ULO with 2.0 kPa CO_2 showed better firmness in comparison with fruits stored in CA (Table 8, *P≤0.001*). Amount of fungal decay caused by *Neofabraea* decreased in Dayton and Zarya Alatau in all three seasons and in Santana and Sultanat in two seasons when the fruit was stored in ULO instead of CA (Table 8, *P≤0.001*). However, the higher CO_2 caused skin injury and flesh browning in Zarya Alatau (Fig. 6).

Table 8. Storability of eight apple cultivars grown in an organic orchard evaluated as percentage of decayed fruit and deterioration in firmness, SSC (soluble solids content) and TA (titratable acid) using three different storage methods. Values are means of three years with three replicates of thirty fruit each.

Cultivars	Storage methods	Appearance (1-10)	Firmness ($kg.cm^{-1}$)	SSC^{Y} (°Brix)	TA^{Y} ($g.mL^{-1}$)	Decay (%)
Agra	CA	5.5 b^{z}	4.6 b	10.9 b	0.6 a	6.3 a
	ULO 1^{x}	6.4 ab	5.1 a	11.8 a	0.5 b	6.0 a
	ULO 2^{x}	6.7 a	5.2 a	11.8 a	0.5 b	5.7 a
Eir	CA	6.2 a	4.2 a	12.0 a	0.8 a	2.4 a
	ULO 1	6.5 a	4.2 a	11.5 a	0.9 a	2.3 a
	ULO 2	7.2 a	4.3 a	12.0 a	0.7 a	2.3 a
Ella	CA	5.9 a	9.3 a	12.4 a	0.9 a	2.3 a
	ULO 1	5.8 a	9.0 a	12.4 a	0.7 b	2.0 a
	ULO 2	5.8 a	9.1 a	12.5 a	0.6 b	1.0 a
Dayton	CA	7.5 a	7.4 a	12.5 b	0.8 a	2.1 a
	ULO 1	6.7 a	7.3 a	12.6 b	0.7 a	2.1 a
	ULO 2	6.9 a	7.6 a	13.1 a	0.7 a	0.8 b
Santana	CA	7.9 a	7.4 a	12.0 a	0.9 a	5.3 a
	ULO 1	7.6 a	7.4 a	11.8 a	0.8 a	4.7 ab
	ULO 2	7.7 a	7.3 a	12.2 a	0.8 a	2.6 b
Zarya Alatau	CA	7.0 a	7.3 a	11.8 b	0.7 a	1.2 a
	ULO 1	7.2 a	7.7 a	12.2 a	0.7 a	0.5 b
	ULO 2	7.1 a	7.4 a	12.5 a	0.6 a	1.3 a
Delorina	CA	6.7 a	7.7 a	12.8 a	0.7 a	2.7 a
	ULO 1	6.9 a	7.8 a	12.9 a	0.6 a	2.5 a
	ULO 2	7.0 a	7.6 a	12.9 a	0.6 a	2.4 a
Sultanat	CA	8.0 a	7.0 a	12.9 b	0.8 a	1.0 a
	ULO 1	8.0 a	6.8 a	12.9 b	0.7 b	0.9 a
	ULO 2	8.1 a	7.2 a	13.7 a	0.8 a	0.2 b

*X. Ultra low oxygen (*ULO: 1.0 kPa O_2 and 1.0 kPa CO_2 and 1.0 kPa O_2 and 2.0 kPa CO_2*). Y. SSC= Solid soluble concentration, TA= titratable acidity. Z. Bars for each cultivar with the same letter are not statistically different at P≤0.001. Values are means of three years, three storage methods, three replicates of thirty fruits each.*

The main benefits of CA and ULO are that the storage period can be prolonged; fruit quality is maintained and fewer problems with physiological disorders are encountered (Brackman *et al*., 1994; Graell *et al*., 1997; López *et al*., 2000). Our results similarly indicate that CA storage is very beneficial for retaining fruit quality, with a large improvement especially in firmness and TA. Even more pronounced effects were obtained for incidence of fungal decay, which was significantly lower in CA-stored fruit of all cultivars compared to fruit in regular air storage.

Storage of these cultivars in a different range of pCO_2 achieved slight additional improvements in some cultivars (Fig. 7), but high CO_2 can also increase disorders. Thus, ULO 1 (1.0 kPa O_2 and 1.0 kPa CO_2) decreased fungal decay and improved flavor quality (SSC:TA ratio) in Zarya Alatau while ULO 2 (1.0 kPa O_2 and 2.0 kPa CO_2) caused flesh browning in this cultivar. Fruits of Dayton and Sultanat responded well to ULO 2 since storage decay decreased and flavor quality improved in comparison with ULO 1 and CA. ULO 2 also decreased storage decay in Santana. In conclusion, CA (2.0 kPa O_2 and 2.0 kPa CO_2) can be recommended for Agra, Eir, Ella and Delorina; ULO 1 (1.0 kPa O_2 and 1.0 kPa CO_2) for Zarya Alatau and ULO 2 (1.0 kPa O_2 and 2.0 kPa CO_2) for Dayton, Santana and Sultanat.

Fig. 6. Skin injury in Zarya Alatau due to high CO_2 level in ULO storage chamber.

AGRA

In air **In ULO**

Delorina

In air In ULO

EIR

In air In ULO

ELLA

In air In ULO

Zarya Alatau

Fig. 7. Fruit color and appearance after shelf life, followed different storage methods

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Appendix 1.

ULO storage chambers

Appendix 2.

Fruit were kept for shelf life

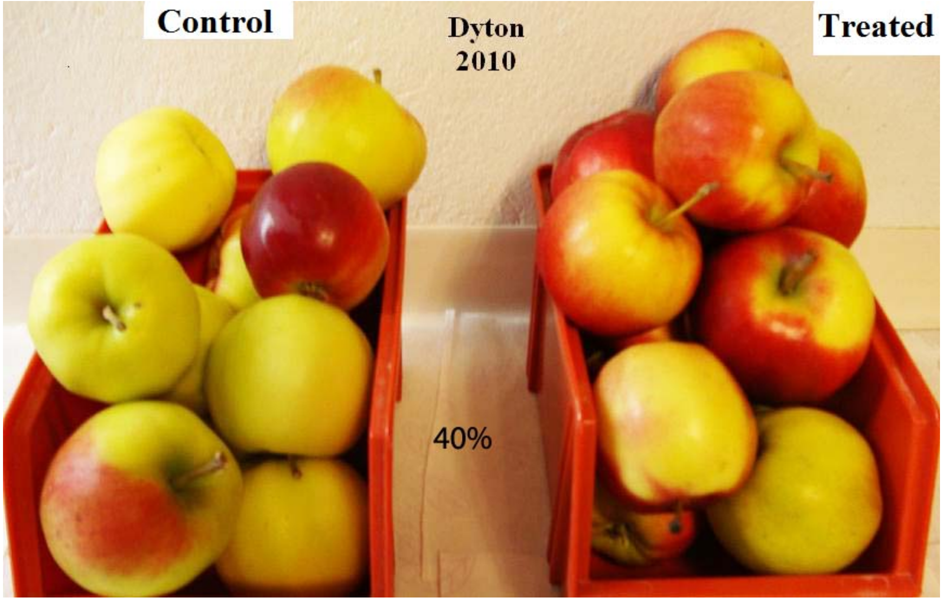
Appendix 3.

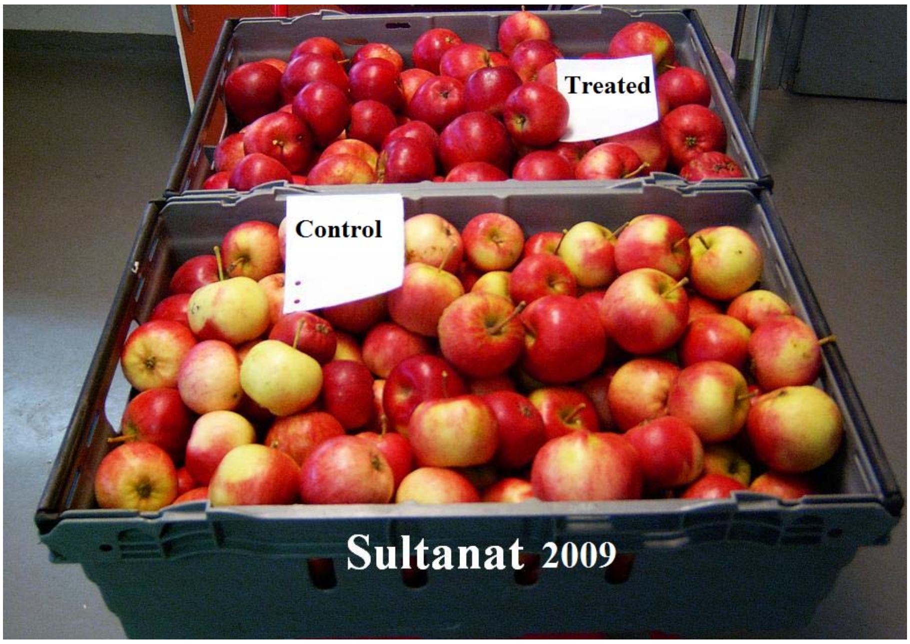
Starch hydrolysis during fruit maturation

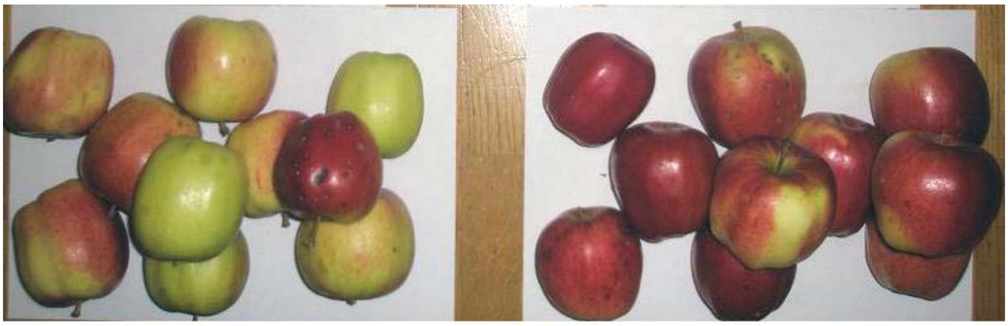
Appendix 4.

Orchard ground covering with white textil

Appendix 5.







Control **Treated**

Delorina 2008

Covering the orchard floor and adjusting vegetative growth in the summer improve fruit color in apples grown in an organic orchard.