



Comparing ripening and storage characteristics of 'Oded' peach and its nectarine mutant 'Yuval'

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ABSTRACT

A peach-to-nectarine mutant 'Yuval', a white, melting-flesh, cling-stone fruit was compared over three seasons with its peach progenitor 'Oded', an early season cultivar, to study ripening and storage characteristics. Twenty-four genome-spanning single sequence repeats markers showed the identity of the peach with its nectarine mutant at the DNA level. There was no difference in cell size at harvest between the 'Oded' peach and 'Yuval' nectarine, although 'Oded' peach was 24% larger by weight than 'Yuval'. The 'Oded' peaches were also less acidic, and had less soluble solids than the 'Yuval' nectarine at harvest. Fruit were stored at two temperatures, 5 °C and 0 °C. Softening was faster in the fruit of both cultivars stored at 5 °C than 0 °C. At 3 d ripening at 20 °C after cold storage, there was more expressible juice at 5 °C than 0 °C in the fruit of both cultivars. 'Oded' peaches developed internal browning and wooliness at 3 d ripening at 20 °C after 5 and 7 weeks 5 °C storage, and had lower expressible juice than 'Yuval' nectarines. Cold storage at 0 °C plus ripening reduced flesh browning, woolly texture and flesh bleeding incidence in 'Oded' fruit compared to ripening after storage at 5 °C. Flesh browning and woolly texture incidence was lower in the 'Yuval' nectarines than 'Oded' peaches. Overall, the data suggest that 'Oded' and 'Yuval' are genetically similar and 'Yuval' conserves several fruit and ripening characteristics that usually come with peach-to-nectarine mutations. Furthermore, 'Yuval' nectarine is comparatively more resistant to chilling injury (flesh browning and woolly texture) than 'Oded' peach after prolonged storage.

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1. Introduction

Peaches and nectarines are highly perishable; they ripen and deteriorate quickly at ambient temperature (Lurie and Crisosto, 2005). Therefore, low temperature storage (0–5 °C) is used to slow the ripening processes as well as decay development during storage and/or shipment (Crisosto et al., 1999; Lurie and Crisosto, 2005). However, if the fruit are held too long at a low temperature they will not ripen properly when rewarmed and will develop chilling injury (CI). The manifestations of CI in peaches and nectarines include defective cell wall disassembly and the development of a dry, woolly rather than soft, juicy texture (Lurie and Crisosto, 2005).

Nectarines arose as peach mutants, and their inheritance pattern is consistent with the glabrous skin characteristic controlled by a single recessive gene (Blake, 1932). Most aspects of nectarine trees, leaves, and flowers are indistinguishable from those of peach; how-

ever, peach researchers have noted differences between peaches and nectarines that extend beyond lack of pubescence. These differences include fruit size, shape, firmness, external color, aroma, flavor, and disease resistance (Wen et al., 1995a,b). It addition, it has been reported that nectarines have better storage characteristics than peaches (Crisosto et al., 1999).

The nectarine character is controlled by a recessive gene, *g*, which determines the glabrous character of the fruit skin. It has been mapped to linkage group 5, but has no close linkage with other markers (Dirlewanger et al., 1998). The hypothesis is that nectarines are phenotypes for a minor, non-lethal mutation, which is further characterized by including closely linked genes that have other roles. Alternatively, it is possible that the nectarine phenotype may arise as an alteration in a single regulatory gene that controls the expression of other genes. This interpretation is consistent with genetic evidence and molecular characterization of the two known classes of glabrous mutations in *Arabidopsis thaliana* (L.) Heynh., glabrous (*g11*) and transparent testa glabrous (*ttg*) (Walker et al., 1999; Maes et al., 2008). It is also consistent with the pleiotropic effect of the mutation, which alters a number of organoleptic and

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morphological characteristics of the fruit. The organoleptic characteristics of organic acid and sugar content have been mapped to diverse linkage maps in peach and are not found associated with the G locus (Dirlewanger et al., 1999).

A nectarine mutant 'Yuval' arose in 2002 within an 'Oded' peach population from a commercial orchard in central Israel. Because the peach cultivar 'Oded' was of high quality, the mutation of interest was propagated. The objective of this study was to compare ripening and storage characteristics of the nectarine with its peach progenitor. This isogenic material provides an opportunity to define physiological attributes of the peach and its peach to nectarine mutation.

2. Materials and methods

2.1. Plant material and treatments

The experiments were carried out with an early-season variety peach [*Prunus persica* (L.) Batsch 'Oded'] and its early-season nectarine mutant [*P. persica* (L.) Batsch, var. nectarine 'Yuval'] for three seasons from 2007 to 2009. The peach and nectarine fruit were harvested from a commercial orchard in Israel. Four hundred and twenty fruit were harvested for each cultivar. The fruit were weighed individually and divided into two lots in addition to sampling at harvest, and harvest after 3 d ripening at 20 °C. One lot was stored immediately in 0 °C, and starting from the 3rd week, the fruit were removed from cold storage every two weeks for up to seven weeks and subsequently held at 20 °C for 3 d for ripening after each removal. The second lot was stored immediately at 5 °C, the fruit were removed from cold storage weekly for three weeks and again after five and seven weeks, and subsequently held at 20 °C for 3 d for ripening after each removal. At the time of observation, 12–15 fruit from each treatment were examined. On the days of removal and after 3 d of ripening each fruit was weighed individually to determine weight loss.

2.2. Endogenous ethylene production

Ethylene production by the peach and nectarine fruit at harvest was determined by gas chromatograph (GC; Varian 3300, Walnut Creek, CA, USA) with an FID detector and an alumina column. Each fruit was individually sealed in a jar (600 mL) for 1 h at 20 °C, and a 5 mL gas sample was taken with a syringe and loaded onto a GC for analysis.

2.3. Firmness, expressible juice, soluble solids content (SSC) and titratable acidity (TA)

Physiological parameters were measured following the protocol described earlier (Zhou et al., 2000a). In brief, firmness was measured on two pitted sides of each fruit using a penetrometer fitted with an 8-mm diameter plunger. After firmness was determined, the fruit was cut into two halves and woolliness was estimated both by visual observation and organoleptically. Juicy fruit with no signs of woolliness were classed as healthy.

The amount of expressible juice was determined by removing a tissue plug weighing about 2 g from each fruit with a cork borer, passing it through a 5 mL syringe into an eppendorf tube, centrifuging and separately weighing the juice and solids (Lill and van der Mespel, 1988).

A wedge-shaped slice (approximately 5 g) was removed from each fruit in the replicates and the pooled sample was passed through an electric juicer (Moulinex, type 753 France) for the measurement of soluble solids content (SSC) and titratable acidity (TA) by a digital refractometer (Atago, Tokyo, Japan) and titration,

respectively. The TA was determined by titration of 2 mL juice to pH 8.2 with 0.1 N NaOH and expressed as percentage of malic acid.

2.4. Fruit cell size measurement

Fruit sections about 1 cm in from the peel were examined under transmitted light microscopy and photographed using a Nikon Eclipse 50i microscope and a Nikon Digital Sight DS-L1 camera system. The cell size was measured using ImageJ, Java-based image processing program.

2.5. Flesh browning (FB), woolly texture (WT) and flesh bleeding (FBL) indices

FB, WT and FBL indices determined as flesh or pit cavity browning, fruit with no juice upon squeezing, and flesh with reddening, respectively, were evaluated visually on fruit following cold storage plus 3 d ripening at 20 °C after cutting individual fruit longitudinally in half.

FB and FBL were scored on a 5-grade scale, according to flesh browning or reddening area as follows: 1, no browning or reddening; 1.5, affected area <5%; 2, affected area ≥5% and <25%; 2.5, affected area ≥25% and <50%; and 3.0, affected area ≥50%. Results were expressed as an index calculated as (%): $((\sum \text{FB or FBL level} \times \text{number of fruit at the FB or FBL level}) / (3 \times \text{total number of fruit in the treatment}) \times 100)$.

WT was also scored according to a 5-grade scale, according to amount of juice released upon squeezing, as follows: 1, very juicy; 1.5, moderate juicy; 2, less juicy; 2.5, small amount of juice; and 3.0, almost no juice. Results were expressed as a WT index calculated as WT index (%): $((\sum \text{WT level} \times \text{number of fruit at the WT level}) / (3 \times \text{total number of fruit in the treatment}) \times 100)$.

2.6. DNA extraction

DNA was extracted from 5 g of young expanding leaves of the peach and its nectarine mutant using a chloroform-isopropanol precipitation protocol as described by Foolad et al. (1995). In short, purification steps involved incubation with RNase (10 mg/mL), followed by extraction with chloroform-isoamyl alcohol (24:1), precipitation with two thirds volume of isopropyl alcohol, two washes with a solution of 76% ethanol and 10 mM ammonium acetate, and dissolving in sterile double-distilled water.

2.7. 'Oded' peach and 'Yuval' nectarine DNA molecular analysis

DNA molecular analysis was carried out on leaf DNA as described previously (Peace et al., 2005). SSR (simple sequence repeat) marker systems were employed to provide marker profiles that, in the context of the present study, were useful in verifying the parentage of 'Oded' peach and its nectarine mutant 'Yuval'. SSR assays were performed according to Dirlewanger et al. (2002) using polyacrylamide gels and silver staining (Promega Corporation, Madison, WI). The list of genome-spanning markers used in 'Oded' and 'Yuval' differentiation is shown in Table 1.

3. Results

3.1. Ethylene production and cell size measurement

There was no difference in ethylene production between the 'Oded' peach and 'Yuval' nectarine fruit at harvest. The ethylene levels in the 'Oded' and 'Yuval' fruit were 0.67 $\mu\text{L kg}^{-1} \text{h}^{-1}$ and 0.62 $\mu\text{L kg}^{-1} \text{h}^{-1}$, respectively, at harvest. Furthermore, no difference in cell size was observed between the two cultivars at harvest

Table 1

List of genome-spanning markers for 'Oded' peach–'Yuval' nectarine differentiation. All markers were monomorphic. The markers with a line through them did not yield any amplification product.

Linkage group	Top	Middle	Bottom
1	UDP96-018	BPPCT021	BPPCT028
2	UDP98-025	BPPCT013	BPPCT030
3	BPPCT007	BPPCT039	UDP96-008
4	CPPCT005	UDP95-003	F-M endoPG
5	BPPCT026	BPPCT017	BPPCT038
6	UDP98-416	BPPCT009	UDP98-412
7	CPPCT022	CPPCT033	EPPCU5176
8	BPPCT033	CPPCT006	EPPCU4726

(data not shown). However, the 'Oded' fruit were 24% bigger by weight than the 'Yuval' fruit at harvest in all three seasons (Table 2).

3.2. Firmness, expressible juice, soluble solids content, titratable acidity and weight loss

There was no consistent difference in firmness between 'Oded' peach and 'Yuval' nectarine firmness at harvest over the three seasons (Table 2). Soluble solids content and titratable acidity were higher in 'Yuval' nectarines than in 'Oded' peaches. Following storage at both 0 and 5 °C the differences in soluble solids content and titratable acidity seen at harvest in the two cultivars remained, with 'Yuval' nectarines still having higher values (data not shown).

Weight loss was similar during 0 °C storage for both cultivars (Fig. 1A). However, at 5 °C storage weight loss of 'Yuval' nectarines greatly exceeded that of 'Oded' peaches. Furthermore, cracks developed on the epicarp of 'Yuval' nectarines after 7 weeks of cold storage at 5 °C which were much less on 'Oded' fruit (data not shown). Firmness of fruit stored at 0 °C changed very little over 7 weeks of storage and was similar for both cultivars (Fig. 2A). When storage was at 5 °C the fruit retained their firmness for the first two weeks of storage, and then began to soften in storage. 'Oded' peaches softened in storage to a greater extent than 'Yuval' nectarines. During shelf life following storage the fruit from 0 °C storage softened to a firmness of between 7 and 14 N, while the fruit from 5 °C softened to between 5 and 8 N. These values were lower than the softening that occurred when fruit were held for 3 d at 20 °C without storage; 'Oded' fruit softened to 24.9 N and 'Yuval' fruit softened to 27.1 N.

At 3 d ripening at 20 °C after cold storage, there was more expressible juice at 5 °C than 0 °C in the fruit of both cultivars for the first 3 weeks (Fig. 3). However, after 5 weeks 'Oded' peaches had more expressible juice in fruit ripened after 0 °C than 5 °C. In addition, after 7 weeks' cold storage 'Oded' peaches from 5 °C storage developed very little expressible juice (19% of ripe fruit with no storage), while the same fruit from 0 °C still had 43% expressible juice, 83% of that found in fruit ripened without storage (Fig. 3). Expressible juice from 'Yuval' nectarines ripened for 3 d after 0 °C

Table 2

Ripeness parameters at harvest of 'Oded' peaches and 'Yuval' nectarines from three years. Standard deviation is indicated.

Cultivar	Firmness (Newton)	SSC (%)	TA (%)	Weight (g)
2009				
Oded	59.8 ± 1.6	11.8 ± 0.9	0.43 ± 0.04	140 ± 14
Yuval	45.8 ± 1.1	14.2 ± 0.7	0.53 ± 0.03	106 ± 6
2008				
Oded	49.7 ± 1.2	12.2 ± 0.5	0.52 ± 0.03	139 ± 12
Yuval	51.3 ± 1.1	13.7 ± 0.4	0.63 ± 0.04	104 ± 3
2007				
Oded	48.2 ± 1.2	11.5 ± 0.5	0.38 ± 0.03	140 ± 10
Yuval	49.7 ± 1.1	12.3 ± 0.4	0.54 ± 0.03	106 ± 5

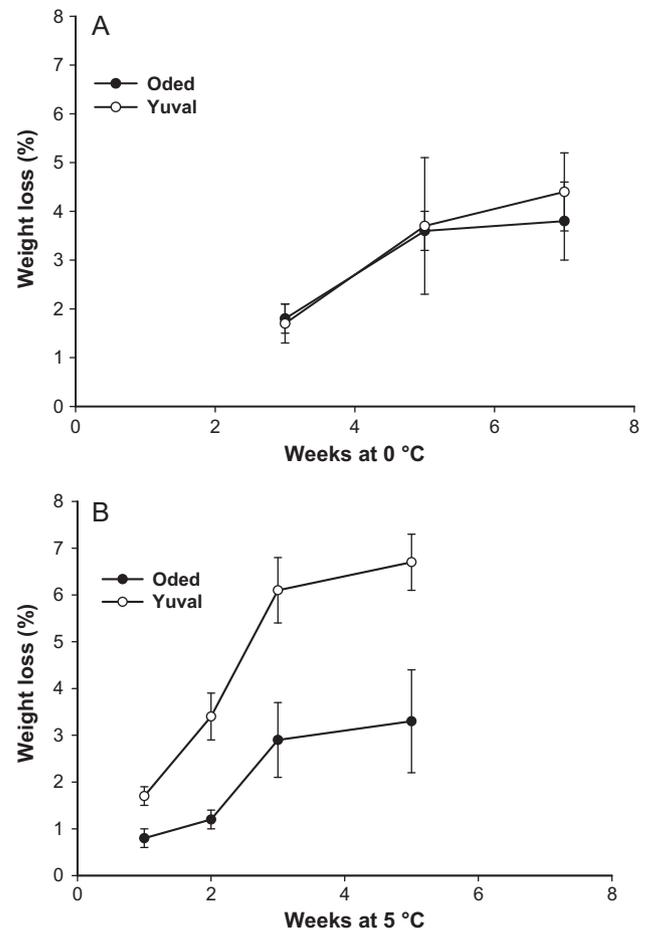


Fig. 1. Weight loss of 'Oded' peaches and 'Yuval' nectarines during cold storage at (A) 0 °C and (B) 5 °C. Standard deviations are indicated.

storage did not change appreciably from 1 through 7 weeks of storage (Fig. 3A).

3.3. Flesh browning (FB), woolly texture (WT) and flesh bleeding (FBL) indices

The cold storage at 0 °C plus ripening reduced the FB, WT and FBL incidence in both cultivars compared to storage at 5 °C (Table 3). FB and WT incidence was lower in the 'Yuval' nectarine than 'Oded' peach at ripening after storage. There was no flesh browning in 'Yuval' nectarines following either storage temperature, while there was minor woolliness development after 7 weeks at both storage temperatures, and minor FBL after storage for 7 weeks at 5 °C.

Table 3

Flesh browning, woolly texture and flesh bleeding indices in 'Oded' peach and 'Yuval' nectarine fruit after storage at 0 °C or 5 °C plus shelf life (3 d at 20 °C).

Time of sampling	0 °C		5 °C	
	Oded	Yuval	Oded	Yuval
Flesh browning index (%)				
5 wk	0	0	12	0
7 wk	5	0	67	0
Woolly texture index (%)				
5 wk	38	0	85	0
7 wk	22	11	100	5
Flesh bleeding index (%)				
5 wk	0	0	6	0
7 wk	0	0	17	10

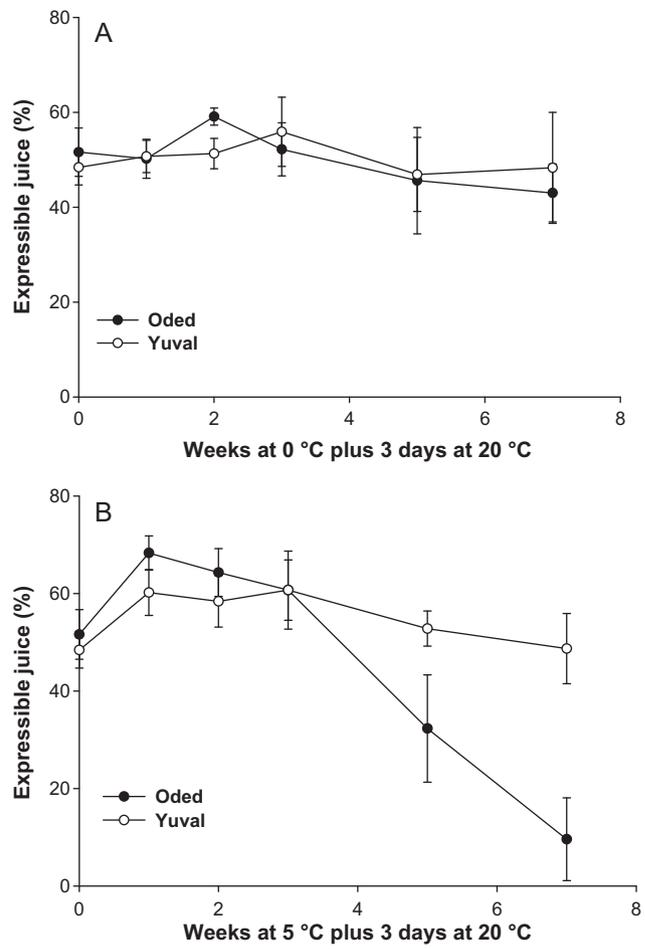
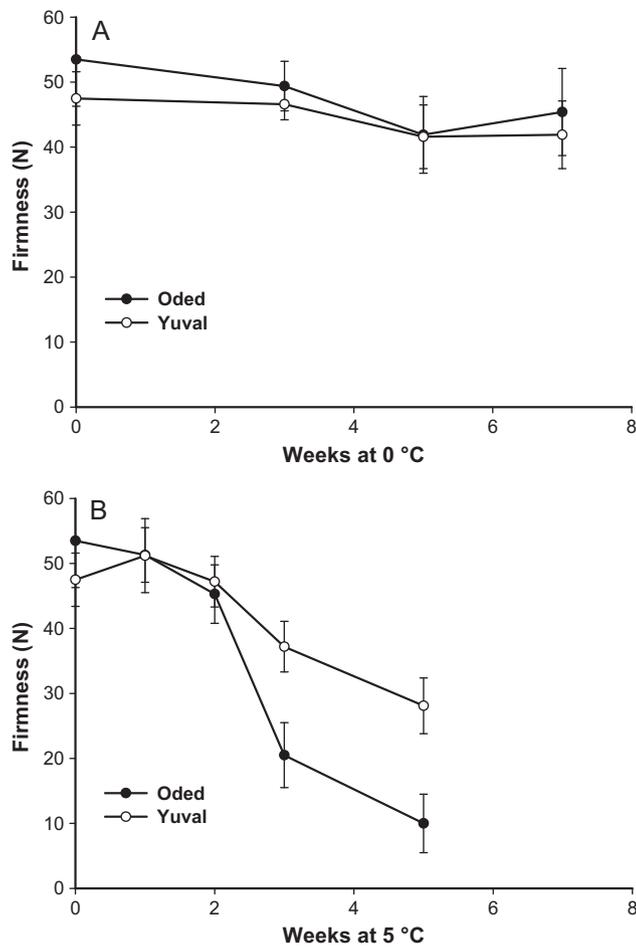


Fig. 2. Firmness of ‘Oded’ peaches and ‘Yuval’ nectarines stored at (A) 0 °C and (B) 5 °C. Standard deviations are indicated.

Fig. 3. Expressible juice from ‘Oded’ peaches and ‘Yuval’ nectarines after storage at (A) 0 °C or 5 °C and 3 d at 20 °C. Standard deviations are indicated.

3.4. Single sequence repeat (SSR) marker analysis

A genome-spanning set of SSR markers from the Pop-DG map was used to distinguish and confirm similarity between ‘Oded’ peach and its nectarine mutant ‘Yuval’ (Dirlewanger et al., 2004; Ogundiwin et al., 2009). This linkage map was constructed from an intraspecific peach progeny population and covers 818 cM of the peach genome. A total of 24 SSR markers were chosen, three per

linkage group and have been used in PCRs (Table 1, Fig. 4). For each linkage group, one marker was chosen close to the top, one close to the bottom, and one in the middle. Out of 24 markers, 21 were monomorphic while the remaining 3 did not yield any amplification product. All markers were found in both cultivars, substantiating the suggestion that ‘Oded’ and ‘Yuval’ are genetically similar. The G locus which differentiates peach from nectarine has not been mapped in the Pop-DG map, but is indicated on the T × E *Prunus*

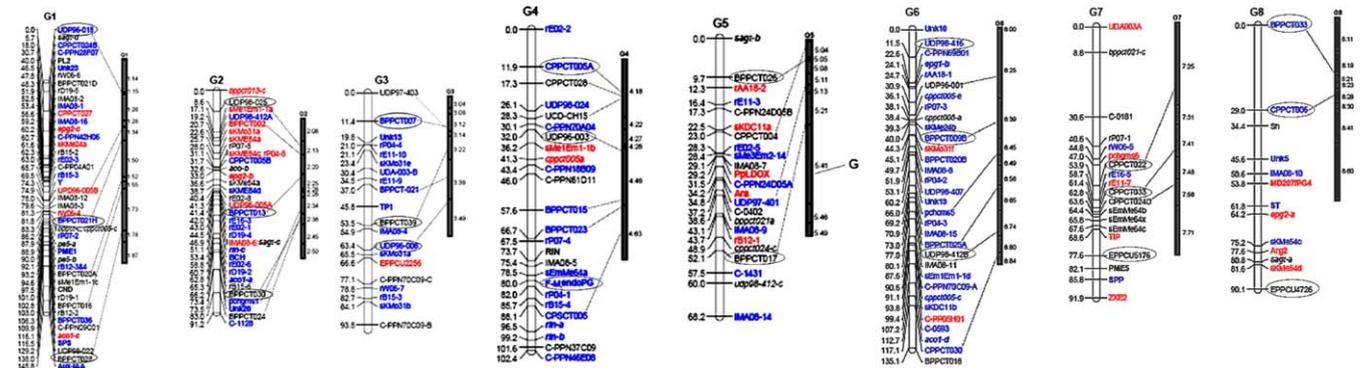


Fig. 4. Location of the 24 genome-spanning single sequence repeats (SSRs) markers (Table 1) that were used to compare and differentiate ‘Yuval’ nectarine and its peach progenitor ‘Oded’ on a Pop-DG map (Ogundiwin et al., 2009). Open vertical bars represent linkage groups of the Pop-DG, while vertical solid bars represent linkage groups of the T × E *Prunus* reference map (Dirlewanger et al., 2004; Howard et al., 2005). Positions of SSR markers on the T × E map corresponding to the Pop-DG map are connected by dotted lines. Genetic markers are to the right side of each linkage group of Pop-DG, genetic distances (cM) are to the left. Markers used to verify identity of ‘Oded’ and ‘Yuval’ are circled. The G locus for peach to nectarine is found on the lower part of linkage group 5 on the T × E map, but has not been located on the Pop-DG map.

reference map in Fig. 4 (Dirlewanger et al., 2004; Ogundiwin et al., 2009).

4. Discussion

The current comparison of a peach-to-nectarine mutant and its peach progenitor included fruit ripening and storage characteristics at 0 and 5 °C. These two storage temperatures were used in this study since chilling injury symptoms usually develop faster and are more severe when fruit are stored at 5 °C rather than at 0 °C (Anderson, 1979; Lill et al., 1989; Crisosto et al., 1999). Both the peach and its nectarine mutant studied here were harvested at the same ripening (pre-climacteric) stage as they had about same levels ($0.60 \mu\text{L kg}^{-1} \text{h}^{-1}$) of ethylene production, and firmness of $50.5 \pm 4.5 \text{ N}$. In all three seasons fresh weight was reduced in 'Yuval' nectarine compared to its peach progenitor 'Oded' (Table 2). The degree of the weight reduction is similar to a previous observation by Wen et al. (1995b), who reported that the nectarine TBN identified from a limb of a 2-year-old 'Tropical Beauty' peach tree was 78% as heavy as its peach progenitor. Wen et al. (1995b) identified the TBN nectarine mutant as a glabrous mutant.

There were no differences in cell size between the 'Oded' peach and its nectarine mutant 'Yuval', so the nectarine may have a reduced cell number, reduced intercellular spaces or both relative to the peach progenitor. Bradley (1959) found that differences in peach fruit size among trees of the same cultivar are due to differences in cell count, with cell size having a minor effect. Furthermore, Scorza et al. (1991) reported that large-fruited peach cultivars ('Loring' and 'Suncrest') had up to 3.7 times more cells than small-fruited cultivars ('Bailey' and 'Booney County'). Thus, the reduced fruit size accompanying the peach-to-nectarine mutant described in this study may be because of cell number, intercellular space, or both.

The soluble solids content level was consistently greater in the nectarine mutant examined here at harvest (Table 2), however, these values are determined based on osmotically active solutes rather than on sugars *per se* (Wen et al., 1995b). Furthermore, following storage at 0 and 5 °C there was more soluble solids content in the 'Yuval' nectarine fruit compared to the 'Oded' fruit (data not shown). This could be because of higher specific gravity of the nectarine mutant, since increase in apple sugar concentration with increase in fruit specific gravity has been reported (Faust, 1989).

The lower level of titratable acidity in 'Oded' peach compared to 'Yuval' nectarine fruit at harvest (Table 2) and following storage at 5 °C (data not shown) may be due to an alteration in some aspect of malic acid (the major organic acid in peaches and nectarines) synthesis, metabolism, or vacuolar compartmentalization (Yen, 1987; Campbell and Koch, 1989; Wen et al., 1995b).

The increase in weight loss with increase in duration of the cold storage is partially due to the vapor pressure deficit in the storage rooms. Although both rooms had 90% relative humidity, the higher temperature of 5 °C would give a higher vapor pressure deficit than the room at 0 °C, and this would lead to greater weight loss. At removals during the 5 °C cold storage, 'Yuval' fruit had more weight loss compared to 'Oded' fruit (Fig. 1B), which might be due to microcracks on the peel of 'Yuval' nectarines. After 7 weeks of cold storage at 5 °C visible cracks appeared on the peel of 'Yuval' and not on 'Oded' peaches (data not shown). However, there was no difference in weight loss between the two cultivars at removals during the 0 °C cold storage (Fig. 1A).

There was no consistent difference between 'Oded' peach and 'Yuval' nectarine firmness at harvest over the three seasons (Fig. 2), even though fruit of both cultivars were harvested at the same commercial harvest stage over the three seasons, as evident from fruit weight and ground color. This inconsistency in firmness may

be because of environmental variations (i.e. temperature, chilling hours) over the three seasons. At the end of 5 weeks at 5 °C, 'Oded' fruit were less firm than 'Yuval' fruit (Fig. 2B), but after 3 d ripening at 20 °C, fruit from both cultivars softened to similar softness. Furthermore, at removals after 0 °C there was no difference in firmness between the two cultivars (Fig. 2A). However, at 3 d ripening at 20 °C after 0 °C storage 'Oded' fruit softened more than 'Yuval' fruit (data not shown). Fruit from both storage regimes softened during shelf life and did not show the inhibited softening known as leatheriness. The greater softening of the peach fruit compared to nectarine has no clear and obvious explanation. It was evident at removals after prolonged cold storage of 5 and 7 weeks at 5 °C that the nectarine mutant fruit were firmer than its peach progenitor (Fig. 2B).

Chilling injury or internal breakdown are the terms used to describe the physiological disorder symptoms that develop during fruit ripening after prolonged low-temperature (2–8 °C) storage. These disorders include changes in the fruit flesh such as woolliness, browning, bleeding, and lack of flavor or the presence of off flavors (Smith, 1934; Anderson, 1979; Dodd, 1984; Hartmann, 1985; Crisosto et al., 1997, 1999). Compared to juicy peach and nectarine fruit, woolliness has been found associated with a decrease in endo-polygalacturonase (endo-PG) activity and an increase in pectin methylesterase (PME) activity (Lurie and Crisosto, 2005). It has been suggested that changes in pectin metabolism cause woolliness either by cell fluids forming calcium-pectate gel complexes with high molecular weight pectin in the middle lamella (Ben Arie and Lavee, 1971; Zhou et al., 2000b; Lurie and Crisosto, 2005), or that the decreased intercellular adhesion in woolly fruit reduces cell rupture during biting and chewing, preventing release of cellular contents (King et al., 1989; Brummell et al., 2004; Lurie and Crisosto, 2005). Lurie and Crisosto (2005) have suggested that the flesh browning disorder may be related to tissue deterioration or senescence, which leads to changes in membrane permeability and the interaction between phenols and polyphenol oxidase, which are generally found in separate compartments in the cell. There has been almost no research conducted on the causes of flesh bleeding. It has been reported in some studies, along with details of other disorders appearing in fruit, but with no further details. Lurie (unpublished data) has suggested that it may be a consequence of tissue senescence, since it is inversely correlated with decrease in organic acids in the tissue.

High levels of expressible juice are an indicator of normal ripening and lack of woolliness, and as fruit become woolly the expressible juice decreases. Based on our work with different cultivars, it was found that peach and nectarine fruit having less than 40% expressible juice based on fresh weight basis had a woolly texture (Zhou et al., 2000a, 2001a,b). In the present study, during ripening of 3 d at 20 °C, there was more expressible juice in both cultivars after 5 °C cold storage compared to 0 °C cold storage, except at 5 and 7 weeks in the case of 'Oded' peaches. The peach fruit after 5 and 7 weeks were woolly as determined visually and organoleptically and had lower expressible juice (i.e. < 40%; Fig. 3) compared to 'Yuval' nectarines that were healthy. Overall, these results support the findings of Crisosto et al. (1999) who reported that nectarines have better storage and shipping characteristics than peaches, and that storage can be longer at 0 °C than at 5 °C.

Woolliness and flesh browning were the major chilling injury symptoms in the 'Oded' peach as described by visual texture and flesh browning indices, respectively, and had higher indices values at ripening after 5 and 7 weeks cold storage at 5 °C than 0 °C cold storage (Table 3). Furthermore, at ripening after cold storage, 'Yuval' nectarine fruit had lower woolly texture values and flesh browning indices than its peach progenitor 'Oded' (Table 3). Flesh bleeding as a consequence of chilling injury was only observed at ripening after 5 °C cold storage. The 'Oded' fruit had higher flesh

bleeding values at ripening after 5 °C cold storage than at ripening of 0 °C cold storage (Table 3). These results are again consistent with findings of Crisosto et al. (1999) that nectarines were more resistant to chilling injury (woolliness, flesh browning and bleeding) than peaches. Thus, 'Yuval' nectarine fruit were more resistant to chilling injury than its peach progenitor 'Oded'.

In order to differentiate and compare the 'Oded' peach and its nectarine mutant 'Yuval' at the DNA level, 24 genome-spanning markers (Table 1) were utilized and have been localized on the Pop-DG map (Fig. 4), and were found to be monomorphic. Thus, the analysis confirmed the identity between the isogenic cultivars except for the nectarine (glabrous skin, G) locus. For technical reasons, 3 markers did not yield any amplification product.

In the past there have been several attempts to characterize the nature of peach-to-nectarine mutants, which has been problematic because of the difficulty in establishing confirmed peach progenitors (Wen et al., 1995b). A few isogenic peach-to-nectarine comparisons have been reported (Oberle and Nickolson, 1953; Wen et al., 1995b). Furthermore, Fogel and Faust (1975) reported three dissimilar types of nectarine mutants differing in their surface structure, although their peach progenitors were not known. In this study, we had isogenic material that provided an opportunity to define ripening and storage (physiological) attributes of the peach and its peach to nectarine mutation.

Thus, to our knowledge, no one has compared isogenic material of peach-to-nectarine mutants at the DNA level, and for ripening, and storage characteristics of prolonged periods of two different low temperature exposures. Here, we have reported that the 'Oded' peach fruit were less acidic, had less soluble solids and were about 24% larger (weight) than its nectarine mutant 'Yuval' fruit at harvest. In terms of resistance to chilling injury for prolonged periods of cold storage 'Yuval' fruit were more resistant than 'Oded' fruit. Thus, the comparison studied here brings us closer to elucidate the pleiotropic effects of the peach-to-nectarine mutation. Further studies are in progress to investigate the molecular attributes at the level of genes and proteins using transcriptome and proteome analysis of this isogenic material ('Oded' peach-to-'Yuval' nectarine mutation).

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References

Anderson, R.E., 1979. The influence of storage temperatures and warming during storage on peach and nectarine fruit quality. *J. Am. Soc. Hortic. Sci.* 104, 459–461.

Ben Arie, R., Lavee, S., 1971. Pectic changes occurring in Elberta peaches suffering from woolly breakdown. *Phytochemistry* 10, 531–538.

Blake, M.A., 1932. The J.H. Hals as a parent in peach crosses. *Proc. Am. Soc. Hortic. Sci.* 29, 131–136.

Bradley, M.V., 1959. Mean cell size in the mesocarp of mature peaches of different sizes. *Proc. Am. Soc. Hortic. Sci.* 29, 131–136.

Brummell, D.A., Dal Cin, V., Lurie, S., Crisosto, C.H., Labavitch, J.M., 2004. Cell wall metabolism during the development of chilling injury in cold-stored peach fruit: association of mealiness with arrested disassembly of cell wall pectins. *J. Exp. Bot.* 55, 2041–2052.

Campbell, C.A., Koch, K.E., 1989. Sugar/acid composition and development of sweet and tart carambola fruit. *J. Am. Soc. Hortic. Sci.* 114, 455–457.

Crisosto, C.H., Mitchell, F.G., Zhiguo, J., 1999. Susceptibility of chilling injury of peach, nectarine and plum cultivars grown in California. *HortScience* 34, 1116–1118.

Crisosto, C.H., Johnson, R.S., DeJong, T., Day, K.R., 1997. Orchard factors affecting postharvest stone fruit quality. *HortScience* 32, 820–823.

Dodd, M.C., 1984. Internal breakdown in plums. *Deciduous Fruit Grower* 34, 255–256.

Dirlewanger, E., Cosson, P., Tavaud, M., Aranzana, M.J., Poizat, C., Zanetto, A., Arus, P., Laigret, F., 2002. Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). *Theor. Appl. Genet.* 105, 127–138.

Dirlewanger, E., Moing, A., Rotham, C., Svanella, L., Pronier, V., Guye, A., Plomion, C., Monet, R., 1999. Mapping QTLs controlling fruit quality in peach (*Prunus persica* (L.) Batsch). *Theor. Appl. Genet.* 98, 18–31.

Dirlewanger, E., Pronier, V., Parvery, C., Rotham, C., Guye, A., Monet, R., 1998. Genetic linkage map of peach (*Prunus persica* (L.) Batsch) using morphological and molecular markers. *Theor. Appl. Genet.* 97, 888–895.

Dirlewanger, E., Graziano, E., Joobeur, T., Garriga-Calderé, F., Cosson, P., Howard, W., Arus, P., 2004. Comparative mapping and marker-assisted selection in Rosaceae fruit crop. *Proc. Natl. Acad. Sci. U.S.A.* 101, 9891–9896.

Faust, M., 1989. *Physiology of Temperate Zone Fruit Trees*. Wiley, New York.

Fogel, H.W., Faust, M., 1975. Ultrastructure of nectarine fruit surfaces. *Proc. Am. Soc. Hortic. Sci.* 100, 434–439.

Foolad, M.R., Arulsekar, S., Becerra, V., Bliss, F.A., 1995. A genetic map of *Prunus* based on an interspecific cross between peach and almond. *Theor. Appl. Genet.* 91, 262–269.

Hartmann, P.E.Q., 1985. Research on woolliness in peaches and nectarines during the 1984–85 season. *Deciduous Fruit Grower* 35, 194–198.

Howard, W., Yamamoto, T., Dirlewanger, E., Testolin, R., Cosson, P., Cipriani, G., Montforte, A.J., Georgi, L., Abbott, A.G., 2005. Mapping with a few plants: using selective mapping for microsatellite saturation of the *Prunus* reference map. *Genetics* 171, 1305–1309.

King, G.A., Henderson, K.G., Lill, R.E., 1989. Ultrastructural changes in the nectarine cell wall accompanying ripening and storage in a chilling-resistant and chilling-sensitive cultivar. *N. Z. J. Crop Hortic. Sci.* 17, 337–344.

Lill, R.E., O'Donoghue, E.M., King, G.A., 1989. Postharvest physiology of peaches and nectarines. *Hortic. Rev.* 11, 413–452.

Lill, R.E., van der Mespel, G.T., 1988. A method for measuring the juice content of mealy nectarines. *Sci. Hortic.* 36, 267–271.

Lurie, S., Crisosto, C.H., 2005. Chilling injury in peach and nectarine. *Postharvest Biol. Technol.* 37, 195–208.

Maes, L., Inzé, D., Goossens, A., 2008. Functional specialization of the TRANSPARENT TESTA GLABRA1 network allows differential hormonal control of laminal and marginal trichome initiation in Arabidopsis rosette leaves. *Plant Physiol.* 148, 1453–1464.

Oberle, G.D., Nickolson, J.O., 1953. Implications suggested by a peach to nectarine sport. *Proc. Am. Soc. Hortic. Sci.* 62, 323–326.

Ogundiwin, E.A., Peace, C.P., Gradziel, T.M., Parfitt, D.E., Bliss, F.A., Crisosto, C.H., 2009. A fruit quality gene map of *Prunus*. *BMC Genomics* 10, 587.

Peace, C.P., Crisosto, C.H., Gradziel, T.M., 2005. Endopolygalacturonase: a candidate gene for freestone and melting flesh in peach. *Mol. Breed.* 16, 21–31.

Scorza, R., May, L.G., Purnell, B., Upchurch, B., 1991. Differences in number and area of mesocarp cells between small- and large-fruited peach cultivars. *J. Am. Soc. Hortic. Sci.* 116, 861–864.

Smith, W.H., 1934. Cold storage of Elberta peaches. *Ice Cold Storage* 37, 54–57.

Walker, A.R., Davison, P.A., Bolognesi-Winfield, A.C., James, C.M., Srinivasan, N., Blundell, T.L., Esch, J.J., Marks, M.D., Gray, J.C., 1999. The TRANSPARENT TESTA GLABRA1 locus, which regulates trichome differentiation and anthocyanin biosynthesis in Arabidopsis, encodes a WD40 repeat protein. *Plant Cell* 11, 1337–1350.

Wen, I., Koch, K.E., Sherman, W.B., 1995a. Comparing fruit and tree characteristics of two peaches and their nectarine mutants. *J. Am. Soc. Hortic. Sci.* 120, 101–106.

Wen, I.C., Sherman, W.B., Koch, K.E., 1995b. Heritable pleiotropic effects of the nectarine mutant to peach. *J. Am. Soc. Hortic. Sci.* 120, 721–725.

Yen, C.R., 1987. Assimilate partitioning and enzymes of organic acid metabolism in fruit of calamondin and low-acid grapefruit. PhD diss., Univ. of Florida, Gainesville.

Zhou, H.-W., Lurie, S., Lers, A., Khatchitski, A., Sonogo, L., Ben Arie, R., 2000a. Delayed storage and controlled atmosphere storage of nectarines: two strategies to prevent woolliness. *Postharvest Biol. Technol.* 18, 133–141.

Zhou, H.-W., Ben Arie, R., Lurie, S., 2000b. Pectin esterase, polygalacturonase and gel formation in peach pectin fractions. *Phytochemistry* 55, 191–195.

Zhou, H.-W., Lurie, S., Ben Arie, R., Dong, L., Burd, S., Weksler, A., Lers, A., 2001a. Intermittent warming of peaches reduces chilling injury by enhancing ethylene production and enzymes mediated by ethylene. *J. Hortic. Sci. Biotechnol.* 76, 620–628.

Zhou, H.-W., Dong, L., Ben-Arie, R., Lurie, S., 2001b. The role of ethylene in the prevention of chilling injury in nectarines. *J. Plant Physiol.* 158, 55–61.