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PRELIMINARY STUDIES OF PHYSIOLOGICAL AND MORPHOLOGICAL INDICATORS OF POTENTIAL POOR QUALITY IN LATE SEASON NEW ZEALAND 'HASS' AVOCADOS

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The harvest season for 'Hass' avocados in New Zealand is from August to March. Typically after storage, fruit harvested in January to march tend to develop more disorders such as stem end rots, body rots and diffuse flesh discoloration than fruit harvested in October and November. The increase in disorders presents a problem to exporters of New Zealand avocados where they can be forced to exit a market prematurely due to poor quality fruit. By identifying poor quality fruit at harvest, it should be possible to prevent it from being exported. To do this, a physiological or morphological marker of advanced maturity that relates to when disorder levels are likely to be commercially unacceptable is needed. While the dry matter content of the fruit is a useful measure of the minimum maturity required for harvest, it appears less useful for determining when fruit can be considered to be over-mature. In this study, changes in carbohydrates in the flesh and water content of the seed of 'Hass' avocados during the December to March period were determined. These fruit characteristics measured at harvest were correlated with the ripe fruit quality of fruit from the same harvests after 4 weeks of storage. In general, a decrease in the incidence of sound fruit after storage was associated with a decrease in carbohydrates as fruit matured. The potential for compositional changes in late season fruit to be used as markers of increased susceptibility to postharvest disorders and rots will be discussed.

Keywords: carbohydrates, disorders, mannoheptulose, MRI, rots, seed, water

ESTUDIOS PRELIMINARES DE LOS INDICADORES FISIOLÓGICOS Y MORFOLÓGICOS DE LA POTENCIAL BAJA CALIDAD EN PALTAS HASS COSECHADAS A FINALES DE TEMPORADA EN NUEVA ZELANDA

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La temporada de cosecha de la palta Hass en Nueva Zelanda comienza en agosto y finaliza en marzo. Habitualmente, la fruta cosechada entre enero y marzo tiende a desarrollar más desórdenes (pudrición del pedúnculo, podrido de pared y decoloración difusa de la pulpa) que la fruta cosechada en octubre y noviembre. El incremento de estos desórdenes representa un problema para los exportadores, quienes se ven obligados a retirarse prematuramente del mercado por la baja calidad del fruto. Se podría evitar su exportación por medio de la identificación de fruta de baja calidad durante la cosecha. Para realizar esto, se necesita un indicador fisiológico o morfológico del nivel de madurez, el cual permita señalar el momento en que cierto nivel de un determinado desorden, originará fruta inaceptable a nivel comercial. El contenido de materia seca es un parámetro útil para determinar el nivel mínimo de madurez, sin embargo, es menos beneficioso para determinar posibles niveles que sobrepasen la madurez. En el presente estudio, se determinaron, entre diciembre y marzo, los cambios en los niveles de carbohidratos en la pulpa y el contenido hídrico en la semilla de paltas Hass. Estos parámetros medidos durante la cosecha, fueron correlacionados con la calidad de la fruta madura de las mismas cosechas, luego de 4 semanas de almacenamiento. En general, la disminución en la calidad de fruta sana luego del almacenamiento, estuvo asociada con la disminución de carbohidratos en la fruta al madurar en el árbol. Se discutirá el potencial uso de los cambios en la composición de la fruta cosechada en la última parte de la temporada, como indicadores de mayor susceptibilidad a los desórdenes poscosecha y las pudriciones.

1. Introduction

The harvest season for ‘Hass’ avocados in New Zealand is from August to March. Typically after storage, fruit harvested in January to March tend to develop more disorders such as stem end rots, body rots and diffuse flesh discolouration than fruit harvested in October and November (Dixon *et al.*, 2003). The increase in disorders presents a problem to exporters where they can be forced to exit a market prematurely because of poor quality fruit. By identifying potential poor quality fruit at harvest, it should be possible to prevent it from being exported. To do this, a physiological or morphological marker of advanced maturity that relates to when disorder levels are likely to be commercially unacceptable is needed.

Maturity in avocado fruit is usually discussed in terms of the dry matter or water content of the fruit flesh. While the dry matter content of the fruit is a useful measure of the minimum maturity required for harvest based on eating quality of the fruit, dry matter appears less useful for determining when fruit are over-mature (Hofman *et al.*, 2000).

Potential markers of fruit maturity that may be indicative of ripe fruit quality in more mature fruit include the carbohydrate status of the fruit flesh and the water status of the seed. In particular for carbohydrate status, the role of the 7-carbon sugar mannoheptulose and its sugar alcohol perseitol are of interest as they are the dominant sugars present in avocado. Both carbohydrate and seed water status have been researched recently as fruit attributes that change during fruit development (Bertling and Bower, 2005; Kalala *et al.*, 2005). However, neither has yet been specifically associated with fruit quality.

In this study, changes in carbohydrates in the flesh and water content of the seed of ‘Hass’ avocados during the December to March period were determined. These fruit characteristics measured at harvest were correlated with the ripe fruit quality of fruit from the same harvests after 4 weeks of storage.

2. Methods

Fruit
Avocado fruit (cultivar 'Hass' on 'Zutano' seedling rootstocks) were harvested from one orchard located in the Eastern Bay of Plenty at Opotiki (Orchard 1) between 1/12/2004 and 3/2/2004 and one orchard located in the Far North region (Orchard 2) between 1/12/2004 and 11/3/2005. At each harvest 150 fruit were collected, 50 fruit for determination of soluble solids content (SSC), individual sugar contents and whole fruit magnetic resonance imaging for seed water, and 100 fruit for storage and quality assessment. Fruit were assessed or put into storage the day after harvest.

Soluble solids content
Individual unripe fruit were cut into quarters and the peel, seed and seed coat removed. The pieces of fruit flesh were passed through a juice extractor (Breville JE95 Juice Fountain) and the separated solids discarded. The extracted liquid was centrifuged in 2 mL Eppendorf tubes (16000 g, 10 min) and SSC measured on an aliquot of the aqueous layer using a digital refractometer (Atago, Japan).

Sugar analysis
From the same aqueous extract that was used for SSC measurement, a 1 mL sample was added to 4 mL ethanol and stored at –20°C until analysed for individual sugar composition by gas chromatography. The identity of mannoheptulose and perseitol were confirmed by gas chromatography-mass spectroscopy using authentic standards (Industrial Research Ltd, Wellington)

Magnetic resonance imaging (MRI)
Transverse and longitudinal images (slice thickness of 3.3 mm) through individual fruit were collected using a GE 1.5T echospeed MR scanner (Mercy Radiology, Auckland). Fruit were imaged in batches of approximately 10 fruit using a torso phased array coil. Proton density images (for water content) and the spin-spin relaxation images (for interaction of water with other compounds) gave similar images and differences in images are discussed simply as water content. Within MR images, the higher the water content (proton density) the lighter the image appears. In addition to seed water, other seed conditions such as embryo growth and seed splitting were recorded from the images.

Fruit storage and quality assessment
Fruit were stored at 4°C ± 0.5°C, 85% RH for 28 days and then ripened at 20°C ± 1°C, 65% RH. Once the fruit had reached at least a minimum eating softness determined by hand-feel, equivalent to a Firmometer measurement of 85 with a 300 g weight, the fruit were assessed for the ripe fruit disorders stem end rot, vascular browning, brown patches and diffuse flesh discolouration. All disorders were quantified for severity according to the scales described in the New Zealand Avocado Industry Council fruit assessment manual 2003 (Dixon, 2003). The incidence of sound fruit when ripe was determined as all fruit for which there was no individual disorder at > 5% severity.

Differences among harvests for each orchard were assessed statistically by analysis of variance (ANOVA) using MINITAB version 13.31, with disorder incidence data square root transformed prior to analysis.

3. Results

Soluble solids at harvest

The SSC at harvest of fruit from both orchards was in the range 7 to 9 °Brix, although the patterns of change between harvests differed for each orchard (Figure 1).

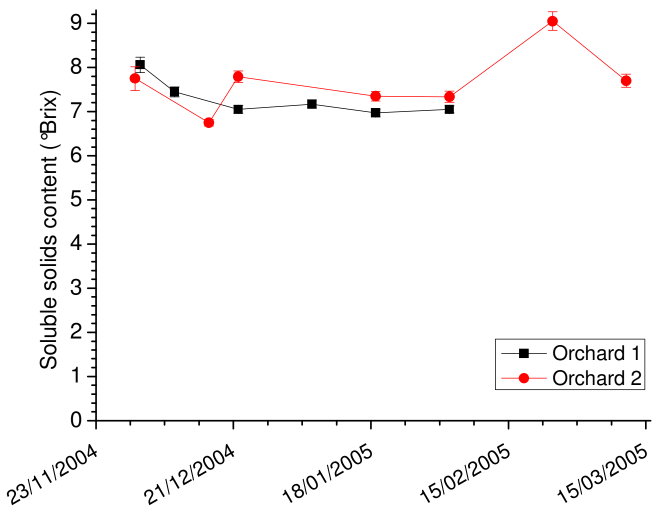


Figure 1. Soluble solids content at-harvest of 'Hass' avocado fruit harvested from two orchards between December 2004 and March 2005. Values are the means of 20 fruit, ± the standard error of the mean.

Figura 1. Contenido de sólidos solubles al momento de la cosecha del aguacate 'Hass' cosechados en dos plantaciones entre Diciembre del 2004 y Marzo del 2005. Los valores constituyen un promedio de 20 frutas, ± el error estándar de la media.

The SSC in fruit from Orchard 1 declined from 1/12/2004 until 22/12/2004 after which there was little further change, with a SSC of about 7 °Brix for the remaining harvests. The SSC in fruit from Orchard 2 tended to be between 7.5 and 8 °Brix for most harvests, but with a lower SSC (6.8 °Brix) in the fruit harvested on 16/12/2004 and higher SSC (9.1 °Brix) on 24/2/2005. The sample of fruit from Orchard 2 on 11/3/2005 showed the increase in SSC at 24/2/2005 to be specific to that harvest and not to be a consistent change in the fruit.

Composition of sugars

The pattern of change in sugars over the harvest period differed for the fruit from the 2 orchards (Figure 2). Overall, there was a greater total sugar content in fruit from Orchard 1 (9-16 mg/mL) than from Orchard 2 (7-9 mg/mL) (Figure 2). While there was a trend for an overall decline in total sugars in fruit from Orchard 2, there was a peak in total sugars in fruit from Orchard 1. In fruit from both orchards, the main sugar was mannoheptulose and the peak in Orchard 1 sugars was largely the result of changes in mannoheptulose. The concentration of mannoheptulose in fruit from Orchard 2 was in the range 2 to 5 mg/mL whereas in fruit from Orchard 1 the concentration of mannoheptulose increased from about 4 mg/mL at the start of December to 10 mg/mL at the end of December and declined throughout January to about 7 to 8 mg/mL by February. The reason for this difference in the concentration of mannoheptulose between orchards is not known, but it may provide a method of segregating orchards.

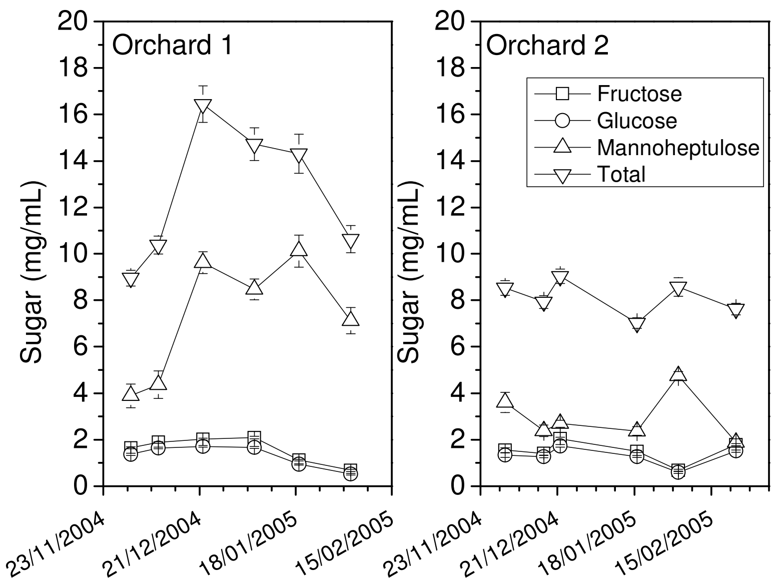
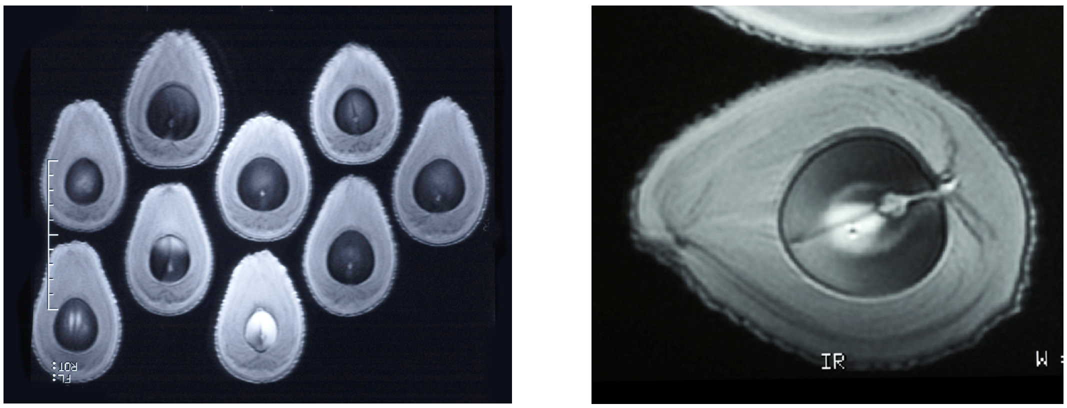


Figure 2. Total and individual sugars in an aqueous fraction from 'Hass' avocado fruit at harvest. Fruit from 2 orchards harvested between December 2004 and March 2005. Values are the means of 20 fruit, ± the standard error of the mean.
Figura 2. Azucares totales e individuales en la fracción acuosa de aguacates ‘Hass’ al momento de la cosecha. Fruta proveniente de 2 plantaciones cosechada entre Diciembre del 2004 y Marzo del 2005. Los valores constituyen el promedio de 20 frutas, ± el error estándar de la media.

In comparison with mannoheptulose, the concentrations of glucose and fructose were low at about 2 mg/mL or less, with only trace amounts of sucrose. In fruit from Orchard 1 there was a trend for the concentrations of fructose and glucose to be about the same for the first 4 harvests, then to be lower in the last 2 harvests. Fruit from Orchard 2 had concentrations of glucose and fructose that varied between harvests in the range 0.6 to 2 mg/mL. Perseitol, a 7-carbon sugar alcohol, was present at levels of 2 to 3 mg/mL, with no consistent trend with time. The data for perseitol and minor sugars are not presented.

Magnetic resonance images of whole fruit
Magnetic resonance images of whole avocado fruit provided clear images of the internal structure of the fruit, including the seed, embryo, flesh and skin (Figure 3A). Among fruit from a single harvest there were differences in the overall seed

water between fruit and also differences in water content within individual seeds, allowing the embryo to be distinguished from the rest of the seed. The water content of the cotyledonary tissues of individual seeds was in the range 20 to 50%, with up to one third of fruit in a single harvest having seeds containing water at the higher end of this range (Table 1). Seeds with 20% water appeared dark in the MR images whereas seeds with 50% water appeared white.



A B

Figure 3. Longitudinal magnetic resonance images of 'Hass' avocado fruit at harvest (A) and one fruit showing radicle emergence (B).
Figura 3. Imágenes de resonancia magnética longitudinal de aguacate ‘Hass’ al momento de la cosecha (A) y una fruta mostrando la emergencia de la radícula (B).

Table 1. Incidence (%) of ‘Hass’ avocado fruit with seeds with high water content (about 40% – 50%) at harvest.
Tabla 1. Incidencia (%) de frutos de aguacate ‘Hass’ con alto contenido de agua en la semilla (aproximadamente entre el 40% -50%) al momento de la cosecha.

Orchard 1		Orchard 2	
Harvest date	High water seeds (%)	Harvest date	High water seeds (%)
1/12/204	6	2/12/204	0
9/12/2004	10	16/12/2004	10
22/12/2004	5	22/12/2004	0
6/1/2005	24	19/1/2005	n.d.[1]
19/1/2005	n.d.[1]	3/2/2005	0
3/2/2005	30	24/2/2005	25

[1] n.d. = not determined

Embryo water content was variable and not related to seed water content. There was a general trend for embryo water content to increase as the harvest season progressed, seen by the increasing lightness of the embryo in the MR images. Seed splitting and radical emergence were clearly visible in the MR images, but were only present in fruit from the final harvest from Orchard 2 (Figure 3B).

Disorders

In general, the incidence of sound fruit was high, often exceeding 90% (Table 2). Fruit from Orchard 1 had the highest incidence of sound fruit, with a decrease in sound fruit occurring only at the last harvest in February. By contrast, the incidence of sound fruit from Orchard 2 was between 65 and 94%, with no trend with harvest. While the incidence of disorders was high, the severity of disorders was very low (data not shown), hence the high incidence of sound fruit when the sound/disordered threshold was at a severity of 5%.

Table 2. Incidence (%) of disorders in ripe 'Hass' avocado fruit from 2 orchards harvested between December 2004 and February 2005. Fruit evaluated after 28 days of storage at 4°C then at 20°C until ripe.

Tabla 2. Porcentaje de incidencia de desordenes en fruta madura de aguacate 'Hass' proveniente de 2 plantaciones cosechadas entre Diciembre del 2004 y Febrero del 2005. La fruta fue evaluada después de 28 días de almacenamiento a 4°C y luego 20°C hasta completar su maduración.

Harvest date	Disorder incidence (%)				
	Stem end rot	Vascular browning	Body rot	Diffuse flesh discolouration	Sound fruit
	Orchard 1				
1/12/2004	3.0^{a}	1.0^{a}	19.0^{a}	0.0	98.0^{a}
9/12/2004	4.0^{a}	3.0^{a}	44.4^{b}	0.0	97.0^{a}
22/12/2004	3.0^{a}	5.0^{a}	38.0^{bc}	1.0	95.0^{a}
6/1/2005	4.0^{a}	0.0^{a}	32.0^{ab}	0.0	99.0^{a}
19/1/2005	2.0^{a}	2.0^{a}	25.0^{ac}	0.0	98.0^{a}
3/2/2005	17.2^{b}	19.2^{b}	68.7^{d}	0.0	65.7^{b}
	Orchard 2				
2/12/2004	3.0^{a}	3.0^{a}	23.0^{a}	0.0	94.0^{a}
16/12/2004	19.4^{b}	20.4^{b}	55.1^{b}	3.1	65.3^{b}
22/12/2004	9.0^{ab}	12.0^{ab}	47.0^{b}	0.0	90.0^{ac}
19/1/2005	5.0^{a}	6.0^{a}	66.0^{b}	0.0	69.0^{b}
3/2/2005	20.0^{b}	10.0^{ab}	52.0^{b}	1.0	87.0^{ac}
24/2/2005	10.2^{ab}	9.2^{ab}	58.2^{b}	0.0	76.5^{bc}

Values in a column for each orchard not sharing a common letter differ at P=0.05.
Sound fruit are fruit having no individual disorder with severity greater than 5%.

The disorders in the ripe fruit were mainly rots, with both stem end rots and rots on the body of the fruit. There was little or no diffuse flesh discoloration in fruit from either orchard, irrespective of harvest. Stem end rot and vascular browning in fruit from Orchard 1 only increased markedly at the final harvest (from < 6% to about 18%), at which time the incidence of body rots was also greatest at 69% (Table 2). In addition to a greater incidence of disorders, there was also a greater severity of disorder in fruit from the final harvest (data not shown). The greater incidence and severity resulted in an overall lower incidence of sound fruit at Harvest 6 (66%) compared with the 95-99% sound fruit incidence for fruit from

Harvests 1 to 5. For fruit from Orchard 2, there was no clear trend with harvest for any individual disorder incidence, or overall sound fruit incidence.

Relationship between at harvest measurements and disorders when ripe

A lower incidence of sound fruit was associated with lower total sugars and mannoheptulose and increased incidence of high water seeds (Figure 4). However, based on scatter plots of sound fruit against the at-harvest measurements, it appears that the associations are not linear and are not strong, with one sample of fruit being a marked outlier in each instance. This outlier was not the same batch of fruit in each plot.

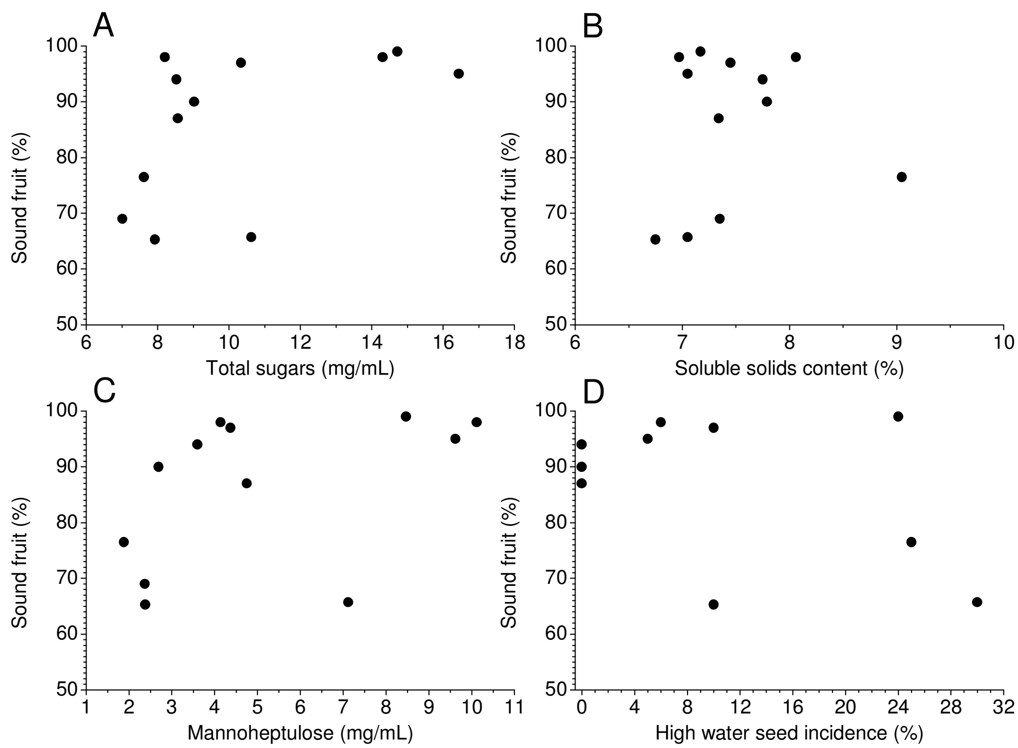


Figure 4. Relationship in 'Hass' avocado between the incidence of ripe fruit that were sound after 28 days of storage at 4ºC and ripening at 20°C, and the content at harvest of A) total sugars, B) soluble solids, C) mannoheptulose and D) incidence of seeds with high water content. Fruit from 2 orchards, 6 harvests per orchard.

Figura 4. Relaciones entre la incidencia de fruta madura sin desorden después de 28 días de almacenada a 4°C y madurada a 20°C y el contenido de A) azucares totales, B) sólidos solubles, C) mannoheptulose y D) presencia de alto contenido de agua en la semilla.

The most marked association was between the overall high level of mannoheptulose and the high sound fruit incidence in fruit from Orchard 1 compared with the lower mannoheptulose and lower sound fruit incidence in fruit from Orchard 2. While there was no overall association between SSC and disorder incidence, the low incidence of sound fruit when ripe and a high incidence of disorder in fruit immediately out of storage for fruit harvested on

16/12/2004 from Orchard 2 did not fit the trend for other harvests, but did correspond with a low SSC at-harvest.

4. Discussion.

The observations described suggest that total sugars, or the most abundant sugar mannoheptulose, or seed water content, measured at harvest may provide an indication of the propensity for disorders to develop in the fruit after storage. Further research on changes in 7-carbon sugars in late season fruit may better define the relationship between disorders and the carbohydrate status of the fruit. Magnetic resonance imaging confirmed that the longer the fruit remained on the tree, the greater the development of the seed to the point at which germination occurred. However, germination occurred very late in the season and is unlikely to provide a marker for poor quality, whereas the change in cotyledon or embryo water status may be more useful.

The search for a single marker of poor quality in late season fruit may be too simplistic an approach for the range of fruit quality problems that may occur. Potential disorders include rots at the stem end or in the skin on the main body of the fruit, flesh discoloration or the development of off-flavours (rancidity) in the ripe fruit, and the source of these problems may be diverse.

The main disorders found in this trial were rots occurring in the skin or at the stem end of the fruit, with a comparatively low incidence of the physiological disorder diffuse flesh discoloration or incomplete ripening. In future, it may be more appropriate to examine the skin and the flesh just below the skin in more detail when trying to predict rots, and the seed or fruit flesh characteristics may be more appropriate for predicting physiological disorders of the fruit flesh.

The low incidence of diffuse flesh discoloration in the present trial is likely to be the result of fruit having only been stored for 4 weeks. Four weeks is insufficient time at 4°C for the fruit to develop to the stage at which diffuse flesh discoloration occurs as the fruit ripen. The propensity for diffuse flesh discoloration to occur after storage may therefore be dependent on the pre-climacteric period at harvest, which declines the longer fruit are left on the tree. Diffuse flesh discoloration also depends on fruit being stored for a sufficiently long time, possibly so that biochemical changes associated with ripening occur in storage.

It is possible that a clearer picture of the patterns of physiological or morphological changes indicative of increased disorder susceptibility would be obtained by evaluating fruit over a wider harvest window (September to March). In addition, storing the fruit for a longer period to the point at which physiological disorders become more frequent would help to clarify changes in susceptibility through the harvest season. Overall, the incidence of rots in the trial was high, although the severity of the rots was very low, resulting in the overall high incidence of sound fruit when the sound/disordered threshold was at a severity of 5%. However, the high incidence of low severity rots when ripe suggests that if

the fruit had been stored at a temperature higher than 4°C, or for longer than 4 weeks, there would likely have been a larger number of more severe rots.
The presence of differences between orchards is illustrated by the data, even though only 2 orchards were examined. There were markedly different sugar profiles between the 2 orchards, with levels of mannoheptulose and perseitol up to 2 fold higher in fruit from Orchard 1 than those from Orchard 2. While there was an overall higher disorder incidence in the fruit from Orchard 2 than from Orchard 1, the majority of the disorders were rots. There is a high rot pressure on avocados grown in New Zealand, due in part to the long period of fruit growth (fruit may remain on the tree for 15 months) and also the climate that is conducive for rot growth (Everett, 2002). When conducting trials it is common to see large differences in rot incidence among orchards, irrespective of the time in the harvest season.

It is concluded that while there was some indication of an association between total sugars, mannoheptulose or seed water content at harvest and the incidence of sound fruit after storage, no definitive marker of advanced fruit maturity, or susceptibility to disorder, has been identified. In the future, research into identifying the risk of disorder in late season fruit will focus on fruit features more closely associated with specific disorders.

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