Factors Affecting Mass Loss of Apples

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Kate Marie Maguire

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"We have not succeeded in answering all your problems. The answers we have found have only served to raise a whole set of new questions. In some ways we feel as confused as ever but we believe we are confused now on a higher level and about more important things."

Anon.

Executive Summary

Mass loss from harvested apples causes direct loss in returns to growers and marketers of fruit. This thesis characterises the process of mass loss in harvested apples, exploring the effects of various factors on water vapour permeance of the fruit, a measure of the ease with which water escapes from the fruit.

Values of permeance of 'Braeburn' and 'Pacific Rose'™ apples were roughly twice those of 'Cripps Pink' and 'Granny Smith'. Permeance of 'Braeburn' and Pacific Rose'™ apples increased with later harvest date whilst values for 'Cripps Pink' and 'Granny Smith' remained relatively constant. There were small differences in mean permeance of apples from different regions. Some growers produced more fruit with high water vapour permeances than others. There was no relationship between maturity indicators tested and the water vapour permeance of the fruit. Fruit from the inner regions of trees and with high numbers of fruit in contact had high permeances. Variation in water vapour permeance around the surface of the fruit had no pattern with respect to blush or sun/shade sides, nor was there any relationship with cuticular thickness. Rather, variation in water vapour permeance of fruit was linked to the extent of cuticular micro-cracking. A model was developed which explains the water vapour permeance based on the proportion of fruit surface which is cracked. Artificial stretch applied to pieces of fruit skin increased cracking and permeance. Strain in the cuticle during growth and development of the fruit created a reticulate crack network. Micro-cracking could be important in determining susceptibility to mass loss and shrivel after harvest. Permeance of 'Braeburn' apples decreased after harvest; the extent of this decrease was greater for low relative humidity and high temperature and for fruit with high initial levels of micro-cracking. Bruising caused by impact damage on

'Braeburn' apples increased water vapour permeance of fruit only very slightly.

A conceptual model is presented which summarises relationships between fruit attributes, environmental conditions and processes which contribute to overall mass loss of apples. A composite mathematical model from previous models developed in the thesis is presented which describes total water loss as determined by the level of micro-cracking in the fruit cuticle, time after harvest, relative humidity and temperature of the storage environment. A number of suggestions for minimised mass loss in the apple industry are presented based on three strategies: minimisation of permeance, reduction of driving force for water loss and segregation of lines of high risk and applying appropriate handling regimes. The composite model could be used to explore a range of alternative handling and marketing scenarios in terms of total mass loss.

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α_1	arc length of skin disc normal	m
α_2	arc length of skin disc under stress	m
a	non-linear regression parameter	
A	surface area of the fruit system	m^2
A^b	area of bruising of the fruit system	m^2
A^{ck}	area of cracking of the fruit system	m^2
A^{cut}	area of intact cuticle of the fruit system	m^2
A^{pores}	area of pores of the fruit system	m^2
A^{tot}	total area of the fruit system	m^2
a_w	water activity of fruit	%
b	non-linear regression parameter	
°C	degrees Celsius	
c	non-linear regression parameter	
c.	approximately	
CO_2	carbon dioxide	
C^t	time conversion for days into seconds (86,400)	$s \cdot day^{-1}$
ΔM	total mass loss	kg
ΔM_{H_2O}	total water loss	kg
Δp_{H_2O}	difference in water vapour partial pressures between environment and inside of fruit	Pa
$\Delta p_{H_2O(1)}$	Δp_{H_2O} for the first set of postharvest conditions	Pa
$\Delta p_{H_2O(2)}$	Δp_{H_2O} for the second set of postharvest conditions	Pa
$\Delta p_{H_2O}^{bl}$	gradient of partial pressure of water vapour through boundary layer	Pa
$\Delta p_{H_2O}^{ck}$	gradient of partial pressure of water vapour through cracks	Pa
$\Delta p_{H_2O}^{icut}$	gradient of partial pressure of water vapour through the	Pa

	inner cuticle	
$\Delta p^{s}_{H_2O}$	gradient of partial pressure of water vapour through crack system	Pa
Δp_j	partial pressure difference for diffusion of species *j*	Pa
Δx	thickness of barrier	m
Δx^{b}	permanent deformation of bruise	m
Δx^{bl}	thickness of boundary layer	m
Δx^{ck}	thickness of cracks	m
Δx^{d}	deformation in centre of skin disc from stretching	m
Δx^{icut}	thickness of the inner cuticle	m
Δx^{flesh}	thickness of flesh at centre of a skin disc in side view	m
Δx^{m}	thickness of centre of a skin disc in side view	m
Δx^{s1}	thickness of side one of a skin disc in side view	m
Δx^{s2}	thickness of side two of a skin disc in side view	m
Δx^{skin}	thickness of visible skin at the centre of a skin disc in side view	m
d	day	
d^{b}	diameter of bruised area	m
d^{f}	diameter of fruit	m
df	degrees of freedom	
D_j	diffusivity of species j	$m^2 \cdot s^{-1}$
e	non-linear regression parameter	
ERH	Equilibrium relative humidity	%
γ	pyschrometric constant (equals 67 Pa $\cdot$ $^{o}C^{-1}$ at 20 ^{o}C)	Pa $\cdot$ $^{o}C^{-1}$
g	gram	
h	hour	
H_2O	water	
h^{b}	bruise depth	m
j	gaseous species	

J	joule	
k	proportion of cracking	
K	kelvin	
L	radius of skin disc	m
L	litre	
m	metre	
M	mass of fruit	kg
min	minute	
mol	mole	
$\%M_{H_2O}$	water loss as a percentage of total mass	%
n	amount of gas	mol
N_{H_2O}	mole fraction of water in the solution	
P	probability and statistical significance of F or T test	
Pa	pascal	
p^{tot}	total pressure in a system	Pa
$P_0^{'}$	initial water vapour permeance of fruit	$mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$
$P_{H_2O}^{'}$	water vapour permeance of fruit surface	$mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$
$P_{H_2O}^{'ck}$	water vapour permeance of cracks	$mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$
$P_{H_2O}^{'cut}$	water vapour permeance of undamaged cuticle	$mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$
$P_{H_2O}^{'pores}$	water vapour permeance of pores or lenticels	$mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$
$P_{H_2O}^{'s}$	water vapour permeance of crack system	$mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$
$P_{H_2O}^{air}$	effective permeability of air to water vapour	$mol \cdot m \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$
$p_{H_2O}^{e}$	partial pressure of water vapour in air	Pa
$p_{H_2O}^{f}$	partial pressure of water vapour in fruit	Pa
$P_{H_2O}^{icut}$	permeability of the inner cuticle to water vapour	$mol \cdot m \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$
$p_{H_2O}^{sat}$	saturated partial pressure of pure water	Pa
$p_{H_2O}^{sat}(T)$	saturated partial pressure of water vapour at temperature (T)	Pa

$p^{sat}_{H_2O}(T_e)$	saturated partial pressure of water vapour at T_e	Pa
$p^{sat}_{H_2O}(T_f)$	saturated partial pressure of water vapour at T_f	Pa
$p^{sat}_{H_2O}(T_w)$	saturated partial pressure of water vapour at T_w	Pa
$p^{soln.}_{H_2O}$	partial pressure of water in steady state with the solution	Pa
$P_j^{'}$	permeance of a barrier to gas species *j*	$mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$
P_j	permeability of a material to species j	$mol \cdot m \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$
$P_t^{'}$	water vapour permeance of fruit at time *t*	$mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$
$P_{t_1}^{'}$	water vapour permeance at the end of the first set of postharvest conditions	$mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$
$P_{t_2}^{'}$	water vapour permeance at the end of the second set of postharvest conditions	$mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$
R	gas constant = 8.314	$m^3 \cdot Pa \cdot mol^{-1} \cdot K^{-1}$
R^2	proportion of total variation explained by regression	%
r_{CO_2}	specific rate of respiration	$mol \cdot kg^{-1} \cdot s^{-1}$
RH	relative humidity	%
$r^{'}_{H_2O}$	rate of water loss in a system	$mol \cdot s^{-1}$
$r^{'bl}_{H_2O}$	rate of transfer of water vapour through the boundary layer	$mol \cdot s^{-1}$
$r^{'ck}_{H_2O}$	rate of transfer of water vapour through the cracks	$mol \cdot s^{-1}$
$r^{'icut}_{H_2O}$	rate of transfer of water vapour through the inner cuticle	$mol \cdot s^{-1}$
$r^{'s}_{H_2O}$	rate of transfer of water vapour through the crack system	$mol \cdot s^{-1}$
$r_j^{'}$	rate of gas transfer of species *j* in a system	$mol \cdot s^{-1}$
$r^{'}_{mass}$	rate of mass loss in a system	$kg^{-1} \cdot s^{-1}$

$rr\%$	respiration as a percentage of total mass loss	%
s	strain in skin	
s	seconds	
SED	standard error of the difference	
S_j	solubility of gaseous species within a fluid	$mol \cdot m^{-3} \cdot Pa^{-1}$
θ_1	angle required for calculating arc lengths of skin disc with no strain	
θ_2	angle required for calculating arc lengths of skin disc when strained	
t	time	d
T	temperature	^oC
t_0	time at beginning of the first set of conditions	d
t_1	time at end of the first set of conditions	d
$T_{(1)}$	temperature during the first set of conditions	^oC
t_2	time at end of second set of conditions	d
$T_{(2)}$	temperature during the second set of conditions	^oC
T_e	temperature of environment or air	^oC
T_f	temperature of fruit	^oC
™	trade mark	
T_w	temperature of wet bulb	^oC
v	velocity of air	$m \cdot s^{-1}$
V	volume	m^3
V^b	volume of bruised flesh	m^3
w/w	weight per weight	

Chapter 1

General Introduction

1.1 BACKGROUND

New Zealand's economy relies heavily on the export of primary products, which comprise approximately 60% of total exports (New Zealand Official Year Book 1997). For the year ending 30 June 1996, horticultural exports (fruits, nuts, vegetables, roots and tubers) were $1027 million (F.O.B.; New Zealand Official Year Book 1997), this represents 5.4 % of total New Zealand exports. Export is extremely important for our horticultural industry; 80-85% of total horticultural production is exported (Bollard 1996).

Fresh fruit in the year ending 30 June 1996 had an export value of $735 million (F.O.B.), 71.6 % of all horticultural exports. Of this the export of fresh apples had a value of $338.6 million, 33 % of horticultural exports. This is the second most valuable single horticultural export after Kiwifruit at 40%. The export trade in apples has developed since the first successful export of apple and pears was made in 1899 to Britain by Canterbury Fruit Growers (Bollard 1996).

New Zealand apples have a premium quality image and hence ENZA is a significant player on the international market. The value of our export crop of fresh apples is dependent on maintaining this premium quality image, which is principally based on the aesthetic qualities of the fruit. This is despite New Zealand supplying only about 2 % of the world's supply of apples (Pipfruit Research and Development Strategic Planning Group 1996). The primary constraint to delivering a premium quality fresh apple product remains the

distance of New Zealand from our main markets of Europe and North America.

New Zealand's position in the southern hemisphere gives it an advantage in exporting out of season produce to countries in the northern hemisphere. However, with improved storage procedures and technologies there is also often competition with carry over of apples from northern hemisphere production (Bollard 1996). The maintenance and improvement of the premium quality image is essential to retain our markets.

Excessive mass loss can render fruit unsaleable as a result of decreased turgidity (Sastry 1985) which, in fruits can result in a shrivelled appearance (Hatfield and Knee 1988; Kader 1992; Wills et al. 1989). Even in the absence of visible wilting, water loss can cause undesirable textural and flavour changes (Ben-Yehoshua 1987; Kader 1992; Wills et al. 1989). It also imposes a physiological stress on the produce which increases susceptibility to disease and decay (Woods 1990). These processes cause additional losses in crop value. Only a 5 % loss of apple mass may cause the development of a shrivelled appearance (Banks pers. comm.).

In 1996, mass loss and its associated disorder, shrivel, cost the New Zealand apple industry $8 million (King pers. comm.). Shrivel is particularly a problem in 'Braeburn' apples, which now comprise 41% of pipfruit exports from New Zealand. There is an urgent need to develop strategies to overcome this problem and to mitigate its effects on returns to pipfruit growers.

Fresh fruit continuously release water vapour into the surrounding atmosphere by transpiration. They also expend organic materials, primarily sugars, in respiration. Both of these processes cause a loss in saleable mass after harvest

(Sastry 1985) and thus a direct loss in returns to growers.

Water loss is the major contributor to mass loss in apples (Banks 1994). Two determining factors which influence the water loss are the barrier properties of the fruit (reflected in the fruit's water vapour permeance) and the surface area of the fruit. The cuticle plays an important role in the regulation of water loss. The thickness, structure and chemical composition of the cuticle vary greatly among commodities and among developmental stages (Kader 1992). The third influencing factor is the driving force for water loss which is a property which is defined by environmental physics.

Anecdotal evidence suggested a high level of variability in the development of shrivel and levels of mass loss between apples within a carton indicating variability in water vapour permeance of individual fruit. Much of this thesis is devoted to characterisation of sources of variation in water vapour permeance within populations of fruit. Systematic study of this issue has enabled development of both conceptual and quantitative models that will help the industry to devise strategies to tackle the problem of mass loss.

1.2 RESEARCH OBJECTIVES AND STRUCTURE OF THE THESIS

In chapter 2, a review of relevant literature is presented, covering the two processes which contribute to mass loss: water loss and respiration.

Chapters 3 to 9 were written more concisely as papers for publication in Journal of American Society of Horticulture, Journal of Experimental Botany, Postharvest Biology and Technology, and New Zealand Journal of Crop and Horticultural Science. These chapters are formatted to the style of the journal to which the papers will be submitted. At the beginning of these chapters a note indicates the journal style used.

Chapters 3 to 9 present investigations of the variation in water vapour permeance in populations of apples. In chapter 3 the effects of cultivar, harvest date, tree, and on the water vapour permeance of apple fruit are examined. Development of a simple mathematical model predicting mass loss is presented in chapter 3 and its limitations are discussed. Chapter 4 investigates variation in water vapour permeance associated with region, maturity and position on the tree. In addition, variation in water vapour permeance and cuticle around the surface of the fruit is explored. Chapter 5 examines more closely the link between cuticular micro-cracking and water vapour permeance of fruit. A model is developed which describes the water vapour permeance of fruit in terms of the intact fruit cuticle and cuticular micro-cracking.

Chapters 6 and 7 explore further the issue of cuticular micro-cracking and its potential contribution to water vapour permeance. Chapter 6 explores a possible mechanism for cracking by artificially inducing strain. Chapter 7 takes

a broader view of fruit development and growth, incorporating issues developed in Chapters 5 and 6. It includes previously unpublished data which were collected by other researchers which, in combination with data and observations collected by the author of this thesis, is used to explore fruit skin growth and its structural integrity.

Chapter 8 reports the effects of relative humidity and time after harvest on the water vapour permeance of 'Braeburn' apples. A model was developed which describes permeance changing with time as influenced by initial permeance and the driving force for water loss. The results are discussed in relation to the model developed in chapter 5. Chapter 9 explores the possibility that water vapour permeance might be affected by cuticular damage associated with impacts that are an inevitable feature of the harvesting process.

Chapter 10 is a general discussion, drawing together information presented in the thesis into conceptual and quantitative models of water vapour permeance and the consequent mass loss. A second part of the discussion investigates control strategies which could be used by the industry to minimise total mass loss and the occurrence of the disorder shrivel.

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Chapter 2

Literature Review

2.1 INTRODUCTION

Water loss of fruits and vegetables has been reviewed many times (Ben-Yehoshua 1987; Burton 1982; Sastry 1985; Sastry et al. 1978; Thompson 1992; Van Den Berg 1987; Wills et al. 1989; Woods 1990). In this review, I examine the slightly broader issue of what fruit and environmental factors affect the mass loss of apples.

Banks (1994) showed that loss of mass of apples through respiration was less than 10% of total mass loss in conditions typical of postharvest handling for the New Zealand industry (*c.* 0 °C and 85% relative humidity). At humidities closer to saturation, the contribution of water loss to total mass loss is reduced and respiration may then become a significant part of the total.

2.2 WATER LOSS

Transpiration by fruits involves the diffusion of water vapour from the fruit into the surrounding environment. Diffusion is a spontaneous process leading to the net movement of a material from one region to an adjacent one, from high concentration to low concentration (Nobel 1991a). Water loss from horticultural products is governed by the steady state solution of Fick's first law of diffusion (Nobel 1991a):

$$r_{H_2O}^{'} = \Delta p_{H_2O} A P_{H_2O}^{'} \qquad (2.1)$$

where:

r'_{H_2O} = rate of water loss from product (mol · s^{-1})

Δp_{H_2O} = difference in partial pressure of water vapour between the environment and inside the fruit (Pa)

A = surface area of fruit (m^2)

P'_{H_2O} = effective permeance of the fruit surface to movement of water vapour under prevailing conditions (mol · s^{-1} · m^{-2} · Pa^{-1})

Eq. 2.1 illustrates that rate of water loss is dependent on barrier properties of the fruit surface (P'_{H_2O}), surface area of the fruit, and driving force (Δp_{H_2O}). Any difference in rate of water loss of fruits relates to change in one or more of these factors. Consideration of Eq. 2.1 therefore provides the basis for development of all strategies for minimising water loss.

2.2.1 Permeance to water vapour

The fruit skin is a barrier to the diffusion of water vapour but it has to be sufficiently permeable to exchange of oxygen and carbon dioxide for normal aerobic respiration to occur (Lendzian and Kerstiens 1991). Permeance to water vapour characterises the ease with which water vapour can escape from fruit. Permeance of fruit can be determined using a rearrangement of Eq. 2.1:

$$P'_{H_2O} = \frac{r'_{H_2O}}{A\ \Delta p_{H_2O}} \qquad \textbf{(2.2)}$$

The determinations involve estimating rate of water loss, surface area and the driving force for water loss. It is this latter variable that is the most difficult to measure accurately; driving force data often have significant errors associated with them (Gaffney et al. 1985).

A generic fruit skin includes four layers of tissue (Bell 1937): a coating of epidermal hairs (absent in mature apples), cuticle, epidermis and hypodermis. The barrier properties of epidermis and hypodermis are very poor because they are all cellular materials; unmodified cellulose walls are very permeable to water migration (Burton 1982). Water is also able to diffuse freely through the semi-permeable membranes surrounding cell vacuoles and cells (Burton 1982). If these were the only barriers to transpiration the surface would become desiccated rapidly. The cuticle is the outermost layer of the fruit skin for a mature fruit. It is an extracellular layer that covers leaves, primary stems, flowers, petioles, fruits, hairs, and even glands (Lendzian and Kerstiens 1991). The cuticle is the barrier which prevents excessive loss of water by evaporation from the plant to its environment (Holloway 1982a).

The structure and development of the protective layers of the apple were described by Bell (1937) on 'McIntosh' apples. The cuticle was present on the ovary before full bloom. By full bloom, the cuticle was continuous and about 1.4 μm thick, though it was thicker at the bases of the hairs. It thickened progressively during development of the fruit until it completely filled the hair base and was continuous except over lenticels; it reached 23 μm thick by harvest time. The cuticle extended between epidermal cells to the hypodermis, as 'cuticular pegs'. There is great variation in the thickness and development of the cuticle from locality to locality (Bell 1937).

Several researchers have investigated the barrier properties of apple fruit skins. Various units for expressing the water vapour permeance of fruit have been

used through time. In Table 2.1, published values have been converted to a common set of units using formulae published by Banks et al. (1995). The measured values of permeance range from 0.6 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ for 'Granny Smith' apples (Nobel 1975) to 145.1 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ for cultivar 'Russet' (Lentz and Rooke 1964), a 240-fold range. Even within cultivars, there was a great variation in estimates published; for 'Golden Delicious' fruit the average values ranged from 16.6 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ (Pieniazek 1943) to 70 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ (Wells 1962). The values in the table are average values. From recent unpublished data it appears that permeance of individual fruit within a cultivar may vary by up to seven fold. Thus, the huge range in these values for individual fruit indicates that P'_{H_2O} is likely to be a key determinant of relative rates of mass loss of harvested apples and which fruit within a given population are likely to develop shrivel.

Conditions under which these measurements are taken are important. Boundary layers of still moist air around the fruit need to be broken up so that a true measure of the skin barrier properties is obtained (Chau et al. 1988; Nobel 1975; Woods 1990). Gaffney et al. (1985) used a modelling approach to determine the errors involved when variables were not included and the conditions under which the most accurate measures of permeance can be obtained at 5 and 25 °C. They determined that the most accurate measurements were obtained using high air flows past the apples at 25 °C and a relative humidity of 50-70%.

Table 2.1: Estimates of water vapour permeance from previously published and recent unpublished data. For values expressed originally on a percentage mass basis but with no information on the mass of the fruit used, fruit mass was assumed to be 0.16 kg. Surface area was estimated using Eq. 2.3 (Section 2.2.2) when this information was not given.

Cultivar	P'_{H_2O} (nmol · s^{-1} · m^{-2} · Pa^{-1})	Source
'Baldwin'	14.6	Pieniazek 1943
'Bankcroft'	33.8	Lentz and Rooke1964
'Boskoop'	60.0	Kessler and Stoll 1953
'Braeburn'	22.4	Banks 1994
	23.0	Banks 1995
'Bramley's Seedling'	28.8	Kidd and West 1931
	21.6	Smith 1932
	29.5	Smith 1933
'Calville Blanc'	22.9	Gae 1955
'Cortland Apples'	16.8	Lentz and Rooke 1964
'Cox's Orange Pippin'	42.8	Smith 1933
	30.9	Johnson 1976
'Golden Delicious'	46.7	Kessler and Stoll 1953
	36.5	Pieniazek 1942a
	16.6	Pieniazek 1943
	33.6	Smock and Neuburt 1950
	70.2	Wells 1962
'Granny Smith'	14.8	Banks 1994
	0.6	Nobel 1975
'Gravenstein'	2.4	Nobel 1975
'Grimes Golden'	63.0	Wells 1962
'Jonathan'	26.7	Kessler and Stoll 1953
	0.8	Nobel 1975
	32.4	Wells 1962
'McIntosh'	16.7	Lentz and Rooke 1964
	20.6	Pieniazek 1942a
	12.0	Pieniazek 1943

'Northern Spy'	18.1	Lentz and Rooke 1964
'Red Delicious'	35.8	Lentz and Rooke 1964
	9.2	Chau et al. 1988
'Rhode Island Greening'	32.8	Lentz and Rooke 1964
	16.3	Pieniazek 1942a; Pieniazek 1942b
'Royal Gala'	23.5	Banks 1994
'Russet'	145.1	Lentz and Rooke 1964
'Sandow'	32.9	Lentz and Rooke 1964
'Worcester Pearmain'	70.0	Burton 1982
'Yellow Bellflower'	21.3	Allen and Pentzer 1935
'Yellow Transparent'	21.5	Pieniazek 1943

2.2.1.1 Cuticle versus pores

In leaves, water loss can occur from the cell wall lining the intercellular spaces adjacent to the stomata through which water vapour is lost. Mature apples have few remaining stomata but they do have lenticels which replace some of the functions of stomata in that they act as gas exchange pores (Burton 1982). Pieniazek (1944) determined that lenticular transpiration accounted for 21% of total transpiration in 'Golden Delicious' and 'Baldwin', 8% in 'Turley' and 19% in 'Canada Red'. This represented a considerable amount of water loss in some varieties. However, 5 to 10 times more water was typically lost directly through the cuticle. The number and appearance of the lenticels alone was a poor indicator of permeance for a cultivar (Pieniazek 1944). Rather, it is the barrier properties of the cuticle which comprise the main influence on transpiration.

2.2.1.2 Cuticular thickness

Many researchers (Kamp 1930; Pieniazek 1944; Schonherr 1976b; Smith 1933) have tried to link cuticular thickness with the effectiveness of the cuticle as a

barrier to water. Kamp (1930) found no correlation between rates of cuticular transpiration of leaves from many species and cuticle thickness. Schonherr (1976b) found that with isolated cuticles (i.e. removed from the plant) from citrus and pear leaves again showed no relationship between the permeance and thickness. Pieniazek (1944) looked at the relationship between cuticle thickness and transpiration rate for several lots of 'McIntosh' apples. One group had a 30% higher transpiration rate but appeared to have no significant differences in cuticular thickness. In addition to this, cultivar differences in transpiration of 'McIntosh', 'Baldwin', 'Golden Delicious' and 'Rhode Island Greening' bore no relationship with cuticle thickness. Smith (1933) concluded that in 'Cox's apples cuticular thickness was not a significant factor in determining rate of water loss. Meyer (1944) suggested that although no apparent relationships had been found between cuticular thickness and transpiration rates, this might be due to the presence of cracks and breaks in the cuticle which may have overriding influences and obscure any effect of the cuticle thickness. Shutak and Schrader (1948) found a high correlation between cutin thickness on the green side of fruit and the cracking of fruit skin. Possibly, fruit with thicker cuticles crack more, thereby increasing transpiration rate, countering any relationship between cuticle thickness and water loss that might otherwise be found.

2.2.1.3 Cuticular composition and structure

Chemically the cuticle can be characterised by two specific groups of lipid substances by their solubility in polar solvents: insoluble polymeric cutins (which constitute the framework of the membrane) and soluble cuticular lipids

(Holloway 1982a; which can appear on the surface as epicuticular wax or embedded within the cuticle as intracuticular wax). The structure of the membrane varies considerably according to species and stage of development (Holloway 1982a).

The polar and charged groups in the plant cuticle can play a role in sorption and transport of gases and vapours such as water (Lendzian and Kerstiens 1991).

2.2.1.3.1 Cutin

Cutin is a three-dimensional polymer of various long-chain substituted aliphatic acids. Further details of the composition of cutins are reviewed in Holloway (1982b). Permeability of the polymer matrix with the soluble cuticular lipids removed has been shown to depend on pH and counter-ions (Schonherr 1976a) and on water activity (Schonherr and Schmidt 1979). This can be explained by the presence of polar functional groups, some of which dissociate. The polymer matrix has been found to be 20 to 1,500 more permeable than an intact cuticle (Schonherr and Lendzian 1991; Schonherr and Merida 1981).

2.2.1.3.2 Soluble cuticular lipids

Soluble cuticular lipids are a characteristic feature of plant cuticles. Evidence from electron microscopy show that aerial surfaces of all higher plants carry a partial or continuous coverage of amorphous wax (Baker 1982). Thick wax layers are commonly found on fruit surfaces e.g. *Malus* spp., *Prunus* spp. and *Citrus* spp. and these often develop into soft mounds of semi-crystalline wax or crusts (Fig. 2.1).

Soluble cuticular lipids have a marked effect on the barrier properties of cuticle membranes to water. Schonherr and Lendzian (1991) found that increase in water permeability of a factor of 20 when the soluble cuticular lipids were removed from the cuticle of tomato fruit. Permeability of cuticles from the bulb scales of onion was increased 1,542 times with similar treatment (Schonherr and Merida 1981). These large effects show that the soluble cuticular lipids have an important role as the main barrier to the transport of water though plant cuticles.

Although a wide variety of components have been extracted from plant surfaces, most are only present as minor components. Principal components of plant epicuticular waxes are fatty acids, aldehydes, alcohols (primary and secondary), alkyl esters, long chain alkanes (C_{17}-C_{35}), ketones, beta-diketones, and cyclic triterpenes (Baker 1982).

The contribution of soluble cuticular lipids to overall resistance depends upon structure as well as chemical composition. Reapplication of extracted soluble cuticular lipids to the cuticle matrix only restored up to 5% of the formerly observed barrier properties of the intact cuticle (Lendzian and Kerstiens 1991).

Waxes on plants have been reported to comprise three distinct fractions: a crystalline zone, a solid amorphous zone, and a mobile amorphous zone (Reynhardt and Riederer 1994). The three fractions show fundamental differences in their chemical composition and in the molecular arrangement of the wax monomers. The cuticular wax can be viewed as a permeable matrix,

the amorphous phase, and impermeable flakes embedded within this matrix, the crystalline phase (Riederer and Schreiber 1995).

Fig. 2.1. Wax layer on adaxial surface of *Pistacia vera* leaf x c. 8800 similar to layers found on fruit surfaces e.g. *Malus* spp. (Baker 1982).

The mobilities of the diffusing molecules are reduced by the impermeable flakes by two mechanisms: (i) they decrease the volume of the amorphous phase available for diffusion and, (ii) they create a tortuous pathway for diffusing molecules, leading to an increased path length of diffusion. It is not only the degree of crystallinity, but also the geometrical arrangement of the crystalline domains which are thought to be important for the efficiency of the barrier (Schreiber et al. 1996).

2.2.1.3.3 Ultrastructure

Schonherr and Riederer (1988) demonstrated pronounced functional asymmetry of cuticular membranes and that the barrier to water vapour transfer

was in the outer surface of the cuticle. A functional model of the cuticle was developed by Schonherr and Riederer (1988) and Riederer and Schreiber (1995). The cuticle consists of an 'inner volume' including cuticular pegs, and 'outer volume' element or skin with a cover of epicuticular waxes providing the barrier properties of the cuticle (Riederer and Schreiber 1995).

The functional model is conceptually very similar to a fine structure model previously developed from polarised light and transmission electron microscopy (Sitte and Rennier 1963; Wattendorff and Holloway 1980). The cuticle can be seen to consist of a layer of epicuticular wax layers on the top, then a cuticular proper which is usually a thinner section of the cuticle with an ordered crystalline appearance with polarised light indicating the presence of waxes (Sitte and Rennier 1963). The inner layer is an amorphous matrix termed the cuticular layer which has be shown to contain a substantial proportion of cutin (Wattendorff and Holloway 1980). De Vries (1968) observed cuticles on mature 'Golden Delicious' apples with transmission light microscopy. These cuticles appeared to have two layers with a thinner layer on the outer surface with a crystalline nature and a thicker amorphous layer toward the inner surface.

2.2.1.4 Changes in permeance during development

Changes in cuticle structure as the fruit grows lead to changes in permeance during development. Pieniazek (1943) studied the changes in permeance during the development of apples. The fruit were picked at weekly intervals and permeances were obtained within 24 h. The author did not mention air flow

over the apples so it is difficult to determine if boundary layer effects on permeance measurements were taken into account. Following an extensive search of the literature, this appears to be the only previously published research in this area. The permeance of extremely immature apple fruit is high initially and decreases towards the time of commercial harvest (Fig. 2.2).

2.2.1.5 Factors influencing permeance of fruit at harvest

2.2.1.5.1 Time of harvest

Permeance of apples to water vapour increases as the fruit maturity changes during the commercial harvest period. Pieniazek (1943) found that permeance of fruit increased as harvest date was delayed, although the effect may have been overestimated since fruit were picked at the time indicated on the graph, then stored until the last fruit had been picked before measurements were taken (Fig. 2.3). The observed effect could have been linked to maturity differences in the fruit, an environmental effect because of the longer period on the tree or to drying out of the fruit surface after picking.

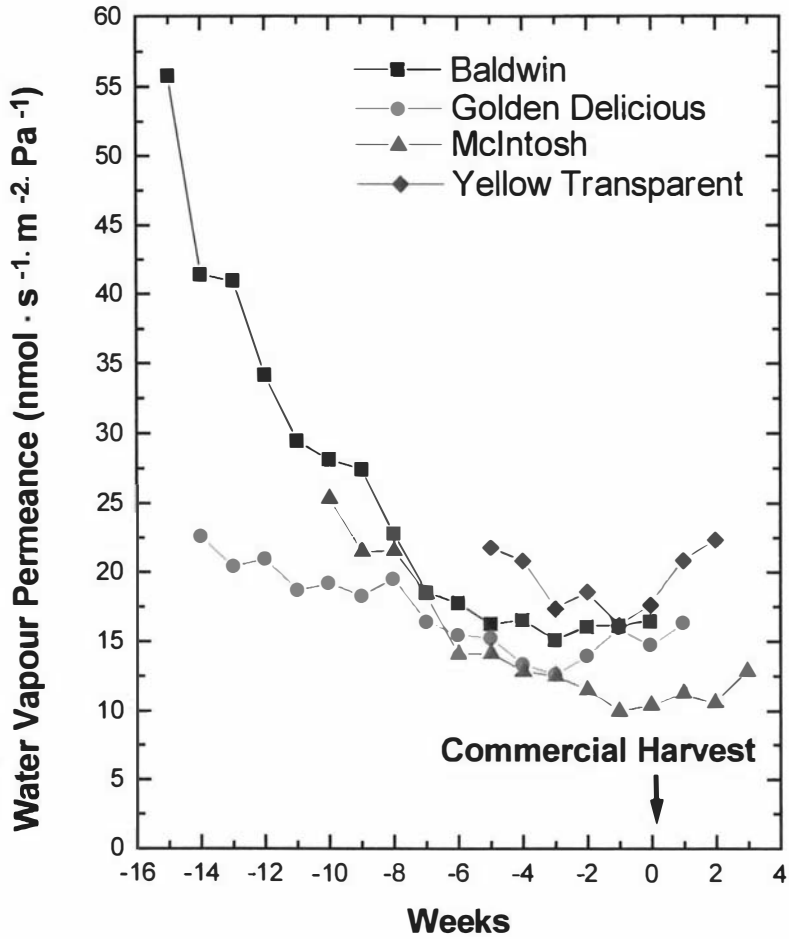

Fig. 2.2. Changes in water vapour permeance during development of four cultivars of apple (calculated from Pieniazek 1943).

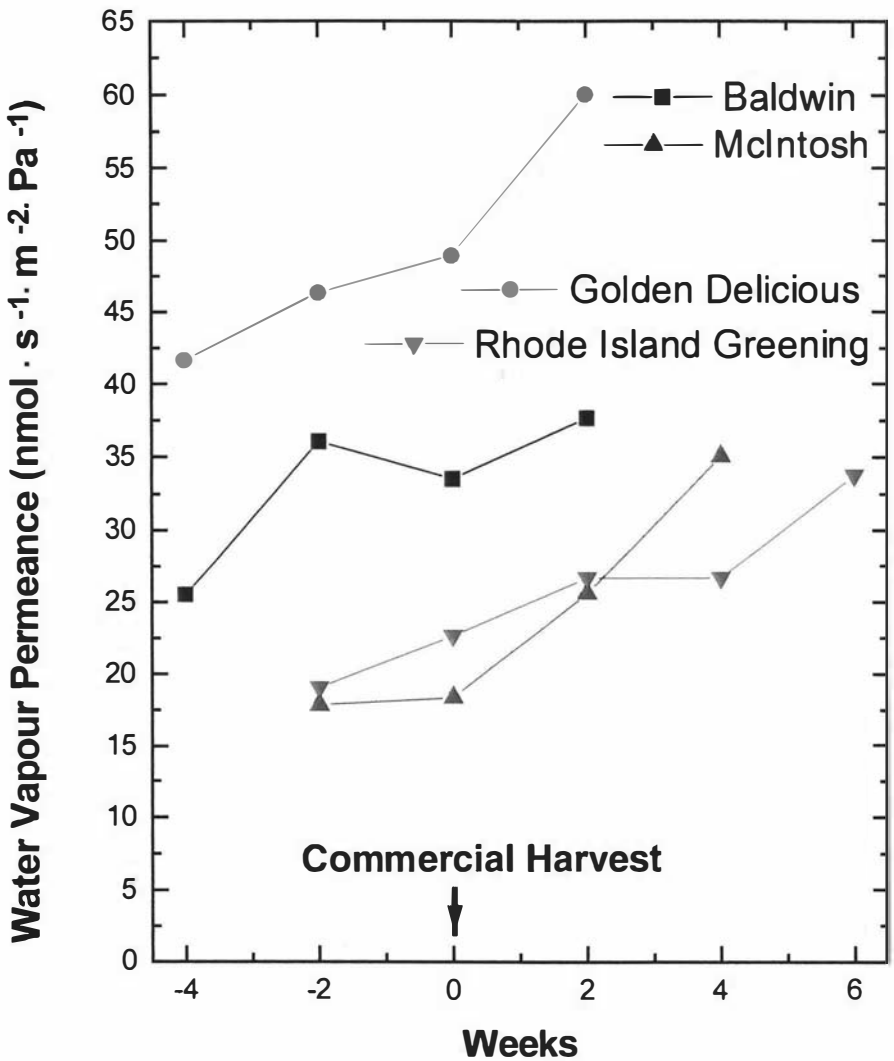

Fig. 2.3. Relationship between water vapour permeance and picking date for 'Baldwin', 'McIntosh', 'Golden Delicious' and 'Rhode Island Greening' (calculated from Pieniazek 1943).

2.2.1.5.2 Maturity

Burton (1982) reported that, in potato tubers, differing stages of maturity have differing permeances because of changes in the nature and structure of the outer layers due to development. Sastry (1985) stated analagously that immature and overmature fruit have been found to transpire more rapidly than mature fruit, but this is not supported by solid evidence. Woods (1990) stated that permeance drops as fruit mature. Pieniazek (1943) used background colour and firmness measurements to characterise maturity of apples and measured their transpiration rates. Early cultivars ('Yellow Transparent' and USDA Seedling varieties) showed 47% and 24% increases, respectively, in water vapour permeance between the green and well-coloured stages of fruit maturation. The later cultivars 'Duchess' and 'Wealthy' showed only 4 and 5% changes with fruit maturation respectively. Although there were no statistics presented on these data it would be safe to assume from the variation observed from other data that a difference of only 4 or 5% could only be of marginal significance.

2.2.1.6 Postharvest factors influencing permeance of fruit

2.2.1.6.1 Mechanical damage

Cuts and stem punctures break the barrier to water vapour transfer and expose the cellular material beneath the cuticle which are poor barriers to water vapour diffusion (Burton 1982). Any cuts incurred by the fruit after harvest would increase water vapour permeance (Sastry 1985) and result in increased water loss. Bruising damages the surface of the fruit and has been claimed to increase water loss (Wills et al. 1989).

Cuticular cracking or skin cracking (as distinct from fruit cracking) are characterised by the presence of numerous minute superficial cracks on the fruit surface (Opara et al. 1997). This disorder develops before harvest (Meyer 1944; Verner 1935) and during storage (Goode et al. 1975, Mezzetti 1959).

Meyer (1944) investigated development of cracks in the skin of 'Golden Delicious'. The skin of this variety developed much the same as other varieties until the fruit were about 16 mm diameter. The epidermis then underwent considerable periclinal division and, from that time on, was very irregular. The cuticle was also uneven. When the fruit were approximately 50 mm in diameter, cracks made their appearance in the cuticles. Subsequent enlargement of the fruit caused the cracks to extend and widen. Mature apples, 76 mm in diameter, generally had a severely cracked cuticle, with many of the epidermal and some hypodermal cells unprotected by either cuticle or cork.

The skin of 'Winesap' went through a similar type of development, except the cuticle rarely became cracked. This is perhaps because 'Winesap' did not reach the same size as 'Golden Delicious' and therefore the cuticle was not subjected to so great a strain. In addition the cuticle of 'Winesap' was not as thick as that of 'Golden Delicious'. Similar observations on 'McIntosh' apples were made by Tukey and Young (1942).

Shutak and Schrader (1948) found that the badly cracked 'York Imperial' apples had deeply indented cutin. Tetley (1930) found that varieties with uneven cutin, which penetrated between cells were more subject to cracking

than other varieties. There are contradictory reports in the literature over which side of the fruit cracks: sun or shade (Shutak and Schrader 1948; Tetley 1930; Verner 1935 and Verner 1938). Despite this, authors agree that the side where cracking is more common has thicker, inelastic cuticle (Opara 1993).

Visai et al. (1989) quoted unpublished data which showed that cracked 'Neipling Stayman' apple fruit had less gibberellic acid (GA)-like substances than intact ones. Taylor and Knight (1986) applied GA_4 and GA_7 on 'Cox's Orange Pippin', 'Discovery' and 'Golden Delicious'. The treatment increased the plasticity of the fruitlet skin. It changed the cuticular morphology by reducing the size of cuticular pegs; the effect on cuticular cracking was not reported.

Russetting is another physiological disorder of apple fruit skin and influences mass loss. Apple fruit with russet have been reported to shrivel first on the affected side (Pieniazek 1944). Russetting is observed with varying degrees of severity. More abundant russetting has deeper cork formation and numerous cracks present which increase water loss from the fruit substantially. Pieniazek (1944) studied severely russetted 'Baldwin' and 'Delicious'; the difference in transpiration compared to non-russetted fruit was 47% and 67%, respectively. Russetting of a superficial nature, such as that seen on 'Golden Delicious' has less effect on transpiration, increasing P'_{H_2O} by only 8% (Pieniazek 1944). Lentz and Rooke (1964) reported that water vapour permeance of a cultivar called 'Russet' was 4 to 6 times higher than the other cultivars they had studied. Baker (1931) found that transpiration through the corky tissue of russetted regions of 'Grimes' apples was considerably greater than through the

normal epidermis and cuticle. Verner (1935) used an aqueous dye solution (Acid fuchsin and methyl blue) fed into the cut ends of small branches. Penetration of the dye into nearby fruit was observed. The movement of the dyes indicates a movement of vascular sap into the tissue beneath these regions of russetting, scab lesions or cracks and that the passage of water out of them by transpiration was more rapid than elsewhere.

2.2.1.6.2 Relative humidity

The structure of fruit cuticle resembles a continuous hydrophobic polymer membrane. The gas permeabilities of hydrophobic polymer films are known to increase with increased relative humidity (Barrie 1968). Permeance to water vapour of the skin has been considered to be independent of environmental modification (Sastry and Buffington 1982). However, several researchers have reported an increase in permeance to water vapour associated with increasing relative humidity or decreasing Δp_{H_2O} (Fockens and Meffert 1972; Lentz and Rooke 1964; Sastry et al. 1978; Shirazi and Cameron 1993 and Smith 1933). Lentz and Rooke (1964) reported that P'_{H_2O} of apples cultivars 'Red Delicious', 'Rhode Island Greening', 'Bankcroft' and 'Sandow' were very dependent on the Δp_{H_2O} whereas the cultivars 'McIntosh', 'Northern Spy' and 'Cortland' were less so (Fig. 2.4); they did not use surface temperature in calculation of Δp_{H_2O} which would have limited accuracy of estimation of P'_{H_2O} (Sastry and Buffington 1982). However, the levels of magnitude of the difference in water vapour permeance they found were far greater than could be accounted for by their failure to take surface temperature into account. They reported that the surface temperature of their fruit was within 0.03 °C of the circulating air. This would have led to only a 0.2% error in P'_{H_2O} if Δp_{H_2O} had been 606 Pa or 4%

error in more humid air with a Δp_{H_2O} of only 38 Pa. (calculations not shown). Fockens and Meffert (1972) found that the permeance of 'Jonathan', 'Laxton's Superb', 'Lombartscalville', 'Golden Delicious' and 'Belle de Boskoop' increased by 70 to 100% when the relative humidity was increased from 70% to close to saturation (the temperature at which this was completed was not reported).

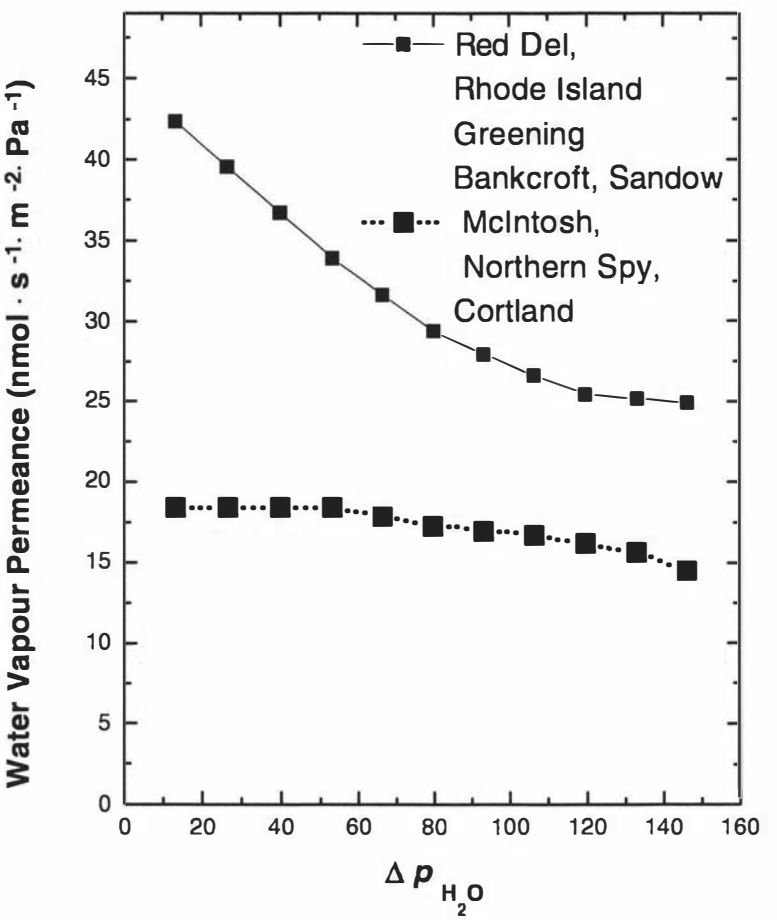

Fig. 2.4. Effect of difference in water vapour partial pressure on the water vapour permeance of apple cultivars (Lentz and Rooke 1964).

Schonherr (1976a) reported that the water vapour permeability of isolated cuticles and cuticular matrices (cuticles with the soluble cuticular lipids removed) increased with increasing water content through sorption. Chamel et al. (1991) found that isolated tomato, apple and pepper fruit cuticles sorb increasing amounts of water with increasing atmospheric water vapour pressure. The water vapour permeability of isolated cuticles from citrus leaves and from eggplant fruit also demonstrated a dependence on the water vapour content of the air (Schonherr and Schmidt 1979; Fig. 2.9). All of these pieces of evidence suggest that the permeance of the cuticle to water vapour responds to the water vapour content of the air.

Despite this evidence, a number of studies have assumed that the permeance of skin is not affected by the water vapour pressure it is exposed to (Chau et al. 1988; Gaffney et al. 1985; Sastry 1985). Accurate prediction of rate of water loss from apples using Eq. 2.1 would depend upon knowledge of the dependence of P_{H_2O} upon Δp_{H_2O}; at present this information is not available.

2.2.1.6.3 Temperature

Schonherr et al. (1979) reported that the water permeability of isolated cuticles increased with increasing temperature. They also found that temperatures higher than 45 ^{o} C caused irreversible structural changes in isolated cuticular membranes, which led to an increase in water permeability. Eckl and Gruler (1980) attributed the temperature effect on water permeability to a phase transition and reorientation of soluble cuticular lipids, which led to development of hydrophilic holes in the barrier. The irreversible membrane

alterations were thought to be due to a recrystallisation of soluble cuticular lipids upon cooling that resulted in a structure different from the original one, with partial preservation of the hydrophilic holes in the barrier.

Schreiber and Schonherr (1990) argued that the increase in water permeability of plant cuticles above transition temperature is caused by the increase in disorder at the interface of the polymer matrix and the soluble cuticular lipids. The phase transition mid-point for isolated cuticles from apple fruit was 54.5 ^{o}C. There is no evidence in the existing literature for this effect occurring *in vivo*. For physiological temperatures (0 - 30^{o} C) the changes in permeance appear to be significant with permeance of citrus leaves increasing 300% for temperatures between 10^{o} C and 30^{o}C. Referring to Eq. 2.10, Δp_{H_2O} is required to calculate P'_{H_2O}. Separating the effects of relative humidity and temperature would be complex because temperature is important in calculating Δp_{H_2O} (Eqs. 2.3-2.6).

2.2.1.6.4 Waxing

Edible surface coatings are applied to fruits or vegetables to improve cosmetic features of the crop (sheen, perceived depth of colour) and to reduce deterioration by suppressing water loss or by achieving modified atmosphere (MA) benefits (Banks et al. 1997). The use of waxing to prevent mass loss has been studied since 1930's (Smock 1936). Recently there has been a substantial increase in research activity on surface coatings (Baldwin 1995; Gontard et al. 1996; Hagenmaier and Baker 1995; Hagenmaier and Shaw 1992; Mannheim and Soffer 1996; Nussinovich and Lurie 1995). Waxing decreases the fruit's permeance to water vapour by adding a layer through which the water

molecules must move. However, there is inevitably some effect on other aspects of gas exchange of the treated commodity (Banks et al. 1997).

Waxing changes the permeance of the skin to O_2 and CO_2 which can lead to modification of internal atmosphere composition and, if sufficiently severe, results in anaerobiosis, fermentation and development of off-flavours (Hagenmaier and Shaw 1992; Mannheim and Soffer 1996). Thus, there is risk associated with coating fruit for the benefits of reduced water vapour and improved cosmetic appearance.

2.2.2 Surface area

Size of a fruit or vegetable has a significant effect on its water loss (Ben Yehoshua 1987). Correcting water loss for surface area allows errors due to variation in size and shape to be accounted for (Woods 1990). Surface area of a fruit can be estimated for apples using the following equation developed by Clayton et al. (1995):

$$A = 0.058M^{0.685} \quad \textbf{(2.3)}$$

where: A is the surface area (m^2) and M is the mass of the fruit (kg). This power law relationship between mass and area indicates that surface area of the fruit increases with increasing fruit mass. Jackson et al. (1971) found that smaller apples shrivelled before the larger fruit. Smaller apples possess a larger area to volume or mass ratio and will lose more moisture on a per unit mass basis (Ben Yehoshua 1987; Sastry 1985).

2.2.3 Driving force (Δp_{H_2O})

Dry air is a mixture of about 78 % nitrogen and 21 % oxygen, with carbon dioxide 0.034 %, argon (0.934%) and other minor constituents comprising the remaining 1 % (Wills et al. 1989). Moist air consists of a mixture of dry air and water vapour. If water is placed in an enclosure containing dry air, water molecules enter the vapour phase and the air eventually becomes saturated with water vapour. The amount of water vapour can vary from virtually zero to a maximum that is dependent on temperature and pressure (Thompson 1992). The amount of water vapour in the air can be described in different ways. Relative humidity (*RH*) is probably the most well known term for quantifying the amount of water vapour in air. *RH* is a ratio, usually expressed as a percentage, of partial pressure of water vapour actually in the air ($p^{e}_{H_2O}$; Pa) to the saturation partial pressure at the environmental temperature ($p^{sat}_{H_2O}(T_e)$; Pa).

$$RH = \frac{p^{e}_{H_2O}}{p^{sat}_{H_2O}(T_e)} \times 100 \qquad \textbf{(2.4)}$$

The partial pressure of water vapour in the intercellular spaces can be assumed to be very close to saturation at the fruit temperature (Burton 1982). The amount of water vapour of air in typical storage environments is generally less than this, to an extent that depends upon temperature and relative humidity of the air. Following the laws of diffusion, water vapour moves out of the fruit into the environment, from high concentration to low concentration. The driving force for the loss of water is therefore the difference in the partial pressures between the fruit and the environment (Δp_{H_2O} ;Pa):

$$\Delta p_{H_2O} = p^{f}_{H_2O} - p^{e}_{H_2O} \quad \textbf{(2.5)}$$

where $p^{f}_{H_2O}$ (Pa) is the partial pressure of water vapour in the fruit.

Lines of constant relative humidity on a psychrometric chart are curved; a given relative humidity at different temperatures represents different partial pressures of water vapour in the air and therefore produces differing driving forces for water loss for fruit at a given temperature.

The partial pressure of water vapour at the fruit surface can be estimated using an equation that describes the curved saturation line on the psychrometric chart, such as that derived by Tetens (1930):

$$p^{sat}_{H_2O}(T) = 611\exp\left(17.27\left(\frac{T}{T+237.3}\right)\right) \quad \textbf{(2.6)}$$

where $p^{sat}_{H_2O}(T)$ (Pa) is the saturated water vapour pressure at temperature T (°C).

The partial pressure of water vapour in air can be obtained using probes which measure relative humidity and air temperature, or by using a psychrometer (wet and dry bulb thermometer). The psychrometer measures air and dew point temperatures which, together, can be used to determine the partial pressure of water vapour in the air.

The following equations can be used to estimate $p^{e}_{H_2O}$ from the basic measurements. Eq. 2.7 is for relative humidity probes where *RH* is the relative humidity expressed as a percentage and T_e (°C) is the environmental temperature.

$$p^{e}_{H_2O} = 611\exp\left(17.27\left(\frac{T_e}{T_e+237.3}\right)\right) \times \frac{RH}{100} \quad \textbf{(2.7)}$$

Eq. 2.8 can be used as an alternative for calculating $p^{e}_{H_2O}$ if dew point temperature (T_W; °C), air temperature (T_e; °C), saturated water vapour pressure ($p^{sat}_{H_2O}(T_w)$; Pa) at dew point temperature (T_W) and the psychrometric constant (γ; which has a value of 67 Pa $^{O}C^{-1}$) are known.

$$p^{e}_{H_2O} = p^{sat}_{H_2O}(T_w) - \gamma(T_e - T_w) \quad \textbf{(2.8)}$$

From these relationships it can be seen that the main factors influencing driving force for water loss are fruit temperature and relative humidity and temperature of the environment.

2.2.3.1 Fruit temperature and water vapour partial pressure

Temperature of the product surface is a major determinant of the driving force for water loss. The temperature of an object is the net result of all the ways that energy can enter or leave it (Nobel 1991b). Energy can enter and leave by both sensible heat and latent heat transfer.

2.2.3.1.1 Sensible heat transfer

There are three mechanisms of sensible heat transfer: radiation, conduction and convection (Monteith and Unsworth 1990; Nobel 1991b).

Infrared or thermal radiation is absorbed from the environment. Any object with a temperature above absolute zero (0 K) emits thermal radiation (Nobel 1991b). Thus, every object within the store emits and absorbs thermal radiation, including the product.

Heat can be conducted from one body to a cooler one in contact with it by molecular and/or electronic collisions (Monteith and Unsworth 1990; Nobel 1991b). Thus heat can be conducted across air by the random thermal collisions of the gas molecules.

Convective heat transfer occurs when such turbulent gas movement is in contact with a body (Monteith and Unsworth 1990; Nobel 1991b). Convectional heat transfer can be separated into free and forced convection. Free convection, or natural convection, occurs when heat is transferred from the body to the air surrounding it. This causes this air to warm, expand, and decrease in density; this more buoyant, warmer air then moves upward and thereby moves heat away from the fruit (Nobel 1991b). This occurs when there is very little air movement. Forced convection is caused by air movement (wind) moving the warmer air away from the product (Nobel 1991b). As the speed of the air movement increases, more heat is dissipated by forced convection.

It is generally accepted that heat is conducted away from the product into the boundary layer and is then removed by free and forced convection (Nobel 1991b).

2.2.3.1.2 Evaporative cooling

The mechanism of latent heat loss is evaporative cooling. The latent heat of vaporisation of water at 100 °C is 2.26 $MJ \cdot kg^{-1}$ or 40.7 $kJ \cdot mol^{-1}$; this is the highest heat of vaporisation of any known liquid per unit mass (Nobel 1991c).

At 20 °C, each mole of water evaporated requires 44.2 kJ, meaning that a substantial heat loss by a plant organ accompanies the evaporation of water in transpiration. For an apple which loses 36 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (with diameter of 0.07 m in an air flow of 0.25 $m \cdot s^{-1}$ at 20 °C and 50% relative humidity; Δp_{H_2O}=1.2 kPa, permeance = 30 $nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$), the energy needed to evaporate this amount of water could lower the temperature by 0.6 °C (Gaffney et al. 1985). This reduction in surface temperature reduces the partial pressure of water vapour at the fruit surface and therefore reduces the total driving force for water loss.

2.2.3.1.3 Heat of respiration

Since fresh fruits and vegetables are living, they continue the process of respiration, a composite and complex process that involves conversion of carbohydrates and oxygen into carbon dioxide, water, and heat. The heat it produces can accumulate in the product and raise product temperature (Gaffney et al. 1985; Sastry 1985; Woods 1990) unless it is removed. This increase in temperature raises the water vapour partial pressure at the surface of the product and, in turn, raises the total driving force for water loss.

Actual fruit surface temperature is therefore a balance between conduction, convection and radiant heat transfer, evaporative cooling, and respiratory heat gain. Packaged apples in a coolstore at 0.92 °C, with a bulk store relative humidity of 85-90% had a surface temperature of 1.45 °C (Amos 1995).

2.2.3.1.4 Dissolved solutes

Use of Eq. 2.4 to determine the partial pressure of water vapour at the surface

of the fruit relies upon the assumption that the water being evaporated is pure. Water present within fruits and vegetables contains dissolved substances that lower the equilibrium partial pressure of the water vapour. This phenomenon was described by the French chemist, Francois Raoult, who found that the ratio of the solvent partial pressure of a solution of dissolved solutes to that of a pure solvent is proportional to the mole fraction of solvent in the solution. Raoult's law, as this relation is called, can be written in the following way for aqueous solutions (Atkins 1990):

$$p_{H_2O}^{soln.} = N_{H_2O}\, p_{H_2O}^{sat} \qquad (2.9)$$

where $p_{H_2O}^{soln.}$ is the partial pressure of water vapour in steady state with the solution, N_{H_2O} is the mole fraction of water in the solution and $p_{H_2O}^{sat}$ is the saturated water vapour partial pressure of pure water. This effect lowers the partial pressure of water vapour at the surface of the fruit and thus lowers the driving force for water loss. N_{H_2O} is also known as the 'water activity' (a_w) of the fruit.

This effect could be quantified for fruits if the molar concentrations of the substances dissolved in the water near the surface could be determined. Gaffney et al. (1985) attempted to determine the vapour pressure lowering effect of dissolved substances through the freezing point depression effect that the dissolved substances have on fruit sap. They suggested that N_{H_2O} was 0.981. However, this approach took no account of the compartmentalisation of total dissolved substances within the tissue. Their estimate therefore probably represents an extreme lower limit for the value of a_w at the fruit surface.

As an alternative, Rooke and van den Berg (1985) determined the equilibrium relative humidity (*ERH*) of the produce, at which there was no mass loss or gain from the produce, i.e. at which there was no driving force. From Eq. 2.7 it follows that the *ERH* of air in contact with tissue at a given water activity, is a_w × 100%. For whole apples, *ERH* was found to be 99%, whereas *ERH* for the cell contents of apples was 97.5 to 98.8 (Rooke and van den Berg 1985). The first figure was probably a slight overestimate of the true *ERH* because no adjustment was made for respiratory mass loss. The latter figures are analagous to the values presented by Gaffney et al. (1985).

The water activity at the fruit surface is therefore close to 0.99. Given the influential effects of minor temperature variations, the water vapour partial pressure at the fruit surface can be approximated with sufficient accuracy as the saturated water vapour pressure at the fruit surface temperature ($p_{H_2O}^{sat}(T_f)$).

2.2.3.2 Packaging effects

Packaging interferes with free air movement and diffusive flux of water vapour around fruit and can therefore profoundly influence effective Δp_{H_2O}. One mechanism by which this occurs is through influence on effective permeance of the boundary layer (the zone of gas around the fruit within which there is limited turbulent mixing). This occurs because thickness of the boundary layer is inversely related to air velocity (Nobel 1975):

$$\Delta x^{bl} = \frac{2.8\sqrt{\frac{d^f}{v}} + \frac{0.25}{v}}{1000} \qquad \textbf{(2.10)}$$

where:

Δx^{bl} = effective boundary layer thickness (m)

d^f = diameter of fruit (m)

v = velocity of air ($m \cdot s^{-1}$)

Thus, the rate of moisture loss from a fruit can be affected by boundary layer permeance as well as by skin permeance. The effective permeance of the boundary layer can be estimated from its thickness using the diffusion coefficient of water vapour in air.

The boundary layer adds an extra resistance to diffusion of water vapour from the fruit. Extra resistances to diffusion slow rates of water loss; as velocity of air past the fruit increases, rate of water loss increases (Gaffney et al. 1985; Nobel 1975).

In addition to the added resistances of the boundary layer and the packaging itself, packaging around the product can have a profound effect on the actual water vapour partial pressure of the environment which determines diffusion of water vapour from the product. The presence of packaging reduces the flow of air within a package. Reducing the air flow around the product allows air in the immediate environment to become more saturated with water vapour. This increases the water vapour partial pressure of the environment considerably, thereby reducing water loss.

Packaging with more ventilation is less effective at disrupting the air flow around the product and thus allows greater values of Δp_{H_2O}. Fruit in such packaging has higher rates of water loss because of the higher air flows. Liners within cartons reduce the airflow around the fruit until it is negligible.

Fruit continue to humidify the air within the liner until it is virtually fully saturated. This lowers Δp_{H_2O} to a very low level. However, there is still a small driving force because the temperature of the product is not the same as the surrounding air. Under these conditions the rate of water loss is minimised because of the extremely low Δp_{H_2O}.

2.2.3.3 Relative humidity and temperature of the environment

Many of the issues summarised here have been explored in more detail by Amos who developed a model for water vapour and heat transfer in apple cool stores (Amos 1995). Lowering the temperature of the environment reduces fruit temperature. This lowers the partial pressure of water vapour at the fruit surface. Therefore, coolstorage is very effective at reducing the driving force for water loss.

2.2.3.3.1 Sources of moisture

Increasing the relative humidity of the air by adding moisture also reduces the driving force for water loss. In coolstorage situations, most of the moisture input is through open doors (Amos 1995). The presence of people also introduces moisture into cool stores due to exhalation of moist air (Amos 1995). Product moisture loss is also an important moisture source within a coolstore.

2.2.3.3.2 Sinks for moisture

Cooling an apple store to approximately 0 °C requires that the evaporator coils are cooler than this; their cool surfaces provide a sink for water vapour, which forms frost on the evaporator coils. The temperature of the surrounding structure also plays a part in the balance of water vapour within cool stores

(Amos 1995). When the temperature of the structure drops below the dew point (wet bulb temperature) of the surrounding air, moisture condenses out of the air onto the structure. Conversely, when the temperature is above the dew point, then moisture which had condensed out when temperature was below dew point would then evaporate into the air again (Amos 1995).

Relative humidity within a cool store is therefore a balance between product moisture loss, inputs from warm moist air from ambient air (door opening) or people, evaporation from surrounding structures and frost forming on the evaporator coils, condensation onto surrounding structures, and absorption by packaging or bins.

Amos (1995) found that the packaging was an important sink for water vapour. Absorption of moisture by cardboard buffered the air against rapid fluctuations in moisture content. The cardboard contained more than 100 times more water than was held in the air: small changes in cardboard moisture content had a large effect on relative humidity.

2.3 RESPIRATION

Respiration is the process by which complex materials such as starch, sugars and organic acids are broken down to simpler molecules such as CO_2 and H_2O, with the concurrent production of energy and other molecules which can be used by the cell for synthetic reactions (Burton 1982; Wills et al. 1989). These reactions are essential to maintain biochemical processes, cellular organisation and membrane integrity of living cells.

Respiration can occur in the presence or absence of oxygen; aerobic or anaerobic respiration, respectively (Wills et al. 1989). Most of the energy required by fruits and vegetables is supplied by aerobic respiration (Dadzie 1992). Mass loss from fruits and vegetables comes from the release of CO_2 during respiration. Respiration is a highly temperature dependent process (Dadzie 1992; Cheng et al. 1998). Factors that influence respiration will therefore influence the amount of mass lost through this process.

The relative importance of respiration to total mass loss changes with the humidity of the environment. As humidity increases the rate of water loss decreases so total mass loss decreases and the percentage loss due to respiration increases. For a 'Royal Gala' apple with a permeance of 20 $nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ with a surface area of 0.016 m^2 at 0° C and 90% relative humidity which is a typical storage environment, only up to 5% of the total mass loss is due to respiration. This increases to 30% in a relative humidity of 99% (Banks 1994).

2.4 DISCUSSION

Many diverse factors affect mass loss in apples. Their inter-relationships in determining water loss are summarised diagrammatically in Fig. 2.5. From this review, it is clear that areas of uncertainty that limit ability to predict rate of water loss in a given environment include:

- cultivar differences
- growing conditions of the fruit including; regional, and grower, tree, and position within canopy effects

- physiological maturity of fruit
- effect of time of harvest
- impact of mechanical damage and micro-cracking of cuticle
- influence of time after harvest
- effect of relative humidity
- temperature effects
- effect of external coatings
- composition and structure of cuticle

This thesis reports, integrates and evaluates findings of investigations of the first eight of these.

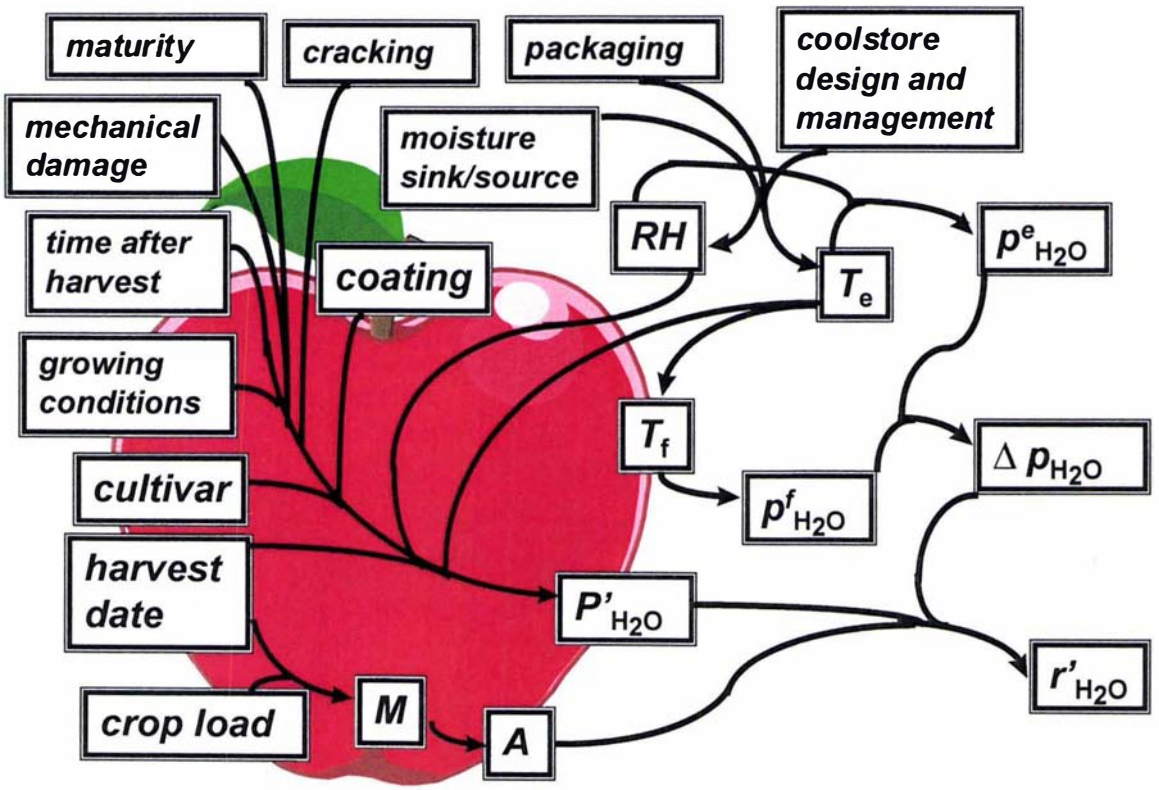

Fig. 2.5. Conceptual model of factors influencing rate of water loss of harvested apples.

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Chapter 3

Harvest Date and Cultivar Effects on Water Vapour Permeance in Apples

Harvest Date and Cultivar Effects of Water Vapour Permeance in Apples

Kate M. Maguire, Nigel H. Banks

Centre for Postharvest and Refrigeration Research, Massey University, Private Bag 11 222, Palmerston North

Alexander Lang

HortResearch, Palmerston North Research Centre, Private Bag 11 030, Palmerston North

Ian L. Gordon

Institute of Molecular Biosciences, Massey University, Private Bag 11 222, Palmerston North

ADDITIONAL INDEX WORDS: *Malus domestica*, water loss, weight loss, cuticle

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Style: This chapter is in the style of Journal of American Society of Horticultural Science.

ABSTRACT. There is large variation in skin water vapour permeance among different horticultural crops, and within populations of fruit of a given cultivar. Such variation masks treatment effects in experimental work which, in turn, makes it difficult to identify the best strategies for controlling water loss. The current work quantified contributions from various sources to total variation in water vapour permeance of 'Braeburn', 'Pacific Rose™', 'Granny Smith' and 'Cripps Pink' apples *(Malus domestica* Borkh). In a study on 'Braeburn' fruit from eight orchards in Central Otago, over 50% of the total variation in permeance was associated with harvest date. This was the result of a large increase in water vapour permeance from 16.6 to 30.2 (SED = 1.24, df = 382) nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ over the 8 week experimental harvest period. Fruit to fruit differences accounted for 22 % of total variation in permeance. Variation associated with experimental error assessed by repeated measurements was less than 1%. Interaction between harvest date and orchard effects explained 7% of the total variation, indicating that fruit from the different orchards responded in differing ways to advancing harvest date. Respiration rate increased from 93.7 to 178.2 (SED = 10.12, df = 382) nmol $\cdot kg^{-1} \cdot s^{-1}$. The mass loss from respiration as a percentage of total mass loss during the measurement period (at 20 °C and approximately 60% *RH*) was 3.04 ± 0.11 across all harvest dates. In a second study of fruit of four apple cultivars, almost 30% of the total variation in water vapour permeance was associated with cultivar differences. The mean water vapour permeance for 'Braeburn', 'Pacific Rose™' , 'Granny Smith' , 'Cripps Pink' fruit was 44, 35, 17 and 20 (SED = 6.1, df = 598) nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ respectively. Over 20% of the total variation was associated with harvest date. This was the result

of a large increase in water vapour permeance from 21 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ at first harvest to 46 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ (SED = 7.5, df = 398) at final harvest, 10 weeks later, on average across all four cultivars. There was large fruit to fruit variation in water vapour permeance accounting for 25% of the total variation in permeance values. An interaction of harvest and orchard effects explained over 20% of the total variation, indicating that permeance of fruit from different cultivars increased by differing amounts with advancing harvest. Water vapour permeance in 'Pacific Rose'™ and 'Braeburn' increased substantially with later harvest but values for 'Granny Smith' and 'Cripps Pink' remained relatively constant. A simple mathematical model was developed to predict mass loss from 'Braeburn' fruit. Based on these findings, it appears that it would be worthwhile to increase the stringency of measures to control mass loss in 'Braeburn' and 'Pacific Rose'™ apples, particularly those harvested late in the season.

3.1 Introduction

Apples lose mass through the processes of transpiration and respiration. Only a five percent loss in mass can cause apples to develop an unattractive shrivelled and wilted appearance and affect texture (Hatfield and Knee, 1988). In a constant environment, the effective permeance of the fruit surface to water vapour under prevailing conditions (P_{H_2O} ; $mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$) can be calculated from rate of water loss (r_{H_2O} ; $mol \cdot s^{-1}$) using the steady state solution of Fick's first law of diffusion (Nobel, 1991):

$$P_{H_2O} = \frac{r_{H_2O}}{\Delta p_{H_2O} A} \qquad (3.1)$$

provided Δp_{H_2O} (the difference in partial pressure of water vapour between the environment and inside the fruit; Pa) and A (the surface area of the fruit; m^2) are known.

P_{H_2O} is a measure of the ease with which water vapour can escape from fruit. In Table 3.1, some published average values for P_{H_2O} of apples have been converted to a common set of units using formulae published by Banks et al. (1995). The measured values of permeance range from 0.6 $nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ for 'Granny Smith' apples (Nobel, 1975) to 145.1 $nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ for cultivar 'Russet' (Lentz and Rooke, 1964), a 240-fold range. Even within cultivars, there was great variation in published values; for 'Golden Delicious' fruit, the average values

ranged from 16.6 nmol · s^{-1} · m^{-2} · Pa^{-1} (Pieniazek, 1943) to 70 nmol · s^{-1} · m^{-2} · Pa^{-1} (Wells, 1962). The current work quantified contributions of respiration to total mass loss of 'Braeburn' fruit and variance in P'_{H_2O} from a number of sources in four cultivars of apples - 'Braeburn', 'Pacific Rose'™, 'Granny Smith' and 'Cripps Pink', to facilitate development of strategies for management of mass loss in storage for the New Zealand apple industry.

3.2 Materials and methods

HARVEST DATE, ORCHARD AND TREE EFFECTS ON WATER VAPOUR PERMEANCE OF 'BRAEBURN' FRUIT.

Six visually unblemished fruit were picked from each of four trees from eight orchards in the Otago region, New Zealand on each of five harvest dates (a total of 960 fruit). Fruit were sampled randomly from trees. The first harvest was 28 March 1995; subsequent harvests were at two week intervals so that the interval between first and last harvests was 8 weeks. P'_{H_2O} was calculated for each fruit using Eq. 3.1. Within 48 h of picking, fruit were placed in an air flow of ≈ 3 m · s^{-1} and rate of mass loss from each fruit was determined twice over a 16 h period using a balance (0.001 g Model PM1206; Mettler Toledo, Switzerland). An average relative humidity was determined by wet and dry bulb temperature readings (thermistor probes CM type, U bead, ± 0.2 °C; Grant Instruments, Cambridge, U.K.). Skin temperature was logged (Squirrel model 1206, Grant Instruments, Cambridge, U.K.) using thermistor probes (FF type, U bead, ± 0.2 °C; Grant Instruments, Cambridge, U.K.) inserted under the skins of several fruit.

Average values for Δp_{H_2O} were calculated using psychrometric relationships (Monteith and Unsworth, 1990a; Monteith and Unsworth, 1990b; Tetens, 1930). Surface area was estimated using an equation developed by Clayton et al. (1995).

Respiration rates were determined by measurement of the change in partial pressure of CO_2 within 1200 mL opaque containers over 60 min at 20 °C. Fruit were equilibrated to 20 °C and 60% relative humidity for 24 h before measurements. Rates of mass loss were corrected for respiration to provide estimates of rates of water loss.

CONTRIBUTION OF RESPIRATION TO TOTAL MASS LOSS OF 'BRAEBURN' FRUIT.

The percentage contribution to total mass loss made by respiration was modelled using an equation developed by Cheng et al. (1998) which relates specific respiration rate (r_{CO_2} ; $mol \cdot kg^{-1} \cdot s^{-1}$) of 'Braeburn' apples to temperature (T; °C):

$$r_{CO_2} = \left(20.8 + \left(2.3\,T\right) + \left(0.131\,T^2\right)\right) \times 10^{-9} \qquad \textbf{(3.2)}$$

Rate of water loss was estimated using:

$$r'_{H_2O} = P'_{H_2O} A \left(p^{sat}_{H_2O}(T) \left(1 - \frac{RH}{100} \right) \right) \qquad \textbf{(3.3)}$$

where $p^{sat}_{H_2O}(T)$ is the saturated water vapour pressure at air temperature (T; °C) and RH is the relative humidity of the air. We assumed the fruit to have a water vapour permeance of 30 $nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$, an initial mass ($M$) of 0.16 kg, a surface area (A) of 0.016 m^2 and a surface temperature the same as air temperature. Total mass loss (r'_{mass} ; $kg \cdot s^{-1}$) was modelled as:

$$\dot{r}_{mass} = \left(0.012\, \dot{r}_{CO_2}\, M\right) + \left(0.018\, \dot{r}_{H_2O}\right) \quad \textbf{(3.4)}$$

assuming a respiratory quotient of unity and taking account of the molecular weights of carbon and water. Respiration as a percentage of total mass loss ($rr\%$) was:

$$rr\% = \frac{\left(0.012\, \dot{r}_{CO_2}\, M\right)}{\dot{r}_{mass}} \times 100 \quad \textbf{(3.5)}$$

HARVEST DATE, CULTIVAR AND TREE EFFECTS ON WATER VAPOUR PERMEANCE OF APPLE FRUIT.

Five visually unblemished fruit were picked from each of ten 'Braeburn', 'Pacific Rose'™, 'Cripps Pink' and 'Granny Smith' trees at Fruit Crops Unit research orchard at Massey University, Palmerston North, New Zealand on each of six harvest dates (a total of 1200 fruit). Variation associated with maturity was minimised by selecting fruit for the study on the basis of uniform background colour at the first harvest (19 March 1996). Subsequent harvests were made at two week intervals so that the interval between first and last harvests was 10 weeks. $\dot{P}_{H_2O}$ was calculated for each fruit using Eq. 3.1. Mass loss was measured within 48 h of picking and was not corrected for respiration.

HARVEST DATE EFFECTS ON WATER VAPOUR PERMEANCE OF APPLE FRUIT.

Five visually unblemished fruit were harvested from of ten 'Braeburn' trees from the Fruit Crops Unit research orchard at Massey University, Palmerston North, New Zealand on each of five harvests (a total of 250 fruit). Variation associated

with maturity was minimised by selecting fruit for the study on the basis of uniform background colour at the first harvest (31 March 1997). Subsequent harvests were made at 2 week intervals; the interval between first and final harvests was 8 weeks. P'_{H_2O} was calculated for each fruit using Eq. 3.1. Mass loss was measured within 48 h of picking. Respiration rates for these fruit were determined as for the previous experiment and total mass loss was corrected for respiration.

Data were analysed using a cross-nested model in the GLM procedure of SAS (1988). Contributions of variance components were calculated from mean squares corrected for model effects.

3.3 Results

HARVEST DATE, ORCHARD AND TREE EFFECTS ON WATER VAPOUR PERMEANCE OF 'BRAEBURN' FRUIT.

Values for P'_{H_2O} from the entire sample were highly variable. There was a five fold difference between the lowest and highest measured water vapour permeance. Fruit to fruit variation explained 22% of the total variance (Fig. 3.1). A further 65% of the variation was associated with harvest date. Across all orchards, permeance increased from an average of 16.6 at first harvest to 30.2 (SED = 1.24, df = 382) $nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ at final harvest 8 weeks later. A further 4% of total variation in permeance was associated with orchard effects. Interaction of harvest and orchard effects accounted for 7% of total variation (Fig. 3.1), indicating that the permeance of fruit

from the different orchards increased by differing amounts with advancing harvest date. P'_{H_2O} of fruit from the different orchards had increased at final harvest to 1.5 to 2.3 fold the values at first harvest (Fig. 3.2). Tree effects explained a further 1% of the total variation (Fig. 3.1). Only the final 1% was associated with measurement error.

CONTRIBUTION OF RESPIRATION TO TOTAL MASS LOSS OF 'BRAEBURN' FRUIT.

Across all orchards, respiration rate at 20 °C increased from 93.7 to 178.2 (SED = 10.12, df = 382) nmol $\cdot kg^{-1} \cdot s^{-1}$ over the 8 week period between initial and final harvests. For these fruit at 20 °C , 60% relative humidity, mass loss due to respiration as a percentage of total mass loss remained approximately constant at 3.04 ± 0.11 across all harvest dates. At 20 °C and 60% relative humidity, the estimated percentage from the model of mass loss from respiration was quite similar to this, at 2.7% (Fig. 3.3). Temperature effects on respiration as a percentage of total mass loss were predicted to be negligible; relative humidity was the main influencing variable (Fig. 3.3).

HARVEST DATE, CULTIVAR AND TREE EFFECTS ON WATER VAPOUR PERMEANCE OF APPLE FRUIT.

Values for P'_{H_2O} from the entire sample were extremely variable, with fruit to fruit variation and measurement error explaining 25% of the total variance (Fig. 3.4). A further 30% of total variation in permeance was associated with cultivar. Mean values of P'_{H_2O} for 'Braeburn' (43.8 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$; SED = 6.1, df = 598),

and 'Pacific Rose'™ (35.4 nmol · s^{-1} · m^{-2} · Pa^{-1}) were nearly twice those of 'Granny Smith' and 'Cripps Pink' (17.3 and 20.5 nmol · s^{-1} · m^{-2} · Pa^{-1}, respectively). A further 21% of the variation was associated with harvest date (Fig. 3.4). Across all cultivars, permeance increased from an average of 21.4 at first harvest to 46.4 (SED = 7.5, df = 398) nmol · s^{-1} · m^{-2} · Pa^{-1} at final harvest 10 weeks later. The final 22% of total variation was due to an interaction of harvest and cultivar effects (Fig. 3.4), indicating that the permeance of fruit of the different cultivars increased by differing amounts with advancing harvest. The P'_{H_2O} of 'Braeburn' increased nearly 4-fold and that of 'Pacific Rose'™ doubled between first and final harvests whilst values for 'Granny Smith' and Cripps Pink' remained fairly constant (Fig. 3.5).

HARVEST DATE EFFECTS ON WATER VAPOUR PERMEANCE OF APPLE FRUIT.

Averaged across fruit from all trees, permeance of 'Braeburn' apples increased almost two fold from an average of 11.5 at first harvest to 29.4 nmol · s^{-1} · m^{-2} · Pa^{-1} (SED = 1.46, df = 98) at final harvest 8 weeks later.

3.4 Discussion

The work on 'Braeburn' fruit described in this paper was completed over three seasons . The increase in water vapour permeance with later harvest date of these fruit was consistent over all years. This phenomenon was also consistent in different regions, orchards and trees. Thus, regardless of origin, it seems that 'Braeburn' fruit harvested late in the season would be more prone to shrivel in storage than early harvested 'Braeburn' fruit.

The first study showed that there was very small error associated with repeated measurements, indicating that the method used was reliable, and that only one set of measurements per fruit would be required to characterise P'_{H_2O} . The error term in analysis of variance of permeance data could be mainly attributed to the natural variation in fruit. Variation between the trees was also small. This indicates that water vapour permeance is a fruit characteristic and the influences of whole tree physiology and genetic variation within a cultivar were minimal. Whilst samples should be drawn to reflect an entire population under study, there appears to be no need to develop sampling strategies to systematically collect information from large numbers of different trees.

'Braeburn' and 'Pacific Rose'™ fruit had higher P'_{H_2O} than 'Granny Smith' and 'Cripp's Pink' fruit, particularly in the case of later harvests. 'Braeburn' and 'Pacific Rose'™ fruit would therefore be more prone to shrivel in storage than 'Granny Smith' and 'Cripp's Pink' fruit, especially if they were picked late in the

harvest season. Contrasting trends with harvest date among cultivars have also been observed in previous work. Pieniazek (1943) recorded that fruit from 'Golden Delicious', 'McIntosh', 'Yellow Transparent' and 'Rhode Island Greening' cultivars all increased in P'_{H_2O} with later harvest whereas permeance of 'Baldwin' fruit remained constant.

Since visibly blemished fruit were excluded from the study, variation in permeance arising from blemishes presumably relates to variation in the physical and or chemical properties of the cuticle. For example, 'Granny Smith' and 'Cripps Pink' fruit are both noted for the development of greasiness in late harvested fruit, which can be related to changes in the wax components on the surface of the fruit (Morice and Shorland, 1973). It may be that the development of this wax prevents the increase in P'_{H_2O} observed in 'Pacific Rose'™ and 'Braeburn' in which the fruit surface of late-harvested fruit remains 'non greasy' to the touch. Further work is needed to establish the cause of the increase in P'_{H_2O} with later harvest.

Fruit to fruit variation in permeance in the first two experiments was large: although fruit from earlier harvest would on average be expected to be less prone to shrivel than their late-harvested counterparts there would still be fruit harvested during this period which would shrivel sooner than others. This explains the variability of levels of shrivel found within commercial packs; the cause for this fruit variation needs to be identified. Presumably this relates to variation in the

physical or chemical properties of the cuticle.

The chemical components of the cuticle which provide the barrier properties are soluble cuticular lipids (Schonherr and Lendzian, 1981; Schonherr and Merida, 1981). Variation in these soluble cuticular lipids and their molecular structure could produce a variation in the measured water vapour permeance of the fruit. Riederer and Schneider (1990) determined that there was no correlation with the water vapour permeance of isolated cuticles from *Citrus* leaves and the chemical composition of the soluble cuticular lipids. Rather, it is thought to be the structure of crystalline soluble cuticular lipids which determine the barrier properties of the cuticle (Riederer and Schneider, 1990). Physical properties such as cuticle thickness (Kamp, 1930) or the presence of micro-cracking (Meyer, 1944) may also influence the water vapour permeance of the fruit.

The simple mathematical model presented here can be used to estimate the percentage mass loss through respiration in commercial storage of New Zealand 'Braeburn' apples (*c.* 0 ^{o}C and 90% relative humidity). Even at such high humidity, respiration was predicted to account for only 7% of total mass loss. Strategies for reducing total mass loss during storage should therefore be directed at reducing their water loss as this is the dominant component.

The model can be used to predict the impact of delays in pre-cooling or the effect of polyliners on total mass loss of a fruit, though its predictions are only very

approximate. Being based on individual fruit rather than packages, it takes no account of the fact that neither cooling nor equilibration of humidity are instantaneous processes. The latter would be affected by water sinks in the cartons such as packaging material buffering the increase in relative humidity from moisture loss of the fruit (Merts 1996).

The model ignores effects of the boundary layer within which there is no turbulent mixing. The thickness of the boundary layer is inversely related to air velocity (Nobel, 1975). Packaging materials (trays and liners) interfere with free air movement and thus influence the thickness of the boundary layer, and hence the true driving force for water loss.

Water vapour permeance of the fruit was fixed at 30 $nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ for each model run at all levels of relative humidity. Smith (1930) found that water vapour permeance declines after harvest to reach a steady level. Several researchers (Fockens and Meffert, 1972; Lentz and Rooke, 1964; Sastry et al., 1978; Smith, 1933) have reported that P'_{H_2O} increases with decreasing Δp_{H_2O}. In addition, we modelled respiration rate of the fruit as being constant during the 8 week period. In reality, duration and magnitude of a climacteric would be affected by the temperature at which the fruit is held (Kidd and West, 1930).

Clearly, accurate definition of response of these processes to all of these factors would require considerable further work. Their inclusion into the model should

enhance the accuracy of its predictions provided that values for all of the input variables were known. Nevertheless, the simple model presented here has enabled some useful first predictions of relative importance of transpiration and respiration to be made.

Assuming that the fruit has a permeance of 30 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ then it would lose about 5 % of its initial mass if instantly pre-cooled and then stored at 0 °C and 90 % *RH* for 8 weeks, then given a shelf life period of 7 days at 20 °C and 60 % *RH* (Fig 3.6). Delaying pre-cooling for one day in an environment at 20 °C and 60 % *RH* would increase the total mass loss to 5.4 %. If this delay was extended to 4 days the total mass loss would then rise to 6.7 % (Fig 3.6). Amounts of mass loss that cause shrivel in individual fruit range from 5-10 % (Banks pers. comm., 1998) so any delay in pre-cooling could result in some fruit being close to developing shrivel. If these fruit had polyliners in the cartons which allowed humidities of 97 % at all temperatures, the mass loss in 8 weeks storage and 7 days shelf life (20 °C and 60 %) with no delay in pre-cooling would be 3.9 %. However, use of polyliners can substantially reduce the efficiency of pre-cooling, slowing the rate of fruit cooling (Merts 1996). Under such conditions, potential to reduce weight loss is considerably reduced and apples such as 'Cox's Orange Pippin' and 'Braeburn' can soften rapidly (Mitchell, 1992).

Although strategies for management of mass loss in storage can be developed on the information reported in this work there is still a large amount of fruit to fruit

variation which remains unexplained. Further work quantifying the influence of soluble cuticular lipids, and physical properties of the cuticle such as thickness and presence of micro-cracking, on water vapour permeance may help to define ways in which the production environment could be managed to reduce susceptibility to mass loss after harvest.

In conclusion, this study has provided information to suggest that 'Pacific Rose'™ and 'Braeburn' apples, particularly those harvested late in the picking season, should be highly prone to shrivel because of their high permeance to water vapour. These fruit should be passed through the marketing chain quickly, with optimal temperature and humidity control, to minimise the chances of shrivel developing in the fruit.

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Table 3.1. Average estimates of water vapour permeance for different cultivars of apples calculated from published data

Cultivar	Permeance ($nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$)	Source
'Baldwin'	15	Pieniazek 1943
'Bankcroft'	34	Lentz and Rooke1964
'Cortland Apples'	17	Lentz and Rooke 1964
'Cox's Orange Pippin'	43	Smith 1933
	31	Johnson 1976
'Golden Delicious'	47	Kessler and Stoll 1953
	37	Pieniazek 1942a
	17	Pieniazek 1943
	34	Smock and Neuburt 1950
	70	Wells 1962
'Granny Smith'	1	Nobel 1975
'Gravenstein'	2	Nobel 1975
'Grimmes Golden'	63	Wells 1962
'Jonathan'	27	Kessler and Stoll 1953
	1	Nobel 1975
	32	Wells 1962
'McIntosh'	17	Lentz and Rooke 1964

	21	Pieniazek 1942a
	12	Pieniazek 1943
'Northern Spy'	18	Lentz and Rooke 1964
'Red Delicious'	36	Lentz and Rooke 1964
	9	Chau et al. 1988
'Rhode Island Greening'	33	Lentz and Rooke 1964
	16	Pieniazek 1942a; Pieniazek 1942b
'Russet'	145	Lentz and Rooke 1964
'Sandow'	33	Lentz and Rooke 1964
'Yellow Transparent'	22	Pieniazek 1943

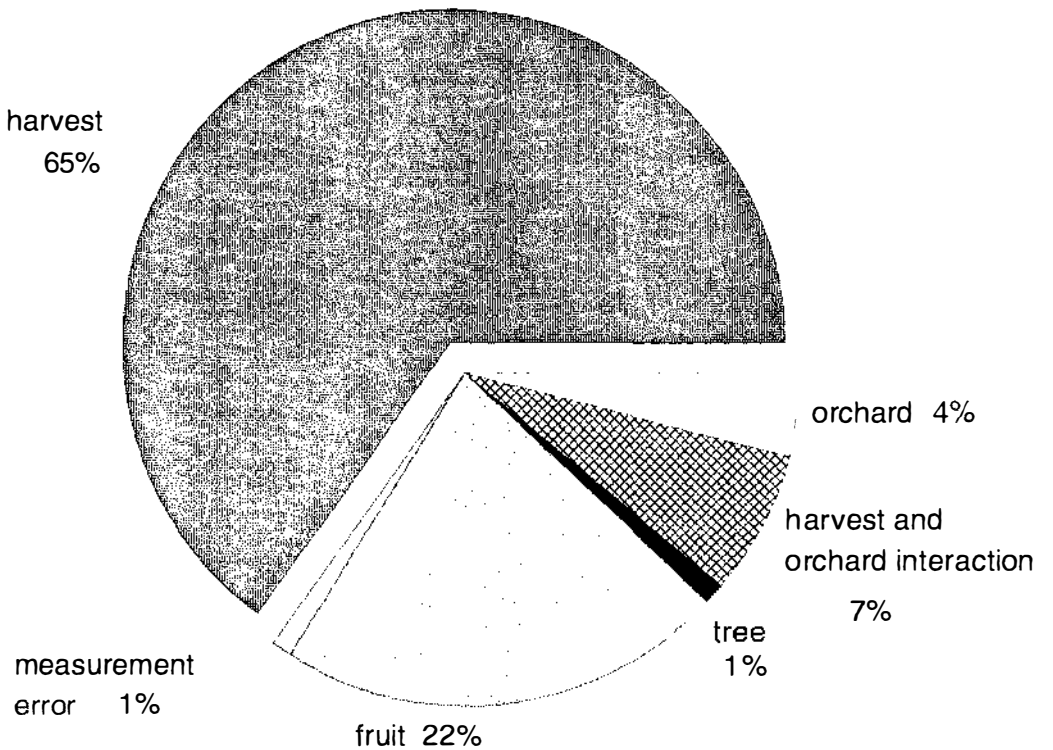

Fig. 3.1. Percentage of total variation in fruit water vapour permeance of 'Braeburn' apples sampled from eight orchards on five harvest dates over a 8 week period.

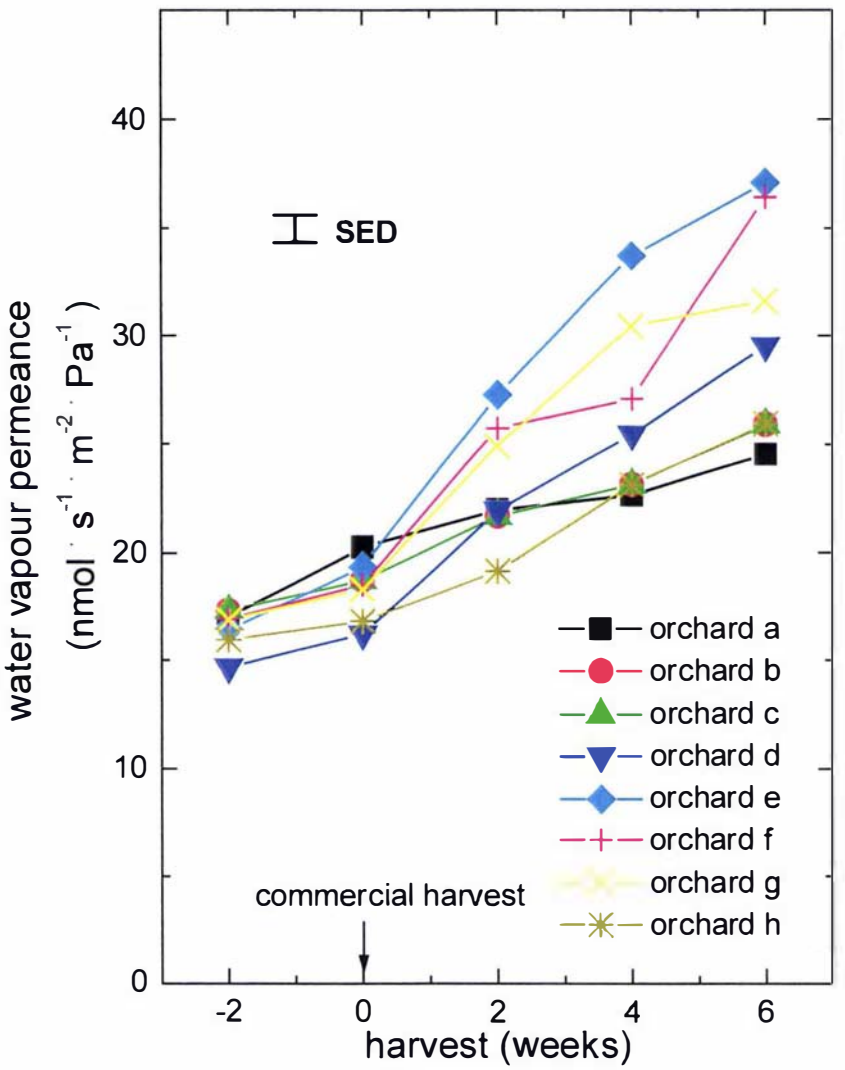

Fig. 3.2. Water vapour permeance of fruit from eight orchards harvested at different times relative to start of the commercial harvest season (0 weeks; SED = 1.32, df = 46).

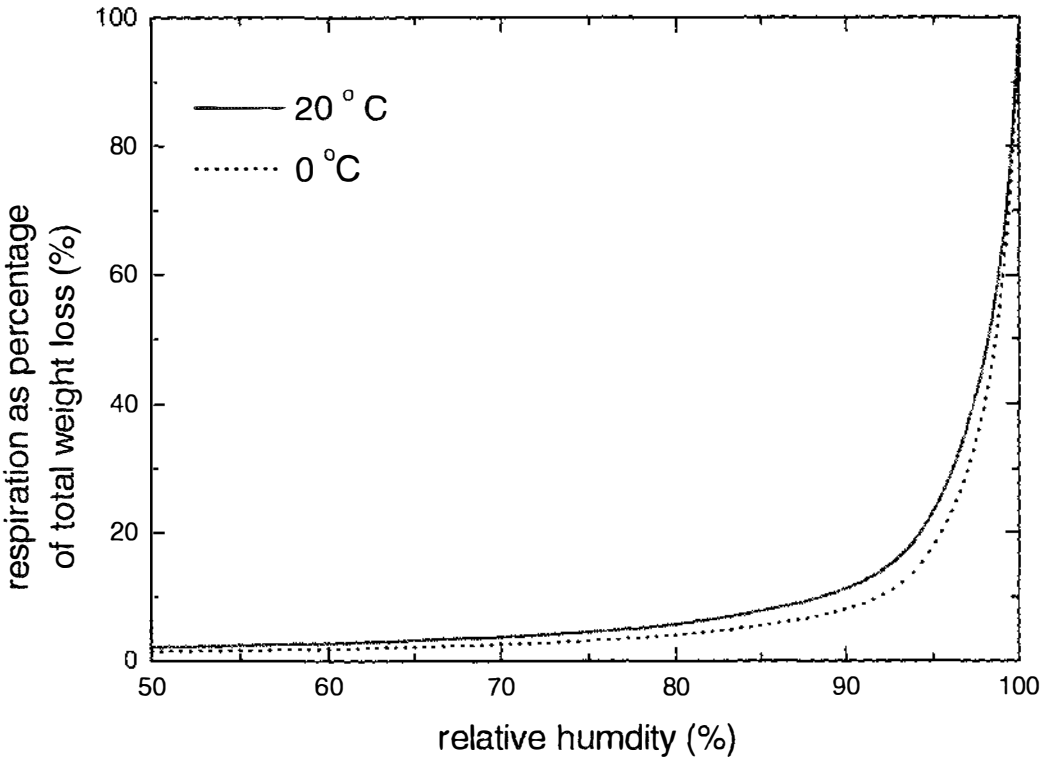

Fig. 3.3. Predicted relationship between respiration as percentage of total mass loss and relative humidity for 'Braeburn' apples using the model described in Eqs. 3.1-3.5.

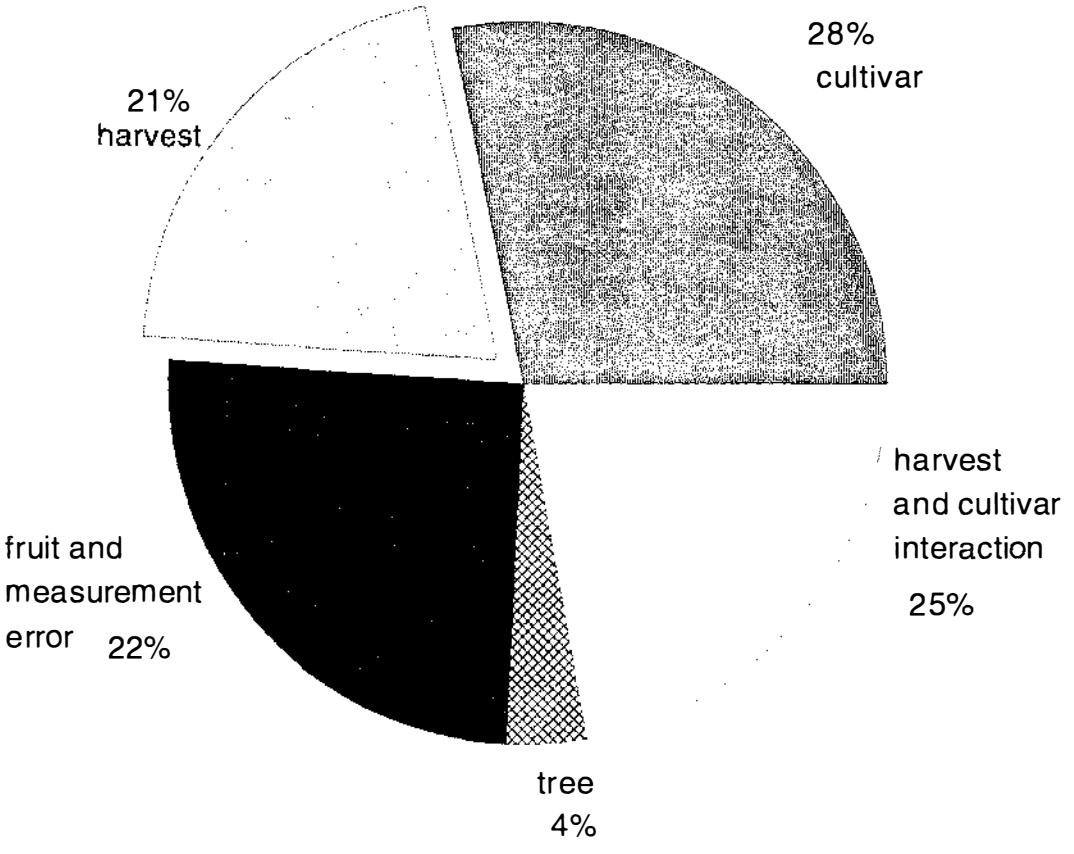

Fig. 3.4. Percentage of total variation in fruit water vapour permeance of four cultivars of apples ('Braeburn', 'Pacific Rose', 'Granny Smith', and 'Cripps Pink') sampled on 6 harvest dates over a 10 week period.

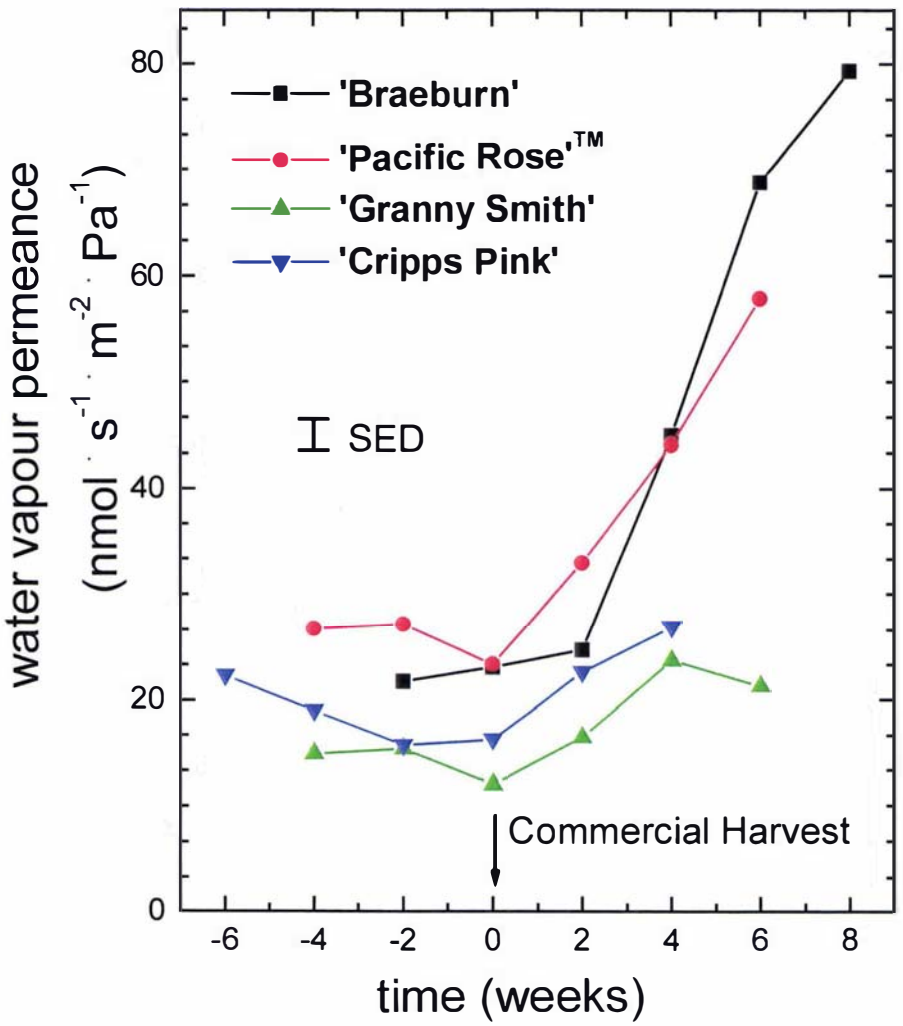

Fig. 3.5. Water vapour permeance of fruit from 'Braeburn', 'Pacific Rose'™, 'Cripps Pink' and 'Granny Smith' trees harvested at different times relative to start of the commercial harvest season for each cultivar (0 weeks; SED = 2.88, df = 98).

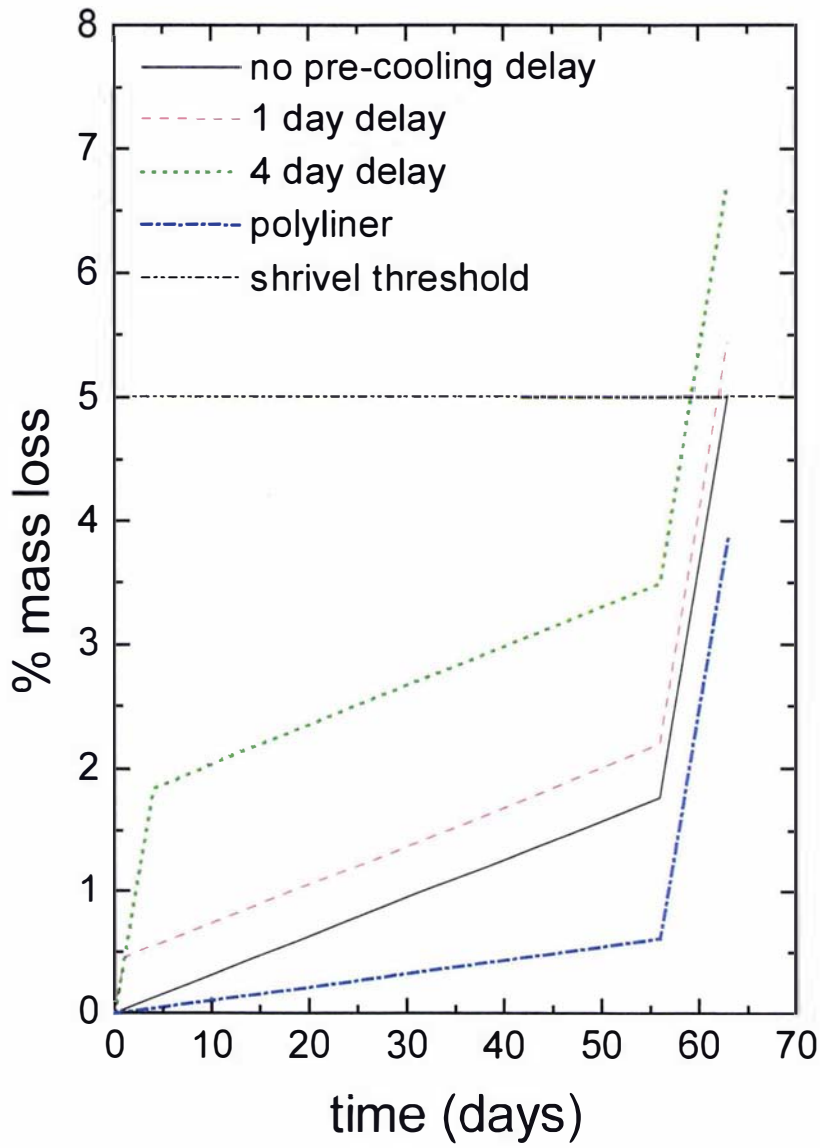

Fig. 3.6. Predicted mass loss from a 'Braeburn' fruit in several different postharvest scenarios using model the model described in Eqs. 3.1-3.5

Chapter 4

Sources of Variation in Water Vapour Permeance of Apple

Sources of variation in water vapour permeance of apple

Kate M. Maguire[a], Nigel H. Banks[a], Alexander Lang[b]

[a]*Centre for Postharvest and Refrigeration Research, Massey University, Private Bag 11 222, Palmerston North*

[b]*HortResearch, Palmerston North Research Centre, Private Bag 11 030, Palmerston North*

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Abstract

There can be large fruit to fruit variation in water vapour permeance within a population of fruit. The current work quantified the following contributions to variation in apple fruit (*Malus domestica* Bork.) water vapour permeance: regional and grower effects in 'Braeburn', maturity and position effects within the canopy in 'Granny Smith' and 'Pacific Rose'™ , the effect of fruit to fruit contact areas in 'Braeburn', 'Fuji', 'Pacific Rose'™ and 'Red Delicious', the difference between sun and shade sides of fruit in 'Royal Gala', and variation over the surface of individual 'Braeburn' fruit. In a study on 'Braeburn' fruit from six growers in each of five apple growing regions of New Zealand, there were highly significant grower differences and marginal regional differences. Mean values for water vapour permeance of 'Braeburn' fruit from Otago, Hastings and Wairarapa, Waikato and Nelson were 17.2, 22.2, 23.0, 24.3 and 24.8, respectively (SED = 4.32, df = 478) $nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$. The water vapour permeance of the fruit was extremely variable within each grower line (48% of total variation in population of fruit). In a second study on 'Granny Smith' and 'Pacific Rose'™, there were no relationships between four fruit maturity indicators (starch, background colour, firmness and soluble solids) and water vapour permeance, though fruit from the inner canopy had mean water vapour permeance values 75 and 40% greater than those from the outer canopy. Fruit of 'Braeburn' and 'Pacific Rose'™ with large numbers of adjacent fruit (3 and 4 contact points) had 57 and 51 % higher water vapour permeance, respectively, than those with less contact points. Numbers of

contacts did not influence water vapour permeance of 'Red Delicious' or 'Fuji' fruit. The areas of contacts in 'Red Delicious' had a mean water vapour permeance 30% higher than other areas. There was no significant difference between sun and shade sides of water vapour permeance of 'Royal Gala' fruit. Neither was cuticle thickness related to fruit water vapour permeance. There was no pattern of water vapour permeance of 'Braeburn' fruit with respect to blush or vertical displacement (shoulder or cheek). However, there were large variations among fruit within a population. Whilst some of this variation has been shown to be associated with position within the canopy in which fruit developed and numbers of adjacent fruit, a large proportion of this variation seems to be non-systematic, random error. Further work is required to determine differences in physical and chemical composition of the cuticle that lead to this variation in water vapour permeance.

Keywords: mass loss; cuticle; water loss; *Malus domestica*

4.1 Introduction

Apples lose mass through the processes of transpiration and respiration. Only a five percent loss in mass can cause apples to develop an unattractive shrivelled and wilted appearance and affect texture (Hatfield and Knee 1988). Water vapour permeance (P'_{H_2O} ; $mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$) is a measure of the ease with which water vapour can escape from fruit. For apples with typical values for P'_{H_2O} and respiration rate, kept in typical postharvest conditions for the New Zealand apple industry (0 °C and 85% relative humidity), about 93% of the total mass loss would be water loss (Maguire et al. 1998).

In a constant environment, the effective permeance of the fruit surface to water vapour under prevailing conditions can be calculated from rate of water loss (r'_{H_2O} ; $mol \cdot s^{-1}$) using the steady state solution of Fick's first law of diffusion (Nobel 1991):

$$P'_{H_2O} = \frac{r'_{H_2O}}{\Delta p_{H_2O} A} \qquad \textbf{(4.1)}$$

provided Δp_{H_2O} (the difference in partial pressure of water vapour between the environment and inside the fruit; Pa) and A (the surface area of the fruit; m^2) are known.

In a previous study, Maguire et al. (1998) found a five-fold variation in water vapour permeance for 'Braeburn' fruit from one region in New Zealand. When harvest, orchard and tree effects were accounted for, 20% of the total variation was attributable to fruit to fruit differences. Pieniazek (1944) determined

that lenticular transpiration accounted for 8-20% of total transpiration in 'Golden Delicious', 'Baldwin', 'Turley' and 'Red Canada'. This represents an important route for water loss in some varieties. However, 5 to 10 times more water is typically lost directly through the cuticle so that the number and appearance of the lenticels alone is probably a poor indicator of permeance for a cultivar (Pieniazek 1944). Fruit to fruit variation must therefore be due to differences in the fruit cuticle, the main barrier to water loss.

The large fruit to fruit variation found in previous work helps explain the variability of severity of shrivel found within commercial packs. It is believed within the industry that fruit from some regions are more prone to severe shrivel and there are also reported to be differences between growers within the regions. Based upon a storage trial, Jackson et al. (1971) reported that fruit from the outer and upper zones of 'Cox's Orange Pippin' trees were less likely than those from the inner zone to become shrivelled. In addition, some observations indicate that the green side of fruit shrivel first (Banks pers. comm.) indicating that there might be variation in the water vapour permeance around the fruit.

Pieniazek (1943) and Maguire et al. (1998) found that water vapour permeance of some varieties of apples increased markedly as harvest date was delayed within the commercial harvest period. This effect could potentially be due to advance of maturity in these fruit, though Pieniazek (1943) reported that maturity influences on the water vapour permeance of 'Yellow Transparent', 'Duchess' and 'Wealthy' were small. There appears to be little knowledge as to whether maturity *per se* is an important source of variation in fruit water vapour

permeance.

This work investigated sources of variation in water vapour permeance of apples such as regional, grower and position on tree effects, maturity, contact with other fruit while growing, variation in water vapour permeance around the fruit surface of individual fruit and differences associated with sun/shade sides of the fruit and cuticle thickness.

4.2 Method and Materials

Regional and grower differences

Forty visibly unblemished, commercially packed 'Braeburn' fruit were selected from each of 6 growers from the following New Zealand regions; Hawkes Bay, Wairarapa, Waikato, Nelson and Otago. All fruit were harvested in the 3rd week of commercial harvest for each region.

Within 48 h of picking, fruit were placed in an air flow of $\approx 3\ m \cdot s^{-1}$ and the rate of mass loss from each fruit was determined over a 16 h period using a balance (0.001 g Model PM1206; Mettler Toledo, Switzerland). An average relative humidity was determined by wet and dry bulb temperature readings (thermistor probes CM type, U bead, $\pm$ 0.2 ^{o}C; Grant Instruments, Cambridge, U.K.). Skin temperature was logged during the period of measurement by recording output (Squirrel model 1206, Grant Instruments, Cambridge, U.K.) of thermistor probes (FF type, U bead, $\pm$ 0.2 ^{o}C; Grant Instruments, Cambridge, U.K.) inserted under the

skin of several sample fruit. Averages were used to calculate Δp_{H_2O} using psychrometric relationships (Monteith and Unsworth 1990a, Monteith and Unsworth 1990b, Tetens 1930). Surface area was calculated using an equation from Clayton et al. (1995). P'_{H_2O} was calculated for each fruit using Eq. 4.1 without correcting rates of mass loss for respiration.

Data were analysed using a nested model in the GLM procedure of SAS (1988). Contributions of variance components were calculated from mean squares corrected for model effects.

Maturity and position within canopy

Twenty-five fruit were picked from one tree each of 'Granny Smith' (27 May 96) , and 'Pacific Rose'™ (22 May 96; Fruit Crops Unit research orchard Massey University, Palmerston North, New Zealand) from each of 4 sections of the canopy based on aspect and position: outer south east, inner south east, outer north west, and inner north west. Water vapour permeance determinations were made as described above.

Four maturity attributes were measured for each fruit. Hue angles of background colour of 'Granny Smith' apples were recorded using chromameter (Model CR-100, Minolta Camera Ltd.; Osaka, Japan). Firmness was measured twice per fruit with 'Kiwifirm' (a non-destructive firmness measuring device, Industrial Research Ltd.; New Zealand) on blush and shade sides. Fruit were cut through the equator and placed face down in iodine solution (0.2% (w/w) elemental iodine dissolved in 0.9% (w/w) aqueous potassium iodide) for 1 min

then evaluated using the Generic Starch-Iodine Index Chart for Apples (1 = 100% starch stained, 8 = 0% starch stained; Cornell University, 1993). Total soluble solids were estimated by refractometer (Atago N-20; Japan) on samples of juice collected when fruit were cut.

Data were analysed using a fixed effects, two factor (aspect and position) factorial model in the GLM procedure of SAS (1988). Simple and multiple regression were used from the regression procedure of SAS (1988) to investigate the relationships between maturity indicators and water vapour permeance.

Fruit to fruit contact areas

Ten fruit were picked for each of the following numbers of fruit-to-fruit contact points: 0, 1, 2, 3 or 4. Fruit were either all from the same spur or from adjacent spurs. The experiment was repeated for one tree of each of four cultivars 'Braeburn' (5 May 97), 'Fuji' (18 April 97), 'Pacific Rose'™ (22 May 97) and 'Red Delicious' (24 March 97; Fruit Crops Unit research orchard Massey University, Palmerston North, New Zealand). Water vapour permeance was determined as described above. Data were analysed using a completely random model in the GLM procedure of SAS (1988).

Areas of fruit to fruit contact were recorded for each of seven 'Red Delicious' fruit picked 29 March 97. The permeance of a small area (200 mm^2) on the area of contact was determined 3 times using a null balance porometer (model 1600 Li-Cor; Lincoln, Nebraska, USA; Fig. 4.1 a); this measurement was repeated on adjacent areas that had not been in contact with other fruit (6 measurements per

fruit). Data were analysed using a completely random model in the GLM procedure of SAS (1988).

Sun versus shade

Thirty 'Royal Gala' apples were harvested (6 March 97) from the Fruit Crops Unit research orchard, Massey University, Palmerston North, New Zealand. Fruit were equilibrated to 20 °C , 60% relative humidity for 8 h. Water vapour permeance was estimated as described above. Fruit were carefully dipped to the mid line in a paraffin-based wax (Mobil Wax 2305™) so as to coat just the sun or shade side of the fruit (as determined by intensity of blush). Ten fruit were assigned to each treatment with ten fruit remaining unwaxed as controls. Fruit were allowed to equilibrate for 4 h then water vapour permeance was remeasured. Surface area was corrected for waxed fruit. The data were analysed using a completely random model in the GLM procedure of SAS (1988).

Cuticle Thickness

Partial cores were cut from the cheek of apples used in the sun versus shade experiment (sun or shade sides) with a sharp cork borer (6 mm diameter). Hand sections (≈ 5 μm thick) were then cut with a sharp blade to give cross sections of the skin, which were mounted on microscope slides in 80% glycerol and viewed with a light microscope (Microlux 11; Kyowa, Japan) connected via a video camera to a computer with Video Trace (Leading Edge Pty. Ltd.; Australia), an image measurement system software package. Cuticle thickness was measured 30 times for each fruit from several sections. A regression of water vapour

permeance on cuticle thickness was performed using the regression procedure of SAS (1988).

Variation of permeance over the surface of 'Braeburn' apples.

Five 'Braeburn' apples with particularly high water vapour permeance (19.8 ± 2.78 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$) and five with particularly low water vapour permeance (10.3 ± 0.42 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$) were selected from a mid-season harvest at the Fruit Crops Unit research orchard, Massey University, Palmerston North, New Zealand. The water vapour permeance of a 200 mm^2 area on the fruit was determined using a null balance porometer (model 1600 Li-Cor; Lincoln, Nebraska, USA; Fig. 4.1 a) at 12 positions on each fruit surface (3 longitudinal and 4 latitudinal displacements in factorial combination; Fig. 4.1 b). The sequence of readings was random. Data were analysed using a random effects, blocked, 2 factor (radial and vertical displacement), factorial model in the GLM procedure of SAS (1988). The initial water vapour permeance grouping was used as the blocking factor.

4.3 Results

Regional and grower differences

There were marginally significant regional differences in mean water vapour permeance of 'Braeburn' fruit ($P < 0.1$). The mean water vapour permeance for fruit from Otago was 17.2 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$, Hastings and Wairarapa were 22.2 and 23.0 and Waikato and Nelson fruit had the greatest water

vapour permeances at 24.3 and 24.8 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ (SED = 4.32, df = 478), respectively. Grower differences were highly significant ($P < 0.0001$), with some growers producing large numbers of fruit with high water vapour permeance (Fig. 4.2). Regional effects explained 11% of the total variation, and grower effects 41%. The remainder of the total variation (48%) was due to unexplained fruit variability and experimental error.

Maturity effects on water vapour permeance

Fruit from the inner canopy of 'Granny Smith' and 'Pacific Rose'™ had mean water vapour permeances about 75% and 40%, respectively, greater than those from the outer canopy ($P < 0.0001$). However, both simple and multiple regressions of water vapour permeance upon the measured maturity indicators explained no more than 20% of the variation in water vapour permeance and were not pursued further.

Fruit to fruit contact areas

Number of contact areas had a significant influence on the whole fruit water vapour permeance of both 'Braeburn' and 'Pacific Rose'™ ($P < 0.05$; R^2 = 39 and 84 % respectively; Fig. 4.3). In 'Pacific Rose'™ this equated to nearly a 50% greater mean water vapour permeance for fruit with four contacts compared to those with only 0 or 1 (Fig. 4.3). No such relationships were found for 'Red Delicious' and 'Fuji'.

The contact areas of 'Red Delicious' fruit had about a 30% higher water vapour permeance, as determined by the porometer, than the other areas of the

fruit ($P < 0.0003$; 66 and 50 $nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ respectively; SED = 3.84, df = 40).

Sun versus shade and cuticle thickness

There were no significant differences between sun or shade sides in terms of water vapour permeance or cuticle thickness of 'Royal Gala' fruit. Neither was there any relationship between cuticle thickness and water vapour permeance of the whole fruit.

Variation of permeance over the surface of 'Braeburn' apples.

Fruit selected for high initial fruit permeance had an average permeance from the twelve readings of 39 $nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ which was very highly significantly different ($P < 0.0001$) from those fruit with a low initial whole fruit permeance (16 $nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$; SED = 4.04, df = 118). Upon initial examination, the twelve readings around the surface of the fruit appeared to be dependent on the radial position with respect to the blush ($P < 0.05$). However, on further investigation, this was found to be related to the data for one fruit which had a mean permeance on the blush side nearly 40% greater than the other areas of the fruit. Upon removal of data from this fruit, the readings had no pattern with respect to the blush of the fruit; neither was there a significant response to the vertical displacement of the reading (shoulder, cheek and bottom).

4.4 Discussion

The substantial variation in the water vapour permeance of 'Braeburn' fruit among grower lines both within and among regions suggests that fruit from some grower lines in all of the major apple grower regions of the country except Otago would have greater problems than others with shrivel development. This is consistent with variation in shrivel development found from fruit in commercial storage (King pers. comm.). These differences presumably relate to the effects of genetics and the growing environment, including rootstocks, soils, and microclimates. There was extreme variation in some regions e.g. in the Wairarapa 34 out of 40 fruit sampled from one grower had permeances higher than the upper quartile whilst none of the fruit from another grower in the same region had permeances of this level. As with previous work (Maguire et al. 1998), the variation between individual fruit was large, indicating that very localised variations in the developmental history of individual fruit are important in overall levels of variation.

There was no consistent relationship between fruit maturity and the water vapour permeance for the 'Pacific Rose' ™ and 'Granny Smith' fruit that we studied, indicating that there was no strong physiological link between development of the fruit tissue and permeance. Position on the tree did influence water vapour permeance of 'Pacific Rose'™ and 'Granny Smith' , with fruit from the more exposed areas of the canopy (outer areas) having a lower water vapour permeance than those from the inner areas of trees. This difference was not related

to maturity but presumably could be linked to many environmental factors which differ between these zones, such as light intensity, relative humidity and temperature.

For 'Pacific Rose' ™ and 'Braeburn' fruit, numbers of fruit to fruit contact points during growth did influence the water vapour permeance of fruit: areas of contact had higher water vapour permeance than non-contact areas. This indicated that fruit from unevenly thinned 'Pacific Rose'™ and 'Braeburn' trees would be more prone to shrivel, and has implications for thinning of young fruit. It would be important to minimise numbers of possible contacts, presumably with both other fruit and with branches. On the other hand, the potential contribution to whole fruit permeance arising from the elevated permeance of the small area of the contact site itself was quite small in 'Red Delicious'. This would not be sufficient to explain the very substantial variation in water vapour permeance of individual fruit which can vary 2 to 5 fold within a given population, particularly when the limited area of these contact points is taken into account. At this point, it is not clear if the elevated permeance in fruit with multiple contact points was linked to a direct contribution of the contact areas themselves or if there was some other physiological basis for whole fruit variation in permeance resulting from fruit developing in clusters.

'Braeburn' fruit which had high water vapour permeance had a uniformly high permeance over a large proportion of the fruit surface. This indicates that individual fruit with a high water vapour permeance do not have areas of extremely high permeance against a background of generally low water vapour

permeance. There was no trend in water vapour permeance around the surface of the fruit i.e. blush versus the green sides of the fruit. This result is supported by the finding that there was no differential in water vapour permeance between sun and shade sides of 'Royal Gala' fruit. Thus, variability in permeance seems to occur at the level of the whole fruit, with the entire surface of some fruit being higher in water vapour permeance than others. This difference, whether it results from environmental or internal influences linked to number of adjacent fruit or position in the canopy, presumably relates to variation in physical structure or chemical composition of the cuticle.

There was no correlation between cuticle thickness and whole fruit water vapour permeance for 'Royal Gala' fruit. This is consistent with findings from a number of previous studies (Kamp 1930; Pieniazek 1944; Schonherr 1976; Smith 1933) which could establish no link between cuticular barrier properties to water and cuticle thickness. Meyer (1944) suggested that the lack of apparent relationships between cuticular thickness and transpiration rates might be due to the presence of cracks and breaks in the cuticle which may have overriding influences and obscure any effect of the cuticle thickness. In addition, variation in cuticular permeance could derive from differences in the soluble cuticular lipids that are thought to provide the barrier properties of the cuticle (Riederer and Schneider 1990). It seems to be the crystalline structure rather than chemical composition of the soluble cuticular lipids which determines the barrier properties of isolated cuticles from *Citrus* leaves (Riederer and Schneider 1990). A combination of the barrier properties of the soluble cuticular lipids, coupled with the extent to which they are

breached by cracks, seems likely to account for variation in permeance observed in the current study.

Overall, we have shown that there are large variations in the proportions of fruit with very high permeance between grower lines that may to some extent be influenced by the region in which the fruit are grown. There was significant variation in permeance associated with position within the canopy in which fruit developed and numbers of adjacent fruit. However, only minor amounts of this variation could be attributable to the higher permeance of contact points in the fruit surface and negligible amounts derived from amounts of blush or fruit maturity or cuticular thickness. The potential to explain this variation through physical structure and chemical composition of the cuticle should be explored.

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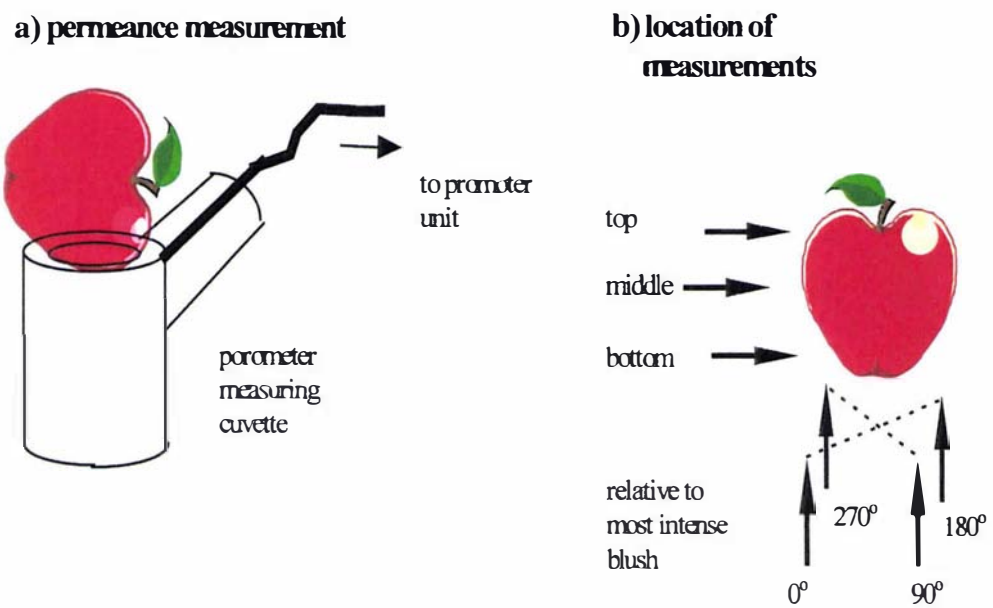

Fig. 4.1. Methods used to characterise variation in P'_{H_2O} around the surface of 'Braeburn' apples. Permeance was determined on limited areas with (a) a null balance porometer (b) on 12 positions on the surface of each fruit.

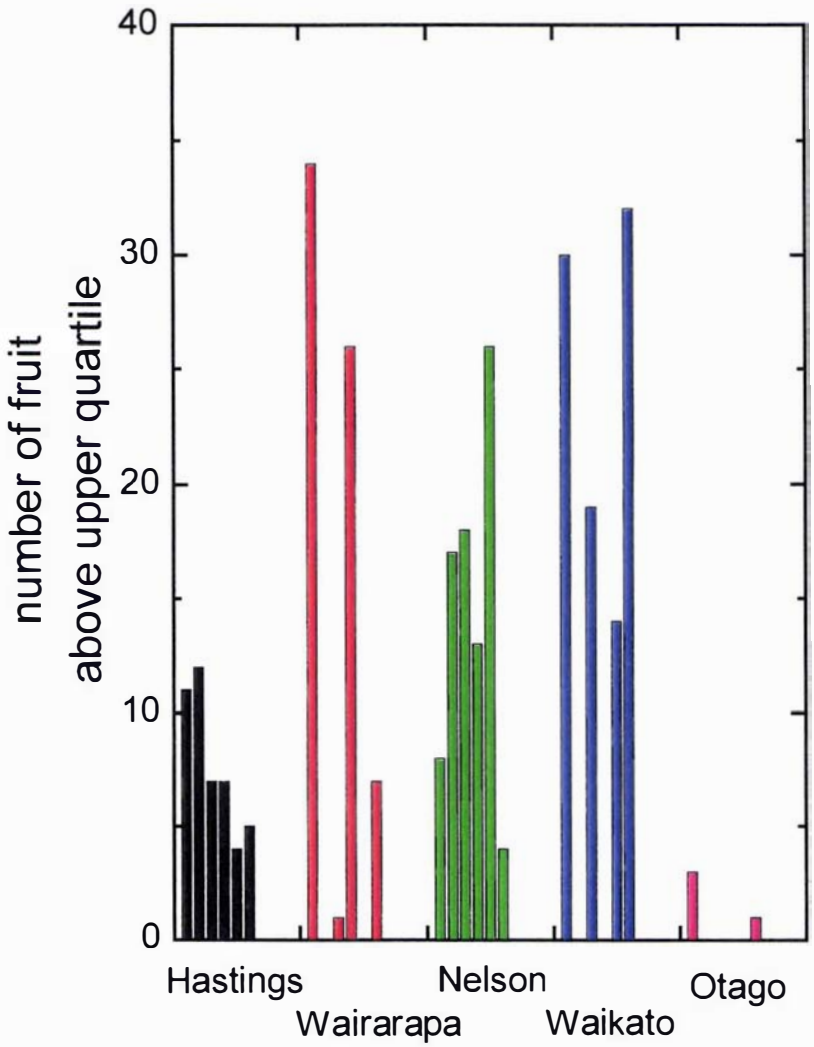

Fig. 4.3. Numbers of fruit out of 40 sampled from each of 6 grower lines within the 5 fruit growing regions in New Zealand with water vapour permeance above the upper quartile value for the whole experiment (26 nmol $\cdot$ s^{-1} $\cdot$ m^{-2} $\cdot$ Pa^{-1}).

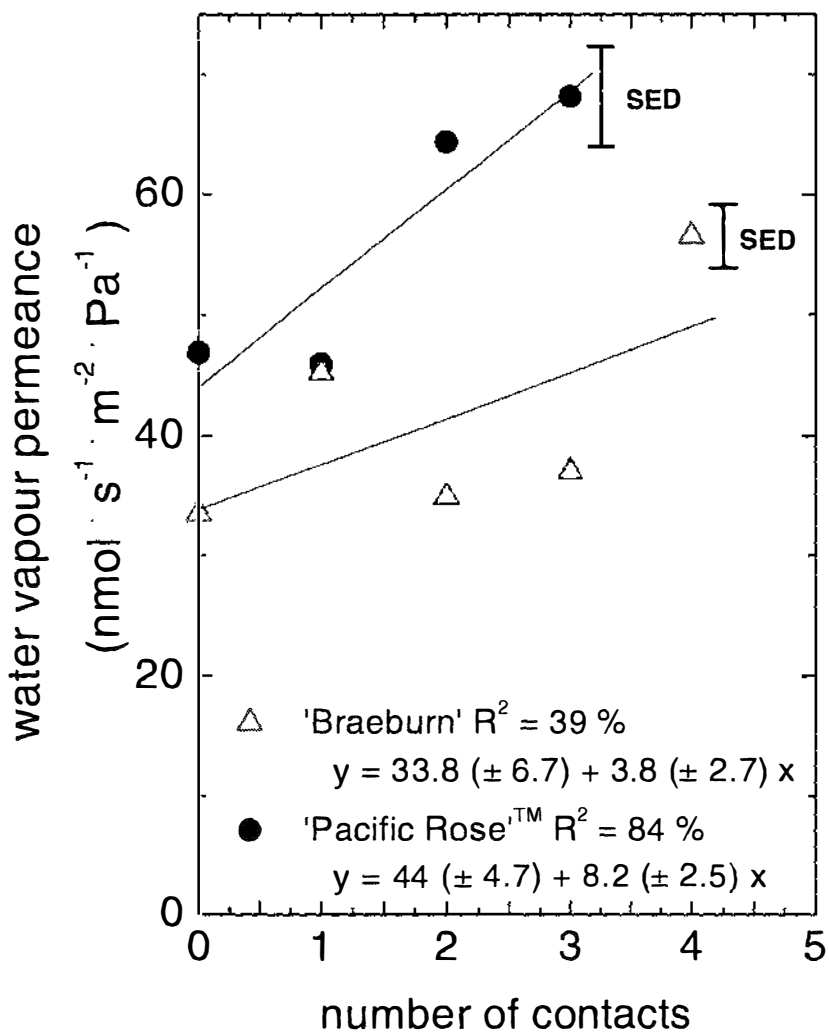

Fig. 4.3. Mean water vapour permeance related to points of contact with other fruit at harvest for 'Braeburn' (SED = 5.3, df = 18) and 'Pacific Rose' ™ (SED = 8.4 nmol · s^{-1} · m^{-2} · Pa^{-1}, df = 18) fruit.

Chapter 5

Relationship between Water Vapour Permeance of Apples and Micro-Cracking of the Cuticle

Relationship between water vapour permeance of apples and microcracking of the cuticle.

Kate M. Maguire[a], Alexander Lang[b], Nigel H. Banks[a], Alastair Hall[b], Doug Hopcroft[c], Raymond Bennett[c]

[a]*Centre for Postharvest and Refrigeration Research, Massey University, Private Bag 11 222, Palmerston North*

[b]*HortResearch, Palmerston North Research Centre, Private Bag 11 030, Palmerston North*

[c]*Keith Williams Microscope Unit, HortResearch, Palmerston North Research Centre, Private Bag 11 030, Palmerston North*

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Abstract

Water vapour permeance is important in determining the rate of water loss from fruits. In this study, the potential to explain variation of water vapour permeance by variation in area of cuticular micro-cracking was investigated using scanning electron microscopy and confocal microscopy. While there was considerable variation in cracking around the surface of the fruit as determined by data obtained with the confocal microscope (coefficient of variation = 44%) there was no obvious pattern in relation to blush. A model based on diffusion of gases was developed and used to explain scanning electron microscope data (R^2 = 51%). This model included terms for intact cuticle, cuticle which was cracked in the outer layer, and boundary layer effects. The model was based upon the effective permeability of air to water vapour (analogous to diffusivity) the depth of the boundary layer (0.5 mm), the depth of the crack in the outer cuticle (8 μm) and the depth of the remaining inner cuticle (8 μm). The model predicted that the permeability of the inner cuticle to water vapour was 1.3 ± 0.75 pmol $\cdot$ m $\cdot$ s^{-1} $\cdot$ m^{-2} $\cdot$ Pa^{-1} and the water vapour permeance of intact cuticle was 11.5 ± 5.4 nmol $\cdot$ s^{-1} $\cdot$ m^{-2} $\cdot$ Pa^{-1}. Cracks were calculated to be 130 times more permeable than intact cuticle. The proportion of cracking is a very important determining factor in the fruit water vapour permeance. This study has provided a model which quantifies the contribution cracks make to variation in the permeance of whole apples.

Keywords: *Malus domestica,* water loss, weight loss

5.1 Introduction

Water vapour permeance (P'_{H_2O}; $mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$) is a measure of the ease with which water vapour can escape from fruit. In a constant environment, effective P'_{H_2O} under prevailing conditions can be calculated from rate of water loss (r'_{H_2O}; $mol \cdot s^{-1}$) using the steady state solution of Fick's first law of diffusion (Nobel, 1991):

$$P'_{H_2O} = \frac{r'_{H_2O}}{\Delta p_{H_2O} A} \qquad \textbf{(5.1)}$$

provided Δp_{H_2O} (the difference in partial pressure of water vapour between the environment and inside the fruit; Pa) and A (the surface area of the fruit; m^2) are known.

Permeability of a material to gas j, (P_j; $mol \cdot m \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$) is related to permeance of the barrier made of that material by:

$$P_j = P'_j \Delta x \qquad \textbf{(5.2)}$$

where Δx is the thickness of the barrier (m; Banks et al., 1995).

In a previous study, Maguire, Banks et al. (1998) found a five-fold variation in values of water vapour permeance for individual, blemish free 'Braeburn' fruit (*Malus domestica* Borkh.) from one region in New Zealand. Twenty percent of the total variation was attributed to fruit to fruit differences. The

large fruit to fruit variation in permeance to water would contribute to variability in water loss between fruit within a population (Eq. 5.1). In turn, this could explain the variability within commercial packs of 'Braeburn' of severity of shrivel , a disorder which results from weight loss (Hatfield and Knee, 1988) which mostly comprises water loss (Maguire, Banks et al., 1998).

Structure, rather than chemical composition *per se*, of the soluble cuticular lipids (soluble cuticular lipids) has been shown to determine the barrier properties of isolated cuticles from *Citrus* leaves (Riederer and Schneider, 1990). Variation in soluble cuticular lipids and their molecular structure could presumably be responsible for variation in the measured water vapour permeance of apples. Physical properties such as cuticle thickness or the presence of micro-cracking may also influence the water vapour permeance of fruits. Meyer (1944) and Faust and Shear (1972) have studied skin of 'Golden Delicious' apples and found cracks appearing on the surface early in the growing season and by the end of the season the cracks were larger and had formed networks on the surface. Roy et al. (1994) suggested that the cracks occurred as a result of fruit expansion during development and ripening.

Diffusion of gases through microcracks can be described by combining Eq. 5.1 and 5.2 (Nobel, 1991):

$$r_j = \frac{P_j \Delta p_j A}{\Delta x} \qquad \textbf{(5.3)}$$

Maguire, Holmes et al. (1998) found that micro-cracks did not fully penetrate

the cuticle. If we suppose that water vapour has to diffuse through an inner layer of cuticle at the base of the crack with an effective depth of Δx^{icut} (m) then through a crack with an effective depth of Δx^{ck} (m) and an effective cross-section A^{ck} (m^2) and thereafter across a boundary layer of effective length Δx^{bl} (m) and total cross-section A^{tot} (m^2; Fig. 5.1) then the rate of water loss through inner layer of cuticle ($r^{icut}_{H_2O}$; $mol \cdot s^{-1}$) can be expressed as:

$$r^{icut}_{H_2O} = \frac{P^{icut}_{H_2O} \Delta p^{icut}_{H_2O} A^{ck}}{\Delta x^{icut}} \quad \textbf{(5.4)}$$

where $P^{icut}_{H_2O}$ is the permeability of water vapour in the cuticle ($mol \cdot m \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$) and $\Delta p^{icut}_{H_2O}$ is the driving force for water vapour movement through the inner cuticle. The rate of water loss through the crack ($r^{ck}_{H_2O}$; $mol \cdot s^{-1}$) and the boundary layer ($r^{bl}_{H_2O}$; $mol \cdot s^{-1}$) can be expressed analogously:

$$r^{ck}_{H_2O} = \frac{P^{air}_{H_2O} \Delta p^{ck}_{H_2O} A^{ck}}{\Delta x^{ck}} \quad \textbf{(5.5)}$$

$$r^{bl}_{H_2O} = \frac{P^{air}_{H_2O} \Delta p^{bl}_{H_2O} A^{tot}}{\Delta x^{bl}} \quad \textbf{(5.6)}$$

where $P^{air}_{H_2O}$ is the effective permeability of air to water vapour ($mol \cdot m \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$; analogous to diffusivity) and $\Delta p^{ck}_{H_2O}$ and $\Delta p^{bl}_{H_2O}$ are the driving forces for water vapour movement through the crack and boundary layer, respectively .The rate of water loss through the cracks must equal the rate of water loss through the boundary layer and if the difference in concentration of water vapour through the

whole system ($\Delta p^{s}_{H_2O}$) is equal to $\Delta p^{bl}_{H_2O} + \Delta p^{icut}_{H_2O} + \Delta p^{ck}_{H_2O}$ then the rate of water loss from the crack system ($r^{'s}_{H_2O}$; $mol \cdot s^{-1}$) can be written as:

$$r^{'s}_{H_2O} = \Delta p^{s}_{H_2O} \frac{P^{air}_{H_2O} P^{icut}_{H_2O} A^{ck} A^{tot}}{P^{air}_{H_2O} A^{tot} \Delta x^{icut} + P^{icut}_{H_2O} A^{tot} \Delta x^{ck} + P^{icut}_{H_2O} A^{ck} \Delta x^{bl}} \quad \textbf{(5.7)}$$

Water vapour permeance of the crack system ($P^{'s}_{H_2O}$; $mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$) can be expressed as:

$$P^{'s}_{H_2O} = \frac{P^{air}_{H_2O} P^{icut}_{H_2O} A^{tot}}{P^{air}_{H_2O} A^{tot} \Delta x^{icut} + P^{icut}_{H_2O} A^{tot} \Delta x^{ck} + P^{icut}_{H_2O} A^{ck} \Delta x^{bl}} \quad \textbf{(5.8)}$$

If movement of water through the intact cuticle was negligible and the proportion of cracking (k) was equal to A^{ck} / A^{tot} then $P^{'s}_{H_2O}$ becomes:

$$P^{'s}_{H_2O} = \frac{P^{air}_{H_2O} P^{icut}_{H_2O}}{P^{air}_{H_2O} \Delta x^{icut} + P^{icut}_{H_2O} \Delta x^{ck} + P^{icut}_{H_2O} k \Delta x^{bl}} \quad \textbf{(5.9)}$$

Including a term for water vapour permeance through the intact cuticle ($P^{'cut}_{H_2O}$; $mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$) the model for fruit water vapour permeance ($P^{'}_{H_2O}$) becomes:

$$P^{'}_{H_2O} = \frac{k P^{air}_{H_2O} P^{icut}_{H_2O}}{P^{air}_{H_2O} \Delta x^{icut} + P^{icut}_{H_2O} \Delta x^{ck} + P^{icut}_{H_2O} k \Delta x^{bl}} + (1-k) P^{'cut}_{H_2O} \quad \textbf{(5.10)}$$

Variable Δx^{bl} (m) can estimated using Nobel (1975):

$$\Delta x^{bl} = \frac{2.8\sqrt{\frac{d^f}{v}} + \frac{0.25}{v}}{1000} \quad \textbf{(5.11)}$$

where d^f is the diameter of the apple (m) and v is the velocity of air flow past the apple ($m \cdot s^{-1}$).

Permeability can be defined as (Nobel, 1991):

$$P_j = D_j S_j \quad \textbf{(5.12)}$$

where D_j is the diffusivity ($m^2 . s^{-1}$) and S_j is the solubility ($mol \cdot m^{-3} \cdot Pa^{-1}$) of the species j. In the case of a gas dissolved in a fluid, solubility is equal to the ratio of concentration to partial pressure. If we consider the ideal gas law (Nobel, 1991):

$$p^{tot}V = nR(T + 273.15) \quad \textbf{(5.13)}$$

where R is the gas constant ($8.314\ m^3 \cdot Pa \cdot mol^{-1} \cdot K^{-1}$), p^{tot} is the partial system pressure (Pa), V is the gas volume (m^3), n is the amount of gas (mol) and T is temperature (^oC). On this basis, solubility can be quantified as:

$$S = \frac{n}{Vp^{tot}} = \frac{1}{R(T + 273.15)} \quad \textbf{(5.14)}$$

For a gaseous system, effective permeability of the system to another gaseous species can be calculated by combination of Eqs. 5.12 and 5.14:

$$P_j = \frac{D_j}{R(T + 273.15)} \quad \textbf{(5.15)}$$

The permeability of air to water vapour ($P_{H_2O}^{air}$) at 20 °C is therefore 9.93 $nmol \cdot m \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$. An apple with diameter of 0.07 m in an air stream with velocity of 3 $m \cdot s^{-1}$ has a boundary layer (Δx^{bl}) 0.5 mm thick. From Maguire, Holmes et

al. (1998) the typical depth of the cracks (Δx^{ck}) is 8 μm with a cuticle thickness of approximately 16 μm leaving 8 μm of the cuticle for the water vapour to diffuse through beneath the crack (Δx^{icut}).

This study used scanning electron microscopy and confocal microscopy to characterise variation in micro-cracking in 'Braeburn' apples and to explore the relationship between micro-cracking and water vapour permeance in these fruit.

5.2 Materials and Methods

'Braeburn' (120) fruit were harvested 12 May 97 from Fruit Crops Unit, Massey University, Palmerston North. Fruit were handled by stalks with minimal contact with the fruit surface. Within 48 h of picking, fruit were placed in an air flow of ≈3 $m \cdot s^{-1}$ and rate of weight loss from each fruit was determined over a 16 h period using a balance (0.001g Model PM1206; Mettler Toledo, Switzerland) P'_{H_2O} was calculated for each fruit using Eq. 5.1 without correction for respiration. An average relative humidity was determined by wet and dry bulb temperature readings (thermistor probes CM type, U bead, ± 0.2 °C; Grant Instruments, Cambridge, U.K.). Skin temperature was logged during the period of measurement by recording (Squirrel model 1206, Grant Instruments, Cambridge, U.K.) thermistor probes (FF type, U bead, ± 0.2 °C; Grant Instruments, Cambridge, U.K.) inserted under the skin of several sample fruit. Averages were used to calculate Δp_{H_2O} using psychrometric relationships

(Tetens, 1930; Monteith and Unsworth, 1990a; Monteith and Unsworth, 1990b). Surface area was calculated using the relationship with fruit weight derived by Clayton et al. (1995).

Four fruit were selected with water vapour permeance between 4 and 8 nmol · $s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ (low P'_{H_2O}) and four fruit between14 to 15 nmol · $s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ (mid P'_{H_2O}) and a further five fruit between 23 and 30 nmol · $s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ (high P'_{H_2O}).

A small piece of fruit surface, 2 mm square and 1 mm thick, was then cut for microscopy from the centre of the blushed area on each fruit. Skin samples were placed in primary fixative overnight at room temperature (3% glutaraldehyde + 2% formaldehyde in 100 mol · m^{-3} phosphate buffer pH 7.2; Karnovsky, 1965) then given three buffer washes (100 mol · m^{-3} phosphate buffer pH 7.2), dehydrated in a graded ethanol series (25, 50, 75, 95, 2 × 100%) and critical point dried using liquid CO_2. They were then mounted on specimen stubs and sputter coated with gold prior and examined by scanning electron microscope (Model Stereoscan 250, Mark 3, Cambridge Instruments; Cambridge, England). Two micrographs (200 × mag) were taken of each sample as systematically spaced fields (as per the "2" dots on a gaming die).

Four fruit were selected with water vapour permeance between 8 and 9 nmol · $s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ (low P'_{H_2O}) and a further six fruit were selected with water vapour permeance between 22 and 23 nmol · $s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ (high P'_{H_2O}). A roughly circular flap (≈ 20 mm diameter) of skin was removed by making a grazing cut

with a sharp blade at the cheek of the fruit at four evenly spaced equatorial positions (approximately 90 °) starting at centre of blush. These samples were placed on a microscope slide with a drop of immersion oil in the centre. Each sample was viewed at 160 × magnification with a confocal microscope (model TCS 4D, Leica; Cambridge, England) in reflection mode (krypton argon laser; laser wavelength 488 μm; detector RSP580). Several optical sections were used to obtain a complete picture of the surface of each sample.

Acetate sheets were laid over each micrograph. Areas of micro-cracks were traced carefully with a permanent black marker onto the acetate. The sheets were placed in a leaf area meter (model 3100 Li-Cor; Lincoln, Nebraska, USA; resolution 1 mm^2). An average of 10 area measurements was obtained for each micrograph.

The model represented in Eq. 5.10 was fitted to data on proportion of cracking determined from scanning electron micrographs and measured water vapour permeance with the NLIN procedure of SAS (1988) assuming $P_{H_2O}^{air}$ = 9.93 nmol · m · s^{-1} · m^{-2} · Pa^{-1} , Δx^{bl} = 0.5 mm, Δx^{ck} = 8 μm and Δx^{icut} = 8 μm. Crack area determined from the confocal micrographs was analysed using a completely random model in the GLM procedure of SAS (1988).

5.3 Results

The mean values for proportion of cracking were 0.004, 0.025 and 0.072 (SED = 0.018, df = 14) for fruit in the low, medium and high $P_{H_2O}^{'}$ groups. The

model fitted (Eq. 5.10) accounted for 51% (Fig. 5.2) of the variation in P_{H_2O} the fitted value for permeance of the intact cuticle ($P_{H_2O}^{cut}$) was 11.5 ± 5.4 nmol · s^{-1} · m^{-2} · Pa^{-1}, and permeability of water vapour in the inner cuticle ($P_{H_2O}^{icut}$) was 1.3 ± 0.75 pmol · m · s^{-1} · m^{-2} · Pa^{-1}.

Cracking on fruit examined with the confocal microscope from the high water vapour permeance group (9.4% of the surface area) was significantly different to the low water vapour permeance group (4.6% of the surface area; SED = 1.17, df = 38). There appeared to be no pattern to the variation in cracking around the fruit surface. From the confocal data the coefficient of variation for the data was 44% indicating that there is considerable variation between samples around the fruit surface. The crack areas from this experiment were consistent with those generated on similar samples using the scanning electron microscope (open circles; Fig. 5.2).

5.4 Discussion

The data showed considerable variation in proportion of cracking with each water vapour permeance group, leading to only a marginal fit (R^2 = 51%) of the model. This variation could have arisen from a number of sources. The sample of fruit on which cracking was quantified was an extremely small proportion of the whole fruit surface. The fitted model assumes that this small area was indicative of the cracking over the entire surface. Failure in this assumption would in itself, be expected to result in considerable variation around

the fitted line for the model. Additional variation could have arisen due to differences in each fruit or within fruit for the variables in the model i.e. crack depth (Δx^{ck}), cuticle depth ($\Delta x^{icut} + \Delta x^{ck}$), boundary layer depth (Δx^{bl}), permeability of water vapour in the partial cuticle ($P_{H_2O}^{icut}$) and the permeance of the intact cuticle ($P_{H_2O}^{'cut}$). At a cracking level (k) of 0.05, a 20% increase in $P_{H_2O}^{icut}$ led to a 7% increase in the predicted $P_{H_2O}^{'}$, a 20% increase in $P_{H_2O}^{'cut}$ led to a 12% increase whereas a 20% increase in Δx^{icut} led to a 7% decrease.

Values for permeance of intact isolated cuticles have been obtained by Becker et al. (1986) for tomato fruit (*Lycopersicon esculentum* Mill.; 56.8 $nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$), capsicum fruit (*Capsicum annuum* L.; 37.12 $nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$) and for eggplant fruit (*Solanum melongena* L.; 8.92 $nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$). With the exception of eggplant, these fruits appear to have slightly more permeable intact cuticles than apple fruit, but on the whole, the figure obtained by model fitting for the water vapour permeance of intact cuticle of apple fruit of 11.5 ± 5.4 $nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ was very comparable. The coefficients of variation for data in literature are often 30-40%, indicating that there is substantial variation possible in $P_{H_2O}^{'cut}$. Haas and Schonherr (1979) concluded that this variation was due to differences in structure and composition of the soluble cuticular lipids. In addition, the fitted model did not take into account lenticel contributions which in some fruit can contribute up to 20% of the total water loss from fruit (Pieniazek, 1944). Thus the fitted value for $P_{H_2O}^{'cut}$ may be an overestimate of the true value.

There are no published values for 'Braeburn' apples but the range for matrix membranes from several diverse species were from 0.01 to 3 pmol · m · s^{-1} · m^{-2} · Pa^{-1} (calculated from Becker et al., 1986).The permeability of water vapour in the cuticle under the cracks ($P_{H_2O}^{icut}$; 1.3 ± 0.75 pmol · m · s^{-1} · m^{-2} · Pa^{-1}) appears to be near the middle of this range. Despite the fact that our data was obtained from whole fruit permeances and Becker et al. (1986) used isolated cuticles, the estimates obtained from the model are physiologically sound.

If the depth of cuticle under the crack (Δx^{icut}) was 8 μm then the permeance of this is 162.5 nmol · s^{-1} · m^{-2} · Pa^{-1} the ratio of permeance of cuticle under the crack to intact cuticle would be 14. The ratios of permeance for the matrix membrane to that of the isolated intact cuticle are 20 for tomato fruit cuticle (Schonherr and Lendzian, 1981) and 1542 for onion bulb scales (Schonherr and Merida, 1981). This indicates that the cuticle beneath the crack is likely to be made mostly of the matrix membrane i.e. cutin matrix with very little soluble cuticular lipids.

The striking feature of this work was the close agreement of the two methods for quantifying crack area. The results from the confocal microscope obtained on fresh samples were highly consistent with the scanning electron microscope data obtained on chemically fixed and dehydrated samples. This indicates that the cracks viewed with the scanning electron microscope were not due to artifacts of the chemical fixation and dehydration during sample preparation but, rather, they are reasonably representative of cracks present in the fresh cuticle.

There was a 5 fold variation in the water vapour permeance of whole fruit within the experiments; this variation would not be explained by the possible 0.2 or 0.3 fold variation expected in permeance of an intact cuticle (Schonherr, 1982). On the other hand, the evidence presented here demonstrates that such variation could be explained by variation in both area and depth of cracks. The permeance of cracks was approximately 130 times more permeable than the cuticle; changes in the proportion of cracking could create a large effect on whole fruit water vapour permeance. For a fruit with very little cracking, variation in the barrier properties of the intact cuticle became an important influence. For the model presented here, if k was 0.05 and the $P'^{cut}_{H_2O}$ was increased by 20% then the consequent increase in P'_{H_2O} was predicted to be 12%. In contrast if the fruit already had a high k of 0.2 then the increase in $P'^{cut}_{H_2O}$ would result in only a 4% change in P'_{H_2O}.

Maguire, Banks et al. (1998) reported values for water vapour permeance of up to 70 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ on fruit with no visible blemish. We can use this model to predict the proportion of cracking area expected on the surface of the fruit. Assuming all other variables were as above, then the model predicted that the area of cracking would be 42% of the total area of the fruit surface.

Maguire, Banks et al. (1998) found that the water vapour permeance of 'Braeburn' fruit increased at each successive harvest throughout the commercial harvest season, a change that could be linked to a greater proportion of micro-cracking in the cuticle. Roy and co-workers (1994)

suggested that micro-cracks occurred as a result of fruit expansion during development and ripening. As fruit remain on the tree longer, their mass increases through expansion, and fruit water vapour permeance increases, presumably caused by fracture resulting from excessive strain.

Overall, this study has provided a credible model by which cracks contribute to variation in the permeance of whole apples. This involves fracture of the outer layer most resistant to water loss to reveal inner cuticle with poorer barrier properties. The proportion of the whole fruit surface covered in cracks then accounts for much of the variation in whole fruit permeance. Useful further work could include quantification of the potential contribution of lenticels to whole fruit permeance developed as an analogous component of the model.

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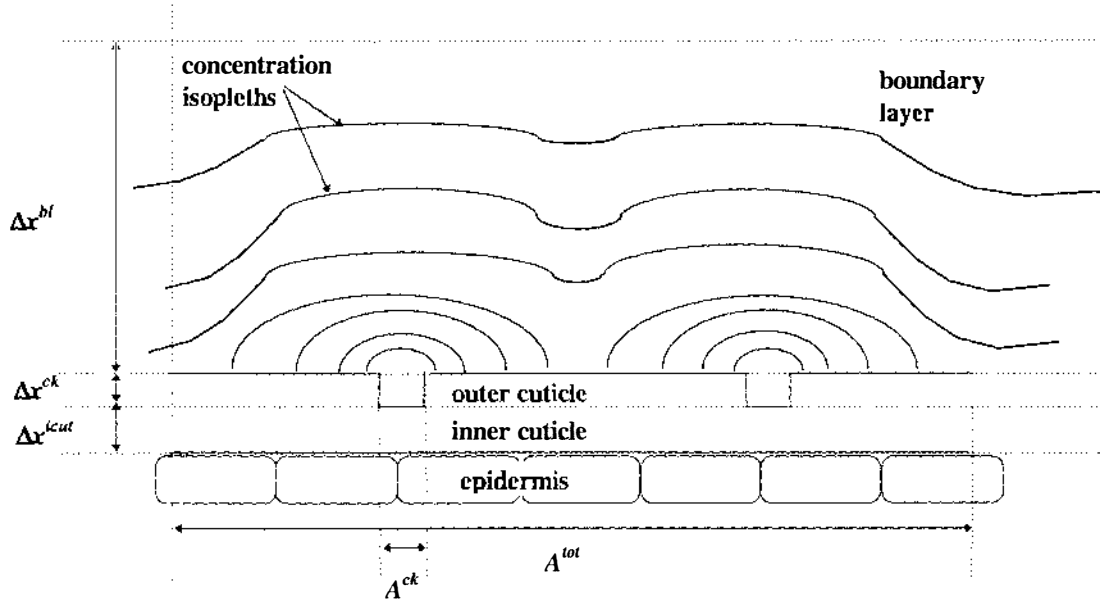

Fig. 5.1. Diagrammatic representation of concentration contours of water vapour outside microcracks in a fruit cuticle

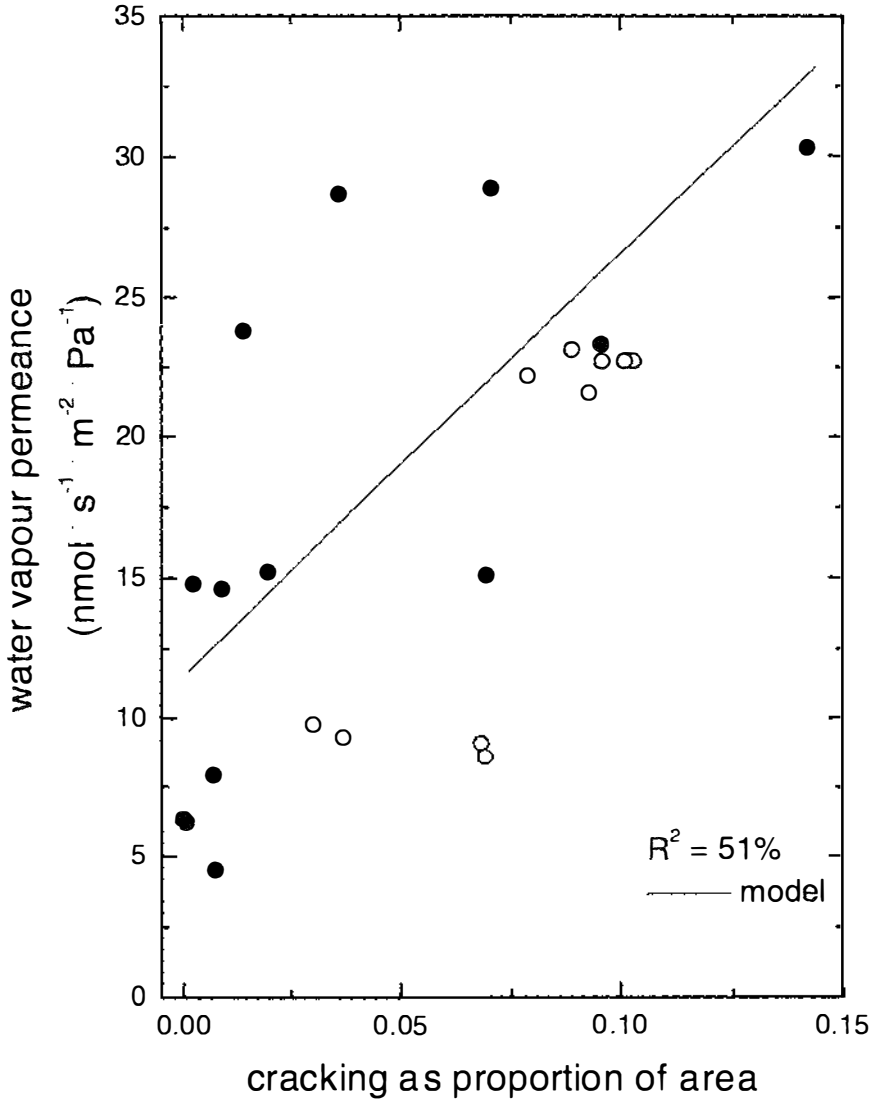

Fig. 5.2. Relationship between water vapour permeance and mean cuticular microcracking for each fruit expressed as a proportion of area to total cuticular area (● scanning electron microscope, ❍ confocal microscope).

Chapter 6

Stretching affects cuticle integrity and water vapour permeance of 'Braeburn' apples

Stretching affects cuticle integrity and water vapour permeance of 'Braeburn' apples.

[1]Kate Maguire, [2]Sandy Lang, [1]Nigel Banks, and [2]Alastair Hall

[1]Centre for Postharvest and Refrigeration Research and Department of Plant Science, Massey University, Private Bag 11 222, Palmerston North.

[2]HortResearch, Palmerston North Research Centre, Private Bag 11 030, Palmerston North

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ABSTRACT

Variation in the water vapour permeance of 'Braeburn' apples is largely attributable to variation in the extent of micro-cracking of the cuticle. We have investigated the effects of 2 dimensional stretching of fruit skin to mimic effects of fruit growth on water vapour permeance and microcracking in the cuticle. A linear strain of c. 2% increased the water vapour permeance of fruit skins by an average of 60% of the initial value of unstretched skin. There was a permanent effect of strain on water vapour permeance; when all pressure had been released, final water vapour permeance was on average 20% higher than initial unstrained values. Apple skin showed a plastic ductile behaviour, with permanent deformation occurring as shown by hysteresis in the stress-strain plot. Cracking measured on electron micrographs of samples increased during stretching from 6% to 14% of total area, through a widening of existing cracks and initiation of new cracks. These findings support the proposition that natural microcracking in the cuticle develops due to growth expansion of the fruit. This seems particularly likely to occur when periods of rapid growth outstrip the fruit's ability to lay down sufficient new cuticular material to accommodate stretch.

Key words: *Malus domestica*, mass loss, water loss.

6.1 INTRODUCTION

Maguire, Banks et al. (1998) found a c.2-fold increase in water vapour permeance of 'Braeburn' fruit between those harvested early and those harvested later. In a separate study, variation in water vapour permeance of 'Braeburn' apples was linked to the extent of cracking in the fruit's cuticle (Maguire, Lang et al., 1998) . Meyer (1944) and Faust and Shear (1972) studied skin of 'Golden Delicious' apples and found cracks appearing on the surface early in the growing season; by the end of the season the cracks were larger and had formed networks on the surface. Roy et al. (1994) suggested that such cracks occurred as a result of fruit expansion during development and ripening.

We argued that such turgor driven fruit expansion might be mimicked in vitro by application of elevated pressures to the underside of a disc of skin in a sealed cylinder. In this work, we investigated the effects of 2 dimensional stretching, achieved in this way, on the water vapour permeance of skin and the micro-cracking of the cuticle.

6.2 MATERIALS AND METHODS

Late harvested, export packed 'Braeburn' fruit from Hastings, New Zealand were removed from 16 weeks cold storage (0 ^{o}C) and equilibrated to 20 ^{o}C for 48 h. One disc of skin and some flesh (radius = 11.5 mm) was removed from

each fruit with a sharp cork borer and most of the flesh was excised with a sharp blade.

Each disk was viewed under a binocular microscope and careful measurements of the major dimensions (Δx^{s1}, Δx^{s2} and Δx^{m}; Fig. 6.1) were made using a calibrated eye piece graticule. Height Δx^{flesh} was estimated as the average of Δx^{s1} and Δx^{s2}, and Δx^{skin} was calculated as Δx^{m}- Δx^{flesh} .

One at a time, the skin discs were sealed into the experimental apparatus shown in Fig. 6.2 developed from a method for stretching skin described by Vincent (1992). This included a null balance porometer (model 1600; Li-Cor, Lincoln, Nebraska, USA) , a linear variable displacement transducer (LVDT; model ms2-100; Sensotech, Columbus, Ohio, USA), a mercury manometer and a screw-driven syringe. The syringe was used to create a positive air pressure in the closed system which slightly distended the skin, stretching it uniformly in 2 dimensions. As the skin was stretched, the LVDT measured the extent of deformation (Δx^{d}). A particular deformation was maintained by continually adjusting the pressure. At the same time, the porometer measured permeance. Average values were calculated from 5 readings at 30 s intervals. Pressure was recorded using the mercury manometer at the end of each period. Strain was in all cases kept within the range experienced by the fruit during its normal cycles of expansion and contraction on the tree (2-3% linear strain diurnally; Lang, 1990).

Strain in the skin (s), can expressed as the fractional linear extension

which can be calculated using the arc lengths α_1 and α_2 in Fig. 6.1:

$$s = \frac{\alpha_2 - \alpha_1}{\alpha_1} \tag{6.1}$$

Values for α_1 and α_2 are given by:

$$\alpha_1 = \left(\frac{L}{Sin\,\theta_1\ Cos\,\theta_1} \right) \theta_1 \tag{6.2}$$

$$\alpha_2 = \left(\frac{L}{Sin\,\theta_2\ Cos\,\theta_2} \right) \theta_2 \tag{6.3}$$

and the values of θ_1 and θ_2 required can be calculated as:

$$Tan\,\theta_1 = \frac{\Delta x^{skin}}{L} \tag{6.4}$$

$$Tan\,\theta_2 = \frac{\Delta x^{skin} + \Delta x^{d}}{L} \tag{6.5}$$

Strain can therefore be rewritten as:

$$s = \left(\frac{\theta_2 \left(Sin\,\theta_1\ Cos\,\theta_1 \right)}{\theta_1 \left(Sin\,\theta_2\ Cos\,\theta_2 \right)} \right) - 1 \tag{6.6}$$

After stretching, a small piece 2 mm square and 1 mm thick was excised from each disc for microscopy. A similar sample was taken from the same fruit as an unstretched control. These samples were placed in primary fixative (3% glutaraldehyde + 2% formaldehyde in 100 mol · m^{-3} phosphate buffer pH 7.2; Karnovsky, 1965) overnight at room temperature then through a series of three buffer washes (100 mol · m^{-3} phosphate buffer pH 7.2). Samples were then dehydrated in a graded ethanol series (25, 50, 75, 95, 100 and 100%) and critical point dried using liquid CO_2. They were then mounted on

specimen stubs and sputter coated with gold before examination by scanning electron microscope (Model Stereoscan 250 mark 3; Cambridge Instruments, Cambridge, England). A series of five micrographs (200 × mag) was taken of each sample as systematically spaced fields (as per the "5" dots on a gaming die).

Acetate sheets were laid over each micrograph. Areas of micro-cracks were traced carefully with a permanent black marker onto the acetate. The sheets were passed through a leaf area meter (model 3100; Li-Cor, Lincoln, Nebraska, USA; resolution 1 mm^2). Five area measurements were obtained for each micrograph. Crack area was analysed using a nested model in the GLM procedure of SAS (1988).

6.3 RESULTS

The water vapour permeance of the samples of skin increased by 60 %, (range 20%-140%) with an increase in linear strain to c. 2% (Fig. 6.3a). Some of this change in water vapour permeance was a permanent alteration i.e. the water vapour permeance did not return to the original level when strain was released (Fig. 6.3a). The ratio of water vapour permeance after a strain of c.2% to the initial water vapour permeance value was greater than unity for all fruit, indicating a permanent increase in water vapour permeance of the skin discs (Fig. 6.4a). The permanent increase in water vapour permeance expressed as a ratio of permeance after stretching divided by that before stretching declined with each increase in strain (Fig 6.4a).

The stress-strain curves were dominated by a plastic response (the ascending line curved and the descending line was straight and did not return to a zero strain; Fig. 6.3b).

Stretching the skin had a highly significant effect ($P < 0.0001$) on the area of cracks measured from the micrographs (Fig. 6.4b). The epicuticular wax of the fruit appeared smooth but had numerous surface cracks that formed an interconnected network on the surface of the fruit (Fig. 6.5a). The average area of cracking for the total sample from this unstretched piece of skin was 6% of the total area of skin shown in the photograph. After stretching (Fig. 6.5b) the surface cracks were both more numerous and wider. The average area of cracking for the total sample for the stretched piece of skin was 14%. Straining induced an increase in area of cracking which ranged from a 20 to 650% of initial values (Fig. 6.4b).

6.4 DISCUSSION

These results demonstrate that excessive strain can exacerbate cracking in 'Braeburn' apple cuticle, and suggests a mechanism by which natural cracks could form during periods of rapid fruit growth. Given the previously published link between the water vapour permeance of 'Braeburn' apples and the amount of cracking found on the fruit surface (Maguire, Lang et al., 1998), this provides a possible mechanism by which water vapour permeance could increase with fruit growth.

Water vapour permeance of the skin discs increased 60 % with only a 2% increase in strain. Consider a transect 100 units long, comprising 90 units of intact cuticle and a crack 10 units wide. Application of 2% strain to this region would increase total transect length to 102 units. If this increase in length came about exclusively from an increase in crack width (from 10 to 12 units), this would represent a 20% increase in crack width and thus approximately a 20% increase in crack area. If the relationship between increasing crack width and increase in water vapour permeance was linear, and water transfer through the cuticle was dominated by movement through the cracks, this would correspond to approximately a 20% increase in water vapour permeance. However, as noted, the increase in permeance was higher than this i.e. up to 140% in some fruit, this would indicate the development of new cracks or deepening existing cracks.

The possibility that cracks seen on the scanning electron micrographs might have been due to an artifact of sample preparation cannot be ruled out. Jeffree and Read (1991) suggested that such artifacts can develop due to shrinkage during critical-point drying or to mechanical damage from the chemical processing. However, we are reasonably confident that these cracks are indeed naturally present in the surface of the fruit as we recently have shown similar cracks to be present in 'Braeburn' apple cuticle on fresh samples with no fixation at all using confocal microscopy (Maguire, Lang et al., 1998). This proposition is supported by work by Roy et al. (1994) on 'Golden

Delicious' apples using cryo-fixation, which is known for its ability to preserve delicate structures of the epicuticular waxes.

When stress was released, the strain on the skin did not return to zero, indicating a permanent change in the dimensions of the skin i.e. diameter of skin disc. This would presumably have been due to widening of existing cracks and the development of new cracks and, possibly, a plastic deformation of intact areas of cuticle. It is difficult to tell from the stress-strain graph at which point the new cracks began to form. However in Fig. 6.3a it appears that a sharp increase in permeance occurred at approximately 0.5% strain and this could possibly coincide with the initiation of new cracks.

The cuticle comprises a thin (20 μm) visually amorphous layer on the outer surface of the epidermis. As fruit growth occurs this must expand by plastic straining. Presumably, during this time, thickness and integrity are maintained by the secretion of new cuticular material. Developing apple fruit commonly experience a 2-3% change in volume within a single diurnal cycle (Lang, 1990). Under more extreme weather conditions, this could easily reach a 5% volume change, which would correspond to a 1.6% linear strain in the skin. A marked increase in water vapour permeance on the first increase of pressure was experienced in all fruit at a strain of approximately 0.5%. This equates to a volumetric change which would commonly be experienced with normal diurnal variation in fruit size.

Overall, these findings support the proposition that cracks develop

because of the natural growth expansion of the fruit, particularly during short periods of especially rapid growth, where this outstrips the ability of the fruit to lay down sufficient new cuticular material. Increase in area of cracks would lead to an increase in permeance of the skin and explain why late harvested fruit have higher permeance than those harvested earlier.

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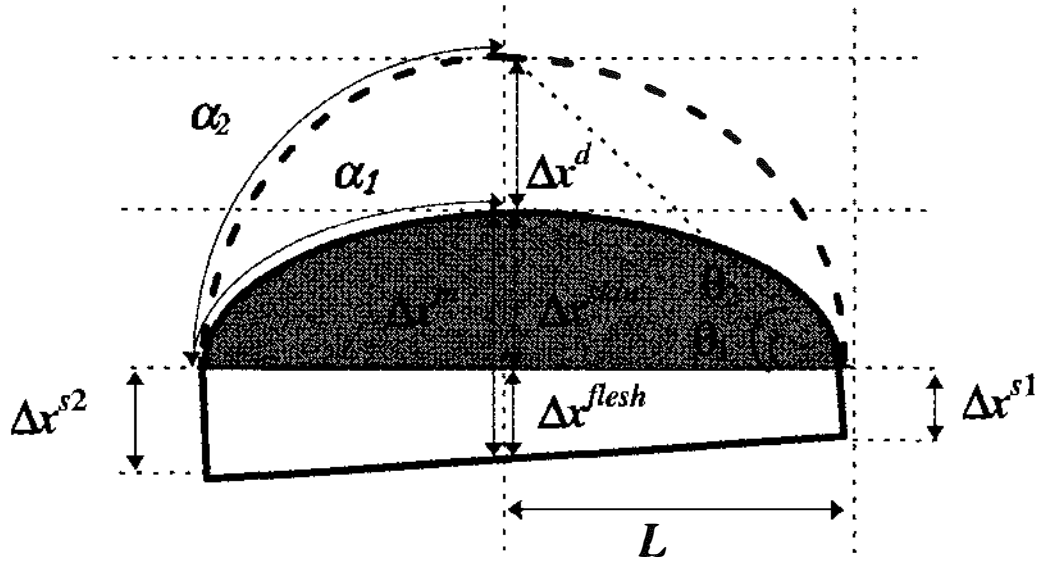

FIG. 6.1. Schematic diagram of the side view of a skin section with measured and calculated variables.

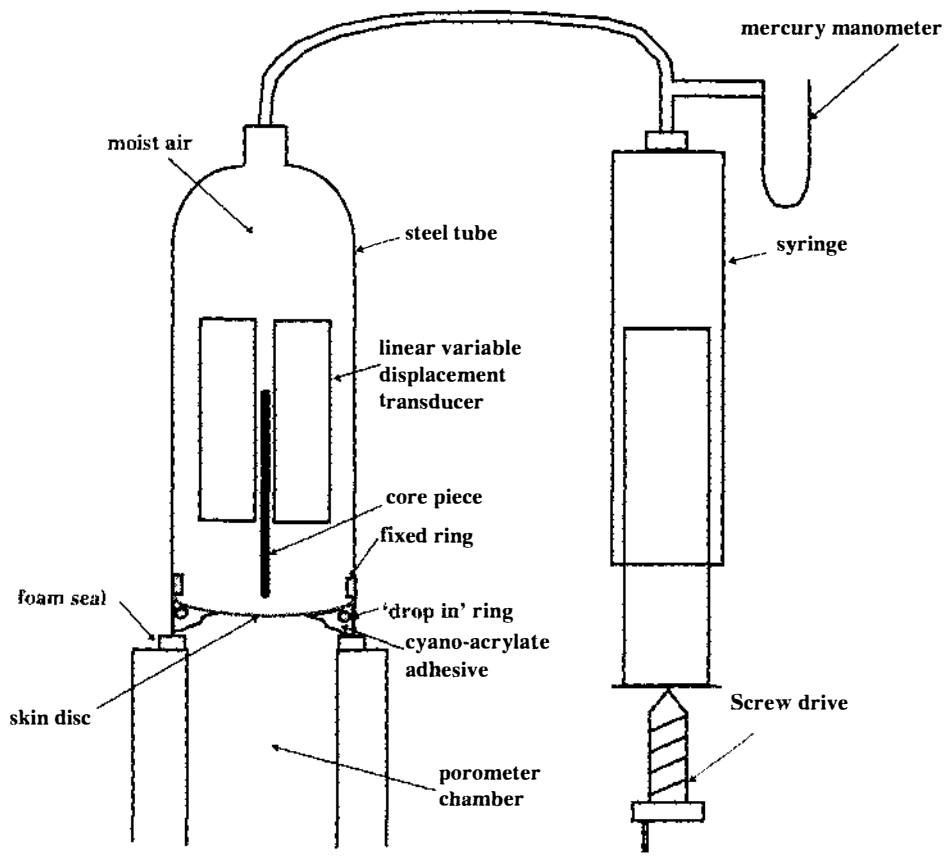

FIG. 6.2. Schematic diagram of experimental apparatus for stretching apple skin developed from a method described by Vincent (1992).

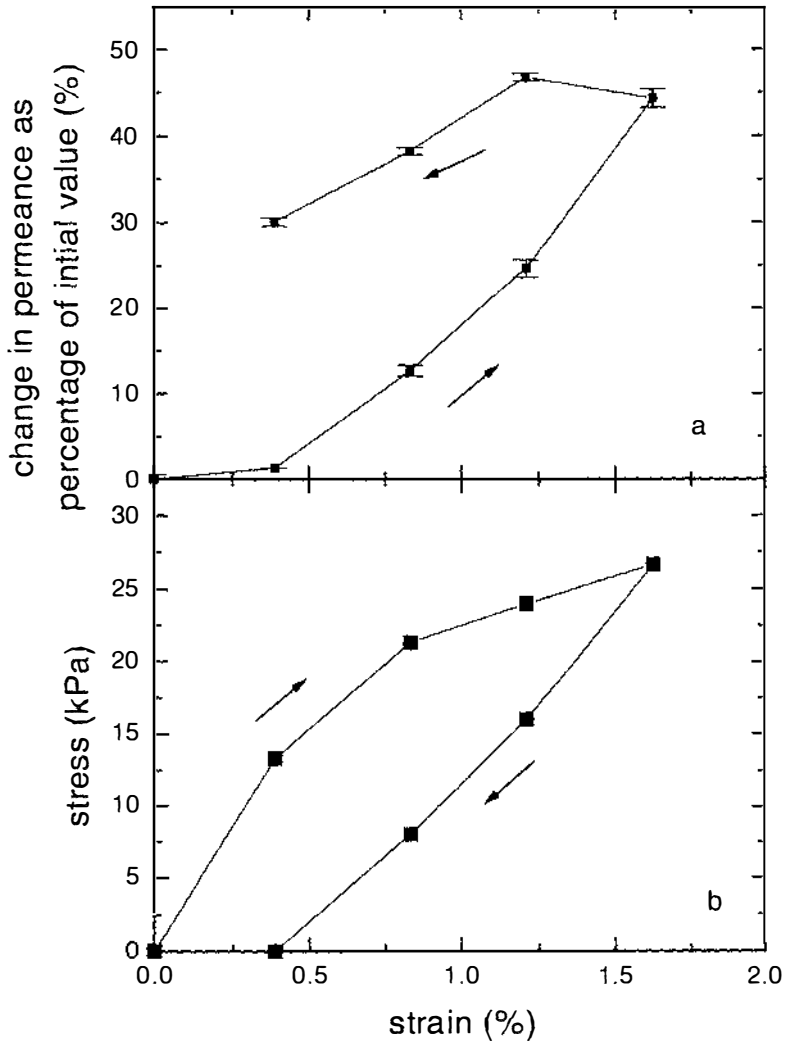

FIG. 6.3. a) Changes in water vapour permeance (as a percentage of initial value) with variation in strain for a representative piece of skin from a 'Braeburn' apple and b) stress-strain curve for the same piece of skin.

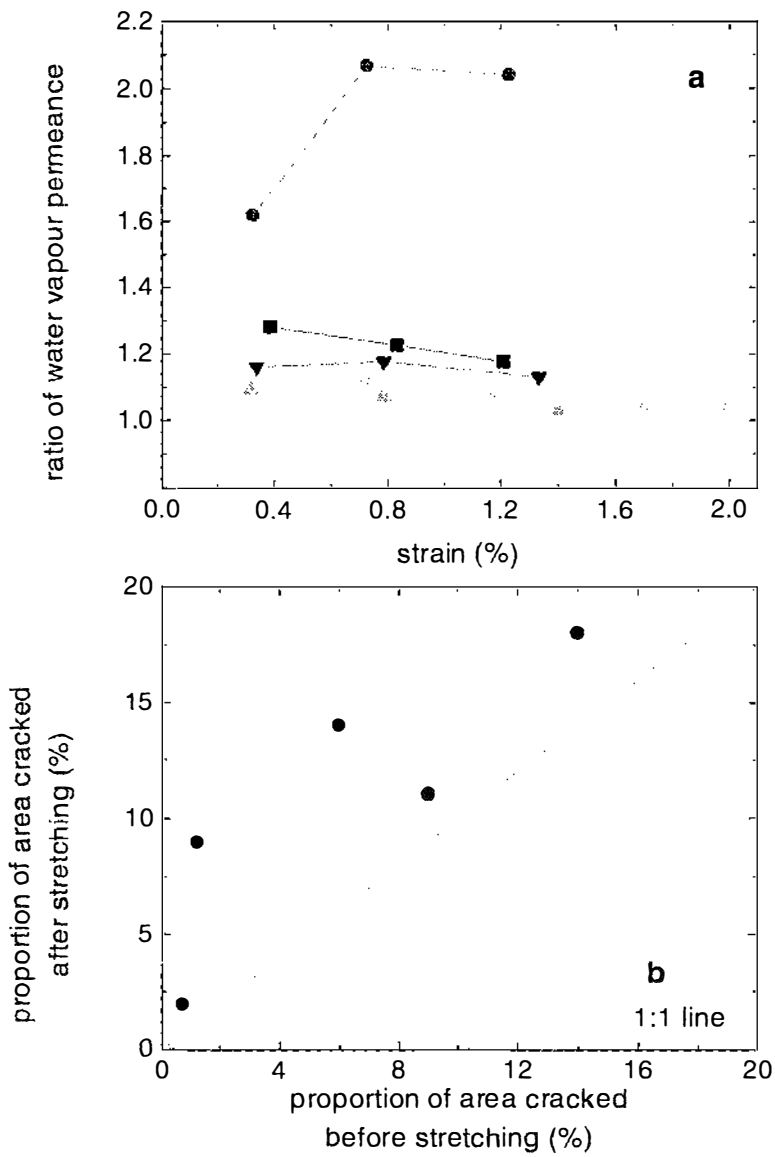

FIG. 6.4. Effect of stretching skin on a) ratio of water vapour permeance before and after stretching at different values of strain and b) proportion of crack area (SED for both x and y variables = 0.05), for 'Braeburn' apples.

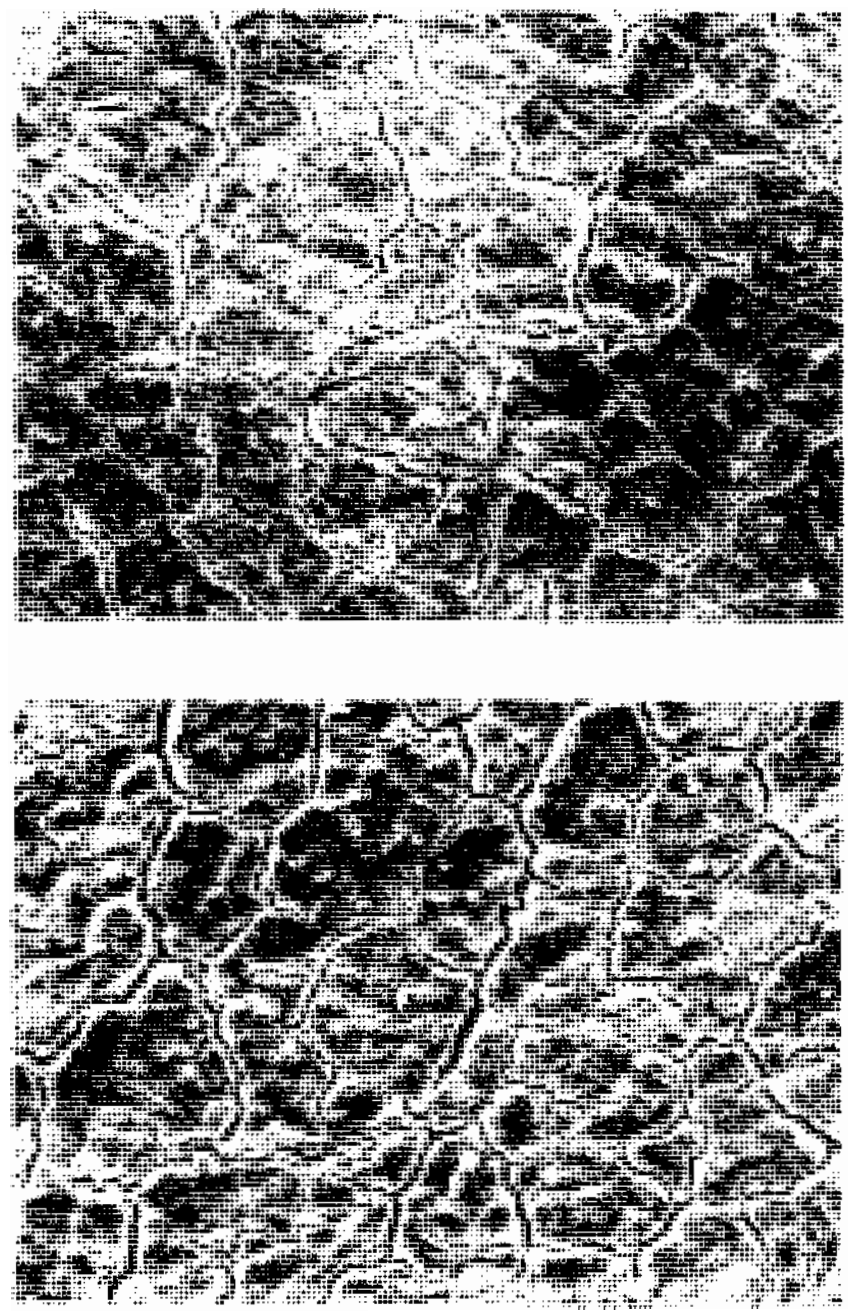

FIG. 6.5. Micrographs of 'Braeburn' apple skin mag c. 200 × for a) unstretched skin and b) stretched skin the examples (selected from single fruit with crack areas of 9% before and 15% after, typical of the 50 micrographs involved in the study).

Chapter 7

Growth and Development of Apple Skin and the Maintenance of its Structural and Functional Integrity

The growth and development of apple skin and the maintenance of its structural and functional integrity

[1]Kate Maguire, [2]Connor Holmes, [2]Alexander Lang, [1]Nigel Banks

[1]Centre for Postharvest and Refrigeration Research, Massey University, Private Bag 11-222, Palmerston North, New Zealand

[2]The Horticulture and Food Research Institute of New Zealand Ltd, Private Bag 11 030, Palmerston North, New Zealand

Style: The chapter is in the style of the Journal of Experimental Botany

ABSTRACT

This study explores factors involved in development and disorders of apple skin through micro-anatomical examination of skin development in 'Red Delicious' apples from bloom to near commercial harvest. Fruit surface area increased throughout development Area expansion of the skin was accommodated both by an increase in epidermal cell number and by an increase in their individual plan areas. The epidermal cells change from "portrait" to "landscape" height to width ratios during fruit development. This change was partly the result of a 3-fold increase in cell width but also of a c. 40% reduction in cell height. The total surface area of the epidermal cell increased initially and then after about 60 days after full bloom remained roughly constant. Cuticle thickness increased linearly nearly 10-fold during the period of fruit growth. Cuticular microcracks formed an interconnecting network, isolating tiny islands of intact cuticle on 'Braeburn' apples, each island covering a few (6-8) epidermal cells. The cracks did not penetrate the cuticle fully, but generally had similar depths and rounded apices. The last stages of growth in fruit surface area are mainly due to expansion and deformation of existing cells. The cuticle accommodates the fruit growth by plastic deformation and secretion of new material. When fruit growth outstrips the ability to lay down new cuticle, and the strain becomes too great, the outer, wax-rich layer of the cuticle fractures, forming a reticulate crack network. This cracking isolates tiny islands of intact cuticle as the fruit continues growing.

This effect could be important in development of russet before harvest and in determining susceptibility to mass loss and shrivel after harvest.

Key words: *Malus domestica*, micro-cracking, water loss, russet, epidermis.

7.1 INTRODUCTION

The skin of a fruit performs two important functions. Biologically, it provides a barrier between the delicate tissues inside of the fruit and the external aerial environment (Bell, 1937; Holloway, 1982; Burton, 1982; Lendzian and Kerstiens, 1991). Commercially, the skin is the visible 'attractive' layer of the fruit with lustre, colour and presence or absence of imperfections contributing to purchase decisions by the consumer (Wills et al., 1989).

The outer two layers of a mature apple are, in total, about 40 μm thick (Bell, 1937). About 30 % this thickness is made up of close fitting tile-like epidermal cells, and the rest is a waterproof overlying layer: the cuticle. Immediately beneath the outer layers in a mature apple is the first of the thin-walled parenchymatous cells of the flesh.

After fruit set, fruit growth is very rapid, the ovary increasing its volume about 20,000-times over the 140 day (d) period from bloom to commercial harvest (De Vries, 1968). During the growth period, the surface area of the skin must increase its area 750-fold so as to keep pace. If it fails to expand at a sufficient rate it will suffer damage at either a gross level - fruit or

skin cracking (tearing of the epidermis; Lang and Thorpe, 1988) or at a fine level - microcracking (strain fractures appearing in the outer surface of the cuticle; Roy et al., 1994; Opara et al., 1997). Both conditions lead to local increases in water loss (Meyer, 1944; Maguire, Lang et al., 1998a).

Fruit cracking is significant in apple (e.g. stem-end splitting in 'Royal Gala') but is not as severe a problem as it is in some stone fruit (e.g. cherries) and berry fruit (e.g. grapes) (Opara et al., 1997). Skin cracks are normally repaired within 3 to 5 days by the differentiation of a phellogen (a cork cambium). The suberised cork cells which result, restore the skin's barrier properties but unfortunately also impart a rough or 'russetted' appearance (Simons and Aubertin, 1959). For a while, however, the skin's barrier functions are compromised, water loss rates are high (Meyer, 1944) and the opportunity for pathogen invasion is offered. Russetting is viewed favourably in some species and cultivars ('Beurre Bosc' and 'Abbe Fetel' pears and 'Tankei' and 'Rock' melons) but significant russet in apples, which normally appears early in the season, is a commercially important cause of fruit rejection (Skene, 1982). The commercial importance of microcracking in apple is probably limited to enhanced fruit water loss (Maguire, Lang et al., 1998a) which, during storage, leads to significant market losses not only through crop mass loss but also through spoilage due to loss of lustre and wrinkling of the skin (Wills et al., 1989). In some fruit species (e.g. grapes) cuticular microcracking can increase the incidence of pathogen invasion (Blaich et al.,

1984).

This study explores factors involved in development and disorders of apple skin through micro-anatomical examination of skin development in 'Red Delicious' apples from bloom to near commercial harvest.

7.3 MATERIALS AND METHODS

'Red Delicious' and 'Braeburn' (data not shown) fruit were harvested from Fruit Crops Unit, Massey University, Palmerston North at regular intervals after fruit set from December 1994 to March 1995. Fruit diameters, mass, volume and surface area were recorded. Partial cores were cut from the cheek of apples with a sharp cork borer (6 mm diameter). Hand sections (≈ 5 μm thick) were then cut with a sharp blade to give vertical sections of the skin. The sections were mounted on microscope slides in 80% glycerol and viewed with a light microscope (Model Microlux 11, Kyowa; Japan) connected via a video camera (Model TK1280E, JVC; Japan) to a computer with Video Trace (Leading Edge Pty. Ltd.; Australia), an image measurement system software package. Dimensions of epidermal cells and thickness of fruit cuticle were measured. Areas of the roughly hexagonal epidermal cells presented at the fruit surface (plan areas) were calculated, as those of circles with the same diameter, as measured in transverse section. Total area was calculated assuming cell shape was cylindrical.

Small pieces 2 mm square and 1 mm thick were cut from mature 'Braeburn' fruit. These samples were placed in primary fixative (3%

glutaraldehyde + 2% formaldehyde in 100 mol · m^{-3} phosphate buffer pH 7.2; Karnovsky, 1965) overnight at room temperature then given three buffer washes (100 mol · m^{-3} phosphate buffer pH 7.2). Samples were then dehydrated in a graded ethanol series (25, 50, 75, 95, 2 × 100%) and critical point dried using liquid CO_2. They were then mounted on specimen stubs and sputter coated with gold prior to examination by scanning electron microscope (Model Stereoscan 250 Mark 3, Cambridge Instruments; Cambridge, England).

Samples were removed from specimen stubs and re-hydrated in primary fixative under vacuum for 2 h to remove air. The samples were then placed in a series of buffer washes followed by a further fixation in osmium tetroxide (1% in 100 mol · m^{-3} phosphate buffer, pH 7.2) and another series of buffer washes. Samples were then dehydrated in a graded ethanol series (25, 50, 75, 95, 2 × 100%) then infiltrated and embedded in "Procure 812"™ epoxy resin at room temperature. The samples were cured in fresh resin in silicone resin moulds at 60 °C for 48 h. One micron sections were cut from the trimmed block, heat mounted onto glass slides and stained with 0.05% toluidine blue. The slides were then viewed and photographed with a light microscope (Model Axioplan; Zeiss, Germany).

7.3 RESULTS

After a short lag following petal fall, fruit surface area increased rapidly and steadily throughout development (Fig. 7.1 a). Area expansion of the skin was

accommodated both by an increase in epidermal cell number (Fig. 7.1 b) and by an increase in their individual plan areas (Fig. 7.2). Somewhat more than half of the total increase in epidermal cell number and of plan areas (Fig. 7.2) occurred in the first half of the season.

The epidermal cells remained roughly isodiametric when viewed from above but altered in shape when the skin was viewed in transverse section. Here, the cells change from "portrait" to "landscape" during fruit development as their aspect ratio (height/width) reduced from about 2 to about 0.5 (Fig. 7.2). This shape change was partly the result of a 3-fold increase in cell width but also of a c. 40% reduction in cell height (Fig. 7.2). Cell width increased rapidly after bloom at first, but slowed later in the season (data not shown), whereas cell height increased early on but declined from around d 60 after bloom (Fig 7.2). The total surface area of the epidermal cell (Fig 7.3) increased initially and then at about 60 days after full bloom remained relatively constant.

Cuticle thickness increased linearly nearly 10-fold during the period of fruit growth (Fig. 7.4; R^2 = 94 %). There was a 785 fold increase in cuticle volume (fruit surface area × cuticle thickness) throughout the study, much of this increase occurring later in the season (data not shown).

Cuticular microcracks formed an interconnecting network isolating tiny islands of intact cuticle on 'Braeburn' apples, each island covering a few (6-8) epidermal cells (Fig. 7.5 a). The cracks did not penetrate the cuticle fully, but

generally had similar depths and rounded apices (Fig. 7.5 b).

7.4 DISCUSSION

It is apparent that from the conservation of total surface area of epidermal cells after d 60 (Fig. 7.3) that the increase in fruit surface area after that time was achieved by a mainly cell deformation (Fig. 7.2), rather than cell expansion and cell division (Fig. 7.1). It appears that the material of the cell wall loses its extensibility at this stage and growth-induced straining of the skin causes parts of the adjoining vertical (anticlinal) walls to detach and be reoriented so as to form extensions of the outer (periclinal) wall. This seems likely to have a significant influence on structural and functional integrity of the overlying cuticle.

While these cellular changes were going on beneath it, the non-living cuticle must have expanded to accommodate them. In the horizontal plane the cuticle appeared to be isotropic. The cuticle apparently accommodated fruit growth by plastic deformation with the cuticular layer 'flowing' so as to adjust itself to the expanding surface beneath. All else being equal, plastic deformation would cause the cuticle to become thinner. As this was clearly not the case, growth must have been accompanied by the secretion of new cuticular material by the epidermal cells.

With this understanding, it seems likely that the cuticle must have been in a state of more-or-less continuous tension. It follows that failure at any point due, say, to mechanical or chemical (spray) damage or to an excessive

expansion rate, would release the tension which had built up. This would have released stored mechanical energy so that a crack, once initiated, would have tended to 'run', explaining the reticulate nature of the cracking observed (Fig 7.5 a).

Maguire, Lang, et al. (1998b) were able to induce and increase microcracking by applying artificial strain to pieces of 'Braeburn' apple skin. Leaving aside skin damage associated with the use of spray chemicals and that induced by microorgansisms (Opara et al., 1997), it would seem plausible to interpret the remainder of cuticular microcracking as probably growth induced, a failure of the cuticle to accommodate the strains associated with tissue expansion beneath. This being the case, microcracking would likely be associated with periods of especially rapid area expansion of the fruit. The 'Red Delicious' fruit in this study experienced just such a period of expansion 50-60 d after bloom (Fig.7.1). Apple fruit are particularly prone to russet (Walter, 1967) suggesting that the two processes are perhaps causally linked.

The cracks started from the outside of the cuticle and worked inward, which is consistent with expansion-induced damage. Given that the cracks propagated to a similar depth throughout those cuticles observed, this indicates that the cuticles were anisotropic in cross-section. There were at least 2 layers of materials with differing properties when strained. The outer layer, which was thinner was less strainable and more brittle than the inner layer. The rounding off at the point of the cracks indicated that, although more brittle, the

outer layer was still plastic.

Fine structure models developed from polarised light and transmission electron microscopy (Sitte and Reiner, 1963; De Vries, 1968; Wattendorff and Holloway, 1980) suggest that there is a thin outer layer of the cuticle termed 'cuticle proper', which shows high crystallinity associated with waxes. This overlays a thicker more amorphous layer which contains cutin termed 'cuticular layer'. In addition Schonherr and Riederer (1988) and Riederer and Schreiber (1995) developed functional models for the cuticle independently. In both models, the cuticle consisted of an inner volume with negligible barrier properties and an outer volume which imparted the barrier to water movement and was suggested to contain waxes.

Fatigue cracks in metal occur with strains after approximately 10^5 cycles (Thomas; pers comm.). The fruit has a diurnal cycle of expansion and contraction (Lang ,1990) that would produce approximately 150 cycles per season. This would suggest that these cracks are a brittle fracture rather than that induced by fatigue.

Two other factors may also be involved in strain fractures of the cuticle, both possibly underlying the observed regional and seasonal variation in the incidence of cuticular microcracking related disorders. First, the visco-elastic properties of most materials, and of organic polymers in particular, are well known to be reduced at low temperatures when they become markedly stiffer and less extensible(King and Cather, 1988). This is also true of grape

skin where the very thick cuticle forms an important structural component (Lang and During, 1990). Low temperature during a period of rapid fruit growth would therefore be a potentially damaging combination. We also know that diurnal cycles of fruit water status (Lang, 1990) result in rates of fruit expansion during the evening and at night 2-3 times greater than those calculated from the average growth rate of the fruit. Significantly, these short bursts of expansion growth also occur during the cooler part of the day.

A further possible mechanism that could contribute to cuticular fracture is illustrated in Fig. 7.6 and related to the tendency for epidermal cells to deform as parts of their adjoining periclinal walls became detached and reoriented to form part of the outer surface. The cuticle apparently adhered reasonably well to the cell wall (*viz.* cuticular detachment during sectioning for microscopy was rare). The small region (between a,b and shaded dark in Fig. 7.6) would therefore have been subject to particularly high rates of strain with the result that failure would have been more likely in these regions. This interpretation is consistent with the observation that cuticular microcracks tend to follow the line of the periclinal cell walls rather than running across the surface of the epidermal cells (Figs. 7.5 a and b).

In a young, rapidly-growing fruit, a network of interconnecting microcracks in the cuticle, isolated 'islands' of cuticle would be left behind by the expanding cells with the result that continued fruit growth would expose increasing areas of flesh to the outside (Fig 7.7), possibly leading to the

development of russet.

Overall, this study has shown that the last stages of fruit growth are mainly due to expansion and deformation of existing cells. The cuticle accommodates the fruit growth by plastic deformation and secretion of new material. When the fruit growth outstrips the ability to lay down new cuticle and the strain becomes too great the outer wax rich layer of the cuticle fractures which releases built up mechanical energy forming a reticulate crack network. This cracking isolates tiny islands of intact cuticle as the fruit continues growing and provides a possible causal mechanism for russet, which develops during growth and cracks which affect susceptibility to mass loss and shrivel after harvest.

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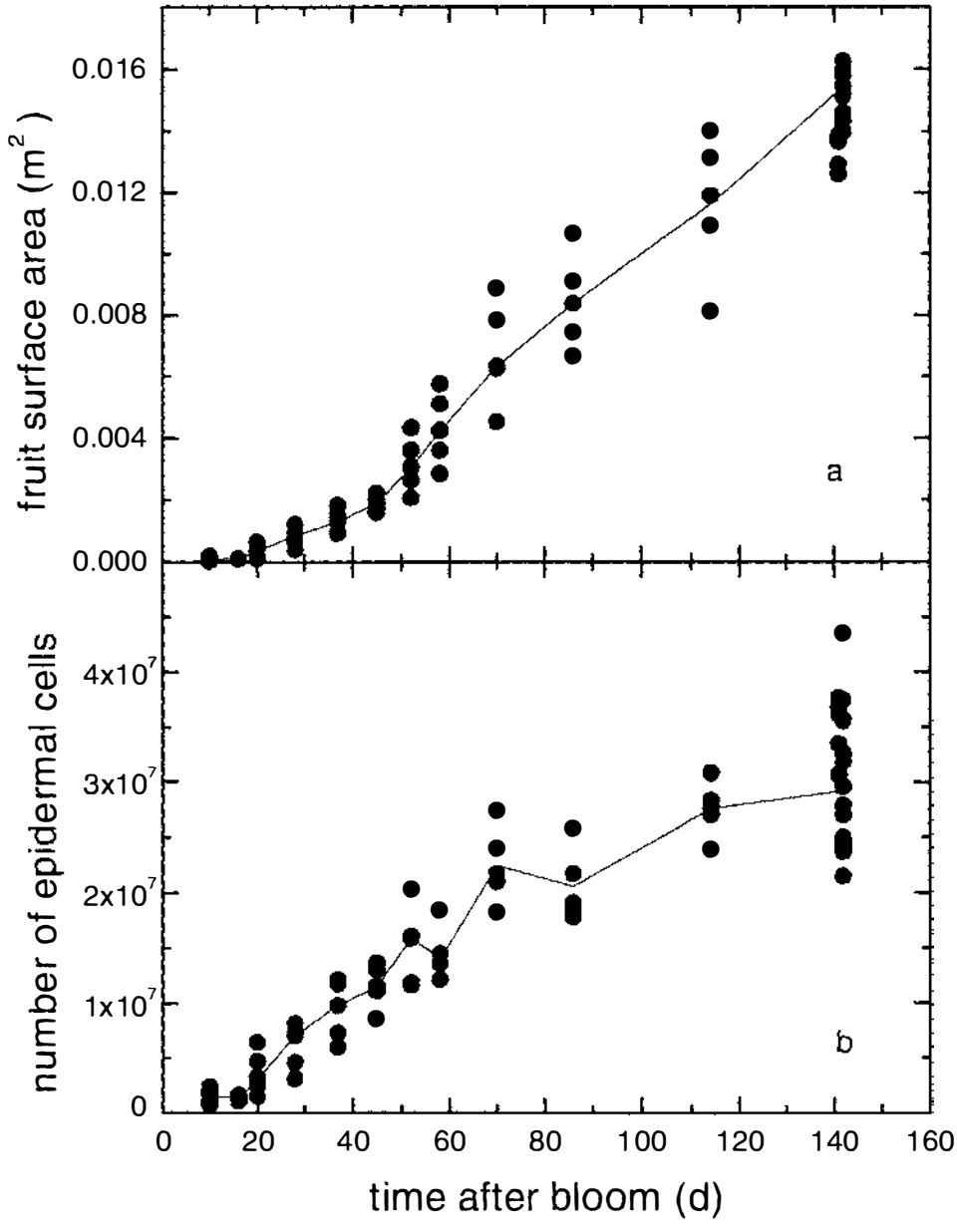

FIG. 7.1. Increases in a) fruit surface area and b) epidermal cell number of 'Red Delicious' from just after bloom to near harvest. Lines connect median values at each time.

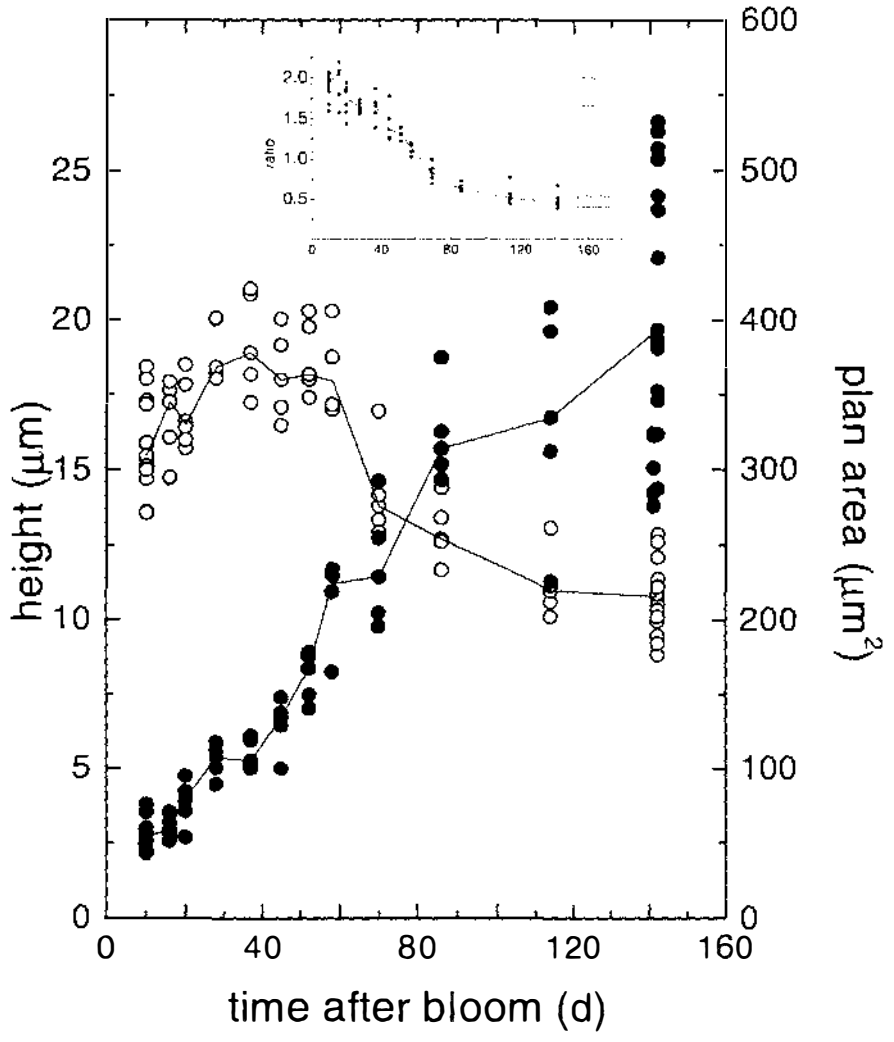

FIG. 7.2. Changes in plan areas (●) and height (○) of epidermal cells of 'Red Delicious' apples from just after bloom to near harvest. The inset shows changes in the aspect ratio (height by width) for the same period. Lines connect median values at each time.

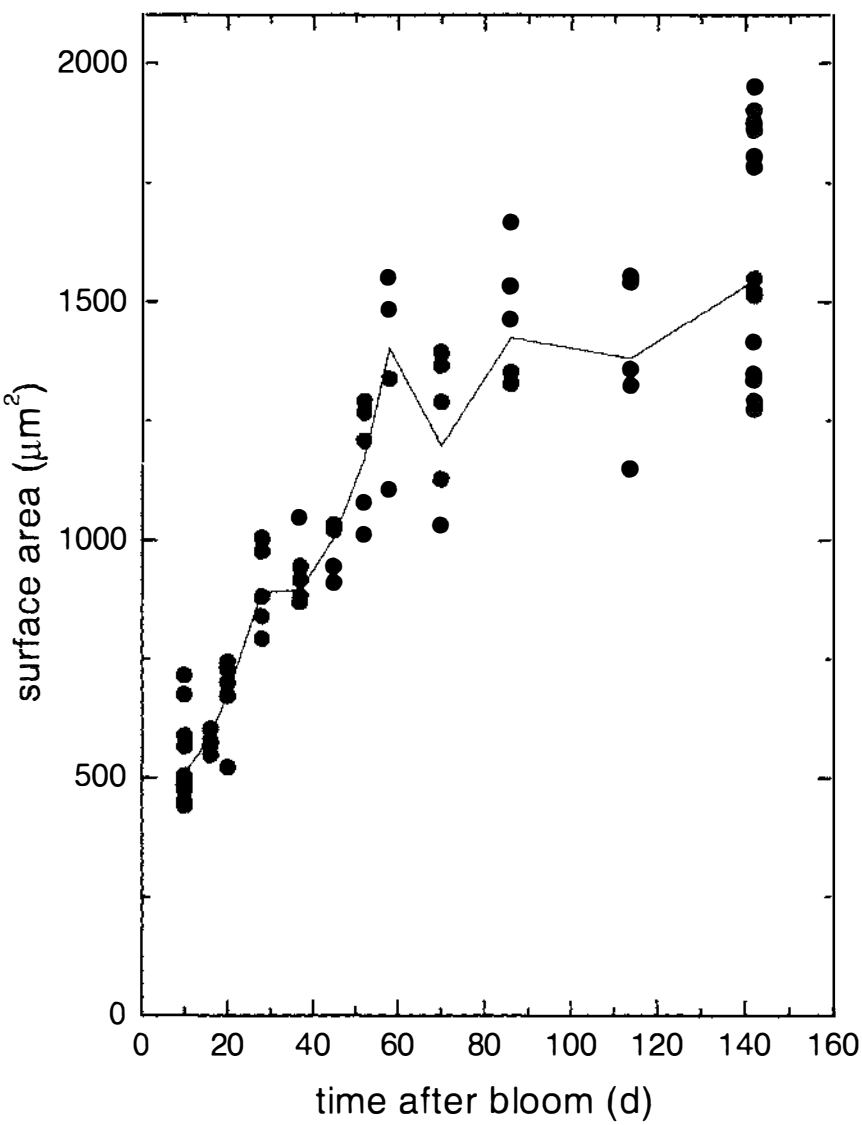

FIG. 7.3. Change in epidermal cell wall surface area (including top, bottom and side walls) in 'Red Delicious' apples from just after bloom to near harvest. Lines connect median values at each time.

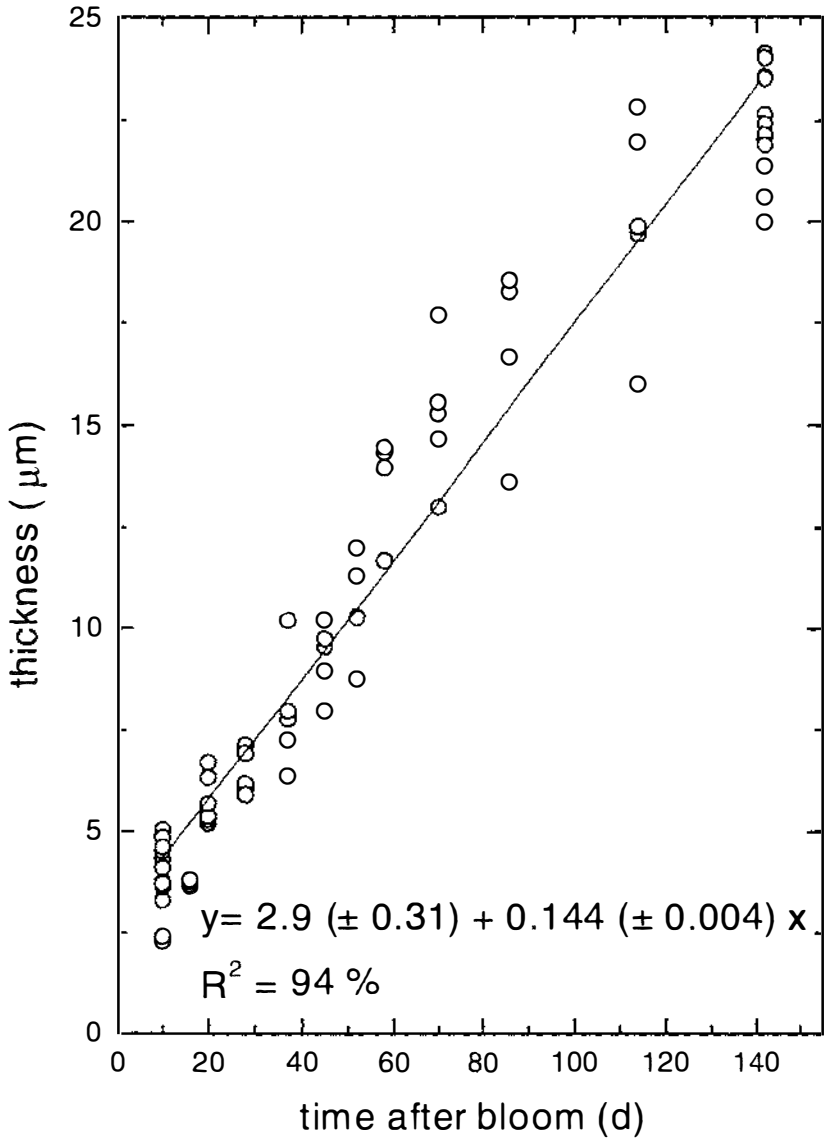

FIG. 7.4. Changes in thickness of cuticles of 'Red Delicious' apples from just after bloom to near harvest.

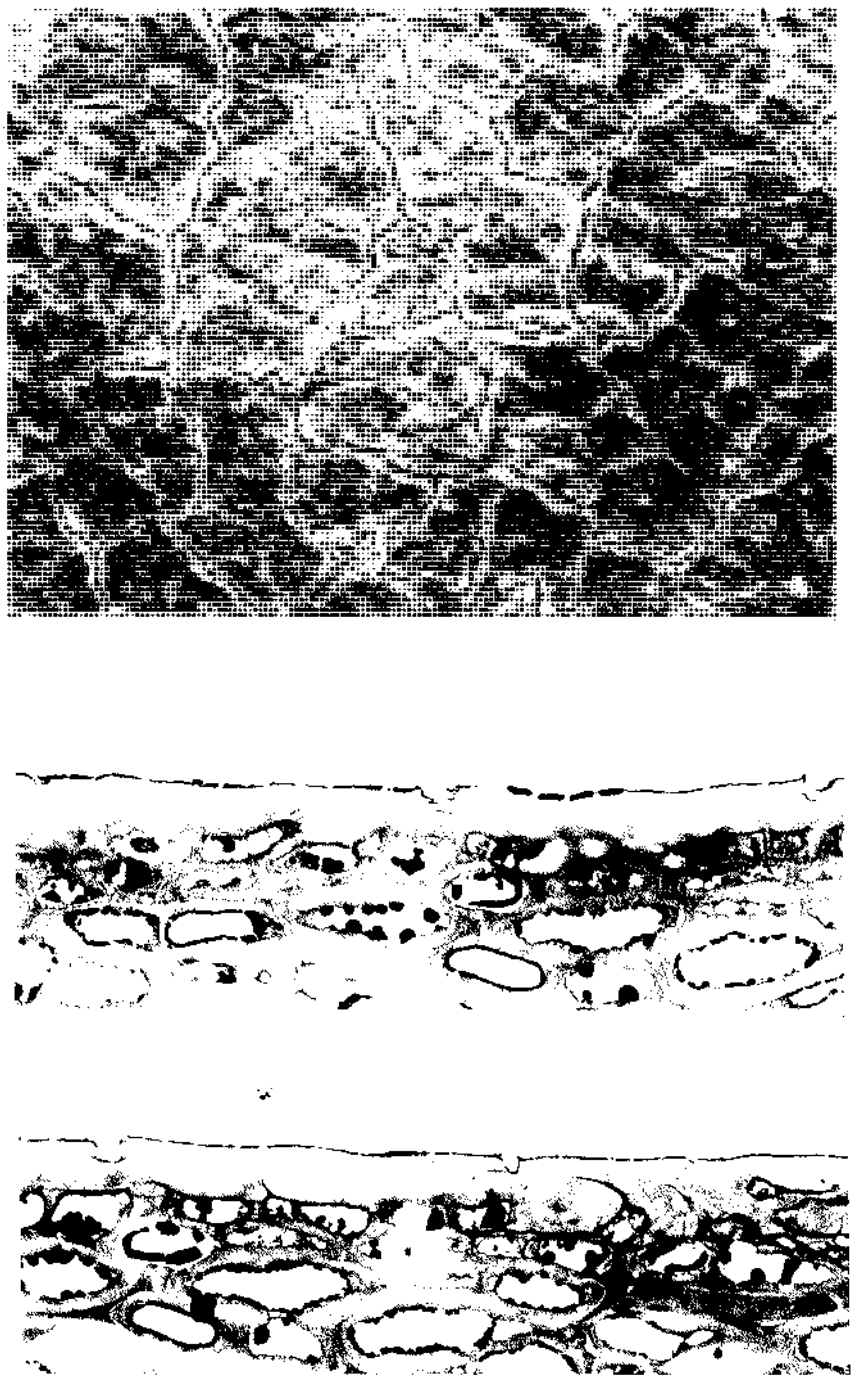

FIG. 7.5. a) Scanning electron micrograph (mag 200 ×) and b) light micrograph (mag 500 ×) of transect from a region of microcracked cuticle of 'Braeburn' apple.

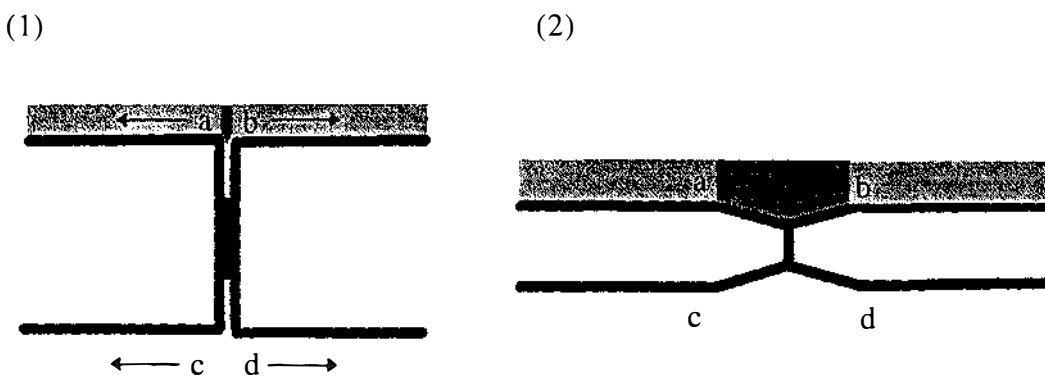

FIG.7.6. Schematic diagram showing the detachment, deformation and reorientation of a part of the anticlinal cell walls of adjoining epidermal cells and the region (shaded dark) of the cuticle likely to suffer particularly high rates of strain.

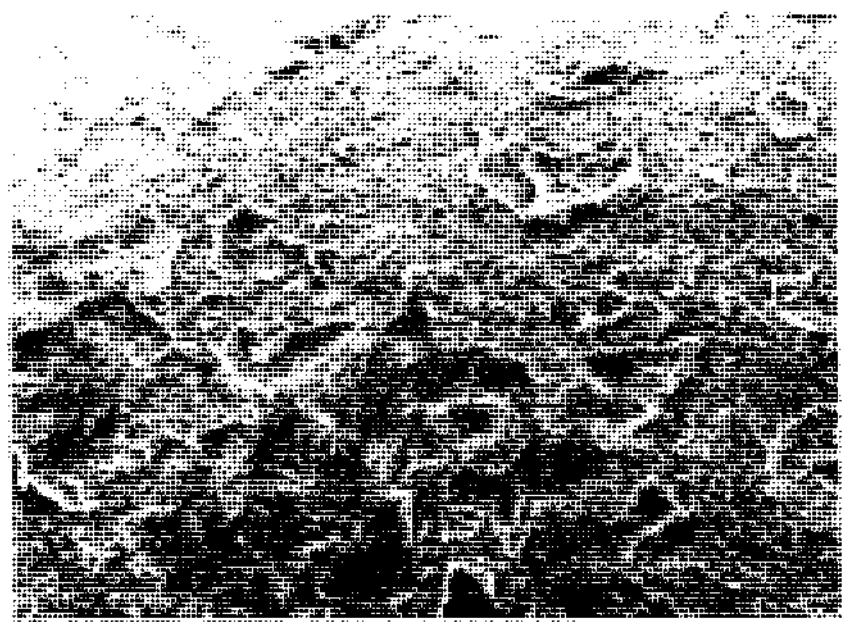

FIG. 7.7. Scanning electron micrograph of a region of russetted epidermis of 'Braeburn' apple (mag 50 ×).

Chapter 8

Effects of Relative Humidity and Time after Harvest on the Water Vapour Permeance of 'Braeburn' Apples

Effects of relative humidity and time after harvest on the water vapour permeance of 'Braeburn' apples.

Kate Maguire[a], Nigel Banks[a], and Sandy Lang[b]

[a]*Centre for Postharvest and Refrigeration Research and Department of Plant Science, Massey University, Private Bag 11 222, Palmerston North.*

[b]*HortResearch, Palmerston North Research Centre, Private Bag 11 030, Palmerston North*

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Style: This chapter is in the style of the Postharvest Biology and Technology Journal

Abstract

Transpiration is the dominant process in the rate of total mass loss in harvested apples. To facilitate prediction of rate of water loss of apples in different environments, the current work characterised changes in barrier properties of 'Braeburn' apples as affected by time (up to 30 days), relative humidity (8 levels) and temperature (2 levels). Water vapour permeance decreased with time in all cases but this was more pronounced for fruit in the lower humidity environments and for those individual fruit with higher initial values for permeance. Permeance was slightly (5%) higher at 20 °C than at 5 °C (19 and 18 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$, respectively; SED = 0.164, df = 301). The decline in permeance with time was greater for fruit at 5 °C than fruit at 20 °C. Fruit at 20° C and high driving force had greater decline in permeance for the same percentage mass loss . There was a strong linear relationship between driving force and the steepness of decline in relationship between water vapour permeance and percentage mass loss. A tentative conceptual and mathematical model was developed to explain the dependence of permeance at a give time on the driving force for water loss, initial permeance and temperature. The model should be valuable in explaining changes in permeance with time after harvest in analogous studies and in exploring likely effects of alternative storage and marketing scenarios on total mass loss in commercial fruit handling.

Keywords: *Malus domestica*, water loss, mass loss, relative humidity, permeance

8.1 Introduction

Only a five percent loss in mass can cause an apple to develop an unattractive shrivelled and wilted appearance and affects texture (Hatfield and Knee, 1988). Harvested apples lose mass through the processes of transpiration and respiration. Maguire, Banks et al. (1998) showed that loss of mass of apples through respiration was less than 10% of total mass loss in conditions typical of postharvest handling for the New Zealand industry; water loss was the dominant contributing process.

The cuticle forms the outermost layer of the fruit skin, and is the barrier which prevents excessive loss of water by evaporation from the plant to its environment (Holloway, 1982). In a constant environment the permeance of the fruit surface to water vapour (P'_{H_2O} ; $mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$) under prevailing conditions can be calculated from rate of water loss (r'_{H_2O} ; $mol \cdot s^{-1}$) using the steady state solution of Fick's first law of diffusion (Nobel, 1991):

$$P'_{H_2O} = \frac{r'_{H_2O}}{\Delta p_{H_2O} A} \qquad (8.1)$$

provided Δp_{H_2O} (Pa; the difference in partial pressure of water vapour between the environment and inside the fruit) and A (m^2; the surface area of fruit) are known. Similarly, rate of water loss from apples can be predicted by rearrangement of Eq. 8.1 providing fruit and environmental characteristics are known. Prediction of mass loss in storage would allow management decisions

regarding a population of fruit to be made, enabling fruit with better final quality to reach the consumer.

Water vapour permeance is a measure of the ease of movement of water molecules through the barrier posed by the outer layers of the fruit. Using an electrical analogy, it can be considered to comprise of three components operating in parallel (Nobel, 1991): the cuticle, cracks in the cuticle and pores or lenticels. This relationship can be represented by:

$$P'_{H_2O} = \frac{A^{ck}}{A^{tot}} P'^{ck}_{H_2O} + \frac{A^{cut}}{A^{tot}} P'^{cut}_{H_2O} + \frac{A^{pores}}{A^{tot}} P'^{pores}_{H_2O} \qquad \textbf{(8.2)}$$

where: A is the area and P'_{H_2O} is the water vapour permeance of the component, with *ck* the superscript for cracks, *cut* for cuticle, *pores* for pores and *tot* for total area.

Schonherr and Riederer (1988) demonstrated pronounced asymmetry of cuticular membranes, with the barrier of cuticular waxes located near the outer surface, whilst the large part of the cuticle adjacent to the inner surface had negligible barrier properties and resembled a polymer matrix (in functional properties; Riederer and Schreiber, 1995)

The functional model is conceptually very similar to fine structure models developed from polarised light and transmission electron microscopy studies (De Vries, 1968; Sitte and Rennier, 1963; Wattendorff and Holloway, 1980). All of these studies indicated a layered structure with a thin, highly structured, crystalline zone at the fruit surface coupled with an inner, thicker, amorphous layer beneath.

Previous work has shown that fruit with high permeances have large numbers of cracks in the cuticle (Maguire, Lang et al., 1998). Maguire, Holmes et al. (1998) have observed cracks in the cuticle of mature 'Braeburn' apples that did not penetrate the cuticle fully and only occur in the outer region.

Schonherr (1976a) reported that the water vapour permeability of cuticular matrices (cuticles with the soluble cuticular lipids removed) increased with increasing water content through sorption. Chamel et al. (1991) found that isolated tomato, apple and pepper fruit cuticles absorb increasing amounts of water with increasing atmospheric water vapour pressure. Schonherr and Schmidt (1979) found that water vapour permeability of isolated cuticles from citrus leaves and from eggplant fruit demonstrated a dependence on the water vapour content of the air. Water vapour permeance of whole fruit, increased with increasing relative humidity or decreasing Δp_{H_2O} (Fockens and Meffert, 1972; Lentz and Rooke, 1964; Sastry et al., 1978; Shirazi and Cameron, 1993; Smith, 1933). Lentz and Rooke (1964) reported that P'_{H_2O} of apples cultivars 'Red Delicious', 'Rhode Island Greening', 'Bankcroft' and 'Sandow' were very dependent on the Δp_{H_2O} whereas the cultivars 'McIntosh', 'Northern Spy' and 'Cortland' were less so. Fockens and Meffert (1972) found that the permeance of 'Jonathan', 'Laxton's Superb', 'Lombartscalville', 'Golden Delicious' and 'Belle de Boskoop' increased when the relative humidity was increased.

Assuming that the level of cuticular water sorption, and hence

permeance, eventually comes to steady state with the surrounding environment, it might be expected that rate of decline of permeance in harvested apples would reduce with time after harvest as the transfer process approached steady state. On this basis, environments with greater drying power (high Δp_{H_2O}) would cause larger drops in permeance than those with low Δp_{H_2O}. However, many researchers still assume that the permeance of skin is not affected by water vapour pressure in the environment (Chau et al., 1988; Gaffney et al., 1985; Sastry, 1985). Before accurate predictions can be made with Eq. 8.1, the relationships between water vapour permeance, time, Δp_{H_2O} and temperature need to be characterised. The current work examined the effects of different levels of Δp_{H_2O} on whole fruit P'_{H_2O} of harvested 'Braeburn' fruit over a period of 30 days storage at two temperatures.

8.2 Materials and Methods

'Braeburn' fruit (200, mass 160-190 g) were picked on 15 April 1997 from at the Fruit Crops Unit, research orchard of Massey University, Palmerston North. P'_{H_2O} was calculated for each fruit using Eq. 8.1. Fruit were placed in an air flow of $\approx 3\ m \cdot s^{-1}$ and, after 6 h equilibration, rate of mass loss from each fruit was determined over a 16 h period using a balance (0.001 g Model PM1206; Mettler Toledo, Switzerland) and was not corrected for respiration. An average relative humidity was determined by wet and dry bulb temperature readings (thermistor probes CM type, U bead, $\pm$ 0.2 ^{o}C; Grant

Instruments, Cambridge, U.K.). Skin temperature was logged during the period of measurement by recording (Squirrel model 1206, Grant Instruments, Cambridge, U.K.) thermistor probes (FF type, U bead, ± 0.2 °C; Grant Instruments, Cambridge, U.K.) inserted under the skin of several sample fruit. Averages were used to calculate Δp_{H_2O} using psychrometric relationships (Monteith and Unsworth, 1990a; Monteith and Unsworth, 1990b; Tetens, 1930). Surface area was calculated using an equation from Clayton et al. (1995).

Fruit were then divided into 20 groups of 10, each group containing individuals with a similar water vapour permeance. One fruit from each group was randomly assigned to each of 10 treatments so that each treatment contained 20 fruit with a range of water vapour permeances. Each treatment group was placed in one of 10 identical pieces of apparatus, each of which comprised an 18 L plastic food storage container with wet and dry bulb recording thermometers, a fan to circulate air, and a small hole to prevent pressure and CO_2 build up (Fig. 8.1). Treatments, comprising different levels of relative humidity were achieved using paper sachets with either 0, 5, 10, 15, 25, 50, 75, 100, 125 or 150 g of silica gel. The silica gel was replaced daily to maintain a constant humidity within the containers. All containers were held in a room at 20 °C.

Following a 24 h equilibration period, fruit were removed and individually weighed at 20 °C on days 0, 1, 2, 3, 4, 7, 8, 15, 16, 31 and 32 (0.001 g sensitivity Mettler, Switzerland) before being returned to their

containers. Fruit temperature was recorded for 5 fruit per box using an infrared thermometer (± 0.1 °C; model 110.2L; Irricrop Technologies Pty Ltd, Australia). Wet and dry bulb temperatures (±0.2 °C; thermistors) were logged continually (every 60 min; Model CR10; Campbell Scientific Inc, USA). Respiration rates were determined by measuring the increase in CO_2 partial pressure with time inside each box, during a brief period in which the boxes were totally sealed. Water vapour permeances, corrected for respiration, were determined, using Eq. 8.1, within the equilibrated box environment on days 0, 1, 3, 7, 15, and 31. The experiment was repeated at 5 °C.

A non-linear curve of the following form, was fitted for each fruit.

$$P'_t = a(t+1)^b \qquad \textbf{(8.3)}$$

where: P'_t is the permeance at time t, t is the time in days, and a and b are parameters of the equation representing the y intercept and steepness of decline in P'_t respectively. Non linear curve fits were generated using NLIN procedure in SAS (SAS Institute 1988) using the DUD method. Parameters a and b were then analysed using a GLM (general linear model) procedure in SAS (SAS Institute 1988) to determine the effects of Δp_{H_2O} and the initial water vapour permeance of each fruit.

8.3 Results

The humidities that resulted from the presence of silica gel at 20 °C were 65, 70, 82, 86, 88, 90, 91, and 94 % whilst those which developed at 5

^{o}C were 72, 73, 74, 81, 84, 90, 91 and 93 %. The two highest humidities at both temperatures (not reported) were omitted from analysis due to the difficulty in obtaining consistent data.

Water vapour permeance of all fruit declined with time but this was more pronounced for fruit in the lower humidity environments (Fig. 8.2 a). There was a strong linear relationship between *b* values and the driving force for water loss (Δp_{H_2O}; Pa) at both 20 ^{o}C and 5 ^{o}C (R^2 = 81 % and 55 %, respectively; Fig. 8.3 a and b). This decrease in water vapour permeance was also more pronounced for individual fruit with higher initial water vapour permeances ($P < 0.0001$), irrespective of the environment they were in (R^2 = 65 and 21%, respectively; Fig. 8.3 c and d).

Average estimates of *a* were strongly related to average initial water vapour permeance at both 20 ^{o}C and 5 ^{o}C, irrespective of driving force for mass loss (R^2 = 90 % and 97 %, respectively; Fig.8.4).

Average values for *a* were slightly (5%) higher at 20 ^{o}C than at 5 ^{o}C (19 and 18 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$, respectively; SED = 0.164, df = 301). Average values for *b* were 20% smaller at 20 ^{o}C than at 5 ^{o}C (-0.069 to -0.086, respectively; SED = 0.004, df = 301).

Values for *b* obtained for plots of water vapour permeance versus the percentage loss of mass from the fruit for the fruit at 20^{o} C (Fig. 8.2 b) were increasingly negative at high levels of Δp_{H_2O} (R^2 = 34%). There was a strong linear relationship between Δp_{H_2O} and the steepness of decline in relationship

between water vapour permeance and percentage mass loss (data not shown, P < 0.0001).

The variable *a* was closely related to permeance at time 0 and *b* was strongly, linearly related to driving force for water loss, permeance at day 0 and with a strong effect of temperature. We can describe the dependence of the permeance at time *t* (P'_t) on the driving force of water loss and initial permeance.

$$P'_t = P'_0(t+1)^{\{c\Delta p_{H_2O} P'_0(e-T)\}} \quad \textbf{(8.4)}$$

where: P'_0 is permeance measured on day 0, *t* is time in days, *T* is temperature (°C), and *c* and *e* are the new model parameters. The new combined model replaced the original *b* with the product of *c*, Δp_{H_2O}, P'_0 and (*e*-*T*). Fig. 8.5 shows the new model on data shown previously in Fig. 8.2. The model fitted well with the exception of the occasional fruit, which had also been poorly described by the individual component models (e.g. Fig 8.5, fruit held at 20 °C, mid Δp_{H_2O}). Overall, the R^2 was 98 %; values for *c* and *e* were -490 (SE = 236, df = 1629) and 35.85 (SE = 0.98), respectively.

8.4 Discussion

Freshly picked fruit have high turgor and the cracks on their surfaces may be presumed to have a maximum area and thus contribute maximally to fruit permeance. As the fruit remains in storage, loss of water would result in turgor reduction and some closing of cracks and hence a decrease in their

fractional area and a decline in overall fruit permeance. Lower relative humidity would accelerate mass loss and lead to more rapid reductions in turgor and water vapour permeance.

If the reduction in water vapour permeance is a response to turgor loss then plots of water vapour permeance versus percentage total mass loss for fruit held in differing environments should lie along the same line regardless of the relative humidity at which fruit are kept. However, as shown in Fig 8.3 b fruit held in low relative humidity environments again had a steeper reduction in water vapour permeance than those in high relative humidity environments. If the decline in water vapour permeance was solely a turgor response this line would be horizontal. The significant slope indicates that there is some other physicochemical response to the environment occurring.

The cuticular layer (the inner volume) is thought to mainly consist of the polymer matrix with a small number of waxes throughout (Wattendorff and Holloway, 1980). Thus, where there are cracks in the outer layer of the cuticle there would be little barrier to the absorption of water into the cuticle and water content beneath the cracks might be expected to be particularly sensitive to the surrounding relative humidity. Polymer matrix membranes have polar functional groups (Schonherr, 1982) which provide sites for hydration, particularly ester and free hydroxyl groups (Chamel et al., 1991). As the humidity increases the water content of the matrix also increases, through adsorption of water molecules to the polar groups (Chamel et al., 1991). This

hydration can create tortuous hydrophilic pores if the polar functional groups are located close enough to each other. The polar pores increase the mobility of water diffusion through the matrix membrane (Schonherr, 1976a). The response of polymer matrix membranes to water activity (relative humidity) has been observed with many cuticles (Schonherr, 1976b; Schonherr and Schmidt, 1979; Schonherr and Merida, 1981).

If the cracks were the only components in terms of both area and permeance that were responding to the environment then water vapour permeance would be expected to drop very rapidly initially and then level out at a steady lower level when all the cracks had closed up. However, water vapour permeance continued to decline over quite extended periods, an effect that might be due to changes in the water vapour permeance of the outer cuticle.

Water movement into the cuticle is more difficult when it is intact i.e. not cracked (Schonherr and Riederer, 1988). However, absorption of water by intact cuticles has been documented for cuticular membranes of tomato, pepper and 'Golden Delicious' apple fruit (Schonherr and Schmidt, 1979). The ability of the cuticle to respond to the relative humidity is dependent on the soluble cuticular lipids, their physical arrangement and resultant transport properties based upon the existence of polar pores through the hydrophobic materials (Schonherr and Schmidt, 1979). The data presented here would be consistent with the proposition that the arrangement of soluble cuticular lipids in the

'Braeburn' apples in this study allowed the formation of some polar pores in the intact cuticle which responded to changes in relative humidity.

The effect of temperature occurred over and above that due to Δp_{H_2O} (driving force for water loss) and is consistent with increases in permeance of isolated cuticles from *Citrus* leaves associated with increasing temperatures reported by Schonherr et al (1979). This effect was linked to changes in the structure of the soluble cuticular lipids. The extent of the response in the current work was smaller than that reported for the isolated cuticle study. This could be due interactions with some of the other variables which determine the water vapour permeance of fruit cuticles (e.g. cracks and pores). However, with only two temperatures included in the experimentation the shape of the relationship between water vapour permeance and temperature is uncertain; the temperature model presented here needs to be validated at a range of temperatures.

In this paper, we have characterised the effects of Δp_{H_2O} and temperature on changes in permeance of 'Braeburn' apples with differing values for initial permeance with time after harvest. We have developed a tentative conceptual and mathematical model which explains these changes. This should be valuable in explaining changes in permeance with time and environmental conditions after harvest in analogous studies and in exploring likely effects of alternative storage and marketing scenarios on total mass loss.

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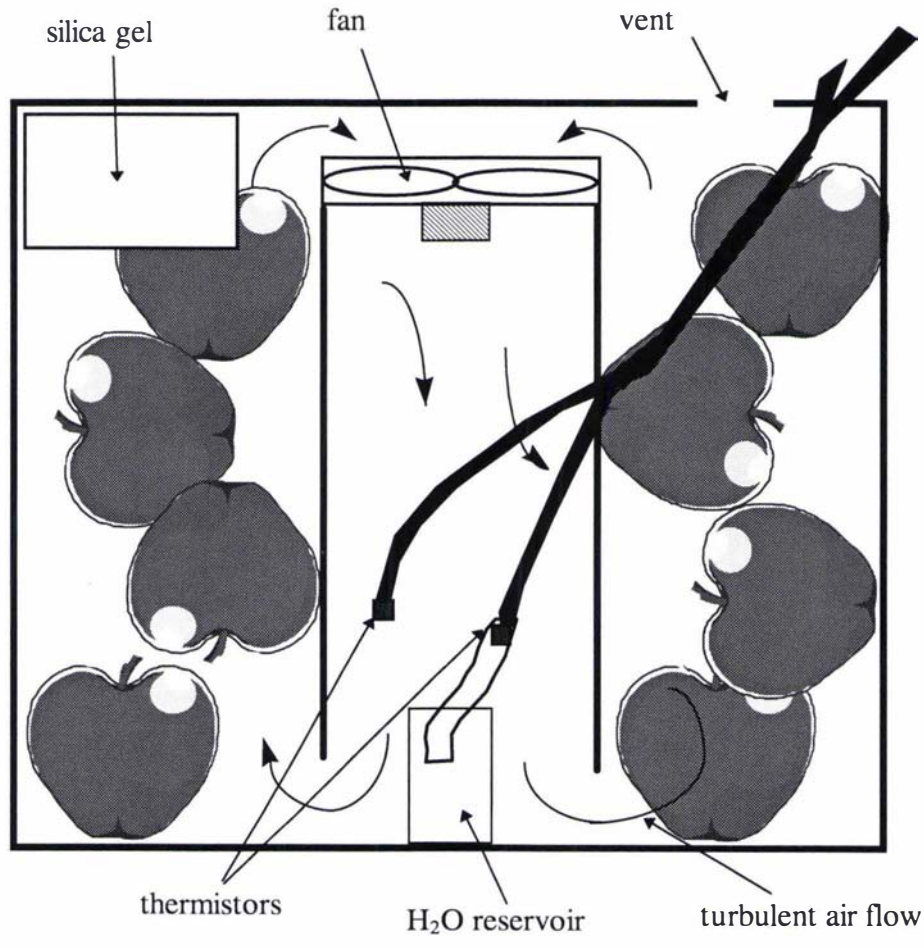

Fig. 8.1. Schematic diagram of experimental apparatus used to expose fruit to different humidity environments at either 20 °C or 5 °C.

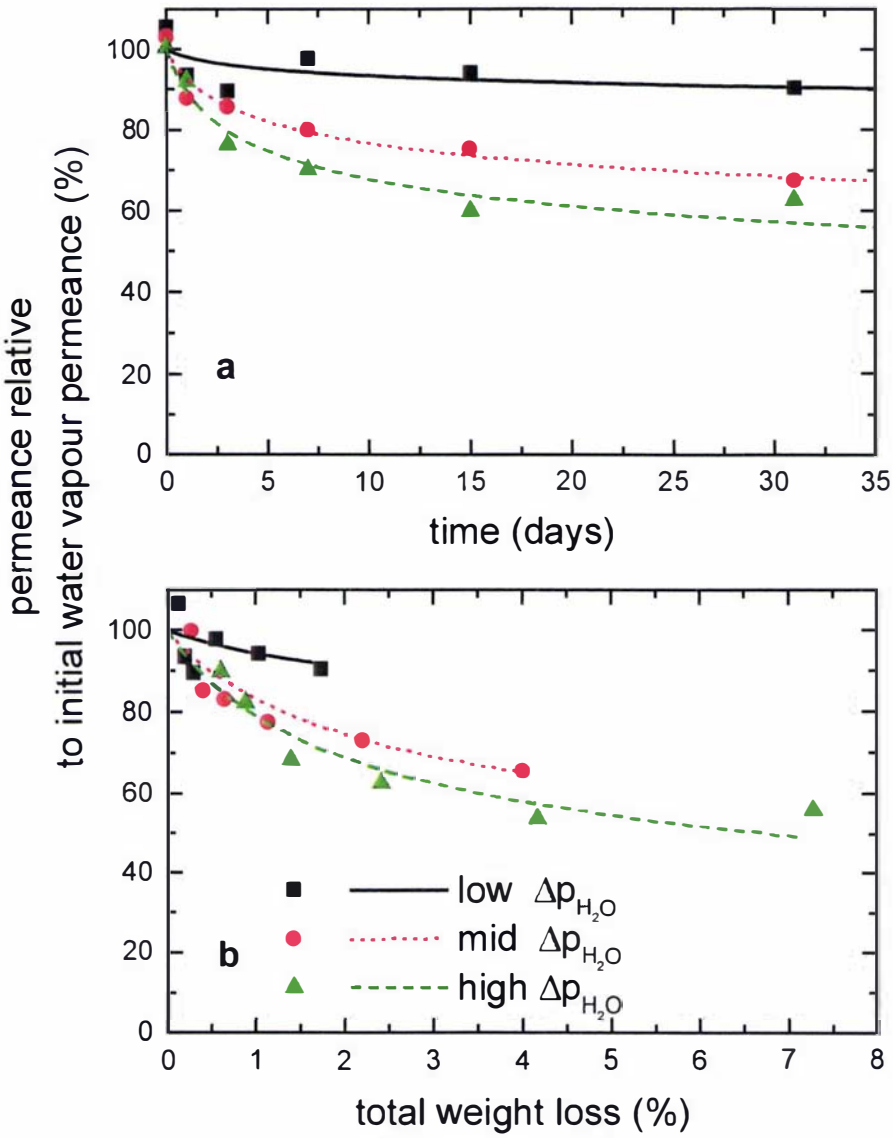

Fig. 8.2. Relationship between water vapour permeance relative to initial permeance and a) time and b) total mass loss as percentage of initial mass for 'Braeburn' apples at 20 °C.

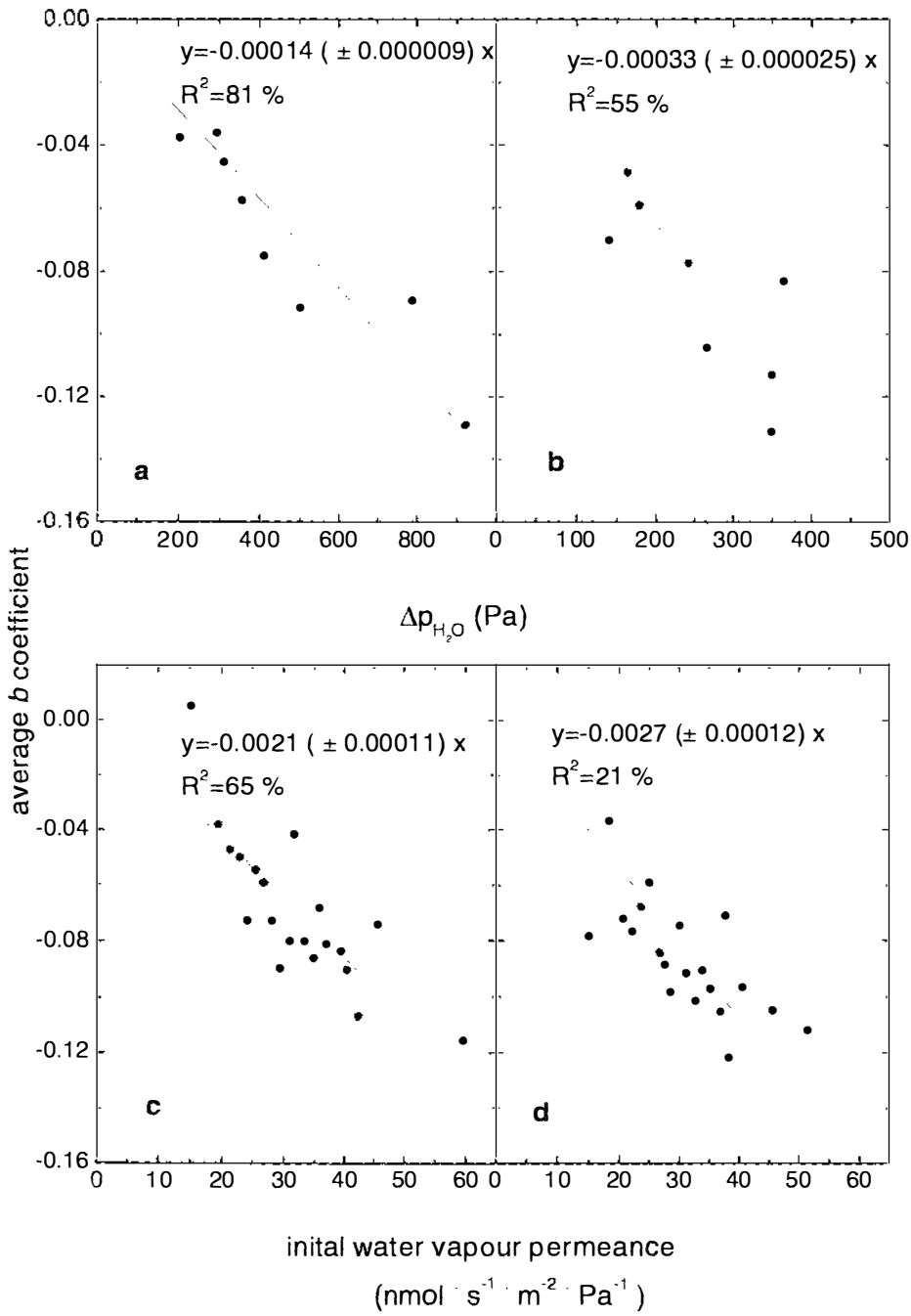

Fig. 8.3. Relationships between average estimates of curve coefficient *b* and a) and b) Δp_{H_2O} at 20 °C and at 5 °C, respectively and c) and d) initial water vapour permeance of fruit at 20 °C and at 5 °C for 'Braeburn' apples.

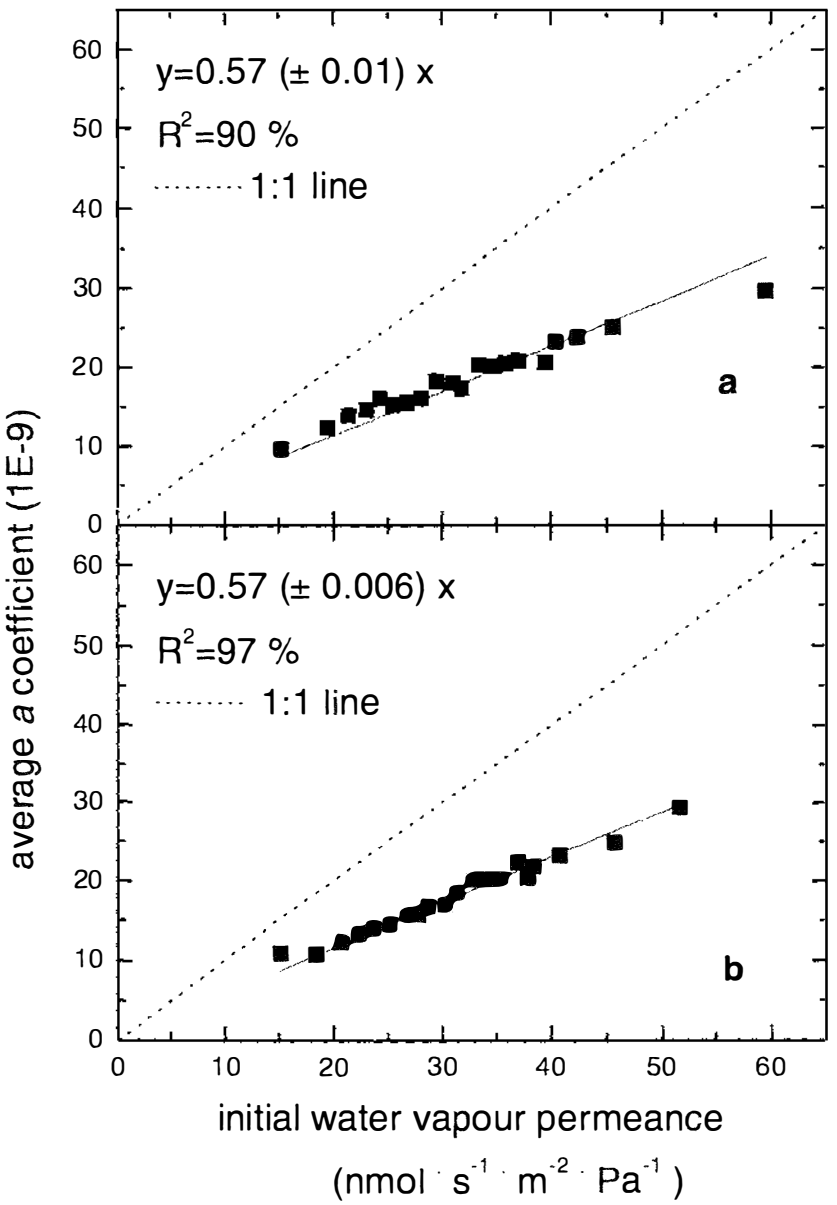

Fig.8.4. Linear dependence of average estimate of curve coefficient *a* upon initial water vapour permeance for 'Braeburn' apples kept at a) 20 °C and b) 5 °C.

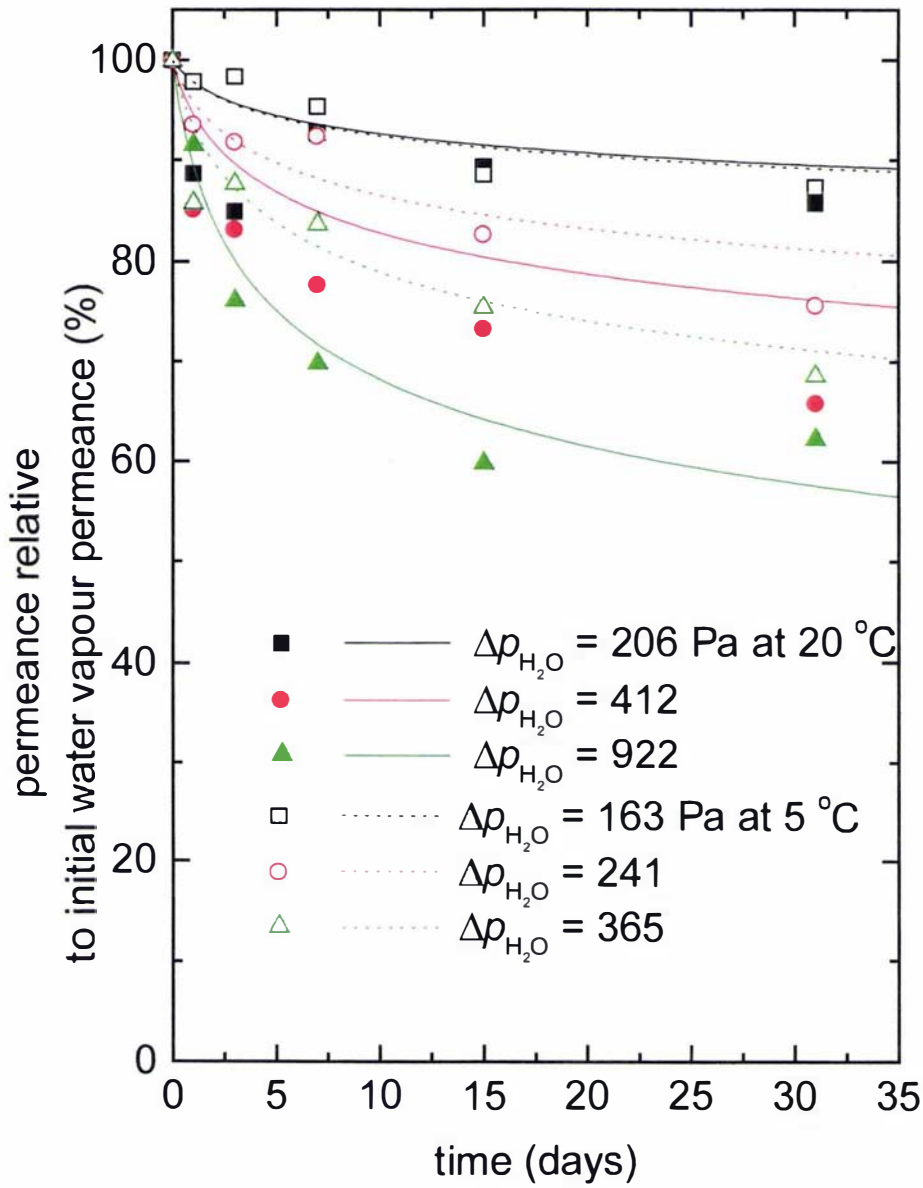

Fig. 8.5. Relationships between time and the ratio of water vapour permeance to initial permeance for individual 'Braeburn' fruit in different environments, fitted curves were generated using Eq. 8.4.

Chapter 9

Effect of Bruising on the Water Vapour Permeance of 'Braeburn' Apples

SHORT COMMUNICATION

Effect of bruising on the water vapour permeance of 'Braeburn' apples.

KATE M. MAGUIRE, NIGEL H. BANKS

Centre for Postharvest Refrigeration Research, Massey University, Private Bag 11 222, Palmerston North

ALEXANDER LANG

HortResearch, Palmerston North Research Centre, Private Bag 11 030, Palmerston North

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Style: This chapter is in the style of a short communication for the New Zealand Journal of Crop and Horticultural Science.

Abstract

'Braeburn' fruit (*Malus domestica* Bork.) were subjected to impact energies of up to 1.97 J to determine the effect of bruising on their water vapour permeance. There was a linear relationship between bruise volume and impact energy (R^2 = 93%), and a strong power relationship between bruise area and impact energy (R^2 = 97 %). Change in water vapour permeance was linearly related to bruise area (R^2 = 87 %). However, the mean increase in water vapour permeance for the most severe bruise treatment was only 5.8 % (SED = 1.75, df = 20), which is small compared to the natural variation in water vapour permeance within a population of fruit. The effects of bruising on visual and other aspects of quality are likely to be much more detrimental to perceived fruit quality than its effects via increased water loss.

Keywords *Malus domestica*; water loss; mass loss; permeance; cuticle; bruising; impact damage

9.1 INTRODUCTION

Apples lose mass through the processes of transpiration and respiration which cause a loss in saleable mass and thus a direct loss in returns at point of sale. Excessive mass loss can also result in a shrivelled appearance (Wills *et al.* 1989 ; Hatfield and Knee 1988) and in extreme cases this renders fruit unsaleable (Sastry 1985). Even in the absence of visible wilting, water loss can cause undesirable textural and flavour changes (Wills *et al.* 1989; Ben-Yehoshua 1987). Mechanical injuries have been reported to accelerate water loss (Kader 1992) as well as providing loci for fungal infection, stimulating respiration and ethylene production and being unsightly. Similarly Wills et al. (1989) reported that bruising damaged the surface organisation of tissue and allows a greater escape of water vapour through the damaged area. Other than these brief references, we could find nothing in the literature that characterised the effects of bruising on water loss of apple fruit. This work describes a simple exploratory study to quantify the effects of impact bruising on the water vapour permeance of 'Braeburn' apples.

9.2 MATERIALS AND METHODS

'Braeburn' fruit (mass 0.18 kg) were removed from storage after approximately 3 months. Water vapour permeance (P'_{H_2O}; $mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$) was calculated for 77 fruit using the method described in Maguire, Banks et al. (1998). Mass loss was not

corrected for respiration since this is negligible under the low humidity conditions in which these measurements are made (Maguire, Banks et al. 1998). Initial permeance was used as a basis for blocking (11 blocks); within each block, fruit were allocated at random to seven treatments. A hard, smooth hockey ball weighing 0.157 kg and with diameter of 72 mm was dropped 0, 40, 80, 160, 320, 640 or 1280 mm down a pipe onto fruit which were supported on top of several moulded papier-mâché trays to prevent bruising on the opposite side (Mowatt 1997). Corresponding impact energies were: 0, 0.062, 0.123, 0.246, 0.493, 0.986, 1.97 J. Drops were completed in random order onto a green area of fruit so that bruising was readily visible. Fruit were left for approximately 5 h at 20 °C to allow bruise development and then P'_{H_2O} was determined again. Diameter of the bruise (d^b; mm), depth (h^b; mm) of the bruised volume, and permanent deformation (Δx^b; mm) were measured (Fig: 1). Bruise area (A^b; mm^2) was calculated as:

$$A^b = \pi\left(\frac{d^b}{2}\right)^2 \tag{9.1}$$

and bruise volume (V^b; mL) was calculated using the equation developed from Schoorl and Holt (1981):

$$V^b = \frac{\frac{\pi h^b}{24}\left(3\left(d^b\right)^2 + 4\left(h^b\right)^2\right) + \frac{\pi \Delta x^b}{24}\left(3\left(d^b\right)^2 + 4\left(\Delta x^b\right)^2\right)}{1000} \tag{9.2}$$

Values for percentage change in P'_{H_2O} were analysed using a randomised complete block model with GLM procedure of SAS (1988).

results in cuticle cracking and plastic deformation of intact cuticle.

We conclude that although bruising results in small increases in fruit water vapour permeance, at the levels of bruising tolerated within the New Zealand apple industry the influence of this increase would not contribute substantively to overall total mass loss. It seems likely that an actual breach of the fruit cuticle such as the cuts and punctures, associated with impacts with rough or sharp objects (rough wood bins, stem ends etc.), would be required for substantive change in water vapour permeance to result from mechanical damage during fruit handling.

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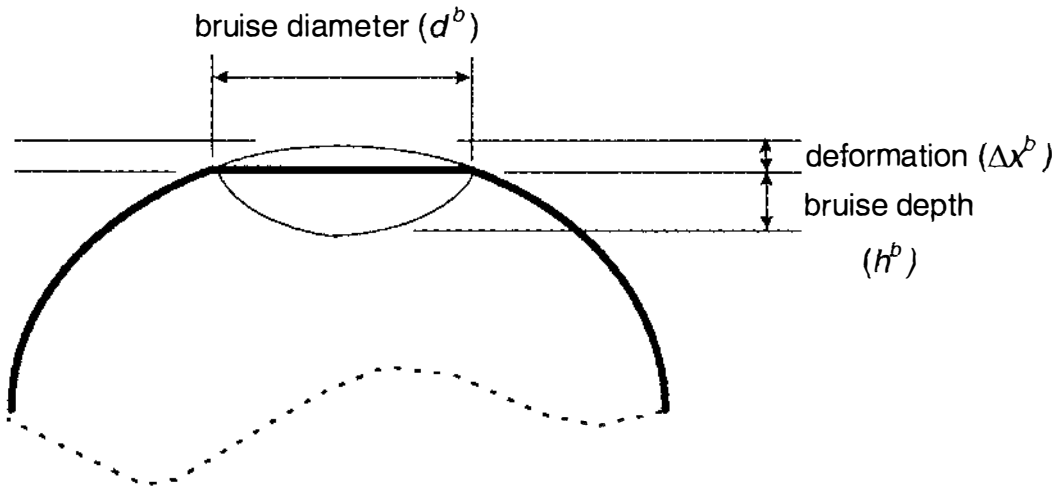

Fig. 9.1 Idealised bruise showing symbols used in bruise volume and area determination.

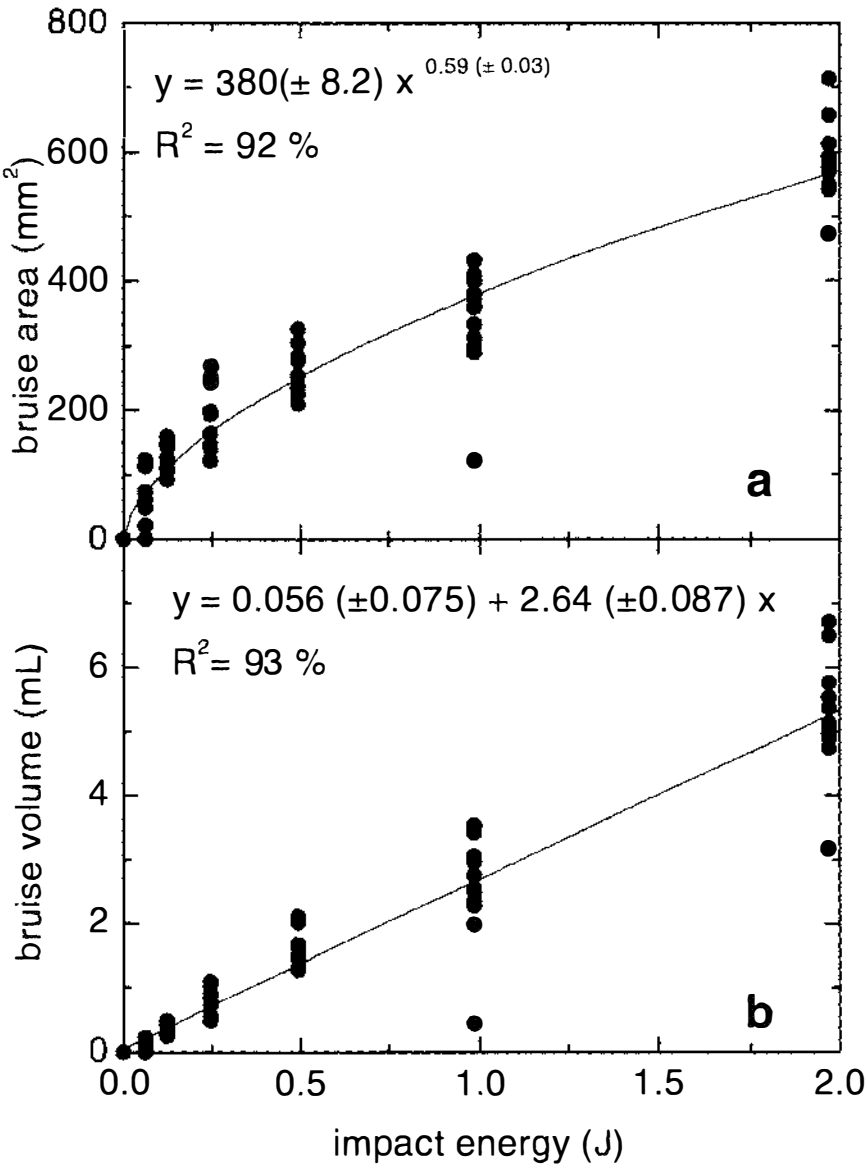

Fig. 9.2 Relationship between a) bruise area and impact energy and b) bruise volume and impact energy, for 'Braeburn' apples.

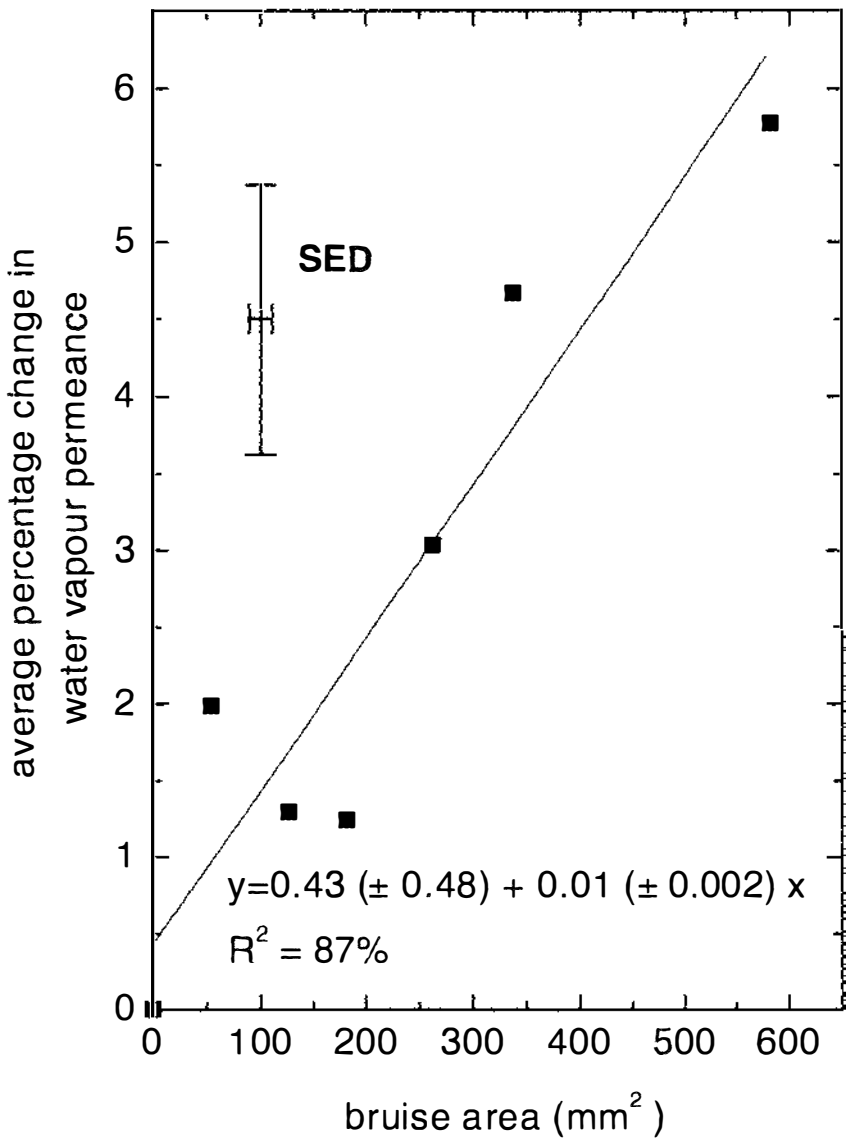

Fig. 9.3 Relationship of average (n = 11) percentage change (SED = 1.75, df = 20) in water vapour permeance and bruise area (SED = 21.4, df = 20), in 'Braeburn' apples at 20 °C.

Chapter 10

General Discussion

10.1 INTRODUCTION

In this thesis, influences of the diverse factors which affect mass loss in apples have been investigated. In this chapter, interactions of these factors are considered in terms of their implications for knowledge in this area and the ways in which this knowledge might be used to achieve beneficial outcomes for the NZ pipfruit industry. The discussion is based around an overall conceptual model of the mass loss process presented in Fig. 10.1.

10.2 TOTAL MASS LOSS

Total mass loss from fruit is most often expressed as a percentage of original mass; percentage mass loss increases as the total mass loss increases. Total mass loss at any particular time is the integral of rate of mass loss ($\dot{r}_{mass}$; kg · s^{-1}) over the different periods following harvest. It is possible to represent this mass loss as a generic function that enables prediction of the total mass loss at any particular point in the postharvest handling sequence provided rate of mass loss is known for each stage:

$$\Delta M = \int_0^t \dot{r}_{mass} \cdot dt \qquad \textbf{(10.1)}$$

Rate of mass loss alters during the different periods following harvest through changes in both the rate of water loss and the rate of carbon loss.

10.2.1 Rate of water loss

The rate of water loss at any given stage is determined by driving force for water loss (Δp_{H_2O}), barrier properties of the fruit (P'_{H_2O}) and fruit surface area (A).

10.2.1.1 Water Vapour Permeance

The water vapour permeance of the fruit skin is a measure of the ease of movement of water molecules through the barrier posed by the outer layers of the fruit. It can be considered to comprise three components in parallel: cuticle, cracks in the cuticle and pores or lenticels. Using electrical network analogy (Banks et al. 1993; Nobel 1991), this relationship can be represented by:

$$P'_{H_2O} = \frac{A^{cut}}{A^{tot}} P'^{cut}_{H_2O} + \frac{A^{ck}}{A^{tot}} P'^{ck}_{H_2O} + \frac{A^{pores}}{A^{tot}} P'^{pores}_{H_2O} \qquad \textbf{(10.2)}$$

where: A is the area and P'_{H_2O} is the water vapour permeance of the component, with *ck* the superscript for cracks, *cut* for cuticle, *pores* for pores and *tot* for total area, respectively. Differences in fruit water vapour permeance are caused by variation in each of the above components.

The permeance of intact cuticle is dependent on the chemical composition and physical structure of deposits of soluble cuticular lipids (section 2.2.1.3.2). The permeance of this composite material sets the baseline level of permeance

for a given fruit. Breaches in this layer, represented by cracks and pores, add substantially to overall permeance (section 5.4). The parallel model of diffusion of water vapour from the fruit as shown in Eq. 10.2 makes clear that affecting permeance of intact areas of cuticle would only have a significant effect on overall permeance if contributions to total permeance from cracks and pores are low. In one early study, lenticular transpiration was shown to represent an important route for water loss in some cultivars (section 2.2.1.1). From the current work, it appears that area of cracks in the cuticle is the primary contributor to the large fruit to fruit variation in permeance found in this work because micro-cracks are approximately 130 times more permeable than the cuticle (section 5.4). Thus, changes in the proportion of cracking can substantially affect whole fruit water vapour permeance (section 5.3) and it is in this area that much of the remainder of this discussion is focused.

Average values for water vapour permeance of New Zealand grown cultivars of apples can vary 2 to 3 fold (section 3.3).Genetic differences between cultivars could presumably relate to quite different cuticular composition, thickness, orientation of materials, levels of cracking and numbers of pores. These inherent differences would interact with growing conditions (section 4.4) and with harvest date to give the final level of permeance of the harvested fruit. Differences in composition could result in cuticles on fruit of various cultivars having quite different properties in terms of tendency to crack. These differences would interact with effects of those growing conditions that have

been shown to exacerbate fruit cracking including sudden changes in soil moisture, rainfall, high relative humidity, temperature and exposure to sunlight as well as cultural factors such as root stock, mineral nutrition, chemical sprays, pruning and thinning (Opara et al. 1997).

As cracking seems to be caused through stress on the fruit (section 6.3 and 7.4), it seems likely that both cuticular elasticity and growth stretch would affect incidence and severity of cuticular cracking; both of these may depend upon production practices. There has been very little quantification of what conditions do cause cracking and their subsequent effects on water vapour permeance, this would be a very valuable area for further work for the apple industry.

Water vapour permeance of 'Braeburn' apples increased significant amounts with delayed harvest (section 3.3) but it seems that maturity *per se* was not the cause of this increase (section 4.3). Changes in the wax composition of the cuticle may have contributed to this effect (section 3.4) but it seems that the major contributor was an increase in cracking, plastic deformation and fractures (section 6.4). With delayed harvest, fruit mass increases through expansion, causing further strain in the fruit skin. Cracking may be exacerbated by cooler temperatures, since visco-elastic properties of most materials, and of organic polymers in particular, are reduced at low temperatures when they become markedly stiffer and less extensible (King and Cather 1988). If this

was true then a smaller increase in strain during the cooler temperatures of autumn may cause a greater proportion of cracking than would occur during summer. Cultivars e.g. 'Cripps Pink' and 'Granny Smith' did not have an increase in water vapour permeance with later harvest date, this could have been due to them having more elastic cuticles that could strain further before cracking than cuticles of 'Braeburn' or 'Pacific Rose'™.

Growing conditions and production practices may provide simple control solutions such as changing a production practice that might reduce water vapour permeance of the fruit and hence mass loss. Further investigations of the growing conditions on water vapour permeance would be valuable.

Only minor effects of temperature on whole fruit permeance were observed in chapter 8, much smaller than those previously reported for isolated cuticles (section 2.2.1.6.3). Further work with a wider range of temperatures would be required before firm conclusions could be drawn regarding differences in response to temperature for permeance to water vapour of fruit cuticles and intact astomatus leaf cuticles. However, these differences in response are consistent with permeance of fruit cuticle being affected by cracks and pores whereas isolated cuticles in the earlier work comprised astomatus, intact cuticle.

Water vapour permeance values of both isolated and native cuticles have been

reported to be affected by relative humidity (section 2.2.1.6.1). The current study did not investigate this effect directly but did explore the interaction of Δp_{H_2O} with time after harvest. The data obtained suggested that water content of the cuticle equilibrated with the postharvest environment, changing the permeance of the intact cuticle and the inner cuticle beneath cracks.

The water vapour permeance of the fruit declined after harvest due to a loss in turgor reducing the area of cracks quickly (section 8.4). Moisture could be adsorbed or desorbed in the inner cuticle beneath the cracks and change the water vapour permeance of the crack system. As the storage period continued the influence of moisture entering the intact areas of the cuticle could be seen (section 8.4).

These above conclusions were reached for 'Braeburn' apples. The extent of the responses or shape of the curve may differ for different cultivars with the genetic differences resulting in different cuticular composition, thickness, orientation of material and the level of cracking. For a cultivar with an inherently low level of cracking we might expect the steepness of the initial reduction to be lessened. If the cuticular composition and orientation of material differed greatly from 'Braeburn' it could be possible that the permeance over the postharvest period declined as the turgor reduced and then remained more or less constant as the intact cuticle could perhaps be unresponsive to relative humidity. If this was so then water vapour permeance

of fruit in the different environments would differ in this curve only because of differences in initial cracking levels rather than response of the cuticle per se to the relative humidity.

10.2.1.2 Surface area

The power law relationship between weight and area indicates that surface area of the fruit would increase with increasing fruit mass (Eq. 2.3; Fig. 10.2 a). Conversely, surface area to mass ratio would decline with increasing fruit size (Fig. 10.2 b). On a per fruit basis, large fruit with large surface area would lose water more rapidly than small fruit with a smaller surface area with the same permeance (Fig. 10.3 and Fig. 10.4 b). On the other hand, the proportional rate of weight loss would be greater for small than for large fruit because of their greater surface area to mass ratio (Fig. 10.4 a). This could be important for the apple industry as it indicates that, on a per carton basis and all other things being equal, cartons of small fruit would lose weight more rapidly than cartons of large fruit. If conditions were such that a carton of count 125 apples with a uniform permeance of 30 $nmol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$ would lose 300 g in a certain amount of time, Fig. 10.5 shows how much weight other cartons with different counts would lose. As fruit size decreases, with an increase in count from 82 to 168, the predicted amount of weight loss by water loss on a per carton basis would increase 25% from 261 to 327 g.

10.2.1.3 Driving force (Δp_{H_2O})

In chapter 2 the physics and factors affecting driving force for water were

outlined. The relationship of the amount of water in the air can be described by Eq. 2.6. At 20 °C the amount of water the air can hold is 280 % more than it can hold at 0 °C (Fig. 10.6 a). As temperature increases, so does the potential for high Δp_{H_2O}. In addition, the amount of moisture required to humidify the air to a certain level is far greater. Fig 10.6 b highlights within each temperature the effect of relative humidity on the extent of the driving force for water loss. This has implications for the storage of fruit to minimise water loss. Rate of water loss, for a constant fruit permeance, is directly proportional to the Δp_{H_2O} (Eq. 2.1), minimisation of water loss would require reduction of Δp_{H_2O} which would be achieved by lowering fruit and air temperature and elevation of the environmental relative humidity.

In section 8.4 and 10.2.1.1 it was suggested that the water vapour permeance may increase with decreasing Δp_{H_2O}. The extent of this effect is not expected to override the influence of minimising the Δp_{H_2O} on rate of water loss. Using data previously reported (Lentz and Rooke 1964) on the variation of permeance over the range of Δp_{H_2O} below 200 Pa (Fig. 10.7 a) the influence of Δp_{H_2O} on the rate of water loss has been predicted (Fig. 10.7 b). The 30% variation in water vapour permeance of the fruit did not cause a substantial increase in the predicted rate of water loss at low Δp_{H_2O}.

10.2.2 Rate of carbon loss

Rate of carbon loss is directly proportional to the rate of respiration for the fruit. It has been suggested that under typical postharvest conditions for the New Zealand apple industry, carbon loss is only likely to account for 7% of the total mass loss (section 3.4) though, clearly, this figure would vary, based upon the effects of the various factors that influence either respiration, water loss or both. Given the climacteric nature of apples, rate of carbon loss would be expected to be higher for fruit harvested after onset of the climacteric than those harvested earlier. Based on previously published relationships between respiration rate and temperature, respiratory contributions to absolute mass loss would be expected roughly to double with every 10 ^{o}C rise in temperature (Cheng et al. 1998; Dadzie 1992; Eaks 1978; Mitchell 1992a). Kidd and West (1930) found that temperature also influences the onset of the climacteric rise in respiration rate. A decrease in temperature increased the time in the pre-climacteric period and lessened the extent of the climacteric rise.

10.2.3 Prediction of water loss

A prediction model of fruit water loss has been developed for 'Braeburn' apples by combining the two models represented in Eqs. 5.10 and 8.4, which enables prediction of the water loss in a given environment over the postharvest period, for apples with a certain proportion of micro-cracking in their cuticle.

The permeance at the end of the storage in the first environment at time t_1 (P'_{t_1} ; $mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$) is then determined by :

$$P'_{t_1} = P'_0(t_1 + 1)^{\left(c\Delta p_{H2O(1)} P'_0 (e - T_{(1)})\right)} \quad \textbf{(10.3)}$$

where: P'_0 is the initial permeance as defined by the micro-cracking and Eq. 5.10, c and e are the model parameters (-490 and 35.85, respectively) determined in chapter 8, $\Delta p_{H_2O(1)}$ and $T_{(1)}$ are the Δp_{H_2O} and temperature prevailing in the first environment. Each of these components of the model has been developed under constant conditions; neither has been tested under variable conditions such as would be expected to occur within the handling chain from producer to consumer. Two possible methods have been developed to allow for a series of conditions to be modelled.

In the first method, the model is assumed to be completely additive. The water vapour permeance at the end of the first set of conditions (P'_{t_1}) becomes the initial permeance value for the next set:

$$P'_{t_2} = P'_{t_1}(t + 1)^{(c\Delta p_{H2O(2)} P'_1 (e - T_{(2)}))} \quad \textbf{(10.4)}$$

where P'_{t_2} is the permeance during the second set of conditions and $\Delta p_{H_2O(2)}$ and $T_{(2)}$ are the driving force and temperature prevailing in the second environment.

The initial component describes the rapid reduction in P'_{H_2O} which occurs over the first initial period within a set of conditions for freshly harvested fruit. Rate

of decline in P'_{H_2O} then reduces considerably after the first few days. This initial decline in permeance is probably related to a reduction in fruit turgor reducing the area of cracks. Implicit in the conceptual model associated with method one is that this reduction in turgor occurs with every move into new conditions. This approach represents the lower limit for permeance as it exaggerates the initial rate of permeance decay with each transfer to a new environment.

The second method treats decline in permeance through the second phase as though the second set of conditions had applied throughout but calculates final permeance as though the decline through the second phase was added to the end point of the first phase:

$$P'_{t_2} = P'_{t_1} + \left[P'_0 (t+1)^{(c \Delta p_{H_2O(2)} / b \cdot (e \cdot T_{(2)}))} \right]_{t_1}^{t_2} \qquad \textbf{(10.5)}$$

Transfer to a third set of conditions can be modelled analogously using either approach.

Total water loss (ΔM_{H_2O}) can be calculated by integrating rate of water loss and integrating with respect to time for each phase.

$$\Delta M_{H_2O} = \int r'_{H_2O} \, .dt \qquad \textbf{(10.6)}$$

Rate of water loss can be calculated by substituting Eq. 10.4 or 10.5 into Eq. 2.1

$$\Delta M_{H_2O} = \int P_t' A \Delta p_{H_2O} .dt \quad \textbf{(10.7)}$$

For method one:

$$\Delta M_{H_2O} = \frac{C' P_0' A \Delta p_{H_2O(1)}}{(cP_0' \Delta p_{H_2O(1)}(e - T_{(1)})) + 1}\left[(t_1 + 1)^{(cP_0' \Delta p_{H_2O(1)}(e - T_{(1)}))+1} - (t_0 + 1)^{(cP_0' \Delta p_{H_2O(1)}(e - T_{(1)}))+1}\right]$$
$$+ \frac{C' P_{t_1}' A \Delta p_{H_2O(2)}}{(cP_{t_1}' \Delta p_{H_2O(2)}(e - T_{(2)})) + 1}\left[(t_2 + 1)^{(cP_{t_1}' \Delta p_{H_2O(2)}(e - T_{(2)}))+1} - (t_1 + 1)^{(cP_{t_1}' \Delta p_{H_2O(2)}(e - T_{(2)}))+1}\right] \quad \textbf{(10.8)}$$

For method two:

$$\Delta M_{H_2O} = \frac{C' P_0' A \Delta p_{H_2O(1)}}{(cP_0' \Delta p_{H_2O(1)}(e - T_{(1)})) + 1}\left[(t_1 + 1)^{(cP_0' \Delta p_{H_2O(1)}(e - T_{(1)}))+1} - (t_0 + 1)^{(cP_0' \Delta p_{H_2O(1)}(e - T_{(1)}))+1}\right]$$
$$+ \frac{C' P_0' A \Delta p_{H_2O(2)}}{(cP_0' \Delta p_{H_2O(2)}(e - T_{(2)})) + 1}\left[(t_2 + 1)^{(cP_0' \Delta p_{H_2O(2)}(e - T_{(2)}))+1} - (t_1 + 1)^{(cP_0' \Delta p_{H_2O(2)}(e - T_{(2)}))+1}\right] \quad \textbf{(10.9)}$$

C^t is a correction for time units and equals 86,400. The subscripts of (1) and (2) refer to the set of conditions that the variables relate to. Percentage of mass loss through water loss can be calculated from:

$$\% M_{H_2O} = \frac{\Delta M_{H_2O}}{M} \times 100 \quad \textbf{(10.10)}$$

We can use this model to further explore the issues raised from the simple mathematical model developed (section 3.4). The simple model assumed that water vapour permeance was constant in all conditions and over the entire period of storage. The more complex model described above accounts for these changes with time and different conditions. It was used to investigate the influence of delaying pre-cooling from 1 day to 4 days on the water vapour permeance of fruit and the percentage water loss with fruit of 0.16 kg with surface area of 0.016 m^2 and proportion of cracking at 0.01 and 0.3, two

extremes that yield initial permeance values of 13 and 55 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$, respectively (section 5.3). The conditions during the delay before pre-cooling were set at 20 °C and 60 % *RH*, the coolstorage conditions were 0.5 °C and 90 % *RH*, and pre-cooling was assumed to be instantaneous, then a 7 day shelf-life period at the same conditions for the delay before pre-cooling. Total storage time was 3 months and 7 days.

The model predicted that water vapour permeance of all fruit would decline sharply during the delays before pre-cooling (Fig 10.9); subsequent decline in permeance during coolstorage was considerably slower. The declines in permeance of the highly cracked fruit were predicted to be much larger than those from the slightly cracked fruit. The 4 day delay before pre-cooling resulted in water vapour permeance of both high and low initial permeance fruit dropping to a lower level than the 1 day delay in pre-cooling even after the intervening 3 month period in cool storage.

Whilst this lower level of permeance may initially appear to be advantageous to the industry, the total amount of weight loss occurring in these treatments was much higher (Fig. 10.10). Both of these effects were related to the much greater decline predicted to occur before pre-cooling than in the subsequent coolstorage phase. Fruit with a high proportion of cracking had a particularly steep rate of water loss during the first phase. Interestingly, the divergence of the lines predicted by the two models was greater for the 4 day delay before

pre-cooling. This was because the first method used the permeance at the end of the delay period as the initial permeance for the next set of conditions, whereas the second model presented uses the original initial permeance, the drop in permeance at the end of the four day delay in pre-cooling was greater than that for a one day delay this created a greater differential in the decline in permeance for the four day delay than the one day.

Banks (pers. comm.) has found shrivel often occurring in fruit at a 5% total mass loss. If this amount of mass was set as a threshold at which fruit might be expected to develop shrivel then times taken for the fruit to begin to shrivel can be predicted for the alternative scenarios. High permeance fruit with a 4 day delay in precooling, stored for 86 days at 0 °C, 90% *RH*, then held at 20 °C and 60% *RH* were predicted to shrivel at 65 to 90 days, whilst with a 1 day delay before pre-cooling and similar storage environments the high permeance fruit could develop shrivel on removal of coolstorage at 90 to 97 days. This highlights the need to minimise delays before pre-cooling the fruit after harvest, particularly for populations of fruit with high permeance. None of the low permeance fruit reached the threshold for shrivel within the prediction period. This illustrates the fundamental importance of permeance in the outcome of commercial fruit handling regimes. It is particularly important to note that average levels of permeance in a population may not be the critical issue for acceptability of lines of fruit after a period of storage. Rather, even a small proportion of fruit with a very high permeance could render an entire line

unacceptable if, as at present, there is no way to segregate these fruit from the remainder of the line.

The model presented here is a considerable over simplification of the complex reality it is intended to reflect; the potential to make further improvements in this model is outlined in Section 10.5. Nevertheless, the model presented here has enabled some useful predictions of effects of delay in pre-cooling on fruit with a range of values for water vapour permeance. Predictions are likely to more closely reflect reality than those made with the simple model developed in chapter three and could be used by industry to explore the approximate outcomes of a range scenarios.

10.3 MINIMISATION OF WATER LOSS

The representation of calculation of mass loss given in Eq. 10.1 signals that the more that can be done to minimise the components of $\dot{r}_{mass}$ at all times after harvest, the lower final mass loss will be. In this regard, two broad strategies for reducing rate of water loss can be identified using Eq. 2.1. One would involve reducing the driving force for water loss (Δp_{H_2O}) by increasing the $p^{e}_{H_2O}$ or decreasing $p^{f}_{H_2O}$. The second would involve enhancing the barrier property of the fruit (or reducing $\dot{P}_{H_2O}$).

In addition, Eq. 10.1 emphasises the role of time in this whole process: fruit that shrivel excessively with long term storage are likely to be perfectly

marketable if they can be delivered to market before shrivel develops. This indicates that an additional strategy of segregating lines of fruit prone to shrivel from those that are not could be effective in maximising the proportion of the crop that can be delivered to lucrative overseas markets without excessive risk of product failure.

These three broad strategies for reducing mass loss and shrivel are summarised in Fig. 10.8. All of these possibilities are explored below.

10.3.1 Minimise fruit permeance

Low permeance fruit take longer to lose an amount of water that would make them shrivel than those with high permeance. The influences of production practices on fruit permeance are largely unexplored at this point but it is clear that avoiding cuticular cracking is central to this strategy as cracks impair the barrier properties of the cuticle. Sudden changes in water status of the tree have been associated with development of cracks (Opara et al. 1997). Reduced irrigation of 'Braeburn' trees at Massey University's Fruit Crops Unit has resulted in fruit with lower rates of mass loss in coolstorage (Kalili et al., unpublished). Thus, it seems likely that witholding irrigation for a period immediately before harvest may better maintain barrier properties of the fruit skin, though the impact of such a practice on yield would have to be assessed before it could be recommended for commercial application. Fruit from trees of low crop load are likely to be particularly at risk to cracking because of their

large growth potential (Volz et al. 1993).

Early harvest could be a second strategy for avoiding cracking that develops as fruit growth outstrips cuticular capacity for stretching without damage. The effects on permeance of delays in harvest for some cultivars, such as 'Braeburn', are very large (Section 2.2.1.5.1). Thus, whilst the potential for harvesting fruit early is limited by the need for fruit to reach export colour standards before they can be harvested, harvesting as early as possible would be an effective way to reduce risk of shrivel. These benefits could be maintained by ensuring that mechanical damage at harvest did not increase permeance.

Once fruit are harvested, permeance could be reduced by the addition of an edible surface coating (Section 2.2.1.6.4). It seems likely that such materials achieve most of their effects through covering areas in the cuticle that have poor barrier properties, such as cracks. This may explain the reduction in variance of permeance achieved by waxing reported by Banks et al. (1997). It appears that most of the potential benefit can be achieved with substantially diluted wax coatings. This allows mass loss to be reduced without excessive risk of suffocating the fruit, which can occur if heavy deposits of full strength coatings are applied.

Temperature effects have not been shown in *in vivo* research but if permeance

of apple cuticle was similarly temperature dependent to that of isolated cuticles (section 2.2.1.6.3), then coolstorage could have benefits over and above those achieved by reducing Δp_{H_2O} (see below). Conversely, there may be additional risks associated with high temperature treatments for quarantine treatments if critical phase change temperatures were exceeded.

10.3.2 Reduce Δp_{H_2O}

Many factors can be manipulated to reduce Δp_{H_2O}. Essentially, these factors operate to reduce fruit temperature or elevate environment relative humidity. These determine the difference in water vapour pressures between the fruit surface and the surrounding environment that constitutes the driving force for water loss. Minimising fruit temperature and maximising environmental *RH* therefore combine to minimise rate of fruit mass loss through transpiration. Reducing fruit temperature also slows mass loss due to carbon loss associated with respiration, though this benefit only becomes significant when rates of water loss are very low (section 3.4).

10.3.3 Reduce fruit temperature

Timely and rapid reduction of fruit surface temperature to a low and constant level is a key strategy for reducing rates of water loss. Standard approaches to achieving this objective include: precooling after harvest with minimum delay and maintaining fruit at a constant low temperature (Mitchell 1992b).

10.3.4 Elevate environment *RH*

At a given temperature, Δp_{H_2O} is minimised when the air is saturated with water vapour. Standard approaches to elevating environment relative humidity include use of well insulated coolstores and a low temperature differential between the evaporator surface and store air, both features that need to be considered at the time a coolstore is being planned as well as during the day to day management of the store (Amos 1995).

The benefit of increased relative humidity in reducing rate of water loss may thus, to some extent, be ameliorated by increases in water vapour permeance at high relative humidity (Section 2.2.1.6.2). Accurate predictions of rates of water loss would therefore require characterisation of the extent to which relative humidity influences P'_{H_2O}. Regardless of the final permeance of a given apple in a particular environment, there is no suggestion from data in this study or the literature that the increase in permeance would be sufficient to nullify the benefits of increased relative humidity (section 10.2.1.3; Fig 10.7 b). Elevating storage environment relative humidity will therefore remain a key strategy for reducing fruit water loss.

Elimination of sinks for moisture would comprise the next step. In terms of coolstore structure and storage bins, this could be dealt with by pre-soaking or supplying additional moisture to the system during equilibration of coolstores

after loading (e.g. by adding water to the floor). In addition, providing barriers to water loss in packaging can substantially reduce Δp_{H_2O} and thus reduce water loss. If packaging materials are themselves confirmed to be a significant sink for moisture (Tanner pers. comm.), then this might be tackled by selection of alternative materials or by addition of a sacrificial source of water.

The extent to which Δp_{H_2O} can be modified is limited due to the physical nature of the relationships i.e. addition of more moisture to already saturated air does not influence water loss. Storage in high humidity can induce mealiness in certain varieties and loss of strength in cardboard causing carton collapse. Thus, alternative strategies should be followed in fruit destined for different markets (Fig. 10.8).

10.3.5 Segregation

This strategy holds major potential for maximising the proportion of marketable fruit whilst controlling the risk of market failure. Essentially, it would involve developing a system to identify lines of 'at risk' fruit and using this to segregate fruit into different categories that could be matched to market niche and storage environments.

Surface area of fruit is a determining factor of water loss (Eq. 2.1). Surface area of the fruit is linked to the fruit mass (Eq. 2.9). Coupled with mass, and for fruit of a given permeance, it affects the time required for fruit in a given environment to lose a certain proportion of its mass by transpiration (section

10.2.1.2). On an individual fruit basis, large fruit lose more water than smaller fruit. However, when considering a full 18.5 kg carton of fruit, cartons with larger fruit (i.e. low count number) would lose mass more slowly than those with smaller fruit and higher count number (section 10.2.1.2). On this basis, it would appear that there is a larger margin for error in storing large than small fruit. However, these calculations assume equal permeance for all fruit and, at this time, there is no definitive data concerning dependence of permeance upon fruit size. It would, therefore, be premature to segregate fruit for different durations of storage on the basis of fruit size.

On the other hand, it is very clear that there is enormous variation in individual fruit to fruit permeance values (section 3.3). If such variation could be clearly linked to another attribute that could readily be measured non-destructively (e.g. colour) then this would provide the basis for segregation of individual fruit into different permeance categories. This would be a particularly valuable tool in that the maximum proportion of the crop could be marketed with very low risk. Unfortunately, there is no reason to believe that such an indicator exists and it seems likely that permeance (and thus risk of shrivel) could be most effectively assessed directly by determining rate of mass loss in a standardised environment. Such measurements would be completely impracticable in the commercial segregation of individual fruit.

This approach might prove feasible on a batch basis although under these

circumstances it would be less effective in retaining the maximum proportion of the crop or minimising the risk. Individual lines of fruit could be sampled, their relative susceptibilities to water loss determined, and decisions made on their suitability for alternative markets or storage regimes. Highest permeance fruit could either be routed to short term markets or given additional treatments (waxing, carton liners or pallet wraps) to reduce mass loss and then despatched to medium term markets (Fig 10.8). For both short and medium term market fruit, Δp_{H_2O} should be minimised to reduce water loss. In contrast, fruit destined for long term markets would need to have additional measures in place to restrict development of mealiness during storage. These could include coupling low Δp_{H_2O} with additional technologies to retard ripening (e.g. controlled or modified atmosphere storage) or optimising, rather than maximising, storage relative humidity.

10.4 FURTHER WORK

It is clear from the discussion that there is considerable scope for further research in factors affecting mass loss from apples.

The current study has established a link between cuticular micro-cracking and fruit water vapour permeance. There is an extensive amount of literature investigating causes of cuticular cracking (Opara et al. 1997), but very little of this literature links back to shrivel or mass loss of fruit. Studies on what growth conditions influence cuticular micro-cracking and hence mass loss in

storage may lead to cultural methods which could be used to produce fruit with minimised water vapour permeance and hence reduction of mass loss in storage.

The incidence and severity of cuticular micro-cracking would seem to depend on cuticular elasticity and growth stretch. In addition to cultivar differences and effects of growing conditions, it may be possible to reduce cracking by application of growth hormones, such as giberellic acid (Taylor and Knight 1986). These effects could usefully be investigated further as another possible form of control of water vapour permeance of fruit.

In the chapter 8 a model was developed to describe the decline in water vapour permeance in relation to the fruit initial water vapour permeance, which represents all the variables expressed in Eq. 10.3 and Δp_{H_2O} (driving force for water loss). The potentially important interaction of temperature was included, but only two temperatures were examined in this experiment. It would be useful to establish more fully the effects of temperature on the relationship between water vapour permeance and time so that better predictions of postharvest environment of fruit mass loss can be made. Coupling such a study with an exploration of relative humidity effects would enhance the range of conditions under which useful predictions might be made with the model.

The model developed in chapter 8 is based on fruit remaining within one set of conditions. This is unlikely to happen in an industry situation. With the more complex model developed in chapter 10, two options of describing the change in permeance with new conditions (Fig 10.9) were presented. To enable accurate predictions to be made with the model, knowledge of changes in permeance in dynamic conditions (relative humidity and temperature) would be beneficial. The model was developed on one line of fruit harvested at one time, exploring the effects of grower and harvest date would enable the model to be more widely applicable and more accurate. The inclusion of boundary layer effects, packaging materials and cooling would also enhance the accuracy of predictions from this model.

Respiration was not included in the model. Although carton loss associated with respiration causes only a small proportion of mass loss at lower humidities, it can represent 10% or more in fruit packaged with very high humidities. Although more accurate predictions of total mass loss would require this component to be added to the model for storage under very high humidities, mass loss is unlikely to be a major concern under such conditions. Nevertheless, a complete model would take account of these effects and this would require characterisation of the relationship between respiration rate and those environmental variables that may be varied for each cultivar of intrest.

10.5 CONCLUSIONS

Mass loss in pipfruit is driven by a series of well documented physical phenomena coupled with some less well understood physiologically moderated features of the fruit. A number of straightforward steps can be taken to reduce mass loss, and its associated problem of shrivel, based around three broad strategies:

- reducing fruit permeance to water
- reducing diffusional driving force for escape of water from the fruit
- segregating lines of fruit on the basis of permeance to water and allocating them to appropriate storage and marketing regimes.

Overall, it appears that there would be potential to reduce mass loss from fruit using a combination of these strategies.

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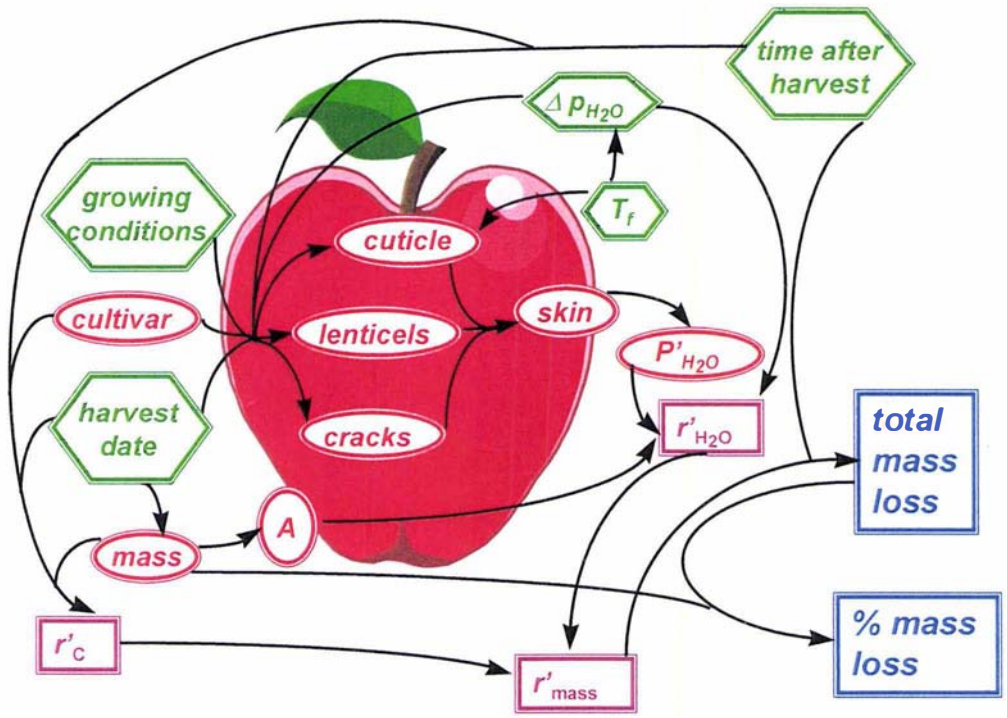

Fig. 10.1. Conceptual model of factors affecting total mass loss. Final outcomes are in blue boxes. Processes which influence this are represented in purple boxes. Fruit attributes are in red ovals, and environmental factors are in green hexagons.

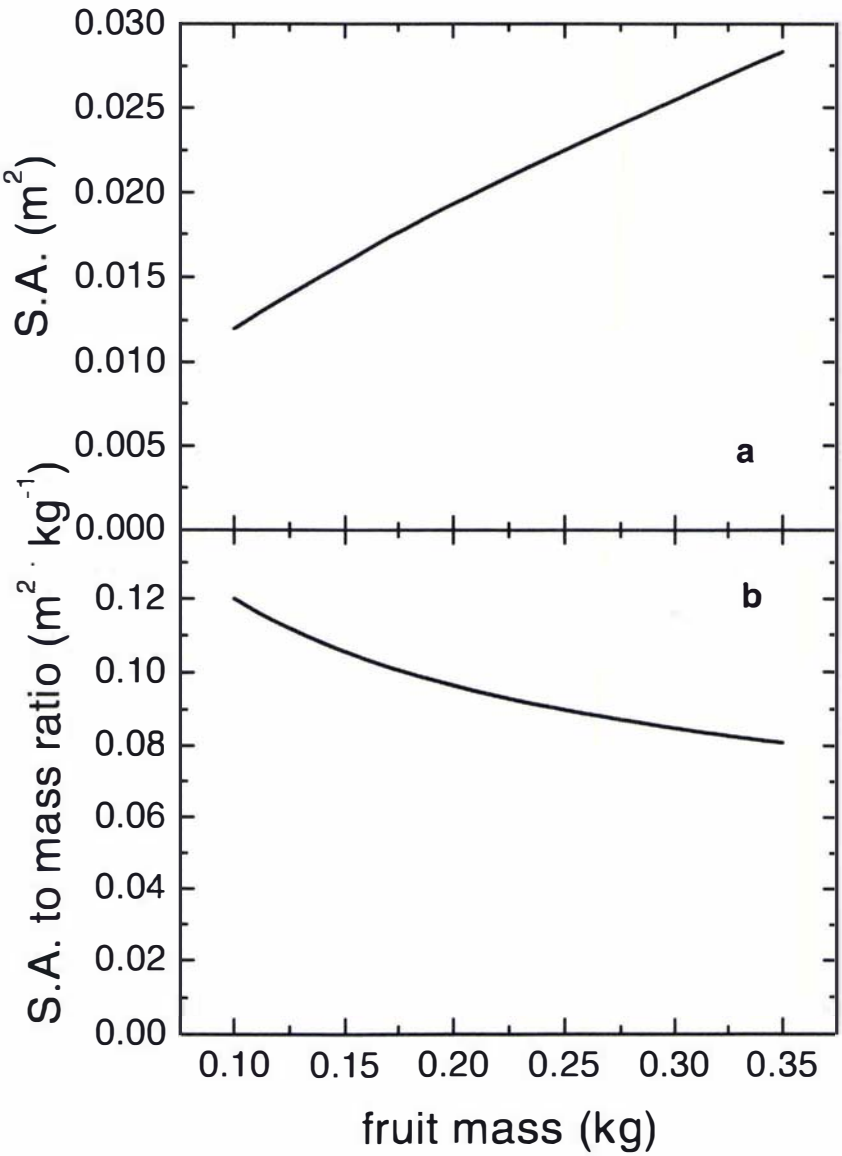

Fig. 10.2. Dependence on fruit mass of a) surface area and b) surface area to mass ratio.

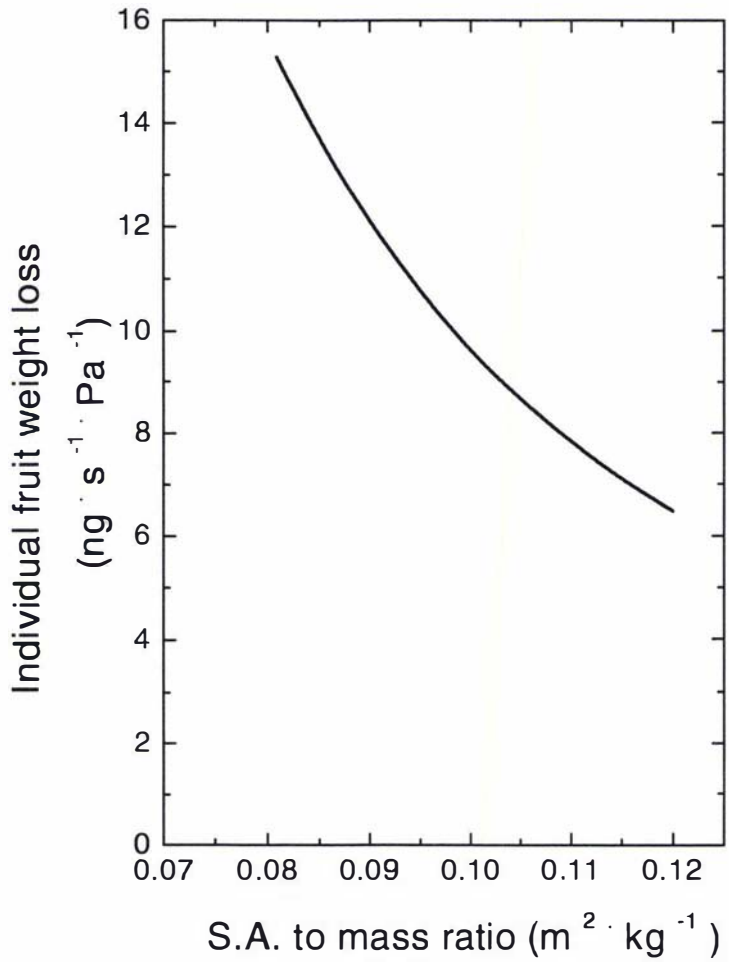

Fig. 10.3. Predicted relationship between weight loss and surface area (S.A.) to mass ratio of fruit with a typical value for permeance of 30 nmol · s^{-1} · m^{-2} · Pa^{-1}. The range of SA to mass ratio displayed is equivalent to a range in fruit mass of 0.1 to 0.35 kg.

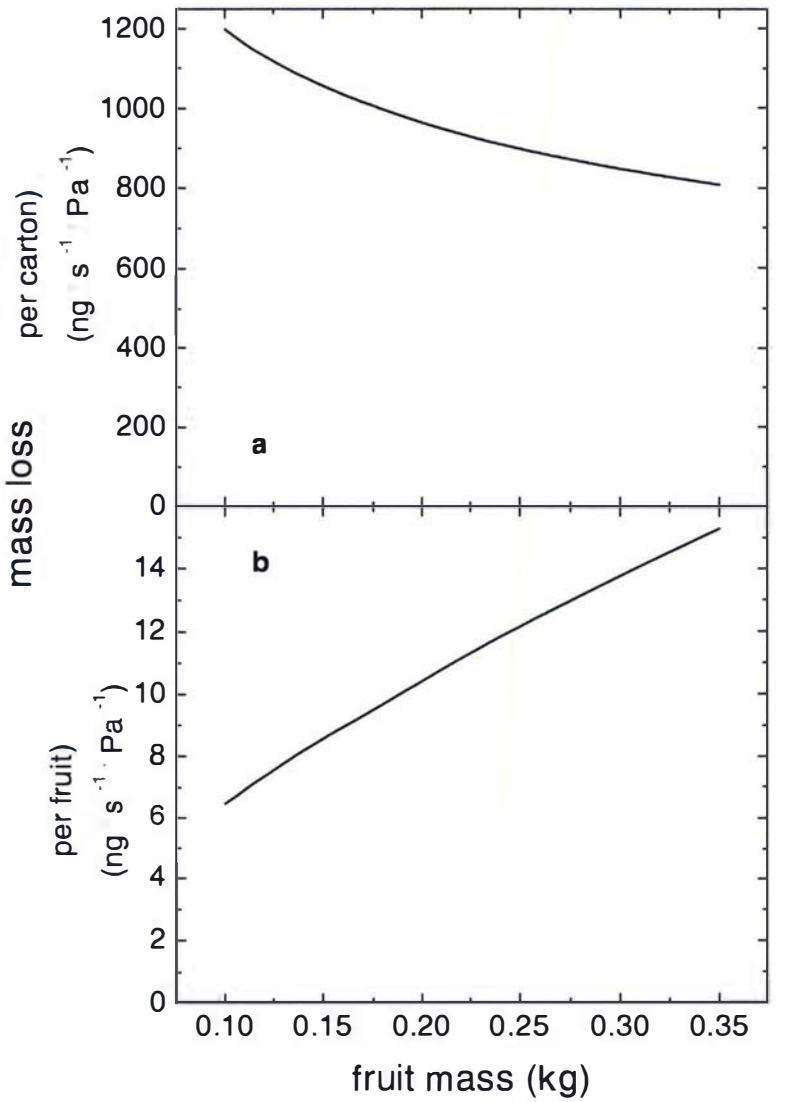

Fig. 10.4. Predicted relationship between weight loss and mass of fruit of a) a 18.5 kg carton of apples and b) a individual fruit with a typical value for permeance of 30 nmol $\cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$.

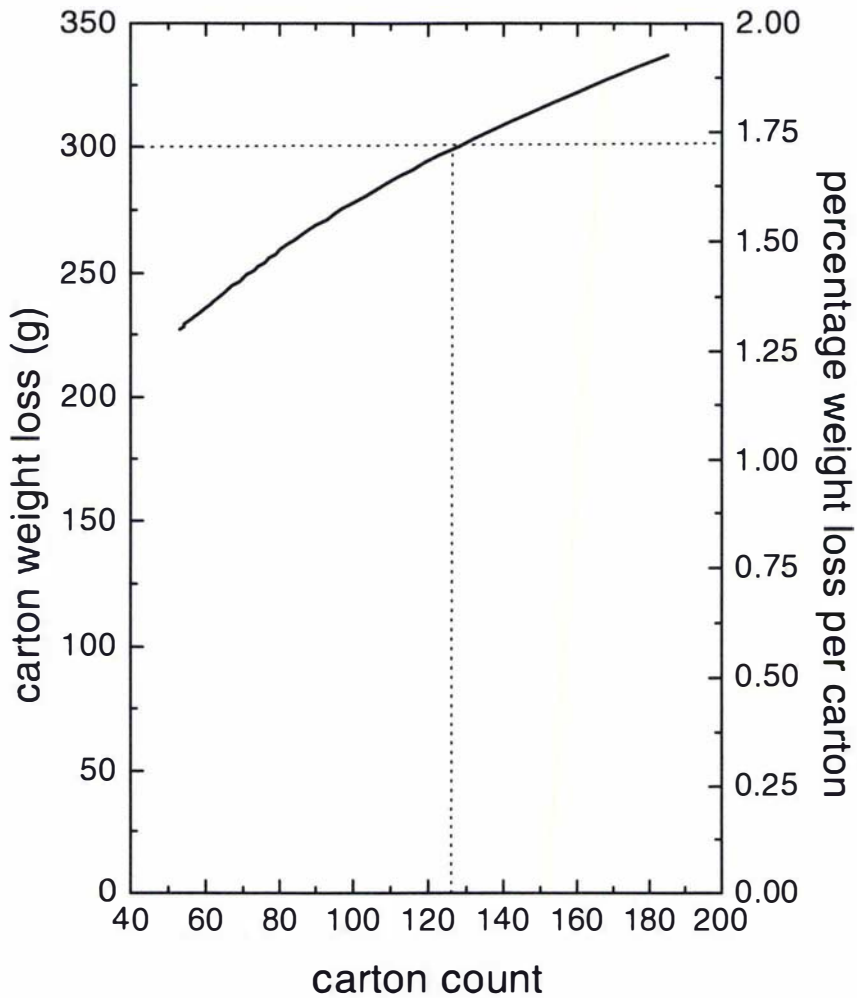

Fig. 10.5. Predicted relationship between count size of fruit and weight loss of 18.5 kg of apples with uniform permeance to water if environmental conditions and duration where such that a carton of count 125 fruit lost 300 g.

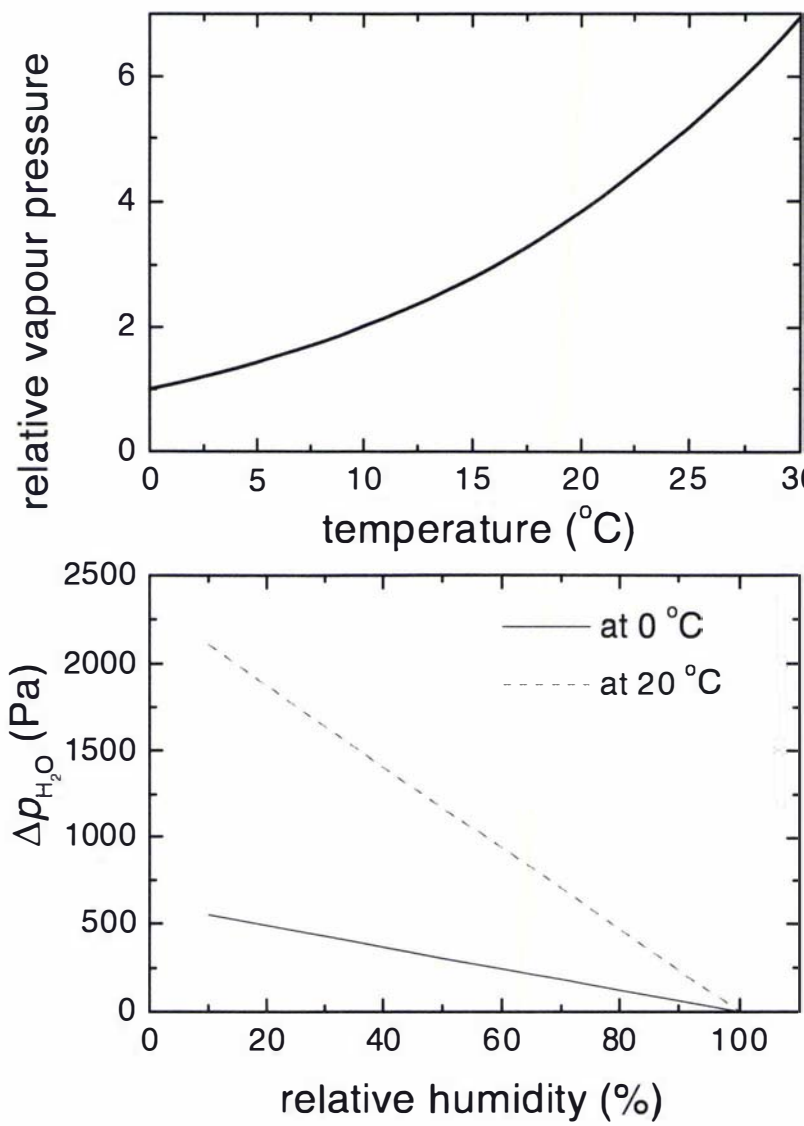

Fig. 10.6. a) Influence of temperature on relative saturated vapour pressure for air and b) influence of relative humidity on the driving force for water loss.

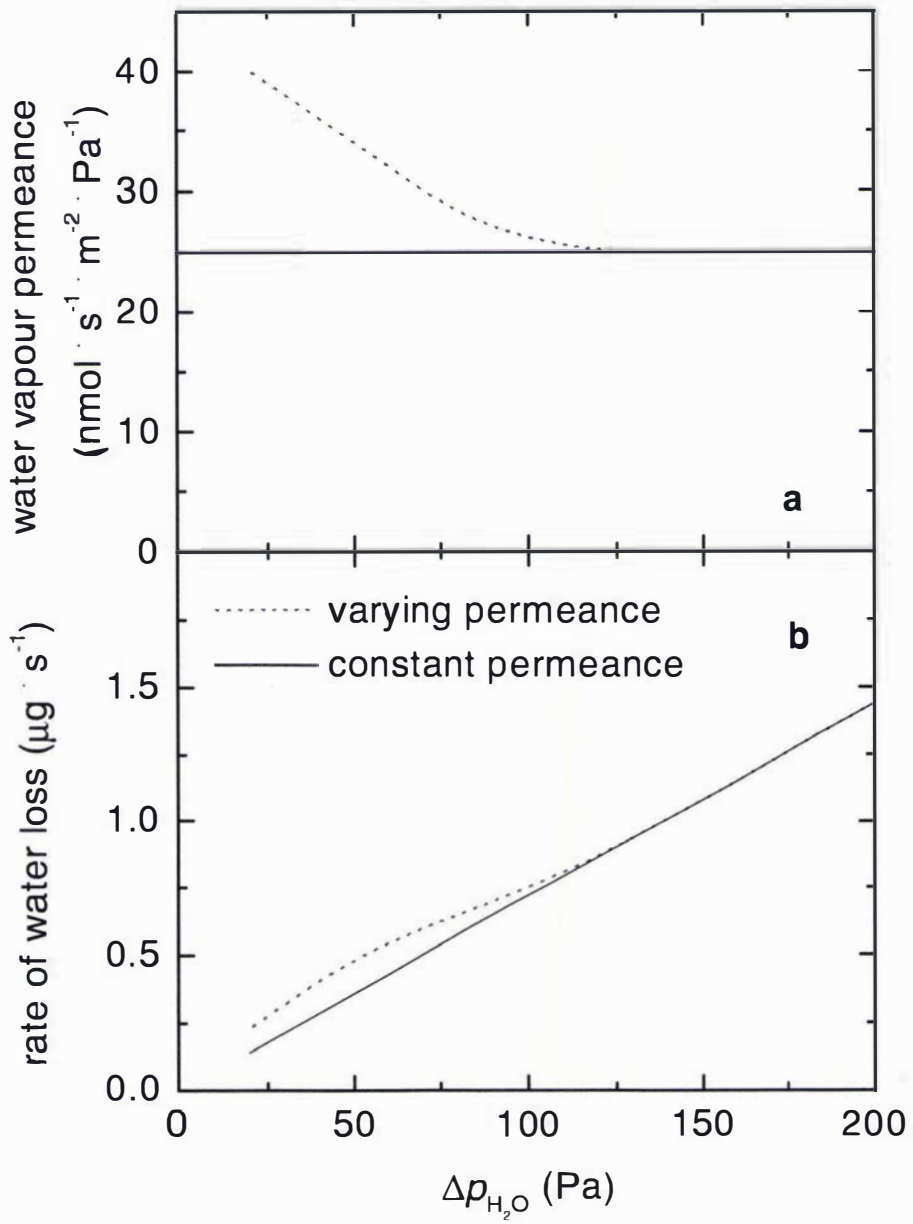

Fig. 10.7. a) Effect of Δp_{H_2O} on the permeance to water vapour (data from Lentz and Rooke 1964) and b) predicted effect of Δp_{H_2O} on the rate of water loss from an apple fruit.

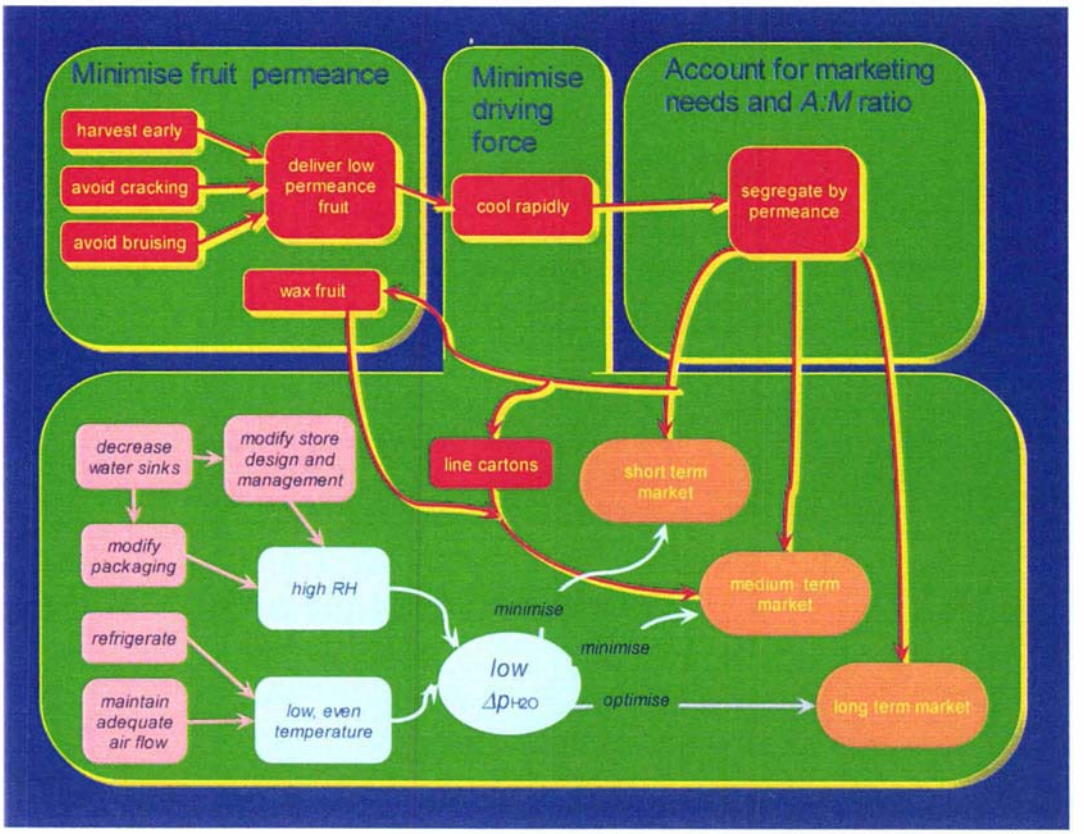

Fig. 10.8. Conceptual diagram of potential strategies available to the pipfruit industry for reducing mass loss.

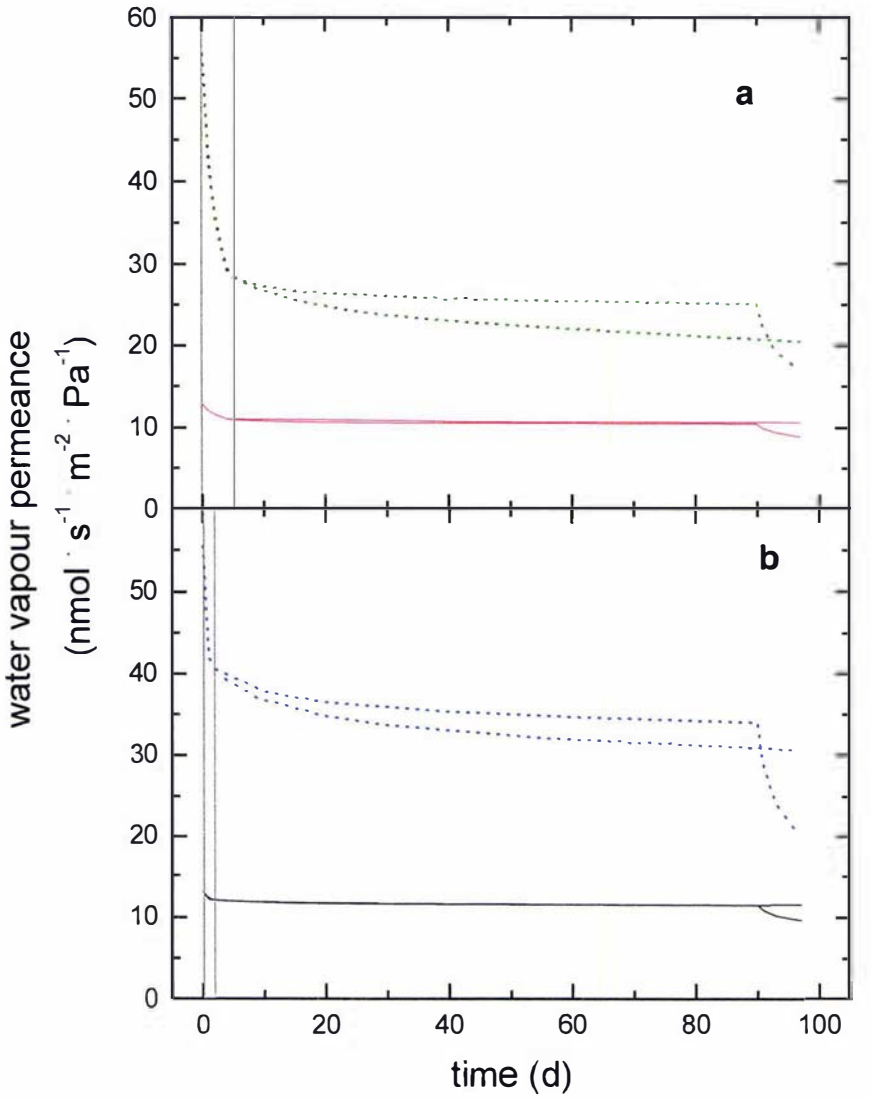

Fig. 10.9. Prediction of water vapour permeance of fruit with high permeance (dotted line) and low permeance (solid line) changes with time in storage with a) 4 days delay in pre-cooling and b) 1 day delay in pre-cooling. Periods of pre-cooling are shaded.

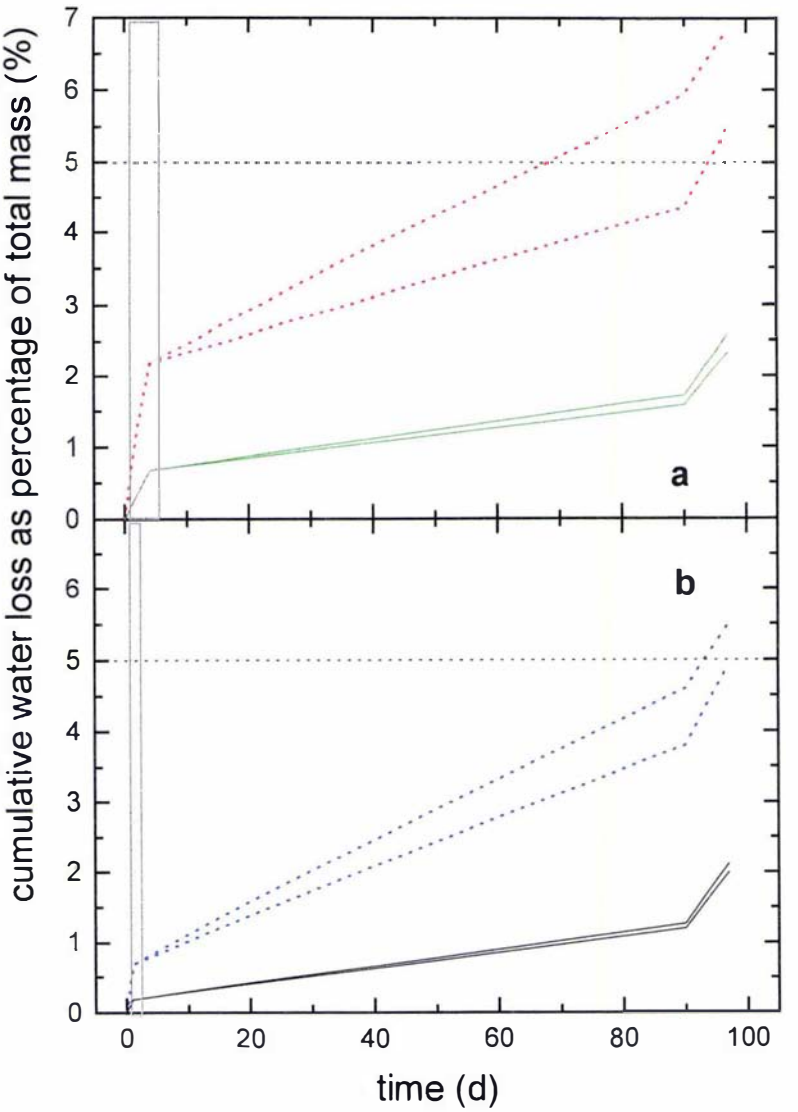

Fig. 10.10. Prediction of water loss as percentage of total mass of fruit with high permeance (dotted line) and low permeance (solid line) changes with time in storage with a) 4 days delay in pre-cooling and b) 1 day delay in pre-cooling. Periods of pre-cooling are shaded.