Internal Lower Oxygen Limits of Apple Fruit

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In memory of my mother

and

to Kathryn, Tim, Daniel, Elissa and Andrew

Abstract

The optimum atmosphere for a crop, with respect to oxygen, lies just above the lower oxygen limit (*LOL*), at which maximum benefits in relation to fruit quality are achieved and below which fruit quality is compromised by fermentation. In contrast to previous work, *LOL*s in this study were estimated on the basis of steady-state internal atmospheres (*LOL*^{i}s) as well as external atmospheres (*LOL*^{e}s) as it is the internal O_2 partial pressure ($p^i_{O_2}$, Pa), close to equilibrium with the cytosol, that mediates important physiological processes. The study tested whether *LOL*^{i}s of 'Cox's Orange Pippin' and 'Braeburn' apples were affected by temperature, elevated CO_2, and physiological age.

Two types of *LOL*s were identified: the anaerobic compensation point (*ACP*) and the fermentation threshold (*FT*). *ACP* was described in terms of plots of the internal CO_2 ($p^i_{CO_2}$) versus internal ($p^i_{O_2}$) and external ($p^e_{O_2}$) O_2, and *FT* in terms of plots of both a measure of the respiratory quotient (RQ_{ia}) and ethanol (EtOH) concentration versus $p^i_{O_2}$ and $p^e_{O_2}$. Mathematical solutions for estimating *ACP* and *FT* based on the RQ_{ia} (FT_{RQ}), and a statistical 'bootstrap' procedure suitable for estimating all *LOL*s and their bias-corrected 95% confidence intervals, are described.

LOL^{i}s of postclimacteric fruit of both cultivars tended to increase slightly between 0° and 28°C and sharply at 32°C. *LOL*^{i}s ranged between 0.5 kPa and 2.2 kPa $p^i_{O_2}$; values for FT^i_{RQ} and FT^i_{EtOH} tended to be higher than for ACP^i. Elevated $p^e_{CO_2}$ (0 to 8 kPa at 0° and 20°C) did not significantly affect *LOL*^{i}s at 20°C, but increases in FT^i_{RQ} and FT^i_{EtOH} occurred for fruit at 0°C. A small decrease in O_2 uptake and RQ_{ia} was measured for fruit in 2 to 8 kPa $p^e_{CO_2}$ at 20°C. No consistent changes in *LOL*^{i}s were observed for either cultivar in relation to physiological age (preclimacteric, climacteric, or postclimatceric fruit at 0° or 20°C).

In contrast to ACP^i, ACP^e increased markedly with temperature, resulting from its dependence on both skin permeance and respiration rate (both of which change with time fruit are in storage). Consequently, use of *LOL*^{i}s, rather than *LOL*^{e}s is recommended for optimising atmospheres for both sealed packages and controlled atmosphere storage, to minimise risk of fermentation.

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List of Symbols and Abbreviations

A	=	fruit surface area (m^2)
AAT	=	alcohol acyltransferase
ACC	=	1-aminocyclopropane-1-carboxylic acid
Acet	=	acetaldehyde
ACP	=	anaerobic compensation point
ACP^e	=	external anaerobic compensation point
ACP^i	=	internal anaerobic compensation point
ADP	=	adenosine diphosphate
ADH	=	alcohol dehydrogenase
AMP	=	adenosine monophosphate
ANOVA	=	analysis of variance
ATP	=	adenosine triphosphate
ATP-PFK	=	ATP phosphofructokinase
b	=	number of bootstrap samples
BBD	=	'Braeburn' browning disorder
BBD^i	=	internal 'Braeburn' browning disorder
BCa	=	bootstrap bias corrected 95% confidence interval
Ca	=	calcium
CA	=	controlled-atmosphere
cDNA	=	complementary deoxyribonucleic acid
C_2H_4	=	ethylene
C_2H_6	=	ethane
CI	=	confidence interval
CN^-	=	cyanide ion
CO_2	=	carbon dioxide
$c^{i}_{O_2,H_2O}$	=	concentration of O_2 in water (mol m^{-3})
c^{T}_{j,H_2O}	=	concentration of gas j in water at temperature T (mol m^{-3})
$c^{T}_{O_2,H_2O}$	=	concentration of O_2 in H_2O at a given temperature, T (mol m^{-3})

CoA	=	coenzyme A
'COP'	=	'Cox's Orange Pippin'
CV	=	coefficient of variation
$dp_{C_2H_6} / dt$	=	rate of change of ethane partial pressure (Pa s^{-1})
$\Delta p_{C_2H_4}^{\Delta t}$	=	difference in partial pressure of C_2H_4 between initial and final measurements over time Δt (Pa)
$\Delta p_{CO_2}^{\Delta t}$	=	difference in partial pressure of CO_2 between initial and final measurements over time Δt (Pa)
Δp_j	=	difference in partial pressures of gas j between internal and external atmospheres (Pa)
$\Delta p_{O_2}^{\Delta t}$	=	difference in partial pressure of O_2 between initial and final measurements over time Δt (Pa)
Δt	=	difference in time between initial and final sampling (s)
ε	=	cortical tissue porosity (m^3 m^{-3})
EC	=	energy charge
EFE	=	ethylene forming enzyme
EP	=	extinction point
$error_{wvp}^{sat,T}$	=	percentage error (%) due to the dilution effect of water vapour pressure at temperature T
EtAc	=	ethyl acetate
EtOH	=	ethanol
ETS	=	electron transport system
Eq(s).	=	equation(s)
F	=	fruit firmness (N)
F1,6-P_2	=	fructose 1,6-bisphosphate
F2,6-P_2	=	fructose 2,6-bisphosphate
F6P	=	fructose 6-phosphate
f_{CO_2}	=	flow rate of CO_2 (mm^3 s^{-1})
FF	=	fruit firmness (N)
Fig(s).	=	figure(s)
f_{N_2}	=	flow rate of N_2 (mm^3 s^{-1})

f_{O_2}	=	flow rate of O_2 ($mm^3\ s^{-1}$)
FT	=	fermentation threshold
FT^e	=	external fermentation threshold
FT^i	=	internal fermentation threshold
FT_{Acet}	=	fermentation threshold based on acetaldehyde accumulation
FT_{EtAc}	=	fermentation threshold based on ethyl acetate accumulation
FT_{EtOH}	=	fermentation threshold based on ethanol accumulation
FT^e_{EtOH}	=	external fermentation threshold based on ethanol accumulation
FT^i_{EtOH}	=	internal fermentation threshold based on ethanol accumulation
FT_{RQ}	=	fermentation threshold based on respiratory quotient
FT^e_{RQ}	=	external fermentation threshold based on respiratory quotient
FT^i_{RQ}	=	internal fermentation threshold based on respiratory quotient
gas	=	gas phase
g	=	gram
$init\ p_{C_2H_6}$	=	initial partial pressure of ethane in the fruit's internal atmosphere (Pa)
h	=	hour
H°	=	background skin colour hue angle
H_2O_2	=	hydrogen peroxide
IA	=	internal atmosphere
K	=	potassium
k_l	=	$p^i_{O_2}$ at which $r^T_{O_2}$ is half maximal (Pa)
k_f	=	a fruit constant ($mol\ kg^{-1}\ s^{-1}\ Pa^{-1}$)
k_x	=	constant with number x
kg	=	kilogram
kgf	=	kilogram force
K_m	=	Michaelis-Menten constant (units of substrate eg. Pa)
kPa	=	kilopascal
L	=	background skin colour lightness
l	=	liter

LDH	=	lactate dehydrogenase
liq	=	liquid phase
LOLs	=	lower oxygen limits
$LOL^e s$	=	external lower oxygen limits
LOL^is	=	internal lower oxygen limits
M	=	fruit mass (kg)
m	=	metre
M_a	=	mass of non-infiltrated wedge in air (kg)
MA	=	modified-atmosphere
MAP	=	modified-atmosphere packaging
Mg	=	magnesium
M_i	=	apparent mass of infiltrated wedge submerged in water (kg)
µl	=	microliter
µmol	=	micromole
mm	=	millimetre
mmol	=	millimole
M_n	=	apparent mass of non-infiltrated wedge submerged in water (kg)
mol	=	mole
M_R	=	relative molecular mass
mRNA	=	messenger ribonucleic acid
n	=	number of fruit or items in a sample
N	=	newton
N_2	=	nitrogen
N_3^-	=	azide ion
NAD^+	=	adenine dinucleotde (oxidised form)
NADH	=	adenine dinucleotide (reduced form)
NADPH	=	nicotinamide adenine dinucleotide phosphate
nmol	=	nanomole
N_j	=	mole fraction of gas species *j* (%, mol mol^{-1} , µl l^{-1})
N_{CO_2}	=	mole fraction of CO_2 (mol mol^{-1})

$N_{CO_2,core}$	=	mole fraction of CO_2 in the core cavity (mol mol^{-1})
$N_{CO_2,room}$	=	mole fraction of CO_2 in the room (mol mol^{-1})
N_{O_2}	=	mole fraction of O_2 required (mol mol^{-1})
$N_{O_2,core}$	=	mole fraction of O_2 in the core cavity (mol mol^{-1})
$N_{O_2,room}$	=	mole fraction of O_2 in the room (mol mol^{-1})
NR	=	nitrogen respiration
NS	=	not significant
N_{std}	=	mole fraction of gas or vapour in the standard (µl l^{-1})
N_{stock}	=	mole fraction of stock standard (µl l^{-1})
O_2	=	oxygen
O_2^-	=	super oxide free-radicals
p	=	probability or level of significance of a statistical test
P	=	phosphorus
Pa	=	pascal
PCK	=	pyruvate carboxykinase
PDC	=	pyruvate decarboxylase
PEPC	=	phosphoenol pyruvate carboxylase
PDH	=	pyruvate dehydrogenase
pH	=	measure of a solutions concentration of hydrogen ions
P_i	=	inorganic orthophosphate
PK	=	pyruvate kinase
PP_i	=	inorganic pyrophosphate
$p_{H_2O}^{sat,T}$	=	saturated water vapour partial pressure at temperature T (Pa)
p_j^e	=	partial pressure of gas j in the external atmosphere (Pa)
$P_{C_2H_6}$	=	fruit permeance to ethane (mol s^{-1} m^{-2} Pa^{-1})
P_{CO_2}	=	fruit permeance to CO_2 (mol s^{-1} m^{-2} Pa^{-1})
p_{CO_2}	=	partial pressure of CO_2 (Pa)
$p_{CO_2}^e$	=	external (or package) partial pressure of CO_2 (Pa)
$p_{CO_2}^i$	=	internal partial pressure of CO_2 (Pa)

P_j = skin or fruit permeability to gas j (mol s^{-1} m m^{-2} Pa^{-1})

$P_j^{'}$ = skin or fruit permeance to gas j (mol s^{-1} m^{-2} Pa^{-1})

p_{O_2} = partial pressure of O_2 in the intercellular air space (Pa)

$p_{O_2}^{e}$ = external (or package) partial pressure of O_2 (Pa)

$p_{O_2}^{t}$ = internal partial pressure of O_2 (Pa)

p_{tot} = total system partial pressure (Pa)

$P_{O_2}^{'}$ = fruit permeance to O_2 (mol s^{-1} m^{-2} Pa^{-1})

PPi-PFK = pyrophosphate phosphofructokinase

Q_{10} = temperature coefficient (= [rate of O_2 uptake at (T+10°C)] / [rate of O_2 uptake at T])

r^2 = square of the correlation coefficient (r), or proportion of the total variability in the y-values that can be accounted for by the independent variable x.

R = gas constant (8.3143 m^3 Pa mol^{-1} K^{-1})

r_{CO_2} = specific rate of transfer of CO_2 between internal and external atmospheres (mol kg^{-1} s^{-1})

$r_{C_2H_4}$ = specific rate of transfer of C_2H_4 between internal and external atmospheres (mol kg^{-1} s^{-1})

ρ_{juice}^{20} = density of fruit juice at 20°C (kg m^{-3})

ρ_{fruit}^{20} = density of cortical tissue of fruit at 20°C (kg m^{-3})

$\rho_{H_2O}^{20}$ = density of water at 20°C (kg m^{-3})

r_j = specific rate of transfer of gas j between internal and external atmospheres (mol kg^{-1} s^{-1})

r_{O_2} = specific rate of transfer of O_2 between internal and external atmospheres (mol kg^{-1} s^{-1})

$r_{O_2}^{ACP^i}$ = rate of transfer of O_2 for the system at the ACP^i (mol s^{-1})

$r_{O_2}^{air,\,T}$ = specific rate of transfer of O_2 in air at temperature T (mol kg^{-1} s^{-1})

$r_{O_2}^{air,\,0}$ = specific rate of transfer of O_2 in fruit in air at 0°C (mol kg^{-1} s^{-1})

$r_{O_2}^{air}$	=	rate of transfer of O_2 for the system in air (mol s^{-1})
$r_{O_2}^{T}$	=	rate of transfer of O_2 for the system at temperature T (mol s^{-1})
$r_{O_2}^{T}$	=	specific rate of transfer of O_2 at temperature T (mol kg^{-1} s^{-1})
$r_{O_2}^{max,T}$	=	specific maximum rate of O_2 uptake when $p_{O_2}^{i}$ is non-limiting, at temperature T (mol kg^{-1} s^{-1})
$r_{O_2}^{max,T}$	=	rate of transfer of O_2 for the system when $p_{O_2}^{i}$ is non-limiting at temperature T (mol s^{-1})
RQ	=	respiratory quotient
RQB	=	respiratory quotient breakpoint
RQ_{ia}	=	respiratory quotient based on internal atmospheres
s	=	second
SAM	=	S-adenosylmethionine
SDH	=	succinate dehydrogenase
se	=	standard error
sed	=	standard error of the difference between means
sem	=	standard error of the mean
s_{O_2,H_2O}^{T}	=	solubility of O_2 in H_2O at a given temperature, T (mol m^{-3} Pa^{-1})
SSC	=	total soluble solids content (%, ° Brix)
t	=	time (s)
T	=	fruit temperature (°C)
TCA	=	tricarboxylic acid cycle or Krebs cycle
V_h	=	volume of submerged portion of hook (m^3)
V_{jar}	=	volume of respiration jar (m^3)
V_{net}	=	net volume, [jar volume - fruit volume] (m^3)
V_{stock}	=	volume of stock gas (m^3)
V_{std}	=	total volume of combined standard (m^3)
V_w	=	volume of wedge (m^3)

Chapter 1

General Introduction

1.1 Background to project

Apples are now New Zealand's leading horticultural crop, with the traditional leader, kiwifruit, continuing a downward trend (Witters, 1995). For the year ended 30 June 1995, horticultural exports were $1436.1 million (7.1% of total New Zealand exports) which was a 15% increase ($168.3 million) over 1994. The 54% increase ($168.6 million) from apple exports represented a significant proportion of the overall increase. Interestingly, returns from pear exports increased 115%, and while pears are the next largest pipfruit crop, they provide only 2.6% compared to export earnings from apples. The large increase in export earnings from apples was primarily due to buoyant prices in the 1995 season following a smaller than expected crop after massive hail damage to the Hawke's Bay crop.

Although the volume of New Zealand apples is comparatively small compared to total world apple production, ENZA New Zealand (International) is one of the most significant individual players in international marketing of apples. New Zealand apples maintain a premium quality image, and the development of new varieties will provide exciting marketing opportunities in the future. However, a primary constraint to delivering an even better quality fresh apple product remains the distance of New Zealand from our markets, particularly those in Europe and North America.

In addition to traditional cool storage, controlled atmosphere storage [CA, achieved using atmospheres of 1 to 2 % oxygen (O_2) and 0 to 5% carbon dioxide (CO_2)] has been used internationally, particularly for apples, to extend storage life and the marketing period for the crop (Meheriuk, 1993). In New Zealand, CA storage is used for apples destined for both export and the local market. CA storage of New Zealand export apples by ENZA New Zealand (International), largely occurs during shipping to Europe for cultivars 'Cox's Orange Pippin', 'Royal Gala', 'Braeburn', and smaller quantities of other cultivars (Fig. 1.1). Land based CA is used primarily for 'Red Delicious', 'Royal Gala' and 'Fuji'. Small quantities of

some cultivars ('Royal Gala', 'Gala', 'Braeburn' and 'Golden Delicious') are exported in folded, but unsealed, polymeric film carton liners. The liners are used for both reducing weight loss and modified atmosphere (MA) effects. The quantity of 'Royal Gala' and 'Gala' exported in polymeric liners is likely to increase in the future.

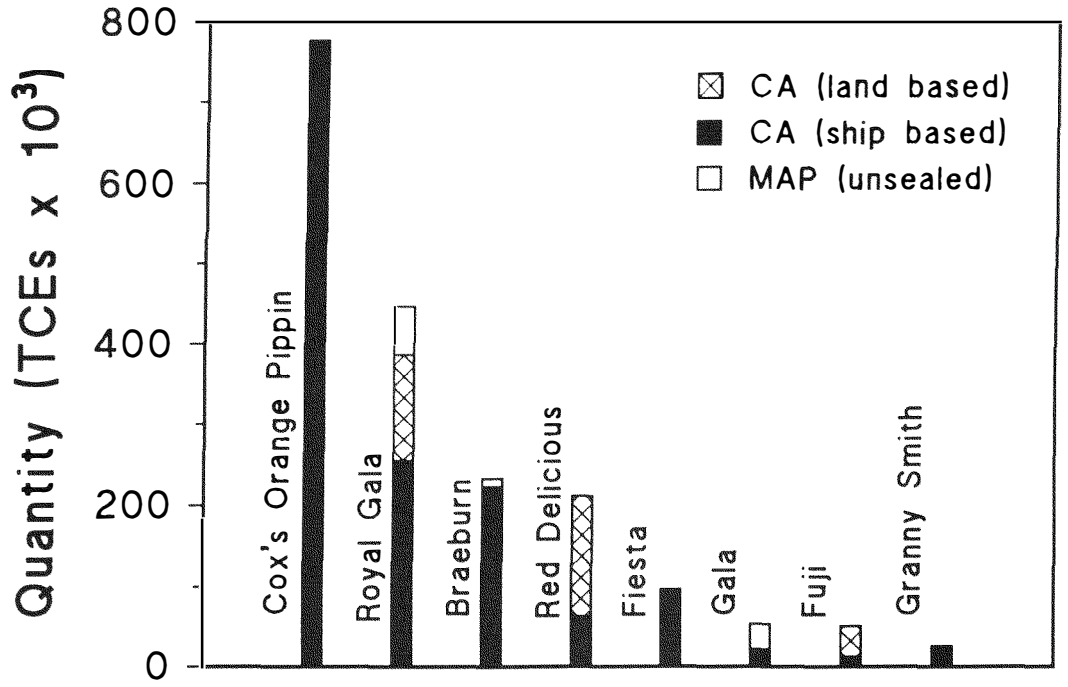


Figure 1.1 Quantities (Tray carton equivalents, TCEs) of some New Zealand apple cultivars stored in controlled atmospheres (CA) or in unsealed polymeric film carton liners (MAP) during 1995 by ENZA New Zealand (International).

Modified atmosphere packaging (MAP) generally, is likely to be utilised more in the future as temperature compensating polymeric films with variable permeability characteristics (Anon, 1992; Challis and Bevis, 1992), active packaging using O_2, CO_2 scavengers/emitters (Day, 1991), and development of "sense-and-respond" packages (Cameron *et al.*, 1995), become available. The atmosphere in MAPs can be modified by flushing the package with the desired gas atmosphere before sealing and/or utilising the metabolic activity of the enclosed crop to modify the package

atmosphere over a few hours during storage. Internationally, MAP has become a major growth area for both whole and minimally processed fresh products.

There is increasing interest in use of edible skin coatings (already used for some lines of apples on the local market in New Zealand), mainly to improve fruit appearance and reduce water loss, but internal atmosphere (IA) is also being modified by these treatments (Baldwin *et al.*, 1995).

While CA storage is an additional cost to the apple industry over and above cool storage, it is increasingly being used by the New Zealand apple industry for both export and local market:

- for improved storage potential,
- to allow longer marketing periods, and
- to alleviate pressure on packhouse facilities, by allowing grading and packing to be spread throughout the season.

In general for horticultural crops, MAP has advantages over traditional cool storage, including (Hewett, 1990):

- extended distribution radius internally,
- opportunity for sea freight rather than air freight,
- reduced labour and wastage at retail level,
- favourable economics due to reduction in handling and distribution of unwanted or low grade produce, and
- excellent branding opportunities for product differentiation.

While CA and MA may slow ripening and senescence and reduce some physiological disorders (Hewett and Thompson, 1989), they may also enhance gas and temperature-related physiological disorders such as low temperature breakdown, low-oxygen (O_2) or alcohol injury and high carbon dioxide (CO_2) injury, core flush, brown heart and 'Braeburn browning disorder'. If O_2 levels are too low or CO_2 levels too high, critical tolerance limits may be reached, below which the fruit begin to ferment (Cameron *et al.*, 1995). Similarly, for MAP, exposure to higher than ideal storage temperatures during distribution and retailing may result in fermentation and development of off-flavours, or growth of organisms prejudicial to consumer health (Cameron *et al.*, 1995). Therefore, it is critical that lower O_2 limits (*LOL*s), below which fermentation is initiated, are identified for optimising storage atmospheres for

a crop. Knowledge of *LOL*s can be used for a range of postharvest techniques including CA, MAP, edible skin coatings, and gas and heat treatments used for pest disinfestation.

To understand the relationship between *LOL*s and optimum storage atmospheres for crops such as apples, first it is important to understand the way key variables of gas exchange directly or indirectly affect fruit physiology.

1.2 Generalized model for gas exchange in apples

The postharvest behaviour of apples is the consequence of complex interactions of environmental stimuli such as temperature, relative humidity, and atmospheric composition, and the inherited biochemical and biophysical characteristics of the fruit. The interrelationships are further complicated as physiological behaviour of apples changes markedly as apples ripen and senesce. Many of these variables are mutually interdependent and their interactions can be modelled. The following schematic model (Fig. 1.2, modified from Dadzie, 1992, and Banks *et al.*, 1993) illustrates the relationships between:

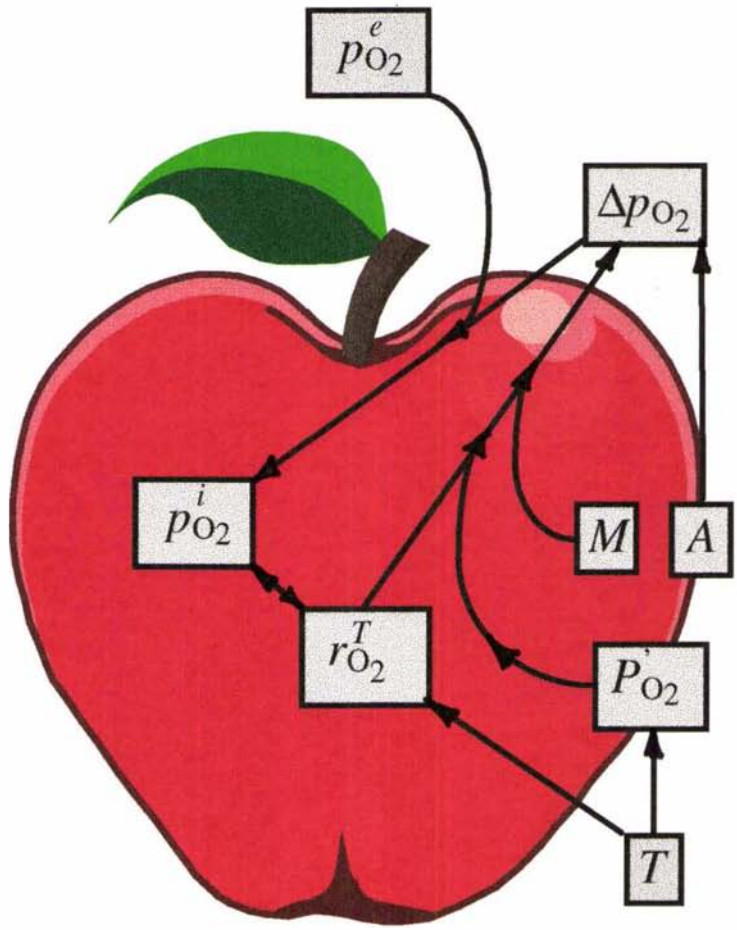
1) environmental variables;

- temperature (T, °C),
- partial pressure of oxygen external to the fruit ($p^{e}_{O_2}$, Pa), and

2) fruit variables;

- surface area (A, m^2),
- fruit mass (M, kg),
- skin permeance to O_2 ($P^{'}_{O_2}$, mol s^{-1} m^{-2} Pa^{-1}),
- O_2 uptake (r_{O_2}, mol kg^{-1} s^{-1}),
- CO_2 evolution from aerobic and anaerobic respiration (r_{CO_2}, mol kg^{-1} s^{-1}), and
- internal partial pressure of O_2 ($p^{i}_{O_2}$, Pa).

$$\Delta p_{O_2} = p^{i}_{O_2} - p^{e}_{O_2} = \frac{r_{O_2} M}{P^{'}_{O_2} A} \qquad (1.1)$$



$$r^{max,T}_{O_2} = r^{max,0}_{O_2} \, Q_{10}{}^{(0.1T)} \qquad (1.2)$$

$$r^{T}_{O_2} = r^{max,T}_{O_2} \left(\frac{p^{i}_{O_2}}{k_1 + p^{i}_{O_2}} \right) \qquad (1.3)$$

$$r_{CO_2} = RQ^{\infty} \, r^{max}_{O_2} \left(\frac{p^{i}_{O_2}}{k_1 + p^{i}_{O_2}} + \frac{10^{-10}}{\left(k_2 + p^{i}_{O_2}\right)^{k_3}} \right) \qquad (1.4)$$

Figure 1.2 Schematic model illustrating the interdependence of various environmental and fruit variables resulting in the gradient between external and internal atmospheres of apples.

O_2 diffuses from a region of high partial pressure outside the fruit ($p^e_{O_2}$) to that of lower partial pressure within the intercellular air spaces of apple cortical tissue ($p^i_{O_2}$). The gradient in partial pressure of O_2 (Δp_{O_2}) between $p^e_{O_2}$ and $p^i_{O_2}$ (Eq. 1.1, see Fig. 1.2), is directly proportional to the rate of O_2 uptake by cortical cells (r_{O_2}) and mass of the fruit (M), and inversely proportional to skin permeance to O_2 (P'_{O_2}) and the surface area of the fruit (A).

When an apple is at steady-state, and given M and A are constant, the diffusion of O_2 into the fruit and uptake of O_2 by respiration and other oxidative processes are equal, and Δp_{O_2} is constant. For apples with the same r_{O_2} and P'_{O_2}, the ratio M/A (Eq. 1.1) for a larger fruit would be comparatively higher compared to a smaller fruit, and consequently Δp_{O_2} of the larger fruit would be comparatively higher.

The interdependencies of the factors can be illustrated by considering some common postharvest handling scenarios. First, consider warm apples being placed into a cool store. Decreasing T directly affects r_{O_2} (Eq. 1.2) such that the maximum rate of r_{O_2} when O_2 is not limiting ($r'^{max.\,T}_{O_2}$) decreases. As r_{O_2} decreases, Δp_{O_2} decreases slightly, thereby increasing $p^i_{O_2}$. r_{O_2} is also directly affected by $p^i_{O_2}$ (Eq. 1.3). An increase in $p^i_{O_2}$ has the effect of slightly increasing r_{O_2}, thereby increasing Δp_{O_2}. This cycle of interactions continues until fruit temperature and r_{O_2} equilibrate, and a new steady-state in gas exchange is reached. It can be seen that changes in $p^i_{O_2}$ interact with r_{O_2} as both cause and effect in the model. The converse of the above interactions occurs when cool apples are warmed. It is possible increasing T could also increase P'_{O_2} of the fruit skin. This effect would in turn moderate the interaction of increasing T on r_{O_2}, $p^i_{O_2}$ and Δp_{O_2}.

Secondly, under CA and MA conditions $p^e_{O_2}$ is lowered. As r_{O_2} initially continues at the same rate, Δp_{O_2} is maintained and $p^i_{O_2}$ begins to decrease, which in turn has an inhibitory effect on r_{O_2}. Eventually, $p^i_{O_2}$ and r_{O_2} reach a new dynamic equilibrium, and diffusion of O_2 into the fruit equals uptake by respiration. Under normal CA or MA handling strategies, reduction in $p^e_{O_2}$ may occur simultaneously with cooling, such that effects of T on r_{O_2}, $p^i_{O_2}$ and Δp_{O_2} are superimposed on effects of reducing $p^e_{O_2}$. Gas exchange for fruit in MAP is more complex than for CA as diffusion

through the polymeric film (which, like apple skin, is typically more permeable to CO_2 than O_2), creates a further gradient in atmospheres external to the fruit, arising from an additional set of analogous interactions.

During cool storage, apples may undergo a respiratory climacteric during which r_{O_2} increases, after which it may decrease again during the postclimacteric phase. The effects on gas exchange of the climacteric would be analogous to the effect of increased T on r_{O_2}. Depending on cultivar and storage conditions (eg. relative humidity), P'_{O_2} may increase (e.g. 'Cox's Orange Pippin'; see Table 7.2) or decrease (e.g. 'Granny Smith'; Dadzie, 1992) during cool storage. If $P'_{\bullet_2}$ decreases during storage, this would increase $\Delta p_{\bullet_2}$, decrease $p^i_{O_2}$ and consequently lower r_{O_2}.

Apples in CA or MA may be exposed to levels of $p^e_{O_2}$ where $p^i_{O_2}$ may drop below *LOL*s and fermentation or anaerobic respiration is initiated. Depending on respiratory substrate, the respiratory quotient [RQ, or ratio of CO_2 production (r_{CO_2}) to r_{O_2}] for apples is typically just above 1.0. During aerobic respiration, r_{CO_2} and r_{O_2} have similar values across a range of $p^i_{O_2}$. However, as anaerobic respiration increases, r_{CO_2}, RQ and the internal partial pressure of CO_2 ($p^i_{CO_2}$) increase markedly (Fig 1.3a). The relationship between $p^i_{O_2}$ on r_{CO_2} of aerobic and anaerobic processes is described by Eq. 1.4 (see Fig. 1.2), where RQ^{∞} is the RQ when $p^i_{O_2}$ is not limiting. The zone of transition between aerobic and anaerobic respiration is the zone of the critical *LOL*s, and the primary interest of the current study.

1.3 Optimum internal atmospheres and lower oxygen limits

The principal aim in exploiting the beneficial effects of CA and MA on apple physiology at a given temperature, is to minimise aerobic respiratory metabolism and associated metabolic processes (including for apples, ethylene biosynthesis and its effects on fruit ripening). This may be achieved by both reducing O_2 and elevating CO_2 to levels within critical tolerance points. Quantification of appropriate external

atmospheres for individual crops has been the subject of numerous studies since the early work of Kidd and West (1927) with apples. More recently the value of quantifying CA and MA effects based on internal atmospheres (IA) has been promoted (Banks *et al.*, 1993). The rationale for this approach is that it is the atmosphere within the crop present in the intercellular air spaces, that mediates all responses to MA regimes. From Eq. 1.1 it is clear that IAs of apples may differ one from the other due to differences in P'_{O_2} and r_{O_2} of individual fruit, even though the external atmosphere is identical. Consequently, identifying the *LOL* based on IAs accounts for fruit to fruit variation in P'_{O_2} and r_{O_2}, and is more likely to represent the *LOL* for a population of apples than one based on external atmospheres.

Composition of the optimum IA represents a compromise between the benefits accruing from lowered aerobic respiration and the risk of deleterious effects associated with fermentation (resulting from either from low $p^i_{O_2}$ or elevated $p^i_{CO_2}$, Fig. 1.3a). Thus, optimum $p^i_{O_2}$ would be expected to lie in a region just above the $p^i_{O_2}$ at which fermentation is initiated. This point may be termed the internal fermentation threshold (FT^i) and may be identified using values of RQ (FT^i_{RQ} , Fig. 1.3b) and ethanol (EtOH) accumulation (FT^i_{EtOH}, Fig. 1.3c). Below the FT^i, the sum of $p^i_{CO_2}$ arising from aerobic and anaerobic components of respiration (described by Eq. 1.4) would decline to a minimum value at $p^i_{\bullet_2}$ termed the internal anaerobic compensation point (ACP^i), then increase again steeply. Optimum $p^i_{CO_2}$ depends on tissue sensitivity to both positive and negative effects of CO_2. Crop responses to CO_2 have been reviewed by Kader (1993), but at present there are no clearly identified criteria by which an optimum level can be identified. In the broader framework of long term storage and the inherent physiological susceptibilities of a crop to physiological disorders, IA is optimised when overall rate of deterioration is reduced to a minimum.

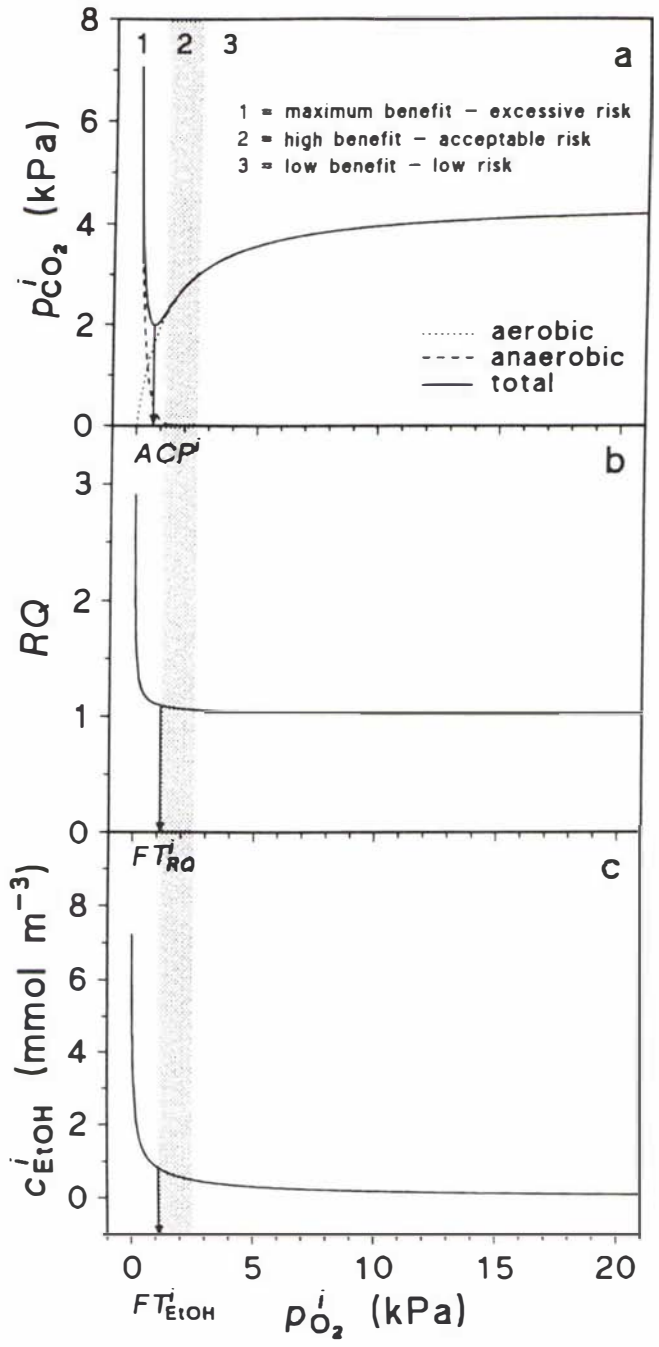


Figure 1.3 Relationship between internal partial pressure of O_2 ($p^i_{O_2}$) and a) internal partial pressure of CO_2 ($p^i_{CO_2}$) from aerobic and anaerobic respiration, b) respiratory quotient (RQ) and (c) internal concentration of ethanol (c^i_{EtOH}) based on data for 'Cox's Orange Pippin' apples at 24°C. Different zones (1 to 3) are identified representing different benefits to firmness and colour change, and risks of storage disorders and off-flavour development for storage atmospheres, and arrows indicate approximate $p^i_{O_2}$ for LOL^i s (ACP^i, FT^i_{RQ} and FT^i_{EtOH}).

1.4 Research objectives and structure of the thesis

Dadzie (1992) and Dadzie *et al.* (1993) modelled the difference between ACP^i and ACP based on external atmospheres (ACP^e) in an analogous way to that of the difference between $p^i_{O_2}$ and $p^e_{O_2}$:

$$ACP^e = ACP^i + \frac{r^{ACP^i}_{O_2} M}{P'_{O_2} A} \qquad (1.5)$$

In quantifying the relationship between ACP^i and ACP^e and the likely impact of temperature on ACP^e, Dadzie *et al.* (1993) assumed that ACP^i was unaffected by either temperature or respiration rate. The question that the current study focused on was whether for two physiologically contrasting apple cultivars [cvs. 'Cox's Orange Pippin' ('COP') and 'Braeburn'], LOL^is, like LOL^es, vary with respect to environmental stimuli and physiological state. Specifically, the null hypotheses tested were that LOL^is are constant with respect to:

- temperature,
- elevated external partial pressures of CO_2 ($p^e_{CO_2}$), and
- physiological age and state of the fruit.

In chapter 2, a review of relevant literature is presented. First a brief account of respiratory metabolism is presented as it is the key metabolic process associated with gas exchange. A review of literature on physiological and biochemical effects of modified atmospheres on aerobic and anaerobic respiration, and studies elucidating the aerobic-anaerobic transition is presented next. The second part of the literature review focuses on gas diffusion and pathways of gas exchange in bulky plant organs and factors affecting IAs, other than respiration.

Chapter 3 details materials and methods used in the study. For key methods, the theoretical basis, empirical characterisation, and discussion on approaches taken to minimise inherent variability associated with quantifying a variable, are presented.

Chapters 4 to 8 were written more concisely as papers for publication in Postharvest Biology and Technology and New Zealand Journal of Crop and Horticultural Science, then reformatted in the style of the thesis. At the time of writing, chapter 4 has been published, chapters 5, 6 and 7 are ready for submission, and chapter 8 has been submitted.

Objective mathematical definitions of *LOL*s have not been published previously. Consequently an early consideration in the research was to develop a quantitative, conceptual framework for identifying *LOL*s as functions of both external and internal partial pressure of O_2 for apples. In chapter 4, objective mathematical and statistical methods for determining various *LOL* measures are described, and their usefulness as a means for optimising storage atmospheres discussed. Supplementary to the published paper, the theoretical development of an alternative method of defining the *FT* is presented and discussed.

The primary research goals outlined above, are the focus of chapters 5, 6 and 7 respectively. Measurement of IAs was clearly a key technique used in the study. Sampling from steady-state atmospheres in surface chambers adhered to the fruit, and from the core cavity, were the principal methods used to estimate IAs. An alternative method used, which was invasive but nondestructive (and thus useful for storage studies), involved cannulating fruit from which repeated samples of IAs could be removed from the core cavity or cortex. In chapter 8, the physiological consequences of cannulation is quantified, and its usefulness discussed.

Chapter 9 is a general discussion drawing together data demonstrating the pivotal role of skin permeance and respiration rate in determining gradients between external and internal atmospheres, routes for gas exchange, the benefits and risks associated with optimisation of storage atmospheres for apples using LOL^i s versus $LOL^e s$, implications of the study in relation to the implementation of CA and MA for apples, a suggested system for using IAs to modulate CA storage atmospheres, and potential areas for extending the research.

In addition to the primary research objectives, I contributed to an industry funded [ENZA New Zealand (International)] collaborative study involving Massey

University and HortResearch Ltd., investigating factors affecting the incidence of 'Braeburn browning disorder'. As this involved a significant portion of time during the course of the Ph.D project, and was indirectly related to the study, brief industry reports are included as appendices.

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Chapter 2

Literature Review

2.1 Introduction

Postharvest research and technology is largely aimed at maintaining quality and prolonging the storage life of whole or minimally processed crops. This has traditionally been achieved through optimising handling procedures and using cold storage and/or modifying or controlling the storage atmosphere, to delay ripening and senescence. As a consequence much postharvest research has been aimed at understanding the metabolic changes associated with ripening, physiological disorders which may be initiated pre- or postharvest, and the way in which temperature and storage atmosphere affect these metabolic changes.

The start of modern postharvest physiology was intimately involved in elucidating the respiratory behaviour of fruit. Preoccupation with fruit respiration stemmed from the perception that respiration was a measure of metabolic health and fruit integrity (Laties, 1995). In 1924, as a consequence of studying the postharvest behaviour of apples in cold storage, Kidd and West characterised the ripening-related respiration phenomenon they termed the 'climacteric' (Kidd and West, 1925). At the same time Blackman commenced a study of the influence of O_2 tension both on the course of respiration and on the extent of glycolysis and fermentation in cold stored fruit. Recently, Laties (1995) reviewed the valuable contribution made by these pioneers of postharvest physiology; attempting to explain the respiratory climateric still remains a challenge to physiologists (Blanke, 1991).

Inherent in early studies of respiration was the definition of the O_2 level at which aerobic respiration was extinguished in favour of fermentation, which Blackman termed the 'extinction point' (Blackman, 1928). The first part of the following literature review centres on respiratory metabolism, the aerobic-anaerobic transition and atmosphere modification effects. The effects of temperature, elevated CO_2 and physiological age on the aerobic-anaerobic respiratory transition are briefly reviewed and discussed further in chapters 5, 6 and 7 respectively.

Differences in concentration of gases between the external and internal atmosphere of a crop are the driving force of diffusion-mediated transfer of gases

(Nobel, 1991, pp. 10-11). The magnitude of these differences may vary markedly between crops and individuals within a population of a crop as a consequence of differences in the rate of respiration and permeance of the fruit surface to gases. The second part of the literature review concentrates on gas diffusion between the external and internal atmospheres and within the crop and its implications for optimising storage atmospheres using lower oxygen limits.

To avoid repetition, review of literature describing methods used for sampling internal atmospheres and rates of transfer of gases, and discussion of the relative merits of different methods, is presented in chapter 3. A review of methods for estimating and monitoring changes in internal atmospheres of apples is also presented in the introduction of chapter 8.

2.2 Respiratory metabolism

2.2.1 Introduction

Respiration refers to the oxidative breakdown of carbohydrates and organic acids, to simpler molecules such as CO_2 and H_2O. Concurrently, energy is produced in the form of adenosine triphosphate (ATP), and other molecules which can be used by the cell for synthetic reactions, maintenance of cellular organisation and membrane integrity, preservation of ion gradients and protein turnover (Blanke, 1991; Hardenburg *et al.*, 1986; Kader, 1987; Wills *et al.*, 1989).

For detached plant organs, respiration occurs at the expense of substrates accumulated during development. Hence, respiration rates of non-growing plant tissues are often regulated by substrate supply, even in tissues with large carbohydrate reserves (Lambers *et al.*, 1983). Not all energy released in respiration is transferred into a usable form. The potential efficiency of respiration is about 40% (the potential energy yield depending on the substrate), and the remaining energy is lost as heat (Salisbury and Ross, 1992, p. 278)]. It was this understanding that respiration was the source of metabolic heat that provided the rationale for the early

work on cold storage of Franklin Kidd and Charles West at the Low Temperature Research Station at Cambridge, England (Laties, 1995).

In general, respiration rate may be used as a guide to the potential storage life of a crop and is often used as a monitor of the efficiency of postharvest methods of maintaining fruit quality (Fidler and North, 1971; Kader, 1992; Wills *et al.*, 1989). Within a given fruit species, a high respiration rate is commonly associated with a short storage life and may indicate the rate at which the fruit is deteriorating in quality and food value (Phan *et al.*, 1975).

Respiration is a broad term and can refer to metabolism occurring in "normoxic" atmospheres (normal O_2 partial pressure in air, $\cong$ 21 kPa O_2) in which aerobic respiration occurs, hypoxic (< 21 kPa O_2) and anoxic (total absence of O_2) atmospheres, in which there may be a transition from aerobic to anaerobic respiration below a critical lower O_2 limit for the tissue. Ricard *et al.* (1994) contend that even under experimental conditions believed to be anoxic, traces of O_2 may be still be present. Therefore, it has been recommended the term anoxia should be restricted to situations where fermentation can be considered to be the only source of ATP, because mitochondrial respiration has become nearly nil (Ricard *et al.*, 1994). Roberts *et al.* (1992) used the term 'deep hypoxia' for atmospheres often stated in reports to be anoxic.

Mitochondrial respiration is eliminated by the absence of O_2 since, without O_2 (which is the final electron acceptor in mitochondrial respiration), the mitochondrial electron transport chain can not operate. This results in a switch to anaerobic or fermentative metabolism in which pyruvate is reduced to lactate and/or decarboxylated to form acetaldehyde which is further reduced to ethanol. The accumulation of anaerobic metabolites may result in the development of off-flavours and tissue breakdown (Kader, 1986; Kader *et al.*, 1989).

The following review considers respiration under aerobic and anaerobic conditions, and the biochemical and physiological basis of atmosphere modification on respiratory metabolism.

2.2.2 Aerobic respiration

The reductive fixation of atmospheric CO_2 into a carbohydrate in chloroplast photosynthesis, can serve as the free energy source for respiration, sucrose and starch being the principle sources of respiratory substrates in plants (Salisbury and Ross, 1992). Glycolysis and the pentose phosphate pathway are the two main pathways of carbohydrate metabolism in plants (Stryer, 1988). The pentose phosphate pathway (also known as the pentose shunt, the hexose monophosphate pathway, or the phosphogluconate oxidative phosphate pathway) occurs in the cytosol and generates reducing power in the form of nicotinamide adenine dinucleotide phosphate (NADPH) when glucose 6-phosphate is oxidised to ribose 5-phosphate (Stryer, 1988). Glycolysis also occurs in the cytosol and is a pathway shared by both aerobic and anaerobic respiration.

2.2.2.1 Glycolysis

Glycolysis was a term introduced in 1909 to mean breakdown of sugar to ethanol but now generally refers to the degradation of hexoses to pyruvate (Salisbury and Ross, 1992). In aerobic organisms under normoxic conditions, glycolysis (also termed the Embden-Meyerhof-Parnas pathway) is the prelude of the tricarboxylic acid cycle (TCA, also termed the citric acid or Krebs cycle) and the electron-transport pathway, the latter processes occurring in the mitochondria. In addition to the primary role of generating adenosine triphosphate (ATP), glycolysis and the TCA cycle form molecules that may be utilised in the synthesis of other plant components as illustrated in Fig. 2.1.

If the carbohydrate glucose is used as the respiratory substrate, under both aerobic and anaerobic conditions, it is first broken down in the cytosol into two molecules of puruvate in a process that requires no O_2 and releases no CO_2 (Fig. 2.2).

In glycolysis, two molecules of the oxidised electron acceptor nicotinamide adenine dinucleotide (NAD^+) are reduced to NADH (+ $2H^+$). Each NADH can subsequently be oxidised by O_2 in the mitochondrion so that NAD^+ is regenerated and two molecules of ATP are formed from adenosine diphosphate (ADP) and

inorganic orthophosphate (P_i) as a result of transfer of electrons. From Fig. 2.1 it can be seen that NADH is formed at only one step in glycolysis. Although some ATP is formed, it is required to phosphorylate either glucose or fructose, and fructose 6-phosphate to fructose -1,6 bisphosphate, a step catalysed by ATP-phosphofructokinase (ATP-PFK).

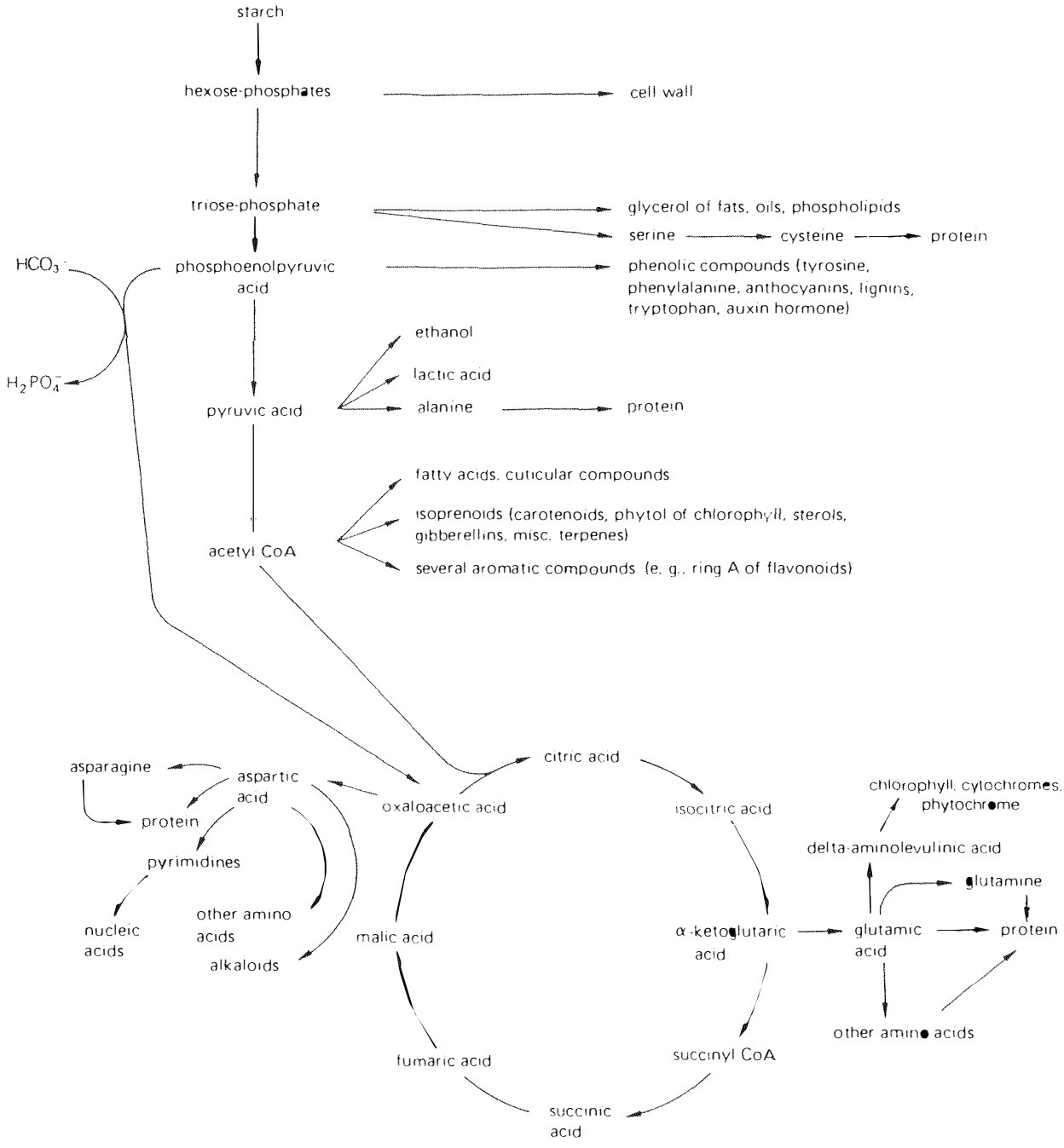

Figure 2.1 Simplified pathways of glycolysis and the TCA cycle to show their relationship to other synthetic processes (From Salisbury and Ross, 1992).

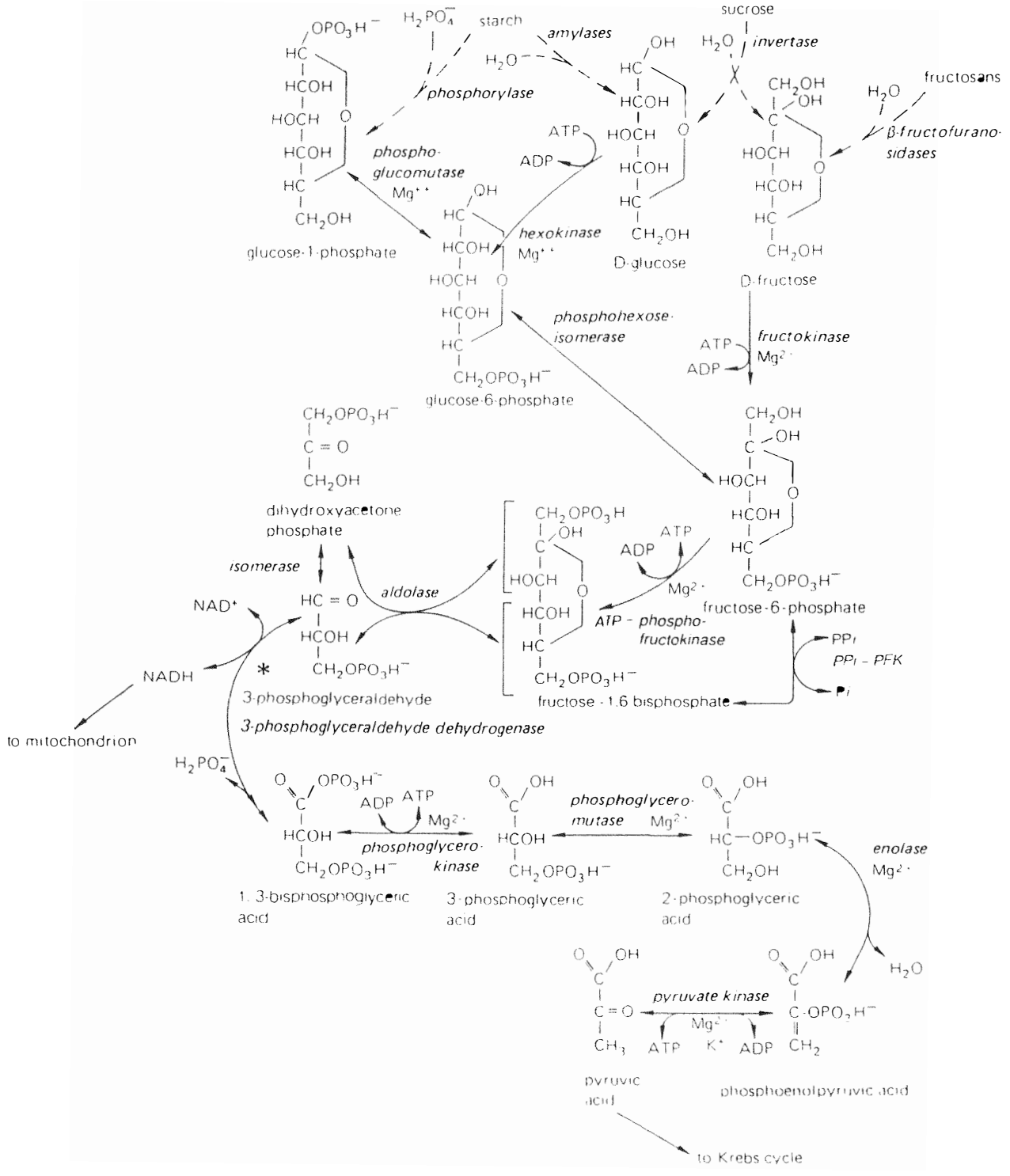

Figure 2.2 Reactions of glycolysis. Names of enzymes and metal ion or ions promoting the activity of enzymes are shown. Reactions indicated by dashed arrows are degradative. (From Salisbury and Ross, 1992).

Another route by which fructose 6-phosphate can be converted to fructose 1-6-bisphosphate was reported by Carnal and Black (1983), and is catalysed by pyrophosphate phosphofructokinase (PPi-PFK). Current evidence suggests the ATP-PFK route is involved in "maintenance respiration" and the PPi-PFK route is more adaptive and can increase and decrease depending on developmental processes and environmental conditions (Black *et al.*, 1987). From an energy utilisation viewpoint, the ATP-PFK route requires two ATPs whereas the PPi-PFK route uses only one ATP. Fructose 1,6-bisphosphate is split to form two three-carbon phosphorylated sugars which are then oxidised to pyruvic acid yielding two ATP from each triosphosphate. The net yield of ATPs to this point in glycolysis is therefore two or three depending on whether the ATP-PFK or PPi-PFK route is used.

Overall glycolysis can be summarised by the equation:

$$\text{glucose} + 2\,NAD^{+} + 2\,ADP^{2-} + 2\,H_2PO_4^{-} \rightarrow$$
$$2\,\text{pyruvate} + 2\,NADH + 2\,H^{+} + 2\,ATP^{3-} + 2\,H_2O \qquad (2.1)$$

Under anaerobic conditions, further oxidation of NADH and pyruvate in mitochondrial respiration is not possible, and as they begin to accumulate anaerobic respiration or fermentation is activated (see 2.3.3).

2.2.2.2 Tricarboxylic acid cycle and the electron transport system

In aerobic conditions, pyruvic acid is decarboxylated and combined with coenzyme A (CoA) to form acetyl CoA (Stryer, 1988). This involves a phosphorylated form of thyamine (Vitamin B_1), and is catalysed by a complex of enzymes collectively termed pyruvate dehydrogenase (PDH). Two hydrogen atoms are removed from pyruvic acid and combined with NAD^{+} yielding NADH. The reactions involved in the TCA cycle are illustrated in Fig. 2.3, and occur in the mitochondrial matrix. The release of CO_2 in the TCA cycle accounts for that measured when estimating respiration rate of plant tissue, but no O_2 is absorbed during any TCA cycle reactions.

The TCA cycle removes some electrons from organic acid intermediates, transferring them to either NAD^+ (to form NADH) or ubiquinone (to form ubiquinol). Also one molecule of ATP is formed from ADP and Pi during the conversion of succinyl coenzyme A to succinic acid. Another function of the TCA cycle is the formation of carbon skeletons used to synthesise amino acids. The overall reactions of the TCA cycle can be written as:

$$2 \text{ pyruvate} + 8\ NAD^+ + 2 \text{ ubiquinone} + 2\ ADP^{2-} + 2\ H_2PO_4^- + 4\ H_2O \rightarrow$$
$$6\ CO_2 + 2\ ATP^{3-} + 8\ NADH + 8\ H^+ + 2 \text{ ubiquinol} \qquad \textbf{(2.2)}$$

When NADH and ubiquinol are oxidised they yield ATP, but as neither can combine directly with O_2 to form H_2O, their electrons are transferred via several intermediate compounds forming the electron carrier system on the inner membrane of the mitochondria. The carriers are arranged in four main protein complexes and O_2 serves as the ultimate electron acceptor, mediated by cytochrome oxidase, in a process termed oxidative phosphorylation (Stryer, 1988). As electrons flow down a potential gradient in the electron transport chain, protons are pumped across the inner mitochondrial membrane through a mitochondrial ATPase coupled with ATP synthesis (chemiosmotic coupling). This is the major source of ATP in aerobic organisms and generates 32 of the 36 ATP molecules that are formed when glucose is completely oxidised to CO_2 and H_2O.

Concentrations of CO_2 in fruit cells are partly regulated by the cytosolic enzymes phosphoenol pyruvate carboxylase (PEPC), recycling of some respiratory CO_2 may occur before it is released through the fruit surface, and possibly by phosphoenol pyruvate carboxykinase (PCK; Blanke, 1991). PEPC has been found in apples (Blanke and Lenz, 1989) and preclimacteric avocado (Blanke and Notton, 1991; Clark *et al.*, 1961). The distribution of PEPC is uneven, being concentrated in the seeds and perivascular tissue such as the hypodermal and inner core vascular tissue of apple and avocado; it is very efficient with a low K_m (Blanke, 1991).

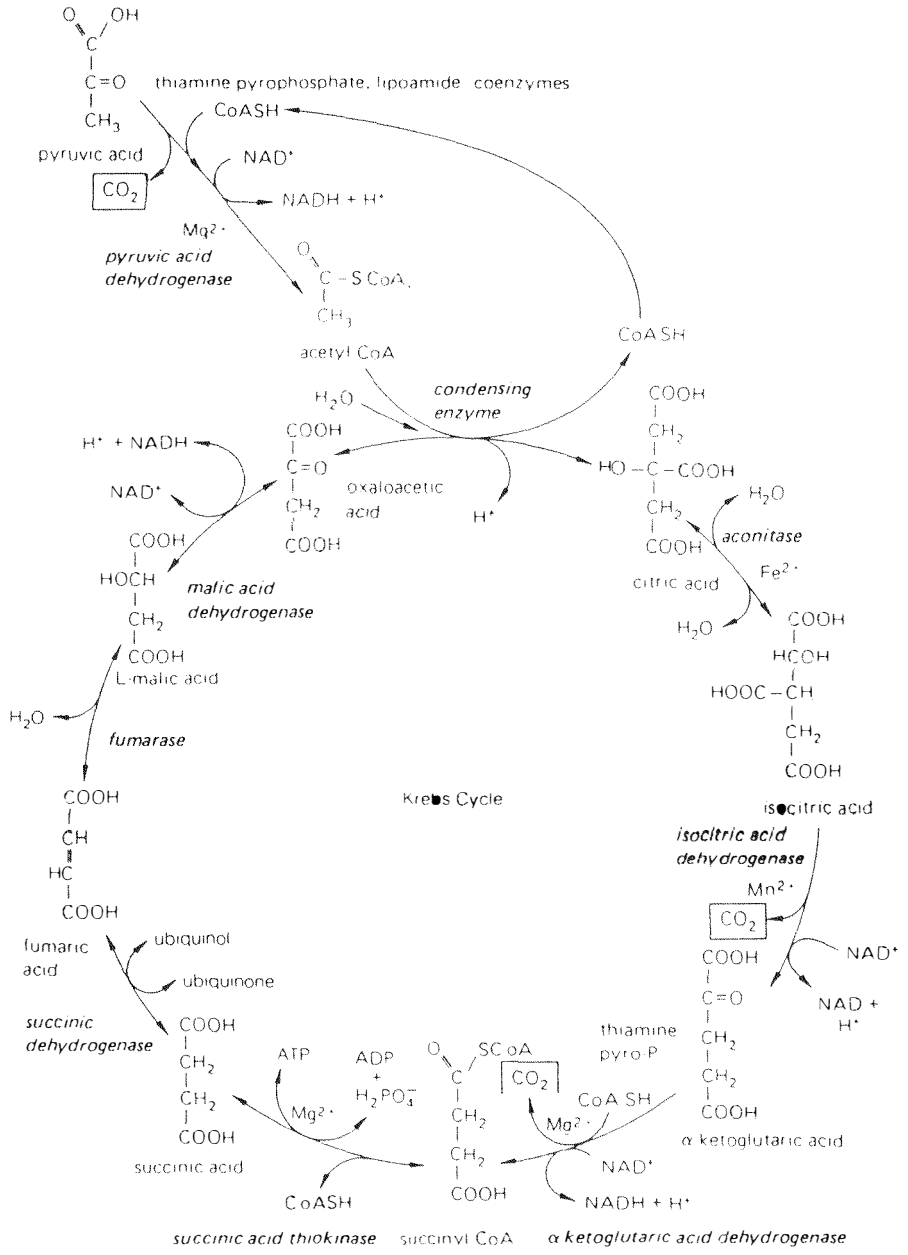

Figure 2.3 Reactions of the TCA cycle, including enzymes and coenzymes. The oxidative reduction of pyruvic acid is also included. (From Salisbury and Ross, 1992).

2.2.2.3 Cyanide-resistant respiration

Aerobic respiration of most organisms is strongly inhibited by some negative ions, particularly cyanide (CN^-) and azide (N_3^-), which complex with the iron of cytochrome oxidase (Stryer, 1988). However, in most plants, poisoning of cytochrome oxidase has only a small effect on limiting respiration, and the respiration that continues is termed cyanide-resistant (cyanide-insensitive) respiration. This alternative short-branch in the electron-transport chain involves ubiquinol, and also allows transport of electrons to O_2, but the alternative oxidase has a much lower affinity for O_2 than does cytochrome oxidase. As little or no oxidative phosphorylation is coupled to the alternative pathway, it leads mainly to the production of heat and produces only one ATP when linked to the oxidation of mitochondrial NADH, whereas the cytochrome pathway yields three ATP molecules. The alternative pathway is believed to act as an overflow mechanism to remove electrons when the cytochrome pathway becomes saturated by rapid glycolysis (Lambers, 1985). The role the cyanide-resistant pathway may play in the respiratory climacteric is discussed in section 2.2.4.1.

2.2.2.4 Respiratory quotient (RQ) and the effects of reduced O_2 and elevated CO_2

Respiration quotient (*RQ*) is the volumetric or molar ratio of the amount of CO_2 produced to O_2 simultaneously consumed during respiration (Burton, 1982; Blanke, 1991). When aerobic respiration is operating in a state of dynamic equilibrium, and with hexose as the respiratory substrate, the *RQ* should be unity. However the composition of crops determines the substrates used in respiration, and estimates of *RQ* can give an indication of the principal substrate (Wang, 1990). The *RQ* is near 1 for carbohydrate substrates, < 1 for lipids, > 1 for organic acids (1.33 for malic acid), and much higher rates usually indicate anaerobic respiration or senescent decay (Blanke, 1991). *RQ* generally increases with ripening and senescence indicating increased metabolism of organic acids, which have more O_2 per carbon atom than carbohydrates and lipids. However, *RQ* values are typically averages as different

types of substrate may be metabolised simultaneously (Devlin and Witham, 1983). Values for *RQ* at 10°C of a number of crops have been published by Platenius (1943).

Kidd and West (1925, 1938) reported that the *RQ* of apples, ripened in air at 22.5°C, increased slightly during the climacteric, which Burton (1982) attributed to increased utilization of malic acid. An even higher rise in *RQ* due to malate decarboxylation during the climacteric was reported by Neal and Hulme (1958) and Hulme and Rhodes (1971). This increase in *RQ* with the climacteric may not be a universal phenomenon in climacteric fruits (Hartmann, 1962). Palmer (1971) reported the *RQ* of preclimacteric banana decreased during the climacteric upswing in respiration rate but increased again to the initial value at the peak. The drop in *RQ* may reflect a short period of utilization of lipids (Tucker and Grierson, 1987), or alternatively, be affected by solublisation of CO_2 that would occur in response to elevated $p^{i}_{CO_2}$.

Decreased external O_2 atmospheres depress the rate of respiration of crops, but above the critical lower O_2 limit, this is thought to occur without change in *RQ* (Beaudry *et al.*, 1992; and Banks *et al.*, 1993b). However, elevated CO_2 by itself or in conjunction with decreased O_2 depresses *RQ* (Fidler, 1950; Fidler and North, 1967; Metlitskii *et al.*, 1972). In a study using postclimacteric 'Cox's Orange Pippin' apples, Fidler and North (1967) reported that relative to air, storage in 5% CO_2 reduced CO_2 production by 45% and O_2 uptake by 25% in levels of O_2 from 1.5% to 16%. Wang (1990) suggested the reduction in *RQ* for fruit in elevated CO_2 was possibly due to an increase in the fixation of CO_2 into organic acids in the fruit, a reduced catabolism of organic acids resulting in a higher organic acid retention, as reported for CA stored apples and pears (Kollas, 1964; Li and Hansen, 1964). CO_2 is mainly fixed into malic acids in apples (Allentoff *et al.*, 1954a, 1954b), but also into citric and pyruvic acids (Murata and Miniamide, 1970). Temperature may also affect *RQ* as observed by Fidler and North (1967) who attributed the effect to a higher temperature coefficient for O_2 uptake than for CO_2 production.

2.2.2.5 *Respiration rate and the effects of reduced O_2 and elevated CO_2 on aerobic respiration*

Reduction of the partial pressure of O_2 surrounding fruit decreases respiration and this effect becomes more marked as the partial pressure of O_2 drops below 10 kPa (Banks *et al.*, 1993b). The relationship between respiration rate and either the internal or external partial pressure of O_2 of crops has been modelled using the Michaelis-Menten equation (Andrich *et al.*, 1991; Banks *et al.*, 1993b; Cameron *et al.*, 1995; Dadzie, 1992; Peppelenbos *et al.*, 1993; Solomos 1982, 1985). Below the critical lower O_2 limit, CO_2 production increases markedly as anaerobic respiration increases and aerobic respiration decreases.

Published data on the effect of elevated CO_2 on O_2 uptake are contradictory, with no effect, an increase or decrease in respiratory activity reported depending on the crop and the partial pressure of CO_2 (Cameron *et al.*, 1995; Kerbel *et al.*, 1988; Kidd, 1916; Kidd and West, 1927, 1933; Kubo *et al.*, 1990; Li and Kader, 1989; Talasila *et al.*, 1992; Thornton, 1933; Young *et al.*, 1962). Joles *et al.* (1994) noted that the effect of elevated CO_2 has often been assumed and even incorporated into some models (Lee *et al.*, 1991; Song *et al.*, 1992). Evidence that elevated CO_2 has no or little effect in reducing respiration rate and O_2 uptake has been reported for bananas (Young *et al.*, 1962), and mushrooms (Peppelenbos *et al.*, 1993). Joles *et al.* (1994) reported that partial pressures of $CO_2 < 17$ kPa did not affect O_2 uptake for raspberries, and Beaudry (1993) that partial pressures of $CO_2 > 20$ kPa resulted in only a small reduction in O_2 uptake of blueberries. Gran (1993) reported that 12 to 15 kPa CO_2 in package headspace increased O_2 uptake compared to packages where CO_2 had been diminished by a CO_2 absorber. However, this interpretation assumes the package atmospheres were at steady-state. If not at steady-state, scrubbing CO_2 from the package atmosphere would increase $p^e_{O_2}$ as the package volume decreased.

Reduction in respiration rate results in a reduction in energy availability to tissue. A quantitative indication of change in energy available to tissue is given by the energy charge [EC: the ratio of adenylitic phosphate held in high energy bonds (2/3 ATP + 1/2 ADP) to the total adenylitic phosphate (ATP + ADP + AMP)] (Pradet and

Raymond, 1983). For nearly all active cells , EC values are between 0.8 and 0.95, but substantially lower values are found in anaerobic cells.

2.2.2.6 *Mode of action of reduced O_2 and elevated CO_2 on aerobic respiration*

The effects of O_2 on fruit include (1) a diminution of aerobic respiration; (2) delay in the climacteric onset of the rise in ethylene; and (3) a decrease in the rate of ripening (Blackman, 1954; Burg and Burg, 1967; Fidler *et al.*, 1973; Kader, 1986; Kanelis *et al.*, 1991; Mapson and Robinson, 1966; Smock, 1979; Solomos, 1982; Yang and Chinnan, 1988a, b).

Blackman (1954) observed that the respiratory isotherms of r_{O_2} as a function of $p^e_{O_2}$ are biphasic in nature, with an initial gradual decrease at relatively high $p^e_{O_2}$, followed by a rapid decline as $p^e_{O_2}$ approached zero. The isotherm for r_{CO_2} follows that for r_{O_2} except at $p^e_{O_2}$ approaching zero when r_{CO_2} rapidly increased due to fermentation.

The biphasic nature of the response of r_{O_2} as a function of $p^e_{O_2}$ has been attributed to (Knee, 1991a; Solomos, 1994):

1. The existence of a regulatory enzyme(s) that perceive(s) the $p^e_{O_2}$ and exert(s) feedback inhibition on the initial steps of glucose oxidation, thus lowering aerobic respiration (Blackman, 1954; Solomos, 1982; Tucker and Laties, 1985);
2. The effect of low skin and tissue permeance to the diffusion of O_2 (Chevillotte, 1973; James, 1953); and
3. The presence of a terminal "oxidase" with a much lower affinity for O_2 than that of cytochrome oxidase (Mapson and Burton, 1962).

Resistance to diffusion of O_2 in apples and avocados has been demonstrated to be unlikely to explain the biphasic nature of the O_2 isotherm (Solomos, 1982, 1988; Tucker and Laties, 1985). Neither cytochrome oxidase nor the alternative terminal oxidase are likely to explain the biphasic response as the K_m for cytochrome oxidase is about 0.05 μM (< 0.1 kPa $p^i_{O_2}$, Knee, 1991b), and the alternative oxidase is 10- to 15-fold higher (Douce, 1985; Siedow, 1982; Solomos, 1977; Tucker and Laties,

1985). Solomos (1994) suggested that if a lower affinity terminal oxidase was to account for detectable decrease in r_{CO_2}, its K_m would have to be > 4 μM.

Both Burton (1982) and Tucker and Laties (1985) have suggested more than one oxidase directly or indirectly affects respiration. Knee (1991b) suggested that there may be mechanisms whereby fruit cells sense hypoxic conditions and limit respiration rate so as to minimise the production of anaerobic metabolites. These views make fruit respiration a consequence of other metabolic processes. Neither the nature of the "residual oxidases" nor their participation in plant respiration is known to any degree of certainty. They are thought to be cytosolic, with low affinity for O_2 (Solomos, 1988, 1994). The inhibition of these residual oxidases is thought to exert a feedback restraint on the initial steps of glucose oxidation. Solomos (1994) noted that though this explanation is not compatible with the most likely known regulatory mechanisms of plant respiration, it is the most likely explanation of the effects of low O_2 on respiration.

Tucker and Laties (1985) reported evidence for a dual role of O_2 in avocado, where depending on the rate of reduction in O_2, the respiratory isotherm was either mono- or biphasic. Rapid reduction in O_2 resulted in a monophasic isotherm with a K_m appropriate for cytochrome oxidase, whereas slow reduction in O_2 resulted in a biphasic isotherm and perhaps allowed for intermediation of biochemical processes, or regulation of gene expression. Laties (1995) suggested the net effect of the slow depletion of O_2 was "that the negative feedback banks the respiratory fire before the operation of the terminal oxidase is curtailed with attendant anaerobiosis".

Tucker and Laties (1985) also suggested that the low affinity arm of the biphasic respiration isotherm has nothing to do with the effect of O_2 on ethylene synthesis or action, as biphasicness was evident equally in preclimacteric and climacteric fruit. Observed effects of hypoxia on ethylene biosynthesis and action may be indirect through suppressing 1-aminocyclopropane-1-carboxylic acid synthase and/or synthesis of transducer(s) of ethylene action (Solomos, 1994).

Knee (1980) reported the response of respiration of apples to diminished O_2 concentrations, or the return to air from hypoxic atmospheres, was delayed by 1 to 4 days depending on the time and direction of transfer. This delay is longer than was required for the internal atmosphere of the fruit to reach physical equilibrium with

the external atmosphere and suggested that though respiration is mediated by cytochrome oxidase, its rate is influenced by some O_2 requiring process whose effects take time to become apparent. Though the response is slow compared to protective mechanisms in animals to hypoxia/anoxia, it is evidence for adaptation to hypoxic atmospheres. Similar delays in restoration of respiratory activity have been observed in other fruit (Rahman *et al.*, 1993). The low-O_2-induced poststorage respiratory suppression of bell pepper fruit was partly explained by the reduced oxidative capacity of mitochondria. (Rahman *et al.*, 1995). Recently, Chervin *et al.* (1996) also speculated as to whether fruit adapt to low O_2, as do some plants and animals (Hochachka, 1991). A clearer understanding of the mechanisms within fruit signaling changing O_2 atmospheres may be exploited to facilitate extended storage either independently or in conjunction with existing storage practices.

2.2.2.7 Effect of reduced O_2 and elevated CO_2 on glycolysis and TCA cycle

Much of the research on the effects of controlled atmospheres (CA) and modified atmospheres (MA) on crops has been directed towards the empirical determination of optimum storage atmospheres. More recently, attention has been given to understanding the physiological and biochemical modes of action of reduced O_2 and elevated CO_2, and concise reviews have been published by Kader (1986) and Wang (1990).

Of the glycolytic enzymes likely to be affected by CA/MA atmospheres, ATP-phosphofructokinase (ATP-PFK) would be a logical enzyme to target as it has been described as a key regulatory enzyme (Turner and Turner, 1980). Similarly, Smyth *et al.* (1984) reported that PPi-phosphofructokinase (PPi-PFK) is a key regulatory enzyme. Kerbel *et al.* (1988) reported a reduction in O_2 uptake and ethylene (C_2H_4) production of pear fruit in air +10 % CO_2 over 4 to 6 days at 20°C compared to fruit in air, a reduction in levels of fructose 1,6-bisphosphate (F1,6-P_2), and activity of ATP-PFK and PPi-PFK declined while levels of fructose 6-phosphate (F6P) and fructose 2,6-bisphosphate (F2,6-P_2) increased. This suggested an inhibitory effect of CO_2 at the site of action of both these enzymes, which may account for the reduction

in O_2 uptake. None of the other glycolytic enzymes showed any change in activity with enhanced CO_2 levels. Rapid and substantial increase in levels of F6P and F1,6-P_2 have been shown to accompany the rise in respiration that occurs during ripening of climacteric fruit (Solomos and Laties, 1974) and under anoxic conditions (Faiz Ur-Rahman *et al.*, 1974).

PPi-PFK is a freely reversible enzyme (in contrast with ATP-PFK which only functions in the glycolytic direction) and it can function in the glycolytic or the gluconeogenic direction. Although PPi-PFK is reversible it is the only enzyme along with ATP-PFK that is capable of converting F6P to F1,6-P_2. If PPi-PFK is to function in the glycolytic direction then an adequate supply of PPi must be present. Although not measured in the study with whole pear fruit (Kerbel *et al.*, 1988), Huber (1986) verified that substantial pools of PPi exists in various plant tissues including fruits and it has also been shown that F2,6-P_2 favours PPi-PFK activity in the glycolytic direction (but does not affect ATP-PFK). F2,6-P_2 also increased as a result of increased CO_2 concentrations. It is possible that the elevated CO_2 concentrations led to either an inhibition of existing ATP-PFK and PPi-PFK and/or the inhibition of synthesis of ATP-PFK and PPi-PFK. Supression of protein synthesis is supported by the decrease in total extractable protein in the CA treated fruit.

A similar study by Kerbel *et al.* (1990), with suspension-cultured pear cells gave complementary results to the whole fruit study (Kerbel *et al.*, 1988). O_2 consumption and C_2H_4 were both reduced by elevated CO_2 concentrations and glycolytic intermediates and enzymes that showed significant changes were the same as those in intact pear fruit. Elevated CO_2 reduced loss of F6P and substantially reduced levels of F1,6-P_2 . Substantial reduction in the activities of ATP-PFK and PPi-PFK occurred. Both studies suggest that although further elucidation of the mode of control needs to be made, elevated CO_2 has an inhibitory effect on both ATP and PPi-dependent phosphofructokinases which could account for changes in levels of F6P and F1,6-P_2 and the observed reduction in respiration.

Regulation of glycolysis by ATP-PFK is also pH dependant (Turner and Turner, 1980). It is possible that levels of CO_2 above 5% could lower intracellular pH (Bown, 1985) and CO_2 may also have a direct effect on metabolic activities (Mitz, 1979). CO_2 may also affect levels of controlling intermediates and cofactors. Plant ATP-PFK can

be inhibited by ATP, ADP, PEP, 2PGA, 3PGA, gluconate 6-P, citrate and malate (Turner and Turner, 1980).

Low-O_2 atmospheres (0.5% and 2%) were reported to increase F2,6-P_2 compared to air in carrot shreds, and activated PPi-PFK, but not ATP-PFK, towards glycolysis (Kato-Noguchi and Watada, 1996). As ATP-PFK activity may be restricted under very low O_2 atmospheres due to reduced ATP supply, the PPi-PFK route of glycolysis may be an energy saving mechanism (Black *et al.*, 1987).

Higher organic acid retention in CA-stored apple and pear fruits than in air-stored fruits has been reported by Kollas (1964) and Li and Hansen (1964). There are numerous reports of loss of titratable acidity of fruit in cold storage being inhibited when stored under CA. In apples and pears this is largely due to the reduced loss of malic acid. However, other organic acids can be affected by CA storage including citric, succinic, citramalic, shikimic, quinic, oxalacetic, pyruvic, tartaric, and a-ketoglutaric acids (Wang, 1990). This decreased loss in organic acids has been suggested to be due to either an increase in CO_2 fixation, an inhibition of respiratory metabolism, or a lower consumption of the acid under CA (Wang, 1990).

Hess *et al.*, 1993 measured ATP-PFK and PPi-PFK activities in preclimacteric avocados exposed to air, 0.25% O_2, 20% O_2 + 80% CO_2, or 0.25% O_2 + 80% CO_2 for 2 days at 20°C. No differences were found in CA treated fruit compared to fruit in air when the extracted enzymes were assayed at the optimum pH of 8.0. However, when assayed at pH equivalent to cytoplasmic levels for fruit in CA treatments, ATP-PFK activity showed a 9% and 23% reduction at pH 6.7 and 6.3 respectively; PPI-PFK did not show any activity at these pH values. A reduction in cytoplasmic pH associated with low O_2 or high CO_2 may therefore decrease ATP-PFK activity and PPI-PFK may not be functioning at all.

McGlasson and Wills (1972) indicated that low O_2 (3% O_2, 97% N_2) caused significant increase in pyruvate, oxaloacetate and α-ketoglutarate levels in banana fruit stored for 1 to 4 days at 20°C. They suggested that low O_2 limited the operation of the TCA cycle between pyruvate and citrate, and between α-ketoglutarate and succinate. Elevated CO_2 resulted in inhibition of succinate dehydrogenase and consequently increased accumulation of succinic acid in pears and apples (Frenkel and Patterson, 1973; Monning, 1983). Alternatively, succinate accumulation in hypoxic fruit

[which has been correlated with CO_2-induced core breakdown in apples (Hulme, 1956)], may result from increased phosphoenolpyruvate carboxykinase activity and a partial reductive TCA cycle (Nanos *et al.*, 1994). Shipway and Bramlage (1973) note that CO_2 may have a marked controlling effect on apple mitochondrial activity through structural or conformational changes and not on succinate dehydrogenase activity alone. They also found that CO_2 above 6% suppressed succinate, fumarate, pyruvate, a-ketoglutarate, citrate, and $NADH/H^+$ oxidations, but stimulated malate oxidation. Ranson *et al.* (1957, 1960) showed that the conversion of succinate into malate and fumarate was markedly inhibited by CO_2 concentrations above 10%, but conversion of fumarate into malate was not affected. At higher CO_2 concentrations, conversion of pyruvate into citrate and other TCA cycle acids was also depressed, suggesting an inhibitory effect of CO_2 on pyruvic oxidase or condensing enzyme. McGlasson and Wills (1972) also reported that low O_2 limited the operation of the TCA cycle between pyruvate and citrate, and 2-oxoglutarate and succinate in green bananas.

During CA storage, CO_2 may be incorporated into malate, lowering intracellular pH and affecting activity of glycolytic enzymes and mitochondrial function. The propensity of fruit to assimilate CO_2 and incorporate it into particular organic acids, and ability to accumulate respiratory end products, may be correlated with fruit tolerance to low O_2 and elevated CO_2 and fruit quality after storage.

2.2.3 Anaerobic respiration

In the 1870s Louis Pasteur described fermentation in yeast (Pasteur, 1872) and in 1897 Eduard Buchner made a serendipitous discovery that the addition of sucrose to cell-free extracts of yeast resulted in its rapid fermentation into alcohol (Buchner, 1897). In so doing they demonstrated for the first time that fermentation could occur outside living cells. Pasteur had recognised that in air ($\approx$ 21 kPa O_2), yeast not only curtailed alcohol production, but also consumed much less sugar than was metabolised anaerobically. This phenomenon was termed the 'Pasteur effect' by Otto Warburg, and was originally thought to be the result of the resynthesis of sugar from a considerable portion of glycolytic product or intermediate for tissue in air

(Krebs, 1972). Harden and Young in 1905 discovered a group of enzymes which they termed zymase; and coined the term 'zymasis' to refer to enzymic conversion of glucose into CO_2 and alcohol under hypoxic or anoxic conditions (Stryer, 1988). Fermentative metabolism allows both the reoxidation of NADH and ATP production, though the rate of ATP production is much lower than in aerobic respiration. Much of the recent literature on anaerobic metabolism in plants relates to flooding tolerance and is the subject of reviews by Davies (1980), Kennedy *et al.* (1992), Perata and Alpi (1993) and Ricard *et al.* (1994). The mechanism of regulation of anaerobic respiration is yet to be fully understood.

2.2.3.1 Function of anaerobic respiration and biochemical pathways

Mitochondrial respiration is greatly affected by the absence of O_2, as without O_2 cytochrome oxidase is totally inhibited (Kennedy *et al.* 1987) and the pyridine nucleotides reduced in glycolysis and the TCA cycle can not be reoxidised through the mitochondrial electron transport chain (Perata and Alpi, 1993). Consequently, NADH and pyruvic acid synthesised in glycolysis accumulate. Anaerobic respiration consumes NADH and pyruvic acid so that glycolysis can proceed and allow the continued production of ATP through substrate phosphorylation (Ke *et al.*, 1995).

It is not certain whether the fermentative pathways are fully turned off under aerobic conditions or at what level of hypoxia they become functional since anaerobic metabolites may be reoxidised by aerobic metabolism (Ricard *et al.*, 1994). A study using carrot tissue by Leshuk and Saltveit (1991) indicated that when the atmosphere is rapidly changed from an aerobic to an anaerobic environment, anaerobic respiration is initiated before the aerobic component of total respiration is reduced.

Both low O_2 and/or high CO_2 concentrations may cause accumulation of ethanol and acetaldehyde and lactic acid in fruits and vegetables (Chang *et al.*, 1983; Ke *et al.*, 1995). Pyruvic acid is mainly decarboxylated to form acetaldehyde (catalysed by pyruvic acid decarboxylase, PDC) then rapidly reduced by NADH to ethanol (catalysed by alcohol dehydrogenase, ADH). It may also be reduced by NADH to

lactic acid (catalysed by lactic acid dehydrogenase, LDH, Fig. 2.4). Low O_2 and elevated CO_2 increased activities of PDC and ADH of sweet potato, 'Bartlet' pear, lettuce, avocado and strawberry (Chang *et al.*, 1983; Nanos *et al.*, 1992; Kanellis *et al.*, 1991; Ke *et al.*, 1993, 1994, 1995). Increases in activities of PDC, ADH and LDH found in maize, barley and rice in low O_2, are thought to be due to increased transcription and translation resulting in new messenger ribonucleic acid (mRNA) synthesis and *de novo* synthesis of the corresponding enzyme proteins (Gerlach *et al.*, 1982; Good and Crosby, 1989; Kelley, 1989). Other metabolites reported to accumulate under anaerobic conditions in plants include alanine, succinate, malate and shikimate, and a complete list has been published by Crawford (1982).

Alanine is another major fermentation product in plants (Reggiani *et al.*, 1988), whose carbons are derived from glycolytic pyruvic acid (Smith and ap Rees, 1979). In white spruce cell suspensions and in barley root tissue, alanine aminotransferase was induced by anaerobic treatment (Good and Crosby, 1989), in agreement with the role of alanine synthesis as an alternative pathway of pyruvic acid metabolism (Ricard *et al.*, 1994). In some plants NADH may enable accumulation of malic acid and glycerol when O_2 is limiting (Crawford, 1982), though Ricard *et al.* (1994) suggested recent evidence establishes that malate does not increase but slowly decreases under anoxia.

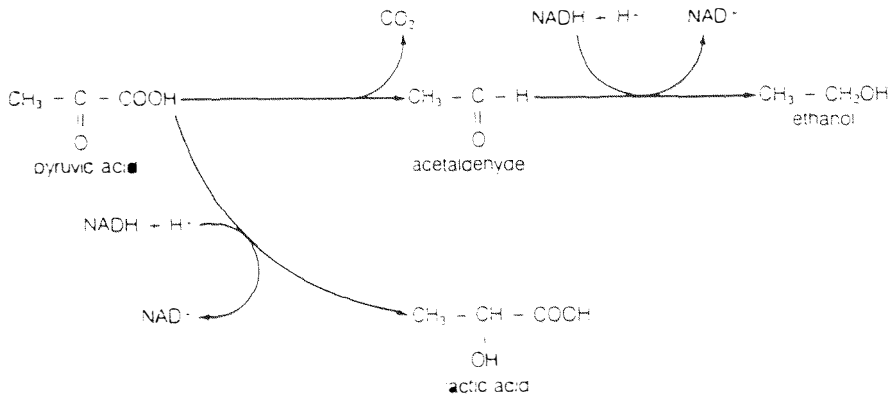

Figure 2.4 Fermentation of pyruvic acid to form ethanol or lactic acid (From Salisbury and Ross, 1992).

2.2.3.2 Regulation of anaerobic respiration

Fermentative metabolism may be regulated by two mechanisms: molecular control (also called "coarse control") of the levels of PDC, ADH and LDH, and metabolic control (also called "fine control") of the functions of the enzymes (Chang *et al.*, 1983; Ke *et al.*, 1995). The induction of ADH, PDC, and/or LDH is considered to account for the accumulation of anaerobic products. However, Ke *et al.* (1995) found increased concentrations of acetaldehyde, ethanol and lactate of avocado fruit under low O_2 without marked increase in levels of PDC, ADH and LDH. They concluded that molecular induction of the anaerobic enzymes was not the major mechanism for regulating fermentative metabolism. Similarly, Roberts *et al.* (1989) reported ethanol production correlated with ADH activity when the enzyme level was very low, but at high enzyme level, ethanol accumulation was independent of ADH activity. Clearly, when enzyme level is low, induction of an anaerobic enzyme is required through molecular control (transcription and/or translation). However, if the enzyme level is high (as in the case of ADH in avocado fruit even in air, Ke *et al.*, 1995), then metabolic control of the functions of the enzymes (changes in pH, substrate concentration, cofactors, inhibitors) may be more important regulators.

Ke *et al.* (1995) proposed the following model for the mode of action of low O_2 on avocado fruit (Fig. 2.5). Low O_2 reduces NADH flux through the electron-transport system (ETS), resulting in reduced NAD and ATP, while NADH increases. Cytoplasmic pH decreases, pyruvate dehydrogenase (PDH) activity is partially reduced and pyruvic acid flux through the TCA cycle is reduced. PDC and LDH activities are enhanced and a new ADH isozyme induced. PDC activity is enhanced by the decrease in pH and increase in pyruvic acid concentration is directed into production of acetaldehyde. Although decreased pH slightly inhibits ADH, which is abundant in avocado, increased acetaldehyde and NADH and decreased NAD drive the ADH reaction resulting in ethanol accumulation. Increased pyruvate and NADH and decreased NAD and ATP also activate LDH and drive production of lactic acid as an additional end product.

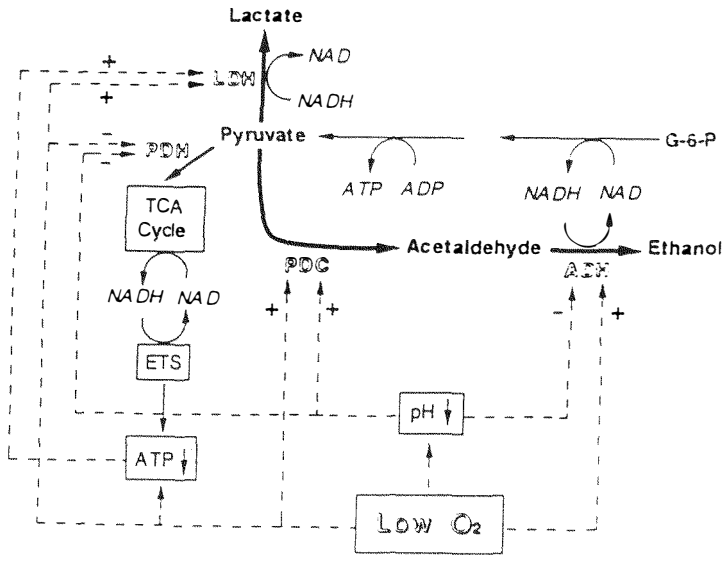

Figure 2.5 Regulation of anaerobic respiration of 'Hass' avocado in low O_2 atmospheres (From Ke *et al.*, 1995).

Davies (1980) proposed a theory of regulation of fermentative metabolism based on changes in cytosolic pH which included the following steps. At the onset of anaerobiosis, cytosolic pH is higher than neutral and PDC, which has an acidic pH optimum, is not active. NADH from glycolysis increases and is recycled by the lactic fermentation pathway, resulting in a fall in cytosolic pH as lactic acid accumulates. The fall in pH progressively inhibits LDH and activates PDC and the subsequent production of ethanol. The drop in pH also enhances the inhibition of LDH by ATP (Davies and Davies, 1972). Therefore, Davies *et al.* (1974) suggested LDH and PDH act as a metabolic pH-stat regulating fermentative metabolism.

Evidence for the Davis-Roberts hypothesis of a decrease in cytosolic pH and rapid accumulation of NADH after the onset of anaerobiosis supported Davies' hypothesis as has been reported by Roberts *et al.* (1984) and Morrell *et al.* (1990). Exposure to reduced O_2 and/or elevated CO_2 resulted in a decrease in cytoplasmic pH in avocado (Hess *et al.*, 1993), 'Barlet' pears (Nanos and Kader, 1993) and lettuce (Siriphanich and Kader, 1986). Recently, Rivoal and Hanson (1994) noted that evidence for and against the Davies-Roberts hypothesis is largely correlative, involving different

species, organs and experimental conditions. Using a genetic approach, over-expressing a barley LDH complementary deoxyribonucleic acid (cDNA) in tomato root cultures, they demonstrated that, consistent with the Davies-Roberts hypothesis, lactate fermentation was still transient.

The Davies-Roberts hypothesis has been challenged by studies in which cytosolic acidification followed more closely the time course of decrease of nucleoside triphosphates rather than lactic acid production (Saint-Ges *et al.*, 1991). This suggested that the acidification may result from both inhibition of proton pumping at low ATP concentration and proton release through ATP hydrolysis. Chervin *et al.* (1996) speculated that as the energy charge falls as the partial pressure of O_2 is reduced, cells may not be able to energise the pumps that maintain pH and other ionic gradients across the tonoplast and plasmalemma, and explain why cytoplasmic pH undergoes a transient acidic shift. Bufler and Bangerth (1982) suggest that acidification of the cytoplasm may result from a release of malic acid from the vacuole to enter the malate decarboxylation system. Not all plants show lactic fermentation preceding production of ethanol (Andreev and Vartapetian, 1992). Leshuk and Saltveit (1991), in a study using carrot disks exposed to rapid reduction of O_2 concentration, found there was a momentary decrease in pH that was coincident with the start of ethanol accumulation, after which pH increased again, and both lactic acid and ethanol continued to accumulate during the experiment. If cytoplasmic acidification is limited and controlled it may serve as a signal for adaptive changes that limit further cytoplasmic acidification . To this end, Blanke (1991) suggested CO_2 pulsing may be beneficial in inducing tolerance through limited acidification.

2.2.3.3 *Responses of crops to metabolites of anaerobic respiration*

Below the threshold of fermentation, ethanol may accumulate in fruit at a rate up to 25 nmol kg^{-1} s^{-1} (≈ 100 mg kg^{-1} per day; Knee, 1991b). Ethanol may be further metabolised to ethyl acetate, catalysed by alcohol acyltransferase (AAT). Blanpied (1983) reported accumulation of ethanol resulted off-flavours in apples when ethanol

accumulated at about 12.5 nmol kg^{-1} s^{-1} (≈ 50 mg kg^{-1} per day). After a brief period of anoxia, the fruit may metabolise ethanol at up to 5 nmol kg^{-1} s^{-1} (≈ 20 mg kg^{-1} per day) at 10°C (Knee, 1991b).

The role of ethanol in induction of anoxia injuries in plants has been debated for a long period. Jackson *et al.* (1982) concluded that for flooded plants, ethanol does not play a major role and only very high, non-physiological ethanol concentrations exogenously applied can mimic the effects of anoxia. Current evidence points to acetaldehyde as the agent causing fruit injury during anaerobiosis (Lurie and Pesis, 1992; Monk *et al.*, 1987; Perata and Alpi, 1991; Smagula and Bramlage, 1977), which, together with ethanol, appears to affect the secondary metabolism and developmental processes of plant tissue (Saltveit, 1989).

Induction of ADH at the same time as PDC, may be a critical adaptive mechanism preventing the accumulation of acetaldehyde during anoxia, as mutants of maize which do not have ADH activity cannot survive anoxia (Kennedy *et al.*, 1992).

Recent work has demonstrated that short-term exposure to atmospheres that induce anaerobic respiration may be beneficial to fruit storage life (Burdon *et al.*, 1994). Direct applications of ethanol and acetaldehyde are capable of both retarding ripening in peaches and nectarines (Lurie and Pesis, 1992), and tomato (Pesis and Marinansky, 1993) and ethylene production in tomatoes (Saltveit and Mencarelli, 1988). Acetaldehyde has also been found to increase soluble solids and decrease acidity in grapes (Pesis and Frenkel, 1989), increase aroma volatiles in feijoa (Pesis *et al.*, 1991), and reduce disorders in a range of fruits (Pesis *et al.*, 1993).

Ethanol may also play a protective role against oxidative stress. Exposure to ethanol vapours has been found to alleviate chilling-induced injury in excised cucumber cotyledons (Saltveit, 1994), and ethanol injection and vapour treatment of 'Granny Smith' apples controlled superficial scald (Scott *et al.*, 1995). Alleviation of chilling injury was believed to occur by inducing stomatal closure resulting in sufficient elevated CO_2 atmospheres to mimic known effects of low O_2 and/or high CO_2 controlled atmospheres on reducing chilling injury (Saltveit, 1994). Exposure of cucumber cotyledon discs to ethanol solutions and anaerobic atmospheres induced accumulation of ethanol in the tissue and also alleviated chilling injury. The mechanism alleviating chilling injury symptoms may involve a role of alcohol in

removing hydrogen peroxide (H_2O_2) peroxidatically by catalase. Catalase acts as a peroxidase in converting H_2O_2 to H_2O in oxidising ethanol and other substrates as H-donors. The oxidation of ethanol in this system yields acetaldehyde. Catalase can be inhibited by the presence of super oxide free-radicals (O_2^-, Kono and Fridovich, 1982) which may be produced when tissue is experiencing oxidative stress. Kono and Fridovich (1982) reported that ethanol prevents and reverses the slow inhibition of catalase by O_2^-, and thus ethanol may perform a protective role in a similar way as superoxide dismutase in quenching free radicals (Scandalios, 1993). Recently, Burmeister and Dilley (1995) suggested that a 'scald-like' disorder of 'Empire' apples, exacerbated by high CO_2 and low temperature (chilling injury), resulted from a free-radical catalysed oxidation of certain amino residues essential for the activity of enzymes in the fruit. CO_2 is known to facilitate metal ion-catalysed oxidation of amino acids at metal binding sites for iron, manganese and copper (Stadtman, 1993). Exposure to chilling temperatures also results in the formation of partially reduced O_2 species, including O_2^- (Burmeister and Dilley, 1995).

Short-term stress treatments (either by inducing anaerobiosis or exogenously applying ethanol vapours) from which the fruit may recover, may provide alternative methods for maintaining postharvest quality of fruit. These treatments may induce protective mechanisms against the effects of oxy-radicals in cells, and could be used either by themselves or in conjunction with current storage technologies.

2.2.4 Respiration rates of crops and changes with physiological age

Aerobic respiration is highly temperature dependent, the relationship being expressed as the temperature coefficient (Q_{10}: the relative increase in rate for a 10°C rise in temperature). Between 5° and 25°C the Q_{10} of fruit varies between 1.5 and 2.5 (Blank, 1991). Respiration rates of crops may also differ with botanical structure (Debney *et al.*, 1980). High respiration rates are typical for young tissues such as apical stems (asparagus), partly developed flower buds (broccoli, globe artichoke), developing seeds (green peas, green beans) and immature fruits (sweet corn). By comparison, low respiration rates are typical of storage organs such as roots (carrots,

sweet potatoes), underground stems (potatoes), bulbs (onion) and mature fruits (apples, pears). Intermediate respiration rates occur in unripe fruits (cucumbers, zucchini) and most leafy vegetables (Hardenburg *et al.*, 1986). However, factors other than respiration *per se* influence the storage potential of a crop such as growing environment (temperature, light, other stresses), stage of maturity at harvest (preclimacteric, climacteric, postclimacteric), gas exchange variables (skin permeance to respiratory gases), resistance to bruising and pre- and postharvest handling methods, susceptibility to low temperatures and other physiological and pathological storage disorders, and postharvest storage atmospheres (CO_2, O_2, CO, C_2H_4 and other volatiles).

Kidd and West (1925) discovered that there was a burst of respiratory activity in apples, which they termed the 'climacteric'. They saw this as a critical stage in the life of a fruit, associated with a period of reorganisation prior to ripening. In apples, and many other fruits, the respiratory climacteric may occur for both fruit attached to the tree and those that have been harvested, and is also accompanied by autocatalytic ethylene production (Kidd and West, 1925). Factors reported to influence the onset of the climacteric include temperature and atmospheric composition (Kidd and West, 1930). Decreased O_2 atmospheres tended to delay the climacteric and may have depressed its intensity, whereas O_2 atmospheres above ambient levels may have increased and hastened the onset of the climacteric (Rhodes, 1970). Elevated CO_2 tended to delay and depress the climacteric in apples and pears. Ethylene is known to hasten the onset of the climacteric (Burg and Burg, 1962; Melford and Prakash, 1986).

Crops have been classified as 'climacteric' or 'non-climacteric' on the basis of their respiratory and ethylene production profiles during maturation and ripening (Biale and Young, 1981). Examples of climacteric crops include apple, pear, peach, nectarine, avocado, banana, mango, and tomato, which are harvested physiologically mature but unripe. These fruit manifest a decline in respiration after harvest (preclimacteric minimum) followed by a rise (climacteric rise) at the onset of ripening to a peak (climacteric peak) followed by a decline (postclimacteric). Non-climacteric crops include citrus, grapes, cherry, strawberry, pepper, squash, and

tamarillo. The respiration and ethylene production of non-climacteric crops declines steadily after harvest (Biale and Young, 1981).

Not all fruit exhibit an ethylene-induced climacteric on ripening, and ethylene may elicit a respiratory response without concomitant ripening (Biale and Young, 1981). Recently, Shellie and Saltveit (1993) reported the absence of a respiratory climacteric in muskmelons on the vine but autocatalytic ethylene production was excessive during ripening. Similar results were found for tomato (Saltveit, 1993). By contrast, picked fruit manifested an increase in respiratory CO_2 in conjunction with increased ethylene during the climacteric. Hence, it was argued that it was the ethylene climacteric and not the respiratory climacteric that was responsible for ripening. Tingwa and Young (1975) suggested there may be a tree factor that dissipates on picking, or a wound signal involved in initiating the respiratory response. Respiratory stimulation can be induced by exogenous ethylene in nonclimacteric oranges in a concentration dependent manner (Biale and Young, 1981), and by a pulse of ethylene or propylene in preclimacteric avocados, without initiating autocatalytic ethylene production (Sarrett and Laties, 1991). However, in climacteric fruit, the nature and magnitude of the respiratory response was not dependent on ethylene concentration. Laties (1995) concluded that the respiratory response can be separated from the ripening-related ethylene climacteric, and that it remains an unanswered question as to whether the climacteric is the consequence of ripening-related anabolism with its attendant energy requirement, or whether the ripening syndrome entails the lifting of one or more specific rate-limiting restraints on the respiratory process.

The contribution of the cyanide-resistant pathway has been found to increase during the climacteric of apples (Monning, 1983) and avocados (Solomos and Laties, 1976). Tucker and Laties (1984) suggested the cyanide-resistant pathway may, during the climacteric, play a role of controlled uncoupling of oxidative phosphorylation, and may operate when electron flow or glycolytic flux exceeds the capacity of the acceptor-regulated cytochrome pathway, and in particular when substrate supply is excessive (Theologis and Laties, 1978; Lambers *et al.*, 1983).

2.3 Aerobic-anaerobic respiratory transition

2.3.1 Introduction

Estimation of the extinction point (EP) of anaerobic respiration (also termed nitrogen respiration, and zymasis) of plant tissue has historically been of great interest to plant biochemists, and is inextricably linked to the rich history of the elucidation of glycolysis and fermentation and the development of modern postharvest physiology (Laties, 1995). The EP as a measure of tolerance limits of a plant tissue is also of practical interest to postharvest physiologists involved in optimising atmospheres for fruit and vegetable storage using skin coatings, controlled atmosphere storage, and modified atmosphere packaging, or for optimising quarantine treatments involving atmosphere modification (Banks *et al.*, 1996; Kader *et al.*, 1989; Ke and Kader, 1992; Leshuk and Saltveit, 1990).

Recently, Yearsley *et al.* (1996) discussed the relationship between different methods of determining, and definitions of, the aerobic-anaerobic transition which were collectively termed 'Lower O_2 limits' (*LOL*s). Objective mathematical descriptions of the different *LOL*s were presented, and the relative merits of defining *LOL*s on the basis of external atmospheres (LOL^e) or internal atmospheres (LOL^i) are discussed. The following section reviews the historical development of concepts associated with the aerobic-anaerobic transition.

2.3.2 Extinction point of anaerobic respiration

Blackman (1928), Blackman and Parija (1928), and Parija (1928), working with 'Bramley's Seedlings' apples, referred to *LOL*s as the anaerobic extinction point. They defined the anaerobic extinction point as the theoretical point at which there is just enough oxygen entering cells to convert the terminal substrate of aerobic respiration to final oxidation products and there is no longer any anaerobic respiration. One physiological index of the anaerobic extinction point was

considered to be the O_2 level when there is minimum production of CO_2, and presumably no longer any accumulation of alcohol in the tissues (Blackman, 1928). Although Blackman and Parija could not specify the substrates and products of anaerobic and anaerobic respiration at this time, the definitions showed insight as to the nature of the aerobic-anaerobic respiratory transition. However, we now know the anaerobic extinction point occurs at a higher level of O_2 than that at which CO_2 production from aerobic and anaerobic respiratory processes is minimal. This is further discussed in chapter 4.

Thomas and Fidler (1933) followed Blackman and Parija's concepts and defined the 'extinction point' (EP) as the "concentration of oxygen at which the anaerobic respiration of apples becomes extinguished". Using Blackman (1928) and Parija (1928) description of the CO_2 component of anaerobic respiration as nitrogen respiration (NR), they defined the EP of NR as the "concentration of oxygen that extinguishes NR". Thomas and Fidler (1933) refer to Stitch (1891) and several workers quoted by him, that determined the EP of anaerobic respiration by measuring the effect of lowered oxygen concentration on the respiratory quotient of certain plant tissues. Thomas and Fidler (1933) adopted an alternative method for estimating the EP. Based on Fidler's earlier research (Fidler, 1933), they measured the accumulation of ethyl alcohol, acetaldehyde and other compounds as a measure of "zymastic products". Consequently, they redefined the EP of NR as "that concentration of oxygen at which the alcohol number becomes zero". James (1953) provided another definition of the extinction point in terms of a narrow range of O_2 concentrations in which respiration dropped off sharply as fermentation gained ascendancy.

2.3.3 Anaerobic compensation point

Boersig *et al.* (1988) noted that the analysis of ethanol by Thomas and Fidler was by steam distillation followed by oxidation in sulphuric acid, redistillation and titration of the resulting acetic acid (Fidler, 1934). This method is far less sensitive than modern chromatographic techniques which have detected ethanol as a normal constituent of apples and other fruits held in aerobic conditions (Nursten, 1970). They concluded that defining the EP on the basis of ethanol accumulation was

untenable, and proposed the concept of the anaerobic compensation point (*ACP*). They defined the *ACP* as "the O_2 concentration at which evolution of CO_2 is minimum" (Boersig *et al.*, 1988).

The traditional steady-state method used for estimating *LOL*s (EP and *ACP*), involved measuring CO_2 concentrations of plant tissue held in storage in several O_2 concentrations (Blackman, 1928; Boersig *et al.* 1988; Thomas and Fidler, 1933). Leshuk and Saltveit (1990) describe a non-steady-state method for the rapid determination of the *ACP* of plant tissue. With this method the rate of CO_2 production was measured for tissue exposed to an exponentially declining O_2 concentration, and plotting CO_2 production versus O_2 concentration. While this method was certainly quicker than the traditional approach, Leshuk and Saltveit (1990) noted that the rate of O_2 reduction had a significant effect on the *ACP* (because of the delay in the response of the tissue to the changing external O_2 atmosphere). A steady-state method based on modified atmosphere packages has been extensively used on research undertaken at Michigan State University (Beaudry *et al.* 1992; Beaudry and Gran, 1993; Gran, 1993; Joles *et al.*, 1994; Talasila *et al.*, 1994). With this system, package O_2 level was controlled by altering either the permeability, surface area, or thickness of the film or the weight of the packaged crop. The *LOL* was specified as the breakpoint observed in increases in respiratory quotient (*RQB*), ethanol, or both in the headspace (Cameron *et al.*, 1995). The disadvantage of this system arises from the different durations the crop requires to achieve steady-state. For apples, this may be from a few days to weeks depending on temperature (Gran, 1993). This may result in differences in physiological age of the fruit, and at higher temperatures the development of pathological disorders can be problematical. Also, it is difficult to control the level of CO_2 in MA packages other than absorbing it with a CO_2 scrubber.

The fundamental problem associated with the methods described, is that they estimate *LOL*s on the basis of the external O_2 level, whereas it is the O_2 concentration in the cytosol to which tissues respond most directly, and which is close to equilibrium with the partial pressure of O_2 in the intercellular air spaces. Estimation of *LOL*s on the basis of the external partial pressure of O_2 has been recognised as a major limitation in the estimation of the *LOL* of the tissue itself (Banks *et al.*, 1993a;

Cameron *et al.*, 1995; Dadzie *et al.*, 1993). In contrast, determining *LOL*s on a steady-state internal atmosphere basis more closely estimates the true tissue *LOL* than values estimated from external or package atmospheres, and provides a more mechanistic basis for models used to predict fruit response to controlled or modified atmospheres. It is this difference in approach and the lack of empirical information on the effect of temperature, elevated CO_2 and physiological age on internal *LOL*s that is the primary justification for the present study.

2.3.4 Effect of temperature on *LOL*s

Studies on the effects of temperature on LOL^es have been reported for a range of crops (Platenius, 1943), for apples (Gran and Beaudry, 1993a and 1993b), and the effect of temperature and $p_{CO_2}^e$ for blueberry fruit (Beaudry, 1993; Beaudry *et al.*, 1992; Cameron *et al.*, 1994 and raspberry fruit (Joles *et al.*, 1994). In general, LOL^es based on *RQ* or ethanol accumulation, were observed to increase with increasing temperature.

Dadzie *et al.* (1993) used Fick's First Law of Diffusion, to argue that the relationship between LOL^i and LOL^e is analogous to the difference between internal and external atmospheres. For *ACP*, the relationship can be described by the equation;

$$ACP^e = ACP^i + \frac{r_{O_2}^{ACP^i}}{P_{O_2}^{'} A} \qquad (2.1)$$

where:

A = fruit surface area (m^2)

$P_{O_2}^{'}$ = skin permeance to O_2 (mol s^{-1} m^{-2} Pa^{-1})

$r_{O_2}^{ACP^i}$ = rate of uptake of O_2 for the system at the ACP^i (mol s^{-1}).

From this relationship Dadzie *et al.* (1993) predicted that for a fixed ACP^i, ACP^e should be proportional to maximum respiration rate ($r_{O_2}^{max}$) and inversely proportional to skin permeance. The effect of temperature on ACP^e would be expected to be

significant due to the power law relationship between $r_{O_2}^{max}$ and temperature. Thus, for a fruit of given surface area and skin permeance, ACP^i would increasingly differ from ACP^e as temperature increased (Beaudry *et al.*, 1992; Cameron *et al.*, 1995; Dadzie *et al.*, 1993). Beaudry *et al.* (1992) suggested the marked increase in *RQB* based on $p_{O_2}^e$, for blueberry fruit, resulted from a larger increase in rate of O_2 uptake compared to ease of diffusion of O_2 through the skin [which Cameron *et al.* (1995) noted is relatively temperature independent]. Banks *et al.* (1993a) commented that the strong dependence of ACP^e on both temperature and P_{O_2} has importance consequences for the design of MAP systems, as MAPs are susceptible to temperature abuse (Cameron *et al.*, 1995). This would be particularly true for cultivars of apples with low or markedly variable P_{O_2} such as 'Braeburn' (Dadzie, 1992). It follows from Eq. 2.1 that effects of variation in P_{O_2} on internal atmospheres for apples in CA at storage temperatures would be smaller as r^{max} is minimised under such conditions.

The effects of temperature on *LOL*'s have not been reported previous to the present study (chapter 5). As the steady-state model of Dadzie *et al.* (1993) assumed ACP^i was constant with temperature, it was considered important to test this assumption to refine the generalized model presented in chapter 1.

2.3.5 Effect of elevated CO_2 on *LOL*s

In general, fermentative metabolism is reported to be enhanced by low external partial pressure of O_2 and elevated external partial pressure of CO_2 ($p_{CO_2}^e$, Pa), long exposure to CA and more advanced developmental stages (Ke *et al.*, 1993). Modifications of individual atmosphere components interact such that crop tolerance to elevated $p_{CO_2}^e$, decrease as $p_{O_2}^e$ decreases, and tolerance to low $p_{O_2}^e$ decreases as $p_{CO_2}^e$ increases (Beaudry and Gran, 1993; Kader *et al.*, 1989).

The effect of elevated $p_{CO_2}^e$ on O_2 uptake (and indirectly its effects on $LOL^e s$) has been discussed in section 2.2.2.3, where it was noted the evidence has been contradictory, with no effect, an increase or a decrease in respiratory activity reported depending on the crop and the partial pressure of CO_2. Gran (1993) investigated the

effect of temperature on the LOL^es of apples using modified-atmosphere packages and found that 12 to 15 kPa CO_2 in package headspace increased O_2 uptake compared to packages where CO_2 had been diminished by a CO_2 absorber, but the effect on LOL^es was not discussed. There are no reports of the effect of $p^{\ell}_{CO_2}$ on LOL^is, and this is the principal subject of the study reported in chapter 5.

2.3.6 Effect of physiological age on *LOL*s

LOL^es of 'Bramley's Seedlings' and 'Newton Wonder' apples was reported to increase as they aged (Fidler, 1933; Thomas and Fidler, 1933). At 23 °C, green apples, shortly after harvest, or stored at low temperature for a brief period, formed no alcohol in 2.5% O_2, which was the level of O_2 at which CO_2 production was minimal. As the apples aged, the threshold value rose to 5% O_2 based on CO_2 production and 9 to 10% O_2 based on alcohol accumulation, for 'Bramley's Seedlings' and above 21% O_2 for yellow 'Newton Wonder' apples. Kidd and West (1937) also reported that the threshold O_2 level for alcohol formation was different for different stages of maturity of apples. They did not detect alcohol in immature fruits even at 0.5% O_2 while ripe and yellow apples produced appreciable quantities of alcohol even in air.

Fidler (1933), noted varietal differences in the shifts in extinction point (EP) of anaerobic respiration as apples aged. He suggested that "the first shift in EP may be a sign of incipient disorganisation" of "the respiration centres", and that the date on which the seasonal shift in EP begins is "determined by the impress made by a complex of significant environmental factors incident on the fruit while it is still on the tree". Dilley *et al.*(1964) demonstrated that the capacity for aerobic respiration decreased with age in apple fruit, whereas the capacity for anaerobic respiration remained constant during senescence. Therefore, as apples ripen, the ratio of anaerobic to aerobic respiration increases, and the ability of the fruit to recover from hypoxic stress may be reduced. Similar evidence has been reported for pears where preclimacteric pears seemed less stressed and had greater potential for posthypoxic recovery than pears of more advanced physiological age (Nanos *et al.*, 1992). Recently, Moriguchi and Romani (1995) reported that the physiological (climacteric)

state of avocado affected the stress capacity of the mitochondria of the fruit, anaerobiosis being more harmful to mitochondria in riper fruit. Boersig *et al.*, (1988) found that ACP^e increases as pear tissue ages, and *ACP* of pear cell suspensions increased as the diffusion coefficient of the medium decreased. The foregoing evidence suggests a strong link exists between physiological age and LOL^es. However, except for the study of Boersig *et al.*, (1988) using cell suspensions, there are no reports of changes in LOL^is with ageing whole fruit. It is possible that LOL^es change as a result of changes in LOL^is and/or change in respiration rate and skin permeance. It would be interesting to know which of these factors is involved so that they could be built into the generalized model, and is the subject of the study reported in chapter 7.

2.4 Effects of decreased O_2 and elevated CO_2 on ethylene biosynthesis and action

Decreased O_2 atmospheres can markedly decrease ethylene production and the sensitivity of crops to ethylene, and under anoxia, ethylene synthesis ceases in apples (Gane, 1934) and pears (Hansen, 1942). Two points in the biosynthetic pathway of ethylene synthesis are dependent on O_2. Adams and Yang (1979) demonstrated that 1-aminocyclopropane-1-carboxylic acid (ACC) accumulated in fruit in nitrogen but was efficiently converted to ethylene when the tissue was incubated in air. Yang and Hoffman (1984) demonstrated oxygen was required in the conversion of 5-methylthioribose-1-phosphate and 2-keto-4-methylthiobutyrate.

Reduction in ethylene production by high CO_2 has been reported in numerous crops (Wang, 1990). Blanke (1991) concluded that CO_2 inhibits ethylene synthesis at two points, formation of ACC from S-adenosylmethionine (SAM) and the synthesis of ethylene from ACC. In the latter reaction CO_2 played a dual role as it can also stimulate the *in vivo* activity of ethylene forming enzyme (EFE); Bufler (1984). Thus in the autocatalytic production of ethylene, ethylene stimulates its own synthesis and

consequently promotes climacteric CO_2 production which in turn antagonises ethylene biosynthesis.

Wang (1990), attempted to explain the apparent contradictory observation that CO_2 can both stimulate and inhibit the conversion of ACC to ethylene on the basis of the response being observed in different tissues and at different CO_2 concentrations. Inhibition of ethylene production by CO_2 primarily occurs in fruits, while stimulation of ethylene was mostly observed in photosynthetic vegetative tissues or leaf disks. The concentration of CO_2 producing an inhibitory effect on ethylene production were much higher than those inducing a stimulatory effect, the saturation point for stimulation of ACC conversion to C_2H_4 being about 0.1% CO_2 (Wang, 1990). Therefore, in ripening fruits and other climacteric type tissues, endogenous CO_2 concentrations produced by respiration often exceed that which is required for the activation of the enzyme.

The actual mechanism of the effect of CO_2 on ethylene action is clearly complex and not fully understood (Sisler, 1991). Burg and Burg (1967) reported that CO_2 is a competitive inhibitor of ethylene action and postulated that it acts at the binding site of the ethylene receptor. Sisler and Wood (1988) did not believe that the inhibitory effect of high CO_2 concentrations involved the binding site, yet noted that in some instances it was one of the most practical inhibitors of ethylene action. High CO_2 inhibition of ethylene biosynthesis and action, suppresses general metabolism and delays the onset of the ripening process. Consequently all reactions associated with ripening are delayed, including rise in respiration, autocatalytic ethylene production, rapid acid catabolism, synthesis of ripening enzymes, softening, and changes in the pectic substances in the cell wall (Wang, 1990).

The efficacy of CA is frequently explained on the basis of effects on ethylene synthesis and action (Kubo *et al.*, 1990). However, CA effects on fruit that produce minimal ethylene (such as bell peppers) suggested that the effect of low O_2 on mitochondrial oxidative capacity, resulting in the impairment of mobilisation of key substrates, may also explain the beneficial effects of CA in reducing respiration rate (Rahman *et al.*, 1995).

2.5 Gas exchange of bulky plant organs

2.5.1 Introduction

Movement of water vapour, O_2, CO_2 and ethylene (C_2H_4) and other volatiles through the intercellular air space and cuticle of bulky plant organs has significant physiological consequences for respiratory activity, ripening and ultimately the storage potential of crops. The great majority of cells in the detached plant organ are respiring, withdrawing O_2 from the internal atmosphere and evolving into it CO_2 and other volatiles. Mechanisms for gas exchange in plants must represent a compromise between the need for adequate diffusion of respiratory gases and ethylene, and minimising the loss of water vapour and consequent desiccation (Ben-Yehoshua and Cameron, 1989). The intercellular system and skin form barriers to the diffusion of gases that result in differences between the external and internal atmospheres. Understanding the nature and quantifying the significance of these and other barriers is critical in determining the response of a crop to intentional modification of these atmospheres, to reduce metabolic activity and enhance storage potential. Modification of internal atmospheres by manipulation of skin permeance through application of skin coatings also requires knowledge of the variation in skin and tissue permeance of individuals within the population of a crop (Banks *et al.*, 1993b). It is not surprising then that there has been considerable effort on the part of postharvest physiologists to understand avenues of gas exchange in bulky plant organs (Burg and Burg, 1965).

This review considers the principles of gas exchange in bulky plant organs, and central issues relating to avenues of gas exchange and sources of variation that impinge on the estimation of lower oxygen limits of crops. Methodology associated with estimation of gas variables (internal atmospheres, permeance, rates of flux of gases) has been reviewed by Ben-Yehoshua and Cameron (1989), and methods used in this study are discussed in chapters 3 and 8.

2.5.2 Laws of gas diffusion

Gas exchange is a spontaneous process, the result of passive diffusion down concentration gradients from areas of high concentrations to areas of lower concentrations. It results from the random kinetic motion of molecules and does not require the direct utilization of metabolic energy of the organ (Nobel, 1991). Given there is no pressure-driven mass flow, and each gas species in a mixture acts independently of all other gases, the rate of movement depends on the properties of the gas molecule at a given thermal energy, the magnitude of the concentration gradient, and properties of any barriers such as thickness, surface area, density, and molecular structure (Barrer, 1951). Increasing the temperature increases the average velocity of all the molecular-sized particles and increases the rate of diffusion. The Q_{10} [temperature coefficient; the rate of process at temperature (T) + 10 °C / rate of process at T, Nobel (1991), p. 144] for diffusion of many gases is about 1.03. Solutes in water have values of 1.2 to 1.4 for diffusion, as increased temperature breaks hydrogen bonds in water so solutes can diffuse more readily and viscosity of water is decreased while permeability of water to solutes is increased (Salisbury and Ross, 1992, p. 43).

Fick (1855) examined diffusion quantitatively, and his First Law of Diffusion deals with diffusion involving planar fronts of uniform concentration. The diffusive flux density of a gas j is the amount crossing a certain area per unit time (J_j, mol m^{-2} s^{-1}). Fick deduced that the concentration gradient is the 'force' leading to net molecular movement. The gradient in concentration of species j (c_j, mol m^{-3}) in the x-direction is $-\partial c_j/\partial x$ (mol m^{-4}). The minus sign indicates the direction of net diffusion is towards regions of lower concentration. The resulting flux density is proportional to concentration gradient (Nobel, 1991):

$$J_j = -D_j \frac{\partial c_j}{\partial x} \qquad \textbf{(2.1)}$$

where: D_j = the diffusion coefficient of species j (m^2 s^{-1}).

Assuming steady-state flow, with no storage in the distance x, it is possible to integrate Equation 2.1 from one point in the system to another as follows:

$$J_j = \frac{(c_{j1} - c_{j2})\, x}{D_j} \qquad \textbf{(2.2)}$$

where: c_{j1} = concentration at its highest point, point 1 (mol m^{-3})

c_{j2} = concentration at its lowest point, point 2 (mol m^{-3}).

The diffusion coefficient of a gas varies with both concentration and temperature. Diffusion coefficients for gases in air under standard atmospheric pressure (101325 Pa) and 20 °C are; water vapour, 2.42 x 10^{-5} m^2 s^{-1}; O_2, 1.95 x 10^{-5} m^2 s^{-1}; and CO_2; 1.51 x 10^{-5} m^2 s^{-1} (Nobel, 1991, p18). For a plant organ at dynamic physiological equilibrium, the rate of production of a gas must equal its rate of loss from the organ and the rate of uptake of a gas must equal its rate of diffusion into the organ. In other words, the flux density becomes equal to the rate of production or uptake respectively. The difference in concentration (c_{j1} - c_{j2}) can be expressed simply as Δc_j, and the distance (x) can be combined with the diffusion coefficient to give the permeance (P_j', m s^{-1}):

$$J_j = \Delta c_j \; P_j' \qquad \textbf{(2.3)}$$

Perrneance is the inverse of resistance.

Fick's First Law can be simplified into a form useful to describe gas diffusion in plant tissues, and has been used extensively in studies of gas exchange in fruit (Banks *et al.*, 1996; Ben-Yehoshua *et al.*, 1963; Burg and Burg, 1965; Burton, 1974, 1978; Cameron, 1982; Cameron and Reid, 1982; Cameron and Yang, 1982; Cameron *et al.*, 1995; Dadzie, 1992; Marcellin, 1974; Trout *et al.*, 1942).

If we use partial pressures of gas species j (p_j, Pa) rather than concentrations for expressing driving forces, as recommended by Banks *et al.* (1995), and use the term 'rate of transfer' rather than 'flux density', we can quantify the difference in partial pressure between the internal and external atmosphere using the equation (Banks *et al.*, 1993a):

$$\Delta p_j = p_j^i - p_j^e = \frac{r_j M}{P_j' A} \quad \textbf{(2.4)}$$

where:

A	=	fruit surface area (m^2)
Δp_j	=	difference in partial pressures of gas j between internal and external atmospheres (Pa)
M	=	fruit mass (kg)
p_j^i	=	partial pressure of gas j in the internal atmosphere (Pa)
p_j^e	=	partial pressure of gas j in the external atmosphere (Pa)
P_j'	=	skin permeance to gas j (mol s^{-1} m^{-2} Pa^{-1})
r_j	=	rate of transfer of gas j between internal and external atmospheres (mol kg^{-1} s^{-1}).

Permeability (P_j, mol s^{-1} m m^{-2} Pa^{-1}) is related to permeance (P_j', mol s^{-1} m^{-2} Pa^{-1}) for a barrier of thickness Δx, (m) by:

$$P_j' = \frac{P_j}{\Delta x} \quad \textbf{(2.5)}$$

Permeance is a useful concept for postharvest research as many barriers are either heterogeneous or of unknown thickness (Banks, *et al.*, 1995). Permeability is the product of diffusion coefficient (or diffusivity) and solubility (s_j, mol m^{-3} Pa^{-1}) of a gas in a medium:

$$P_j' = \frac{D_j s_j}{\Delta x} \quad \textbf{(2.6)}$$

Banks *et al.* (1995) observed, that it may be difficult to separate the permeance of fruit skins into the diffusivity and solubility components, as diffusion through the skin involves parallel transfer through pores and cuticle.

The application of Fick's First Law to plant systems is only valid if the gas of interest can diffuse much more readily through the pulp of the organ than through the

skin, and that the thickness of the skin is a negligible component of the dimensions of the organ (Brooks, 1937, Burg and Burg, 1965; Burton, 1950, 1974; Cameron, 1982; Cameron and Reid, 1982, Cameron and Yang, 1982). If this is not true, and the internal concentration of a gas is estimated from the centre of the crop, then the gradient would be overestimated, and a three-dimensional version of Fick's First Law may be required (Solomos, 1987). Variation in atmospheres within bulky tissues is discussed in section 2.5.4.

Rates of gas diffusion in bulky plant organs are largely determined by the respiration rate, stage of maturity, physiological age, crop mass and volume, pathways and barriers to diffusion, properties of the gas, partial pressure of the gas in the atmosphere external to the crop, magnitude of the difference in partial pressure across barriers, temperature and relative humidity (Burton, 1982; Banks, 1984a, b; Smith and Stow, 1984). Numerous models that include some of the above variables have been published for the purpose of predicting the atmosphere within polymeric film packages surrounding a crop (Banks, 1985; Banks, *et al.*, 1989; Cameron, 1989; Cameron, *et al.*, 1989, 1995; Christie, *et al.*, 1995; Emond, *et al.*, 1991; Henig and Gilbert, 1975; Kader *et al.*, 1989; Kok and Raghavan, 1985; Mannapperuma *et al.*, 1989; Solomos, 1982, 1987; Zagory and Kader, 1988; Wade and Graham, 1987; Yang and Chinnan, 1988. More recently, Merts (1996) has developed a comprehensive model for optimising modified atmosphere packages for apples that uses fruit internal atmospheres rather than package atmospheres for calculating respiration rates. Such a model would be potentially more representative of the variables that affect O_2 uptake in the tissue of the packaged crop, though limited by the accuracy of the values for skin and tissue permeability as well as for O_2 uptake (Cameron *et al.*, 1995).

2.5.3 The skin as a barrier to gas diffusion, and pathways for gas exchange

It is generally recognised that the skin (compared to the flesh) of a bulky plant organ such as a fruit represents the primary barrier to gas diffusion (Burg and Burg, 1965; Cameron and Yang, 1982; Trout *et al.*, 1942).

2.5.3.1 The structure of the skin

The skin has an epidermal layer of cells which excrete a relatively thick cuticle on the outside. Most of the cuticle comprises cutin, a diverse group of complex hydrophobic polymers principally of esters of 16- and 18-carbon monocarboxylic acids that have two or three hydroxyl groups (Nobel, 1991, p 4). The remainder of the cutin is soluble cuticular lipids, wax-like substances containing long-chain fatty acids esterified with long-chain monohydric alcohols, embedded within the cutin matrix, and pectin polysaccharides attached to the cell wall. The primary function of this complex surface is to restrict transpiration and protect against pathogens and minor mechanical damage (Lendzien and Kirsteins, 1991; Salisbury and Ross, 1992, p. 314).

The continuity of the cuticle may be interrupted by pores (as for apples and potatoes), or cuticle may contain no pores (as for tomatoes and capsicums). In young apples, the skin contains stomata and trichome bases which are transformed into lenticels (Tetley, 1930, 1931), and epidermal breaks in mature fruit (Clements, 1935). Tetley (1930, 1931) observed transformation of stomata and trichome bases into lenticels resulted from stretching on the epidermis and impregnation of the hypodermal cells which form beneath the aperture with tannin-like substances. Tetley also observed that cork cambium may develop below the aperture, extending some distance into the hypodermis, and force its way up through the aperture.

Park (1990) reported a relationship between the formation of blockage within lenticels of apples and the rapid increase in resistance to gas exchange during development of the ethylene climacteric. In mature apples, four types of lenticels each with different porosities, could be classified according to hypodermal differentiation below the aperture (Park, 1990). On average 42% of lenticels of 'McIntosh' apples were open. Adams (1975) reported lenticels on potato may also be open or closed . There does not appear to be a correlation between lenticel size and porosity in apples (Kidd and Beaumont, 1925) or potatoes (Banks and Kays, 1988), or the resistance of individual lenticels and their dimensions.

While the number of lenticels remain unchanged during fruit development, the density of lenticels appears to be cultivar- and maturity-dependent (Tetley, 1931; Clements, 1935). Regardless of cultivar, it was observed that two-thirds of the lenticels

were distributed over the calyx one-third of the fruit surface, and most of open lenticels on the equatorial portion or at the pedicel end. Clements (1935) reported the density of pores on apple fruit as 0.018 lenticels per mm^2. As the number of stomata does not change as the fruit surface expands, the density of pores decreases. The total number of pores may vary markedly between different fruit. For example, for apples the number of pores per unit surface area decreases to 0.04-0.4 per mm^2 (Blanke, 1987; Krapf, 1961; Marcellin, 1958), and 3-5 per mm^2 in avocado fruit (Blanke and Bower, 1990).

2.5.3.2 Permeance of the skin to gases

The permeance of the fruit skin to gases has been reported to vary with physiological age, for example permeance of apple skin to O_2 , but not CO_2, increased as a function of ripening time, to reach a maximum near harvest (Andrich *et al.*, 1989, 1990). Park (1990) reported resistance to gas diffusion in 'McIntosh' apples changed with fruit ontogeny. Resistance to gas diffusion was lowest one month after full bloom, then increased during growth showing the highest value nearest optimum maturity, after which there was a decrease and a levelling off as fruit fully matured and ripened on the tree. The rapid increase and subsequent decrease in the resistance near full maturity appeared to be related to ethylene production.

Dadzie (1992) reported values of skin resistance to CO_2 and ethane (C_2H_6) for eight cultivars of freshly harvested New Zealand apples, and found them to be cultivar-dependent. 'Braeburn' apples had the highest skin resistance to CO_2 (average approximately 28,000 s cm^{-1}, or in terms of permeance, approximately 0.2 nmol s^{-1} m^{-2} Pa^{-1}), and 'Royal Gala' the lowest (7000 s cm^{-1} or approximately 0.6 nmol s^{-1} m^{-2} Pa^{-1}). However, there was also a large degree of variation within cultivars, particularly for 'Braeburn' apples. This variability between fruit within a population has important ramifications for optimising storage atmospheres and coating treatments in that it affects differences in composition between internal and external atmospheres and consequently lower oxygen limits based on external atmospheres (Banks, 1985).

2.5.3.3 *Routes for gas exchange through the skin*

There is comparatively little information on the relative contribution of potential routes for gas exchange and reported evidence has often been contradictory. Potential routes are (1) cuticle, (2) air filled spaces (stomata, lenticels and cracks) within the cuticle and (3) pedicel and/or floral ends, or a combination of these routes.

In the absence of pores, the transport of gases and vapours through cuticles occurs as activated diffusion, that is a combination of sorption, diffusion and desorption. The process of transport through cuticles has been reviewed recently by Lendzian and Kirsteins (1991). For astomatous, or aporous fruit such as tomato or capsicum, the routes for gas exchange are more restricted than for porous organs. Cameron and Yang (1982) estimated 94%, 81% and 67% of C_2H_4, CO_2 and water vapour in tomato fruit occurred through the stem scar and cuticular permeance to water vapour was 10^3 times greater than permeance to CO_2, O_2 and C_2H_4. The calyx opening of apples has also been reported to contribute to gas exchange (Cameron and Reid, 1982; Marcellin, 1974; Markley and Sando, 1931a, b). Cameron (1982) reported the calyx provided 42% of the C_2H_4, 24% of the CO_2 and 2% of the water vapour diffusion in 'Golden Delicious' apples.

For porous crops, Burton (1982) postulated stomata or lenticels were the principal sites of gas exchange, as gases find the pathway with least resistance to diffusion, and many reports support this view (Burg and Burg, 1965; Burton, 1965; Burton and Wigginton, 1970; Wigginton, 1973) though Hall *et al.* (1954) contended that lenticels on the skin of apples played no part in gas exchange. Early experimental evidence of diffusion through pores was reported by Devaux (1891), who forced gas through submerged pumpkins. Devaux suggested O_2 enters the pumpkin primarily through pores and CO_2 exits primarily through the cuticle, a view supported by studies by Marcellin (1974). Metlitskii *et al.* (1972), reported for apples that 20% of O_2 diffused through lenticels and 80% through the cuticle.

The effective permeance of pores on a fruit surface to gases is proportional to the diffusion coefficients of gases (Nobel, 1983). If diffusion of all gases were through pores, then we might expect permeances of the fruit to be to of the order: water vapour $> O_2 > CO_2$. However, permeance to water vapour has been reported to be one or two

orders of magnitude greater than permeance to O_2 (Cameron and Reid, 1982). Data presented in the current study indicated that apple skin was differentially permeable to O_2 and CO_2, and the higher permeance to CO_2 suggested that it moves through additional routes to that of O_2.

Earlier studies of Pieniazek (1944) found no correlation between cuticle thickness and rate of transpiration and lenticular transpiration accounts for < 30% of total water loss, cuticular transpiration accounting for the rest. Blocking of lenticels on apples with paraffin only reduced weight loss by 8% to 25%, while removal of the cuticle increased weight loss by 7% to 88%, suggesting water vapour diffuses primarily through the cuticle. Horrocks (1964) in a study of waxes on apple found permeabilities of water vapour of both apple tissue and isolated peel were similar. Schönherr (1976) found diffusion of water in citrus, pear and onions to be determined by the permeability coefficient for the cuticular waxes, and independent of cuticle thickness (Schönherr and Schmidt, 1979). The permeability of 'Valencia' orange skin to water vapour has been reported to be approximately 53, 55 and 63 times higher respectively than to O_2, CO_2 and C_2H_4 (Ben-Yehoshua *et al.*, 1985) and waxing fruit to block the stomatal pores had a significantly greater effect on decreasing permeability to O_2, CO_2 and C_2H_4 than to water vapour. Burg and Kosson (1983) proposed that water vapour moves preferentially through a liquid water phase formed at intercellular spaces and the cuticle where water conductance is 60-fold greater than gas conductance, whereas gases move through air-filled pores of the epidermal layer.

Thus, the permeance of the skin to water vapour may be one or two orders of magnitude higher than permeance to O_2 (Cameron and Reid, 1982) and permeance to CO_2 has been reported to be higher, the same or lower than permeance to O_2 (Cameron and Reid, 1982; Dadzie, 1992). Banks *et al.* (1993b) suggested the discrepancy in relative permeances of gases and vapours can be explained on the basis of the cuticle having differential permeance in the order: water vapour >> CO_2 >> O_2. Thus diffusion of gases through fruit skins is likely to involve parallel transfer through several phases, pores and cuticle, in which effective diffusivity and solubility of the gas may differ substantially (Banks *et al.*, 1993b; Ben-Yehoshua *et al.*, 1985; Cameron *et al.*, 1982; Lendzien and Kirsteins, 1991). Banks *et al.* (1993b) modelled the effect of different pore areas on the resistance of a model fruit, assuming a ratio of cuticular resistance to water vapour, O_2 and CO_2 of 1.1×10^{-5}: 1.0: 0.033. When the area of

pores was high, virtually all the gas exchange was predicted to occur through the pores, overall fruit resistance was low and resistance to CO_2 was slightly greater than to O_2. Conversely, when the area of pores was low, overall resistance was greater and the fruit skin less resistant to CO_2 than to O_2, owing to the contribution of the cuticle to diffusion of CO_2.

2.5.4 Gas diffusion in fruit flesh

Diffusion of CO_2 and C_2H_4 for preclimacteric cantaloupe (Lyons *et al.*, 1962), bananas and mangoes (Burg and Burg, 1962), were well characterised by Fick's First Law, whereas climacteric avocado (Ben-Yehoshua *et al.*, 1963), stored apples (Trout *et al.*, 1942), and postclimacteric bananas (Leonard and Wardlaw, 1941), were not. This suggests barriers to gas diffusion other than the skin existed for the latter fruit. Thus, whether or not diffusion in a crop meets the assumptions of Fick's First Law depends on anatomical components of the diffusion pathway, which can change with physiological age and environmental factors.

Both the volume and continuity of the intercellular air spaces may determine the effective diffusivity of a gas within a plant tissue. A recent study of the structure of apple parenchyma, indicated that cells increased in size from the periphery to the centre (Khan and Vincent, 1990). Cells on the periphery consisted of radially flatterned or spherical cells with spherical intercellular spaces whereas interior cells were radially elongated and organised in radial columns. Between the columns were radially elongated spaces up to 3 mm long and 100-200 μm wide. During ripening and senescence there is a progressive decrease in cell adhesion (Knee and Bartley, 1981), leading to enlargement of the intercellular spaces. Once O_2 has diffused across the skin it can diffuse within the fruit through these intercellular channels and/or in the fluid/solid phase of the cellular matrix. Two models may be proposed for diffusion through these components; a parallel model (diffusion either in air channels or the fluid/solid matrix) and series model (diffusion through air channels and fluid/solid matrix in turn; Rajapakse *et al.*, 1990). Theoretical estimates of the contribution of these two pathways have been compared with empirically determined estimates of effective diffusivity for apples, Asian pears, and nectarines (Rajapakse *et*

al.,1990). They concluded that O_2 diffusion takes place in a combination of series and parallel modes in the intercellular spaces and fluid/solid matrix, with the parallel mode increasingly dominant in more open textured tissue.

Gradients in O_2 concentration across the cortex of apples are generally small due to the relatively large intercellular air space volume (Burton, 1982; Burg and Burg, 1965; Trout *et al.*, 1942). However, Solomos (1987) reported gradients in CO_2 between the surface of peeled apples and the core cavity. Rajapakse *et al.* (1990) report considerable variation in diffusive resistance of 'Braeburn' and 'Cox's Orange Pippin' apples, and 'Hosui' and 'Kosui' Asian pears and this was reflected in O_2 gradients between tissues immediately beneath the skin and the core cavity. Effective diffusivity of the tissue varied with cultivar and were broadly consistent with intercellular space volume of the tissue. For example, very dense organs such as potatoes have low porosity (Soudain, P. and Phan Phuc, A., 1979) and also have low permeabilities to gas diffusion (Banks and Kays, 1988). Dadzie (1992) found large O_2 and CO_2 differences between the equator and calyx end of freshly harvested 'Gala', 'Royal Gala', 'Braeburn' and 'Cox's Orange Pippin' and smaller differences in 'Golden Delicious', 'Red Delicious', 'Granny Smith' and 'Splendour' apples at 20°C. Similar differences were found between the equator and the calyx end shoulder, but differences between the core cavity and the equator were comparatively small except for 'Braeburn' and 'Cox's Orange Pippin' apples. Tissues at the calyx end of 'Braeburn' and 'Granny Smith' apples consistently had lower O_2 but higher CO_2 and C_2H_4 levels than any other position on the fruit and tissues at the equator had higher O_2 and lower CO_2 and C_2H_4 than other parts of the fruit.

Dadzie (1992) postulated that gradients in atmospheres throughout apple cortex may relate to localised variation in intercellular air space volume. This is possible as the porosity of tissue from the stem-end and equator of 'Braeburn' and 'Cox's Orange Pippin' (Yearsley, data not published) and 'Golden Delicious' (Soudain, P. and Phan Phuc, A., 1979) were found to be greater than those of tissue from the calyx-end. Furthermore, gradients in CO_2 and O_2 may develop around the vascular bundles and seeds in apple (Brändle, 1968; Henze, 1969a, 1969b) and avocado (Burg and Burg, 1965; Burton, 1982). Thus, there is ample evidence suggesting homogeneity of internal atmospheres can not be assumed. This has important

implications for modelling gas exchange in fruits and the effects of CA/MA on their physiology. It may also provide information that partly explains the pattern of development of atmosphere-related disorders in fruit (Dadzie, 1992; Soudain, P. and Phan Phuc, A., 1979). In the present study, because heterogeneity in internal atmospheres of postclimacteric 'Braeburn' and 'Cox's Orange Pippin' apples was likely to exist, lower internal O_2 limits were estimated on the basis of both subcuticular (surface chamber) and core cavity atmospheres.

Gradients in atmospheres of avocado (Ben-Yehoshua *et al.*, 1963), stored apples (Trout *et al.*, 1942), and postclimacteric bananas (Leonard and Wardlaw, 1941), may have resulted from changes in the diffusion pathway as the fruit senesced. As internal tissue softened with lost cell turgor, the intercellular air space may have diminished or the tissue became water soaked as membranes lost their functionality, reducing diffusivity. Similarly, in a study using 'Red Gold' nectarines, Rajapakse *et al.* (1990) found flesh firmness, intercellular space volume significantly lower in ripe compared to unripe fruit and the gradient in O_2 atmosphere 1.7 times as great. This may have arisen through blockage of the intercellular air space in ripe fruit affecting O_2 diffusivity as the diffusion coefficients of gases in solution is of the order of 10^4 lower than in the gas phase (Nobel, 1991). Rodriguez *et al.* (1989) in studies using avocado and peach also correlated loss of flesh firmness with decreases in permeance to O_2, CO_2, and C_2H_4.

It may be necessary when optimising storage atmospheres for some crops to consider the permeance of both the skin and internal tissues when determining external lower O_2 limits (see section 2.3.6). Also, because of changes in respiration rate and skin and flesh permeance as fruit ripen during storage, Wollin *et al.* (1985) suggested it may be necessary to continuously change the external atmospheric composition in long-term CA/MA storage to accommodate changes in susceptibility of the crop to anaerobiosis.

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Chapter 3

General Materials and Methods

3.1 Introduction

This chapter describes and discusses the materials and techniques used in this study. In places, attention has been given to the theoretical basis of the estimation of variables, and experimental characterisation of the variability inherent in the procedure. The results of experiments characterising the equilibration of surface chamber atmospheres have been included as this was the primary method used for monitoring IAs of apples, and obtaining the steady-state atmosphere used for estimating internal lower O_2 limits (*LOL*'s). In the discussion section, potential sources of variability in sampling and measuring internal atmospheres (IAs), estimating rates of transfer of gases, and the benefits and disadvantages of particular methods are detailed. In subsequent chapters (written in the format of scientific papers), methods appropriate to the study are described in concise form.

3.2 Fruit supply and ethylene pretreatments

3.2.1 Fruit supply

Early harvest, preclimacteric apples (*Malus domestica* Borkh., cultivars 'Cox's Orange Pippin' ('COP') and 'Braeburn') were harvested from a Hawkes Bay orchard (Carmel Orchard, Algernon Road, Hastings, N.Z.) for the study during the 1993, 1994 and 1995 seasons. For each cultivar, a single block of mature trees was used. Fruit were selected from the inner canopy of trees, and fruit with extremes of maturity or blush were avoided in an endeavour to select a population of fruit with less variation in skin permeance. Fruit were graded to size (count size 125) using sizing rings (N.Z. Fruitgrowers Federation, Palmerston North, N.Z.), and quality by visual inspection to exclude fruit with blemishes to the surface. The fruit were

transported to Massey University for random allocation to treatments and pretreatment.

3.2.2 Ethylene pretreatment

Unless the experiment required preclimacteric or climacteric apples, the apples were induced into their respiratory climacteric by pretreating them with ethylene (C_2H_4) before cool-storage. The fruit were either sealed in a 2 m^3 temperature-controlled cabinet or sealed in a polymeric-film/metal-foil laminate bag at 20°C. Hydrated lime (4 kg) was enclosed in the cabinet or bag to absorb CO_2, and pure C_2H_4 injected to give a final partial pressure of 10.24 Pa C_2H_4. The levels of C_2H_4 and CO_2 were monitored during the treatment, and C_2H_4 generally maintained within ± 0.5 Pa of desired values and CO_2 was maintained below 0.1 kPa. After 12 h, the chamber or bag was vented and fruit were kept at 20°C for a further 48 h. Respiration and ethylene production of a random sample of 20 fruit were monitored after 48 h. By this time the apples were showing a marked increase in respiration rate and ethylene production indicative of the climacteric. The fruit were then placed in cartons in a cool-store until required for the experiments.

3.3 Controlled temperature fruit storage

Fruit were kept at storage temperatures in cool-stores at the Massey University Plant Growth and Fruit Crops Units. 'COP' apples were stored at 2°C in perforated polymeric film bags, and 'Braeburn' at 0°C without bags. Fruit were removed from cool-storage and equilibrated at 20°C before use in experiments. Experiments were conducted in 1.0 m^3 controlled temperature cabinets (McAlpine Spaceline, McAlpine, Auckland, N.Z.).

3.3.1 Temperature monitoring

For each experiment, thermistors (CM-UU-V5-1T, Grant Inc., Cambridge, U.K.) were inserted beneath the skin of 2 to 4 fruit. The fruit were located on either side of each shelf of the cabinet where treatments were positioned. A further thermistor was placed in the centre of the cabinet to monitor cabinet air temperature. Temperatures were logged as the mean of three 10 minute readings each 30 minutes using a Grant Squirrel data logger (Model 1205, Grant Inc., Cambridge, U.K.).

Small variations in temperatures existed for fruit at different positions (Table 3.1). Fruit at top right and bottom left were similar and slightly warmer than fruit at top left and bottom right. However, mean differences were not more than 0.5°C and generally < 0.3°C. For each experiment, treatments were randomly allocated to position within the cabinet, to ensure observed effects were not caused by variation in fruit temperature due to position within the cabinet.

The rate of cooling of fruit from 20°C to 0°C, and the effect of frequent door opening for internal atmosphere sampling was characterised for 'COP' and 'Braeburn' apples enclosed in controlled atmosphere bags (Fig. 3.1 a and b). For both cultivars, skin temperature was within 0.5°C of the required storage temperature after 20 h and had cooled to 0°C after 44 h. Rapid changes in cabinet air temperature resulted from opening of the cabinet doors required for frequent sampling of internal atmosphere to characterise the equilibration time of surface chambers (see section *3.5.1.2*). The normal experimental protocol did not require this frequency of sampling. Treatments were placed in controlled temperature cabinets and left overnight before the first gas atmosphere sampling was made. As a consequence the rate of cooling was more rapid than that described in Fig. 3.1 a and b. Cooling was also interrupted by cyclical periods of defrosting of the evaporator and these defrost periods are seen as the less marked increase and decrease in cabinet air temperature in Fig. 3.1 a and b.

Table 3.1 Fruit skin and cabinet air temperatures [means and standard errors of means (sem)] for 'Braeburn' fruit in different positions in a 1.0 m^3 controlled temperature cabinet.

Treat. temp. (°C)	Skin temperature (°C)								Cabinet air temp. (°C)	
	Top right		Top left		Bottom right		Bottom left			
	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem
0	0.2	0.08	0.0	0.08	0.2	0.08	0.2	0.07	0.2	0.10
4	4.2	0.01	4.1	0.01	4.1	0.01	4.2	0.01	4.1	0.14
8	8.0	0.01	7.9	0.01	8.0	0.01	8.1	0.01	7.9	0.02
12	11.8	0.01	11.8	0.01	11.9	0.01	12.1	0.06	12.0	0.01
16	15.9	0.01	15.9	0.01	16.0	0.01	16.0	0.01	15.9	0.02
20	20.2	0.01	19.8	0.01	20.0	0.01	19.8	0.01	19.8	0.02
24	24.1	0.01	23.7	0.01	23.8	0.01	24.1	0.01	23.8	0.02
28	28.5	0.01	27.9	0.01	28.1	0.01	28.1	0.01	28.2	0.02
32	32.4	0.02	32.1	0.02	32.3	0.02	32.2	0.02	32.4	0.02

3.3.2 Relative humidity monitoring

The relative humidity (RH) of CA bag atmospheres was monitored during one experiment at both 0° and 20°C, using a Grant squirrel data logger (Model 1205, Grant Inc., Cambridge, U.K.) by enclosing RH sensors (1H3602, Hy-Cal Engineering, El Monte, CA, U.S.A.), and thermistor probes (FF-U-V5 -2, Grant Inc., Cambridge, U.K.) to correct the RH sensor signals for air temperature, in CA bags containing 'Braeburn' fruit at 0 and 20°C. Mean RH (± standard error of the mean) within controlled atmosphere (CA) containers for 'Braeburn' fruit at 0 and 20°C was 90.2 ± 0.54 % and 94.0 ± 0.54 % respectively.

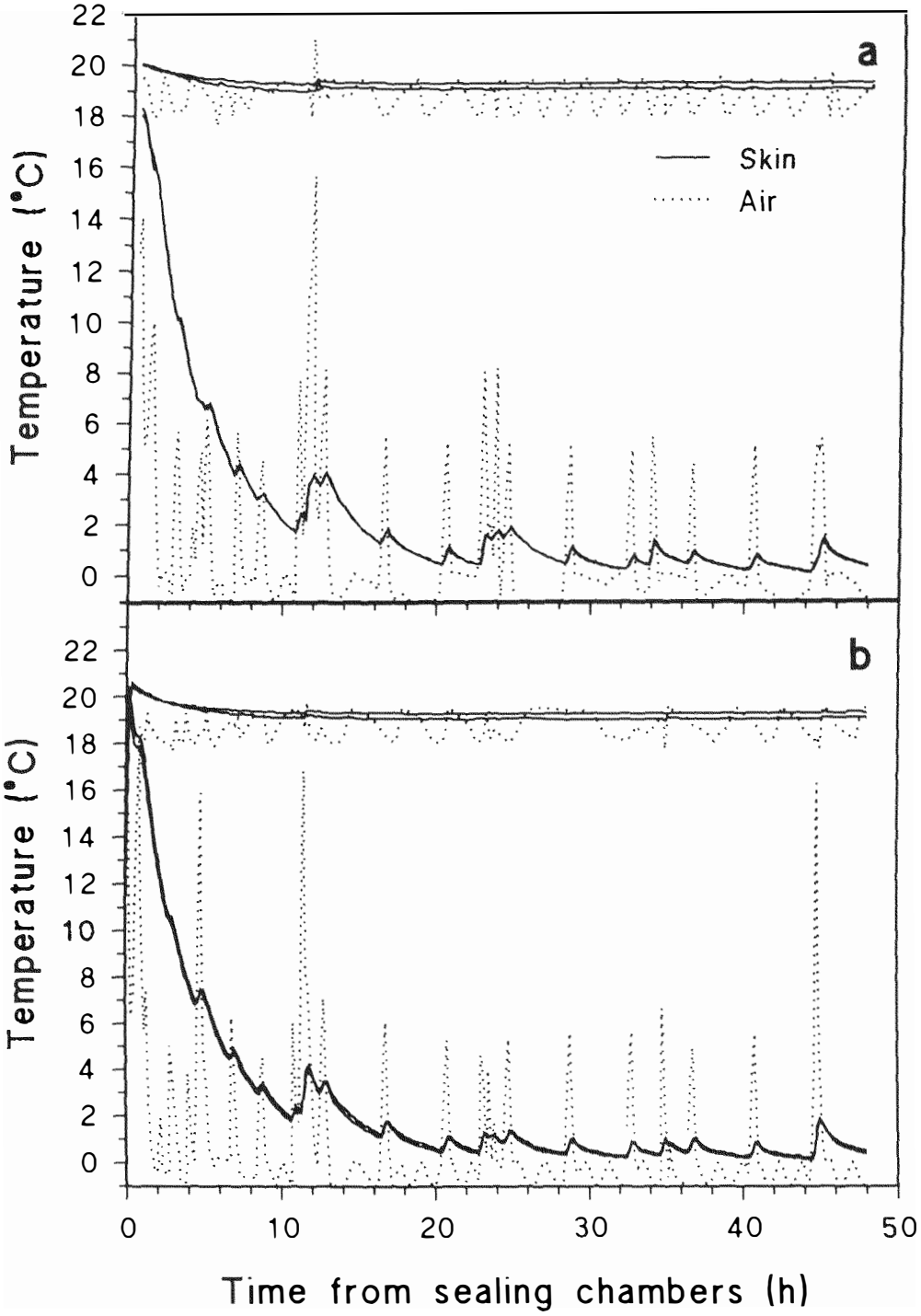

Figure 3.1 Cooling of (a) 'Cox's Orange Pippin' and (b) 'Braeburn' apples sealed in controlled atmosphere bags in a controlled temperature cabinet set at 0°C. Values represent fruit skin temperature for two fruit at the same height but either side of the cabinet and air temperature at the centre of the cabinet.

3.4 Gas mixing, controlled atmosphere system and treatments

3.4.1 Gas mixing

Controlled atmosphere gas mixtures were produced by metering the flow of dry air, food grade CO_2 and O_2-free N_2 from cylinders of compressed gases (NZIG, Palmerston North, N.Z.). For mixtures with O_2 above 21%, a cylinder of compressed certified gas mixture of 30% O_2 (balance N_2) was used (NZIG, Special Gases, Wellington). The compressed gases were controlled with two-stage regulators (NZIG GPT 270, NZIG, Special Gases, Wellington, N.Z.) to 344.75 kPa (gauge) and with single-stage regulators (R55-26030W Master Pneumatic-Detroit, Inc., U.S.A.) to 34.48 kPa (gauge). Flows of the low pressure gases were then metered with fine metering valves (Nupro S series, Nupro Co., Willoughby, Ohio, U.S.A.)) into a manifold, and the resulting mixed gases were conducted through 6 mm external diameter (OD) polyethylene tubing at a total flow of 1.7×10^3 mm^3 s^{-1} (16.7 mm^3 s^{-1} $\approx$ 1 cm^3 min^{-1}) into the controlled temperature cabinet(s). Each gas mixture was humidified by being bubbled through water in humidifiers within the cabinets before passing into the CA bags. There was sufficient tubing within the cabinets, and through bubbling the mixtures through water at the cabinet temperature, for the gas mixtures to equilibrate to the required treatment temperature.

Flow rates of air, pure CO_2 and O_2-free N_2 were corrected for the dilution effect of water vapour at different temperatures. The saturated water vapour partial pressure was calculated using the following equation (Tetens, 1930; Murry, 1967):

$$p_{H_2O}^{sat.T} = 611\, e^{[17.27\,(T/T+237.3)]} \qquad \textbf{(3.1)}$$

where: $p_{H_2O}^{sat.T}$ = saturated water vapour partial pressure at temperature T (Pa)

T = air temperature (°C) .

The percentage error due to water vapour was calculated using the following equation:

$$error_{\text{wvp}}^{T} = \left(\frac{p_{\text{H}_2\text{O}}^{sat,T}}{p_{tot}} \right) 100\ \% \tag{3.2}$$

where: $error_{\text{wvp}}^{sat,T}$ = percentage error (%) due to the dilution effect of water vapour pressure at temperature T

p_{tot} = total system partial pressure (Pa).

Required flow rates of component gases were calculated (assuming saturated water vapour pressure) using the following equations:

$$f_{\text{O}_2} = \left(N_{\text{O}_2} + \left(\frac{error_{wvp}^{sat,T}\ N_{\text{O}_2}}{100} \right) \right) \left(\frac{100}{21} \right) f_{total} \tag{3.3}$$

$$f_{\text{CO}_2} = \left(N_{\text{CO}_2} + \left(\frac{error_{wvp}^{sat,T}\ N_{\text{CO}_2}}{100} \right) \right) f_{total} \tag{3.4}$$

$$f_{\text{N}_2} = f_{total} - \left(f_{\text{O}_2} + f_{\text{CO}_2} \right) \tag{3.5}$$

where: f_{CO_2} = flow rate of CO_2 ($mm^3\ s^{-1}$)

f_{N_2} = flow rate of N_2 ($mm^3\ s^{-1}$)

f_{O_2} = flow rate of O_2 ($mm^3\ s^{-1}$)

N_{CO_2} = mole fraction of CO_2 required (mol mol^{-1})

N_{O_2} = mole fraction of O_2 required (mol mol^{-1}).

Flow rates were measured using an electronic flow meter (J&W Scientific ADM 1000), an analysis of the gas mixture composition was made using an IRGA and O_2 electrode (mol mol^{-1} = % x 10^{-2}). Final levels in experiments were checked as described in section 3.7.1.

3.4.2 Controlled atmosphere system

The CA system used a continuous flow of humidified gas mixtures flowed through the CA bags in which fruit were kept. Each CA bag had a different mixture that was controlled by individual gas mixers as detailed in section 3.6.1. The bags were constructed of Tuflex (Trigon N.Z. Ltd., Hamilton, N.Z.), a clear laminate which was easily heat sealable and to which silicon rubber septa easily adhered. Enclosed within the close-fitting CA bags, were plastic containers with partitions to hold six apples. Each container had a perforated false floor beneath which were enclosed a Tyvek (DuPont, New Zealand Ltd., Auckland, N.Z.) sachets containing 50 g of soda lime (self indicating 1.2-2.0 mm granules, Ajax Chemicals, Auburn, NSW, Australia) as a CO_2 absorber and 50 g of Purafil (Papworth Engineering, Cambridge, N.Z.) as an C_2H_4 absorber. CO_2 was absorbed in experiments where levels of CO_2 were not being investigated, to ensure that observed effects were not caused by CO_2 accumulation.

The CA mixtures entered the top of the CA bags and flowed out from the bottom of the bags through a 2 m length of 6 mm OD tubing to the outside of the controlled temperature cabinet. The CA bags (10 per experiment) were randomly positioned on two levels within the cabinet. Samples of the atmosphere within CA bags and within surface chambers on the apples they contained were taken by inserting the needle of a sampling syringe through silicon rubber septa adhered to the CA bags above the surface chambers.

3.4.3 Controlled atmosphere treatments

Ten CA treatments were used to characterise the effects of the level O_2 and CO_2 on internal lower O_2 limits (LOL^i) of apples. The range of external O_2 atmospheres used depended on the cultivar of apple and treatment temperature, and was chosen using a steady-state model based on experimental data of Dadzie (1992). A decription of this model is presented in detail in chapter 5.2.3. Validation of this model and comparison with the model of Dadzie *et al.* (1996) is presented in chapter 5.4.5 and discussed in chapter 5.5.

3.5 Measurement of internal atmospheres

Methods used in sampling internal atmospheres (IAs) have been reviewed by Banks (1983), Solomos (1987), Ben-Yehoshua and Cameron (1988), and Dadzie (1992). Two methods of sampling IAs were used in the studies of internal lower O_2 limits (*LOL*'); surface chambers and direct sampling from the core cavity. The surface chamber method (Banks and Kays, 1988) was non-invasive and non-destructive, and is discussed in some detail in this section. Direct sampling was invasive and destructive, and therefore used as an alternative method to surface chambers at the end of an experiment.

An alternative invasive technique, cannulation, was investigated as a method for monitoring changes in apple IA, and is the subject of chapter 8.0.

3.5.1 Surface chambers

Surface chambers were used extensively in the studies of *LOL*'. The chambers were constructed from 1500 mm^3 clear glass screw cap autosampler vials, 23 mm high, 12 mm OD and 9-10 mm ID (Alltech, Auckland, N.Z.). The vials were cut with a diamond tipped saw approximately 12 mm from the bottom. Bottom pieces were discarded and the top with the screw cap opening used to construct the chambers. 1000 mm^3 disposable syringes (Monoject, Sherwood Medical, St. Louis, U.S.A.) were cut at the 0.25 ml graduation mark and the lower part with the luer taper glued into the screw cap opening of each vial with 24 h cure epoxy resin (Araldite®, Ciba-Geigy, Auckland, N.Z.). A mean volume (± standard error of mean) of 935 ± 7.0 mm^3 was measured for 20 representative chambers. The syringe tip was sealed with the rubber plunger from the syringe, and a water seal applied above the plunger as a precaution against leaks (Fig. 3.2a).

For some experiments the chamber design was modified, to reduce further the possibility of permeation of gas through plastic syringe components of the chambers. These components were replaced with the luer sleeve from a 50 mm x 2 mm OD cannula (Phoenix, U.S.A.) from which the needle had been removed. A small length of plastic tube was slipped over the luer sleeve to extend its length, a silicon rubber

septum was inserted inside the luer sleeve and a water seal applied above it (Fig. 3.2b).

The surface chambers, with rubber septa removed, were adhered to the skin of apples at an equatorial position above a lenticel(s) using 5 minute cure epoxy resin (Araldite®, Ciba-Geigy, Auckland, N.Z.). Epoxy-resin was suitable for use at 0°C. The chambers were typically applied to areas of skin that did not show blush. As a further precaution against leakage through the skin-adhesive-chamber interface, a light coating of silicon grease (Molycote 111, Dow Corning Australia Pty. Ltd.) was applied over the cured adhesive and just onto both skin and chamber. The spread of the adhesive was kept to a minimum to maximise the surface area of skin beneath the surface chambers and minimise the local effect the adhesive might have on permeation of gas through the skin adjacent to the chamber. The mean skin surface area (± standard error of the mean) not covered by adhesive and available for gas exchange was estimated for 20 typical chambers as 49.0 ± 0.83 mm^2.

After the epoxy resin had cured (typically left 4 to 6 h at 20°C), the chambers were flushed with laboratory air using a 6.0 x 10^4 mm^3 syringe (Omnifix, B.Braun, Welsungen, AG., Germany). Rubber septa were carefully inserted into the top of the chambers using a tool that released pressure from the chamber as it was being inserted, and the water seal applied ensuring no air bubbles were trapped.

3.5.1.1 Gas tightness of surface chambers

The gas tightness (O_2 leakage) of the surface chambers was tested by Tanner (1995). Six replicates each of the two types of surface chamber (one with plastic and the other with metal components) were adhered onto glass microscope slides using 24 h cure epoxy resin (Araldite®, Ciba-Geigy, Auckland, N.Z.). The sealed chambers were flushed with O_2 free N_2 and an initial measurement of chamber O_2 atmosphere made by sampling from the chambers with a N_2 flushed gas-tight syringe (Hamilton, 100 mm^3). A final reading was made after 24 h. It was calculated that for the chambers to come to 99.9% equilibrium with the external O_2 atmosphere, would take 434 and 374 days respectively for chambers with plastic components and metal components respectively. Therefore, there was no advantage in using the more

expensive chamber construction using metal components. Care needed to be taken that no air bubbles were trapped in the water seal, otherwise slight negative pressures in chambers might potentially have leakage into the chamber or into the syringe when removing a sample from the chamber.

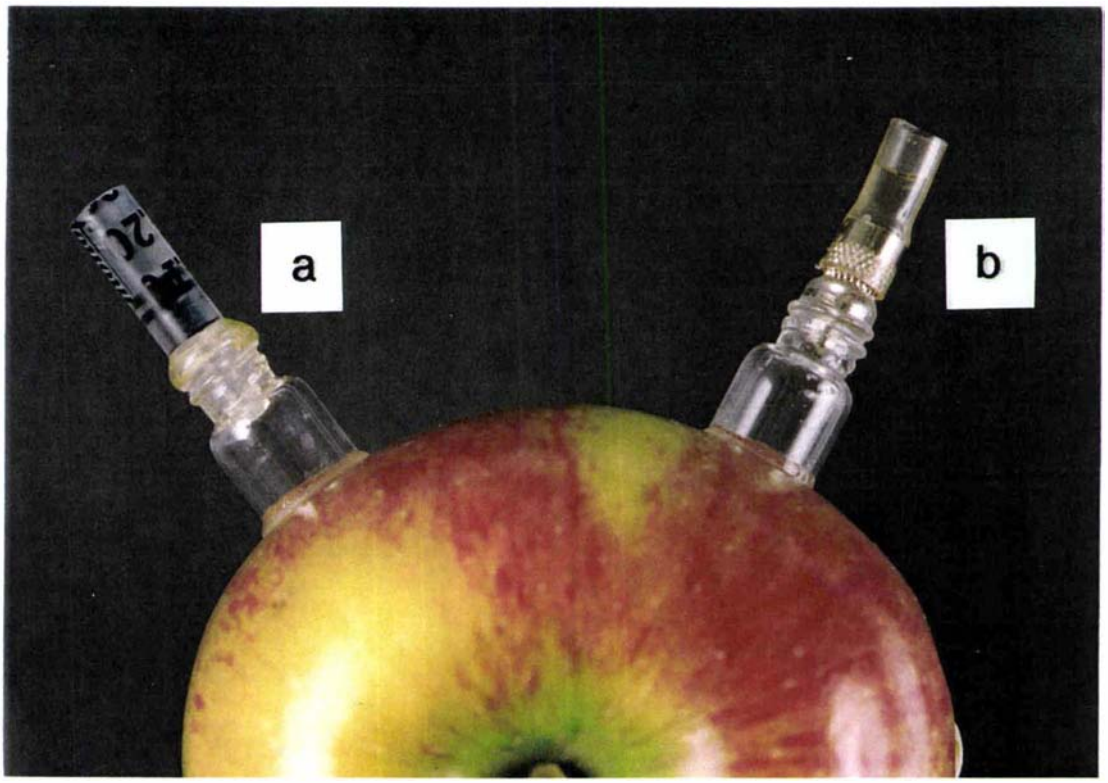

Figure 3.2 Surface chambers with (a) plastic syringe and (b) metal components.

3.5.1.2 Equilibration of surface chambers to the internal atmosphere of apples

The equilibration of surface chambers was characterised for postclimacteric, non-ethylene treated 'Cox's Orange Pippin' ('COP') and 'Braeburn' apples. Five apples of similar mass and internal C_2H_4 partial pressure were randomly selected for each of four treatments; 0°C and 20°C in air and either 2kPa O_2 ('COP') or 5 kPa O_2 ('Braeburn'). Surface chambers were adhered to the fruit at an equatorial position and left at 20°C overnight. The surface chambers were flushed with 5.0×10^4 mm^3 dry air, then each chamber was sealed with a septum and water seal every 120 s, and

the seal time noted. When all the chambers were sealed, the fruit were enclosed in CA bags, each bag enclosing an additional apple with a thermistor probe (CM-UU-V5-1T, Grant Inc., Cambridge, U.K.) inserted beneath the skin surface. The CA bags were heat sealed and connected to the appropriate CA gas mixture supply within controlled temperature cabinets, and the time of connection noted. CA mixtures were humidified and allowed to flow (total flow 1.7 x 10^3 mm^3 s^{-1}) for 2 h prior to connection to the CA bags. The atmosphere of the CA bags and surface chambers were sampled at frequent intervals by removing 60 mm^3 samples with a gas-tight syringe (Hamilton, 100 mm^3), and using an injection volume of 50 mm^3. The time for the apples to equilibrate to the storage temperature was also determined.

At the conclusion of the experiments the surface chambers with epoxy resin still attached were removed from the fruit. The volume of the surface chambers was estimated from the mass of water required to fill each chamber, and the surface area available for gas exchange beneath the chamber estimated from the mean of two measurements of internal diameter of the epoxy resin seal.

3.5.1.3 Results

Fruit of similar mass were used for treatments within cultivars, but the mean mass of 'Braeburn' apples was greater than for 'COP' fruit (Table 3.2). The area of skin available for gas diffusion beneath the chambers were similar between treatments, as were chamber volumes, and are unlikely to account for the marked differences in chamber atmospheres between fruit and treatments as they came to equilibrium.

Differences in individual fruit respiration rate and skin permeance (and number of functional lenticels beneath the chamber) are more likely to account for differences in fruit response to a treatment. This was particularly noticeable in treatments where the fruit equilibrated at 20•C were placed at 0°C in either air or low O_2 atmospheres. For these fruit, there was an initial rise in chamber $p^i_{CO_2}$ during the first 5 to 15 h at 0°C (Figs. 3.3a, 3.4a). This initial rise probably resulted from a lag in the reduction of r_{CO_2} as warm fruit cooled, and was more marked for 'COP' than 'Braeburn', presumably because 'COP' have higher r_{CO_2}. A similar response was detected in the

decrease in $p_{O_2}^i$ in fruit placed at 0°C in air (Figs. 3.3b and 3.4b), but not at 0°C in low O_2 atmospheres (Figs. 3.5b, 3.6b).

For both cultivars at 0°C in air, all fruit tested had come to approximately steady state between 60 to 90 h (Figs. 3.3, 3.4). 'Braeburn' apples responded more slowly than 'COP' in coming to equilibrium, and there may have been both physiological and physical components to equilibration judging from the variation in response of individual fruit (Fig. 3.6b).

The CA bags reached the required external O_2 ($p_{O_2}^e$) within 6 h and remained approximately constant throughout the experiment (Figs. 3.5, 3.6, 3.9, 3.10). For 'COP' apples in low $p_{O_2}^e$ atmospheres at 0°C, $p_{CO_2}^i$ equilibrated within approximately 48 h (Fig. 3.5a) and $p_{O_2}^i$ with 24 to 48 h (Figs. 3.5b). However, for 'Braeburn' apples the equilibration time for $p_{CO_2}^i$ was approximately 70 h (Fig. 3.6a), and for $p_{O_2}^i$ varied between 24 and 120 h (Fig. 3.6b). In general, there was less variation in the equilibrium values of $p_{O_2}^i$ for fruit in low $p_{O_2}^e$ than there was for fruit in air, and similar or less variation in $p_{CO_2}^i$.

For fruit at 20°C, the time for chambers to reach equilibrium was less than for fruit at 0°C. Possible reasons for a more rapid equilibration at 20°C are that there was no need for physiological equilibration, and diffusivity may have been greater at the higher temperature. With the exception of one fruit, 'COP' apples in air had come to equilibrium within 40 h, and 'Braeburn' within 50 h (Figs. 3.7, 3.8). Chamber atmospheres of 'Braeburn' apples at 20°C in air continued to drift slightly with time after coming to quasi steady state. For fruit at 20°C in air, the equilibration times represent 'physical equilibration' of the surface chambers, whereas equilibration times for fruit whose r_{CO_2} is adjusting to temperature and/or low $p_{O_2}^e$, represent a combination of physical and 'physiological equilibration'.

For 'COP' and 'Braeburn' apples at 20°C and in low $p_{O_2}^e$, there was an overshoot in chamber $p_{CO_2}^i$ followed by a reduction as the low $p_{O_2}^e$ atmosphere affected r_{CO_2} (Figs. 3.9a, 3.10a). There was a small but distinct overshoot in reduction of $p_{O_2}^i$ for 'COP' and 'Braeburn' apples that equilibrated quickly (Figs. 3.9b, 3.10b). For those fruit that equilibrated slowly, physiological equilibration was masked by slow physical equilibration. This overshoot effect would be more apparent if the scale for

$p^{i}_{CO_2}$ and $p^{i}_{O_2}$ were the same. Taking into account the overshoots, the physiological equilibration occurred between 80 and 100 h at 20°C, and low $p^{e}_{O_2}$. The difference between $p^{e}_{O_2}$ and $p^{i}_{O_2}$ was greater for 'Braeburn' than 'COP' apples as a result of 'Braeburn' apples lower skin permeance (Figs. 3.9b, 3.10b). The difference between physical equilibrium and physiological equilibrium for whole 'COP' apples (about 4 days for physiological equilibrium) in either lowered or raised O_2 atmospheres has been reported by Knee (1991b).

Table 3.2 Fruit mass, skin surface area beneath chambers and chamber volume for postclimacteric 'Cox's Orange Pippin' ('COP') and 'Braeburn' apples used in surface chamber equilibration time experiments. Values represent means and standard errors of means (n=5).

Cultivar	Treatment	Fruit mass ($kg \times 10^{-3}$)		Skin surface area (mm^2)		Chamber volume (mm^3)	
		mean	sem	mean	sem	mean	sem
'COP'	0°C, air	154.01	2.861	49.20	0.740	934.2	15.44
	0°C, 2 kPa	156.32	2.655	50.66	1.070	934.2	16.52
	20°C, air	154.96	4.290	49.31	2.349	936.6	7.32
	20°C, 2 kPa	153.43	3.956	46.76	1.334	935.0	13.20
'Braeburn'	0°C, air	182.33	3.481	51.97	0.777	937.0	10.19
	0°C, 5 kPa	173.46	5.379	54.01	1.262	927.0	12.80
	20°C, air	184.66	2.444	50.19	1.377	927.8	14.13
	20°C, 5 kPa	178.83	0.676	51.87	1.043	952.0	14.72

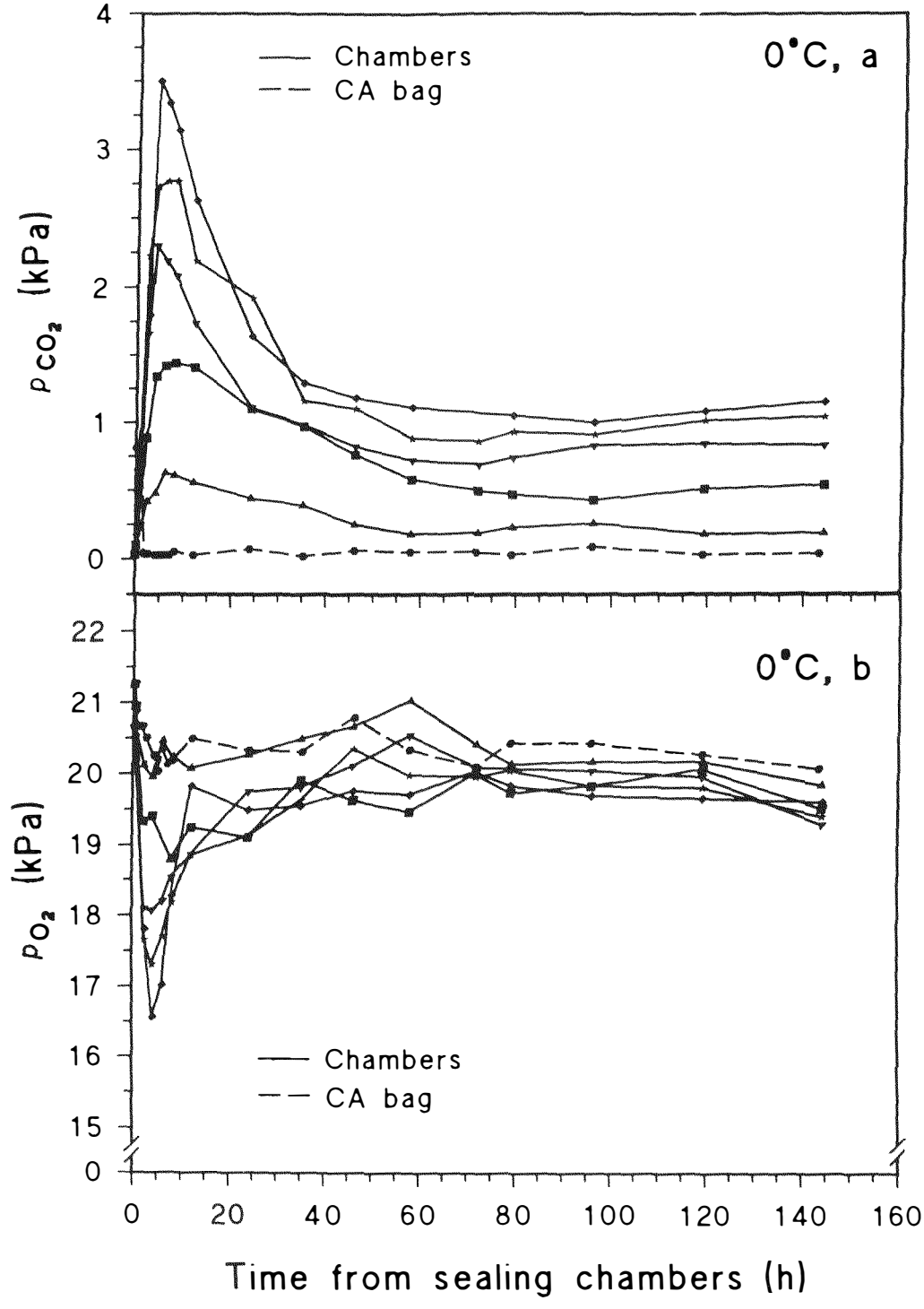

Figure 3.3 Equilibration of (a) p_{CO_2} and (b) p_{O_2} in surface chambers adhered to five 'Cox's Orange Pippin' apples in air at 0°C. Chambers were adhered to fruit at 20°C at an equatorial position, the fruit sealed in CA bags through which humidified air circulated, and CA bags placed in a controlled temperature cabinet at 0°C.

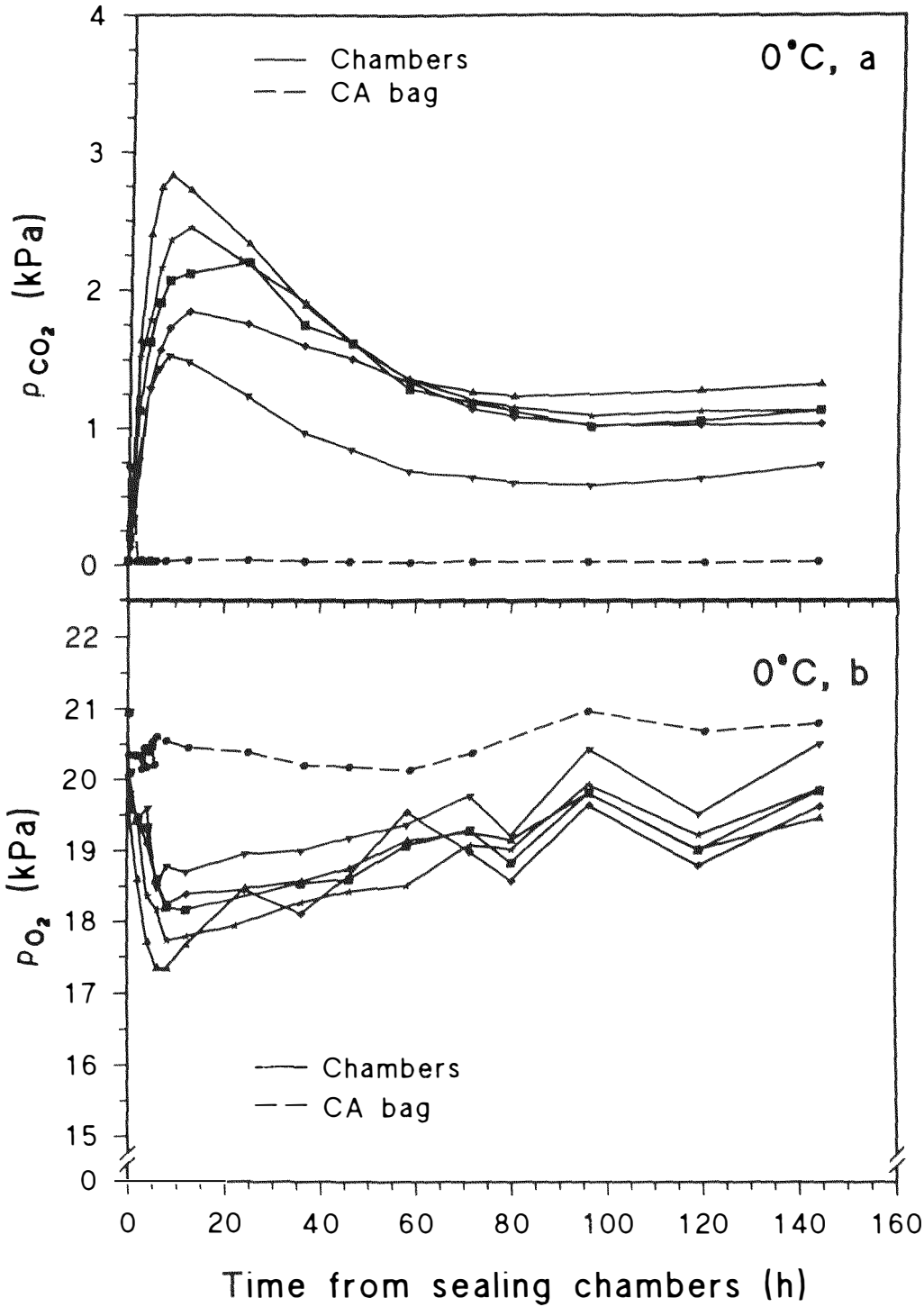

Figure 3.4 Equilibration of (a) p_{CO_2} and (b) p_{O_2} in surface chambers adhered to five 'Braeburn' apples in air at 0°C. Chambers were adhered to fruit at 20°C at an equatorial position, the fruit sealed in CA bags through which humidified air circulated, and CA bags placed in a controlled temperature cabinet at 0°C.

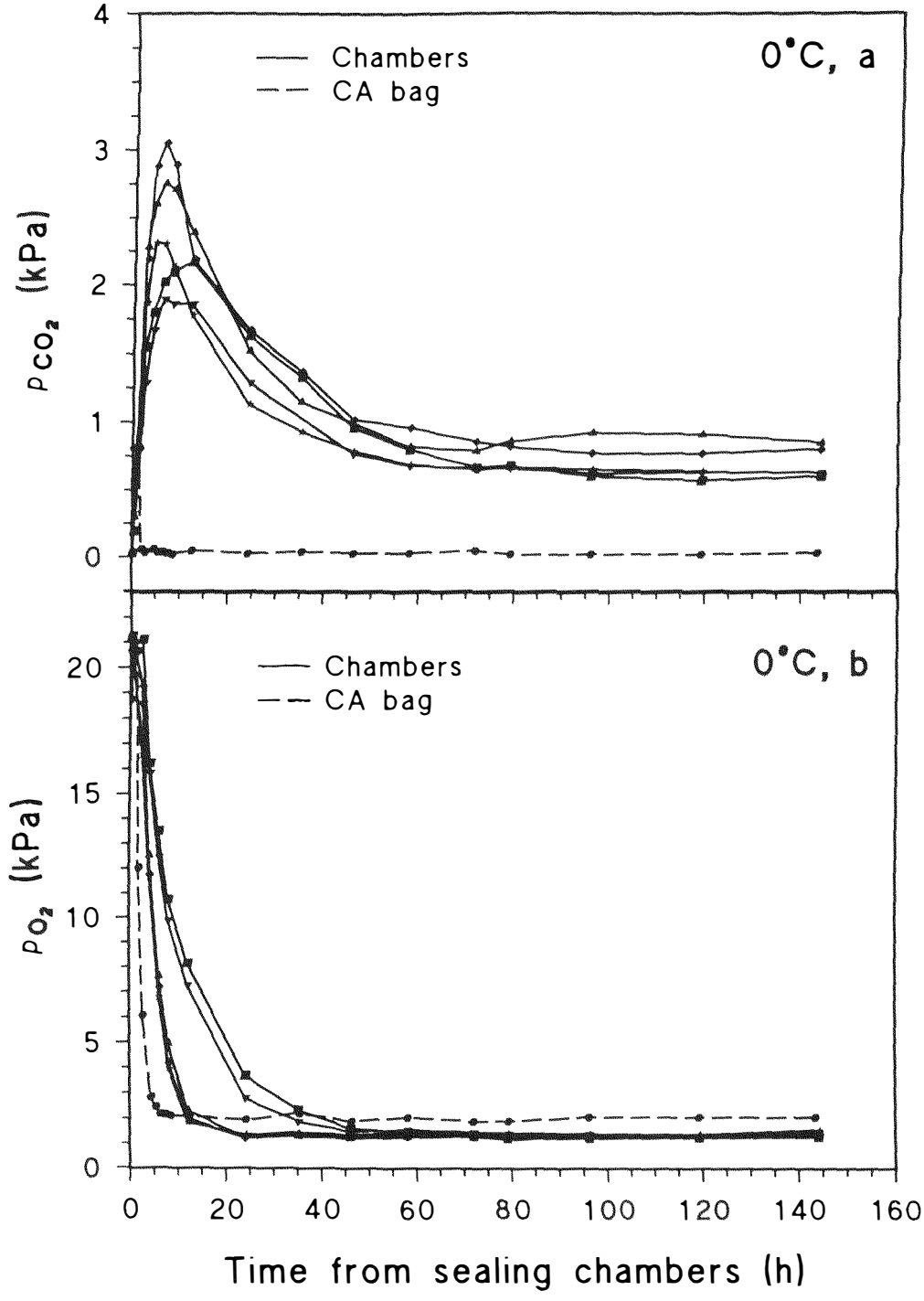

Figure 3.5 Equilibration of (a) p_{CO_2} and (b) p_{O_2} in surface chambers adhered to five 'Cox's Orange Pippin' apples in 2 kPa O_2 at 0°C. Chambers were adhered to fruit at 20°C at an equatorial position, the fruit sealed in CA bags through which a humidified 2 kPa O_2 atmosphere (balance N_2) circulated, and CA bags placed in a controlled temperature cabinet at 0°C.

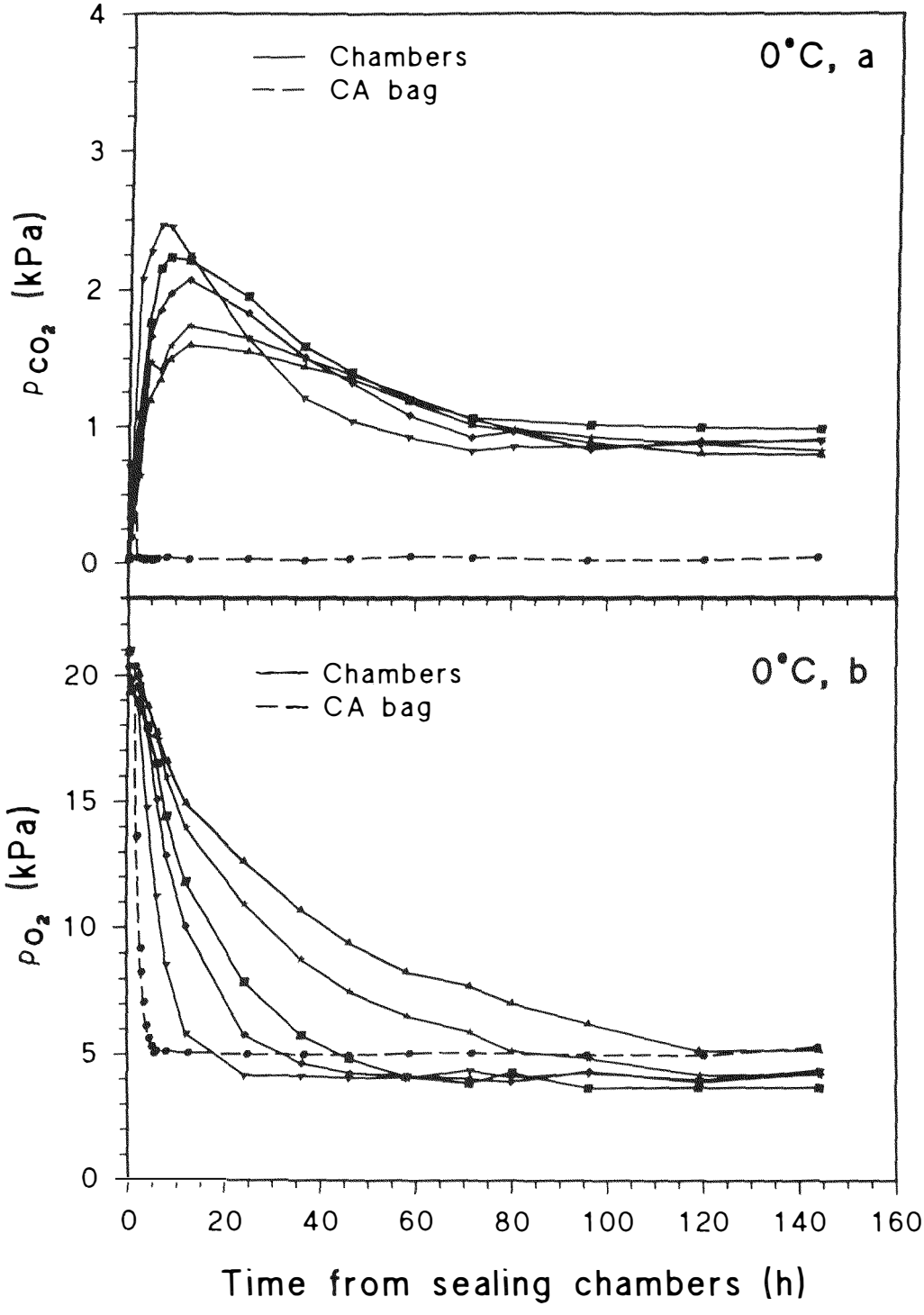

Figure 3.6 Equilibration of (a) p_{CO_2} and (b) p_{O_2} in surface chambers adhered to five 'Braeburn' apples in 5 kPa O_2 at 0°C. Chambers were adhered to fruit at 20°C at an equatorial position, the fruit sealed in CA bags through which a humidified 5 kPa O_2 atmosphere (balance N_2) circulated, and CA bags placed in a controlled temperature cabinet at 0°C.

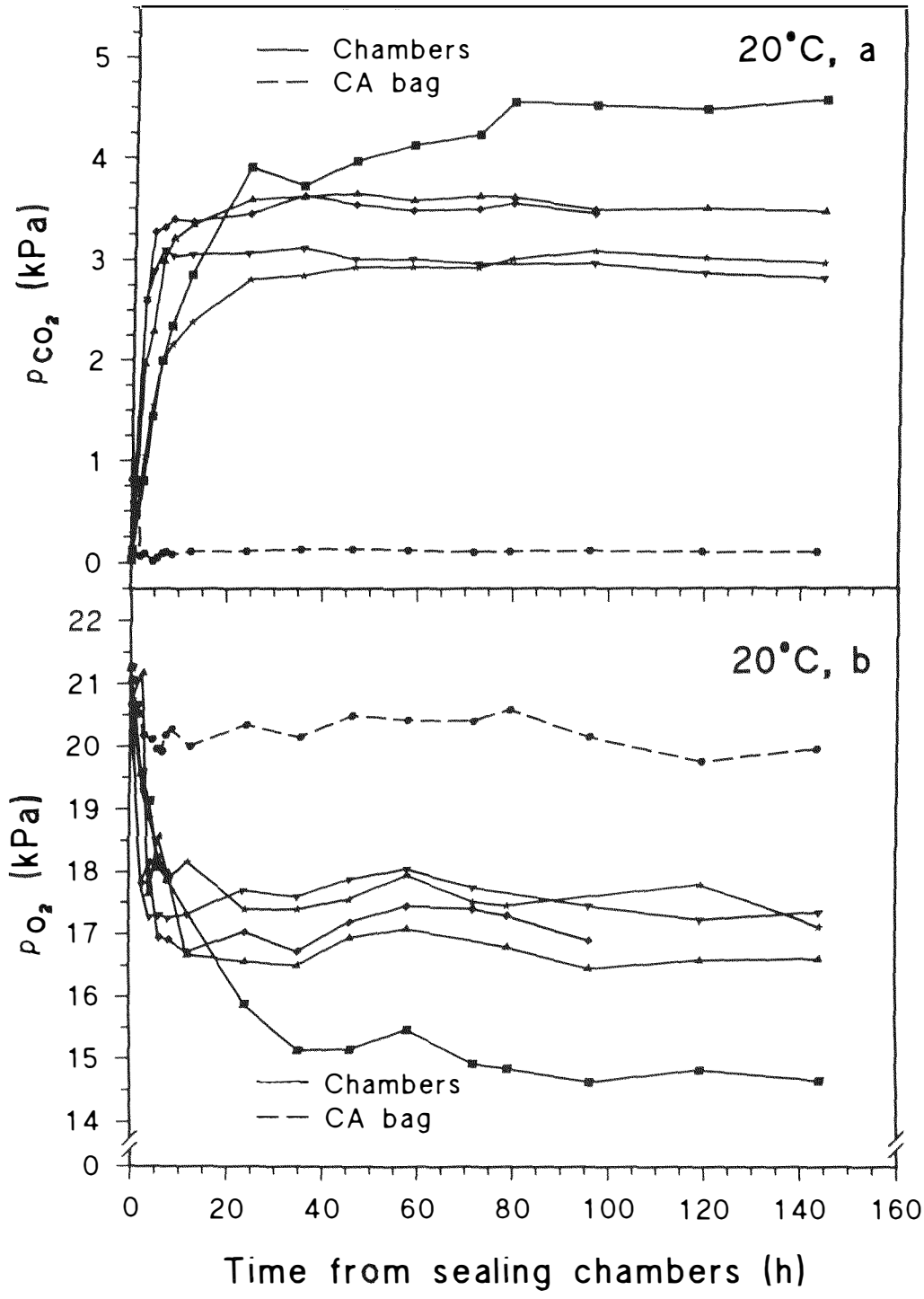

Figure 3.7 Equilibration of (a) p_{CO_2} and (b) p_{O_2} in surface chambers adhered to five 'Cox's Orange Pippin' apples in air at 20°C. Chambers were adhered to fruit at 20°C at an equatorial position, the fruit sealed in CA bags through which humidified air circulated, and CA bags placed in a controlled temperature cabinet at 20°C.

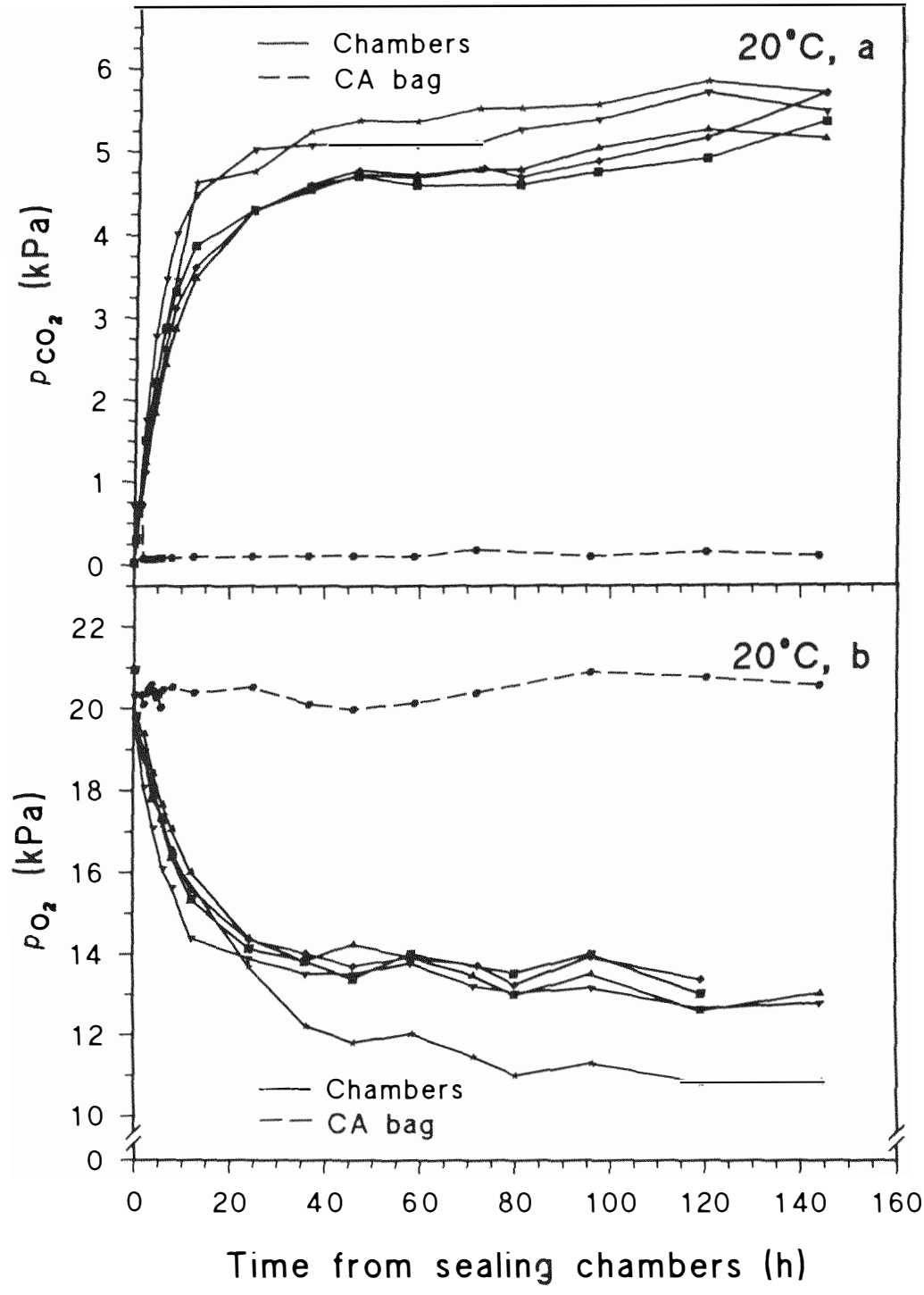

Figure 3.8 Equilibration of (a) p_{CO_2} and (b) p_{O_2} in surface chambers adhered to five 'Braeburn' apples in air at 20°C. Chambers were adhered to fruit at 20°C at an equatorial position, the fruit sealed in CA bags through which humidified air circulated, and CA bags placed in a controlled temperature cabinet at 20°C.

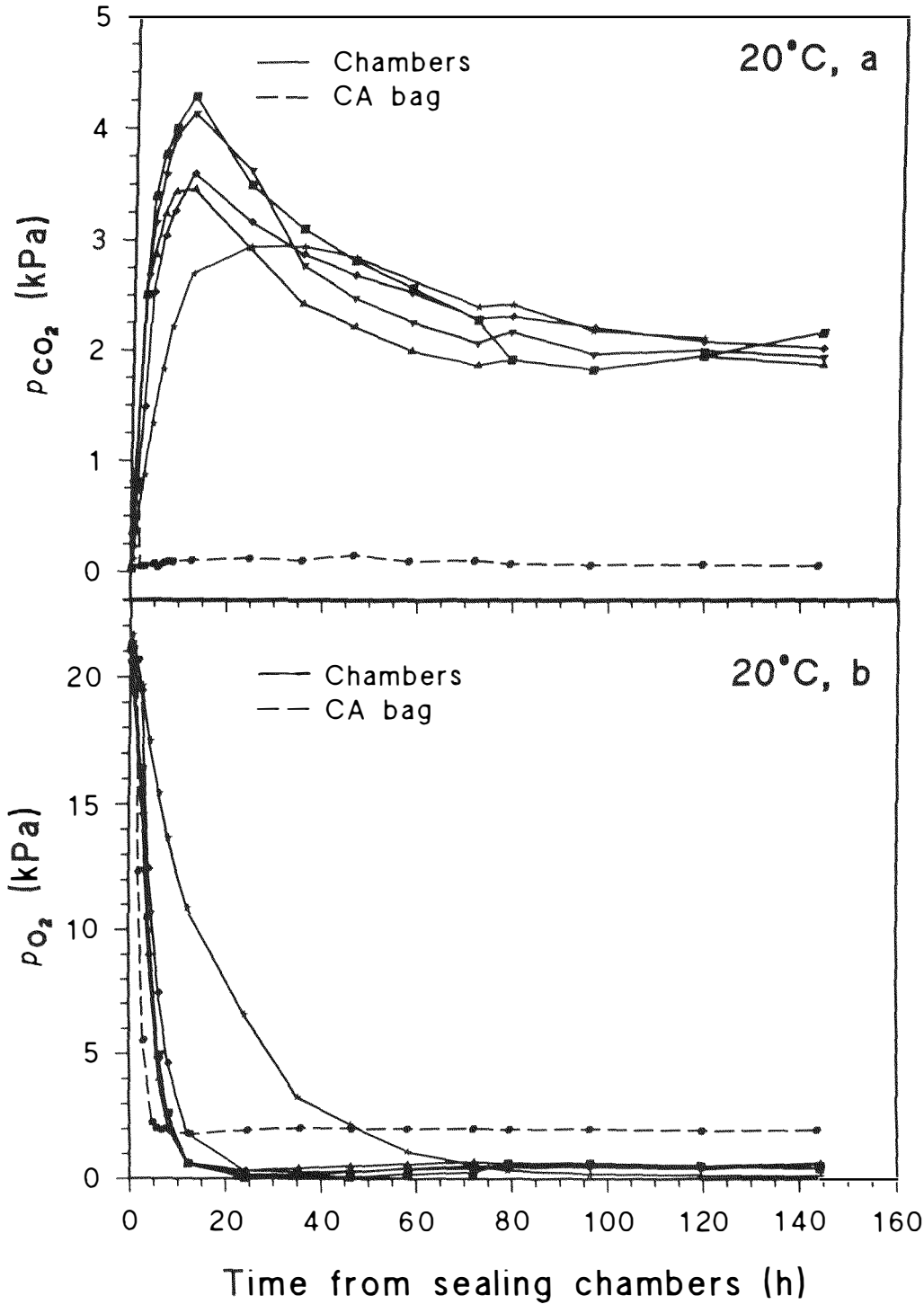

Figure 3.9 Equilibration of (a) p_{CO_2} and (b) p_{O_2} in surface chambers adhered to five 'Cox's Orange Pippin' apples in 2 kPa O_2 at 20°C. Chambers were adhered to fruit at 20°C at an equatorial position, the fruit sealed in CA bags through which a humidified 2 kPa O_2 atmosphere (balance N_2) circulated, at 20°C.

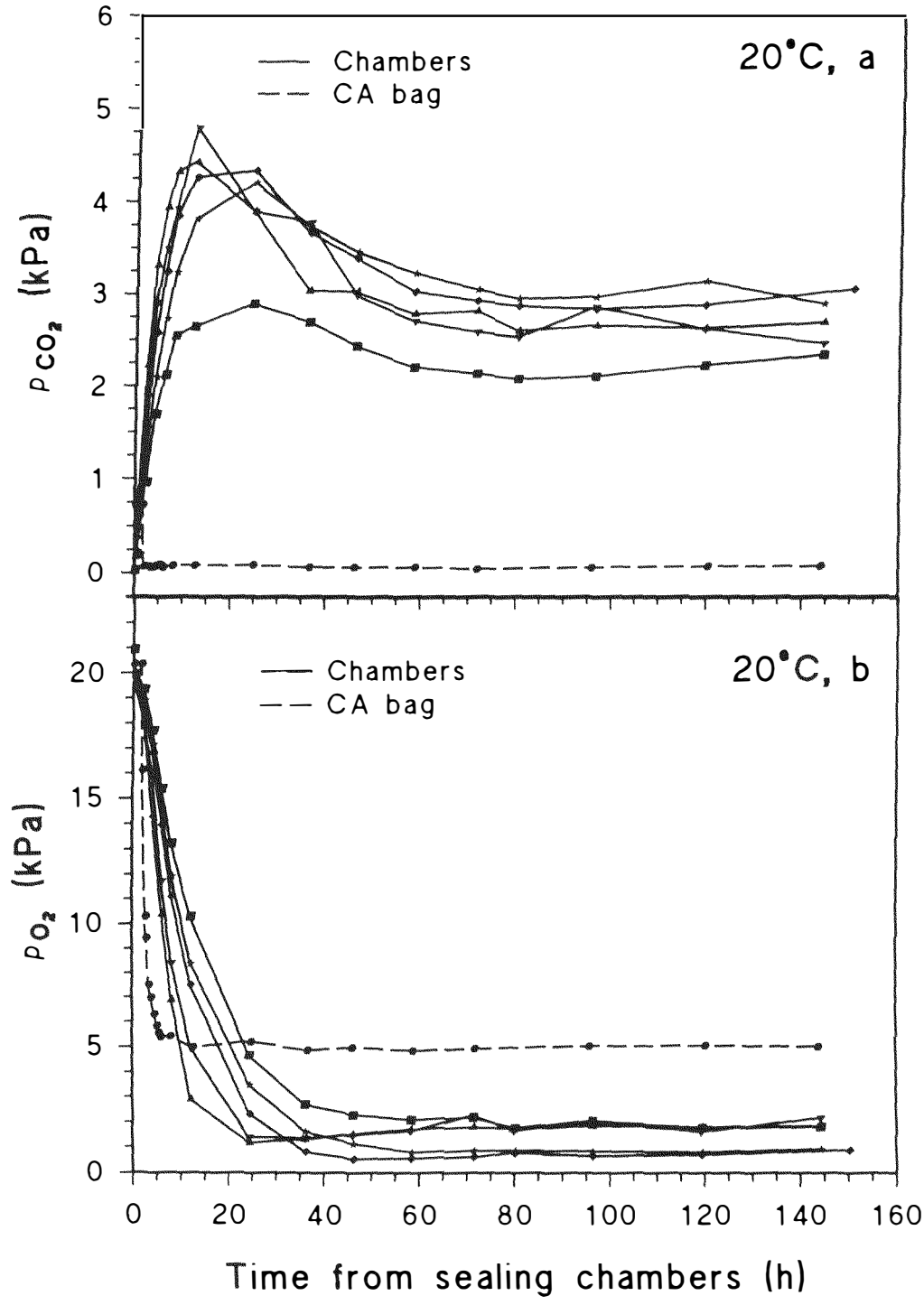

Figure 3.10 Equilibration of (a) p_{CO_2} and (b) p_{O_2} in surface chambers adhered to five 'Braeburn' apples in 5 kPa O_2 at 20°C. Chambers were adhered to fruit at 20°C at an equatorial position, the fruit sealed in CA bags through which a humidified 5 kPa O_2 atmosphere (balance N_2) circulated, and CA bags placed in a controlled temperature cabinet at 20°C.

3.6.2 Direct sampling from the core cavity

IAs were sampled destructively from the core cavity of apples for estimating skin permeance to CO_2 and O_2 and for characterising the IA of fruit held in CA. During sampling of the core cavity atmosphere, apples were submerged in water so as to prevent mass flow of the external atmosphere into the fruit. The sample was taken using a 3000 mm^3 syringe (Monoject, Sherwood Medical, St. Louis, U.S.A., 3 ml disposable syringe) fitted with a blunt 50 mm x 1 mm cannula into which was inserted an entomological pin. The pin prevented tissue blocking the needle as it was inserted through the distal end of the fruit into the core. By retracting the inserted cannula slightly, the pin head fixed in tissue would remain *in situ*, and the sample could be slowly withdrawn from the core into the syringe. The sample syringe was withdrawn, needle removed and syringe capped and stored under water. Subsamples were withdrawn from the syringe using gas-tight glass syringes (Hamilton, 100 mm^3), that had had their dead volume replaced with water. The subsample was used for analysis of CO_2, O_2 and C_2H_4 composition.

For apples under CA, the CA containers were first sealed then inverted under water, and the fruit removed and sampled without them coming into contact with the laboratory atmosphere.

3.7 Measured gas variables

When the internal partial pressure of O_2 ($p^i_{O_2}$, Pa) and CO_2 ($p^i_{CO_2}$, Pa) are at steady-state and homogenous throughout the fruit, then their rates of transfer between the internal and external environment are governed by Fick's Law of diffusion (see Eq. 2.4; Burg and Burg, 1965). Therefore, the key gas variables to measure are the internal and external partial pressures, rate of transfer and permeance of the physiologically important gases, primarily oxygen (O_2) and carbon dioxide (CO_2). In this section, the methods of analysis of these variables are described.

3.7.1 Gas analysis

All gas samples are measured on instruments with detectors/sensors that respond to the absolute amount of gas measured in the gas sample (= n mol).

Mole fraction composition of O_2 and CO_2 was determined using an O_2 electrode (Citicell C/S type, City Technology Ltd., London, U.K.) in series with a miniature infra-red CO_2 transducer with electronic linearisation (Analytical Development Company, Hoddeston, U.K.), with O_2-free N_2 as carrier gas (flow rate 580 $mm^3\ s^{-1}$). Output signals from the CO_2 transducer and O_2 electrode were analysed using integrators (Hewlett Packard, models 3394A, 3395 and 3396) using either peak height or peak area modes.

Unlike O_2 analysis gas chromatography, detection of O_2 with the O_2 electrode is rapid (< 30 s) and is not influenced by argon and the response between 0% and 21% has been shown to be linear (Banks, 1986). A calibration curve was prepared using eight O_2 standards ranging from 0.0% to 20.95%. A coefficient of variation (CV) for repeated injections of standards ranged from 0.78 to 2.35 using peak height data. Linear regression relationship between peak height and mole fraction of O_2 (N_{O_2}, %) was: Peak height = 2.61 N_{O_2} + 0.12 (r^2 = 0.99). Values for CV of peak height and peak area measurements of the 20.95% O_2 standard were 0.6% and 0.64% respectively. High levels of CO_2 can acidify the O_2 electrode and result in overestimation of O_2. This effect was characterised using three replicate 100 mm^3 injections of a range of CO_2 mixtures in O_2 free N_2 (0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, and 10.0 % CO_2). No response was measured in mixtures of 0.0, 0.5 % CO_2. Between 1.0 and 10.0 % CO_2 responses that were equivalent to between 0.03 and 0.14 % O_2 at 10 % CO_2, but with no consistent linear increase with increasing CO_2. When levels of CO_2 above 0.5 % were present in gas samples, the O_2 sensor was calibrated with an O_2 standard with an appropriate amount of CO_2 present as described in section 3.2.2.1.

A calibration curve was prepared for CO_2 for 5 CO_2 standards ranging from 0.035% to 9.96%. Linear regression relationship between peak height and mole fraction of CO_2 (N_{CO_2}, %) was: Peak height = 0.39 N_{CO_2} + 0.06 (r^2 = 0.99). In general the response of the CO_2 transducer was more stable than the O_2 electrode.

Mole fraction composition of ethylene (C_2H_4) was determined by flame ionisation gas chromatography (Philips PU4500, Philips Activated Alumina 2 m x 4.0 mm glass column, gas flow rates of 500, 580, and 5830 $mm^3 s^{-1}$ O_2-free N_2 carrier, H_2 and air respectively, and 130, 150 and 200°C for column, injector and detector temperatures). The CV of ten repeat injections of a 9.7 $\mu l l^{-1}$ C_2H_4 standard using 1000 mm^3 and 90 mm^3 injection volumes was lower using peak heights (0.78% and 1.28% respectively) compared to peak areas.

Mole fraction composition of the anaerobic volatiles, ethanol (EtOH), acetaldehyde (Acet) and ethyl acetate (EtAc) were measured using flame ionisation gas chromatography (Varian 3400 GLC; Econocap Carbowax column, 30m x 0.32 mm ID, film thickness 0.25 µm, Alltech Associates, Inc., Illinois, U.S.A.; gas flow rates of 20.8, 500 and 6666 $mm^3 s^{-1}$ for O_2-free N_2, H_2 and air respectively, and 130, 150 and 300°C for column, injector and detector temperatures, respectively.

3.7.2 Calibration standards

Calibration of gas analysis instruments were made using either volumetrically prepared certified gas mixtures, or mixtures freshly prepared in the laboratory at the time of the experiment.

3.7.2.1 CO_2 and O_2 standards

Calibration standards for CO_2 were either 2.02 ± 0.04% CO_2 (in 5.05% O_2, 1.97 $\mu l l^{-1}$ C_2H_4, balance N_2) or 9.96 ± 0.02% CO_2 (in 19.8% O_2, 9.7 $\mu l l^{-1}$ C_2H_4, balance N_2) certified gas mixtures (NZIG, Special Gases, Wellington, N.Z.).

Calibration standards for O_2 were prepared from a cylinder of compressed instrument grade air composed of 20.98% O_2, balance N_2 (NZIG, Special Gases, Wellington, N.Z.), or for lower O_2 standards, the certified gas mixtures noted above were used. A humidified 0.5% CO_2 volumetrically prepared standard was used for respiration experiments. This was prepared by injecting 9400 mm^3 of pure CO_2 from a cylinder of compressed food grade CO_2 (NZIG, Wellington, N.Z.), into a 1.815 x 10^{-3} m^3 jar, and the jar atmosphere pressurised with addition of 0.185 x 10^{-3} m^3 of air.

Ambient level of CO_2 was assumed to be 0.033%. The atmosphere of the standard bottle was humidified by injecting a drop of water into the jar and stirring the jar atmosphere.

A fresh standard was made when the bottle partial pressure has decreased to that near atmospheric pressure. All atmosphere samples from fruit at different temperatures were equilibrated to the temperature of the calibration gas (laboratory air temperature) before injecting into analysers.

3.7.2.2 *Ethylene (C_2H_4) and ethane (C_2H_6) standards*

Two commercially prepared certified gas mixtures were used as standards for C_2H_4 analysis depending on amounts present in samples (NZIG, Special Gases, Wellington, N.Z.), either:

1.97 ± 0.1 µl l^{-1} (in 2.02% CO_2, 5.03% O_2 balance N_2) or,

9.7 ± 0.3 µl l^{-1} (in 9.96% CO_2, 19.8% O_2 balance N_2).

A freshly prepared 1.0 µl l^{-1} C_2H_6 standard was prepared for estimating fruit permeance using ethane efflux as described below. First a 1000 µl l^{-1} C_2H_6 stock standard was prepared by injecting 2000 mm^3 pure C_2H_6 (from a lecture bottle of pure compressed C_2H_6, NZIG, Special Gases, Wellington, N.Z.) into a sealed jar (1.815×10^{-3} m^3, Agee) and the jar atmosphere pressurised with addition of 0.185×10^{-3} m^3 of air. After stirring the atmosphere, a 2000 mm^3 subsample was removed from the stock standard and injected into a separate standard jar (1.815×10^{-3} m^3, Agee) that had been flushed with compressed dry air. Finally, the jar was pressurised with addition of 0.185×10^{-3} m^3 of air, and used as a 1.0 µl l^{-1} C_2H_6 standard.

3.7.2.3 *Ethanol, acetaldehyde, ethyl acetate and ethylene analysis*

Separate gaseous stock standards (5000 µl l^{-1}) were first prepared using redistilled ethanol, acetaldehyde and ethyl acetate kept at 0°C. For each compound, a sealed jar

of known volume (1.815 x 10^{-3} m^3, Agee) containing a magnetic stirrer bar was used as follows:

1) 1 x 10^5 mm^3 air was removed using a 6.0 x 10^4 mm^3 syringe (Omnifix, B.Braun, Welsungen, AG., Germany),
2) a known aliquot of liquid compound was added to the jar using glass syringes (Hamilton, 25 or 50 mm^3 gas tight syringes) through a rubber septum in the jar lid,
3) the compound was allowed to volatilise, aided by stirring with the magnetic stirrer,
4) the jar was brought back to atmospheric pressure by inserting a syringe needle through the septum,
5) the jar atmosphere was pressurised with addition of 0.185 x 10^{-3} m^3 of air, and the jar atmosphere stirred for a further period at room temperature.

The calculation of the volume of liquid ethanol (EtOH (*liq*)) required to make up a 5000 µl l^{-1} EtOH (*gas*) stock standard was made as follows, and assumes EtOH behaves as an Ideal Gas:

$$n = \frac{p_{tot}\ V}{R\ (T + 273.15)} \tag{3.6}$$

where:

n	=	absolute amount of gas in a given sample (mol)
p_{tot}	=	total pressure in system (Pa)
R	=	gas constant (8.3143 m^3 Pa mol^{-1} K^{-1})
T	=	temperature (°C)
V	=	volume (m^3)

Therefore, the number of moles of gas in 1.815 x 10^{-3} m^3 at 20°C (293.15 K) and 111653 Pa is:

$$n = \frac{111653(\text{Pa})\quad 1.815\ x10^{-3}(\text{m}^{-3})}{8.3142(\text{m}^3\,\text{Pa}\,\text{mol}^{-1}\,\text{K}^{-1})\ 293.15(\text{K})} = 0.08314\,\text{mol} \tag{3.7}$$

For the gaseous stock standard, the mole fraction of EtOH (*gas*) required at 20°C is 5 x 10^{-3} mol mol^{-1}, and the number of moles of EtOH (*gas*) in this amount in 1.815 x 10^{-3} m^3 is:

$$n_{EtOH}^{20} = 5 \times 10^{-3} (\text{mol mol}^{-1}) \quad 0.08314 (\text{mol}) = 4.157 \times 10^{-4} \text{mol} \tag{3.8}$$

The mass of EtOH in 4.157 x 10^{-4} mol is:

$$M = n M_R = 4.157 \times 10^{-4} (\text{mol}) \quad 46.0688 (\text{g mol}^{-1}) = 1.915 \times 10^{-5} \text{kg} \tag{3.9}$$

where: M = mass of EtOH (*gas*)

M_R = relative molecular mass of EtOH (C_2H_5OH = 46.0688 g mol^{-1})

The volume of EtOH at ●°C required to volatilise to produce 1.915 x 10^{-5} kg at 20°C is:

$$V = \frac{M}{\rho_{EtOH}^{0}} = \frac{1.915 \times 10^{-5} (\text{kg})}{0.80625 \times 10^{3} (\text{kg m}^{-3})} = 23.75 \times 10^{-9} \text{m}^3 = 23.750 \text{ mm}^3 \tag{3.10}$$

Using the same procedure, the volume of acetaldehyde and ethyl acetate at 0°C required to make 5000 µl l^{-1} stock standards was calculated:

acetaldehyde (CH_3CHO, M_R = 44.053, ρ^0 = 834.0 kg m^3) = 21.95 mm^3

ethyl acetate ($CH_3COOC_2H_5$, M_R = 88.106, ρ^0 = 902.0 kg m^3) = 40.60 mm^3

From the 5000 µl l^{-1} stock standards, a combined standard containing 100 µl l^{-1} of EtOH, Acet and EtAc and 50 µl l^{-1} C_2H_4 was prepared in a sealed jar (1.825 x 10^{-3} m^3, Agee) as follows:

1) 4.0 x 10^{-5} m^3 of each of EtOH, Acet and EtAc 5000 µl l^{-1} stock standards were transferred from the stock standard jars to the combined standard jar using separate 6.0 x 10^4 mm^3 syringes (Omnifix, B.Braun, Welsungen, AG., Germany),
2) 100 mm^3 of C_2H_4 from a lecture bottle of 99.9% pure compressed C_2H_4 (NZIG, Special Gases, Wellington, N.Z.) was injected into the combined standard jar using a glass syringe (Hamilton, 100 mm^3 gas tight syringe).
3) the jar atmosphere was pressurised with addition of 0.185 x 10^{-3} m^3 of air, and stirred with the aid of a magnetic stirrer.

The volume of each stock standard added to the combined standard jar was calculated by the following equation:

$$V_{stock} = \frac{N_{std}\ V_{std}}{N_{stock}} \qquad \textbf{(3.11)}$$

where:
N_{stock} = mole fraction of stock standard (5000 µl l^{-1})
N_{std} = mole fraction of required in the standard (100 µl l^{-1})
V_{stock} = volume of stock gas required (m^3)
V_{std} = total volume of combined standard (m^3).

3.7.3 Conversion of units for amounts of gas in samples

Results of analyses were reported by integrators as mole fractions (Nobel, 1991; p. 71) either, % for CO_2 and O_2 or µl l^{-1} for C_2H_4, ethanol, acetaldehyde and ethyl acetate. Although concentration differences (mol m^{-3}) are the prime driving forces for diffusion-mediated gas exchange (Nobel, 1991; p. 409), Banks *et al.* (1995) recommend the use of partial pressures as units for driving force for permanent gases in the gas phase. Consequently we have expressed O_2 and CO_2 as partial pressures but report anaerobic volatiles as concentrations.

3.7.3.1 *Converting mole fractions to partial pressures*

Conversions of mole fraction amounts were made by adjusting for the total system pressure using the following equation:

$$p_j = N_j \, p_{tot} \tag{3.12}$$

where: N_j = mole fraction of gas species j (% or $\mu l \, l^{-1}$)

p_j = partial pressure of gas species j (Pa)

p_{tot} = total system partial pressure (Pa)

The total system partial pressure was approximated as the laboratory atmospheric pressure measure with an electronic altimeter/barometer (Barigo, GmbH, D-7330, Villimgen-Schwenningen, Switzerland). Some data were adjusted using 0900 h measurements of atmospheric pressure recorded at the Palmerston North Airport (National Institute of Water and Atmospheric Research, Wellington, N.Z.).

3.7.3.2 *Calculation of concentrations of dissolved O_2 in equilibrium with of partial pressures of O_2*

Oxygen in the intercellular air space dissolves in water in the interstices of the cell wall from where it diffuses through the cell wall, plasmalemma and symplasm. The diffusivity of gases are about 10^4 times greater in the gas phase than in water (Foust *et al.*, 1980). The concentration of dissolved O_2 at equilibrium at a given temperature is described by Henry's Law, were calculated from its partial pressure in the gas phase and its solubility in water (Lendzian and Kirsteins, 1991):

$$c^{T}_{O_2,H_2O} = s^{T}_{O_2,H_2O} \, p_{O_2} \tag{3.13}$$

where: $c^{T}_{O_2,H_2O}$ = concentration of O_2 in H_2O at a given temperature, T (mol m^3)

p_{O_2} = partial pressure of O_2 in the intercellular air space (Pa)

$s^{T}_{O_2,H_2O}$ = solubility of O_2 in H_2O at a given temperature, T (mol m^{-3} Pa^{-1})

The solubility of O_2 in a medium at a given temperature, is equivalent to the Henry's Law constant. Henry's Law is accurate only for relatively low concentrations and pressures, and for gases that do not react significantly with the solvent. Lowering the temperature increases the solubility of O_2 so that the solution concentration corresponding to a given partial pressure would be 50% higher at 5° than at 25°C.

3.7.2.3 Converting mole fractions to concentrations

The accumulation of anaerobic volatiles (ethanol, acetaldehyde and ethyl acetate) was expressed in terms of concentrations using the following equation:

$$c^{T}_{j,\text{air}} = \frac{N_j \; p_{tot}}{R \; (T + 273.15)} \qquad \textbf{(3.14)}$$

where: $c^{T}_{j,\text{air}}$ = concentration of gas j in air at temperature T (mol m^{-3})

N_j = mole fraction of gas j ($\mu l \; l^{-1}$)

3.7.4 Rates of transfer of physiological gases

3.2.4.1 Rate of respiration

Cellular respiration occurs in mitochondria and is the source of ATP production (other than in cells where functional chloroplasts are more abundant) through oxidative phosphorylation (Nobel, 1991), indirectly driven by the strong thermodynamic tendency for O_2 to be reduced. Under aerobic conditions, and depending on the substrate, the respiratory quotient (RQ) or ratio of rate of CO_2 released to O_2 consumed (r_{CO_2}/r_{O_2}) is normally be close to unity (Blanke, 1991).

Apples are climacteric fruit and during the climacteric rise in respiration, CO_2 production and O_2 uptake can increase by 50 to 100% apparently without any rise in *RQ* (Fidler and North, 1967).

Pretreating apples with C_2H_4 and subsequent cool-storage resulted in inducing a climacteric rise in r_{CO_2}. Postclimacteric fruit were removed from cool-storage at different times for use in the experiments. The physiological status of fruit removed from cool-storage over time was monitored by estimating r_{CO_2} and ethylene production ($r_{C_2H_4}$) of a random sample from the population of fruit removed. Fruit removed from cool-storage were brought to physiological equilibrium by storing them in air in a dark constant temperature cabinet for at least 24 h. Except when r_{CO_2} was being estimated for fruit at a range of temperatures, r_{CO_2} was estimated for fruit equilibrated at either 0° or 20°C.

Two methods are typically used for estimating r_{CO_2} for whole plant organs, flow through (steady state) and closed systems (non-steady state). For the flow through system, the plant organ of known mass is enclosed in a container through which a metered flow of humified air of known flow rate is passed. The composition of CO_2 (and O_2, though rarely due to inaccuracies in measuring the small uptake of O_2,) in the inlet and outlet streams are measured and r_{CO_2} calculated on the basis of the crop's mass, air flow rate and change in CO_2 or O_2 (Burg and Burg, 1965; Cameron, 1982; Kader, 1987; Reid *et al.*, 1973; Solomos, 1989). The flow through system can also be used for estimating ethylene production rate and water vapor flux (Ben-Yehoshua and Cameron, 1988).

The closed system for estimating r_{CO_2} requires the plant organ of known mass to be sealed in a container of known volume, and the CO_2 (and O_2) composition of the jar atmosphere measured at the beginning and end of a specific period of time. This system can also be used for estimating ethylene production rate. Estimates of r_{CO_2} using this system have been reported for many studies (Banks 1984a, b; Burg and Burg, 1965; Cameron, 1982; Rajapakse *et al.*, 1990). This non-steady state method has limitations in that accumulation of CO_2 and ethylene can influence r_{CO_2}. However, these effects can be minimised by ensuring the measurement period is such that these gases do not accumulate to physiologically significant levels in the container.

The closed system was used in the present study. Single fruit were sealed in respiration jars (5.8 x 10^{-4} m^3 Agee jars) of known volume that had been completely covered in black tape to exclude light. The jars were left for approximately 5 minutes during which time the partial pressure of water vapour in the jar would reach near saturation as a result of fruit transpiration. At that time (t=0), two samples of the jar atmosphere were removed by flushing and slightly overfilling 1000 mm^3 syringes (Becton Dickinson Medical Products Pte Ltd., Singapore, or Monoject, Sherwood Medical, St. Louis, U.S.A., 1 ml Tuberculin disposable syringe). One sample was used for measuring CO_2 by infra-red gas analysis (IRGA), and the second for C_2H_4 by gas chromatography. For CO_2, exactly 1000 mm^3 was injected into the IRGA which had been calibrated with a humidified 0.5% CO_2 volumetrically prepared standard. Before removal of final samples (t=1800 s or 3600 s for fruit at 20 and 0°C respectively) the jar atmosphere was mixed by flushing with a 1 x 10^4 mm^3 syringe. Final CO_2 levels in respiration jars were not permitted to exceed 0.3%.

The r_{CO_2} was estimated using the following equation:

$$r_{CO_2} = \frac{\left(V_{jar} - \frac{M}{\rho^{20}_{fruit}} \right) \Delta p^{\Delta t}_{CO_2}}{R\,(T + 273.15)\,M\,\Delta t} \qquad \textbf{(3.15)}$$

where:

$\Delta p^{\Delta t}_{CO_2}$ = difference in partial pressure of CO_2 between initial and final measurements over time Δt (Pa)

M = fruit mass (kg)

ρ^{20}_{fruit} = fruit density at 20°C (kg m^{-3})

r_{CO_2} = specific rate of transfer of CO_2 between internal and external atmospheres (mol kg^{-1} s^{-1})

R = gas constant (8.3143 m^3 Pa mol^{-1} K^{-1})

T = fruit temperature (°C)

Δt = difference in time between initial and final sampling of CO_2 (s)

V_{jar} = volume of respiration jar (m^3)

A calculation of the percentage of total variance due to fruit and method of estimating of r_{CO_2} was made using postclimacteric 'Braeburn' apples at 20°C. Two estimates of r_{CO_2} were made on each of 15 fruit, 5 h apart. An analysis of variance was performed on the raw data using the PROC GLM procedure of the SAS system (SAS, 1990). The fruit accounted for 93.5% of the variance and the r_{CO_2} method 6.5% of the variance.

3.7.4.2 Ethylene production rate

Ethylene production ($r_{C_2H_4}$, mol kg^{-1} s^{-1}), like r_{CO_2}, is an indicator of the maturity and ripeness of apples. Ethylene production in apples increases about 1000 fold at the same time as the climacteric increase in r_{CO_2} (Reid *et al.*, 1973). The $r_{C_2H_4}$ was estimated for the same fruit for which r_{CO_2} was determined, as described above using the following equation:

$$r_{C_2H_4} = \frac{\left(V_{jar} - \frac{M}{\rho_{fruit}^{20}}\right) \Delta p_{C_2H_4}^{\Delta t}}{R\,(T + 273.15)\,M\,\Delta t} \qquad \textbf{(3.16)}$$

where: $\Delta p_{C_2H_4}^{\Delta t}$ = difference in partial pressure of C_2H_4 between initial and final measurements over time Δt (Pa)

$r_{C_2H_4}$ = specific rate of transfer of C_2H_4 between internal and external atmospheres (mol kg^{-1} s^{-1})

A calculation of the percentage of total variance due to fruit and method of estimating of $r_{C_2H_4}$ was made using the same fruit and method described for r_{CO_2} above (section 3.7.4.1). The fruit accounted for 96.3% of the variance and the $r_{C_2H_4}$ method 3.7% of the variance.

3.7.5 Estimating fruit skin permeance to gases

The two methods described below estimate whole fruit permeance to either ethane (non-steady state method) or CO_2 and O_2 (steady state method) and approximate the skin permeance.

3.7.5.1 Permeance to ethane

Permeance of apples to ethane ($P'_{C_2H_6}$, mol s^{-1} m^{-2} Pa^{-1}) was estimated using the non-steady state ethane efflux method (Banks, 1985). The following procedure was used:

1) individual apples of known mass, equilibrated to 20 ± 0.5°C, were sealed in glass jars with quick release lids (approximately 1.1 x 10^{-3} m^3),
2) 1000 mm^3 sample of ethane from a lecture bottle of compressed pure C_2H_6 (NZIG, Special Gases, Wellington, N.Z.) was injected into each jar through a septum in the lid, and the jar atmosphere stirred for 60 s with the aid of a magnetic stirrer,
3) jars were placed in a dark controlled temperature cabinet at 20 ± 0.5°C overnight to allow the C_2H_6 to equilibrate throughout the apples,
4) a single jar with fruit enclosed was removed to a fume hood and the atmosphere of the jar stirred again for 60 s,
5) a 1000 mm^3 sample of the jar atmosphere was removed with a 1000 mm^3 syringe (Becton Dickinson Medical Products Pte Ltd., Singapore) and injected into a dilution jar (0.481 x 10^{-3} m^3),
6) the fruit was quickly removed from the sealed jar, rotated in front of a fan to disturb the boundary layer around the fruit for approximately 5 s, then sealed in a glass jar of known volume (1.098 x 10^{-3} m^3) with a magnetic stirrer operating,
7) upon sealing the jar a timer was started and two 1000 mm^3 samples removed simultaneously with syringes after each of 30, 60, 90, 120 and 150 s (during the linear phase of C_2H_6 efflux); the needles of the syringes were inserted into a rubber bung to reduce leaks,

8) samples were analysed for the mole fraction of C_2H_6 present by gas chromatography using the same analysis conditions as for C_2H_6 described above, and the procedure repeated for the other fruit.

Permeance to C_2H_6 was calculated as:

$$P'_{C_2H_6} = \left(\frac{V_{net} \left(\frac{dp_{C_2H_6}}{dt} \right)}{init\ p_{C_2H_6}\ A} \right) \left(\frac{1}{R\ (T + 273.15)} \right) \qquad \textbf{(3.17)}$$

where:

	$init\ p_{C_2H_6}$	=	initial partial pressure of ethane in the fruit's internal atmosphere (Pa)
	$dp_{C_2H_6} / dt$	=	rate of change of ethane partial pressure (Pa s^{-1})
	R	=	gas constant (8.3143 m^3 Pa $mol^{-1}K^{-1}$)
	T	=	fruit temperature (°C)
	V_{net}	=	net volume = jar volume - fruit volume (m^3)

The $init\ p_{C_2H_6}$ was estimated from the amount of C_2H_6 (µl l^{-1}) in the dilution jar multiplied by the dilution factor (481). The rate of efflux of C_2H_6 from the fruit ($dp_{C_2H_6} / dt$) was estimated by linear regression and only fruit for which the regression had an $r^2 \geq 0.97$ were used. Fruit volume was estimated from fruit mass and mean density (see section 3.8.6). Fruit surface area was estimated as described in section 3.8.5.

3.7.5.2 Permeance to CO_2 and O_2

Permeance values of apples to CO_2 and O_2 (P'_{CO_2} and P'_{O_2}, mol s^{-1} m^{-2} Pa^{-1}) were estimated from measures of the internal and external partial pressures of CO_2 and O_2 and respiration rate (r_{CO_2}) for apples at steady-state. P'_{CO_2} was calculated using the following equation:

$$P'_{CO_2} = \frac{r_{CO_2} M}{A\left(\left(N_{CO_2,core} - N_{CO_2,room}\right) p_{tot}\right)} \quad (3.18)$$

where: $N_{CO_2,core}$ = mole fraction of CO_2 in the core cavity (mol mol^{-1})

$N_{CO_2,room}$ = mole fraction of CO_2 in the room (mol mol^{-1}), typically 3.5 x 10^{-4} mol mol^{-1}.

This steady-state method assumes that the IA composition was homogenous throughout the fruit (Burg and Burg, 1965). Assuming $RQ = 1$, P'_{O_2} was calculated using r_{CO_2}:

$$P'_{O_2} = \frac{r_{CO_2} M}{A\left(\left(N_{O_2,room} - N_{O_2,core}\right) p_{tot}\right)} \quad (3.19)$$

where: $N_{O_2,core}$ = mole fraction of O_2 in the core cavity (mol mol^{-1})

$N_{O_2,room}$ = mole fraction of O_2 in the room (mol mol^{-1}), typically 0.2095 mol mol^{-1}, assuming air was dry.

Fruit area was estimated as described in section 3.8.5.

3.8 Measures of other fruit attributes

3.8.1 Fruit tissue firmness

Fruit firmness (F, N; the force required to penetrate the cortical tissue) of apples equilibrated to 2°C, was measured with a press-mounted (Black and Decker electric drill press) penetrometer (0-12 kgf Effegi, fitted with an 11.1 mm diameter head; Facchini, Alfonsine, Italy). A 1 mm thick slice of skin/outer cortex was removed

from opposite sides of apples at an equatorial position as sites for penetration of the penetrometer head. Fruit firmness was determined as the mean value of firmness measurements at these two sites for 20 apples randomly selected from the population used for an experiment. Penetrometer was calibrated using a 3 decimal place top loading balance (Mettler FM 460 Delta range, Mettler Instrumente AG Greifensee-Zürich, Switzerland). The penetrometer values were converted to newtons (N) by multiplying values in kg by 9.807 (Soule, 1985).

3.8.2 Fruit total soluble solids content

Total soluble solids content (SSC, %) of apples equilibrated to 20°C was determined using a hand held refractometer (0-20% Atago, Atago Co., Ltd., Tokyo, Japan) calibrated at 20°C to 0% with distilled water. Measurements were made by mixing a drop of juice from each of the two penetrometer wound sites in the penetrometer, and the mean determined for 20 randomly selected apples from a population used for an experiment.

3.8.3 Starch index

The pattern of starch hydrolysis to soluble sugars was determined using the starch iodine test (Reid *et al.*, 1982). Each of 20 randomly selected apples per experiment was cut transversely at the equator, and one half was placed, cut surface down, for 3 minutes in a solution of potassium iodide and iodine (10 g potassium iodide, 2.5 g iodine dissolved in 1.0 l of water). The resulting pattern of stained tissue on the cut surface was scored using charts on a scale of 0 to 6, where 0 indicated the least and 6 the most starch to sugar conversion. Index charts specific for N.Z. 'Cox's Orange Pippin' and 'Braeburn' cultivars were used (ENZA New Zealand (International)).

3.8.4 Fruit background skin colour

The background skin colour, determined as lightness (L) and hue angle (H°) was measured using a chromameter (Minolta, CR-200, Minolta Camera Co., Ltd.). The

measurements were made for two areas of skin (non-blushed areas) on each of 20 randomly-selected apples from a population used for an experiment. The chromameter was calibrated with a green calibration tile (Minolta Camera Co., Ltd.). The L*C*H° chromatic measurement system was used (as distinct from L^*,a^*,b^* values) as it is considered more descriptive of the perception of colour by the human eye (M°Guire, 1992).

3.8.5 Fruit surface area

Fruit surface areas were estimated from fruit mass using the formula (Clayton *et al.*, 1995):

$$A = 0.0581\ M^{0.685} \tag{3.20}$$

where: A = fruit surface area (m^2)

M = fruit mass (kg).

3.8.6 Cortical tissue porosity and density

Tissue porosity (ε, $m^3\ m^{-3}$) and tissue density (ρ, $kg\ m^{-3}$) were estimated using 5 mm wide longitudinal wedges of tissue from which the skin had been removed using a method based on the Archemedies' principle (Raskin, 1983). A wedge of tissue was suspended from a hook beneath an analytical balance (Mettler AE200 Mettler Instrumente AG Greifensee-Zürich, Switzerland) and the volume of the tissue determined:

$$V_w = \frac{(M_a - M_n) - V_h}{\rho_{H_2O}^{20}} \tag{3.21}$$

where: M_a = mass of non-infiltrated wedge in air (kg)

M_n = apparent mass of non-infiltrated wedge submerged in water (kg)

$\rho_{H_2O}^{20}$ = density of water at 20°C (kg m^{-3})

V_h = volume of submerged portion of hook (m^3).

V_w = volume of wedge (m^3).

Wedges of tissue were vacuum infiltrated with water (a final pressure close to 0 kPa was achieved in three minutes, maintained for a further three minutes, then rapidly released and the slices left submerged for two to three minutes until completely infiltrated). The apparent mass of infiltrated wedges submerged in water was determined and ε calculated:

$$\varepsilon = \frac{\left(\frac{M_i - M_n}{V_w}\right)}{\rho_{H_2O}^{20}} \qquad \textbf{(3.22)}$$

where: ε = tissue porosity (m^3 m^{-3})

M_i = apparent mass of infiltrated wedge submerged in water (kg).

Cortical tissue density was calculated as:

$$\rho = \frac{M_a}{V_w} \qquad \textbf{(3.23)}$$

3.9 Discussion

Minimising sources of errors was thought to be particularly critical when measuring low O_2 atmospheres in these studies, because of the large difference in O_2 levels between air and many of the samples. Leakage of O_2 into or from sampling syringes could result from poorly maintained syringes, or syringes containing samples that were left for long periods before analysis, particularly plastic syringes

(Blankenship and Hammett, 1987). As a result of slight negative pressures in surface chambers compared to atmospheric pressure, when gas samples were taken from surface chambers, it was critical to withdraw the syringe needle through a water seal to prevent uptake of atmospheric O_2. Water sucked into the syringe needle was expelled before injecting the sample into the analyser.

Errors in measuring composition of gas samples could arise if the sample was not equilibrated to the temperature of the standard gas used to calibrate instruments (Banks and Cleland, 1993). For example, a sample at 0°C immediately injected into an analyser calibrated with a standard at 20°C would result in an over-estimate of the actual amount by 298.15/273.15 = 1.0915. Banks and Cleland (1993) noted that equilibration of samples to laboratory temperature is normally rapid due to the thermal mass of the injection syringe being the predominant determinant of sample temperature. In practice a number of glass gas-tight syringes kept at laboratory temperature were used in rotation when samples were taken from fruit in treatments in which temperature differed markedly from that of the laboratory.

The IRGA was sensitive to water vapour, as well as CO_2, and it was considered important for standards to have similar water vapour pressures as samples, particularly when measuring small changes in CO_2 over time as in the estimation of respiration rates. The response of the CO_2 transducer to CO_2 was inherently non-linear, and the signal was linearised electronically. Although a linear curve was fitted for levels of CO_2 between 0 and 10 %, it was also thought to be important to use standards close to the expected values for samples.

The partial pressure of O_2 in surface chambers was reduced by the partial pressure of water vapour which was assumed to always be at saturated equilibrium. The O_2 electrode was not sensitive to water vapour and always responded to the actual partial pressure of O_2 irrespective of the partial pressure of O_2 present in the sample. However the O_2 electrode was affected by the level of CO_2 in samples. High levels acidified the sensor membrane and resulted in over-estimation of O_2 levels. This effect was minimised by calibrating the O_2 electrode with an O_2 standard that incorporated partial pressures of CO_2 close to that expected in samples.

Both non-invasive (surface chambers) and invasive (direct sampling and cannulation) steady state methods of measuring IAs were used in these studies. The

surface chamber method was time consuming to apply, depended on a perfect seal between the fruit skin and the chamber, and required 60 to 96 h to come to steady state. However, surface chambers were considered suitable for monitoring changes in IAs in postclimacteric apples under CA. Given the seal between a glass surface and chamber is perfect, it was calculated that it would take 434 days for the chambers to come to 99.9% equilibrium with external O_2 atmosphere. Therefore, given there is a perfect seal between skin and chambers, the exchange of gases between fruit and chamber would be exclusively through the skin beneath chambers. More rapid equilibration times may have been possible, if a chamber of similar volume but wider diameter had been used on fruit. For the surface chambers used in these studies, the amount of adhesive covering the skin around the chamber was kept to a minimum (approximately a 3 mm wide annulus) to minimise any effect on altering subculticlar IAs. A chamber of larger diameter would also have reduced the ratio of skin surface beneath the chamber to adhesive covered skin surface. For fruit at 20°C in air, the equilibration times represented 'physical equilibration' of the chambers as the fruit were already at equilibrium physiologically. However, for fruit whose rate of respiration was adjusting to either a reduction in fruit temperature or low $p_{O_2}^{e}$, equilibration times represented a combination of physical and 'physiological equilibration' as the time to reach physiological equilibrium was greater than that to achieve physical equilibrium. Because of this lag in reaching steady state, the surface chamber method would not be as suitable for fruit in which rates of respiration are changing rapidly (such as fruit in climacteric phase, and/or for fruit at higher storage temperatures) as the atmosphere of surface chambers may only reach a quasi-steady state. Occasionally surface chambers did not adhere well to the skin and fell off or were easily knocked off the fruit. The IAs of these fruit were sampled at the end of the experiment by direct sampling from the core cavity along with those with surface chambers still attached. A comparison of the surface chamber method and a cannulation technique for monitoring IAs is presented and discussed in some detail in chapter 8.0.

Errors in measuring the difference in partial pressures of CO_2 and O_2 in respiration jars arise unless the partial pressure of water vapour in the jars has come to a saturated equilibrium before sampling. If not at equilibrium, over-estimation of

initial partial pressures compared to final measurements result as water vapour dilutes the composition of gas mixtures in proportion to its own concentration. This effect is dependent upon relative humidity (RH) and temperature (ASHRAE, 1986); errors would be expected to become increasingly large as fruit temperature increases. Banks and Cleland (1993) estimated the error associated with dilution for CO_2 as about 1.5% of the true value for a hypothetical fruit of 0.16 kg in a volume of 1.6 x 10^{-4} m^3 at 25°C. This error is small in comparison to other sources of variation, such as poor sealing of respiration jars, changes in the response of sensors (instrument calibration), and loss of sample through absorption of gases into plastic syringe components if samples are not measured immediately. However, the error associated with dilution for O_2 measurements would be substantial such that for an initial reading of $p^e_{O_2}$ in an atmosphere at 50% RH and 25°C, the increase in water vapour partial pressure due to transpiration, would cause a depression in p_{O_2} at the time of the final measurement greater than that due to O_2 uptake. In addition to the errors associated with water vapour pressure, the change in $p^e_{O_2}$ relative to initial atmosphere values is small compared to that for $p^e_{CO_2}$ and may result in unreliable measurement of O_2 uptake. For these reasons O_2 uptake is seldom attempted using the static system adopted for estimating r_{CO_2}. The O_2 electrode was not suitable for measuring the small changes in O_2 uptake during respiration measurements.

SI units have been adopted throughout (Downs, 1988; Salisbury, 1991), as recommended by Banks *et al.* (1995) for postharvest gas exchange research. Mole fractions are easily converted to partial pressures which provide a more meaningful measure of driving force in gas exchange. Expressed as kPa, the values are similar enough to % values to be immediately meaningful. Units for rates of transfer of gases are typically reported in postharvest literature as the volume or mass of a gas produced or consumed per unit crop mass and time (ml kg^{-1} s^{-1}). The rates are then often converted to the mass of gas produced or the mass evolved per unit crop mass and time (mg kg^{-1} s^{-1}) to remove the effect of temperature on the volume of gas. However, on a mass basis, values for the rate of CO_2 released and O_2 consumed in respiration would appear quite different because of the differences in their molecular weights (mass of CO_2 released would be 44/32 times as great as the mass of O_2 consumed per unit time). Banks *et al.* (1995) recommend that rates of transfer be

expressed in absolute terms per unit mass of fruit (mol kg^{-1} s^{-1}) as this allows physiologically meaningful comparisons to be made, and will facilitate more realistic models of gas exchange.

The two methods are described in section 3.7.5 for estimating fruit permeance. The non-steady state ethane efflux method was developed by Cameron and Yang (1982). Ethane has a similar molecular weight to N_2 and O_2, has diffusion properties similar to C_2H_4 and O_2, and under normal conditions is not significantly metabolised by the fruit. The Cameron and Yang (1982) method is time consuming, requiring several hours for ethane to equilibrate in the fruit and measurement during the efflux phase. Banks (1985) modified this method to reduce the time taken to estimate efflux kinetics but still requires the lengthy equilibration phase, and is the technique used in these studies. Both methods require an accurate estimate of fruit skin area, assume ethane is uniformly distributed throughout the cortex, and the skin is the principle barrier to efflux of ethane from the fruit. Knee (1991a) reported a rapid ethane efflux technique where the loading and efflux phases are reduced to 20 minutes. This technique requires an accurate estimate of the internal volume of the fruit accessible to ethane. If the cortex is not a significant barrier to efflux of ethane, values for $P'_{C_2H_6}$ would approximate skin permeance to ethane. While this is a reasonable assumption for apple tissue, it would not be for some fruit with low porosity (Cameron, 1982). Also ethane would not be suitable for use with fruit with high lipid contents, such as avocado, due to its high solubility in lipids (Solomos, 1987).

The steady-state method for estimating fruit permeance requires accurate measurement of IAs, respiration rate, and fruit surface area (Ben-Yehoshua and Cameron, 1988). Estimates of P'_{CO_2} and P'_{O_2} using IAs of the core cavity represent whole fruit permeance. Skin permeance could be estimated using this method if IA composition was homogeneous or if subcuticular IAs were used. The steady-state method has been used successfully for a wide variety of gases and tissues (Burg and Burg, 1965; Cameron, 1982; Cameron and Reid, 1982; Dadzie, 1992), and was the preferred method in these studies as it was less time consuming.

In conclusion, for these studies it was necessary to measure very low partial pressures of O_2. Given the high ambient partial pressure of O_2, careful attention was

given to identifying and minimising potential sources of error in sampling procedures, sensor characteristics and procedures for estimating gas variables. The O_2 electrode was used primarily because of its rapid response and selectivity of O_2 without interference from argon, water vapour or anaerobic volatiles. However, it was necessary to calibrate with O_2 standards incorporating CO_2 at partial pressures similar to that being measured in fruit, particularly anaerobic fruit with low $p^{i}_{O_2}$ and high $p^{i}_{CO_2}$ to correct for the signal enhancement effect of high partial pressures of CO_2 on the O_2 electrode. The surface chamber method for estimating IAs was suitable for use in these studies, but direct sampling from the core cavity using cannulae may be more appropriate for fruit storage studies where repeated measurement of relative differences in IAs between treatments is required.

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Chapter 4

Determination of Lower Oxygen Limits of Apple Fruit

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4.1 Abstract

Knowledge of the lower oxygen limit (*LOL*) is critical for optimising the gaseous storage environment for fruits. The optimum storage atmosphere occurs just above the *LOL* at which aerobic respiration is at the lowest level which can be achieved without development of anaerobic metabolism. Measures of *LOL* based on a fruit's internal atmosphere, rather than external or package atmospheres, estimate the true *LOL* as these account more directly for differences in respiration rate and skin permeance of individual fruit. Two measures of *LOL* were considered: the anaerobic compensation point (*ACP*) and the fermentation threshold (*FT*). The *ACP* was described in terms of plots of the internal partial pressure of CO_2 versus internal O_2 ($p^i_{O_2}$) and external O_2 ($p^e_{O_2}$) partial pressures. The *FT* was described in terms of plots of both the respiratory quotient (*RQ*) and ethanol concentration versus $p^i_{O_2}$ and $p^e_{O_2}$, and occurred at higher p_{O_2} than the *ACP*. Mathematical solutions for estimating the *ACP* and the *FT* based on the *RQ* (FT_{RQ}) are described. A statistical 'bootstrap' procedure is described for estimating the *FT* based on ethanol concentration (FT_{EtOH}) and was also suitable for estimating all other *LOL*s and their confidence intervals.

*LOL*s were estimated for 'Cox's Orange Pippin' apples (*Malus domestica*, Borkh) at 24°C using controlled atmospheres (CA). The steady-state internal partial pressures of O_2, CO_2 and concentrations of acetaldehyde, ethyl acetate and ethanol were estimated non-invasively by sampling the headspace of 1000 mm^3 glass surface chambers sealed to the equatorial surface of the apples. *LOL*s estimated on a $p^i_{O_2}$

basis were on average 1.69 kPa, 1.94 kPa and 2.10 kPa p_{O_2} lower for *ACP*, FT_{RQ} and FT_{EtOH} respectively than those estimated relative to $p^e_{O_2}$. The bootstrap 95% confidence limits for internal *ACP* (*ACP*^{i}) were 0.70 to 0.78 kPa $p^i_{O_2}$ whilst for internal FT_{RQ} (FT^i_{RQ}) the interval was 1.04 to 1.20 kPa $p^i_{O_2}$ and for internal FT_{EtOH} (FT^i_{EtOH}) 0.68 to 0.87 kPa $p^i_{O_2}$. Bootstrap estimates were similar, though typically higher, than mathematically fitted estimates.

Determining *LOL*s on a steady-state internal atmosphere basis estimates the true *LOL* more accurately than those estimated from external or package atmospheres, and provides a more mechanistic basis for models used to predict fruit responses to CA. As the FT_{RQ} represents the critical point at which fermentation occurs it was considered the safest estimate of the true *LOL* for optimising storage atmospheres.

Keywords: Malus domestica; Internal atmosphere; Anaerobic compensation point; Fermentation threshold; RQ breakpoint; Skin permeance; Bootstrap

4.2 Introduction

Knowledge of the lower oxygen limit (*LOL*) of apples is critical for optimising the gaseous storage environment using controlled atmospheres (CA), modified atmosphere packaging (MA) or skin coatings to retard the crop's physiological deterioration, or using low oxygen (O_2), high carbon dioxide (CO_2) and/or high temperatures for insect disinfestation. As the level of O_2 in a storage atmosphere is reduced there is a depression in the rate of respiratory CO_2 evolution (r_{CO_2}, mol kg^{-1} s^{-1}) until the *LOL* is reached. It is generally accepted that the optimal storage atmosphere for a given temperature is at an O_2 partial pressure (p_{O_2}, Pa) above the point where accumulation of the products of anaerobic respiration resulting in off-flavours and degradation of the tissue are likely (Banks *et al.*, 1993a).

Different approaches have been used in the literature for defining *LOL*s. Blackman (1928) used the term 'Extinction Point' (*EP*), defined as the threshold O_2 concentration at which all anaerobic respiration was just extinguished. Thomas and Fidler (1933) defined the *EP* as the lowest O_2 concentration at which ethanol (EtOH)

production ceased. Boersig *et al.* (1988), studying the aerobic-anaerobic transition zone in pear fruit and pear fruit cell suspension cultures, proposed an alternative concept, the 'Anaerobic Compensation Point' (*ACP*). They defined the *ACP* as the O_2 concentration at which CO_2 production was minimal. Cameron *et al.* (1989), Cameron (1990), Beaudry *et al.* (1992), Gran and Beaudry (1993a) and Beaudry (1993) used the concept of the 'Respiratory Quotient Breakpoint' (*RQB*) which Beaudry (1993) defined as the p_{O_2} below which the steady-state respiratory quotient (*RQ*) began to increase as O_2 level decreased. From a physiological perspective, two classes of *LOL* can be conceived: the *ACP*, and measures of the 'Fermentation Threshold' (*FT*, which includes the points defined above as *EP* and *RQB*).

Different methods of determining the variously defined *LOL*s have been reported. Traditional methods involved measuring the rate of CO_2 production at different levels of p_{O_2}. Leshuk and Saltveit (1990) noted that this only approximates the true *ACP* since it must be calculated from respiration measurements taken at discrete levels of p_{O_2}. They proposed a method for rapidly estimating the *ACP* by measuring the rate of CO_2 production from tissue exposed to an exponentially declining p_{O_2}. Cameron *et al.* (1989), Cameron (1990), Beaudry *et al.* (1992), Gran and Beaudry (1993a), used packages of plastic film of known permeability and containing different masses of fruit to obtain a range of steady-state external p_{O_2} ($p^e_{O_2}$, Pa) within the package. They determined *RQB* by measuring steady-state p_{O_2} and p_{CO_2} of the packages and calculating respiration rates and *RQ*s.

Objective mathematical definitions of p_{O_2} at which *LOL*s occur have not been reported, and published values for *LOL*s using the methods described above, are based on external p_{O_2} (LOL^e) rather than internal atmospheres (LOL^i). However, the O_2 concentration to which tissues respond most directly is that in the cell sap which is close to steady-state with the p_{O_2} of the fruit's internal atmosphere ($p^i_{O_2}$, Pa). Consequently, the objective of this study was to develop a quantitative, conceptual framework for identifying *LOL*s as functions of both internal and external p_{O_2} for apples. Objective mathematical and statistical methods for determining various *LOL* measures are described and their usefulness as a means of optimising storage atmospheres discussed.

4.3 Theoretical background

When $p^i_{O_2}$ and $p^i_{CO_2}$ are at steady-state and homogenous throughout the fruit, then their rates of transfer between the internal and external environment are governed by Fick's Law of diffusion (Burg and Burg, 1965):

$$\Delta p_j = p^i_j - p^e_j = \frac{r_j M}{P^{'}_j A} \quad (4.1)$$

where:

A	=	fruit surface area (m^2)
Δp_j	=	difference in partial pressures of gas j between internal and external atmospheres (Pa)
M	=	fruit mass (kg)
p^i_j	=	partial pressure of gas j in the internal atmosphere (Pa)
p^e_j	=	partial pressure of gas j in the external atmosphere (Pa)
$P^{'}_j$	=	skin permeance to gas j (mol s^{-1} m^{-2} Pa^{-1})
r_j	=	rate of transfer of gas j between internal and external atmospheres (mol kg^{-1} s^{-1})

Therefore, variation in $p^i_{O_2}$ and $p^i_{CO_2}$ between apples results from differences in respiration rate, skin permeance and fruit surface area to mass ratio (Cameron and Reid, 1982; *Dadzie et al.*, 1993; Banks *et al.*, 1993b).

The relationship between r_{CO_2} and both $p^i_{O_2}$ and $p^e_{O_2}$ involves two physiological processes: aerobic and anaerobic respiration (Boersig *et al.*, 1988). For aerobic respiration, r_{CO_2} is increasingly suppressed as p_{O_2} is decreased and the relationship can be described by a Michaelis-Menten equation (Andrich *et al.*, 1991; Solomos, 1982, 1985; Dadzie, 1992; Banks *et al.*, 1993b). For anaerobic respiration, r_{CO_2} declines rapidly as p_{O_2} increases above zero, intercepting the curve for aerobic respiration and approaching zero at a p_{O_2} close to the *FT*. Banks *et al.*, (1993b) modelled the two respiratory components using the equation:

$$r_{CO_2} = RQ^{\infty} \; r_{O_2}^{max} \left(\frac{p^i_{O_2}}{k_1 + p^i_{O_2}} + \frac{10^{-10}}{\left(k_2 + p^i_{O_2}\right)^{k_3}} \right) \qquad \textbf{(4.2)}$$

RQ^{∞} and $r_{O_2}^{max}$ were the respiratory quotient and r_{O_2} when $p^i_{O_2}$ was unlimiting, and k_1, k_2 and k_3 coefficients of the equation. A similar approach can be used to define the relationship between $p^i_{CO_2}$ and p_{O_2} (either external or internal). In this case, using the Michaelis-Menten equation for the aerobic component and an exponential function for the anaerobic component:

$$p^i_{CO_2} = k_4 \; e^{k_5 \, p_{O_2}} + \frac{k_6 \; p_{O_2}}{(k_1 + p_{O_2})} \qquad \textbf{(4.3)}$$

If we define the *ACP* as the p_{O_2} when $p^i_{CO_2}$ is minimal, an objective estimate of the *ACP* can be made as $p^i_{CO_2}$ is minimal when the first differential of Eq. 4.3 is zero:

$$\frac{dp^i_{CO_2}}{dp_{O_2}} = k_4 \; k_5 \; e^{k_5 \, p_{O_2}} + \frac{k_6 \; k_1}{(k_1 + p_{O_2})^2} \qquad \textbf{(4.4)}$$

or;

$$(k_1 + p_{O_2})^2 \; e^{k_5 \, p_{O_2}} + \frac{k_6 \; k_1}{k_4 \; k_5} = 0 \qquad \textbf{(4.5)}$$

For a given set of data, this expression can be solved using the Gauss-Newton method (Gallant, 1987).

As a consequence of the difference between internal and external atmospheres (Eq. 4.1), the *ACP* can be defined in relation to both $p^i_{O_2}$ and $p^e_{O_2}$. The internal and external *ACP* (ACP^i and ACP^e) can be related by Fick's law (Dadzie *et al.*, 1993):

$$ACP^e = ACP^i + \frac{r_{O_2}^{ACP^i} \, M}{P_{O_2} \, A} \qquad \textbf{(4.6)}$$

From this relationship Dadzie *et al.* (1993) predicted that for a fixed ACP^i, ACP^e should be proportional to maximum respiration rate ($r_{O_2}^{max}$) and inversely proportional to skin permeance. The difference between ACP^e and ACP^i should be large for high rates of respiration and low values of skin permeance. Temperature has a significant influence on *LOL*s due to the power law relationship between $r_{O_2}^{max}$ and temperature. Thus, for a fruit of given surface area and skin permeance, the difference between ACP^i and ACP^e should be increasingly greater as temperature increases (Dadzie *et al.*, 1993; Beaudry *et al.*, 1992), though this effect may be somewhat moderated if fruit skin permeance is responsive to temperature in a similar way to polymeric films (Cameron *et al.*, 1995). However, given that the majority of gas diffusion in fruits is known to occur through pores (Banks *et al.*, 1993b), the extent of this moderation is likely to be small because of the low dependence of diffusivity in the gas phase on temperature (Cameron *et al.*, 1995).

A quantity (RQ_{ia}) proportional to RQ can be calculated from the ratio of the differences in partial pressures of the respiratory gases between internal and external atmospheres:

$$RQ_{ia} = \left(\frac{p_{CO_2}^i - p_{CO_2}^e}{p_{O_2}^e - p_{O_2}^i} \right) = \frac{P_{O_2}^{'}}{P_{CO_2}^{'}} RQ \qquad \textbf{(4.7)}$$

Depending on substrate, the RQ of apples remains close to unity (Blanke, 1991) until p_{O_2} approaches the RQB, below which $p_{CO_2}^i$ increases markedly due to anaerobic respiration. A number of functions can be used to describe the relationship between RQ and p_{O_2}. We have used the function:

$$RQ_{ia} = k_7 (p_{O_2})^{k_8} + k_9 \qquad \textbf{(4.8)}$$

Defining the FT_{RQ} as the p_{O_2} when the RQ_{ia} rises to 0.1 times the asymptotic value reached at high p_{O_2}:

$$FT_{RQ} = \left(\frac{0.1\ k_9}{k_7} \right)^{\frac{1}{k_8}} \qquad \textbf{(4.9)}$$

Using this criterion, FT_{RQ} for internal (FT^{i}_{RQ}) and external (FT^{e}_{RQ}) p_{O_2} can be estimated depending on the p_{O_2} values used to determine the coefficients in Eq. 4.8.

Another estimate of the FT, can be determined by plotting ($p^{i}_{CO_2} + p^{i}_{O_2}$) - ($p^{e}_{CO_2} + p^{e}_{O_2}$) against p_{O_2} (see supplementary section 5.7 for a discussion on how this measure compares to RQ_{ia}). This plot is analogous to that of RQ_{ia} but with values close to zero until the onset of fermentation. Similar curves, though described by different functions, exist for the relationship between the concentration of the anaerobic volatiles acetaldehyde (Acet), ethyl acetate (EtAc) and ethanol (EtOH) and p_{O_2}. As the FT marks the onset of anaerobic fermentation it occurs at similar but higher p_{O_2} than the ACP.

An alternative approach to estimating *LOL*s mathematically from fitted curves, is to base estimates on visual assessment of *LOL*s by a panel of assessors using unscaled and untitled graphs. When the number of assessors is small, estimation of average *LOL*s using traditional statistical methods can be unsatisfactory. However, for data sets with few samples a technique called 'bootstrap' offers an attractive alternative to the constraints of traditional parametric theory. This is an empirical methodology introduced by Efron (1979) for assessing the variability or statistical error in an estimate on the basis of the data at hand. Bootstrap samples (also called pseudodata) are obtained by resampling the original observations in such a way that the stochastic structure of the data is preserved. These samples are then used to estimate the parameter of interest. The attraction of this methodology is that it requires little knowledge of distributional assumptions or statistical modelling, and can be applied in an automatic way to any situation, no matter how complicated. The technique is useful for estimation of parameters using small samples. Recent developments of bootstrapping, including computation of bootstrap confidence intervals, are summarised by Efron and Tibshirani (1993).

4.4 Materials and methods

4.4.1 Fruit supply

Freshly harvested, preclimacteric 'Cox's Orange Pippin' apples (*Malus domestica* Borkh.; 125 count apples; mean mass 0.145 kg) were obtained from a Hawkes Bay orchard, N.Z. The fruit were pretreated with 10.24 Pa ethylene at 20°C ($p^e_{CO_2}$ was maintained below 0.1 kPa) for 12 hours, stored at 20°C for a further 48 hours, then packed on trays and enclosed in perforated polyethylene bags in cartons and stored at 2°C. Fruit that had entered the climacteric were used for subsequent experiments, and equilibrated to 24±0.5°C for 24 hours before experimentation.

4.4.2 CA treatments

Fruit were randomly allocated to treatments and a 1000 mm^3 glass surface chamber (9mm diameter) was adhered over a lenticel to each fruit at an equatorial position using 5 minute cure epoxy adhesive (Araldite®, Ciba-Geigy, Auckland, N.Z.). The surface chambers were flushed with room air, sealed with a rubber septum and a water seal added above the septum and the skin-adhesive-glass interface lightly covered with silicone grease (Molycote 111, Dow Corning Australia Pty. Ltd.) to prevent leaks. Six fruit were placed in each of 10 plastic containers with perforated false floors that enclosed Tyvek sachets containing 50g of CO_2 absorber (soda lime) or ethylene absorber (Purafil). CO_2 was absorbed to ensure that observed effects were not caused by accumulation of CO_2 in the experimental system. The containers were sealed inside close-fitting 'Tuflex' barrier bags (Trigon NZ Ltd., Hamilton, N.Z.) through which the gas mixtures flowed and held stored in the dark in a controlled temperature cabinet (Fig. 4.1).

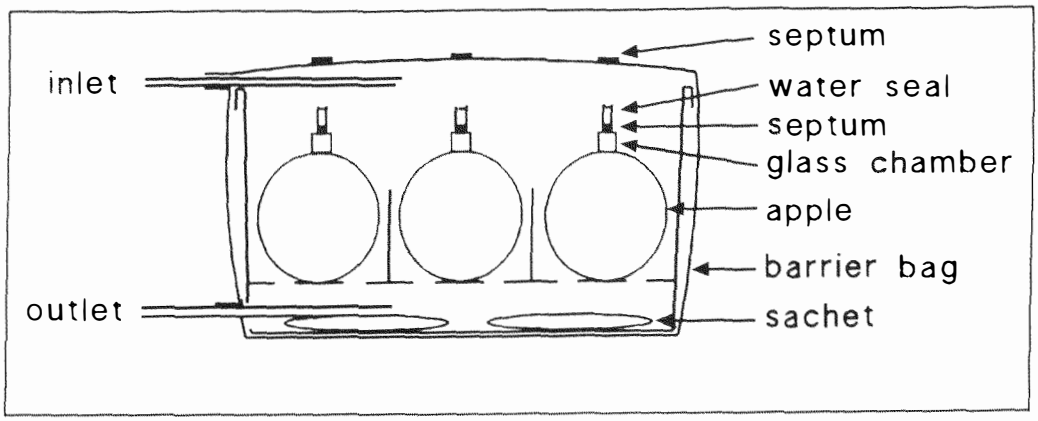

Figure 4.1 Schematic diagram of a CA bag with container holding apples with glass surface chambers adhered to the skin at an equatorial position. Withdrawal of internal atmospheres from the chambers was made through silicone rubber septa attached to the CA bag.

A suitable range of values of $p_{O_2}^e$ for the CA treatments was identified using a steady-state model based on experimental data of Dadzie (1992). The $p_{O_2}^e$ used ranged between 0.0 kPa and 30 kPa, balance nitrogen (N_2), and decreased geometrically towards zero. Gas atmospheres were generated by mixing O_2-free N_2 with either dry air or a 30% O_2 in N_2 mixture from cylinders of compressed gas. Gas flow was controlled by two-stage regulators and precision needle valves (Nupro S series, Nupro Co., Willoughby, Ohio, U.S.A.), and humidified at the experimental temperature before passing into CA containers (total flow 1.7 x 10^3 mm^3 s^{-1}).

4.4.3 Gas measurement and analysis

Steady-state $p_{O_2}^i$ was estimated as the equilibrated O_2 partial pressure in the surface chambers after 86-90 hours exposure to the CA treatments (Dadzie 1992). Gas samples were removed by gas tight syringe (Hamilton 100 mm^3) from the headspace of each surface chamber through a septum on the CA bags above the chamber and immediately analysed. Values for $p_{O_2}^i$ and $p_{CO_2}^i$ were determined using an O_2 electrode (Citicell C/S type, City Technology Ltd., London, U.K.) in series

with a minature infra-red CO_2 transducer (Analytical Development Company, Hoddesdon, U.K.), with O_2-free N_2 as carrier gas (flow rate 580 $mm^3\ s^{-1}$).

The steady-state concentrations of acetaldehyde, ethyl acetate and ethanol (c^l_{Acet}, c^l_{EtAc}, c^l_{EtOH}, mol m^{-3}) in the surface chambers were measured using flame ionization gas chromatography (Varian 3400 GLC; Econocap Carbowax column, 30m x 0.32 mm ID, film thickness 0.25µm, Alltech Associates, Inc., Illinois, U.S.A; gas flow rates of 20.8, 500 and 6666 $mm^3\ s^{-1}$ for O_2-free N_2, H_2 and air respectively, and 130, 150 and 300°C for column, injector and detector temperatures, respectively.

4.4.4 Respiration and ethylene production rates

Fifteen fruit randomly selected from the same population of experimental fruit were used to determine r_{CO_2} and C_2H_4 production ($r_{C_2H_4}$, mol $kg^{-1}\ s^{-1}$) approximately 18 and 120 hours after CA treatments were imposed. C_2H_4 partial pressures were determined by flame ionization gas chromatography (Philips PU4500, Philips Activated Alumina 2 m x 4.0 mm glass column, gas flow rates of 500, 580, and 5830 $mm^3\ s^{-1}$ O_2-free N_2 carrier, H_2 and air respectively, and 130, 150 and 200°C for column, injector and detector temperatures).

4.4.5 Skin permeance

Permeance of the fruit's skin to ethane ($P'_{C_2H_6}$, mol $s^{-1}\ m^{-2}\ Pa^{-1}$) was estimated using ethane efflux (Banks, 1985), calculated as:

$$P'_{C_2H_6} = \left(\frac{V_{net} \left(\frac{dp_{C_2H_6}}{dt} \right)}{init\ p_{C_2H_6}\ A} \right) \left(\frac{1}{R\,(T + 273.15)} \right) \qquad \textbf{(4.10)}$$

where: $init\ p_{C_2H_6}$ = initial partial pressure of ethane in the fruit's internal atmosphere (Pa)

$dp_{C_2H_6}/dt$ = rate of change of ethane partial pressure (Pa s^{-1})

R = gas constant (8.3143 m^2 Pa mol^{-1} K^{-1})

T = fruit temperature (°C)

V_{net} = net volume (m^3)

Fruit surface areas were estimated from fruit mass using the formula (Clayton *et al.*, 1995):

$$A = 0.0581\ M^{0.685} \qquad \textbf{(4.11)}$$

where: A = fruit surface area (m^2)

M = fruit mass (kg)

4.4.6 Tissue porosity and cortical tissue density

Tissue porosity (ε, m^3 m^{-3}) and tissue density (ρ, kg m^{-3}) were estimated using 5 mm wide longtitudinal wedges of tissue from which the skin had been removed. A wedge of tissue was suspended from a hook beneath an analytical balance (Mettler AE200) and the volume of the tissue determined:

$$V_w = \frac{(M_a - M_n) - V_h}{\rho_{H_2O}} \qquad \textbf{(4.12)}$$

where: M_a = mass of non-infiltrated wedge in air (kg)

M_n = apparent mass of non-infiltrated wedge submerged in water (kg)

ρ_{H_2O} = density of water (kg m^{-3})

V_h = volume of submerged portion of hook (m^3).

V_w = volume of wedge (m^3)

Wedges of tissue were vacuum infiltrated with water (a final pressure close to 0 kPa was achieved in three minutes, maintained for a further three minutes, then rapidly released and the slices left submerged for two to three minutes until completely infiltrated). The apparent mass of infiltrated wedges submerged in water was determined and ε calculated:

$$\varepsilon = \frac{(M_i - M_n) / V_w}{\rho_{H_2O}} \quad (4.13)$$

where: ε = tissue porosity ($m^3\ m^{-3}$)

M_i = apparent mass of infiltrated wedge submerged in water (kg).

Tissue density was calculated as:

$$\rho = M_a / V_w \quad (4.14)$$

4.4.7 Experimental design and analysis

Ten CA treatments each with a sample of six fruit were used. When $p^i_{O_2}$ and $p^i_{CO_2}$ were at steady-state, plots of $p^i_{CO_2}$, RQ_{ia}, c^i_{EtOH} versus $p^i_{O_2}$ and $p^e_{O_2}$ were made. Curves were fitted to mean data weighted by the inverse of the variance using the PROC NLIN procedure of the SAS system (SAS, 1990). The functions describing each plot were used to estimate ACP^i, ACP^e, FT^i_{RQ} and FT^e_{RQ} using Eqs. 5 and 9.

Unsealed, untitled graphs of the plots described above were submitted to 23 panellists. Each panellist was asked to hand-draw curves through the data then mark on the curves either the minimum point for *ACP* type curves, or the point where the curve began to rise above the baseline for *FT* type curves. 1000 bootstrap samples (b=1000) were obtained by random sampling with replacement from the original observations. Bootstrap means and bias corrected (BCa) 95% confidence intervals (Efron, 1987) were calculated using Gauss software (Gauss, 1993).

4.5 Results

Values for $p^{i}_{O_2}$ and $p^{i}_{CO_2}$ in the surface chambers held in CA treatments had reached steady-state within 48-96 hours for all treatments (data not shown). Mean $p^{e}_{CO_2}$ was maintained within the range of 0.07 to 0.18 kPa and $p^{e}_{C_2H_4} \leq 0.07$ Pa. Mean r_{CO_2} and $P_{C_2H_6}$ were similar to those reported by Dadzie (1992) for N.Z. 'Cox's Orange Pippin' (Table 4.1). Estimates of cortical tissue density and tissue porosity indicate that the fruit were highly porous (Table 4.1).

4.5.1 $p^{i}_{CO_2}$ as a function of $p^{i}_{O_2}$ and $p^{e}_{O_2}$

Anaerobic CO_2 production increased markedly as $p^{i}_{O_2}$ decreased below 2.0 kPa, reaching a maximum at the lowest levels of $p^{i}_{O_2}$ (near 0.0 kPa; Fig. 4.2a). However, when considering $p^{i}_{CO_2}$ in relation to $p^{e}_{O_2}$, the onset of anaerobic CO_2 production occurred at a higher p_{O_2} and the exponential increase in $p^{i}_{CO_2}$ was more gradual (Fig. 4.2b). The relationship for aerobic respiration was well described by the second term in Eq. 4.3. (Michaelis-Menten equation) with a marked response at low $p^{i}_{O_2}$ but becoming less marked with increased $p^{i}_{O_2}$ levels. The $p^{i}_{O_2}$ when $p^{i}_{CO_2}$ was half-maximal (k_1) was 1.20 kPa (Fig. 4.2a). The increase in $p^{i}_{CO_2}$ was more gradual with increasing $p^{e}_{O_2}$ than with increasing $p^{i}_{O_2}$ as indicated by the higher value for $p^{e}_{O_2}$ when $p^{i}_{CO_2}$ was half-maximal of 6.18 kPa (Fig. 4.2b). For 'Cox's Orange Pippin' apples at 24°C, the estimate of the ACP^{i} derived using Eq. 4.5 was lower than the bootstrap estimate (Table 4.2). However, the estimate of the ACP^{e} derived using Eq. 4.5 was significantly higher than the bootstrap estimate (Table 4.2). The standard error of means of bootstrap estimates of the ACP indicate variation of the ACP was greater when estimated using $p^{e}_{O_2}$ than $p^{i}_{O_2}$ (Table 4.2).

Table 4.1 Biophysical measurements (means ± standard errors of means (sem)) for subsamples of 'Cox's Orange Pippin' apples from the same population of fruit used to estimate lower oxygen limits for fruit in controlled atmospheres at 24°C.

Measurement	n	Mean ± sem	Units
Fruit temperature during experiment	4	24.3 ± 0.04	°C
Skin background colour (L)[1,2]	15	69.0 ± 0.57	
Skin background colour (H°)[1,2]	15	104.4 ± 0.23	
Respiration rate (r_{CO_2})[3,4]	15	0.167 ± 0.0061	μmol kg^{-1} s^{-1}
Ethylene production rate ($r_{C_2H_4}$)[3,5]	15	1.542 ± 0.0407	nmol kg^{-1} s^{-1}
Density of cortical tissue (ρ)[1]	7	870 ± 3.0	kg m^{-3}
Tissue porosity (ε)[1]	7	0.174 ± 0.0004	m^3 m^{-3}
Skin permeance to ethane ($P'_{C_2H_6}$)[1,6]	13	0.189 ± 0.0024	nmol s^{-1} m^{-2} Pa^{-1}

[1] Determined at approximately 20°C.

[2] Determined using a Minolta Chromameter (CR-200); L=lightness, H°=Hue angle.

[3] Determined at 24±1°C.

[4] For r_{CO_2}, 1 μmol kg^{-1} s^{-1} = 158.4 mg kg^{-1} h^{-1} (Banks *et al.*, 1995).

[5] For $r_{C_2H_4}$, 1 nmol kg^{-1} s^{-1} = 0.1008 mg kg^{-1} h^{-1} (Banks *et al.*, 1995).

[6] For $P'_{C_2H_6}$ at 20°C, 1 nmol s^{-1} m^{-2} Pa^{-1} = 2.4368 x 10^{-4} cm s^{-1} (Banks *et al.*, 1995).

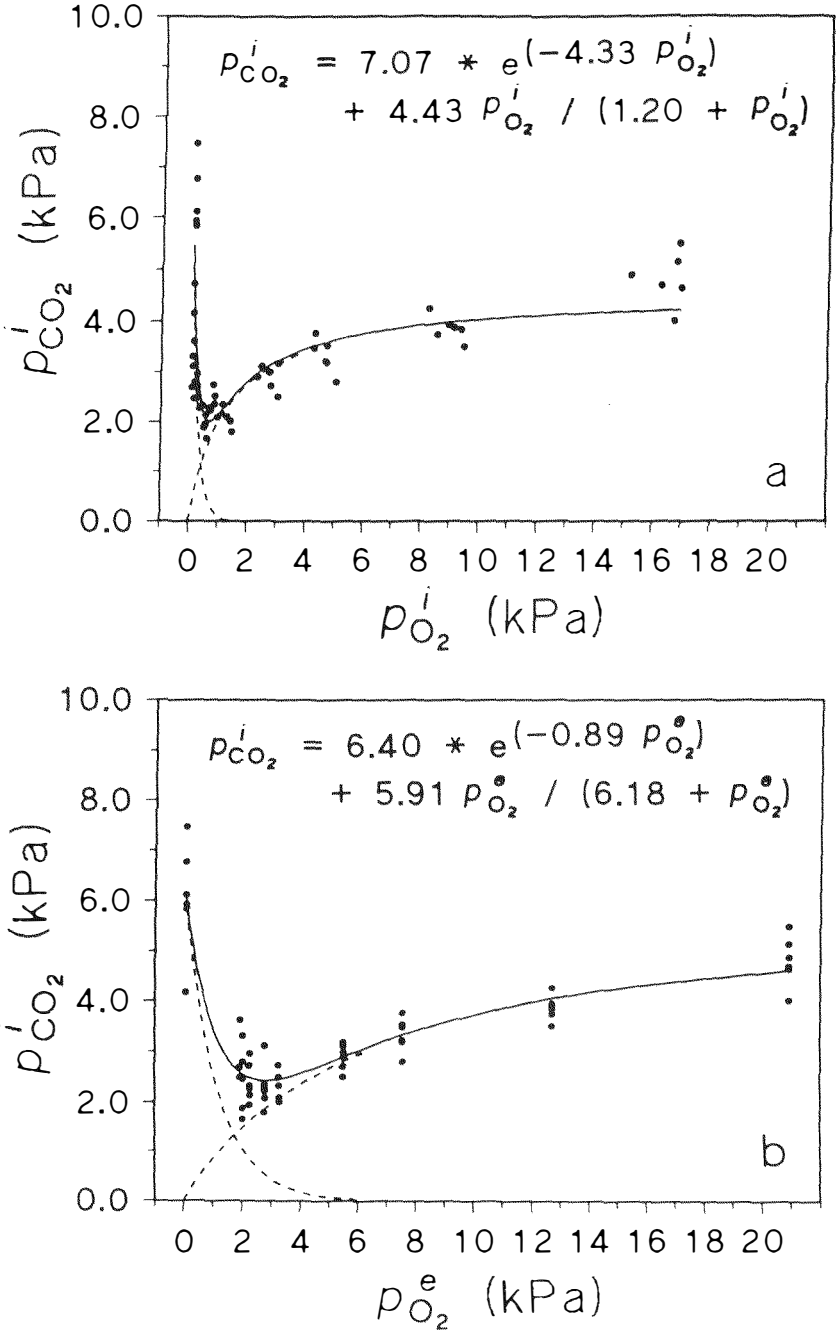

Figure 4.2 Relationship between $p^i_{CO_2}$ and (a) $p^i_{O_2}$ and (b) $p^e_{O_2}$ of individual 'Cox's Orange Pippin' apples after 86 to 90 hours at 24°C in $p^e_{O_2}$ ranging from 0.0 kPa to < 30.0 kPa. The solid line was fitted by nonlinear regression using Eq. 4.3 ($r^2 = 0.59$ and 0.90 for graphs a and b respectively) and represents the sum of the dotted lines which characterise the anaerobic and aerobic components of the relationship.

Table 4.2 Fitted and bootstrap estimates of means and standard errors of means (sem), lower and upper 95% bias corrected (BCa type) confidence intervals (CI) of steady-state lower oxygen limits of 'Cox's Orange Pippin' apples held in controlled atmospheres at 24°C.

	Steady-state lower oxygen limits (kPa)					
	ACP^i	FT^i_{RQ}	FT^i_{EtOH}	ACP^e	FT^e_{RQ}	FT^e_{EtOH}
Fitted	0.70[1]	0.84[2]		2.85[1]	2.99[2]	
Bootstrap	0.74	1.12	0.76	2.43	3.06	2.86
sem	0.019	0.040	0.049	0.083	0.078	0.074
CI (BCa)						
lower	0.701	1.042	0.681	2.261	2.863	2.742
upper	0.778	1.195	0.866	2.575	3.183	3.037

[1] Fitted using Eq. 4.6.

[2] Fitted using Eq. 4.9.

4.5.2 RQ_{ia} as a function of $p^i_{O_2}$ and $p^e_{O_2}$

The mean asymptotic RQ_{ia} of the fruit was marginally above 1.0 (Figs. 4.3a and 4.3b). RQ_{ia} increased very gradually as $p^i_{O_2}$ and $p^e_{O_2}$ decreased to about 2.0 kPa and 4.0 kPa respectively. Below these values there was an increasingly steep response in RQ_{ia} linked to the marked rise in $p^i_{CO_2}$ resulting from anaerobic respiration as seen in Figs. 4.2a and 4.2b. Estimates of FT^i_{RQ} and FT^e_{RQ} using Eq. 4.9 gave lower values than the bootstrap estimates (Table 4.2). The standard error of means of bootstrap estimates of the FT_{RQ} indicate variation of the FT_{RQ} was greater when estimated using $p^e_{O_2}$ than $p^i_{O_2}$ (Table 4.2).

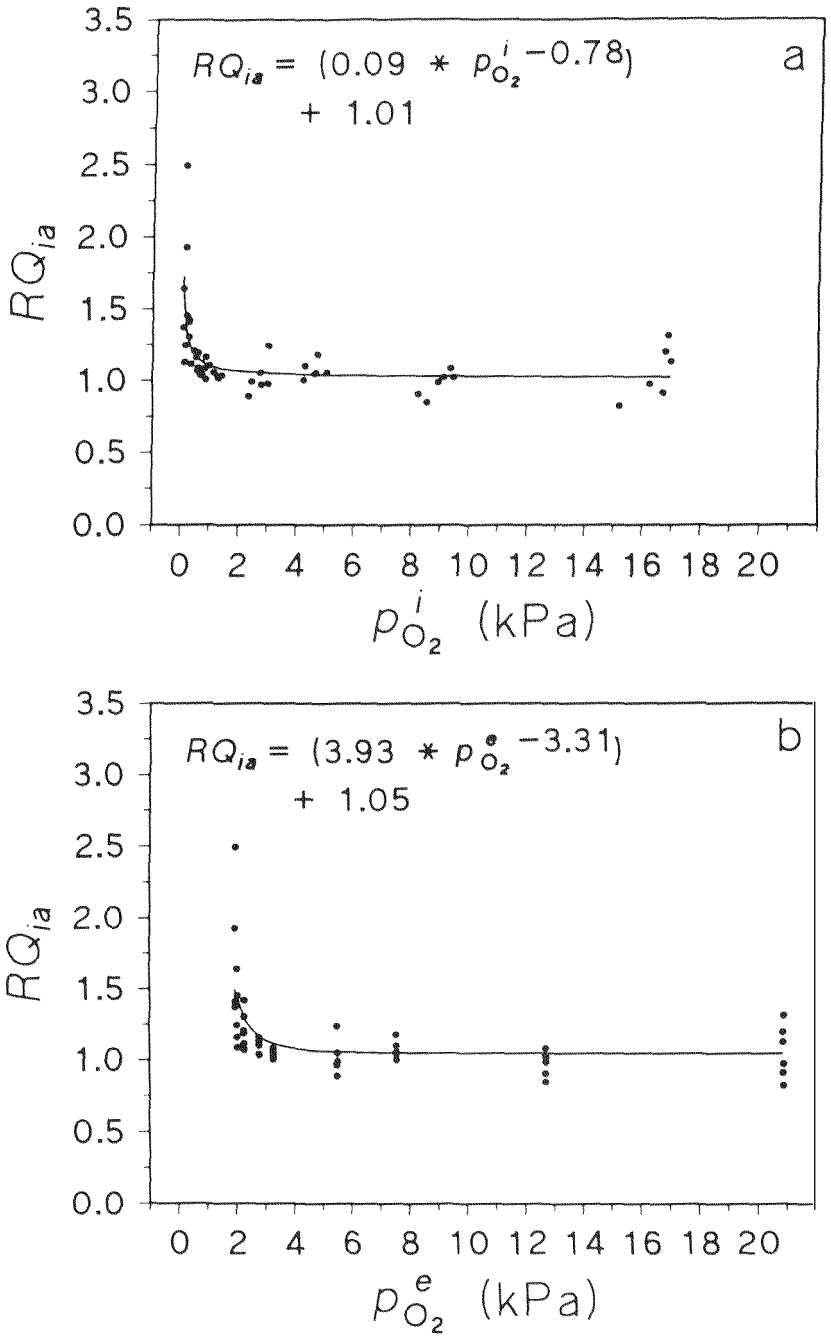

Figure 4.3 Relationship between respiratory quotient [RQ_{ia}; calculated as $(p^{i}_{CO_2} - p^{e}_{CO_2}) / (p^{e}_{O_2} - p^{i}_{O_2})$] and (a) $p^{i}_{O_2}$ and (b) $p^{e}_{O_2}$ of individual 'Cox's Orange Pippin' apples after 86 to 90 hours at 24°C in $p^{e}_{O_2}$ ranging from 0.0 kPa to < 30.0 kPa. The solid line was fitted by nonlinear regression using Eq. 4.8 ($r^2 = 0.48$ for both graphs a and b).

4.5.3 c^{i}_{Acet} c^{i}_{EtAc} and c^{i}_{EtOH} as a function of $p^{i}_{O_2}$ and $p^{e}_{O_2}$

Acetaldehyde in the headspace of surface chambers on fruit was only detected in CA treatments with $p^{e}_{O_2} < 2.8$ kPa. c^{i}_{Acet} increased marginally as mean $p^{e}_{O_2}$ decreased reaching a maximum of 2.4 mmol m^{-3} at the lowest level of $p^{e}_{O_2}$ (0.04 kPa; data not presented). c^{i}_{EtAc} (data not presented) and c^{i}_{EtOH} (Figs. 4.4a and 4.4b) remained below 2.2 mmol m^{-3} and 4.3 mmol m^{-3} respectively until $p^{e}_{O_2}$ decreased below 2.04 kPa. Below 1.0 kPa $p^{i}_{O_2}$ and 2.0 kPa $p^{i}_{O_2}$ both c^{i}_{EtAc} and c^{i}_{EtOH} in particular, increased markedly. The relationship between c^{i}_{EtOH} and p_{O_2} was approximated by the equation:

$$c^{i}_{EtOH} = p_{O_2}^{-k_{10}} + k_{11} \qquad (4.15)$$

The mean estimate of the FT^{i}_{EtOH} using bootstrap sampling was lower than the estimate of the FT^{i}_{RQ} and similar to the bootstrap estimate of the ACP^{i} (Table 4.2). In contrast the bootstrap estimate of the FT^{e}_{EtOH} was similar to the bootstrap estimate of FT^{e}_{RQ}. The standard error of the mean was larger for estimates of FT^{e}_{EtOH} than FT^{i}_{EtOH}.

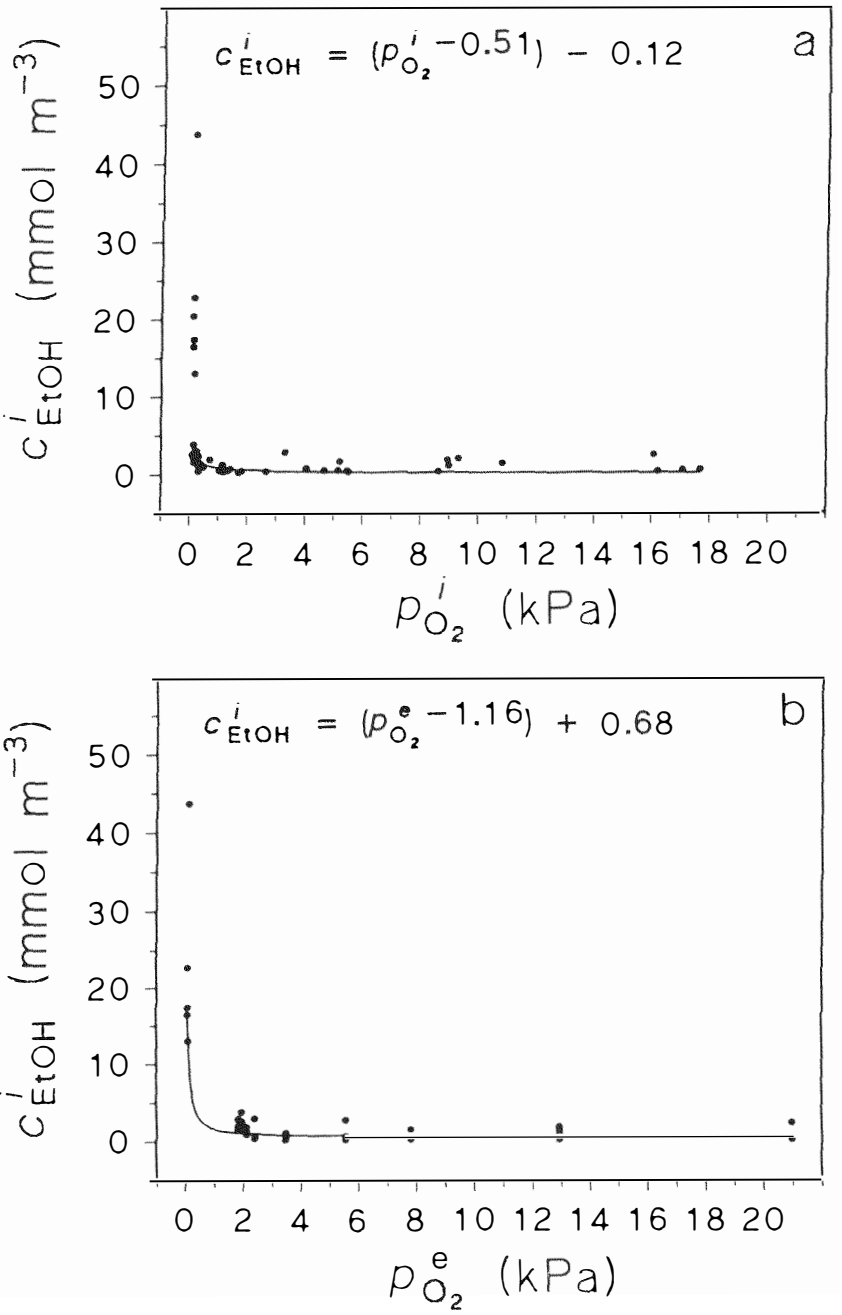

Figure 4.4 Relationship between internal ethanol concentration (c^i_{EtOH}) and (a) $p^i_{O_2}$ and (b) $p^e_{O_2}$ of individual 'Cox's Orange Pippin' apples after 86 to 90 hours at 24°C in $p^e_{O_2}$ ranging from 0.0 kPa to < 30.0 kPa. $r^2 = 0.98$ for both graphs a and b.

4.6 Discussion

Eq. 4.5 provided a robust, objective means of estimating *ACP*. Due to the variation in internal atmospheres of apples in the same $p_{O_2}^e$ (Figs. 4.2 to 4.4) curves were fitted to mean values weighted using the inverse of the variance to emphasise critical data surrounding the *LOL*. The procedure for estimating the FT_{RQ} required the arbitrary selection of a critical value above the asymptotic RQ_{ia} of aerobically respiring fruit. In our study we selected a critical value of 10% as a point that was visually identifiable as a distinct deviation of the fitted curve from the asymptote. Beaudry (1993) arbitrarily selected 20% in a study of the effect of elevated CO_2 on the *RQ* of blueberry fruit. Extensive exploration of alternative approaches failed to yield objective mathematical solutions for FT_{EtOH}. Therefore, subjective visual estimation of the various parameters related to the *LOL* from graphs using a panel of assessors combined with bootstrap methodology was used. In general, bootstrap estimates of *ACP*s and *FT*s were significantly higher than those mathematically derived from fitted curves.

Variation in the internal atmosphere of individual apples (as seen in Figs. 4.2 to 4.4) results from variation in respiration rate, skin permeance and fruit surface area as described by Eq. 4.1. The variance of steady-state internal atmospheres for apples for the same $p_{O_2}^e$, tended to decrease as $p_{O_2}^i$ decreased towards the *LOL*s then increased markedly as $p_{O_2}^i$ fell below the *LOL*. Assuming $\dot{P}_{O_2}$ and $\dot{P}_{CO_2}$ were unaffected by $p_{O_2}^e$, the change in variance would have resulted from the supressive effect of decreasing $p_{O_2}^i$ on aerobic r_{CO_2} above the *LOL* and stimulatory effect on anaerobic r_{CO_2} below the *LOL*. The decrease in aerobic $p_{CO_2}^i$ with diminishing $p_{O_2}^e$ was modelled using the Michaelis-Menten equation (Dadzie *et al.*, 1993). The p_{O_2} at which $p_{CO_2}^i$ was half-maximal (k_1 in Eq. 4.2) was approximately five times higher for the relationship with $p_{O_2}^e$ than with $p_{O_2}^i$ due to the difference in partial pressure between internal and external atmospheres. Conseqently, the estimates of LOL^i were lower than LOL^e as predicted by Eq. 4.6 (1.69 kPa, 1.94 kPa and 2.10 kPa p_{O_2} lower for *ACP*, FT_{RQ} and FT_{EtOH}, respectively, based on bootstrap estimates). This was largely due to differences in r_{CO_2} and $\dot{P}_{CO_2}$ and $\dot{P}_{O_2}$ of individual fruit and would be significant for apple cultivars with low skin permeance (e.g. 'Braeburn') or high r_{CO_2} (e.g. 'Cox's

Orange Pippin'). The magnitude of the difference would also depend on temperature and physiological stage of the fruit in relation to their climacteric. Discrepancies between empirically determined estimates of ACP^e and those determined using Eq. 4.6, can be explained by the larger absolute variability in data around the estimates of the LOL^e (as indicated by larger standard errors of means, Table 4.2). Consequently, defining *LOL*s of fruit on the basis of their steady-state internal atmospheres would be a less variable and more accurate estimate of the true *LOL* than when defined on the basis of external or package atmospheres. Although more expensive to operate, the CA system we used allowed non-invasive sampling of internal atmospheres and direct control over the rate of establishment of the required atmospheres (eliminating the differences in time to establish steady-state conditions that occur with package systems).

Bootstrap estimates of the *ACP* were on average 0.38 kPa $p^i_{O_2}$ and 0.63 kPa $p^e_{O_2}$ lower than those for the FT_{RQ}. Conceptually, the *ACP* and *FT* define different points in the aerobic-anaerobic transition zone. The *ACP* occurs below the onset of CO_2 production resulting from anaerobic respiration, whereas the *FT* is likely to be more representative of p_{O_2} where CO_2 and anaerobic volatiles markedly increase due to fermentation in cortical tissue. Therefore, for modelling optimum storage atmospheres, the *FT* is conceptually more conservative and safer estimate than the *ACP* as it indicates at what level of O_2 fermentation is induced. The 'fermentation threshold' is an appropriate term for this steady-state lower oxygen limit.

It was anticipated that the increase in c^i_{EtOH} would occur at similar $p^i_{O_2}$ to the FT^i_{RQ}. While low c^i_{EtOH} was detected in some fruit above the FT^i_{EtOH}, the increase in c^i_{EtOH} in surface chambers that occurred below the FT^i_{RQ} was more marked than the increase in RQ_{ia}. Gran and Beaudry (1993a) also found that increase in package headspace concentrations of ethanol in 'Law Rome' apples at 0°C occurred below the *RQB*. The difference in the *LOL* determined by ethanol accumulation and *RQB* also increased with increasing temperature (Gran and Beaudry, 1993b). It is possible, in the current work, that chamber c^i_{EtOH} had not reached equilibrium and that tissue c^i_{EtOH} was underestimated. Therefore, the equilibration time for c^i_{EtOH} needs to be quantified to determine if it is larger than for O_2 and CO_2. Alternatively, such data

may have arisen because the internal levels of ethanol were themselves still in a state of rapid change.

To conclude, objective mathematical procedures can be used to estimate the ACP and FT_{RQ} but are highly dependent on fitting appropriate curves to data and the arbitrary choice of critical value in the case of the FT_{RQ}. Use of subjective visual assessments was time consuming but, using a trained panel and bootstrap sampling, valid estimates of all parameters indicative of the LOL and their confidence intervals proved possible. The FT, by definition, represents the critical LOL below which fermentation occurs. The ACP, as predicted from theoretical considerations, was shown to occur at lower p_{O_2} than the FT. Therefore, estimating LOL's using FT's should provide the safest estimates of the true LOL of a population of fruit. However, as the LOL is the consequence of the interplay of a diversity of physiological and biochemical processes and may change with temperature, physiological age, and duration of exposure to hypoxic atmospheres, it should still be used cautiously for optimising storage atmospheres. Final identification of the optimum storage atmosphere would require integration of information on LOLs with information on the susceptibility of the fruit to physiological disorders and the effect of atmospheres on aroma production and other sensory attributes. Using the methods described in this paper, we are currently investigating the effects of temperature, elevated $p^{\varepsilon}_{CO_2}$ and physiological status in relation to the climacteric, on estimates of internal LOL for N.Z. 'Cox's Orange Pippin' and 'Braeburn' apples.

4.7 Supplementary results and discussion

The following was results and discussion not presented in the published paper. Some considerable effort was expended in developing the concept presented below before it was decided that it did not contribute further information than could be provided by the RQ_{ia} procedure for estimating the fermentation threshold (*FT*).

4.7.1 $p_{(i\text{-}e)}$ as a function of $p^{i}_{O_2}$

An alternative quantitative approach to identify the transition between aerobic and anaerobic respiration was considered based on the sum of the internal partial pressures of O_2 and CO_2 less the sum of external partial pressures of O_2 and CO_2, which we termed $p_{(i\text{-}e)}$:

$$p_{(i-e)} = (p^{i}_{O_2} + p^{i}_{CO_2}) - (p^{e}_{CO_2} + p^{e}_{O_2}) \qquad (4.16)$$

or,

$$p_{(i-e)} = (p^{i}_{O_2} - p^{e}_{O_2}) + (p^{i}_{CO_2} - p^{e}_{CO_2}) \qquad (4.17)$$

The relationship between $p_{(i\text{-}e)}$ and $p^{i}_{O_2}$ was similar to that of RQ_{ia} and $p^{i}_{O_2}$ but with values in the aerobic portion of the curve close to zero rather than unity. The relationship between RQ_{ia} and $p_{(i\text{-}e)}$ is developed below. We considered earlier (section 5.2.1) how RQ_{ia} which is proportional to RQ can be calculated from the ratio of the differences in partial pressures of the respiratory gases between internal and external atmospheres. From Fick's First Law of Diffusion:

$$r_{CO_2} = P_{CO_2} A (p^{i}_{CO_2} - p^{e}_{CO_2}) \qquad (4.18)$$

and,

$$r_{O_2} = P_{O_2} A (p^{e}_{O_2} - p^{i}_{O_2}) \qquad (4.19)$$

If RQ is,

$$RQ = \frac{r'_{CO_2}}{r'_{O_2}} \tag{4.20}$$

then RQ_{ia}, which is proportional to RQ; will be:

$$RQ_{ia} = \left(\frac{P'_{CO_2}}{P'_{O_2}} \right) \left(\frac{p^i_{CO_2} - p^e_{CO_2}}{p^e_{O_2} - p^i_{O_2}} \right) \tag{4.21}$$

Rearranging Eq. 4.21 we obtain the equation,

$$(p^i_{CO_2} - p^e_{CO_2}) = \left(\frac{RQ_{ia} \, (p^e_{O_2} - p^i_{O_2}) \, P'_{O_2}}{P'_{CO_2}} \right) \tag{4.22}$$

and substituting equation (4.22) into equation (4.17), we derive the equation:

$$p_{(i-e)} = (p^i_{O_2} - p^e_{O_2}) + \left(\frac{RQ_{ia} \; P'_{O_2} \; (p^e_{O_2} - p^i_{O_2})}{P'_{CO_2}} \right) \tag{4.23}$$

Solving for the common factor we obtain the equation,

$$p_{(i-e)} = (p^e_{O_2} - p^i_{O_2}) \left(RQ_{ia} \frac{P'_{O_2}}{P'_{CO_2}} - 1 \right) \tag{4.24}$$

Comparison of the relationships of $p^i_{CO_2}$, RQ_{ia} and $p_{(i-e)}$ as a function of $p^i_{O_2}$ are illustrated in Fig. 4.5. The curves are based on model predictions using Eqs. 4.3, 4.7 and 4.16 respectively for an apple with $P'_{O_2}/P'_{CO_2} = 0.73$, $P'_{O_2} = 1.9 \times 10^{-10}$ mol s^{-1} m^{-2} Pa^{-1} and $r'^{air}_{O_2} = 1 \times 10^{-8}$ mol s^{-1}.

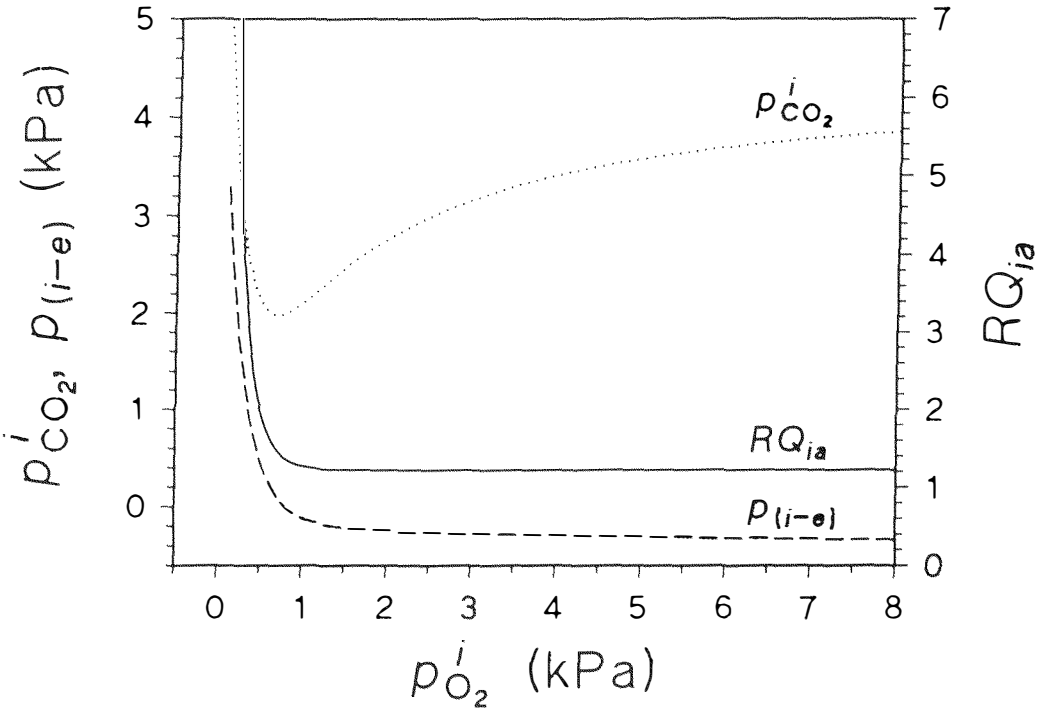

Figure 4.5 Relationship between $p^i_{CO_2}$, RQ_{ia} and $p_{(i-e)}$ as a function of $p^i_{O_2}$ for an apple with $P'_{O_2}/P'_{CO_2} = 0.73$, $P'_{O_2} = 1.9 \times 10^{-10}$ mol s^{-1} m^{-2} Pa^{-1} and $r'^{air}_{O_2}$ = 1 x 10^{-8} mol s^{-1}.

As $p^i_{O_2}$ decreased to levels near the *FT*, there was a diminution of respiration, seen in Fig 4.5 as a decrease in $p^i_{CO_2}$. Consequently, as $p^e_{O_2}$ and $p^i_{O_2}$ decreased, values of Δp_{O_2} or ($p^e_{O_2}$ - $p^i_{O_2}$) became smaller and $p_{(i-e)}$ became decreasingly negative. The increase in $p_{(i-e)}$ was small at higher $p^i_{O_2}$ with a gentle upswing as $p^i_{O_2}$ decreased to near the *FT*, then increased markedly as fermentation commenced. In contrast, RQ_{ia} changed little as $p^i_{O_2}$ decreased from higher values to those near the *FT*, and the upswing in RQ_{ia} at the initiation of fermentation was more rapid than that of $p_{(i-e)}$. The transition between aerobic and anaerobic respiration was more sharply defined when determined by RQ_{ia} compared to $p_{(i-e)}$ and was therefore judged to be a better measure for estimating the *FT*.

Estimates for P'_{O_2}/P'_{CO_2} for 'COP' and 'Braeburn' apples used in experiments in 1995, were 0.97 and 0.72 respectively for fruit at 20°C, and approximately 1.0 for fruit at 0°C. Assuming P'_{O_2}/P'_{CO_2} was constant as $p^e_{O_2}$ varied between 0 and 21 kPa, and RQ_{ia} was constant until the onset of fermentation, then $p_{(i-e)}$ changed in

proportion to ($p^{e}_{O_2}$ - $p^{i}_{O_2}$). As a consequence, the difference between $p_{(i\text{-}e)}$ and RQ_{ia} at different values of $p^{i}_{O_2}$ would not be 1, and asymptotic values of $p_{(i\text{-}e)}$ were not always equal to zero.

A major problem associated with $p_{(i\text{-}e)}$ was finding an objective mathematical procedure for estimating $p^{i}_{O_2}$ at the *FT*. A numerical and separate statistical approach were considered.

The numerical method required finding $p^{i}_{O_2}$ (x_1) when the slope of $p_{(i\text{-}e)}$, or $dp_{(i\text{-}e)}/dp^{i}_{O_2} = -1$. The coordinates of $p_{(i\text{-}e)}$ when the slope was -1 was (y_1,x_1) [Fig. 4. 6]. The asymptote of $p_{(i\text{-}e)}$ at $p^{i}_{O_2}$ above the *FT* was y_{as}. The *FT* was considered to be the $p^{i}_{O_2}$ at which a tangent to $p_{(i\text{-}e)}$ that touched (y_1,x_1) intersected the asymptote (x_{FT}):

$$x_{FT} = x_1 + \left(\frac{y_{as} - y_1}{\text{slope}} \right) \qquad \textbf{(4.25)}$$

The problem with this approach was that $p_{(i\text{-}e)}$ is a term with units and the *y* intercept, and hence the scale of the *y* axis varied considerably between data sets. As a result, the point on the curve where the slope was -1 was dependent on chosen units.

The statistical method estimated the *FT* as the $p^{i}_{O_2}$ at which the asymptote of the upper 95% confidence interval intersected the curve of mean $p_{(i\text{-}e)}$. The *FT* estimated by this method was highly dependent on the variability of the empirical data. The more variable the data, the lower was the value of $p^{i}_{O_2}$ at the *FT*. However, to avoid risk of fruit fermenting, we would require a higher value for the estimate of *FT* with a more variable data set. Therefore $p_{(i\text{-}e)}$ was not considered a useful measure for estimating the *FT*, but RQ_{ia} (and also ethanol accumulation, as discussed in section 4.4.3) was considered satisfactory.

The relationship between RQ_{ia} and $p^{i}_{O_2}$ for apples at various $P^{'}_{O_2}$ and /or $r^{'air}_{O_2}$ is illustrated in Fig. 4.7. For apples with the same respiration rate in air, asymptotic values of RQ_{ia} would be greater for fruit with a higher $P^{'}_{O_2}$ (Fig. 4.7a). For an apple with higher $P^{'}_{O_2}$, the increase in RQ_{ia} resulting from fermentation would occur at marginally higher $p^{i}_{O_2}$. However, this will be offset somewhat by a more gradual increase in RQ_{ia} than a fruit with lower $P^{'}_{O_2}$. These counterbalancing effects of $P^{'}_{O_2}$

on RQ_{ia} may result in the FT^{i}_{RQ}, as estimated by Eq. 4.9, remaining fairly constant. A similar effect was seen for apples with the same $P^{'}_{O_2}$ but different $r^{'air}_{O_2}$ with the exception that higher $r^{'air}_{O_2}$ resulted in lower asymptotic values for RQ_{ia} (Fig. 4.7b). While changes in $P^{'}_{O_2}$ and $r^{'air}_{O_2}$ affect the asymptotic values of RQ_{ia}, neither are likely to markedly alter the FT^{i}_{RQ}. Therefore, RQ_{ia} was considered a relatively robust measure for estimating the FT.

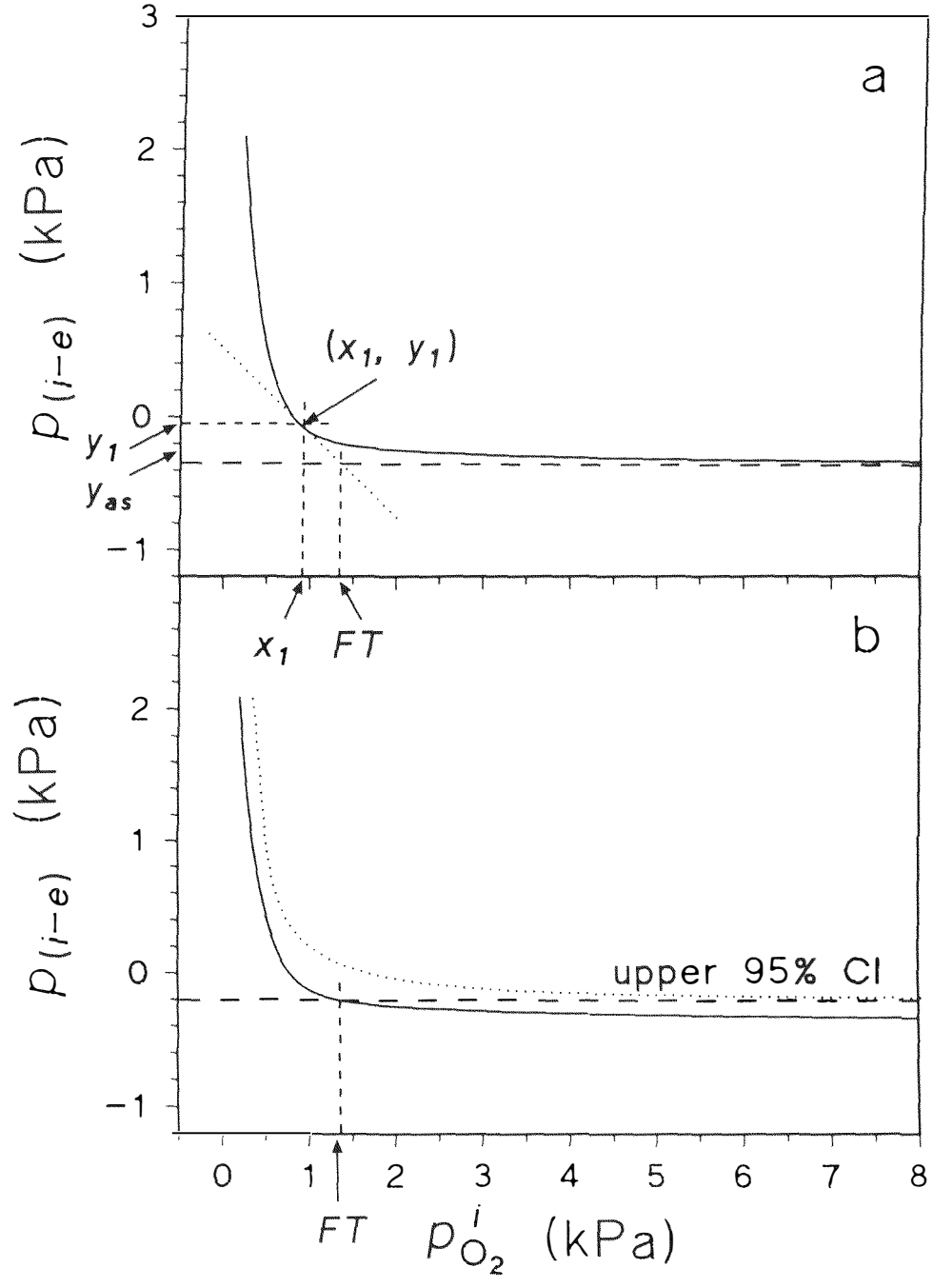

Figure 4.6 Mathematical estimation of the Fermentation Threshold (*FT*) based on the relationship between $p_{(i-e)}$ as a function of $p^i_{O_2}$ using (a) a numerical method (where $FT = x_1 + [(y_{as} - y_1)/\text{slope}]$), and (b) a statistical method (where FT = $p^i_{O_2}$ at the intercept of the asymptote of the upper 95% confidence interval (CI) and the curve of $p_{(i-e)}$ Vs $p^i_{O_2}$.

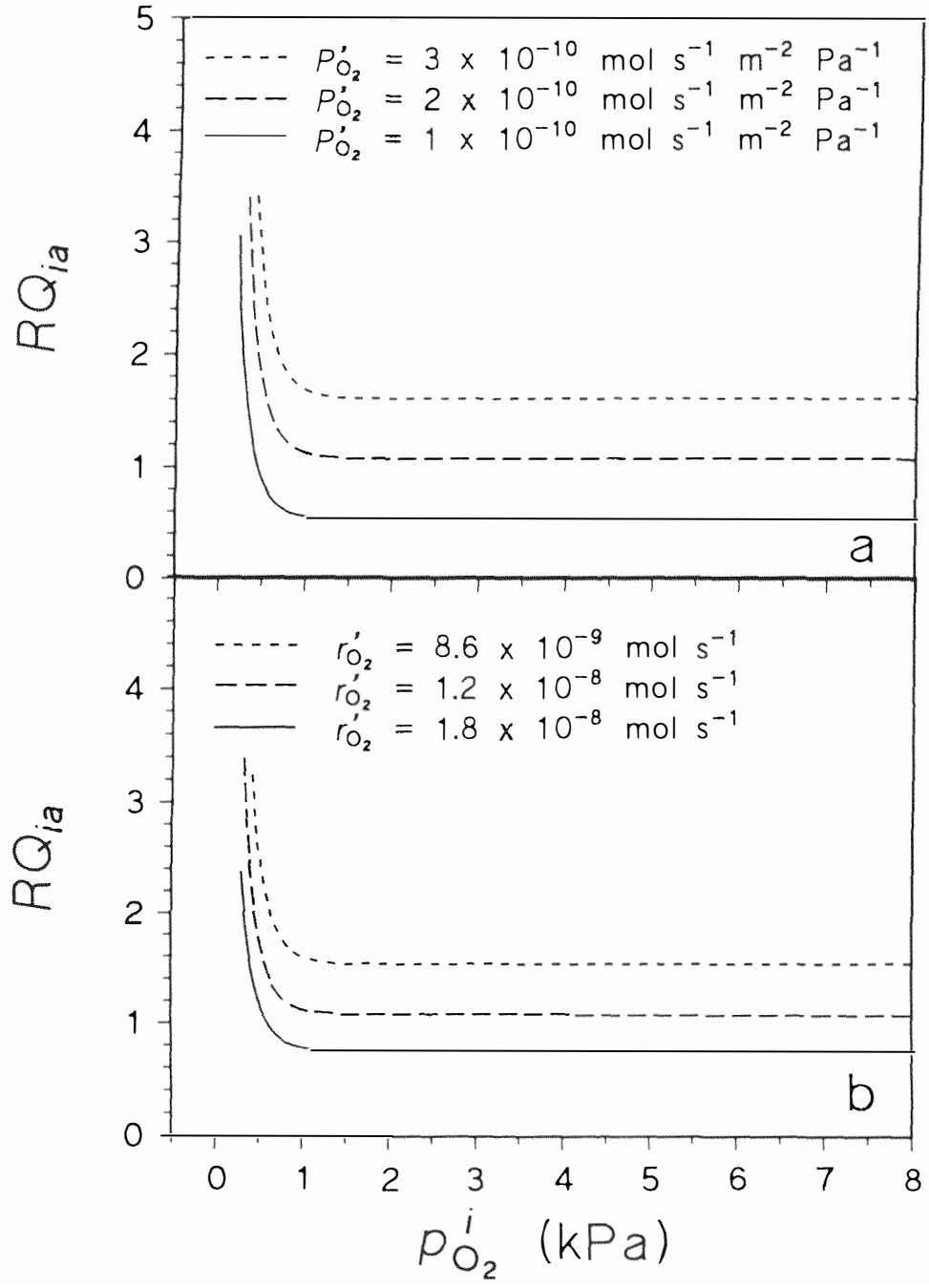

Figure 4.7 The relationship between the respiratory quotient of apples based on internal atmospheres (RQ_{ia}) and $p^i_{O_2}$ for various levels of (a) skin permeance to O_2 (P'_{O_2}) for fruit with the same respiration rate in air ($r'^{air}_{O_2}$) and (b) $r'^{air}_{O_2}$ for fruit with the same P'_{O_2}.

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Chapter 5

Temperature Effects on the Lower Internal Oxygen Limits of Apple Fruit

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5.1 Abstract

Exposure of packaged crops in modified atmospheres (MA) to elevated temperatures can cause the partial pressure of oxygen inside the crop ($p^i_{O_2}$) to fall below critical internal lower O_2 limits (LOL^is), resulting in anaerobic fermentation and loss of quality. In this study LOL^is of apple cultivars 'Cox's Orange Pippin' ('COP') and 'Braeburn' tended to increase with temperature between 0° and 32°C in a complex manner. Consequently the assumption of some steady-state models that the internal anaerobic compensation point (ACP^i) is constant with temperature is simplistic. Two types of LOL^i were estimated from both external chamber and core cavity atmospheres: the anaerobic compensation point (ACP^i), and the fermentation threshold based on the respiratory quotient (FT^i_{RQ}) and on ethanol (EtOH) accumulation (FT^i_{EtOH}). Estimates of LOL^is based on chamber and core cavity atmospheres were similar, and ranged between 0.5 kPa and 1.3 kPa $p^i_{O_2}$ for ACP^i; 0.8 kPa and 2.2 kPa $p^i_{O_2}$ for FT^i_{RQ}, and 0.5 kPa and 2.0 kPa $p^i_{O_2}$ for FT^i_{EtOH}, for fruit temperatures between 0° and 28°C. Values for LOL^is estimated at 32°C were notably higher than those between 0° and 28°C. In general, estimates of dissolved O_2 concentration ($c^i_{O_2,H_2O}$) at ACP^i and FT^i_{RQ} tended to decrease with increasing temperature for 'Braeburn' apples but changed little for 'COP' apples, except at 32°C. On average, estimates of ACP^i were higher for 'Braeburn' than 'COP' apples. Values for FT^i_{RQ} were also higher for 'Braeburn' than for 'COP' apples based on chamber atmospheres but similar for core atmospheres. The effect of temperature on

diffusion coefficients and solubility were considered to have little effect on LOL^is except for solubility at higher temperatures, but differences in tissue porosity may explain differences in LOL^is between cultivars.

Results of the current study indicate temperature effects on LOL^is would not be significant except for MA packages destined for markets with ambient temperatures in excess of 28°C. In contrast, values of ACP for external atmospheres (ACP^e) calculated from ACP^i were strongly influenced by respiration rate and fruit permeance to O_2 and increased in a curvilinear relationship with temperature. Optimum MA package atmospheres based on LOL^es will need to be assessed for the highest temperature the sealed packages are likely to be exposed to for significant durations. Recommendations for optimum storage atmospheres based on LOL^is or LOL^es must be adjusted for the susceptibility of a crop to gas and temperature-related disorders that may develop during long term storage.

Keywords: Malus domestica; Cox's Orange Pippin; Braeburn; Internal atmosphere; Anaerobic compensation point; Fermentation threshold; RQ breakpoint; Skin permeance; Ethane efflux; Respiration rate; Ethylene production rate; Bootstrap

5.2 Introduction

Controlled atmosphere storage (CA) and modified atmosphere packaging (MAP) potentially can extend the shelf life of fresh horticultural crops. CA storage is typically at the recommended storage temperature of the crop (Kader *et al.*, 1989). However, exposure of MAP to higher than recommended storage temperatures may occur during exporting, distribution and retailing of the crop. As temperature increases, rate of O_2 uptake of the enclosed crop increases, but the permeability of polymeric film to O_2 and CO_2 in passive MAP systems does not increase to the same extent (Cameron *et al.*, 1995). Consequently, partial pressures of O_2 in both the package ($p^e_{O_2}$, Pa) and in the crop's internal atmosphere ($p^i_{O_2}$) decrease, whilst those of CO_2 ($p^i_{CO_2}$ and $p^e_{CO_2}$ respectively) increase. Exposure to elevated temperatures can result in internal partial pressure of O_2 ($p^i_{O_2}$) falling below the critical internal lower

O_2 limit (LOL^i), at which anaerobic fermentation is initiated. Fermentation can result in loss of crop quality by accumulation of volatiles causing off-flavours, and may prejudice consumer safety (Hintlian and Hotchkiss, 1986; Kader, *et al.*, 1989; Nguyen-the and Carlin, 1994). Therefore, the optimum design for passive MAP systems is constrained by the highest temperature likely to be encountered for any significant duration after sealing the package. Cameron *et al.* (1995) developed a model to predict the likely effect of temperature on package atmospheres, but acknowledged that accuracy of models was reliant on availability of empirical data. Objective empirical estimates of lower oxygen limits (*LOLs*) of a crop are required to enhance model predictions of optimum storage atmospheres.

Fermentation thresholds [based on respiratory quotient breakpoint (*RQB*) and ethanol (EtOH) accumulation] have been determined for some crops on the basis of external or package atmospheres using MAPs (Beaudry *et al.*, 1992; Beaudry and Gran, 1993; Joles *at al.*, 1994; Talasila *et al.*, 1994). Analogous to these external lower O_2 limits (LOL^e), Yearsley *et al.* (1996a) presented mathematical descriptions of *LOL*s based on internal atmospheres (IA) estimated experimentally for apples in CAs. Two internal *LOL*s (LOL^i) were described: the internal Anaerobic Compensation Point (ACP^i), and internal Fermentation Threshold (FT^i). Optimum IA composition minimises aerobic respiration without inducing anaerobic respiration, and would be expected to occur at a $p^i_{O_2}$ just above the internal FT^i (Banks *et al.*, 1993). In general, LOL^es based on *RQ* or ethanol accumulation, have been observed to increase with increasing temperature for apples (Gran and Beaudry, 1993a and b), blueberries (Beaudry, 1993; Beaudry *et al.*, 1992; Cameron *et al.*, 1994) and raspberries (Joles *et al.*, 1994). However, the effect of temperature on LOL^is does not appear to have been estimated for any crop.

Dadzie *et al.* (1993) used Fick's First Law of Diffusion to argue that the relationship between LOL^i and LOL^e is analogous to the difference between internal and external atmospheres. For *ACP* the relationship can be described by the equation:

$$ACP^e = ACP^i + \left(\frac{r_{O_2}^{ACP^i}}{P'_{O_2} A} \right) \quad \textbf{(5.1)}$$

where: A = fruit surface area (m^2)

P_{O_2} = skin permeance to O_2 (mol s^{-1} m^{-2} Pa^{-1})

$r_{O_2}^{ACP^i}$ = rate of uptake of O_2 for the system at the ACP^i (mol s^{-1})

From this relationship Dadzie *et al.* (1993) predicted that for a fixed ACP^i, ACP^e should be proportional to maximum respiration rate ($r_{O_2}^{max}$), and inversely proportional to skin permeance. The effect of temperature on ACP^e should be significant due to the power law relationship between $r_{O_2}^{max}$ and temperature. Thus, for a fruit of given surface area and skin permeance, ACP^e would increasingly differ from ACP^i as temperature increases (Beaudry *et al.*, 1992; Cameron *et al.*, 1995; Dadzie *et al.*, 1993). The steady-state model of Dadzie *et al.* (1993) assumed ACP^i was constant with temperature. In this study, this assumption was tested using two physiologically contrasting cultivars of apples, 'Cox's Orange Pippin' (COP) and 'Braeburn'. 'COP' apples have a relatively high respiration rate and moderate skin permeance, whereas 'Braeburn' fruit have a low respiration rate and low but highly variable skin permeance.

5.3 Materials and methods

5.3.1 Fruit supply, initial measurements, treatments and storage

Freshly harvested, preclimacteric 'Cox's Orange Pippin' and 'Braeburn' apples (*Malus domestica* Borkh.; 125 count; mean mass 0.144 kg for 'COP' and 0.145 kg for 'Braeburn') were obtained from a Hawkes Bay orchard, N.Z. Fruit were held at 20°C overnight and initial measures of fruit mass, fruit firmness (on pared surfaces on opposite sides of the fruit at the equator using a Bryce 0 to 12 kgf press-mounted penetrometer fitted with an 11 mm head), soluble solids content (of combined drops of juice from the firmness test sites using an Atago 0 to 20% refractometer), background green skin colour (at two locations per fruit using a Minolta CR-200

chromameter measuring lightness and hue angle, Minolta Camera Co., Ltd., Osaka, Japan), were made on 20 fruit. Fruit were treated with 10.24 Pa (≈ 100 µl l^{-1}) ethylene at 20°C for 12 h ($p^{e}_{CO_2}$ was maintained below 0.1 kPa using hydrated lime) in a 1.3 m^{3} constant temperature chamber, then ventilated in air at 20°C for a further 48 h. Trays of fruit were randomly allocated to temperature treatments and stored in cartons, at either 2°C for 'COP' (enclosed in perforated polymeric film bags used commercially for this cultivar to reduce water loss), or 0°C for 'Braeburn' (without bags). Postclimacteric fruit were used for all treatments to minimise variation resulting from differences in fruit respiration.

Fruit were removed from cool store and equilibrated in air at 20 ± 0.5°C for 24 h before six blemish free fruit were randomly allocated to each of 10 CA treatments. Additionally, 20 fruit were allocated for maturity assessments and 15 fruit were stored in a perforated polymeric film bag (to reduce weight loss) at the treatment temperature for estimating respiration and ethylene production rates during the experiment.

5.3.2 Temperature treatments, temperature and relative humidity monitoring

Experiments were conducted in 1.0 m^{3} controlled temperature cabinets (Spaceline, Muller-McAlpine, Auckland, N.Z.). Treatment temperatures used for 'COP' were 0, 12, 24, and 32°C, and for 'Braeburn' 0, 4, 8, 12, 16, 20, 24, 28 and 32°C. As temperature treatments could not be applied simultaneously, they were applied in randomised order over time.

Fruit temperature was monitored in four fruit, one fruit on the left and right sides on each of two levels in the cabinet using a Grant Squirrel datalogger (Model 1205, Grant Inc., Cambridge, U.K.). Calibrated thermistor probes (FF-U-V5 -2, Grant Inc., Cambridge, U.K.) were inserted beneath the skin of each apple at an equatorial position through a 2 mm diameter hole cut tangentially through the cortex to just beneath the skin. Cabinet air temperature was monitored at the centre of the cabinet.

Fruit and air temperatures were logged every 30 minutes. Mean temperature for monitored fruit varied ≤ 0.3°C between fruit in any position within the cabinet.

Relative humidity (RH) was monitored in CA containers enclosing 'Braeburn' fruit at 0°C and 20°C, by inserting RH sensors (IH3602, Hy-Cal Engineering, El Monte, C.A., U.S.A), and thermistor probes to correct the RH sensor signals for air temperature. Mean RH (± standard error of the mean) within CA containers for 'Braeburn' fruit at 0° and 20°C was 90.2 and 94.0 ± 0.54 % respectively.

5.3.3 CA system and model for estimating CA atmospheres

Ten CA treatments, each containing six fruit, were used for each temperature treatment. Metered, humidified gas mixtures flowed through each CA container (as described by Yearsley *et al.* (1996a)) with a total flow of 1.7 x 10^3 mm^3 s^{-1}. Mean CA container $p^e_{O_2}$ was maintained within ± 0.2 kPa of required $p^e_{O_2}$ for treatments ≤ 10 kPa $p^e_{O_2}$ and ± 0.5 kPa for treatments > 10 kPa $p^e_{O_2}$. Mean $p^e_{CO_2}$ of the CA containers was maintained within the range of 0.02 to 0.28 kPa and the highest $p^e_{C_2H_4}$ = 0.97 Pa, for both cultivars over the range of temperature treatments. For CA treatments resulting in $p^i_{O_2}$ around the LOL^is, $p^e_{CO_2}$ and $p^e_{C_2H_4}$ were physiologically insignificant.

The range of O_2 atmospheres used depended on the cultivar and treatment temperature, and were identified using a steady-state model adapted from Dadzie *et al.* (1993) and based on experimental data of Dadzie (1992). The model (model 1) predicted a range of $p^e_{O_2}$ for CA treatments between 0 kPa and 30 kPa that would result in steady-state $p^i_{O_2}$ between 0.09 kPa and 20 kPa.

A geometrically increasing range of $p^i_{O_2}$, weighted towards lower $p^i_{O_2}$, was calculated using the equation:

$$p^i_{O_2} = e^{(3 + (-0.6\ step))} \qquad (5.2)$$

where: $step$ = number between 0 and 10.

The rate of transfer of O_2 at temperature T (°C) was estimated using the Michaelis-Menten equation:

$$r_{O_2}^{T} = r_{O_2}^{max,T} \left(\frac{p_{O_2}^{i}}{k_1 + p_{O_2}^{i}} \right) \quad \textbf{(5.3)}$$

where: k_1 = $p_{O_2}^{i}$ at which $r_{O_2}^{T}$ is half maximal (Pa)

$r_{O_2}^{T}$ = rate of transfer of O_2 at temperature T (mol kg^{-1} s^{-1})

$r_{O_2}^{max,T}$ = maximum rate of O_2 uptake when $p_{O_2}^{i}$ is non-limiting, at temperature T (mol kg^{-1} s^{-1})

T = temperature (°C),

and the maximum rate of O_2 uptake at T when $p_{O_2}^{i}$ was not limiting was calculated as:

$$r_{O_2}^{max,T} = r_{O_2}^{max,0} \, Q_{10}^{(0.1T)} \quad \textbf{(5.4)}$$

where: Q_{10} = temperature coefficient (= [rate of O_2 uptake at (T+10°C)] / [rate of O_2 uptake at T])

$r_{O_2}^{max,0}$ = maximum rate of O_2 uptake when $p_{O_2}^{i}$ is non-limiting, at 0°C (mol kg^{-1} s^{-1}).

The rate of transfer of O_2 for the system at T was calculated using the equation:

$$r_{O_2}^{'T} = r_{O_2}^{T} M \quad \textbf{(5.5)}$$

where: M = fruit mass (kg)

$r_{O_2}^{'T}$ = rate of transfer of O_2 for the system at temperature T (mol s^{-1}).

Model 1 predictions of $p_{O_2}^{s}$ required to achieve a particular level of $p_{O_2}^{i}$ were made using the equation:

$$p_{O_2}^{e} = p_{O_2}^{i} + \left(\frac{r_{O_2}^{'T}}{P_{O_2}^{'} A} \right) \tag{5.6}$$

Values used for variables in model 1 were based on Dadzie (1992):

A = 1.46×10^{-2} m^2

k_1 = 2.03×10^{3} Pa

P = 1.01325×10^{5} Pa

$P_{O_2}^{'}$ = 1.02×10^{-9} mol s^{-1} m^{-2} Pa^{-1} for 'COP' apples

0.27×10^{-9} mol s^{-1} m^{-2} Pa^{-1} for 'Braeburn' apples

Q_{10} = 1.91

$r_{O_2}^{0}$ = 2.48×10^{-8} mol kg^{-1} s^{-1}.

Dadzie *et al.* (1996) modelled $p_{O_2}^{i}$ as a function of $p_{O_2}^{e}$ using the following equation:

$$p_{O_2}^{i} = 0.5 \left(-\left(k_1 + \left(\frac{r_{O_2}^{'max,T}}{P_{O_2}^{'} A} \right) - p_{O_2}^{e} \right) + \left(\left(k_1 + \left(\frac{r_{O_2}^{'max,T}}{P_{O_2}^{'} A} \right) - p_{O_2}^{e} \right)^2 + 4 k_1 p_{O_2}^{e} \right)^{0.5} \right) \tag{5.7}$$

where: $r_{O_2}^{'max,T}$ = rate of transfer of O_2 for the system when $p_{O_2}^{i}$ is non-limiting at temperature T (mol s^{-1}).

Rearranging and solving the equation to express $p_{O_2}^{e}$ as a function of $p_{O_2}^{i}$ resulted in the following equation (model 2):

$$p_{O_2}^{e} = \left(p_{O_2}^{i} + k_1 + \left(\frac{r_{O_2}^{'max,T}}{P_{O_2}^{'} A} \right) \right) \left(\frac{p_{O_2}^{i}}{k_1 + p_{O_2}^{i}} \right) \tag{5.8}$$

Model 2 was fitted to experimental data using empirically determined values for fruit and gas variables presented in Table 5.3.

5.3.4 Gas measurement and analysis

Fruit allocated to temperature treatments each had a 1000 mm^3 glass surface chamber adhered over lenticels at an equatorial position (Yearsley *et al.*, 1996a.) Steady-state IAs were estimated as the equilibrated $p^i_{O_2}$ and $p^i_{CO_2}$ in surface chambers after 86-90 h exposure to CA treatments (Yearsley, 1996). Gas samples were removed by syringe (Gas-tight 100 mm^3, Hamilton Company, Nevada, U.S.A.) from the headspace of each surface chamber through a septum on the CA bags above the chamber. The percentage composition of O_2 and CO_2 were immediately determined using an O_2 electrode (Citicell C/S type, City Technology Ltd., London, U.K.) in series with a miniature infra-red CO_2 transducer (Analytical Development Company, Hoddeston, U.K.), with O_2-free N_2 as carrier gas (flow rate 580 $mm^3\ s^{-1}$). Mole fraction values for IA composition were converted to $p^i_{O_2}$ and $p^i_{CO_2}$ by adjusting for atmospheric pressure (Barigo electronic altimeter/barometer, GmbH, D-7330, Villimgen-Schwenningen, Switzerland).

Steady-state concentrations of acetaldehyde, ethyl acetate and ethanol (c^i_{Acet}, c^i_{EtAc}, c^i_{EtOH}, mol m^{-3}) in the surface chambers were measured using flame ionization gas chromatography (Varian 3400 GLC; Econocap Carbowax column, 30m x 0.32 mm ID, film thickness 0.25µm, Alltech Associates, Inc., Illinois, U.S.A.; gas flow rates of 20.8, 500 and 6666 $mm^3\ s^{-1}$ for O_2-free N_2, H_2 and air respectively, and 130, 150 and 300°C for column, injector and detector temperatures, respectively).

5.3.5 Respiration and ethylene production rates

Fifteen fruit randomly selected from the same population of experimental fruit were used to determine r_{CO_2} and C_2H_4 production ($r_{C_2H_4}$, mol $kg^{-1}\ s^{-1}$) at treatment temperatures approximately 18 and 120 h after CA treatments were imposed (Yearsley *et al.*, 1996b). The r_{CO_2} and $r_{C_2H_4}$ of apples in air were modelled as a power function of temperature using Eq. 5.4 and substituting CO_2 and C_2H_4 for O_2 (Dadzie *et al.*, 1993).

5.3.6 Skin permeance, cortical tissue porosity and density

Permeance of the fruit's skin to ethane ($P^{'}_{C_2H_6}$, mol s^{-1} m^{-2} Pa^{-1}) was estimated using ethane efflux (Banks, 1985; Yearsley *et al.*, 1996a). Fruit surface areas were estimated from fruit mass (Clayton *et al.*, 1995). Tissue porosity (ε, m^3 m^{-3}) and tissue density (ρ, kg m^{-3}) were estimated using 5 mm wide longitudinal wedges of tissue from which the skin had been removed (Yearsley *et al.*, 1996a).

5.3.7 Estimation of *LOL*^{i}s based on partial pressures and dissolved oxygen

A "bootstrap" statistical procedure was used to estimate *LOL*^{i}s using steady-state $p^{i}_{O_2}$ and $p^{i}_{CO_2}$ of surface chambers (Yearsley *et al.*, 1996a). Plots of $p^{i}_{CO_2}$, RQ_{ia}, and c^{i}_{EtOH} versus $p^{i}_{O_2}$ were made and unscaled, untitled graphs of the plots described above submitted to 15 trained panellists. Each panellist was asked to hand-draw curves through the data then identify ACP^{i} and FT^{i}. 1000 bootstrap samples (b = 1000) were obtained by random sampling with replacement from the panellists' original observations. Bootstrap means and bias corrected (BCa) 95% confidence intervals of means were calculated (Efron, 1987; Yearsley, 1996) using Gauss software (Gauss, 1993). Estimates of *LOL*^{i}s based on dissolved O_2 concentration were calculated directly from partial pressures using the following equation (Lendzian and Kirsteins, 1991):

$$c^{i,T}_{O_2,H_2O} = s^{T}_{O_2,H_2O}\ p^{i}_{O_2} \qquad \textbf{(5.9)}$$

where:

$c^{i,T}_{O_2,H_2O}$ = internal concentration of O_2 in H_2O at a given temperature, T (mol m^{-3})

$p^{i}_{O_2}$ = internal partial pressure of O_2 of the *LOL*^{i} (Pa)

$s^{T}_{O_2,H_2O}$ = solubility of O_2 in H_2O at a given temperature, T (mol m^{-3} Pa^{-1}).

5.3.8 Estimation of ACP^e from ACP^i

The maximum rate of O_2 uptake of the system at T ($r_{O_2}^{'max,\,T}$, mol s^{-1}) was calculated using the equation:

$$r_{O_2}^{'max} = r_{O_2}^{'air}\left(\frac{k_1 + 20950}{20950}\right) \tag{5.10}$$

where: $r_{O_2}^{'air}$ = rate of transfer of O_2 for the system in air (mol s^{-1}).

Values of $r_{O_2}^{'air}$ were calculated using Eqs. 5.4 and 5.5, and assumed RQ was unity. Values of k_1 were estimated from plots of $p_{CO_2}^i$ versus $p_{O_2}^i$ at various T (data not shown). Rate of O_2 uptake of the system at the ACP^i ($r_{O_2}^{'ACP^i}$, mol s^{-1}) was calculated using the equation:

$$r_{O_2}^{'ACP^i} = \frac{r_{O_2}^{'max} ACP^i}{k_1 + ACP^i} \tag{5.11}$$

ACP^e was calculated using Eq. 5.1 from bootstrap estimates of ACP^i obtained using combined IA data from surface chambers and core cavity for 'Braeburn' apples. ACP^e was calculated at T between 0° and 28°C, and for fruit with low and high $P_{O_2}^{'}$ (0.1 and 0.3 nmol s^{-1} m^{-2} Pa^{-1} respectively, and A = 0.0151 m^2, M = 0.14 kg).

5.3.9 Statistical analysis

Regression analyses of relationships between LOL^is, mean fruit temperature and time in storage were conducted (for 'Braeburn' apples) using the PROC REG procedure of the SAS system (SAS, 1990). Data were weighted with either the inverse of the standard error of means or time in storage. They were also adjusted for time in storage using inverse of the standard error of means as weights. Fruit

maturity, cortical tissue porosity and density and $P_{C_2H_6}^{'}$ data were analysed as a randomised block model using the PROC GLM procedure (SAS, 1990) as were data for r_{CO_2} and $r_{C_2H_4}$, but using repeated measures.

5.4 Results

Mean chamber $p_{C_2H_4}^{c}$ during ethylene pretreatment was 10.9 and 10.8 Pa for 'COP' and 'Braeburn' respectively with $p_{CO_2}^{c}$ maintained below 0.1 kPa for both cultivars. Estimates of r_{CO_2} and $r_{C_2H_4}$ for the fruit 48 h after pretreatment indicated the fruit had commenced the climacteric phase prior to cool storage (data not shown).

5.4.1 Fruit maturity

'Braeburn' apples were 13.9 N firmer and had 1% higher soluble solids content than 'COP' at harvest (Table 5.1). For both cultivars, fruit firmness decreased rapidly and soluble solids increased slightly within the first 3 to 8 weeks of storage. Fruit firmness and soluble solids content differed significantly for some treatments during the experimental period, but the differences were small physiologically. For both cultivars, background skin colour became lighter (L) and hue angle (H°) decreased (changed from green to green-yellow) with increasing time in storage (Table 5.1). There were statistical differences over storage time for L and H°, but only small changes occurred during the period over which fruit were removed from storage for experiments.

Table 5.1 Fruit firmness, soluble solids content, and background colour lightness (L) and hue angle (H°) for 'Cox's Orange Pippin' and 'Braeburn' apples from a Hawkes Bay orchard stored at 2°C and 0°C respectively for various durations. Fruit were removed from cool store and maintained at 20°C for 24 h prior to measurement at 20°C. Values represent least squares means, and within cultivars, treatments with different letters (a, b, c) are significantly different ($p = 0.05$, n=20).

Cultivar and Time in storage	Treatment temperature	Fruit firmness	Soluble solids content	Background colour	
(weeks)	(°C)	(N)	(%)	(L)	(H°)
'Cox's Orange Pippin'					
0	Prestorage	70.8 a	10.9 a	69.5 a	109.9 a
1.9	24	NE[1]	NE	69.0 a	104.4 a
3.3	0	48.9 b	11.9 b	69.5 a	103.9 b
9.4	32	41.2 c	11.7 b	72.3 b	100.5 c
10.4	12	39.9 c	11.6 b	72.7 b	100.8 c
'Braeburn'					
0	Prestorage	84.7 a	11.9 a	63.7 a	102.1 a
8	28	63.7 b	13.0 bc	65.0 a	100.7 ab
13	24	59.8 bc	13.0 bc	68.0 b	99.7 ab
14	12	60.2 bc	13.2 c	69.1 bc	99.6 ab
15	8	61.0 bc	12.8 b	67.8 b	97.8 b
16	16	61.2 b	13.3 c	68.9 bc	97.4 b
19	4	58.1 cd	12.8 b	69.4 bc	99.6 ab
20	32	56.2 cd	12.9 bc	68.3 b	98.6 b
23	0	55.5 d	13.1 c	70.1 bc	98.6 b
24	20	57.6 cd	12.9 bc	69.7 bc	99.1 b
Storage time comparisons (ANOVA p values)					
'Cox's Orange Pippin'		0.0001	0.0001	0.0001	0.0001
'Braeburn'		0.0001	0.0001	0.0001	0.0004

[1] NE = not estimated

5.4.2 Skin permeance to ethane (P'_{C2H6})

Mean estimates of $P'_{C_2H_6}$ for 'COP' apples ranged between 0.218 and 0.236 nmol s^{-1} m^{-2} Pa^{-1}, and were twice as permeable to ethane as 'Braeburn' apples for which values lay between 0.102 and 0.121 nmol s^{-1} m^{-2} Pa^{-1} (Table 5.2). Values were similar to those reported by Dadzie (1992) for these cultivars. For each cultivar, $P'_{C_2H_6}$ did not change significantly with increasing time in storage.

5.4.3 Cortical tissue porosity (ε) and density (ρ)

Cortical tissue porosity of 'COP' apples was 1.2 times as high as for 'Braeburn' apples, when averaged over all assessment times (Table 5.2). Cortical tissue density of 'COP' apples was marginally lower than for 'Braeburn', and neither porosity nor density of 'COP' fruit varied markedly during storage. For 'Braeburn' fruit (which were stored longer than 'COP'), porosity decreased slightly from 0.142 m^3 m^{-3} to 0.132 m^3 m^{-3} from 8 to 14 weeks in storage, then increased again to 0.152 m^3 m^{-3} after 24 weeks storage. For both cultivars, cortical tissue density decreased proportionally with increasing porosity (Fig. 5.1). Individual apples in the population sampled varied markedly in porosity and density. The range in estimates of porosity and density was greater for 'Braeburn' than 'COP' apples (0.165 and 0.045 m^3 m^{-3}, and 60.0 and 40.0 kg m^{-3} respectively).

5.4.4 Respiration rate (r_{CO2}) and ethylene production (r_{C2H4}) as a function of fruit temperature

For both 'COP' and 'Braeburn' apples, r_{CO_2} and $r_{C_2H_4}$ increased with increasing fruit temperature between 0°C and either 24° or 28°C, and then decreased (Fig. 5.2). Estimates of r_{CO_2} for 'COP' were higher than for 'Braeburn' apples and increased more steeply with temperature. Estimates of $r_{C_2H_4}$ for 'COP' were higher than for 'Braeburn' apples at 0°C, but similar or lower at higher temperatures.

Table 5.2 Cortical tissue porosity (ε), density (ρ) and skin permeance to ethane ($P_{C_2H_6}$) for 'Cox's Orange Pippin' and 'Braeburn' apples from a Hawkes Bay orchard stored at 2°C and 0°C respectively for various durations. The fruit were removed from cool storage and stored at 20°C for 24 h prior to estimating ε and ρ and 7 days before estimating $P_{C_2H_6}$. Values represent least squares means and, within cultivars, treatments with different letters are significantly different (p = 0.05, n=15).

Cultivar and Time in storage (weeks)	Treatment temperature (°C)	Cortical tissue porosity (ε) ($m^3\ m^{-3}$)	Cortical tissue density (ρ) ($kg\ m^{-3}$)	Skin permeance to ethane ($P_{C_2H_6}$)[1] ($nmol\ s^{-1}\ m^{-2}\ Pa^{-1}$)
'Cox's Orange Pippin'				
1.9	24	0.174 a	867 a	0.218 a
3.3	0	0.176 a	863 a	NE[2]
9.4	32	NE	NE	0.236 a
10.4	12	0.170 a	866 a	0.234 a
'Braeburn'				
8	28	0.142 ab	898 c	0.109 a
13	24	0.140 bc	899 bc	0.102 a
14	12	0.132 c	911 ab	0.109 a
15	8	0.138 bc	907 ab	0.112 a
16	16	0.140 bc	903 bc	0.116 a
19	4	0.142 ab	905 bc	0.117 a
20	32	0.149 a	893 c	NE
23	0	0.148 a	898 bc	0.109 a
24	20	0.152 a	891 c	0.121 a
Storage time comparisons (ANOVA p values)				
'Cox's Orange Pippin'		0.1730	0.0090	0.8720
'Braeburn'		0.0001	0.0001	0.5899

[1] For $P_{C_2H_6}$ at T (°C), 1 $mol\ s^{-1}\ m^{-2}\ Pa^{-1}$ = $((T + 273.15) / 1.203 \times 10^{-3})$ $cm\ s^{-1}$ (Banks *et al.*, 1995).

[2] NE = not estimated.

Fitted values for Q_{10} for r_{CO_2} of 'COP' were greater than for 'Braeburn' (2.44 compared to 2.28) but lower for $r_{C_2H_4}$ (2.12 compared to 2.55). It was not possible to characterise the transition from increasing to decreasing r_{CO_2} and $r_{C_2H_4}$ at higher temperatures due to insufficient data for temperatures around the optima. As r_{CO_2} and $r_{C_2H_4}$ were not estimated for 'COP' for the same range of temperatures as for 'Braeburn', more data would be required to be confident of the temperature relationships for 'COP' apples. [For r_{CO_2}, at T (°C), 1 mol kg^{-1} s^{-1} = 1.5839 x 10^{8} mg kg^{-1} h^{-1} or (T + 273.15) / 3.341 x 10^{-11} p_{tot} ml kg^{-1} h^{-1} where p_{tot} (Pa) is the total pressure in the system; for $r_{C_2H_4}$ at T (°C), 1 mol kg^{-1} s^{-1} = (T + 273.15) / 3.341 x 10^{-14} p_{tot}, µl kg^{-1} h^{-1} (Banks *et al.*, 1995)].

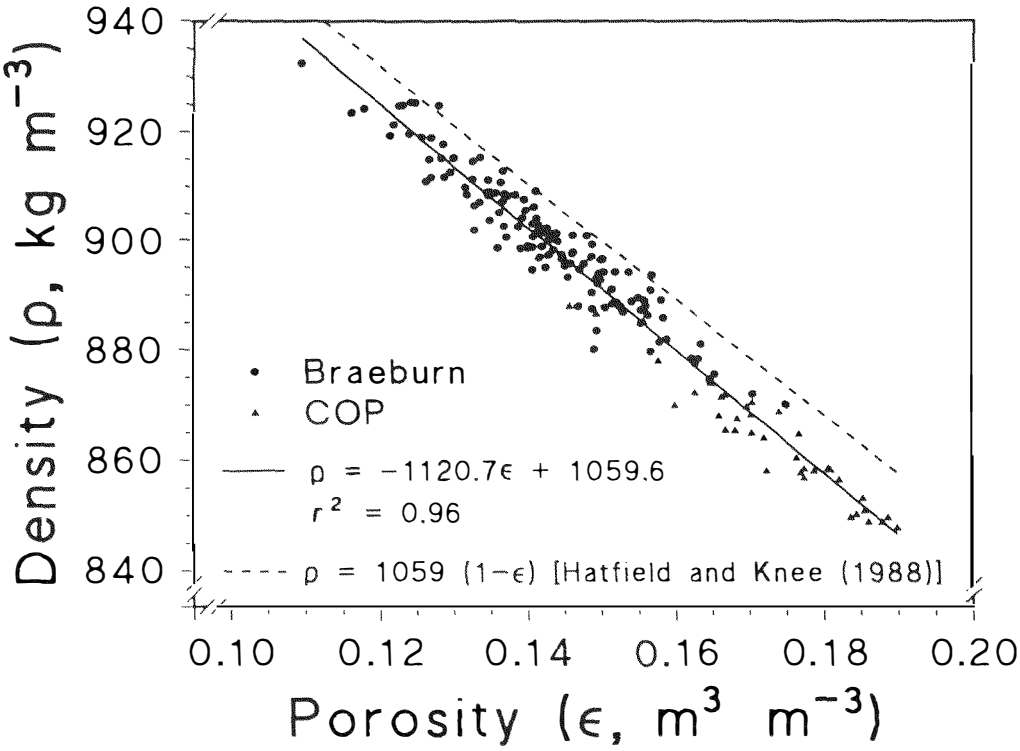

Figure 5.1 Cortical tissue density (ρ) as a function of porosity (ε) for 'Cox's Orange Pippin' and 'Braeburn' apples from a Hawkes Bay orchard stored at 0°C and 2°C respectively for periods up to 24 weeks, and held at 20°C for 24 h before estimation.

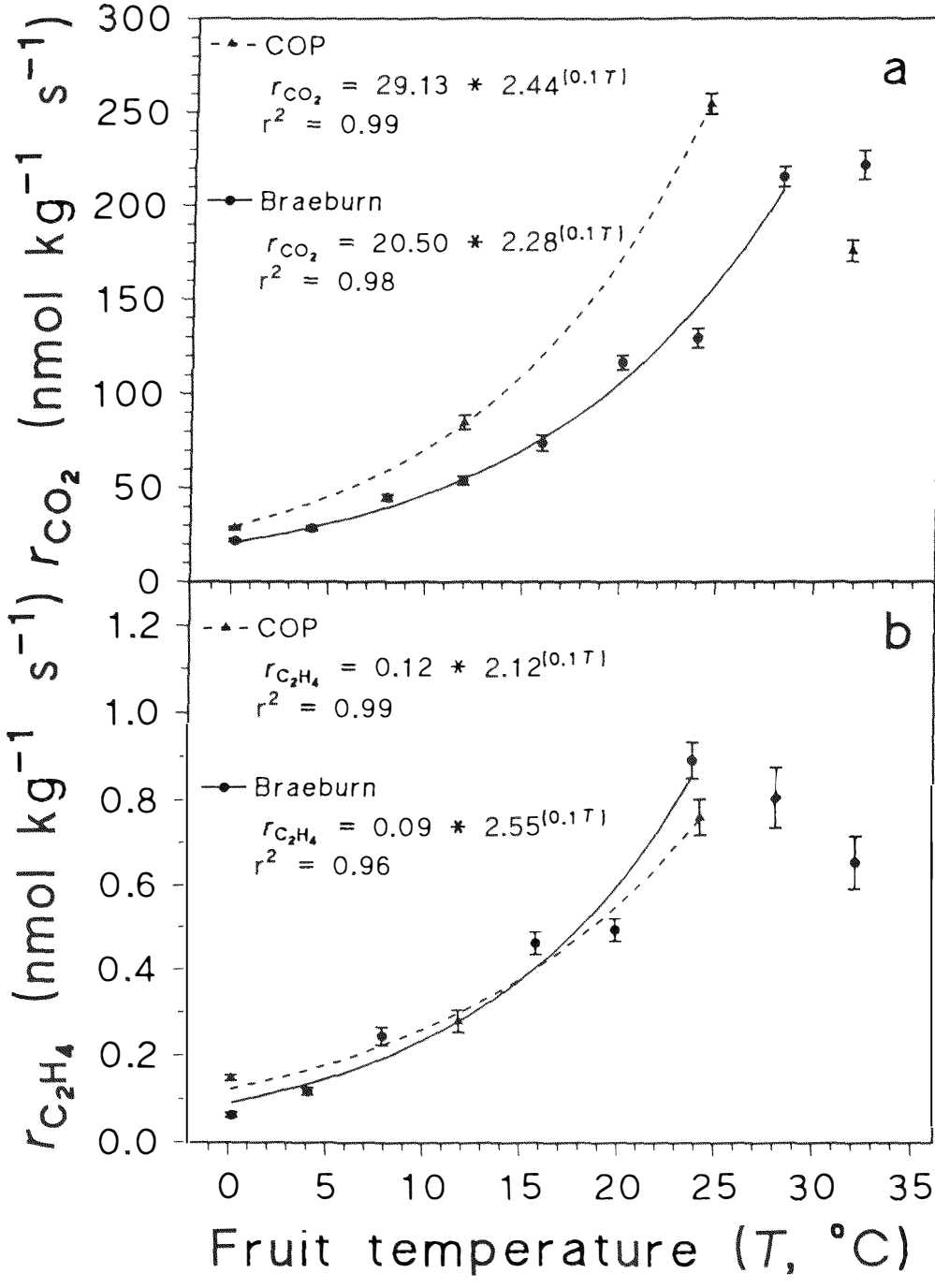

Figure 5.2 Respiration rate (r_{CO_2}) [a] and ethylene production ($r_{C_2H_4}$) [b] as a function of fruit temperature for 'Cox's Orange Pippin' and 'Braeburn' apples from a Hawkes Bay orchard stored at 0°C and 2°C respectively for periods up to 24 weeks. Fruit were removed from cool storage and equilibrated at the treatment temperature for 24 to 48 h before assessment. Estimates represent least squares means and standard error bars (n=15).

5.4.5 Verification of steady-state model 1 predictions

For both cultivars at 0°C, model 1 (Eq. 5.6) predictions of $p_{O_2}^e$ for a given $p_{O_2}^i$ were close to empirical data (Fig. 5.3). However, at 24°C model 1 underestimated $p_{O_2}^e$, particularly for 'Braeburn' apples.

For a given $p_{O_2}^i$, model 2 (Eq. 5.8) predicted $p_{O_2}^e$ increased proportionally with increasing $r_{O_2}^{'max,T}$ and decreasing $P_{O_2}^{'}$ (data not shown). For fruit at 0°C, model 2 predictions were close to empirical data for 'COP' and slightly overestimated $p_{O_2}^e$ for 'Braeburn' fruit for $p_{O_2}^i \geq 3$ kPa. For fruit at 24°C, model 2 underestimated $p_{O_2}^e$ for both cultivars for $p_{O_2}^i \leq 1.5$ kPa, but overestimated $p_{O_2}^e$ for $p_{O_2}^i \geq 1.5$ kPa for 'COP' and between 1.5 and 12.0 kPa for 'Braeburn' fruit.

5.4.6 LOL^is as a function of temperature

Bootstrap means were similar to means of original panellists' data (data not shown). However, as bias corrected 95% confidence intervals could be calculated for bootstrap means of LOL^is, the latter were used for regression analyses. As the bootstrap procedure did not assume data to be normally distributed, BCa 95% confidence intervals were not necessarily symmetrical about the means (Figs. 5.4 to 5.7). As there were insufficient data to perform valid regressions for 'COP' fruit, only results of regressions for 'Braeburn' fruit are presented. Regressions were weighted using the inverse of the standard error as calculated by the bootstrap procedure, and the influence of temperature was adjusted statistically for the time fruit had been in cool storage. Polynomial effects of temperature up to the third power were tested for data from temperature treatments up to 28°C.

Chamber and core cavity atmospheres were similar but not identical; neither was consistently greater than the other (data not presented). Consequently, LOL^is were similar when estimated using chamber and core atmospheres, except at temperatures ≥ 24°C where LOL^is based on core atmosphere tended to be higher. However, for 'Braeburn' apples at treatment temperatures ≤ 18°C, LOL^is based on core atmospheres were lower than or equal to those based on chamber atmospheres.

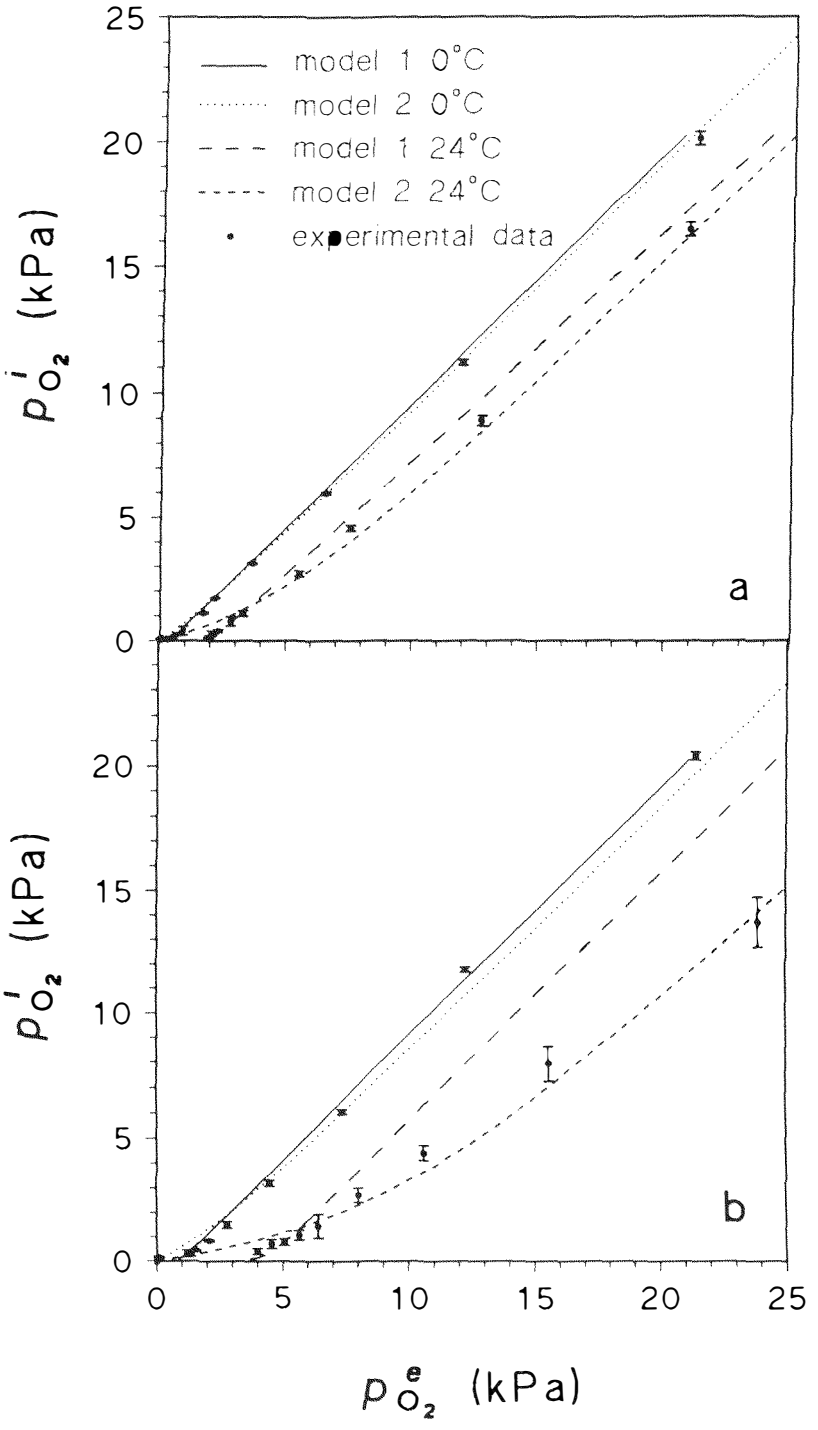

Figure 5.3 Steady-state model 1 (Eq. 5.6) and model 2 (Eq. 5.8) predictions of $p^e_{O_2}$ calculated from $p^i_{O_2}$ and experimental data for (a) 'Cox's Orange Pippin' and (b) 'Braeburn' apples at 0 and 24°C. Values for fruit and gas variables used in model 1 are presented in section 5.3.3 and for model 2 in Table 5.3. Experimental data represent means and standard error bars (n = 6).

Table 5.3 Values for fruit and gas variables used for model 2 predictions of $p_{O_2}^e$ as a function of $p_{O_2}^i$ using Eq. 5.8 for 'Cox's Orange Pippin' ('COP') and 'Braeburn' apples.

Variables	Units	'COP'	'Braeburn'
A	m^2	1.50×10^{-2}	1.51×10^{-2}
M	kg	1.45×10^{-1}	1.46×10^{-1}
k_1	Pa	2.03×10^{3}	2.33×10^{3}
$P_{O_2}^i$	mol s^{-1} m^{-1} Pa^{-1}	2.90×10^{-10}	1.10×10^{-10}
$r_{O_2}^{i\,max\,0}$	mol s^{-1}	3.50×10^{-9}	3.20×10^{-9}
Q_{10}		2.20	2.10

5.4.6.1 ACP^i and ACP^e as a function of temperature

Bootstrap estimates of ACP^i based on chamber atmospheres (chamber ACP^i) and core atmospheres (core ACP^i) were on average 0.24 kPa and 0.83 kPa $p_{O_2}^i$ higher for 'Braeburn' than 'COP' apples respectively.

For 'COP' apples, chamber ACP^i increased from 0.54 kPa O_2 at 0°C to 0.69 kPa $p_{O_2}^i$ at 24°C, then rose markedly to 1.14 kPa at 32°C (Fig. 5.4). Marginally higher values were found for core ACP^i at 24° and 32°C (0.80 and 1.3 kPa $p_{O_2}^i$ respectively, Fig. 5.5). Concentrations of dissolved O_2 in water (c_{O_2,H_2O}^i) calculated from $p_{O_2}^i$ using Eq. 5.9 were similar at 0° and 12°C, lower at 24°C and highest at 32°C, for both chamber and core ACP^i (Figs. 5.4 and 5.5).

For 'Braeburn' apples, there was no significant relationship between chamber ACP^i and temperature based on $p_{O_2}^i$ between 0° and 28°C (Fig. 5.6). However, a marked increase in chamber and core ACP^i was observed at 32°C. A cubic relationship was found between core ACP^i based on $p_{O_2}^i$ and temperature ($r^2 = 0.93$, Fig. 5.7), and also for chamber and core ACP^i based on c_{O_2,H_2O}^i and temperature ($r^2 =$ 0.60 and 0.89, Figs. 5.6 and 5.7 respectively). With the exception of 8°C, chamber

ACP^{i} based on $p^{i}_{O_2}$ increased from 0.85 kPa to 1.03 kPa $p^{i}_{O_2}$ then remained at marginally below this value up to 28°C before rising sharply to 1.63 kPa $p^{i}_{O_2}$ at 32°C. Chamber ACP^{i} based on $c^{i}_{O_2,H_2O}$ was approximately 18.0 to 19.0 mmol m^{-3} between 0° and 4°C, then decreased irregularly to 11.9 mmol m^{-3} as temperature increased to 28°C, rising sharply again to 18.4 mmol m^{-3} at 32°C (Fig. 5.6). Core ACP^{i} based on $p^{i}_{O_2}$ decreased from 0.84 kPa to 0.64 kPa O_2 between 0 and 8°C, then rose to 1.11 kPa at 28°C, and markedly to 2.34 kPa at 32°C (Fig. 5.7). Core ACP^{i} based on $c^{i}_{O_2,H_2O}$ decreased steadily from 17.9 to 13.6 mmol m^{-3} between 0° and 28°C (with the exception of 24°C), then increased at 32°C to 26.5 mmol m^{-3}.

Values of ACP^{e} calculated from bootstrap estimates of ACP^{i} were greater than ACP^{i}, increasing more markedly as temperature increased, and particularly at low $P^{'}_{O_2}$ (Fig. 5.8). A power law function was fitted to each curve. For ACP^{i}, $p^{i}_{O_2} = 7.05 \times 10^{-7} \times 9.71^{(0.1T)} + 0.83$ ($r^2 = 0.92$). For ACP^{e} for a fruit with low $P^{'}_{O_2}$, $p^{e}_{O_2} = 0.15 \times 4.08^{(0.1T)} + 2.04$ ($r^2 = 0.99$), and for a high $P^{'}_{O_2}$, $p^{e}_{O_2} = 0.10 \times 3.30^{(0.1T)} + 1.12$ ($r^2 = 0.98$).

5.4.6.2 FT^{i}_{RQ} as a function of temperature

Bootstrap estimates of chamber FT^{i}_{RQ} were on average 0.28 kPa O_2 higher for 'Braeburn' than 'COP' apples, but similar for core FT^{i}_{RQ}.

For 'COP' fruit, chamber and core FT^{i}_{RQ} were between 0.3 and 1.0 kPa O_2 (between 2.5 and 22.4 mmol m^{-3}) higher than equivalent ACP^{i} (Figs. 5.4 and 5.5). Chamber FT^{i}_{RQ} increased between 0.81 and 1.36 kPa O_2 and core FT^{i}_{RQ} between 1.55 and 2.00 kPa O_2 with increasing temperature between 0° and 32°C. Chamber $c^{i}_{O_2,H_2O}$ increased from 17.4 to 20.2 mmol m^{-3} between 0° and 12°C, then decreased to 15.1 mmol m^{-3} at 24°C and 15.4 mmol m^{-3} at 32°C (Fig. 5.4). Core $c^{i}_{O_2,H_2O}$ decreased markedly from 33.0 to 20.0 mmol m^{-3} between 0° and 24°C, then increased again to 23.4 mmol m^{-3} at 32°C (Fig. 5.5).

For 'Braeburn' apples, a quadratic relationship was found between chamber FT^{i}_{RQ} and temperature based on $p^{i}_{O_2}$ ($r^2 = 0.56$, Fig. 5.6), and a cubic relationship for core

FT^i_{RQ} ($r^2 = 0.93$, Fig. 5.7). No significant relationship between chamber or core FT^i_{RQ} and temperature based on $c^i_{O_2\text{-}H_2O}$ was found. Chamber FT^i_{RQ} based on $p^i_{O_2}$ followed a similar trend with increasing temperature as ACP^i, but with chamber values 0.14 to 0.76 kPa O_2 higher (Fig. 5.6). Results for core FT^i_{RQ} as a function of temperature were variable. However, there was a general trend for FT^i_{RQ} to increase as temperature increased to 24°C above which it declined (Fig. 5.7).

5.4.6.3 Chamber FT^i_{EtOH} as a function of temperature

For both cultivars, c^i_{Acet} and c^i_{EtAc} accumulation was highly variable (data not presented). For 'COP' fruit, ethanol accumulation was measured for some treatments, but there was insufficient data to indicate a relationship with temperature (Fig. 5.4). For 'Braeburn' fruit, FT^i_{EtOH} increased from 1.17 to 2.08 kPa O_2 as fruit temperature increased from 0 to 32°C, in a quadratic relationship ($r^2 = 0.73$, Fig. 5.5). However, the results for ethanol accumulation between temperature treatments varied considerably.

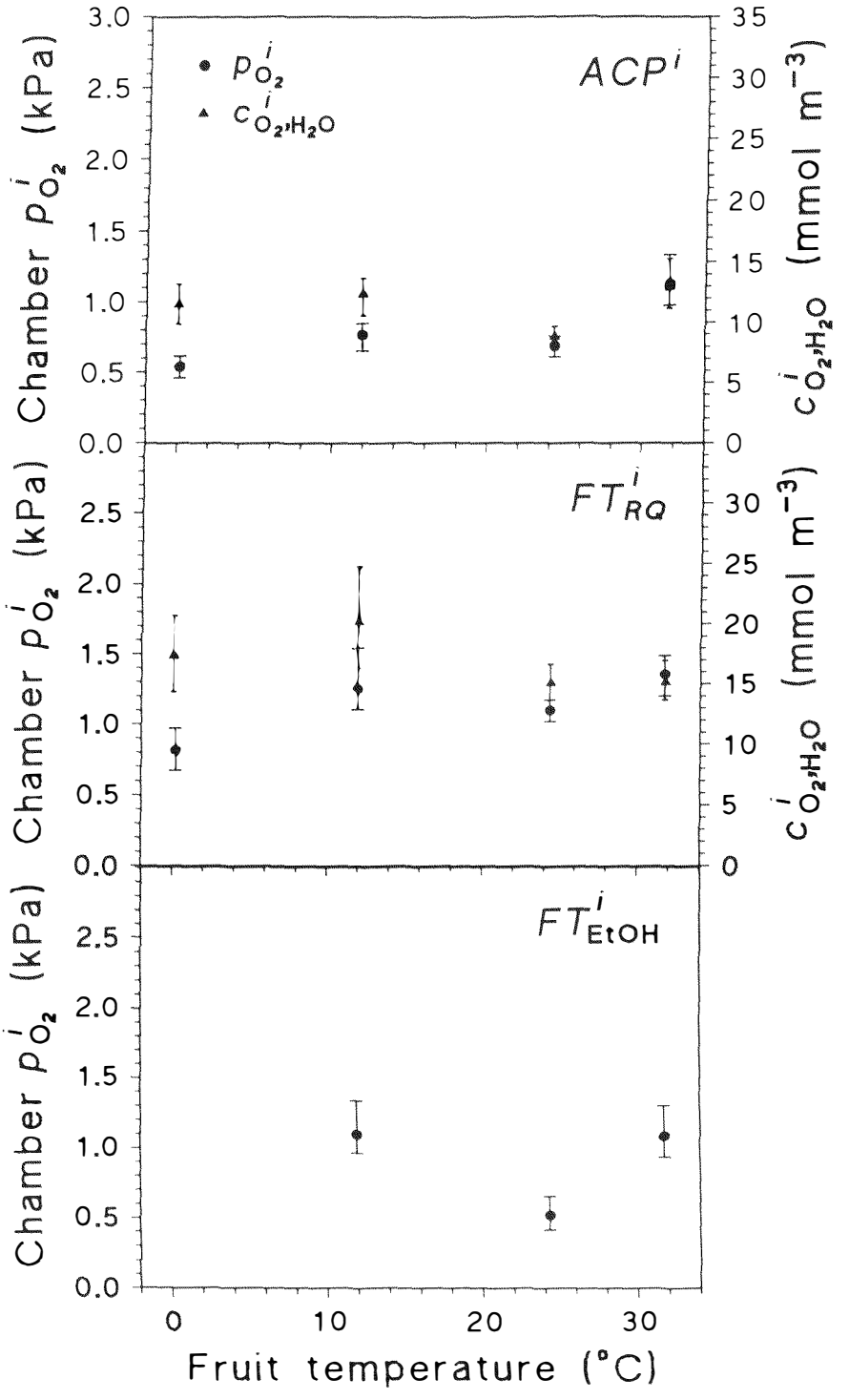

Figure 5.4 Bootstrap estimates of steady-state internal lower oxygen limits (ACP^i, FT^i_{RQ}, and FT^i_{EtOH}) based on partial pressures of surface chambers and equivalent dissolved oxygen concentrations for 'Cox's Orange Pippin' apples as a function of fruit temperature. Values represent bootstrap means and 95% bias corrected (BCa type) confidence intervals, b = 1000).

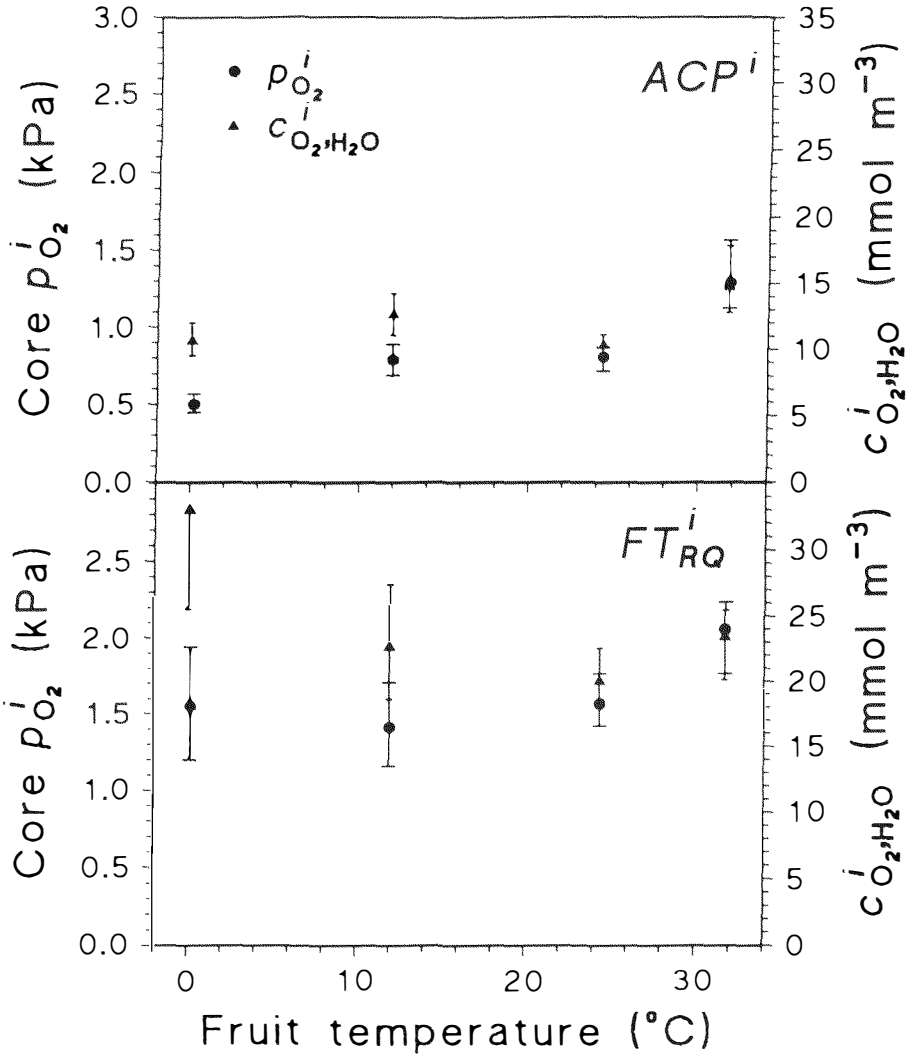

Figure 5.5 Bootstrap estimates of steady-state internal lower oxygen limits (ACP^{i} and FT^{i}_{RQ}) based on partial pressures in the core cavity and equivalent dissolved oxygen concentrations for 'Cox's Orange Pippin' apples as a function of fruit temperature. Values represent bootstrap means and 95% bias corrected (BCa type) confidence intervals, b = 1000).

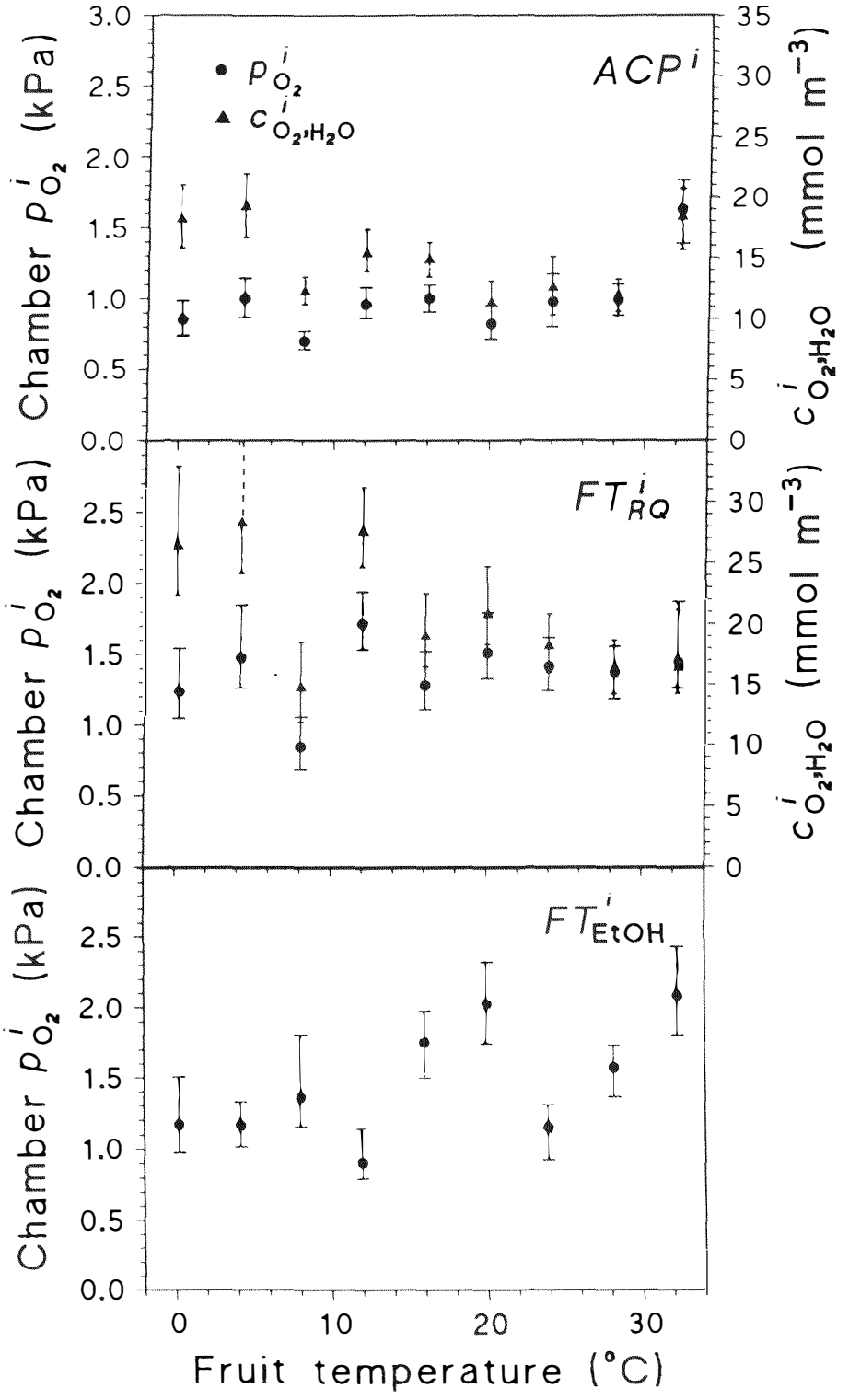

Figure 5.6 Bootstrap estimates of steady-state internal lower oxygen limits (ACP^i, FT^i_{RQ}, and FT^i_{EtOH}) based on partial pressures in surface chambers and dissolved oxygen concentrations for 'Braeburn' apples as a function of fruit temperature. Values represent bootstrap means and 95% bias corrected (BCa type) confidence intervals, b = 1000).

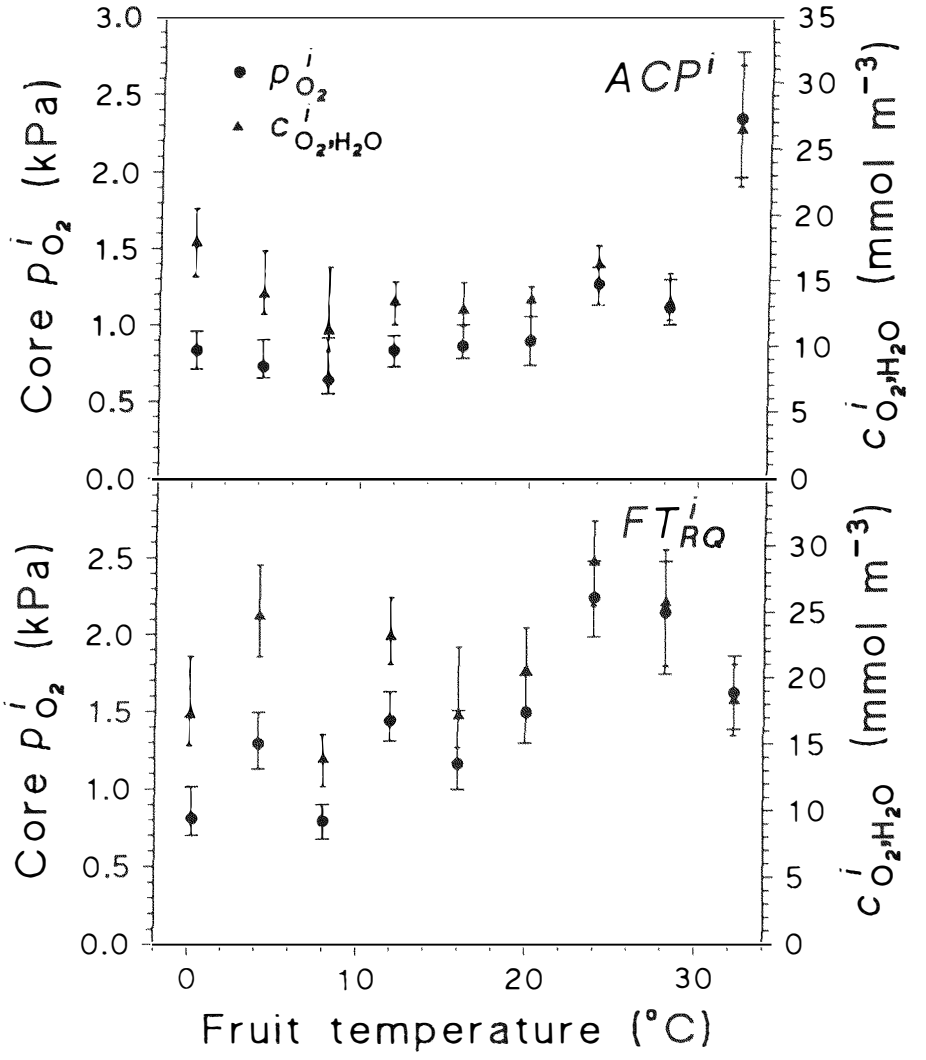

Figure 5.7 Bootstrap estimates of steady-state internal lower oxygen limits (ACP^i and FT^i_{RQ}) based on partial pressures in the core cavity and equivalent dissolved oxygen concentrations for 'Braeburn' apples as a function of fruit temperature. Values represent bootstrap means and 95% bias corrected (BCa type) confidence intervals, b = 1000).

5.5 Discussion

Model 2 predictions of $p^e_{O_2}$ required to achieve particular levels of $p^i_{O_2}$ (Eq. 5.8) accounted for the effect of both temperature and $p^i_{O_2}$ on respiration, whereas model 1 only accounted for the effect of temperature (Eq. 5.6). Consequently, model 2 predictions were closer to experimental values than those of model 1, particularly for apples at 24°C. Discrepancies between empirical data and model 2 predictions still occurred, and suggested the physiological processes involved were probably more complex than that described by the single rate constant (k_1). A number of reports suggest more than one oxidase directly or indirectly affects respiration (Burton, 1982; Knee, 1991; Tucker and Laties, 1985). Regulatory enzyme(s) with lower affinity for O_2 may sense hypoxic conditions and lower respiration by feedback inhibition of the initial steps of glucose oxidation (Solomos, 1994). Some improvement in the fit of model 2 to experimental data (data not shown) was achieved by including a second rate constant (k_2) using the following equation:

$$p^e_{O_2} = \left(p^i_{O_2} + k_1 + \left(\frac{r^{max,T}_{O_2}}{P^{'}_{O_2} A} \right) \right) \left(\left(a \left(\frac{p^i_{O_2}}{k_1 + p^i_{O_2}} \right) \right) + \left((1-a) \left(\frac{p^i_{O_2}}{k_2 + p^i_{O_2}} \right) \right) \right) \quad \textbf{(5.12)}$$

where: a = constant describing the proportion of total O_2 uptake governed by k_1 and k_2

However, improved fit was only achieved by arbitrarily setting values for k_1, k_2 and a as there were no empirical data to suggest what these values should be. More experimental evidence is required before the increased complexity caused by adding a second rate constant to model 2 can be justified.

Bootstrap estimates (means) of LOL^is varied in a complex manner in relation to temperature. Although statistically significant effects were found for FT^i_{RQ} and FT^i_{EtOH} based on chamber and core partial pressures for 'Braeburn' apples, the relationships are difficult to explain physiologically. In general for both 'COP' and 'Braeburn' apples ACP^is and FT^i_{RQ} s based on chamber atmospheres did not increase significantly as temperature increased between 0° and 28°C, but increased noticeably

at 32°C. There was more evidence for an increase in ACP^is and FT^i_{RQ}s with temperature when they were estimated using core cavity data. The variation in estimates at 32°C may have resulted from inhibition of enzymes at this temperature as indicated by the suppression of r_{CO_2} and $r_{C_2H_4}$ at this temperature (Fig. 5.2).

It was anticipated LOL^is might change as a function of temperature in a complex way resulting from the effects of temperature on diffusion of O_2 through the intercellular air space, solubility of O_2 in water in the interstices of the cell wall, and diffusivity through the cell wall, plasmalemma, symplasm, and mitochondrial membranes and matrix. Once O_2 has permeated through the pores in the skin, it can diffuse through the fruit via intercellular air spaces and/or in the fluid/solid phase of the cellular matrix both in parallel and in series (Rajapakse *et al.*, 1990). If diffusion of O_2 was severely restricted within fruit by low tissue porosity or poor continuity of intercellular air spaces, or if a higher respiration rate in some areas raised the O_2 requirement of tissue, we might expect an increase in LOL^is for cultivars with lower porosity.

In this study, cortical tissue porosity was greater and tissue density lower in 'COP' than in 'Braeburn' apples, though the relationships between density and porosity were similar, and these cultivar differences may have resulted in higher LOL^is estimated for 'Braeburn' fruit. However, lower respiration rate and Q_{10} of 'Braeburn' compared to 'COP' fruit, would tend to have attenuated the effects of lower tissue porosity of 'Braeburn' on LOL^is.

Porosity can also be estimated using the density of apple juice (Hatfield and Knee, 1988):

$$\rho^{20}_{fruit} = \rho^{20}_{juice} \ (1 - \varepsilon) \qquad \textbf{(5.13)}$$

where:

ρ^{20}_{juice} = density of fruit juice at 20°C (kg m^{-3})

ρ^{20}_{fruit} = density of fruit at 20°C (kg m^{-3}).

For a juice density of 1059 kg m^{-3} (Hatfield and Knee, 1988), the slope of the relationship between density and porosity was similar, but values of fruit density higher, than data from the current study (Fig. 5.1). The difference between the slopes probably represents a difference in juice density. Values of juice density between 1040 and 1055 kg m^{-3} have been obtained for New Zealand 'Braeburn' apples (R. Harker, unpublished data), and using a juice density of 1040 kg m^{-3} resulted in the same slope as the line fitted to the experimental data. Differences in porosity and interconnectivity of intercellular air spaces have been observed in different tissue zones of apples (Soudain and Phan Phuc, 1979), and these may result in heterogeneity of IAs (Dadzie *et al.*, 1993). As these factors can also vary with stage of fruit ripeness and the degree to which the intercellular air spaces are filled with cell sap from breakdown of cell walls and membranes (Burton, 1982, Kader *et al.*, 1989; Rajapakse *et al.*, 1990), LOL^ts may vary slightly between fruit in a population or for an individual fruit as it ages.

The magnitude of the effect of the cell wall and plasmalemma on O_2 is difficult to estimate due to variation in cell size and geometry and the area of cell wall exposed to intercellular air spaces (Solomos, 1987). The boundary layer of the cell wall exposed to the intercellular air space would be greater in thickness than that of the plasmalemma where cytoplasmic streaming causes mechanical mixing in the cytosol. However, given diffusion of O_2 in undisturbed cytosol is likely to be 10^4 lower than in the intercellular air space (Nobel, 1991), the effect of the cell wall boundary layer is a less significant barrier. Diffusion over the relatively small distances from cell wall to mitochondrion are assumed non-limiting, and would increase as the kinetic energy of dissolved O_2 molecules increased with increasing temperature.

In contrast to the effect of temperature on diffusivity, solubility of O_2 in the cell sap decreases markedly with increasing temperature. Consequently, if solubility primarily limited the availability of O_2 to the terminal oxidase, we would expect LOL^ts to increase as a function of temperature such that $c^t_{O_2,H_2O}$ at the LOL^t would either remain constant or increase with increasing temperature. However, Figs. 5.4 to 5.7 indicate $c^t_{O_2,H_2O}$ equivalent to partial pressure estimates of ACP^t and FT^t_{RQ} tended to decrease with increasing temperature for 'Braeburn' apples or were similar for 'COP', with an exception at 32°C. Solubility of O_2 also decreases with

increasing soluble solids content of the cell sap, which may further reduce diffusivity of O_2 as fruits ripen (Leonard, 1939), but neither of these factors influencing solubility appear to explain the changes in LOL^is observed in this study.

Temperature may also influence LOL^is through altering cytoplasmic pH and by the effect of pH on rate constants of oxidative metabolism (Gibson, 1953). Knee (1980) speculated that higher temperatures may lower the affinity of cytochrome oxidase for O_2. If this were correct, the effect would be to raise LOL^is at higher temperatures. The influence of these factors on the results of the current study are difficult to estimate.

It was anticipated LOL^is might not change as a function of temperature to the same extent as LOL^es have been reported to, as LOL^is are independent of skin permeance and the resulting Δp_{O_2} between internal and external atmospheres. Dadzie *et al.* (1993) demonstrated that for a fixed ACP^i, ACP^e was linearly related to r^{max} at fixed temperature, but showed a strong curvilinear dependence on temperature. The magnitude of the response of ACP^e to temperature was inversely proportional to P'_{O_2}. The current study indicated ACP^i of 'Braeburn' apples showed little increase with temperature, only increasing very slightly above 20°C (Fig. 5.8). By contrast, calculated values of ACP^e showed a marked increase with temperature due to the power law relationship between r^{max} and temperature (Figs. 5.2 and 5.8). The Δp_{O_2} between ACP^i and ACP^e was greater for fruit with lower P'_{O_2}. Beaudry *et al.* (1992) suggested the marked increase in RQB (estimated using $p^e_{O_2}$) for blueberries, resulted from a larger increase in rate of O_2 uptake compared to diffusion of O_2 through pores in the skin [which Cameron *et al.* (1995) noted is relatively temperature independent]. As diffusion of O_2 in through the skin of apples is likely to occur through pores, the same mechanism would explain the divergence in values of LOL^es from LOL^is with increasing temperature, and would be accentuated in apples with low P'_{O_2}.

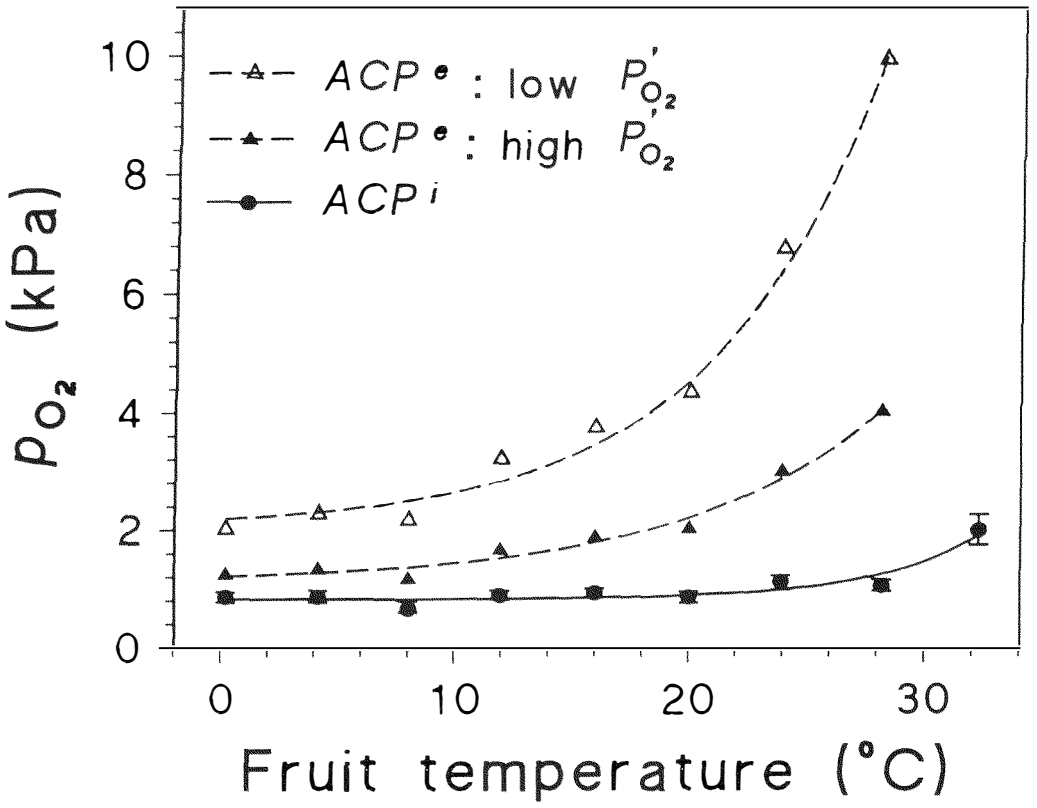

Figure 5.8 Bootstrap estimates of steady-state internal and external lower oxygen limits (ACP^i and ACP^e) of 'Braeburn' apples as a function of fruit temperature, based on combined chamber and core partial pressure data. Values of ACP^i represent bootstrap means and 95% bias corrected (BCa type) confidence intervals (b = 1000), and ACP^e for a fruit with low and high fruit permeance to oxygen (P'_{O_2}) calculated from ACP^i using Eq. 5.1.

The strong dependence of LOL^es on both temperature and P'_{O_2}, has important consequences for the design of MAP systems (Banks *et al.*, 1993). Although not particularly variable in the current study, natural variation in P'_{O_2} of apples both within and between cultivars, can result in a large variation in Δp_{O_2} between internal and external atmospheres (Dadzie, 1992). For apples in CA at storage temperatures, r^{max} is minimised such that the effect of variation in P'_{O_2} on Δp_{O_2} would also be minimised. The optimum storage atmosphere (based on external atmospheres) for a population of apples that avoids anaerobiosis would be just above the FT^e_{RQ} of the

fruit with the highest P'_{O_2}. This would also be true for MAPs under isothermal cool storage conditions. However, there is a likelihood that MAPs may experience periods of exposure to higher than storage atmospheres during distribution and retailing of the crop. During prolonged exposure, fruit in the population with low P'_{O_2} and/or higher r_{CO_2} may accumulate fermentation products. This may be accentuated by differential effects of elevated temperature on P'_{O_2} and skin permeance to CO_2 (P'_{CO_2}) that may not be accounted for if tolerance limits are only determined at cold storage temperatures. If anaerobic conditions are experienced for very short periods the fruit may be able to metabolise fermentation products (Knee, 1991). For MAPs that could experience higher temperatures for significant periods while sealed, the optimum package atmosphere would be dictated by the highest temperature the MAP will be exposed to. Ideally, MAPs may use different permeability characteristics for the same crop going to different markets. Optimising atmospheres for MAPs is therefore more complex than for CA.

The strong dependence of ACP^e on both P'_{O_2}, and the relationship between r_{O_2} and temperature, lessens the usefulness of ACP^e for optimising storage atmospheres, and illustrates the need to consider the response of individual fruit to $p^e_{O_2}$ rather than the population average. By contrast, LOL^is are independent of the effects of P'_{O_2}, but may be influenced by variation in the diffusivity of gases in the cortex and the effect of temperature on solubility and intracellular processes.

In conclusion, estimates of LOL^is varied as a function of temperature in a complex manner but suggested LOL^is were not greatly influenced by temperature except at temperatures > 28°C. Given the relationships were not consistent, there was little justification for altering steady-state model 1 to account for the effect of temperature on LOL^is. For both cultivars, FT^i_{RQ} tended to be higher than ACP^i, and more accurately indicated the onset of fermentation (Yearsley *et al.*, 1996a). Higher values of LOL^is for 'Braeburn' apples compared to 'COP', may have reflected the lower cortical tissue porosity of 'Braeburn'. Despite differences in cortical tissue density and porosity, ACP^i and FT^i_{RQ} were similar within cultivars based on surface chamber and core atmospheres. Other studies indicate that even if diffusion of O_2 through the cortex is not limiting, temperature effects on LOL^is may still arise from the complex interaction of processes at the cellular level. Increasing temperature increases O_2

uptake, may raise rate constants of oxidases, decreases solubility, but also increases diffusivity coefficients of O_2 between the cell wall and mitochondria. In contrast to LOL^is, LOL^es increased markedly with temperature, particularly for fruit with low skin permeance. Therefore, identifying LOL^is of apples is valuable for predicting minimum safe storage atmospheres due to their consistency compared to LOL^es, which are strongly influenced by respiration rate and skin permeance. Other factors such as physiological age and susceptibility to disorders may interact with the effect of temperature in long term storage, and knowledge of these is required to qualify LOL^is to optimise fruit quality during CA/MA storage.

5.6 Acknowledgements

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Chapter 6

Effect of Elevated Carbon Dioxide on the Lower Internal Oxygen Limits of Apple Fruit

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6.1 Abstract

The effect of elevated CO_2 between 0 and 8 kPa on steady-state lower internal O_2 limits (LOL^i) was estimated for postclimacteric 'Cox's Orange Pippin' ('COP') and 'Braeburn' apples at 0° and 20°C. Three LOL^is were estimated from surface chamber atmospheres: the anaerobic compensation point (ACP^i), the internal fermentation threshold based on the respiratory quotient (FT^i_{RQ}), and the fermentation threshold based on ethanol (EtOH) accumulation (FT^i_{EtOH}). ACP^i for both cultivars and temperatures, remained constant at 0.5 kPa for 'COP' and 0.8 to 1.0 for 'Braeburn' apples for $p^e_{CO_2}$ between 0 and 8 kPa. Variation in FT^i_{RQ} and FT^i_{EtOH} with $p^e_{CO_2}$ was more complex than for ACP^i with both small increases and decreases with increasing $p^e_{CO_2}$ but no consistent trend evident at 20°C for either cultivar. In contrast, at 0°C FT^i_{RQ} and FT^i_{EtOH} were 0.2 to 0.8 kPa $p^i_{O_2}$ higher at 8 kPa than at 0 kPa $p^e_{CO_2}$ (with the exception of FT^i_{RQ} for 'COP'). A small decrease in O_2 uptake (estimated from the difference in external and internal O_2 atmospheres) was observed at $p^e_{CO_2}$ between 2 and 8 kPa at 20°C. Elevated $p^e_{CO_2}$ slightly lowered the respiratory quotient (RQ_{ia}, estimated from the ratio of differences between external and internal atmosphere levels of p_{CO_2} and p_{O_2}) of 'COP' in 8 kPa CO_2 and 'Braeburn' in 2 to 8 kPa CO_2 at 20°C, and more markedly in 8 kPa CO_2 at 0°C. The RQ_{ia} of 'COP' and 'Braeburn' apples was slightly and markedly higher respectively at 0° compared to 20°C. The lower RQ_{ia} of 'Braeburn' at 20°C compared to that of 'COP' indicated a higher contribution of cuticular diffusion of CO_2 compared to that

through pores for 'Braeburn' apples. This study indicates the tolerance of 'COP' and 'Braeburn' apples to low O_2 levels may be affected by levels of CO_2. This may be an important consideration when optimising storage atmospheres, particularly for apples with low skin permeance such as 'Braeburn' in which relatively high $p^i_{CO_2}$ may accumulate and which are susceptible to CO_2-related disorders. At low storage temperatures, increased solubility of CO_2 may lower the tolerance of apples with high $p^i_{CO_2}$ exposed to low O_2 atmospheres. Alternatively, for apples in modified atmosphere packages exposed to higher than normal storage temperatures, increased respiration may elevate $p^i_{CO_2}$ to levels that reduce tolerance to diminished package O_2.

Keywords: Malus domestica; Internal atmosphere; Anaerobic compensation point; Fermentation threshold; RQ breakpoint; Skin permeance; Ethane efflux; Respiration rate; Ethylene production rate; Bootstrap

6.2 Introduction

Substantial benefits in maintaining postharvest quality of fresh fruits and vegetables can accrue from storage in low O_2 and/or elevated CO_2 atmospheres at levels within the fruit's tolerance limit, as an adjunct to reducing crop temperature (Kader *et al.*, 1989). Tolerance of crops to modified atmospheres can vary markedly depending on cultivar, strain, physiological age, initial quality, temperature, rate of establishment of atmospheres and duration of exposure. In general, under severe stress, fermentative metabolism is enhanced by low external partial pressure of O_2 ($p^e_{O_2}$, Pa) and elevated external partial pressure of CO_2 ($p^e_{CO_2}$, Pa), long exposure to CA and more advanced developmental stages (Ke *et al.*, 1993). Effects of modification of individual atmosphere components interact: tolerance to elevated $p^e_{CO_2}$ decreases as $p^e_{O_2}$ is decreased whilst tolerance to low $p^e_{O_2}$ decreases as $p^e_{CO_2}$ is increased (Beaudry and Gran, 1993; Kader *et al.*, 1989).

Lower O_2 limits (*LOL*s) of crops are typically reported in relation to external or package atmospheres (LOL^e). However, those based on internal atmospheres (LOL^i), are likely to more accurately estimate the true *LOL* as they account for variation in

respiration rate (r_{CO_2}, mol kg^{-1} s^{-1}) and skin permeance to O_2 and CO_2 (P'_{O_2}, P'_{CO_2}, mol s^{-1} m^{-2} Pa^{-1}) of individual fruit (Yearsley *et al.*, 1996a). Two types of LOL^i can be described (Yearsley *et al.*, 1996a): the internal anaerobic compensation point [ACP^i; or internal O_2 partial pressure ($p^i_{O_2}$, Pa) at which internal CO_2 partial pressure ($p^i_{CO_2}$) from aerobic and anaerobic respiration is minimal], and the internal fermentation threshold below which anaerobic respiration is initiated [FT^i]. This can be established as the $p^i_{O_2}$ at which the respiratory quotient based on internal atmospheres (RQ_{ia}) rises, say, 10% above the asymptotic value obtained at higher $p^i_{O_2}$, or, alternatively, the $p^i_{O_2}$ below which ethanol accumulation increases marking the initiation of fermentation. Optimum internal atmosphere (IA) composition lies just above the internal FT^i (Banks *et al.*, 1993a).

The effects of temperature on LOL^es have been reported for apples (Gran and Beaudry, 1993a and 1993b), blueberries (Beaudry, 1993; Beaudry *et al.*, 1992; Cameron *et al.*, 1994), and raspberries (Joles *et al.*, 1994), and these show strong curvilinear dependence on temperature. In contrast, Yearsley *et al.* (1996c) showed that LOL^is of apples only increased marginally with temperature, and the effects were not always consistent.

The direct and/or indirect suppression of aerobic respiration by $p^e_{O_2}$ has been widely reported, and the effects of $p^e_{O_2}$ on the enzymatic rate of O_2 uptake (r_{O_2}) have been modelled using the Michaelis-Menten equation (Cameron *et al.*, 1995; Dadzie *et al.*, 1993; Peppelenbos *et al.*, 1993). The effects of elevated $p^e_{CO_2}$ on O_2 uptake (and its effects on LOL^es) have often been studied but the resulting evidence has been contradictory, with no effect, an increase or a decrease in respiratory activity reported depending on the crop and the partial pressure of CO_2 (Cameron *et al.*, 1995; Kerbel *et al.*, 1988; Kidd, 1916; Kidd and West, 1927, 1933; Kubo *et al.*, 1990; Li and Kader, 1989; Thornton, 1933; Young *et al.*, 1962). Evidence that elevated CO_2 has no or little effect in reducing r_{CO_2} or r_{O_2} has been reported for bananas by Young *et al.* (1962), and for mushrooms by Peppelenbos *et al.* (1993). Joles *et al.* (1994) reported that $p^e_{CO_2} < 17$ kPa did not affect r_{O_2} or the LOL^e for raspberries, and Beaudry (1993) that $p^e_{CO_2} > 20$ kPa resulted in only a small reduction in r_{O_2} of blueberries. Gran (1993) investigated the effect of temperature on the LOL^es using modified-atmosphere packages and found that 12 to 15 kPa CO_2 in package

headspace increased r_{O_2} compared to packages where CO_2 had been diminished by a CO_2 absorber, but the effect on LOL^es was not discussed.

There are no reports of the effect of $p^e_{CO_2}$ on LOL^is, and this is the principal subject of this study of postclimacteric 'Cox's Orange Pippin' and 'Braeburn' apples and using a controlled-atmosphere (CA) method (Yearsley *et al.*, 1996a). In addition, a relative estimate of r_{O_2} for a fruit at steady-state in CA can be determined from the difference between the external and internal partial pressures of O_2 (Δp_{O_2}, Pa). Using Fick's First Law of diffusion, for a fruit of given mass (M, kg) and surface area (A, m^2), Δp_{O_2} is proportional to the rate of transfer of O_2 (r_{O_2}, mol kg^{-1} s^{-1}) and inversely proportional to permeance of the skin to O_2 (P'_{O_2}, Burg and Burg, 1965). Given that over the short period an apple comes to steady-state in CA [estimated to be approximately 48-96 h by Dadzie (1992)], fruit surface area, mass and skin permeance are not likely to change markedly. Therefore, the r_{O_2} would be proportional to Δp_{O_2} (Dadzie *et al.*, 1996), or:

$$r_{O_2} = k_f \, \Delta p_{O_2} \qquad (6.1)$$

where: k_f = a fruit constant (mol kg^{-1} s^{-1} Pa^{-1}).

Thus, we might usefully use Δp_{O_2} to give some preliminary indication of the effect of $p^e_{CO_2}$ on r_{O_2}, and this was attempted in this study.

6.3 Materials and methods

6.3.1 Fruit supply, initial measurements, treatments and storage

Freshly harvested, commercially graded and packed, preclimacteric 'Cox's Orange Pippin' and 'Braeburn' apples (*Malus domestica* Borkh.; 125 count; mean mass 0.152 kg for 'COP' and 0.149 kg for 'Braeburn') were obtained from a Hawkes Bay

orchard, N.Z. The fruit were held at 20°C overnight and fruit mass, fruit firmness (0 to 12 kgf Effegi press-mounted penetrometer fitted with an 11 mm head), soluble solids content (0 to 20% Atago refractometer), and background skin colour (Minolta CR-200 Chromameter measuring lightness (L) and hue angle (H°), Minolta Camera Co., Ltd.) measured for 20 fruit. Fruit were treated with approximately 10 Pa ethylene at 20°C for 12 h ($p^e_{CO_2}$ was maintained below 0.1 kPa using hydrated lime) in a 1.3 m^3 constant temperature chamber, then ventilated in air at 20°C for a further 48 h during which time, respiration and ethylene production rates increased, indicating fruit had become climacteric. Trays of fruit were randomly allocated to temperature and CO_2 treatments and stored in cartons, at either 2°C for 'COP' (enclosed in perforated polyethylene bags), or 0°C for 'Braeburn' (without bags). Postclimacteric fruit were used for subsequent experiments to minimise variation resulting from differences in fruit respiration.

Fruit used for each temperature treatment were removed from cool storage and equilibrated in air at 20 ± 0.5°C for 24 h before blemish-free fruit were randomly allocated to treatments, 6 fruit to each of 10 CA treatments. As the different $p^e_{CO_2}$ treatments were in a randomised order over time, physiological changes to fruit in storage were monitored. For each removal of fruit from cool-storage, an additional 20 fruit were allocated for maturity assessments, and 15 fruit were stored in a perforated polyethylene bag at the treatment temperature for estimating respiration and ethylene production rates during the experiment.

7.3.2 CA and elevated CO_2 treatments

Experiments were conducted in 1.0 m^3 controlled temperature cabinets (Spaceline, Muller-McAlpine, Auckland, N.Z.). Each experiment used 10 CA containers, each containing six fruit, and each with a different level of $p^e_{O_2}$ but common nominal $p^e_{CO_2}$ CO_2 treatments used for both cultivars were 0, 2, 4, 6, and 8 kPa CO_2 at 20°C (cabinet thermostat set at 19.8•C), and 0 and 8 kPa CO_2 at 0°C.

Metered, humidified gas mixtures flowed through each CA container (total flow = 1.7 x 10^3 mm^3 s^{-1}) as described by Yearsley *et al.* (1996a). The values of $p^e_{O_2}$ used

depended on cultivar and treatment temperature; these were identified using a steady-state model (Badzie, 1992) that predicted $p^e_{O_2}$ between 0.0 kPa and 30 kPa required to produce levels of $p^i_{O_2}$ decreasing geometrically between 20 kPa and 0.09 kPa (Yearsley *et al.*, 1996c). Mean container $p^e_{O_2}$ was controlled within ± 0.2 kPa at low values and ± 0.5 kPa at the highest values, and $p^e_{CO_2}$ ± 0.3 kPa, of required levels. Mean external partial pressures of ethylene ($p^e_{C_2H_4}$) within CA containers were < 1.0 Pa at the highest $p^e_{O_2}$ and 20°C, and ≤ 0.08 Pa at 0°C.

Mean temperature for monitored fruit varied ≤ 0.3°C between fruit in any position within the cabinet. Mean relative humidity within similar CA containers had been previously measured to be ≥ 90% (Yearsley *et al.*, 1996c).

6.3.3 Gas measurement and analysis

Fruit allocated to CA treatments each had a 1000 mm^3 glass surface chamber adhered over lenticels at an equatorial position as described by Yearsley *et al.* (1996a). Steady-state $p^i_{O_2}$ and $p^i_{CO_2}$ were estimated as the equilibrated $p^i_{O_2}$ and $p^i_{CO_2}$ in the surface chambers after 86-90 h exposure to the CA treatments (Yearsley, 1996). Gas samples were removed by gas tight syringe (Hamilton 100 mm^3) from the headspace of each surface chamber through a septum on the CA bags above the chamber and immediately analysed. Mole fractions of O_2 and CO_2 were determined using an O_2 electrode in series with a miniature infra-red CO_2 transducer (Yearsley *et al.*, 1996a), and converted to $p^i_{O_2}$ and $p^i_{CO_2}$ by adjusting for atmospheric pressure (Yearsley *et al.*, 1996b).

Steady-state concentrations of acetaldehyde, ethyl acetate and ethanol (c^i_{Acet}, c^i_{EtAc}, c^i_{EtOH}, mol m^{-3}) in the surface chambers were measured using flame ionization gas chromatography (Yearsley *et al.*, 1996a).

6.3.4 Respiration and ethylene production rates

Fifteen fruit randomly selected from the same population of experimental fruit were used to determine r_{CO_2} and C_2H_4 production ($r_{C_2H_4}$, mol kg^{-1} s^{-1}) approximately

68 and 120 h after CA treatments were imposed, as described by Yearsley *et al.* (1996b).

6.3.5 Skin permeance, cortical tissue porosity and density

Permeance of the fruit's skin to ethane ($P'_{C_2H_6}$, mol s^{-1} m^{-2} Pa^{-1}) was estimated using ethane efflux (Banks, 1985), on a random subsample of 15 fruit at the beginning and end of experiments with each cultivar, and calculated as described by Yearsley *et al.*, 1996a. Tissue porosity (ε, m^3 m^{-3}) and tissue density (ρ, kg m^{-3}) were estimated using 5 mm wide longitudinal wedges of tissue from which the skin had been removed as described by Yearsley *et al.* (1996a).

6.3.6 Estimation of LOL^is

The "bootstrap" statistical procedure (Yearsley *et al.*, 1996a) was used to estimate LOL^is from steady-state $p^i_{O_2}$ and $p^i_{CO_2}$of surface chambers. Plots (without scales or titles) of $p^i_{CO_2}$, RQ_{ia}, and c^i_{EtOH} versus $p^i_{O_2}$ were submitted to 15 trained panellists, who hand-drew curves through the data then identified ACP^i and FT^i. 1000 bootstrap samples were obtained by random sampling with replacement from the panellists' original data. Bootstrap means and bias corrected (BCa) 95% confidence intervals around means (Efron, 1987) were calculated using Gauss software (Gauss, 1993). As the bootstrap procedure did not assume data to be normally distributed, confidence intervals generated were not necessarily symmetrical about means.

6.3.7 Statistical analysis

Regression analysis of relationships between LOL^is of fruit from both cultivars at 20°C, mean $p^e_{CO_2}$ and time in storage were performed using PROC REG of the SAS system (SAS, 1990). Regressions were weighted with the inverse of the bootstrap standard error of means, and either with or without adjustment for time in storage.

When adjusted for time, limited degrees of freedom prevented fitting polynomial models beyond quadratic effects. Consequently, regression models were not adjusted for time when storage time effects were not significant. Despite high r^2 values, polynomial effects models were not always significant, indicating there was not enough evidence to make statistically justifiable inferences about the relationship between LOL^is and $p^e_{CO_2}$. Analyses of variance of fruit maturity, cortical tissue porosity, density, r_{CO_2}, $r_{C_2H_4}$ and $P'_{C_2H_6}$ data were performed using PROC GLM of the SAS system (SAS, 1990). Parameters were estimated for functions fitted to Δp_{O_2} and RQ_{ia} using Fig.P software (Fig.P, 1991).

6.4 Results

6.4.1 Fruit maturity

'COP' and 'Braeburn' apples had similar fruit firmness and soluble solids content at harvest (Table 6.1). Fruit firmness for both cultivars decreased rapidly and soluble solids content increased slightly within the first 10 weeks of storage. Though values differed significantly for some treatments during the experimental period, differences were considered small physiologically. Background skin colour became lighter and hue angle decreased (changed from green to green-yellow) for both cultivars consistent with increasing ripeness (Table 6.1). Statistically significant differences occurred for L and H° during storage, but changes were generally small during the experimental period.

6.4.2 Cortical tissue porosity (ε), density (ρ) and skin permeance to ethane (P'_{C2H6})

'COP' apples had slightly higher average cortical tissue porosity and lower density than 'Braeburn' apples (Table 6.2). Cortical tissue porosity and density at different storage times differed significantly for 'COP' but not 'Braeburn' fruit, but the trend over time for 'COP' fruit was not consistent. There was a linear relationship for both

cultivars between cortical tissue porosity and density, such that fruit with higher porosity had lower density (Fig. 6.1). Individual apples in the population sampled varied markedly in porosity and density. 'Braeburn' apples had greater variation in estimates of porosity and density than 'COP' apples (ranges of 0.07 and 0.05 m^3 m^{-3}, and 69 and 47 kg m^{-3} respectively). The relationship between cortical tissue density and porosity for 'Braeburn' apples was similar to that reported by Yearsley *et al.*, 1996c, but 'COP' apples were denser relative to their porosity compared to fruit from the same orchard from the previous season.

Mean $P'_{C_2H_6}$ was approximately 1.7 times higher for 'COP' than 'Braeburn' fruit, and did not change markedly with time in storage (Table 6.2). Values were similar to those reported for the previous season for apples from the same orchard (Yearsley *et al.*, 1996c).

6.4.3 Respiration rate (r_{CO2}) and ethylene production (r_{C2H4})

There was a tendency for r_{CO_2} and $r_{C_2H_4}$ of both cultivars and at both 0° and 20°C to increase with time in storage, although the differences were not always significantly different between each time (Table 6.3). The changes were consistent with those expected for apples ripening in cool storage.

Table 6.1 Fruit firmness, soluble solids content, and background colour [lightness (L) and hue angle (H°)], for 'Cox's Orange Pippin' and 'Braeburn' apples from a Hawkes Bay orchard stored at 2°C and 0°C respectively for various durations. The fruit were removed from cool storage and stored at 20°C in air for 24 h prior to measuring variables at 20°C. Values represent least squares means and within cultivars, treatments with different letters are significantly different ($p = 0.05$, n=20).

Cultivar and Time in storage (weeks)	Treatment T; $p^e_{CO_2}$ (°C; kPa)	Fruit firmness (N)	Soluble solids content (%)	Background colour	
				(L)	(H°)
'Cox's Orange Pippin'					
0.0	Prestorage	74.1 a	11.4 a	68.2 c	108.6 a
1.6	0; 0	66.7 b	12.4 bc	69.4 c	106.0 b
4.7	20; 2	53.9 c	12.7 bc	59.5 bc	103.5 cd
5.7	20; 4	53.4 c	12.3 bc	69.0 c	103.9 c
6.6	0; 8	62.7 c	12.4 bc	68.5 c	102.8 cd
9.6	20; 6	45.4 d	12.2 b	70.8 ab	101.8 de
10.6	20; 8	47.2 d	12.6 bc	69.5 bc	101.6 de
11.6	20; 0	46.5 d	12.8 c	71.5 a	101.2 e
'Braeburn'					
0.0	Prestorage	75.4 a	11.2 a	67.6 c	108.3 a
12.0	20; 2	58.9 bc	11.8 c	69.7 a	100.2 cd
13.9	0; 0	54.8 cd	11.8 c	69.6 b	101.4 bcd
15.0	20; 6	56.9 bc	11.5 ab	70.0 a	102.3 bc
16.0	20; 0	54.6 cd	11.8 cb	70.7 ab	101.8 bc
21.0	20; 4	55.8 bc	12.0 ab	71.7 b	100.0 d
22.0	20; 8	51.5 d	11.4 ab	70.6 ab	100.3 cd
23.0	0; 8	51.4 d	12.2 bc	70.7 ab	100.8 bcd
Storage time comparisons (ANOVA p values)					
'Cox's Orange Pippin'		0.0001	0.0002	0.0001	0.0001
'Braeburn'		0.0001	0.0022	0.0013	0.0001

Table 6.2 Cortical tissue porosity (ε), density (ρ), and mean (± standard error of means) skin permeance to ethane ($P_{C_2H_6}$) for 'Cox's Orange Pippin' and 'Braeburn' apples from a Hawkes Bay orchard stored at 2°C and 0°C respectively for various durations. The fruit were removed from cool storage and stored at 20°C in air for 24 h prior to measuring variables at 20°C, except $P_{C_2H_6}$ which was estimated after 7 days. Values represent least squares means and within cultivars, treatments with different letters are significantly different ($p = 0.05$, n=15).

Cultivar and Time in storage (weeks)	Treatment T; $p^{e}_{CO_2}$ (°C; kPa)	Cortical tissue porosity (ε) ($m^3\ m^{-3}$)	Cortical tissue density (ρ) ($kg\ m^{-3}$)	Skin permeance to ethane ($P_{C_2H_6}$)[1] mean ± sem ($nmol\ s^{-1}\ m^{-2}\ Pa^{-1}$)
'Cox's Orange Pippin'				
0.0	Prestorage	NE[2]	NE	NE
1.6	0; 0	0.155 abcd	883.5 b	0.301 ± 0.0107
4.7	20; 2	0.148 d	893.0 a	NE
5.7	20; 4	0.156 a	884.4 b	NE
6.6	0; 8	0.150 bcd	890.2 a	NE
9.6	20; 6	0.153 abcd	887.5 ab	NE
10.6	20; 8	0.155 abc	884.9 b	NE
11.6	20; 0	0.156 a	883.2 b	0.272 ± 0.0116
'Braeburn'				
0.0	Prestorage	NE	NE	NE
12.0	20; 2	0.134 a	904.5 a	0.165 ± 0.0077
13.9	0; 0	0.139 a	898.3 a	NE
15.0	20; 6	0.136 a	902.2 a	NE
16.0	20; 0	0.137 a	902.0 a	NE
21.0	20; 4	0.140 a	898.6 a	NE
22.0	20; 8	0.133 a	901.4 a	NE
23.0	0; 8	0.139 a	896.3 a	0.170 ± 0.0072
Storage time comparisons (ANOVA p values)				
'Cox's Orange Pippin'		0.0412	0.0155	
'Braeburn'		0.3812	0.4599	

[1] For $P_{C_2H_6}$ at T (°C), 1 $mol\ s^{-1}\ m^{-2}\ Pa^{-1} = ((T + 273.15) / 1.203 \times 10^{-3})\ cm\ s^{-1}$ (Banks *et al.*, 1995).

[2] NE = not estimated.

Table 6.3 Estimates of respiration rate (r_{CO_2}) and ethylene production ($r_{C_2H_4}$) for 'Cox's Orange Pippin' ('COP') and 'Braeburn' apples from a Hawkes Bay orchard stored at 2°C and 0°C respectively for various durations. Fruit were removed from cool storage, equilibrated at 0°C or 20°C (T) in air and r_{CO_2} and $r_{C_2H_4}$ estimated after approximately 4 days. Values represent overall least squares means adjusted for day effects. Within cultivars and treatment temperatures, $p^{e}_{CO_2}$ treatments with different letters are significantly different (T tests (LSD), alpha = 0.05, n=30).

Cultivar and Time in storage (weeks)	Treatment T; $p^{e}_{CO_2}$ (°C; kPa)	r_{CO_2}[1] (nmol kg^{-1} s^{-1})	$r_{C_2H_4}$[2] (nmol kg^{-1} s^{-1})
'Cox's Orange Pippin'			
1.6	0; 0	25.1 b	0.139 b
4.7	20; 2	170.6 c	1.164 c
5.7	20; 4	178.0 a	1.414 b
6.6	0; 8	30.6 a	0.170 a
9.6	20; 6	181.6 a	1.694 a
'Braeburn'			
12.0	20; 2	119.7 b	0.971 a
13.9	0; 0	16.6 b	0.052 b
15.0	20; 6	129.1 a	0.957 a
16.0	20; 0	108.4 c	0.966 a
21.0	20; 4	128.5 a	0.917 ab
22.0	20; 8	132.4 a	0.830 b
23.0	0; 8	19.4 a	0.093 a
Storage time comparisons (ANOVA p values)			
'COP'	0°C	0.0001	0.0001
	20°C	0.0005	0.0001
'Braeburn'	0°C	0.0001	0.0001
	20°C	0.0001	0.0758

[1] For r_{CO_2}, at T (°C), 1 mol kg^{-1} s^{-1} = 1.5839 x 10^{8} mg kg^{-1} h^{-1} or ((T + 273.15) / 3.341 x 10^{-11} p_{tot}) ml kg^{-1} h^{-1}, where p_{tot} (Pa) is the total pressure in the system (Banks *et al.*, 1995).

[2] For $r_{C_2H_4}$ at T (°C), 1 mol kg^{-1} s^{-1} = ((T + 273.15) / 3.341 x 10^{-14} p_{tot}) µl kg^{-1} h^{-1} (Banks *et al.*, 1995).

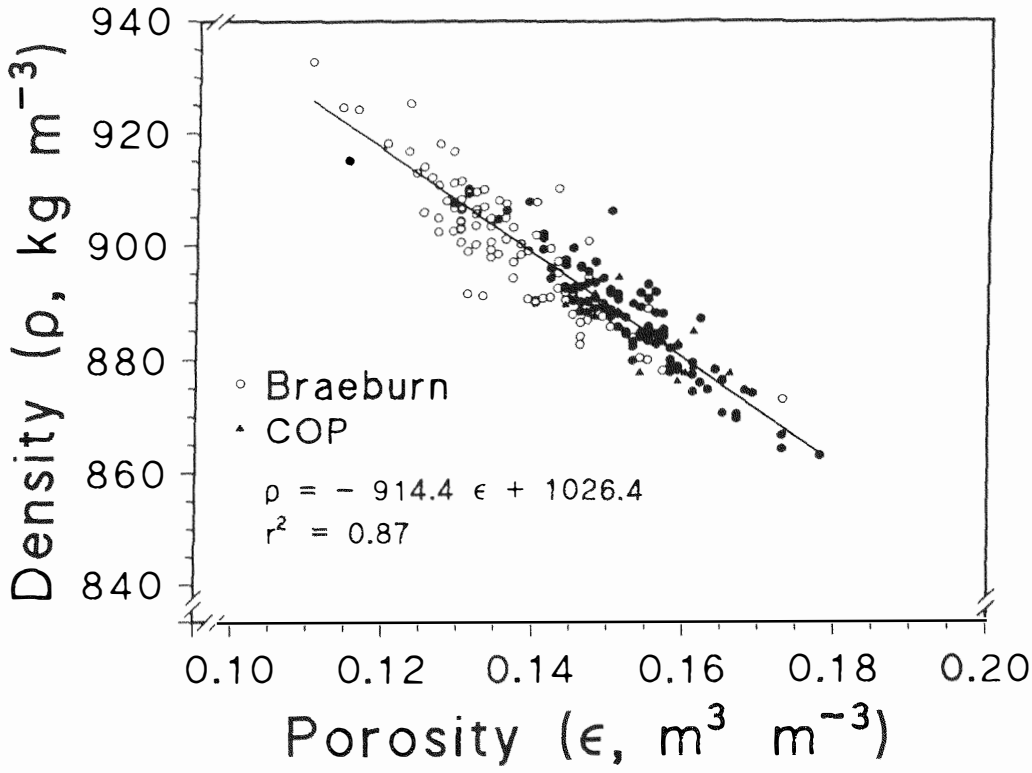

Figure 6.1 Cortical tissue density (ρ) as a function of porosity (ε) for 'Cox's Orange Pippin' and 'Braeburn' apples from a Hawkes Bay orchard stored at 2°C and 0°C respectively for periods up to 23 weeks, and held at 20°C for 24 h before estimations were made.

6.4.4 *LOL*^{i}s as a function of p^e_{CO2}

Bootstrap estimates of mean *LOL*^{i}s based on core atmospheres were only slightly higher for 'COP' and approximately 0.4 kPa higher for 'Braeburn' than those based on surface chamber atmospheres (data not shown). However, as overall trends in core and surface chamber *LOL*^{i}s were similar, only data based on surface chambers are presented. Though statistically significant polynomial fits of *LOL*^{i}s as a function of $p^e_{CO_2}$ were found for some data, they were small and not considered physiologically significant.

6.4.4.1 ACP^i as a function of p^e_{CO2}

Bootstrap estimates of means ACP^i remained essentially constant (approximately 0.52 kPa O_2) for 'COP' apples at 20°C and $p^e_{CO_2}$ between 0 and 8 kPa (Fig. 6.2). At 0°C, ACP^i decreased from 0.67 to 0.48 kPa O_2 with increase in $p^e_{CO_2}$ from 0 to 8 kPa CO_2 (Fig. 6.2). For 'Braeburn' apples at 20°C, ACP^i decreased slightly between 0 and 4 kPa $p^e_{CO_2}$ then increased at 6 and 8 kPa $p^e_{CO_2}$ (Fig. 6.3; mean over all levels of $p^e_{CO_2}$ was 0.92 kPa O_2). For 'Braeburn' at 0°C, ACP^i was constant at 0.88 kPa O_2 at 0 and 8 kPa $p^e_{CO_2}$ (Fig. 6.3). On average, ACP^i was 0.31 and 0.40 kPa O_2 higher for 'Braeburn' than 'COP' apples at 0° and 20°C respectively (Figs. 6.2 and 6.3).

6.4.4.2 FT^i_{RQ} as a function of p^e_{CO2}

There was a slight increase in FT^i_{RQ} as $p^e_{CO_2}$ increased from 0 to 4 kPa for 'COP' fruit at 20°C , and a decrease in FT^i_{RQ} at 6 and 8 kPa $p^e_{CO_2}$ (Fig. 6.2; overall mean = 1.21 kPa O_2). At 0°C, FT^i_{RQ} increased from 0.94 to 1.32 kPa as $p^e_{CO_2}$ was increased from 0 to 8 kPa (Fig. 6.2). 'Braeburn' apples had a more complex relationship between FT^i_{RQ} and $p^e_{CO_2}$ than 'COP' at 20°C. There was an indication FT^i_{RQ} decreased between 0 and 2 kPa $p^e_{CO_2}$ but then increased again as $p^e_{CO_2}$ increased to 8 kPa (Fig. 6.3). When averaged over all p_{CO_2} treatments, FT^i_{RQ} for 'COP' (0.69 kPa O_2 , Fig 6.2), but not 'Braeburn', apples at 20°C were higher than ACP^i.

6.4.4.3 FT^i_{EtOH} as a function of p^e_{CO2}

In general, estimates of FT^i_{EtOH} were higher than ACP^i (Figs. 6.2 and 6.3), but the change in FT^i_{EtOH} with $p^e_{CO_2}$ was quite different to that for ACP^i and FT^i_{RQ}. The relationship between FT^i_{EtOH} and $p^e_{CO_2}$ for fruit at 20°C was complex, particularly for 'COP', and there was evidence for 'Braeburn' that FT^i_{EtOH} was higher for apples in 2 to 8 kPa $p^e_{CO_2}$ compared to 0 kPa. Estimates of FT^i_{EtOH} at 20°C for 'Braeburn' apples were higher than for 'COP'. As with FT^i_{RQ} at 0°C, FT^i_{EtOH} was higher for fruit at 8

than 0 kPa $p^e_{CO_2}$, particularly for 'Braeburn' apples. For both cultivars, c^i_{Acet} and c^i_{EtAc} accumulation in surface chambers was highly variable (data not shown).

6.4.5 Δp_{O2} as a function of p^i_{O2} and p^e_{CO2}

The relationship between Δp_{O_2} and $p^i_{O_2}$ was modelled using the Michaelis-Menten equation:

$$\Delta p_{O_2} = \frac{k_1 \, p^i_{O_2}}{k_2 + p^i_{O_2}} \qquad (6.2)$$

Estimates and standard errors of k_1 and k_2 are presented in Table 6.4. There was a large range in Δp_{O_2} for fruit at higher values of $p^i_{O_2}$ but the range decreased as $p^i_{O_2}$ decreased (Figs. 6.4 and 6.5). Over all treatments, there was a small decrease in fitted values for Δp_{O_2} as $p^i_{O_2}$ decreased from approximately 18 kPa to 5 kPa; below approximately 5 kPa $p^i_{O_2}$, Δp_{O_2} rapidly decreased.

There was little difference in Δp_{O_2} of 'COP' apples at $p^e_{CO_2}$ of 0 or 2 kPa at 20•C and $p^i_{O_2}$ > approximately 5 kPa and (Fig. 6.4). However, as $p^e_{CO_2}$ increased from 4 to 8 kPa, there was a small decrease in Δp_{O_2} compared to fruit in 0 kPa $p^e_{CO_2}$. For 'COP' at 0°C, at both 0 and 8 kPa $p^e_{CO_2}$, Δp_{O_2} values were similar for fruit with $p^i_{O_2}$ between approximately 20 and 5 kPa.

The Δp_{O_2} of 'Braeburn' apples at 20°C and $p^i_{O_2}$ > approximately 5 kPa, were markedly higher than for 'COP' (Fig. 6.4). The substantial decrease in Δp_{O_2} at low $p^i_{O_2}$, tended to commence at higher $p^i_{O_2}$ for 'Braeburn' compared to 'COP' apples (Figs. 6.4 and 6.5), as generally seen in higher k_2 for 'Braeburn' apples (Table 6.4). 'Braeburn' apples at 20°C and 0 kPa $p^e_{CO_2}$ had the highest Δp_{O_2}; values for fruit in 2, 4, 6 and 8 kPa $p^e_{CO_2}$ were similar but lower than for fruit in 0 kPa $p^e_{CO_2}$ (Fig. 6.4). Similarly, Δp_{O_2} for 'Braeburn' apples at 0°C was higher in fruit at 0 than at 8 kPa $p^e_{CO_2}$ but similar in 0 and 8 kPa $p^e_{CO_2}$ for 'COP' (Fig. 6.5).

6.4.6 RQ_{ia} as a function of p^i_{O2}, temperature and p^e_{CO2}

The relationship between RQ_{ia} and $p^i_{O_2}$ was modelled using the equation:

$$RQ_{ia} = (k_3\ p^{i\ k_4}_{O_2}) + k_5 \qquad \textbf{(6.3)}$$

Estimates of parameters and standard errors are presented in Table 6.5. Difficulties in obtaining significant parameters prevented them being used to calculate FT^i_{RQ}.

RQ_{ia} remained close to unity for 'COP' apples at 0° and 20°C and 0, 2, 4, and 6 kPa $p^e_{CO_2}$ until initiation of fermentation at low $p^i_{O_2}$ (Figs. 6. 6 and 6.7). RQ_{ia} was lower for 'COP' fruit in 8 kPa $p^e_{CO_2}$ and 0°C. Asymptotic levels of RQ_{ia} of 'Braeburn' apples at 20°C were < 1 and lower than for 'COP'. At both 0 and 20°C, RQ_{ia} was marginally lower at 2, 4, 6 and 8 kPa $p^e_{CO_2}$ compared to 0 kPa.

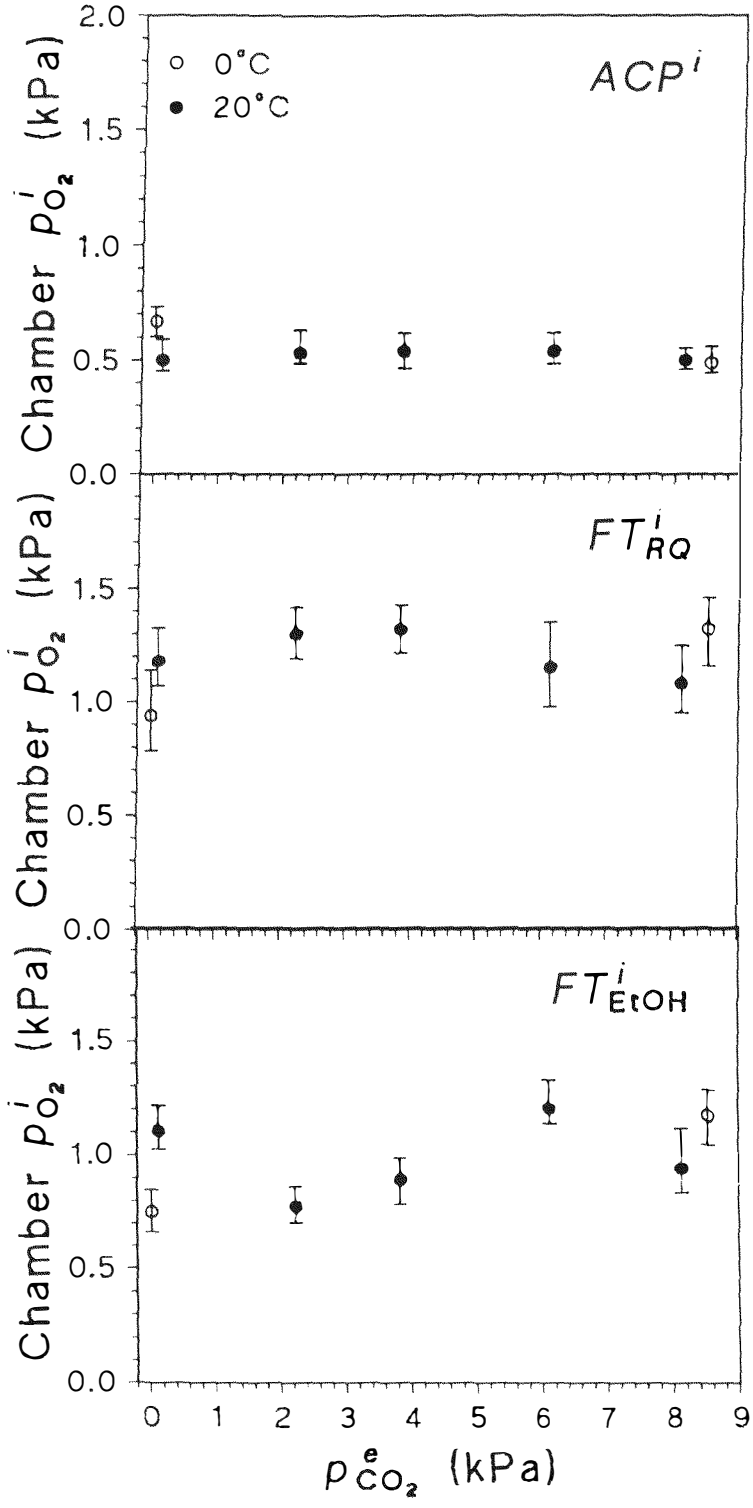

Figure 6.2 Bootstrap estimates of steady state internal lower oxygen limits (ACP^i, FT^i_{RQ}, and FT^i_{EtOH}) based on partial pressures of surface chambers for 'Cox's Orange Pippin' apples as a function of external partial pressure of CO_2 ($p^e_{CO_2}$). Values represent bootstrap means and 95% bias corrected (BCa-type) confidence intervals).

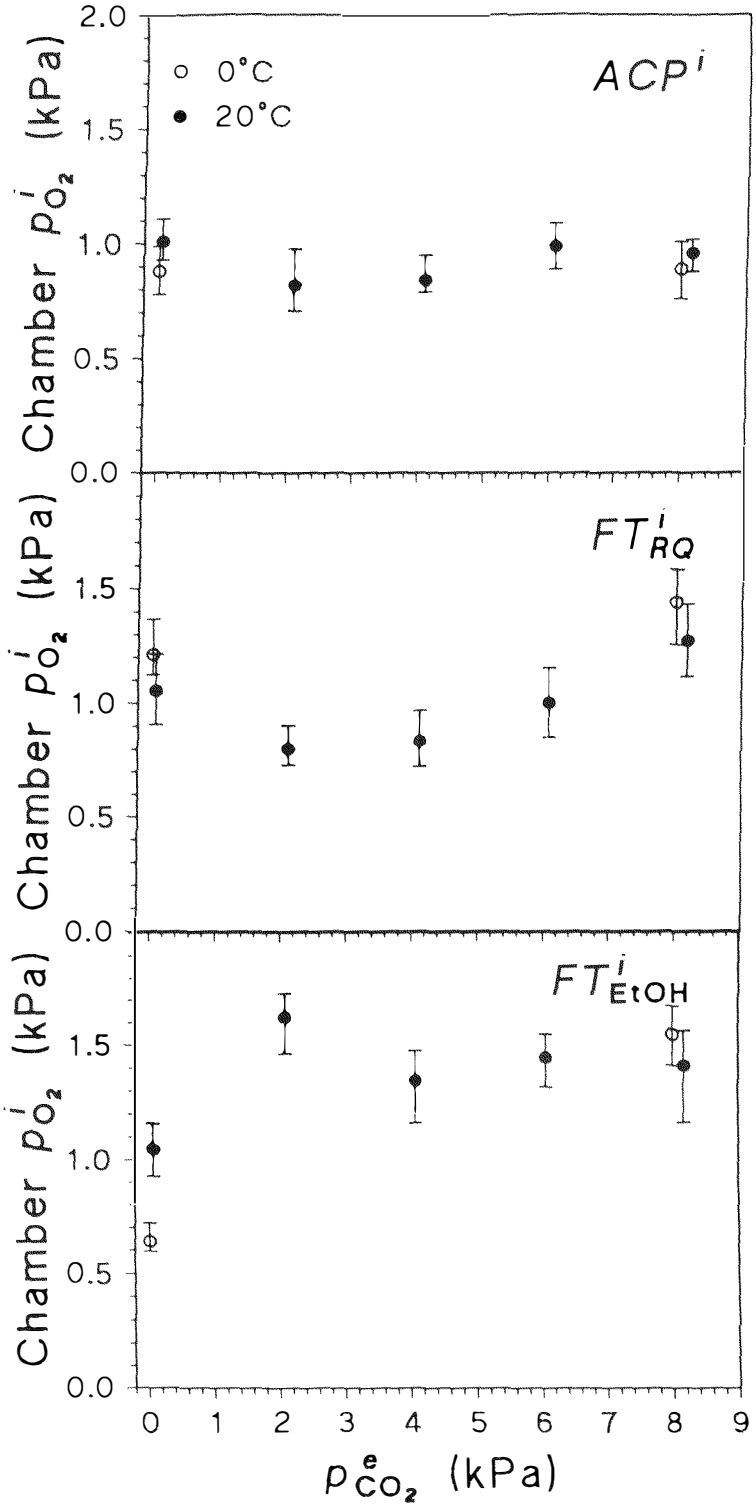

Figure 6.3 Bootstrap estimates of steady state internal lower oxygen limits (ACP^i, FT^i_{RQ}, and FT^i_{EtOH}) based on partial of surface chambers for 'Braeburn' apples as a function of external partial pressure of CO_2 ($p^e_{CO_2}$). Values represent bootstrap means and 95% bias corrected (BCa-type) confidence intervals).

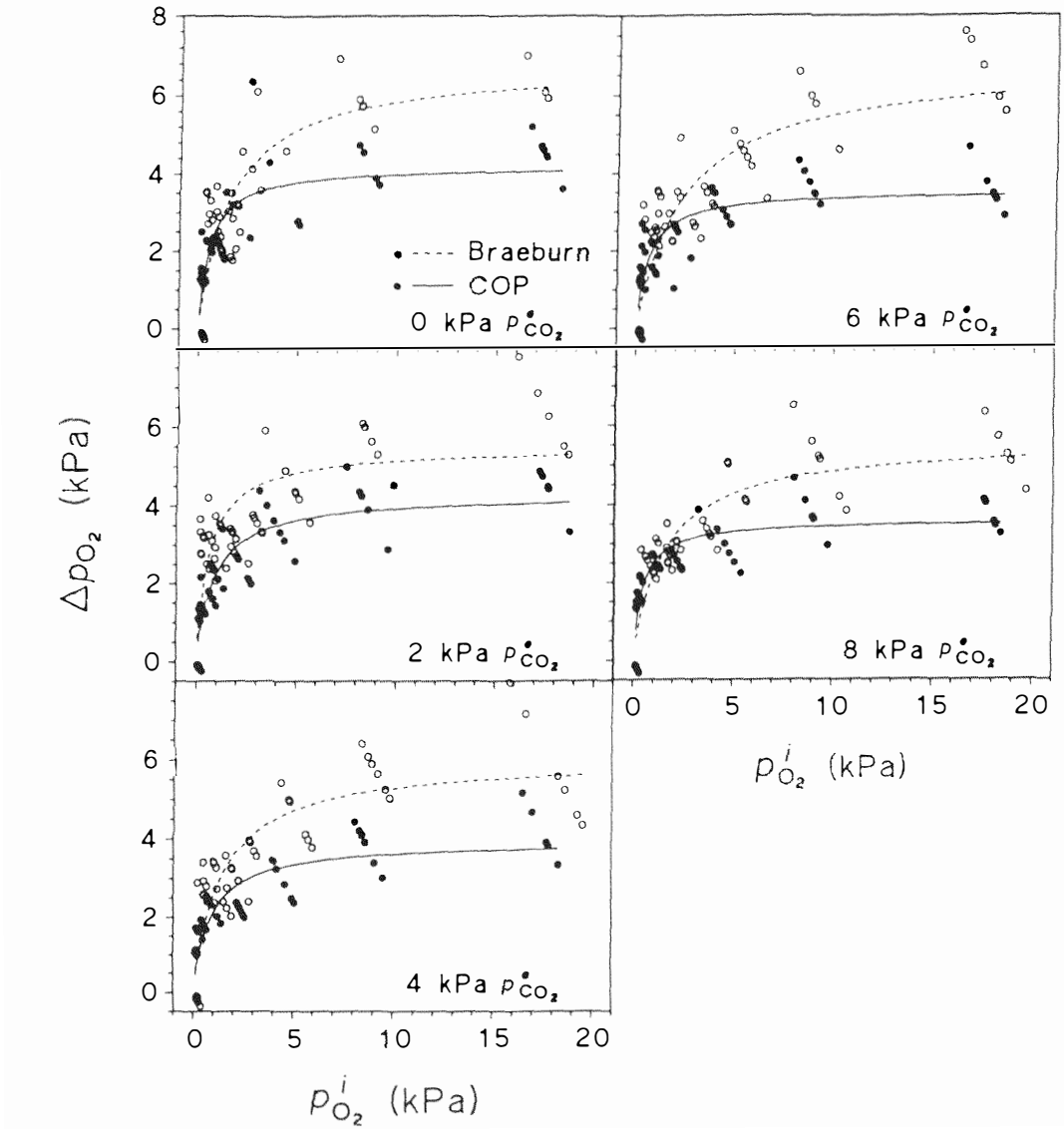

Figure 6.4 Difference between external and internal (chamber) steady-state partial pressure of O_2 ($\Delta p_{O_2} = p^e_{O_2} - p^i_{O_2}$) as an estimate of differences in respiration rate in 'Cox's Orange Pippin' (COP) and 'Braeburn' apples at 20°C, stored in various external partial pressures of O_2 and either 0, 2, 4, 6, or 8 kPa external partial pressures of CO_2 ($p^e_{CO_2}$), balance N_2. For estimates of parameters of the curves see Table 6.4.

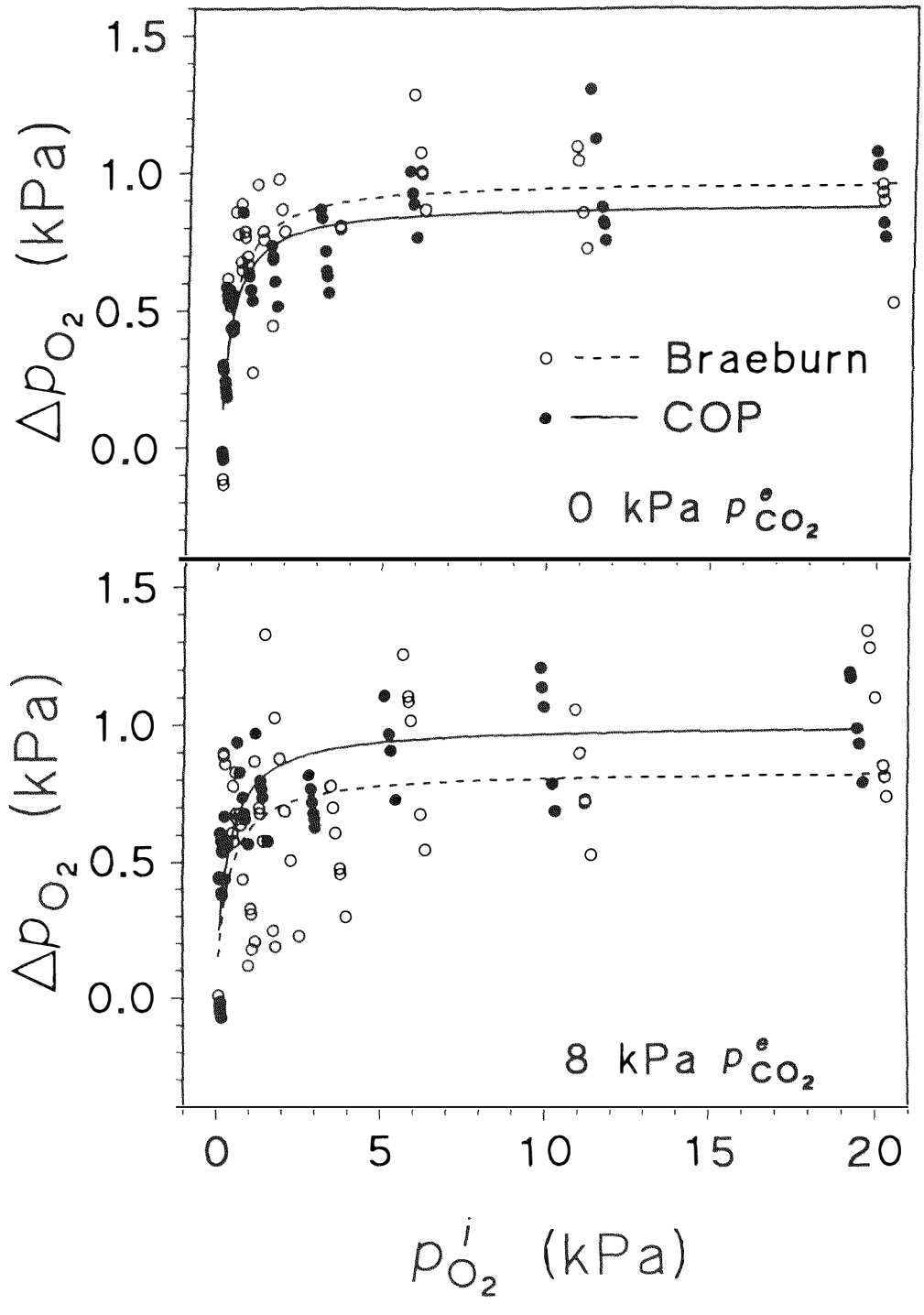

Figure 6.5 Difference between external and internal (chamber) steady-state partial pressure of O_2 ($\Delta p_{O_2} = p^e_{O_2} - p^i_{O_2}$) as an estimate of differences in respiration rate in 'Cox's Orange Pippin' (COP) and 'Braeburn' apples at 0°C, stored in various external partial pressures of O_2 and either 0 and 8 kPa external partial pressures of CO_2 ($p^e_{CO_2}$), balance N_2. For estimates of parameters of the curves see Table 6.4.

Table 6.4 Estimates of parameters and standard errors (se) for Eq. 6.2 describing the relationship between the difference in external and internal (chamber) steady-state partial pressures of O_2 (Δp_{O_2}) and internal partial pressure of O_2 for 'Cox's Orange Pippin' ('COP') and 'Braeburn' apples at 20° and 0°C (T) at various external CO_2 partial pressures ($p^e_{CO_2}$).

T (°C)	$p^e_{CO_2}$ (kPa)	k_1	se	k_2	se	r^2
'COP'						
20	0	4.11	0.227	0.55	0.112	0.748
20	2	4.27	0.231	0.90	0.166	0.777
20	4	3.91	0.204	0.86	0.177	0.776
20	6	3.53	0.205	0.58	0.134	0.668
20	8	3.58	0.177	0.45	0.089	0.712
0	0	0.89	0.040	0.31	0.064	0.747
0	8	1.00	0.053	0.33	0.077	0.610
'Braeburn'						
20	0	6.75	0.517	1.54	0.333	0.731
20	2	5.47	0.347	0.65	0.168	0.512
20	4	5.98	0.370	1.35	0.286	0.710
20	6	6.76	0.525	2.32	0.502	0.710
20	8	5.56	0.323	1.54	0.289	0.734
0	0	0.98	0.059	0.34	0.093	0.614
0	8	0.84	0.074	0.36	0.161	0.291

Table 6.5 Estimates of parameters for plots and standard errors (se) of the respiratory quotient (RQ_{ta}) as a function of internal (chamber) steady-state partial pressure of O_2 using Eq. 6.3, for 'Cox's Orange Pippin' ('COP') and 'Braeburn' apples at 20° and 0°C (T), and at various external CO_2 partial pressures ($p^e_{CO_2}$).

T (°C)	$p^e_{CO_2}$ (kPa)	k_3	se	k_4	se	k_5	se	r^2
'COP'								
20	0	0.52	0.294	-0.48	0.185	0.78	0.255	0.624
20	2	0.18	0.136	-1.02	0.388	1.03	0.123	0.628
20	4	0.06	0.070	-1.54	0.566	1.03	0.105	0.617
20	6	0.11	0.075	-1.13	0.298	0.98	0.082	0.618
20	8	0.20	0.170	-1.06	0.420	0.74	0.153	0.528
0	0	0.28	0.236	-0.47	0.264	0.95	0.198	0.449
0	8	0.54	0.343	-0.36	0.202	0.19	0.295	0.605
'Braeburn'								
20	0	0.37	0.165	-1.02	0.195	0.70	0.151	0.780
20	2	0.03	0.010	-1.94	0.146	0.69	0.024	0.921
20	4	0.03	0.020	-2.52	0.401	0.58	0.033	0.830
20	6	0.11	0.066	-2.05	0.349	0.67	0.074	0.768
20	8	0.10	0.027	-1.94	0.164	0.66	0.023	0.923
0	0	0.02	0.039	-1.64	0.810	1.09	0.060	0.460
0	8	0.46	0.450	-0.32	0.279	0.46	0.405	0.253

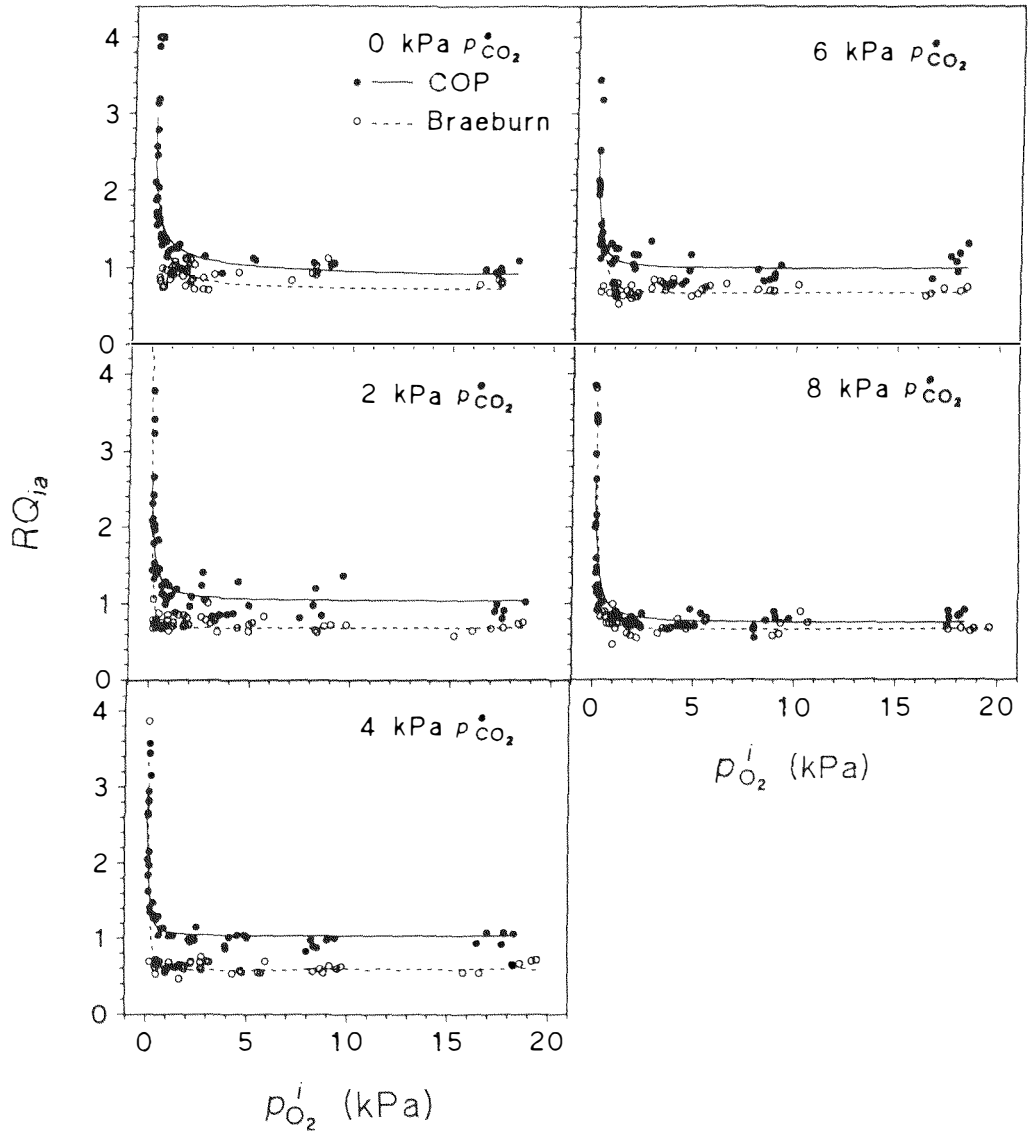

Figure 6.6 Respiratory quotient (RQ_{ia}) as a function of the internal (chamber) steady-state partial pressure of O_2 ($p^i_{O_2}$) for 'Cox's Orange Pippin' (COP) and 'Braeburn' apples at 20°C, stored in 0, 2, 4, 6 or 8 kPa external partial pressure of CO_2 ($p^e_{CO_2}$), balance N_2. For estimates of parameters of the curves see Table 6.5.

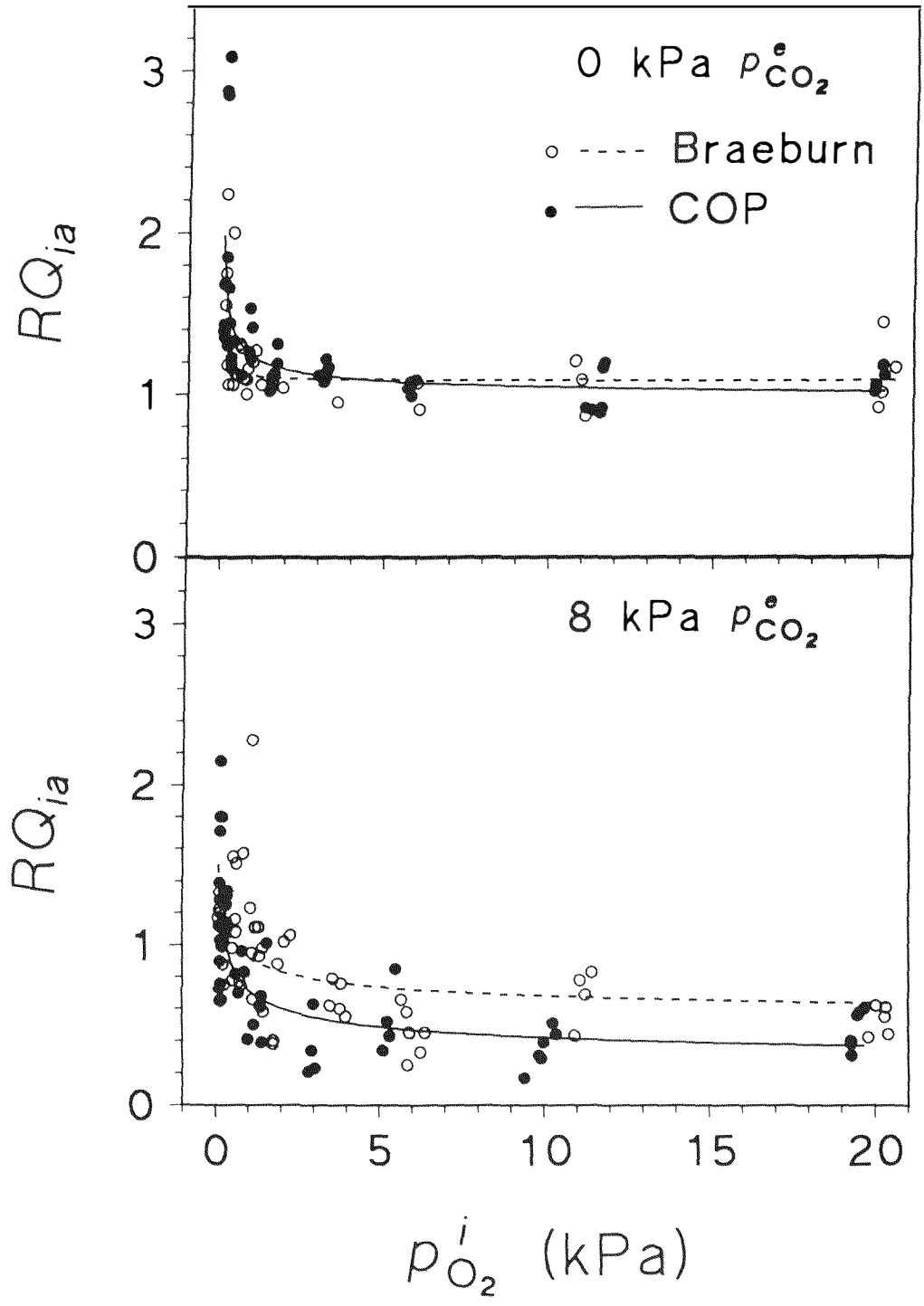

Figure 6.7 Respiratory quotient (RQ_{ia}) as a function of the internal (chamber) steady-state partial pressure of O_2 ($p^i_{O_2}$) for 'Cox's Orange Pippin' (COP) and 'Braeburn' apples at 0°C, stored in 0 and 8 kPa external partial pressure of CO_2 ($p^e_{CO_2}$), balance N_2. For estimates of parameters of the curves see Table 6.5.

6.5 Discussion

For 'COP' apples at 0°C there was a marked increase in FT^{l}_{RQ} and FT^{l}_{EtOH}, but not ACP^{l}, in response to elevated $p^{e}_{CO_2}$. A similar, but not so marked increase (except for chamber FT^{l}_{EtOH}) occurred for 'Braeburn' fruit at 0°C. Other than that, this study indicated that although variation in *LOL*'s occurred for 'COP' and 'Braeburn' apples at 20°C, they were not markedly affected by levels of $p^{e}_{CO_2}$ between 0 to 8 kPa CO_2. There was no evidence that the levels of $p^{e}_{CO_2}$ used in the study cause CO_2-induced fermentation over the short period the apples were exposed to CA treatments.

The pattern of changes in FT^{l}_{EtOH} at 20°C was the converse of that for FT^{l}_{RQ}. Given that these should both be reflections of the same physiological transition point, it seems safest to conclude that there was no major effect of $p^{e}_{CO_2}$ on fermentation thresholds at 20°C. On the other hand, the difference in response of FT^{l}_{EtOH} may have reflected the likelihood that EtOH levels in the tissue would have been continually accumulating with time. Since the chambers would inevitably have lagged behind changes occurring within the fruit, the concentrations of EtOH sampled from the chambers may not have been at steady-state. The marked increase at 8 kPa $p^{e}_{CO_2}$ in FT^{l}_{RQ} and FT^{l}_{EtOH} at 0°C may have been a consequence of the higher solubility of CO_2 at the lower temperature, and this might have accentuated the effect of a given $p^{e}_{CO_2}$ on fruit tolerance to low $p^{l}_{O_2}$. Alternatively, since the vapour pressure of EtOH above aqueous solutions would be highly temperature sensitive, the onset of fermentation may be more difficult to detect by measuring vapour phase levels of EtOH in fruit kept at the temperatures used in the experiment. Therefore, it is possible that the accumulation of EtOH in chambers did not reflect tissue concentrations because either values for chamber EtOH were not at steady-state or were not measured with great accuracy.

Both 'COP' (as a consequence of high respiration rate, Table 6.3) and 'Braeburn' (as a consequence of low skin permeance, Table 6.2) apples have high $p^{l}_{CO_2}$ compared to other cultivars (Dadzie, 1992). Both cultivars are susceptible to CO_2-related physiological storage disorders (core flush and internal browning in 'COP'

(Meheriuk *et al.*, 1994), and the brown-heart like 'Braeburn browning disorder' in 'Braeburn' (Banks, N.H., unpublished data). No visible external or internal damage was observed in experimental fruit, even for those in anoxic atmospheres for up to 7 days, atmospheres of 8 kPa CO_2 were well above the level of CO_2 used for commercial CA cool-storage of apples. Brief exposures to potentially harmful levels of CO_2 may be tolerated for short periods (Ke and Kader, 1992). However, it is probable that damage would develop in fruit during longer storage periods in such high CO_2 atmospheres.

Higher LOL^is for 'Braeburn' compared to 'COP' apples at approximately 0 kPa, and for fruit at 20° compared to 0°C, were consistent with those reported for the same cultivars from the previous season (Yearsley *et al.*, 1996c). As suggested in that work, increases in LOL^is as a response to increasing temperature, and lower LOL^is for 'COP' apples could have been a consequence of higher tissue porosity for 'COP' as well as the effect of increasing temperature lowering the solubility of gases. As cortical tissue porosity and density did not change significantly with time in storage for 'Braeburn' fruit, and the small changes for 'COP' were not consistent with time, they were unlikely to explain changes in LOL^is over time. The lower porosity and higher density of 'Braeburn' fruit may reduce diffusivity of O_2 through the cortex, lowering the tolerance and increasing LOL^is of 'Braeburn' compared to 'COP' fruit. The slightly lower gradient in the linear relationship between density and porosity for 'Braeburn' compared to 'COP' apples (Fig. 6.1), may have resulted from incomplete infiltration of some 'Braeburn' slices, particularly those with lower porosity. The larger gradients in O_2 partial pressure (Δp_{O_2}) for 'Braeburn' compared to 'COP' fruit at 20° (Fig. 6.4) were probably a consequence of lower skin permeance (Table 6.2) and possibly lower porosity (Fig. 6.1), as the respiration rate of 'Braeburn' was lower that for 'COP' (Table 6.3). These differences were attenuated at 0°C because of the lower respiration rate at that temperature. As LOL^is should be independent of skin permeance, these differences would not have contributed to variation in LOL^is between cultivars.

Variation in Δp_{O_2} resulted from differences in respiration rate and skin permeance of individual apples, such that a fruit with high respiration rate and/or low skin permeance had higher Δp_{O_2} and lower $p^i_{O_2}$. The lower Δp_{O_2} of apples at 0°C

compared to 20°C was primarily a consequence of lower respiration rate of fruit at the lower temperature. In general, elevated $p^{e}_{CO_2}$ had a small effect on reduced r_{O_2} (as reflected by Δp_{O_2}) for both cultivars at 20°C compared to fruit in 0 kPa $p^{e}_{CO_2}$. Although the fitted curve for 'COP' at 0°C, 8 kPa $p^{e}_{CO_2}$ was slightly higher than that for the 0 kPa $p^{e}_{CO_2}$ treatment, the change in Δp_{O_2} was too small, and the variation in 'Braeburn' data at 8 kPa $p^{e}_{CO_2}$ too variable, to indicate a significant effect of elevated $p^{e}_{CO_2}$ on r_{O_2} at 0°C. The change in apparent Michaelis-Menten constant (values of k_2 in Table 6.4) was not consistent with change in p_{CO_2}. However, the higher values of k_2 for 'Braeburn' apples indicated depression of $p^{i}_{O_2}$ had a more marked effect on r_{O_2} of 'Braeburn' compared to 'COP', suggesting involvement of oxidases with lower affinity in 'Braeburn' than 'COP' or differences in gradients in $p^{i}_{O_2}$ within the flesh associated with differences in interconnectedness of intercellular spaces.

A number of studies with apples indicate that elevated CO_2 may reduce *RQ* (Fidler and North, 1967; Metlitskii *et al.*, 1972). The slight lowering of RQ_{ia} at elevated $p^{e}_{CO_2}$ observed in this study suggested there was a larger effect of $p^{e}_{CO_2}$ on r_{CO_2} than r_{O_2}, and resulted in smaller Δp_{CO_2} compared with Δp_{O_2}. Elevated CO_2 (5%) has also been reported to reduce r_{CO_2} to a larger extent than r_{O_2} of postclimacteric 'COP' (Fidler and North, 1967). This may result from increased fixation of CO_2 into organic acids, and reduced catabolism of organic acids (Murata and Miniamide, 1970). The higher RQ_{ia} of 'Braeburn' at 0° compared to 20°C may have resulted from a higher temperature coefficient for r_{O_2} compared to r_{CO_2} (Fidler and North, 1967). However, RQ_{ia} is not an absolute measure of *RQ*, and depends both on *RQ* and the relative permeance of the fruit skin to CO_2 and O_2. Therefore, lower RQ_{ia} for 'Braeburn' compared to 'COP' apples at 20°C (Fig. 6.6, and k_5 values in Table 6.5), indicated 'Braeburn' fruit had a higher permeance to CO_2 relative to O_2 compared to 'COP' apples, as has been previously reported (Yearsley *et al.*, 1996d). This may have indicated a higher permeability of the cuticle to CO_2 than O_2, and/or a larger component of diffusion occurred through the cuticle compared to pores for 'Braeburn' compared to 'COP' apples (Banks *et al.*, 1993b). The slightly lower RQ_{ia} for 'COP and particularly 'Braeburn' apples at 20° compared with 0°C and 0 kPa $p^{e}_{CO_2}$ (compare Figs. 6.6 and 6.7), might also be explained by differential effects of

temperature on diffusion through cuticles and pores. With increasing temperature, cuticular diffusion would increase more than diffusion through pores which is relatively temperature-independent (Cameron *et al.*, 1994). As O_2 diffusion in apples is dominated by pores, and diffusion of CO_2 occurs through pores and cuticle (Banks *et al.*, 1993b), increasing temperature would have a greater effect on increasing P_{CO_2} than P_{O_2}, resulting in decreased RQ_{ta}.

CO_2-related disorders include browning and cavities in defined areas of cortex and core tissue injury (Meheriuk *et al.*, 1994; Padfield, 1969), 'scald-like' symptoms purported to result from free-radical-catalysed oxidation of proteins and other macromolecules (Burmeister and Dilley, 1995), and interactions with low temperature injury (Padfield, 1969). CO_2-related disorders may develop at $p^i_{O_2}$ and $p^i_{CO_2}$ above the onset of fermentation, reducing mitochondrial function and enhancing other oxidative processes. Therefore, it is critical to integrate information on storage disorders with *LOL*'s to establish optimum storage atmospheres for apple cultivars.

In conclusion, for postclimacteric 'COP' and 'Braeburn' apples, effects of elevated $p^e_{CO_2}$ on fruit metabolism were not strongly reflected in an increase in *LOL*' s of either cultivar at 20•C. The increase in FT^i_{RQ} and FT^i_{EtOH} of fruit kept in 8 kPa $p^e_{CO_2}$ at 0°C may have resulted from the higher solubility of CO_2 at the lower temperature. Elevated $p^e_{CO_2}$ resulted in a small reduction in r_{O_2} (as measured by Δp_{O_2}) at 20•C, but there was less evidence of an effect at 0°C. RQ_{ta} was lower for apples at 20°C and 8 kPa compared to 0 kPa $p^e_{CO_2}$ and more markedly so for apples at 0°C. As both 'COP' and 'Braeburn' apples are susceptible to CO_2-related disorders during cool-storage in elevated CO_2, recommendations for optimum storage atmospheres based on *LOL*'s would need to be adjusted for the potential to develop disorders.

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Chapter 7

Effect of Physiological Age on the Lower Internal Oxygen Limits of Apple Fruit

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7.1 Abstract

The effects of physiological age on steady-state lower internal O_2 limits (LOL^i) were estimated for 'Cox's Orange Pippin' ('COP') and 'Braeburn' apples at 0° and 20°C. Two LOL^is were estimated from both external chamber and core cavity atmospheres: the anaerobic compensation point (ACP^i), and the internal fermentation threshold (FT^i_{RQ}). Mean ACP^i of 'COP' fruit at 0° and 20°C were similar and varied between 0.5 and 0.8 kPa $p^i_{O_2}$. Values for mean FT^i_{RQ} of 'COP' fruit were slightly higher than ACP^i and varied between 0.6 and 1.0 kPa $p^i_{O_2}$. A similar range of values for ACP^i and FT^i_{RQ} were estimated for 'Braeburn' fruit at 0°C, but values at 20°C were higher for pre- and postclimacteric fruit. Although significant physiological changes, commensurate with time in storage, were observed in both cultivars for fruit firmness, soluble solids content, background skin colour, starch index, respiration rate and ethylene production rate, no consistent changes in LOL^is of either cultivar were observed in relation to physiological age. Tissue porosity of 'COP' increased significantly with time in storage and may partially explain the lack of increase in LOL^is with age. Higher LOL^is of preclimacteric 'Braeburn' at 20°C may have resulted from poor connectivity of the intercellular air spaces, as the tissue could only be partially infiltrated, and higher LOL^is of postclimacteric 'Braeburn' from loss of cellular homeostasis. The results contrast with studies that indicate LOLs based on atmospheres external to the fruit (LOL^es) increase with physiological age. Controlled and modified atmospheres optimised using LOL^is rather than LOL^es are less likely to

be affected by physiological changes associated with ripening and senescence at storage temperatures.

Keywords: Malus domestica; Cox's Orange Pippin; Braeburn; Maturity; Internal atmosphere; Anaerobic compensation point; Fermentation threshold; Skin permeance; Respiration rate; Ethylene production rate; Bootstrap

7.2 Introduction

The extent to which modification of the storage atmosphere benefits postharvest quality of a crop depends on cultivar and strain, physiological age, initial quality, level of O_2 and CO_2, temperature and duration of exposure (Kader, 1989). Numerous empirical studies have estimated the tolerance or lower oxygen limits of apples on the basis of external or package atmospheres (LOL^es; Blackman, 1928; Gran and Beaudry, 1993a and 1993b; Thomas and Fidler, 1933). However, it is the concentrations of O_2 and CO_2 in the cytosol, close to equilibrium with the partial pressures of O_2 and CO_2 in the intercellular air spaces, that directly affect physiological processes. Therefore, lower O_2 limits based on internal atmospheres (LOL^is) are likely to more accurately estimate the tissue *LOL* (Yearsley *et al.*, 1996a). The difference between LOL^is and LOL^es is a consequence of the differences in partial pressure of CO_2 and O_2 between internal and external atmospheres, which are directly proportional to respiration rate and inversely proportional to skin permeance to gas exchange. Therefore, LOL^is should be less susceptible to fluctuation as they account for differences between fruit in respiration rate and skin permeance (Banks *et al.*, 1993; Yearsley *et al.*, 1996a). For example, temperature has been demonstrated theoretically and empirically to have a marked effect on LOL^es of apples (Badzie *et al.*, 1993; Gran and Beaudry, 1993b), but had little effect on LOL^is of postclimacteric 'Cox's Orange Pippin' ('COP') and 'Braeburn' apples, except at temperatures ≥ 28°C (Yearsley *et al.*, 1996c).

Physiological age of apples has also been reported to affect LOL^es (Fidler, 1933; Kidd and West, 1937; Thomas and Fidler, 1933), and Fidler (1933), noted varietal differences in the shifts in the extinction point (*EP*) of anaerobic respiration as apples

aged. He suggested the first shift in *EP* may indicate "incipient disorganisation" of "the respiration centres", and seasonal effects resulting from a complex of environmental factors affecting fruit on the tree. Boersig *et al.* (1988) found ACP^e increased as pear tissue aged, and *ACP* of pear cell suspensions decreased as the diffusion coefficient of the medium increased. Preclimacteric pears have been reported to be less stressed by low O_2 and to have greater potential for posthypoxic recovery than pears of more advanced physiological age (Nanos *et al.*, 1992). Diminution of aerobic but not anaerobic CO_2 evolution rate was observed in senescing 'Red Delicious' and 'McIntosh' apples (Dilley *et al.*, 1964).

The foregoing evidence suggests a strong link between physiological age and LOL^es. However, there are no reports of changes in LOL^is with ageing of whole fruit, and it might be expected that the rapid respiratory and compositional changes that occur at the climacteric should affect the aerobic-anaerobic transition for climacteric fruits such as apples. It might also be anticipated that LOL^is would increase for ageing whole apples as a consequence of senescence, diminished maintenance of cellular integrity, and lower diffusivity of O_2 in the tissue as intercellular air spaces become blocked and/or connectivity reduced (Rajapakse *et al.*, 1990). These propositions were tested in this study for two physiologically contrasting cultivars of apple, 'COP' and 'Braeburn' at 0° and 20°C, and the implications of the results for optimising controlled atmospheres (CA) and modified atmospheres (MA) based on LOL^is and LOL^es discussed.

7.3 Materials and methods

7.3.1 Fruit supply, initial measurements, treatments and storage

Freshly harvested, preclimacteric 'COP' and 'Braeburn' apples (*Malus domestica* Borkh.; mean mass 0.145 kg for 'COP' and 0.184 kg for 'Braeburn') were obtained from a Hawkes Bay orchard, N.Z. The fruit were held at 20°C overnight and initial measures of fruit mass, fruit firmness (0 to 12 kgf Bryce press-mounted penetrometer

with an 11 mm head), soluble solids content (0 to 20% Atago refractometer); starch content (using ENZA New Zealand (International) starch-iodine index charts), background skin colour (Minolta CR-200 Chromameter measuring lightness (L), and hue angle (H°), Minolta Camera Co., Ltd., Japan) made on 20 fruit. Trays of fruit were randomly allocated to treatments and climacteric and postclimacteric treatments stored in cartons, at either 2°C for 'COP' (enclosed in perforated polymeric film bags), or 0°C for 'Braeburn' (without bags). Preclimacteric fruit were not cool-stored, but used 72 h after harvest.

7.3.2 Selection of experimental fruit

The three physiological ages required for this study (nominally classified as preclimacteric, climacteric and postclimacteric stages) were selected on the basis of the steady-state partial pressure of ethylene of surface chambers ($p^{i}_{C_2H_4}$). Fruit used for each treatment were equilibrated in air at 20 ± 0.5°C for 24 h, and 1000 mm^3 glass surface chambers (9 mm diameter) were adhered to the skin at an equatorial position on 120 blemish-free fruit using epoxy adhesive (5 minute cure Araldite®, Ciba-Geigy, Auckland, N.Z.), and sealed as described by Yearsley *et al.* (1996a). Approximately 24 h after sealing the chambers, $p^{i}_{C_2H_4}$ in each chamber was measured, and 95 fruit with the closest $p^{i}_{C_2H_4}$ selected, and randomly allocated to treatments.

7.3.3 Controlled atmosphere treatments

Experiments were conducted in 1.0 m^3 controlled temperature cabinets (Spaceline, Muller-McAlpine, Auckland, N.Z.). Each experiment used 5 CA containers each enclosing six fruit at both 0° and 20°C. Metered, humidified gas mixtures flowed through each CA container (total flow 1.7 x 10^3 mm^3 s^{-1}), and the partial pressure of O_2 external to the fruit ($p^{e}_{O_2}$, Pa) used in each container identified using a steady-state model (Dadzie, 1992) as described by Yearsley *et al.* (1996a). Mean $p^{e}_{O_2}$ was controlled within ± 0.2 kPa of that required. Within the CA containers, Tyvek® (DuPont, New Zealand Ltd., Auckland, N.Z.) sachets containing 50 g of both CO_2

absorber (soda lime, Ajax chemicals, Auburn, NSW, Australia) and C_2H_4 absorber (Purafil, Papworth Engineering, Cambridge, N.Z.) controlled the partial pressure of CO_2 external to the fruit ($p^e_{CO_2}$) ≤ 0.12 kPa and external partial pressure of ethylene ($p^e_{C_2H_4}$) ≤ 0.51 Pa. Mean temperature for monitored fruit varied ≤ 0.3°C between fruit in any position within the cabinet. Mean relative humidity within CA containers had been previously measured to be $\geq 90\%$ (Yearsley *et al.*, 1996c).

7.3.4 Gas measurements and analysis

Fruit allocated to CA treatments each had a surface chamber adhered at an equatorial position. Steady-state internal partial pressures of O_2 ($p^i_{O_2}$) and CO_2 ($p^i_{CO_2}$) were estimated as the equilibrated $p^i_{O_2}$ and $p^i_{CO_2}$ in the surface chambers after 86-90 h exposure to the CA treatments (Yearsley, 1996). Gas samples were removed by gas-tight syringe (Hamilton 100 mm^3) from the headspace of each surface chamber through a septum on the CA bags above the chamber and immediately analysed. Values for percentage composition of O_2 and CO_2 were determined using an O_2 electrode in series with a miniature infra-red CO_2 transducer (Yearsley *et al.*, 1996a). Mole fraction values for O_2 and CO_2 were converted to $p^i_{O_2}$ and $p^i_{CO_2}$ by adjusting for atmospheric pressure (Yearsley *et al.*, 1996b).

7.3.5 Respiration and ethylene production, skin permeance, cortical tissue porosity and density

Fifteen fruit randomly selected from the same population of experimental fruit were used to determine respiration rate (r_{CO_2}, mol kg^{-1} s^{-1}) and C_2H_4 production ($r_{C_2H_4}$, mol kg^{-1} s^{-1}) and internal atmosphere (IA) composition of the core cavity ($p^i_{O_2}$ and $p^i_{CO_2}$) approximately 68 h after CA treatments were imposed, as described by Yearsley *et al.* (1996b). Values for permeance of fruit to CO_2 and O_2 (P'_{CO_2} and P'_{O_2}, mol s^{-1} m^{-2} Pa^{-1}) were calculated using Fick's First Law of Diffusion (assuming RQ = 1; Banks *et al.*, 1995):

$$P_j^{'} = \frac{r_{CO_2} M}{\Delta p_j A} \qquad (7.1)$$

where:

A	=	fruit surface area (m^2)
Δp_j	=	difference between internal and external partial pressure of gas j (Pa)
M	=	fruit mass (kg)
$P_j^{'}$	=	fruit permeance to gas j (mol s^{-1} m^{-2} Pa)
r_{CO_2}	=	rate of transfer of CO_2 between internal and external atmospheres (mol kg^{-1} s^{-1}).

Fruit surface area was estimated from fruit mass ($A = 0.0581\ M^{0.685}$; Clayton *et al.*, 1995). Tissue porosity (ε, m^3 m^{-3}) and tissue density (ρ, kg m^{-3}) were estimated for fruit used for maturity assessments using 5 mm wide longitudinal wedges of tissue from which the skin had been removed, as described by Yearsley *et al.*, 1996a.

Results are presented in SI units but may be converted to other units as follows: For r_{CO_2}, at T (°C), 1 mol kg^{-1} s^{-1} = 1.5839 x 10^8 mg kg^{-1} h^{-1}, or ((T + 273.15) 3.341x 10^{-11} p_{tot}) ml kg^{-1} h^{-1}, where p_{tot} (Pa) is the total pressure in the system; for $r_{C_2H_4}$ at T (°C), 1 mol kg^{-1} s^{-1} = ((T + 273.15) / 3.341 x 10^{-14} p_{tot}) µl kg^{-1} h^{-1}; and for $P_{CO_2}^{'}$ and $P_{O_2}^{'}$ at T (°C), 1 mol s^{-1} m^{-2} Pa^{-1} = ((T + 273.15) / 1.203 x 10^{-3}) cm s^{-1} (Banks *et al.*, 1995).

7.3.6 Estimation of *LOL*^{i}s

The "bootstrap" statistical procedure (Yearsley *et al.*, 1996a) was used to estimate *LOL*^{i}s using steady-state $p_{O_2}^{i}$ and $p_{CO_2}^{i}$ of surface chambers and core cavity. Plots of $p_{CO_2}^{i}$ and relative respiration quotient calculated from IA data [RQ_{ia}; estimated as ($p_{CO_2}^{i} - p_{CO_2}^{e}$)/($p_{O_2}^{e} - p_{O_2}^{i}$)] versus $p_{O_2}^{i}$ were made, and graphs without axis titles or scales submitted to 15 trained panellists. Each panellist was asked to hand-draw curves through the data then mark on the curves the minimum point to identify ACP^{i}, or the point where the curve began to rise above the baseline to identify FT^{i}. 1000 bootstrap samples were obtained by random sampling with replacement from the

panellists' original observations, both for chamber and core data independently and on combined chamber and core data sets. Bootstrap means and bias-corrected (BCa) 95% confidence intervals around means (Efron, 1987) were calculated using Gauss software (Gauss, 1993). As the bootstrap procedure did not assume data to be normally distributed, the confidence intervals generated were not necessarily symmetrical about the mean.

7.3.7 Statistical analysis

Analyses of variance of fruit maturity variables, cortical tissue porosity and density, r_{CO_2}, $r_{C_2H_4}$, P'_{CO_2} and P'_{O_2} data were performed using the PROC GLM procedure of the SAS system (SAS, 1990).

7.4 Results

7.4.1 Descriptors of physiological age of apples

Significant physiological changes commensurate with time in storage were observed for fruit firmness, soluble solids content, background skin colour and starch index for both cultivars (Table 7.1). Chamber $p^i_{C_2H_4}$ increased for each population of fruit removed from storage over time (data not shown), as did estimates of $r_{C_2H_4}$ (Table 7.2). The $r_{C_2H_4}$ of 'COP' apples continued to increase significantly with time in storage, for fruit at both 0° and 20°C (Table 7.2). In contrast, r_{CO_2} remained the same for fruit at 0°C but had increased for fruit at 20°C after 1.3 weeks and remained constant at 14.4 weeks cool storage (Table 7.2). The $r_{C_2H_4}$ of 'Braeburn' apples also increased significantly at both experimental temperatures after 2.4 weeks cool storage but was lower after 11.3 weeks cool storage. The r_{CO_2} of 'Braeburn' at both temperatures increased as fruit became climacteric and either remained higher for fruit at 0°, or decreased again for fruit at 20°C.

Table 7.1 Fruit firmness, soluble solids content, background colour [lightness (L) and hue angle (H°)], and starch index, for 'Cox's Orange Pippin' and 'Braeburn' apples from a Hawkes Bay orchard stored at 2°C and 0°C respectively for various durations. The fruit were removed from cool storage and stored at 20°C in air for 24 h prior to measuring variables at 20°C. Values are least squares means and within cultivars, treatments [preclimacteric (Preclim.), climacteric (Clim.), and postclimacteric (Postclim.)] with different letters are significantly different ($p = 0.05$, n = 20).

Cultivar and time in storage (weeks)	Treatment (stage)	Fruit firmness (N)	Soluble solids content (%)	Background colour (L)	Background colour (H°)	Starch index[1]
'Cox's Orange Pippin'						
0.0	Prestorage	62.8 a	10.6 a	69.1 a	110.4 a	3.2
0.4	Preclim.	63.6 a	11.1 b	69.5 a	110.6 a	3.6
1.3	Clim.	62.5 a	12.0 c	70.5 a	107.4 b	4.7
14.4	Postclim.	35.3 b	12.0 c	75.5 b	97.0 c	6.0
'Braeburn'						
0.0	Prestorage	76.9 a	10.3 a	66.9 a	110.6 a	2.6
0.4	Preclim.	79.2 a	10.4 a	66.1 ab	110.1 a	2.8
2.4	Clim.	66.8 b	11.4 b	65.5 b	107.7 b	5.1
11.3	Postclim.	68.8 b	11.2 b	66.7 ab	106.3 c	6.0
Storage time comparisons (ANOVA p values)						
'Cox's Orange Pippin'		0.0001	0.0001	0.0001	0.0001	
'Braeburn'		0.0001	0.0004	0.1853	0.0001	

[1] Based on ENZA New Zealand (International) index charts; 0 = completely stained (high starch content), 6 = no staining (least starch content).

Table 7.2 Estimates of respiration rate (r_{CO_2}), ethylene production ($r_{C_2H_4}$), and skin permeance to CO_2 and O_2 (P'_{CO_2} and P'_{O_2}) for 'Cox's Orange Pippin' ('COP') and 'Braeburn' apples from a Hawkes Bay orchard stored at 2°C and 0°C respectively for various durations. Fruit were removed from cool storage, equilibrated at 0°C or 20°C in air and variables estimated after 3 days. Values represent least squares means. Within cultivars, treatments [preclimacteric (Pre.), climacteric (Clim.) and postclimacteric (Post.)] with different letters are significantly different (T tests (LSD), alpha = 0.05, n=15).

Cultivar and storage (weeks)	Treatment [T (°C); stage]	r_{CO_2}[1] (nmol kg^{-1} s^{-1})	$r_{C_2H_4}$[1] (pmol kg^{-1} s^{-1})	P'_{CO_2}[1] (nmol m^{-2} s^{-1} Pa^{-1})	P'_{O_2}[1] (nmol m^{-2} s^{-1} Pa^{-1})
'Cox's Orange Pippin'					
0.4	0; Pre.	28.9 a	0.8 a	0.183 a	0.195 a
1.3	0; Clim.	29.7 a	47.7 b	0.190 a	0.199 a
14.4	0; Post.	28.2 a	183.1 c	0.269 b	0.264 b
0.4	20; Pre.	93.8 a	0.01 a	0.273 a	0.264 a
1.3	20; Clim.	160.4 b	460.5 b	0.280 a	0.270 a
14.4	20; Post.	158.2 b	1192.1 c	0.348 b	0.334 b
'Braeburn'					
0.4	0; Pre.	10.3 a	0.01 a	0.071 a	0.076 a
2.4	0; Clim.	15.6 b	36.1 b	0.073 a	0.091 a
11.3	0; Post.	15.6 b	26.4 c	0.108 b	0.088 a
0.4	20; Pre.	55.0 a	0.05 a	0.156 a	0.109 a
2.4	20; Clim.	118.8 c	454.4 b	0.169 ab	0.123 ab
11.3	20; Post.	98.3 b	426.9 b	0.186 b	0.135 b
Storage time comparisons (ANOVA p values)					
'COP'	0°C	0.2974	0.0001	0.0001	0.0002
	20°C	0.0001	0.0001	0.0001	0.0001
'Braeburn'	0°C	0.0001	0.0001	0.0001	0.1452
	20°C	0.0001	0.0001	0.0042	0.0182

[1] For conversion to other units see section 7.3.5.

7.4.2 Cortical tissue porosity (ε), density (ρ)

On average, 'COP' apples had a higher cortical tissue porosity and lower density than 'Braeburn' apples (Table 7.3). Preclimacteric 'Braeburn' apple wedges were particularly difficult to completely infiltrate (data omitted), though this would not have affected the estimation of density. Neither porosity nor density of 'Braeburn' fruit changed significantly with time in storage. However, postclimacteric 'COP' were significantly more porous and less dense than preclimacteric and climacteric fruit.

A linear relationship existed between porosity and density (Fig. 7.1) for both cultivars, and differences in the variables between cultivars were clearly larger than between physiological stages within cultivars.

7.4.3 Fruit permeance to CO_2 (P'_{CO_2}) and O_2 (P'_{O_2})

Fruit permeance increased with time in cool storage, with a significant increase for 'COP' fruit between 1.3 and 14.4 weeks in storage, and for 'Braeburn' between 0.4 and 11.3 weeks (Table 7.2). Overall, estimates of P'_{CO_2} and P'_{O_2} for both cultivars were higher for fruit at 20° compared to 0°C. On average, 'COP' had similar P'_{CO_2} and P'_{O_2} at 0° and 20°C , whereas 'Braeburn' fruit were 1.4 times more permeable to CO_2 than O_2 at 20°C though values were similar at 0°C. Averaging over storage times, 'COP' apples were approximately 2.6 times more permeable to CO_2 and O_2 than 'Braeburn' at 0°C, and 1.8 and 2.4 times more permeable to CO_2 and O_2 respectively at 20°.

7.4.4 LOL^is as a function of physiological age

Steady-state chamber and core cavity $p^i_{O_2}$ and $p^i_{CO_2}$ were similar on average (data not shown), and resulted in LOL^is based on chamber and core atmospheres that were not significantly different at any physiological stage. Consequently, LOL^is based on combined chamber and core data are presented (Fig. 7.2).

Table 7.3 Cortical tissue porosity (ε) and density (ρ), for 'Cox's Orange Pippin' and 'Braeburn' apples from a Hawkes Bay orchard stored at 2°C and 0°C respectively for various durations. The fruit were removed from cool storage and stored at 20°C in air for 24 h prior to measuring variables at 20°C. Values are least squares means and within cultivars, treatments with different letters are significantly different ($p = 0.05$, n = 15).

Cultivar and Time in storage (weeks)	Treatment (stage)	Cortical tissue porosity (ε) ($m^3 m^{-3}$)	Cortical tissue density (ρ) ($kg\ m^{-3}$)
'Cox's Orange Pippin'			
0.0	Prestorage	NE[1]	NE
0.4	Preclimacteric	0.160 a	871.5 a
1.3	Climacteric	0.156 a	868.6 a
14.4	Postclimacteric	0.175 b	860.4 b
'Braeburn'			
0.0	Prestorage	NE	NE
0.4	Preclimacteric	NCI[2]	900.2 a
2.4	Climacteric	0.125 a	905.5 a
11.3	Postclimacteric	0.127 a	902.8 a
Storage time comparisons (ANOVA p values)			
'Cox's Orange Pippin'		0.0001	0.0059
'Braeburn'		0.0001	0.1593

[1] NE = not estimated.

[2] NCI = not completely infiltrated.

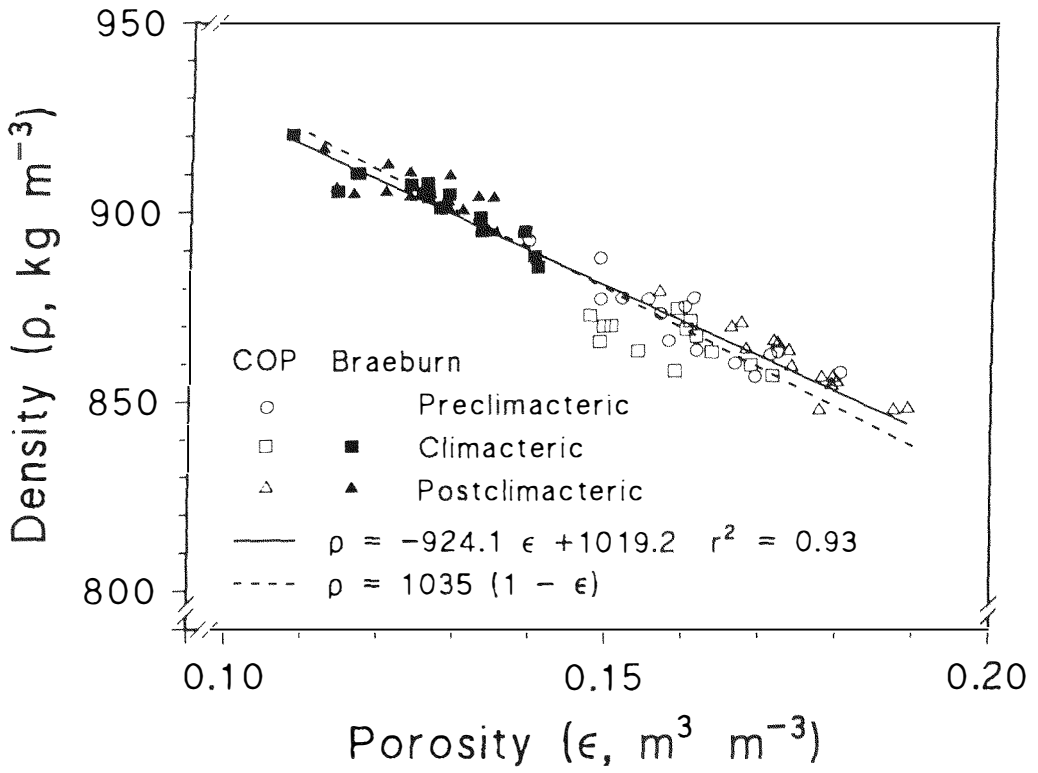

Figure 7.1 Cortical tissue density (ρ) as a function of porosity (ε) for 'Cox's Orange Pippin' (COP) and 'Braeburn' apples of different physiological stages. Fruit were from a Hawkes Bay orchard and either not stored (preclimacteric) or stored at 2°C and 0°C respectively for up to 14.4 weeks. Fruit were equilibrated for 24 h at 20°C before estimations were made. The solid line is the best fit to the data and the dashed line represents the relationship between ρ and ε based on juice density of 1035 kg m^{-3} (Hatfield and Knee, 1988).

7.4.4.1 ACP^i as a function of physiological age

Bootstrap means of ACP^is for 'COP' ranged between 0.55 and 0.75 kPa $p^i_{O_2}$. ACP^i did not shift significantly as a function of physiological age of 'COP' apples at 20° (Fig. 7.2, comparisons based on bootstrap 95% confidence intervals). However, at 20°C, there was an indication that ACP^is of postclimacteric 'COP' may have been slightly lower than apples at earlier stages. Fruit temperature did not significantly affect estimates of ACP^i for 'COP' apples.

ACP^i of 'Braeburn' apples ranged between 0.59 and 0.69 kPa $p^i_{O_2}$ at 0°C and 0.62 and 1.0 kPa $p^i_{O_2}$ at 20°C (Fig. 7.2). As with 'COP', ACP^i of 'Braeburn' apples did not shift significantly as a function of physiological age for fruit at 0°C, but at 20°C, pre- and postclimacteric fruit had significantly higher ACP^i than climacteric fruit at both 20° and 0°C.

7.4.4.2 FT^i_{RQ} as a function of physiological age

In general, for both cultivars and temperatures, estimates for FT^i_{RQ} were higher than those for ACP^i. FT^i_{RQ} for 'COP' apples at 0°C declined from 0.93 kPa to 0.65 kPa $p^i_{O_2}$ as fruit aged, and FT^i_{RQ} of preclimacteric fruit was significantly higher than for climacteric and postclimacteric fruit (Fig. 7.2). FT^i_{RQ} for 'COP' at 20°C, was generally similar to that at 0°C except for preclimacteric fruit which was significantly lower.

FT^i_{RQ} of 'Braeburn' apples at 0°C did not change significantly as fruit aged, and means varied between 0.72 kPa and 0.84 kPa $p^i_{O_2}$(Fig. 7.2). However, at 20°C FT^i_{RQ} was lower for climacteric compared to preclimacteric or postclimacteric fruit.

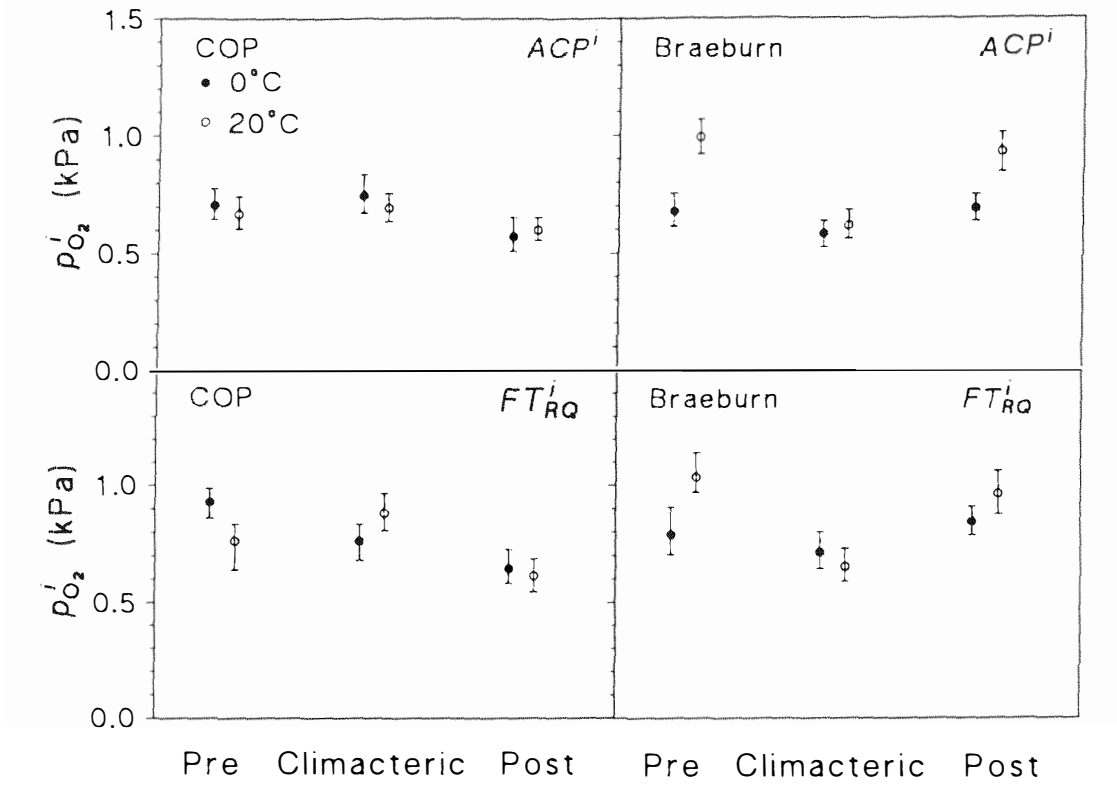

Figure 7.2 Bootstrap estimates of steady state internal lower oxygen limits (ACP^i and FT^i_{RQ}) based on partial pressures of O_2 and CO_2 of surface chambers and core cavities for 'Cox's Orange Pippin' and 'Braeburn' apples at 0° and 20°C for preclimacteric (Pre), climacteric and postclimacteric (Post) stages. Values represent bootstrap means and 95% bias corrected (BCa type) confidence intervals.

7.5 Discussion

Three distinct physiological stages of the fruit were identifiable based on $p^t_{C_2H_4}$, r_{CO_2} and $r_{C_2H_4}$ of fruit selected at different times in storage (Table 7.2), and indicated that the description of each stage as preclimacteric, climacteric and postclimacteric, was reasonable. Changes in fruit firmness, soluble solids, background skin colour and starch index, while not always significantly different between stages, were consistent with increasing physiological age and ripening during cool storage.

No significant differences in ACP^t of 'COP' apples were observed between temperatures or physiological age of the fruit. The slight downward trend in FT^t_{RQ} of postclimacteric 'COP' suggested fruit of that stage may have been more tolerant of hypoxia than those at earlier stages. In contrast, ACP^t and FT^t_{RQ} of pre- and postclimacteric 'Braeburn' were higher than climacteric fruit, particularly for fruit at 20°C, indicating that they were less tolerant of hypoxia than climacteric fruit. While temperature has a marked effect on LOL^ts, Yearsley *et al.* (1996c) reported the effect of temperature on LOL^ts was small except at temperatures higher than those used in this study. In the current work, LOL^ts for 'Braeburn' were similar at 0°C and 20°C for climacteric fruit. However, the contrasting finding for pre- and postclimacteric fruit indicates an interaction between effects of temperature and physiological age; inherent differences in fruit physiology were magnified at the higher temperature.

Physiological age may affect LOL^ts resulting from changes in the diffusion pathway of O_2 from the skin to mitochondria, solubility of O_2, or uptake of O_2 by oxidative processes. Each of these factors is assessed below as a potential explanation for the changes in LOL^ts observed in this study.

Diffusivity of gases in fruit tissue can decline as some fruits age (Ben-Yehoshua *et al.*, 1963; Rajapakse *et al.*, 1990), probably due to clogging of the intercellular air space as a result of cellular leakage (Blanke, 1991). However, if a decrease in diffusivity of gases in the cortex had occurred, significant gradients in CO_2 and O_2 between surface chamber and core cavity atmospheres would have developed and measured porosity would have declined. Gradients in internal atmosphere composition were not observed in the current study for chambers attached at an equatorial position, though it is not possible to rule out such gradients for other zones

of the fruit (Dadzie, 1992). Cortical tissue porosity of 'COP' was higher than 'Braeburn' (0.16 to 0.18 and 0.12 to 0.13 respectively), and for 'COP', actually increased significantly during storage. An increase in porosity of 'COP' apples with time in storage has been previously reported by Hatfield and Knee (1988). Values for porosity were marginally lower than those reported by Rajapakse *et al.* (1990) estimated using a similar method, and for New Zealand grown fruit of the same cultivars and similar firmness. The relationship between cortical tissue density and porosity can be estimated using the density of apple juice (Hatfield and Knee, 1988; see Eq. 5.13). Using this approach, and a value for juice density of 1035 kg m^{-3}, resulted in a relationship similar to that of the line fitted to the experimental data (Fig. 7.1). Increases in porosity of 'COP' fruit may explain the lack of increase in LOL^is with storage time and the slight downward trend in FT^i_{RQ}. They also suggest that connectivity of the intercellular air spaces remained high.

Increase in porosity of 'COP' apples may also explain the significant decrease in fruit firmness during storage (Table 7.3), and probably resulted from cell separation associated with the onset of the climacteric rise (Knee, 1991). Storage conditions, particularly RH, have been reported to affect diffusion properties of apple cortex, (Lidster, 1990; Hatfield and Knee, 1988; Wilkinson, 1965). It is possible that high RH during storage, particularly for 'COP' apples (enclosed in perforated polymeric film bags within cartons), assisted in maintaining cell turgor and porosity.

High LOL^is of postclimacteric 'Braeburn' were not related to porosity which did not change between 2.4 and 11.4 weeks in storage. However, as Burton (1982) noted, fruit with high tissue porosity can still be poorly aerated depending on the connectivity of the intercellular air spaces. While connectivity of the intercellular air space was not directly measured, inability to completely infiltrate preclimacteric 'Braeburn' tissue wedges suggested lack of connectivity, and may explain the high LOL^is for this stage. The decrease in LOL^is of climacteric 'Braeburn' might then have been a result of improved connectivity of the intercellular air spaces. If connectivity of tissue continued to increase with storage time, it would be expected that LOL^is would have remained low. The lack of increase in LOL^is of climacteric apples of both cultivars suggested that diffusivity of gases within the tissue was adequate to meet increased O_2 uptake during the respiratory climacteric. However,

as LOL^is increased in postclimacteric 'Braeburn', processes other than tissue diffusivity, such as reduced solubility of O_2 or loss of tight metabolic control leading to increased O_2 demand, may have been involved. It is possible that disorganisation of mitochondrial activity (Fidler, 1933), postulated by Romani (1987) to commence with the respiratory climacteric may have led to loss of tight metabolic control and contributed to the increased LOL^is.

Changes in cell composition, such as increasing soluble solids may also affect the availability of O_2 in the cytosol, as Leonard (1939) demonstrated that the solubility of O_2 decreases with increasing sugar concentration in solution. While a significant increase in soluble solids content was observed in this study (Table 7.1), and might have contributed to a shift in LOL^is, the effect was likely to be small compared to that of changes in diffusivity of O_2 within the cortex.

Skin permeance should not affect LOL^is, but it would be expected to have a significant effect on LOL^es (Dadzie *et al.*, 1993). Park *et al.*, (1993) reported skin permeance can change markedly during fruit maturation: permeance decreased then increased rapidly in 'McIntosh' apples, with the lowest permeance occurring just prior to, or coincident with, the ethylene climacteric. This change in permeance might result in an upward shift in LOL^es near the climacteric and may partly explain reports of a shift in LOL^es to higher levels of O_2 with increasing age of pipfruit (Boersig *et al.*, 1988; Fidler, 1933; Kidd and West, 1937; Thomas and Fidler, 1933). In the current study, P'_{CO_2} of early harvested 'COP' and 'Braeburn' apples were similar to those reported by Dadzie (1992). Interestingly, both P'_{CO_2} and P'_{O_2} increased significantly with storage time for both cultivars, and particularly for 'COP' (Table 7.2), and may have resulted from maintenance of a high relative humidity (RH) during storage, particularly for 'COP' (Lidster, 1990). Values for ACP^e, calculated from bootstrap estimates of ACP^i (Dadzie *et al.*, 1993; see Eq. 5.1), and estimates of r_{CO_2} (corrected for O_2 level effects) and P'_{O_2} (Table 7.2), were higher than ACP^i. The difference between values of ACP^i and ACP^e became increasingly large as 'Braeburn' fruit matured, and markedly for 'COP' fruit at 20°C. Increases in ACP^e were largely due to increases in respiration, whereas the decrease in ACP^e for postclimacteric 'COP' due to a marked increase in permeance.

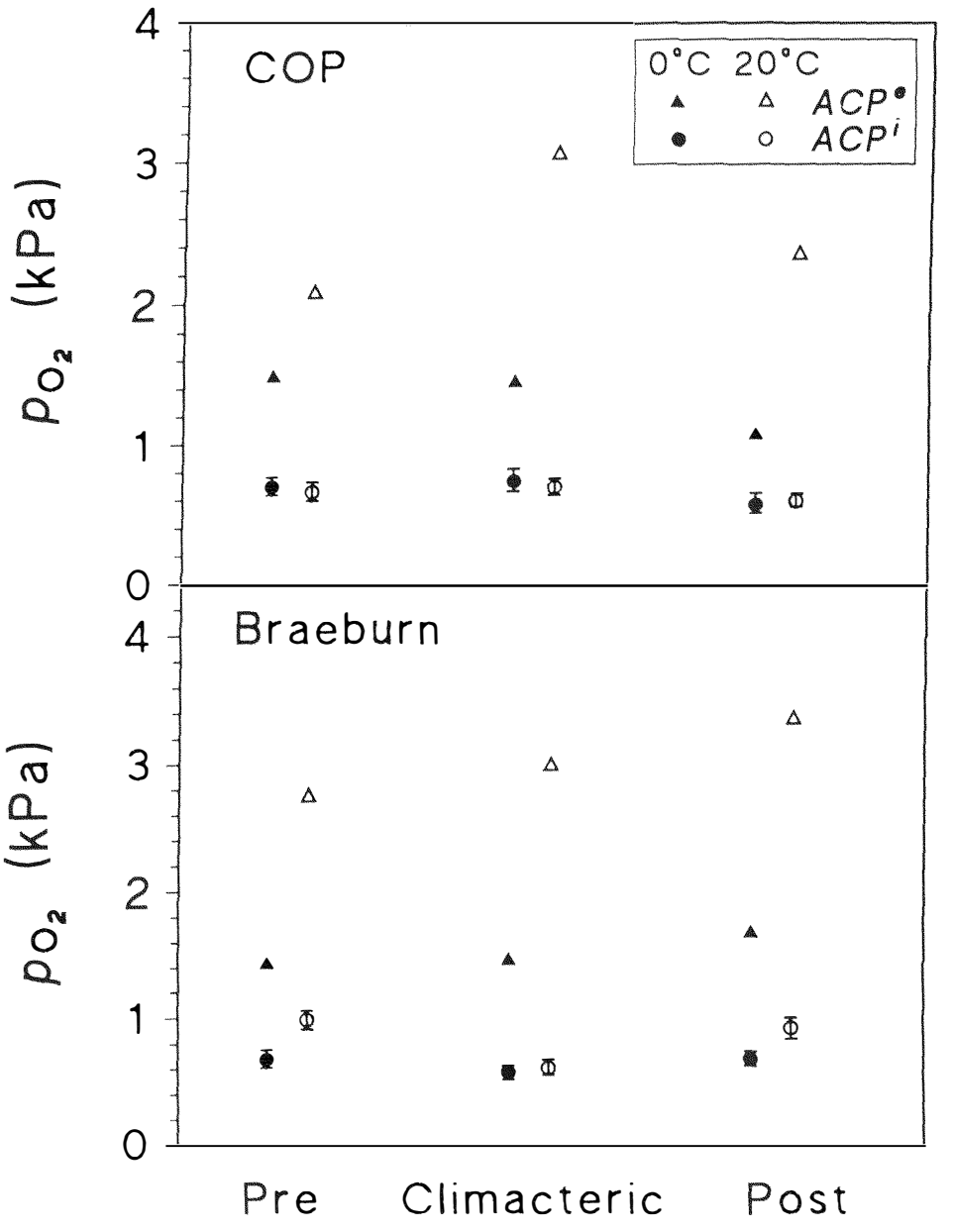

Figure 7.3 Steady-state external anaerobic compensation point (ACP^e) calculated from bootstrap estimates of internal anaerobic compensation point (ACP^i) for 'Cox's Orange Pippin' (COP) and 'Braeburn' apples at 0°C and 20°C and preclimacteric (Pre), climacteric and postclimacteric (Post) stages, based on combined chamber and core partial pressure data. Values of ACP^i represent bootstrap means and 95% bias corrected confidence intervals, and ACP^e were calculated using r_{CO_2} and P'_{O_2} values in Table 7.2.

Increased temperature has a marked affect on raising LOL^es (Dadzie *et al.*, 1993). This effect would be enhanced for apples with lower skin permeance such as 'Braeburn', compared to fruit with higher skin permeance such as 'COP'. Interestingly, in this study, both P'_{CO_2} and P'_{O_2} of 'Braeburn' apples were higher at 20°C compared to fruit at storage temperatures, but the increase in P'_{CO_2} was greater. Higher P'_{CO_2} compared to P'_{O_2} suggested CO_2 diffused through the skin by additional routes to that of O_2. Increased temperature would have had a similar and smaller effect on CO_2 and O_2 diffusion through pores (Cameron *et al.*, 1995). Pores are probably the dominant routes for CO_2 and O_2, diffusion to an extent that depends upon skin porosity (Banks *et al.*, 1993). However, CO_2 may also diffuse through the cuticle, and the larger increase in P'_{CO_2} implied temperature had a comparatively larger effect on the contribution of cuticular diffusion of CO_2.

These results have important implications for apples in MA packages that may be exposed to high temperatures out of storage. When optimising MA package atmospheres, particularly for cultivars with low skin permeance, effects of higher temperatures on $p^i_{CO_2}$ and $p^i_{O_2}$ resulting from higher respiration rates, and differences in skin permeance to CO_2 and O_2, must be accounted for to avoid fermentation. The effect of physiological age on raising LOL^is is more likely to be significant in climacteric fruits such as European and Asian pears, stonefruit and avocados which have low tissue porosity, and are more susceptible to flooding of the intercellular spaces as they age, compared to many cultivars of apples. High variability in skin permeance of individual fruit, variation in populations of the same cultivar from different seasons and orchards, and effects of temperature on respiration rate and differential permeance of the skin would interact to cause large variation in LOL^es. In contrast, LOL^is are less likely to be affected by temperature (Yearsley *et al.*, 1996c), level of external CO_2 (Yearsley *et al.*, 1996d) or physiological age. Storing of a crop in CA, close to but always just above its LOL, may therefore be achieved with greater reliability by monitoring fruit IA during storage if a practical method could be developed. However, both LOL^es and LOL^is relate to a fruit's respiratory metabolism. While this is clearly linked to many aspects of a fruit's storage behaviour, there may be other, more direct, effects of MAs on fruit physiology. For this reason, Wollin *et al.* (1985) suggested it may be necessary to continuously

change the external atmospheric composition in long-term CA to account for changing response to hypoxic conditions as fruit age in storage. Such an approach would clearly be much more difficult to achieve with MA packages.

In conclusion, LOL^is for 'COP' apples changed only slightly as fruit aged and may have reflected increases in cortical tissue porosity. In general, LOL^is of 'Braeburn' apples were higher than for 'COP', particularly for pre- and postclimacteric fruit at 20°C. Higher LOL^is of pre- and postclimacteric 'Braeburn' may have resulted from poor connectivity of the intercellular air spaces, and lack of tight metabolic control of cellular processes, respectively. Skin permeance to CO_2 and O_2 was greater at 20° than 0°C for both cultivars but, for 'Braeburn' fruit, greater increases in permeance to CO_2 relative to O_2 indicated increasing temperature had a greater effect on the permeance of the cuticle than pores. This would have important consequences for optimising atmospheres on the basis of LOL^os for MA packages, but would be less important for fruit stored in CA; it would not directly affect optimum atmospheres based on LOL^is.

7.6 Acknowledgements

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Chapter 8

Cannulation as a Technique for Monitoring Changes in Apple Internal Atmosphere

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8.1 Abstract

Determination of internal atmosphere (IA) composition and respiration rate (r_{CO_2}) of fruits and vegetables is required to characterise their response to modified atmospheres and surface coatings. Cannulation is a simple, invasive technique for repeated sampling of the atmosphere inside apples. In this study, cannulae inserted and sealed aseptically into apples did not result in phytotoxic responses or pathological infection. For preclimacteric/climacteric 'Braeburn' fruit monitored for 9 days at 20°C, r_{CO_2} was increased, and for postclimacteric fruit both r_{CO_2} and ethylene production ($r_{C_2H_4}$) were enhanced by cannulation. No significant differences between control fruit and those with multiple cannulae inserted were detected for r_{CO_2} or $r_{C_2H_4}$ of postclimacteric 'Cox's Orange Pippin' and 'Braeburn' apples at 20°C. For fruit that were not at physiological equilibrium, cannulation allowed a more accurate estimate of IAs of apples than chambers adhered to the fruit surface. Enhanced r_{CO_2} of cannulated preclimacteric/climacteric apples resulted in increased internal partial pressure of CO_2 and reduced O_2 compared to controls though this effect was absent in postclimacteric apples. Cannulation is particularly suitable for measuring IAs of postclimacteric fruit and for fruit storage trials, and more suitable than external chambers for repeated sampling of IAs of fruit under conditions when fruit metabolism is changing rapidly.

Key words: Malus domestica; External chambers; Modified atmosphere; Respiration; Ethylene production

8.2 Introduction

Accurate measurement of internal atmosphere (IA) composition and respiration of fruits and vegetables is required to study responses to modified atmospheres (MAs) and controlled atmospheres (CAs) during storage (Banks *et al.*, 1993). Modification of IAs can be achieved using polymeric film wraps, surface coatings or CAs. Variation in IAs between individual fruit results from differences in respiration rate, skin permeance to gas diffusion, and fruit surface area to mass ratio (Banks *et al.*, 1993; Cameron *et al.*, 1995; Dadzie *et al.*, 1993). Measurement of the difference in partial pressures of gas between internal and external atmospheres provides information required for estimation of fruit skin permeance to oxygen and carbon dioxide.

Several methods are available for sampling IAs. Because it is destructive, direct sampling by syringe from the core cavity or intercellular air space (Banks, 1983; Blanpied, 1968; Saltveit, 1982) is only suitable when a single sample is required. For repeated measurements, samples can be taken from small chambers fixed to the fruit which have been allowed to equilibrate with the IA (Banks and Kays, 1988; Cameron, 1982; Dadzie, 1992; Rajapakse *et al.*, 1990; Yearsley *et al.*, 1996). This technique has the advantage of being non-destructive and non-invasive. It is more suitable for crops with a highly porous surface, because of the period required for chambers to equilibrate with the IA. Also, chamber atmospheres may not equate with those at the centre of the crop due to gradients through the tissue (Dadzie, 1992). Chambers can also become dislodged from the crop during storage, and there can be uncertainty as to whether or not individual chambers have developed leaks. Cannulation is an alternative technique that allows repeated direct sampling from either the core cavity or artificial cavities within cortex tissue (Banks, 1983; Burg and Burg, 1965; Knee, 1980; Reid *et al.*, 1973; Solomos, 1989; Trout *et al.*, 1942). Cannulation is an invasive technique and concerns as to altered metabolism due to wounding have been raised by Burton (1974) and Kahal (1974). Although these effects have been shown to be minor in climacteric bananas (Banks, 1983) there has been no thorough study of the physiological effects of cannulation in apples.

In this paper we characterise these effects and discuss the suitability of an optimised cannulation procedure using a single cannula (experiment 1) or multiple cannulae (experiment 2) in either preclimacteric/climacteric and/or postclimacteric 'Cox's Orange Pippin' ('COP') and 'Braeburn' apples (*Malus domestica* Borkh.).

8.3 Materials and methods

8.3.1 Experiment 1: Surface chambers and cannulation

8.3.1.1 Fruit supply

Six cartons of freshly harvested, preclimacteric 'Braeburn' apples (commercially graded to size count 125; and with a mean mass of 0.157 kg) were obtained from an orchard in the Auckland region and transported to Massey University, Palmerston North, N.Z.

8.3.1.2 Treatments

Trays of fruit from the cartons were randomised, three cartons kept at 20°C, and a further three placed in 0°C storage for two months to repeat the treatments on what was presumed to be postclimacteric fruit. Fruit brought out of 0°C storage were first equilibrated to 20°C before treatments were applied. Three groups of 20 blemish-free fruit were randomly selected from the three cartons, and the following treatments applied:

1) controls (not treated, randomly selected at each sampling time for destructive sampling),
2) chambers (9 mm diameter glass chambers of approximately 1000 mm^3 volume adhered over a lenticel on each fruit at an equatorial position), and
3) on a single batch of fruit, chambers and cannulae
   a) chambers (adhered as for treatment 2) and,

b) cannula inserted aseptically into the core cavity as described below (see Fig. 8.1a).

Chambers were adhered to the surface using epoxy adhesive (24 hour cure Araldite®, Ciba-Geigy, Auckland, N.Z.). After attaching chambers and cannulae, fruit were left at 20°C in the dark for 24 hours before any measurements were taken. Rates of respiration and ethylene production (postclimacteric fruit only), and internal atmosphere composition were estimated 1, 2, 3, 5, 7 and 9 days after treatment. At the end of the experiment the fruit were cut open to assess internal damage.

8.3.2 Experiment 2: Multiple cannulation

8.3.2.1 Fruit supply

Preclimacteric 'COP' and 'Braeburn' (mean mass 0.145 and 0.184 kg respectively) were harvested on 28/2/95 and 27/3/95 respectively from a Hawkes Bay orchard, N.Z. Fruit were pretreated with 10.24 Pa ethylene at 20°C for 12 hours to induce a respiratory climacteric, then vented at 20°C for a further 48 hours. 'COP' fruit were enclosed in perforated polyliners in cartons and stored at 2°C, and 'Braeburn' fruit were stored in cartons without polyliners at 0°C.

8.3.2.2 Treatments

After 24 days in cool storage, fruit were removed and equilibrated at 20°C in the dark for 48 hours. Respiration and ethylene production were determined on thirty blemish free fruit, and twenty fruit with closely matching rates selected from these were randomly allocated to two treatments, ten fruit per treatment:

1) controls (non-cannulated), and

2) multiply cannulated fruit.

Multiply cannulated fruit had one cannula sealed into the core cavity as described below and three shorter (20 mm) cannulae sealed into 25 mm deep holes cut obliquely into mid cortex tissue at the stem-end shoulder, equatorial region and blossom-end shoulder as illustrated (Fig. 8.1a). The fruit were enclosed in a

perforated polybag to reduce weight loss and stored at 20°C in the dark. Respiration and ethylene production rates were estimated daily for 5 days, as described below.

8.3.3 Cannulation procedure

A small area (ca. 100 mm^2) at an equatorial position was surface sterilised using 95% ethanol. A core of tissue was removed through from the sterilised skin to the core cavity was using a sterilised 50 mm x 2 mm OD stainless steel needle (Phoenix, U.S.A.). The same sized needles were cut with a tube cutter to produce blunt-ended 35 mm x 2 mm OD cannulae. A sterile blunt cannula was inserted into the hole from which the core of tissue had been removed and sealed in position using 24 hour cure epoxy adhesive. After the adhesive had cured, the skin-adhesive-cannula interface was lightly covered with silicone grease (Molycote 111, Dow Corning Australia Pty. Ltd.). Cannulae were sealed by inserting the luer end of the lower portion (20 mm) of a 1000 mm^3 plastic syringe barrel into the cannula. A triple seal using water between two rubber plunger tips with a further water seal above these in the syringe barrel provided an air-tight seal through which samples of IA could be removed (Fig. 8.1a). A similar sealing system was used with the surface chambers.

8.3.4 Measurement of internal atmospheres

A gas-tight syringe (Hamilton, 100 mm^3) was used to remove 90 mm^3 samples from the headspace of surface chambers and cannulae. Direct sampling of IA from the core cavity of control fruit was achieved by inserting the needle of a 3000 mm^3 syringe into the core cavity and withdrawing a 3000 mm^3 sample while the fruit were submerged in water. A 90 mm^3 subsample was then used for analysis. IA samples were analysed immediately. Values for percentage composition of O_2 and CO_2 were determined using an O_2 electrode (Citicell C/S type, City Technology Ltd., London, U.K.) in series with a miniature infra-red CO_2 transducer (Analytical Development Company, Hoddeston, U.K.), with O_2-free N_2 as carrier gas (flow rate 580 $mm^3\ s^{-1}$). Mole fraction values for IA composition were converted to partial pressures of O_2

($p^i_{O_2}$, Pa) and CO_2 ($p^i_{CO_2}$) by adjusting for atmospheric pressure (Barigo electronic altimeter/barometer, GmbH, D-7330, Villimgen-Schwenningen, Switzerland).

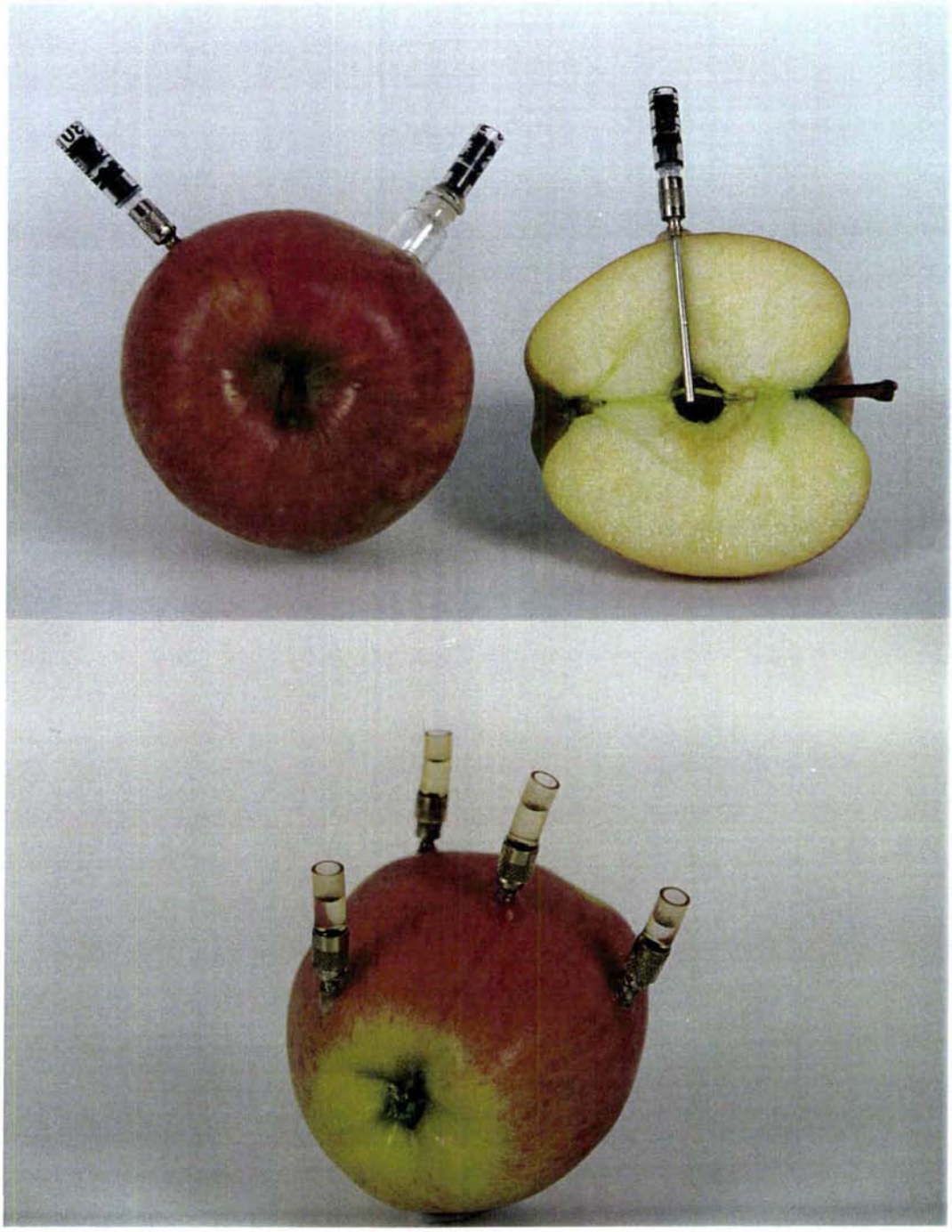

Figure 8.1 'Braeburn' apple with (a) a cannula inserted and sealed into the core cavity and glass chambers adhered to the surface in an equatorial region, and (b) 'Braeburn' apple with cannulae inserted and sealed into the core cavity, mid-cortex region at the stem-end shoulder, equatorial region and blossom-end shoulder.

8.3.5 Estimation of respiration rate and ethylene production rate

Rates of respiration (r_{CO_2}, mol kg^{-1} s^{-1}) and ethylene production ($r_{C_2H_4}$) were determined for all fruit at each sampling period before estimating IA composition. Fruit were sealed in 1.168 x 10^{-3} m^3 containers at 20°C in the dark. Two, 1000 mm^3 samples of the headspace were withdrawn after stirring jar contents, and this procedure was repeated exactly 30 minutes later. One sample was analysed for CO_2 and the other for ethylene. Ethylene partial pressures were determined by flame ionisation gas chromatography (Philips PU4500, Philips Activated Alumina 2 m x 4.0 mm glass column, gas flow rates of 500, 580, and 5830 mm^3 s^{-1} O_2-free N_2 carrier, H_2 and air respectively, and 130, 150 and 200°C for column, injector and detector temperatures).

8.3.6 Data analysis

Where appropriate, untransformed data were subjected to analysis of variance using the PROC GLM procedures of the SAS system (SAS, 1990) for data at each sampling time, and repeated measures analysis with polynomial contrasts was used to examine trends with time.

8.4 Results

8.4.1 Experiment 1: Surface chambers and cannulation

For each of the variables estimated above for preclimacteric/climacteric and postclimacteric fruit, analysis of variance detected highly significant mean polynomial effects of time after treatment up to the fifth power. Thus, while changes with time were demonstrated with a high degree of confidence, the complexity of the changes meant that the polynomial model itself was of little further use in

interpreting the data. The following observations are based on subjective assessment of trends with time.

8.4.1.1 Respiration rate (r_{CO2})

The r_{CO_2} of the sample of fruit with both cannulae and chambers was significantly higher than fruit with only chambers attached throughout the experiment (Fig. 8.2a). However, the relative increase in r_{CO_2} over time was similar to that of fruit with only chambers attached. Interestingly, r_{CO_2} of control fruit were higher than fruit with either cannulae or chambers attached after day 5. The r_{CO_2} of postclimacteric fruit in all treatments showed no particular trends over 9 days (Fig. 8.2b). Cannulated fruit had higher r_{CO_2} than with only chambers on, but were similar to samples of control fruit, or lower than control fruit on days 5 and 9.

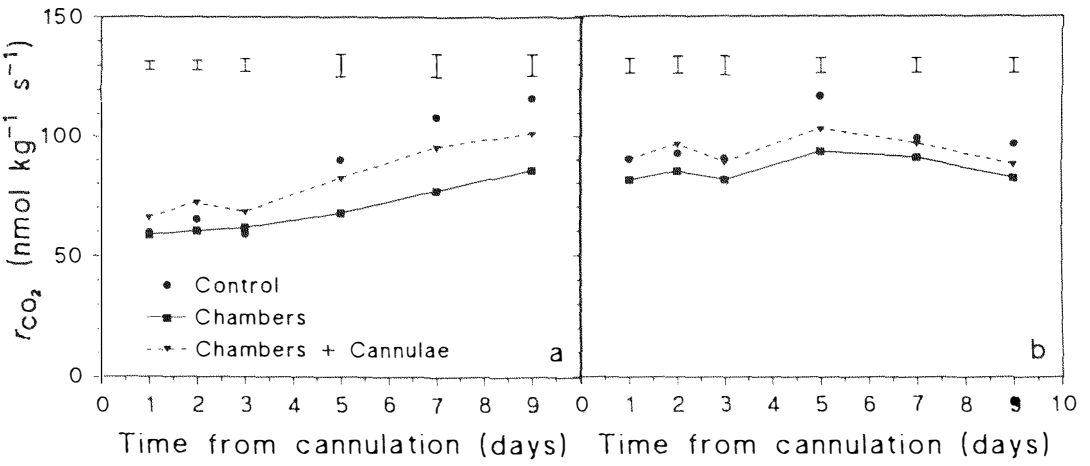

Figure 8.2 Respiration rate (r_{CO_2}) of (a) preclimacteric/climacteric and (b) postclimacteric 'Braeburn' apples at 20°C. Control fruit were not cannulated and a separate group was selected at each sampling time. Fruit in a second treatment had chambers attached to the surface at an equatorial position and those in a third treatment had vials attached to the surface at an equatorial region and cannulae inserted and sealed into the core cavity at an equatorial position. Values represent least squares means and the bars represent 95% confidence intervals (n=20). For r_{CO_2}, 1 nmol kg^{-1} s^{-1} = 0.1584 mg kg^{-1} h^{-1} (Banks *et al.*, 1995a).

8.4.1.2 Internal ethylene partial pressure (p^{i}_{C2H4})

For preclimacteric/climacteric fruit, the number of fruit with $p^{i}_{C_2H_4} \geq 0.1$ Pa increased markedly during the first 5 days for all treatments (Fig. 8.3), and more cannulated fruit than samples of control fruit had core $p^{i}_{C_2H_4} \geq 0.1$ Pa. There was a lag in $p^{i}_{C_2H_4}$ accumulation in chambers (data not presented) and this was seen in fewer fruit with $p^{i}_{C_2H_4} \geq 0.1$ Pa when estimated from chambers on cannulated fruit rather than the core (Fig. 8.3).

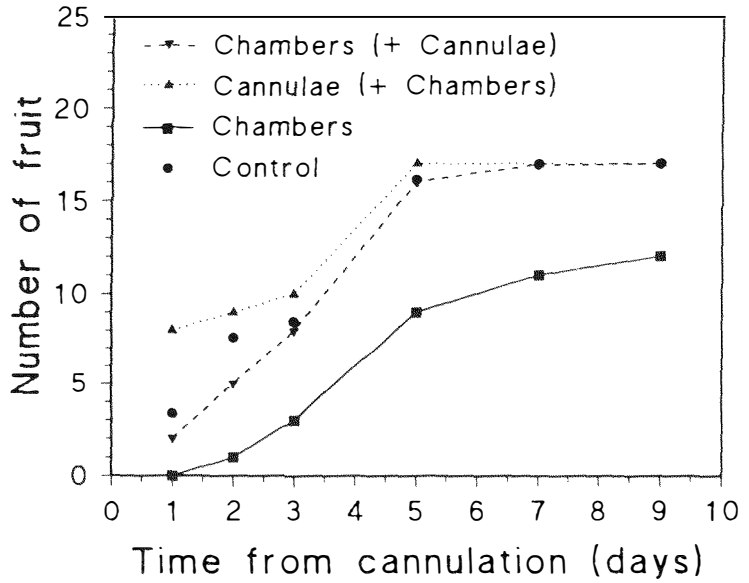

Figure 8.3 Number of fruit with internal ethylene partial pressure ($p^{i}_{C_2H_4}$) ≥ 0.01 Pa for preclimacteric/climacteric 'Braeburn' apples over 9 days at 20°C (n=17). Treatments are described in the caption to Fig. 8.2.

8.4.1.3 Ethylene production rate (r_{C2H4})

The $r_{C_2H_4}$ was estimated for postclimacteric fruit only and increased steadily for all treatments during the first 5 days (Fig. 8.4). Samples of control fruit had significantly higher $r_{C_2H_4}$ than other treatments (except for cannulated fruit on days 1 and 7), and cannulated fruit had significantly higher $r_{C_2H_4}$ than fruit with only chambers attached, (except on days 1 and 3).

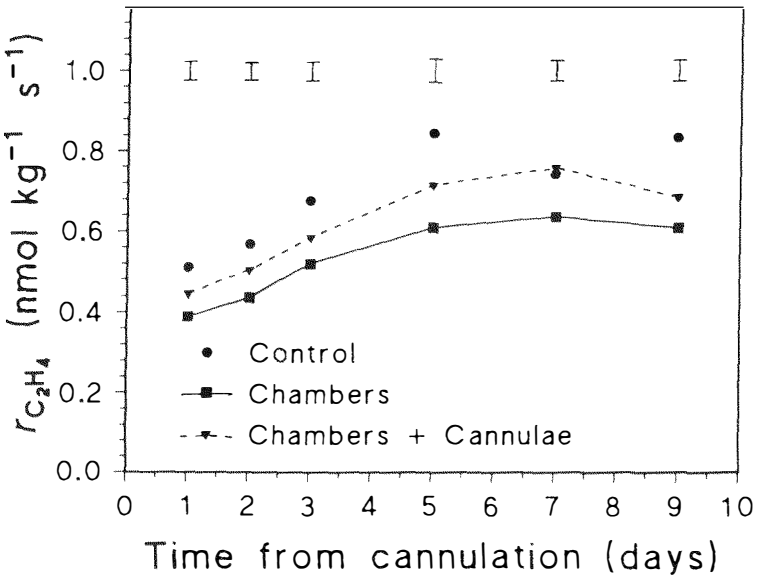

Figure 8.4 Ethylene production rate ($r_{C_2H_4}$) of postclimacteric 'Braeburn' apples at 20°C. Treatments are described in the caption to Fig. 8.2. Values represent least squares means and the bars represent 95% confidence intervals (n=20). For $r_{C_2H_4}$, 1 nmol kg^{-1} s^{-1} = 0.1008 mg kg^{-1} h^{-1} (Banks *et al.*, 1995a).

8.4.1.4 *Internal partial pressures of carbon dioxide (p^{i}_{CO2}) and oxygen (p^{i}_{O2})*

A steady increase in $p^{i}_{CO_2}$ and concomitant decrease in $p^{i}_{O_2}$ over time was observed for preclimacteric / climacteric fruit (Figs. 8.5 a and b). Estimates of core $p^{i}_{CO_2}$ for samples of control fruit and cannulated fruit were not significantly different over the 9 days of the experiment. Core $p^{i}_{CO_2}$ of cannulated fruit was significantly higher than in chambers on the same fruit (except on day 9), but significantly lower than for core $p^{i}_{CO_2}$ of control fruit on days 1 and 3. As with $p^{i}_{CO_2}$, there was a significant gradient between core and chamber $p^{i}_{O_2}$ in cannulated fruit except for days 7 and 9, and at day 9, $p^{i}_{O_2}$ values were similar for all treatments.

Changes in $p^{i}_{CO_2}$ and $p^{i}_{O_2}$ were not as marked for postclimacteric fruit as preclimacteric / climacteric fruit (Figs. 8.5 c and d). Core $p^{i}_{CO_2}$ of control fruit were significantly higher than for cannulated fruit (except on days 2 and 5), and there was a significant gradient between core and chamber $p^{i}_{CO_2}$ on days 1 and 2. There were no significant differences between treatments for $p^{i}_{O_2}$ with the exception that on day 1, chamber $p^{i}_{O_2}$ was lower for cannulated fruit than fruit with only chambers attached.

8.4.1.5 *Physical damage resulting from cannulation*

No tissue damage (oxidative browning, water soaking or pathological lesions) were observed in any fruit as a result of cannulation. The use of epoxy resin to adhere cannulae to the fruit did not cause any visible phytotoxic response in the skin.

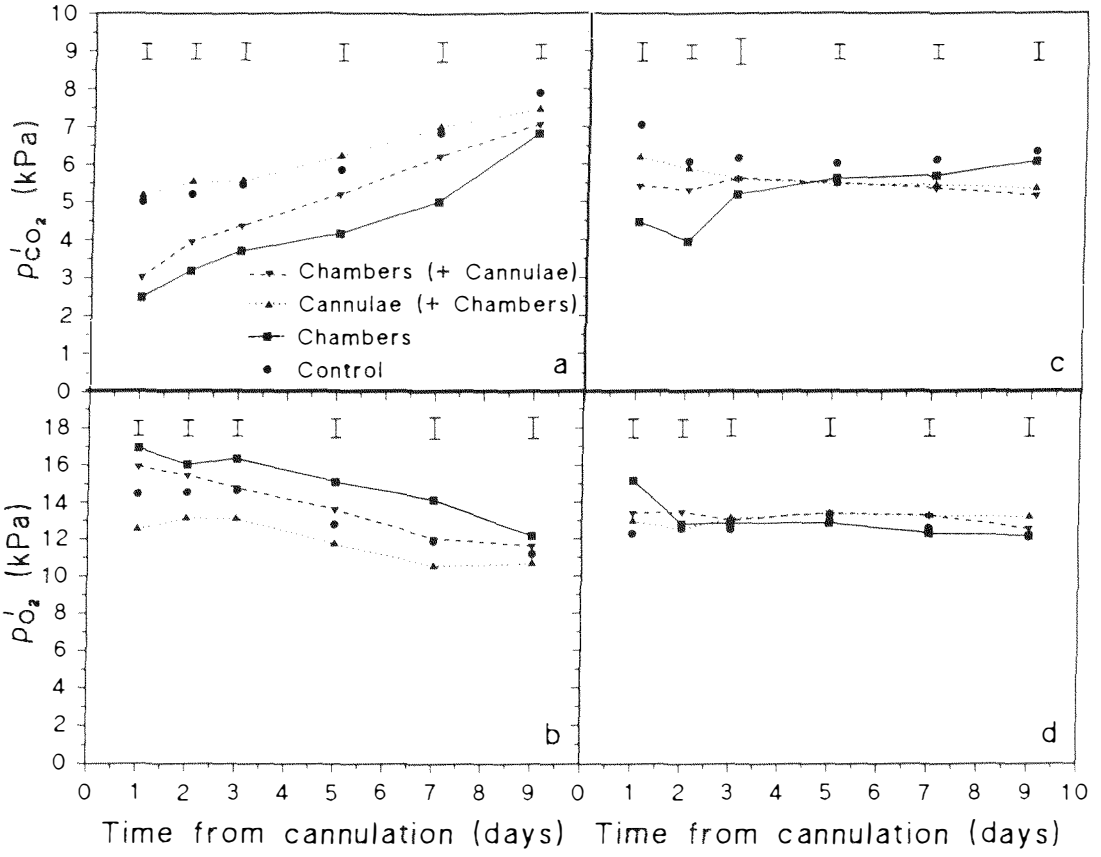

Figure 8.5 Internal partial pressures of carbon dioxide (p'_{CO_2}) and oxygen (p'_{O_2}) of (a and b) preclimacteric/climacteric and (c and d) postclimacteric 'Braeburn' apples at 20°C. Treatments are described in the caption to Fig. 8.2. For treatment 3, values for chambers and cannulae are plotted separately. Values represent least squares means and the bars represent 95% confidence intervals (n=20).

8.4.2 Experiment 2: Multiple cannulation

The only significant mean polynomial effect of time found in r_{CO_2} and $r_{C_2H_4}$ data was a quadratic effect ($p = 0.044$) for $r_{C_2H_4}$ for 'COP' fruit, indicating the polynomial model itself was of little further use in interpreting the data. The following observations are based on subjective assessment of trends with time.

8.4.2.1 Respiration rate

For postclimacteric 'COP' apples, there was a slight trend of increasing r_{CO_2} over 5 days, but no significant differences between control and cannulated fruit (Fig. 8.6a). Estimates of r_{CO_2} of postclimacteric 'Braeburn' were lower than for 'COP' apples and did not increase over time. Control fruit had significantly higher values than multiply cannulated fruit for the first four days (Fig. 8.6c).

8.4.2.2 Ethylene production rate

There was a marked increase in $r_{C_2H_4}$ for both control fruit (2 fold) and multiple cannulated (2.5 fold) postclimacteric 'COP' (Fig. 8.6b). However, there was no significant difference between treatments over 5 days. Although overall $r_{C_2H_4}$ was lower for postclimacteric 'Braeburn' apples, a similar increase occurred for control (2 fold) and multiply cannulated (2.3 fold) fruit (Fig. 8.6d). The $r_{C_2H_4}$ of 'Braeburn' control fruit was higher (significantly on day 2, $p = 0.016$) than multiply cannulated fruit.

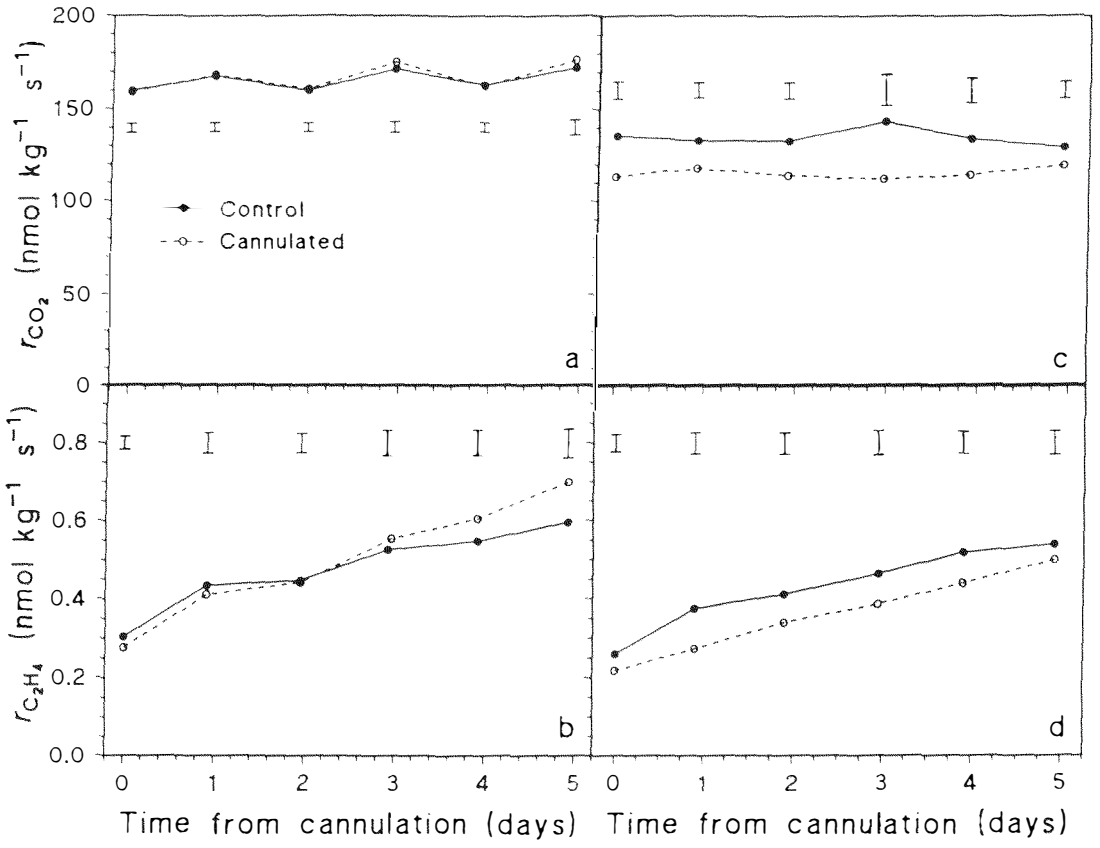

Figure 8.6 Respiration rate (r_{CO_2}) and ethylene production rate ($r_{C_2H_4}$) for postclimacteric 'Cox's Orange Pippin' (a and b) and 'Braeburn' (c and d) apples at 20°C. Control fruit were not cannulated and cannulated fruit had cannulae inserted and sealed into the core cavity, mid-cortex region at the stem-end shoulder, equatorial region and blossom-end shoulder. Values represent least squares means and bars represent 95% confidence intervals (n=10). For r_{CO_2}, 1 nmol kg^{-1} s^{-1} = 0.1584 mg kg^{-1} h^{-1}, and for $r_{C_2H_4}$, 1 nmol kg^{-1} s^{-1} = 0.1008 mg kg^{-1} h^{-1} (Banks *et al.*, 1995a).

8.5 Discussion

Ideally, a procedure for repeated sampling of IA of apple fruit, whether for optimising storage atmospheres or determining response to postharvest treatments, should be simple enough to implement on large numbers of fruit and have no or negligible physical or physiological effects. Cannulation, clearly meets the first criterion of simplicity of application. Cannulae can be simply and rapidly prepared from hypodermic needles of sufficient gauge to allow the needle of a sampling syringe to be inserted.

Physical damage to internal tissue resulting from cannulation was minimal. Aseptic procedure for insertion of cannulae ensured there were no visible pathological effects in either experiment. This same procedure has been used in trials for a number of apple cultivars stored at 0°C for several months without fungal or bacterial lesions developing. No phytotoxic responses of the apple skin to the epoxy resin used to adhere the cannulae in position were observed.

Cannulation stimulated r_{CO_2} of 'Braeburn' apples, resulting in a significant 15% mean increase for preclimacteric/climacteric fruit compared to fruit with only chambers attached, and an 8.8% mean increase for postclimacteric fruit (Figs. 8.2 a and b). The difference in r_{CO_2} between treatments for preclimacteric/climacteric but not postclimacteric fruit increased over time. For preclimacteric/climacteric fruit, the number of fruit which had entered the climacteric increased during the experiment in all treatments, resulting generally in an increase in r_{CO_2}. Interpreting the effect of cannulation was complicated in that the increase in r_{CO_2} for preclimacteric/climacteric control fruit was higher than for cannulated fruit, implying that control fruit samples (though selected randomly at each time period) were not physiologically similar. The data, particularly from direct sampling of control fruit, illustrate the contribution of between fruit variability to overall variance. However, cannulation with its potential for repeated sampling largely overcomes this problem, and fruit with only chambers attached (treatment 2) were the relative control for cannulated fruit (treatment 3). Although there were real physiological differences

between fruit within treatments, these were minimal compared to the effect of treatments on fruit physiology.

Reid *et al.* (1973) found that insertion of a needle to sample the IA of preclimacteric 'COP' apples at 12°C caused a slight increase in r_{CO_2} and $r_{C_2H_4}$, but levels returned to that of presampled fruit after 3 days, without suggestion of starting a continuous period of ethylene production. The increase in r_{CO_2} (Fig. 8.2a), and particularly the marked increase in $p_{C_2H_4}^{i}$ (Fig. 8.3), indicated a high proportion of preclimacteric fruit became climacteric regardless of cannulation over the duration of the experiment. However, cannulation of preclimacteric apples may advance the onset of the climacteric (Fig. 8.3), a feature which would need to be taken into account when designing experiments to be carried out on fruit at 20°C. The extent of this effect is likely to be less marked in studies using fruit at low temperatures. On this basis, cannulation appears to be most suitable for comparative studies of treatments applied to fruit in the climacteric or postclimacteric stage.

In contrast to the results of experiment 1, multiple cannulation of both postclimacteric 'COP' and 'Braeburn' apples did not significantly increase either r_{CO_2} or $r_{C_2H_4}$ over 5 days at 20°C. For 'COP' apples, $r_{C_2H_4}$ of multiply cannulated fruit increased at a slightly higher rate than control fruit (Fig. 8.6b) and this might have become significant over longer periods at 20°C. For 'Braeburn' apples the control fruit had higher r_{CO_2} and $r_{C_2H_4}$. There are no clear reasons why the fruit in the two experiments responded differently to cannulation other than that the fruit were sourced from different regions and times during the season, and that fruit used in experiment 2 had been pretreated with ethylene.

The stimulation of r_{CO_2} in cannulated preclimacteric/climacteric apples resulted in enhanced $p_{CO_2}^{i}$ and diminished $p_{O_2}^{i}$ in the core cavity. However, the differences were not significant except for $p_{O_2}^{i}$ for the first 3 days after cannulation. Similarly, IA composition of cannulated postclimacteric fruit was similar to samples of control fruit after 3 days at 20°C. For apples at physiological steady-state, the IA of chambers typically comes to physical equilibrium within 48 to 96 hours (Rajapakse *et al.*, 1990; Dadzie, 1992). For the preclimacteric/climacteric fruit used in experiment 1, chambers did not appear to approach equilibrium until close to 9 days

when chamber contents finally approached those found in the core. Therefore, cannulation would be more appropriate than surface chambers as a means of monitoring the changes in IA composition in fruit undergoing rapid metabolic change.

In conclusion, insertion of a single cannula into the core cavity of apples resulted in an increase in r_{CO_2}, $r_{C_2H_4}$ at 20°C in preclimacteric/climacteric and postclimacteric 'Braeburn' apples, and may have advanced the climacteric in some preclimacteric fruit. However, for postclimacteric 'COP' and 'Braeburn' apples with four cannulae inserted, no significant differences were detected in r_{CO_2} and $r_{C_2H_4}$ at 20°C. Adhering surface chambers to apples is a non-invasive method suitable for estimating the IA, but the technique requires the fruit to be at steady-state and a period during which the chamber atmosphere comes to physical equilibrium. On surface coated apples, the decrease in surface permeance may result in an undesirably long equilibration time unless an artificial pore is created in the surface beneath the chamber. When the chamber has reached physical equilibrium, chamber IA will underestimate core $p^i_{CO_2}$ and overestimate core $p^i_{O_2}$ as a consequence of atmosphere gradients through the cortex. Cannulation, although an invasive technique, is more suitable than the chamber method for measuring IA composition in fruit where the metabolic rate is changing rapidly. Cannulation is also more suitable for sampling from the core cavity where repeated removal of small volumes ($\leq$ 100 mm^3) may be required to monitor changes in IA and estimating changes in skin permeance.

8.6 Acknowledgements

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Chapter 9

General Discussion

9.1 Project overview

Much of CA and MA literature has focused on optimising storage atmospheres of fruit and vegetables to maximise fruit quality on the basis of external O_2 and CO_2 atmospheres (Kader *et al.*, 1989). In contrast, in the current study, quantitative methods were developed to define critical lower O_2 limits of apples on the basis of internal atmospheres. This approach was based on the concept that LOL^is differ from LOLs expressed in terms of external levels of O_2 in an analogous way to differences in internal and external pressure (Dadzie, 1992; Eq. 5.1). Studies by Dadzie (1992), did not allow precise measurement of LOL^is due to insufficient data at the aerobic-anaerobic respiratory transition. The current study rectified this by characterising changes in $p^i_{CO_2}$ and RQ_{ia} as a function of steady-state $p^i_{O_2}$ using a range of $p^e_{O_2}$ from zero to above ambient levels, weighted towards levels around the aerobic-anaerobic transition zone.

Prior to this study, it had been assumed LOL^is were constant in relation to temperature (Dadzie *et al.*, 1993). If LOL^is are to be used as the preferred basis for optimising storage atmospheres in apples, then it is critical to know if this assumption is true. Additionally, effects of elevated CO_2 and physiological age of apples on LOL^is needed to be quantified.

The current studies have indicated that temperature, $p^e_{CO_2}$ and physiological age have some small effects on LOL^is, but they were not considered sufficiently large to warrant modifying the steady-state model for apples presented by Dadzie *et al.* (1993). While small changes in LOL^is associated with these variables made it difficult to explore possible mechanisms that might affect LOL^is, from a modelling perspective, it considerably simplifies a potentially complex steady-state gas exchange model for apples. However, it can not be assumed that the variables studied would not affect LOL^is of tissues other than that of apples.

Despite the conclusion that temperature effects on LOL^is were small, curves were fitted to ACP^i data based on mean surface chamber and core cavity atmospheres (eg.

Figs. 5.8 and 9.6). Estimates of ACP^e were then calculated for each cultivar from the fitted values of ACP^i to demonstrate differences between ACP^i and ACP^e as a function of temperature.

It would be interesting to test the hypothesis that LOL^is (in contrast to LOL^es) are essentially a constant for a diverse range of plant tissues. This would be particularly interesting for fruit or vegetable tissues with lower cortical diffusivity of O_2, for tissues in which porosity may change markedly during ripening, or for tissues which can be stored under deep hypoxia (eg. persimmons), or do not respond dramatically to ethylene (eg. Nashi).

The following general discussion considers:

1. the pivotal role of skin permeance and respiration rate in determining differences between external and internal atmosphere of apples
2. estimation of, and factors affecting *LOL*s
3. the relationship between LOL^is and LOL^es as a function of temperature and implications for CA / MA storage
4. the potential for use of IAs as a basis for modulating CA store atmospheres
5. recommendations for further research derived from questions raised by the current study, and potential research areas leading to advances in the way CA and MA technologies will be used in the future.

9.2 Difference in partial pressure as a function of skin permeance and respiration rate

The following discussion draws together evidence from the studies to demonstrate the pivotal role of skin permeance and respiration rate in determining differences between external and internal atmospheres of apples. The data confirms the applicability of the general model for gas exchange presented in chapter 1 for apples. Evidence for differential permeance of apple skin to CO_2 and O_2 is also discussed and implications for use of MA / CA and edible skin coatings.

9.2.1 Effect of skin permeance on differences between external and internal partial pressures

The effect of skin permeance on the gradient in partial pressures of CO_2 and O_2 is illustrated in Fig. 9.1, for populations of preclimacteric, climacteric, and postclimacteric 'COP' and 'Braeburn' apples equilibrated in air at 20°C (see section 7.3.5). 'COP' apples had generally higher and a greater range of values for P'_{O_2} and P'_{CO_2} than 'Braeburn'. There was an inverse relationship between permeance values and the gradient in partial pressures for 'COP'. The small cluster of points lower than the main group of points were for preclimacteric fruit which had lower respiration rates. The range in values of permeance tends to increase for climacteric and postclimacteric fruit compared to preclimacteric fruit. Park *et al.* (1993) reported a similar effect of increased permeance as 'McIntosh' apples matured using the ethane efflux method for estimating permeance.

The lower and narrower range of permeance values for 'Braeburn' resulted in markedly higher gradients (particularly of Δp_{O_2}), as the skin of 'Braeburn' apples at 20°C was more permeable to CO_2 than O_2. Though not as distinct as for 'COP', the cluster of points on the lower left of the graph also represent values for preclimacteric fruit.

9.2.2 Differential permeance of the skin to O_2 and CO_2

Differential permeance of the skin to CO_2 and O_2 at 0° and 20°C was explored by plotting P'_{CO_2} against P'_{O_2} (Fig. 9.2). As CO_2 is a larger molecule than O_2 it would be expected that $P'_{CO_2} < P'_{O_2}$ if diffusion were exclusively through pores. Conversely, $P'_{CO_2} > P'_{O_2}$ might indicate CO_2 was diffusing through alternative routes through the cuticle in addition to diffusing through pores (see section 2.4.3.2). Estimation of P'_{O_2} assumed $RQ = 1$. If $RQ>1$ (eg. if the respiratory substrate was organic acids), then r_{O_2} would have been lower than the assumed value, and values of P'_{O_2} presented would have overestimated actual P'_{O_2}. The reverse would be the case if $RQ<1$.

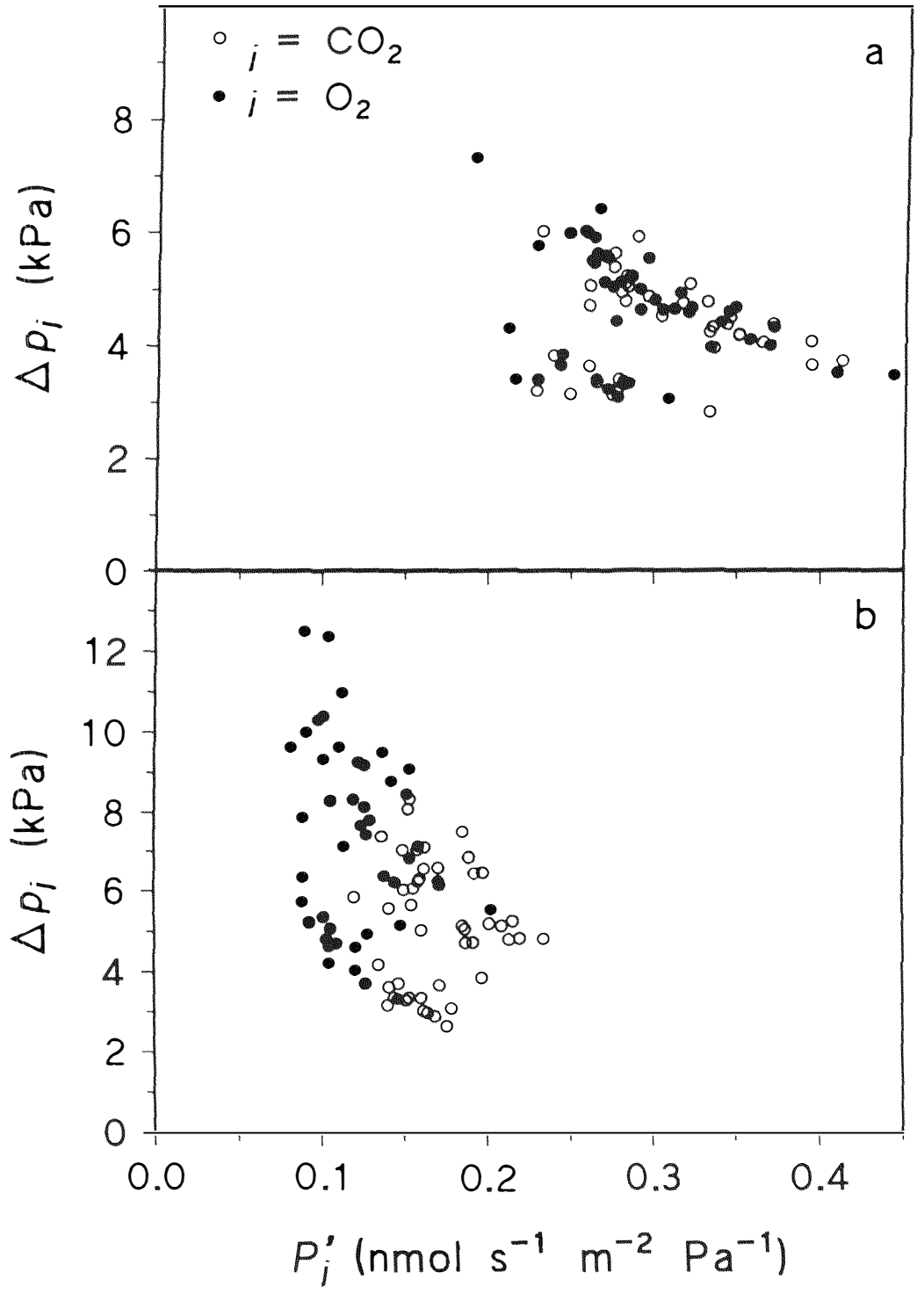

Figure 9.1 Relationship between the difference in partial pressures of O_2 and CO_2 (Δp_{O_2}, and Δp_{CO_2}) and fruit permeance to O_2 and CO_2 (P'_{O_2}, and P'_{CO_2}) respectively for a) 'Cox's Orange Pippin' and, b) 'Braeburn' apples at 20°C in air.

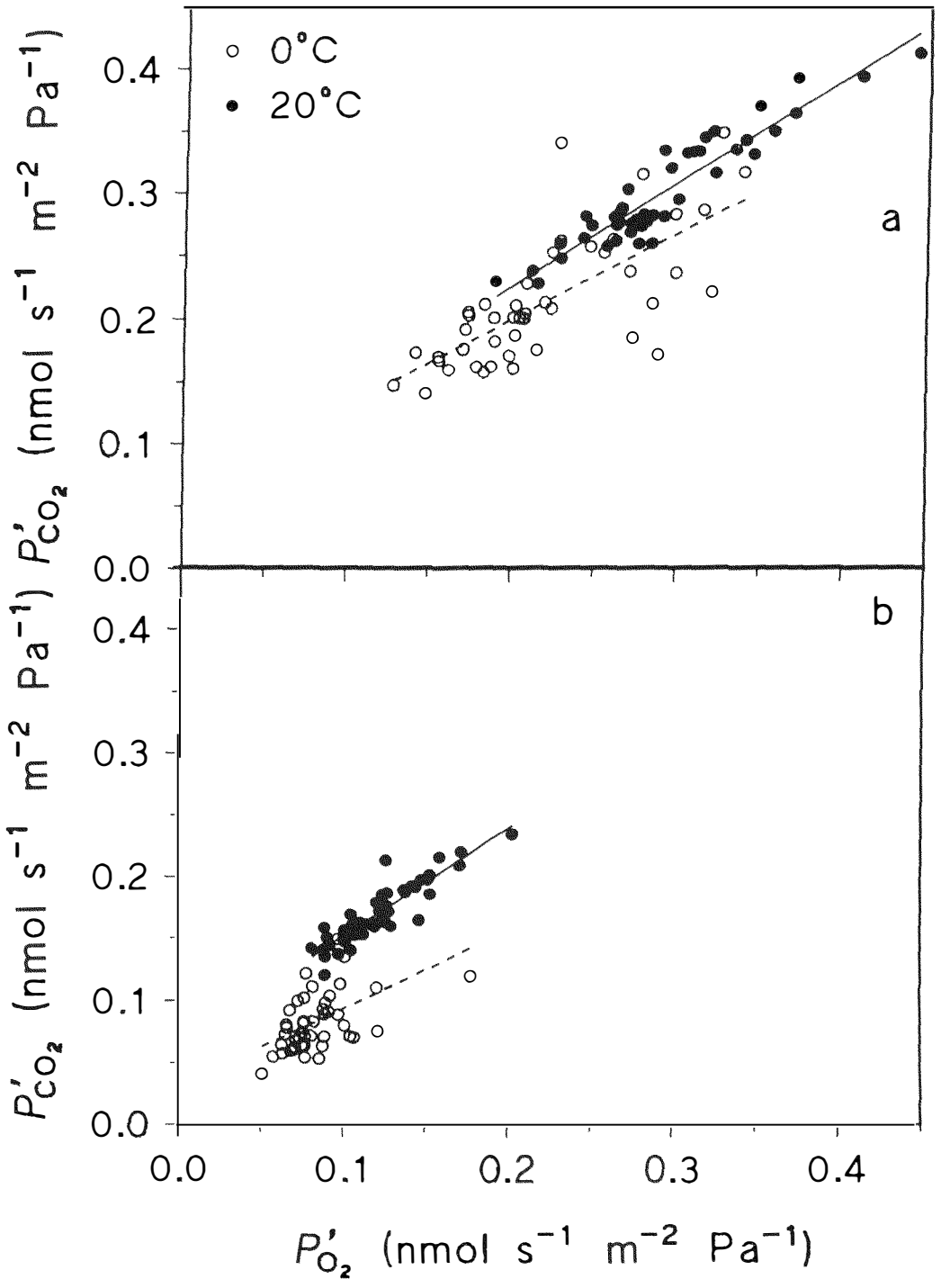

Figure 9.2 Relationship between fruit permeance to CO_2 (P'_{CO_2}) and O_2 (P'_{O_2}) for a) 'Cox's Orange Pippin' and, b) 'Braeburn' apples at 0° and 20°C in air.

Assuming RQ=1, data for both 'COP' and 'Braeburn' at 0°C indicated that for the majority of fruit $P'_{O_2} > P'_{CO_2}$ ($P'_{CO_2} = 0.68\ P'_{O_2} + 0.07$, $r^2 = 0.51$; $P'_{CO_2} = 0.62\ P'_{O_2} + 0.03$, $r^2 = 0.27$, for 'COP' and 'Braeburn' respectively). Therefore, at 0°C, diffusion was probably dominated by pores. For many fruit at 20°C and particularly for 'Braeburn', values of both P'_{CO_2} and P'_{O_2} were higher than at 0°C. The slope of the linear regression in the relationship between P'_{CO_2} and P'_{O_2} was also higher ($P'_{CO_2} = 0.83\ P'_{O_2} + 0.06$, $r^2 = 0.88$; $P'_{CO_2} = 0.88\ P'_{O_2} + 0.06$, $r^2 = 0.81$, for 'COP' and 'Braeburn' respectively), and for many fruit $P'_{CO_2} > P'_{O_2}$. Therefore, at the higher temperature an increased proportion of CO_2 probably diffused through cuticle, particularly for fruit with low permeance values, which would be the fruit with low skin porosity.

The effect of temperature increasing P'_{CO_2} and P'_{O_2} would be of advantage to fruit in MAP, because it would reduce the increase in Δp_{CO_2} and Δp_{O_2}that would otherwise be expected to arise from higher respiration rates as temperature increased. The greater increase in P'_{CO_2} relative to P'_{O_2} at the higher temperature, would not necessarily be advantageous, except perhaps to reduce the potential for CO_2 -induced fermentation if p'_{CO_2} increased above approximately 20 kPa (Kubo *et al.*, 1990). However, this would be unlikely except for a few fruit in MAPs at high temperatures.

Values of P'_{CO_2} and P'_{O_2} of 'COP' fruit, in addition to being higher than for 'Braeburn', also had a greater range. However, because of the inverse relationship between Δp_j and P'_j the significance of variation in P'_j is not the same at different absolute levels of P'_j. The narrower range of P'_j for 'Braeburn' resulted in a larger range in Δp_j compared to the case with 'COP' fruit. Also, the lower P'_j of 'Braeburn' would result in a marked effect of temperature on gradients.

Currently, the effect of temperature on the permeability of polymeric films is not great enough to match the effect of temperature on gradients in fruit with high permeance (Cameron *et al.*, 1995), let alone with permeance values as low as those of 'Braeburn' apples. Consequently, MAPs need to be designed for the maximum temperature they are likely to be exposed to while still sealed if atmospheres likely to induce fermentation are to be avoided.

Surface coatings have been used on apples to improve cosmetic features and reduce deterioration either by suppression of water loss or by achieving MA benefits. Recently, there has been a substantial increase in knowledge of permeability characteristics of edible surface coatings (Avena-Bustillos *et al.*, 1994). Hagenmeier and Shaw (1992) reported materials used for coating fruits and vegetables had permeabilities to CO_2 which were 2 to 8 times greater than for O_2, and which were between 40 and 20,000 times more permeable to water vapour than to O_2. However, regardless of the permeability characteristics of the film, the interaction of a coating with pores and cuticle, in addition to the natural variability in skin permeance, can result in markedly different overall permeance to O_2 (Banks *et al.*, 1993).

Coatings are likely to exacerbate the effect elevated respiration rates associated with high temperature have on depressing $p^i_{O_2}$, and hence the risk of anaerobiosis. Banks *et al.* (1996a) have quantified the relationship between $p^i_{CO_2}$ and $p^i_{O_2}$ for fruit with a tightly adhering coating, and recommended a quantitative approach to optimising surface coatings using plots of $p^i_{CO_2}$ as a function of $p^i_{O_2}$. The extent to which skin permeance to gas diffusion is affected by coatings would largely depend on the degree to which pores are blocked by the coating and how tightly the coating adheres to the fruit surface in the vicinity of the pores (Banks *et al.*, 1993).

Due to pore blockage, internal atmospheres of fruit with tightly adhering coatings are affected more dramatically than those with loosely adhering coatings. A loosely adhering coating, assuming pores are not blocked for diffusion of O_2, would allow opportunity for exchange of gases in the space between the fruit surface and the coating. This would be analogous to individual film wrapping (Ben-Yehoshua, 1985). A coated fruit is likely to have portions of the fruit with tightly adhering coating and other areas with loosely adhering coating, and the proportions of each largely influence the effect on internal atmospheres.

Fruit to fruit variation in skin permeance and maximum respiration rate may have more significant consequences in coated fruits than for MAP, the latter being analogous to a loosely fitting coating. Unlike a MAP, a coating can not be removed from the fruit when they are removed to a higher temperature. Consequently, coatings would need to be optimised for the highest temperature the crop will be exposed to. There may be potential for using the beneficial effects of coatings on

modifying internal atmospheres to slow deterioration of processing grade fruit (Banks *et al.*, 1993). In contrast to fresh fruit, it would be of less concern if a small proportion of the population of processing fruit were below their $LOL^{'}$. Benefits might arise for processing fruits from retention of volatiles (Nisperos-Carriedo *et al.*, 1990), and the potential to reduce refrigeration costs, storing coated fruit for short periods at ambient temperatures.

9.2.3 Effect of respiration rate on partial pressure gradients

The effect of respiration rate on atmosphere gradients is illustrated in Fig. 9.3, for 'COP' and 'Braeburn' at 20°C. For 'COP' (Fig. 9.3a), Δp_{O_2} and Δp_{CO_2} as a function of r_{CO_2} were similar. 'COP' had higher r_{CO_2} than 'Braeburn', but variability within the population was similar. As in Fig. 9.1, the cluster of points on the lower left are for preclimacteric fruit with lower r_{CO_2}. For 'Braeburn' (Fig. 9.3b), generally $\Delta p_{O_2} > \Delta p_{CO_2}$. This was a consequence of $P^{'}_{O_2} < P^{'}_{CO_2}$ for 'Braeburn' at 20°C, and the lower overall permeance for 'Braeburn' relative to 'COP' resulted in greater variability in Δp_{O_2} and Δp_{CO_2}.

9.3 Estimation of *LOL*s

9.3.1 Methods for estimating *LOL*s

Identifying the aerobic-anaerobic transition has been of theoretical and practical interest to plant biochemists studying respiratory behaviour of plants and to postharvest physiologists. Two approaches have been taken in developing methods for estimating *LOL*s, (1) steady-state methods (using CA or MAP), and (2) non-steady-state methods. The advantages and disadvantages of these methods were assessed before undertaking this study, and it was decided to use the steady-state CA method. The non-steady-state method of Leshuk and Saltveit (1990), while more

rapid than steady-state methods, was not suitable as rate of pull-down of O_2 may have significantly affected *LOL*s because of the delay in response of tissues to changing O_2. The steady-state method using MAP has recently been extensively used for estimating LOL^es of fruit (Beaudry *et al.* 1992; Beaudry and Gran, 1993; Gran, 1993; Joles *et al.*, 1994; Talasila *et al.*, 1994). In these studies, package atmospheres were sampled and average, rather than individual fruit response, measured. It would be possible to sample IA of fruit using this method, but the main disadvantage of this system arises from the varying duration different treatments required to achieve steady-state package atmospheres. For apples, this ranged from a few days, to weeks, depending on temperature and the mass of fruit in each package (Gran, 1993). This resulted in differences in physiological age of the fruit by the time steady-state was achieved, rendering the method unsatisfactory for studying the effects of physiological age on LOL^is. Also, at higher temperatures the development of rots can be a problem with this method.

The steady-state CA method used in the study required a gas control system for mixing air and N_2 (and CO_2 for studying effects of $p^e_{CO_2}$), and continuous supply of these gases for the duration of the experiment. While this equipment was relatively expensive, the system allowed greater control over the rate of pull-down to steady-state atmospheres and levels required. In general, surface chamber atmospheres equilibrated within 40 to 100 hours, depending on temperature and cultivar. This resulted in a potential experimental cycle of five to seven days, depending on the objective of the experiment. The value of estimating *LOL*s on the basis of IAs using this method was that it provided a more mechanistic basis for models used to predict fruit response to CA and MA.

Disadvantages of the CA method used were: (1) time taken to set up CA atmospheres, (2) time taken for surface chambers to reach equilibrium, and (3) potential for surface chambers to be dislodged from fruit or develop leaks while in CA.

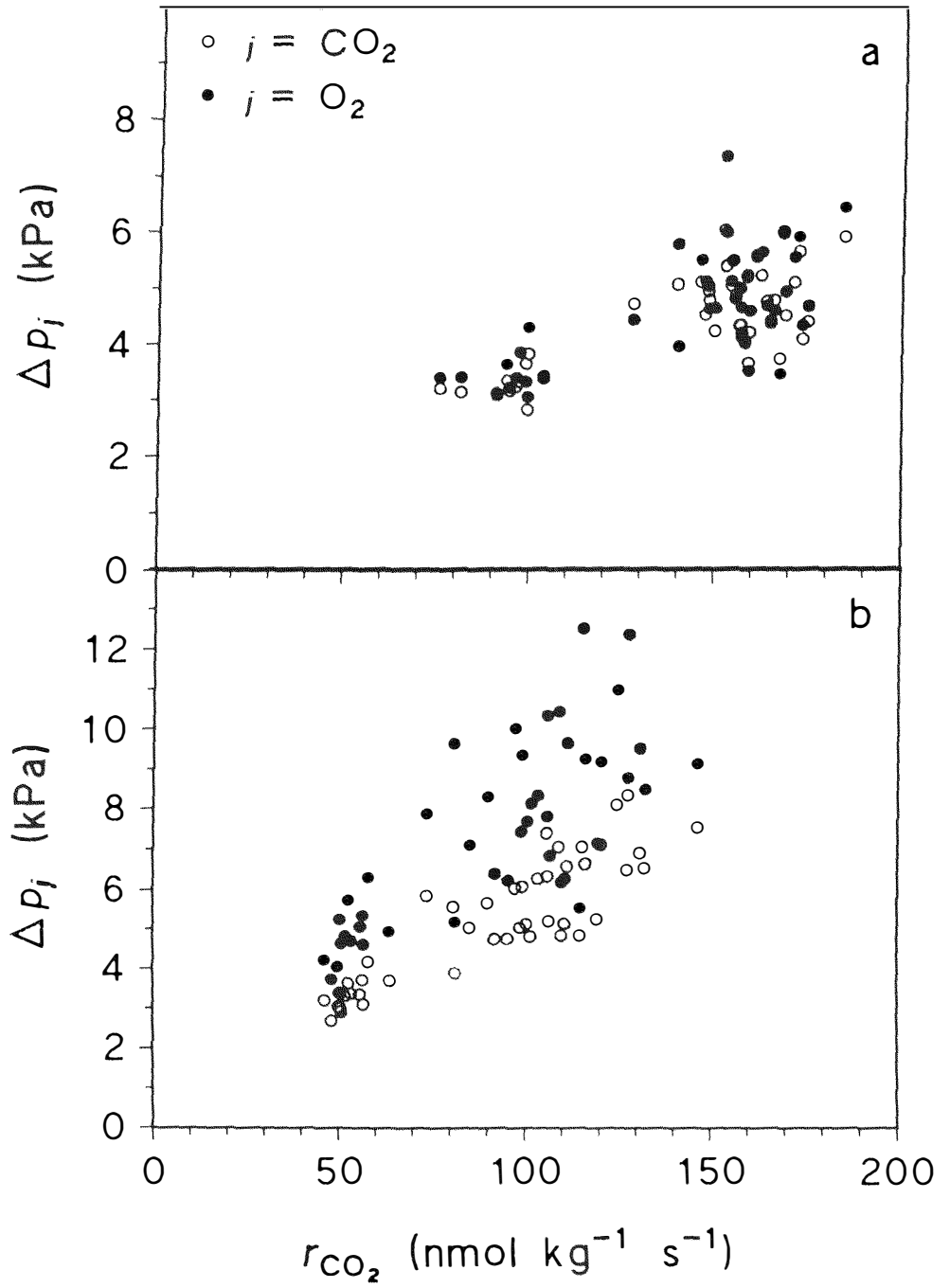

Figure 9.3 Relationship between the difference in partial pressures of O_2 and CO_2 (Δp_{O_2}, and Δp_{CO_2}) and respiration rate (r_{CO_2}) for a) 'Cox's Orange Pippin' and, b) 'Braeburn' apples at 20°C in air.

Cannulating apples to sample from the core cavity, was considered as an alternative method as it had been used extensively in previous studies (Trout *et al.*, 1942; Burg and Burg, 1965; Solomos, 1989; Reid *et al.*, 1973; Knee, 1980, Banks, 1983), but no data in the literature quantifying deleterious physiological effects was available. Consequently, this was investigated (chapter 8), though for reasons of consistency, and because there was some evidence that cannulation could advance the climacteric, this method was not used in studies on *LOL*'s. The cannulation method of estimating IAs, was considered particularly suitable for studies where repeated sampling is required over either short periods under experimental conditions where fruit metabolism is changing rapidly, or long periods (cool storage, CA / MA storage studies etc.). For example, the method has been used extensively with apples in recent studies using fruit coatings, and characterising the effects of hot water disinfestation treatments on rate of decline of $p_{O_2}^i$ (Banks *et al.*, 1996b).

9.3.2 Quantification of *LOL*s

A quantitative conceptual framework such as that developed in this study (chapter 5) for estimating *LOL*s as objective, mathematical, definitions of *LOL*s had not been published previously. This enabled objective comparison of effects of variables on *LOL*s. Of the two types of *LOL* described, the anaerobic compensation point could easily be solved for mathematically, as it represented the $p_{O_2}^i$ when the slope of $p_{CO_2}^i$ derived from aerobic and anaerobic respiration was zero. The precision of this method was contingent on finding adequate functions for and significant fits of curves to the data. A similar approach to Dadzie (1992) was used using the two parameter Michaelis-Menten equation for $p_{CO_2}^i$ derived from aerobic respiration and a two parameter exponential function for $p_{CO_2}^i$ derived from anaerobic respiration (Eq. 4.3). The increased density of data around the aerobic-anaerobic transition validated the use of these functions in modelling $p_{CO_2}^i$ as a function of $p_{O_2}^i$ and $p_{O_2}^e$. However, the range of IA data resulting from inherent variability in fruit respiration and skin permeance, resulted in the need to weight the data using the inverse of the variance of means for fitting parameters. It was often difficult to obtain significant fits for all

parameters. While this might have been improved using functions with a greater number of parameters, this was not considered desirable as it becomes increasing difficult to find physiological and often statistical significance in more complex functions. The compromise in mathematical models (as opposed to statistical and mechanistic models) is always the idealisation of a phenomenon, which may or may not reflect the true cause-and-effect relationships (Sharp and Rykiel, 1991).

Quantification of the fermentation threshold was also contingent on adequate fitting of curves to either RQ_{ia} or c^{i}_{EtOH} data. In addition, an arbitrary value of 10% increase above basal levels, was selected as a reasonable estimate of significant increase in either RQ_{ia} or c_{EtOH}. This was lower than the 20% value used by Beaudry *et al.* (1992) for identifying RQ breakpoint of blueberries, and the 10% value was realistic in terms of what could be observed visually as a significant increase.

The large number of plots to which significant curve fits were required in the study meant use of mathematical estimation of LOL^{i}s was difficult to achieve. Consequently, an alternative statistical approach was considered, that could analyse estimates of LOL^{i}s based on visual assessment by a small trained panel of assessors. To be useful, the statistical method needed to (1) use small sample sizes (n=15), (2) not assume any probability distribution of data, and (3) calculate confidence intervals which could be used to compare treatments. The statistical "bootstrap" procedure had been established in the literature as a robust statistical technique (Efron, 1979, 1987; Efron and Gong, 1983; Efron and Tibshirani, 1993) and met these requirements. The number of bootstrap samples resampled from the raw data was arbitrarily set at 1000. This was a compromise between extra precision gained by increasing the number and computation time. Doubling the number to 2000, did not dramatically improve confidence intervals. Mean bootstrap LOL^{i}s were similar (essentially identical from a physiological perspective and in terms of the resolution of the O_2 / CO_2 sensors) to raw data means. It was also possible to generate bootstrap medians, but as bias corrected (BCa-type) confidence intervals could not be generated, only means were used in the study.

9.4 Factors affecting *LOL*s

Two principal factors, each involving a number of environmental and fruit-related variables, may affect *LOL*s:

1) those influencing the diffusion of O_2 to the site of consumption by either cytosolic oxidases and mitochondrial terminal oxidase(s)
2) those influencing uptake of O_2, modulating respiration or generally affecting enzyme function.

The steady-state CA method used in this study was used to estimate LOL^is for a population of apples. Variability in $p^i_{CO_2}$ and RQ_{ia} as a function of $p^i_{O_2}$ decreased dramatically as $p^i_{O_2}$ decreased. However, at the estimated LOL^is individual fruit still showed differences in $p^i_{CO_2}$ and RQ_{ia}. This indicated LOL^is of individual fruit vary slightly, presumably as a consequence of inherent genetic and physiological differences associated with the factors above. The fruit-related variables discussed below are likely to affect LOL^is of individual fruit, and consequently those estimated on a population basis.

A summary of some environmental and fruit-related variables that affect, or are likely to affect, these two factors above, and which result in variation between LOL^is and LOL^es, is presented in Table 9.1. This study has concentrated on three important variables (temperature, $p^e_{CO_2}$ and physiological age), but it is clear from Table 9.1, that there is considerable uncertainty over the effects of many variables. It was beyond the scope of the present study to investigate the variables involving cell physiology and biochemistry, but the following briefly uses evidence from this and other studies to speculate on mechanisms by which variability in LOL^is may have occurred. Variation in LOL^es involves all variables affecting LOL^is, and in addition, those relating to gas diffusion through the skin. The way these interact and result in differences between LOL^is and LOL^es is discussed in section 9.5.

9.4.1 Diffusion of O_2 to sites of oxidative metabolism

Data from the current study indicated O_2 diffused into apples through pores in the cuticle (see section 9.2.2). Many studies have demonstrated that diffusion through bulky plant organs such as fruit, roots and tubers follows Fick's First Law of Diffusion (Eq. 1.1; Burg and Burg, 1965; Burton, 1974; Cameron and Yang, 1980; Solomos, 1987; Trout *et al.*, 1942). However, diffusion in climacteric avocado (Ben-Yehoshua *et al.*, 1963), stored apples (Trout *et al.*, 1942) and postclimacteric bananas (Leonard and Wardlaw, 1941) could not be described by Fick's First Law. Therefore, for some fruits, there are likely to be factors limiting diffusion of O_2 within the cortex, such as blockage of intercellular air space associated with physiological age or disorders. Diffusion occurs down the O_2 gradient that results from uptake of O_2 within cells. Burg and Burg (1965) showed diffusivity of gases within apples was inversely proportional to total pressure as would be expected from the ideal gas law, and this indicated diffusion of O_2 within apples must be primarily in the gas phase. If diffusion of O_2 within apples were purely in an aqueous phase, then (depending on the rate of O_2 uptake) it has been calculated that the maximum radius that could maintain 1 kPa $p^{l}_{O_2}$ in the core cavity would be approximately 7 mm (Solomos, 1987).

Rajapakse *et al.* (1990) suggested two models of potential routes for gas exchange within the cortex. In the parallel model, diffusion would be through intercellular air channels and/or in the fluid/solid phase of the cellular matrix, and in the series model diffusion would occur through air channels and fluid solid phase in turn. Empirically derived estimates of effective diffusivity fell between values calculated for the two models. This suggested the actual mechanisms for O_2 diffusion in fruit flesh is a combination of those described by both models in which there are several parallel gas and fluid/solid pathways through the intercellular system, but a proportion of the gas pathways are blocked by the fluid/solid matrix, forcing some diffusion in series.

Table 9.1 Summary of effects or likely effects on LOL^es and LOL^is of environmental and fruit-related variables. Symbols: + = increasing level of variable proportionally increases *LOL*; - = increasing level of variable proportionally decreases *LOL*; ? = relationship unknown; 0 = no effects; -? or +? = likely effects. The relative magnitude of the relationships are indicated by the number of + or -.

Variables affecting *LOL*s	LOL^is	LOL^es
Environmental		
Temperature	+	++
Relative humidity	?	- and +
Rate of O_2 pull-down	+?	++?
$p^e_{CO_2}$	- and +	- and +
$p^e_{C_2H_4}$	+?	+?
Fruit-related		
(a) Biophysical		
Mass	+?	+
Surface area	0	-
Surface area / mass	0	-
Diffusivity of gases in cortex	-	-
Skin permeance	0	-
$p^i_{CO_2}$	- and +	- and +
$p^i_{C_2H_4}$	?	+
Solubility	-	-
(b) Physiological		
Respiration	+	++
Age	+ and -	+
Susceptibility to disorders	+?	+?
(c) Biochemical / bioenergetics		
Energy requirements	--	--
Energy charge	- and +	- and +
Anaerobic stress enzymes	?	?
Ionic balance	-?	-?
Cytoplasmic acidification	?	?
"Residual" oxidases	?	?
(d) Biomolecular		
Regulation of anaerobic stress enzymes	?	?

9.4.1.1 Diffusion of O_2 through intercellular spaces

Although it is not always valid to assume atmospheres are homogenous within bulky plant tissues (Cameron and Yang, 1980), gradients within the tissues are often small and assumed to be physiologically insignificant, with adequate diffusion of O_2 within the cortex for aerobic respiration. However, this might not be the case for all apple cultivars (Dadzie, 1992), and variation in intercellular air spaces may vary as fruit age. Knee and Bartley (1981) found that during ripening and senescence there was a progressive decrease in cell adhesion leading to separation of cell columns and enlargement of intercellular spaces. Other studies report midseason apples were found to have larger intercellular spaces and lower density than late season apples (Vincent, 1989).

What variables then might affect diffusion of O_2 up to the point of entry into cells and result in a shift in *LOL*'s? Of the environmental variables, temperature and relative humidity (RH), and of fruit-related variables, length and connectivity of the diffusion pathway, physiological age and physiological disorders are variables that may affect diffusion.

Diffusion coefficients depend inversely on the viscosity of the medium, the rate of diffusion in air being approximately 10^4 higher than in water. Diffusion coefficients of gases in air are inversely proportional to ambient pressure, becoming larger as total pressure in the system drops. While the latter property of gases may be relevant for fruit in hypobaric storage, it is unlikely to have a significant influence under normal refrigerated, CA and MA storage. Rates of diffusion of O_2 as both a gas and as a solute are affected by temperature (Q_{10} for gas is about 1.03 and for solutes 1.3-1.4, see section 2.4.2). Therefore, at higher temperature, O_2 diffuses more rapidly to sites of utilization, but rates of uptake are also markedly affected by temperature, with the Q_{10} for respiration of apples (> 2) being greater than that for diffusion. However, given that pathways for diffusion are adequate to meet the demand for O_2, the effect of temperature *per se* on rate of diffusion is unlikely to shift *LOL*'s.

Detached from the tree apples lose water and this affects permeance to gas diffusion through changes to both skin and cortical structure (Park *et al.*, 1993). Under conditions of high vapour pressure deficits (low RH) fruit volume and

permeance may decrease (Wilkinson, 1965), and at high RH (>95%) the opposite can occur (Lidster, 1990). The lower permeance of apples at low RH was attributed by Wilkinson (1965) to contraction of both skin and flesh or closing of lenticels, and under high RH conditions the increase in permeance to rounding off of cortical cells, facilitating diffusion through the intercellular air channels. Fockens and Meffert (1972) presented a similar model which explained effects of RH on cell shape and arrangement on the basis of changes in turgor pressure. Therefore, under conditions of high RH and considering effects on gaseous diffusion pathways, LOL^is would be expected to remain constant or decrease, and at low RH they may increase.

In the current study, the porosity of cortical tissue increased significantly during storage. A similar result was reported by Hatfield and Knee (1988). Khan and Vincent (1990) have even suggested that size of intercellular spaces can be used to define the age of fruit! Increased porosity may have resulted from either storage in high RH, particularly for 'COP', which were enclosed in a polymeric film carton liner to reduce water loss. Alternatively, increased porosity may have resulted from decreased cell to cell adhesion during ripening and senescence (Knee, 1991).

There is, then, a complex of environmental and physiological age variables that may result in higher porosity. Given that connectivity of the intercellular air channels remained high, increased porosity in apples would result in increased diffusivity of O_2, and no change, or a lowering of LOL^is. Continuity of air channels was not measured in the current study. However, the lack of decrease in LOL^is of 'COP' as fruit aged (section 7.4.4), and slight decrease in FT^i_{RQ} of 'Braeburn' apples suggested higher porosity was also accompanied by no change or an increase in connectivity of air channels.

For other fruits with substantially lower cortical tissue porosity and higher skin permeance to O_2 than apples (eg. nashi and particularly nectarines), the percentage of the total O_2 gradient resulting from the cortex may be substantially higher than for apples. For example, for 'Red Gold' nectarines, the gradient in O_2 across the cortex and skin were 56% and 44% respectively, and porosity (percentage intercellular air space) of unripe and ripe fruit were 8.0% and 3.7% respectively (Rajapakse *et al.*, 1990). It is anticipated that for fruit like nectarines, porosity and continuity of the air channels in the cortex are more likely to affect LOL^is than for apples, because of

decreased diffusivity of O_2 through the cortex, and this may result in an increase in LOL^ts.

9.4.1.2 Diffusion of O_2 in cells

Gaseous O_2 in the intercellular air spaces would dissolve in water in the cell wall, diffuse through plasmalemma to the cytosol where it is consumed by cytosolic oxidases or diffuse through the mitochondrial double membrane to the terminal oxidase(s). Diffusion is extremely fast over the relatively short diffusion pathway into the cell and, assisted by mechanical mixing due to cytoplasmic streaming (which can lead to much more rapid movement than by diffusion), has been assumed to be non-limiting for oxidative processes (Nobel, 1991, p. 19). Solomos (1987) indicated it is difficult to estimate the magnitude of the effect of the cell wall and plasmalemma on diffusion due to variation in cell size and geometry and the area of cell wall exposed to air channels.

So long as the air channels do not become blocked with fluid, it is unlikely that diffusion into the cell would be so limiting as to cause a shift in LOL^ts. As for gaseous diffusion, diffusion of dissolved O_2 into the cell would increase as the kinetic energy of dissolved O_2 molecules increased with temperature. This would tend to limit any increase in LOL^ts with temperature. For fruit in which physiological disorders are present, membrane disfunction may result in leakage of cytosol into intercellular spaces, partially reducing the diffusion pathway to that of a fluid/solid matrix, and shifting LOL^ts to higher $p^t_{O_2}$. Evidence for possibility for whole fruit may be inferred from studies using cultured pear cells where the anaerobic compensation point shifted to higher O_2 levels as the diffusion coefficient of the cell suspensions decreased (Boersig *et al.*, 1988).

Solubilities of gases in liquids are highly temperature dependent as described by Henry's Law (see section *3.7.3.2*), with solubility decreasing more markedly per degree as temperature increases. If LOL^ts are constant with respect to dissolved O_2 concentrations, then this would further exaggerate variation in $LOL^e s$ as temperature increased (Banks *et al.*, 1993). The effect of temperature on solubility of O_2 is likely to be more significant physiologically than for diffusion of O_2. With increasing

temperature, there will be lower cytosolic concentrations of O_2 at the same time as O_2 uptake is increasing. In the current study, we would have expected LOL^is to increase markedly with temperature if solubility of O_2 primarily limited the availability of O_2 to oxidases. However, LOL^is based on partial pressures remained relatively constant, rising only slightly at high temperatures. The dissolved O_2 concentrations calculated from values of partial pressure at different temperatures tended to decrease with temperature, indicating the effect of temperature on solubility of O_2 did not limit O_2 uptake in these studies. Calculation of dissolved O_2 from partial pressures assumed the solvent was water. However, solubility of O_2 also decreases with increasing soluble solids content (Leonard, 1939). This would further exaggerate the effect of temperature on solubility, particularly as fruit age and soluble solids content increases. Again, evidence from the current study indicated this was not a significant factor affecting LOL^is.

In conclusion, of the variables discussed above, only a decrease in diffusion through intercellular spaces due to lack of connectivity, or filling of the air spaces with fluid as some fruits age or disorders occur, is likely to substantially affect LOL^is. In the absence of direct measurement of cytosolic O_2 concentrations using O_2 microprobes, it still remains to be demonstrated whether $p^i_{O_2}$, cytosolic O_2 concentration, or chemical activity of O_2 is the crucial factor in determining LOL^is of the tissue.

9.4.2 Uptake of O_2 by oxidative metabolism

The physiological and biochemical basis of CA / MA effects is still being elucidated. Low O_2 and/or elevated CO_2 atmospheres are considered to delay senescence by directly or indirectly suppressing oxidative metabolism and other degradative processes. These include suppression of respiratory metabolism, ethylene biosynthesis and action, and compositional changes, all of which consume O_2.

Variables that might affect cellular O_2 uptake are temperature, O_2 pull-down time, $p^e_{CO_2}$, $p^e_{C_2H_4}$, solubility, physiological age, susceptibility to disorders, ionic balance, energy charge, cytoplasmic acidification, level and activity of cytosolic "residual

oxidases" and regulation of anaerobic stress enzymes. Although this study did not focus on most of these variables, potential effects on *LOL*'s of some of these are discussed below.

In the current study, the steady-state rate of aerobic and anaerobic respiration (estimated by $p^{i}_{CO_2}$) as a function of $p^{i}_{O_2}$ and $p^{e}_{O_2}$ was described by Eq. 4.3. This used the Michaelis-Menten equation to characterise the aerobic respiratory component. The model currently favoured to explain the biphasic nature of the O_2 isotherm as a function of $p_{\bullet_2}$ (ie. the initial slow decline in respiration at higher p_{O_2}, and rapid reduction as p_{O_2} decreased towards zero), is that regulatory enzymes perceive the level of O_2 and exert a feedback inhibition on the initial steps of glucose oxidation, thus lowering respiration (Blackman, 1954; Solomos, 1982, 1994; Tucker and Laties, 1985). Thus, coefficient k_1 in Eq. 4.3 (which is analogous to the Michaelis-Menten coefficient, K_m) is not related to the activity of the "terminal" cytochrome oxidase *per se*, but probably to a plethora of oxidases that directly or indirectly affect respiration.

Studies by Nanos *et al.* (1994) with pear fruit and cultured cells found that during hypoxia (0.25% O_2), ATP phosphofructokinase (ATP-PFK), pyrophosphate phosphofructokinase (PPi-PFK) and succinate dehydrogenase (SDH) increased while pyruvate kinase (PK) decreased, consistent with the operation of a partially reduced TCA cycle. Cytochrome oxidase activity showed little change, and this was construed to support the hypothesis that, above the fermentation threshold, hypoxia suppresses oxidases with high K_ms for O_2 while not substantially altering rate of oxidation by cytochrome oxidase.

Increasing temperature increases the rate of CO_2 production, and in this study the relationship was tentatively characterised for 'COP' and more extensively for 'Braeburn' (Fig. 5.2). As previously discussed, temperature effects on solubility of O_2 were unlikely to limit availability of O_2 to cytosolic or mitochondrial oxidases or explain the slight upward shift in *LOL*'s at higher temperatures. Knee (1980) speculated that higher temperatures may lower the affinity of cytochrome oxidase (and possibly other oxidases) for O_2. This would have the potential to increase *LOL*'s as temperature increased.

Elevated $p^{e}_{CO_2}$, can inhibit O_2 uptake and ethylene production, and consequently, may lower LOL^{i}s. Evidence from the current study suggested $p^{e}_{CO_2}$ ($\leq$ 8 kPa) had only a small effect on reducing O_2 uptake of 'COP' and a slightly larger effect for 'Braeburn' at 20•C. Changes in LOL^{i}s as a function of $p^{e}_{CO_2}$ were probably not physiologically significant in this study. It was anticipated that increased O_2 uptake of climacteric and postclimacteric apples might have reduced tolerance to hypoxia and increased LOL^{i}s. Lack of significant change in LOL^{i}s of 'COP' as a function of physiological state suggested diffusion of O_2 was sufficient for increased O_2 uptake. Increase in LOL^{i}s for postclimacteric 'Braeburn' may have resulted from reduction in cellular homeostasis. The effect of $p^{e}_{CO_2}$ was more apparent at $p^{i}_{O_2}$ well above LOL^{i}s and was unlikely to cause potential lowering of LOL^{i}s. Kerbel (1988) reported for pears, that CO_2 inhibits ATP-PFK and PPi-PFK, which may account for the reduction in O_2 uptake. Regulation of PPi-PFK is pH dependent (Turner and Turner, 1980) and is affected by cytosolic acidification which may occur at levels of CO_2 above 5% (Bown, 1985). However, high levels of $p^{e}_{CO_2}$ may have a marked negative effect on mitochondrial activity through structural and conformational changes (Shipway and Bramlage, 1973). This would lower availability of ATP for cell maintenance and may result in higher LOL^{i}s. High CO_2 can result in accumulation of succinate which has been correlated with CO_2 -induced breakdown in apples (Hulme, 1956). Increased susceptibility to, and incidence of, CO_2-related disorders, such as Braeburn browning disorder, core-flush and brown heart, are also likely to reduce tolerance to hypoxia and may increase LOL^{i}s.

Recent molecular and biochemical studies indicate significant changes in cell function may occur during hypoxia and anoxia, leading to disturbances in ionic balance of cells, reflected in energy depletion and membrane depolarisation (Sachs *et al.*, 1996). These changes may result in temporary reduction in oxidative and phosphorylative capacities of mitochondria, with restoration occurring after return to air storage, or to an extended period of poststorage respiratory suppression (Rahman *et al.*, 1995). Nanos *et al.* (1992) reported preclimacteric pears seemed less stressed and to have greater potential for posthypoxic recovery than pears of a more-advanced physiological age. Low-O_2 injury in climacteric, but not preclimacteric pears after transfer from hypoxic to normal atmospheres indicated that, consistent with the

hypothesis of Romani (1987), repair or homeostasis begins to decline after the inflection point of the climacteric rise and virtually ceases at the climacteric peak. Therefore, it might be anticipated that *LOL*'s would increase as fruit age on the basis of diminishing ability to maintain cell function or survive hypoxic stress. This may have contributed to higher *LOL*'s observed in the current study for postclimacteric 'Braeburn' apples.

Ionic changes in cells resulting from hypoxia include alterations in cytosolic pH. Chervin *et al.* (1996) speculated that as the energy charge of cells fall as partial pressure of O_2 is reduced, cells may not be able to energise pumps that maintain pH and other ionic gradients across the tonoplast and plasmalemma. This may contribute to a lowering of tolerance of cells of hypoxic atmospheres and increase *LOL*'s. Alternatively, if cytoplasmic acidification is limited and controlled, it may serve as a signal for adaptive changes that limit further acidification. On the basis of this hypothesis, Blanke (1991) suggested CO_2 pulsing of apples may be beneficial in inducing tolerance through limited acidification. In this case, *LOL*'s would decrease as a result of adaptation to hypoxic atmospheres.

Studies by Tucker and Laties (1985) with preclimacteric and climacteric avocado indicated that rate of O_2-pull down may markedly affect respiratory O_2 isotherms. Differences between the isotherms were not accounted for by O_2 gradients across skin and flesh, and it was postulated that slow depletion of O_2 allowed feedback repression of O_2 uptake at relatively high O_2 levels. They noted this would lower *LOL*'s. In contrast, rapid depletion of O_2 resulted in an O_2 isotherm approaching that of a single oxidase having high affinity for O_2. Under these conditions it is anticipated that *LOL*'s would shift to higher levels of O_2, and there would be little opportunity for feedback regulation to reduce O_2 uptake or for fruit to adapt to hypoxia. It would be interesting to investigate whether apples might manifest a similar response to rate of O_2 pull-down. This phenomenon has important implications for the use of gas-flushing of MAP and the rate of establishing hypoxic atmospheres in small CA stores or shipping containers. This would be particularly important if CA were to be established by flushing the store while fruit are being cooled.

During ripening and senescence, a complex of controlled synthetic and degenerative changes, which may or may not be independent of one another, require energy to drive them. When energy production is blocked (eg. with dinitrophenol; Ben-Yehoshua, 1964), ripening is normally also inhibited. While O_2 is not required for the functioning of glycolytic pathway, it is required for the TCA cycle, ET system and pentose phosphate pathway for the production of energy. There will be a minimum energy requirement for tissue under CA or MA, and this may differ between different plant tissues and at different physiological stages. Therefore, fruit with lower energy requirements would have lower LOL^is. It would be useful to include energy requirements into models predicting optimum storage atmospheres.

In conclusion, it is difficult to quantify the importance of many variables that affect oxidative metabolism and may affect LOL^is. Some qualitative indications of likely effects have been discussed. It is anticipated that advances in cell physiology and molecular aspects of the anaerobic-stress response of plants will unravel mechanisms by which fruits are susceptible to or able to tolerate low O_2 atmospheres and explain potential differences in LOL^is of different tissues.

9.5 Relationship between LOL^is and LOL^es as a function of temperature

The rationale for using internal rather than external atmospheres for characterising optimum storage atmospheres was the fact that it is the partial pressure of O_2 and CO_2 in the intercellular air spaces, in equilibrium with the concentration of O_2 and CO_2 in the cytosol, that mediates physiological processes. Just as the physiological state of each fruit varies, so each fruit would have its own LOLs, and consequently optimum storage atmosphere. Therefore, for a population of fruit, the optimum storage atmosphere will be an average, a compromise based on the range of LOL^is of individual fruit in a population.

It was concluded that LOL^is estimate more precisely than LOL^es the true aerobic-anaerobic transition, and the range in LOL^is of individual fruit will be considerably smaller than that of LOL^es for the same population of fruit. Greater variability in

LOL^es for a population of fruit was derived from effects of variability in skin permeance and respiration rate of individual fruit, and resulted in a large range of Δp_{O_2}.

To demonstrate the relationship between LOL^is and LOL^es and the implications for CA and MA storage, it was necessary first to establish that there were no clear relationships between r_{CO_2} and either P_{O_2} or P_{CO_2}. If this was true, then differences in LOL^is and LOL^es as a function of temperature could be tested for any combination of r_{CO_2} and skin permeance.

In Figs. 9.4 and 9.5, r_{CO_2} as a function of P_{O_2} and P_{CO_2} respectively are presented for preclimacteric, climacteric and postclimacteric 'COP' and 'Braeburn' at 0° and 20°C. Although the two cultivars are presented on the same graphs, it should be born in mind that cultivars should be considered independently of each other. There were no clear relationships for any physiological stage or temperature for either cultivar, with the possible exception of climacteric and possibly postclimacteric 'Braeburn'. Consequently, data from postclimacteric 'COP' and 'Braeburn' at various temperatures from 0° to 32°C were used to model the effect of temperature on ACP^e (estimated from ACP^i using Eq. 1.5) at empirically determined high and low estimates of P_{O_2} for each cultivar (Fig. 9.6). A power law equation was used to model the relationship:

$$ACP = a\ b^{(0.1T)} \qquad \textbf{(9.1)}$$

The coefficients a and b for the curves in Fig. 9.6 are presented in Table 9.2.

The ACP^i of both postclimacteric 'COP' and 'Braeburn' apples increased slightly with increasing temperature, with a greater increase for 'Braeburn' than 'COP' (section 5.4.6). The strong dependence of ACP^e on both temperature and P_{O_2} is illustrated by Fig. 9.6. The magnitude of the response of ACP^e to temperature was inversely proportional to P_{O_2} and was clearly cultivar dependent.

The implications of the differences between LOL^is and LOL^es depends on the likely range of temperatures fruit in low O_2 atmospheres will be exposed to. CA storage temperatures are typically isothermal. MAP storage ideally should be

isothermal, but the cool chain may be broken at a number of points during the distribution and marketing of the crop. Depending on the form of transport, sealed MAPs may be exposed to high temperatures during loading and unloading of ship holds or refrigerated shipping containers. Packages destined for tropical countries, where ambient air temperature may exceed 30°C, would be particularly vulnerable. Similar breaks in the cool chain may exist in the retail distribution phase. Using the data in Fig. 9.6, the likely impact of CA and MAP storage for 'COP' and 'Braeburn' can be assessed.

At cool store temperatures of 2• and 0°C for 'COP' and 'Braeburn' respectively typical CA atmospheres of 2 kPa and 2 kPa $p^{e}_{CO_2}$ for 'COP' and 2 kPa $p^{e}_{O_2}$ and 1 kPa $p^{e}_{CO_2}$ for 'Braeburn' are unlikely to result in fermentation, even in fruit with lowest $P^{'}_{O_2}$. On the basis of the data presented in Fig. 9.6, the ACP^{e} of 'COP' apples would not approach 2.0 kPa O_2 until fruit were warmed to between 12.8° and 20°C, and, for 'Braeburn', between 7° and 15°C. It is also evident that if the same fruit were in MAPs, and unless adequate cool chain was maintained throughout the period the fruit were sealed in packages, there would be a relatively high risk of some fruit becoming anaerobic. It was demonstrated in chapter 4 that actual point at which fermentation commences (the fermentation threshold) occured at even higher $p^{e}_{O_2}$ than the ACP^{e}. It would also appear from the relationship between solubility of gases in liquid and temperature that variation in ACP^{e} with temperature would be further exaggerated if ACP^{e} is fixed as a constant O_2 concentration in the liquid phase (Banks *et al.*, 1993; Cameron *et al.*, 1995). However, the data presented in chapter 6 indicated that *LOL*'s expressed in terms of dissolved O_2 concentration tended to decrease as temperature increased. This suggested that calculation of concentrations of O_2 in the liquid phase resulting from $p^{l}_{O_2}$ at the *LOL* may not give a precise estimate of the cytosolic concentration of O_2 at the *LOL*s of the tissue.

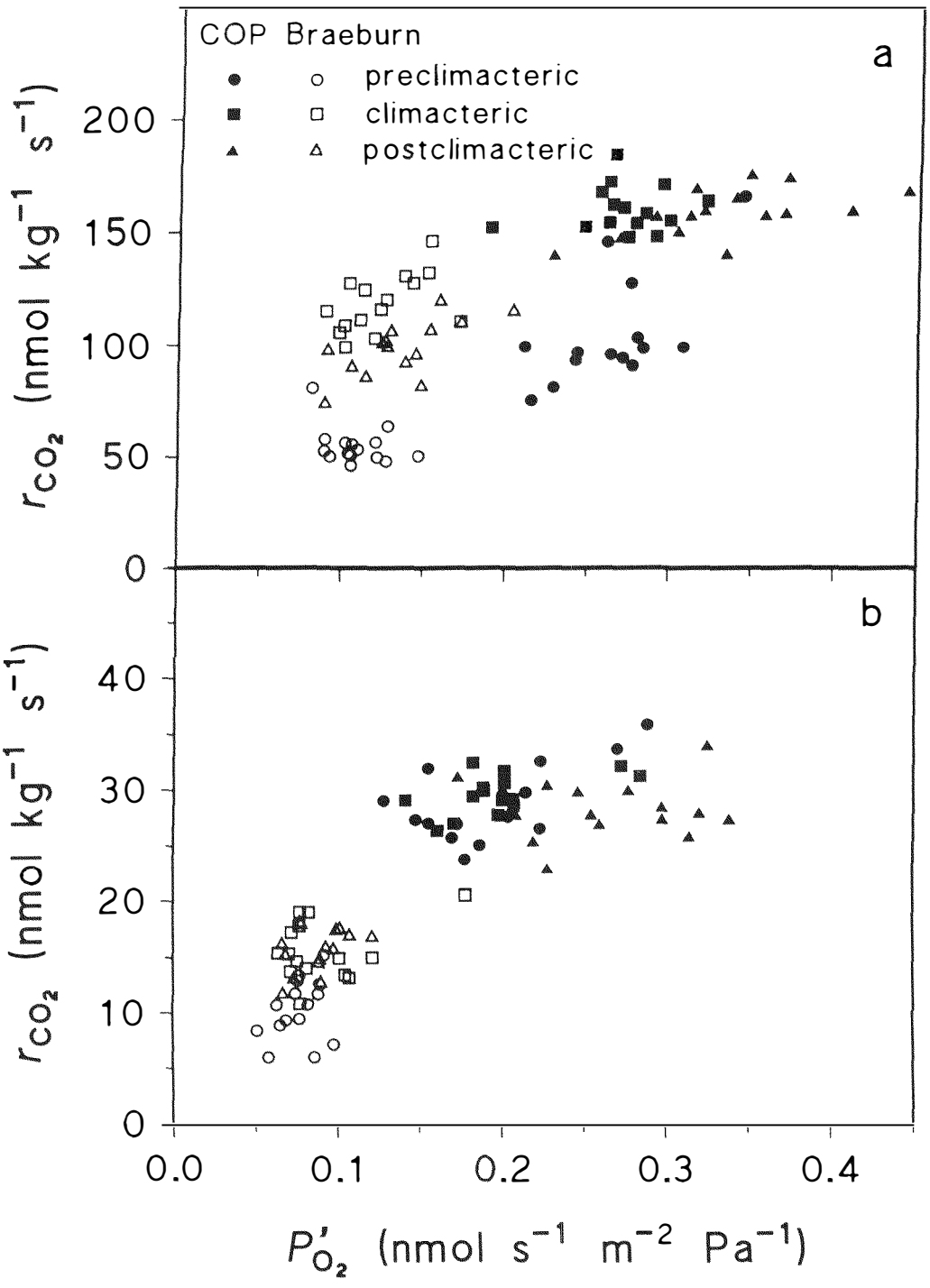

Figure 9.4 Relationship between respiration rate (r_{CO_2}) and fruit permeance to O_2 (P'_{O_2}) for 'Cox's Orange Pippin' ('COP') and 'Braeburn' apples at different physiological stages in air at a) 20°C and, b) 0•C.

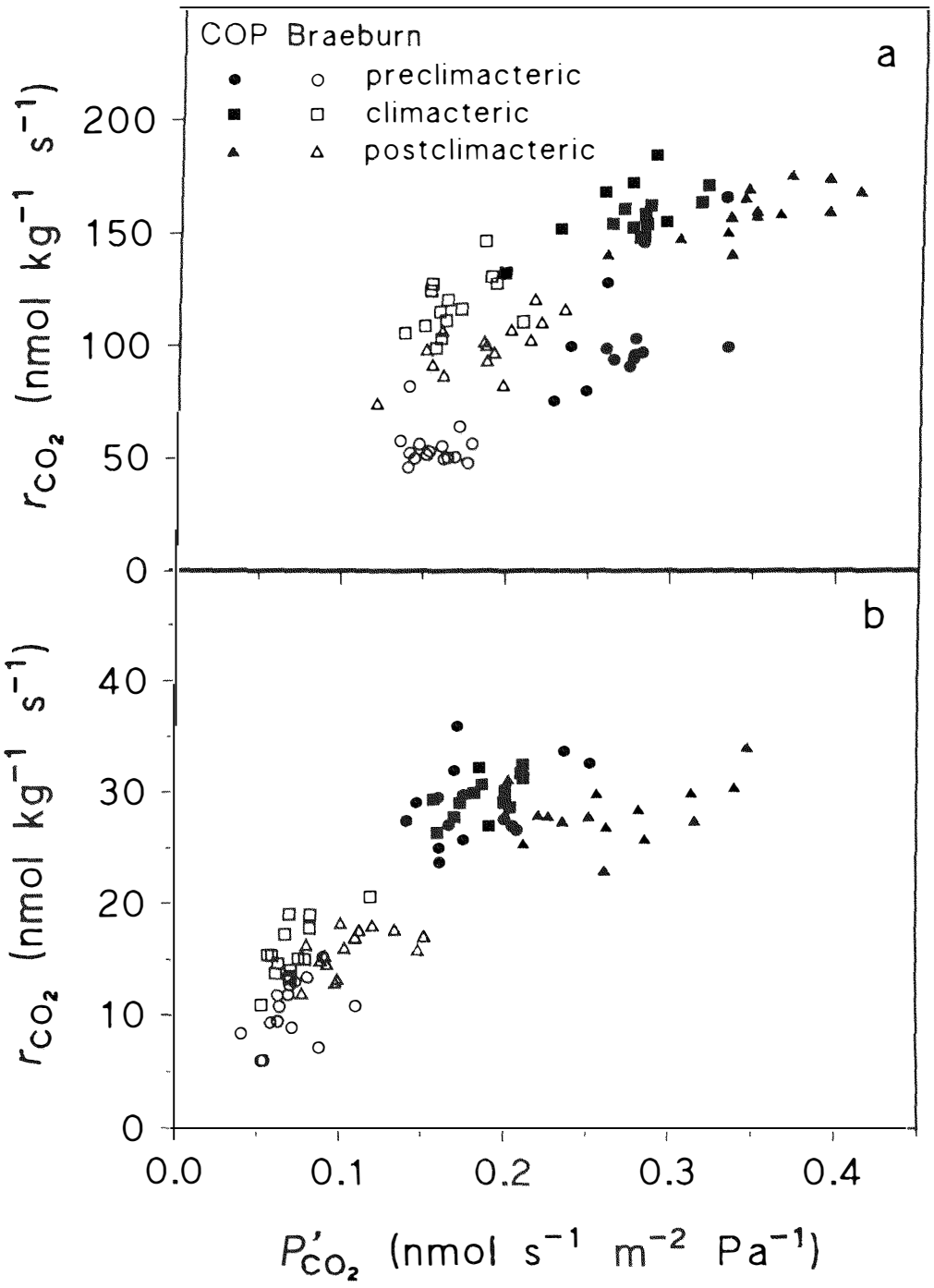

Figure 9.5 Relationship between respiration rate (r_{CO_2}) and fruit permeance to CO_2 (P'_{CO_2}) for 'Cox's Orange Pippin' ('COP') and 'Braeburn' apples at different physiological stages in air at a) 20°C and, b) 0•C.

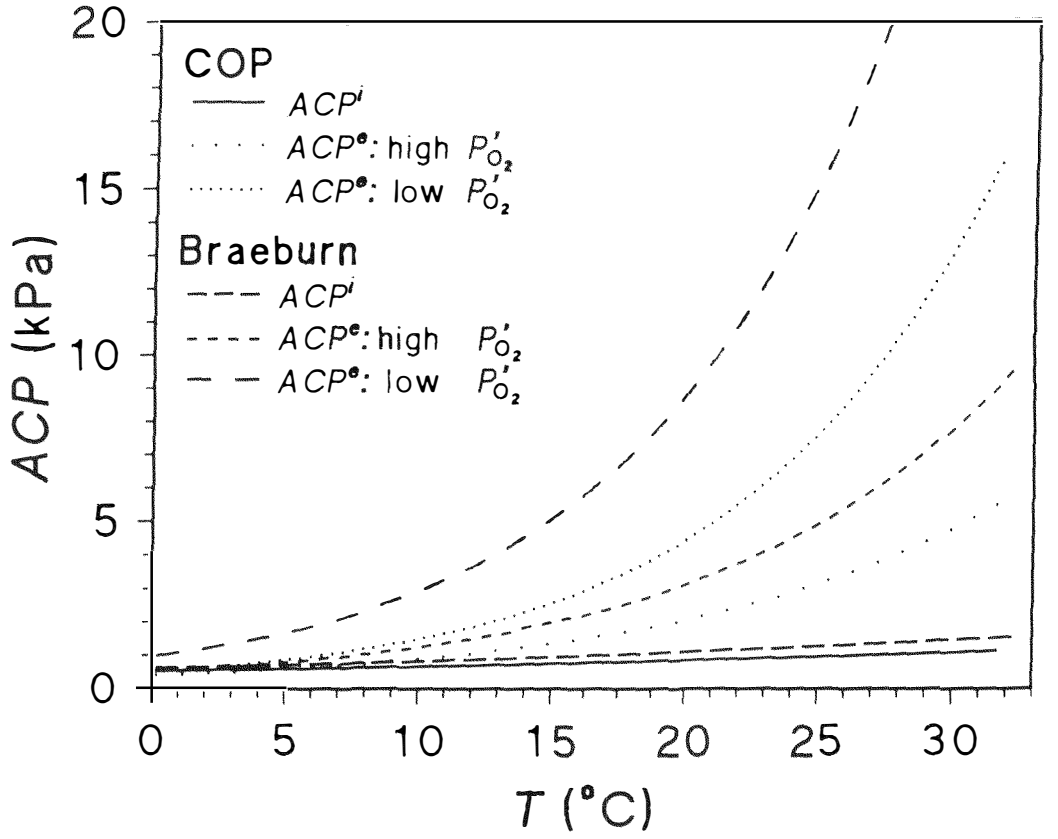

Figure 9.6 Estimates of steady-state ACP^i and ACP^e for 'Cox's Orange Pippin' ('COP') and 'Braeburn' apples as a function of fruit temperature (T) using data from section 5.4.6. Values for ACP^e are estimated from ACP^i using Eq. 1.6, for high and low values of the range of values of fruit permeance to O_2 (P'_{O_2}) estimated for each cultivar. For 'COP'; high and low values used were 0.5 and 0.15 nmol s^{-1} m^{-2} Pa^{-1}, and for 'Braeburn'; 0.2 and 0.05 nmol s^{-1} m^{-2} Pa^{-1}. Data were modelled using the equation $ACP = a\, b^{(0.1T)}$, and coefficients a and b are listed in Table 9.2.

Table 9.2 Coefficients ± standard errors (se) and r^2 values for the relationship between ACP^i, ACP^e and temperature [$ACP = a\ b^{(0.1\ T)}$] for 'Cox's Orange Pippin' ('COP') and 'Braeburn' apples in air at various temperatures, and for fruit with high and low skin permeance to O_2 (P'_{O_2}). For 'COP'; high and low values used were 0.5 and 0.15 nmol s^{-1} m^{-2} Pa^{-1}, and for 'Braeburn'; 0.2 and 0.05 nmol s^{-1} m^{-2} Pa^{-1}.

Cultivar	ACP type	Relative P'_{O_2}	Coefficient ± standard error				r^2
			a	se	b	se	
'COP'	ACP^i		0.52	0.097	1.27	0.093	0.86
	ACP^e	High	0.39	0.225	2.31	0.451	0.96
	ACP^e	Low	0.50	0.330	2.96	0.639	0.98
'Braeburn'	ACP^i		0.62	0.125	1.33	0.113	0.61
	ACP^e	High	0.50	0.168	2.49	0.289	0.94
	ACP^e	Low	0.96	0.303	3.01	0.317	0.98

Estimates of LOL^es are highly dependent on the precision with which the permeance of the skin can be estimated. Two methods were used in this study, the non-steady state ethane efflux method to determine $P'_{C_2H_6}$ (resulting in values similar to P'_{O_2}), and the steady-state method using respiration rate and internal atmosphere data to calculate P'_{O_2} and P'_{CO_2}. The steady-state method was considered the more useful approach for apples as both P'_{O_2} and P'_{CO_2} could be estimated, and the method was less time consuming. Also if a nondestructive technique for estimating IAs were used (such as surface chambers or cannulation), then estimates of P'_{O_2} and P'_{CO_2} could be made for fruit *in situ* in CA or MAPs.

It would be interesting to compare the effect of the different ranges in permeance of 'COP' and 'Braeburn' with other apple cultivars to determine how their ACP^es may be affected by temperature. Dadzie (1992) estimated skin resistance to ethane and r_{CO_2} for eight New Zealand preclimacteric apple cultivars, and the range of values (converted from the original data to $P'_{C_2H_6}$) are presented in Fig. 9.7. It is

apparent from this comparison of cultivars that 'Braeburn' had the lowest $P'_{C_2H_6}$ and range of values and similar range of r_{CO_2} compared to the other cultivars. 'COP' had a medium to low range of $P'_{C_2H_6}$ but the highest values for r_{CO_2}. 'Royal Gala', 'Gala' and 'Splendour' had high $P'_{C_2H_6}$ and a broad range of values and moderate r_{CO_2}. In MAP, these cultivars would be less likely to develop anoxic $p_{O_2}^i$ when subjected to higher temperatures. By contrast, 'Golden Delicious' and 'Red Delicious' had moderate skin permeance but relatively high r_{CO_2}, and would be more susceptible to develop large Δp_{O_2} if MAPs of these cultivars were subjected to higher temperatures. 'Granny Smith' apples had relatively low $P'_{C_2H_6}$ but moderate r_{CO_2} and may respond to temperature in a similar way to 'COP'. However, skin permeance and particularly r_{CO_2} may change markedly as fruit age, and it would be necessary to quantify these changes to be confident of their potential for MAP. Overall, it appears the data for 'COP' and 'Braeburn' can be used as a basis for predicting likely effects of temperature on the behaviour of the other apple cultivars.

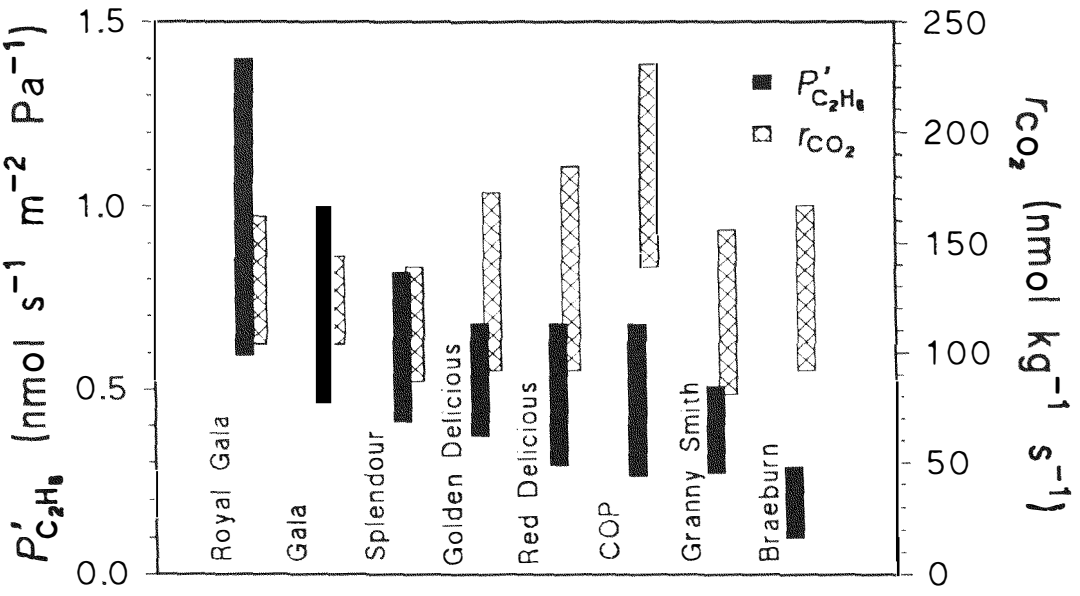

Figure 9.7 Range of estimates of fruit permeance to ethane ($P'_{C_2H_6}$) and respiration rate (r_{CO_2}) at 20°C for a selection of preclimacteric New Zealand apple cultivars. Cultivars are in order of decreasing $P'_{C_2H_6}$ from left to right, and data are converted from Dadzie (1992); n=16.

9.6 Internal atmosphere modulated CA storage of apples (IAMCA)

Ideally, optimum storage atmospheres based on external atmospheres would be changed as fruit age to reflect the changing physiological status of the fruit (Wollin *et al.* 1985). Physiological change associated with the climacteric in apples is greatly attenuated for fruit in CA, due to the inhibitory effect of both low O_2 and low temperature on r_{CO_2}. However, modulating CA atmospheres would attenuate effects on internal atmospheres determined from increasing skin permeance during storage or increasing susceptibility to gas-related disorders.

A system for dynamic control of storage atmosphere has been reported by Wolfe *et al.* (1993) using a controller that determines conditions for producing minimum product respiration. This used a complex algorithm and had the ability to continually improve on its ability to predict changes in a fruit. It may be possible to develop a conceptually much simpler control system for modulating CA atmospheres using feedback from IA composition. Changes in skin and flesh permeance and respiration during CA storage result in slow changes in IAs. IAs may approach values for LOL^is increasing the risk of fermentation. By monitoring IAs adjustments could be made automatically to increase $p^e_{O_2}$ and decrease $p^e_{CO_2}$ to maintain the optimum storage atmosphere at any point in time. IAs could be sampled remotely from a small number of strategically placed "reference fruit" in the CA store.

What might constitute a reference fruit? Should it represent the average, maximum or minimum skin permeance and respiration rate of the population? In a study comparing 'Braeburn' apples from four orchards in two geographically distant growing regions in New Zealand and for fruit from early, middle and late harvests, no differences were found in average P'_{O_2} and P'_{CO_2} between regions, orchards or harvests (see Appendix 5). However, when extreme values were compared, differences between regions and orchards and harvests were observed. Therefore, average values may obscure important information about the relative risks of different populations of fruit in CA becoming anaerobic. To minimise risk of fermentation of some fruit, reference fruit should be those with the lowest permeance in the population.

Reference fruit might be found by estimating permeance and respiration rate from a subsample of the population using the steady-state method. Fruit with low permeance could be created using a skin coating. Although the latter method would be simpler to apply once the type and amount had been determined, it is uncertain how a skin coating might affect natural changes in the properties of lenticels and cuticle as fruit age. Skin coatings tend to block pores (the primary route for O_2 diffusion) and are generally more permeable to CO_2 than O_2. Therefore, coatings may increase the ratio of P'_{CO_2}/P'_{O_2} in comparison with controls. The effect of pore blockage on Δp_{O_2} may vary considerably, and there is evidence that so long as even a small proportion of large pores remain open, Δp_{O_2} may not be excessively affected (N.H. Banks, *pers comm.*). Alternatively, a random selection of fruit could be used for the reference fruit, if it was considered acceptable that a small proportion of fruit with low skin permeance become anaerobic in order that the larger proportion of fruit are not exposed to suboptimal atmospheres.

Given that a suitable form of reference fruit could be found, changes in core cavity atmosphere of these fruit throughout storage could be fed into a model that predicted appropriate $p^e_{O_2}$ and $p^e_{CO_2}$ for the physiological state of the fruit in CA. The $p^i_{O_2}$, $p^i_{CO_2}$ and/or ethanol of reference fruit could be monitored remotely by sensors connected to the core cavity by a cannula. The core cavity atmosphere and that above the sensors would come to equilibrium by passive diffusion through the cannula.

The main components of a IAMCA system are presented in Fig. 9.8, and could operate as follows:

1. The atmosphere ($p^e_{O_2}$ and $p^e_{CO_2}$) of the CA store containing precooled fruit would be monitored as the external atmosphere is rapidly pulled down to a preset level.
2. The final external atmosphere would be achieved through IAMCA control. IAs ($p^i_{O_2}$, $p^i_{CO_2}$, and/or ethanol) of cannulated reference apples would be monitored with CO_2 /O_2 or ethanol sensors by: a) either recirculating the core cavity atmosphere with a small remotely operated pump in the sensor housing, or b) allowing the core cavity atmosphere coming to equilibrium by passive diffusion with the atmosphere surrounding the sensors. The sampling procedure and data capture would be under the control of a data interface and computer.

3. IAs could be compared to preset tolerance values by the computer. Alternatively, average values of IAs might be used to estimate the risk of fermentation or development of storage disorders. Tolerance values might be determined from LOL^is, and the susceptibility of the cultivar to storage disorders.
4. If levels of $p^i_{O_2}$, $p^i_{CO_2}$ or ethanol were outside a predetermined range, the computer programme would gradually adjust levels of $p^e_{O_2}$ and $p^e_{CO_2}$ in the CA store to bring IAs back within the acceptable range.
5. IAMCA software would instruct the CA gas controller to adjust the CA store atmosphere automatically.

As CA stores are typically loaded with fruit from different growers (each with potentially different fruit characteristics), ideally, batches of representative fruit would be randomly selected from each grower. Monitoring individual grower lines may provide a more objective basis for deciding when CA should be broken for particular lines. For CA stores with capability for rapid O_2 pull-down, there is the potential to break CA storage to remove and market specific lines of fruit.

The main problems of the system would be the need to set up reference fruit in the store, whether these fruit truly represent the lowest permeance fruit in the population, and the need to account for the lag in response of fruit to changing external atmospheres.

The benefits of an IAMCA system are that it would account for changes in skin and flesh permeance and respiration rate of fruit throughout CA storage. It may also be possible to factor into the software information on the susceptibility of the cultivar to gas- or temperature- related disorders. Operational constraints may render IAMCA impractical for automated control of store atmospheres. However, it may be suitable as an alarm system to alert CA store operators of the need to manually adjust store atmospheres.

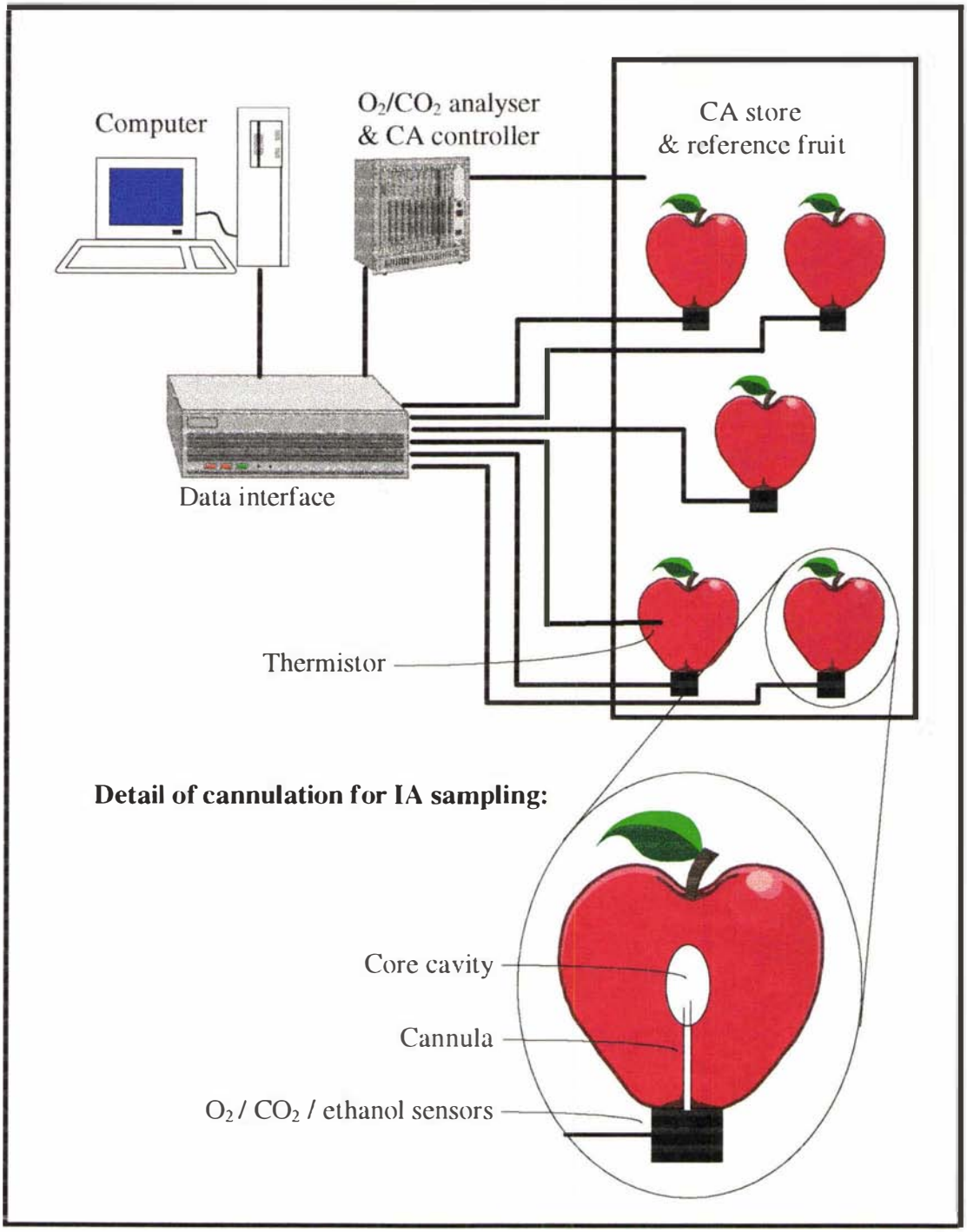

Figure 9.8 Schematic diagramme of a system for internal atmosphere modulation of CA storage atmospheres for apples (IAMCA).

9.7 Recommendations for further research

It is clear from Table 9.1 that there is considerable scope for further research in both modelling biophysical variables and physiological responses of fruit in hypoxic atmospheres, particularly around the aerobic-anaerobic transition. The current study explored quantitative approaches to characterising physiological responses of apples using IAs, in relation to some environmental and fruit-related variables. The biophysical and physiological characteristics of apple cultivars vary widely, and the two cultivars used in the study were sufficiently contrasting to permit qualitative extrapolation of the results to other cultivars. Similar methods used in this study could be used to quantify responses in other fruit or vegetables, particularly those with either low cortical porosity, or whose porosity (and possibly diffusivity to O_2) changes significantly during ripening (as for avocados and stonefruit). Data from these studies would be valuable in extending the general steady-state model for gas exchange outlined in chapter 1, to situations where significant heterogeneity of IAs clearly invalidates use of Fick's First Law of Diffusion.

The current study used fruit of one commercial size (count 125) with relatively constant surface area to mass ratio (A / M). It is possible that larger fruit, with smaller A / M have even larger gradients between external and internal atmospheres. The longer diffusion path from skin to the core cavity may also result in lower diffusivity, significant intracortical gradients, and heterogenous respiration rates throughout the cortex. Some evidence from these studies (data not presented) suggested this may be true, particularly for 'Braeburn'. Alternatively, smaller fruit have a larger A / M and less likely to have significant intracortical gradients. While the size of fruit is likely to affect LOL^es as a result of larger Δp_{O_2}, the effect on LOL^is is unknown. Specifically, it would be interesting to characterise the relationships between LOL^is of apple cultivars of different densities and porosities and at different temperatures, and quantify LOL^is of individual fruit to quantify variability. This might best be achieved using the non-steady state method of Leshuk and Saltveit (1990) and very low O_2-depletion rates. Some preliminary results were obtained for LOL^is in different tissue zones indicating differences in LOL^is might be correlated to porosity of tissue in different zones (data not presented). It would also be interesting

to correlate this information with the incidence and severity of disorders such as core-flush, for different tissue zones throughout the cortex.

It has generally been assumed that the fastest possible exposure of harvested produce to optimal CA conditions is essential for quality preservation in pipfruit over longer storage times (Bohling, 1994; Little and Peggie, 1987). However, beneficial effects on quality of apples may also be achieved with slower pull-down of both temperature and (through utilisation of fruit respiration) O_2 atmospheres (Bohling,1994). For example, development of 'Braeburn browning disorder' (a disorder of 'Braeburn' apples exacerbated by low levels of O_2 and high CO_2) was markedly reduced from 88% to 22% when the time taken to reach a CA atmosphere of 2 kPa CO_2 and 2 kPa O_2 increased from 1 to 16 days (Elgar *et al.*, 1996). The rate of pull-down to anoxic atmospheres in the current studies led to establishment of atmospheres in approximately six hours. Rate of pull-down has been demonstrated to affect respiratory behaviour and ACP^e of carrot slices (Leshuk and Saltveit, 1990, 1991), and pear fruit and cultured pear fruit cells (Boersig *et al.*, 1988). It would be interesting to see if the ACP^i shifts in relation to the rate of pull-down. These studies might be conducted at both isothermal conditions and for decreasing fruit temperature to quantify the effect that the lag in respiration might have on $p^i_{O_2}$ and *LOL*s. Future studies could also include estimation of key TCA cycle acids and level and activities of enzymes of anaerobic respiration.

Knowledge of *LOL*s is critical for optimising storage atmospheres. However, optimum storage atmospheres are not merely those at which aerobic respiration is minimised without increase in ethanol production, but atmospheres that minimise the rate of deterioration in crop quality. It is crop quality (and cost) that determines consumer interest, and little attention appears to have been given to developing models predicting the effect of low O_2 and elevated CO_2 on quality attributes. Solomos (1994) noted that such studies would be useful in identifying the affinity for O_2 of enzymes whose activity is restricted at relatively high p_{O_2}, and that may be involved in the response to O_2 and CO_2 levels. Therefore, there is a need for a broader, holistic model for optimising storage atmospheres, but these need to be undertaken on the basis of internal atmospheres. Conceptually, this would link gas

exchange models characterising effects on respiratory behaviour, and those quantifying:

- rates of physiological deterioration (changes in key crop quality attributes/indices such as firmness, density, soluble solids content, skin colour and incidence and severity of disorders),
- sensory attributes (texture, flavour, and relationships between ethanol content or other flavour volatiles and flavour scores (Ke and Kader, 1992), and
- consumer health (nutritive value, microbiological issues).

The need for this approach in optimising CA storage atmospheres has recently been recognised by the apple industry, and a collaborative project initiated involving HortResearch Ltd., and the Centre for Postharvest and Refrigeration Research at Massey University, is now funded by ENZA New Zealand (International).

Loss of flavour of CA stored apples may become increasingly important as demand for longer storage periods increases. This may be particularly evident in export markets where New Zealand CA stored apples, perceived by consumers as being of high quality, may be purchased along with fresh local apples. Therefore, there is a need to develop commercially viable methods for enhancing flavour volatiles in CA stored fruit, either by anaerobic-shock pretreatments or during CA storage.

Looking to the future, major advances in the way CA and MA technologies are used for pipfruit, are likely to come from four sources:

1. Optimisation of quality using existing and new CA/MA technologies through holistic models. The objective would be to minimise deterioration in apple quality using novel pretreatments (such as low O_2 or high CO_2 shock etc...), and modulated CA storage to enhance the benefits of CA storage specific to the needs of individual cultivars and the changes to their biophysical and physiological attributes during storage.
2. MAP storage. The commercial use of "sense-and-respond" packages is likely to shift emphasis from expensive CA technologies to MAP (Cameron *et al.*, 1993). Sense-and-respond packages would use polymeric films that are strong (resist puncture) and have permeability that changes as a function of temperature or CO_2 / O_2 / ethanol, to prevent the crop fermenting when packages are subjected to temperature abuse. Development of sense-and-respond packages requires not only

technical innovations in polymer chemistry, but empirical data characterising relationships between $p^{i}_{O_2}$ and rates of ethanol (and/or acetaldehyde) biosynthesis, and headspace ethanol concentrations (Beaudry *et al.*, 1993). Significant levels of ethanol and acetaldehyde may accumulate without decreasing sensory quality (Ke and Kader, 1990a 1990b; Ke *et al.*, 1990). Therefore, some degree of anaerobiosis may be tolerated, which in concept allows the design of sense-and-respond packaging systems based on the presence of ethanol and acetaldehyde (Beaudry *et al*, 1993). Characterising rates of off-flavour development relative to the degree and duration of anaerobiosis would also be required. Cameron *et al.* (1993) described a sensing system which would change colour in response to very low levels of ethanol. This might be useful as an indicator that the packaged crop has experienced significant periods of anaerobiosis. However, what is ultimately required is a system whereby small amounts of headspace ethanol can control O_2 permeability of the package.

3. Studies of adaptive responses of tissues to hypoxia and anoxia, similar to those extensively undertaken for animals (Hochachka, 1991) and plants susceptible to flooding (Sachs *et al.*, 1996). It is possible that fruits may adapt to very low $p^{i}_{O_2}$ if the initial exposure is to an atmosphere that is sensed by the tissues as low in O_2, but in which respiration produces enough energy to allow adaptive changes. The stress level, duration and temperature required to promote adaptation would need to be quantified for each crop and storage regime. Pretreatments of this nature for apples [analogous to heat treatments to reduce scald and low temperature injury, Klein and Lurie (1992)], are likely to differ substantially from the current approach of rapid cooling and immediate CA, both of which probably reduce opportunity of fruit to adapt to hypoxia. Such pretreatments may require fruit to be placed in a low $p^{e}_{O_2}$, but well above the final storage $p^{e}_{O_2}$, and at higher temperatures than the final storage temperature, for a few days before transfer to storage atmospheres and temperatures. Preconditioning may allow, for example, transcription of ADH mRNA [as reported for tomatoes at 12 kPa $p^{e}_{O_2}$, Longhurst *et al.* (1994)]. This may result in enhanced ability to convert toxic levels of acetaldehyde to ethanol, which may then be further metabolised to ethyl acetate and flavour volatiles. Chervin *et al.* (1995) suggested a greater emphasis on the

adaptive role of non-glycolytic enzymes induced by hypoxia, and a potential role for chaperonins (mediators of protein conformation and turnover) in response to anoxia, may lead to new approaches to improve options for fruit storage and gas-related disinfestation techniques.

4. Studies of the role of cytosolic calcium ions in the signal transduction pathway leading to anaerobic response, molecular responses to hypoxia and anoxia, and basis of physiological disorders (Sachs *et al.*, 1996). Hypoxia induces expression of anaerobic proteins, while suppressing genes involved in plant development and that eventually lead to plant senescence. Understanding the fundamental molecular regulation of these genes that suppress fruit metabolism and may result in physiological disorders, may provide scope for genetic manipulation of tissue to enhance adaptation to low O_2 atmospheres, lower ripening responses to ethylene, decrease susceptibility to disorders, and augment traditional approaches to pipfruit breeding. The benefits may allow not only improved maintenance of quality for fruits in traditional CA and MA storage, but improve potential disinfestation techniques (whether gas and/or temperature based) by maximising insect mortality while minimising deleterious tissue responses.

The advent of "super-ships" may diminish a major constraint to marketing our fresh pipfruit, that is, the time taken for fresh product to reach our distant markets. However, the New Zealand pipfruit industry faces challenges from increasing volumes of crops competing with apples on supermarket shelves, and improving quality of pipfruit from large volume producers also supplying our traditional markets. If New Zealand is to maintain its competitive edge in marketing premium quality pipfruit, we will need to strengthen existing strategic and applied research programmes, and initiate new collaborative efforts between the industry and science providers.

9.8 Conclusions

Knowledge of *LOL*s is crucial to maximise the benefits of CA and MA storage of apples without risk of some fruit becoming anaerobic. Two types of *LOL* were defined quantitatively; the anaerobic compensation point and the fermentation threshold. The fermentation threshold was defined on the basis of change in respiratory quotient and ethanol accumulation, as a function of the partial pressure of O_2. Objective methods developed for quantifying both measures of *LOL* were considered useful for optimising storage atmospheres. The fermentation threshold by definition indicated the point at which anaerobic fermentation was detectable and occurred at higher partial pressures of O_2. Consequently it was a more conservative measure of *LOL*.

This study has highlighted the practical importance of conceptual and objective quantitative tools for estimating *LOL*s on the basis of the internal atmospheres rather than external or package atmospheres. Internal atmospheres differed from external atmospheres as a consequence of effects of respiration rate and skin permeance, and the relationships were adequately described by Fick's First Law of Diffusion, as evidenced by small intracortical gradients in internal atmosphere composition. LOL^is and LOL^es differed in an analogous way to internal and external atmospheres.

Increasing temperature from 0° to 32°C had little effect on increasing LOL^is of 'COP' and 'Braeburn' apples, except at temperatures > 28°C. Similarly, elevated levels of CO_2 in the storage atmosphere, and physiological age of these cultivars had no or little effect on LOL^is. While cultivars with lower cortical tissue porosity, such as 'Braeburn', are likely to have slightly higher LOL^is, they were relatively constant with respect to the environmental and physiological variables measured in this study. In contrast, LOL^es increased markedly as temperature increased, and the increase was greater for fruit with low skin permeance to O_2 and CO_2, such as 'Braeburn'. The high intra- and intercultivar variability in skin permeance means LOL^is, and in particular the fermentation threshold, should provide the safest estimate of the true *LOL* of a population of apples.

For apples in CA storage at low isothermal temperatures, the effects of skin permeance and respiration rate on the difference between LOL^is and LOL^es are minimised. Changes in skin permeance and respiration rate may occur throughout the storage period resulting in a shift in LOL^e. It may be possible to monitor internal atmospheres in low permeance fruit to modulate CA storage atmospheres, reducing the risk of a proportion of fruit fermenting. It is more likely that apples in MAPs could ferment if sealed packages are exposed to high temperatures. The effect of temperature may be attenuated using sense-and-respond packages, otherwise MA atmospheres should be optimised using internal atmospheres of fruit equilibrated at the highest temperature likely to be experienced for any significant duration.

Overall, this study has demonstrated that an understanding of the dynamics of gas interactions in apple fruit using modelling approaches is a powerful tool for optimising storage atmospheres. This coulpled with the knowledge of the inherent susceptibility of fruit to disorders can be usefully used to maximise the quality of apples stored in CA and MAP.

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Appendix 1

Sample Plots of Data for Assessment of Lower Oxygen Limits

The following graphs are representative of the types of plots made from chamber and core internal atmospheres. For each type of plot two types of graph are presented below:

(a) raw data for all treatments in a graph with axis labels,

(b) raw data for all treatments excluding data for $p^{i}_{O_2} > 13$ kPa, in a graph without axis labels.

Type (b) graphs were presented to a trained panel for assessment of the lower O_2 limits. The graphs presented to the panelists were enlarged and printed one per A4 page in landscape format, to improve clarity and accuracy. Each graph was identified by a 3 digit code, where the first digit represented a treatment, the second a plot type and the third the cultivar. Each panelist was given a set of the same graphs (approximately 80 per set) but in randomised order. One graph was duplicated 5 times and evenly distributed throughout each set as a check on the consistency with which individual panelists assessed the *LOL*s.

The following coded graphs of type (a) and (b) are presented below:

Code: Treatment, Plots type, Cultivar:

920 24°C, chamber $p^{i}_{CO_2}$ versus chamber $p^{i}_{O_2}$, 'COP'

940 24°C, core $p^{i}_{CO_2}$ versus core $p^{i}_{O_2}$, 'COP'

910 24°C, chamber RQ_{ia} chamber $p^{i}_{O_2}$, 'COP'

930 24°C, core RQ_{ia} versus core $p^{i}_{O_2}$, 'COP'

900 24°C, chamber c^{i}_{EtOH} versus chamber $p^{i}_{O_2}$, 'COP'

620 2 kPa $p^{e}_{CO_2}$, 20°C, chamber $p^{i}_{CO_2}$ versus chamber $p^{i}_{O_2}$, 'COP'

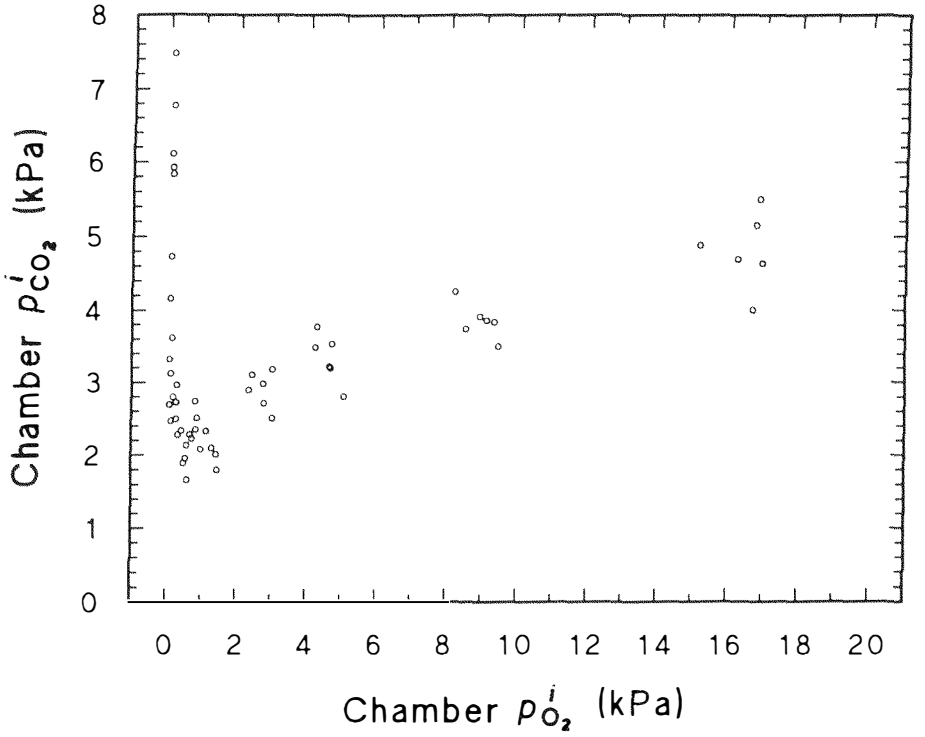
610 2 kPa $p^{e}_{CO_2}$, 20°C, core $p^{i}_{CO_2}$ versus core $p^{i}_{O_2}$, 'COP'

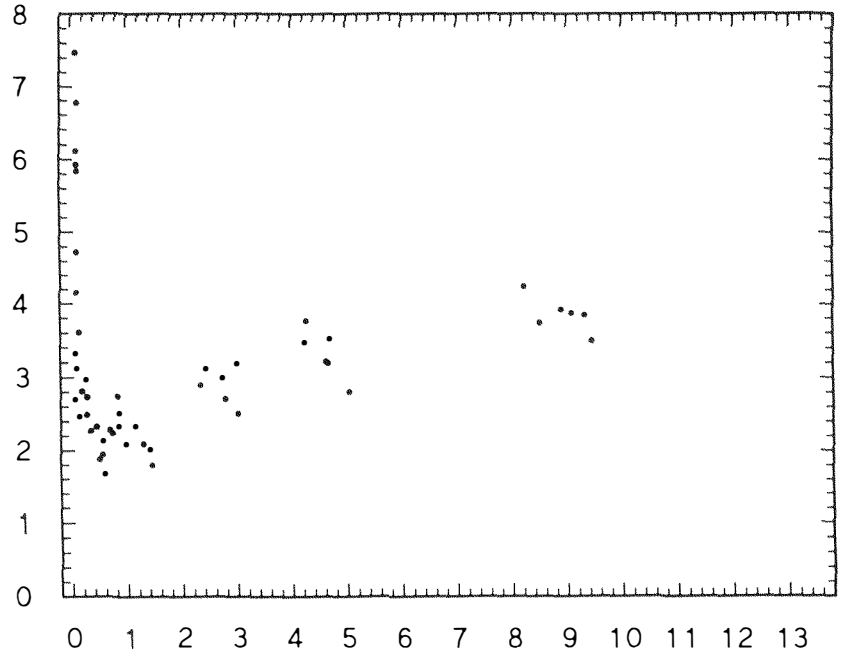
650 2 kPa $p^{e}_{CO_2}$, 20°C, chamber RQ_{ia} chamber $p^{i}_{O_2}$, 'COP'

640 2 kPa $p^{e}_{CO_2}$, 20°C, core RQ_{ia} core $p^{i}_{O_2}$, 'COP'

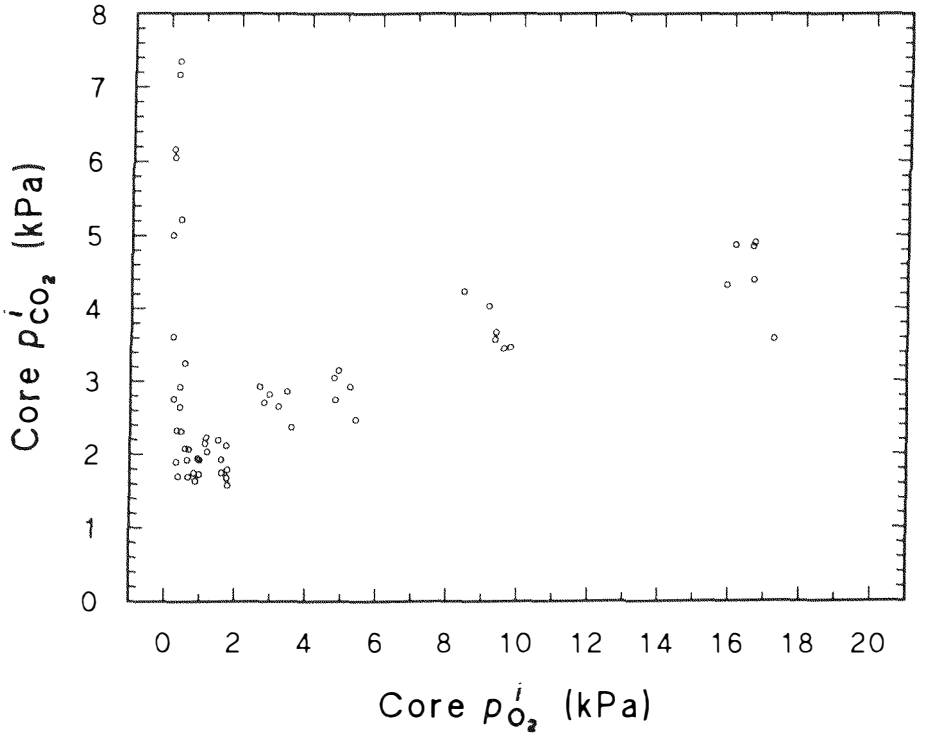
630 2 kPa $p^{e}_{CO_2}$, 20°C, chamber c^{i}_{EtOH} versus chamber $p^{i}_{O_2}$, 'COP'

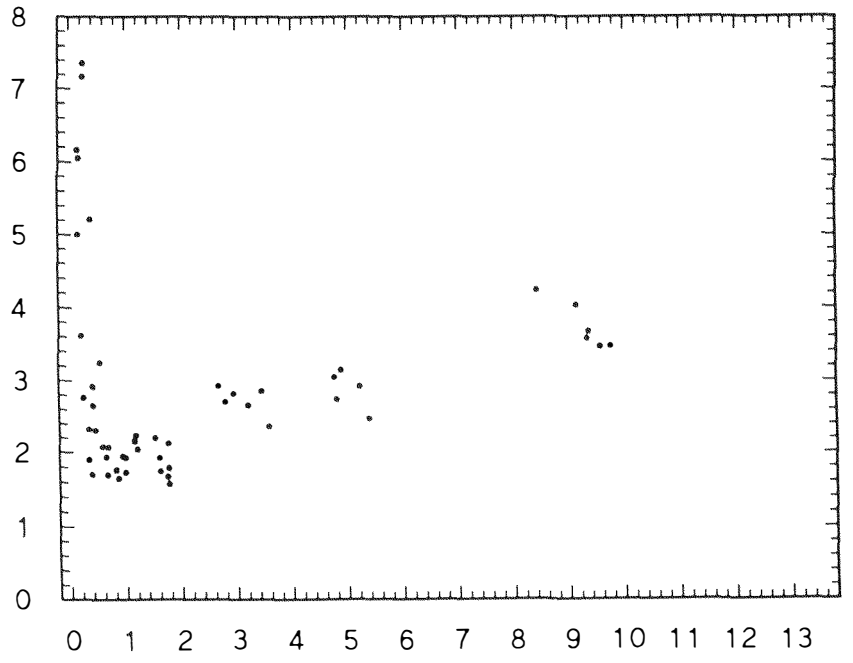
920


Chamber $p^i_{CO_2}$ (kPa)
0
1
2
3
4
5
6
7
8
0 2 4 6 8 10 12 14 16 18 20
Chamber $p^i_{O_2}$ (kPa)

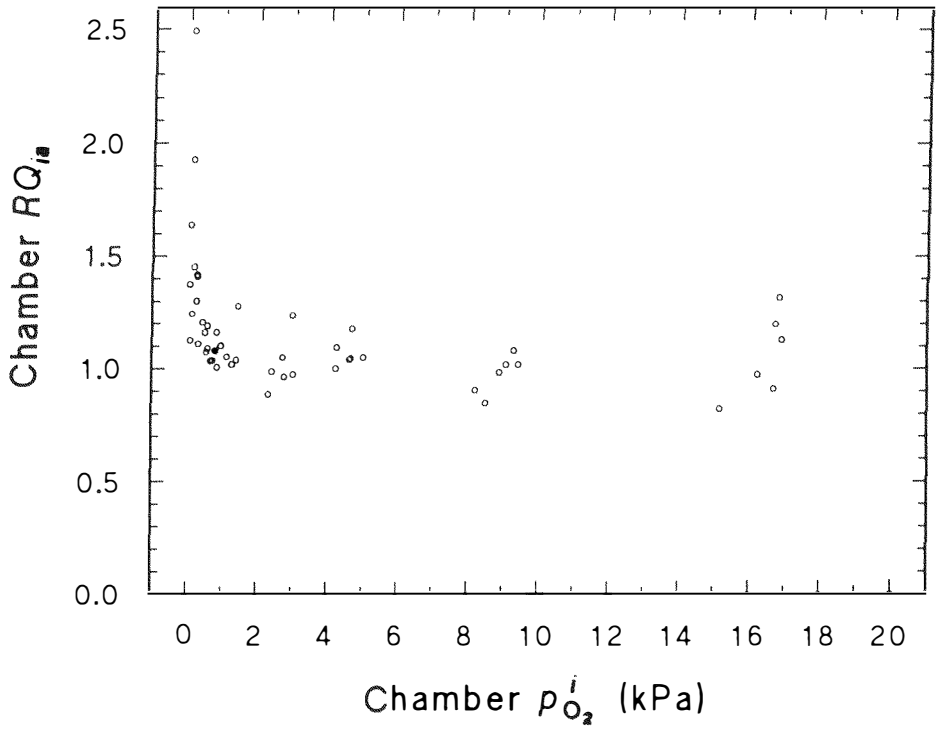

0
1
2
3
4
5
6
7
8
0 1 2 3 4 5 6 7 8 9 10 11 12 13

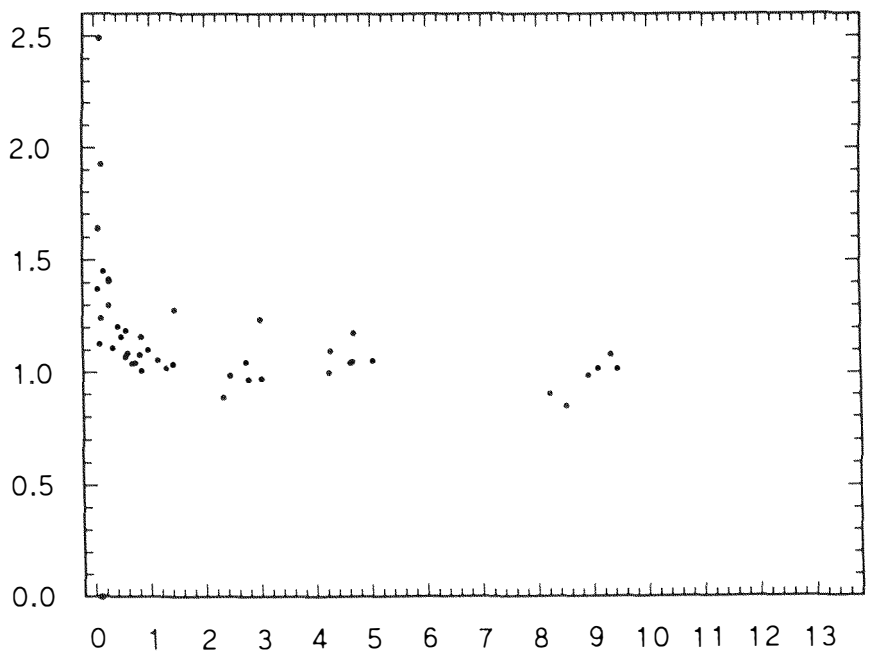
940


Core $p^i_{CO_2}$ (kPa)
0
1
2
3
4
5
6
7
8
0 2 4 6 8 10 12 14 16 18 20
Core $p^i_{O_2}$ (kPa)

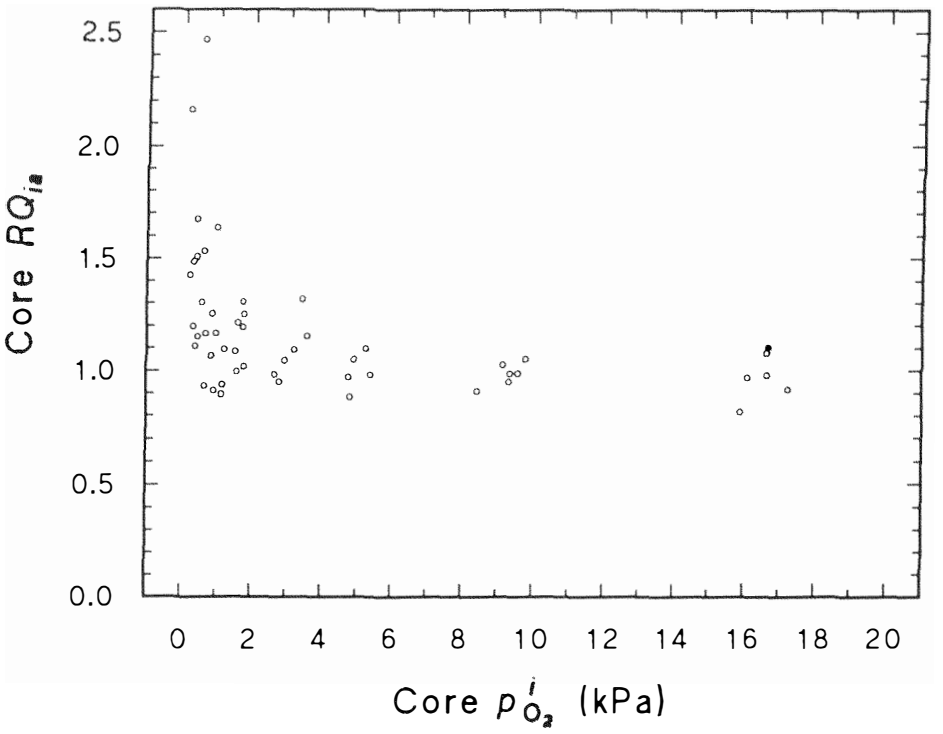

0
1
2
3
4
5
6
7
8
0 1 2 3 4 5 6 7 8 9 10 11 12 13

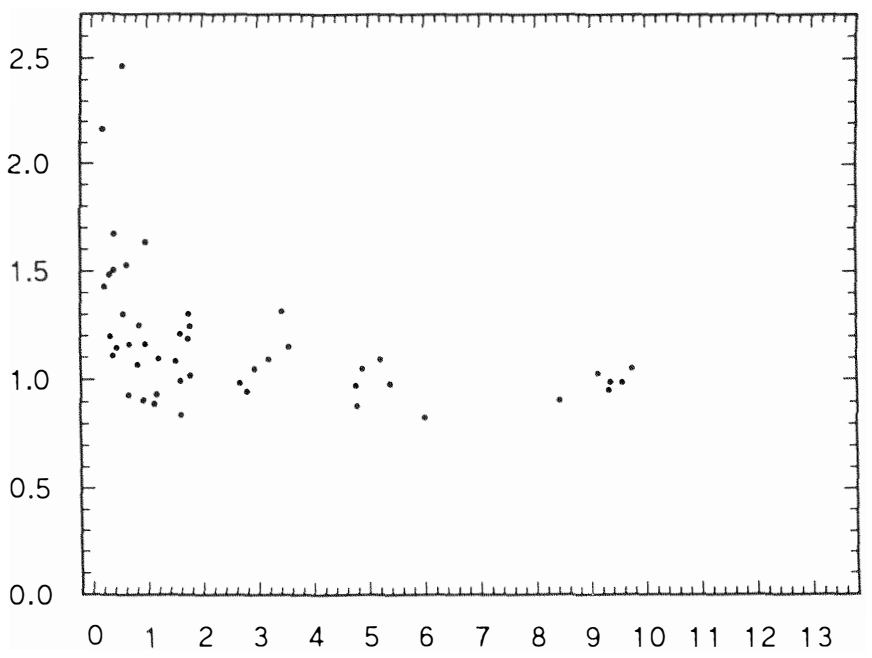
910


Chamber RQ_{ia}
2.5
2.0
1.5
1.0
0.5
0.0
0 2 4 6 8 10 12 14 16 18 20
Chamber $p^i_{O_2}$ (kPa)

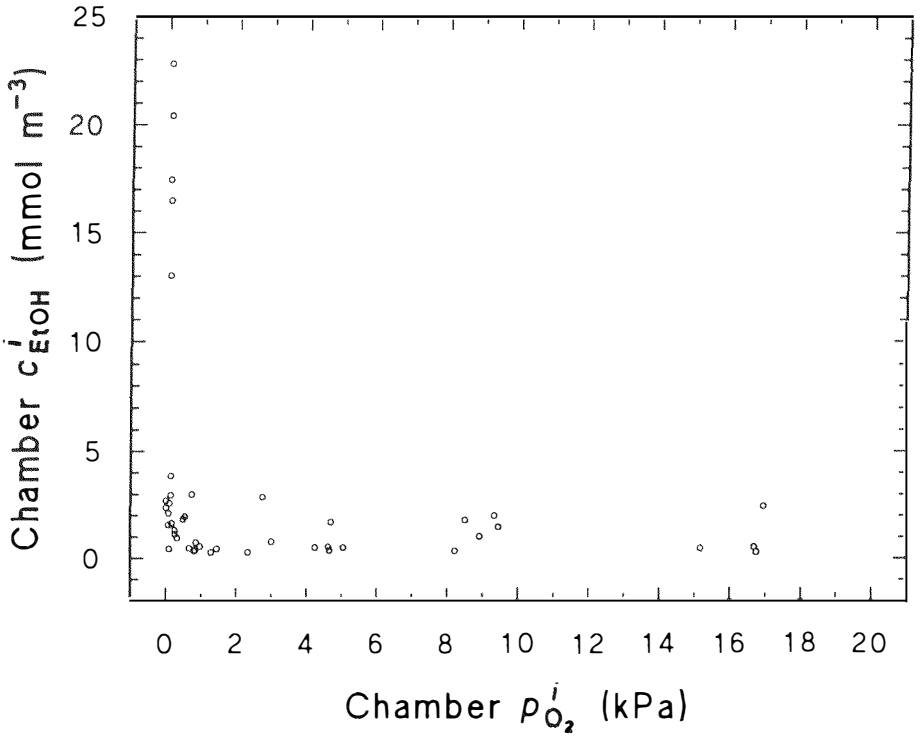

2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13

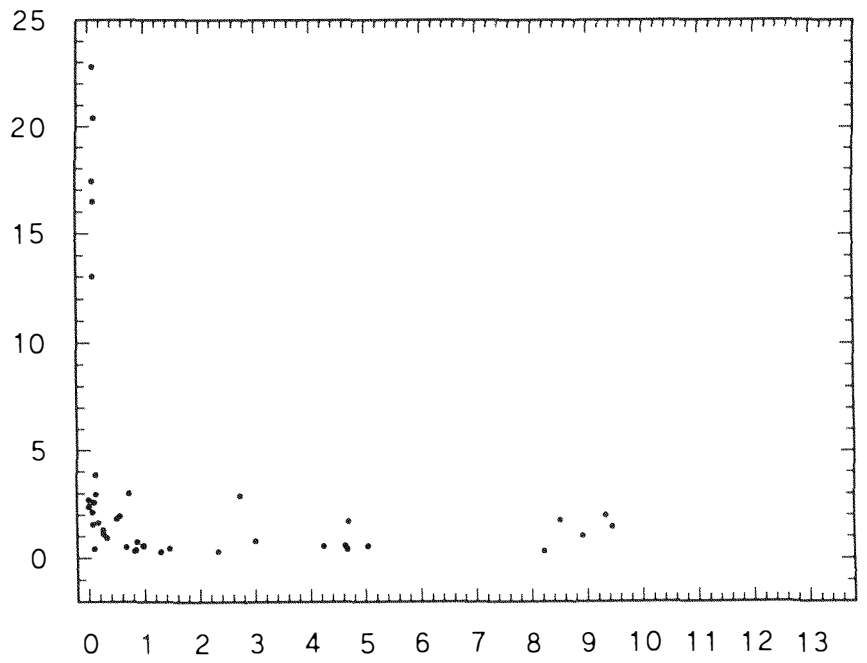
930


2.5
2.0
1.5
1.0
0.5
0.0
Core RQ_{is}
0 2 4 6 8 10 12 14 16 18 20
Core $p^{i}_{O_2}$ (kPa)

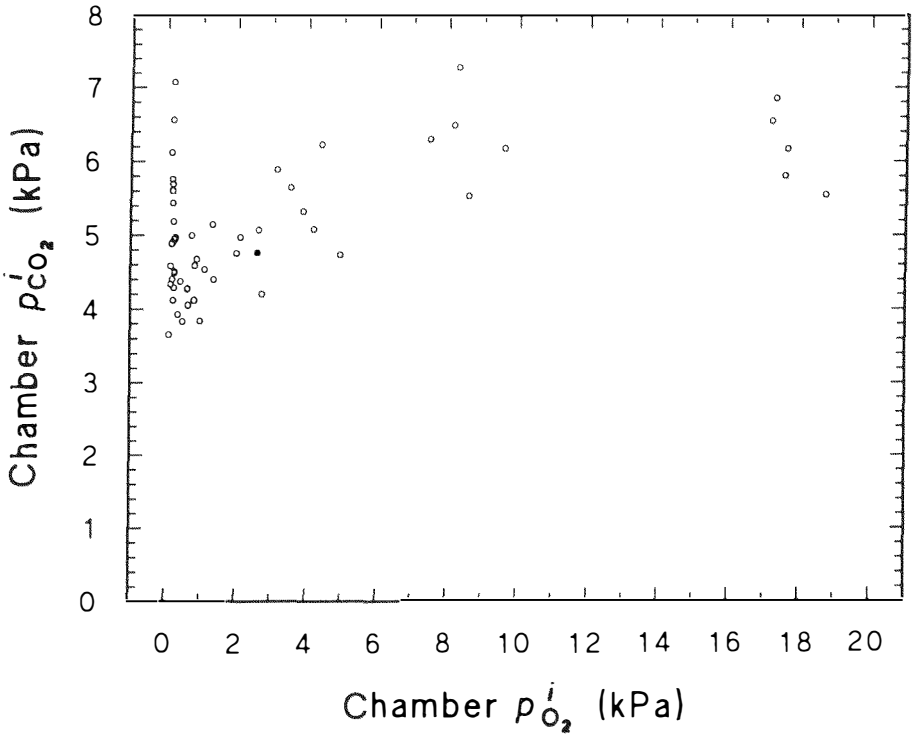

2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13

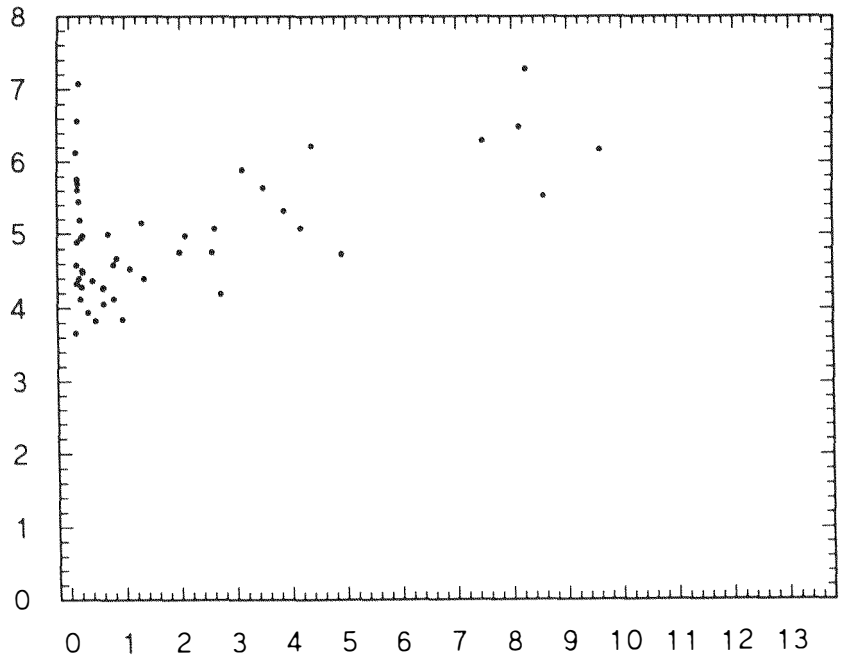
900


25
20
15
10
5
0
Chamber c^i_{EtOH} (mmol m^{-3})
0 2 4 6 8 10 12 14 16 18 20
Chamber $p^i_{O_2}$ (kPa)

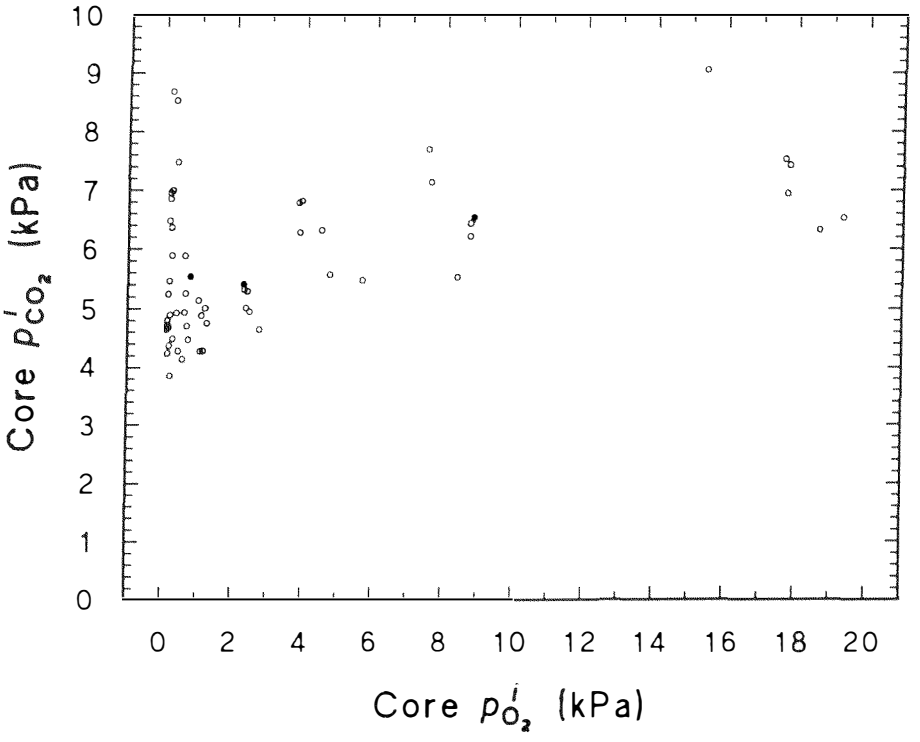

25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13

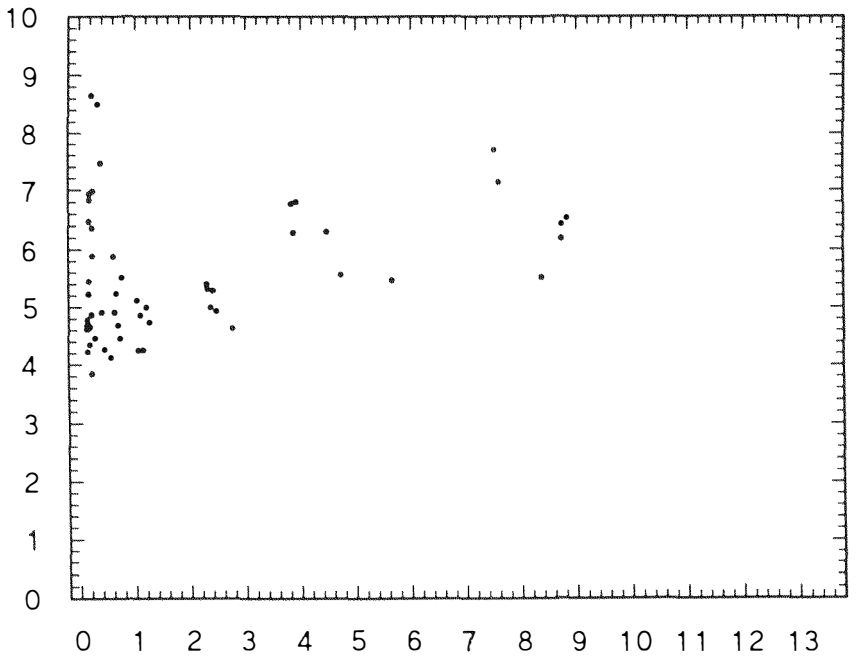
620


Chamber $p^i_{CO_2}$ (kPa)
Chamber $p^i_{O_2}$ (kPa)
0 1 2 3 4 5 6 7 8
0 2 4 6 8 10 12 14 16 18 20

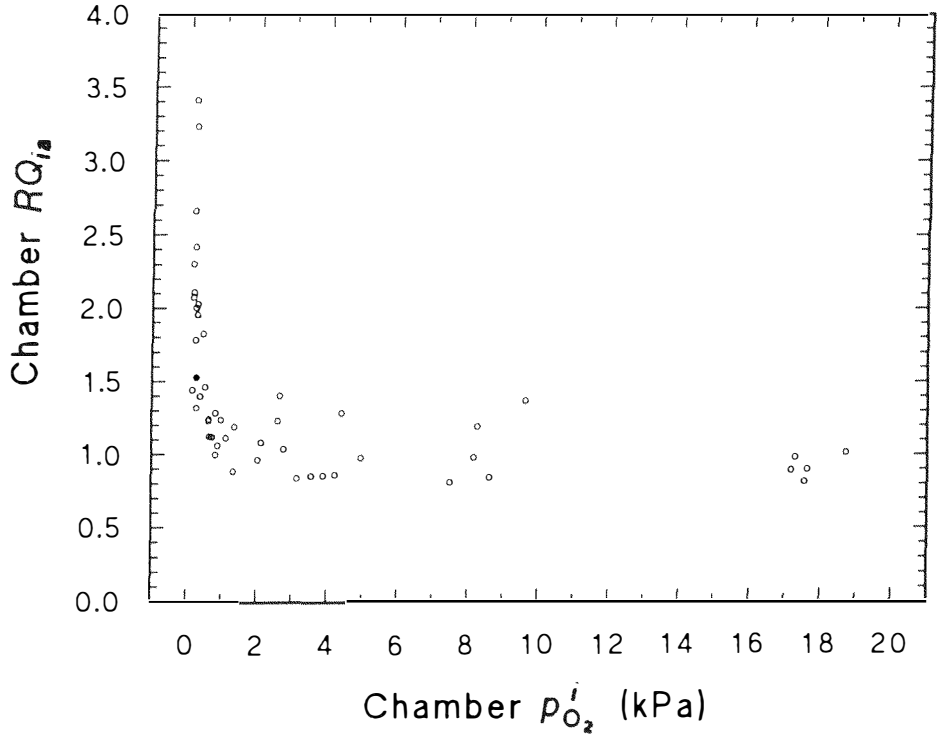

0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 6 7 8 9 10 11 12 13

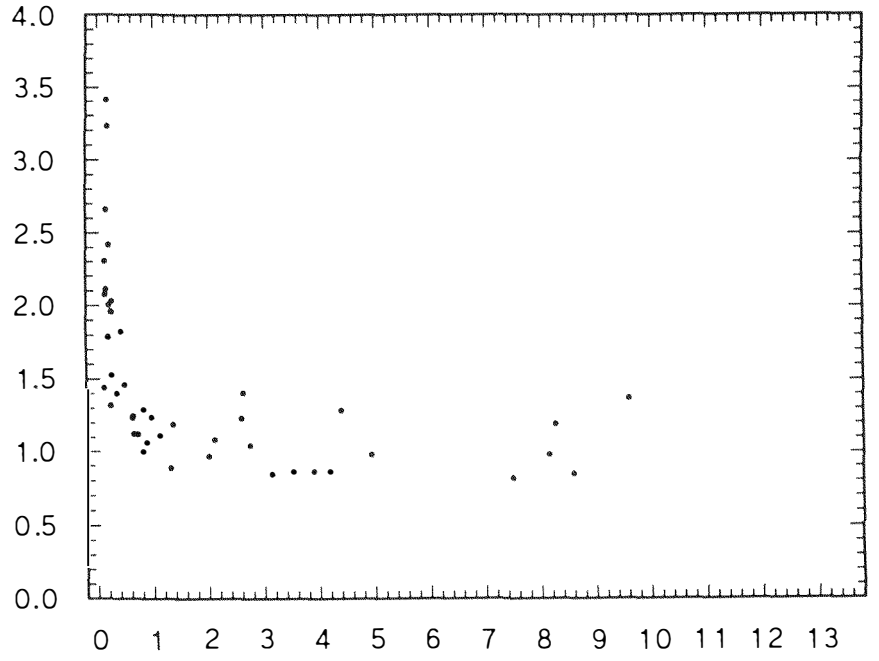
610


Core $p^i_{CO_2}$ (kPa)
Core $p^i_{O_2}$ (kPa)
0 1 2 3 4 5 6 7 8 9 10
0 2 4 6 8 10 12 14 16 18 20

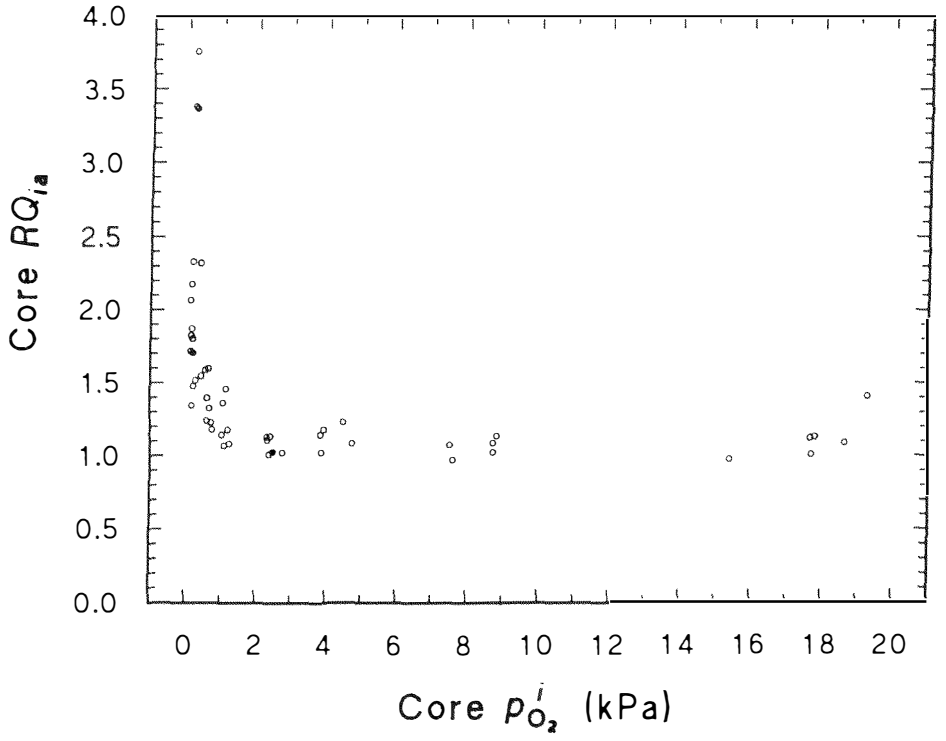

0 1 2 3 4 5 6 7 8 9 10
0 1 2 3 4 5 6 7 8 9 10 11 12 13

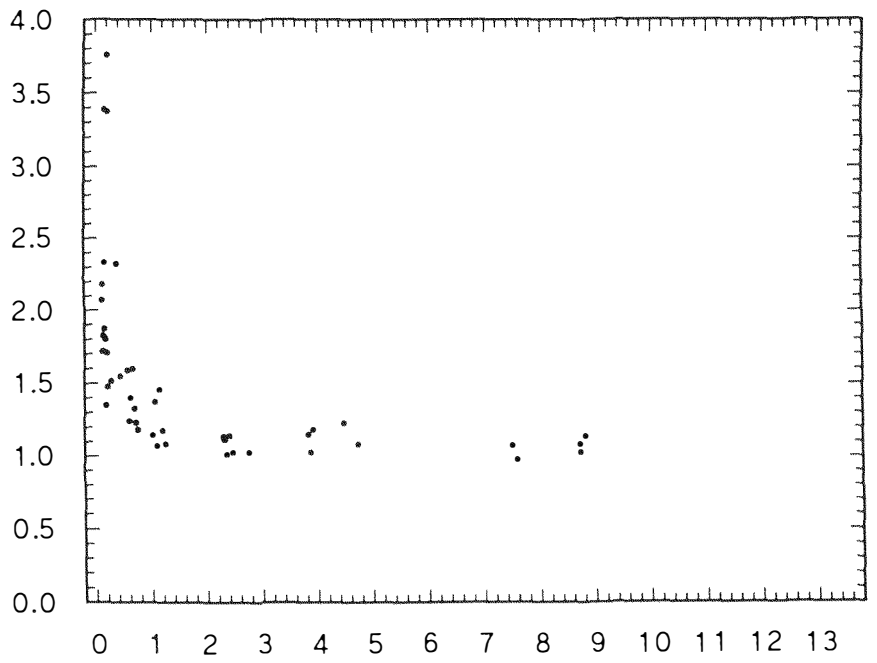
650


4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Chamber RQ_{ia}
0 2 4 6 8 10 12 14 16 18 20
Chamber $p^{i}_{O_2}$ (kPa)

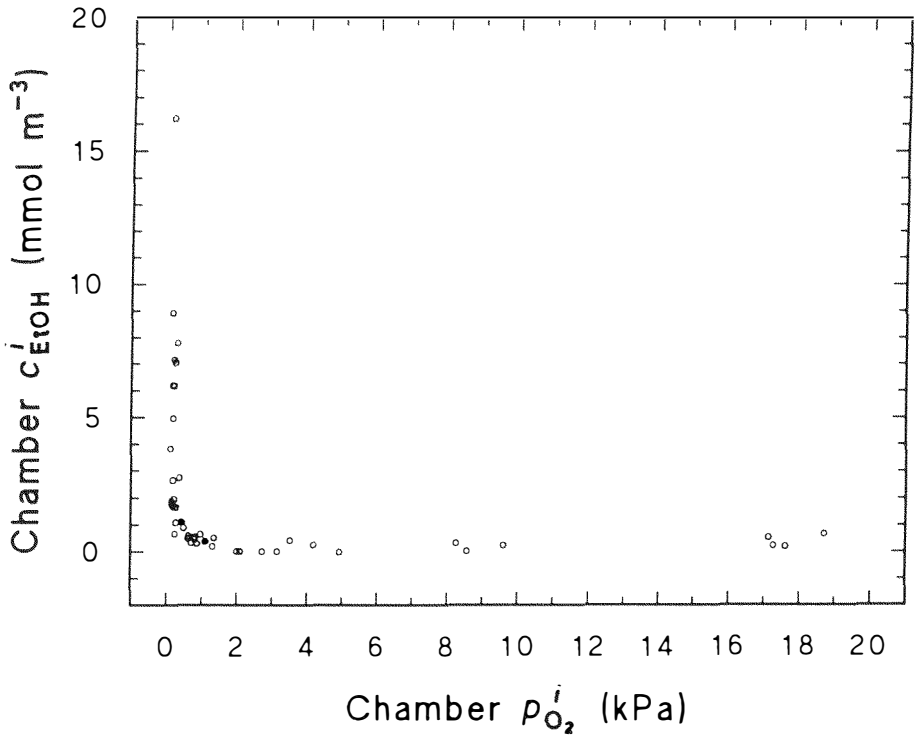

4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13

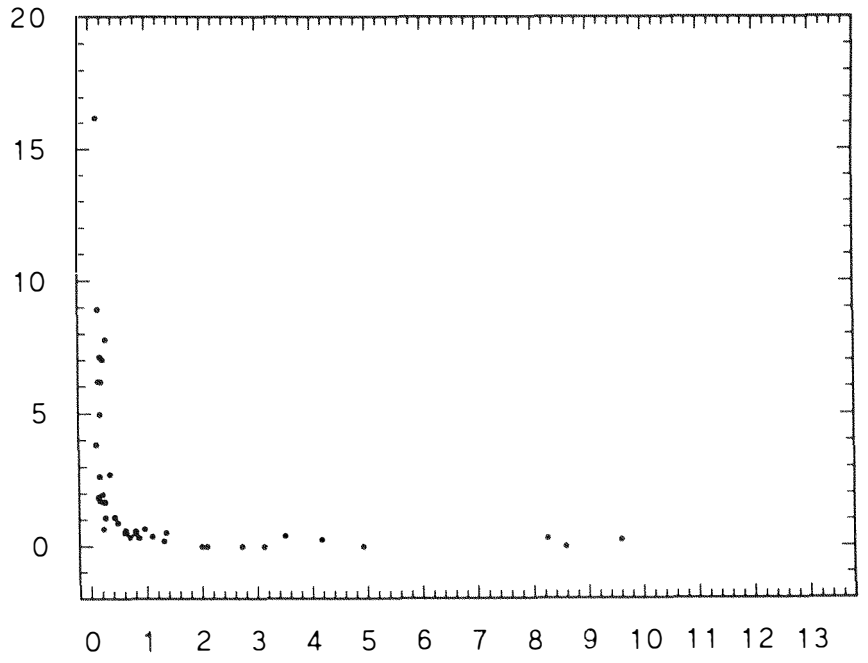
640


4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Core RQ_{ia}
0 2 4 6 8 10 12 14 16 18 20
Core $p^{i}_{O_2}$ (kPa)


4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13

630


20
15
10
5
0
Chamber c^i_{EtOH} (mmol m^{-3})
0 2 4 6 8 10 12 14 16 18 20
Chamber $p^i_{O_2}$ (kPa)


20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13

Appendix 2

Panel Assessment of Lower Oxygen Limits

The following instructions, and data recording sheet were given to panellists for assessing the lower oxygen limits from plots of raw data. Panellists were trained in the assessment procedure, and also briefed on the physiological basis for the selection of lower oxygen limits.

Graphical Estimation of Lower Oxygen Limits

Name:.....

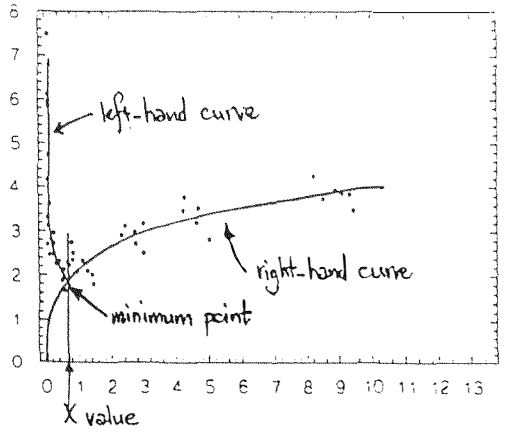
For each graph:

Draw a 'best-fit' curve through the data points as illustrated below. Use a clear plastic ruler to draw lines through linear portions.

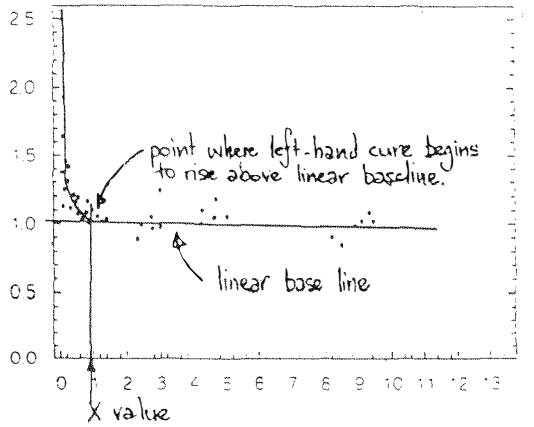
For **type 1** curves, commence drawing the right-hand curve from zero on the X-axis. Draw in the left-hand curve and where left and right-hand curves intersect is the minimum point. Estimate the X value of the minimum point and note it on the data sheet against the graph's code number.

For **type 2** curves, draw in an extended linear baseline, then hand draw in the left-hand curve to join the baseline. Estimate the X value at the point where the left-hand curve begins to rise above the linear baseline and note it on the data sheet against the graphs code number.

Type 1



Type 2



Please complete the job by ..... Note the total time taken to complete the job, then return the sheets to my pigeon-hole outside the office on the 3rd floor of the Ag-Hort building, or contact me on 3569099 x 5539 or 7039.

Thanks for your help.

Chris Yearsley.

Graphical Estimation of Lower Oxygen Limits

Name:..

Total time for job:..............................minutes.

Code	X value	Code	X value	Code	X value

Appendix 3

Gauss Programme to Calculate Bootstrap Estimates of *LOL*'s

The following programme, written by Dr. Siva Ganesh (Statistics Department, Massey University, Palmerston North, New Zealand) was used to calculate "bootstrap" estimates of lower oxygen limits:

1. sample estimates (means, standard deviations, standard errors, medians, maximum and mimimum values),
2. "bootstrap" estimate of means; means, standard errors, minimum and maximum values,
3. "bootstrap" estimate of medians; means, standard errors, maximum and minimum values,
4. acceleration constants (*a* values) for "bootstrap" confidence intervals (for means and medians),
5. confidence intervals (95%), upper and lower percentile, BC type and BCa type for "bootstrap" means, and
6. confidence intervals (95%), upper and lower percentile, BC type and BCa type for "bootstrap"medians.

The programme, written using GAUSS software (3861 VM, Aptech Systems, Inc., Maple Valley, WA, 1994), was stored as [*filename*.gau] and executed on a 33 MHz 486 DX IBM clone with 16 MB RAM, using the command c:\gau *filename*.gau. The data file structure comprised rows panelists (15) and columns as data for different treatments/plot types.

```
NEW;
#LINESON;

LOAD dt[15,4] = filename.dat;  /* to load raw data, 15= No. Panelists, 4= No.
                                  Treatments/plot types */
n=ROWS(dt); p=COLS(dt);
OUTWIDTH 200;
OUTPUT FILE = filename.res RESET;   /* to define output file */
OUTPUT ON;

/* Declare constants */
alpha=0.05/2;          /* Sig.level for CIs */
nboot=1000;            /* No. of bootstrap samples */
```

```
ncdf=nboot/10;          /* No. of cumf points in boot_distn. */
cipct=100*(1-2*alpha);
FORMAT /rd 3,0;
PRINT;
PRINT "Bootstrap Estimates and" cipct "% Confidence Intervals: experimental data:";
PRINT "Number of Bootstrap samples: " nboot;
PRINT;

/* Generating Std.Norm Cum.fns. */
nmin=-4; nmax=5; nint=0.001; nzcdf=(nmax-nmin)/nint;
zz = SEQA(nmin,nint,nzcdf); invzz = CDFN(zz);

/* Upp & Low inverse cum. Std.Norm values ... */
k=1;
DO WHILE k <= nzcdf-1;
IF invzz[k]==alpha; zalphal=zz[k];
 ELSEIF invzz[k]<alpha AND invzz[k+1]>alpha ;
  zalphal=zz[k]+((zz[k+1]-zz[k])*(alpha-invzz[k])/(invzz[k+1]-invzz[k]));
 ELSEIF invzz[k]==(1-alpha); zalphah=zz[k]; BREAK;
 ELSEIF invzz[k]<(1-alpha) AND invzz[k+1]>(1-alpha);
  zalphah=zz[k]+((zz[k+1]-zz[k])*((1-alpha)-invzz[k])/(invzz[k+1]-invzz[k]));
  BREAK;
ENDIF;
k = k+1;
ENDO;

/* Format for output printing - 3 decimals */
FORMAT /rd 5,3;

/* Sample estimates - Raw data */
xt = MEANC(dt); me = MEDIAN(dt);
st = STDC(dt);  se = st/SQRT(n);
mn = MINC(dt);  mx = MAXC(dt);
PRINT "Sample Estimates - Means, Stds, Medians, Min & Max - graphs";
PRINT "Means   " xt';
PRINT "Stds    " st';
PRINT "Stderrs " se';
PRINT "Medians " me';
PRINT "Min     " mn';
PRINT "Max     " mx';
PRINT;

/* Generating the bootstrap samples */
xtb = ZEROS(1,p); meb = ZEROS(1,p);
b=1;
DO WHILE b<=nboot;
  rndrows=FLOOR(n*RNDU(n,1)+ONES(n,1));
  db=SUBMAT(dt,rndrows,0);
/* sample estimates - bootstrap data */
  xtb = xtb|(MEANC(db))';
  meb = meb|(MEDIAN(db))';
b=b+1;
ENDO;
xtb = xtb[2:nboot+1,.]; /* Bootstrap means data */
meb = meb[2:nboot+1,.]; /* Bootstrap medians data */
/* Bootstrap estimates */
mxtb = MEANC(xtb); sxtb = STDC(xtb);
```

```
mmeb = MEANC(meb); smeb = STDC(meb);
minxtb = MINC(xtb); maxxtb = MAXC(xtb);
minmeb = MINC(meb); maxmeb = MAXC(meb);
PRINT "Bootstrap _Mean_ Estimates";
PRINT "Means   " mxtb';
PRINT "Stderrs " sxtb';
PRINT "Min     " minxtb';
PRINT "Max     " maxxtb';
PRINT;
PRINT "Bootstrap _Median_ Estimates";
PRINT "Means   " mmeb';
PRINT "Stderrs " smeb';
PRINT "Min     " minmeb';
PRINT "Max     " maxmeb';
PRINT;

/* Acceleration constants computation */
a1 = 0; a2=0;
/* Means */
k=1;
DO WHILE k <= p;
  da = SUBMAT(dt,0,k);
  mu = ((SUMC((da - MEANC(da))^3))/((SUMC((da - MEANC(da))^2))^(3/2)))/6;
  a1 = a1|mu;
k=k+1;
ENDO;
a1 = a1[2:p+1,.];
PRINT "Accelleration constants (a values) for Bootstrap CIs (Means)";
PRINT "       " a1';

/* Empirical Cumulative function G(s) & Compute Zo ... */
cumf1l=0; cumf1h=0; cumf2l=0; cumf2h=0;  /* Percentile intervals */
cuf1=0; cuf2=0; zo1=0; zo2=0;   /* for BC & BCa intervals */
bc1l=0; bc1h=0; bc2l=0; bc2h=0; bca1l=0; bca1h=0;  /* BC & BCa intervals */

j=1;
DO WHILE j <= p;
/* Boot_Means ... */
  dx1 = SUBMAT(xtb,0,j);
/* To use with Percentile CIs */
  nmin=MINC(dx1); nmax=MAXC(dx1); ncdf=100; nint=(nmax-nmin)/(ncdf-1);
  v1 = SEQA(nmin,nint,ncdf);
  c1 = (COUNTS(dx1,v1))/nboot;  /* Emp. Cdf */
  c2 = CUMSUMC(c1);
  k=1;
  DO WHILE k <= ncdf-1;
    IF c2[k]==alpha; c0l=v1[k];
      ELSEIF c2[k]<alpha AND c2[k+1]>alpha ;
        c0l=v1[k]+((v1[k+1]-v1[k])*(alpha-c2[k])/(c2[k+1]-c2[k]));
      ELSEIF c2[k]==(1-alpha); c0h=v1[k]; BREAK;
      ELSEIF c2[k]<(1-alpha) AND c2[k+1]>(1-alpha) ;
        c0h=v1[k]+((v1[k+1]-v1[k])*(1-alpha-c2[k])/(c2[k+1]-c2[k]));
        BREAK;
    ENDIF;
  k = k+1;
  ENDO;
```

```
  cumf1l = cumf1l|c0l;  cumf1h = cumf1h|c0h;
/* To use with BC & BCa using Std.Zs */
  v0 = xt[j];
  c0 = (COUNTS(dx1,v0))/nboot;
  k=1;
  DO WHILE k <= nzcdf-1;
    IF invzz[k]==c0; z0=zz[k];
      ELSEIF invzz[k]<c0 AND invzz[k+1]>c0 ;
        z0=zz[k]+((zz[k+1]-zz[k])*(c0-invzz[k])/(invzz[k+1]-invzz[k]));
    ENDIF;
  k = k+1;
  ENDO;
  cuf1 = cuf1|c0; zo1 = zo1|z0;
/* BCa intervals */
  zbca1a = z0 + (z0 + zalphal)/(1 - a1[j]*(z0 + zalphal));
  zbca1b = z0 + (z0 + zalphah)/(1 - a1[j]*(z0 + zalphah));
  zzbca1a = CDFN(zbca1a); zzbca1b = CDFN(zbca1b);
  k=1;
  DO WHILE k <= ncdf-1;
    IF c2[k]==zzbca1a; c0l=v1[k];
      ELSEIF c2[k]<zzbca1a AND c2[k+1]>zzbca1a ;
        c0l=v1[k]+((v1[k+1]-v1[k])*(zzbca1a-c2[k])/(c2[k+1]-c2[k]));
      ELSEIF c2[k]==zzbca1b; c0h=v1[k]; BREAK;
      ELSEIF c2[k]<zzbca1b AND c2[k+1]>zzbca1b ;
        c0h=v1[k]+((v1[k+1]-v1[k])*(zzbca1b-c2[k])/(c2[k+1]-c2[k]));
        BREAK;
    ENDIF;
  k = k+1;
  ENDO;
  bca1l = bca1l|c0l;  bca1h = bca1h|c0h;
/* BC intervals */
  zbca = z0 + (z0 + zalphal); zbcb = z0 + (z0 + zalphah);
  zzbca = CDFN(zbca); zzbcb = CDFN(zbcb);
  k=1;
  DO WHILE k <= ncdf-1;
    IF c2[k]==zzbca; c0l=v1[k];
      ELSEIF c2[k]<zzbca AND c2[k+1]>zzbca ;
        c0l=v1[k]+((v1[k+1]-v1[k])*(zzbca-c2[k])/(c2[k+1]-c2[k]));
      ELSEIF c2[k]==zzbcb; c0h=v1[k]; BREAK;
      ELSEIF c2[k]<zzbcb AND c2[k+1]>zzbcb ;
        c0h=v1[k]+((v1[k+1]-v1[k])*(zzbcb-c2[k])/(c2[k+1]-c2[k]));
        BREAK;
    ENDIF;
  k = k+1;
  ENDO;
  bc1l = bc1l|c0l;  bc1h = bc1h|c0h;
/* Boot_Medians ....*/
  dx1 = SUBMAT(meb,0,j);
/* To use with Percentile CIs */
  nmin=MINC(dx1); nmax=MAXC(dx1); ncdf=100; nint=(nmax-nmin)/(ncdf-1);
  v1 = SEQA(nmin,nint,ncdf);
  c1 = (COUNTS(dx1,v1))/nboot;  /* Emp. Cdf */
  c2 = CUMSUMC(c1);
  k=1;
  DO WHILE k <= ncdf-1;
    IF c2[k]==alpha; c0l=v1[k];
```

```
        ELSEIF c2[k]<alpha AND c2[k+1]>alpha ;
          c0l=v1[k]+((v1[k+1]-v1[k])*(alpha-c2[k])/(c2[k+1]-c2[k]));
        ELSEIF c2[k]==(1-alpha); c0h=v1[k]; BREAK;
        ELSEIF c2[k]<(1-alpha) AND c2[k+1]>(1-alpha) ;
          c0h=v1[k]+((v1[k+1]-v1[k])*(1-alpha-c2[k])/(c2[k+1]-c2[k]));
          BREAK;
      ENDIF;
    k = k+1;
    ENDO;
    cumf2l = cumf2l|c0l;  cumf2h = cumf2h|c0h;
  /* To use with BC using Std.Zs */
    v0 = me[j];
    c0 = (COUNTS(dx1,v0))/nboot;
    k=1;
    DO WHILE k <= nzcdf-1;
      IF invzz[k]==c0; z0=zz[k];
        ELSEIF invzz[k]<c0 AND invzz[k+1]>c0 ;
          z0=zz[k]+((zz[k+1]-zz[k])*(c0-invzz[k])/(invzz[k+1]-invzz[k]));
      ENDIF;
    k = k+1;
    ENDO;
    cuf2 = cuf2|c0; zo2 = zo2|z0;
  /* BC intervals */
    zbca = z0 + (z0 + zalphal); zbcb = z0 + (z0 + zalphah);
    zzbca = CDFN(zbca); zzbcb = CDFN(zbcb);
    k=1;
    DO WHILE k <= ncdf-1;
      IF c2[k]==zzbca; c0l=v1[k];
        ELSEIF c2[k]<zzbca AND c2[k+1]>zzbca ;
          c0l=v1[k]+((v1[k+1]-v1[k])*(zzbca-c2[k])/(c2[k+1]-c2[k]));
        ELSEIF c2[k]==zzbcb; c0h=v1[k]; BREAK;
        ELSEIF c2[k]<zzbcb AND c2[k+1]>zzbcb ;
          c0h=v1[k]+((v1[k+1]-v1[k])*(zzbcb-c2[k])/(c2[k+1]-c2[k]));
          BREAK;
      ENDIF;
    k = k+1;
    ENDO;
    bc2l = bc2l|c0l;  bc2h = bc2h|c0h;
  j=j+1;
  ENDO;

  /* Cumf values of <samp_est., ie. G(s), and Inv(G(s)) - Means */
  cuf1 = cuf1[2:p+1,.]; cuf2 = cuf2[2:p+1,.];
  zo1 = zo1[2:p+1,.]; zo2 = zo2[2:p+1,.];
  PRINT "Cumf values of <samp_est., ie. G(s), and Inv(G(s)) - Means";
  PRINT "G(boot) " cuf1';
  PRINT "Zo      " zo1';
  PRINT "Cumf values of <samp_est., ie. G(s), and Inv(G(s)) - Medians";
  PRINT "G(Boot) " cuf2';
  PRINT "Zo      " zo2';
  PRINT;

  /* Confidence Intervals - Means... */
  cumf1l = cumf1l[2:p+1,.]; cumf1h = cumf1h[2:p+1,.];
  bc1l = bc1l[2:p+1,.]; bc1h = bc1h[2:p+1,.];
  bca1l = bca1l[2:p+1,.]; bca1h = bca1h[2:p+1,.];
```

```
/* Confidence Intervals - Medians... */

cumf2l = cumf2l[2:p+1,.]; cumf2h = cumf2h[2:p+1,.];
bc2l = bc2l[2:p+1,.]; bc2h = bc2h[2:p+1,.];

PRINT "Confidence Intervals - Means....";
PRINT "Percentile";
PRINT " Lower  " cumf1l';
PRINT " Upper  " cumf1h';
PRINT "BC type";
PRINT " Lower  " bc1l';
PRINT " Upper  " bc1h';
PRINT "BCa type";
PRINT " Lower  " bca1l';
PRINT " Upper  " bca1h';
PRINT;
PRINT "Confidence Intervals - Medians....";
PRINT "Percentile";
PRINT " Lower  " cumf2l';
PRINT " Upper  " cumf2h';
PRINT "BC type";
PRINT " Lower  " bc2l';
PRINT " Upper  " bc2h';

OUTPUT OFF;
GOTO gan;
gan:
END;
#LINESOFF;
```

Appendix 4

Quantifying the Association between 'Braeburn Browning Disorder' with Mineral Composition and Skin Permeance to Gases of 'Braeburn' Apples

Report to ENZA New Zealand (International) by

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A4.1 Abstract

Previous storage experiments with 'Braeburn' apples from Central Hawkes Bay orchards indicated there were marked differences between orchards in susceptibility to incidence of 'Braeburn Browning Disorder' (BBD). We investigated two factors, mineral composition and skin permeance, that may correlate with tissue susceptibility to physiological disorders in 'Braeburn' apples on fruit from the five Central Hawkes Bay orchards which had been stored in a controlled atmosphere of 2 kPa O_2 and 5 kPa CO_2. We found that there was no clear relationship between incidence of physiological disorders and either mineral composition or skin permeance to ethane. However in this season the CA mixture induced a higher incidence of disorders than had been reported in storage experiments from previous seasons. This confounded our ability to make conclusions about the relationships as originally intended. The experiment demonstrated differences in tissue susceptibility to storage in high partial pressures of CO_2 and indicates the possibility of using high CO_2 atmospheres to screen for susceptible lines that will assist in making marketing decisions.

A4.2 Introduction

'Braeburn Browning disorder' (BBD), formally termed 'brown heart', is a physiological disorder of 'Braeburn' apples, has been identified as a problem since 1978 in trials where the fruit were stored in unperforated polyliners. It has caused significant economic loss in the New Zealand export market, particularly in the last two seasons (4.5% of the national export crop in 1993), and primarily in fruit from the colder growing regions (June and Ahlborn, 1993).

Elevated CO_2 in poorly ventilated controlled atmosphere (CA) stores may induce BBD (Knee, 1973; Fidler *et al.,* 1973; and Padfield, 1969). BBD, core cavities and coreflush can all be induced in by storage in elevated CO_2 atmospheres (2% and 5% CO_2 in 2% O_2) and the severity of the disorders were found to be strongly grower dependent (Elgar and Watkins, 1992).

Studies by Dadzie (1992) indicate a link between skin resistance to gas diffusion, internal CO_2 concentration and coreflush in 'Granny Smith' apples. Park *et al.,* (1993) report positive correlations between skin resistance to gas diffusion in strains of 'McIntosh' apples and incidence of low O_2 injury which develops in CA storage. Compared with other apple cultivars, 'Braeburn' apples have a high and more variable skin resistance (in this report we will discuss skin permeance which is the reciprocal of skin resistance). They also have a high internal CO_2 concentration, particularly at the calyx end where there is a tendency for BBD to be more severe.

In our trial we tested the hypothesis that the marked differences in severity of BBD (as reported in Elgar and Watkins 1992 trial) relate to differences in skin permeability of different lines (populations) of fruit. The fruit were stored in CA to induce the development of BBD. We also investigated whether there were differences in mineral composition in tissue taken from populations of fruit with different severities of BBD.

A4.3 Materials and methods

A4.3.1 Fruit supply and initial fruit measurements

'Braeburn' apples were harvested from five central Hawkes Bay orchards (D988, D1382, C1356, D844, D183) packed on 24/4/93 as 125 count fruit and transported overnight to HortResearch (Auckland). On 21/4/93 thirty fruit from each orchard were randomly selected for estimation of respiration rate and skin permeance. A further fifteen fruit were randomly selected for an initial assessment of fruit maturity by measuring, skin background colour using a Minolta chromameter ($L^*a^*b^*$ converted to L and Hue angle after method of McGuire (1992)), fruit firmness on pared surfaces on opposite sides of each fruit with an electronic pressure tester fitted with an 11.1 mm head (FF, N: EPT-1, Lake City Technical Products Inc., Canada), total soluble solids content on juice from a combined drop of juice from each side of the fruit using a refractometer (SSC, %: Atago 0-20%), and internal ethylene partial pressure of the core cavity using flame ionisation chromatography ($p^i_{C_2H_4}$, Pa: Philips PU4500 fitted with a Philips glass Alumina F1 column, temperatures: column, 130°C; injector, 160°C; detector, 200°C).

A4.3.2 Respiration rates

Estimates of respiration rate (r_{CO_2}, mol kg^{-1} s^{-1}) were made for a total 30 fruit per orchard (10 fruit on each of 3 consecutive days) at 20°C in the dark. Fruit were weighed, placed in 5.5 x 10^5 mm^3 respiration jars and sealed. A 100 mm^3 gas sample was removed from each jar after 1 h and CO_2 composition measured using thermal conductivity gas chromatography (Pye Unicam 304, Alltech CTR1 column, temperatures: column, 32°C; injector, 40°C; detector, 100°C). A 100 mm^3 sample from a sealed jar without fruit (1 blank jar for each batch of 10 fruit) was also measured and the ambient CO_2 concentration subtracted from the values from jars containing fruit before calculating rates. C_2H_4 composition was also measured as above but production rates were too low to report.

A4.3.3 Skin permeance to ethane

Skin permeance to gas diffusion ($P'_{C_2H_6}$, mol s^{-1} m^{-2} Pa^{-1}; atmospheric pressure assumed to be 101325 Pa) was determined on the same fruit used for estimating respiration rates The ethane efflux method of Banks (1985) was used. Each fruit was sealed in a 1.05 x 10^6 mm^3 jar and a 100 mm^3 injection of pure ethane was added to each jar. The atmosphere in each jar was mixed using a 6 x 10^4 mm^3 syringe and left over night to equilibrate at 20°C in the dark. The following morning, the atmosphere was mixed again and a 100 mm^3 sample removed and mixed in a 5.5 x 10^5 mm^3 dilution jar. Each jar was placed in a fume hood, the fruit removed, held in front of a fan to blow away ethane from its boundary layer, inserted into a second 1.087 x 10^6 mm^3 jar with a magnetic stirrer and sealed. A 100 mm^3 sample was taken at 30, 60, 90, 120 and 150 s after sealing.

$P'_{C_2H_6}$ was calculated as:

$$P'_{C_2H_6} = \left(\frac{V_{net} \left(\frac{dp_{C_2H_6}}{dt} \right)}{init\ p_{C_2H_6}\ A} \right) \left(\frac{1}{R\ (T + 273.15)} \right) \qquad \textbf{(A4.1)}$$

where:

	$init\ p_{C_2H_6}$	=	initial partial pressure of ethane in the fruit's internal atmosphere (Pa)
	$dp_{C_2H_6} / dt$	=	rate of change of ethane partial pressure (Pa s^{-1})
	R	=	gas constant (8.3143 m^3 Pa $mol^{-1}K^{-1}$)
	T	=	fruit temperature (°C)
	V_{net}	=	net volume = jar volume - fruit volume (m^3)

Fruit surface area was estimated using the method of Clayton *et al.*, 1995:

$$A = 0.0581\ M^{0.685} \qquad \textbf{(A4.2)}$$

where: A = fruit surface area (m^2)

M = fruit mass (kg).

A4.3.4 Controlled atmosphere storage

All fruit were kept at 20°C until the afternoon of 24/4/93. Four trays of fruit from each orchard (100 fruit per orchard) were randomly assigned to a position within four cartons enclosed in a 0.33 m^3 plastic CA chamber stored at 0°C. An atmosphere of 2 kPa O_2 and 5 kPa CO_2 (1% = 1.0135 kPa), balance N_2, was established in the CA chamber by mixing metered flows of compressed air, oxygen-free N_2 and CO_2 (total flow rate = 3.34 x 10^3 mm^3 s^{-1})) which was saturated with water vapor in a humidifier before entering the chamber. A small fan in the chamber kept the atmosphere mixed. The atmosphere was monitored twice weekly initially and weekly during the later stages of storage by analysing 1 cm^3 gas samples using gas chromatography.

A4.3.5 Evaluation of stored fruit

The fruit were removed from CA storage on 30/10/93, placed in a 20°C room over night then air freighted to Massey University on 1/11/93 for evaluation. Fruit were visually evaluated for the incidence and severity of internal and external BBD, core cavities and CO_2 injury.

External BBD and CO_2 injury were assessed as being either present or absent. Internal BBD and core cavities were assessed using a 0-5 scale after slicing the fruit latitudinally into 5 pieces.

The following evaluation scales were used:

(a) Internal BBD

0 nil

1 1 lesion visible to < 10% area of any slice affected

2 10 to < 30% area of any slice affected

3 30 to < 60% area of any slice affected

4 60 to < 90% area of any slice affected

5 90 to 100% area of any slice affected

(b) Core cavities

0 nil

1 1 cavity visible in any slice

2 1 to 3 cavities near core and/or 1 large cavity in outer cortex of any slice

3 > 3 to 6 cavities near core and/or 2 to 4 large cavities in outer cortex of any slice

4 numerous cavities near core and/or > 4 to 6 large cavities in outer cortex of any slice

5 numerous cavities near core and/or > 6 large cavities in outer cortex of any slice

The incidence of coreflush and rots were also noted.

A4.3.6 Mineral analyses

A bulk sample of peeled, cored and sliced fruit (7 to 10 fruit) from each of internal BBD categories 0, 3 or 5, for each orchard were frozen on 1/11/93. Homogenates of the thawed samples were made using a Waring blender on 3/11/93. Subsamples of homogenate (approximately 2.0×10^8 mm^3) were placed into plastic pots, frozen in a blast freezer at -30°C over-night, then freeze dried over the following 4 days. The freeze-dried samples were ground with a pestle and mortar and a 5 g subsample used for mineral analysis. Mineral analysis (N, P, K, Ca and Mg) were conducted at the Fertilizer and Lime Research Centre (Massey University).

A4.3.7 Statistical analysis

The statistical package SAS (SAS, 1990) was used to perform data analysis. The frequency proceedure was used to analyse disorder data and the General Linear Models procedure for analysis of variance and regressions of other data.

A4.4 Results and discussion

A4.4.1 Initial fruit maturity

The fruit from all orchards used in the experiment were received in excellent condition and although there were some differences in fruit firmness between orchards there were no differences in any of the other estimates of fruit maturity measured (Table A4.1). The values in brackets for hue angle relate to the background green colour of the apples (the higher the value the deeper the green and the lower the value the more yellow the colour). The values for partial pressure of ethylene sampled from the core indicate the fruit was still in the preclimacteric phase.

A4.4.2 Respiration rates and skin permeance

There was no difference between orchards in the respiration rate of fruit and the relatively low rate indicates the fruit were probably still in a preclimacteric phase (Table A4.2).

Differences in estimates of skin permeance were measured between fruit from different orchards (Table A4.2). Fruit from orchards 1, 3 and 5 had the highest skin permeance to ethane. fruit from orchards 2 and 4 had the lowest skin permeance. Skin permeance is the reciprocal of skin resistance, values for which have been reported for 'Braeburn' apples by Dadzie (1992). The average values for estimated skin permeance for fruit from orchards 1 - 5 converted to skin resistance are: 34,429; 36,251; 34,795; 41,153; and 31,823 s cm^{-1} respectively. These values are within the range of those previously reported by Dadzie (1992).

Table A4.1 Estimates of the initial fruit maturity of 'Braeburn' apples: fruit firmness (FF); total soluble solids content (SSC); background fruit colour, lightness (L) and Hue angle (H°); and internal ethylene partial pressure ($p^{i}_{C_2H_4}$), for fruit from five Central Hawkes Bay orchards (n=15). Differences between growers are indicated at $p = 0.05$. Values in brackets are back transformed means from $\log_e$ transformations.

Orchard No.	FF (N)	SSC (%)	L	H°	$p^{i}_{C_2H_4}$ [1] (Pa)
1	99.06 ab	11.75	4.23 (68.72)	4.70 (109.9)	0.012
2	95.14 c	11.58	4.24 (69.21)	4.70 (109.66)	0.011
3	99.15 ab	11.39	4.22 (68.07)	4.69 (109.22)	0.012
4	97.66 bc	11.72	4.24 (69.74)	4.71 (111.24)	0.022
5	101.78 a	11.63	4.25 (70.44)	4.70 (109.75)	0.015
sed	1.924	0.235	0.013	0.010	0.004
p=(orchard)	0.019	NS[2]	NS	NS	NS

[1] Pa x 10.1325 = $\mu l\ l^{-1}$ or ppm. [2] NS = Not significant.

Table A4.2 Estimates of respiration rates (r_{CO_2}) and skin permeance to ethane ($P'_{C_2H_6}$) of 'Braeburn' apples at 20°C harvested from five Central Hawkes Bay orchards before cool storage in a controlled atmosphere (n=30).

Orchard No.	r_{CO_2} [1] ($\mu mol\ kg^{-1}\ s^{-1}$)	$P'_{C_2H_6}$ ($nmol\ s^{-1}\ m^{-2}\ Pa^{-1}$)
1	0.085	0.1224 a
2	0.074	0.1040 bc
3	0.082	0.1196 a
4	0.080	0.1013 c
5	0.081	0.1185 ab
sed	0.0619	0.00737
p= (orchard)	NS[2]	0.0143

[1] $\mu mol\ kg^{-1}\ s^{-1}$ / 0.006313 = $mg\ kg^{-1}\ h^{-1}$. [2] NS = not significant.

A4.4.3 Disorders

A4.4.3.1 Symptoms of the disorders

Internal BBD appeared as circular or elongated areas of medium to dark brown tissue at the calyx end or throughout the cortex and core regions. The size of the lesions depended on the severity of the disorder and ranged from small flecks to large areas where lesions had coalesced. The affected areas were light brown in colour or darker brown watersoaked areas.

Core cavities appeared as lens-shaped cavities (3-5 mm long, 1-3 mm wide) in the core area but also in the cortex in severe cases; the tissue surrounding a cavity was also usually brown. In some fruit large cavities (5-8 mm long, 5-8 mm wide) were present in the cortex along with smaller cavities. There were proportionately fewer BBD lesions present in these fruit.

External BBD appeared as sunken brown discolourations of the skin, associated with brown tissue beneath the skin sometimes with a watersoaked appearance and sometimes associated with CO_2 injury. CO_2 injury appeared as sunken and wrinkled areas of skin with firm tissue beneath the lesions. With CO_2 injury, tissue beneath the skin was not discoloured.

A4.4.3.2 Incidence and severity of the disorders

Fruit from orchard 4 had the highest incidence of internal BBD particularly for the more severe categories of the disorder, and the lowest incidence of fruit without any type of disorder (Fig. A4.1(A)). Fruit from orchard 1 had the greatest number of fruit without any disorder. The other orchards had a similar incidence of internal BBD.

Fruit from orchard 4 had the lowest incidence of cavities (Fig. A4.1(B)). The other orchards had a similar incidence of cavities. There appears to be an inverse relationship between the incidence of internal BBD and cavities. It is possible that this indicates two ways in which the underlying physiological cause of BBD may manifest itself. Localised areas of susceptible tissue may form brown lesions that affect surrounding tissue froming watersoaked or dry areas. In some cases, the tissue forming

dry lesions may collapse to form small cavities which in the outer cortex may expand to form large cavities. In some fruit both processes appear to occur at the same time but fruit with large cavities seldom have severe BBD lesions in the outer cortex. No relationship was observed between the blush side of the fruit and the spatial distibution of internal BBD or cavities.

The incidence of external BBD was highest in fruit from orchard 4, and similar levels were seen in fruit from the other orchards (Fig. A4.2 (A)). No differences in the incidence of CO_2 injury were found between fruit from the five orchards (Fig. A4.2 (B)).

A4.4.4 The relationship between skin permeance, internal BBD and cavities

No statistically significant correlation was found between skin permeance of the 'Braeburn' apples from the five orchards used in this experiment and internal BBD (Fig. A4.3 (A), $r^2 = 0.15$) or cavities (Fig. A4.3 (B), $r^2 = 0.008$).

Fruit from orchard 4 had the highest incidence and severity of internal BBD (Fig. A4.1) and lowest skin permeance (Table A4.2, or highest skin resistance) indicating a tendency for fruit with a lower skin permeance to be more susceptible to internal BBD. Though not statistically significant, the regression of skin permeance and internal BBD and cavities had the highest r^2 values (0.34 and 0.19 respectively).

These data do not make it possible to draw conclusions about any relationship between skin permeance and disorders that may explain the clear differences between orchards that have been observed in previous experiments. The very high levels of disorders probably resulted primarily from storing the fruit in an atmosphere with a high partial pressure of CO_2. The decision to uses this atmosphere was based on the results of previous trials in which a low incidence of internal BBD was found in fruit stored in 2 kPa CO_2 / 2 kPa O_2 (Elgar and Watkins, 1992). It was initially intended to repeat the estimation of respiration rates, skin permeance and also measure internal atmospheres of the fruit removed from storage. However, given the high levels of external BBD and CO_2 injury seen on the fruit when it was removed from CA storage it

was decided that a very high proportion of the fruit would have internal BBD and these measurements would not add any useful extra information.

The experiment demonstrates some variation in the susceptibility of tissue of apples from different orchards to elevated CO_2, and suggests the possibility of using even higher partial pressures of CO_2 at ambient temperatures as a means of screening the susceptibility of different lines of 'Braeburn' apples to developing internal BBD in long term storage.

A4.4.5 Tissue mineral content and the severity of internal BBD

There were no differences in fruit mineral content between orchards except for P and Ca where orchard 4 had marginally higher P and lower Ca (Table A4.3). Neither was there any relationship identified between mineral content and the severity internal BBD with the exception of Mg. Marginally higher amounts of Mg were found in fruit with internal BBD than fruit without any disorder. There are no previous reports indicating high levels of Mg predispose apples to BBD.

Table A4.3 Mineral content (N, P, K, Ca and Mg) of the cortex of 'Braeburn' apples from five Central Hawkes Bay orchards after storage at 0°C for 27 weeks in a controlled atmosphere (2 kPa O_2 and 5 kPa CO_2, balance N_2, flow rate 3.34×10^3 mm^3 s^{-1}, humidified). Mineral analysis was conducted on freeze dried samples from homogenates of cortex tissue of 7-10 fruit per orchard for each of three levels of internal BBD (BBD^i: 0 = no disorder; 3 = moderate; 5 = severe).

Orchard	BBD^i	Mineral analysis (kg kg^{-1} dry weight)[1]				
No.	score	N	P	K	Ca	Mg
1	0	2.01	0.35	7.20	0.81	0.25
	3	2.03	0.59	7.27	0.70	0.28
	5	1.67	0.57	6.49	0.90	0.3
2	0	1.76	0.52	6.94	<0.10	0.23
	3	1.87	0.49	7.26	0.92	0.27
	5	1.65	0.46	6.92	1.61	0.28
3	0	1.57	0.46	5.94	1.03	0.25
	3	1.93	0.57	7.07	0.84	0.27
	5	1.95	0.60	6.94	1.40	0.24
4	0	2.11	0.69	6.69	0.24	0.23
	3	2.00	0.60	7.06	<0.10	0.26
	5	2.00	0.59	6.94	<0.10	0.25
5	0	1.75	0.44	7.21	<0.10	0.24
	3	1.72	0.42	7.88	0.33	0.27
	5	1.70	0.47	7.12	0.39	0.27
sed		0.093	0.033	0.209	0.213	0.008
p= (orchard)		NS[2]	0.020	NS	0.036	NS
p= (BBD^i core)		NS	NS	NS	NS	0.012

[1] 1 kg kg^{-1} = 1 mg g^{-1} [2] NS = not significant.

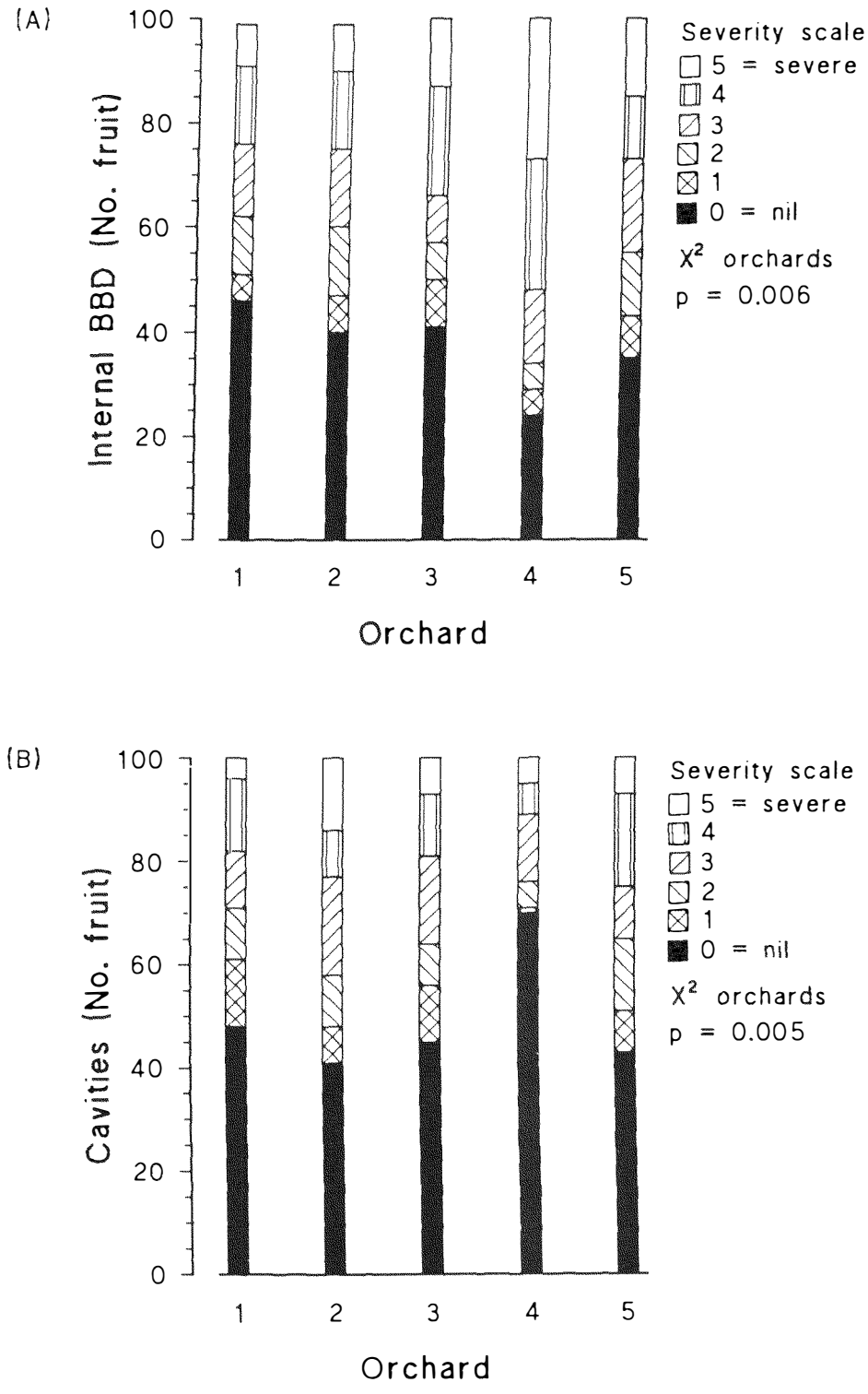

Figure A4.1 Incidence and severity of internal disorders, (A) internal BBD and (B) cavities, in 'Braeburn' apples harvested from five Hawkes Bay orchards and stored at 0°C for 27 weeks in a controlled atmosphere (2 kPa O_2 and 5 kPa CO_2, balance N_2, flow rate 3.34 x 10^3 mm^3 s^{-1}, humidified, n = 100).

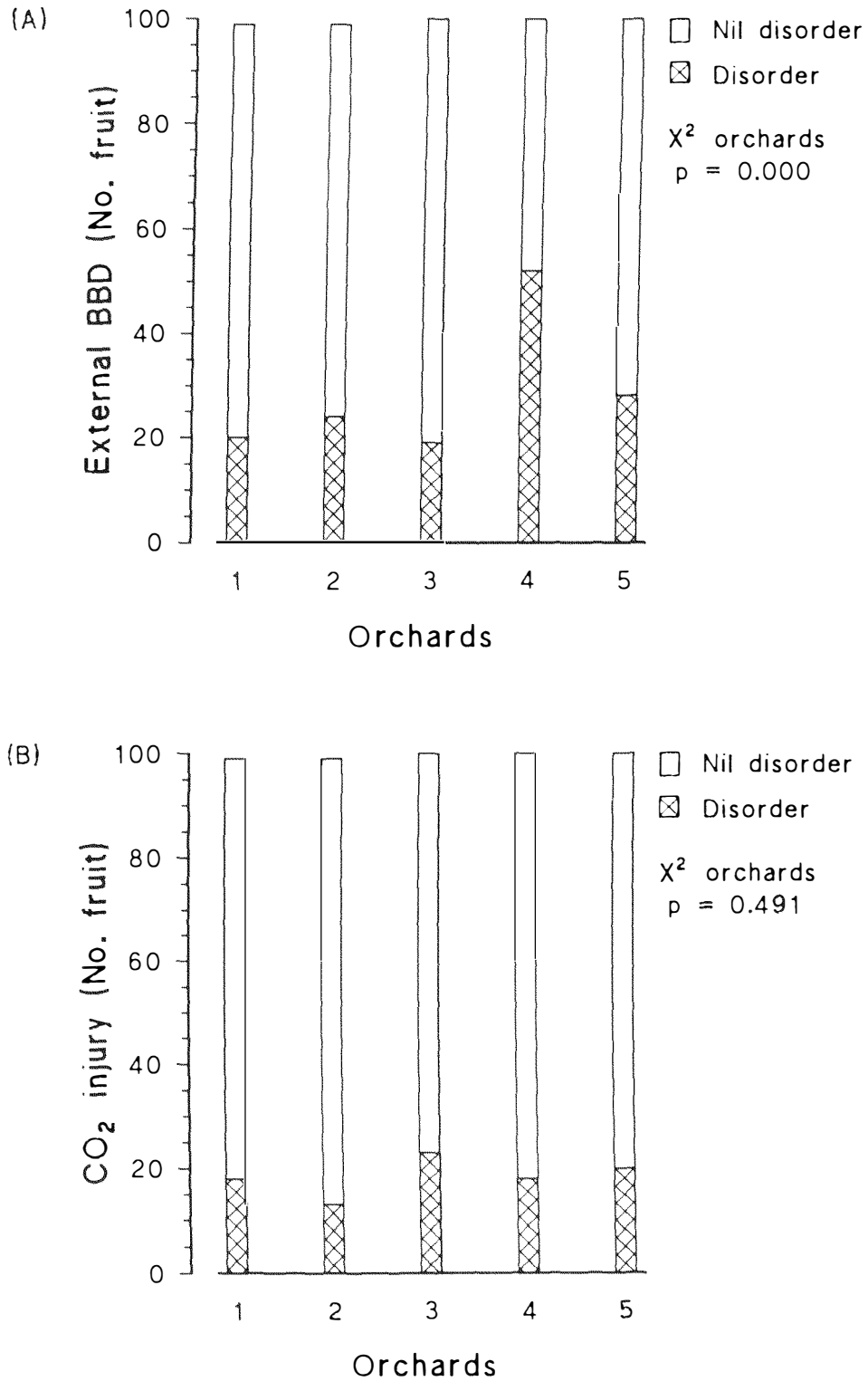

Figure A4.2 Incidence of external disorders, (A) external BBD and (B) CO_2 injury, in 'Braeburn' apples harvested from five Hawkes Bay orchards and stored at 0°C for 27 weeks in a controlled atmosphere (2 kPa O_2 and 5 kPa CO_2, balance N_2, flow rate 3.34 x 10^3 mm^3 s^{-1}, humidified, n = 100).

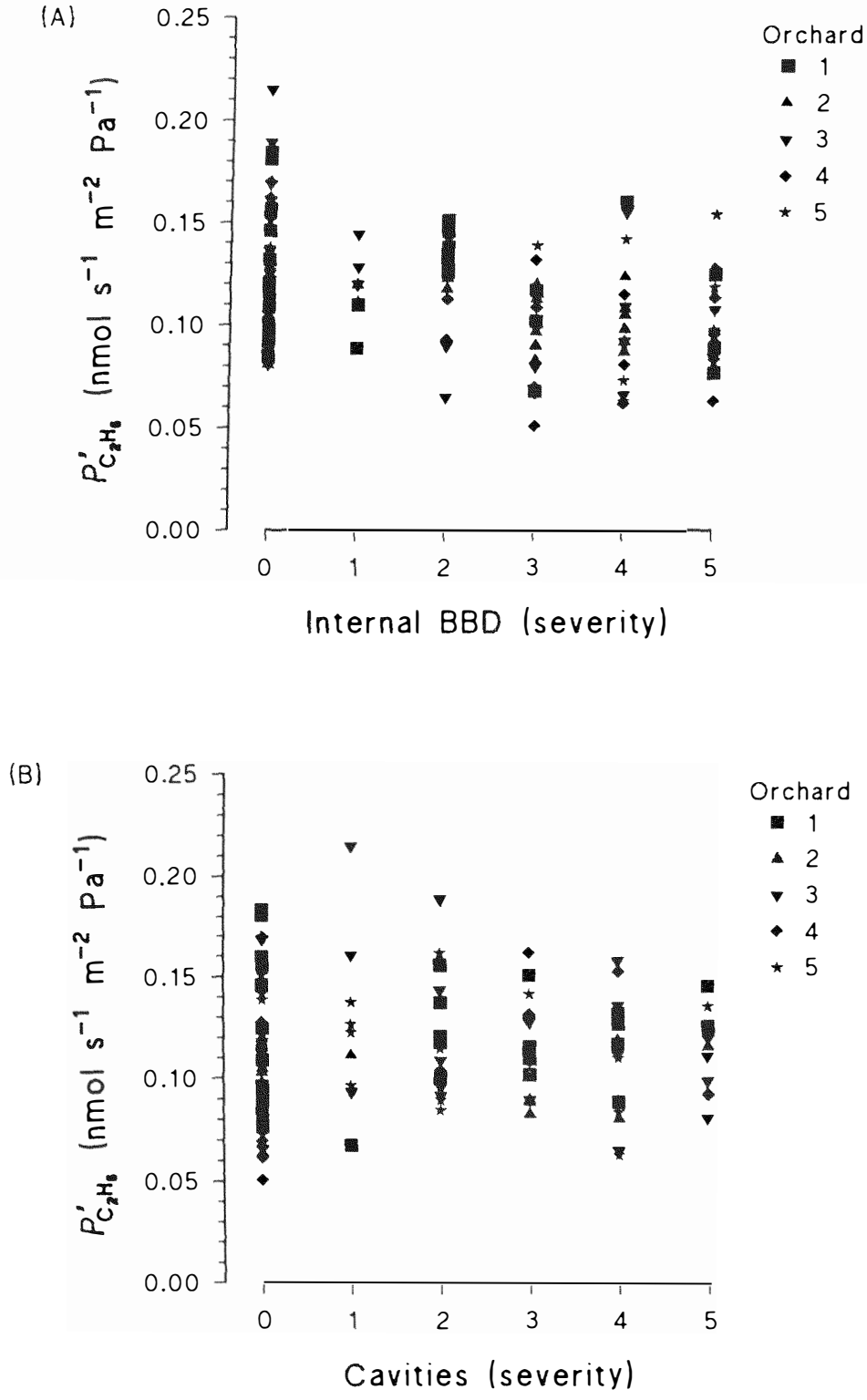

Figure A4.3 Skin permeance to ethane and the severity of (A) internal BBD and (B) cavities for 'Braeburn' apples harvested from five Hawkes Bay orchards and stored at 0°C for 27 weeks in a controlled atmosphere (2 kPa O_2 and 5 kPa CO_2, balance N_2, flow rate 3.34 x 10^3 mm^3 s^{-1}, humidified, n = 100).

A4.5 Conclusions

No relationship was found between skin permeance and the incidence and severity of internal BBD or cavities in 'Braeburn' apples that might clearly explain the marked differences in the susceptibility of different lines of fruit to internal BBD observed in previous storage experiments. The high incidence of internal BBD in fruit from all orchards probably resulted from storage in an atmosphere containing a high partial pressure of CO_2. The relationship between skin permeance and internal atmospheres and internal BBD in 'Braeburn' apples is worthy of further investigation in both air and CA storage. There were no differences between the mineral composition of cortex tissue of fruit with different severities of internal BBD except for Mg which had slightly higher levels in fruit with disorder.

A4.6 Acknowledgements

We would like to thank Chris Watkins (HortResearch, Auckland, and now at Cornell University, Ithaca, New York) for his advice and assistance with fruit assessments, John Elgar (HortResearch, Auckland) for monitoring the CA chamber, Siva Ganesh (Massey University) for statistical advice, and the ENZA New Zealand (International) for their generous support through the Don Sinclair Fellowship.

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Appendix 5

Regional Differences in Gas Exchange Characteristics of 'Braeburn' Apples in Relation to the Incidence of 'Braeburn Browning Disorder'

Report to ENZA New Zealand (International) by

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A5.1 Abstract

There was an absence of either statistically or physiologically significant variation in the average values for gas exchange variables (respiration rate, skin permeance to O_2 and CO_2 and internal partial pressure of O_2 and CO_2) between the two regions. This indicates some other factor(s) are involved in the difference in susceptibility to 'Braeburn Brown Heart' (BBD) between the regions. In this experiment, there was a decrease in susceptibility to the disorder with increasing maturity. However, the average $p^{i}_{CO_2}$ increased with maturity, which may to some extent counteract the decline in susceptibility to BBD with increasing maturity. Very low $p^{i}_{O_2}$ in some Central Otago fruit indicates the potential for some late-harvested fruit to experience fermentation on the tree. Further work should be done to assess the potential for variability in gas exchange characteristics at an individual fruit level to explain varied susceptibility to BBD.

A5.2 Introduction

'Braeburn' apples are susceptible to a physiological disorder 'Braeburn Browning Disorder' (BBD). The disorder has been identified as a problem since 1978 in trials where the fruit were stored in unperforated polyliners. BBD caused significant economic loss in the New Zealand export market, particularly in the 1992 and 1993 seasons (4.5% of the national 'Braeburn' export crop in 1993), and primarily in fruit from the colder growing regions (June and Ahlborn, 1993). However, the disorder does not increase with prolonged cool storage suggesting that it is not a form of chilling injury *per se*.

Elevated CO_2 in poorly ventilated controlled atmosphere (CA) stores may induce brown heart in some apple cultivars (Knee, 1973; Fidler *et al.*, 1973; and Padfield, 1969). BBD and core cavities and coreflush were all induced in 'Braeburn' apples by storage in elevated CO_2 atmospheres (2% and 5% CO_2 in 2% O_2) and the severities of the disorders were found to be strongly grower dependent (Elgar and Watkins, 1992). Reported studies are divided as to whether less mature or more mature fruit are more susceptible to BBD.

Studies by Dadzie (1992) indicate a link between skin resistance to gas diffusion, internal CO_2 concentration and coreflush in 'Granny Smith' apples. Park *et al.* (1993) reported positive correlations between skin resistance to gas diffusion in strains of 'McIntosh' apples and incidence of low O_2 injury which develops in CA storage. Compared with other apple cultivars, 'Braeburn' apples have a high and more variable skin resistance (in this report we discuss skin permeance which is the reciprocal of skin resistance). They also have a high internal CO_2 concentration, particularly at the calyx end where there is a tendency for brown heart to be more severe.

The objective of this study was to determine whether differences in gas exchange characteristics are correlated with regional differences in susceptibility to BBD between fruit from Hawkes Bay and Central Otago.

A5.3 Materials and Methods

A5.3.1 Fruit supply

'Braeburn' apples were harvested from four orchards in Hawkes Bay and Central Otago. Fruit were harvested from at random from upper outer north and inner lower south positions of separate groups of three trees at early, middle and late periods of the commercial harvest. For Hawkes Bay fruit the harvest dates were 22/3/94, 5/4/94 and 19/4/94, and for Central Otago fruit, 29/3/94, 12/4/94 and 27/4/94.

The fruit were sent to Massey University where three gas exchange variables were estimated; respiration rate (r_{CO_2}, mol kg^{-1} s^{-1}), skin permeance to O_2 and CO_2 (P'_{O_2} and P'_{CO_2}, mol s^{-1} m^{-2} Pa^{-1}) and internal partial pressure of O_2 and CO_2 ($p^t_{O_2}$ and $p^t_{CO_2}$, Pa). these fruit were subsequently air freighted to HortResearch, Auckland, by overnight courier for assessment of maturity, and measurement of tissue density, lenticel frequency, cuticle thickness and wax content. An additional sample of 75 fruit from each side of each tree was stored at 0°C for 16 to 21 weeks to be assessed for BBD.

A5.3.2 Gas exchange characteristics

A5.3.2.1 Respiration rates

Respiration rates (r_{CO_2}, mol kg^{-1} s^{-1}) were determined for a subsample of 8 fruit from each side of each tree. Fruit were sealed in 5.8 x 10^5 mm^3 containers at 20°C in the dark and the CO_2 partial pressure of the jars measured after 5 minutes of sealing and again 30 minutes later.

The r_{CO_2} was estimated using the following equation:

$$r_{CO_2} = \frac{\left(V_{jar} - \frac{M}{\rho^{20}_{fruit}}\right) \Delta p^{\Delta t}_{CO_2}}{R\,(T + 273.15)\,M\,\Delta t} \qquad \textbf{(A5.1)}$$

where: $\Delta p^{\Delta t}_{CO_2}$ = difference in partial pressure of CO_2 between initial and final measurements over time Δt (Pa)

M	=	fruit mass (kg)
ρ_{fruit}^{20}	=	fruit density at 20°C (kg m^{-3})
r_{CO_2}	=	specific rate of transfer of CO_2 between internal and external atmospheres (mol kg^{-1} s^{-1})
R	=	gas constant (8.3143 m^3 Pa mol^{-1} K^{-1})
T	=	fruit temperature (°C)
Δt	=	difference in time between initial and final sampling of CO_2 (s)
V_{jar}	=	volume of respiration jar (m^3).

A5.3.2.2 Internal CO_2 and O_2 partial pressures

Values for $p_{O_2}^i$ and $p_{CO_2}^i$ were determined for fruit after their respiration rates had been estimated. Fruit were submerged in water and a 3 x 10^3 mm^3 sample of gas removed from the core cavity. The $p_{CO_2}^i$ and $p_{O_2}^i$ of a 90 mm^3 subsample of this gas was determined using an O_2 electrode (Citicell C/S type, City Technology Ltd., London, UK) in series with a minature infra-red CO_2 transducer (Analytical Development Company, Hoddesdon, UK), with O_2 free N_2 as carrier gas (flow rate 583 mm^3 s^{-1}).

A5.3.2.3 Skin permeance

Values for $P_{CO_2}^{'}$ and $P_{O_2}^{'}$ for each fruit were calculated from estimates of r_{CO_2}, $p_{O_2}^i$, $p_{CO_2}^i$ and fruit surface area (A, m^2) and mass (M, kg) using the following formulae:

$$P_{CO_2}^{'} = \frac{r_{CO_2} M}{A\left(\left(N_{CO_2,\text{core}} - N_{CO_2,\text{room}}\right) p_{tot}\right)} \qquad \textbf{(A5.2)}$$

where: $N_{CO_2,\text{core}}$ = mole fraction of CO_2 in the core cavity (mol mol^{-1})

$N_{CO_2,room}$ = mole fraction of CO_2 in the room (mol mol^{-1}), typically 3.5×10^{-4} mol mol^{-1}.

p_{tot} = total partial pressure of system (equivalent to atmospheric pressure), (Pa)

This steady-state method assumes that the IA composition was homogenous throughout the fruit, that the fruit were approximately spherical (Burg and Burg, 1965). Assuming $RQ = 1$, P'_{O_2} was calculated using r_{CO_2}:

$$P'_{O_2} = \frac{r_{CO_2} M}{A\left(\left(N_{O_2,room} - N_{O_2,core}\right) p_{tot}\right)} \quad \textbf{(A5.3)}$$

where:

$N_{O_2,core}$ = mole fraction of O_2 in the core cavity (mol mol^{-1})

$N_{O_2,room}$ = mole fraction of O_2 in the room (mol mol^{-1}), typically 0.2095 mol mol^{-1}.

Fruit surface area was estimated from fruit mass using the formula (Clayton *et al.*, 1995):

$$A = 0.0581\ M^{0.685}$$

A5.3.2.4 Statistical analysis

The statistical package SAS (SAS, 1990) was used to perform data analysis. The General Linear Models procedure was used for analysis of variance of the gas exchange characteristics for all growers across regions, harvests within grower, and trees within harvest. Paired t-tests were used to investigate differences between positions on the tree for each tree.

A5.4 Results and discussion

A5.4.1 Respiration rates

Average r_{CO_2} for different growers' fruit were all similar with no regional differences apparent (Fig. A5.1). Significant differences between r_{CO_2} of fruit from early, middle and late harvests occurred for four of the eight growers, though the absolute magnitudes of these differences were modest and there was no consistent pattern to the variation (Fig. A5.2).

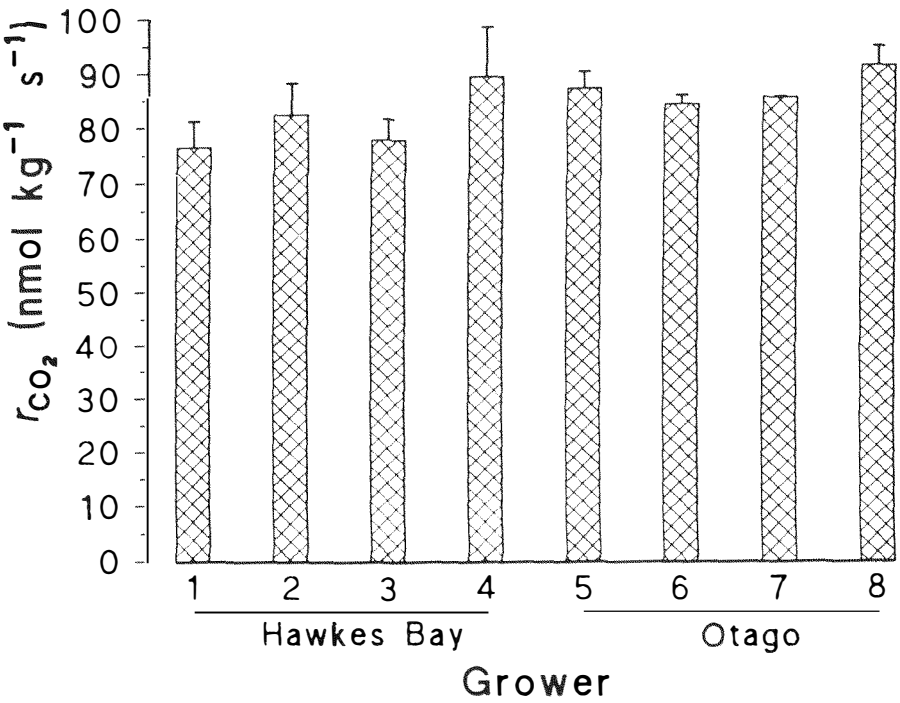

Figure A5.1 Respiration rates of 'Braeburn' apples from eight growers in two regions. Bars represent means and standard error of means of three harvests (early, middle and late), n = 3, $p = 0.375$.

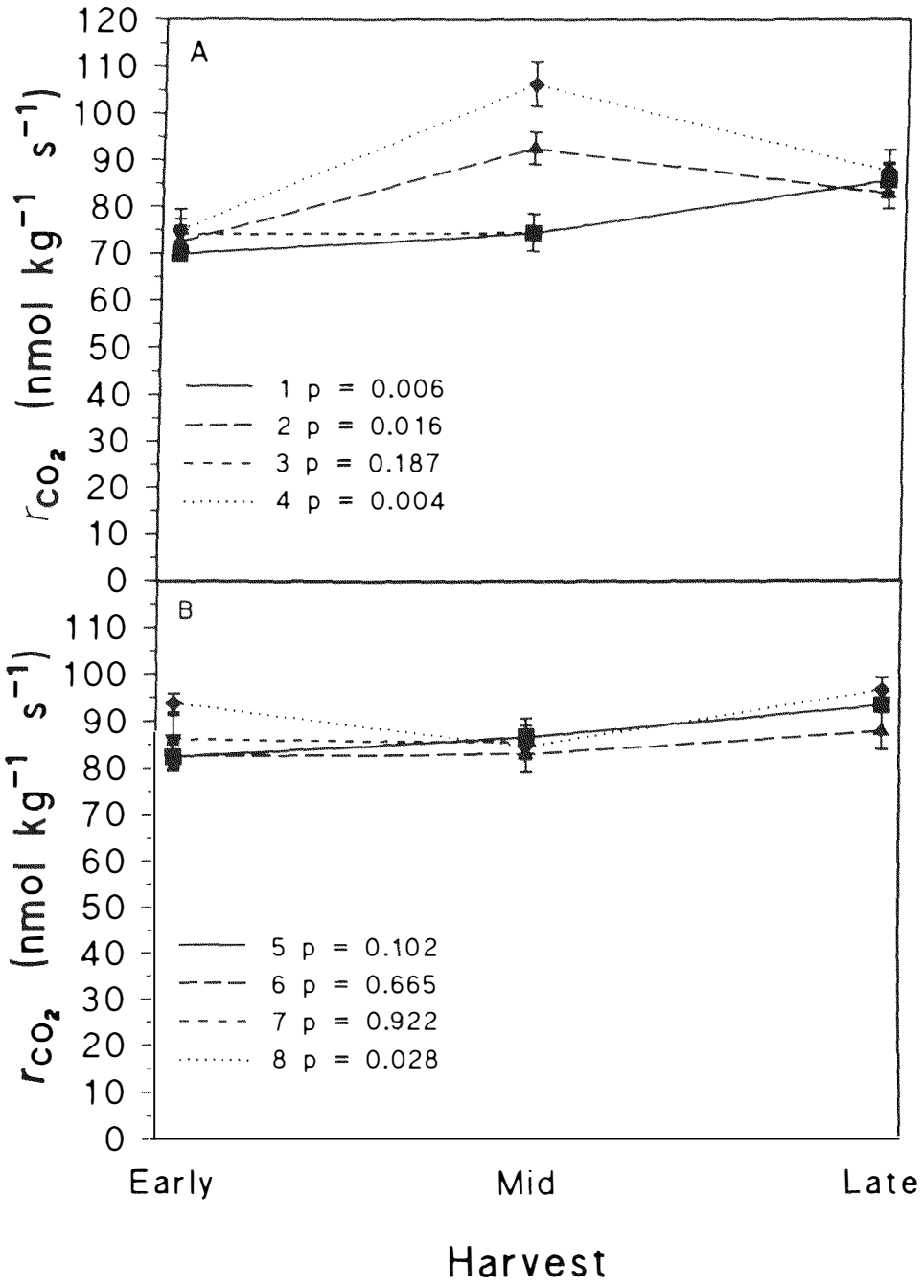

Figure A5.2 Respiration rates of 'Braeburn' apples from (A) Hawkes Bay and (B) Central Otago. Values represent means and standard error of means for early, middle and late harvests, n = 3.

A5.4.2 Skin permeance

There were no significant differences between growers of regions for average P'_{CO_2} or P'_{O_2} (Figs. A5.3 and A5.4). Average values for P'_{CO_2} were higher than for P'_{O_2}. There was some evidence for variation in P'_{CO_2} and P'_{O_2} between fruit from different harvests taken from Hawkes Bay but there was no particular pattern with time (Fig. A5.5). In contrast, fruit from Central Otago exhibited a consistent decline in P'_{O_2} values for latter harvests for three of the four growers (Fig. A5.6).

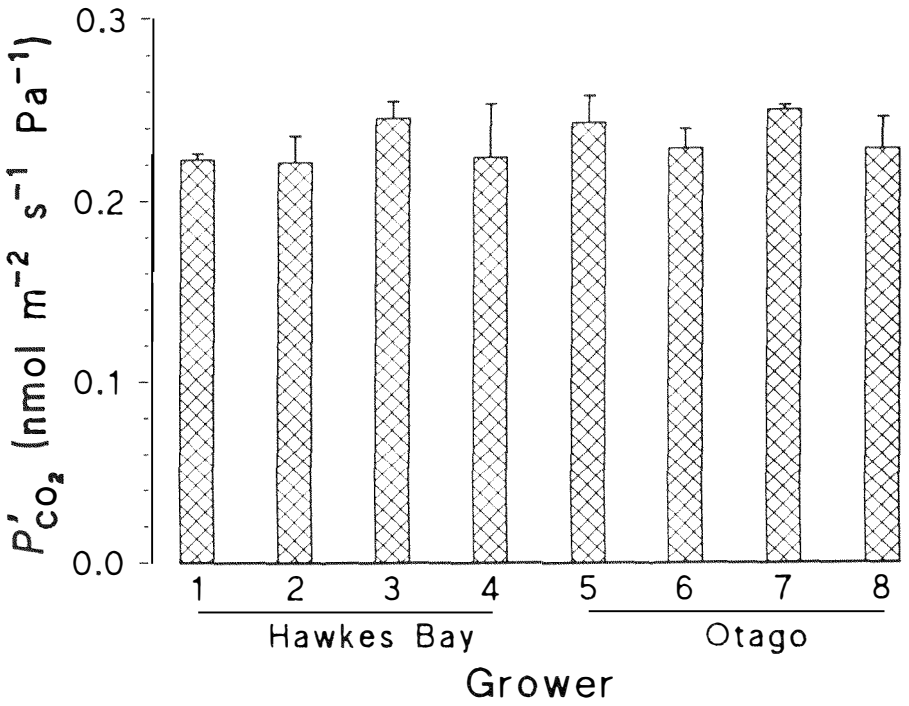

Figure A5.3 Skin permeance to carbon dioxide of 'Braeburn' apples from eight growers in two regions. Bars represent means and standard error of means of three harvests (early, middle and late), n = 3, p = 0.835.

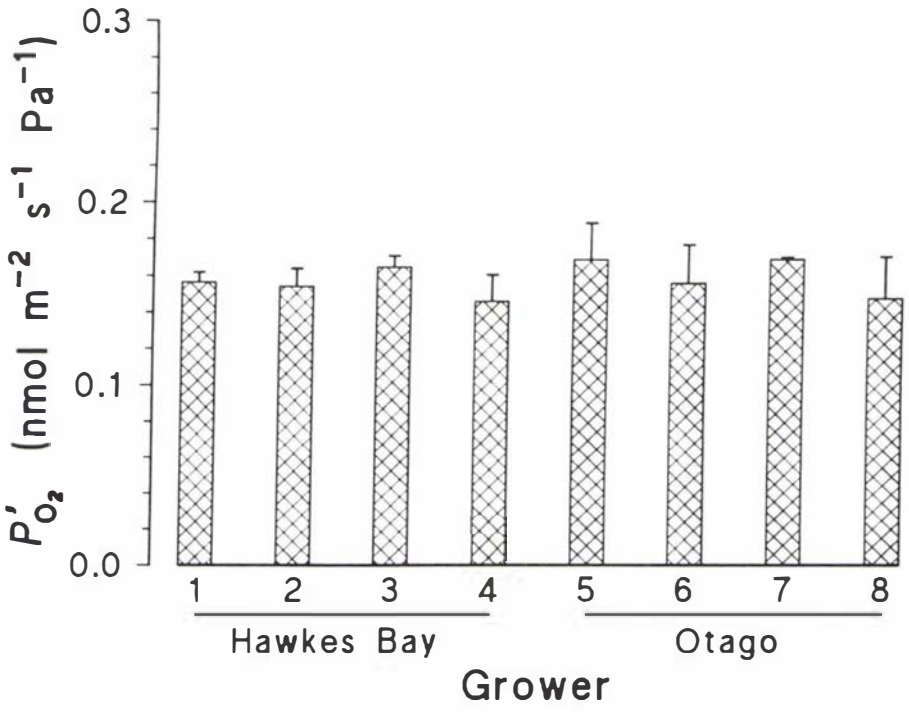

Figure A5.4 Skin permeance to oxygen of 'Braeburn' apples from eight growers in two regions. Bars represent means and standard error of means of three harvests (early, middle and late), n = 3, $p = 0.936$.

A5.4.3 Internal atmospheres

Average values for $p^i_{CO_2}$ and $p^i_{O_2}$ were similar for all growers across regions (Figs. A5.7 and A5.8). However, $p^i_{CO_2}$ increased and $p^i_{O_2}$ decreased for latter harvest for most growers in both regions, particularly for Central Otago growers (Figs. A5.9 and A5.10).

Individual fruit values for $p^i_{CO_2}$ and $p^i_{O_2}$ were hyperbolically related to P'_{CO_2} and P'_{O_2} respectively (Fig. A5.11). The range of values for individual growers was greater for fruit from Central Otago than those from Hawkes Bay (Figs. A5.12 and A5.13). For fruit from Central Otago very low $p^i_{O_2}$ and high $p^i_{CO_2}$ was detected in a few late-harvested fruit (Fig. A5.14). However, significant incidence of the BBD was only detected in early and middle harvested fruit from Central Otago (data not shown, but presented in the HortResearch report on the experiment). Variability in $p^i_{O_2}$ was much greater than $p^i_{CO_2}$, presumably because of the fruit's greater permeance to CO_2 than O_2, particularly in fruit with low overall levels of permeance (Fig. A5.11).

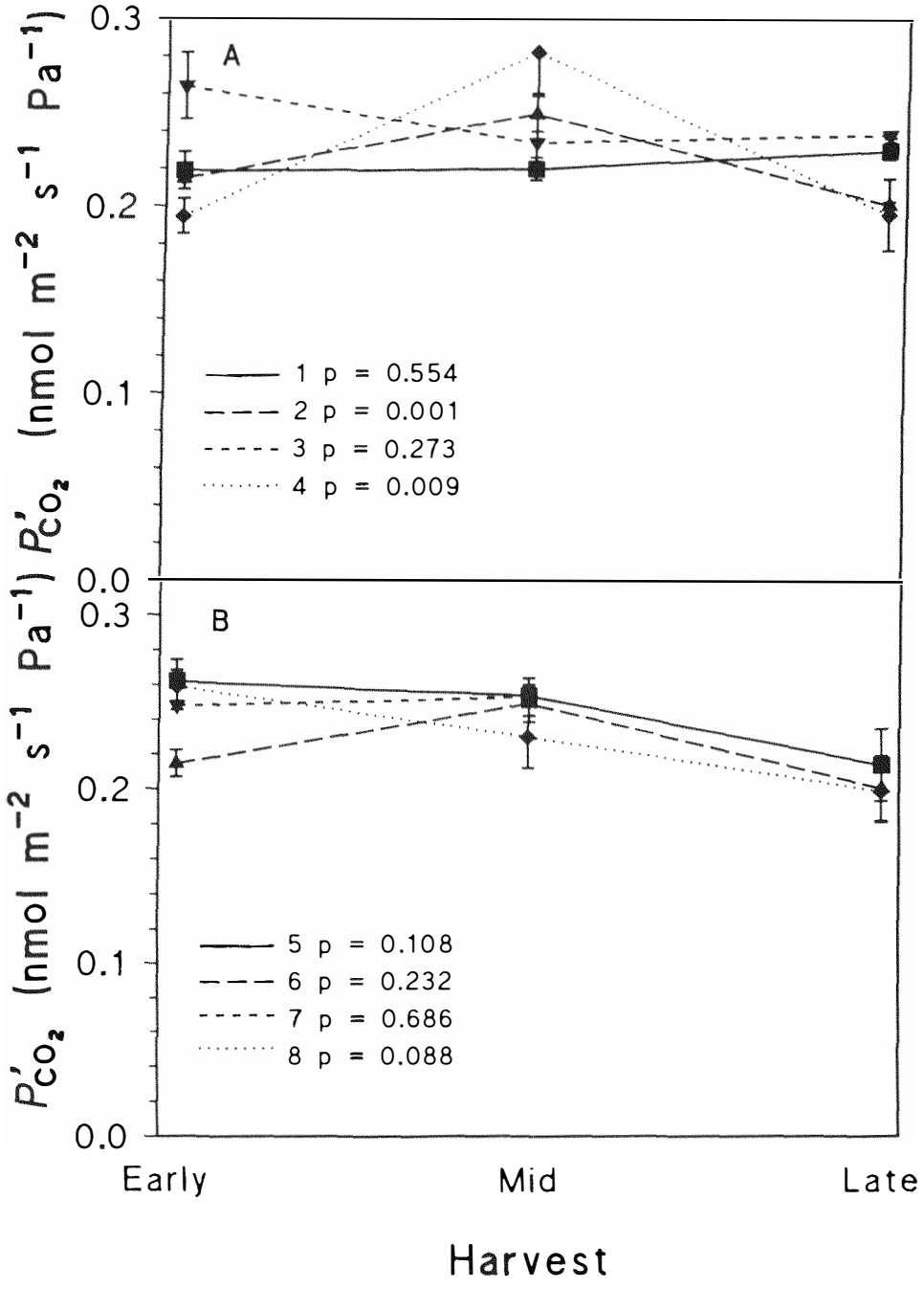

Figure A5.5 Skin permeance to carbon dioxide of 'Braeburn' apples from (A) Hawkes Bay and (B) Central Otago. Values represent means and standard error of means for early, middle and late harvests, n = 3.

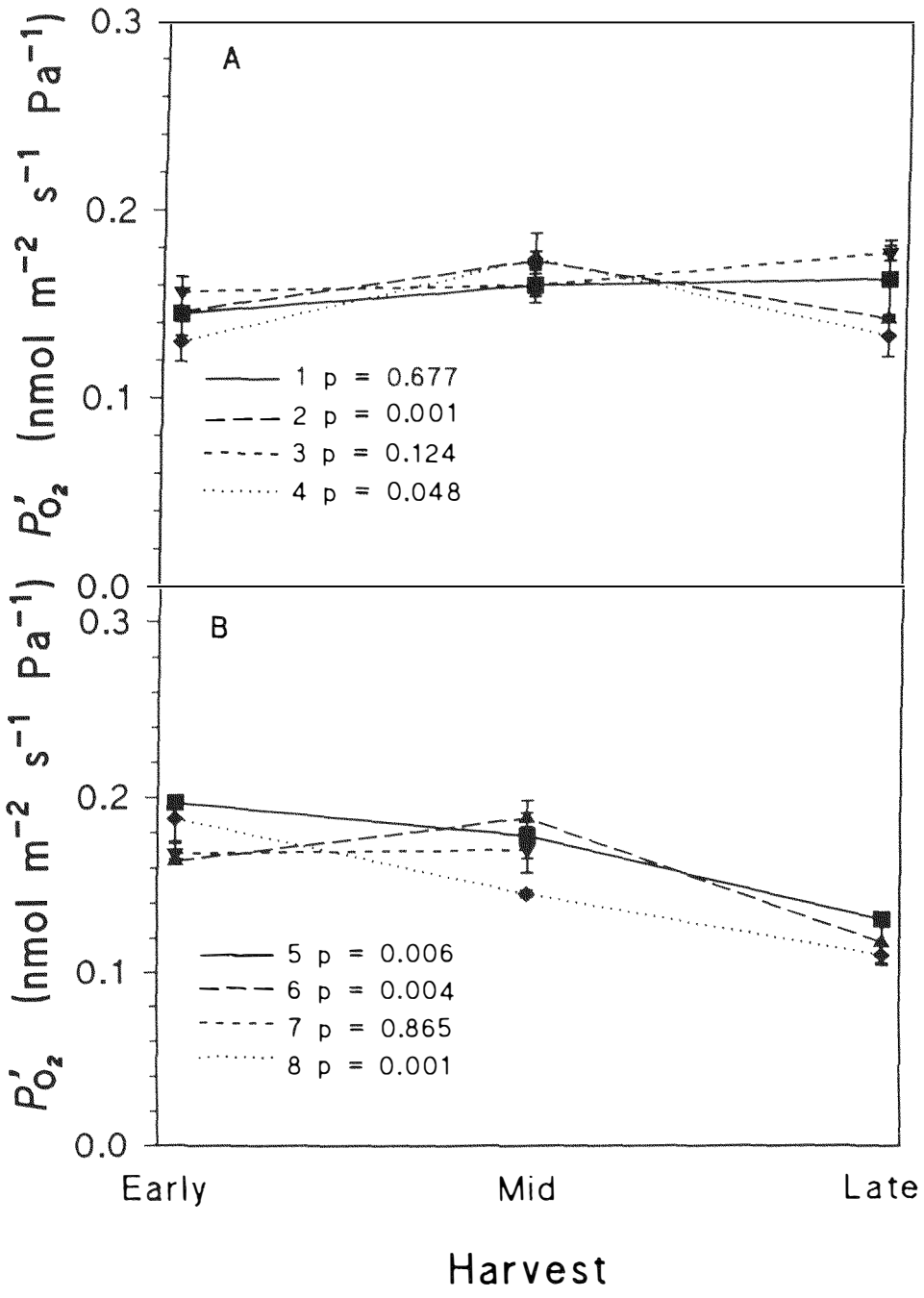

Figure A5.6 Skin permeance to oxygen of 'Braeburn' apples from (A) Hawkes Bay and (B) Central Otago. Values represent means and standard error of means for early, middle and late harvests, n = 3.

Plots of ($p^i_{O_2} + p^i_{CO_2}$) indicate none of the fruit were fermenting. It is possible that at temperatures greater than 20°C, some of these fruit could experience periods of fermentation on the tree. Despite the small variation in r_{CO_2} and skin permeance between harvests, there was a consistent increase in $p^i_{CO_2}$ with later harvests, particularly for Central Otago growers. Decline in susceptibility to BBD with increasing maturity may therefore to some extent be counteracted by increasing $p^i_{CO_2}$ in the more mature fruit.

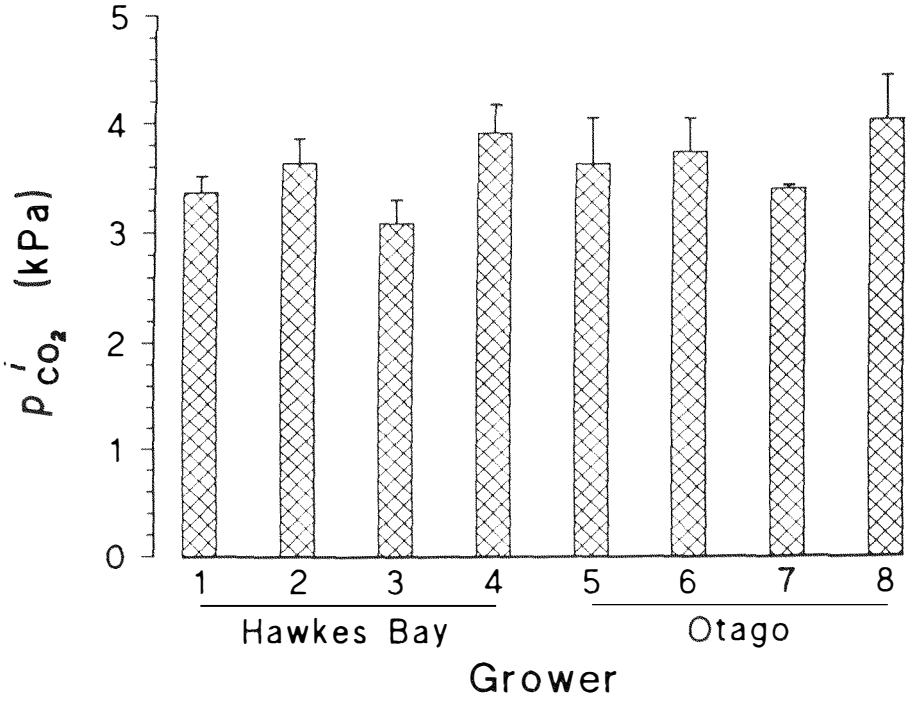

Figure A5.7 Internal partial pressure of carbon dioxide of 'Braeburn' apples from eight growers in two regions. Bars represent means and standard error of means of three harvests (early, middle and late), n = 3, $p = 0.419$.

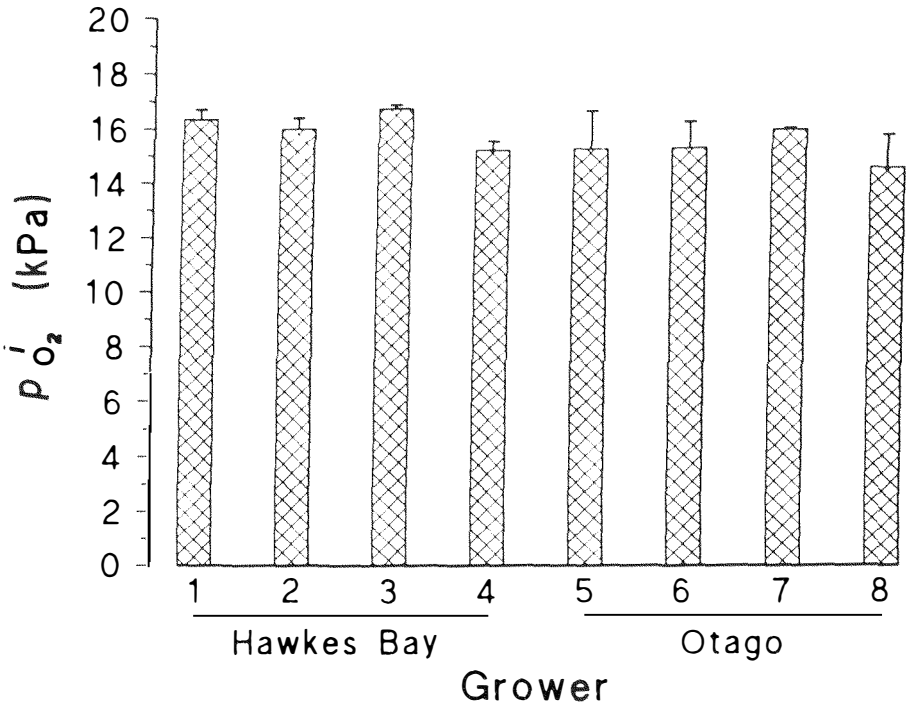

Figure A5.8 Internal partial pressure of oxygen of 'Braeburn' apples from eight growers in two regions. Bars represent means and standard error of means of three harvests (early, middle and late), n = 3, $p = 0.632$.

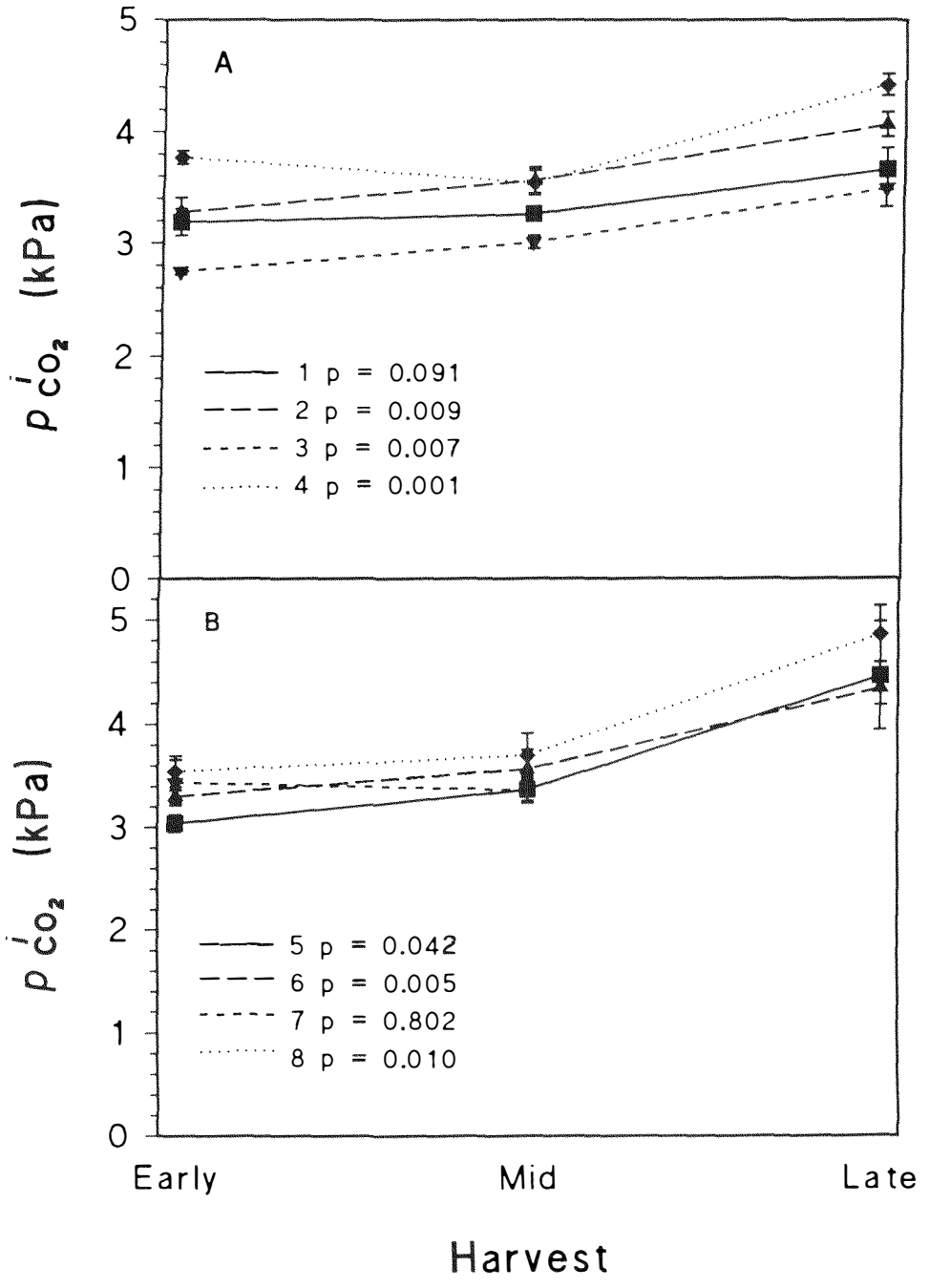

Figure A5.9 Internal partial pressure of carbon dioxide of 'Braeburn' apples from (A) Hawkes Bay and (B) Central Otago. Values represent means and standard error of means for early, middle and late harvests, n = 3.

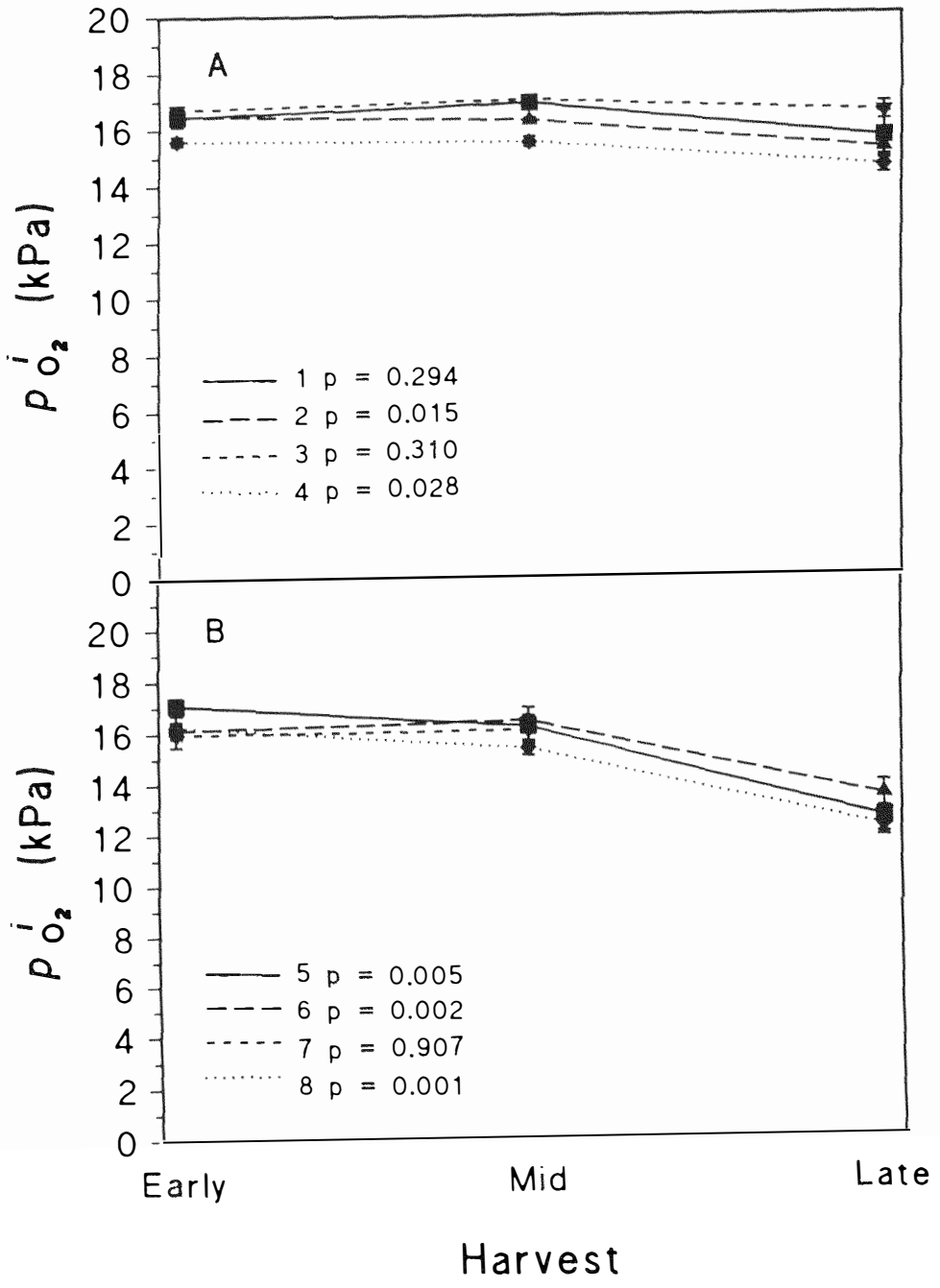

Figure A5.10 Internal partial pressure of oxygen of 'Braeburn' apples from (A) Hawkes Bay and (B) Central Otago. Values represent means and standard error of means for early, middle and late harvests, n = 3.

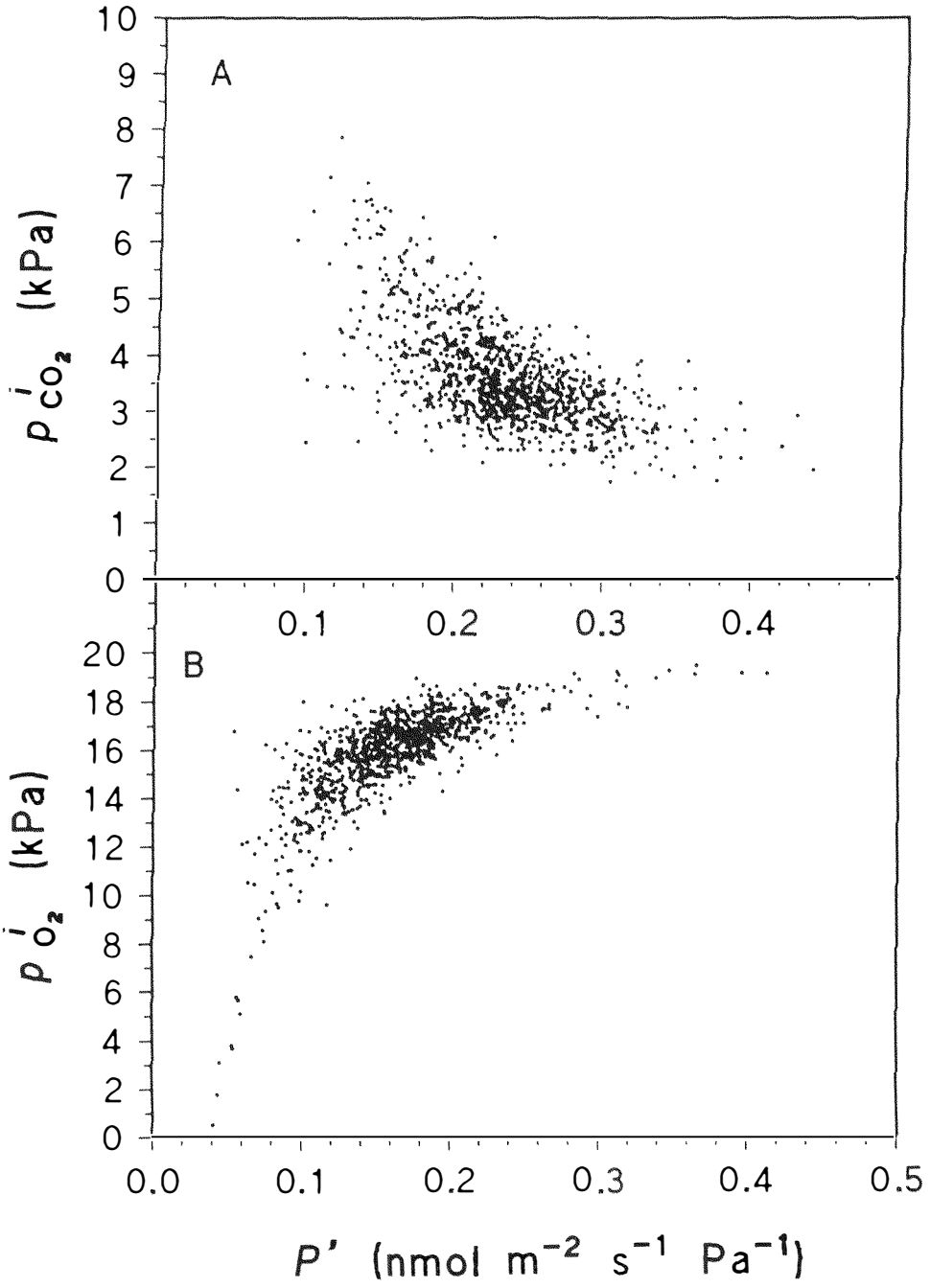

Figure A5.11 Relationship between skin permeance to (A) carbon dioxide and (B) oxygen and internal partial pressures of carbon dioxide and oxygen in 'Braeburn' apples for Hawkes Bay and Central Otago growers.

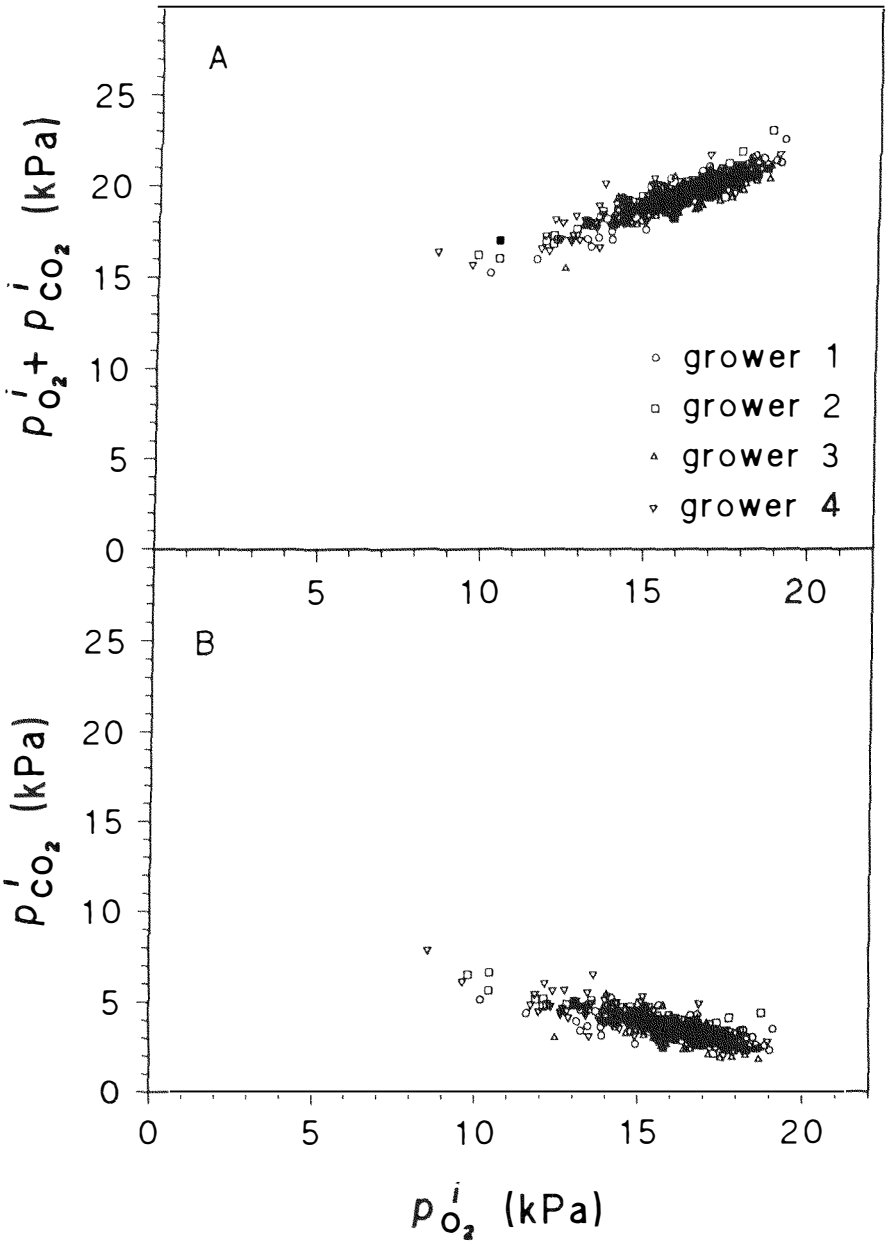

Figure A5.12 Relationship between internal oxygen partial pressure of 'Braeburn' apples and (a) sum of internal atmosphere pressure, (b) internal carbon dioxide partial pressure for all harvests of Hawkes Bay growers.

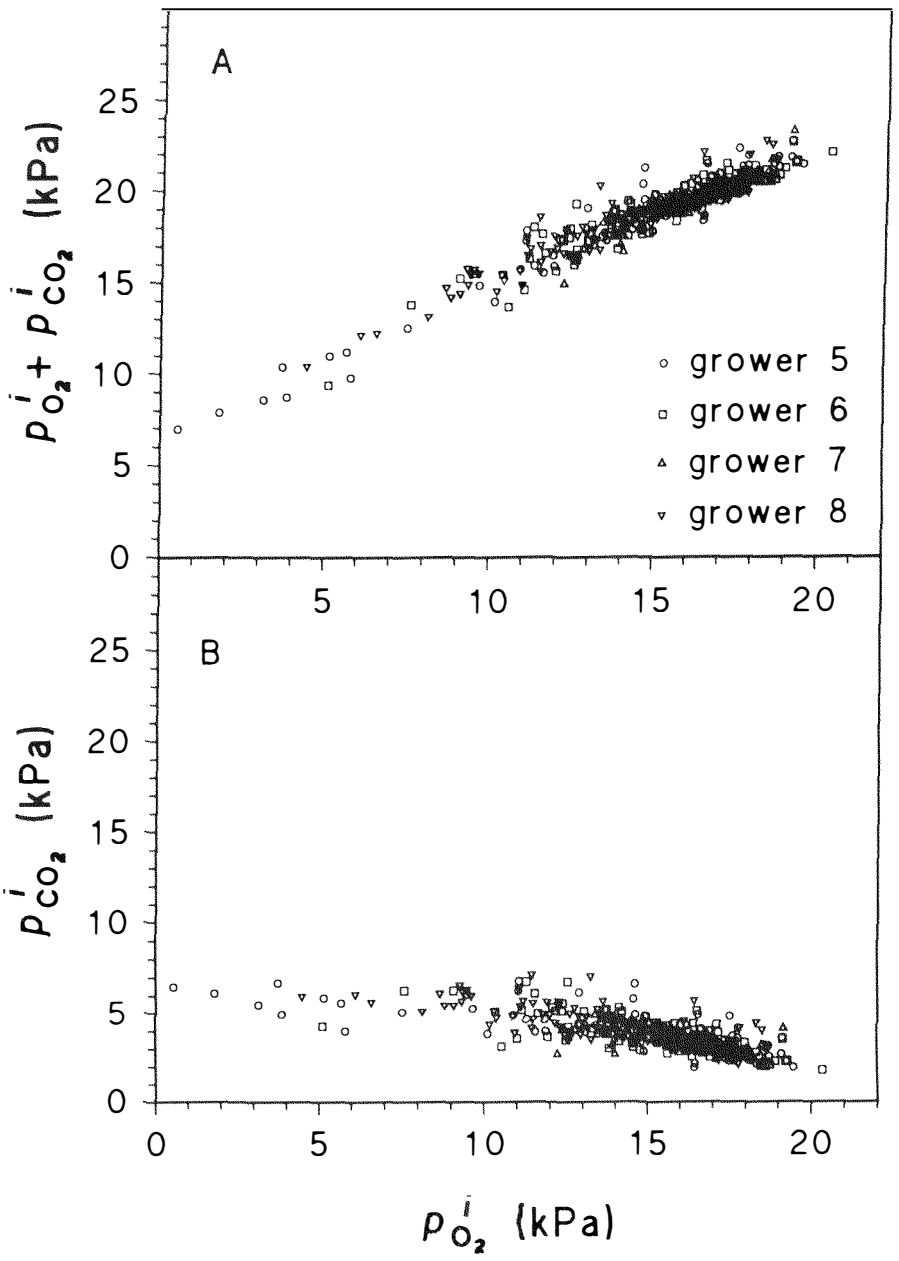

Figure A5.13 Relationship between internal oxygen partial pressure of 'Braeburn' apples and (a) sum of internal atmosphere pressure, (b) internal carbon dioxide partial pressure for all harvests of Central Otago growers.

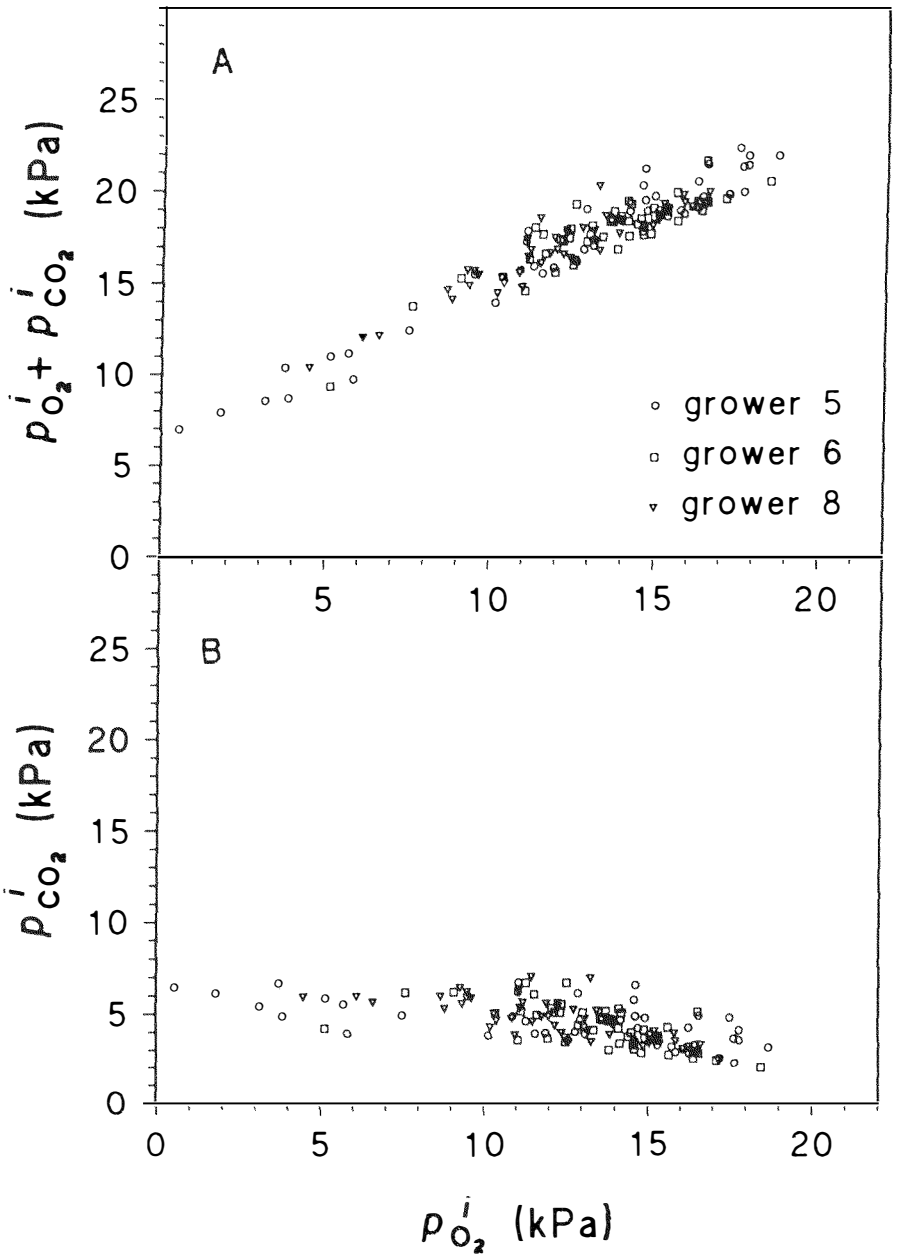

Figure A5.14 Relationship between internal oxygen partial pressure of 'Braeburn' apples and (a) sum of internal atmosphere pressure, (b) internal carbon dioxide partial pressure for late harvested apples from Central Otago growers.

A5.4.4 Differences between trees and fruit position in the canopy

No significant differences were found for the three variables between different trees. There were very highly significant differences in gas exchange characteristics of fruit from upper outer north and lower inner south positions of the tree canopy. However, these average differences were small in absolute terms (typically only 10% of the average value for each variable; data not shown).

A5.5 Conclusions

Absence of either statistically or physiologically significant variation in the gas exchange variables between the two regions indicate some other factor(s) are involved in the difference in susceptibility to BBD. For this experiment, there was a decrease in susceptibility to the disorder with increasing maturity. However, the average $p_{CO_2}^{i}$ increased with maturity, which may counteract the decline in susceptibility with increasing maturity. Very low $p_{O_2}^{i}$ in some Central Otago fruit indicates the potential for some late-harvested fruit to experience fermentation on the tree. If differing susceptibilities to BBD are linked to gas exchange characteristics, it is probably the outliers in the population that would be prone to developing the disorder. Further work should therefore be done to assess the potential for variability in gas exchange characteristics at an individual fruit level to explain varied susceptibility to BBD.

A5.6 Acknowledgements

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