GAS EXCHANGE CHARACTERISTICS AND QUALITY OF APPLES

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ABSTRACT

Atmospheric modification can extend the storage life of harvested fruits and vegetables beyond that which can be achieved with refrigerated air storage alone. Apples are particularly well suited to modified atmosphere (MA) storage and yet the recommended atmospheres for different cultivars of apples vary widely and responses of individual populations of apples to a given treatment can be variable. Part of this variation may be related to the variability in the internal atmosphere composition of individual fruit. This thesis explores the relationships between internal atmosphere composition of apples and factors such as skin resistance to gas diffusion (**R**), respiration, external oxygen concentration ($[O_2]_{ext}$), temperature and artificial barriers, all of which can influence the outcome of a given MA treatment.

Skin resistance to gas diffusion (**R**) values of freshly harvested apples of eight cultivars grown in New Zealand, were obtained using non-steady state and steady state methods at 20±1°C. **R** was cultivar dependent, with freshly harvested Braeburn apples having the highest mean **R** and Royal Gala the lowest. Skin resistance to ethane diffusion ($\mathbf{R}C_2H_6$) was linearly related to skin resistance to ethylene diffusion ($\mathbf{R}C_2H_4$) for individual apples within cultivars. Although there was a large degree of variation in between pairs of **R** values obtained on different apples within each cultivar, individual **R** values within these pairs were very similar to each other. The close relationship between the two independent estimates of **R** confirmed that this was real fruit to fruit variation rather than measurement error. In contrast, estimates of skin resistance to carbon dioxide diffusion ($\mathbf{R}CO_2$) were consistently higher than values for $\mathbf{R}C_2H_4$. There was a curvilinear relationship between $\mathbf{R}CO_2$ and $\mathbf{R}C_2H_6$ in a combined data set for all cultivars, indicating that CO_2 may diffuse through additional routes to those available for O_2, C_2H_4 and ethane (C_2H_6).

Freshly harvested Cox's Orange Pippin apples were respiring nearly twice as fast as Splendour, Granny Smith or Braeburn apples and a third higher than Gala, Royal Gala and Golden Delicious apples. Respiration rate appeared to be independent of $\mathbf{R}C_2H_6$ both within individual cultivars and in a combined data set for all cultivars. On the other hand, there was a declining exponential relationship between $[O_2]_i$ and $\mathbf{R}C_2H_6$ for individual apples and an increasing relationship between $[CO_2]_i$ and $\mathbf{R}C_2H_6$. Thus, the magnitude of **R** affects internal atmosphere composition for a given external atmosphere.

The respiratory and C_2H_4 production responses of Cox's Orange Pippin and Granny Smith apples to reduced O_2 concentrations were characterised by studying the variation in the magnitude of O_2, CO_2 and C_2H_4 concentration differences between the internal and external atmospheres ($\Delta[O_2]$, $\Delta[CO_2]$ and $\Delta[C_2H_4]$) of individual apples maintained in different O_2 atmospheres at 20±1°C. $\Delta[O_2]$ decreased at low O_2 levels, reflecting the decreased rate of O_2 uptake in low O_2 concentrations. Oxygen uptake relative to that in air (***Rel***O_2) approximately followed Michaelis-Menten kinetics, with a half-maximal rate of 2.5% O_2 for $[O_2]_i$ and 7.5% for $[O_2]_{ext}$. A mathematical equation was developed to describe the two physiological processes (ie. anaerobic and aerobic respiration) involved in the relationship between relative rate of CO_2 production (***Rel***CO_2) or internal CO_2 concentration ($[CO_2]_i$) and $[O_2]_{ext}$ or $[O_2]_i$. The equation had two components, each describing one of the two physiological processes.

The relationship between relative rate of C_2H_4 production (***Rel***C_2H_4) or internal C_2H_4 concentration ($[C_2H_4]_i$) and $[O_2]_i$ was more closely described by an exponential rather than a Michaelis-Menten type hyperbolic curve. Nevertheless, the overall shape of the relationship conformed to the expectation that small changes in O_2 concentration would have much greater effect at low $[O_2]_i$ than they do at high $[O_2]_i$. In contrast, the presence of the

skin as a diffusion barrier (**R**) resulted in development of an apparent 'lag phase' in the relationship between ***Rel***C_2H_4 or $[C_2H_4]_i$ and $[O_2]_{ext}$ such that it was no longer described by an exponential type curve and became essentially sigmoidal. These differences are attributable to gradients in gas composition between internal and external atmospheres.

Washing of Granny Smith apples in Tween 20 solutions inhibited development of greasiness. This effect was associated with increased **R**, depressed $[O_2]_i$, lower respiration and increased $[CO_2]_i$ and $[C_2H_4]_i$ in the washed fruit compared to controls. The depression of $[O_2]_i$ in Tween 20 treated fruit was greater than the elevation of CO_2, suggesting that the Tween 20 treatment may have affected CO_2 production and O_2 uptake to different extents or alternatively the Tween 20 deposit on the fruit surface was differentially permeable to these two gases. Washed fruit also remained greener and firmer than controls. Pre-treatment by wiping without using Tween 20 solution had none of these effects but did stimulate weight loss. None of the treatments induced internal browning which is often associated with the development of greasiness in Granny Smith apples.

The relationship between temperature and **R**, internal atmosphere compostion, respiration and rate of C_2H_4 production of eight cultivars of apples was ascertained after equilibrating fruit at temperatures ranging from 0 - 30°C for 72h. **R** appeared to be independent of temperature. $[O_2]_i$ decreased, while $[CO_2]_i$ increased, in response to increasing temperatures and varied with cultivar. Braeburn apples consistently had lower $[O_2]_i$ and higher $[CO_2]_i$ than the other cultivars while the converse applied for Splendour apples. Internal C_2H_4 concentrations ($[C_2H_4]_i$) and rate of C_2H_4 production increased with increasing temperatures to a maximum at 25°C, above which internal concentrations and rates of production declined. The magnitude of decline was cultivar dependent. Compared to the other cultivars, Splendour apples had the least capacity to accumulate and produce C_2H_4. There was a

progressive increase in fruit respiration rate with increasing temperatures, which varied with cultivar. Over all the temperature regimes, Splendour had the lowest average respiration rate while Cox's Orange Pippin apples had the highest. The potential for variability in these gas exchange variables being associated with overall storage life and response to MAs is discussed.

Small gas concentration differences were measured between the equator and calyx end, and between the equator and calyx end shoulder within individual fruit in Golden Delicious, Red Delicious, Granny Smith and Splendour apples at 20±1°C. In contrast, large O_2 and CO_2 concentration differences between the same positions were found in Gala, Royal Gala, Braeburn and Cox's Orange Pippin apples. The differences were much greater than those measured between the core cavity and the fruit surface. Similarly, tissues in the calyx region of Braeburn and Granny Smith apples consistently had lower O_2 but higher CO_2 and C_2H_4 concentrations than any other position on the fruit surface, whilst tissues at the equator had higher O_2 and lower CO_2 and C_2H_4 concentrations than other parts of the fruit. These data falsify the notion that the internal atmosphere of individual apples can be regarded as being homogeneous. The heterogeneous distribution of gases within individual fruit would presumably affect the tendency of individual tissues to develop low-O_2 or high CO_2 disorders, particularly for fruit stored in MAs at elevated temperatures.

A conceptual model is presented which summaries the relationships between fruit $[O_2]_i$ and $[O_2]_{ext}$, **R**, respiration, temperature and artificial barriers. The $[O_2]_i$ of apples are always lower than the $[O_2]_{ext}$ used during MA storage, to an extent which is determined by the respiratory O_2 uptake by the tissues coupled with **R**. With everything else being maintained equal, increased **R** or increased respiration rate therefore depresses $[O_2]_i$ which in turn modifies the extent of response of the crop to a given MA treatment. These variables are therefore all important in determining the fruit's response to atmospheric modification.

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A	=	Fruit surface area (cm^2)
AA	=	Acetaldehyde
$[AA]_{se}$	=	Subepidermal acetaldehyde concentration ($\mu l\ l^{-1}$)
ACP	=	Anaerobic compensation point (% O_2)
$[ACP]_{ext}$	=	Anaerobic compensation point for external oxygen (% O_2)
$[ACP]_i$	=	Anaerobic compensation point for internal oxygen (% O_2)
CA	=	Controlled atmosphere
CO_2	=	Carbon dioxide
$[CO_2]_i$	=	Internal carbon dioxide concentration (%)
$[CO_2]_{initial}$	=	Initial carbon dioxide concentration in jar (%)
$[CO_2]_{final}$	=	Final carbon dioxide concentration in jar (%)
C_2H_4	=	Ethylene
$[C_2H_4]_i$	=	Internal ethylene concentration ($\mu l\ l^{-1}$)
$[C_2H_4]_{initial}$	=	Initial ethylene concentration in jar ($\mu l\ l^{-1}$)
$[C_2H_4]_{final}$	=	Final ethylene concentration in jar ($\mu l\ l^{-1}$)
C_2H_6	=	Ethane
ΔC_{CA}	=	Difference in gaseous concentration (ie. $\Delta[O_2]$, $\Delta[CO_2]$, $\Delta[C_2H_4]$ etc.) between the external and internal atmospheres of a fruit in CA (%)
ΔC_{air}	=	Difference in gaseous concentration (ie. $\Delta[O_2]$, $\Delta[CO_2]$, $\Delta[C_2H_4]$ etc.) between the external and internal atmospheres of a fruit in air (%)
ETOH	=	Ethanol
$[ETOH]_{se}$	=	Subepidermal ethanol concentration ($\mu l\ l^{-1}$)

F	=	Flux or respiration rate ($cm^3\ s^{-1}$)
F_{max}	=	Maximum rate of exchange when O_2 is saturating ($cm^3\ O_2\ s^{-1}$)
F_{air}	=	Flux of gases (ie. O_2, CO_2, C_2H_4 etc) in air ($cm^3\ s^{-1}$)
F_{CA}	=	Flux of gases (ie. O_2, CO_2, C_2H_4 etc) in CA ($cm^3\ s^{-1}$)
FO_2	=	Rate of oxygen uptake ($cm^3\ kg^{-1}\ h^{-1}$)
FCO_2	=	Rate of carbon dioxide production ($cm^3\ kg^{-1}\ h^{-1}$)
FC_2H_4	=	Rate of ethylene production ($\mu l\ kg^{-1}\ h^{-1}$)
h	=	Hour
k'_f	=	a fruit constant (**A/R**) ($cm^3\ s^{-1}$)
K_m	=	Michaelis-Menten constant (% O_2)
MA	=	Modified atmosphere
N	=	Newtons
O_2	=	Oxygen
$[O_2]_i$	=	Internal oxygen concentration (%)
$[O_2]_{ext}$	=	External oxygen concentration (%)
$[O_2]_{initial}$	=	Initial oxygen concentration in jar (%)
$[O_2]_{final}$	=	Final oxygen concentration in jar (%)
R	=	Skin resistance to gas diffusion ($s\ cm^{-1}$)
RCO_2	=	Skin resistance to carbon dioxide diffusion ($s\ cm^{-1}$)
RC_2H_4	=	Skin resistance to ethylene diffusion ($s\ cm^{-1}$)
RC_2H_6	=	Skin resistance to ethane diffusion ($s\ cm^{-1}$)
RelE	=	Relative rate of exchange
RelO_2	=	Relative rate of oxygen uptake
RelCO_2	=	Relative rate of carbon dioxide production
RelC_2H_4	=	Relative rate of ethylene production
*Rel*RQ	=	Relative respiratory quotient
RQ	=	Respiratory quotient
rF_{max}	=	Maximum relative rate of exchange when $[O_2]_i$ is saturating ($cm^3\ O_2\ s^{-1}$)

rK_m	=	Michaelis-Menten constant for the relative rate (% O_2)
s	=	Seconds
Π	=	3.1416
V_{fruit}	=	Fruit volume (cm^3)
V_{jar}	=	Jar volume (cm^3)
W_{fruit}	=	Fruit weight (kg)
T	=	Time

CHAPTER 1

GENERAL INTRODUCTION

Innumerable physiological processes are accelerated (or initiated) in apples at the time of harvest. On removal from the parent plant, the fruit are deprived of their normal supply of water, minerals and simple organic molecules (eg. sugars, hormones) which normally would be translocated to them from other parts of the plant. However, harvested apples are still living as they continue to perform metabolic reactions including those involved in respiration and maintain the physiological system, while being solely dependent on their own reserves and moisture content (Biale, 1975; Pai and Sastry, 1990).

During normal respiration, oxygen (O_2) diffuses inwards via the skin and flesh of the fruit from the external atmosphere to the sites of reaction inside the cells. Utilisation of O_2 at the reaction centres results in a concentration difference (gradient) between the sites of reaction and the external atmosphere, causing more O_2 to diffuse towards the sites of utilisation (Burton, 1982).

Carbon dioxide (CO_2) and ethylene (C_2H_4) produced by respiratory metabolism occur within the cell sap and cause local increases in concentrations. This induces diffusion outwards to regions of lower concentrations through the openings on the surface of the fruit to the external atmosphere (Burton, 1982; Wolfe, 1980). The pattern of the gradient that is established for O_2 diffusion is the reverse of that for CO_2 and C_2H_4 (Burton, 1982; Kader *et al.*, 1989). The flux of gases to and from a fruit is related to the

magnitude of the resistance of its skin and of its flesh to gas diffusion, the respiration rate and the magnitude of the gas concentration difference between the internal and external atmosphere (Burg and Burg, 1965; Burton, 1982).

The problem of gas exchange in harvested apples can be appreciated considering the fact that they lack the blood circulatory system which functions in animals (Rahn *et al.*, 1979) to provide gas exchange. Still, fruit tissues are usually considered to be adequately ventilated. There is continuous gas exchange between the internal and external atmosphere of the fruit (Ben-Yehosua and Cameron, 1988; Burton, 1982). Adequate ventilation of the fruit relies on properties of the gas phase present within fruit tissues. The parenchymatous tissue of a fruit is interlaced with intercellular spaces which in some cases may occupy one fourth of the total volume (Biale, 1960a). The gas phase in the intercellular spaces acts as a continuum which extends throughout the fruit (Burton, 1982; Ben-Yehoshua, 1969; Burg and Burg, 1965; Devaux, 1891; Marcellin, 1974). Any action that results in the clogging of these spaces can impede gas exchange which can lead to fermentation (Ben-Yehoshua *et al.*, 1963; Sacher, 1973). Most cells of a fruit are in direct contact with the gaseous phase in adjacent intercellular spaces commonly referred to as internal atmosphere. This internal atmosphere is generally quite different in composition from that outside the fruit (Burton, 1982; Ben-Yehosua and Cameron, 1988; Phan, 1987). The internal atmosphere of the commodity is affected by factors such as respiration rate, skin resistance to gas diffusion (**R**) temperature, artificial barriers and stage of physiological maturity (Burton, 1982; Cameron and Reid, 1982; Sharples and Johnson, 1987; Zagory and Kader, 1988).

Oxygen concentrations between 1 and 5% have generally been used in controlled atmosphere (CA) of apples (Meheriuk, 1990). These concentrations are measured in the atmosphere surrounding the fruit and it is important to know the concentrations inside the fruit since it is the internal atmosphere that

brings about the reduced rate of deterioration seen in CA stored apples. Furthermore, knowledge of the internal atmosphere composition may also help develop physiological explanations of the mode of action of low-O_2 storage. The development of some postharvest physiological disorders in apples such as internal browning or core flush and brown-heart has been related to the fruit internal atmosphere composition (Hewett and Thompson, 1989). However, direct sampling of gas concentrations in fruit under low-O_2 atmospheres and even under normal atmospheric conditions is subject to many problems and reported results often seem to be erroneous (Knee, 1973; Pekmezci, 1971). Consequently, there is a dearth of information on factors affecting the internal atmosphere composition of apples presumably because of the difficulties in obtaining internal gas samples from fruits.

Most investigators have looked at the impact of the external O_2 ($[O_2]_{ext}$) rather than the internal O_2 concentration ($[O_2]_i$) on fruit respiration (and C_2H_4 production). However, it is the O_2 inside the fruit that is the most direct cause of depressed respiration (and C_2H_4 production) rather than $[O_2]_{ext}$ on which $[O_2]_i$ is dependent. The present investigation focused on $[O_2]_i$ (and $[CO_2]_i$ and $[C_2H_4]_i$) by studying the relationships between internal atmosphere composition of apples and factors such as skin resistance to gas diffusion (**R**), respiration, $[O_2]_{ext}$, artificial barriers and temperature. An attempt was also made to develop conceptual models illustrating these relationships. From these studies, it is hoped that further insight may be provided into the way in which these factors affect the internal atmosphere composition of apples and hence regulate the rate of deterioration of the fruit.

Pertinent literature related to the internal atmosphere composition as well as respiration and gas exchange in fruits including apples ~~are~~ reviewed in the next chapter. As far as possible, this was constructed from results of experiments carried out on apples by other investigators. However, a considerable proportion of the literature relevant to this work comprise results obtained with a variety of other fruits.

CHAPTER 2

LITERATURE REVIEW

2.1 Internal atmosphere of fruits

Gases are present inside fruits either in gaseous phase in the intercellular air spaces, hereafter called the 'internal atmosphere', or they are dissolved in the aqueous phase of the tissue, including the cell contents. Those present in the latter phase are most directly related to the levels of each gas present at the sites of metabolic activity within the cells. The intracellular concentration of a gas is determined by:

1. its concentration in the gaseous phase in the surrounding intercellular spaces i.e. the internal atmosphere in that part of the fruit;
2. the solubility of the gas in the cytoplasm at the prevailing temperature and pressure;
3. its rate of utilisation or production within the cells;
4. the resistance to movement between the intercellular gas phase and the intracellular solution (e.g., that afforded by the cell membrane and cell wall), which is affected by the solubility and diffusivity of the gas in water (Banks, 1981).

2.1.1 Factors affecting internal atmosphere composition

Extensive research has been conducted by various investigators to determine how the internal concentrations of gases in fruits including apples are affected by factors such as temperature, composition of the external

atmosphere, skin resistance to gas diffusion, artificial barriers and stage of maturity. The results of these studies are reviewed briefly in the following sections. However there is still a dearth of information on the importance of these factors on the internal atmosphere composition of apples.

2.1.1.1 Temperature

Temperature is one of the most important single environmental factors affecting the internal atmosphere composition of fruits and other plant organs (Kader, 1987). Decline in internal O_2 concentration and increase in internal CO_2 concentration within bulky plant organs in response to an increase in temperature has been demonstrated for different commodities including apples (Kader *et al.* 1989; Kidd and West, 1925), potatoes (Burton, 1950; Devaux, 1891b), oranges (Eaks and Ludi, 1960) papaya and banana (Leonard and Wardlaw, 1941), peaches and apricots (Maxie and Mitchell, 1974). Claypool (1938) reported that in apples, peaches, nectaries and plums internal CO_2 increased proportionally to increases in temperature. Trout *et al.* (1942) showed that Granny Smith apples in air had an internal O_2 content of 17% at 7°C and only 2% at 29°C. The corresponding CO_2 percentages were 2 and 17 at these two temperatures. Anzueto and Rizvi (1985) reported that apples stored at low temperatures (0, 5°C) had lower internal CO_2 and higher internal O_2 than their counterparts at higher temperatures (20, 25°C). Ladeinde and Hicks (1988) observed that at 0.3% external O_2, temperature did not influence the internal O_2 level of onions, but at higher external O_2 levels an increase in temperature lowered internal O_2 levels. However, when onions were stored at 30°C with external O_2 levels of less than 10%, the internal O_2 level in the bulb was close to zero. Information on the effects of a range of temperatures on the internal atmosphere composition of fruits is limited. In New Zealand quantitative data on the effects of different temperature regimes on the internal atmosphere composition of various cultivars of apples are unavailable.

2.1.1.2 Composition of the external atmosphere

The internal atmosphere composition of fruits and other bulky plant organs is closely related to the external atmosphere. Thus a change in the external atmosphere significantly influences the fruit internal atmosphere (Hulme, 1951; Lalaguna and Thome, 1982; Lidster, 1982; Knee, 1973, 1980, 1990; Williams and Patterson, 1962). Leonard (1947) reported that when bananas were placed at different O_2 levels at 12°C, the internal O_2 concentration was linearly related to the external O_2 concentration. Similar observations were made by Wardlaw and Leonard (1936) for pawpaw and Leonard and Wardlaw (1941) for banana.

Wardlaw (1936) found that there was a close relation between the composition of the internal atmosphere of bananas and pawpaws and that of the atmosphere in which the fruit is stored. The lower limit to which O_2 can be reduced in the external atmosphere without subsequent injury has been empirically determined for large number of fruits and vegetables (Kader, 1980). According to Weichmann (1987) vegetable crops reacting positively to CA conditions usually require a minimum external O_2 content of 1 - 3%. At such concentrations it is possible that the O_2 level inside the tissue is just 0.2% (Kader, 1986). Isenberg (1979) observed that at external O_2 levels below 2% most vegetables react with a sudden increase in internal CO_2. Information on the effect of external atmosphere composition on the internal atmosphere of fruit including apples is limited presumably because direct analysis of internal gas concentration in fruits under low O_2 or high CO_2 atmospheres is subject to many problems (such as contamination of gas samples with air or water during sampling) and often gives results which are obviously in error (Knee, 1973).

2.1.1.3 Skin resistance to gas diffusion

The skin of a fruit presents significant barrier to gas exchange and hence influence the internal atmosphere composition (Burg and Burg, 1965; Burton, 1950; Cameron, 1982; Hardy, 1949; Montero, 1987; Solomos, 1985; Soudain and Phan Phuc, 1979; Ulrich and Marcellin, 1968). For instance, Burg and Burg (1965) observed that internal CO_2 and C_2H_4 concentrations in apples declined considerably when the fruit skin was peeled off. Trout *et al.* (1942) reported that on removal of the skin of stored apples containing low internal O_2, there was an increase in internal O_2 concentration and a decrease in internal CO_2. Variation in skin resistance to gas diffusion between fruit cultivars could result in variation in internal atmosphere composition (Burton, 1982). In New Zealand information on the effects of skin resistance to gas diffusion on the internal gas composition of various locally grown apple cultivars is unavailable.

2.1.1.4 Artificial barrier

Artificial barriers such as coatings, waxes or plastic films or polyliners have been used extensively in commercial and postharvest research (Banks, 1984a, b, 1985a, c, d; Ben-Yehoshua, 1967, 1985; Hardenburg, 1971; Kader, 1980; Rizvi, 1981; Smith *et al.*, 1987). The presence of an artificial barrier to diffusion around fruit may result in reduced internal O_2 and increase CO_2 concentrations, altered water vapour and C_2H_4 concentrations (Smith *et al.*, 1987). The degree to which these factors are altered for a given commodity will depend on species, cultivar, mass:surface area ratio and respiration rate (Banks, 1984a, b, 1985a; Kader, 1980; Smith *et al.*, 1987).

Trout *et al.* (1942) recognised that coating apples reduced internal O_2 and increased internal CO_2 concentrations to levels that could result in anaerobiosis and off-flavours. Wax applications have been shown to increase

internal CO_2 concentrations (Claypool, 1938; Wardlaw, 1936) and to decrease internal O_2 concentrations (Hulme, 1951; Eaks and Ludi, 1960). In some instances the increase in CO_2 has been noted to exceed the decrease in O_2, but usually the reverse is true. The difference would be due to whether or not the internal O_2 was below the anaerobic compensation point (Ben-Yehoshua, 1969; Hall *et al.*, 1955). Ladeinde and Hicks (1988) observed that paraffin wax applied to the root plate of onions caused a dramatic reduction in internal O_2 and elevation of internal CO_2 concentration. Burg and Burg (1965) reported that when the pedicels of tomatoes and peppers were blocked with lanolin paste, the internal CO_2 doubled within 6h as a result of increased resistance to diffusion of CO_2 from the fruit, showing that the main pathway to gas movement was in the stem scar. Cohen *et al.* (1990) reported that postharvest waxing of 'Murcott' tangerine with Britex or Zivdar, resulted in increased internal CO_2, ethanol and consequently off-flavour.

Coating with TAL Pro-long modified internal concentrations of O_2, CO_2 and C_2H_4 and delayed ripening of bananas (Banks, 1983, 1984a, b) and apples (Banks, 1985a). Banks (1984a, b) observed that the depression of O_2 levels inside coated fruit was greater than the elevation of CO_2, suggesting that coating may have affected CO_2 evolution and O_2 uptake to different extents or that the coating deposit on the fruit surface was differentially permeable to these gases.

Using another coating agent (Nutri-save), Elson *et al.* (1985) and Meheriuk and Lau (1988) obtained similar results for Golden Delicious apples and 'd'Anjou pears. Studies by Banks (1984a), indicated that sucrose ester coating applied to bananas had no significant effect on internal C_2H_4 concentration. Ben-Yehoshua (1967) coated oranges, avocados and bananas with a formulation called Tag, and observed slightly higher CO_2 and markedly lower O_2 concentrations in their internal atmosphere as well as lower

respiration rates. Other commercial wax emulsions were found to have a similar effect on internal atmosphere composition (Ben-Yehoshua, 1967; Davis and Harding, 1960).

Early work showing that internal atmosphere modification was dependent on cultivar, coating type and thickness and holding temperature (Trout *et al*., 1953) was confirmed in work on apples (Banks, 1985a; Smith and Stow, 1984). According to Smith *et al.* (1987), relative concentrations of internal O_2 and CO_2 which develop inside coated fruit depends on coating type and cultivar. For instance some cultivars such as Bramley's seedling have relatively high concentrations of natural surface wax which may prevent wetting of the surface by the coating. Consequently the internal gases would be relatively different from another cultivar eg. Spartan which has less natural surface wax (Smith *et al.*, 1987). Ben-Yehoshua (1967) suggested that the effects of skin coating such as Tag (a polyethylene-wax emulsion) and several other commercial waxes (Zivdar, Britex, Flavorseal) on oranges depended on the type of coating and its thickness. Ben-Yehoshua (1967) suggested that the optimal coating should maximally reduce weight loss without creating an injurious atmosphere inside the fruit. He recognised that the internal atmosphere should be within the range in which there is neither a deficiency of O_2 nor an excess of CO_2 during storage life at the range of temperatures to which the fruit would be exposed.

Films are generally extruded plastic or polymeric materials that are used to surround the produce as shrink or stretch wraps, or as sealed loose covers (Smith *et al.*, 1987). The use of polymeric films to extend the postharvest life of fruits and other plant organs through modification of the internal atmosphere of the commodity has increased greatly during the last two decades (Kawada, 1982). This is due mainly to the rapid development of new films and packaging technology, together with changes in produce marketing systems (Kawada, 1982). Films have been employed to restrict water loss in

storage for many years but their use in modified atmosphere packs has been relatively limited (Eaves, 1960; Gerhardt, 1951; Hardenberg, 1971; Mannapperuma *et al.*, 1989; Scott and Roberts, 1966; Tomkins, 1962, 1967; Watkins *et al.*, 1989). Early attempts resulted in only partially modified or anaerobic conditions, presumably because the films used had either excessive or inadequate permeability to O_2 (Allen and Allen, 1960; Scott and Tewfik, 1947). The internal atmosphere of the commodity in a plastic package is known to be influenced by the atmosphere inside the package (Kader *et al.*, 1989; Hudson *et al.*, 1989). However, quantitative data of the concentration of gases inside the produce within packages is limited. Usually when fruits and vegetables are enclosed in a polymeric film, the internal atmosphere becomes modified - depleted in O_2 and enriched in CO_2 (Geeson, 1988). On the other hand Drake *et al.* (1988) claimed that the use of a rigid film package for Delicious apples significantly reduced internal CO_2 and C_2H_4 content of fruit.

2.1.1.5 Stage of maturity

The stage of maturity of a fruit appears to affect the internal gas composition. As plant organs advance towards senescence, the intercellular spaces become clogged with cellular sap consequently reducing the diffusivity of gases and causing a decrease in internal O_2 and increase in internal CO_2 (Burton, 1982; Kader *et al.*, 1989 Trout *et al.*, 1942). Early work by Kidd and West, (1949) indicated that the internal atmosphere of apples was influenced by their physiological stage. For instance in climacteric fruit (eg. banana), during the preclimacteric stage, internal O_2 levels are high while internal CO_2 levels are low. At the peak of the climacteric, internal O_2 decreases while internal CO_2 (and respiration rate ie. CO_2 production) increases. Brooks (1937) also reported that the CO_2 in the internal atmosphere of tomatoes increased with an increase in maturity from the mature green to the pink stage. Reid *et al.* (1973) reported that the internal concentration of CO_2 in preclimacteric Cox's Orange Pippin apples was 2% while the internal C_2H_4 content was about 0.02ppm (at 12°C).

Martin and Juniper (1970) observed that as fruit mature, the cuticle thickens, thus affecting the diffusivity of gas and hence internal gas composition. Some apple cultivars notably Granny Smith, develop a natural coating or become greasy after a period of storage (Huelin and Gallop, 1951a, b; Leake *et al.*, 1989a; Lill *et al.*, 1989; Martin and Juniper, 1970; Trout *et al.*, 1953). This is likely to increase the resistance of the skin to gaseous diffusion and thus affect the internal gas concentrations.

2.1.2 Methods of sampling internal atmosphere

The gas concentration gradient of a given species is an estimate of the chemical potential or driving force of gas diffusion. It is usually estimated from accurate measurement of the internal and external gas concentrations using a method which does not alter the composition (Ben-Yehoshua and Cameron, 1988). Accurate measurement of the internal gas composition is essential for the estimation of skin resistance to gas diffusion and also in estimating the physiological stage of maturity of a fruit (especially apples and fruits with internal cavities).

Several different approaches have been utilised to obtain internal gas samples from fruits and other bulky plant organs and none of these methods is entirely satisfactory. Some of these approaches are summarised briefly in the following sections:

2.1.2.1 Direct sampling method

The direct sampling method is easy, quick and straightforward for obtaining samples from the internal atmospheres of bulky organs such as musk-melon (Lyons *et al.*, 1962) and apple (Blanpied, 1968, Dadzie *et al.*, 1990a, b; Smith, 1947; Lyons and Pratt, 1964; Rajapkse *et al.*,

1989a, b, 1990; Sfakiotakis and Dilley, 1973; Saltveit, 1982; Williams and Patterson, 1962). It consists of inserting a long hollow hypodermic needle through the skin into the tissue or core cavity and slowly extracting gas samples by pulling back the plunger on an attached syringe. The resulting partial vacuum causes gas to flow from the tissue into the syringe (Ben-Yehoshua and Eaks, 1970; Burg and Burg, 1962, 1965; Blanpied, 1968; Lyons and Pratt, 1964; Sfakiotakis and Dilley, 1973; Williams and Patterson, 1962; Forsyth *et al.*, 1973; Lyons *et al.*, 1962; Maxie *et al.*, 1965).

Contamination of the sample with air from outside the tissue may result if the sample is taken while holding the commodity in the air. This can be prevented either by taking samples from produce submerged an aqueous solution (Ben-Yehoshua, 1969; Hulme, 1951; Lyons *et al.*, 1962; Maxie *et al.*, 1974; Sfakiotakis and Dilley, 1973; Smith, 1947; Williams and Patterson, 1962) or by sealing around the needle with wax or Vaseline (Smith, 1947; Hulme, 1951) or cement (Williams and Patterson, 1962). The needles of hypodermic syringes are frequently blocked by fruit tissue when the internal atmosphere of fruits are sampled. This problem can be avoided by taking samples from just beneath the skin of fruit (Burg and Burg, 1962) or a wire filament may be used to prevent blockage of the needle during insertion (Rajapkse *et al.*, 1989a, 1990; Sfakiotakis and Dilley, 1973; Reid *et al.*, 1973; Smith, 1947) or by using a blunted or side-delivery needle (Williams and Patterson, 1962). Blockage can still occur during sampling of fruit with any of these techniques. In the current work, this problem was overcome by using a modification of the 'direct removal' method described by Banks (1983) in which a pin head is fitted into the mouth of a canula which is also fitted to a disposable syringe.

It should be mentioned that most 1 ml disposable syringes have 0.1 ml or more dead space at the tip and in the needle. This is not much of a problem if large samples are taken. However if only very small samples are taken the dead space can cause severe dilution problems. Purging with nitrogen or salt

solution prior to sampling does not solve the problem completely. When only very small volumes of gas are available for sampling, glass syringes with minimal dead space volume should be used for best possible accuracy (Ben-Yehoshua and Cameron, 1988).

Direct measurement of internal gas concentrations is usually difficult or inconvenient and reported results, particularly for O_2, often seem to be erroneous (Knee, 1991a, b). Fidler and North (1967) also found the direct sampling method to be unreliable. However in this research reliable results were easily obtained using a modification of this technique. Similarly various research workers including Banks (1981, 1984a, b, 1986), Banks and Kays (1988), Cameron (1982), Dadzie *et al.* (1990a, b), Rajapkse *et al.* (1989a, b, 1990), Hewett *et al.* (1989) have also obtained reliable results using the direct sampling method.

2.1.2.2 Artificial internal cavity extraction method

Devaux (1891b) while attempting to take gas samples from the internal atmospheres of potatoes, appears to have been the first to have created an artificial internal cavity from which repeated gas samples could be taken. In this technique, which was rediscovered by Wardlaw (1936), a cork borer is used to remove a cylinder of tissue from the plant organ. One end of a short piece of glass tubing is inserted mid way into the cavity created and the other end is sealed with a septum. Samples are withdrawn after the air in the tube equilibrates with the atmosphere inside the organ. Trout *et al.* (1942) critically examined this approach and concluded that equilibrium was established within 24 hour after each sampling. Ben-Yehoshua *et al.* (1963) also used this method for avocado, after showing that the injury involved in establishing the cavity did not affect respiration and gas exchange during the sampling period.

An approach was used by Wardlaw (1936); Kidd and West (1949) and Ekambaram (1922) in which a small section of skin was removed and the tube attached to the outside of the plant organ. Wardlaw and Leonard (1939a, b) sampled the internal atmosphere of banana fruit by placing a volume of air (5.0 ml) in contact with the pulp of the fruit via an insertion and leaving it to equilibrate with the contents of the intercellular spaces. Samples were withdrawn at regular intervals for analysis.

Hulme (1951), Sfakiotakis and Dilley (1973), Smith (1947) and Vendrell (1970) used an alternative approach which involved a combination of the direct sampling and artificial cavity methods. A slightly larger syringe needle was inserted into the organ rather than a piece of glass tubing and covered with a septum before sampling. This method permitted repeated gas samples to be taken from a given depth beneath the skin.

The greatest drawback of the internal cavity methods described is the unmeasured effect of wounding, as wounding will normally increase fruit respiration rate, rate of ethylene production and the degree of water soaking in the tissue (Burton, 1974; Kahl, 1974) any one of which can change diffusion behaviour markedly. In addition, these methods have the disadvantage that they involve considerable mutilation of the tissue of the fruit. Further, they do not take into account the existence of gaseous diffusion gradients between different parts of the tissue (Smith, 1947).

2.1.2.3 External cavity extraction method

The method has been used to study the internal atmosphere of potatoes (Banks and Kays, 1988; Devaux, 1891b), apples (Cameron, 1982; Dadzie *et al.*, 1990a, b; Ekambaram, 1922, Rajapkse *et al.*, 1989a, b, 1990, Solomos, 1989) and other bulky organs such as banana (Banks, 1983) nectarines, (Rajapkse *et al.*, 1990) and tomatoes (Cameron, 1982). In this method, using

gelatin or soft wax (Devaux, 1891b) or vaseline (Cameron, 1982) or PVA glue (Dadzie *et al.*, 1990a, b; Rajapkse *et al.*, 1989a, b; 1990) a small chamber is attached to the surface of a fruit or vegetable and allowed to equilibrate. Cameron (1982) modified the technique by sealing a small chamber to the surface of the fruit using a small amount of vaseline; both the fruit and chamber were held in place by a ring stand and clamps. Samples were taken through the septum. As the plunger of the syringe is moved back and forth, the water in the arm of the chamber is drawn up and down. The method ensures a tight seal, prevents a significant drop in chamber pressure (which could cause mass flow from the fruit), and equilibrates the gas in the syringe with that in the chamber. After the sample is withdrawn, the water level remains elevated in the arm, drawing a very slight vacuum on the skin of the fruit exposed to the chamber.

For fruits with lenticels or other 'holes', this slight vacuum slowly dissipates by net movement of gas from the tissue to the chamber. For fruits such as tomato, which contain no lenticels, a quantity of gas equal to that removed can be injected into the chamber after sampling. Cameron (1982) found that sampling could be initiated about 8 hour after attaching the chamber onto apples and every 2 hour thereafter. A much simpler method developed by Banks and Kays (1988) based on the principles described by Cameron (1982) involves attaching one more sampling chambers to the fruit surface (Dadzie *et al.*, 1990a, b; Rajapkse *et al.*, 1989a, b, 1990). Contents of the chamber on apples have been found to equilibrate with the internal atmosphere of the organ after 40 to 90h equilibration (Dadzie *et al.*, 1990a, b; Rajapkse *et al.*, 1989a, b) and repeated samples can be taken.

Since this method measures the concentration immediately beneath the skin, it is ideal for estimating skin resistance even for organs with substantial flesh resistance (Cameron, 1982, Dadzie *et al.*, 1990a; Rajapkse *et al.*, 1989a, 1990). In addition, wounding of the plant organ is avoided.

Banks (1983) found that this technique may modify the atmosphere under the skin considerably. He found that chambers (7.0 mm diameter) attached to the surface of green banana fruit, after ripening for 4 days at 20°C, the skin of the fruit was yellow except for that covered by the chamber, which remained green. This problem has not been found in apples in which this method is used on a routine basis (Cameron, 1982; Dadzie *et al.*, 1990a, b; Rajapkse *et al.*, 1989a, b, 1990). In fact in this study reliable data were easily obtained using this technique.

2.1.2.4 Vacuum extraction method

Several workers have used vacuum extraction to obtain samples of gases from inside fruits and vegetables. Magness (1920) appears to have been the first to report the use of this method to extract gases from tissue segments of plant organs. He removed tissue segments from the organ of interest with a cork borer and transferred them directly into the extraction chamber, keeping the tissue segments submerged under mercury. Using a series of tubes, valves and a bottle of mercury, the pressure was dropped, causing the internal gases to expand and escape into the mercury where they were eventually collected and analysed.

Many modifications of the apparatus have been developed for tissue segments (Burton and Spragg, 1948; Christensen *et al.*, 1939; Culpepper *et al.*, 1936; Gerhardt and Ezell, 1934; Harley and Fisher, 1930; Whiteman and Schomer, 1945) and for entire organs (Brooks, 1938; Maxie *et al.*, 1965). A partial vacuum can be applied to tissue in air (Blanpied, 1971; Hulme, 1951; Poapst *et al.*, 1974; Smith, 1947), to tissue submerged in an aqueous solution (Denny, 1946; Maxie *et al.*, 1965; Staby and De Hertogh, 1970), or to tissue submerged in mercury (Brooks, 1938; Culpepper *et al.*, 1936; Magness und Diehl, 1924).

Beyer and Morgan (1970) used a saturated solution of ammonium sulphate instead of mercury because it absorbed ethylene relatively slowly and saturated salt solutions are often used in place of the toxic mercury, since they have a relatively low solubility coefficient for gases (Bussel and Maxie, 1966; Denny, 1946; Maxie *et al.*, 1965). Bussel and Maxie (1966) and Beyer and Morgan (1970) developed a more convenient system for vacuum extraction for fruit and for vegetative tissues. The organ or tissue segment was placed in a pressure cooker or desiccator jar half filled with saturated salt solution. An inverted funnel with a septum inserted into the tip was then placed over the tissue and all air from the surface of the tissue and the interior of the funnel was removed. The apparatus was then covered and a vacuum applied. As the pressure was reduced, internal gases expanded and there was a mass flow of gases out of the fruit through the lenticels, stomata, pores, and other regions of low resistance to accumulate in the neck of the inverted funnel. The vacuum was released after a short time and gas samples taken directly through the septum with a hypodermic needle for gas analysis. A similar method was used by Blanpied (1971), Fidler and North (1971) and Staby and De Hertogh (1970).

Gorter and Nadort (1941), using Magness's technique demonstrated that the volume of gas extracted from potato tubers was greater than the volume of intercellular spaces. Solomos (1987) reported that the vacuum extraction method introduces the uncertainty that some of the gases extracted may include not only those present in the intercellular spaces but also those that are dissolved in the cell sap. Beyer and Morgan (1970) reckoned that a vacuum below 100 mm Hg can induce the release of bound and dissolved gases, particularly ethylene, from plant tissue. These gases may alter the concentration of gases normally within the air spaces of tissues due to the differing solubilities of the gases under investigation. The resulting samples from the vacuum extraction method are therefore not representative of the

gaseous atmosphere normally present in the intercellular spaces. Higher estimates of internal ethylene of 'Winesap' apples and cantaloupes have been obtained when comparing vacuum with syringe sampling (Beyer and Morgan, 1970) and at best, estimates of internal gaseous concentrations obtained using this method may only be relative. Thus it is generally suggested that a small vacuum be used for as short a time as possible to avoid extraction of dissolved gases (Ben-Yehoshua and Aloni, 1974; Beyer and Morgan, 1970; Gorter and Nadort, 1941). However Cameron (1982), using a modification of the vacuum extraction method presented by Beyer and Morgan (1970) and Bussel and Maxie (1966) on Golden Delicious apples, obtained internal gas concentrations comparable with the direct sampling and external chamber method he developed.

2.1.2.5 Heat extraction method

Some workers developed a number of methods to extract internal gases by boiling segments of plant organs in alcohol (Burton, 1950; Denny, 1946; Eaks and Ludi, 1960; Willamen and Beaumont, 1928), salt solution (Claypool, 1938), buffer solution (Ulrich and Thaler, 1952) or water (Gerhardt, 1942; Poapst *et al.*, 1974). The gases evolved were entrained in a flow of inert gas and trapped in alkali. However Fidler and North (1971) reported that using these methods they obtained inconsistent results. In a similar method, Jerie *et al.* (1979) removed previously loaded radioactive ethylene from tissue segments by holding the tissue in a closed chamber over boiling water. Cameron (1982) in a series of experiments to test the applicability of the method by Jerie *et al.* (1979) to sampling endogenous C_2H_4, found that the amount of ethylene driven from the tissue increased with time of exposure to the boiling water for up to 30 minutes. Cameron (1982) found the method unsatisfactory except for fruits such as apples which contain exceptionally large amounts of internal ethylene. For other tissues tested, significantly greater amounts of ethylene were found to be released than were extracted by the vacuum extraction or direct sampling methods.

Most authors who have used this method have recognised that the amount of gas liberated from the tissue is far in excess of that originally contained in the intercellular space as determined by other methods (Burton, 1950; Denny, 1947). Although attempts have been made to calculate the original concentration of gases in the intercellular spaces (Burton, 1950), heat extraction methods are not desirable for the estimation of internal gas composition and skin resistance to gas diffusion (Cameron, 1982).

2.1.2.6 Digestion method

In the estimation of the internal atmosphere, Denny (1947) and Eaks and Ludi, (1960) attempted to digest tissue segments in strong alkali solution. The severity and inaccuracy of this method as well as the problems associated with gas solubility and calculation of original gas concentration in the plant organ make this method unacceptable for the estimation of internal gas composition and resistance coefficients (Cameron, 1982).

2.2 Respiration metabolism

Respiration is the metabolic process defined as the oxidative breakdown of complex materials such as starch, sugars and organic acids, to simpler molecules such as CO_2 and H_2O, with the concurrent production of energy and other molecules which can be used by the cell for synthetic reactions (Hardenburg *et al.*, 1986; Forcier *et al.*, 1987; Wills *et al.*, 1981). Such metabolic reactions are essential for maintenance of biochemical processes, cellular organisation and membrane integrity of living cells. Maintaining the supply of adenosine triphosphate (ATP) is the primary purpose of respiration (Kader, 1987).

Respiration takes place in the cells (cytoplasm, mitochondria) of tissues both in light and in the dark (Berrie *et al.*, 1987; Debney *et al.*, 1980).

The rate of respiration of produce is an excellent indicator of the metabolic activity of the tissue and thus is a useful guide to the potential storage life of the produce (Fidler and North, 1971; Wills *et al.*, 1981). Kader *et al.* (1985) contended that the rate of deterioration or perishability of harvested commodities is generally proportional to their respiration rate. The respiration rate of a given commodity differs with plant part, cultivar, area of production, growing conditions and growing season (Hardenburg *et al.*, 1986). According to Debney *et al.* (1980), if different types of produce are classified by their botanical structure, there is a close relationship between structural type and respiration rate. High respiration rates are typical of young tissues such as growing points, (eg. asparagus), partly developed flower buds (broccoli, globe artichoke), developing seeds (green peas, green beans) and immature fruits (sweet corn). Low respiration rates are typical of storage organs such as roots (carrots, sweet potatoes), underground stems (potatoes), bulbs (onions) and mature fruits (apples). Intermediate respiration rates occur in unripe fruits (cucumbers, zucchini) and most leafy vegetables (Debney *et al.*, 1980).

Respiration rate of a commodity is dependent upon various factors related to the produce, which include type of commodity and genotype, stage of development at harvest, weight of commodity, and chemical composition. It is also dependent on environmental factors such as temperature, light, stress, O_2, CO_2, carbon monoxide and C_2H_4 concentrations, and other hydrocarbons such as propylene, acetylene (Debney *et al.*, 1980; Hardenburg *et al.*, 1986; Kader *et al.*, 1989). Respiration is also one of the important factors affecting the internal atmosphere composition of fruits, however there is limited information on the relationship between respiration and internal atmosphere composition of apples. This relationship ~~have~~ has been investigated in the current study.

Respiration can occur in the presence (aerobic respiration) or absence of O_2 (anaerobic respiration, sometimes called fermentation) (Biale, 1960a; Montgomery *et al.*, 1990; Forward, 1965; Wills *et al.*, 1981).

2.2.1 Aerobic respiration

Most of the energy required by fruits and vegetables is supplied by aerobic respiration. Aerobic respiration involves a series of reactions, each of which is catalysed by a specific enzyme and involves oxidative breakdown of complex molecules (certain organic substances such as carbohydrates stored in the tissue) to simpler molecules (Biale, 1960a; ap Rees, 1980; Forward, 1965). Figure 2-1 taken from ap Rees (1980) shows the principal pathways responsible for the respiration of carbohydrate.

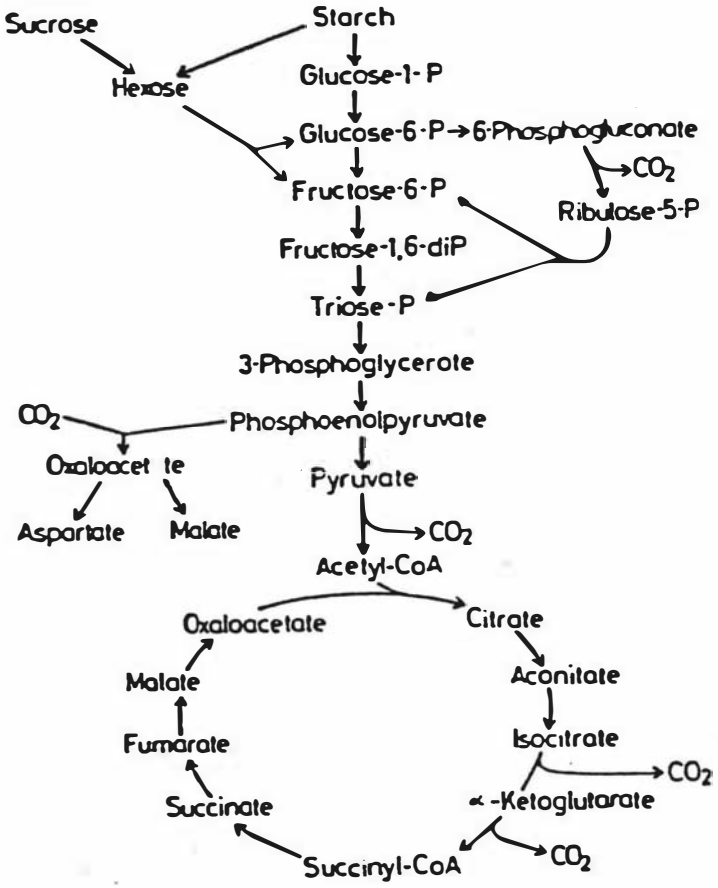

Fig. 2-1. Principal pathways responsible for the respiration of carbohydrate (ap Rees, 1980).

It involves the following three metabolic pathways:

1. Glycolysis, which takes place in the cytoplasm, is the degradative pathway involving a series of reactions, each of which is catalysed by a specific enzyme, in which glucose, glucose-1-phosphate or fructose released by hydrolysis of starch or other reserve polysaccharides is converted to pyruvate (fig. 2-2; Montgomery *et al.*, 1990; Soule, 1985). Glycolysis is accompanied by the formation of ATP, although this is only about a quarter of the ATP that can be derived from the complete oxidation of glucose to CO_2 and water. Glycolysis can proceed either under aerobic or anaerobic (hypoxic) conditions (Montgomery *et al.*, 1990).

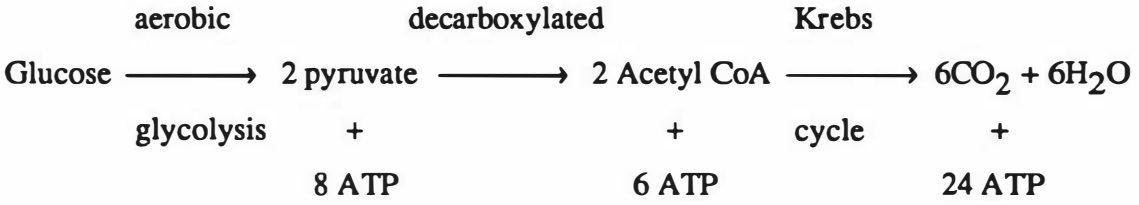

Fig. 2-2. Pathways of aerobic metabolism (Montgomery *et al.*, 1990).

2. Krebs (Citric or Tricarboxylic acid) cycle, is the second major phase in respiration which occurs in the mitochondria. The pyruvate formed by glycolysis is decarboxylated to acetyl CoA, which enters the Krebs cycle by condensation with oxaloacetate, where it is oxidised to CO_2 and water (fig. 2-2). No O_2 is absorbed in any part of the cycle (Kader, 1987; Montgomery *et al.*, 1990; Soule, 1985).

3. Electron transport system, the last major phase of respiration, where low-energy nicotinamide adenine dinucleotide (NAD) is reduced to the high-energy form NADH. Electrons are transferred by a series of intermediate

compounds (i.e. several cytochromes, a quinone, and a riboflavin-containing protein) ultimately to combine with O_2 to form H_2O and produce ATP (Montgomery *et al.*, 1990; Soule, 1985).

2.2.2 Anaerobic respiration

The normal atmosphere is rich in O_2 (20.95%), thus O_2 is available in the plant organ. However, under various storage conditions (such as under low O_2 or high CO_2 enrichment conditions) the amount of O_2 in the atmosphere may be limiting and insufficient to maintain full aerobic metabolism (Wills *et al.*, 1981). Under these conditions the tissue can initiate anaerobic respiration, in which glucose is converted to pyruvate through the process of glycolysis, but the pyruvate produced (rather than going into the Krebs cycle) is metabolised into either lactate (in animals) or acetaldehyde (AA) and ethanol (ETOH)(in plants) (see fig. 2-3) (Wills *et al.*, 1981). Conversion of pyruvate to AA and CO_2 is catalysed by the enzyme carboxylase and the cofactor thiamin pyrophosphate. Acetaldehyde is converted into ETOH by the action of the enzyme alcohol dehydrogenase. Two moles of ATP and 21 k calories of heat energy are produced in anaerobic respiration per mole of glucose (Kader, 1987). Anaerobic respiration produces much less energy per mole of glucose than does aerobic respiration, but it does allow some energy to be made available to the tissue under adverse conditions (Wills *et al.*, 1981).

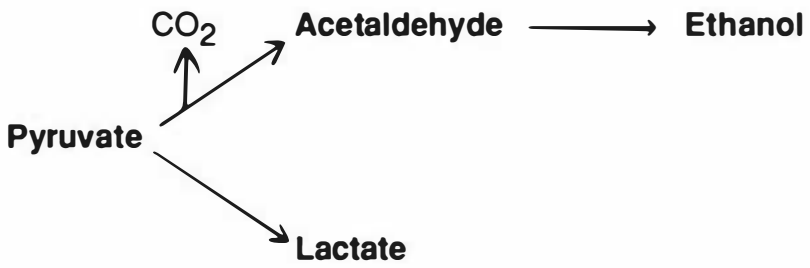

Fig. 2-3. Pathways of anaerobic metabolism (Wills *et al.*, 1981).

2.2.3 Extinction Point (EP) or Anaerobic Compensation Point (ACP)

The external O_2 concentration at which a shift from aerobic to anaerobic respiration occurs is known as the 'Extinction Point' (EP) or anaerobic compensation point (ACP). Figure 2-4 taken from Kader (1987) shows the effects of O_2 concentration on aerobic and anaerobic respiration. The O_2 concentration at EP depends on several factors such as species, cultivar, physiological stage of maturity and temperature (Biale, 1960a; Fidler, 1951; Kader, 1987; Wills *et al.*, 1981).

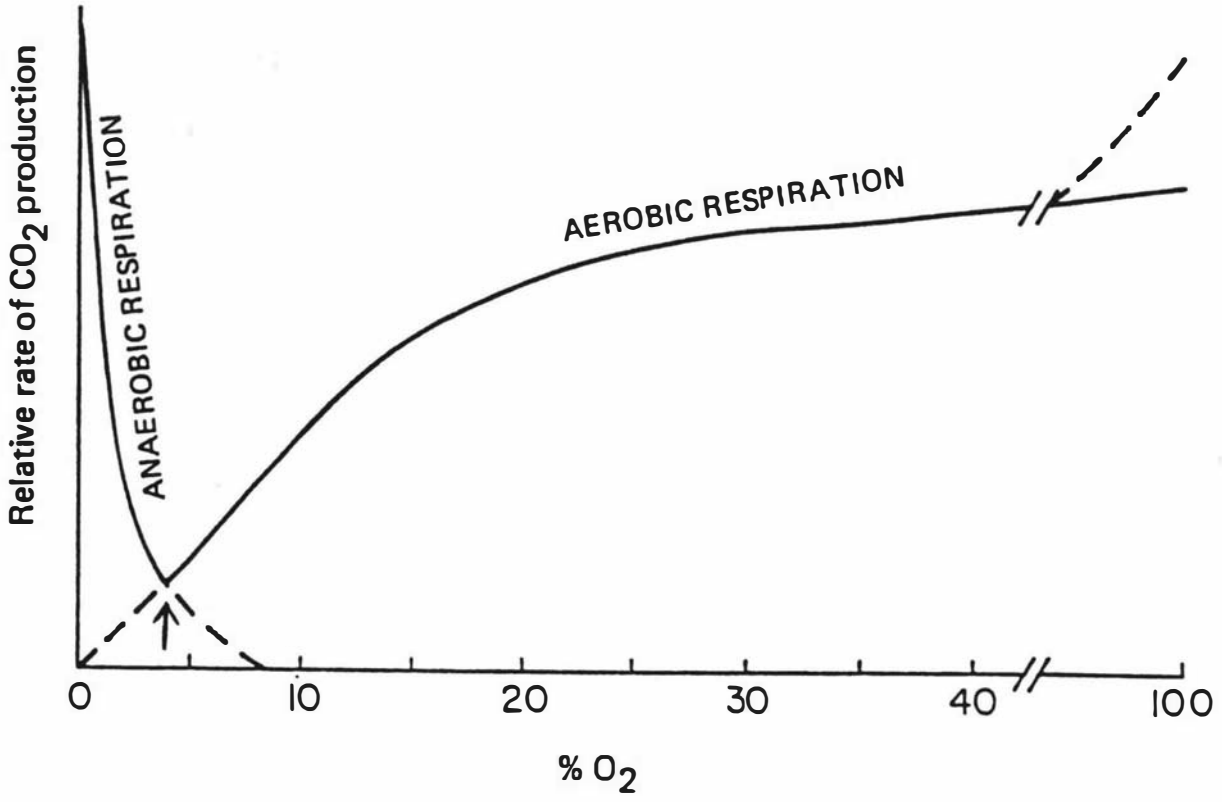

Fig. 2-4. A schematic representation of the effects of O_2 concentration of the external atmosphere on aerobic and anaerobic respiration. The arrow indicates the EP (Redrawn from Kader, 1987).

The transition zone between aerobic and anaerobic respiration was examined by early workers (Blackman, 1928; Fidler, 1934, 1951; Thomas and Fidler, 1933) who reasoned that the salient feature of this transition was the EP. Blackman (1928) working with Bramley's Seedling apples, defined the EP as the threshold O_2 concentration which just extinguishes all aerobic respiration. Thomas and Fidler (1933) approached the concept more empirically by defining the EP as that concentration of O_2 at which alcohol production ceased. Kidd and West (1937) reported that the threshold O_2 concentration 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. Singh (1937) found the critical O_2 concentration for mangoes to be 9.2%. Platenius (1943) determined the critical O_2 concentration to be 1% for spinach and snap beans, 2.5% for asparagus and 4% for peas and carrots when held for several days at 20°C.

Boersig *et al.* (1988) re-examined the aerobic - anaerobic respiratory transition in pear fruit and cultured pear fruit cells. They argued that the use of alcohol production by early workers as an indicator of the shift from aerobic to anaerobic respiration was unacceptable, since ethanol is now known to be a normal constituent of many fruits held under aerobic conditions. In addition the methods of analysis for alcohol used at that time were less sensitive than the more recent chromatographic techniques. Consequently the EP defined by early research workers was an untenable concept based on archaic analytical methods. Boersig *et al.* (1988) therefore suggested the use of CO_2 evolution rather than alcohol production as an indicator of the shift from aerobic to anaerobic respiration. Based on that concept, these authors proposed an alternative terminology, the 'Anaerobic Compensation Point' (ACP), which they defined as the external O_2 concentration at which CO_2 production was minimum. The EP defined by early workers is probably erroneous as aerobic

respiration would continue at some reduced level even below the EP as shown in Kader's diagram (fig. 2-4). This makes the ACP_A^q more sensible term from a physiological point of view.

The ACP shifted to lower O_2 concentrations after extended exposure of the cells to lower O_2 atmospheres and it shifts to higher O_2 concentrations as fruits matured physiologically or as the diffusion coefficient of cell suspensions decreased (Boersig *et al.*, 1988; Fidler, 1951; Thomas and Fidler, 1933).

2.2.4 Respiration quotient (RQ)

Respiration quotient (RQ) is the volume or molecular ratio of the volume of CO_2 produced to O_2 simultaneously consumed during respiration (Devlin and Witham, 1983; Berrie *et al.*, 1987).

The RQ usually gives an indication of the type of respiratory substrate being metabolised as the main source of respiration (Burton, 1982; Salisbury and Ross, 1985; Wills *et al.*, 1981). However a precise identification of the type of substrate being respired by a tissue through RQ values is impossible. If different substrates are being respired simultaneously, the RQ value obtained is only an average of the RQ values of each individual substrate (Devlin and Witham, 1983). The RQ has the greatest advantage of being a pure ratio independent of the amount of respiring material. According to Hackney (1944), alcohol analyses are not necessarily a true index of anaerobic respiration, only determination of the RQ can be regarded as a true index of the nature of respiration.

Metlitskii *et al.* (1972) reported that the RQ generally increases with ripening and senescence of most fruits and vegetables. An increase in RQ indicates an increased use of organic acids rather than carbohydrates or fatty acids as the major substrate for respiration. Since organic acids have more

O_2 per carbon atom than sugars or fatty acids, they therefore require less O_2 consumption for the production of CO_2 (Wills *et al.*, 1981). Neal and Hulme (1958) reported that addition of organic acids to the tissues of post-climacteric apple fruit induced a large increase in CO_2 output and little change in O_2 uptake, thus increasing the RQ values. However this phenomenon was not observed in preclimacteric fruit, suggesting that the ability to utilise organic acids in fruit increased with senescence. Kidd and West (1938) observed that the RQ of Bramley's seedling apples, ripened in air at 22.5°C, rose during the climacteric from 1.02 to 1.25. Apples have a high content of malic acid which is utilised by the fruit in the climacteric stage (Burton, 1982). Similar findings have been reported by other investigators (Burton, 1982; Hulme and Rhodes, 1971; Neal and Hulme, 1958).

The increase in RQ of apples during and after the climacteric, according to Hartmann (1962) is not a universal phenomenon in fruits which exhibit a climacteric phase, but only in those, such as apples and pears in which an acid is respired during the climacteric. For instance, Kidd and West (1925) reported that preclimacteric apples have an RQ of about 1.04, a value consistent with carbohydrate as the main respiratory substrate. This RQ rises to about 1.4 during the respiratory climacteric, a finding consistent with an increased use of organic acids (mainly malic acid, in addition to sugar) as respiratory substrates. Conversely, preclimacteric banana fruit have an RQ of about 1; this drops to about 0.76 during the climacteric rise but is again equal to 1 at the peak of the respiratory climacteric (Palmer, 1971). The value of 0.76 could imply utilization of fat for a short period before carbohydrates once again becomes the dominant substrate (Tucker and Grierson, 1987).

Modified atmosphere (MA) conditions can alter the RQ which in turn will affect the atmosphere created by the respiration of the commodity within the package (Kader *et al.*, 1989; Tomkins, 1965). RQ in apples decreased markedly in gas mixtures containing high CO_2 and low O_2 or high CO_2 alone

compared to that in air storage (Metlitskii *et al.*, 1972; Fidler and North, 1967; Fidler, 1950). According to James (1953) when respiration has settled to a steady state, the RQ is usually determined by the chemical nature of the substrate being consumed. At low O_2 concentrations the results are complex, because anaerobic processes release additional CO_2; but above 5-10% O_2 complete oxidation of the respiratory substrate is likely to dominate. A reduction in RQ value by CA also indicates a retardation of the ripening and senescence of fruit and a reduced catabolism of organic acids, resulting in higher acid retention under CA conditions (Kader, 1986). Very high RQ values usually indicate anaerobic respiration (Kader, 1987).

2.2.5 Respiration patterns of fruits

The pioneering work of Kidd and West, (1922, 1930) made the first major contribution to the study of patterns of respiration in fleshy fruits. They showed that the onset of visible ripening changes of detached unripe apples was marked by an upsurge in the rate of respiration. Kidd and West coined the term 'respiration climacteric' to describe what they saw as a critical phase in the life of the fruit. The climacteric was seen as a period of reorganisation that was a necessary prelude to ripening.

Fruit have been classified as 'climacteric' and 'non-climacteric' on the basis of respiration and C_2H_4 production patterns during maturation and ripening (Biale and Young, 1981; Biale 1960a, 1964; McMurchie *et al.*, 1972). Climacteric fruits, such as apple, banana, mango, pawpaw and avocado, which are harvested fully developed but unripe, exhibit a decline in their respiration rate to a minimum (preclimacteric minimum) before rising (climacteric rise) at the time of onset of ripening to a peak (climacteric peak) after which it declines (post climacteric). Figure 2-5 shows the phases of the climacteric period (Watada *et al.*, 1984). According to Rhodes (1970, 1980a, b) the significance

of the climacteric phase is that it marks the transition from growth and maturation phases in the life of the fruit to the onset of senescence. It is the 'beginning of the end' as Biale (1960b) puts it.

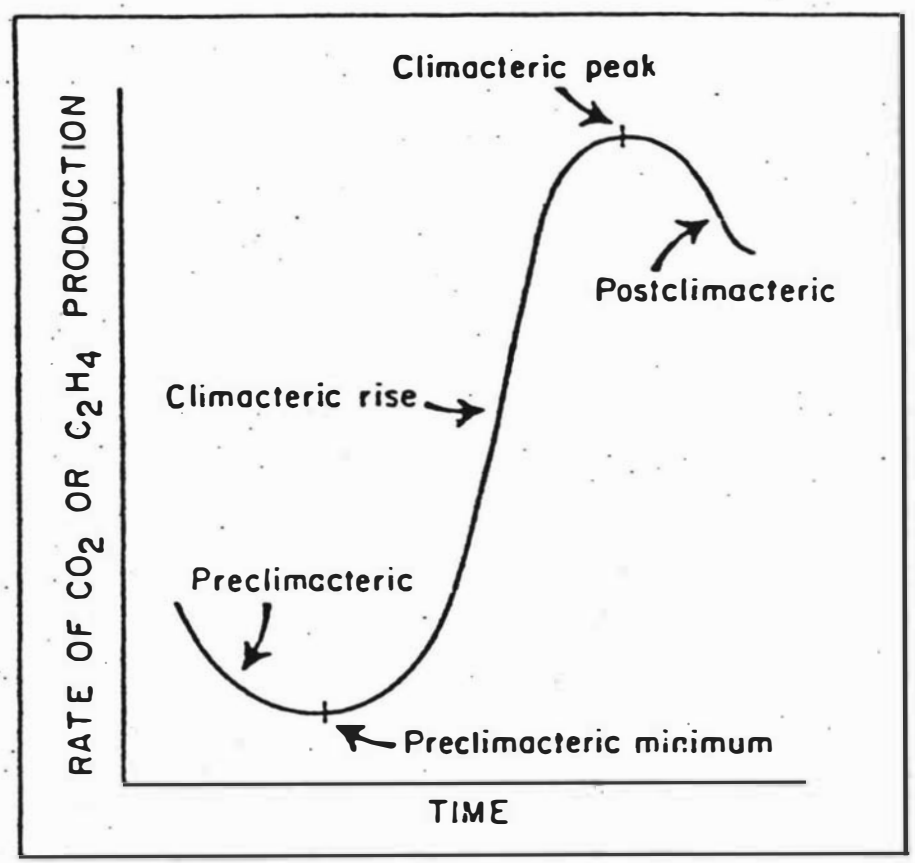

Fig. 2-5. Phases of the climacteric period (Redrawn from Watada, 1984).

Known factors that influence onset of the climacteric in fruits are temperature (lowering the temperature generally delays onset of the climacteric; Kidd and West, 1930), O_2 and CO_2 concentration. Generally O_2 mixtures richer than air tend to hasten the climacteric, those poorer than air to delay it, and sometimes to depress it's intensity (Rhodes, 1970). The climacteric, according to Forward (1965) is an aerobic phenomenon and fails to appear in the absence of O_2. High CO_2 concentrations (5% or 10%) tend to delay and depress the climacteric in apples and pears (and a combination of low O_2 and high CO_2 eliminates it in banana), and the onset of the climacteric is hastened by traces of ethylene (Melford and Prakash, 1986; Peacock, 1972; Pratt and Goeschl, 1969).

An upsurge in respiration occurs in climacteric fruits allowed to ripen while still attached to the plant (except avocado) and in fruits detached after maturity has been reached (Biale, 1964).

In non-climacteric fruits, such as strawberry, citrus fruits and pineapple, respiration and C_2H_4 production continue to decline steadily after harvest (Biale, 1964; Biale and Young, 1981; Rhodes, 1970, 1980a, b). Figure 2-6 taken from Biale (1964) shows the stages in fruit development and maturation and respiratory trends which characterise climacteric and non-climacteric fruits.

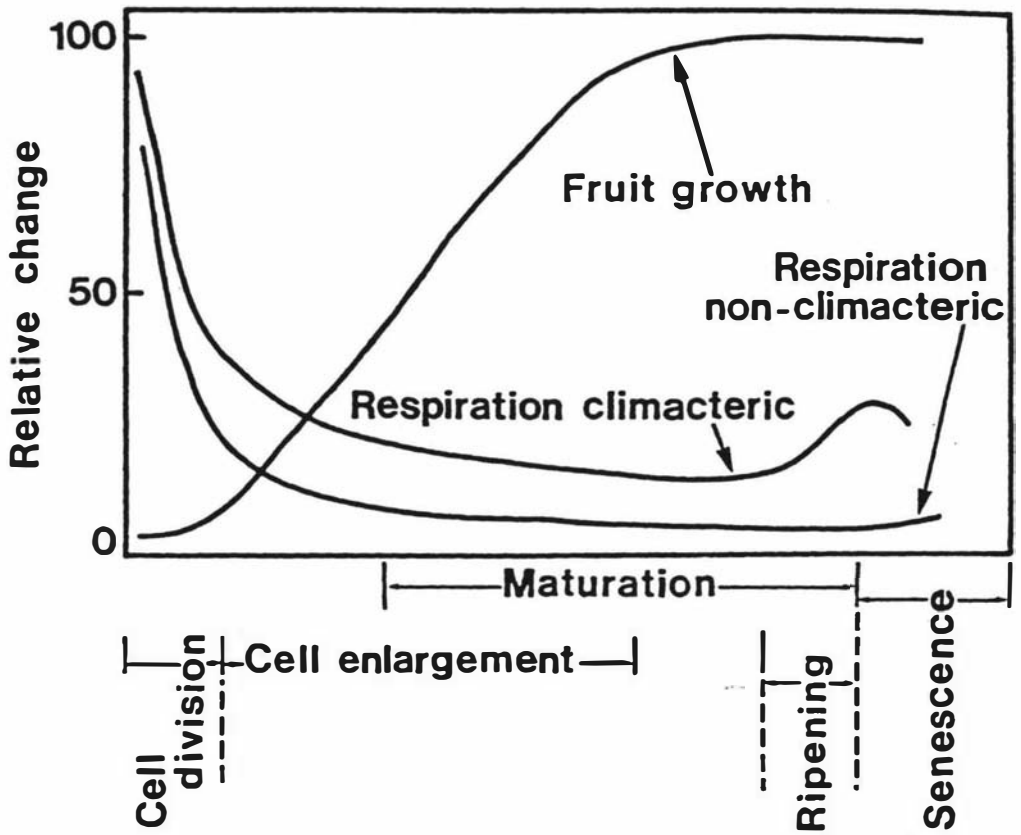

Fig. 2-6. Stages in fruit development, maturation and respiratory trends which characterise climacteric and non-climacteric fruits (Biale, 1964).

Climacteric fruit at the end of growth undergo a large increase in respiration accompanied by marked changes in composition and texture, whereas non-climacteric fruit show no change in respiration that can be associated with distinct changes in composition. Ripening in climacteric fruit is associated with large increase in C_2H_4 production (Biale, 1960b). The increase in respiration and C_2H_4 production can be induced prematurely in climacteric fruit by treating them with a suitable concentration of C_2H_4 or its analogues (such as propylene, acetylene). The ripening process is irreversible once endogenous (autocatalytic) C_2H_4 production increases to a certain level (McGlasson, 1985). In contrast, an unnatural climacteric-like respiratory increase can be induced in nonclimacteric fruit by treating them with C_2H_4 or its analogues (Biale and Young, 1962, 1981). Yet this increased respiration is not accompanied by an increase in endogenous C_2H_4 production, and the respiration rate usually subsides fairly rapidly upon removal of the exogenous C_2H_4. However, C_2H_4 treatment does accelerate senescence in nonclimacteric fruit. Ethylene treatment of climacteric fruit does not change the climacteric patterns and magnitude of respiration and C_2H_4 production, thus response of respiration and C_2H_4 biosynthesis to C_2H_4 are concentration independent. In contrast in nonclimacteric fruit, the magnitude of the respiratory response increases as a function of C_2H_4 concentration, but this increase in respiratory activity is not accompanied by C_2H_4 production. The internal C_2H_4 levels in climacteric fruit can range from low to high, but in nonclimacteric fruit levels are low (Burg and Burg, 1962; McMurchie *et al.*, 1972; Reid and Pratt, 1970; Yang, 1985, 1987).

2.2.6 Methods of estimating respiration rate

Accurate estimation of respiration rate of fruits (including apples) and other plant organs is important in determining the rate of metabolic activity in the plant organ. It is also important in the estimation of skin resistance to gas diffusion. Various methods of estimating respiration rate or rate of flux of bulky

plant organs have been reported but none of them is entirely satisfactory. These methods are either based on direct measurements of CO_2 and/or O_2, or on indirect determination by monitoring pressure or volume variations resulting from CO_2 evolution and O_2 uptake (Forcier *et al.*, 1987). Some of the methods that have been used are briefly reviewed in the following sections.

2.2.6.1 Flow through system

A flow through system involves incubating the plant organ in a sealed container through which is passed a known flow of gas. The exit stream is passed through a column containing a suitable CO_2 absorber, such as sodium hydroxide, which absorbs the respired CO_2. The amount of CO_2 production during a specific duration is determined by subsequent titrimetric or gravimetric analysis of the absorbed material. Alternatively, CO_2 and/or O_2 concentration differences between the inlet and outlet of the container can be determined using a gas chromatograh (TCD) or an infra red gas analyser (IRGA) and the respiration rate calculated on the basis of commodity weight, flow rate, and change in CO_2 or change in O_2 concentration (Burg and Burg, 1965; Cameron, 1982; Kader, 1987; Reid *et al.*, 1973; Solomos, 1989).

According to Ben-Yehoshua and Cameron (1988) the flow through system is especially suited for the measurement of CO_2 and C_2H_4 flux since these gases can be scrubbed from the incoming gas flow so that only the amounts produced by the organ are present in the effluent stream of air. It can also be used for measuring water flux if the vapour pressure or dew point is measured in both inlet and outlet streams.

It is extremely difficult to measure O_2 accurately using this approach, since it is necessary to measure accurately the difference between the inlet and outlet streams, following the relatively small amount of O_2 uptake by the

product. It may be necessary to use very slow flow rates or even a closed system for a given time interval to monitor O_2 flux into the organ (Ben-Yehoshua and Cameron, 1988).

2.2.6.2 Closed system

In this method, samples are sealed in a container and the accumulation of CO_2, and/or depletion of O_2 in the atmosphere of the sealed container are measured after a specific duration usually one or two hours. For a known weight of commodity in a known volume of free space, respiration rate (cm^3 CO_2 or O_2 kg^{-1} h^{-1}) can be calculated (Banks, 1984a, b; Burg and Burg, 1965; Rajapkse *et al.*, 1989a, b, 1990).

The principal limitation of this method is that it is a non equilibrium system, and the depletion of O_2 and accumulation of CO_2 or other gases (especially C_2H_4) may affect the tissue and its respiration rate (Kader, 1987). However these problems can be overcome by keeping the incubation period to the minimum possible and/or by placing CO_2 and C_2H_4 absorbers in the sealed container to absorb these gases if desired. Reliable estimate of respiration using this method have been reported by various authors including Banks (1984a, b), Burg and Burg (1965); Cameron (1982) and Rajapkse *et al.*, 1989a, b, 1990). In the current study, estimates of respiration rates of apples were obtained using the closed system. Conscious of the limitation posed by this technique, incubation period was kept to the minimum possible (approximately one hour).

2.2.6.3 Tissue disc respiration

Studies with tissue discs have facilitated experiments on the effects of exogenous substances on tissue physiology, respiration, metabolic pathways and reaction mechanisms (Parkin, 1987; ap Rees, 1966; Palmer and

McGlasson, 1969; Gude and van der Plas, 1985; Lee *et al.*, 1970; Atta-Aly *et al.*, 1987). In this method whole intact fruit are sanitised or sterilised by washing with 80% ethanol (Atta-Aly *et al.*, 1987) or soaked for a few minutes in a solution of 20% commercial bleach (Parkin, 1987; Saltveit and Mencarelli, 1988). A sterilised cork borer is used to take discs of tissue usually from the equatorial region of the fruit. Fruit discs are resterilised by immersing in ethanol. The cut tissue slices are usually suspended in a buffered iso-osmoticum of sucrose, mannitol or other carbohydrate (Parkin, 1987; Pesis and Ben-Arie, 1986). Disc respiration is then determined in air-saturated mannitol and potassium phosphate. According to Parkin (1987) the rates of O_2 uptake of discs suspended in air-saturated, buffered mannitol are similar to the rates of CO_2 evolution for discs incubated in an air-tight flask as measured by head space gas analysis using gas chromatography. This method requires that all procedures are performed under sterilised conditions (Edwards *et al.*, 1983; Gross and Saltveit, 1982).

The primary limitation of this method is due to the susceptibility of the cut tissue to microbial contamination and decay, which renders it unsuitable for lengthy study (Parkin, 1987; ap Rees, 1966). In the absence of suspending medium, tissue slices are prone to desiccate or re-direct their physiology toward callus formation (Lee *et al.*, 1970), a metabolic activity that may not be representative of the intact tissue during certain periods of its life cycle or be the focus of intended study (Parkin, 1987). Wounding of the tissue is known to increase respiration (Burton, 1974; Kahl, 1974). In view of these limitations, whole fruit were used in this study.

2.3 Gas exchange in fruits

Gas exchange in fruits and other bulky plant organs is by passive process of diffusion: the tendency for a high concentration of one type of molecule to move down a concentration gradient to an area of lower

concentration. Diffusion takes place because of the kinetic energy of gas molecules and does not require the direct expenditure of metabolic energy by the fruit tissue (Rahn *et al.*, 1979). The lower concentration of O_2 inside the fruit brings new O_2 molecules from the external atmosphere, where the concentration is usually higher. Conversely, the concentration of CO_2 inside the fruit causes those molecules to diffuse toward the outside, where the concentration is very low [0.03%] (Burton, 1982). In simple terms gases diffuse according to concentration gradients from regions of high concentration to regions of low concentration (Kader *et al.*, 1989).

Ideally, when there is no pressure-driven mass flow, each gas in a mixture behaves independently of all other gases, diffusing in a direction determined by its own gradient in concentration. The rate at which a gas moves depends on the properties of the gas molecule, the magnitude of the concentration difference, and the physical properties of any intervening barriers, such as thickness, surface area, density and molecular structure (Barrer, 1951).

In fruits and other bulky plant organs, rates of gas diffusion are determined largely by the respiration rate, stage of maturity, physiological age, commodity mass and volume, pathways and barriers for diffusion, properties of the gas molecule, concentration of the gases in the atmosphere surrounding the commodity, magnitude of gas concentration difference across barriers and temperature (Burton, 1982; Banks, 1984a, b; Smith and Stow, 1984).

The properties of a barrier can significantly influence the rate of gas movement. For instance, O_2 moves about 10,000 times more slowly in water than in air for a given concentration difference (Himmelblau, 1965). Movement of a gas directly through a solid (eg. plastic) or liquid film involves adsorption onto the film surface, diffusion across the film and evaporation from the opposite surface. Thus, both solubility and diffusivity are important for diffusion across films (Stannett 1968, 1978).

2.3.1 Laws of gas diffusion

Many of the diffusion processes of relevance to this thesis are governed by Fick's First Law of diffusion, which applies to all gases and has often been used to study gas exchange in fruits and other bulky plant organs (Ben-Yehoshua *et al.*, 1963; Burg and Burg, 1965; Burton, 1974, 1978; Cameron, 1982; Cameron and Reid, 1982; Cameron and Yang, 1982; Marcellin, 1963, 1974; Sastry *et al.*, 1978; Trout *et al.*, 1942) and in leaves (Nobel, 1974, 1983).

Within limits, Fick's first law of diffusion states that the movement or flux of a gas in or out of a plant tissue depends on the concentration drop across the barrier involved, the surface area of the barrier, and the resistance of the barrier to diffusion (Burg and Burg, 1965). A simplified version of Fick's Law can be written as follows:

$$F_j = \mathbf{A} * \Delta C_j / \mathbf{R}_j \qquad [2.1]$$

where the total flux of species $_j$ (ie. O_2, CO_2, C_2H_4, water vapour, etc) per unit time (F_j in $cm^3\ s^{-1}$) is moving across a barrier which has surface area (**A**) (cm^2) and resistance to diffusion of species $_j$ of $\mathbf{R}_j$ (s cm^{-1}). The driving force for the movement depends on the concentration difference across the barrier (ΔC_j).

Some researchers (Brooks, 1937; Burg and Burg, 1965; Burton, 1950, 1974; Cameron, 1982; Cameron and Reid, 1982; Cameron and Yang, 1982), contended that the use of the simplified form of Fick's law to the study of gas exchange of bulky plant organs is only valid as long as the resistance of the tissue is insignificant relative to the resistance of the skin, and that the thickness of the skin is an insignificant part of the fruit diameter. If resistance

within the fruit becomes significant, for example when a fruit softens or becomes water soaked, the equation ceases to be valid, since concentration gradients will then be established between the skin and the fruit centre.

2.3.2 Skin resistance to gas diffusion

Most studies on gas exchange in fruits and other bulky plant organs indicate that the skin represents the primary significant barrier to gas exchange between the commodity and the atmosphere surrounding it (Burg and Burg, 1965; Burton, 1950; Cameron, 1982; Hardy, 1949; Montero, 1987; Solomos, 1985; Soudain and Phan Phuc, 1979; Ulrich and Marcellin, 1968). Hall *et al.* (1954) also contended that the most important cause of resistance to gas diffusion was the skin. Many other workers have reached similar conclusions. According to Burg and Burg (1965), Burton (1982), Soudain and Phan Phuc (1979) and Ulrich and Marcellin (1968) resistance of apple skin to gas diffusion is higher than that of the flesh. Solomos (1987) observed that depending on cultivar, the resistance of the skin of apples is 10 to 20 fold higher than that of the pulp. On the contrary, Ben-Yehoshua *et al.* (1963) observed that the site of resistance in avocado fruit changed during ripening. Their results indicate that before and during the climacteric, the major barrier to gas exchange was in the peel, but the pulp became an important barrier to gas exchange in post-climacteric avocados. The increase pulp resistance probably arose because of the clogging of the air space with cellular sap (Ben-Yehoshua *et al.*, 1963; Sacher, 1973).

Resistance of fruits to gas diffusion has been shown to increase during maturation and ripening. As plant organs advance in the senescence stage, cell walls and membranes begin to breakdown and cell contents fill some of the air spaces. Consequently the resistance of the flesh to gas diffusion may become significant resulting in decrease in internal O_2 and increase in internal CO_2 (Kader *et al.*, 1989; Trout *et al.*, 1942). Resistance to diffusion of CO_2

out of fruits and vegetables increases during the maturation period (Ben-Yehoshua, 1969; Kidd and West, 1949). The period of least resistance to gas diffusion appears to be when the fruit is still immature (Marcellin, 1974), and generally there is a marked increase in resistance to O_2 and CO_2 diffusion shortly after harvest (Burton, 1965; Trout *et al.*, 1942). Resistance to gas diffusion continues to increase during ripening (Ben-Yehoshua, 1987) and in some fruits there is evidence that, during their postclimacteric stage, the resistance to respiratory gases increases substantially (Marcellin, 1974; Leonard and Wardlaw, 1941; Williams and Patterson, 1962).

Changes in water vapour diffusion during maturation of bulky organs have been characterised. Generally rates of water vapour diffusion decline during maturation and ripening, reaching a minimum, in fruits, about the time of the climacteric and increasing thereafter (Pieniazek, 1943; Sastry *et al.*, 1978). A number of workers (Leonard and Wardlaw, 1941; Markley and Sando, 1931a, b; Smith, 1931, 1933) reported that transpiration rates of bulky organs decline sharply in the period immediately after harvest. The peel of some fruit shrivels after prolonged exposures to low vapour concentrations, and there is evidence that this leads to an increase in resistance to diffusion (Ben-Yehosua 1969; Smith, 1931, 1954; Wilkinson, 1965).

Skin resistance to water vapour diffusion has been shown to be much lower than resistance to O_2, CO_2, or C_2H_4 diffusion (Cameron, 1982). Waxes, and not cutins, seem to be the primary barriers to water vapour diffusion through the skin in leaves and bulky organs (Horrocks, 1964; Schonherr, 1976; Soliday *et al.*, 1979). Wilkinson (1965) attempted to determine the effects of relative humidity on the resistance to gaseous diffusion of apples. He found that apples tested under humid conditions showed a two or threefold increase in permeability to gases over a 6 month period. Apples in dry environments, decreased steadily in permeability over the same period, and also shrivelled gradually. However, no immediate or sudden effects were noted.

2.3.3 Avenues to gas exchange

Theoretically, gases diffuse through the pathways that offer least resistance (Kader *et al.*, 1989), i.e., channels filled with air (Burton, 1982; 1974; Burg and Burg, 1965; Solomos, 1987). In leaves, by control of stomatal aperture, the resistance of the epidermal layer to gas diffusion can be altered so that water vapour movement from the tissue can be minimised when adequate CO_2 is present in the tissue. However, in fruits and other bulky plant organs, evidence of the presence of functional stomata or other active control mechanisms of gas exchange is lacking (Clements, 1935; Adams, 1975). These organs have a much lower surface-to-volume ratio than leaves; the distance over which gases diffuse in the tissues is very large, and respiration, not photosynthesis, accounts for the major metabolic source of CO_2 and sink for O_2 (Kader *et al.*, 1989).

Several investigators have attempted to identify the principal avenue of gas exchange in bulky plant organs and many conflicting reports have been presented. For instance, Hall *et al.* (1954) contended that lenticels on the skin of apples play no role in gas exchange. They stated that in mature Granny Smith apples, gas exchange took place by diffusion through the skin and none occurred through the calyx and the lenticels. In contrast the majority of gas diffusion in bulky organs has been shown by other workers in subsequent studies to occur through the lenticels (Burg and Burg, 1965; Burton, 1965; Burton and Wigginton, 1970; Haberlandt, 1914; Wigginton, 1973). The role of lenticels in water vapour loss was noted by Pieniazek (1944) who demonstrated that when every lenticel of apples was blocked with Vaseline, water vapour flux dropped by 8-20% compared to untreated fruit. In Burton's view (1982), lenticels and stomata have only minor importance in water loss because of their sparse distribution in fruit.

In oranges, however, the relative contribution of stomata or lenticels to gas exchange is unclear. Moreshet and Green (1980) studied the functioning of stomata in orange fruit on and off the tree. Their data indicated that the stomata still open and close in response to light and are highly effective in conducting water and CO_2 before harvest in spite of being occluded by natural wax (Albrigo, 1972). However their data indicated that the stomata stop functioning after harvest. Ben-Yehoshua *et al.* (1983, 1985) using scanning electron microscope photomicrographs showed that in Hamlin oranges and Duncan grapefruits, although most stomata were closed, some stomatal pores may be seen as dark open slits between the two guard cells on fruit stored in the dark. Thus it appears that the stomata of harvested oranges are partially open and allow some gas exchange.

In fully matured harvested banana fruit Johnson and Brun (1966) reported that the stomata open in high relative humidity and light and close with low relative humidity and in darkness.

The calyx opening of apples has been shown to contribute to gas exchange (Cameron and Reid, 1982; Marcellin, 1974; Markley and Sando, 1931a, b). For instance, Cameron (1982) observed that the contribution of the calyx to the passage of different gases varied in different fruits. In Golden Delicious apples, the calyx provided for the diffusion of 42% of the C_2H_4, 24% of the CO_2, and only 2% of the water. In tomatoes, the percentages were 94, 81, and 67 respectively.

Many researchers including Burg and Burg (1965), Cameron (1982) Cameron and Reid (1982), Cameron and Yang (1982), Clendenning (1941), have investigated the contribution of the stem scar as an avenue for gas exchange in tomatoes. For example, Burg and Burg (1965) found that sealing the stem scars of green peppers and tomatoes reduced CO_2 emanation by 60%; with cantaloupes and grapefruits a 10% decline was noted. In oranges,

the exchange of CO_2 and C_2H_4 through the stem scar is only twice that of the peel (Barmore and Biggs, 1972). Cameron and Yang (1982) showed that the peel of tomato had 1227-fold more resistance to ethane than the stem scar per unit surface area. Brooks (1937), working with tomatoes noted that the skin of harvested tomato is practically impermeable to gases and any gas exchange is entirely through the stem scar.

2.3.4 Methods of estimating skin resistance to gas diffusion

Several approaches have been utilised by researchers to determine of the numerical value of the resistance of the skin of fruit and other bulky plant organs to gas diffusion, none of which are entirely free of criticisms. Basically there are two main approaches used; these are as follows.

2.3.4.1 Non steady-state approach

Based on the observed net movement of gases moving in opposite directions across the fruit skin, Marcellin (1963, 1974) developed a non steady state approach to measure the resistance of apple fruit to O_2 and CO_2 diffusion. In one system, hydrogen was introduced into the external atmosphere surrounding the apple fruit and he monitored the increase in internal gas volume using a moving liquid index in a glass tube which was confluent with the central locule. This approach is based on the principle that hydrogen moves much more rapidly through both gaseous and solid phases than O_2 or nitrogen. Thus, the hydrogen was observed to move into the fruit much faster than O_2 and nitrogen (assumed to be the primary components of the internal atmosphere) moved out. The moving liquid index ensured that the internal pressure remained near one atmosphere. The measured rate of volume increase combined with the known diffusivity of hydrogen in air was used to estimate the resistance coefficients of O_2. Marcellin, then replaced the hydrogen with pure CO_2 and estimated the resistance to CO_2 by comparison with the results obtained with hydrogen.

In another approach, Cameron and Yang (1982) conscious of the inherent problems associated with using the steady-state method, developed a simple alternative approach for the quantitative measurement of skin resistance based on a kinetic analysis of the efflux of preloaded gases from plant organs which did not require flux to be at steady-state. In essence, the method involves incubating a fruit in an atmosphere containing a known concentration of ethane, and measurement of the increase in concentration of this gas after transferring the fruit to an ethane free container. It is apparent that if there is a high resistance to ethane movement, the measured rate of ethane increase in the jar will be slow and vice versa.

Cameron and Yang (1982) chose ethane because it has similar molecular weight to nitrogen and O_2 and has diffusional properties similar to those of C_2H_4 and O_2. Besides it is neither produced nor metabolised to a significant degree by the tissue under normal conditions.

Cameron and Yang's (1982) method is time consuming since it requires equilibration for several hours during the loading and unloading phases, and several chromatographic analyses are performed in order to establish the efflux kinetics. Banks (1985b) using some of the principles employed by Cameron and Yang (1982), developed a more rapid method, involving several measurements in the first two minutes of efflux, but equilibration during loading was still required. Both of these approaches are certainly time consuming. Based on the same principles, Knee (1991a) devised a much more rapid method for measuring the resistance to gaseous diffusion of bulky plant organs, such as apple fruit. In his method, an apple fruit is incubated in a sealed container in the presence of a measured ethane concentration for a certain time, usually 20 minutes. The fruit is then transferred to another similar container. The ethane concentration which diffuses into the new container is measured after an equal time usually 20 minutes. This method requires an estimate of the internal volume of the fruit, accessible to ethane.

The non steady-state approach to measurement of skin resistance to gas diffusion overcomes some of the drawbacks associated with the steady state method: the system need not be at equilibrium; the diffusion of gases which are not produced in significant amounts (such as C_2H_4 in preclimacteric fruits or in vegetative tissues) can be studied; and the techniques can be designed such that the internal atmosphere need not be sampled. Ethane can be measured quickly and accurately at low concentrations by gas chromatography (Cameron and Yang, 1982; Banks, 1985b). Accurate estimation of fruit surface area is essential for the estimation of skin resistance to gas diffusion using the approach.

Despite these advantages, the primary limitation of the efflux approach in the determination of skin resistance for other physiologically important gases is that suitable analogs are difficult to find. In addition, the use of hydrocarbon gases in tissues with high fat content is not feasible because of their high solubility in lipids. For example, avocado give unrealistically high intercellular volume when ethane is used for measurement (Solomos, 1987). Under certain circumstances because of limited amount of air space within tissues much greater flesh resistance to gas diffusion could occur and this could affect the measured values using the efflux method (Cameron, 1982).

2.3.4.2 Steady-state approach

Resistance of plant tissues and organs to diffusion of O_2, CO_2, and C_2H_4 has been investigated using a steady-state approach (Burg and Burg, 1962, 1965; Burton, 1974, 1978; Cameron, 1982; Cameron and Reid, 1982; Forsyth *et al.*, 1973; Kidd and West, 1949).

The steady-state approach is greatly dependent on accurate measurement of the surface area (cm^2) of the commodity, the production or

consumption rate (ie. respiration rate; $cm^3\ s^{-1}$) of the gas of interest by the organ and the concentration of the gas in the internal and external atmospheres (% by volume/100) (Ben-Yehoshua and Cameron, 1988).

The skin resistance to gas diffusion (**R**; $s\ cm^{-1}$) is then calculated as follows:

$$R = \frac{\text{concentration gradient} * \text{surface area}}{\text{production (or consumption) rate}} \qquad [2.2]$$

The steady state approach relies on the assumption that both internal atmosphere and respiratory rates are at equilibrium, a condition which is difficult to ensure. Furthermore, in some bulky plant organs (e.g. avocado, potato) reliable estimates of the internal atmosphere composition may be difficult to obtain (Banks, 1985b) because of the compactness of the tissues.

In addition, by definition the system must be in a steady state having a constant flux of gas through organ's skin. This is a limiting factor if the resistance of the fruit to O_2 is being measured at a developmental stage when fluxes are most likely not to be at steady-state such as during the climacteric when there are marked changes in the rate of production of CO_2 and C_2H_4 and utilisation of O_2 (Banks, 1985b; Cameron and Reid, 1982).

This approach also requires that the tissue must produce significant levels of the gas of interest. For example, both production rates and internal concentrations of C_2H_4 are often extremely difficult to detect in preclimacteric fruits or in vegetative tissues. Accurate measurement of internal concentration of gases can be difficult. Although internal samples are easily withdrawn from fruits with internal cavities such as apples, cantaloupes, extraction methods

used to determine the internal atmosphere of bulky organs such as avocado or potato can often yield inconsistent or misleading results (Cameron and Reid, 1982).

Another drawback may result if wounding or other perturbations occur which invariably cause non-steady state fluxes (Cameron, 1982). For instance, wounding has the potential to increase rate of respiration, rate of C_2H_4 production and degree of water soaking (Kahl, 1974) any one of which can change diffusion behaviour markedly.

In spite of these limitations the steady state method can be used to obtain useful information on a wide variety of gases and tissues (Burg and Burg, 1965; Cameron, 1982; Cameron and Reid, 1982). In the current study, reliable data were obtained using both the steady state and non steady state methods.

The foregoing review clearly demonstrates that there is a dearth of information on factors affecting the internal atmosphere composition of fruits (including apples) as well as the relationships between these factors and the atmosphere inside the fruit. There is a clear need for further research in this area.

In the ensuing chapters the relationships between skin resistance to gas diffusion, respiration and internal atmosphere composition of apples ~~has~~ have been examined. In addition the relationship between respiration and internal atmosphere composition of apples under varying O_2 atmospheres has been ascertained. The effects of temperature and coating treatments on gas exchange characteristics as well as variation in internal atmosphere composition within single apples with time in storage ~~has~~ have also been investigated.

CHAPTER 3

GENERAL MATERIALS AND METHODS

This chapter describes the techniques, materials and experimental conditions used in this study.

3.1 Fruit supply

Freshly harvested apple, (***Malus domestica*** Borkh.) cultivars, Cox's Pippin Orange, Gala, Royal Gala, Golden Delicious, Red Delicious, Splendour, Braeburn and Granny Smith apples, count 125 were obtained from the New Zealand Apple and Pear Marketing Board in Hastings.

3.2 Measurement of surface area

Fruit surface area (**A**) was estimated (assuming that the fruit geometry approximated a sphere) from an average measurement of the three axial diameters using digital calipers. Using the standard formula for calculating the radius (r) of a sphere, the surface area (cm^2) was obtained as follows:

$$A = 4 . \Pi . r^2 \qquad [3.1]$$

where $\Pi = 3.1416$

3.3 Measurement of gases

3.3.1 Analysis of Carbon dioxide and Oxygen

The O_2 and CO_2 in gas samples were analysed respectively using an O_2 electrode (City Technology Ltd. London); (Banks, 1986) in series with an infra-red CO_2 analyser (Binos-1-2, Leybold-Heraeus). The O_2 electrode was insensitive to argon, an important advantage over the use of a thermal conductivity detector for samples with low O_2 content. Using a gas-tight monoject tuberculin syringe, each gas sample was injected manually into the apparatus in a stream of nitrogen (flow rate of 25 cm^3 min^{-1}) carrier gas flowing through both pieces of equipment and the response to sample injection measured as peak heights using a Hewlett Packard 3393A integrator.

3.3.2 Analysis of Ethylene and Ethane

Ethylene (C_2H_4) and ethane (C_2H_6) concentrations (μl l^{-1}) in gas samples were estimated using either a Varian 3400 or Pye Unicam Series 104 gas chromatograph (GC) fitted with flame ionisation detector (FID) and a stainless steel activated alumina column (80-100 mesh, 1.8m long and 0.32cm diameter). Temperatures of the column, injector and detector were 100°C, 100°C and 150°C respectively. Nitrogen was used as carrier gas with a flow rate of 30 cm^3 min^{-1} and hydrogen and air for the detector (flow rates 30 cm^3 min^{-1} and 300 cm^3 min^{-1} respectively). Samples were injected manually into the GC using a gas-tight or monoject tuberculin disposal syringe.

3.3.3 Analysis of Acetaldehyde and Ethanol

Acetaldehyde (AA) and ethanol (ETOH) (μl l^{-1}) in gas samples were estimated using a Pye Unicam Series GCD gas chromatograph fitted with an FID and a 10% Carbowax 20 m column (80/100 chromosorb WAW, 1.8m long

and 0.32cm in diameter). Temperatures of the column, injector and detector were 80°C, 110°C and 150°C respectively. Nitrogen was used as carrier gas with a flow rate of 30 cm^3 min^{-1} and hydrogen and air for the detector (flow rates 30 cm^3 min^{-1} and 300 cm^3 min^{-1} respectively). Standards were prepared by adding 5µl acteldehyde to 5µl, 95% ethanol in an empty 2.5 litre bottle with a magnetic stirrer. The bottle was placed on a magnetic stirer to mix the contents. A concentration of 799 and 729µl l^{-1} of AA and ETOH (respectively) was obtained.

3.3.4 Measurement of internal atmosphere concentrations

The internal atmosphere concentrations of fruit were measured using the following methods:

3.3.4.1 Direct sampling method

Direct sampling of internal atmospheres was carried out by the method described by Banks (1983). Fruit were submerged in water, a 1.0 ml disposable hypodermic syringe was fitted with a stainless steel canula (16 gauge, 38 mm long, luer-fitting). The dead air space was replaced with saturated sodium chloride solution (NaCl). The mouth of the canula was occluded by a pin head to prevent blockage with tissue during insertion into the fruit. The canula was inserted into the core cavity through the calyx end of the fruit and withdrawn slightly so that the pin head remained at the base of the bore-hole, leaving the canula's mouth open. The internal atmosphere was obtained by withdrawing the plunger slowly. If liquid from the tissue entered the syringe, the sample was discarded. Sampling was completed in less than 25 seconds (s) to avoid any change in respiration and C_2H_4 production due to wounding.

After sample withdrawal, the canula was detached from the syringe and a septum cap was fitted to the syringe tip. A gas-tight syringe, in which the

dead space had been replaced with saturated NaCl solution, was used to take 90µl gas samples by piercing through the septum cap fitted to the 1.0ml hypodermic syringe.

3.3.4.2 External chamber method

A non-destructive method was used for repeated sampling of internal atmospheres (Banks and Kays, 1988).

A 2 ml glass vial from which the bottom had been removed and the top fitted with a 0.4 ml plastic tube was stuck, using polyvinyl acetate adhesive (PVA), onto the fruit surface. Chambers were sealed after allowing the adhesive to dry for 24 hours (h), by inserting a rubber septum into the plastic tube fitted onto each chamber. The well so formed in the plastic tube after sealing the chamber was filled with water to prevent gas leaks through the septum and atmospheric contamination of gas samples during sampling.

3.3.5 Measurement of skin resistance

3.3.5.1 Ethane efflux method

The ethane efflux method described by Banks (1985b) was used to determine skin resistance to gas diffusion. Each fruit was individually placed in a 1000 cm^3 glass 'Le Parfait' storage container (fitted with brass couplings sealed with silicone rubber septa) and injected with 1ml of pure ethane using an hypodermic syringe. After overnight equilibration, samples of the container atmospheres were analysed for ethane content ($C_i(0)$, $\mu l\ l^{-1}$). A stopwatch was started as a single fruit was removed from its jar outside the laboratory (t=0), held in front of a fan for 2-3s, then quickly rushed into the laboratory and sealed in a 1000 cm^3 glass jar (t=14-16s) through which the contents were rapidly being circulated using a diaphragm pump. Samples were taken at 10s intervals for 50s. Samples of the atmosphere inside the jar were analysed for their ethane contents by GC with flame ionisation detector (see 3.3.2).

Skin resistance to gas diffusion was calculated using the following formula (Banks 1985b):

$$R = \frac{C_i(0) * A}{V_e * (dC_e/dt)} \qquad [3.2]$$

where **R** was the resistance of the fruit surface to gas diffusion (s cm^{-1}) and $C_i(0)$ in ($\mu l\ l^{-1}$) was the internal concentration of ethane at time t = 0, **A** was the fruit surface area (cm^2), V_e (cm^3) the external volume, equal to container volume minus fruit volume, and (dC_e/dt) was the rate of change of concentration of ethane in the container ($\mu l\ l^{-1}$).

3.3.5.2 Steady state method

Fruit were weighed and surface area determined as described in 3.2. Fruit respiration (CO_2 evolution) and C_2H_4 production were determined by sealing single fruit in 580cm^3 glass storage jars for 60min at 20°C and measuring the difference in the initial and final gaseous contents of each container. Jars were usually submerged in a water bath at 20°C during the run to ensure both constant temperature and that no gas leakage occurred.

Core cavity O_2, CO_2 and C_2H_4 concentrations were determined by the direct sampling method described in 3.3.4.1.

Skin resistance to gas (ie. CO_2 or C_2H_4) diffusion was calculated from the following equation assuming that composition of the internal atmosphere was uniform throughout the fruit and that the fruit geometry approximated a sphere (Burg and Burg, 1965). For example skin resistance to CO_2 diffusion was calculated as:

$$RCO_2 = \frac{A * ([CO_2]_{core} - [CO_2]_{ext})}{FCO_2} \quad [3.3]$$

where,

RCO_2 = skin resistance to gas diffusion (s cm^{-1})

A = fruit surface area (cm^2)

$[CO_2]_{core}$ = core cavity carbon dioxide concentration (%)

$[CO_2]_{ext}$ = external carbon dioxide concentration (%)

FCO_2 = flux at steady state or respiration rate (cm^3 s^{-1})

3.3.6 Gas mixing and measurement

Fruit were individually placed in containers under continuous flow of humidified air or gas at known flow rates. Precision needle valves were used to mix air and nitrogen to produce the required atmospheres. The composition of the atmospheres were verified daily by taking 1ml gas samples which were analysed as previously described (section 3.3).

3.3.7 Calculation of gas concentrations from chromatograhic data

Peak height data were used to calculate the concentrations of CO_2, C_2H_4 and O_2 as follows:

3.3.7.1 Oxygen, carbon dioxide and ethylene concentrations

A Hewlett Packard 3393A integrator was used to determine the peak height of eluent peaks of O_2, CO_2 and C_2H_4 on the gas chromatograh (section 3.3.1). Sample concentrations were estimated from these areas as outlined below:

3.3.7.2 Oxygen concentration using Oxygen electrode:

Oxygen concentration was calculated as follows:

$$O_2\,(\%) = \frac{\text{Sample peak height} * 20.95}{\text{Mean standard peak height}} \quad [3.4]$$

3.3.7.3 Calculation of the rate of O_2 uptake and CO_2 and C_2H_4 production

The rates of O_2 uptake, CO_2 and C_2H_4 production were calculated as follows:

$$FO_2 = \frac{[O_2]_{initial} - [O_2]_{final}}{100} * (V_{jar} - V_{fruit}) * \frac{1000}{W_{fruit}} * \frac{60}{T} \quad [3.5]$$

$$FCO_2 = \frac{[CO_2]_{final} - [CO_2]_{initial}}{100} * (V_{jar} - V_{fruit}) * \frac{1000}{W_{fruit}} * \frac{60}{T} \quad [3.6]$$

$$FC_2H_4 = \frac{[C_2H_4]_{final} - [C_2H_4]_{initial}}{1000} * (V_{jar} - V_{fruit}) * \frac{1000}{W_{fruit}} * \frac{60}{T} \quad [3.7]$$

where:

FO_2	= rate of oxygen uptake ($cm^3\ kg^{-1}\ hr^{-1}$)
FCO_2	= rate of carbon dioxide production ($cm^3\ kg^{-1}\ hr^{-1}$)
FC_2H_4	= rate of ethylene production ($\mu l\ kg^{-1}\ hr^{-1}$)
$[O_2]_{initial}$	= initial oxygen concentration (%)
$[O_2]_{final}$	= final oxygen concentration (%)
$[CO_2]_{initial}$	= initial carbon dioxide concentration (%)
$[CO_2]_{final}$	= final carbon dioxide concentration (%)
$[C_2H_4]_{initial}$	= initial ethylene concentration ($\mu l\ l^{-1}$)
$[C_2H_4]_{initial}$	= final ethylene concentration ($\mu l\ l^{-1}$)
V_{jar}	= jar volume (cm^3)
V_{fruit}	= fruit volume (cm^3)
W_{fruit}	= fruit weight (kg)
T	= time (h)

3.3.8 Measurement of quality parameters

The following methods were used to assess fruit quality:

3.3.8.1 Firmness

Using a potato peeler, 1 mm of apple skin was removed from two opposite areas on the equatorial surface of the fruit. Fruit firmness (the force required to penetrate the cortical tissue in kg) was measured on both areas using a hand-held Effegi penetrometer fitted with an 11.1 mm diameter probe. Fruit firmness was obtained as the mean of the two measurements taken and converted to newtons (N) by multiplying by 9.807 (Soule, 1985).

3.3.8.2 Soluble solids

Unless described otherwise soluble solids concentration (%) of juice expressed from two opposite sides of the fruit equatorial surface was estimated using a hand-held Atago N-20 refractometer (Model N, McCormick Fruit Tech., brix range from 0 - 20% at 20°C). The refractometer was zeroed using distilled water. The prism surface and the light plate were thoroughly washed and dried with a clean soft tissue paper between each reading. The two readings taken on each fruit were averaged prior to data analysis.

3.3.8.3 Fruit skin colour

Fruit skin background colour was measured at any two green parts on the equatorial surface using a Minolta chromameter (CR-100). Skin colour was expressed as hue angle. Hue angle values decrease as skin colour changes from green to yellow (Little, 1975). Thus high hue angle values indicate an intense green colour. The two readings taken on each fruit were averaged prior to data analysis.

3.3.9 Data analysis

Unless stated otherwise, Statistical Analysis System (SAS) programmes (SAS/STAT User's Guide, 1988) were used to analyse data from each experiment for Analysis of variance (ANOVA), means, standard deviations, and standard errors. Mean comparisons were also carried out by Duncan's Multiple range test at 5% and 1% level of significance (Duncan, 1955). Unless stated otherwise, linear and non linear regression and correlation analysis were also carried out as described by Steel and Torrie (1980).

CHAPTER 4

ESTIMATING SKIN RESISTANCE TO GAS DIFFUSION IN APPLES.

4.1 ABSTRACT

Skin resistance to gas diffusion (**R**) values of freshly harvested apple cultivars, Cox's Orange Pippin, Gala, Royal Gala, Golden Delicious, Red Delicious, Splendour, Braeburn and Granny Smith were obtained using non-steady state (ethane efflux) and steady state methods at 20±1°C.

R was cultivar dependent with freshly harvested Braeburn apples having the highest mean **R** and Royal Gala the lowest. There was a linear relationship between skin resistance to ethane diffusion (RC_2H_6) and ethylene diffusion (RC_2H_4) of individual apples within cultivars. This suggests that RC_2H_6 and RC_2H_4 are indeed very similar. Although there was a large degree of variation in **R** values of individual apples within each cultivar, the close relationship between the two independent estimates of **R** confirmed that this was real fruit to fruit variation rather than measurement error. In contrast, estimates of skin resistance to CO_2 diffusion (RCO_2) and RC_2H_4 estimated concurrently by the steady state method were different and the mean RCO_2 was higher than RC_2H_4. The relationship between RCO_2 and RC_2H_6 in combined data set for all cultivars was hyperbolic, indicating that CO_2 may diffuse through additional routes to those available for O_2, C_2H_4 and ethane (C_2H_6).

Respiration rate of Cox's Orange Pippin apples were nearly twice as fast as Splendour, Granny Smith or Braeburn apples and a third higher than Gala, Royal Gala and Golden Delicious apples. In freshly harvested fruit,

respiration rate appeared to be independent of RC_2H_6 (both within individual cultivars and in a combined data set for all cultivars).

In spite of their intermediate respiration rate, Braeburn apples had lower internal O_2 concentration ($[O_2]_i$) than the other cultivars and this could be related to their high **R** and low intercellular space volume. The mean $[O_2]_i$ in freshly harvested Splendour, Golden Delicious, Gala and Royal Gala apples were about a fifth greater than in Cox's Orange Pippin and half as much again as in Braeburn apples. On the other hand the average internal CO_2 concentration ($[CO_2]_i$) in Braeburn and Cox's Orange Pippin were nearly three fold higher than in Splendour and twice higher than in Gala, Royal Gala and Golden Delicious apples. There was a decreasing exponential relationship between $[O_2]_i$ and RC_2H_6 but an increasing relationship between $[CO_2]_i$ and RC_2H_6 of individual apples for all cultivars. Thus, the magnitude of **R** affects internal atmosphere composition for a given external atmosphere composition.

The rate of C_2H_4 evolution, internal C_2H_4 concentration ($[C_2H_4]_i$) and quality indices of freshly harvested fruits differed between cultivars. Freshly harvested Braeburn apples had the highest mean firmness and the lowest soluble solids content compared to the other cultivars, whilst Splendour apples had the highest soluble solids content and Granny Smith apples were the greenest fruit.

4.2 INTRODUCTION

The skin of apples presents a much greater barrier to gas diffusion than the pulp (Burton, 1978). It therefore plays an important role not only as a mechanical protection for the tissues beneath but also as a partial regulator of the internal atmosphere composition and consequently physiological processes occurring in the fruit (Cameron and Yang, 1982; see chapter 2 for a review of **R** as a factor affecting the internal atmosphere composition of fruit).

Magness and Diehl (1924) contended that the storage quality of apples was closely associated with skin condition and it was important in determining the resistance of the fruit to decay and wilting.

In apples, the large degree of variation in **R** of individual fruit is well known. Kidd and West (1938, 1949) recognised that this, together with variation in the respiratory rates between fruit, caused major differences in the composition of the internal atmospheres of fruit. Burton (1974) emphasised the significance of natural variation in **R** of fruits when setting limits for controlling the composition of the atmosphere within CA stores. Since that time, the concept of skin diffusive resistance and flesh resistance to gas diffusion and their physiological significance in postharvest technology have been further investigated (Banks, 1984, 1985a; Cameron and Reid, 1982; Solomos, 1982, 1987; Soudain and Phan Phuc, 1979). While the mean **R** values of small samples of populations have been reported, the significance of the variation around the mean values and the effects that this variation may have on the physiological response to externally imposed atmospheric regimes have been largely ignored, despite the fact that herein may lie a major component of the cause of variability in quality of fruits stored under such conditions.

Only limited information is available on cultivar variation in **R** (Banks, 1985a; Cameron, 1982; Knee, 1991) though such information could be important in explaining observed cultivar differences in sensitivity to MA or CA conditions. Furthermore, only limited research has been conducted to measure seasonal changes in **R** of fruits even though it is well known that there are seasonal variations in sensitivity to low O_2 (Marcellin, 1974).

Knowledge of **R** may also provide some valuable information on some of the physiological disorders that develop in some apple cultivars during CA storage. Furthermore, knowledge of **R** is needed for studying the nature of

oxidases that may be involved in fruit respiration and also for predicting minimum gas levels that can be safely used in CA storage (Solomos, 1987). Information on **R** (as well as internal atmosphere composition and respiration rates) of different apple cultivars grown in New Zealand is meagre.

The foregoing clearly demonstrate that research is required to quantify apple **R**, since it may help in understanding some of the physiological behaviour of fruit during storage. Therefore, the current research was initiated to estimate **R** of freshly harvested apples of eight cultivars grown in New Zealand, using both the ethane efflux (non-steady state) and steady-state methods (see chapter 2 for a comprehensive review of the two methods).

4.3 MATERIALS AND METHODS

4.3.1 Fruit supply

Freshly harvested apples (***Malus domestica*** Borkh.; count 125; av. weight 148 g) of eight export cultivars (Cox's Orange Pippin, Gala, Royal Gala, Golden Delicious, Red Delicious, Splendour, Braeburn and Granny Smith) were obtained by courier within 48h of commercial mid-season harvest of each cultivar from similar source as previously described in 3.1. Experiments commenced on the day of receiving the fruit. Even-size, blemish-free fruit were selected. All measurements were done at 20±1°C.

4.3.2 Estimation of skin resistance using ethane efflux method

In a series of experiments, the ethane efflux method described by Banks (1985a) was used to estimate **R** (see 3.3.5.1).

4.3.3 Estimation of skin resistance using steady-state method

After estimating **R** using the ethane efflux method, the same fruit were allowed to equilibrate at 20±1°C for 6h before measuring the rate of respiration (CO_2 production) and C_2H_4 production in fruit kept in the dark as previously described in 3.3.5.2.

Core cavity O_2, CO_2 and C_2H_4 concentrations were measured by the direct sampling method (see 3.3.4.1) and gas samples analysed as described in 3.3.1. and 3.3.2.

RCO_2 and RC_2H_4 were then calculated (see 3.3.5.2).

4.3.4 Fruit quality assessment

Fruit firmness, soluble solids content and background colour were measured as described in 3.3.8.1, 3.3.8.2 and 3.3.8.3, respectively.

4.3.5 Experimental design and analysis

Sixteen single fruit replicates from each apple cultivar were used in a completely randomised design. Data were analysed as previously described in section 3.3.9. Mean comparisons were carried out by Duncan's multiple range test at 1% levels of significance (Duncan, 1955; Petersen, 1977), to test cultivar differences. Linear and nonlinear regression analyses (Mead and Curnow, 1983; Steel and Torrie, 1980) were performed using SAS and in some cases CGLE graphics and statistical package (version 3.2, 1991).

4.4 RESULTS

4.4.1 Skin resistance to gas diffusion

Skin resistance to gas diffusion of apples varied with cultivar ($P < 0.01$). Regression analysis of the raw data from each cultivar revealed that plots of the estimates of RC_2H_4 as a function of RC_2H_6 estimated respectively by the steady state and ethane efflux methods were linear (figs. 4-1, 4-2 and 4-3). Estimates of RC_2H_4 and RC_2H_6 using both methods corresponded closely (with coefficient of variation from the regression analyses for Braeburn, Cox's Orange Pippin, Granny Smith, Red Delicious, Royal Gala and Splendour apples being 6.4, 9.3, 7.7, 8.6, 11.4 and 11.4% respectively). In contrast, comparison of RCO_2 and RC_2H_4 of each cultivar estimated concurrently by steady state method indicated that mean estimates of RCO_2 were different and higher than estimates of RC_2H_4 (fig. 4-4). A plot of RCO_2 as a function of RC_2H_6 of individual apples for all cultivars (in a combine set of data) indicated that the relationship was curvilinear (fig. 4-5) and was reasonably closely described by an exponential equation of the form:

$$RCO_2 = a * [1.0 - \exp(-(b * RC_2H_6))] + c \qquad [4.1]$$

where a, b and c are parameters of the equation.

With the exception of Braeburn apples, fruit of the other cultivars which had RCO_2 in the range of approximately 5,000 and 20,000 s cm^{-1} also had RC_2H_6 between about 5,000 and 15,000 s cm^{-1}.

There was large a degree of variation of **R** values of individual apples within each cultivar, as shown by the coefficient of variation presented in Table 4-1. Despite these variations, **R** of Braeburn apples was greater than the other cultivars. The mean **R** of Braeburn apples was approximately three times higher than Royal Gala apples which had the lowest **R**.

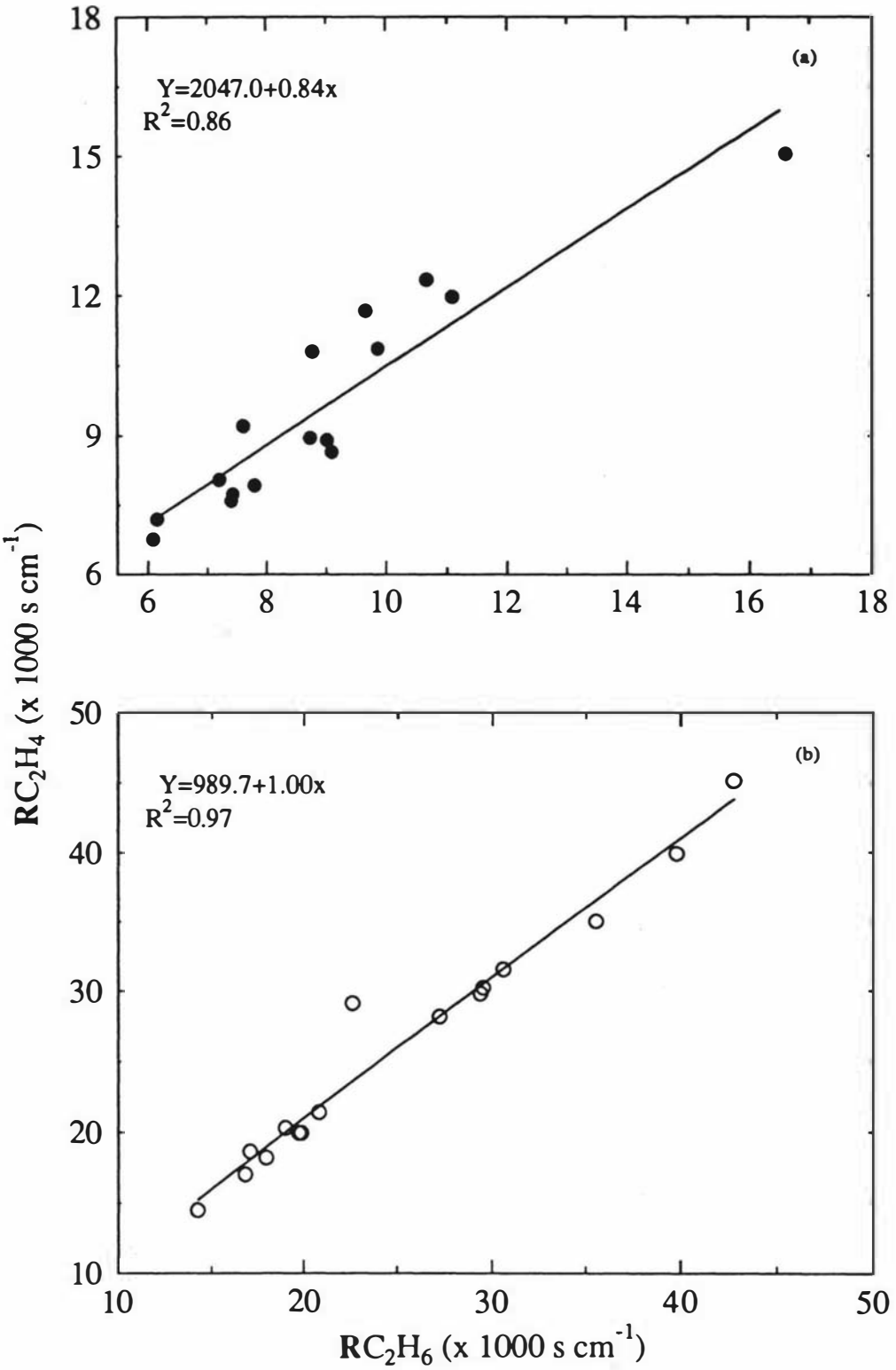

Fig. 4-1. Comparison of RC_2H_6 and RC_2H_4 of individual (a) Cox's Orange Pippin and (b) Braeburn apples estimated by the ethane efflux and steady state methods respectively with fitted regression.

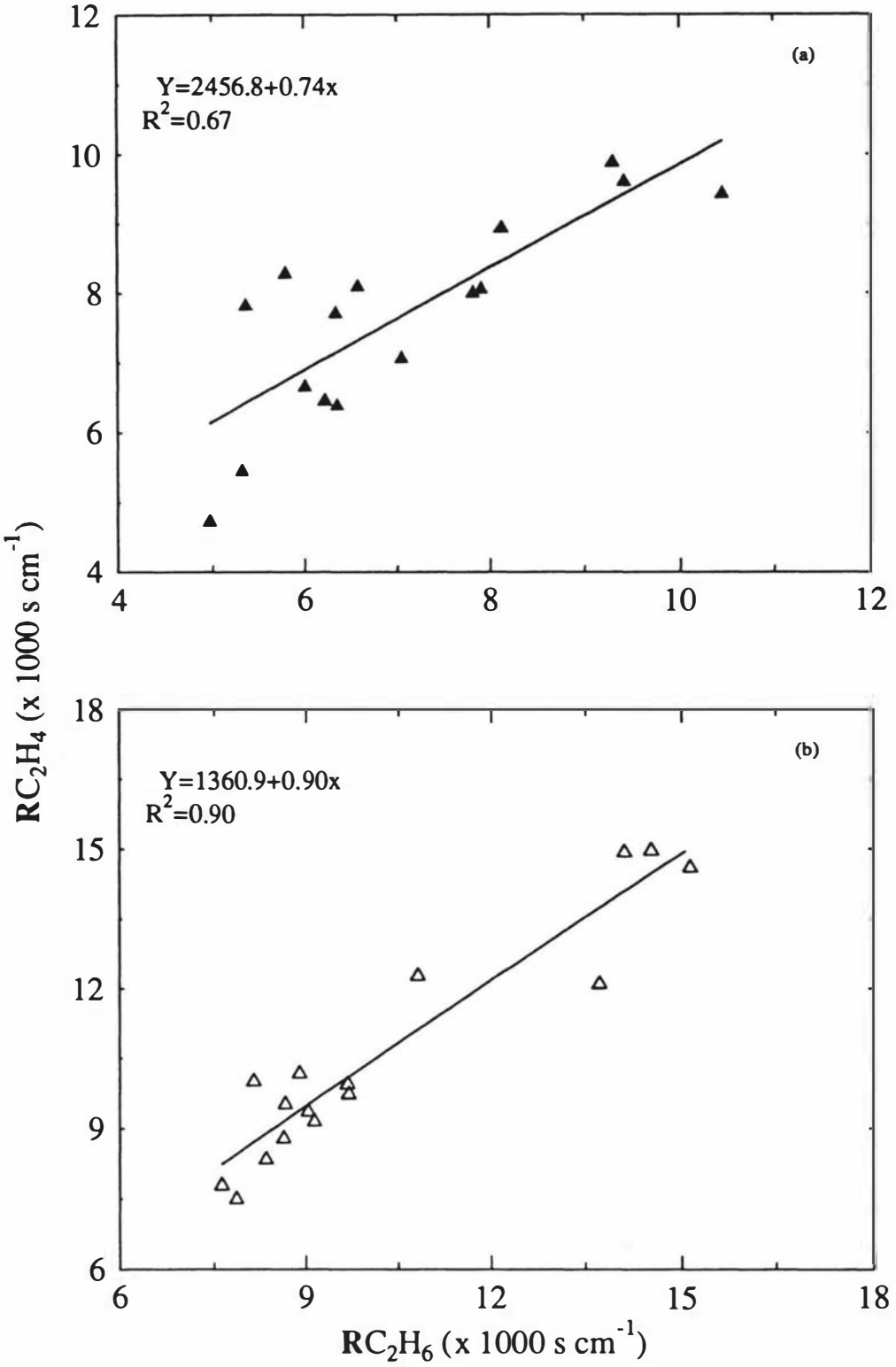

Fig. 4-2. Comparison of RC_2H_6 and RC_2H_4 of individual (a) Splendour and (b) Granny Smith apples estimated by the ethane efflux and steady state methods respectively with fitted regression.

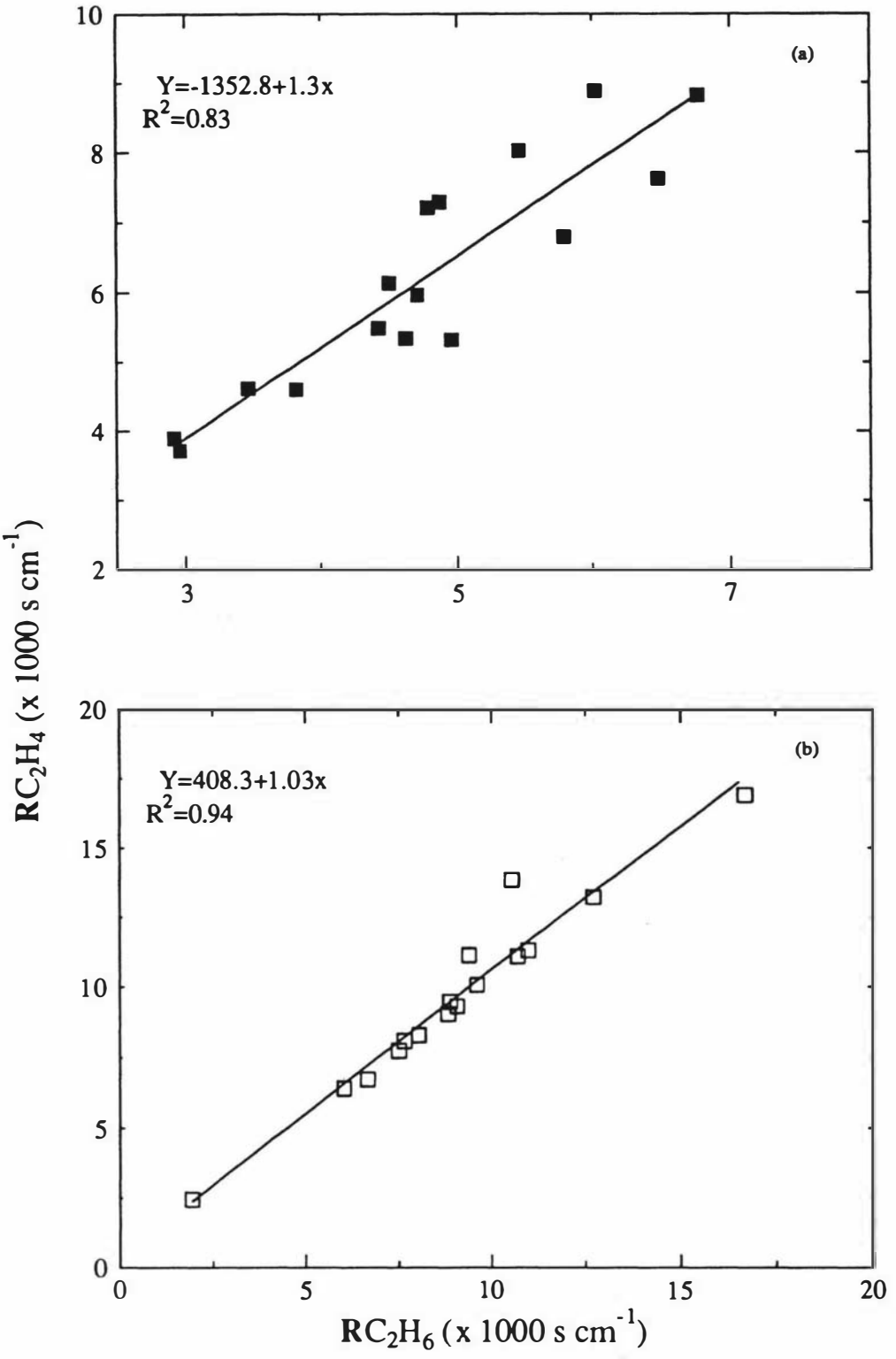

Fig. 4-3. Comparison of RC_2H_6 and RC_2H_4 of individual (a) Royal Gala and (b) Red Delicious apples estimated by the ethane efflux and steady state methods respectively with fitted regression.

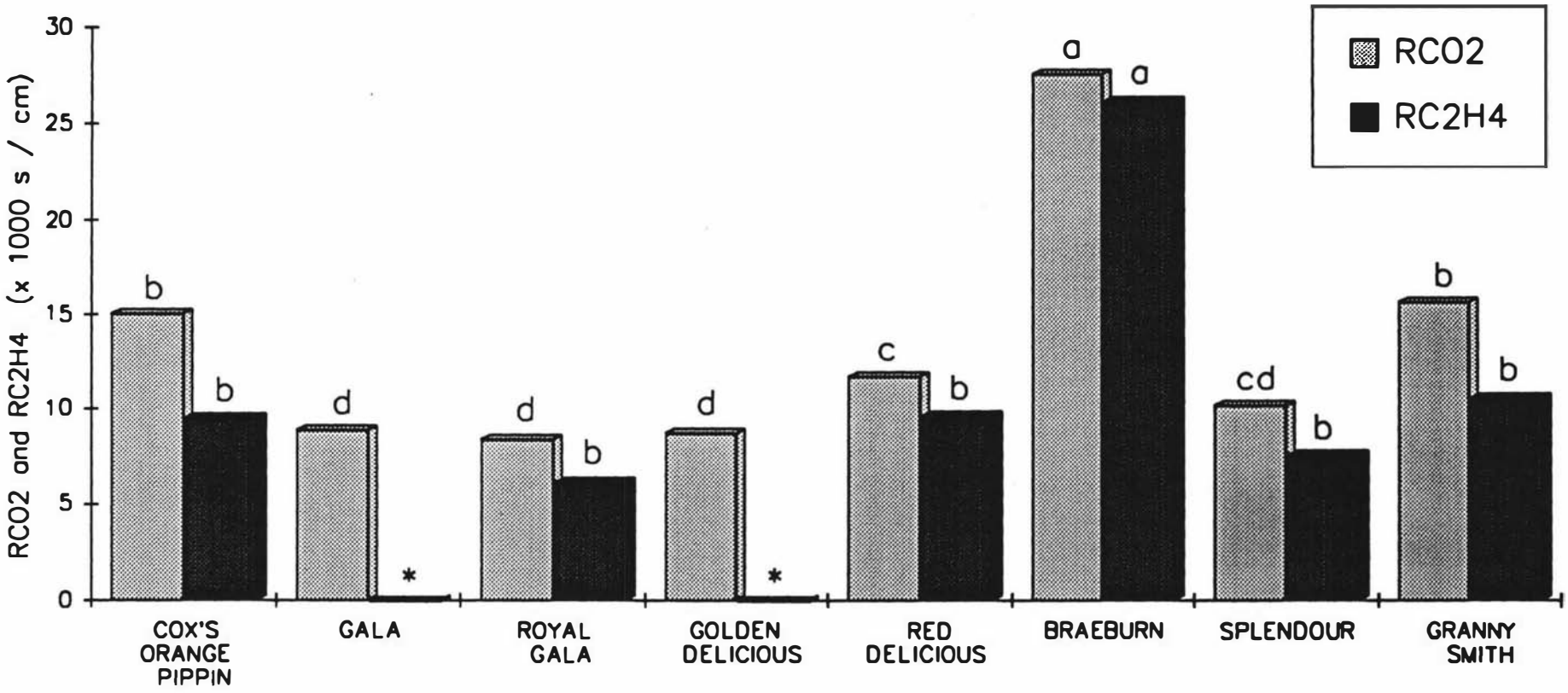

Fig. 4–4. Skin resistance to CO2 and C2H4 diffusion of freshly harvested apples, estimated by the steady state method at 20°C. * indicate no detectable C2H4 at the time of experiment. Letters in common for each gas not significantly different at the 1% level. Mean separation by Duncan's multiple range test.

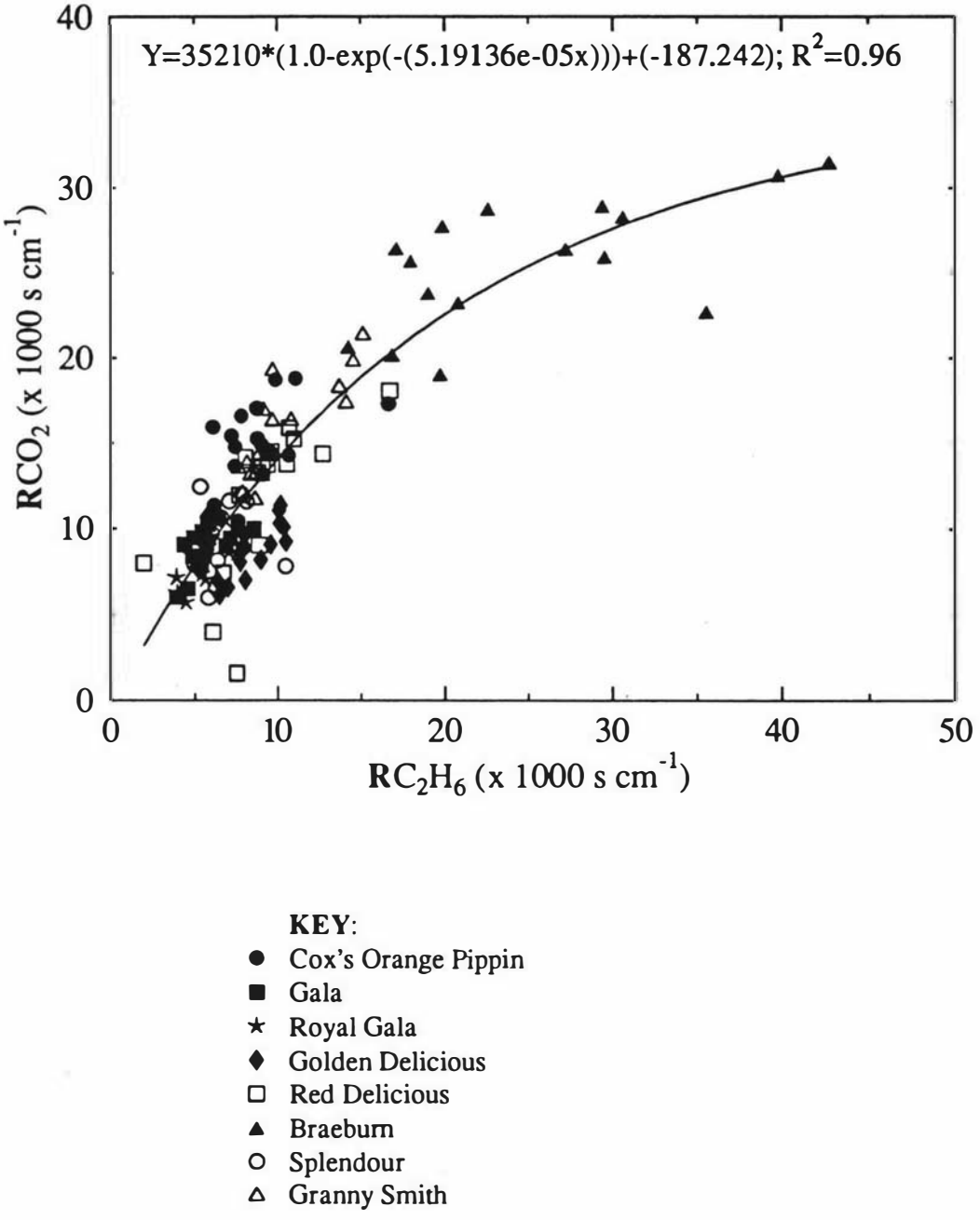

Fig. 4-5. Relationship between RCO_2 and RC_2H_6 of individual apples within cultivar estimated by the steady state and ethane efflux methods respectively. Solid line was fitted by nonlinear regression using equation [4.1].

Table 4-1. Coefficients of variation (ie. standard error as a percentage of the mean) for RC_2H_6, RC_2H_4 and RCO_2 [s cm^{-1}] in different apple cultivars.

	Coefficient of variation (%)		
Cultivar	RC_2H_6	RC_2H_4	RCO_2
Cox's Orange Pippin	28.1	23.8	15.4
Gala	21.2	*	14.8
Royal Gala	19.7	26.4	17.2
Golden Delicious	21.5	*	18.6
Red Delicious	35.0	34.6	38.3
Braeburn	34.5	33.8	14.6
Splendour	22.9	19.1	25.1
Granny Smith	25.2	23.2	18.8

* indicate no detectable C_2H_4 at the time of experimentation, hence no values for the coefficient of variation.

4.4.2 Fruit respiration rate

Fruit respiration rate (CO_2 evolution) differed between cultivars ($P < 0.01$; fig. 4-6). Cox's Orange Pippin apples were respiring nearly twice as rapidly as Splendour, Granny Smith or Braeburn apples and a third as rapidly again as Gala, Royal Gala and Golden Delicious apples (fig. 4-6). An attempt was made to fit an exponential model to describe the apparent declining relationship between respiration rate and RC_2H_6 of individual apples within cultivars (fig. 4-7). The fitted parameters were not significantly different from zero and the visual fit to the data was poor which was attributed to the effects of the wide spread of respiration rates between cultivars with RC_2H_6 between 3,000 and 12,000 s cm^{-1} (fig. 4-7). With the exception of Braeburn apples which had intermediate respiration rate and high RC_2H_6, most of the fruit of the other cultivars which had respiration rates in the range of about 7.5 and 20 cm^3 CO_2 kg^{-1} h^{-1} had RC_2H_6 between approximately 3,000 and 12,000 s cm^{-1}.

4.4.3 Internal O_2 and CO_2 concentrations

Internal O_2 and CO_2 concentrations differed significantly between cultivars ($P < 0.01$; fig. 4-8). The mean $[O_2]_i$ in freshly harvested Splendour, Golden Delicious, Gala and Royal Gala apples were about a fifth greater than in Cox's Orange Pippin and nearly half as high again as in Braeburn apples. On the other hand, the average $[CO_2]_i$ in Braeburn and Cox's Orange Pippin were nearly threefold as high as in Splendour and twice as high as in Gala, Royal Gala and Golden Delicious apples.

A plot of $[O_2]_i$ or $[CO_2]_i$ as a function of RC_2H_6 of individual apples for all cultivars using equation [4.1] indicated a declining exponential relationship between $[O_2]_i$ and RC_2H_6 (fig. 4-9) and an increasing relationship between $[CO_2]_i$ and RC_2H_6 (fig. 4-10). Apart from Braeburn apples, which had

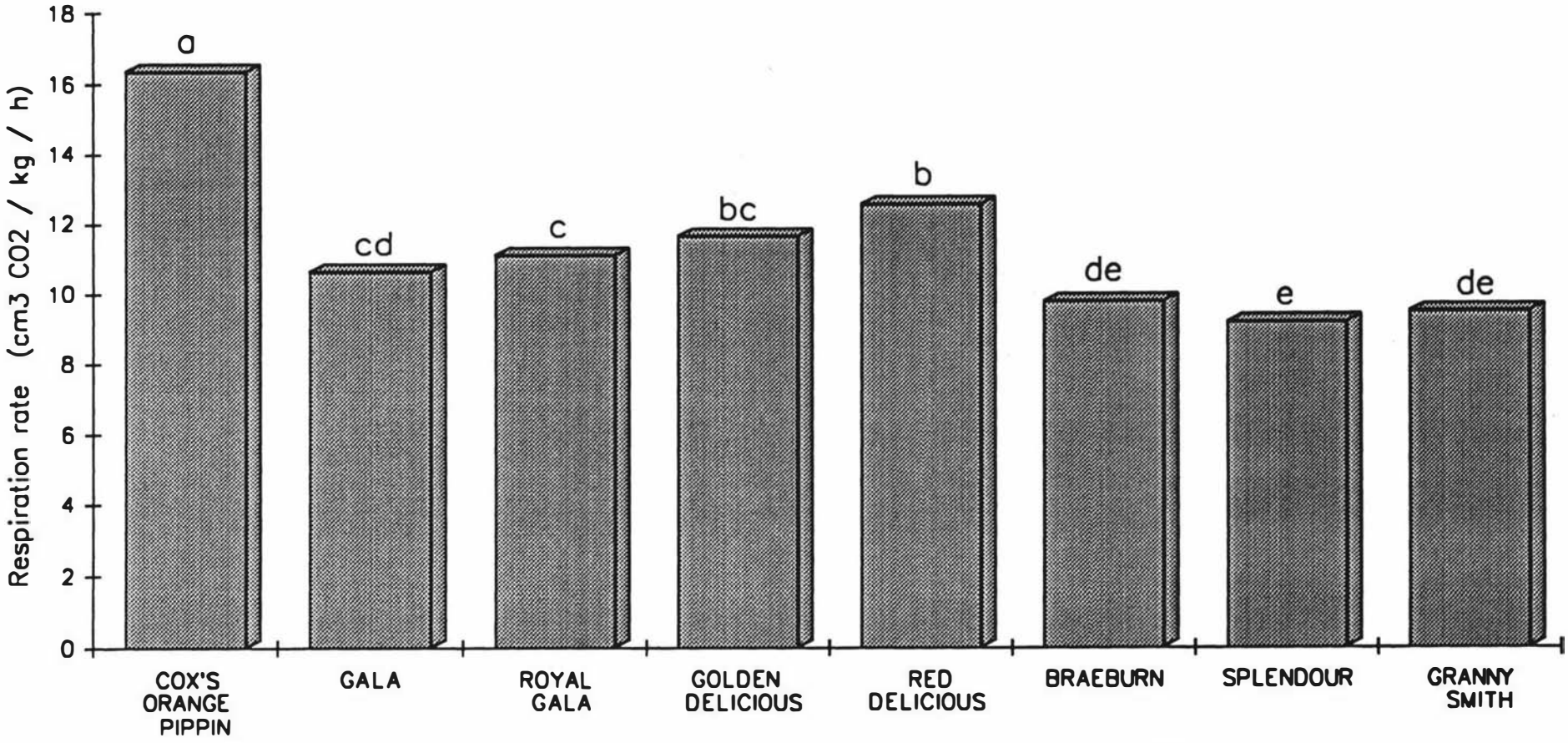

Fig. 4–6. Respiration rates of freshly harvested apples at 20°C. Letters in common not significantly different at the 1% level. Mean separation by Duncan's multiple range test.

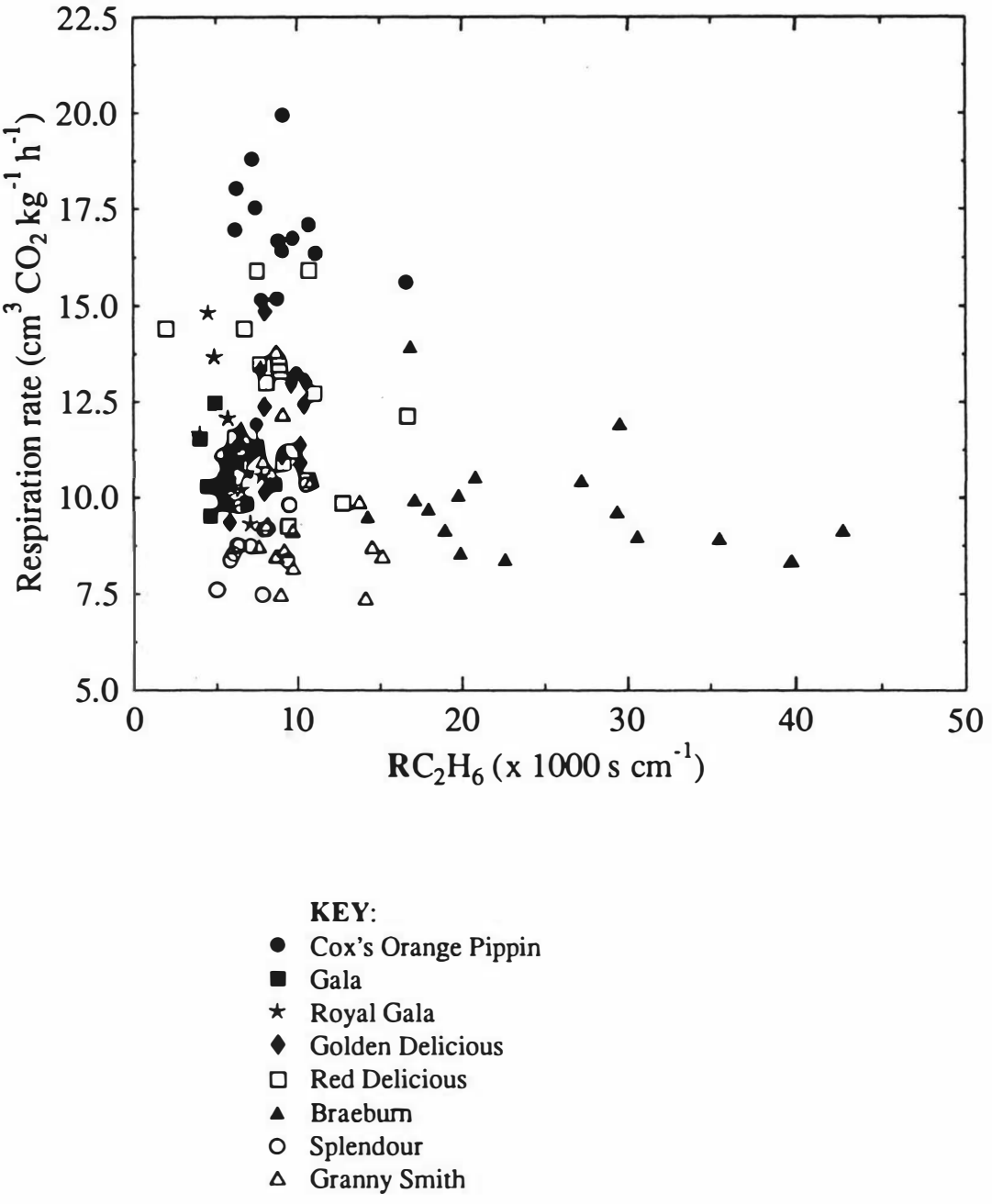

Fig. 4-7. Relationship between respiration and RC_2H_6 of individual apples within cultivar.

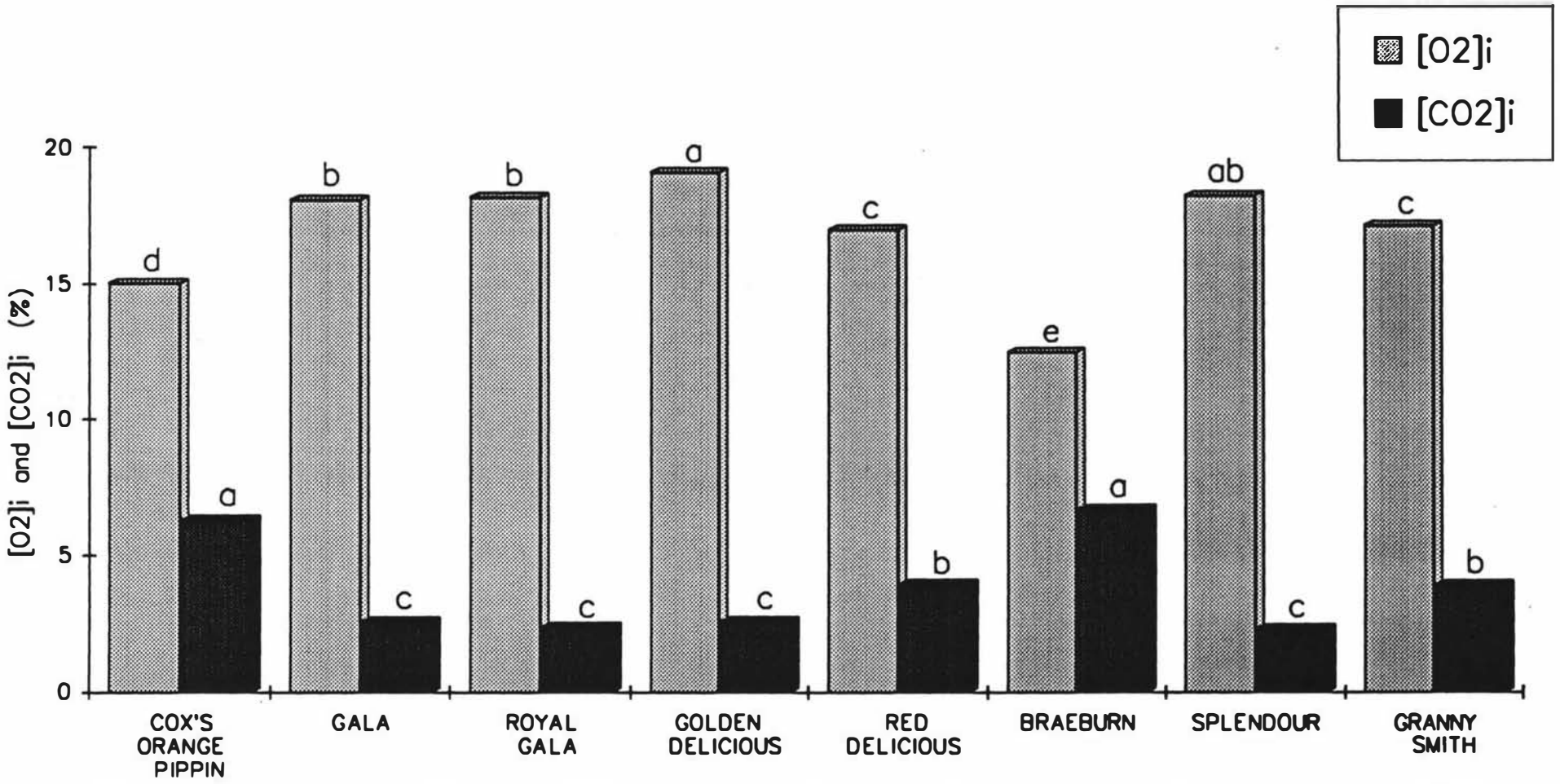

Fig. 4–8. Internal O2 and CO2 concentrations of freshly harvested apples at 20°C. Letters in common for each gas not significantly different at the 1% level. Mean separation by Duncan's multiple range test.

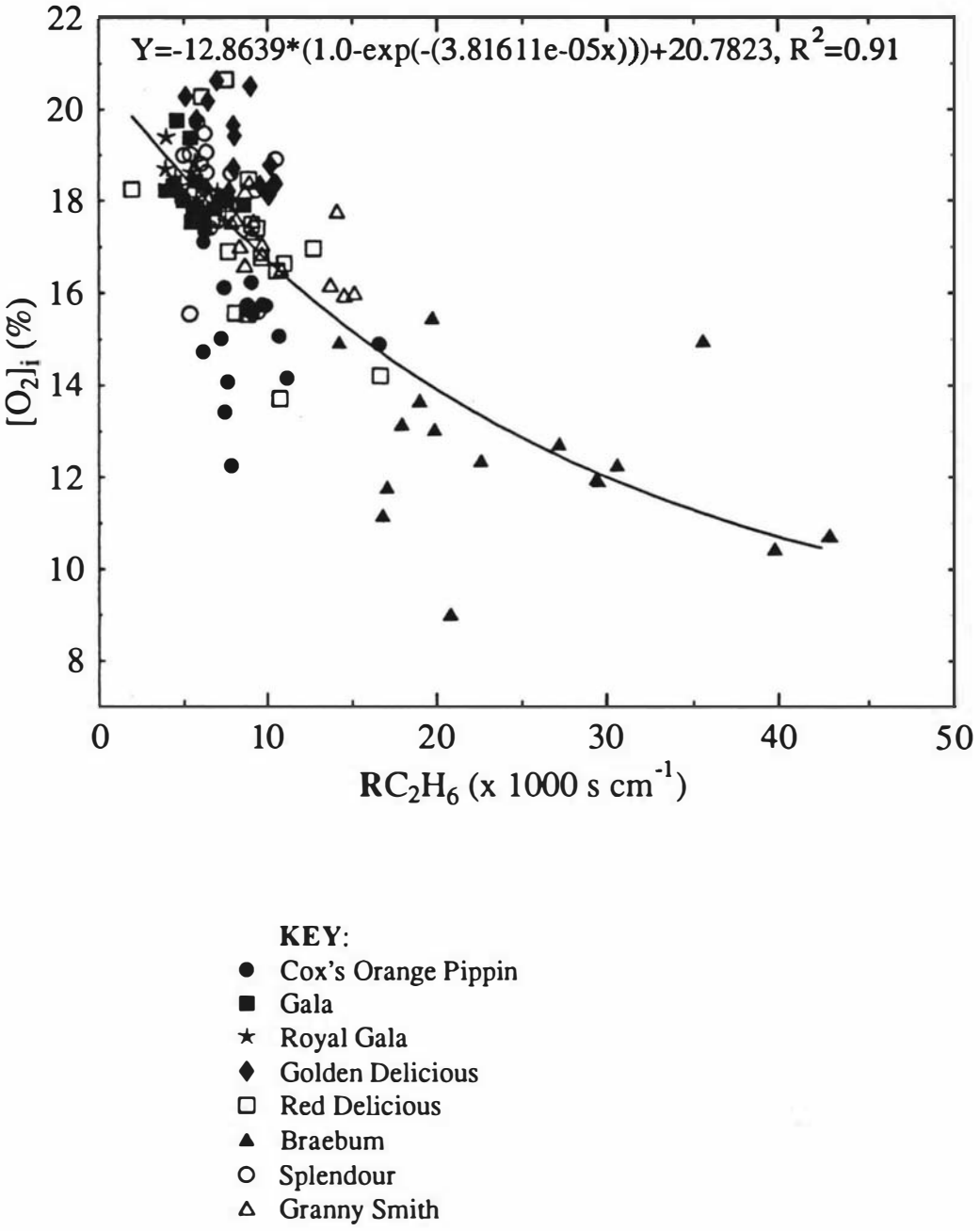

Fig. 4-9. Relationship between $[O_2]_i$ and RC_2H_6 of individual apples within cultivar. Solid line was fitted by nonlinear regression using equation [4.1].

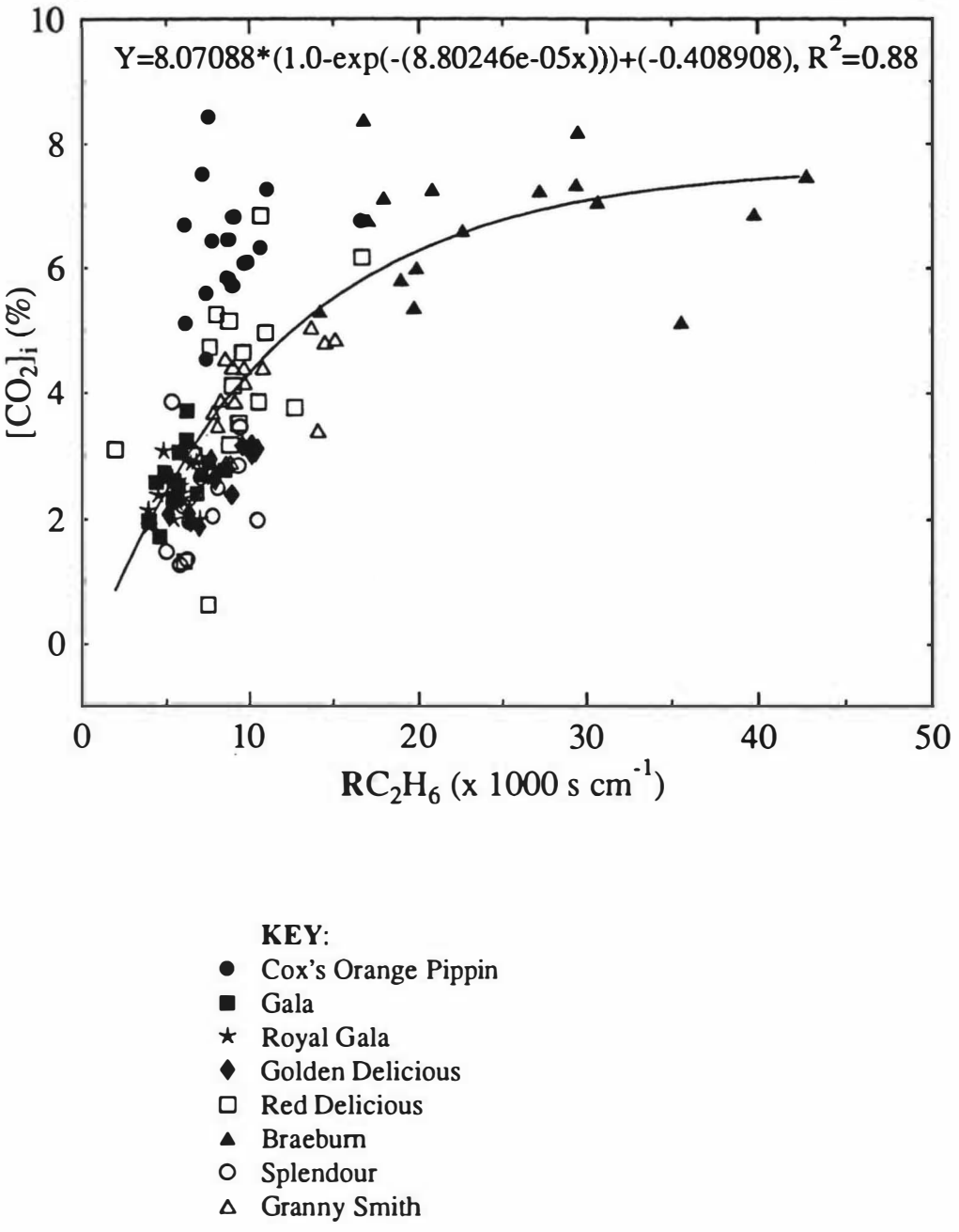

Fig. 4-10. Relationship between $[CO_2]_i$ and RC_2H_6 of individual apples within cultivar. Solid line was fitted by nonlinear regression using equation [4.1]

comparatively low $[O_2]_i$, high $[CO_2]_i$ and high RC_2H_6, the majority of the fruit of the other seven cultivars which had $[O_2]_i$ in the range of approximately 12 and 21% and $[CO_2]_i$ between 1 and 8% had RC_2H_6 between approximately 3,000 and 12,000 s cm^{-1}.

There were no significant relationships between $[O_2]_i$ or $[CO_2]_i$ and respiration rate in a combined data set for all cultivars (fig. 4-11 and 4-12). With the exception of Cox's Orange Pippin apples, most of the fruit of the other cultivars which had respiration rates between about 8 and 14 cm^3 CO_2 kg^{-1} h^{-1} had $[O_2]_i$ in the range of approximately 12 and 21% and $[CO_2]_i$ between approximately 1 and 8%.

A multiple regression relating $[O_2]_i$ or $[CO_2]_i$ to RC_2H_6 and respiration rate was highly significant ($P < 0.0001$), so a stepwise regression was performed. In the case of $[O_2]_i$, when the first variable (ie. RC_2H_6) was added to the stepwise regression model, an R^2 of 0.55 was obtained but when the second variable (ie. respiration rate) was added to the model the R^2 was improved from 0.55 to 0.62. Similarly, in the case of $[CO_2]_i$, addition of the second variable to the regression model improved R^2 from 0.44 to 0.69.

4.4.4 Internal C_2H_4 and C_2H_4 evolution

Internal C_2H_4 concentration and rate of C_2H_4 evolution were cultivar dependent ($P < 0.01$; figs. 4-13 and 4-14). With the exception of Red Delicious and Cox's Orange Pippin apples, most of the cultivars were either in the pre-climacteric stage or just entering the climacteric stage of development, consequently, their $[C_2H_4]_i$ as well as production rates were rather low. In the case of Gala and Golden Delicious apples, there was no detectable C_2H_4 at the time of experimentation.

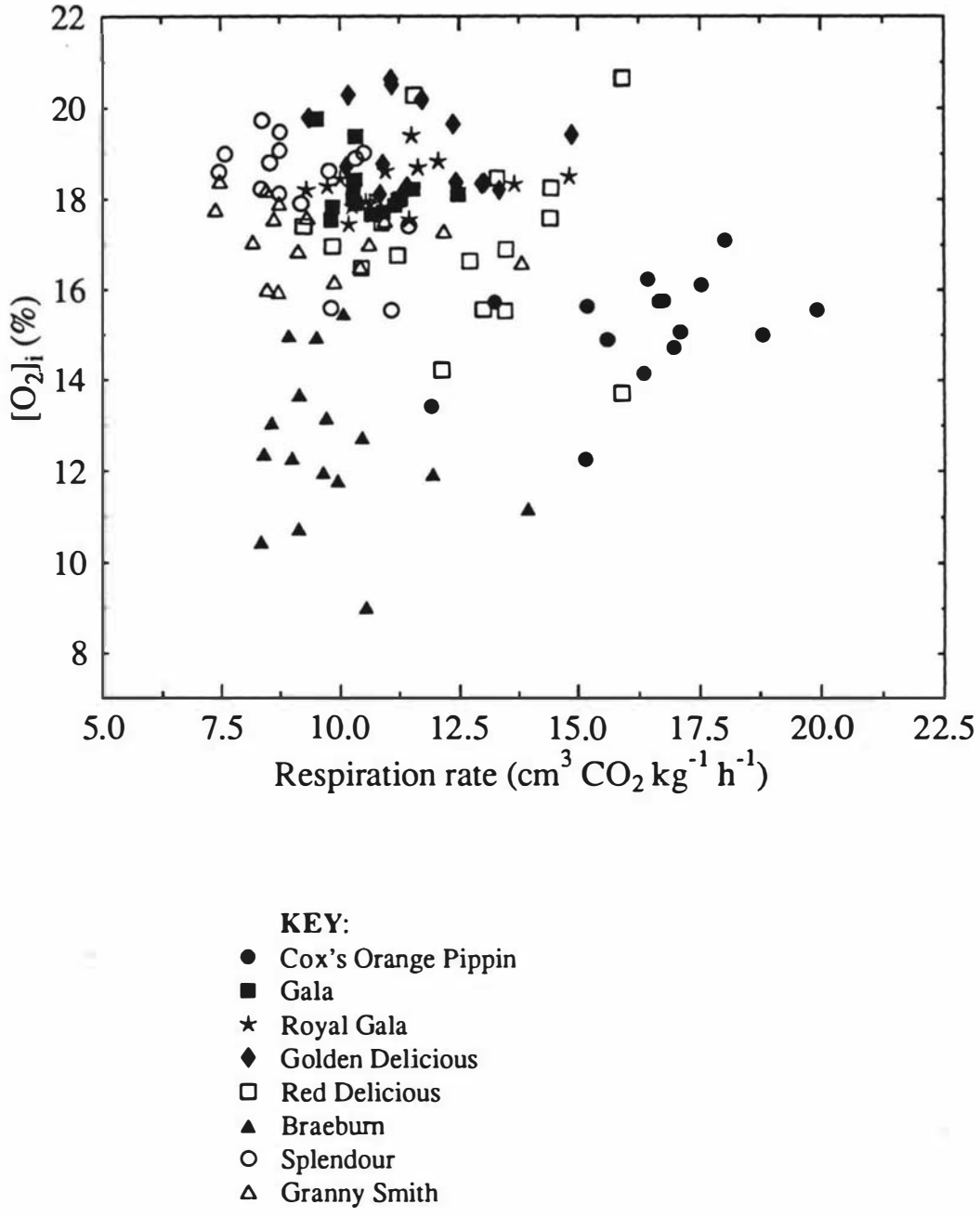

Fig. 4-11. Relationship between $[O_2]_i$ and respiration rate of individual apples within cultivar.

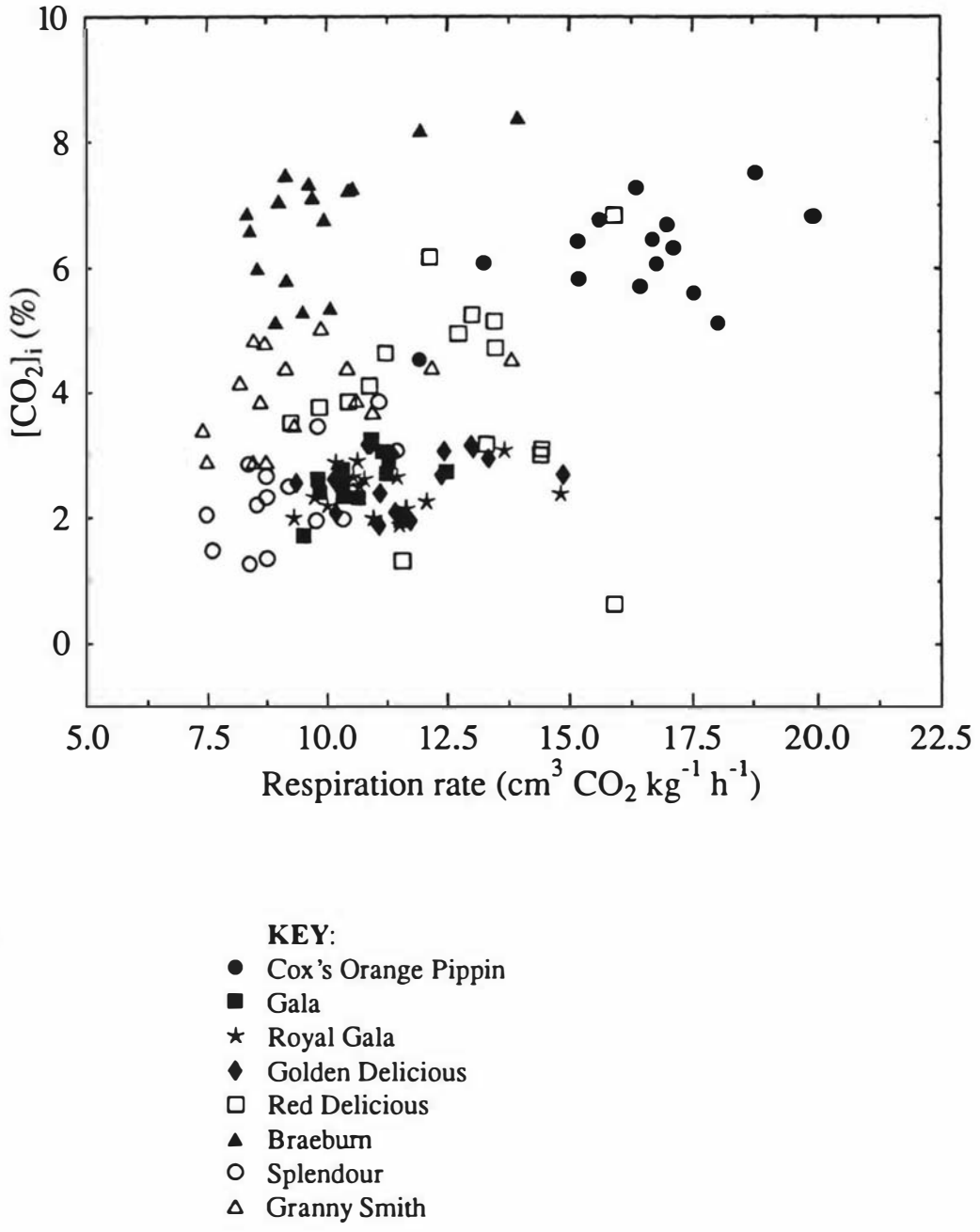

Fig. 4-12. Relationship between $[CO_2]_i$ and respiration rate of individual apples within cultivar.

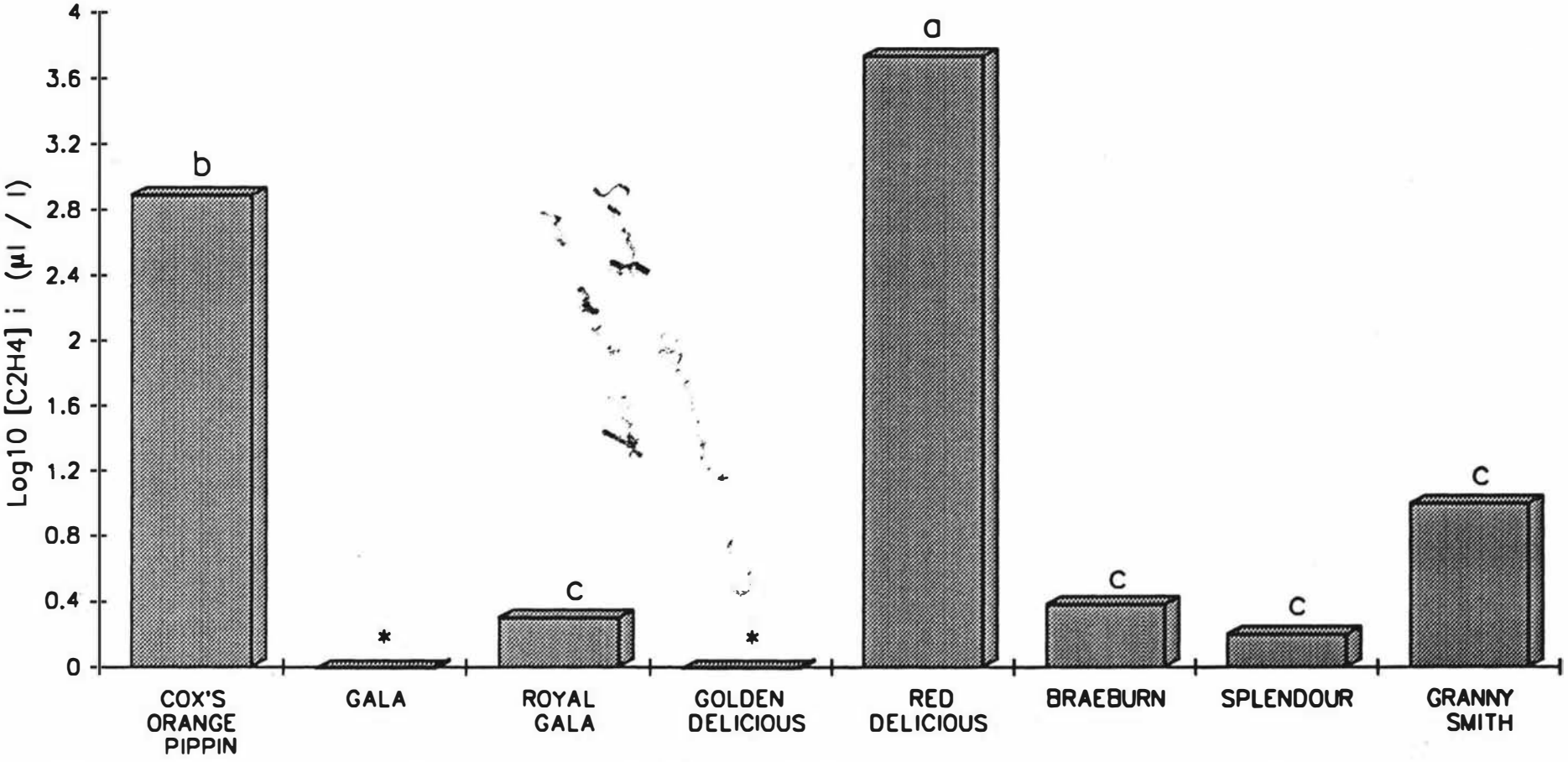

Fig. 4–13. Internal C2H4 concentrations of freshly harvested apples at 20°C. * indicate no detectable C2H4 at the time of experiment. Letters in common not significantly different at the 1% level. Mean separation by Duncan's multiple range test.

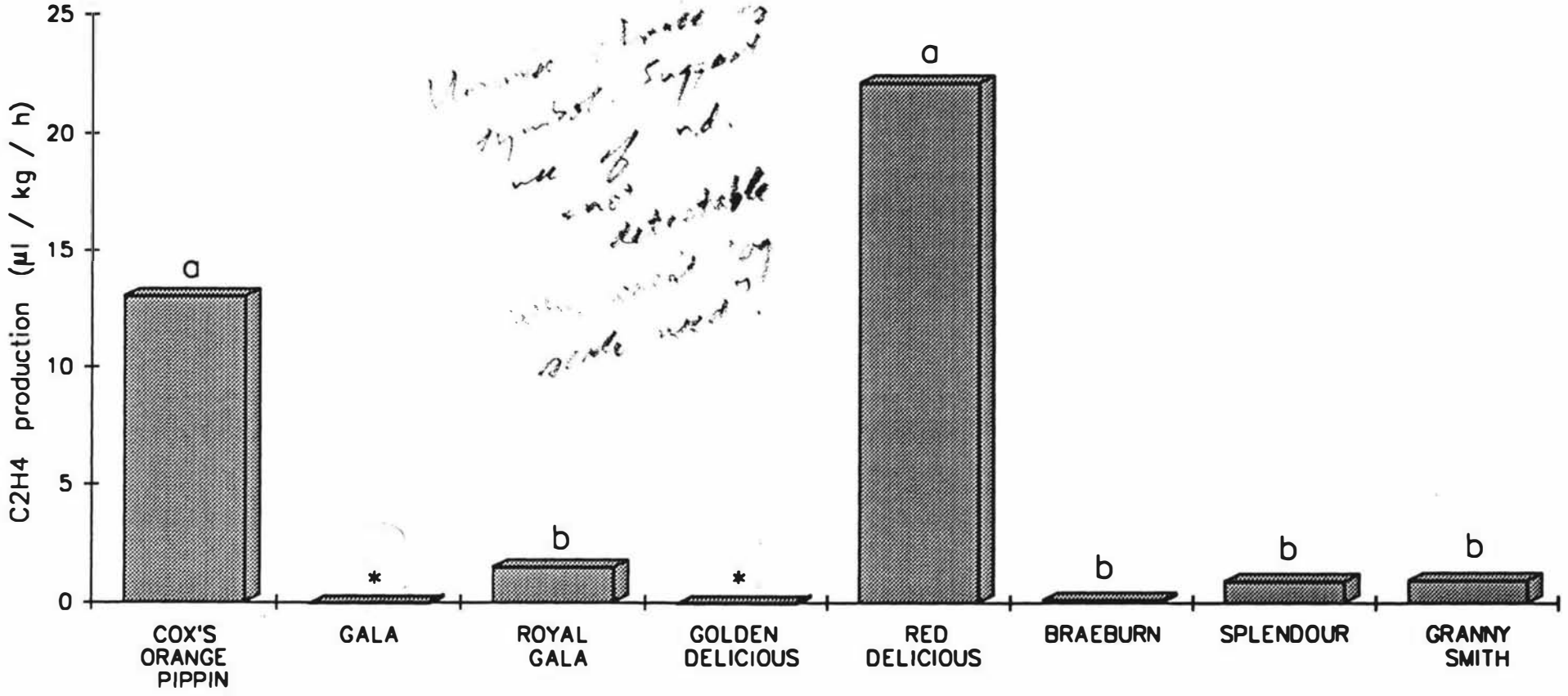

Fig. 4–14. Ethylene production of freshly harvested apples at 20°C. * indicate no detectable C2H4 at the time of experiment. Letters in common not significantly different at the 1% level. Mean separation by Duncan's multiple range test.

4.4.5 Quality indices

Fruit firmness and soluble solids content differed between cultivars ($P < 0.01$; figs. 4-15 and 4-16 respectively). Braeburn apples had the highest mean firmness and the lowest soluble solids content compared to the other cultivars ($P < 0.01$), while Splendour apples had the highest soluble solids content. Background colour (hue angle) measurements were highly cultivar specific ($P < 0.01$; fig. 4-17). As expected, Granny Smith apples were significantly greener than the other cultivars ($P < 0.01$).

4.5 DISCUSSION

The study clearly demonstrates that both the ethane efflux and steady state methods provide similar estimates of resistance of the apple's skin to gas diffusion. The close agreement between RC_2H_6 and RC_2H_4 determined respectively by each method suggest strongly that RC_2H_6 and RC_2H_4 are indeed very similar. These findings are consistent with those reported by Cameron (1982). These results further support the assertion of Cameron (1982) that the efflux method using C_2H_6 allows direct estimation of RC_2H_4 of plant organs. It has been utilised to examine the resistance characteristics of gas diffusion in fruits including apples and tomatoes (Cameron, 1982).

The resistance of tissues and organs to CO_2 and C_2H_4 gases has normally been investigated using the steady state approach (Burg and Burg, 1962, 1965; Burton, 1974, 1978; Cameron, 1982; Kidd and West, 1949). Comparison of the mean RCO_2 and RC_2H_4 of apples estimated by the steady state method (fig. 4-4) indicated that RCO_2 was higher than RC_2H_4. The difference could be due to differences in their molecular weight (44 and 28 for CO_2 and C_2H_4 respectively) which would affect their relative diffusivity in air by a factor of about 20%, similar to the differences observed in this study. It is apparent that CO_2 would be expected to have higher resistance values than

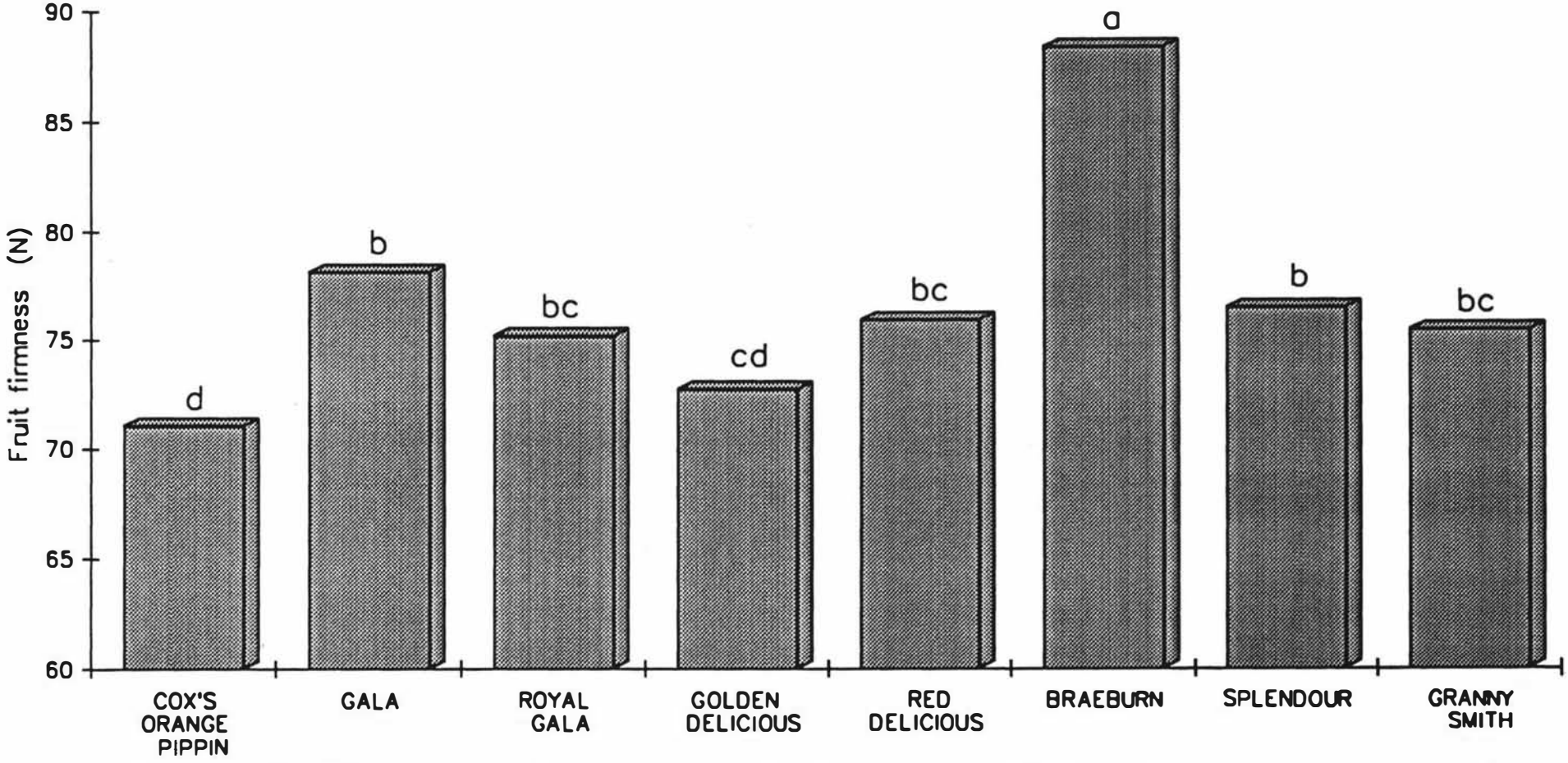

Fig. 4–15. Firmness of freshly harvested apples at 20°C. Letters in common not significantly different at the 1%. Mean separation by Duncan's multiple range test.

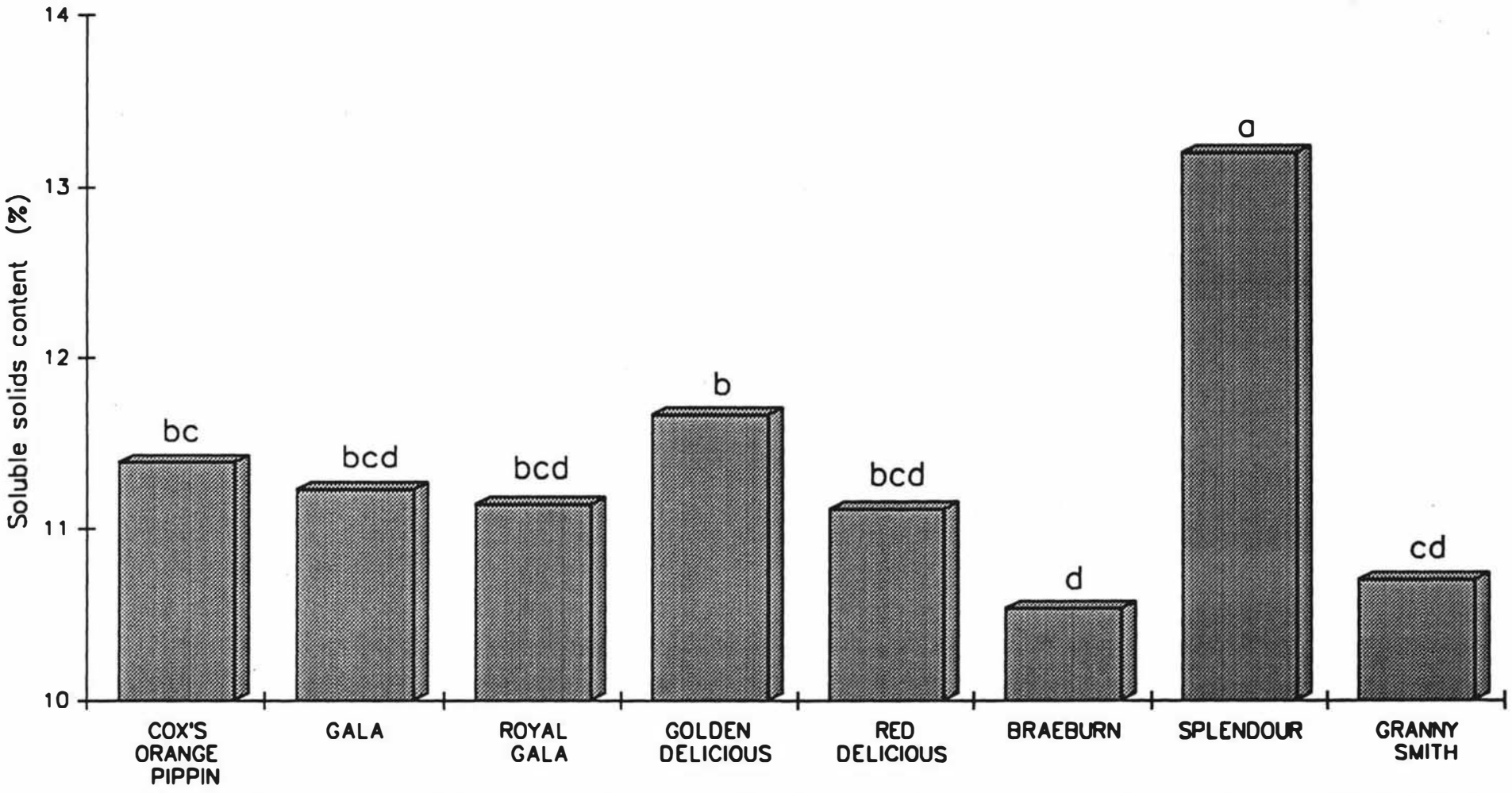

Fig. 4–16. Soluble solids content of freshly harvested apples. Letters in common not significantly different at the 1% level. Mean separation by Duncan's multiple range test.

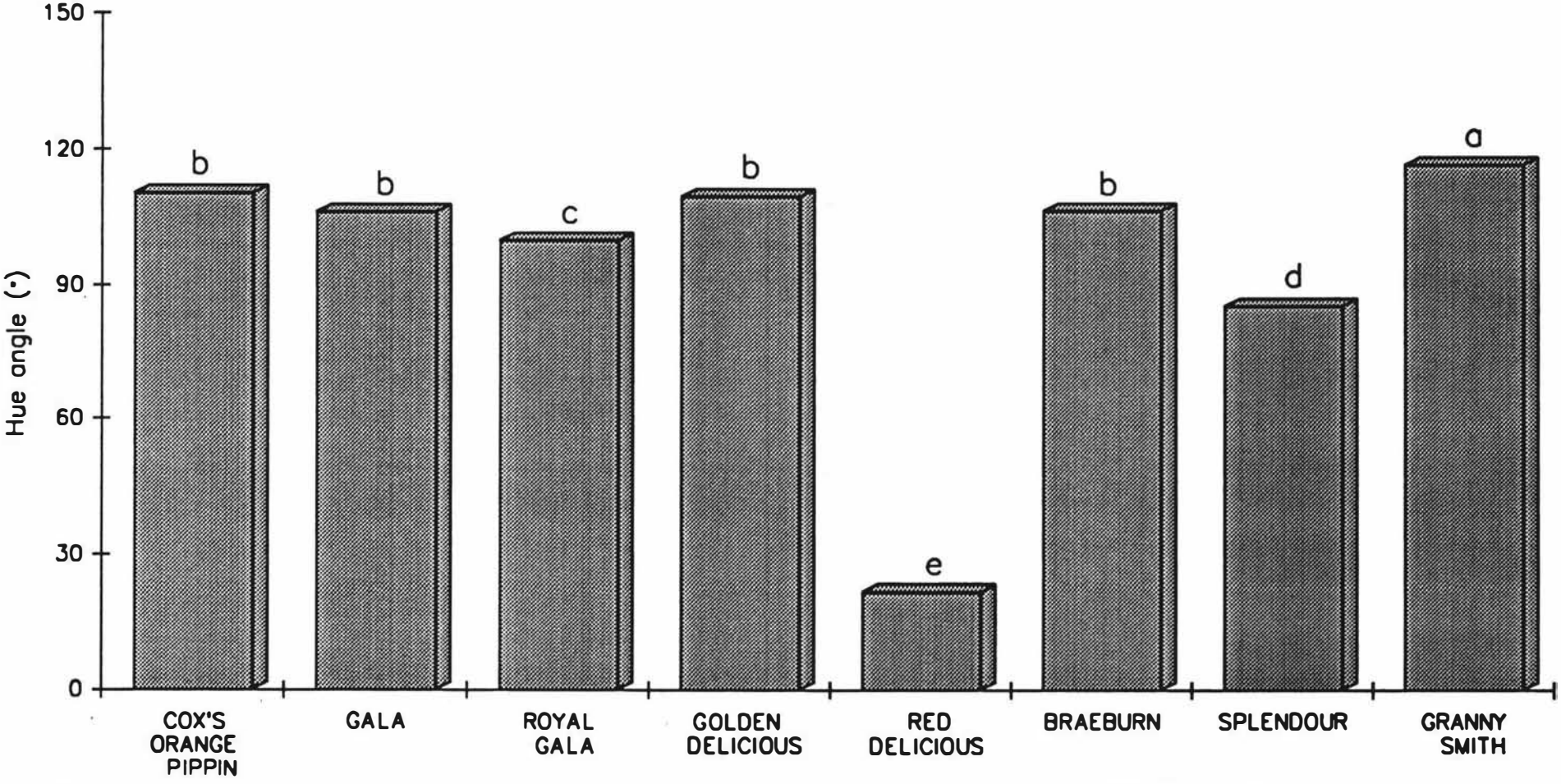

Fig. 4–17. Hue angle values of freshly harvested apples at 20°C. Letters in common not significantly different at the 1% level. Mean separation by Duncan's multiple range test.

C_2H_4, since it should move more slowly in the gas phase. In contrast, although the data presented by Cameron (1982) indicated that the mean RCO_2 and RC_2H_4 of Golden Delicious was 8,000 and 11,500 s cm^{-1} respectively, whilst in Yellow Newton apples it was 9,500 and 13,500 s cm^{-1} respectively he concluded that RCO_2 and RC_2H_4 determined by the steady state method were quantitatively similar.

The exponential relationship between RCO_2 and RC_2H_6 (obtained in this study) supports the concept that CO_2 may diffuse through additional routes to those available for O_2, C_2H_4 and ethane (C_2H_6) diffusion as suggested by other researchers including Banks (1984), Burton (1974) and Marcellin (1974). If it is assumed that CO_2 diffuses through the epidermal cells and cuticle as well as the pores and C_2H_6 only through pores, then reducing the area of pores available for diffusion would have differential effects on the movement of the two gases. As available pore area declined towards zero, so would RC_2H_6 increase towards infinity. In contrast, RCO_2 would increase towards an asymptotic value representative of the resistance of the epidermal cells and cuticular route.

Although there was a large variation in **R** values of individual apples within each variety, the close relationship between the two independent estimates of **R** that this was real fruit to fruit variation rather than measurement error. **R** largely depends on skin characteristics such as thickness of the peel, number and distribution of open lenticels, surface cracks and wax deposits. The skin characteristics are affected by both environmental and genetic factors (Padfield, 1969; Pratt, 1988). Differences in these characteristics among cultivars may have contributed to the large variation in **R** values of individual apples within each cultivar. Variation in **R** of apples, coupled with variation in respiratory rates, will lead to variation in the fruit's internal atmosphere composition which in turn will lead to variability in physiological response. The physiological significance of such variability in apples has been emphasised in

relation to the effects of CA/MA and coating treatments (Banks, 1985b, c; Burton, 1982; Cameron and Reid, 1982). Inherent differences in the resistance of both the skin and internal tissues to gas diffusion may affect the relative effects of CA/MA and coating treatments on O_2-dependent physiological processes (such as, respiration, C_2H_4 production etc.; Burton, 1982). Anatomical differences responsible for differing diffusion resistance, rather than biochemical differences among different fruits and vegetables, may be largely responsible for differences in tolerance to low-O_2 and high CO_2 in CA/MA storage (Burton, 1974). The findings of the current study concur with a more limited study report by Kidd and West (1949) which was based on data obtained with the steady state method.

R was found to differ between cultivars (figs. 4-1, 4-2, 4-3 and 4-4). Braeburn apples had the highest mean **R** compared to the other cultivars. Cultivar differences in **R** could be due to anatomical differences such as differences in size of intercellular spaces near the fruit surface; size, number and distribution of functional lenticels on the fruit surface and thickness and nature of wax deposits of the cuticle etc. As a result of the differences in **R** between apple cultivars, it would be expected that different cultivars would respond differently to CA/MA treatment. These findings are in line with similar findings reported by other researchers including Cameron (1982), Knee (1991), Padfield, (1969), Rajapakse *et al.* (1990).

Cox's Orange Pippin apples had the highest mean respiration rate compared to the other cultivars, while Splendour apples had the lowest mean respiration rate. Rajapakse *et al.* (1990) working with two cultivars of apples, showed that the respiration of Cox's Orange Pippin was higher than Braeburn apples. Similar findings have also been reported by other investigators including Fidler and North (1967, 1971).

Respiration rate of a fruit is often regarded as an index of its rate of deterioration and hence storage potential (Blanke, 1991; Johnson and Ertan, 1983). Within a given fruit species, a high respiration rate is commonly associated with a short storage life. On this basis, it would be expected that a cultivar such as Cox's Orange Pippin apples with high respiration rate would have a higher rate of deterioration and shorter shelf-life than those cultivars such as Splendour with low respiration rate or those with intermediate respiration rates.

Approximate storage life of fruit of different cultivars of apples in air storage (Table 4-2; Personal communication, McLeod (1992); New Zealand Apple and Pear Marketing Board) broadly reflects the respiration rates of the various cultivars (fig. 4-6). It is significant to mention that in addition to respiration, other factors such as resistance to bruising damage, gas exchange characteristics, stage of maturity at harvest as well as adequate preharvest and postharvest handling techniques also affect the storage potential of a cultivar.

The high respiration rate coupled with the high $[CO_2]_i$ and intermediate RCO_2 of freshly harvested Cox's Orange Pippin apples may account for the cultivar's low storage potential, high susceptibility to CO_2 injury and high incidence of postharvests disorders such as core flush or internal browning. As a result of the high $[CO_2]_i$ and high respiration rate of Cox's Orange Pippin apples, it would be expected that exposure to a high CO_2 atmosphere could predispose this cultivar to CO_2 injury. The degree of injury however would depend upon temperature and physiological stage of maturity of the fruit.

Internal atmosphere composition of freshly harvested apples differed among cultivars. The difference in $[O_2]_i$, $[CO_2]_i$ and $[C_2H_4]_i$ of the various cultivars was related to the differences in **R** and fruit respiration rate. Stepwise regression analysis indicated that although both **R** and respiration contributed

Table 4-2. Approximate storage life of fruit of various apple cultivars in refrigerated air storage (McLeod, 1992; Personal communication, New Zealand Apple and Pear Marketing Board) together with ranking from 1 to 8 (ie. high to low) of cultivars based on mean respiration rates (see fig. 4-6).

Cultivar	**Storage life in weeks**	**Ranked respiration rates**
Cox's Orange Pippin	12	1
Red Delicious	18-26	2
Golden Delicious	18	3
Royal Gala	16	4
Gala	16	5
Braeburn	18-26	6
Granny Smith	26	7
Splendour	26	8

to the variation in internal atmosphere composition, **R** contributed more to the variability than respiration. High **R** affects gas movement across the fruit skin and results in lower $[O_2]_i$ and higher $[CO_2]_i$ and/or $[C_2H_4]_i$ for a given respiration rate and external atmosphere composition. Differences in intercellular space volume could contribute to the differences in internal atmosphere composition of the various cultivars. Furthermore, the presence or absence of open calyx could also provide explanation for the differences in internal atmosphere composition between cultivars. Variation in the diffusive properties of the fruit could result in different $[O_2]_i$ and $[CO_2]_i$ within fruit under the same external conditions (Knee and Farman, 1989).

In spite of their intermediate respiration rate, freshly harvested Braeburn apples contained higher $[CO_2]_i$ (similar in Cox's Orange Pippin) and lower $[O_2]_i$ than the other cultivars and this could be associated with their high **R** and low intercellular space volume. Exposing apple cultivars with high **R** and/or high respiration rate, high $[CO_2]_i$ and low $[O_2]_i$ to low-O_2 or high CO_2 regimes or elevated temperatures could predispose the cultivar to various postharvest disorders such as internal browning, CO_2 toxicity or low O_2 injury. Splendour apples on the other hand contained higher $[O_2]_i$ and lower $[CO_2]_i$ compared to the other cultivars. This could presumably be due in part to the fact that fruit of this cultivar often have an open calyx. The possibility that open calyx contributed to this effect was supported by the observation that during internal atmosphere sampling, no resistance to penetration of the syringe into the core cavity (via the calyx) was felt in any of the Splendour apples used in this study. The high $[O_2]_i$ and low $[CO_2]_i$ of Splendour apples could also be linked to their relatively thin skin, numerous visible lenticels, high intercellular space volume and hence easier gas diffusion into and out of the fruit. These variables together with the low respiration rate of Splendour apples provide some explanation for the high storage potential of this cultivar. However it should be mentioned that one important disadvantage of this cultivar is that it is highly susceptible to bruising damage.

There were no significant relationships between $[O_2]_i$ or $[CO_2]_i$ and respiration rate (in a combined data set for all cultivars), suggesting that variation in fruit respiration rate at this one temperature (ie. 20°C) was not an overriding factor in determining $[O_2]_i$ and/or $[CO_2]_i$ of freshly harvested apples and vice versa. For instance Cox's Orange Pippin and Braeburn apples compared to the other six cultivars had higher $[CO_2]_i$ and lower $[O_2]_i$ (fig. 4-8) but then, although Cox's Orange Pippin apples had high rate of respiration (CO_2 production) that was not the case in Braeburn apples. The disparity could be due to anatomical differences as well as differences in **R**.

Internal C_2H_4 concentration is a good index of climacteric fruit maturity (Pratt *et al.*, 1977; Saltveit, 1982; Su *et al.*, 1984; Zagory and Kader, 1988). Based on their $[C_2H_4]_i$, most of the cultivars used in this study were either in the preclimacteric phase or just entering the climacteric stage of maturation, consequently they had not commenced rapid softening. Braeburn apples had the highest mean firmness compared to the other cultivars, while Cox's Orange Pippin had the lowest mean firmness. This may be related to the low intercellular space volume of Braeburn apples (approximately 14.1%; Cox's Orange Pippin 17.4%; Rajapakse *et al.*, 1990). Blanpied *et al.* (1978) showed that nitrogen fertilisation of apple trees decreased the firmness of apples at harvest and after four months storage. These authors also showed that the position of the fruit on the tree affects the firmness. Apples grown in exposed positions that receive much sunlight are generally firmer than well-shaded apples grown on the same tree. Splendour apples were also found to contain more soluble solids than the other cultivars whilst, Granny Smith apples were greener. Background colour (hue angle) measurements were highly cultivar dependent reflecting the differing distribution of the different coloured pigments on the greenest part of the fruit as well as providing some measure of within cultivar variation in maturity.

In conclusion estimates of RC_2H_6 and RC_2H_4 of individual apples using the ethane efflux and steady state methods respectively corresponded closely and provided consistent estimates of RC_2H_6 and RC_2H_4. However the former approach in comparison to the latter was easier to use and did not require that the system be at equilibrium. The efflux method enabled estimation of RC_2H_4 even in cultivars which were in the preclimacteric stage of maturity and not producing detectable quantities of C_2H_4. Estimates of RCO_2 and RC_2H_4 of apples estimated concurrently by the steady state method were different and the mean RCO_2 was consistently higher than RC_2H_4. There was large degree of variation in **R** values of individual apples within each cultivar. Cultivar differences in resistance to gas diffusion may be important in determining sensitivity to CA limits. Knowledge of **R** might be used in conjunction with other physiological data to predict optimum storage conditions for apples. However, when considering optimum CA storage conditions for apples, the combined influence of both the rates of fluxes and the resistance coefficients on internal atmosphere composition must be taken into account.

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CHAPTER 5

RELATIONSHIP BETWEEN APPLE RESPIRATION AND OXYGEN CONCENTRATION IN THE INTERNAL AND EXTERNAL ATMOSPHERES.

5.1 ABSTRACT

Oxygen uptake, CO_2 and C_2H_4 output by Cox's Orange Pippin and Granny Smith apples were characterised by studying variation in the magnitude of O_2, CO_2 and C_2H_4 concentration differences between the internal and external atmospheres ($\Delta[O_2]$, $\Delta[CO_2]$ and $\Delta[C_2H_4]$) of individual apples maintained in different O_2 atmospheres at 20±1°C.

$\Delta[O_2]$ was confirmed to decrease at low O_2 levels, reflecting the decreased rate of O_2 uptake in low O_2 concentrations. Two approaches to analysing these data yielded similar relationships between respiration rate and $[O_2]_i$. Oxygen uptake relative to that in air (***Rel***O_2) followed approximately Michaelis-Menten kinetics, with a half-maximal rate at 2.5% O_2 for internal O_2 ($[O_2]_i$) and 7.5% O_2 for external O_2 concentration ($[O_2]_{ext}$). The difference between the figures derives from the impact of $\Delta[O_2]$ on the availability of O_2 to fruit tissues. This underlines the importance of considering factors affecting the size of $\Delta[O_2]$ when setting limits for CA/MA storage.

A mathematical equation was developed to describe the two physiological processes (ie. anaerobic and aerobic respiration) involved in the relationship between relative rate of CO_2 production (***Rel***CO_2) or internal CO_2 concentration ($[CO_2]_i$) and $[O_2]_{ext}$ or $[O_2]_i$. The equation had two components, each describing one of the two physiological processes.

Fruit contained similar amounts of acetaldehyde (AA) irrespective of $[O_2]_{ext}$. On the other hand, ethanol (ETOH) was only detectable when $[O_2]_{ext}$ was kept at zero.

The relationship between relative rate of C_2H_4 production (***Rel***C_2H_4) or internal C_2H_4 concentration ($[C_2H_4]_i$) and $[O_2]_{ext}$ was sigmoidal. This contrasted with their relationship with $[O_2]_i$ which lacked the apparent 'lag phase' seen in plots against $[O_2]_{ext}$ and were described by an exponential hyperbola. These observations were used as a basis for discussion of the relative affinities of the oxidases involved in C_2H_4 production and respiration.

5.2 INTRODUCTION

Depression of respiration rate induced by low-O_2 concentrations is one of the fundamental effects thought to link reduction of O_2 availability to the slowing of a fruit's physiological deterioration achieved by CA storage. Identifying the relationship between respiration rate and O_2 concentration has therefore been a critical area of research in postharvest physiology since the pioneering work on MAs by Kidd and West (Knee, 1991). Successful characterisation of this relationship would greatly facilitate modelling work aimed at optimising MA packages published recently (Cameron, 1985; Cameron *et al.*, 1989; Emond *et al.*, 1991; Lakin, 1987; Wade and Graham, 1987) because it would permit prediction of modified rates of uptake under a changing atmospheric regime.

The O_2 concentration to which the tissues respond most directly is that in the cell sap ($[O_2]_{cs}$), which is in equilibrium with the O_2 concentration in the fruit's internal atmosphere ($[O_2]_i$). Measuring $[O_2]_{cs}$ is difficult and prone to artifacts (Burton, 1974, 1982; Ben-Yehoshua and Cameron, 1988) but respiration (review of fruit respiration is presented in chapter 2) can be studied as a function of either $[O_2]_{ext}$ or $[O_2]_i$. Studies with $[O_2]_{ext}$ are simpler to

undertake and are most readily applicable to the empirical design of packages. Unfortunately, fruit responses to lowered $[O_2]_{ext}$ can vary widely, depending on crop type and physiological condition as shown for avocados (Tucker and Laties, 1985) and for freshly harvested versus coolstored pears (Boersig *et al.*, 1988). This may be caused by variability of individual fruit skin resistance to gas diffusion (**R**, s cm^{-1}; Burton, 1982). It is this variability, coupled with respiration rate, which determines the difference between the internal and external atmospheres of the fruit ($\Delta[O_2]$) and which therefore determines the $[O_2]_i$ for a given $[O_2]_{ext}$.

This suggests that if the variation in **R** could be eliminated by examining the respiratory response of the tissues to $[O_2]_i$, rather than $[O_2]_{ext}$ then some of the variability in plots of respiration versus O_2 might be overcome. However, characterisation of the relationship between respiration versus $[O_2]_i$ in intact fruit has to date only been attempted indirectly via mathematical models (Andrich *et al.*, 1989, 1991; Banks *et al.*, 1989; Chevillotte, 1973; Solomos, 1985, 1988; Tucker and Laties, 1985) presumably because of the experimental difficulties (such as contamination of gas samples with air or blockage of syringe during internal atmosphere sampling) involved with concurrent monitoring of internal atmosphere and respiration rate in different $[O_2]_{ext}$. Using a combination of a non-invasive technique for monitoring internal atmospheres and the use of fruit immersion (in water) to prevent contamination of direct removal samples, the current research has characterised the respiratory and C_2H_4 production responses of two apple cultivars (Cox's Orange Pippin and Granny Smith) to reduced O_2 concentrations. This involved studying the variation in the magnitude of O_2 and CO_2 (and C_2H_4) concentration differences between the internal and external atmospheres of individual apples maintained in different O_2 atmospheres at 20±1°C.

5.3 Theoretical background

If it is assumed that the concentrations of the respiratory gases, O_2 and CO_2, inside any fruit are at equilibrium and that they are effectively homogeneous throughout the fruit (Andrich *et al.*, 1989, 1991), then their rates of flux between the internal and external atmospheres are governed by Fick's Law of diffusion (Burg and Burg, 1965). A simplified version of this is:

$$F = (A / R) * ([O_2]_{ext} - [O_2]_i) / 100 \qquad [5.1]$$

where

F = flux at steady state or respiration rate ($cm^3\ s^{-1}$)
A = fruit surface area (cm^2)
R = skin resistance to gas diffusion ($s\ cm^{-1}$)
$[O_2]_{ext}$ = external oxygen concentration (%)
$[O_2]_i$ = internal oxygen concentration (%)

If F is related to $[O_2]_{ext}$ or $[O_2]_i$ by the Michaelis-Menten equation ie.

$$F = \frac{F_{max} * [O_2]_i}{K_m + [O_2]_i} \qquad [5.2]$$

where

F_{max} = maximum rate of exchange when O_2 is saturating ($cm^3\ O_2\ s^{-1}$)
K_m = Michaelis-Menten constant for O_2 (%)

then it is possible to express $[O_2]_i$ as a function of $[O_2]_{ext}$ by rearranging and solving the following equation, obtained by substituting equation [5.2] into equation [5.1]:

$$[O_2]_i = [O_2]_{ext} - \frac{\mathbf{R}}{\mathbf{A}} * \frac{F_{max} * [O_2]_i}{K_m + [O_2]_i} \qquad [5.3]$$

As $[O_2]_i$ cannot be a negative number, only the following solution is acceptable:

$$[O_2]_i = (-(K_m + F_{max} * \mathbf{R/A} - [O_2]_{ext}) + ((K_m + F_{max} * \mathbf{R/A} - [O_2]_{ext})^2 + 4 * K_m * [O_2]_{ext})^{0.5}) / 2 \qquad [5.4]$$

Whilst **R** has been shown to change during ripening under certain conditions (Wilkinson, 1965), the ratio of **A/R** is likely to remain approximately constant over short periods of time. Thus, for any given fruit studied for a short period, equation [5.1] may be rewritten as:

$$F = k'_f * \Delta C \qquad [5.5]$$

where

k'_f = a fruit constant (=**A/R**) ($cm^3\ s^{-1}$)

ΔC = difference in gaseous concentration between the external and internal atmospheres (%)

Thus, a relative measure of the effects of different CA mixtures on the gas exchange of a fruit can be obtained simply by studying variation in ΔC in these mixtures. For each fruit, it is possible to quantify the rate of exchange for a particular gas under CA relative to its rate of exchange in air as a ratio of the two:

$$RelE = F_{CA}/F_{air} = (\Delta C_{CA}) / (\Delta C_{air}) \qquad [5.6]$$

where

$RelE$ = relative rate of exchange

F_{CA} = flux in CA at steady state ($cm^3\ s^{-1}$)

F_{air} = flux in air at steady state ($cm^3\ s^{-1}$)

ΔC_{CA} = difference in gaseous concentration between the external and internal atmospheres of a fruit in CA (%)

ΔC_{air} = difference in gaseous concentration between the external and internal atmospheres of a fruit in air (%)

Since this is a relative rather than an absolute measure of the process, an alternative notation for constants involved (ie. rK_m = relative Michaelis-Menten constant and rF_{max} = maximum relative respiration rate) can be used:

$$RelE = \frac{rF_{max} * [O_2]_i}{rK_m + [O_2]_i} \qquad [5.7]$$

where

rF_{max} = maximum relative rate of exchange when $[O_2]_i$ is saturating ($cm^3\ O_2\ s^{-1}$)

rK_m = Michaelis-Menten constant for $[O_2]_i$ (%) for the relative rate

The relationship between $RelCO_2$ and $[O_2]_{ext}$ or $[O_2]_i$, involves two physiological processes, anaerobic and aerobic respiration. A mathematical equation was developed to describe the overall rate of CO_2 production in different O_2 atmospheres:

$$RelCO_2 = [1 / (([O_2] + a)^b)] + \frac{c * [O_2]}{d + [O_2]} \qquad [5.8]$$

The first part of the equation describes a curve which declines with increasing O_2 concentration ($[O_2]$) at a rate determined by the values of a and b, two parameters of the equation. This describes the anaerobic contribution to total CO_2 production. The second part describes the aerobic contribution to CO_2 production using a Michaelis-Menten equation.

5.4 MATERIALS AND METHODS

5.4.1 Fruit supply

Freshly harvested apples (***Malus domestica*** Borkh.) of two cultivars, Cox's Orange Pippin and Granny Smith (count 125; av. weight 148 g) were

obtained as previously described in 3.1. Fruit were stored in air at 0°C until needed. Fruit were removed from storage and held at room temperature for at least 24h before experimentation to ensure complete temperature equilibrium.

5.4.2 Gas measurement and analysis

A 2 ml (9mm diameter) glass vial, from which the bottom had been removed was stuck, using polyvinyl acetate adhesive, onto the equatorial surface (see fig. 5-1) of thirty-six fruit of each cultivar. Sub-epidermal internal gas concentrations were estimated to be the same as the equilibrated gas concentrations in the glass chambers after 40 to 90h (see appendix 1) at 20±1°C in air (Banks and Kays, 1988). Each fruit was individually placed on a tray (in the dark) which was connected to a flow through system (flow rate of approximately 80 ml. min^{-1}) which gave various O_2 concentrations ranging from 0 to 20%. Precision needle valves were used to mix air and nitrogen to the required atmospheres, which were bubbled through water to give a relative humidity of approximately 98%. The required gas concentrations were verified at least three times daily by analysis of gas samples as previously described in section 3.3. After 40 to 90h of equilibration (see appendix 1) of the atmosphere in the glass vial with the fruit sub-epidermal internal gas concentrations at 20±1°C, a gas tight syringe was used to take 90µl gas samples from each glass vial as well as from the tray's headspace and analysed for O_2 and CO_2 (see 3.3.1), C_2H_4 (see 3.3.2), AA and ETOH concentrations (see 3.3.3).

Immediately after sampling, each tray was submerged in water and fruit core cavity gas samples were taken by the direct sampling method (see 3.3.4.1) and analysed for O_2, CO_2, C_2H_4, AA and ETOH as previously described (see 3.3). As the difference between sub-epidermal and core cavity sample composition within each apple was small, internal O_2, CO_2 and C_2H_4 concentrations ($[O_2]_i$, $[CO_2]_i$ and $[C_2H_4]_i$) were estimated as the

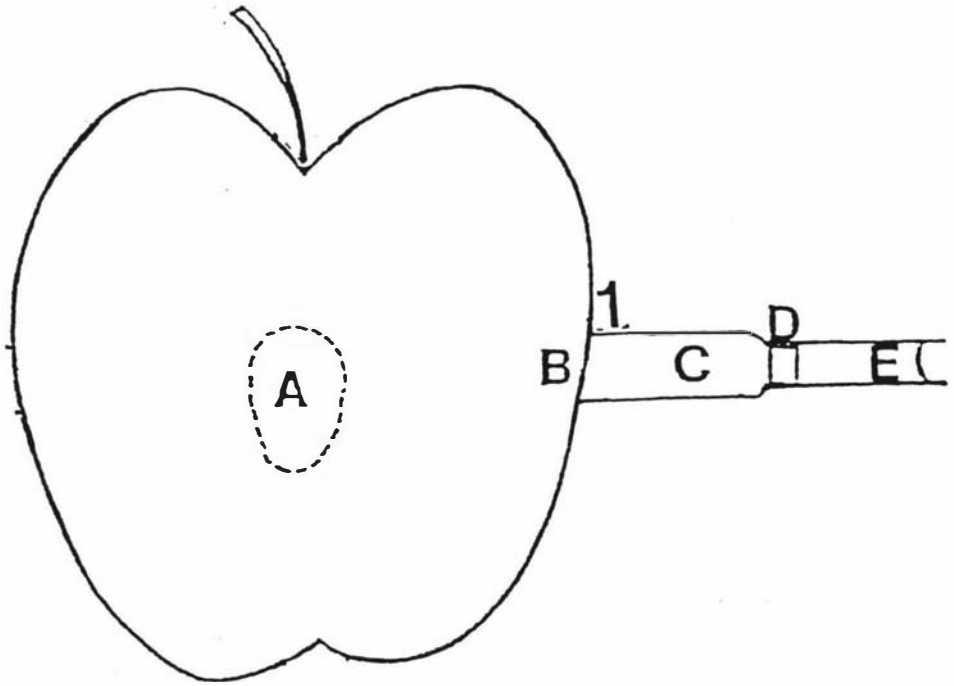

Fig. 5-1. ARRANGEMENT OF GLASS SAMPLING CHAMBER ON THE SURFACE OF AN APPLE FRUIT

A. Core cavity

B. Fruit surface

C. Glass chamber

D. Septum cap

E. Silicone tube with water

1. Equator

average of the sub-epidermal and core cavity gas samples. AA and ETOH were not detected in the core cavity of fruit hence, sub-epidermal concentrations ($[AA]_{se}$ and $[ETOH]_{se}$) are reported.

RelO_2, ***Rel***CO_2 and ***Rel***C_2H_4 were estimated from equation [5.6] and relative respiratory quotient (***Rel***RQ) was estimated as the ratio of ***Rel***CO_2 to ***Rel***O_2.

5.4.3 Experimental design and analysis

Thirty-six single fruit replicates of each cultivar were used in a completely randomised design. Experiments were repeated at least once. Data were analysed as previously described (3.3.9). Nonlinear regressions (Steel and Torrie, 1980) were performed using CGLE graphics and statistical package (version 3.2, 1991) and, in some cases, SAS.

5.5 RESULTS

5.5.1 $[O_2]_i$ as a function of $[O_2]_{ext}$

Assuming a fruit skin resistance of 9,000 and 10,200 s cm^{-1} for Cox's Orange Pippin and Granny Smith apples respectively (see chapter 4), the estimates of $[O_2]_i$ as a function of $[O_2]_{ext}$ obtained using equation [5.4] indicated that there was a nonlinear relationship between the two variables (fig. 5-2). The regression accounted for between 99.0% and 99.5 % of the total variation in $[O_2]_i$ in both cultivars. There was essentially a linear reduction in $[O_2]_i$ as $[O_2]_{ext}$ declined from that in air (20.95%) to about 6% $[O_2]_{ext}$. Below this, the slope of the relationship decreased progressively as $[O_2]_{ext}$ was reduced towards zero. The fitted line indicated that at 18% $[O_2]_{ext}$ the differences between the $[O_2]_{ext}$ and $[O_2]_i$ was about 5.0% for Cox's Orange Pippin and 3.7% for Granny Smith apples. At 2% $[O_2]_{ext}$ these differences

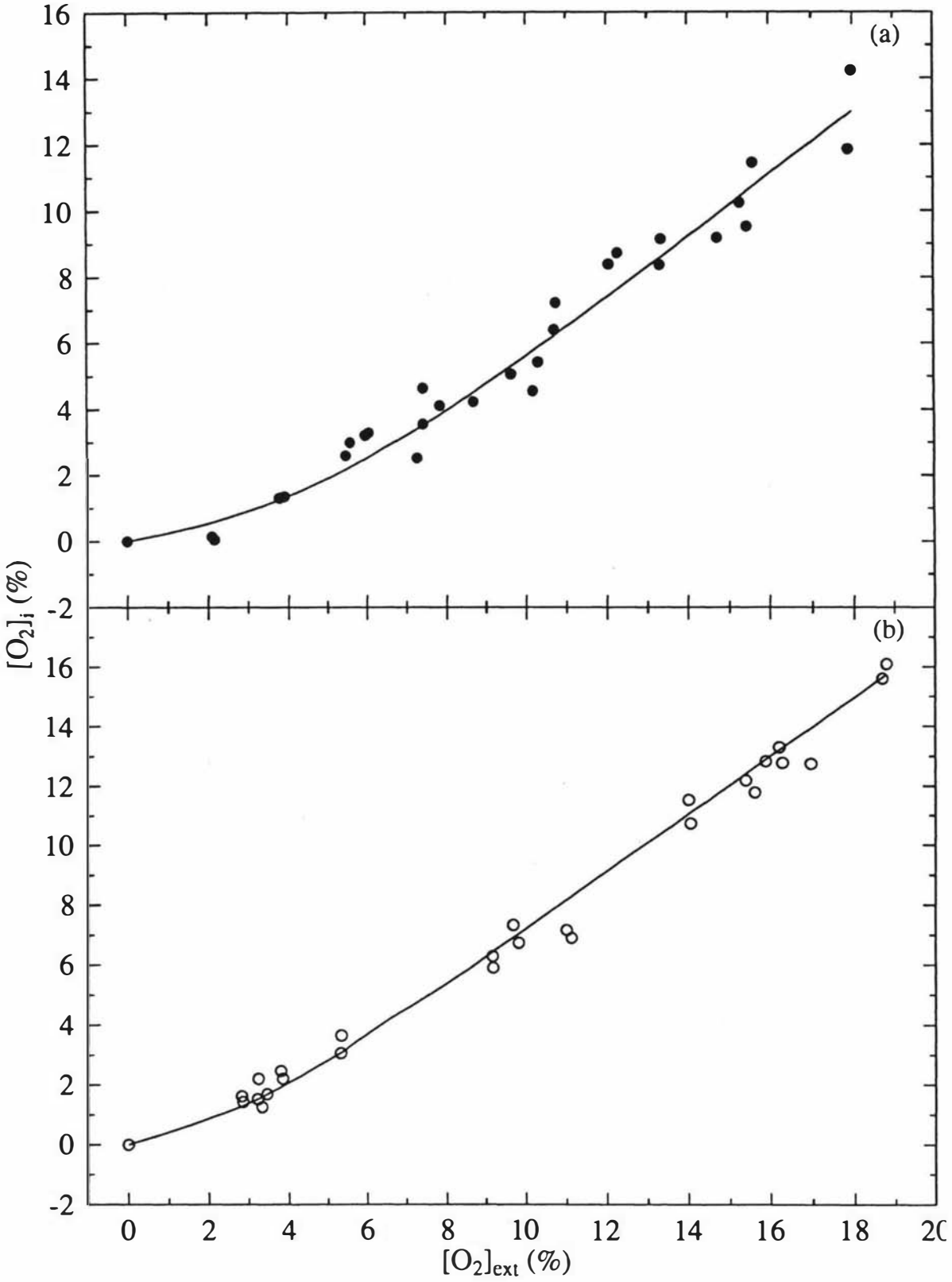

Fig. 5-2. Relationship between $[O_2]_i$ and $[O_2]_{ext}$ of individual (a) Cox's Orange Pippin (R^2=0.99) and (b) Granny Smith (R^2=0.995) apples kept at 20°C. Solid lines were fitted by nonlinear regression using equation [5.4].

had decreased to approximately 1.6% and 1.0% for the two cultivars, respectively. Although the apparent K_m estimated for Granny Smith (obtained using equation [5.4]; Table 5-1) was almost twice that for Cox's Orange Pippin apples the standard error of the Granny Smith value was high and the two did not differ significantly (at P = 0.05). Similarly there was no difference in the F_{max} of these cultivars.

Table 5-1. Apparent K_m and F_{max} values calculated using equation [5.4] for $[O_2]_i$ of Cox's Orange Pippin and Granny Smith apples as a function of $[O_2]_{ext}$.

Cultivar	K_m (%)	F_{max} ($cm^3\ s^{-1}\ fruit^{-1} \times 10^{-4}$)
Cox's Orange Pippin	2.2 ± 0.82	10.9 ± 1.05
Granny Smith	4.2 ± 1.68	7.5 ± 1.00

5.5.2 *Rel*O_2 as a function of $[O_2]_{ext}$ or $[O_2]_i$

Fruit relative rate of O_2 uptake (*Rel*O_2) estimated from equation [5.6] was significantly (P < 0.0001) influenced by $[O_2]_{ext}$ in both cultivars. *Rel*O_2 increased from zero (in an anaerobic atmosphere) towards a value approaching approximately one in air (fig 5-3). The data were closely described by Michaelis-Menten curves (R^2 = 0.98 and 0.94, respectively). Cox's Orange Pippin had higher rF_{max} values than Granny Smith apples (P < 0.05, Table 5-2) but the rK_m values for the two cultivars did not differ significantly (overall mean = 7.55 for rK_m and 1.37 for rF_{max}).

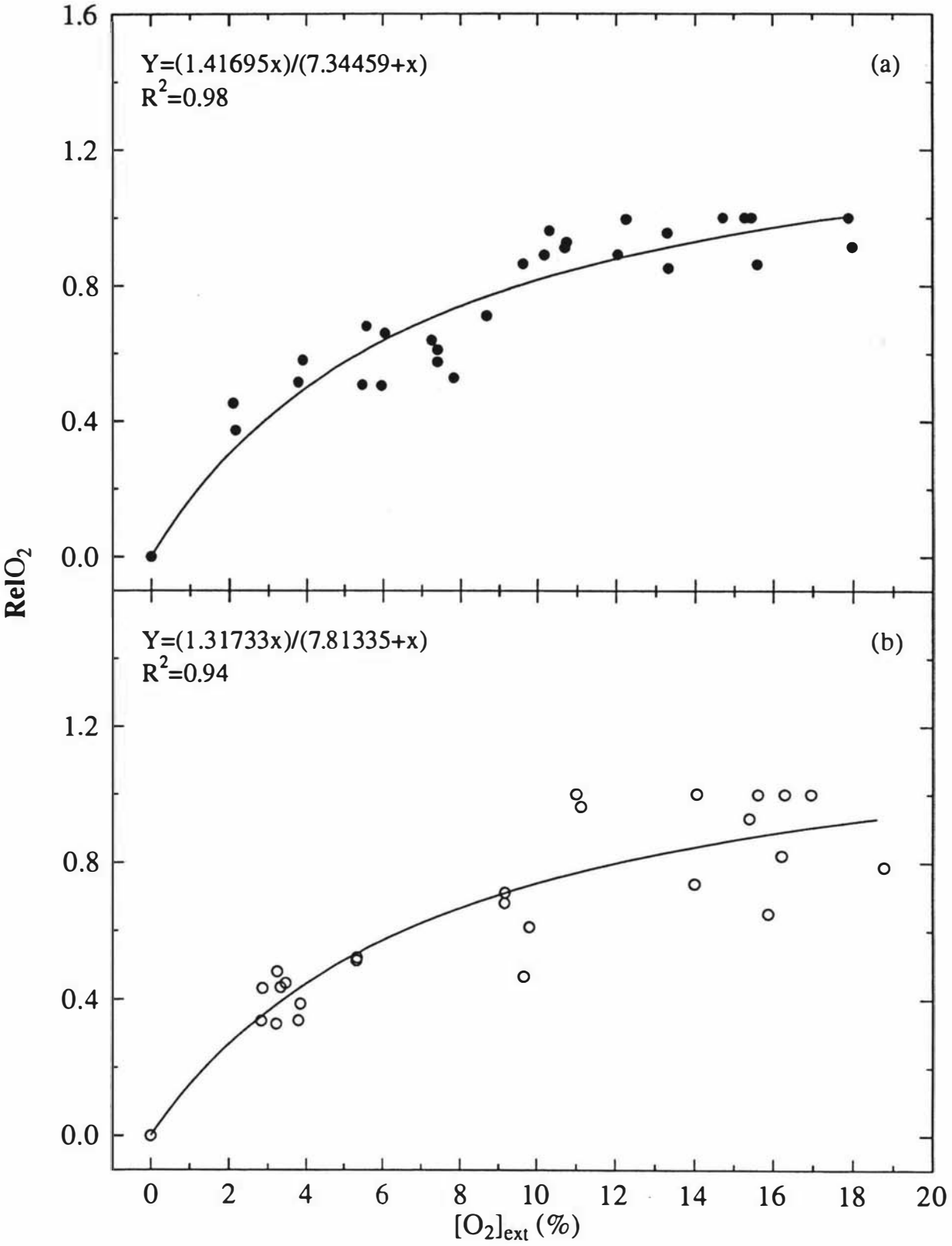

Fig. 5-3. Relationship between **Rel**O_2 and $[O_2]_{ext}$ of individual (a) Cox's Orange Pippin and (b) Granny Smith apples kept at 20°C. Solid lines were fitted by nonlinear regression using equation [5.7].

Table 5-2. rK_m and rF_{max} of equation [5.6] for $Rel O_2$ as a function of $[O_2]_{ext}$ or $[O_2]_i$ fitted by nonlinear regression for Cox's Orange Pippin and Granny Smith apples.

Cox's Orange Pippin	rK_m	rF_{max}
$[O_2]_{ext}$	7.3 ± 1.44	1.42 ± 0.116
$[O_2]_i$	2.1 ± 0.59	1.13 ± 0.088
Granny Smith	rK_m	rF_{max}
$[O_2]_{ext}$	7.8 ± 1.32	1.32 ± 0.150
$[O_2]_i$	2.9 ± 0.80	1.06 ± 0.091

The relationships between ***Rel***O_2 and $[O_2]_i$ followed a similar general form to those with $[O_2]_{ext}$ (fig. 5-4), although the slopes were steeper at low O_2 levels, as reflected in the lower rK_m values (Table 5-2). rK_m and rF_{max} values were similar for $[O_2]_i$ in both cultivars (overall mean = 2.5 and 1.10, respectively).

Average O_2 gradients across the flesh (ie. between the sub-epidermal and core cavity) were significant (0.306 ± 0.052 and 0.462 ± 0.064 for Cox's Orange Pippin and Granny Smith apples respectively). However the relationships between these gradients and $[O_2]_i$ were poorly defined (R^2 = 0.11, $P < 0.05$ and $R^2 = 0.37$, $P < 0.001$ for linear regression respectively) for Cox's Orange Pippin and Granny Smith apples (data not shown).

5.5.3 ***Rel***CO_2 or $[CO_2]_i$ as a function of $[O_2]_{ext}$ or $[O_2]_i$

The relationships between relative rate of CO_2 production (***Rel***CO_2) or internal CO_2 concentration ($[CO_2]_i$) and $[O_2]_{ext}$ (figs. 5-5 and 5-6) were well described by fitted curves obtained by nonlinear regression using equation [5.8]. However, with the spread of data points around the fitted line, coupled with the complexity of the equation (ie. with 4 parameters), the parameter values cannot be claimed to have been accurately estimated in this study.

At zero percent $[O_2]_{ext}$, ***Rel***CO_2 or $[CO_2]_i$ of both Cox's Orange Pippin and Granny Smith apples increased markedly indicating that fruit were respiring anaerobically. Between 2% and 18% $[O_2]_{ext}$, ***Rel***CO_2 of Cox's Orange Pippin apples increased from approximately 0.5 to a maximum value of one, while $[CO_2]_i$ increased from about 3 to 7% over the same range of O_2 concentrations. In contrast in Granny Smith apples ***Rel***CO_2 increased from approximately 0.4 at $[O_2]_{ext}$ of approximately 3% to a maximum value of about 0.9, while $[CO_2]_i$ increased from about 1.5 to 3.5%.

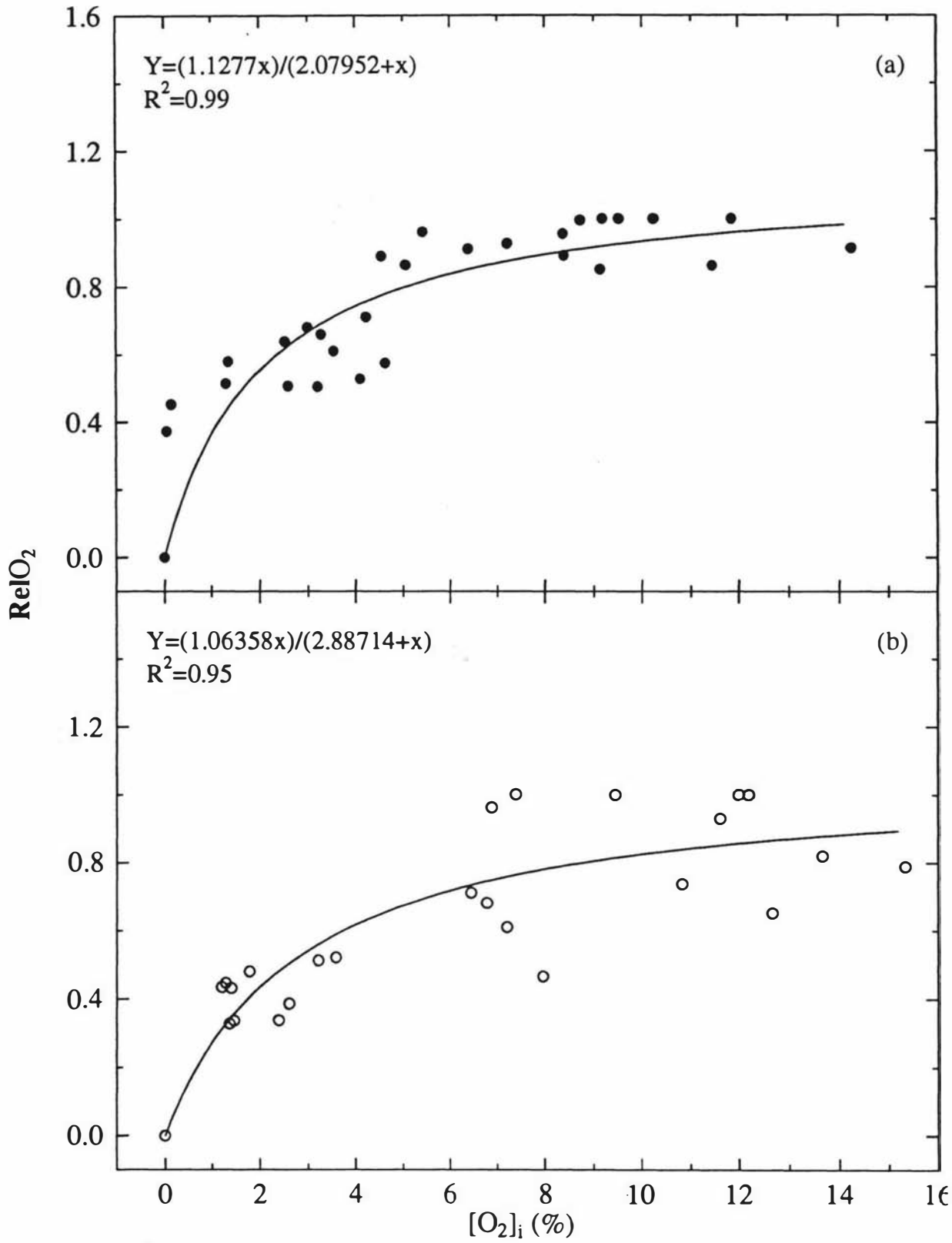

Fig. 5-4. Relationship between $\mathbf{Rel}O_2$ and $[O_2]_i$ of individual (a) Cox's Orange Pippin and (b) Granny Smith apples kept at 20°C. Solid lines were fitted by nonlinear regression using equation [5.7].

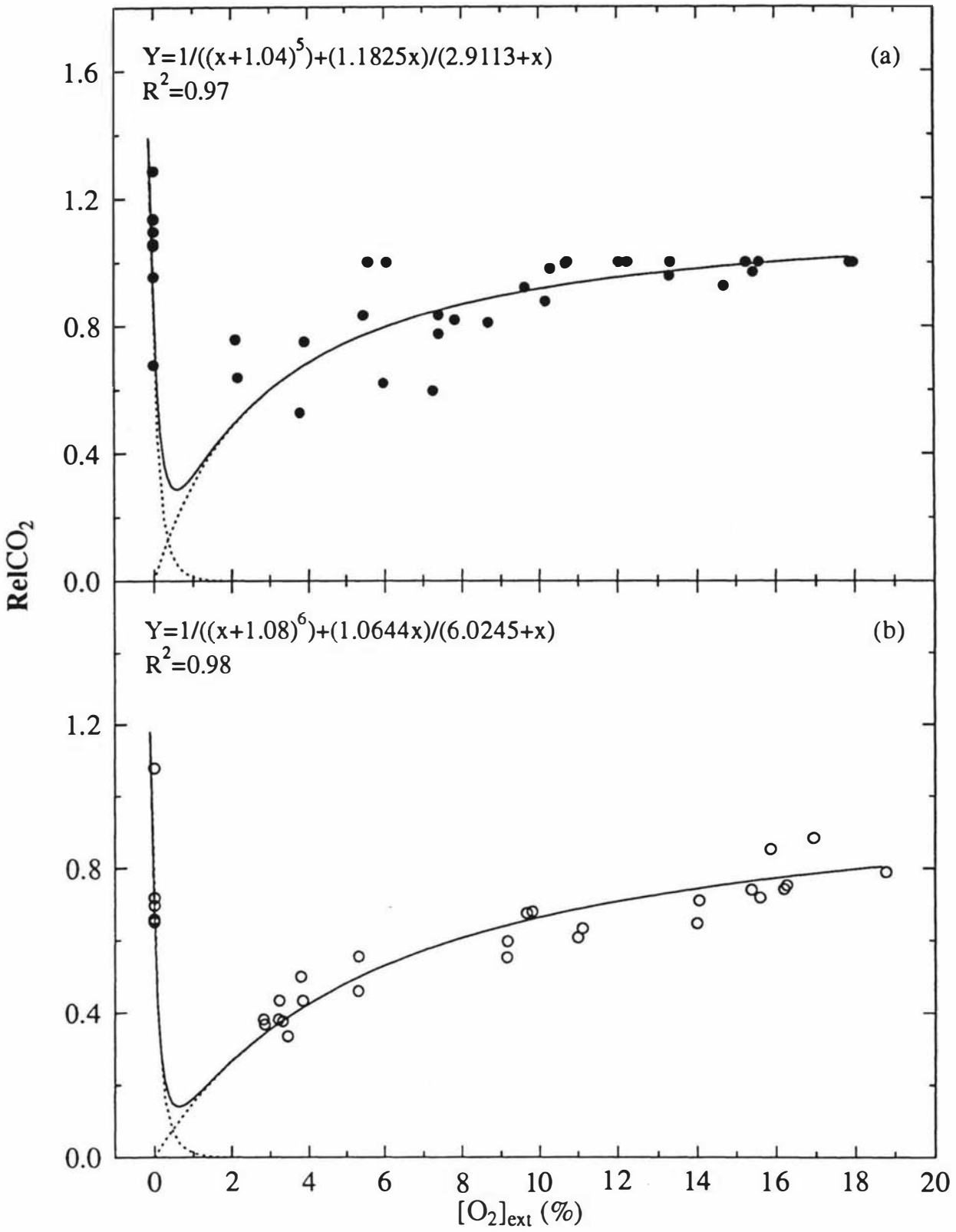

Fig. 5-5. Relationship between **Rel**CO_2 and $[O_2]_{ext}$ of individual (a) Cox's Orange Pippin and (b) Granny Smith apples kept at 20°C. Dotted lines represent individual anaerobic and aerobic components of the relationship. Solid lines describing the composite function were fitted by nonlinear regression using equation [5.8].

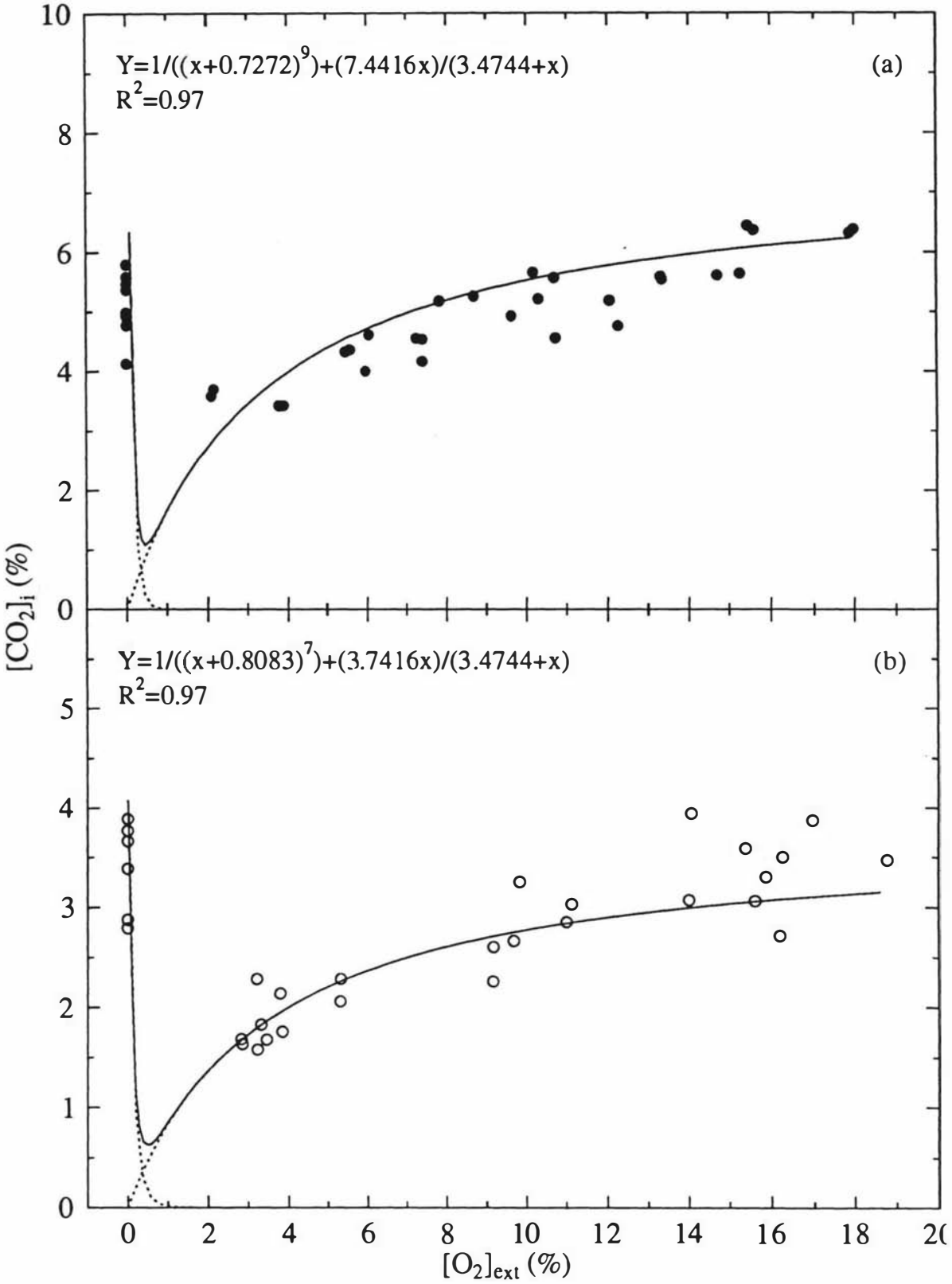

Fig. 5-6. Relationship between $[CO_2]_i$ and $[O_2]_{ext}$ of individual (a) Cox's Orange Pippin and (b) Granny Smith apples kept at 20°C. Dotted lines represent individual anaerobic and aerobic components of the relationship. Solid lines describing the composite function were fitted by nonlinear regression using equation [5.8].

The relationship between ***Rel***CO_2 or $[CO_2]_i$ and $[O_2]_i$ followed a similar general trend to those with $[O_2]_{ext}$ (figs. 5-7 and 5-8). However the shape of the aerobic phase of the curves relating ***Rel***CO_2 and $[O_2]_i$ was steeper (at lower O_2 levels) than with $[O_2]_{ext}$.

5.5.4 *Rel*RQ versus $[O_2]_{ext}$ or $[O_2]_i$

The relationship between relative respiratory quotient (***Rel***RQ) and $[O_2]_{ext}$ (fig. 5-9) was described by an exponential equation of the form:

$$\textit{Rel}RQ = a / [1 + b * \exp(-(c * [O_2]_{ext} + d * [O_2]_{ext} ** 2))] \qquad [5.9]$$

where a, b, c and d are constants of the equation.

RelRQs of Granny Smith apples were approximately constant between 18% and approximately 6% $[O_2]_{ext}$. Below approximately 6% $[O_2]_{ext}$, ***Rel***RQ increased in Granny Smith. In contrast, ***Rel***RQs of Cox's Orange Pippin apples increased continually from 18% $[O_2]_{ext}$ downwards, with the slope increasing towards the lower O_2 levels.

The relationship between ***Rel***RQ and $[O_2]_i$ followed a similar general form to those with $[O_2]_{ext}$ (fig. 5-10) and was described by equation [5.9]. Generally ***Rel***RQs in Cox's Orange Pippin were higher than in Granny Smith apples.

5.5.5 $[AA]_{se}$ or $[ETOH]_{se}$ versus $[O_2]_{ext}$ or $[O_2]_i$

In both Cox's Orange Pippin and Granny Smith apples neither $[O_2]_{ext}$ (fig. 5-11) nor $[O_2]_i$ (fig. 5-12) had a significant (P = 0.05) influence on sub-epidermal acetaldehyde ($[AA]_{se}$). On the other hand there was no detectable

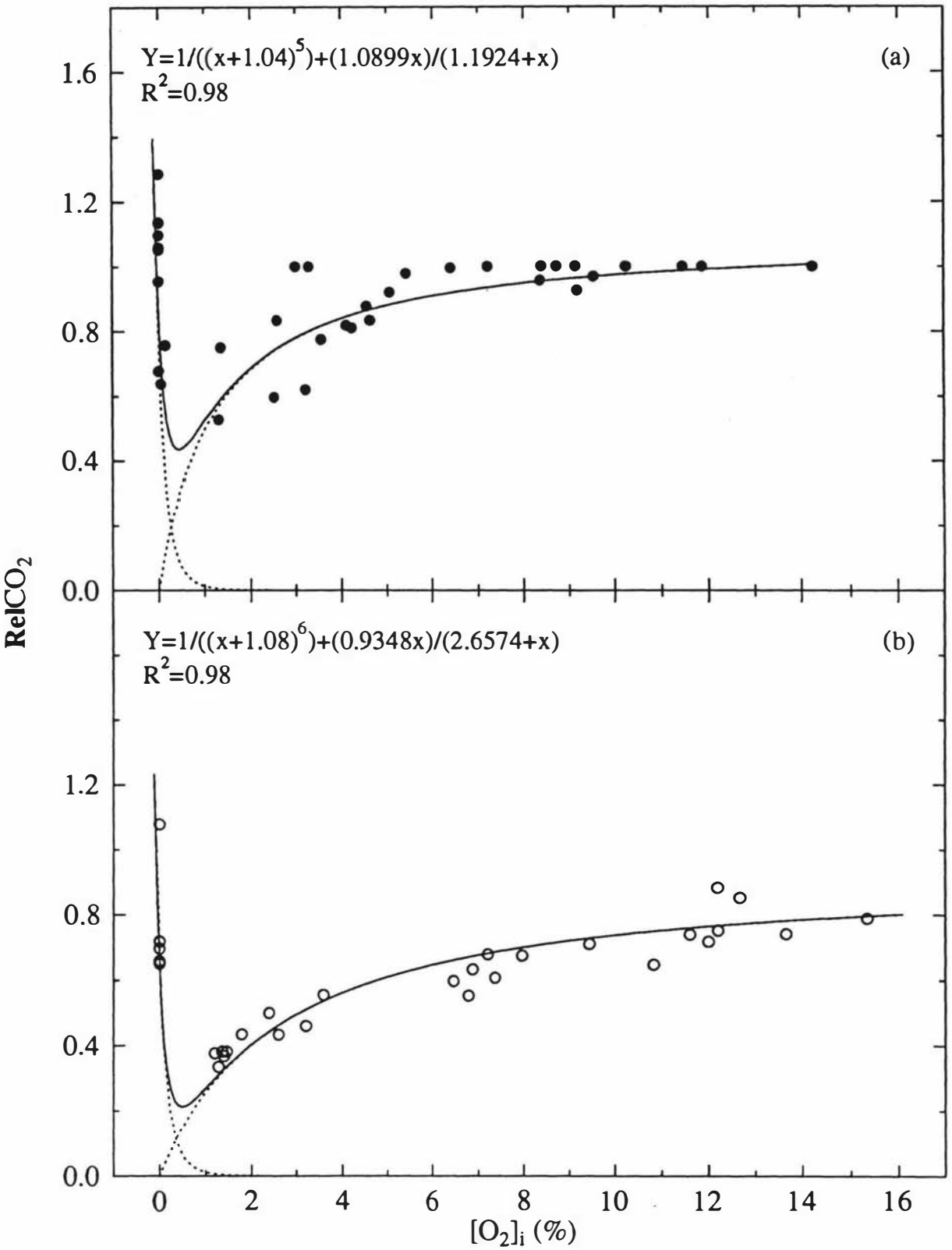

Fig. 5-7. Relationship between **Rel**CO_2 and $[O_2]_i$ of individual (a) Cox's Orange Pippin and (b) Granny Smith apples kept at 20°C. Dotted lines represent individual anaerobic and aerobic components of the relationship. Solid lines describing the composite function were fitted by nonlinear regression using equation [5.8].

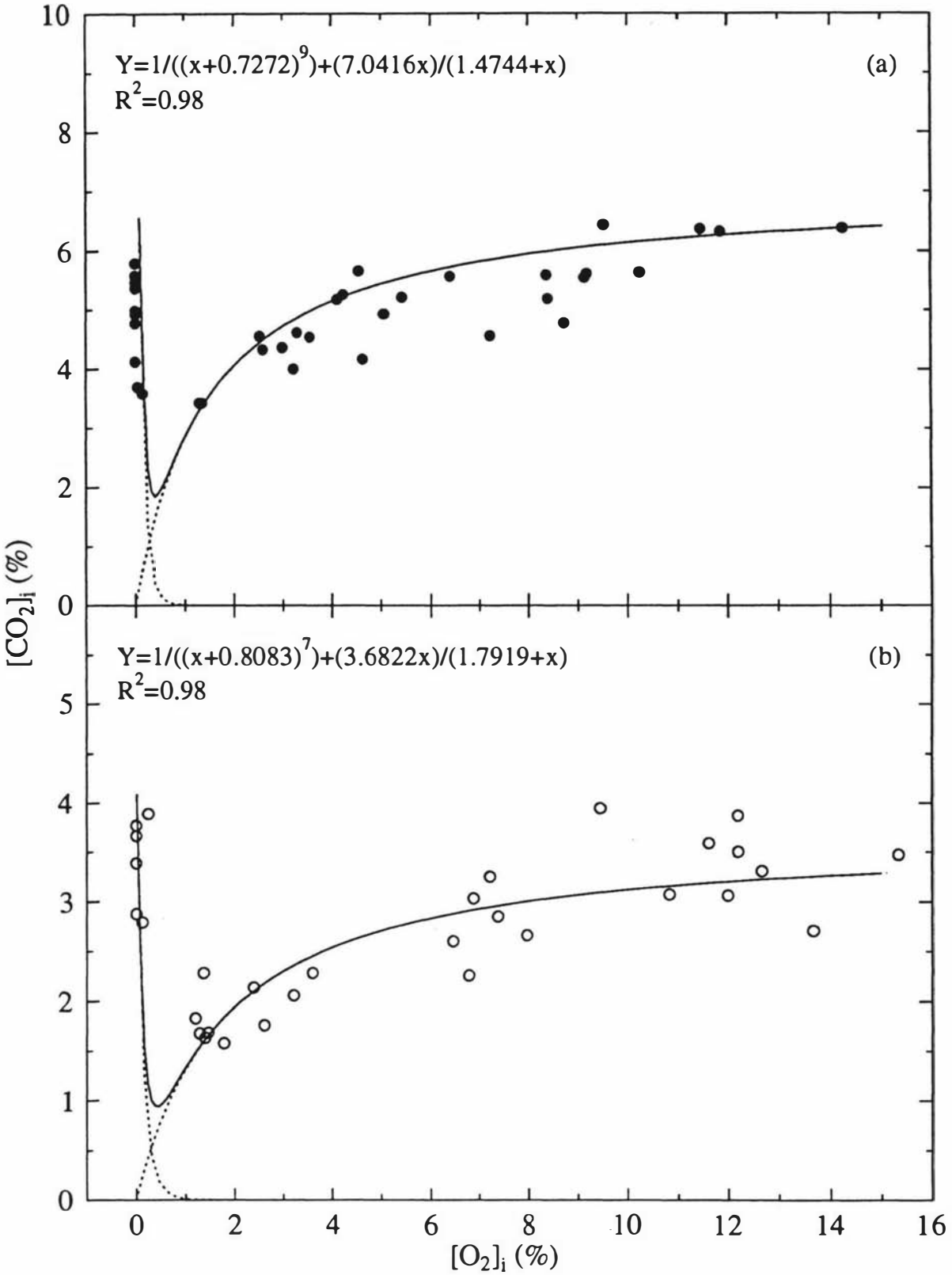

Fig. 5-8. Relationship between $[CO_2]_i$ and $[O_2]_i$ of individual (a) Cox's Orange Pippin and (b) Granny Smith apples kept at 20°C. Dotted lines represent individual anaerobic and aerobic components of the relationship. Solid lines describing the composite function were fitted by nonlinear regression using equation [5.8].

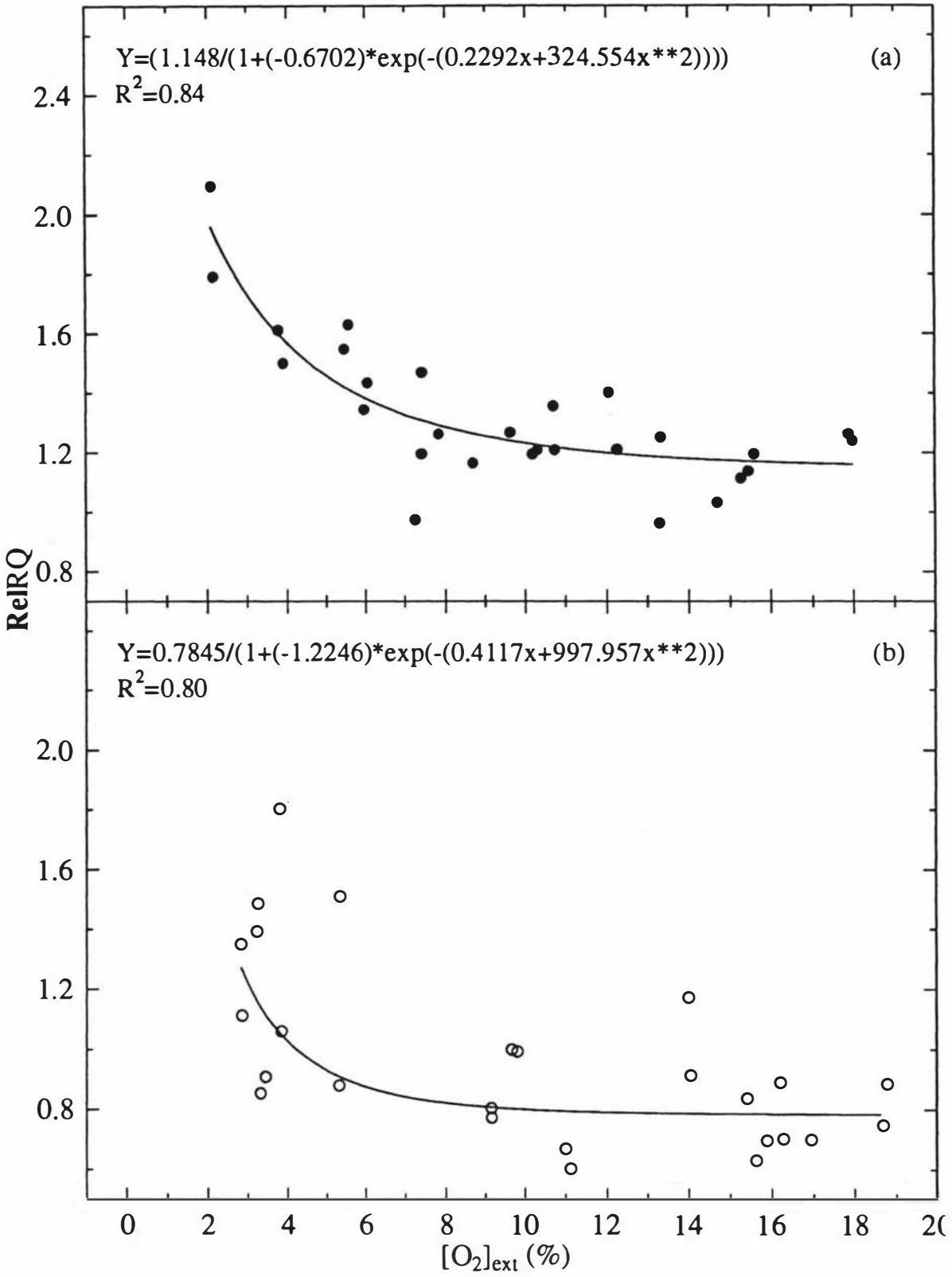

Fig. 5-9. Relationship between **Rel**RQ and $[O_2]_{ext}$ of individual (a) Cox's Orange Pippin and (b) Granny Smith apples kept at 20°C. Solid lines were fitted by nonlinear regression using equation [5.9].

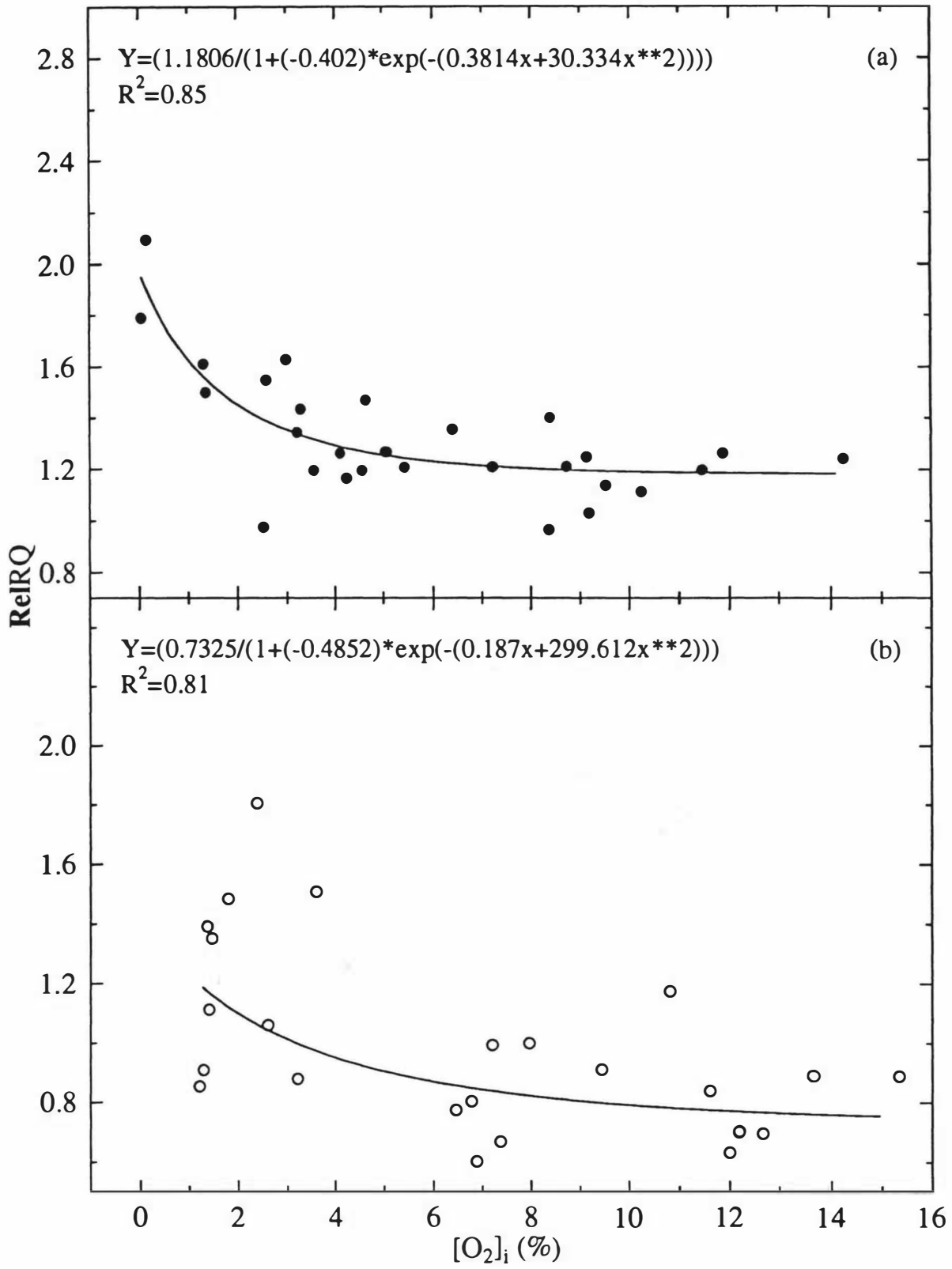

Fig. 5-10. Relationship between **Rel**RQ and $[O_2]_i$ of individual (a) Cox's Orange Pippin and (b) Granny Smith apples kept at 20°C. Solid lines were fitted by nonlinear regression using equation [5.9].

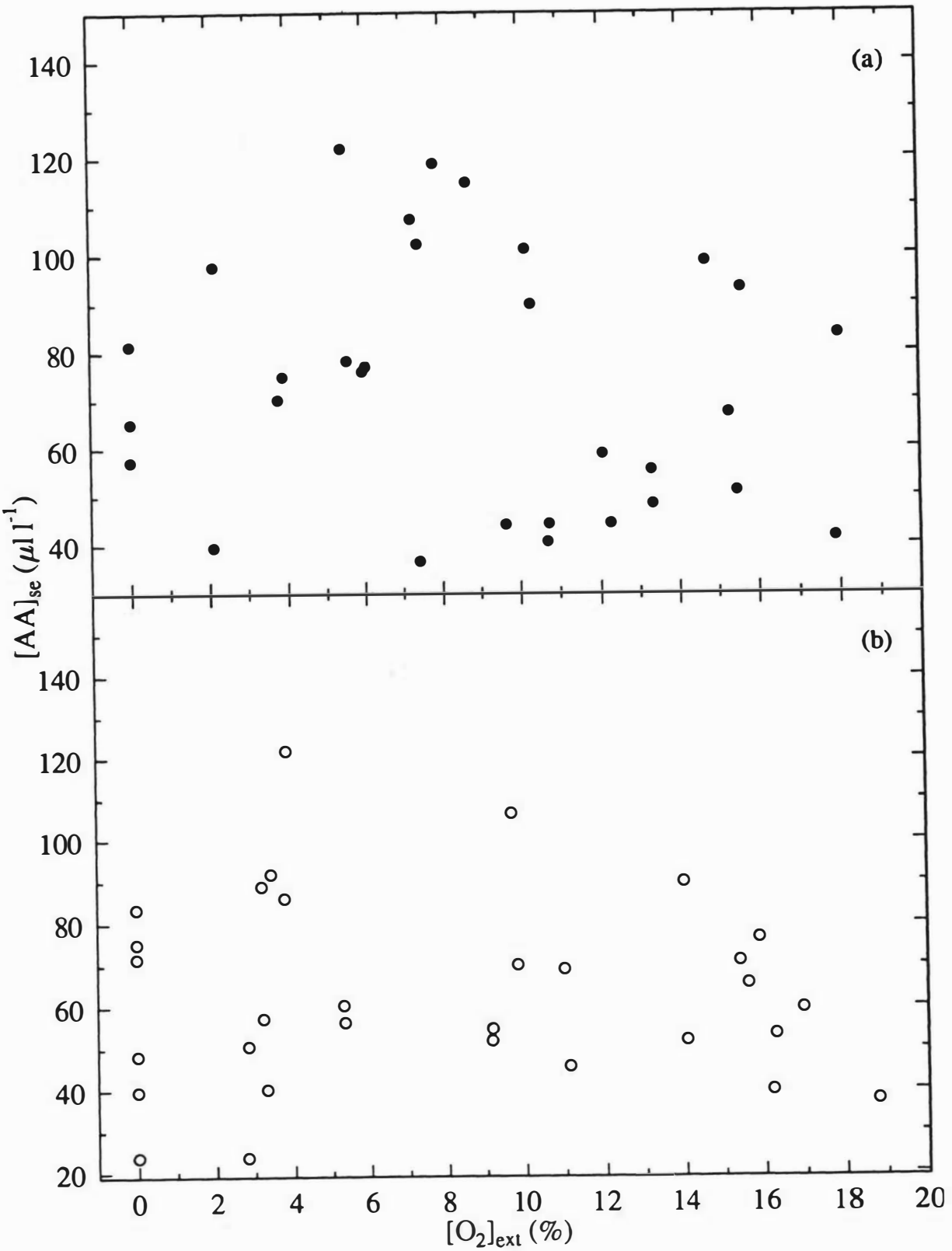

Fig. 5-11. Relationship between $[AA]_{se}$ and $[O_2]_{ext}$ of individual (a) Cox's Orange Pippin and (b) Granny Smith apples kept at 20°C.

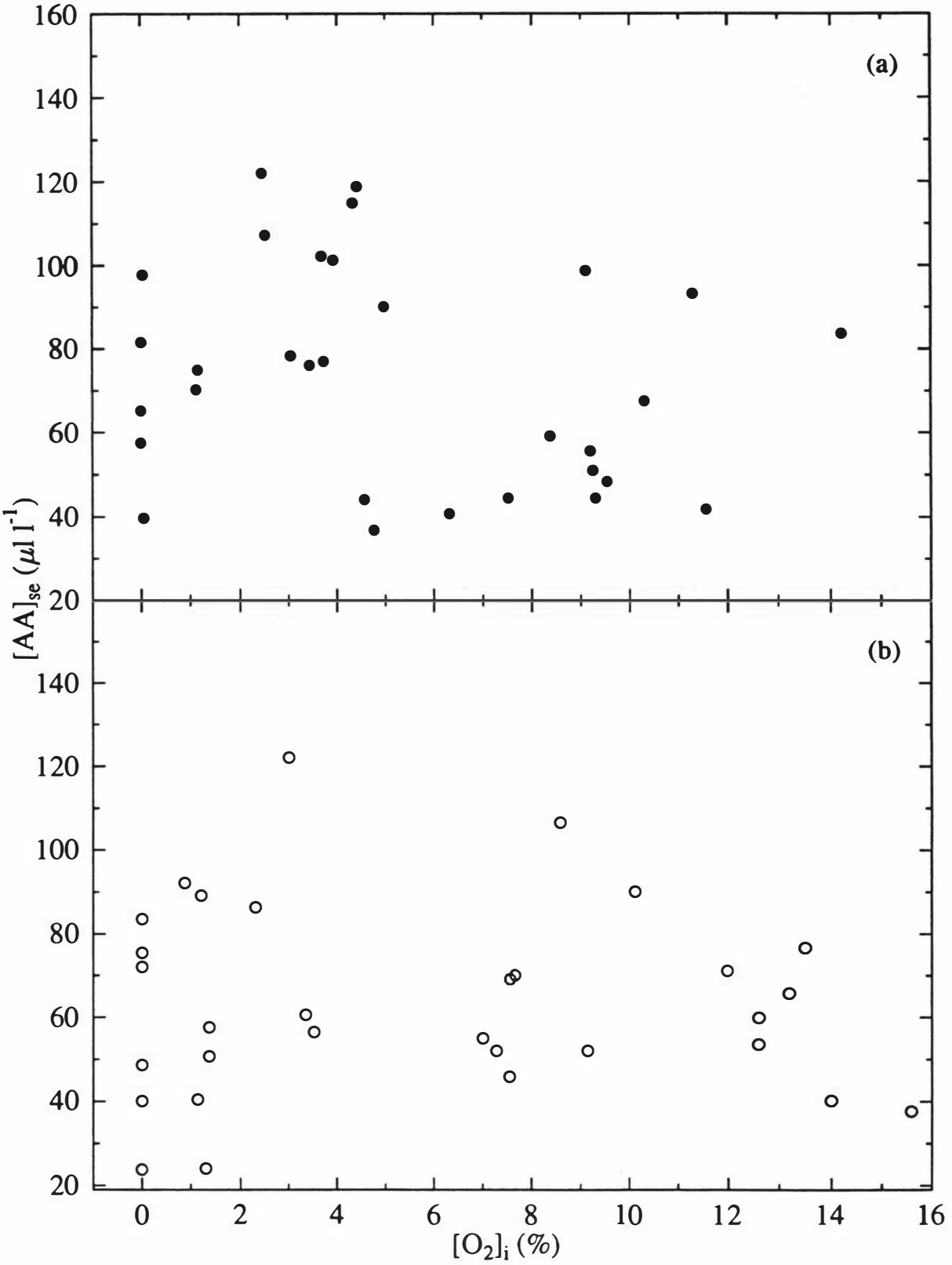

Fig. 5-12. Relationship between $[AA]_{se}$ and $[O_2]_i$ of individual (a) Cox's Orange Pippin and (b) Granny Smith apples kept at 20°C.

sub-epidermal ethanol ($[ETOH]_{se}$) except in fruit kept in a zero % $[O_2]_{ext}$ (fig. 5-13). A plot of $[ETOH]_{se}$ versus $[O_2]_i$ (fig. 5-14) showed similar relationship to those with $[O_2]_{ext}$. Surprisingly, neither AA nor ETOH was detectable in the core cavity of fruit of Cox's Orange Pippin and Granny Smith apples irrespective of the levels of $[O_2]_{ext}$ or $[O_2]_i$.

5.5.6 *Rel*C_2H_4 or $[C_2H_4]_i$ as a function of $[O_2]_{ext}$ or $[O_2]_i$

Fruit relative rates of C_2H_4 production (*Rel*C_2H_4) and internal C_2H_4 concentration ($[C_2H_4]_i$) were significantly ($P < 0.0001$) influenced by the level of O_2 concentration and differed between the two cultivars. A plot of the relationship between *Rel*C_2H_4 or $[C_2H_4]_i$ and $[O_2]_{ext}$ (figs. 5-15 and 5-16) was well described by a logistic type equation of the form:

$$Rel C_2H_4 = a / [1.0 + b * \exp(-c * [O_2]_{ext})] \qquad [5.10]$$

where a, b, and c are constants of the equation.

At zero percent $[O_2]_{ext}$, *Rel*C_2H_4 and $[C_2H_4]_i$ of both Cox's Orange Pippin and Granny Smith apples was zero. However, as $[O_2]_{ext}$ rose from 0 to about 2% there was a 'lag phase' during which there was little response of *Rel*C_2H_4 or $[C_2H_4]_i$ of Cox's Orange Pippin and Granny Smith apples to $[O_2]_{ext}$. However between approximately 2% and 6 or 8% $[O_2]_{ext}$ there was a phase of rapid increase in *Rel*C_2H_4 or $[C_2H_4]_i$ in response to increasing $[O_2]_{ext}$. Above approximately 8% $[O_2]_{ext}$, *Rel*C_2H_4 or $[C_2H_4]_i$ reached an asymptote.

The $[O_2]_{ext}$ value at which *Rel*C_2H_4 was half of the upper asymptote (of the fitted line) was approximately 4.2 and 3.1% for Cox's Orange Pippin and Granny Smith apples respectively.

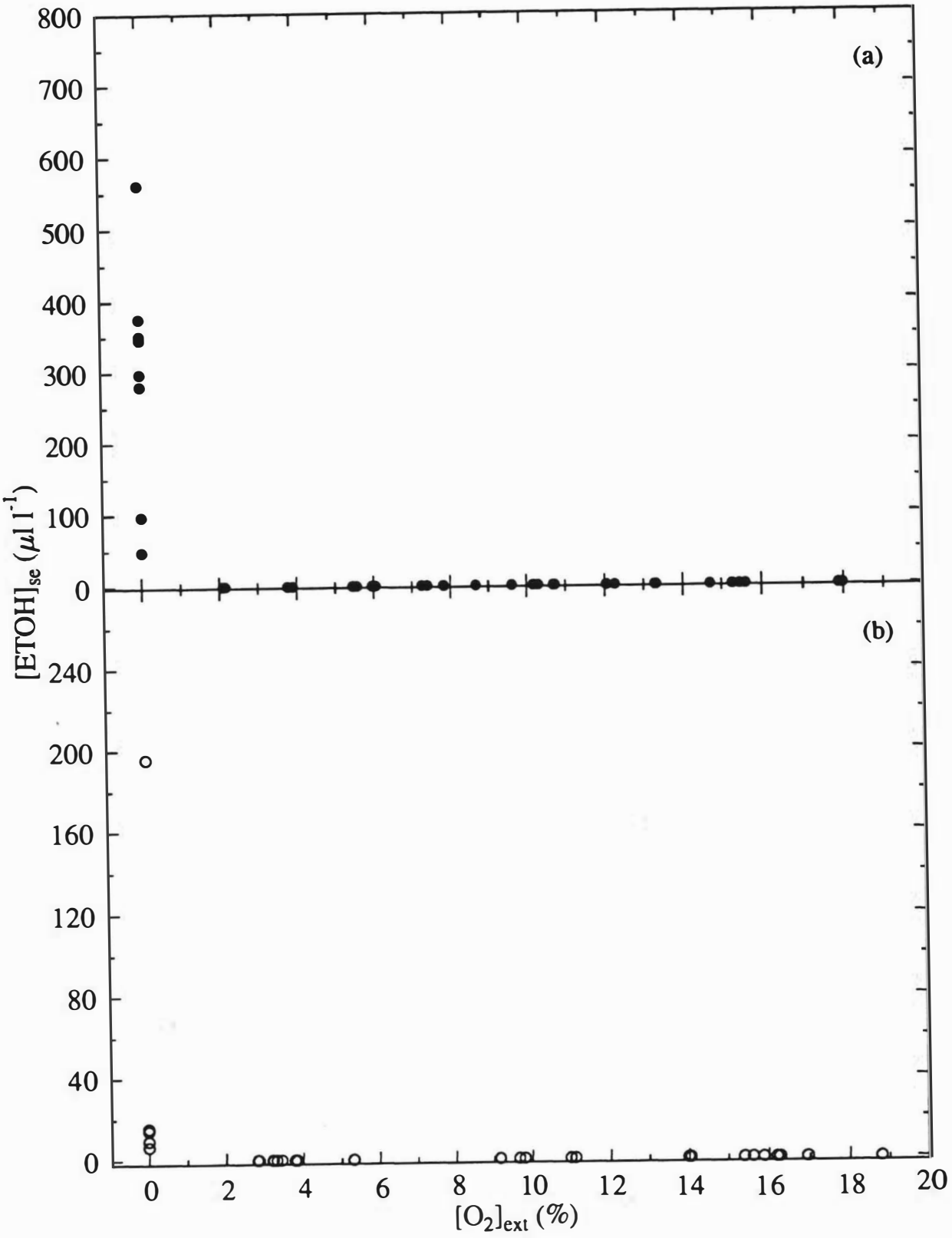

Fig. 5-13. Relationship between $[ETOH]_{se}$ and $[O_2]_{ext}$ of individual (a) Cox's Orange Pippin and (b) Granny Smith apples kept at 20°C.

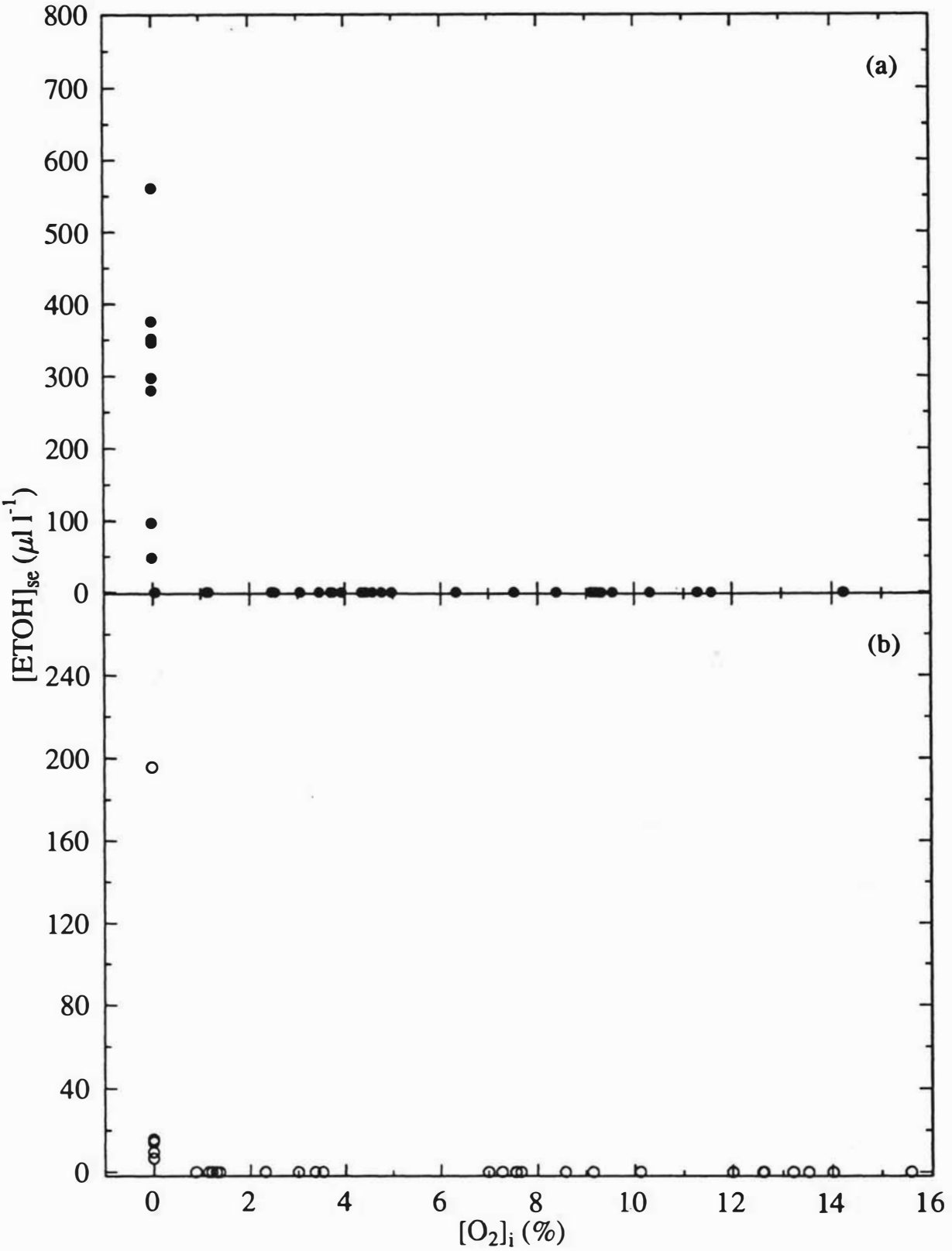

Fig. 5-14. Relationship between $[ETOH]_{se}$ and $[O_2]_i$ of individual (a) Cox's Orange Pippin and (b) Granny Smith apples kept at 20°C.

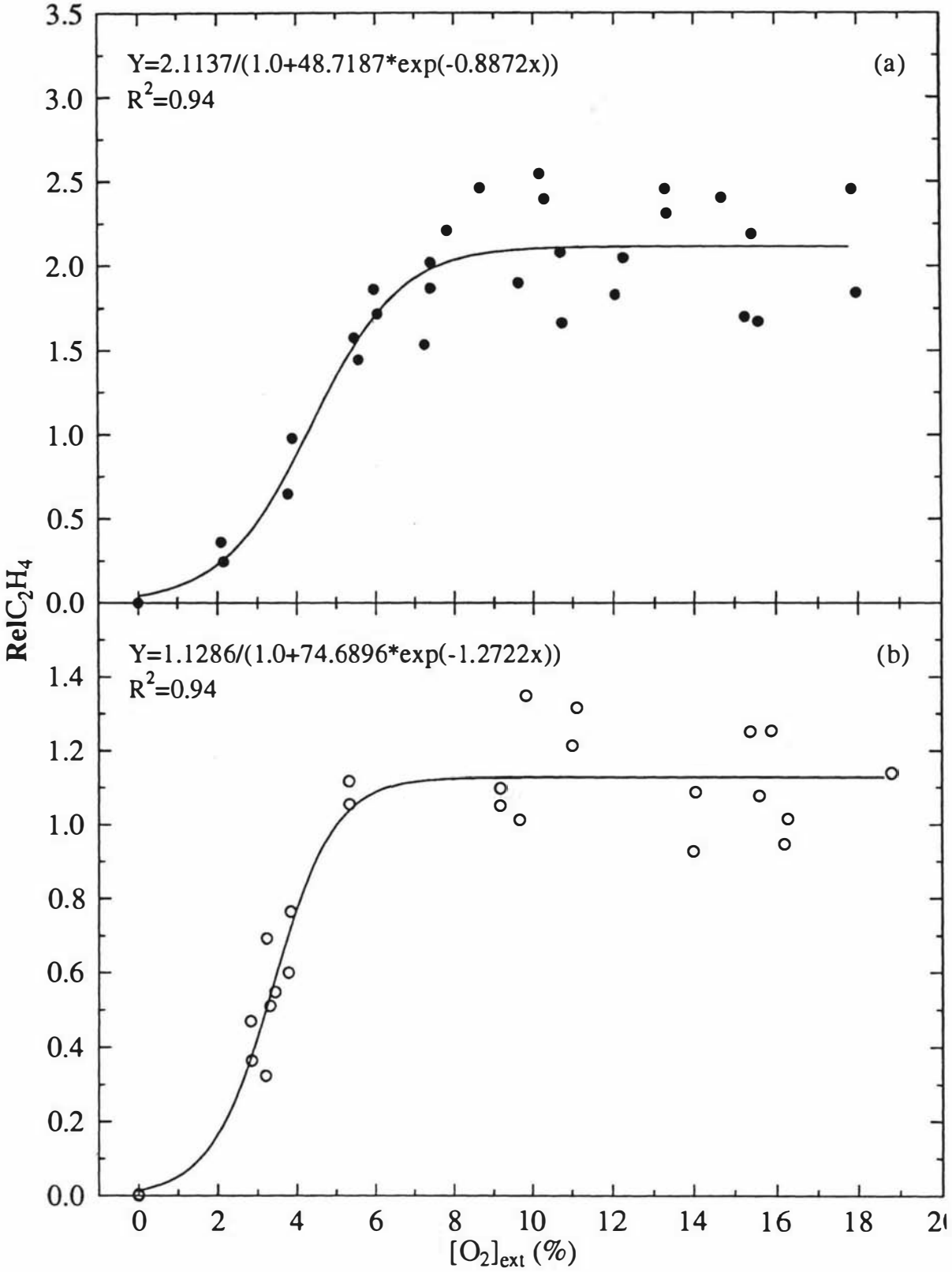

Fig. 5-15. Relationship between **Rel**C_2H_4 and $[O_2]_{ext}$ of individual (a) Cox's Orange Pippin and (b) Granny Smith apples kept at 20°C. Solid lines were fitted by nonlinear regression using equation [5.10].

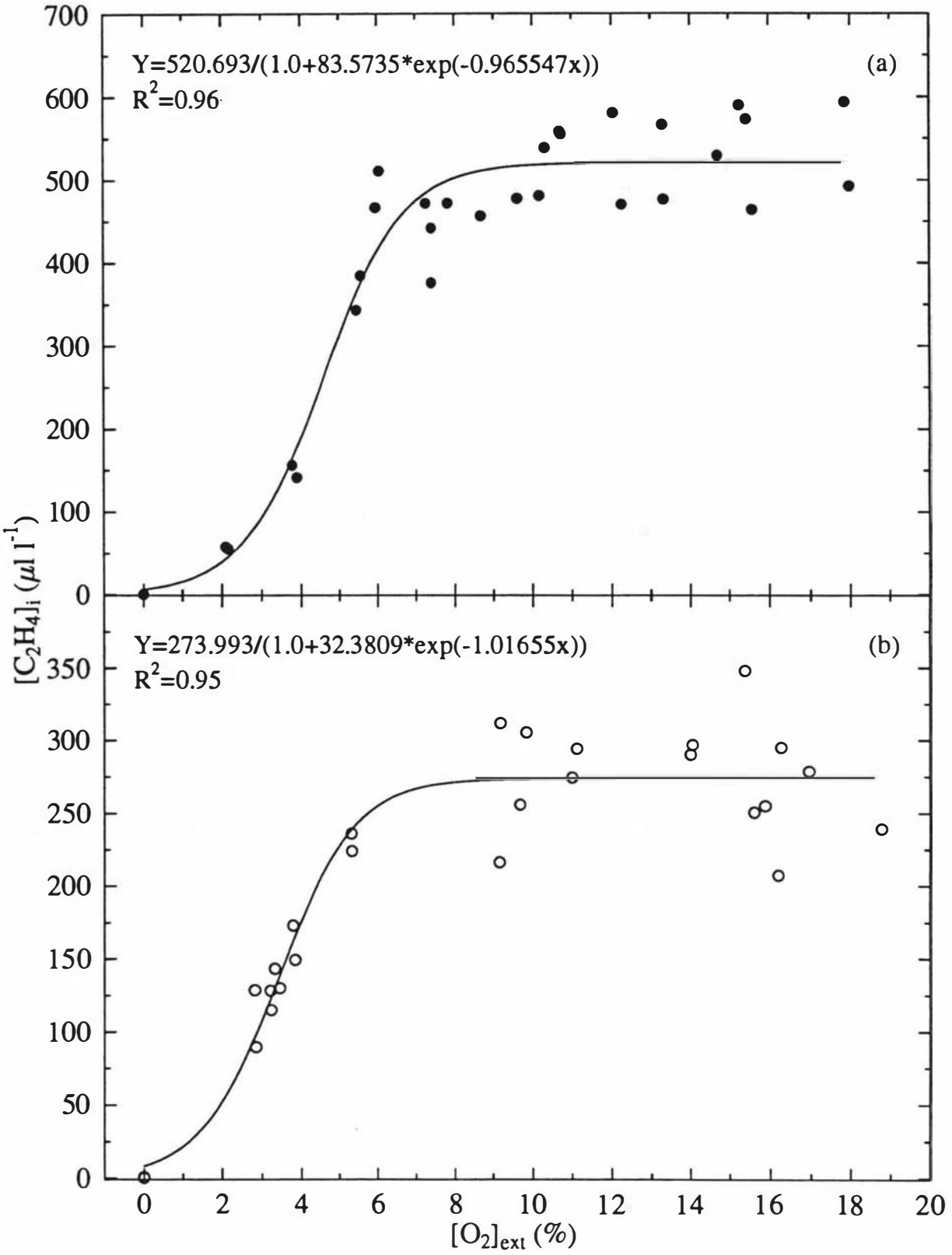

Fig. 5-16. Relationship between $[C_2H_4]_i$ and $[O_2]_{ext}$ of individual (a) Cox's Orange Pippin and (b) Granny Smith apples kept at 20°C. Solid lines were fitted by nonlinear regression using equation [5.10].

In contrast, the relationship between ***Rel***C_2H_4 or $[C_2H_4]_i$ and $[O_2]_i$ (figs. 5-17 and 5-18) was well described by an exponential type equation (rather than the Michaelis-Menten equation (equation [5.7])) of the form:

$$\textit{Rel}C_2H_4 = a * [1.0 - \exp(-b * [O_2]_i)] \qquad [5.11]$$

where a and b are constants of the equation.

Between 0% and approximately 6% $[O_2]_i$ there was a phase of rapid increase in ***Rel***C_2H_4 or $[C_2H_4]_i$ of Cox's Orange Pippin and Granny Smith apples in response to increasing $[O_2]_i$. Above about 6% $[O_2]_i$, ***Rel***C_2H_4 or $[C_2H_4]_i$ reached a plateau. Generally, Cox's Orange Pippin had higher ***Rel***C_2H_4 and $[C_2H_4]_i$ than Granny Smith apples.

The $[O_2]_i$ value at which ***Rel***C_2H_4 was approximately half the upper asymptote (of the fitted line) was approximately 1.6 and 1.4% for Cox's Orange Pippin and Granny Smith apples respectively.

5.6 DISCUSSION

Internal O_2 concentration ($[O_2]_i$) in individual apple fruit (ie. Cox's Orange Pippin and Granny Smith apples) closely mirrors $[O_2]_{ext}$, with the slope of the relationship approaching the theoretical maximum of 1 as O_2 availability becomes increasingly non-limiting at high $[O_2]_{ext}$. At low $[O_2]_{ext}$, the reduced $[O_2]_i$ decreased the rate of respiration and decreased the difference between the internal and external atmosphere composition. Whilst the nonlinear regression (equation [5.4]) accounted for a large proportion of the overall variation in $[O_2]_i$, variability in the estimates of the O_2 contents of the two atmospheres (ie. in air and in CA) limited the accuracy of this approach for characterising the relationship between respiration and $[O_2]_i$. An increased density of points in the lower quadrant of the graph could clearly have assisted in this regard.

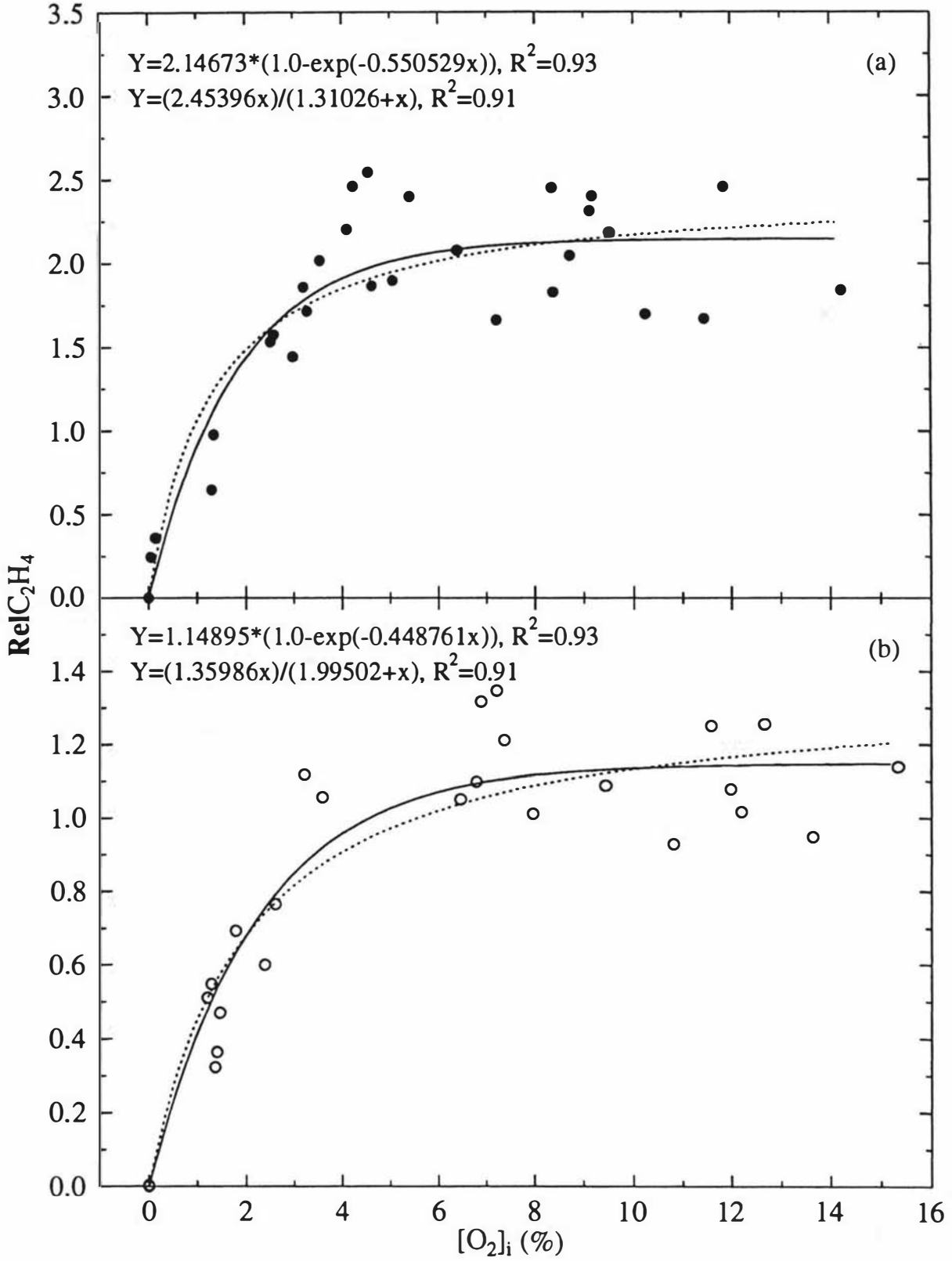

Fig. 5-17. Relationship between **Rel**C_2H_4 and $[O_2]_i$ of individual (a) Cox's Orange Pippin and (b) Granny Smith apples kept at 20°C. Solid lines were fitted by nonlinear regression using an exponential model (equation [5.11]). Dashed lines were fitted using the Michaelis-Menten model (equation [5.7]).

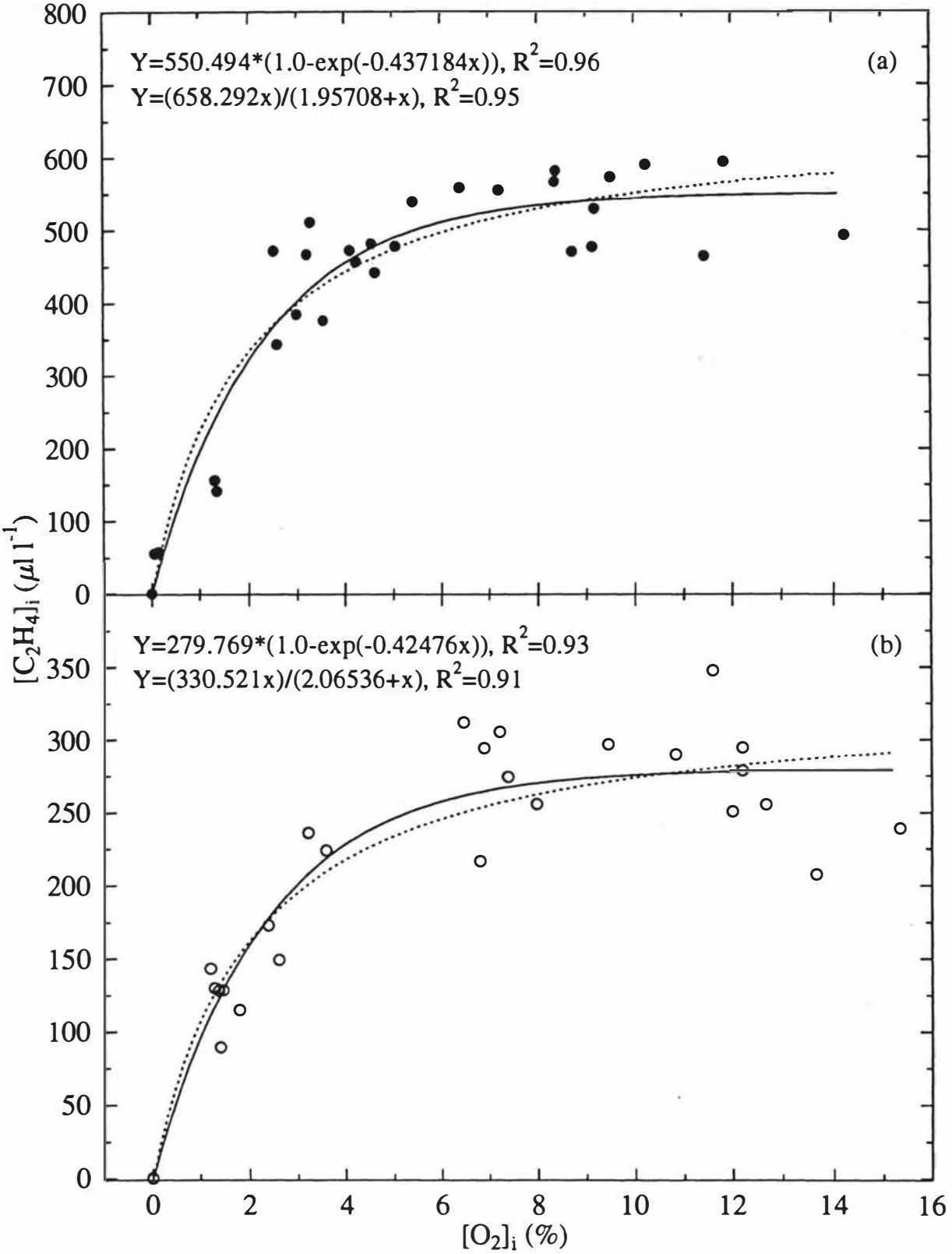

Fig. 5-18. Relationship between $[C_2H_4]_i$ and $[O_2]_i$ of individual (a) Cox's Orange Pippin and (b) Granny Smith apples kept at 20°C. Solid lines were fitted by nonlinear regression using an exponential model (equation [5.11]). Dashed lines were fitted using the Michaelis-Menten model (equation [5.7]).

The alternative approach outlined in equation [5.6] has permitted confirmation that the relationship between $Rel O_2$ and O_2 conforms at least approximately to the hyperbolic Michaelis-Menten kinetics as suggested by previous investigations on apples (Andrich *et al.*, 1991; Solomos, 1982, 1985), and avocados (Tucker and Laties, 1985). This contrasts with the linear pattern of respiratory O_2 uptake versus $[O_2]_i$ reported for apples in a sealed system in which $[O_2]_{ext}$ levels were in continuous decline by Banks *et al.* (1989). Their data also contrast with those of Tucker and Laties (1985) obtained on a similar, dynamic system, although in the latter case CO_2 was allowed to accumulate within the system and its contribution to the shape of the curve obtained cannot be ruled out. However, the similar curves obtained by Leshuk and Saltveit (1990) for disks of carrot tissue indicate that the effects of CO_2 within Tucker and Laties' system were probably quite small.

Values for rK_m were about three times as high for plots of $Rel O_2$ versus $[O_2]_{ext}$ as they were for $Rel O_2$ as a function of $[O_2]_i$. The difference was due to the O_2 concentration gradient between internal and external atmospheres ($\Delta[O_2]$). Clearly, this difference would itself depend greatly upon the magnitude of this O_2 concentration gradient. This means that $\Delta[O_2]$ would be affected by both fruit respiration rate (which is itself a function of both developmental stage and temperature) and of **R**, which has been shown to vary between apples of different cultivars and, to a lesser extent, between individual fruit within a cultivar (this subject has been addressed in the preceding chapter). This clearly illustrates the advantages of considering fruit physiological responses to CA/MAs as a function of their internal atmosphere composition rather than the external atmosphere to which they are exposed. Although modelling can be used quite satisfactorily to develop our knowledge in this area (Andrich *et al.* 1991; Banks *et al.*, 1989; Tucker and Laties, 1985) the responses of individual fruit can only be identified if individual data are gathered (as utilised in this study).

Whilst the scatter in the data makes it difficult to be definitive about the exact shape of the curve relating $Rel O_2$ and $[O_2]_i$ (fig. 5-4), nevertheless it is clear that rK_m for this relationship is much higher than that reported for cytochrome oxidase (is < 0.1% O_2 in the gas phase; Burton, 1978; Butt, 1991; Knee, 1991). This is supported by the similar values for apparent K_m for respiration derived by describing $[O_2]_i$ as a function of $[O_2]_{ext}$ (fig. 5-2). Similar conclusions have been reported by other researchers (Knee, 1973; Solomos, 1985, 1988; Theologis and Laties, 1978).

Chevillotte (1973) attributed the deviation of this relationship from what might be expected for cytochrome uptake, to the development of zones of anaerobiosis within individual fruit cells. If the effect was solely mediated by inhibition of the efficiency of uptake by a terminal oxidase then we would predict that any depression of respiration rate would be accompanied by ethanol accumulation, which was not observed in this study. In contrast, Tucker and Laties (1985) proposed a mechanism which involved at least two steps: a rapid response which involved inhibition of O_2 uptake by a terminal oxidase (thought to be cytochrome oxidase) followed only later by a response which involved inhibition of glycolytic flux. The apparent $K_m O_2$ for the latter was much higher than for direct inhibition of uptake by cytochrome oxidase and accounted for the different responses of their avocados in rapid and slow O_2 depletion experiments. This mechanism might also account for the delayed response of apples to a reduced O_2 atmosphere reported by Knee (1980).

Interestingly, rF_{max} values were also higher for $[O_2]_{ext}$ than for $[O_2]_i$. Since rF_{max} theoretically must be the same in both approaches this difference presumably represents the relative accuracy with which the Michaelis-Menten curve fits the two sets of data.

The difference between sub-epidermal O_2 ($[O_2]_{se}$) and core cavity O_2 concentration ($[O_2]_{core}$) would itself be predicted to be affected by $[O_2]_i$ as, like relative rate of exchange (***RelE***) it should be directly proportional to respiration rate. The weak relationships between the gradients (across the flesh) and $[O_2]_i$ identified in Cox's Orange Pippin and in Granny Smith apples were presumably a reflection of the limitations in accuracy of this approach given the other factors involved (eg. flesh porosity, initial respiration rate, measurement error).

The difference in estimate of the Michaelis-Menten constants obtained when considering $[O_2]_i$ or $[O_2]_{ext}$ confirm the importance of considering factors affecting the size of this difference (such as **R** and respiration rate) when setting limits for MA storage. The magnitude of this concentration difference would be substantially affected by natural or imposed variation in **R** (such as development of greasiness, waxing, coating and packaging) and particularly by variation in respiration rate achieved by exposing fruit to different temperatures (see chapter 7 for details on this subject) or inherent in fruit of different cultivars, production regimes or developmental stages.

One of the measurable effects of reduced $[O_2]_{ext}$ in an atmosphere is on CO_2 evolution, which gives an indication of the rate of metabolic activity (Ben-Yehoshua *et al.*, 1963; Isenberg, 1986; Wollin *et al.*, 1985). ***Rel***CO_2 and $[CO_2]_i$ of Cox's Orange Pippin or Granny Smith apples declined in response to diminishing $[O_2]_{ext}$ and $[O_2]_i$. However under anoxic conditions, ***Rel***CO_2 and $[CO_2]_i$ increased, suggesting that anaerobic respiration was predominant. Carbon dioxide was produced by the fruit even in the absence of O_2. These findings are consistent with those report by other investigators including ap Rees (1980, 1985), Blackman (1954), Kader (1986) and Isenberg (1986) who indicated that the relative release of CO_2 decreased as the available O_2 decreased from the amount in air (ie. 20.95%) to approximately 3% (depending on the commodity). In this range of O_2 concentrations, the Krebs cycle is the

predominant energy-generating system functioning in the organ and the bulk of the CO_2 is evolved from this system (Isenberg, 1986). Anaerobic CO_2 release from pyruvate and accompanying acetaldehyde and ethanol release in this range of O_2 concentration are insignificant due to indirect inhibition effects of O_2 on glycoysis. When O_2 becomes more severely limiting somewhere below approximately 3% there is a rapid rise in CO_2 evolution. At this point, the glycolytic system is no longer inhibited by O_2, and takes over the energy supplying function in place of the Krebs cycle (ap Rees, 1980, 1985). In this region (ie. below 3%), the O_2 concentration is low enough to allow significant anaerobic CO_2 release from pyruvate and accompanying ethanol production (ap Rees, 1985; Kader, 1987; Isenberg, 1986; Lambers, 1985).

The relationship between ***Rel***CO_2 or $[CO_2]_i$ and $[O_2]_{ext}$ or $[O_2]_i$ was described mathematically as the composite of its two component processes (anaerobic and aerobic respiration) as suggested by previous investigations on apples (Biale and Young, 1962; Blackman, 1954; Boersig *et al.* 1988; Cameron, 1985; Kader, 1987; Leshuk and Saltveit, 1990; Solomos, 1988, Wills *et al.*, 1981). An attempt was made to estimate the anaerobic compensation point (ACP) for both $[O_2]_{ext}$ ($[ACP]_{ext}$) and $[O_2]_i$ ($[ACP]_i$); (ACP defined as the $[O_2]_{ext}$ concentration at which CO_2 production was minimum; Boersig *et al.* 1988). Whilst the scatter in the data makes it difficult to be definitive about the estimate of the ACP, nevertheless the fitted curve indicated that $[ACP]_{ext}$ (for $[O_2]_{ext}$) was about 0.5% and $[ACP]_i$ (for $[O_2]_i$) was approximately 0.3% in both Cox's Orange Pippin apples and Granny Smith apples. The minimum $[CO_2]_i$ coincides with the $[ACP]_{ext}$; below this, fermentation or off-flavour will occur and the fruit will be unsaleable. Blackman, (1954), Boersig *et al.*, (1988) and Fidler, (1951) indicated that ACP shifts in relation to duration of low O_2 treatment, physiological age of the fruit, temperature and O_2 diffusion coefficient of the fruit.

RelRQs in Cox's Orange Pippin were generally greater than unity while in Granny Smith apples ***Rel***RQs were mostly lower than one except in fruit kept in O_2 levels lower than approximately 4%. The disparity was presumably related to real differences in the rates of O_2 uptake and CO_2 output as cultivar differences in differential skin resistance to the two gases would cancel in the way that ***Rel***RQ was calculated. Similar variations in respiratory quotient (RQ) have been reported by other investigators including Fidler and North (1971), and Forcier *et al.* (1987). Cameron (1985) also reported RQ's greater than unity for tomato and cherry fruit sealed in packages. Similarly, Hudson *et al.* (1991) also obtained RQ's higher than one for tomatoes in MA packages. An increase in RQ indicates an increased use of organic acids rather than carbohydrates or fatty acids as the major substrate for respiration (ap Rees, 1980, 1985). Organic acids have more O_2 per carbon atom than sugars or fatty acids, therefore requiring less O_2 consumption for the production of a given amount of CO_2. It has also been observed that RQ generally increases with ripening and senescence of most fruits and vegetables (Metlitskii *et al.*, 1972). Other treatments can also induce increases in RQ, such as the acetaldehyde treatment of grapes reported by Pesis and Marinansky (1992).

Respiratory quotient in apples decreased markedly in gas mixtures containing high CO_2 and low O_2 or high CO_2 alone compared to that in the air storage (Metlitskii *et al.*, 1972; Fidler and North, 1967; Fidler, 1950). This was attributed to an increase in the fixation of CO_2 into organic acids in fruit. A reduction in RQ values by CA according to Wang (1990) indicates retardation of the ripening and senescence of fruit and a reduced catabolism of organic acids, resulting in higher acid retention under CA conditions.

Acetaldehyde (AA) was measured in fruit sub-epidermal layer irrespective of the level of $[O_2]_{ext}$ or $[O_2]_i$. On the other hand there was no detectable ETOH until $[O_2]_{ext}$ or $[O_2]_i$ dropped to zero. It was observed that at zero percent $[O_2]_{ext}$ fruit were producing large quantities of ETOH. This is

consistent with what might be expected of these fruit becoming anaerobic: pyruvic acid produced by the process of glycolysis is no longer oxidised but is decarboxylated to form AA, CO_2 and ultimately, ETOH (ap Rees, 1980; Kader, 1987; Montgomery *et al.*, 1990; Pesis and Marinansky, 1992). The accumulation of the products of anaerobic metabolism can lead to cellular breakdown, typically accompanied by internal browning. These findings are in agreement with those of Smith *et al.* (1987) who demonstrated that alcohol formation in apples was associated with $[O_2]_{ext}$ below 1% at low temperatures.

There was no detectable AA or ETOH in the core cavity of either Cox's Orange Pippin or Granny Smith apples, irrespective of the $[O_2]_{ext}$. It may be that at the time of internal atmosphere sampling, AA and ETOH may not have accumulated in sufficient concentrations to be detectable. Further research is required to investigate this surprising finding. Acetaldehyde is a natural aroma component in almost every fruit (Pesis and Frenkel, 1989). Many researchers including Fidler (1933, 1968), Gerhardt and Ezell (1939), Janes and Frenkel (1978), Pesis *et al.* (1991) and Pesis and Avissar (1989, 1990) reported that AA and ETOH are usually found in fruit in trace amounts but they begin to accumulate as fruits begin to ripen and increase during ripening and senescence. Acetaldehyde also accumulates during development of many physiological disorders, although its role in deterioration is not clear (Avissar *et al.*, 1989; Smagula and Bramlage, 1977). Examining the O_2 gradients in fruits in relation to ripening, Biale and Young (1981) indicated in their review article that at the climacteric peak of respiration the O_2 concentration could approach zero at the centre of a fruit. This is consistent with an earlier report by Fidler (1951) that certain apple varieties produce AA and ETOH when they ripen; this implies that they contain anaerobic tissue, even though they have 21% O_2 outside.

It has long been reported that O_2 is necessary for C_2H_4 production in many fruits (including apples) and when O_2 is absent or under anoxia conditions C_2H_4 production ceases because the conversion of 1-aminocyclopropane-carboxylic acid (ACC) to C_2H_4 is inhibited. This results in accumulation of ACC in the tissue, since earlier steps in C_2H_4 biosynthesis from methionine occur in the absence of O_2 (Adams and Yang, 1979; Lürssen *et al.* 1979; Miyazaki and Yang, 1987; Yang, 1985; Yang and Hoffman, 1984). However characterising the relationship between the rates of C_2H_4 production or $[C_2H_4]_i$ and different O_2 concentrations (ie. $[O_2]_{ext}$ or $[O_2]_i$) has not been reported. This study showed that the relationship between ***Rel***C_2H_4 or $[C_2H_4]_i$ and $[O_2]_{ext}$ was sigmoidal, but the relationship with $[O_2]_i$ was exponential. The difference in the nature of response (ie. between ***Rel***C_2H_4 or $[C_2H_4]_i$ and $[O_2]_{ext}$ or $[O_2]_i$) was due to the presence of the effects of **R**. The presence of **R** implies there exists a gradient in gas concentration between the atmosphere inside the fruit and the external atmosphere. The magnitude of this gradient is a function of the skin of the fruit to gas diffusion.

The $[O_2]_{ext}$ values at which ***Rel***C_2H_4 was half of the upper asymptotic value was higher in Cox's Orange Pippin than in Granny Smith apples. These differences could be related to the differences in **R**. In both cultivars, the $[O_2]_{ext}$ values at which ***Rel***C_2H_4 was half the upper asymptotic value was higher than the $[O_2]_i$ value. The disparity is due to the presence of **R**. This further emphasises the point made earlier that fruit response to CA/MA are likely to be more consistent when measured in terms of the internal atmosphere composition rather than that external atmosphere (ie. $[O_2]_{ext}$) to which they are exposed, since it is the O_2 inside the fruit that is the direct cause of inhibition of both C_2H_4 production and $[C_2H_4]_i$ rather than $[O_2]_{ext}$, on which $[O_2]_i$ is dependent.

It is interesting to note that the relationship of ***Rel***C_2H_4 versus $[O_2]_i$ was well described by an exponential type equation (equation [5.12]) than by the Michaelis-Menten equation (equation [5.7]; fig. 5-17). ***Rel***C_2H_4 appeared to

reach an upper asymptotic value at lower $[O_2]_i$ values than might be predicted from equation [5.7]. This may be partly related to the fact that C_2H_4 production from its immediate precursors involves at least two substrates (ACC and O_2) and is therefore not strictly susceptible to Michaelis-Menten analysis. Keeping fruit in low O_2 might be expected to result in accumulation of ACC within the tissue (because the breakdown of ACC to C_2H_4 requires O_2 whilst the cleavage of S-adenosylmethionine (SAM) to yield ACC does not; Adams and Yang, 1979; Lau *et al.*, 1984; Miyazaki and Yang, 1987; Yang, 1985; Yang and Hoffman, 1984). This could stimulate higher levels of C_2H_4 production from a piece of tissue in CA than would otherwise be expected from the same piece of tissue based upon its rate of C_2H_4 production in air. An alternative explanation could be that the rate of C_2H_4 production by the fruit in air was not stable and increased over time. This again could have stimulated C_2H_4 production at lower O_2 levels giving higher rates than might be expected from their rate of production in air. However, the potential interactions of this system at the biochemical level are quite complex and beyond the scope of this study; consequently further work would be required to test this idea.

To conclude, data on the O_2 concentration around and within fruit have been utilised to examine the respiratory behaviour as well as C_2H_4 production of apples in response to O_2 concentrations in the internal or external environment. It is clear that fruit response to CA/MA storage are likely to be more consistent when measured in terms of the internal atmosphere composition (ie. $[O_2]_i$) rather than that external atmosphere (ie. $[O_2]_{ext}$) to which they are exposed, since it is the O_2 inside the fruit that is the direct cause of inhibition of respiration and C_2H_4 production rather than $[O_2]_{ext}$, on which $[O_2]_i$ is dependent.

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CHAPTER 6

GAS DIFFUSION AND QUALITY OF APPLES INFLUENCED BY SURFACTANT.

6.1 ABSTRACT

Granny Smith apples tend to develop greasy skins after extended storage which makes them slippery to touch and reduces their aesthetic appeal. Washing of Granny Smith apples in Tween 20 solutions inhibited development of greasiness. This effect was associated with increased **R**, depressed $[O_2]_i$, lower respiration rates and increased $[CO_2]_i$ and $[C_2H_4]_i$ in the washed fruit compared to controls. The depression of $[O_2]_i$ in Tween 20 washed fruit was greater than the elevation of CO_2, suggesting that the Tween 20 treatment may have affected CO_2 production and rate of O_2 uptake to different extents or alternatively the Tween 20 deposit on the fruit surface was differentially permeable to these two gases.

Washed fruit also remained greener and firmer than controls. Pre-treatment by wiping without using Tween 20 solution had none of these effects but did stimulate weight loss. None of the treatments induced internal browning which is often associated with the development of greasiness in Granny Smith apples.

The positive effects of Tween 20 treatment appeared to be the result of modification of the fruit's gas exchange characteristics. The magnitude of modification achieved by the Tween 20 treatment was dependent upon temperature, period of storage and the concentration of washing mixture applied.

6.2 INTRODUCTION

'Granny Smith' is an important export apple cultivar grown in New Zealand and accounts for more than 35% of total production (New Zealand Apple and Pear Marketing Board, 1986). Since New Zealand is far from export markets, most fruit are stored for some period of time. However, during long term storage, there is often an increase in greasiness on the fruit surface (Martin and Juniper, 1970; Trout *et al.*, 1953). Greasiness develops rapidly at ambient temperatures after removal of fruit from 0°C in air or CA storage (Leake *et al.*, 1989a). This diminishes the aesthetic value and consumer appeal of the fruit as a marketable commodity. Greasiness in Granny Smith apples has been of considerable concern to the New Zealand apple industry over recent years.

Apple fruit cuticle is primarily composed of cutin and waxy materials. Cutin, which is the major part of the cuticle, is insoluble in water and organic solvents (Huelin, 1959). On the basis of their chemistry and physical properties, the waxy materials have been divided into three separate groups namely ursolic acid, wax and oil (Leake *et al.* 1989a; Lill *et al.*, 1989). The onset of greasiness is a natural phenomenon associated with the wax and oil composition of the fruit cuticle (Leake *et al.* 1989a). Major changes in the wax and oil fractions occur during fruit development (Knuth and Stosser, 1987) and during storage (Mazliak and Pommier-Miard, 1963; Metlitskii *et al.*, 1983). Although the wax fraction does not change much after harvest, the oil component continues to increase markedly during storage (Lill *et al.*, 1989, 1991; Leake *et al.*, 1989a; Morice and Shorland, 1973). The oil is the fraction that is liquid at room temperature (Huelin and Gallop, 1951a). Whilst these changes occur generally in apple cultivars such as Gala, Cox's Orange Pippin, Sturmer etc., they appear to occur very markedly in Granny Smith apples. Huelin and Gallop (1951b) showed that the oil fraction of the skin increased to 3 - 4 times its original concentration during storage at 0°C.

Development of the oil fraction increases skin resistance by blocking lenticels, thereby impeding gas exchange, modifying the internal atmosphere of the fruit (Eaks and Ludi, 1960; Huelin and Gallop, 1951a, 1951b; Trout *et al.*, 1942, 1953) with the consequent potential for triggering development of physiological disorders such as coreflush (Little and Minnis, 1978). The fruit also become slippery to touch.

Much research has been undertaken to understand the causes and prevention of the development of greasiness in apples particularly Granny Smith (Huelin and Gallop, 1951a, b; Knuth *et al.*, 1987; Leake *et al.*, 1989a; Markley and Sando, 1931; Martin and Juniper, 1970; Mazliak and Pommier-Miard, 1963; Metlitskii *et al.*, 1983; Trout *et al.*, 1953). Solvents have been used to remove surface wax in apples in an attempt to study the chemical and physical properties of waxes (Horrocks, 1964; Hall, 1966; Lill *et al.*, 1989, 1991). Leake *et al.* (1989a) indicated that development of greasiness may be influenced by time of harvest, storage period and position of fruit in the tree canopy, but fruit size was not a factor.

In a preliminary study (Appendix 2) with Granny Smith apples kept in air at 20°C, it was found that when fruit that had developed greasy surfaces were handled by touching, their $[O_2]_i$ were lower than in non-handled controls. The $[CO_2]_i$ of fruit in both handled and non-handled treatments were high and when these fruit were cut open some had developed internal browning (see Appendix 3). The high $[CO_2]_i$ and $[O_2]_i$ in control fruit compared to the handled fruit suggested that this treatment could have caused blockage of lenticels through the redistribution of grease on the fruit surface. Since O_2 effectively only diffuses via the lenticels (available evidence indicates CO_2 can move through both the cuticle and epidermis [Banks, 1984; Burton, 1974]) any treatment which blocks the lenticels on the fruit surface is likely to affect $[O_2]_i$ more than $[CO_2]_i$. A strong correlation ($r = 0.63$; $P < 0.0006$) was measured

between the severity of internal browning (on a scale of 0 - 3) and $[CO_2]_i$. Based on these findings, it was reasoned that if some form of treatment was applied to the fruit surface to remove some of the surface grease, gas exchange might be enhanced and consequently development of internal browning could be reduced or prevented. Tween 20 solution was therefore chosen.

Tween 20 is a surfactant routinely used in fruit production either as an adjuvant in pesticide formulation or added to agricultural sprays mainly to improve wetting and to enhance penetration of the active ingredient into the plant material (Greene and Bukovac, 1974). Tween surfactant has been used to prevent post storage greening in potato tubers (Poapst *et al.*, 1978; Poapst and Forsyth 1974; 1975). Information on the rate of persistence or disappearance of Tween 20 solution in apples in the field or in storage is unavailable, however it is known to be nontoxic and used in the laboratory as a detergent (Poapst and Forsyth 1974; 1975). It is also used in pharmaceutical preparations as a surfactant for internal use (The Merck index, 1976).

An experiment was conducted in 1989 to investigate whether removing grease from CA stored Granny Smith apples by washing in Tween 20 solution enhanced gas exchange and prevented development of internal browning. Contrary to initial expectation, the results of the experiment showed that redevelopment of greasiness in Granny Smith apples could be inhibited by the Tween 20 treatment, however, this was associated with greater rather than lesser modification of the internal atmosphere of fruit. Based on these results, it was worthwhile to identify the optimum Tween 20 concentration that would prevent development of greasiness without causing excessive modification of internal atmosphere composition of fruit. A further experiment was therefore undertaken in 1990 in which freshly harvested Granny Smith apples were washed in various concentrations of Tween 20 solution before storage at 0°C

and subsequent transfer to 20°C with the objective of preventing development of greasiness and studying the internal atmosphere composition as well as some aspects of fruit quality.

6.3 MATERIALS AND METHODS

6.3.1 Fruit supply

Granny Smith apples (***Malus domestica*** Borkh.; count 125; av. weight 148 g) stored in CA (2% O_2 and 2% CO_2) for approximately 4 months were obtained in 1989 as previously described in section 3.1. Fruit were further stored at 0°C in air for 14 days and subsequently transferred to 20±1°C for 14 days and during that period grease developed on fruit surface.

In a second experiment in 1990, freshly harvested Granny Smith apples (count 125; av. weight 148 g) were also obtained from similar source as mentioned above.

6.3.2 Treatment

6.3.2.1 Experiment 1

Fruit were divided into three lots of 15 and weighed individually before the following treatment application:

1. control (in the light of the results of the preliminary experiment, fruit were handled by the stem (or peduncle)
2. fruit washed gently in Tween 20 solution (0.15% v/v) (Polyoxyethylene (20) - sorbitan monolaurate) and then rinsed under running water
3. fruit wiped with soft tissue paper until they were shiny.

Fruit were further stored at 20±1°C for 22 days to allow grease to redevelop before measurements were made. Fruit were reweighed 1 and 22 days after treatment application.

6.3.2.2 Experiment 2

Four fruit per treatment per assessment time were weighed and washed in 0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2% v/v Tween 20 solution then rinsed under running water. Fruit were then stored at 0°C in air for 0, 8, 16, and 24 weeks and subsequently transferred to 20±1°C for 0, 1, 8 and 15 days before measurements were made.

6.3.3 Assessment of greasiness

In both experiments 1 and 2, greasiness was assessed quantitatively by weighing the fruit on a Mettler AE 200 balance to the nearest 0.001g before and after wiping thoroughly with soft tissue paper. The difference in weight provided a measure of grease weight (mg).

Greasiness was also estimated subjectively in experiment 1 (Leake *et al.*, 1989b) by rubbing fruit against the hand and scoring the degree of greasiness on the following scale: no greasiness (0), slight (1), moderate (2), and severe (3).

6.3.4 Estimation of gas exchange variables

In experiment 1 fruit respiration (CO_2 production) and C_2H_4 production were measured on fruit kept in the dark (see 3.3.5.2).

In both experiments, core cavity O_2, CO_2 and C_2H_4 concentrations were measured by the direct sampling method (see 3.3.4.1).

RCO_2 and RC_2H_4 were also estimated (see 3.3.5.2) on fruit in experiment 1.

6.3.5 Fruit quality assessment

Fruit firmness, soluble solids content and background colour were measured in both experiments 1 and 2 as previously described (see 3.3.8.1, 3.3.8.2 and 3.3.8.3).

Fruit were cut open across the equator and internal browning assessed subjectively by rating (0 - 3) the severity of browning as none, slight, moderate and severe respectively.

6.3.6 Experimental design and analysis

6.3.6.1 Experiment 1

Fifteen single fruit replicates of each treatment were used in a completely randomised design. Data were tested with analysis of variance procedure using General Linear Models (GLM) procedure of SAS (see 3.3.9). Mean comparisons were carried out using the Least significant difference (LSD) procedure at 1% level of significance (Steel and Torrie, 1980; Little, 1981). Data on percentage weight loss were subjected to arcsin transformation (Mead and Curnow, 1983; Little, 1985) prior to statistical analysis and back transformed for presentation.

6.3.6.2 Experiment 2

Within the overall study, there were four sampling times at 0°C, each analysed as a separate experiment. There were four single fruit replicates, eight Tween 20 concentrations and four sampling times at 20°C used in a factorial design within each experiment. Analysis of variance was carried out

using General Linear Models procedure (GLM) of SAS (see 3.3.9). The analysis incorporated quantification of linear, quadratic or cubic effects by regression (Steel and Torrie, 1980; Little, 1981).

6.4 RESULTS

6.4.1 Greasiness and internal browning

6.4.1.1 Experiment 1

Redevelopment of greasiness, in fruit measured objectively as the difference in weight of the fruit before and after rubbing the fruit free from grease, was markedly reduced by washing in Tween 20 solution ($P < 0.01$; fig. 6-1). Compared to the controls, washing of fruit in Tween 20 solution resulted in 58% reduction in the subsequent grease development. There was no significant difference in greasiness between control and wiped fruit (at $P = 0.01$). Similar results were obtained when greasiness were assessed subjectively (fig. 6-2). There was a positive correlation between the objective and subjective methods of measuring greasiness ($r = 0.50$; $P < 0.0007$), however the relationship was poorly defined. No treatment induced internal browning.

6.4.1.2 Experiment 2

Storage of Granny Smith apples at 0°C and subsequent transfer to 20°C enhanced development of greasiness in Granny Smith apples. No significant differences ($P = 0.05$) were observed between treatments during the first 15 days storage at 20°C after harvest and treatment application (fig. 6-3a). However, after 8, 16 or 24 weeks storage at 0°C and especially after subsequent transfer to 20°C, significant ($P < 0.0001$) reductions of grease development were achieved by treatment with Tween 20 solution (fig. 6-3). These effects were greater for the higher Tween 20 treatment concentrations

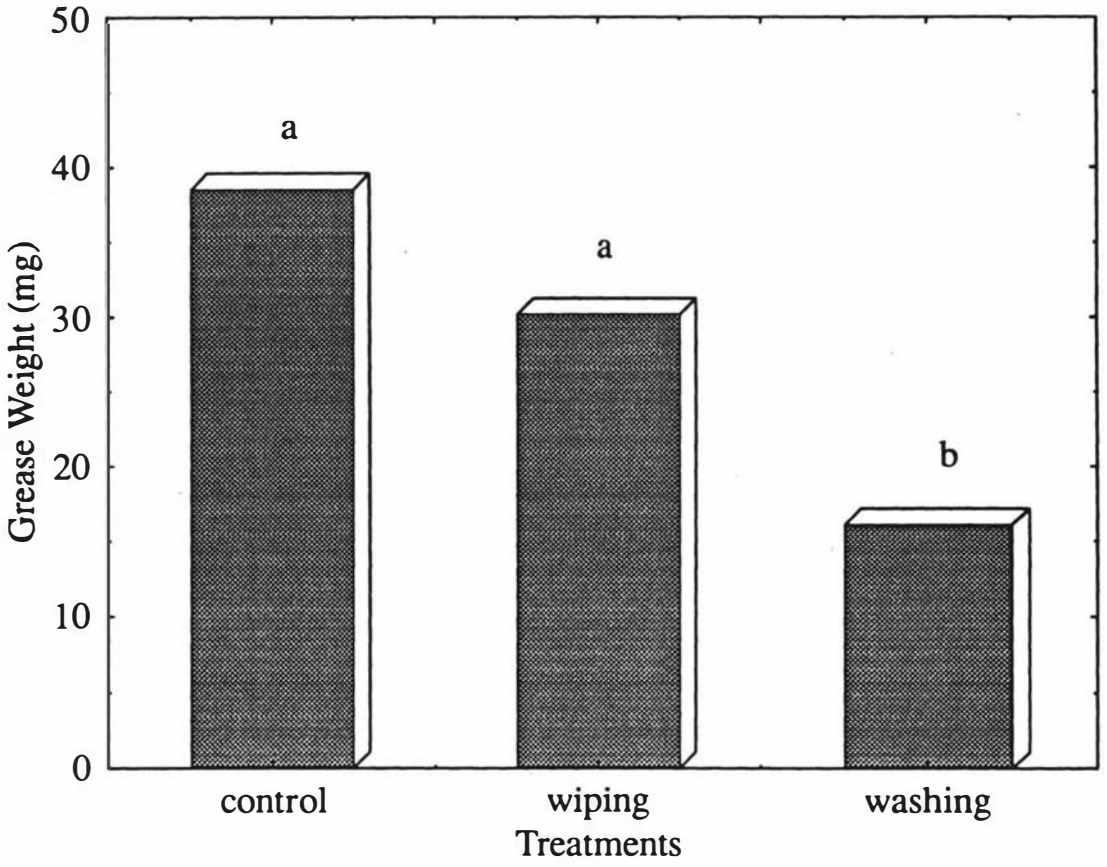

Fig. 6-1. Grease weight of Granny Smith apples after wiping or washing in Tween 20 solution and storage for 22 days at 20°C. Letters in common not significantly different at the 1% level. Mean separation by Least significant difference (LSD) procedure.

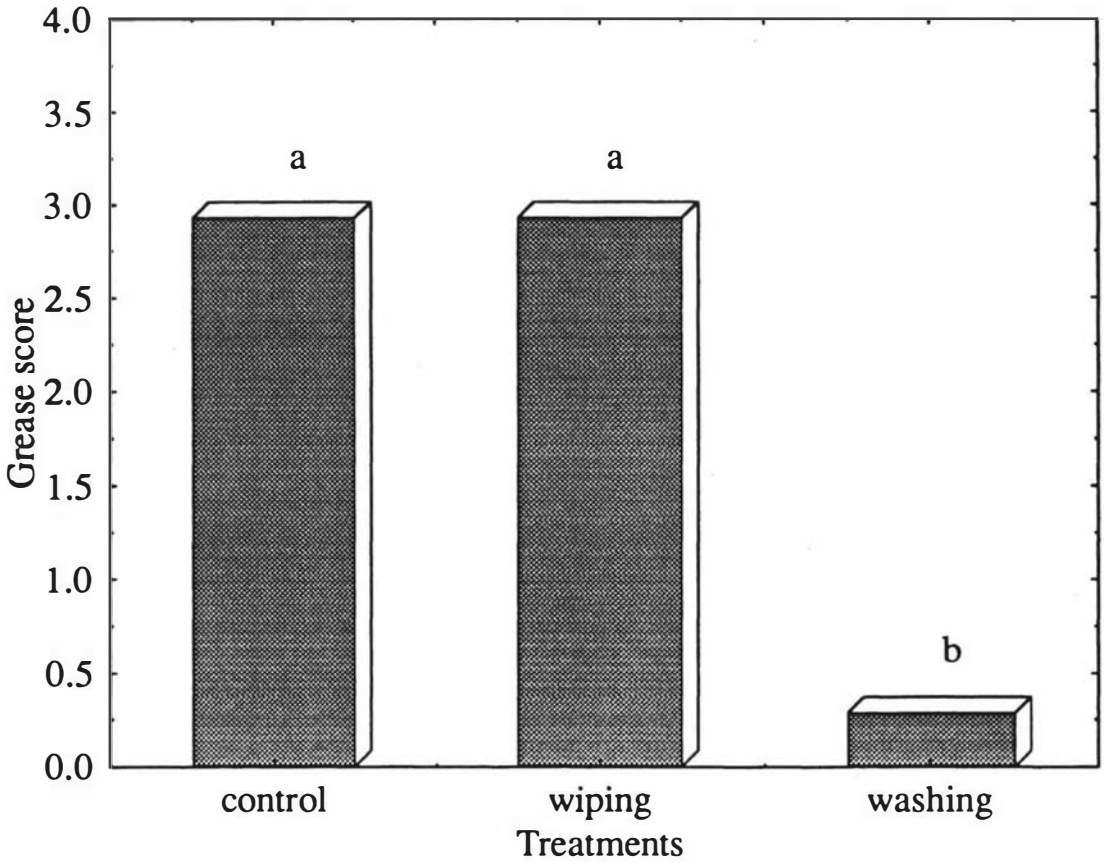

Fig. 6-2. Grease score of Granny Smith apples after wiping or washing in Tween 20 solution and storage for 22 days at 20°C. Letters in common not significantly different at the 1% level. Mean separation by Least significant difference (LSD) procedure.

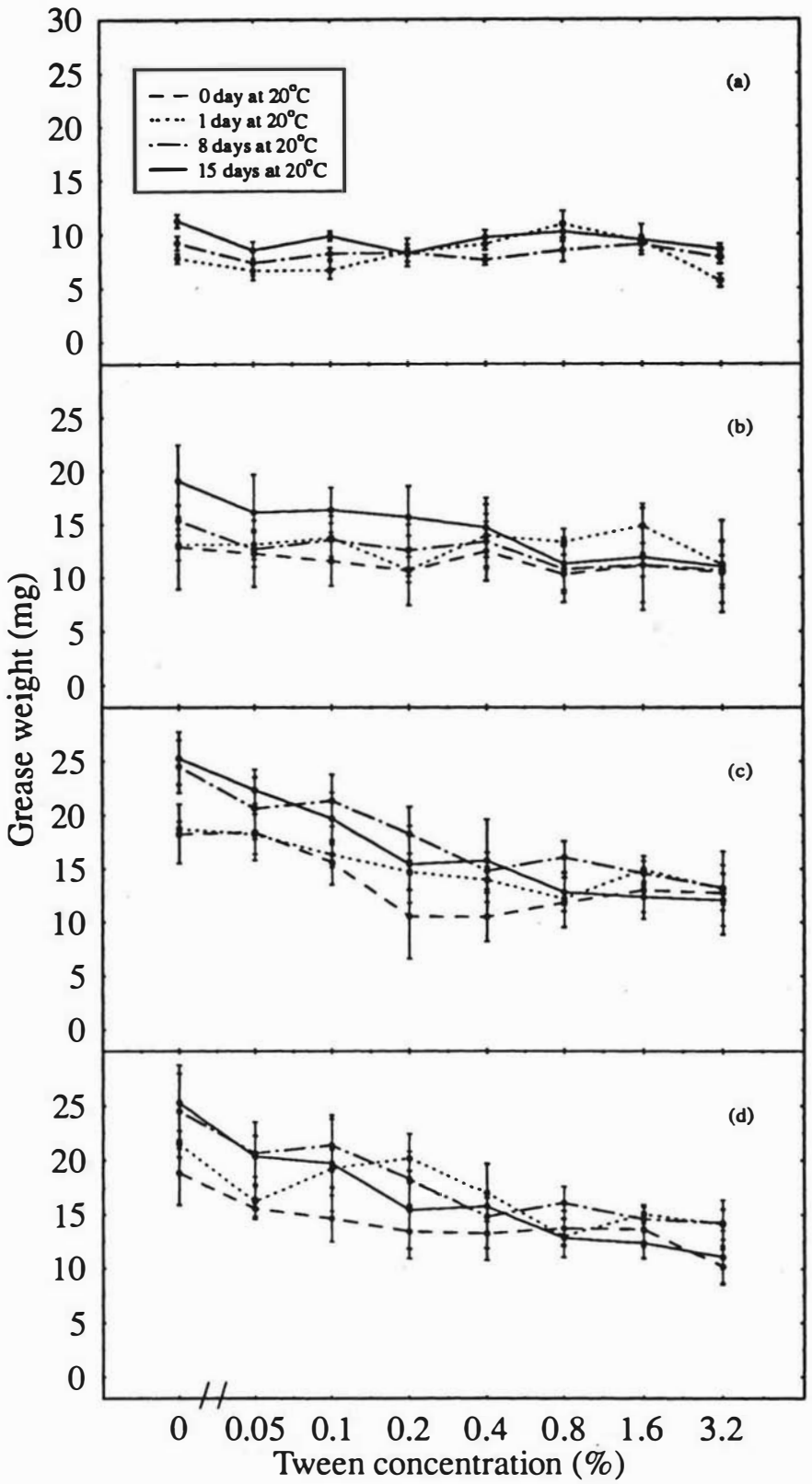

Fig. 6-3. Grease weight of Granny Smith apples after washing in different concentrations of Tween 20 solution, stored (for (a)=0, (b)=8, (c)=16, (d)=24 weeks) at 0°C and subsequent transfer to 20°C (for 0, 1, 8 and 15 days). Vertical bars indicate standard errors of means.

($P < 0.0001$ for the linear effect of $\log_2$(Tween concentration) with no significant quadratic or cubic deviations). Greasiness developed rapidly in control fruit at 20°C, particularly in fruit stored for 16 and 24 weeks at 0°C. This development of greasiness was totally inhibited during storage at 20°C in fruit coated with the highest concentrations of Tween 20, with progressive amounts of inhibition seen at the intermediate levels of treatment. These effects were confirmed by the significance of the interaction of the effects of days and treatment concentration ($P < 0.001$ at 8, 16 and 24 weeks). None of the treatments induced internal browning.

6.4.2 Skin resistance to gas diffusion

Washing of Granny Smith apples in Tween 20 solution increased both RCO_2 and RC_2H_4 ($P < 0.01$; fig. 6-4, experiment 1). Skin resistance to CO_2 diffusion of washed fruit was 33% more than controls and 23% more than wiped fruit. RC_2H_4 of washed fruit was 36% more than controls and 17% more than wiped fruit. The wiping treatment resulted in a mean increase of nearly 13% and 23% respectively for CO_2 and C_2H_4 diffusion compared to the controls.

6.4.3 Respiration rate and ethylene production

Washing of fruit in Tween 20 solution (experiment 1) significantly decreased the rate of CO_2 production from the fruit ($P < 0.01$; fig. 6-5). Tween 20 treated fruit were respiring approximately 36% less than the controls, while wiped fruit were respiring nearly 30% more than the washed ones and only about 8% less than the controls (not significant).

Rate of C_2H_4 evolution was not affected by the treatments (at $P = 0.01$; fig. 6-6), with an overall mean of 29.1 ± 1.9 µl/kg/hr.

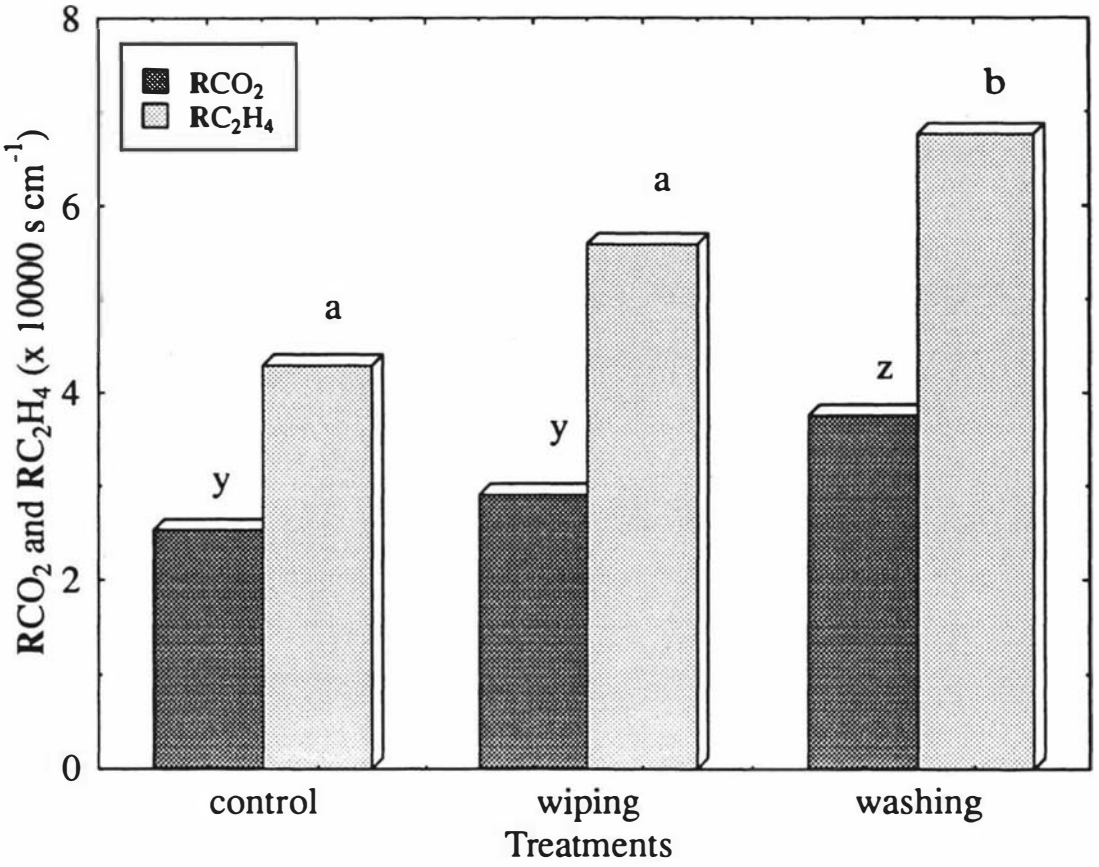

Fig. 6-4. RCO_2 and RC_2H_4 of Granny Smith apples after wiping or washing in Tween 20 solution and storage for 22 days at 20°C. Letters in common for each gas not significantly different at the 1% level. Mean separation by Least significant difference (LSD) procedure.

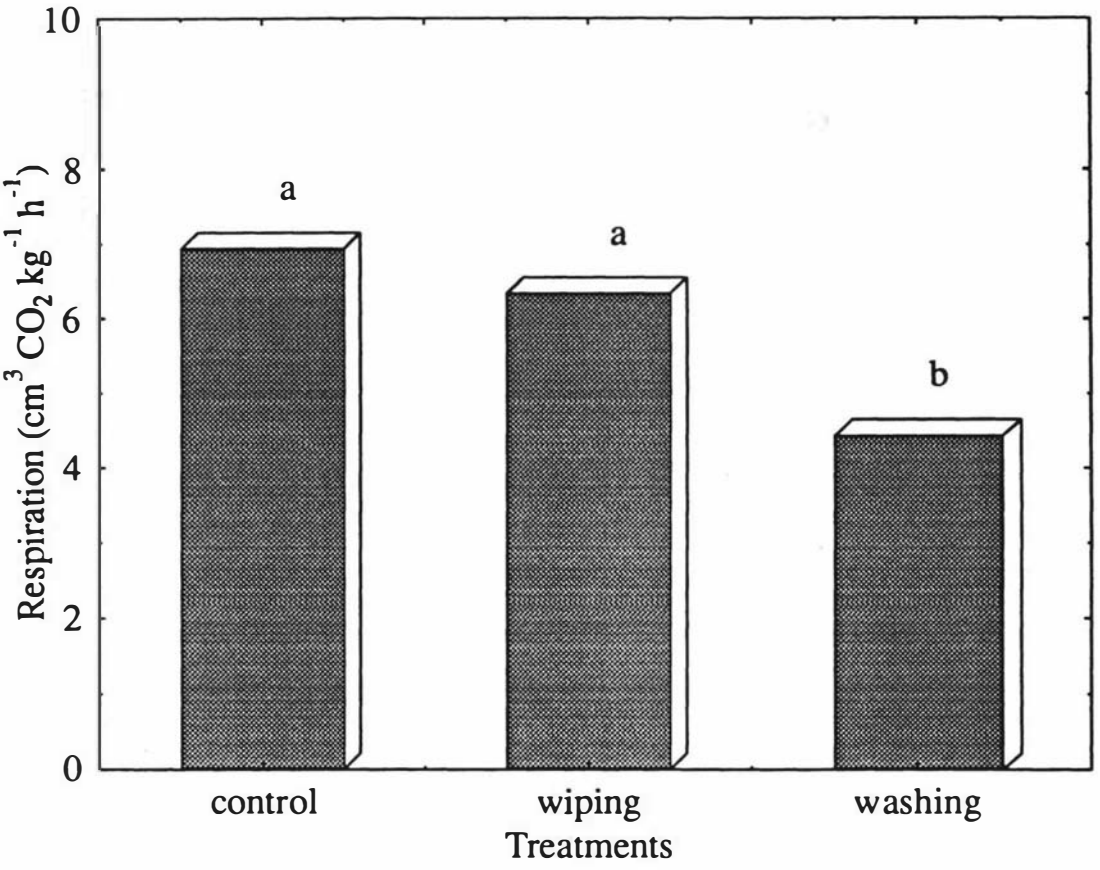

Fig. 6-5. Respiration rate of Granny Smith apples after wiping or washing in Tween 20 solution and storage for 22 days at 20°C. Letters in common not significantly different at the 1% level. Mean separation by Least significant difference (LSD) procedure.

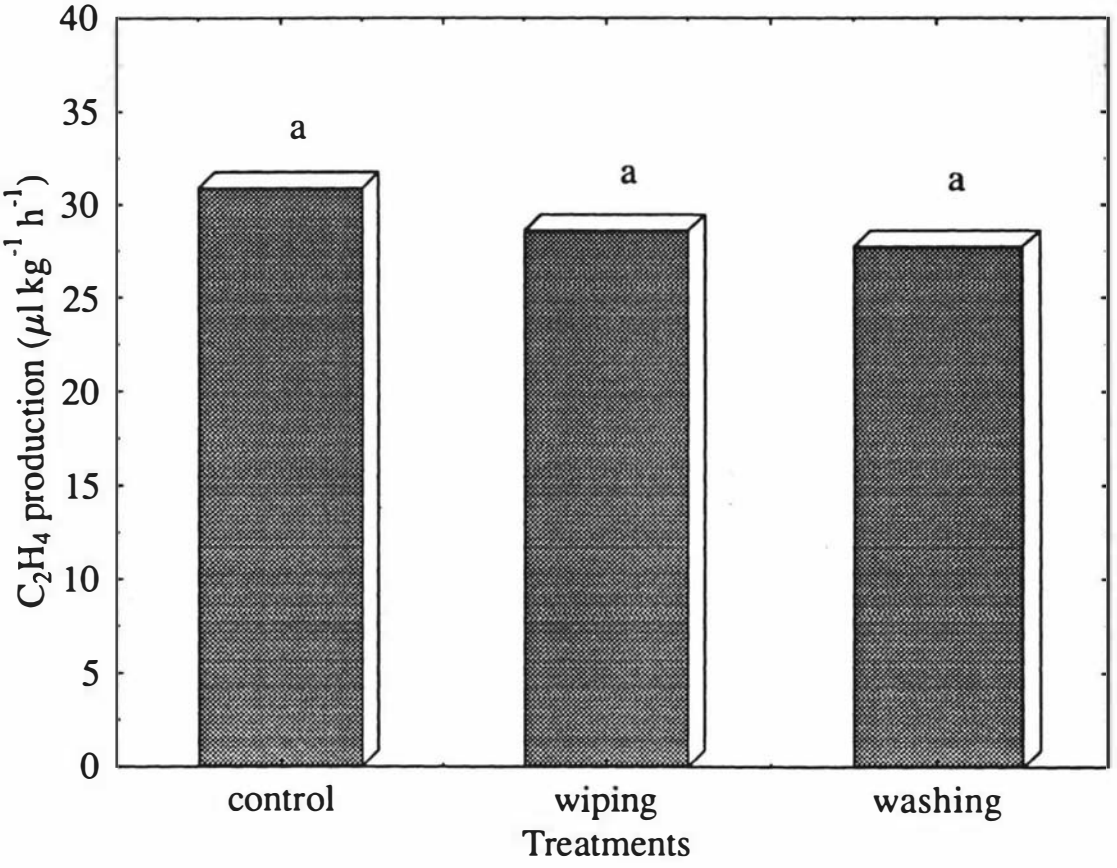

Fig. 6-6. C_2H_4 production of Granny Smith apples after wiping or washing in Tween 20 solution and storage for 22 days at 20°C. Letters in common not significantly different at the 1% level. Mean separation by Least significant difference (LSD) procedure.

6.4.4 Internal gas concentrations

6.4.4.1 Experiment 1

Compared to the control and wiping treatments, washing of Granny Smith apples in Tween 20 solution decreased $[O_2]_i$ by two-thirds (fig. 6-7). There were no significant differences in $[O_2]_i$ between control and wiped fruit ($P < 0.01$). Internal CO_2 was not affected by any of the treatments (at $P = 0.01$; fig. 6-7), with an overall average of $4.8 \pm 0.2\%$. On the other hand the washing treatment significantly increased $[C_2H_4]_i$ ($P < 0.01$; fig. 6-8) by 30% compared to the controls. There were no marked differences in $[C_2H_4]_i$ between control and wiped fruit.

6.4.4.2 Experiment 2

In general during storage at 0°C there was a progressive decline in $[O_2]_i$ with increasing Tween 20 concentration. Fruit washed in various Tween 20 concentrations before storage at 0°C and subsequent transfer to 20°C ($P < 0.001$) had lower $[O_2]_i$ than control fruit and the magnitude of this effect was directly proportional to the $\log_2$ (Tween concentration) (fig. 6-9). Marked depression of $[O_2]_i$ was observed 24 hours after transfer of fruit from cold storage (0°C) to ambient temperature (20°C). The magnitude of depression was more apparent in fruit washed in the highest concentrations of Tween 20. Internal O_2 however increased thereafter.

Similarly, there was a consistent elevation of $[CO_2]_i$ during storage at 20°C in fruit coated with the highest concentrations of Tween 20, with a progressive increase seen at the intermediate levels of treatment ($P < 0.001$ for the linear and quadratic effects of $\log_2$ (Tween concentration), with no significant cubic deviations; fig. 6-10). A similar trend was observed in fruit

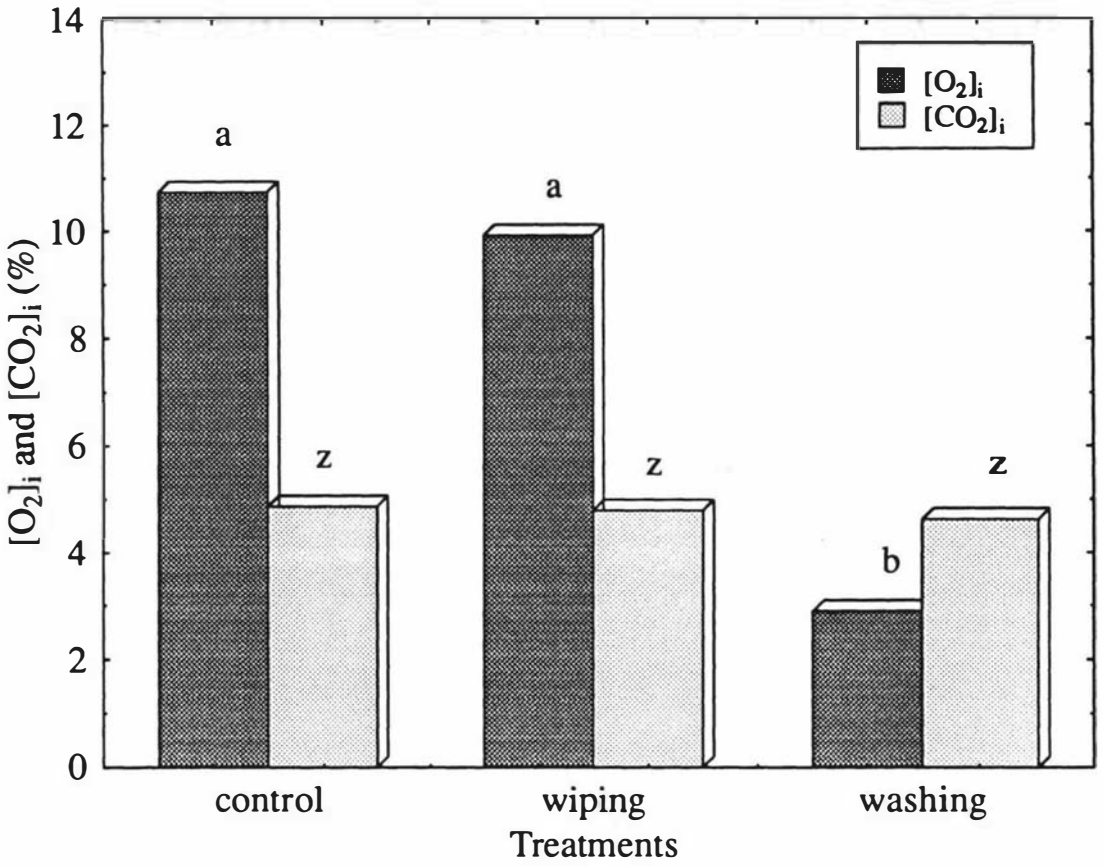

Fig. 6-7. $[O_2]_i$ and $[CO_2]_i$ of Granny Smith apples after wiping or washing in Tween 20 solution and storage for 22 days at 20°C. Letters in common for each gas not significantly different at the 1% level. Mean separation by Least significant difference (LSD) procedure.

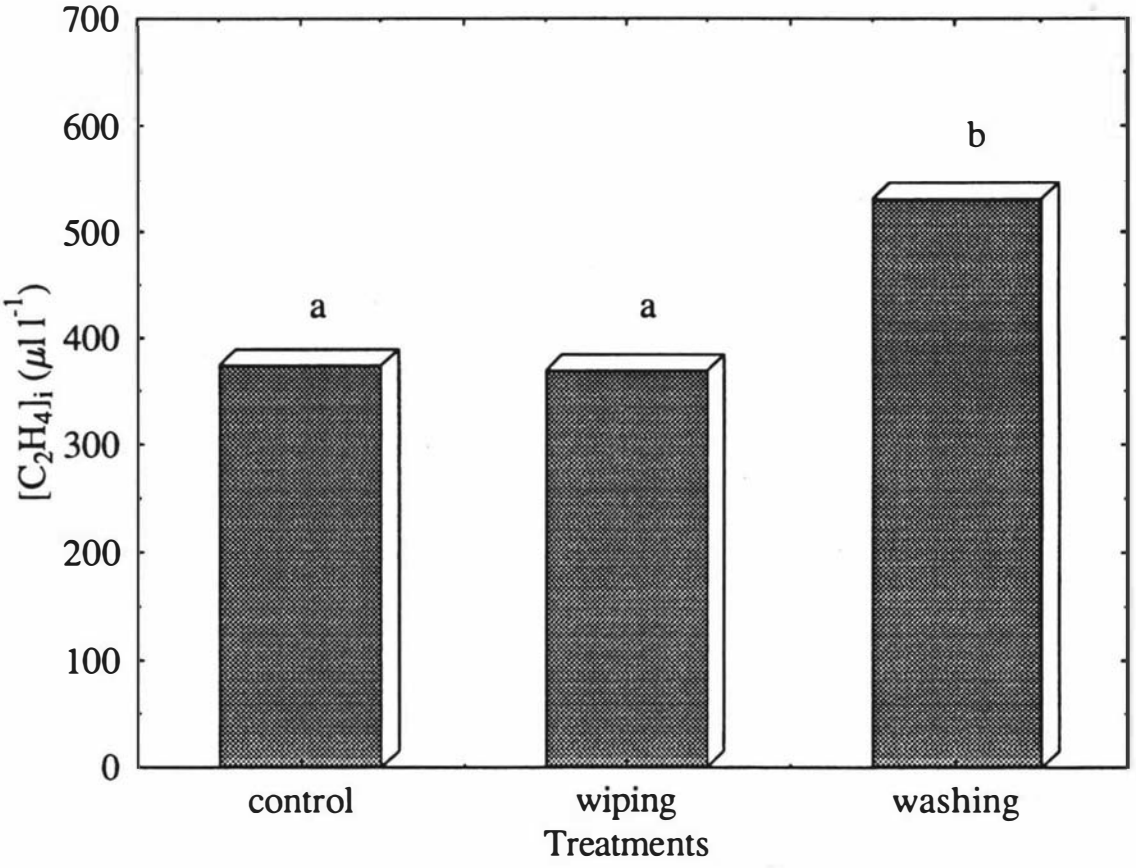

Fig. 6-8. $[C_2H_4]_i$ of Granny Smith apples after wiping or washing in Tween 20 solution and storage for 22 days at 20°C. Letters in common not significantly different at the 1% level. Mean separation by Least significant difference (LSD) procedure.

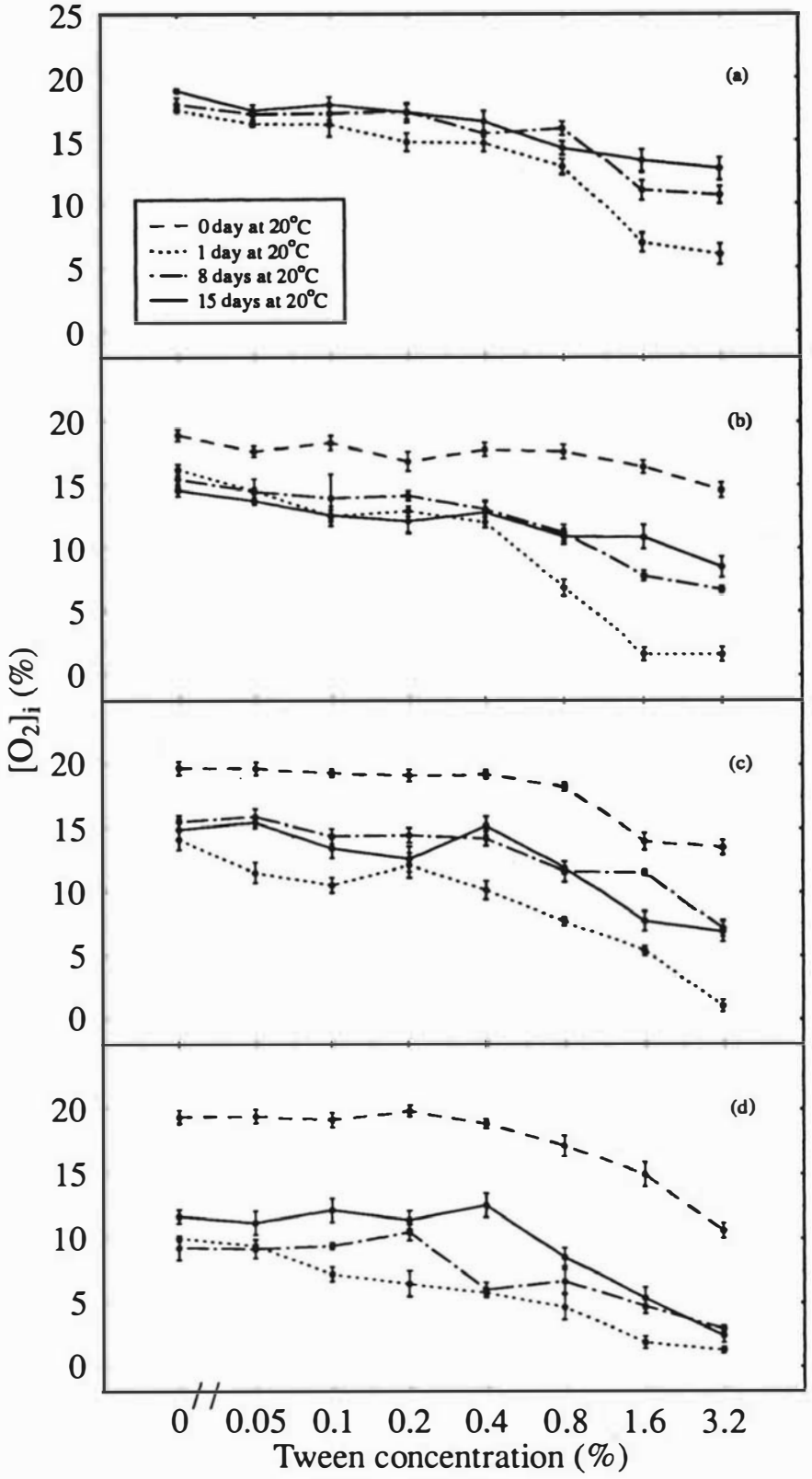

Fig. 6-9. Internal O_2 concentrations of Granny Smith apples after washing in different concentrations of Tween 20 solution, stored (for (a)=0, (b)=8, (c)=16, (d)=24 weeks) at $0^{\circ}C$ and subsequent transfer to $20^{\circ}C$ (for 0, 1, 8 and 15 days). Vertical bars indicate standard errors of means.

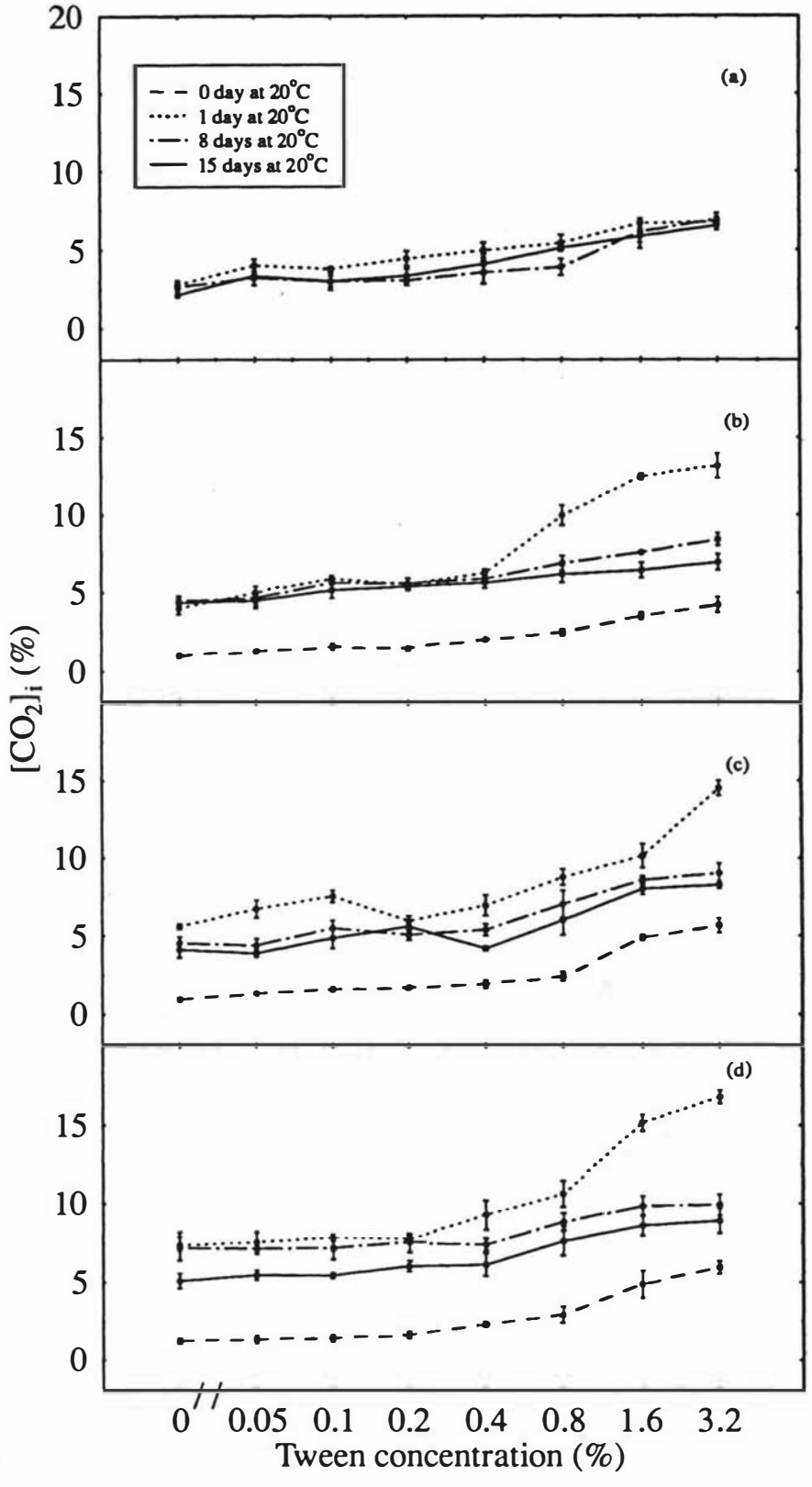

Fig. 6-10. Internal CO_2 concentrations of Granny Smith apples after washing in different concentrations of Tween 20 solution, stored (for (a)=0, (b)=8, (c)=16, (d)=24 weeks) at 0°C and subsequent transfer to 20°C (for 0, 1, 8 and 15 days). Vertical bars indicate standard errors of means.

during storage at 0°C, but their $[CO_2]_i$ was lower than those stored at 20°C. In the first 24 hours after transfer of fruit from 0°C to 20°C, there was a pronounced increase in $[CO_2]_i$. The degree of increase was greater in fruit washed in the higher Tween 20 concentrations. Internal CO_2 concentrations however decreased thereafter at 20°C. The $[CO_2]_i$ in fruit treated with Tween 20 (1.6 or 3.2%) and after 24 weeks of cold storage and 1 day at 20°C were twice the level in similar fruit at stored at 20°C for 15 days.

There was no detectable $[C_2H_4]_i$ in fruit during the first 15 days after harvest and treatment application at 20°C (fig. 6-11). During storage at 0°C $[C_2H_4]_i$ in both control and treated fruit was low, but concentrations increased steadily during storage at 20°C in contrast to the increase then decrease seen for $[CO_2]_i$. Generally, after 8, 16 or 24 weeks of storage at 0°C and subsequent transfer to 20°C, there was a consistent trend of increasing $[C_2H_4]_i$ in fruit washed with the highest concentrations of Tween 20, with progressive increase in $[C_2H_4]_i$ observed at intermediate concentrations of Tween 20.

6.4.5 Percentage weight loss

6.4.5.1 Experiment 1

Twenty-two days after treatment application, water loss was increased by about 25% in fruit wiped with a soft tissue paper compared to control fruit. There was no significant effect of washing with Tween 20 solution on water loss (at $P = 0.05$; fig. 6-12).

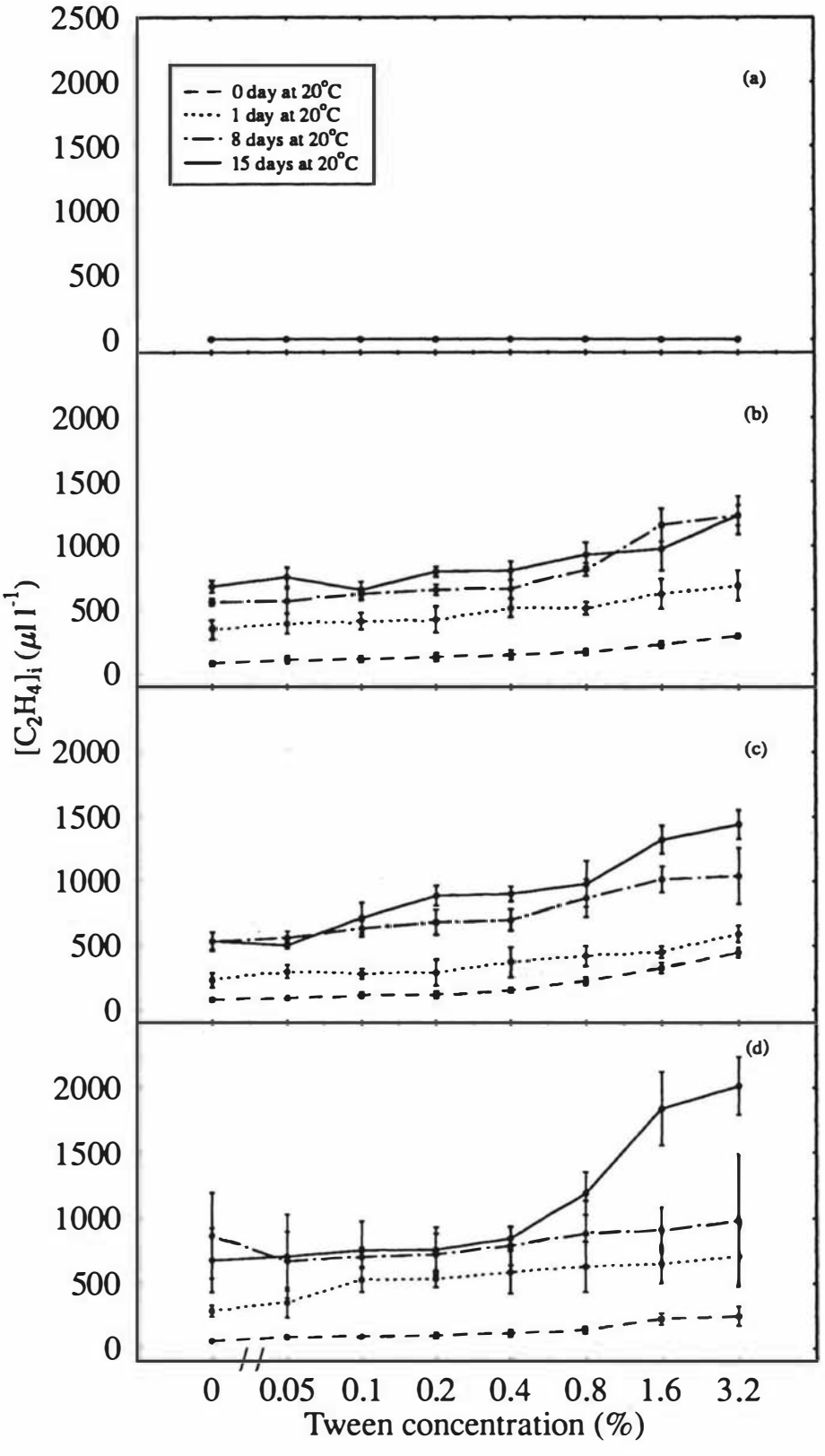

Fig. 6-11. Internal C_2H_4 concentrations of Granny Smith apples after washing in different concentrations of Tween 20 solution, stored (for (a)=0, (b)=8, (c)=16, (d)=24 weeks) at 0°C and subsequent transfer to 20°C (for 0, 1, 8 and 15 days). Vertical bars indicate standard errors of means.

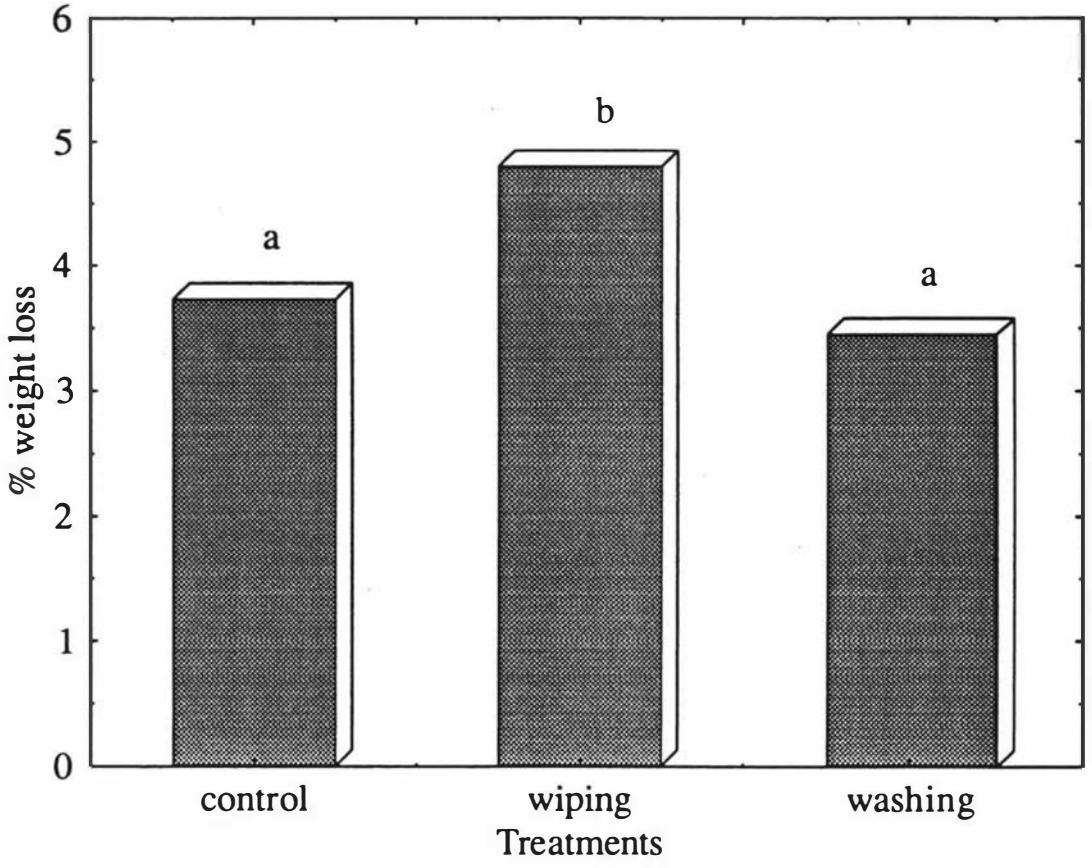

Fig. 6-12. Percentage weight loss of Granny Smith apples after wiping or washing in Tween 20 solution and storage for 22 days at 20°C. Letters in common not significantly different at the 1% level. Mean separation by Least significant difference (LSD) procedure.

6.4.5.2 Experiment 2

Twenty-four hours after treatment application and storage at 20°C, there were no differences in percentage weight loss between controls and Tween 20 treated fruit. Weight loss increased with time of storage at 20°C, with fruit washed in higher Tween 20 concentrations losing more weight (fig. 6-13a). During storage at 0°C it was fruit washed in the highest Tween 20 concentrations that lost most weight and a similar pattern was seen for the elevated rate of weight loss during subsequent storage at 20°C. There was a progressive loss of weight in fruit during the period of storage at 20°C after a period of storage at 0°C with the highest amount recorded on the 15th day at 20°C. Fruit stored for 24 weeks at 0°C and subsequently transferred to 20°C for 15 days lost nearly 6% of their total weight. Generally, percentage weight loss increased with increasing Tween 20 concentrations applied.

6.4.6 Quality indices

6.4.6.1 Experiment 1

After twenty-two days of treatment application and storage at 20°C, control and wiped fruit markedly lost their green colour (fig. 6-14). In contrast, fruit washed in Tween 20 solution retained their green colour (as indicated by the Hue angle values) ($P < 0.01$; fig. 6-15). Fruit treated with Tween 20 were approximately 6% and 9% firmer than control and wiped fruit respectively ($P < 0.01$; fig. 6-16). There were no treatment effects on soluble solids content of fruit (at $P = 0.05$, with overall mean of $11.4 \pm 0.9\%$).

6.4.6.2 Experiment 2

When freshly harvested Granny Smith fruit were washed in various concentrations of Tween 20 solution and stored at 20°C for 1, 8 and 15 days there were no significant (at $P = 0.05$) differences in colour change between

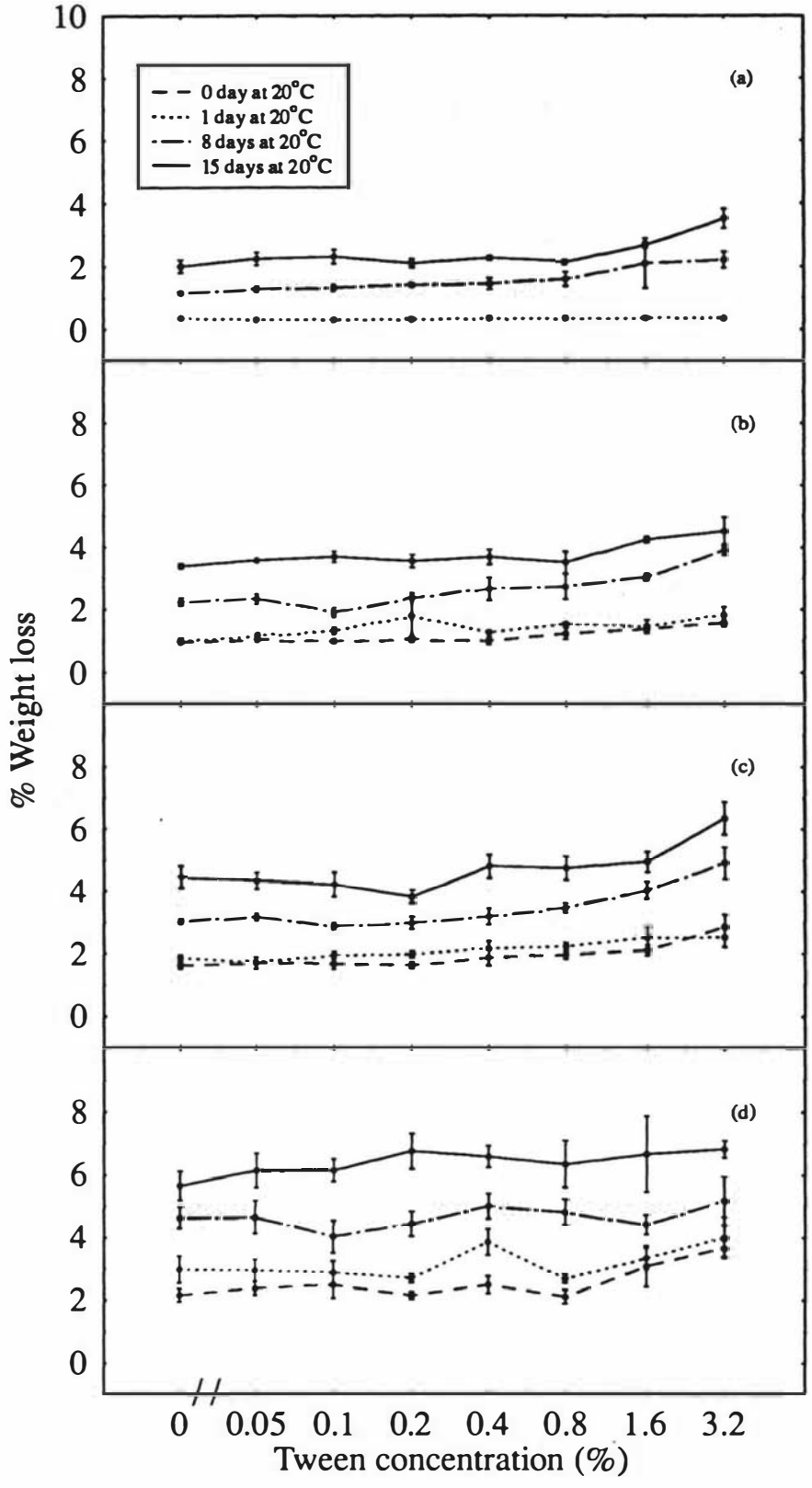

Fig. 6-13. Percentage weight loss of Granny Smith apples after washing in different concentrations of Tween 20 solution, stored (for (a)=0, (b)=8, (c)=16, (d)=24 weeks) at 0°C and subsequent transfer to 20°C (for 0, 1, 8 and 15 days). Vertical bars indicate standard errors of means.

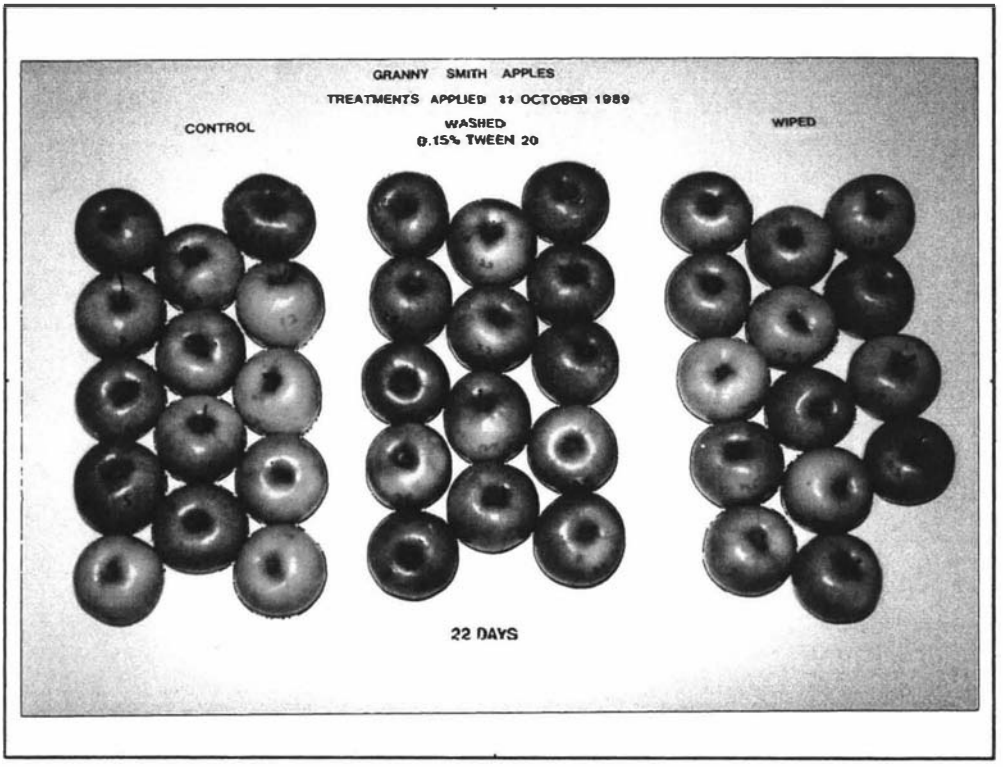

Fig. 6-14. Photograph showing Granny Smith apples after wiping or washing in Tween 20 solution and storage for 22 days at 20°C.

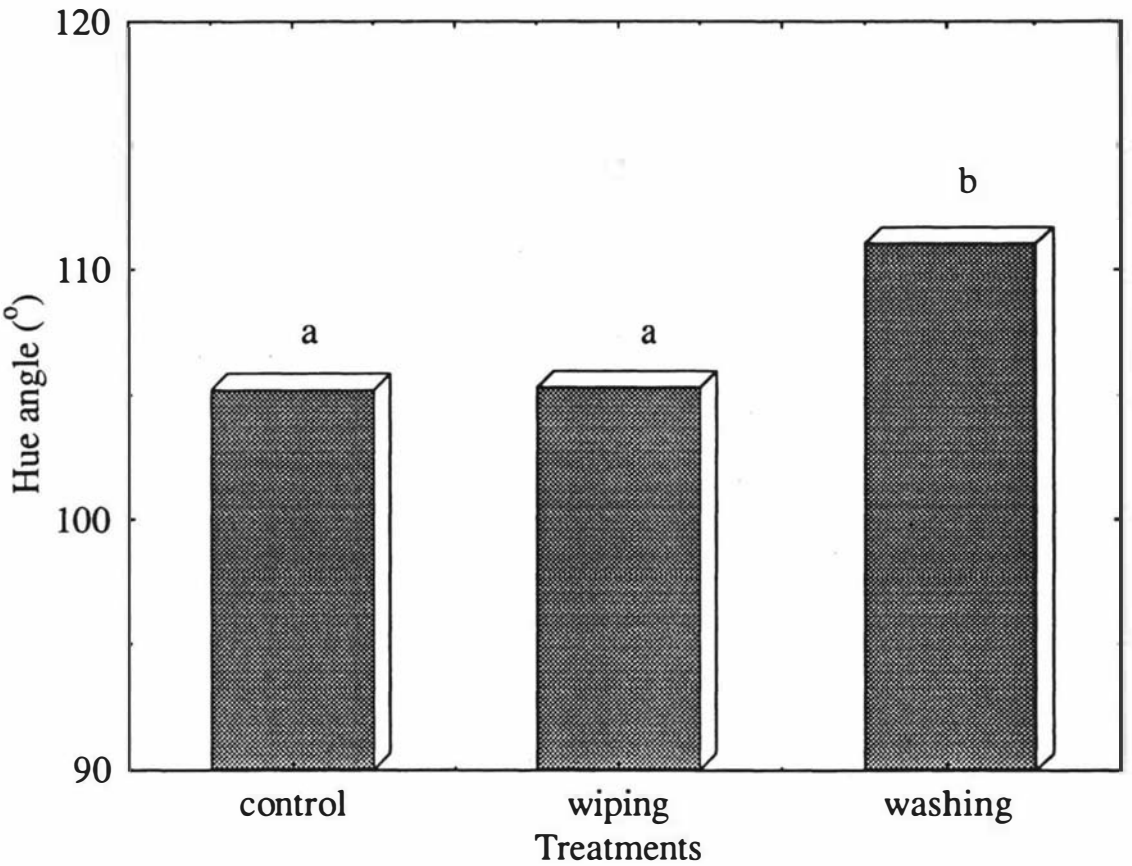

Fig. 6-15. Hue angle of Granny Smith apples after wiping or washing in Tween 20 solution and storage for 22 days at 20°C. Letters in common not significantly different at the 1% level. Mean separation by Least significant difference (LSD) procedure.

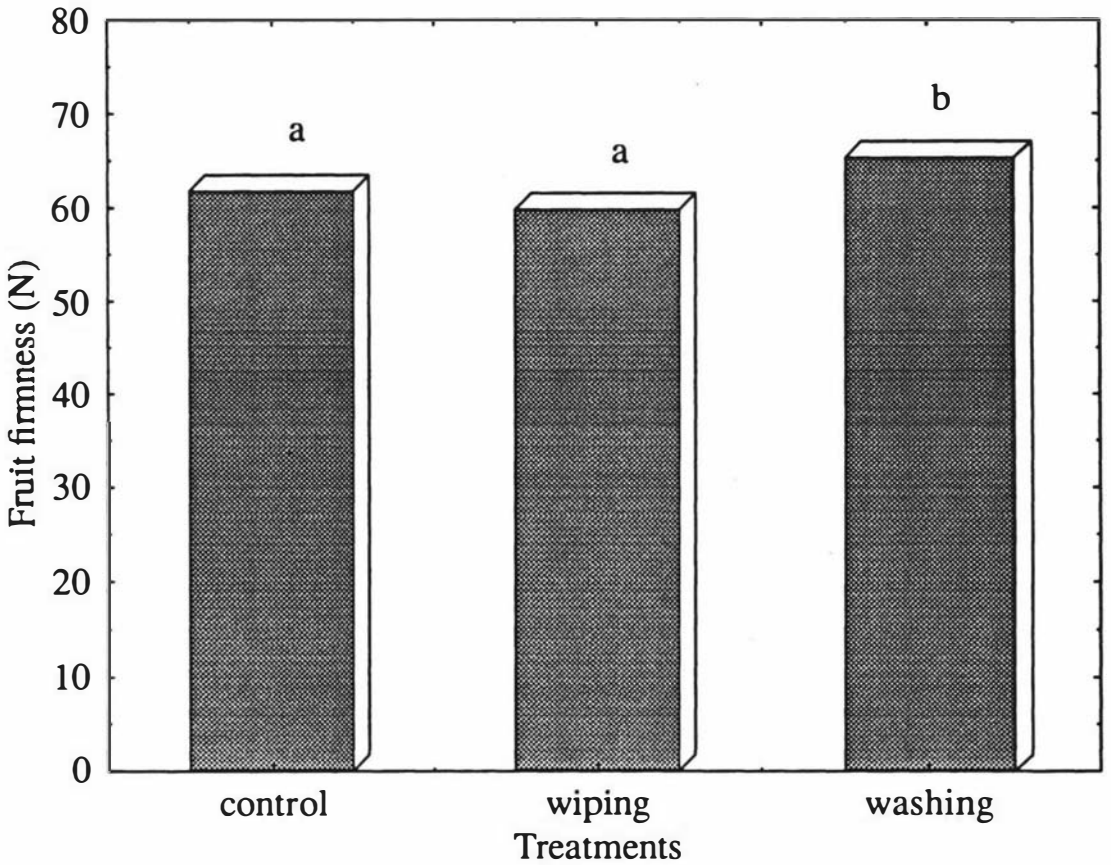

Fig. 6-16. Firmness of Granny Smith apples after wiping or washing in Tween 20 solution and storage for 22 days at 20°C. Letters in common not significantly different at the 1% level. Mean separation by Least significant difference (LSD) procedure.

treatments (fig. 6-17). However hue angle declined during storage of treated fruit at 0°C and especially following subsequent transfer to 20°C. During storage at 0°C, there was a progressive inhibition of degreening in higher treatment concentrations. Fruit washed in high Tween 20 concentrations before storage for 8 or 16 or 24 weeks at 0°C and then stored at 20°C were greener than their respective controls. There was a trend of decreasing loss of greenness with higher treatment concentrations in fruit stored at 0°C for 24 weeks and subsequently transferred to 20°C.

Fruit firmness remained high (mean of 80 Newtons) and approximately constant ($P < 0.05$) during the first 15 days after treatment application and storage at 20°C. However there were consistent trends ($P < 0.001$ for the linear effect of $\log_2$(Tween concentration)), of decreasing loss of firmness with higher Tween 20 concentrations after a period of storage at 0°C and subsequent storage at 20°C (fig. 6-18). Fruit washed in high (1.6 or 3.2%) Tween 20 solution were firmer than the controls during storage at 0°C for 16 or 24 weeks and subsequent storage at 20°C.

There was no marked (not significant at $P = 0.05$) effect of treatments on fruit soluble solids contents during storage at 0°C and subsequent transfer to 20°C (fig. 6-19). However there were significant differences in the overall soluble solids content of fruit stored at 0°C for 8, 16 and 24 weeks and subsequent storage at 20°C ($P < 0.05$). For example soluble solids content of control and most treated fruit increased by the 15th day at 20°C (fig. 6-19a) on the other hand it decreased after 24 weeks at 0°C and 15 days at 20°C (fig. 6-19d).

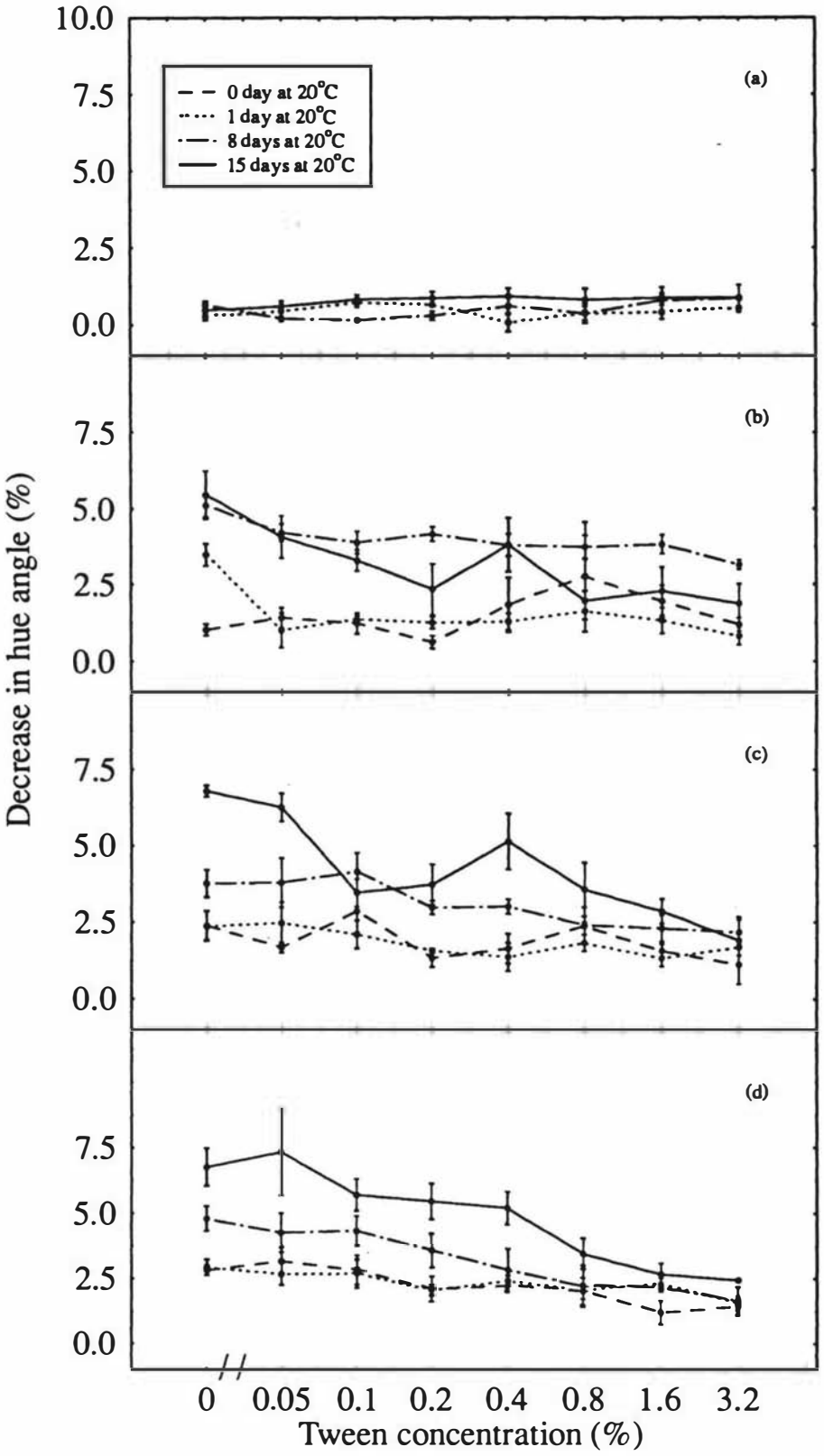

Fig. 6-17. Percentage decrease in hue angle of Granny Smith apples after washing in different concentrations of Tween 20 solution, stored (for (a)=0, (b)=8, (c)=16, (d)=24 weeks) at 0°C and subsequent transfer to 20°C (for 0, 1, 8 and 15 days). Vertical bars indicate standard errors of means.

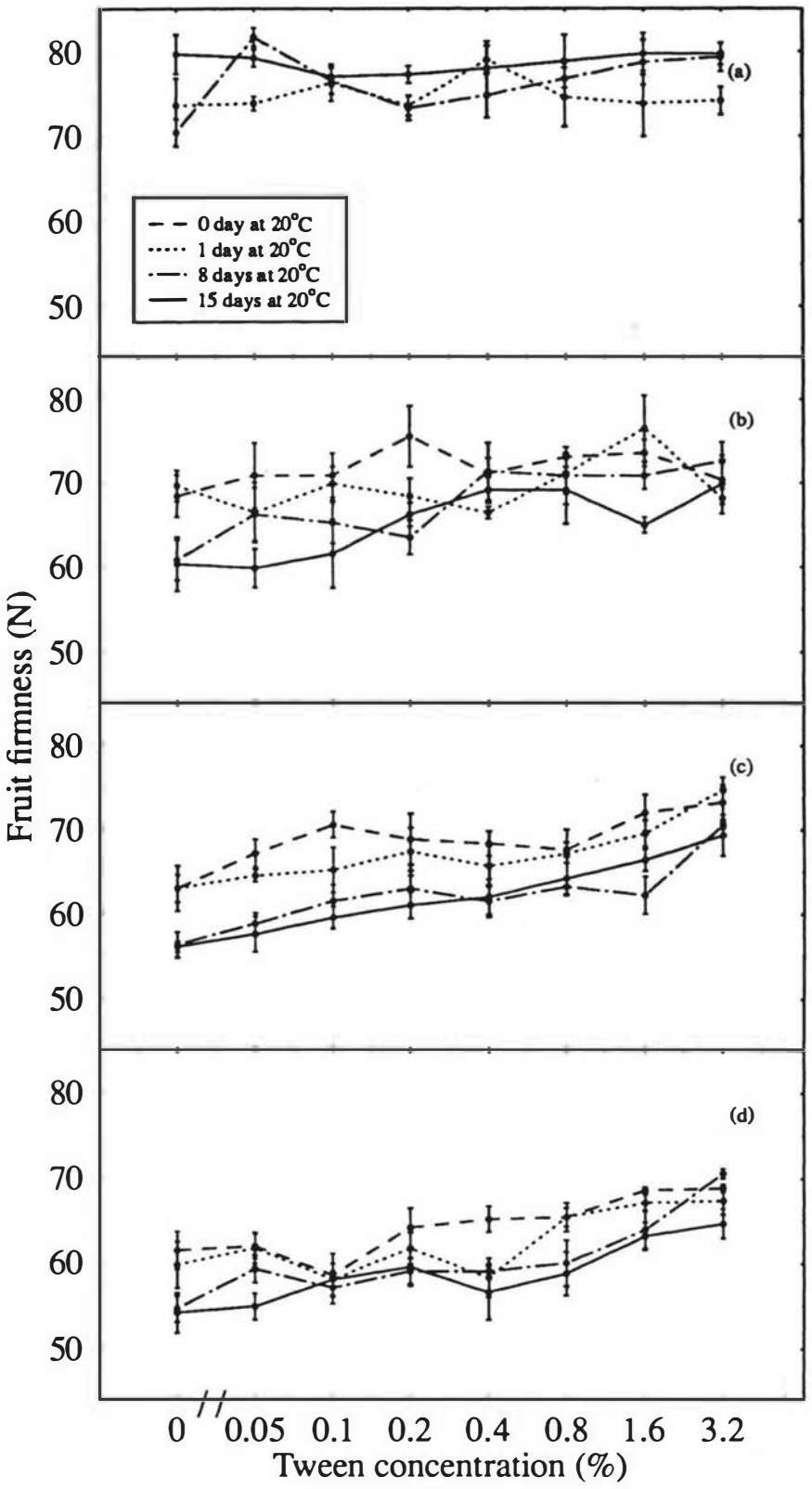

Fig. 6-18. Firmness of Granny Smith apples after washing in different concentrations of Tween 20 solution, stored (for (a)=0, (b)=8, (c)=16, (d)=24 weeks) at 0°C and subsequent transfer to 20°C (for 0, 1, 8 and 15 days). Vertical bars indicate standard errors of means.

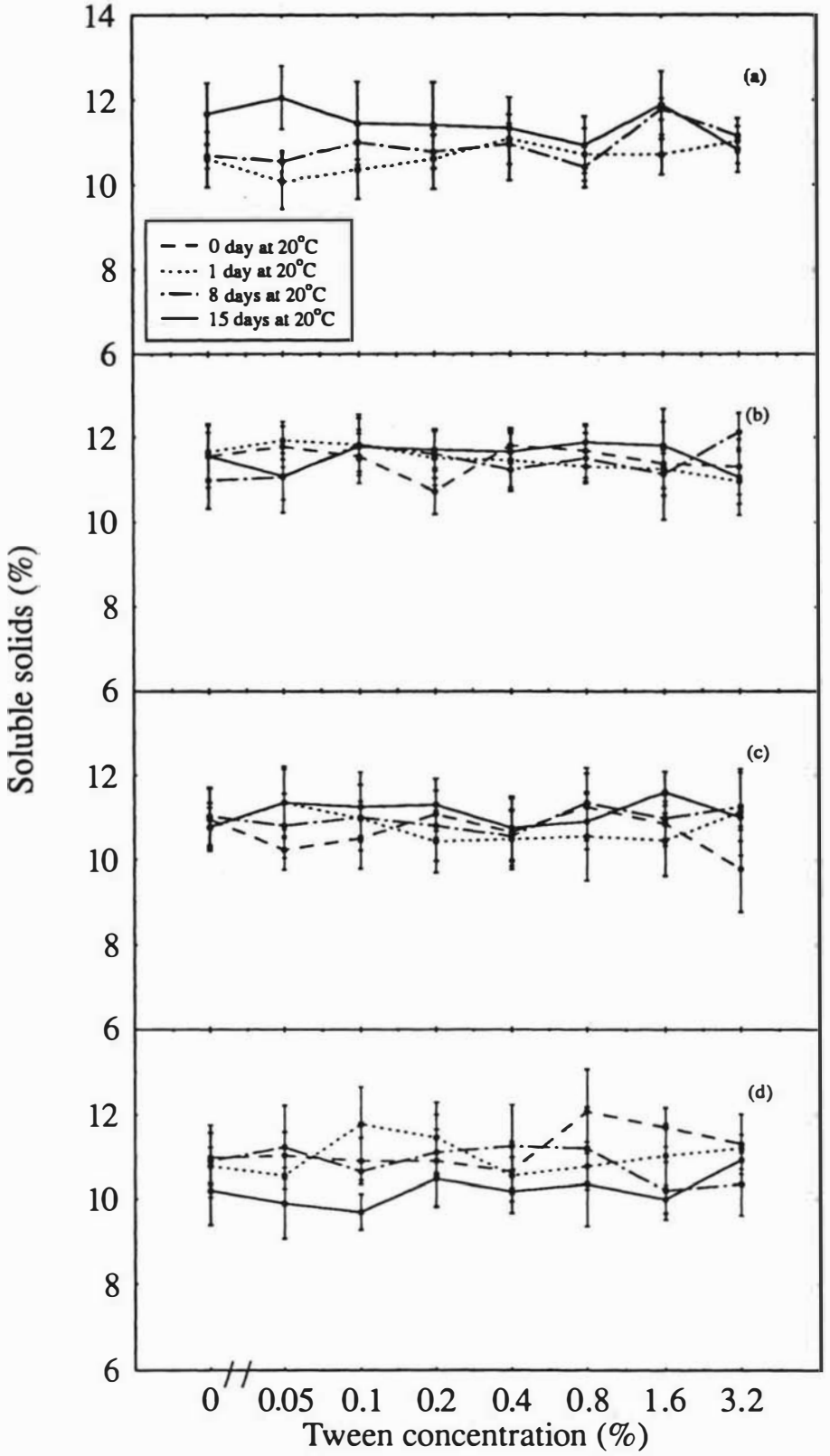

Fig. 6-19. Soluble solids contents of Granny Smith apples after washing in different concentrations of Tween 20 solution, stored (for (a)=0, (b)=8, (c)=16, (d)=24 weeks) at 0°C and subsequent transfer to 20°C (for 0, 1, 8 and 15 days). Vertical bars indicate standard errors of means.

6.5 DISCUSSION

The current study was initiated to examine the effects of Tween 20 solution on removal and/or development of greasiness and effects on browning in Granny Smith apples. Initial results obtained indicated that washing of greasy Granny Smith apple fruit in 0.15% Tween 20 solution markedly inhibited subsequent grease development. Similarly grease development was reduced when freshly harvested fruit were washed in Tween 20 concentrations of 0.8, 1.6 or 3.2% before storage at 0°C for 16 or 24 weeks and subsequent transfer to 20°C. The high Tween 20 concentrations presumably exerted their effects on grease development through modification of the fruit skin surface by blocking pores on fruit surface and consequently modifying fruit internal atmosphere composition. The high temperature storage seems to have exacerbated the development of greasiness in the control and fruit washed in low Tween 20 concentrations.

Data obtained in experiment 1 demonstrated that washing of fruit in 0.15% Tween 20 solution resulted in marked depression of $[O_2]_i$, increased $[C_2H_4]_i$, but had no effect on $[CO_2]_i$. The increase in $[C_2H_4]_i$ and depression of $[O_2]_i$ as a result of the Tween 20 treatment was presumably due to increased **R**. The low $[O_2]_i$ decreased fruit respiration rate. The combined increase in **R** and decreased respiration apparently led to maintenance of $[CO_2]_i$. These findings are in agreement with similar findings by Banks (1985).

The Tween 20 treatment generally increased RCO_2 and RC_2H_4 (fig. 6-4). However, the relative effects of the treatment on the diffusion of these gases were different. RC_2H_4 was higher than RCO_2 in all the treatments (experiment 1). The difference could be due to differences in the routes of diffusion of these two gases. CO_2 may move through both the cuticle and epidermis whilst C_2H_4 like O_2 diffuses only via the lenticels (Banks, 1984; Burton, 1974), so any treatment which blocks or affects the lenticels on the fruit

surface is likely to affect $[C_2H_4]_i$ more than $[CO_2]_i$. Hence RC_2H_4 was higher than that of RCO_2. This finding contrasts with the earlier findings reported in chapter 4. The disparity could be due to the application of the treatments and/or the high amount of grease on the fruit surface which presumably blocked lenticels in the present experiment (experiment 1). The current findings are consistent with the data published by Trout *et al.* (1953) who showed that the type of treatment or material applied to apples can indeed alter the relative effects of skin resistance to these gases since these gases differ in their paths of diffusion.

Modification of internal atmosphere at 20°C was much greater than the modification achieved by the same treatment at 0°C, presumably because of increased respiration at the higher temperature. There was a marked depression of $[O_2]_i$ in both control and treated fruit one day after fruit were transferred from 0°C to 20°C. However after the initial period of depression of $[O_2]_i$ and corresponding increase in $[CO_2]_i$, fruit recovered (or established a new physiological equilibrium at the new temperature), with an increase in $[O_2]_i$ and decrease in $[CO_2]_i$. There was no condensation on the fruit at the time of measurement so, in the absence of grease development, **R** probably remained approximately constant. Therefore changes in internal atmosphere composition presumably reflect change in respiration: adjustment of respiration (to low-O_2) appears to take longer than physical equilibration. A similar finding was reported by Knee (1980) who showed that the response of fruit respiration to changes in O_2 concentration was delayed by 1 to 4 days depending upon the time and direction of change. Knee considered this delay was longer than the time required for equilibration of O_2 concentrations inside and outside the fruit. On the other hand, Tucker and Laties (1985) reported that with avocados only a time interval of 12h was required for re-establishment of a constant respiration rate after a change in O_2 concentration.

The depression of O_2 levels inside washed fruit was greater than the elevation of CO_2, suggesting that washing in Tween 20 solution may have affected CO_2 evolution and O_2 uptake to different extents. It seems more likely that skin resistance to the two gases might have been altered to different extents because the Tween 20 deposit on the fruit surface was differentially permeable to these two gases. In general, washing of fruit with Tween 20 solution increased $[CO_2]_i$, but because the solubility of CO_2 in water and lipids (Mitz, 1979) is about 20 times greater than that of O_2, the increase was usually less than that the decrease for O_2. Furthermore, as mentioned in the preceding page, because of the differences in the path of diffusion of these gases, any modification of the fruit skin surface which blocks pores is likely to have greater effect on $[O_2]_i$ and $[C_2H_4]_i$ than on $[CO_2]_i$. High Tween 20 concentrations (1.6 or 3.2%) resulted in lower $[O_2]_i$ and higher $[CO_2]_i$ and $[C_2H_4]_i$, and were most effective in preventing the development of greasiness in Granny Smith apples. The reduced $[O_2]_i$ were not low enough to significantly result in any internal physiological damage to the fruit. In both studies, in spite of the fact that some of the fruit developed high $[CO_2]_i$, none of the treated fruit developed internal browning. It is interesting to note that in the preliminary experiment the $[CO_2]_i$ which was correlated with in internal browning were lower than those obtained in experiments 1 and 2. This disparity could be due to the fact that development of internal browning was not only dependent on high $[CO_2]_i$, but it may also be maturity or ripeness related. Even though the use of Tween 20 solution elevated $[CO_2]_i$ it delayed ripening through the depression of respiration and $[O_2]_i$.

Poapst *et al.* (1978) working with potatoes indicated that application of Tween surfactant for the prevention of greening in cold-stored potato tubers resulted in high $[CO_2]_i$. They indicated that this was associated with reduced CO_2 permeability caused by the adhering surfactant film. In their studies on potato tubers, Poapst and Forsyth (1975) also reported that the greening control mechanism exerted by Tween surfactant was related to accumulation

of CO_2 within the tuber and suggested that the surfactant formed a temporary film on the tuber's surface. In the current study washing of Granny Smith apples in Tween 20 surfactant depressed $[O_2]_i$ much more than it elevated $[CO_2]_i$. Since, Poapst and Forsyth did not measure the $[O_2]_i$ this raises the strong possibility that their claim that high CO_2 affects greening could in fact be due more to reduced O_2 inside the tubers than to increased CO_2 concentration.

Wiping of Granny Smith apples with tissue paper to remove surface grease resulted in an increase in percentage weight loss over the 22 days after treatment application. The increased weight loss was probably due to the fact that most of the natural coating on the fruit surface had been removed by the wiping treatment. This is consistent with the findings by Hall (1966) who reported that when the surfaces of Granny Smith apples ~~are~~ were wiped with paper wrappers, their transpiration rates increased and this was probably due to removal of surface waxes. Similar findings have also been reported (for oranges, apples and various kinds of leaves) by other investigators including Ben-Yehoshua (1969), Denna (1970), Marshall *et al.* (1936), Pieniazek (1944), Schonherr (1976), Smith (1932), Soliday *et al.* (1979), Trout *et al.* (1953).

Percentage weight loss of washed and control fruit were similar and statistically different from wiped fruit. Similarly, during the first 24h after treatment application percentage weight loss of control and treated fruit were similar. However after a period of cold storage and subsequent transfer to high temperature percentage weight loss of fruit increased. This would be expected from the increase in vapour pressure deficit (at the high temperature) experienced by the fruit (Burton, 1982). It is also possible that the increase in percentage weight loss at the high temperature could be caused by an increased rate of respiration (Inaba and Chachin, 1988). Washed fruit lost weight more rapidly than the controls, with increasing treatment concentration aggravating this effect. The Tween 20 treatment presumably affected weight loss through the removal of the surface wax or alternatively through permanent

penetration of the cuticle or inhibition of grease development. These findings are consistent with those of other investigators including, Horrocks (1964), Huelin and Gallop (1951b), Markley and Sando (1931), Pieniazek (1944), and Richmond and Martin (1959), who demonstrated that use of surfactant to remove surface deposits of wax embedded in apple cuticle increased rates of water loss and accelerated wilting or shrivelling.

Washing of Granny Smith apples in Tween 20 solution markedly reduced colour change. The retention of green colour in washed fruit could be associated with the depressed $[O_2]_i$ and/or depressed respiration rate . Any treatment which retards fruit respiration was associated with retention of colour change (Trout *et al.*, 1953). Studies by other researchers have also shown that the loss of green colour in apples was delayed in MA or low-O_2 atmospheres (Hewett *et al.*, 1989; Kader, 1986; Knee, 1975, 1980). Alternatively, the retention of green colour could also be due to elevation in $[CO_2]_i$ as a result of the Tween 20 treatment. This finding is consistent with that of Burton, (1974) who indicated in a review article that high CO_2 (10%) could retain the green colour of apples.

Fruit washed in Tween 20 solution were firmer than their respective controls. In experiment 2 there was an effect of Tween 20 solution on firmness changes even at 0°C. The effect of Tween 20 solution on firmness changes could presumably be mediated through the depression of $[O_2]_i$ and respiration and/or elevation of $[CO_2]_i$. There are several reports of the use of coating materials, CA or MA to delay loss of firmness in apples (Kader, 1986; Knee, 1980; Trout *et al.*, 1942; Hulme, 1949; Tomkins, 1968) and these are also thought to have been effective through modification of the fruit's internal atmosphere. Results obtained in the current study confirm these assertions.

In conclusion these findings demonstrate that washing of greased Granny Smith apples in 0.15% Tween 20 solution markedly reduced subsequent grease development. Washing freshly harvested fruit in high

Tween 20 concentrations (1.6 or 3.2%) was most effective in preventing the development of greasiness. The Tween 20 concentrations presumably exerted their effects on grease development by blocking pores on fruit surface and consequently modifying fruit internal atmosphere composition and respiration. Fruit were greener and firmer and there was no noticeable internal browning which is often associated with the development of greasiness. These findings indicate that some form of coating treatment could be used to prevent the development of greasiness in Granny Smith apples.

In the commercial situation, coating treatment of this type could easily be incorporated into the packing chain of the grower or pack house. If maximum benefits of the Tween 20 treatment are to be achieved it is essential that the temperature at which fruit are stored are properly controlled in view of the fact that high Tween 20 concentrations tend to exacerbate water loss and modification of gas exchange characteristics of the fruit.

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CHAPTER 7

TEMPERATURE EFFECTS ON INTERNAL ATMOSPHERE COMPOSITION, RESPIRATION, ETHYLENE PRODUCTION AND SKIN RESISTANCE TO GAS DIFFUSION OF APPLES.

7.1 ABSTRACT

The relationship between temperature and internal atmosphere composition, respiration, rate of C_2H_4 production and **R** of Cox's Orange Pippin, Gala, Royal Gala, Golden Delicious, Red Delicious and Splendour apples was ascertained after equilibrating fruit at temperatures ranging from 0 - 30°C for 72h.

There was a progressive decline in $[O_2]_i$ with corresponding increase in $[CO_2]_i$ in response to increasing temperatures. Braeburn apples consistently had lower $[O_2]_i$ and higher $[CO_2]_i$ than the other cultivars. This could be related to their high **R**, low intercellular space volume as well as intermediate respiration rate. In contrast Splendour apples had higher $[O_2]_i$ and lower $[CO_2]_i$ than the other cultivars. The reason could be due to their low **R**, low respiration rate, presence of open calyx and numerous lenticels hence easier gas diffusion.

Between 0 and 10°C both $[C_2H_4]_i$ and rate of C_2H_4 production were low, however concentrations increased rapidly in response to increasing temperature to a maximum at 25°C above which concentrations declined, ~~with further temperature increase~~. The magnitude of decline varied between cultivars. At 30°C the capacity to produce C_2H_4 declined markedly. Splendour apples had the least capacity to accumulate and produce C_2H_4.

Fruit respiration rate increased in response to increasing temperatures. Cox's Orange Pippin apples equilibrated at 30°C were respiring nearly ten times more rapidly than those stored at 0°C. The capacity to produce CO_2 (for all the temperature regimes) was markedly lower in Splendour apples compared to the other cultivars.

RCO_2 and RC_2H_4 appeared to be independent of temperature. However, R varied between cultivar with Braeburn apples having the highest mean RCO_2 and RC_2H_4 compared to the other cultivars.

Braeburn apples were firmer than the other cultivars. Fruit softening was accelerated by high temperatures. Soluble solids content varied with cultivar but was not affected by temperature.

7.2 INTRODUCTION

Temperature is the single most important environmental factor affecting the rate of respiration and hence internal atmosphere composition of fruits including apples (Eaks, 1978; Kader *et al.*, 1985, 1989; Maxie *et al.* 1974). In harvested apples, the rate of respiratory activity as measured by O_2-CO_2 exchange, is an index of the rate of metabolism and hence the length of life of the fruit (Porritt, 1951; Porritt and Lidster, 1978). A depression in $[O_2]_i$ and elevation of $[CO_2]_i$ as well as respiration rate of fruits (including apples) occurs in response to increasing temperature (Kader *et al.*, 1985, 1989; Kidd and West, 1930; Magness, 1920; Magness and Diehl, 1924; Trout *et al.*, 1942). A review of temperature as a factor affecting the internal atmosphere of fruit is present in chapter 2. However, detailed information on the effects of a range of temperatures on the internal atmosphere composition and respiration rate of intact apples of different cultivars is limited or meager.

Information on the effect of temperature on **R** of various apple cultivars is also largely unavailable. Various investigators have measured apple **R** but generally only at one or two temperatures (Banks, 1985; Burg, 1962; Burg and Burg, 1965; Burton, 1974, 1978; Cameron, 1982; Cameron and Yang, 1982; Kidd and West, 1949; Knee, 1991). Information on the effect of various storage temperatures on **R** as well as internal atmosphere composition of various cultivars of apples could help in our understanding of the physiological behaviour of fruit during storage. For instance, Granny Smith apples develop a greasy surface after a period of cold storage and subsequent transfer to ambient temperatures; this increases **R**, impedes gas exchange thus modifying the internal atmosphere of fruit (this subject is addressed in more detail in the preceding chapter). As a result the development of certain physiological disorders such as internal browning may be triggered (Trout *et al.*, 1942). Consequently, knowledge of the effect of varying temperatures on **R** may help in our understanding of the development of some of these physiological disorders that develop in apples during storage.

Review articles on the physiology of C_2H_4 by Abeles (1973), Lieberman (1979), Roberts and Tucker (1985) and Osborne (1978) have presented limited information on various storage temperature-induced effects on $[C_2H_4]_i$ and rate of C_2H_4 production by fruits, particularly apples. This is largely because of the general paucity of information and until recently the lack of understanding of C_2H_4 biosynthesis (Field, 1985; Lyons *et al.*, 1979). Considering the significance of temperature as a major environmental variable and its known influence on many physiological process it is surprising that it has received relatively scant attention. As a corollary, relatively few reports on C_2H_4 physiology include information on the temperature effects on $[C_2H_4]_i$ of apples. In spite of the numerous research that has been conducted to study the accumulation and production of C_2H_4 in apples, there is still a dearth of information on the effects of a range of temperatures on $[C_2H_4]_i$ and C_2H_4 production of intact apple cultivars. Such information may help in our

understanding of the physiological response to varying temperatures by apple cultivars after low temperature storage and subsequent transfer to higher temperatures during the distribution chain.

The current research was therefore initiated to study the effects of a range of temperatures (0 - 30°C) on internal atmosphere composition, respiration rate, C_2H_4 evolution, **R** and some aspects of quality of eight commercial apple cultivars grown in New Zealand.

7.3 MATERIALS AND METHODS

7.3.1 Materials

Freshly harvested fruit of eight commercial export apple (***Malus domestica*** Borkh.) cultivars (Cox's Orange Pippin, Gala, Royal Gala, Golden Delicious, Red Delicious, Splendour, Braeburn and Granny Smith apples; count 125; av. weight 148 g) differing in date of harvest and maturity were obtained as previously described in 3.1. Fruit were stored in air at 0°C for 4 - 6 weeks. Prior to starting each experiment, fruit were taken directly from storage and carefully selected for uniformity in size and freedom from blemishes. A set of eight fruit from each cultivar was placed in the dark in thermostatically controlled temperature cabinets maintained at 0, 5, 10, 15, 20, 25 and 30±1°C respectively for 72h to allow fruit to equilibrate with the appropriate temperatures. A temperature probe was inserted into a representative sample of four fruit to ensure that fruit were at temperature equilibrium with their surroundings before measurements were made. This was because (in chapter 5) respiration took time to reach an equilibrium when fruit were transferred to new O_2 levels, so it was thought that it would be worthwhile to leave fruit for similar period to regain steady state at the new temperature.

7.3.2 Methods

7.3.2.1 Estimation of gas exchange variables

Respiration (CO_2 production) and C_2H_4 production were measured on fruit kept in the dark as previously described in 3.3.5.2.

Core cavity O_2, CO_2 and C_2H_4 concentrations were measured by the direct sampling method (see 3.3.4.1) and gas samples analysed as described in 3.3.

RCO_2 and RC_2H_4 were estimated by the steady-state method (see 3.3.5.2).

7.3.2.2 Fruit quality assessment

Fruit firmness was measured as previously described (see 3.3.8.1). Soluble solids content was also measured with an Atago N-1 hand refractometer (see 3.3.8.2) on a small volume of fruit juice extracted from fruit using a blender (Philips, type HR 2290/A).

7.3.2.3 Experimental design and analysis

Eight randomly selected single fruit replicates of each apple cultivar (completely randomised design) were kept at each of the seven temperature regimes. Experiments were repeated at least once. Data were analysed as previously described in section 3.3.9. Regression analyses were also performed according to the methods described by Steel and Torrie (1980). Methods of comparison of regression lines were undertaken as described by Kleinbaum and Kupper (1978). Mean comparisons were also carried out by the Least significant difference (LSD) procedure at 1% level of significance (Little and Hills, 1978) to test cultivar differences.

7.4 RESULTS

7.4.1 Internal O_2 concentration

There was a consistent decline in fruit $[O_2]_i$ in response to increasing temperatures (figs. 7-1 and 7-2). The magnitude of response varied with cultivar. After equilibration at 0°C, $[O_2]_i$ in all the cultivars was high and similar, however with increasing temperatures concentrations declined. At 25°C the average $[O_2]_i$ of Splendour apples ~~were~~ was nearly 19% as against approximately 12% in Braeburn and 15% in Granny Smith or Red Delicious. For all the temperature regimes studied, Splendour, Gala, Royal Gala and Golden Delicious apples consistently contain higher $[O_2]_i$ (even at 30°C, mean $[O_2]_i$ approximately 18%) than Braeburn or Granny Smith apples (10% and 13% respectively).

Regression analysis revealed that plots of $[O_2]_i$ versus temperature were linear with no significant deviations from the fitted lines ($P < 0.0001$; figs. 7-1 and 7-2). Comparison of the intercepts and slopes of the regression lines relating $[O_2]_i$ of apples to temperature showed that the intercepts for all cultivars were similar at about 20.6% O_2 though differences between cultivars were in many cases statistically highly significant (Table 7-1). On the other hand the slopes of the regression lines were different with a range of 0.09% O_2 $°C^{-1}$ (for Splendour) to 0.34% O_2 $°C^{-1}$ (for Braeburn) (figs. 7-1 and 7-2; Table 7-2).

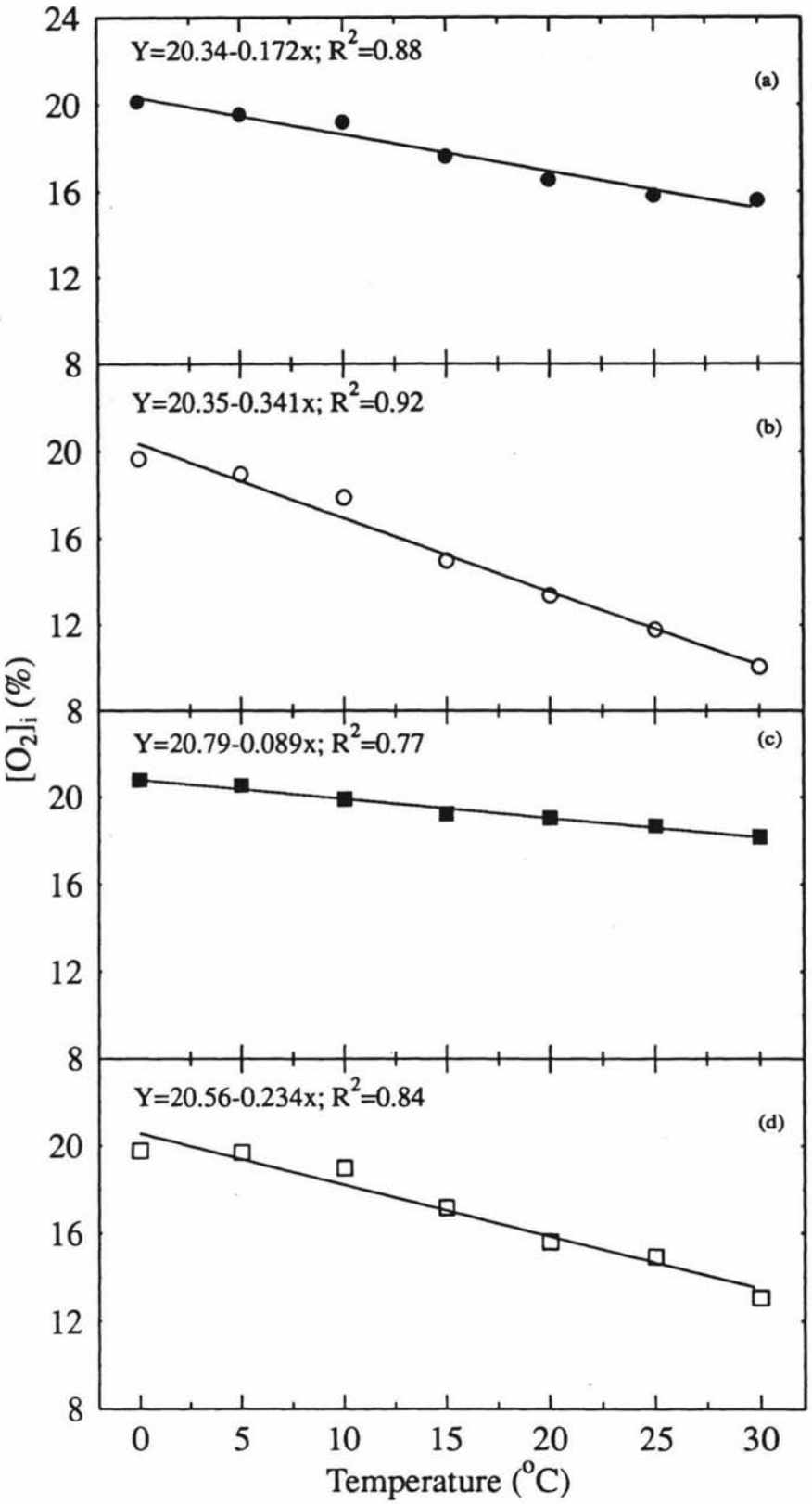

Fig. 7-1. Relationship between temperature and $[O_2]_i$ of (a)=Cox's Orange Pippin (b)=Braeburn (c)=Splendour (d)=Granny Smith apples with fitted regression.

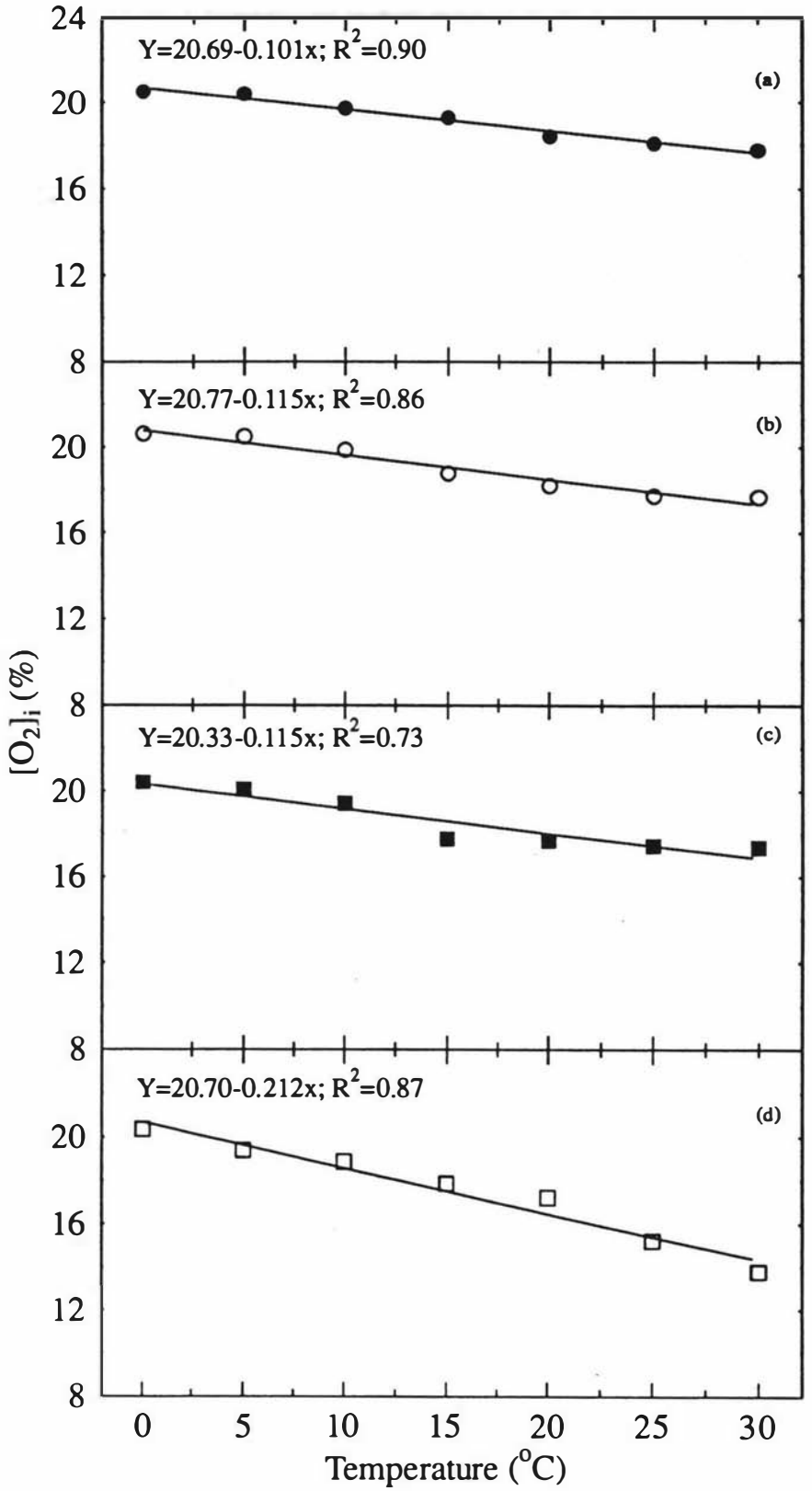

Fig. 7-2. Relationship between temperature and $[O_2]_i$ of (a)=Gala (b)=Royal Gala (c)=Golden Delicious (d)=Red Delicious apples with fitted regression.

Table 7-1. Comparison of significant differences between the intercepts of the regression lines relating $[O_2]_i$ of apples to temperature.

	APPLE CULTIVAR						
Cultivar	COP	G	GD	GS	RD	RG	S
Braeburn (BB)	***	***	***	***	***	***	***
Cox's Orange Pippin (COP)		**	NS	NS	NS	*	**
Gala (G)			NS	***	**	NS	NS
Golden Delicious (GD)				**	*	NS	NS
Granny Smith (GS)					NS	***	***
Red Delicious (RD)						**	***
Royal Gala (RG)							NS

NS, *, **, *** = Nonsignificant or significant at P = 0.05, 0.01, 0.001 respectively.

S = Splendour apple.

Table 7-2. Comparison of significant differences between the slopes of the regression lines relating $[O_2]_i$ of apples to temperature.

	APPLE CULTIVAR						
Cultivar	COP	G	GD	GS	RD	RG	S
Braeburn (BB)	***	***	***	**	***	***	***
Cox's Orange Pippin (COP)		**	*	**	NS	*	***
Gala (G)			NS	***	***	NS	NS
Golden Delicious (GD)				***	***	NS	NS
Granny Smith (GS)					NS	***	***
Red Delicious (RD)						***	***
Royal Gala (RG)							NS

NS, *, **, *** = Nonsignificant or significant at P = 0.05, 0.01, 0.001 respectively.

S = Splendour apple.

7.4.2 Internal CO_2 concentration

Temperature had significant ($P < 0.0001$) effect on $[CO_2]_i$ and the response of fruit $[CO_2]_i$ to temperatures was cultivar dependent (figs. 7-3 and 7-4). At low temperatures (0 - 10°C) $[CO_2]_i$ were low, however concentrations increased progressively in response to increasing temperatures. At 25°C, Braeburn and Cox's Orange Pippin apples contained nearly 8 and 7% CO_2 respectively as opposed to approximately 3% in Splendour, Gala or Royal Gala. At 30°C the $[CO_2]_i$ of Braeburn apples was nearly eight times higher than those at 0°C, compared to a threefold increase over the same temperature range for Splendour.

Regression analysis indicated that fruit $[CO_2]_i$ was linearly related to temperature ($P < 0.001$; figs. 7-3 and 7-4; with no significant quadratic or cubic deviations). Comparison of the intercepts of the regression lines relating $[CO_2]_i$ to temperature indicated cultivar differences ranging from 0.32% CO_2 (in Royal Gala) to 0.97% CO_2 (in Braeburn) and in some cases differences between cultivars were statistically highly significant (Table 7-3). Similarly, the slopes of the regression lines were also different with a range of 0.09% CO_2 $°C^{-1}$ (for Splendour) to 0.27% CO_2 $°C^{-1}$ (for Braeburn) and differences between cultivars were statistically highly significant in some cases (figs. 7-3 and 7-3; Table 7-4).

7.4.3 Fruit respiration rate

Temperature had a pronounced ($P < 0.0001$) effect on rate of respiration and varied with cultivar (figs. 7-5 and 7-6). Respiration rates of fruit were low at low temperatures (0 or 5°C), but rates increased progressively as temperature increased to 30°C. The magnitude of increase differed between cultivars. Splendour apples had the lowest average respiration rate compared to the other cultivars, whilst Cox's Orange Pippin apples had the highest

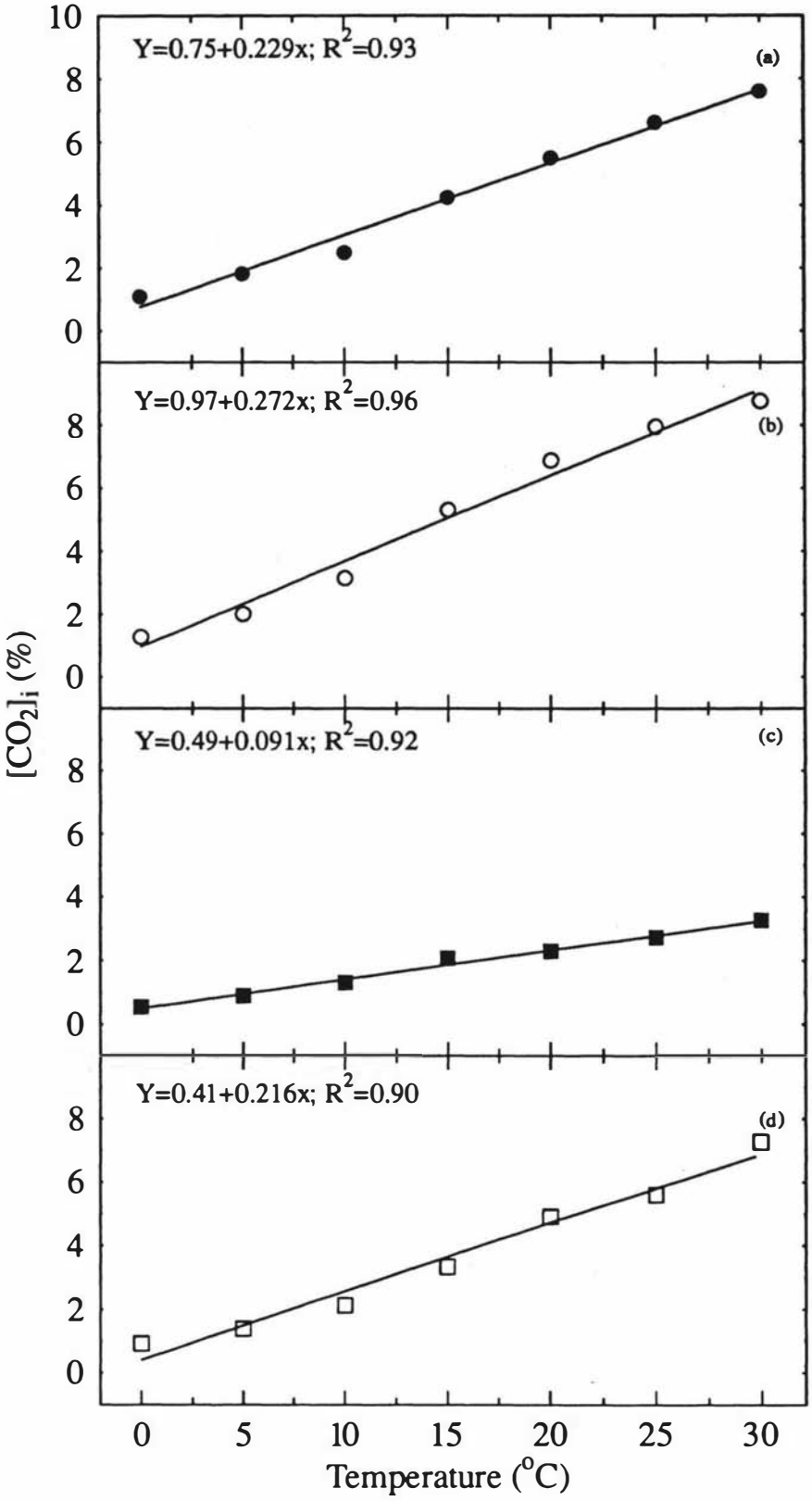

Fig. 7-3. Relationship between temperature and $[CO_2]_i$ concentrations of (a)=Cox's Orange Pippin (b)=Braeburn (c)=Splendour (d)=Granny Smith apples with fitted regression.

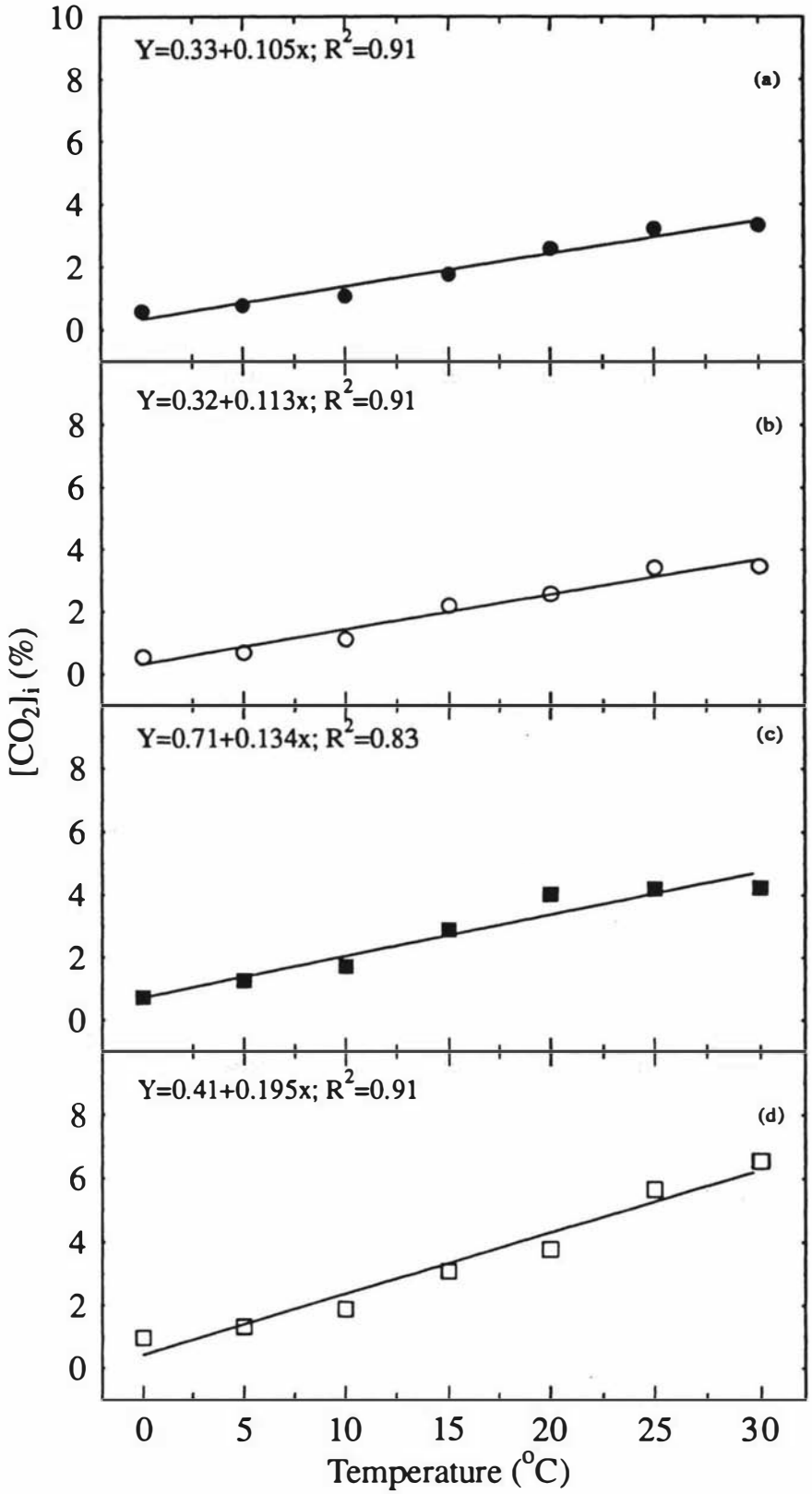

Fig. 7-4. Relationship between temperature and $[CO_2]_i$ of (a)=Gala (b)=Royal Gala (c)=Golden Delicious (d)=Red Delicious apples with fitted regression.

Table 7-3. Comparison of significant differences between the intercepts of the regression lines relating $[CO_2]_i$ of apples to temperature.

	APPLE CULTIVAR						
Cultivar	COP	G	GD	GS	RD	RG	S
Braeburn (BB)	*	***	***	**	***	***	***
Cox's Orange Pippin (COP)		***	***	NS	*	***	***
Gala (G)			NS	***	**	NS	NS
Golden Delicious (GD)				*	NS	NS	*
Granny Smith (GS)					NS	***	***
Red Delicious (RD)						**	***
Royal Gala (RG)							NS

NS, *, **, *** = Nonsignificant or significant at P = 0.05, 0.01, 0.001 respectively.

S = Splendour apple.

Table 7-4. Comparison of significant differences between the slopes of the regression lines relating $[CO_2]_i$ of apples to temperature.

	APPLE CULTIVAR						
Cultivar	COP	G	GD	GS	RD	RG	S
Braeburn (BB)	*	***	***	**	***	***	***
Cox's Orange Pippin (COP)		***	***	NS	NS	***	***
Gala (G)			NS	***	***	NS	NS
Golden Delicious (GD)				***	**	NS	*
Granny Smith (GS)					NS	***	***
Red Delicious (RD)						***	***
Royal Gala (RG)							NS

NS, *, **, *** = Nonsignificant or significant at P = 0.05, 0.01, 0.001 respectively.

S = Splendour apple.

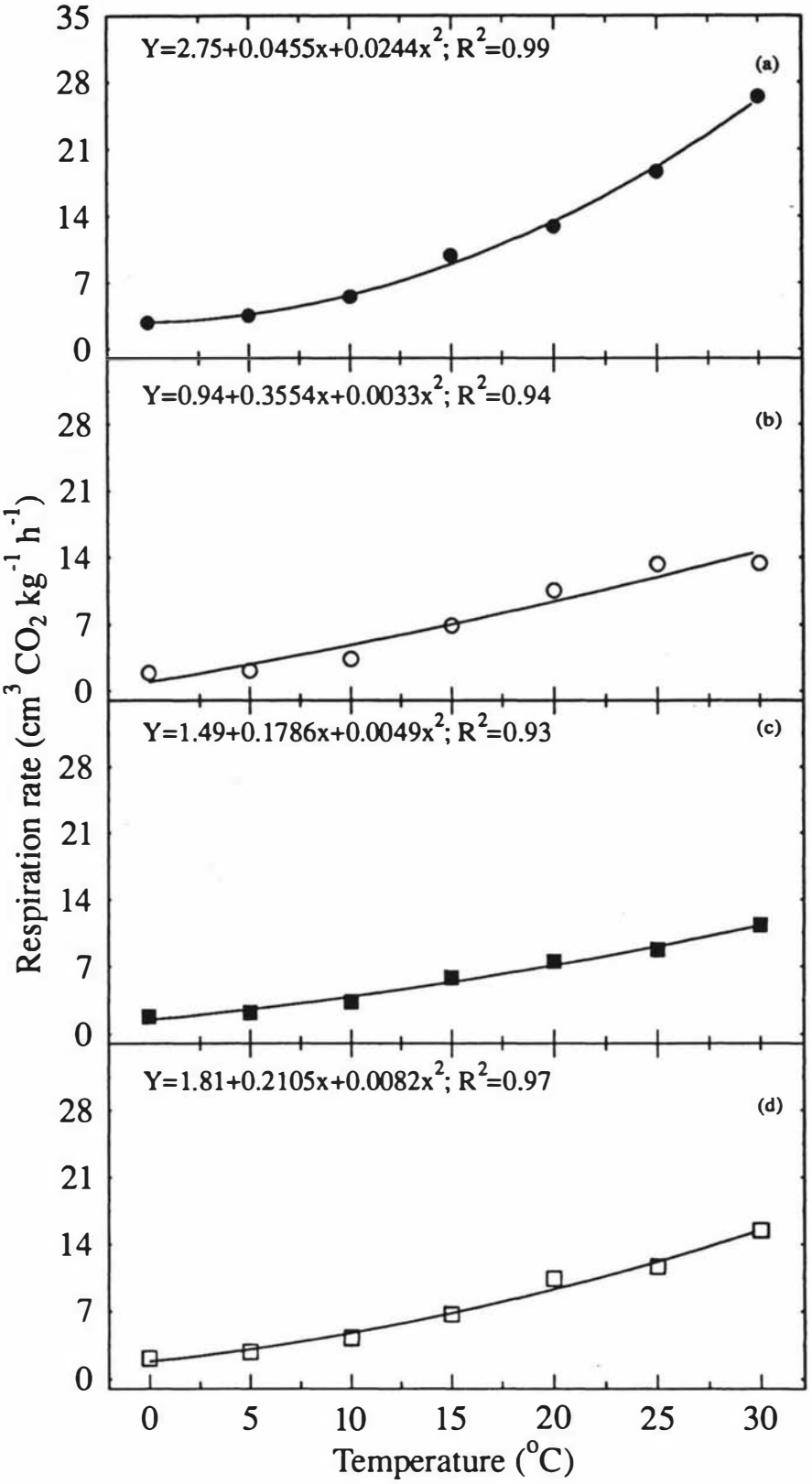

Fig. 7-5. Relationship between temperature and respiration rate of (a)=Cox's Orange Pippin (b)=Braeburn (c)=Splendour (d)=Granny Smith apples with fitted regression.

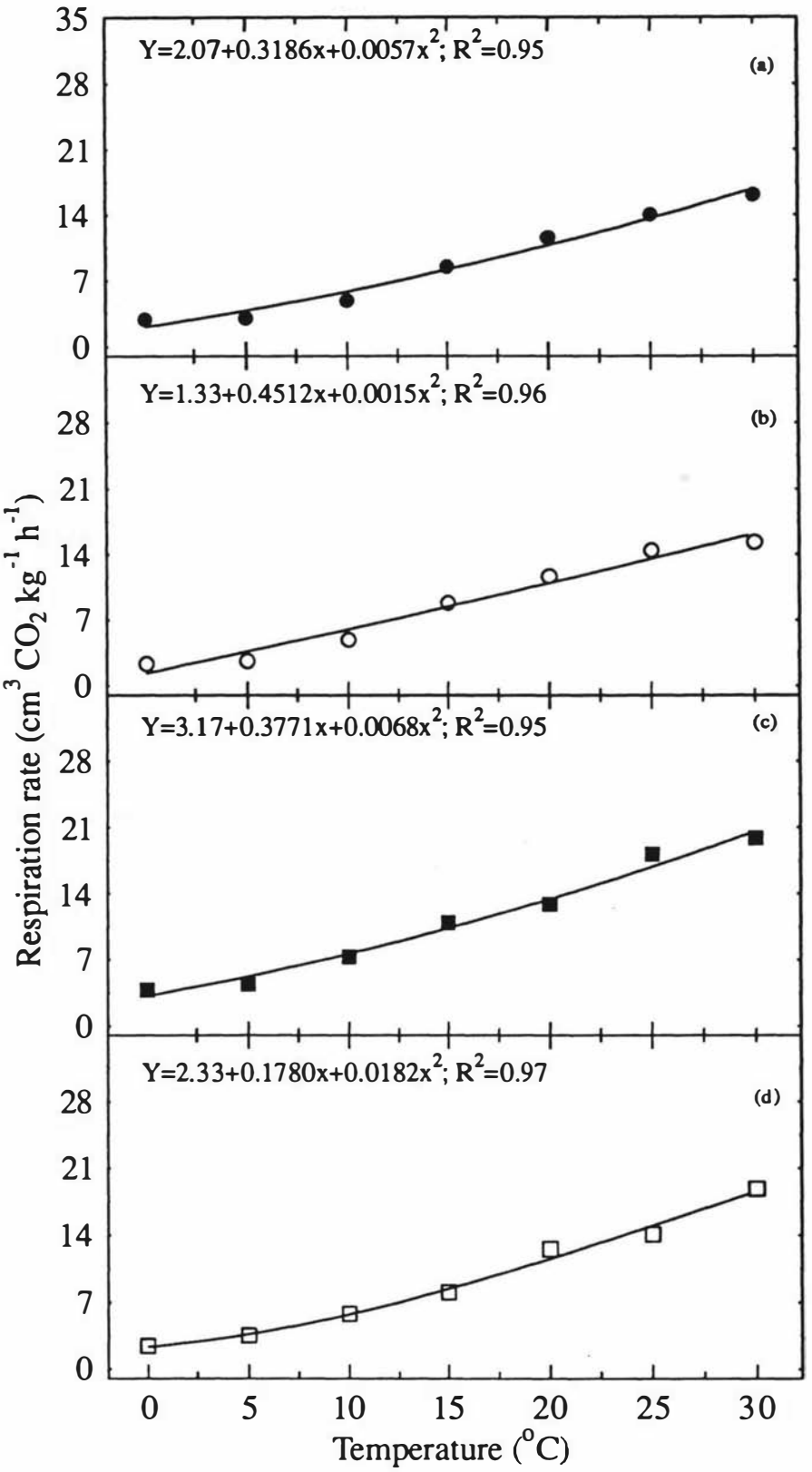

Fig. 7-6. Relationship between temperature and respiration rate of (a)=Gala (b)=Royal Gala (c)=Golden Delicious (d)=Red Delicious apples with fitted regression.

respiration rate. At 30°C, Cox's Orange Pippin apples were respiring over 50% more rapidly than Splendour apples. Cox's Orange Pippin apples equilibrated at 30°C were respiring nearly ten times more than at 0°C.

Regression analysis showed that with the exception of Cox's Orange Pippin apples, in which the response of respiration to temperature was decidedly curvilinear, in most cases the response was largely linear with a minor quadratic component (figs. 7-5 and 7-6).

7.4.4 Internal C_2H_4 concentration

Fruit $[C_2H_4]_i$ were markedly affected by equilibration temperatures with significant linear, quadratic and cubic effects of temperature (Table 7-5). The response pattern differed between cultivars (figs. 7-7 and 7-8). Low equilibration temperatures between 0 and 10°C markedly depressed fruit $[C_2H_4]_i$ ($P < 0.001$). However concentrations increased progressively in response to further increase in temperature to a maximum at 25°C above which internal concentrations declined with further temperature increase. The magnitude of decline varied with cultivar. At 30°C most of the cultivars lost the capacity to accumulate C_2H_4.

Exposing fruit to 30°C significantly ($P < 0.001$) depressed $[C_2H_4]_i$ relative to that found at 25°C. The magnitude of this depression varied with cultivar. For example, Royal Gala apples stored at 30°C were found to contain only about one seventh as much C_2H_4 as at 25°C as opposed to about a third as much as in Cox's Orange Pippin, approximately half as much in Golden Delicious and almost the same amount in Granny Smith.

Of all the cultivars studied Splendour apples had the least capacity to accumulate C_2H_4 over all the temperature regimes. For instance, Splendour apples equilibrated at 0°C and 25°C had mean $[C_2H_4]_i$ of nearly 15 and 66 µl

Table 7-5. Relationship between $[C_2H_4]_i$ and temperature of apples.

Cultivar	Regression equation	Level of significance L	Q	C
Cox's Orange Pippin	$Y=97.45 - 34.47x + 4.93x^2 - 0.12x^3$	**	****	****
Braeburn	$Y=94.65 - 64.39x + 8.24x^2 - 0.20x^3$	***	****	****
Splendour	$Y=17.32 - 2.05x + 0.55x^2 - 0.02x^3$	*	*	**
Granny Smith	$Y=59.06 - 18.96x + 2.37x^2 - 0.05x^3$	*	**	**
Gala	$Y=83.08 - 44.25x + 7.23x^2 - 0.19x^3$	**	****	****
Royal Gala	$Y=57.79 - 35.84x + 6.39x^2 - 0.17x^3$	***	****	****
Golden Delicious	$Y=127.82 - 19.79x + 4.16x^2 - 0.11x^3$	**	****	****
Red Delicious	$Y=98.26 - 18.28x + 2.85x^2 - 0.07x^3$	**	****	****

*, **, ***, **** = significant at P = 0.05, 0.01, 0.001, or 0.0001 respectively.

L, **Q** and **C** = Linear, Quadratic and Cubic

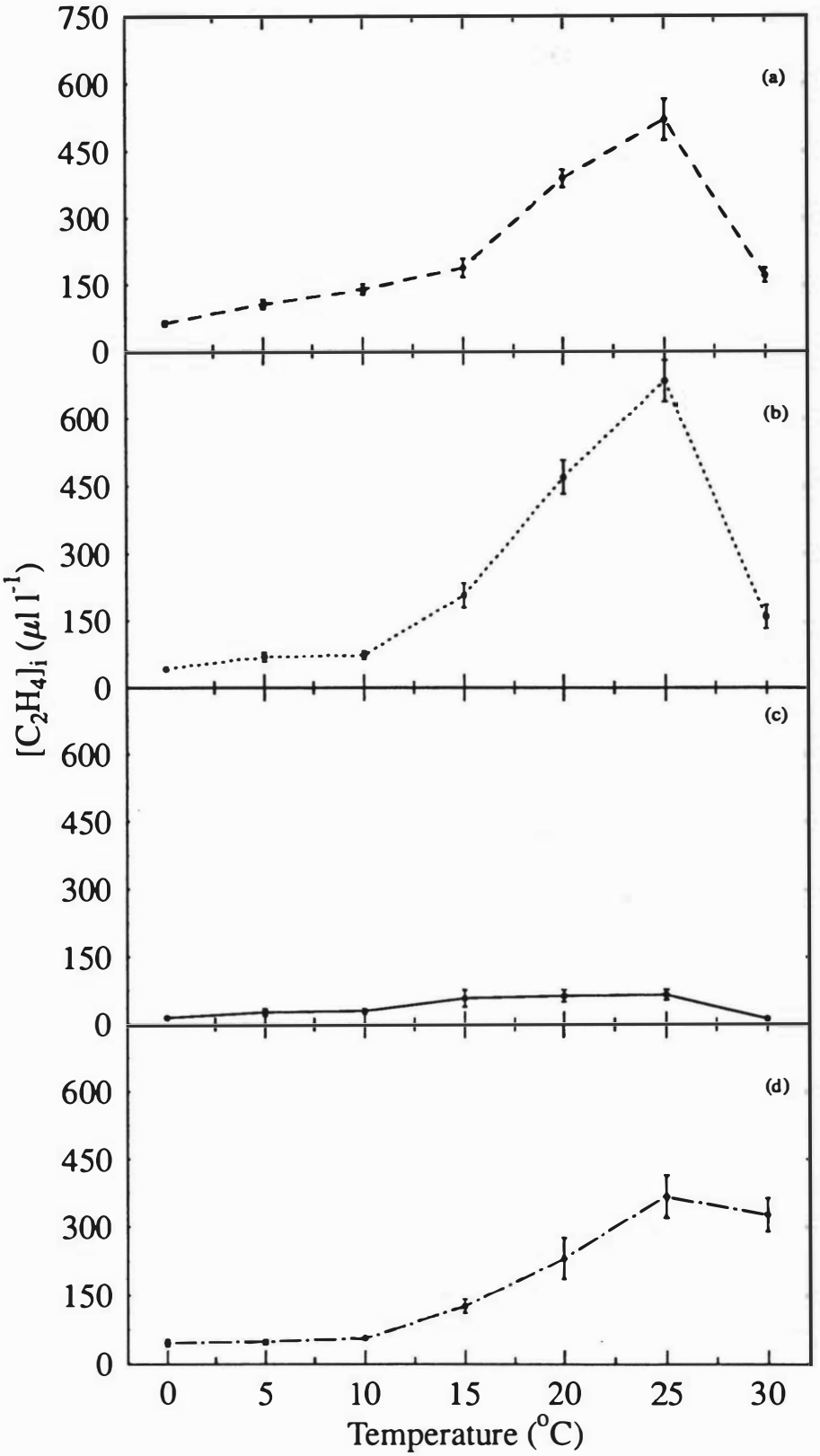

Fig. 7-7. Temperature effects on $[C_2H_4]_i$ of (a)=Cox's Orange Pippin (b)=Braeburn (c)=Splendour and (d)=Granny Smith apples. Vertical bars represent standard error of the means.

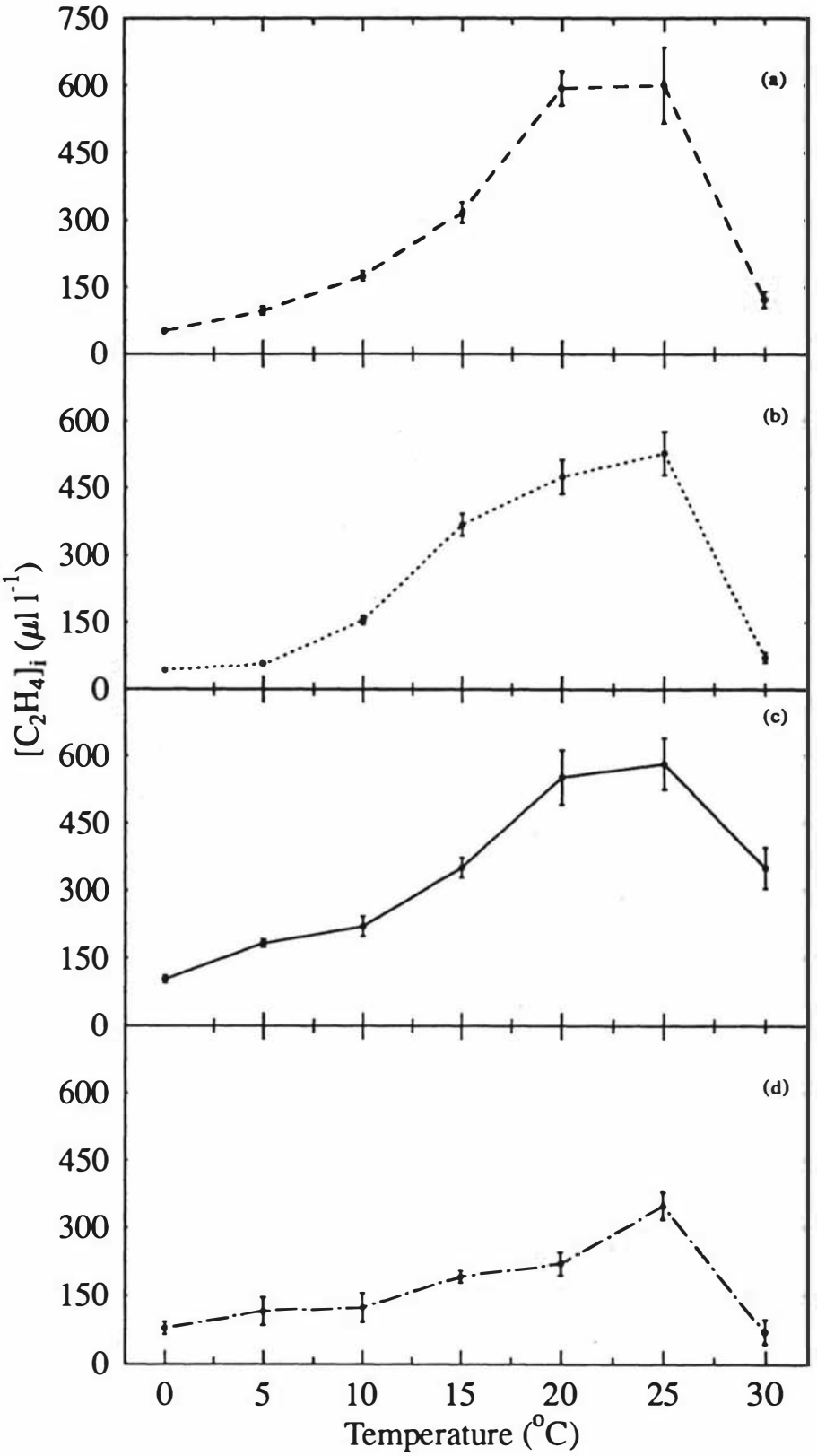

Fig. 7-8. Temperature effects on $[C_2H_4]_i$ of (a)=Gala (b)=Royal Gala (c)=Golden Delicious (d)=Red Delicious apples. Vertical bars represent standard error of the means.

l^{-1} respectively, compared to 43 and 684 µl l^{-1} in Braeburn; 63 and 521 µl l^{-1} in Cox's Orange Pippin; 50 and 600 µl l^{-1} in Gala; and 103 and 583 µl l^{-1} in Golden Delicious.

7.4.5 C_2H_4 production

Temperature had pronounced effect on the rate of C_2H_4 production (Table 7-6; with significant linear, quadratic and cubic effects) and varied with cultivar (figs. 7-9 and 7-10). At low temperatures between 0 and 10°C the rates of C_2H_4 production were low but production increased progressively with increasing temperature to a maximum at 25°C above which production declined. The capacity to produce C_2H_4 obviously declined markedly at 30°C. However the magnitude of decline was cultivar dependent. The rates of C_2H_4 production of Braeburn, Royal Gala, Gala, Cox's Orange Pippin and Granny Smith apples at 25°C were 20, 15, 12 10 and 8 times higher than at 0°C (respectively). At 25°C, Golden Delicious and Gala apples produced nearly twelve times more C_2H_4 than Splendour. At 30°C, Golden Delicious and Gala apples were respectively producing approximately one half and one fifth as much C_2H_4 as at 25°C. Over all the temperature ranges, Splendour apples had the lowest rate of C_2H_4 production compared to the other cultivars.

7.4.6 Skin resistance to gas diffusion

RCO_2 and RC_2H_4, estimated using the steady-state method, were cultivar dependent (figs 7-11, 7-12 and figs. 7-13, 7-14 respectively). Statistically equilibration temperature had no significant (at $P = 0.05$) effect on RCO_2 and RC_2H_4. However there were marked differences in RCO_2 and RC_2H_4 between temperatures in some of the cultivars. For example at 0°C, RCO_2 and RC_2H_4 of Braeburn apples were markedly ($P < 0.01$) lower than at 5 or 10°C. RCO_2 and RC_2H_4 of Braeburn apples over all the temperature

Table 7-6. Relationship between C_2H_4 production and temperature of apples.

Cultivar	Regression equation	Level of significance L	Q	C
Cox's Orange Pippin	$Y=51.69 - 21.59x + 2.98x^2 - 0.07x^3$	**	****	****
Braeburn	$Y=23.51 - 18.71x + 2.26x^2 - 0.05x^3$	***	****	****
Splendour	$Y=12.44 - 2.91x + 0.48x^2 - 0.01x^3$	*	**	***
Granny Smith	$Y=19.65 - 5.44x + 0.67x^2 - 0.01x^3$	*	**	**
Gala	$Y=68.12 - 36.69x + 6.14x^2 - 0.16x^3$	***	****	****
Royal Gala	$Y=43.50 - 32.73x + 5.66x^2 - 0.15x^3$	****	****	****
Golden Delicious	$Y=109.53 - 23.75x + 4.38x^2 - 0.11x^3$	**	****	****
Red Delicious	$Y=33.41 - 9.06x + 1.56x^2 - 0.04x^3$	**	**	***

*, **, ***, **** = significant at P = 0.05, 0.01, 0.001, or 0.0001 respectively.

L, **Q** and **C** = Linear, Quadratic and Cubic

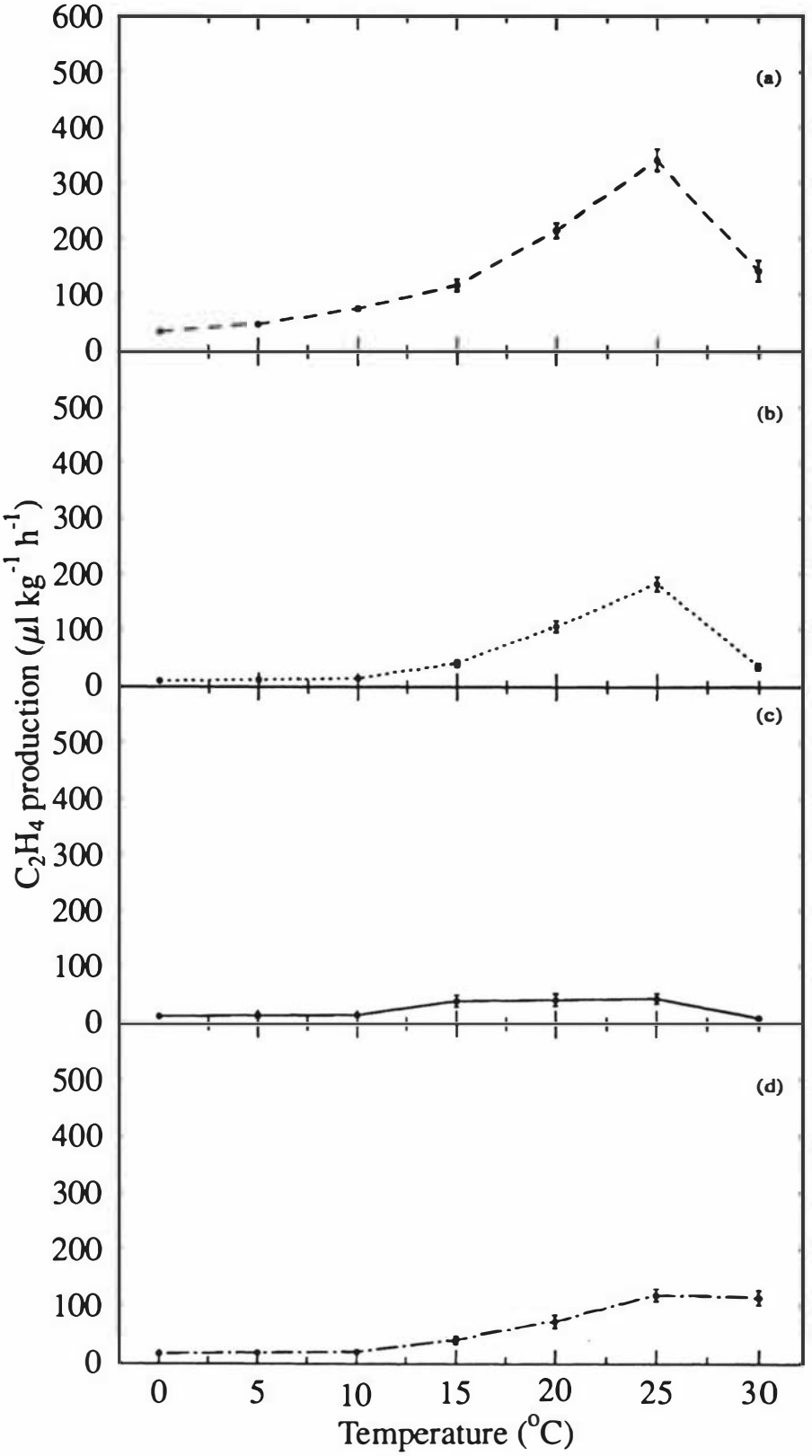

Fig. 7-9. Temperature effects on C_2H_4 production of (a)=Cox's Orange Pippin (b)=Braeburn (c)=Splendour and (d)=Granny Smith apples. Vertical bars represent standard error of the means.

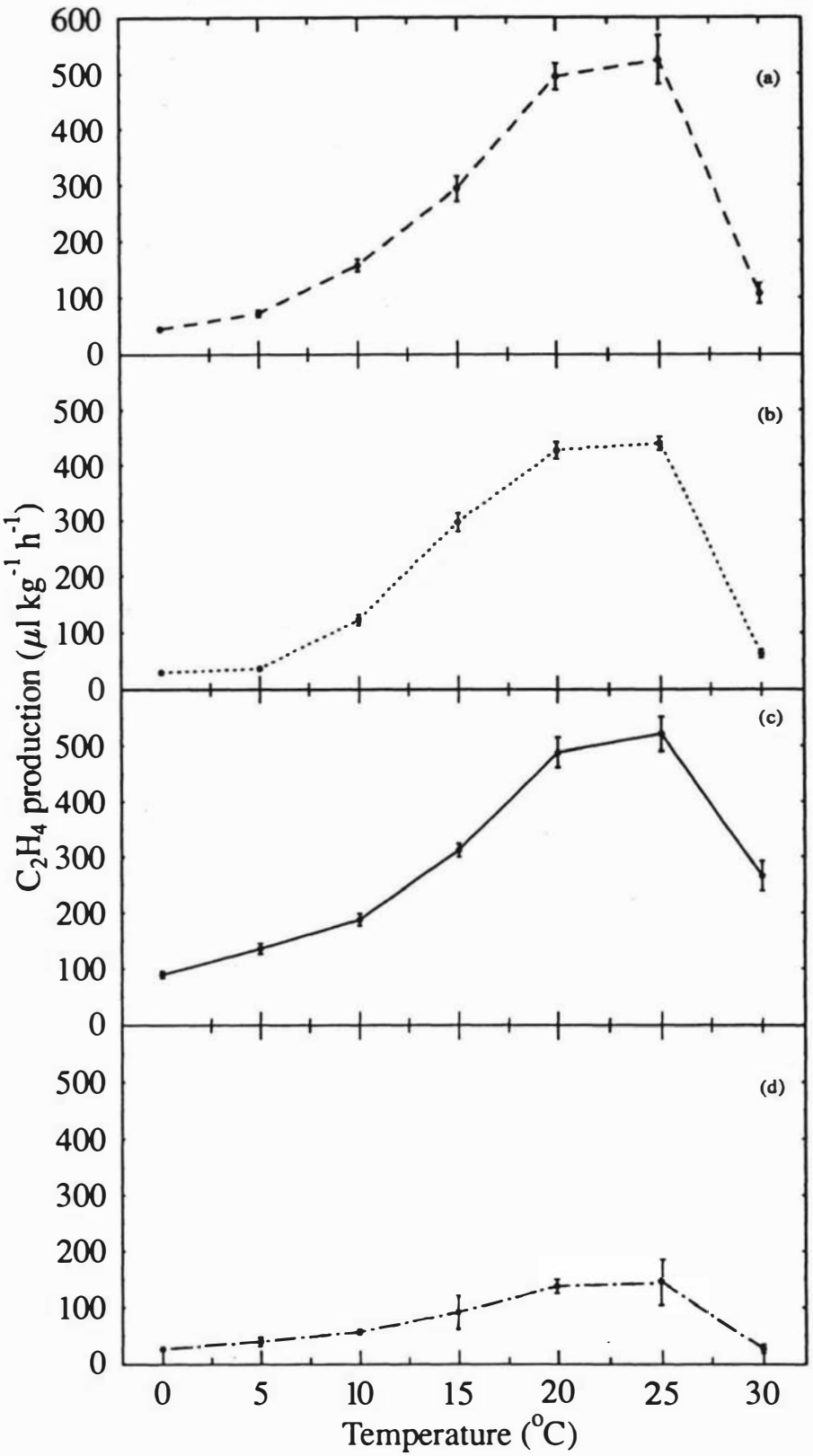

Fig. 7-10. Temperature effects on C_2H_4 production of (a)=Gala (b)=Royal Gala (c)=Golden Delicious and (d)=Red Delicious apples. Vertical bars represent standard error of the means.

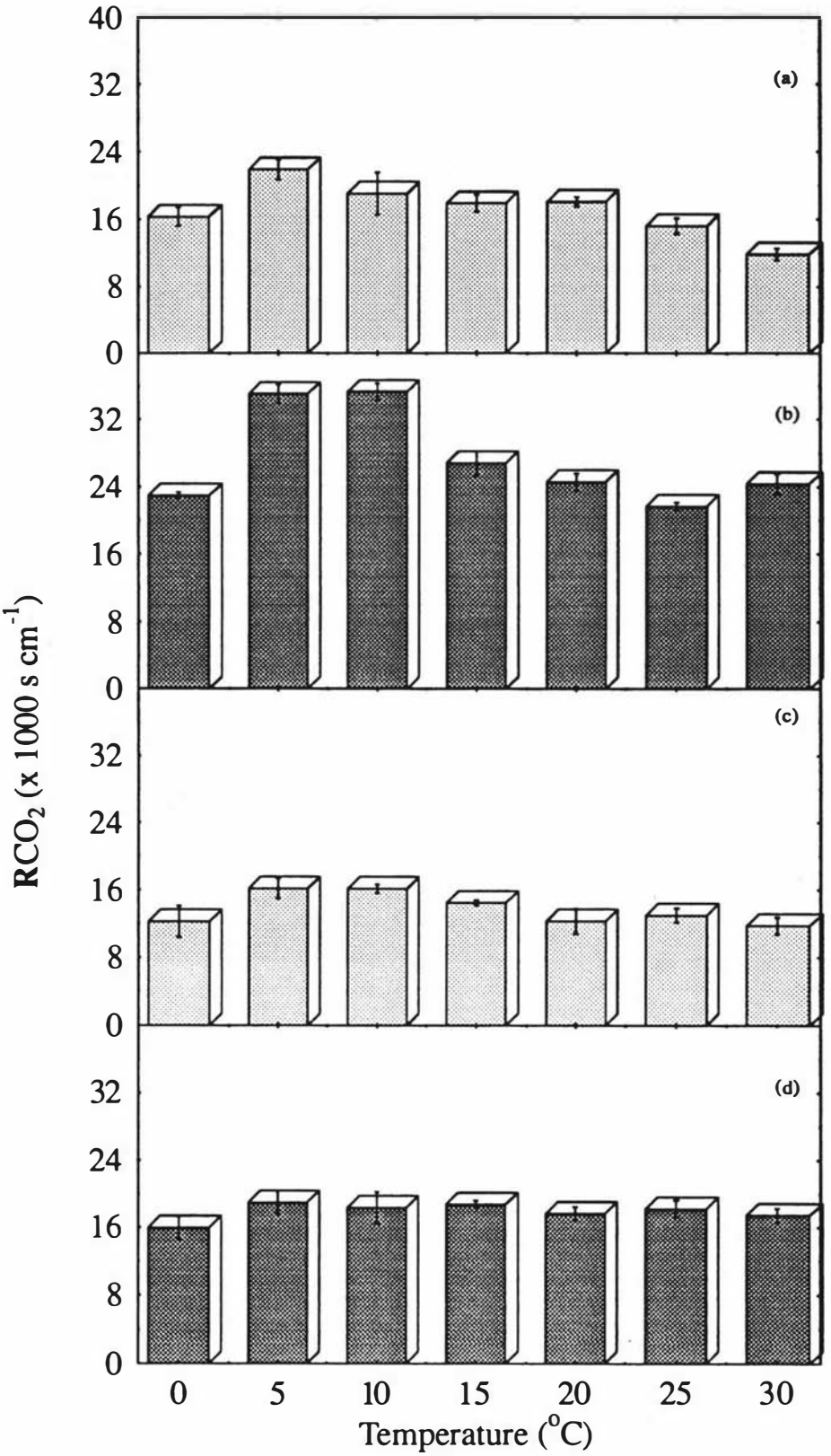

Fig. 7-11. Temperature effects on RCO_2 of (a)=Cox's Orange Pippin (b)=Braeburn (c)=Splendour and (d)=Granny Smith apples. Vertical bars represent standard error of the means.

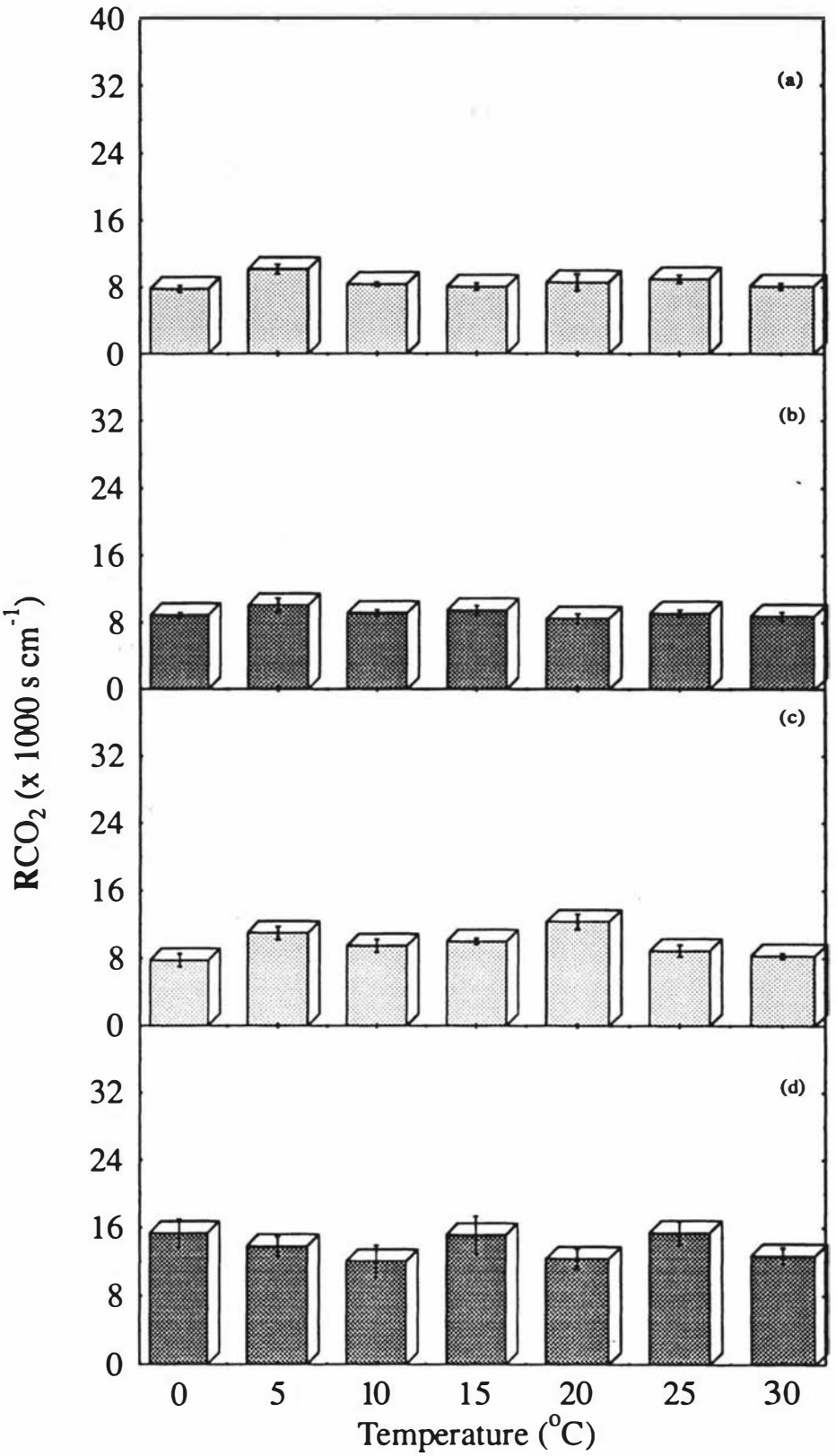

Fig. 7-12. Temperature effects on RCO_2 of (a)=Gala (b)=Royal Gala (c)=Golden Delicious and (d)=Red Delicious apples. Vertical bars represent standard error of the means.

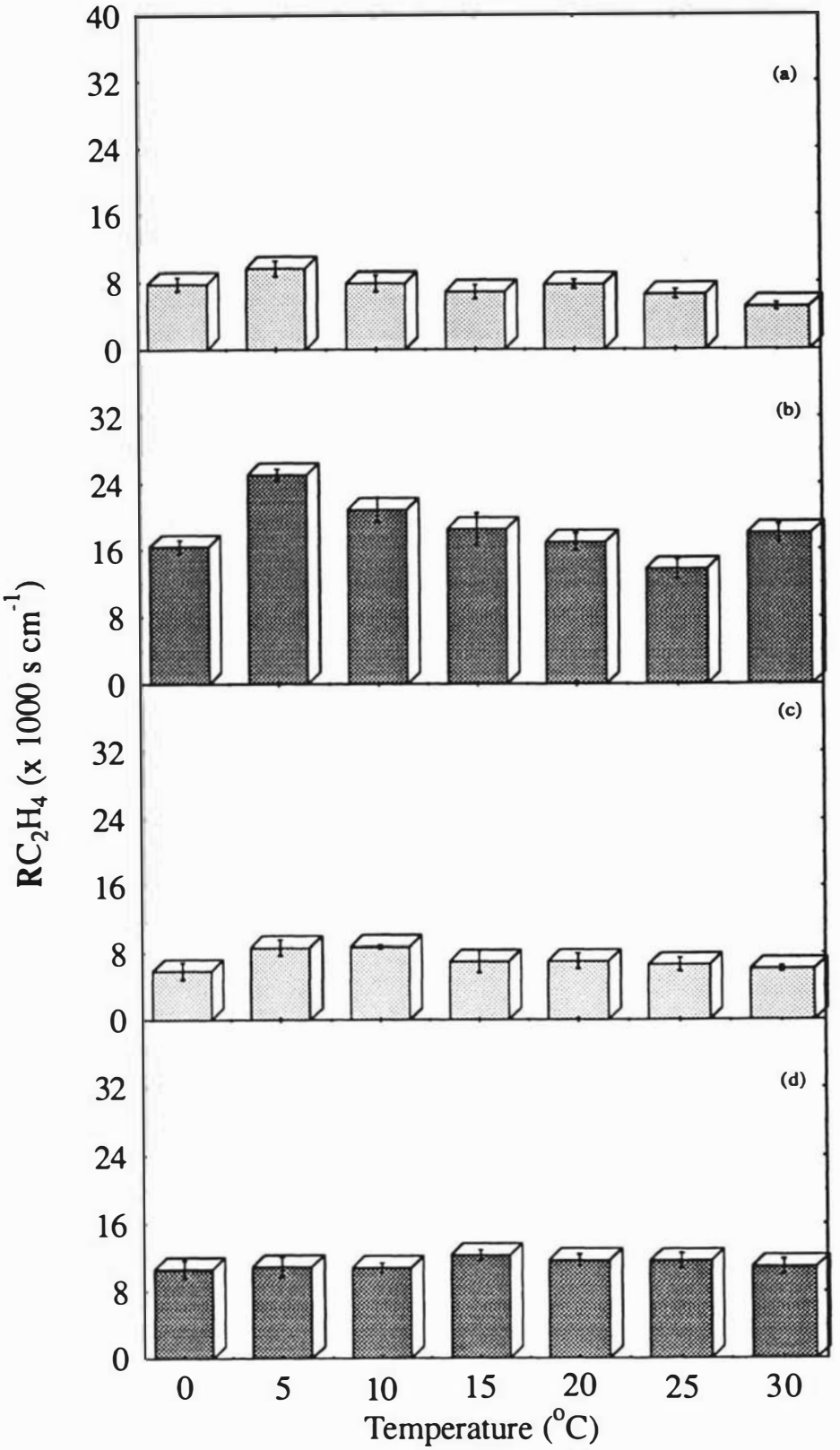

Fig. 7-13. Temperature effects on RC_2H_4 of (a)=Cox's Orange Pippin (b)=Braeburn (c)=Splendour and (d)=Granny Smith apples. Vertical bars represent standard error of the means.

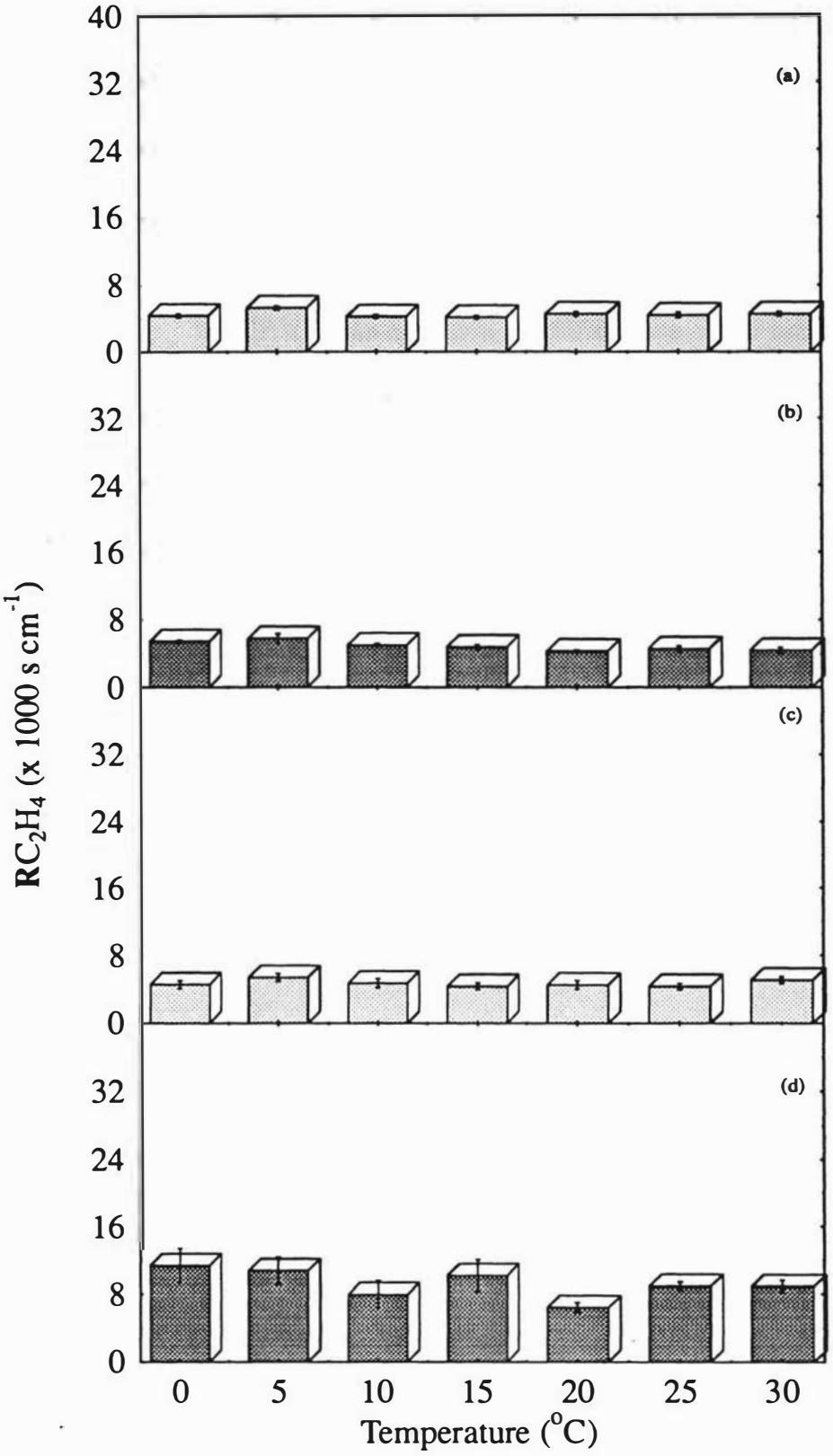

Fig. 7-14. Temperature effects on RC_2H_4 of (a)=Gala (b)=Royal Gala (c)=Golden Delicious and (d)=Red Delicious apples. Vertical bars represent standard error of the means.

regimes were higher than the other cultivars. For instance at 30°C, RCO_2 and RC_2H_4 of Braeburn apples was more than 3 times greater than Gala apples (which had the lowest **R**).

7.4.7 Quality indices

Fruit firmness varied between cultivars and it was significantly influenced by temperature ($P < 0.001$; figs. 7-15 and 7-16). Generally fruit equilibrated at low temperatures were firmer than their counterparts at higher temperatures. Fruit firmness declined as temperature increased. For all the temperatures, Braeburn apples were firmer than the other cultivars. For example, at 30°C, the mean firmness of Braeburn apples was 80 Newtons as against 47 and 42 Newtons in Golden Delicious and Cox's Orange Pippin apples respectively.

Soluble solids differed among cultivars but there was no significant ($P = 0.05$) effect of equilibration temperature on the soluble solids content of apples (figs. 7-17 and 7-18). However there were some differences in soluble solids content between temperatures in some of the cultivars. For example at 0°C the soluble solids content of Golden Delicious and Splendour apples were lower than those at 5°C, while in Royal Gala it was the opposite. Generally Splendour apples contained higher soluble solids than the other cultivars. For instance at 30°C the average soluble solids content of Splendour apples was approximately 14% compared to 12.5% in Red Delicious, 12% in Golden Delicious and Cox's Orange Pippin and 11% in Gala, Royal Gala or Braeburn.

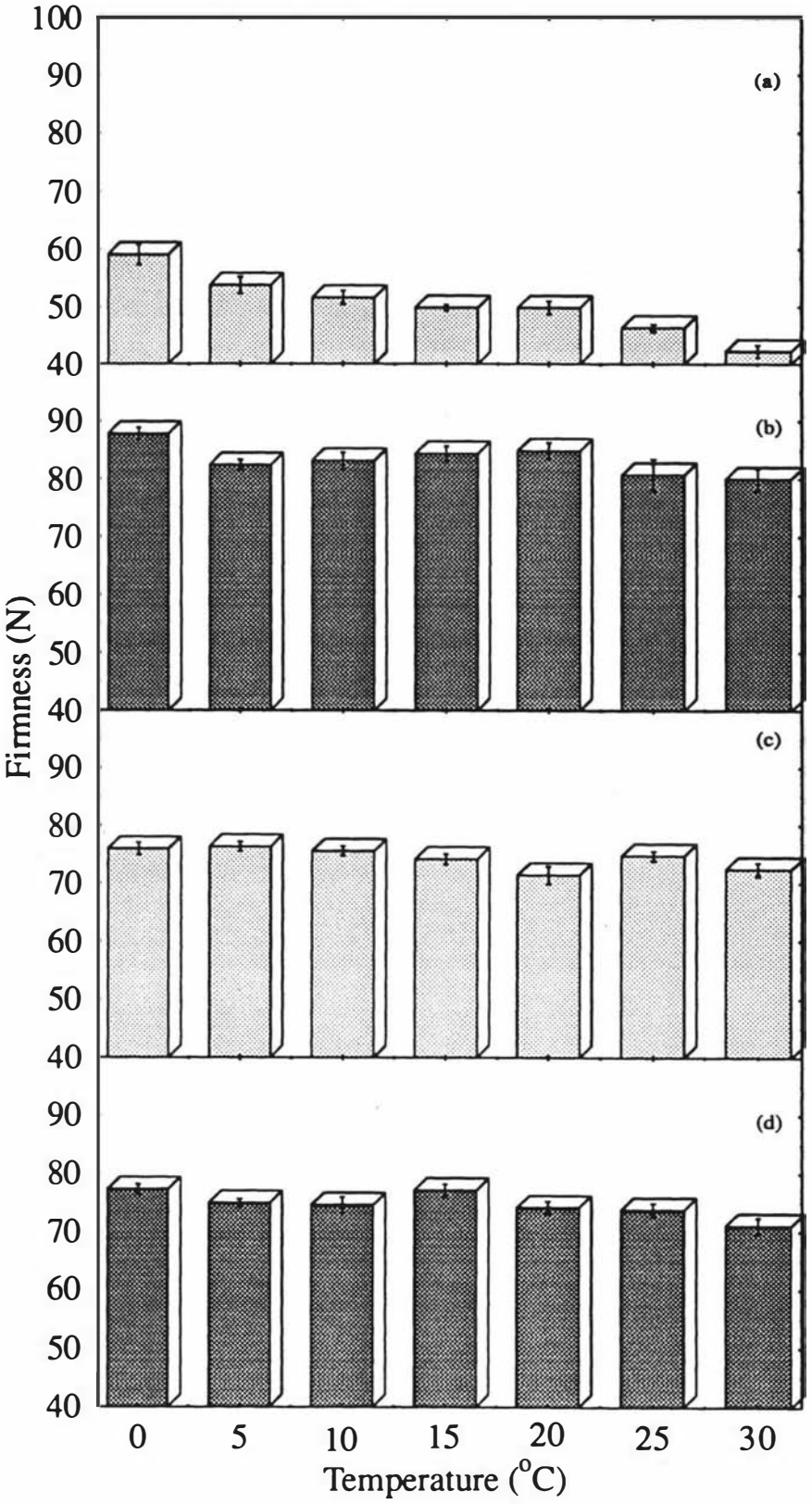

Fig. 7-15. Relationship between temperature and firmness of (a)=Cox's Orange Pippin (b)=Braeburn (c)=Splendour (d)=Granny Smith apples. Vertical bars represent standard error of the means.

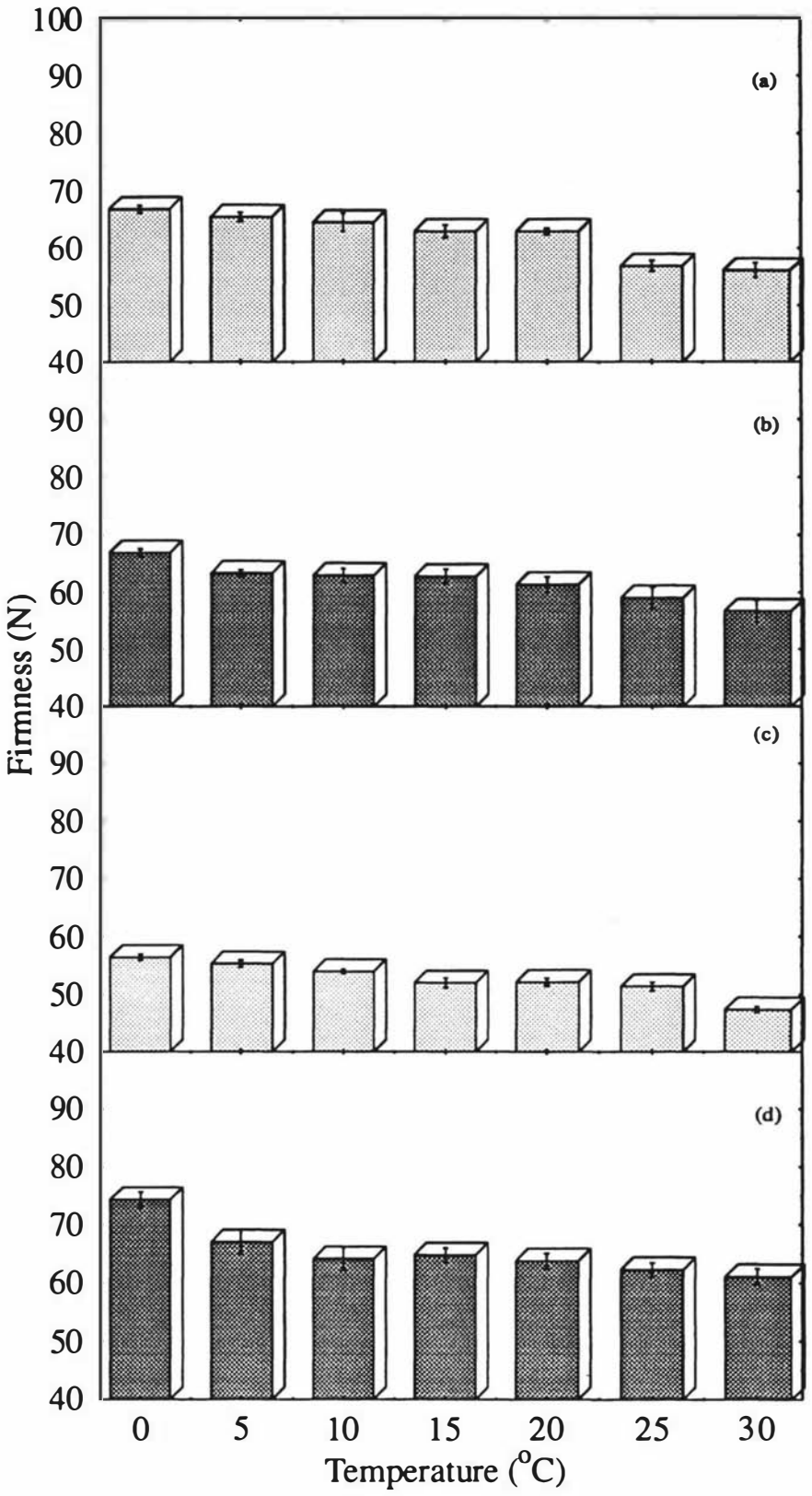

Fig. 7-16. Relationship between temperature and firmness of (a)=Gala (b)=Royal Gala (c)=Golden Delicious and (d)=Red Delicious apples. Vertical bars represent standard error of the means.

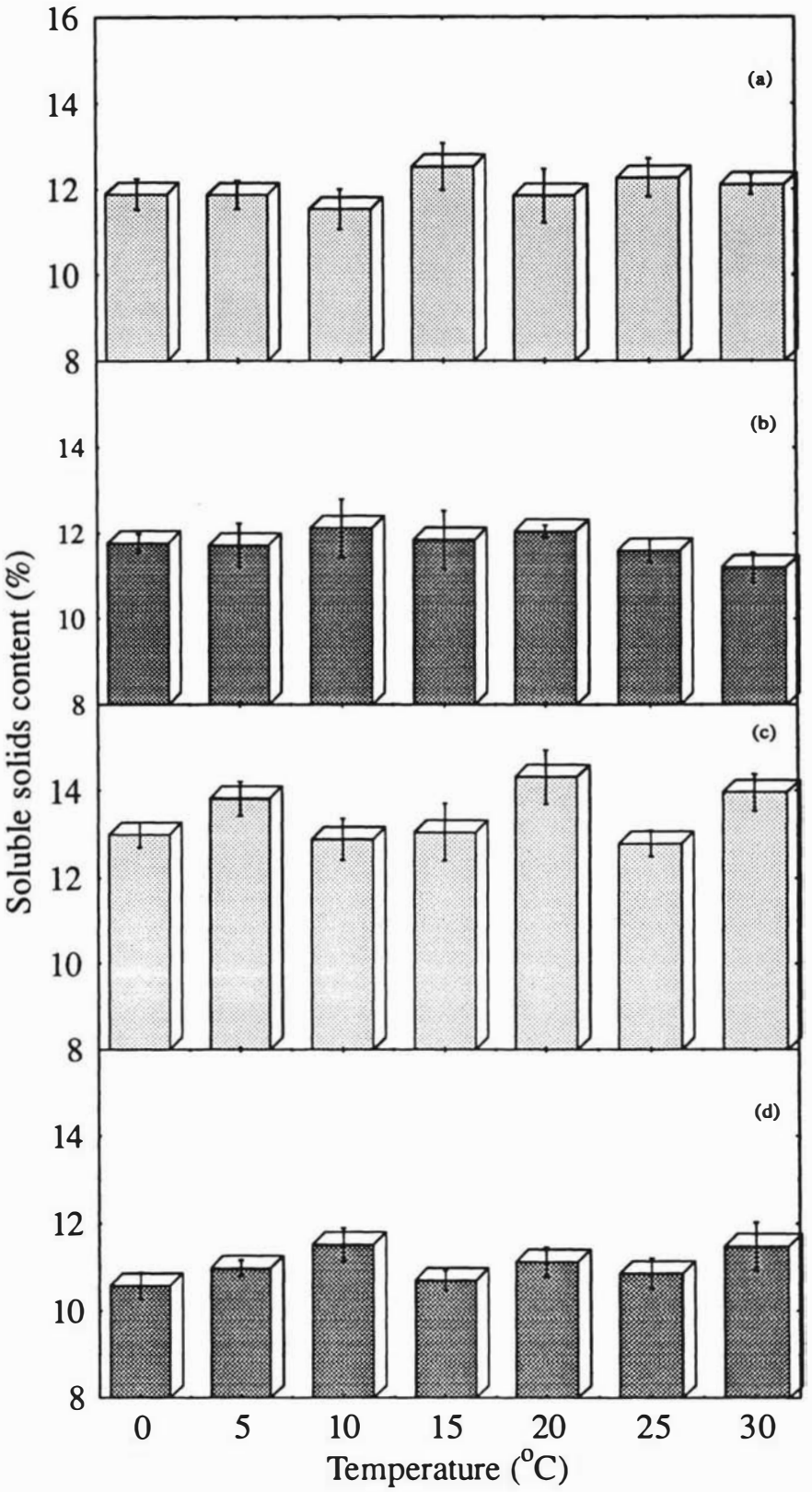

Fig. 7-17. Temperature effects on soluble solids contents of (a)=Cox's Orange Pippin (b)=Braeburn (c)=Splendour and (d)=Granny Smith apples. Vertical bars represent standard error of the means.

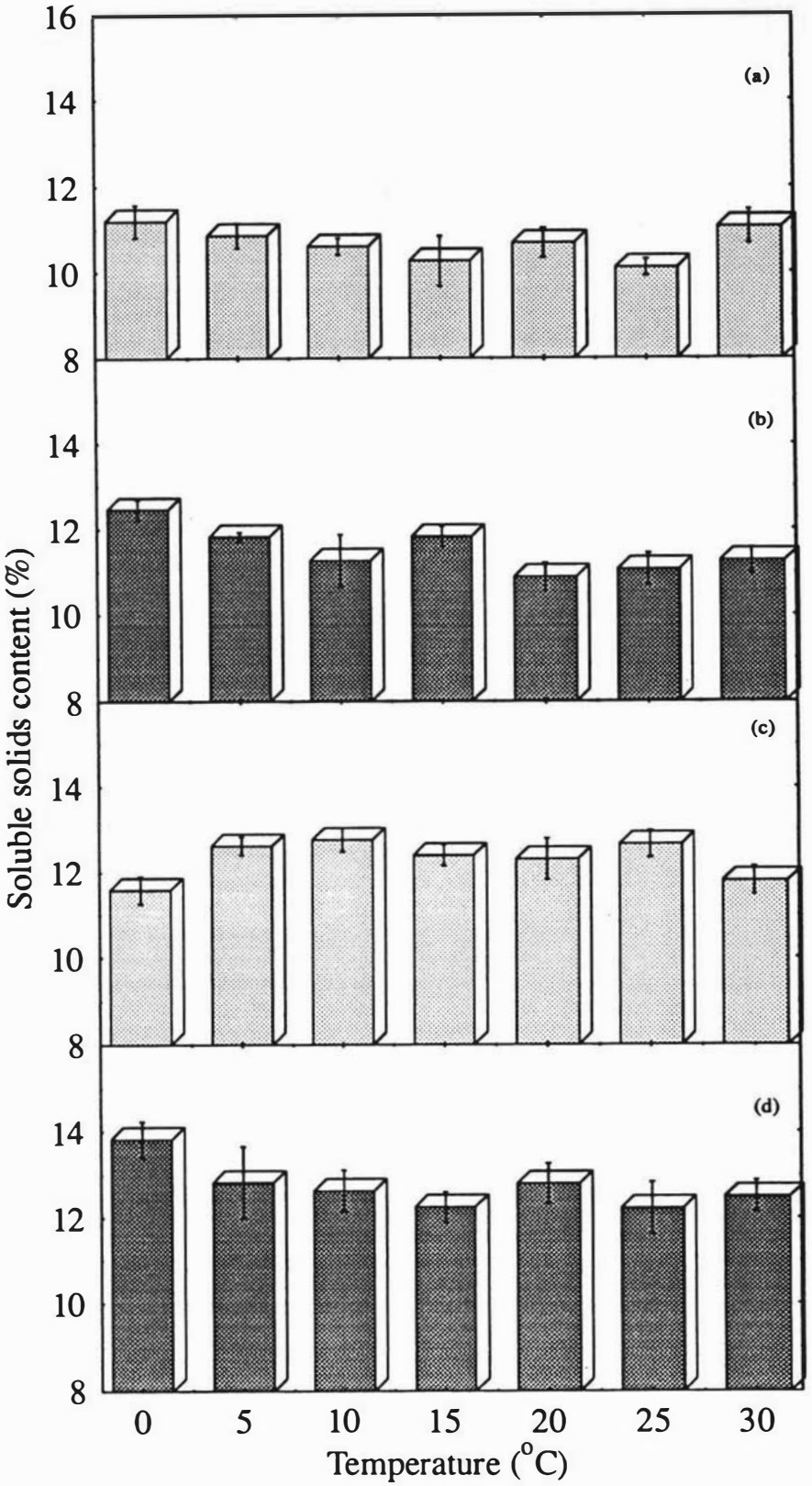

Fig. 7-18. Temperature effects on soluble solids contents of (a)=Gala (b)=Royal Gala (c)=Golden Delicious and (d)=Red Delicious apples. Vertical bars represent standard error of the means.

7.5 DISCUSSION

Temperature markedly influenced the internal atmosphere composition of apples. Results obtained in the present study (figs. 7-1, 7-2 and figs. 7-3, 7-4) clearly demonstrated that the O_2 concentrations inside the fruit decreased with a concomitant increase in $[CO_2]_i$ in response to increasing temperatures. This may be explained primarily on the basis of increased O_2 utilisation in the fruit as temperature increased (ie. Increasing temperatures increase O_2 consumption through respiration and decrease O_2 solubility in the fruit tissues). These findings are consistent with those reported by other investigators including, Anzueto and Rizvi (1985) who reported that apples stored at lower temperatures had lower $[CO_2]_i$ and higher $[O_2]_i$ than their counterparts at higher temperatures. Similarly, as early as 1920, Magness also showed that the O_2 in the intercellular spaces of apples decreases when fruit are held at higher temperatures, and CO_2 within the tissues correspondingly increases.

In Braeburn apples both the intercept and slope of the regression line relating $[O_2]_i$ and $[CO_2]_i$ to temperature were significantly different from the other cultivars. In physiological terms this could be interpreted to mean that Braeburn apples consistently had lower $[O_2]_i$ and higher $[CO_2]_i$ than the other cultivars at all the temperature regimes and the rate of change in $[O_2]_i$ and $[CO_2]_i$ with respect to temperature in Braeburn apples was higher than the other cultivars. The reason for the difference could be due to the fact that Braeburn apples have high **R** and low intercellular space volume (approximately 14.1% Rajapaskse *et al*, 1990) coupled with intermediate respiration rate. High skin resistance therefore affects gas movement across the fruit skin and results in lower $[O_2]_i$ and higher $[CO_2]_i$ for a given respiration rate and external atmosphere composition. In contrast, Splendour apples had higher $[O_2]_i$ and lower $[CO_2]_i$ than the other cultivars. The reason could be attributed to their low **R**, low respiration rate, presence of open calyx and numerous lenticels hence easier gas diffusion.

Temperature had a marked effect on fruit respiration rate which varied between cultivars. Respiration rates of fruit were low after equilibration at 0°C. A low respiration rate at low temperatures indicates an inhibitory effect on the overall metabolic system of the fruit (Wang, 1990). In most cultivars, respiration rates increased linearly in response to increasing temperatures, except in Cox's Orange Pippin apples in which an exponential type of response was observed which is similar to that reported for many crops and forms the basis of temperature quotient (Johnson and Thornley, 1985). Perhaps the increased rate in respiration was partially offset by the decreased O_2 solubility at higher temperatures. This would mean that for a given internal atmosphere composition, O_2 content of the cell sap would be reduced at higher temperatures. At 30°C, Cox's Orange Pippin apples were respiring approximately ten times faster than at 0°C and over 50% more rapidly than Splendour (at 30°C). High temperatures coupled with the high respiration rate and the high $[CO_2]_i$ of Cox's Orange Pippin apples could exacerbate the cultivar's high susceptibility to CO_2 injury and high incidence of postharvest disorders such as core flush or internal browning.

High storage temperatures increase respiration metabolism, probably by enhancing the activity of enzymes involved in the breakdown of respiratory substrates and production of CO_2 (Kader et al., 1985; Knee, 1990). On the contrary low temperatures depress the activity of respiratory enzymes and consequently decline in CO_2 production (Abeles, 1973; Knee, 1990; Wang, 1990). Low temperatures have been shown to reduce respiration rate of apples (Johnson and Ertan, 1983). Hansen (1942) working with pears found that respiration increases in intensity throughout the range of 0 - 40°C. Although CO_2 content was high in the fruit tissue with correspondingly low O_2 content, Hansen indicated that this was not the factor limiting C_2H_4 production at 40°C.

Since the rate of deterioration of harvested apples is inversely related to their rate of respiration, fruit stored at higher temperatures would be expected to have shorter shelf-life because of the increased rate of respiration at high temperatures (Johnson and Ertan, 1983; Kader *et al.*, 1985, 1989). On this basis, exposing cultivars such as Cox's Orange Pippin apples which have high respiration rates to high temperatures could result in shorter shelf-life compared to Splendour apples which have low respiration rates.

Temperature had pronounced effects on $[C_2H_4]_i$ and rate of C_2H_4 production and varied between cultivar. Low equilibration temperatures between 0 and 10°C depressed both $[C_2H_4]_i$ and rate of C_2H_4 production in all the apple cultivars, however concentrations increased in response to increasing temperatures reaching a maximum at 25°C and declined in response to further temperature increase. This is the first time the effects of a range of temperatures on $[C_2H_4]_i$ of different apple cultivars has been characterised. These findings are consistent with those reported by other investigators including Burg (1962) who contended that low storage temperatures depressed rate of C_2H_4 evolution by slowing down the synthesis and/or activity of the enzymes (ie. 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and/or ethylene-forming enzymes (EFE)) necessary for C_2H_4 production. Similar conclusions have be reported by other investigators including Abeles (1973), Field (1985, 1989), Jobling *et al.* (1991) and Knee (1985, 1988). Yu *et al.* (1980), and Klein (1989) reported that storage of fruit at high temperatures inhibited C_2H_4 production at the step of conversion of ACC to C_2H_4 (ie. EFE activity), as opposed to Biggs *et al.* (1988) who indicated that ACC synthase is the primary site affected by high temperatures, with EFE activity only affected secondarily.

Many fruits and a variety of tissues produce low amounts of C_2H_4 at low temperatures, reaching a maximum between 20 and 30°C, and almost none at 40°C (Burg, 1962). This was first indicated by experiments with

comice pear, in which Hansen (1942) found that small amounts of C_2H_4 were produced at low temperatures, large quantities were evolved by the fruit at 20°C but negligible amounts at 40°C. On the other hand McIntosh apples produced maximum amount of C_2H_4 at 32°C, but almost none during one hour at 40°C (Burg and Thimann, 1959). In this research, fruit $[C_2H_4]_i$ and rate of C_2H_4 production increased to a maximum at 25°C however the choice of the temperature ranges did not permit accurate identification of the optimum temperature for $[C_2H_4]_i$ and C_2H_4 production, nevertheless the shape of the graphs (figs. 7-9 and 7-10) indicate that the optimum could lie any where between 20 and 30°C.

Literature dealing with C_2H_4 contains conflicting reports on the optimum temperature for C_2H_4 evolution in apples. For instance, Burg and Thimann (1959) working with apple tissue sections reported that the optimum temperature for C_2H_4 production was 32°C and above this optimum, production declined rapidly. They further indicated that at 40°C and above, C_2H_4 production ceased. However, inhibition was reversible when fruits were transferred to lower temperatures. Mattoo *et al.* (1977) and Yu *et al.* (1980), using apple fruit plugs, reported a slightly lower optimum temperature of 30°C, while Apelbaum *et al.* (1981) obtained an optimum of 29°C when working with apple fruit discs. All of these workers determined the optimum temperature for C_2H_4 evolution on sections of apples. Although I do not dispute these conclusions, it may be that data obtained in experiments with fruit slices, discs, or tissue sections may not necessarily be applicable to the metabolism of the intact fruit. Sliced fruit may well behave differently from whole intact fruit because of the stimulation of C_2H_4 production and respiration by wounding (Abeles, 1973; Rosen and Kader, 1989). Abeles (1973) indicated that an example of a process which is controlled by an increase in the rate of C_2H_4 production is wound-induced protein synthesis. Tissue damage results in an increase in C_2H_4 production (Field, 1985). In addition, the combined effects of

physical wounding and temperature may simply be additive in freshly-cut sections which could increase rate of C_2H_4 production (Field, 1985) and perhaps also affect the optimum temperature.

After storage at 30°C most of the apple cultivars lost much of their capacity to accumulate and produce C_2H_4. The decline in rate of C_2H_4 evolution beyond the maximum suggests a loss of integrity of the C_2H_4-synthesising system (Field, 1981; Saltveit and Dilley, 1978). More specifically the high temperature may perturb membrane structure, leading to increases in activation energy of membrane-bound enzymes and hence the reduced rate of C_2H_4 synthesis (Field, 1981). The decline in rate of C_2H_4 production after reaching the maximum and eventual cessation at higher temperatures suggests that production of C_2H_4 was enzyme dependent (Spencer, 1969). Enzymes are well known to be denatured at high temperatures (Forward, 1960) and this presumably accounted for the decline in capacity to accumulate and produce C_2H_4. In several cases, e.g. apples, pears and bananas, it has been ascertained that CO_2 production has not yet reached its maximum value at the temperature which causes total inhibition of C_2H_4 evolution, suggesting that the effect of temperature is directly on the mechanism of C_2H_4 synthesis (Burg, 1962). Osborne (1978) indicated that as temperature increases the enzymes concerned with C_2H_4 biosynthesis appear to be relatively labile compared with those of the respiratory pathway. The ability of high temperatures to reduce drastically or totally prevent C_2H_4 evolution may be reflected in the failure of many fruits to ripen normally above 30°C to 35°C, if they ripen at all (Burg, 1962; Hansen, 1945; Yu *et al.*, 1980).

Splendour apples compared to the other cultivars had the least capacity to accumulate and/or produce C_2H_4. It has long been known that there are cultivar differences in rates of C_2H_4 production and this could be a determinant of storage life (Nelson, 1940). The formation and accumulation of C_2H_4 in fruit tissues is a vital factor in the natural ripening of apples and consequently in their rate of deterioration (Kidd and West, 1937).

Evidence presented in this study demonstrated that equilibration temperatures (0 - 30°C) had no consistent significant effect on RCO_2 and RC_2H_4 of apples. A temperature change of 5°C had little or no effect on skin resistance, even though production rates and internal gas concentrations changed substantially. This might be expected since resistance to diffusion is a physical parameter and hence should be relatively unaffected by temperature (Cameron and Reid, 1982). Leonard and Wardlaw (1941), in their work with banana, reported that a temperature change of 8°C had little or no effect on R. RCO_2 and RC_2H_4 varied between cultivars, with Braeburn apples having higher RCO_2 and RC_2H_4 (for all the equilibration temperatures) than the other cultivars.

Generally fruit were firmer at low temperatures but increasing temperatures accelerated softening and varied with cultivar. The findings of this study are compatible with those reported by Magness and Diehl (1924) who indicated in their studies with six cultivars of apples that softening of fruits were greatly accelerated by higher temperatures. Similarly, Bourne (1982) reported that with some exception, most horticultural fruits and vegetables showed a decline in firmness with increasing temperature over the range 0 - 45°C. Mitcham and McDonald (1992) also reported that loss of firmness by fruit during storage at high temperatures could be related to moisture loss. Temperature could affect firmness in two ways, first, it could have reversible effect with direct effect on fruit texture and secondly, it could accelerate the process of softening and this is essentially irreversible. Softening of pome fruits including apples are associated with a reduction in middle lamella cohesion and is characterised by solubilisation of pectin (Knee, 1982). Temperature did not affect the soluble solids content of fruit.

It can be concluded that the internal atmosphere composition, C_2H_4 evolution, respiration rate and firmness of apples respond to increasing temperatures. The magnitude of response was cultivar dependent. Fruit $[O_2]_i$ decreased while $[CO_2]_i$ and respiration rates increased in response to increasing temperatures. Internal C_2H_4 and C_2H_4 production in apples reached a maximum at 25°C. At 30°C the ability to produce C_2H_4 declined markedly in most apple cultivars. Temperature did not affect **R** and soluble solids content of apples.

The temperature at which an apple cultivar is stored after harvest and during the postharvest handling chain is a vital factor to the rate of deterioration and hence storage life. Fresh fruits including apples are assuming a position of increasing importance in the diet of the human race. If a supply of this fruit is to be available to the ultimate consumers throughout the year, suitable storage temperatures are essential throughout the distribution network. Not only does proper storage temperature management ensure regular supply, but it also stimulates consumption and stabilises prices for the grower through its effects on quality.

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CHAPTER 8

VARIATION IN INTERNAL ATMOSPHERE COMPOSITION WITHIN SINGLE APPLES

8.1 ABSTRACT

Gas concentration gradients within individual fruit have previously been considered to be negligible. In this work, large O_2 and CO_2 concentration differences were found between the equator and calyx end of Gala, Royal Gala, Braeburn and Cox's Orange Pippin, while smaller differences were found in Golden Delicious, Red Delicious, Granny Smith and Splendour apples at 20±1°C. The average O_2 concentration difference in Gala apples was nearly nine times higher than in Splendour, and eight times higher than in Red Delicious or Granny Smith apples.

Similarly, O_2 and CO_2 concentration differences were also observed between the equator and caylx end shoulder. These concentration differences were much greater than those which have hitherto been measured between the core cavity and the fruit surface of other cultivars. Oxygen and CO_2 concentration differences between the core cavity and the equatorial surface of the fruit were small but statistically significant in cultivars such as Braeburn and Cox's Orange Pippin apples.

Tissues at the calyx region of Braeburn and Granny Smith apples consistently had lower O_2 but higher CO_2 and C_2H_4 concentrations than any other position on the fruit surface, whilst tissues at the equator had higher O_2 and lower CO_2 and C_2H_4 concentrations than other parts of the fruit. Braeburn had higher CO_2 and C_2H_4, and lower O_2 concentrations at the different positions beneath the skin surface compared to Granny Smith apples.

These gas concentration differences at the various positions on the fruit surface may be related to localised variation in intercellular space volume as well as differences in **R** and fruit respiration rate.

Modified atmosphere treatments are effective through the effects they have on fruit internal atmosphere composition. The discovery that internal atmosphere composition in apple fruit is heterogeneous has important implications for the way in which we attempt to model the gas exchange of these fruit and the effects of CA/MA on their physiology. It may also provide a mechanism which explains the pattern of development of atmosphere-related disorders develop within fruit stored in CA/MAs.

8.2 INTRODUCTION

Postharvest deterioration of harvested apples can be delayed in storage atmospheres in which the concentrations of gases surrounding the produce are altered relative to the composition of air (Burton, 1982). The limit of atmospheric modification for maximum benefits depends upon the produce characteristics such as gas diffusion across the skin and flesh tissues, response to low O_2 and/or high CO_2 and the ability of the produce to maintain essential physiological processes at these conditions without causing injuries to internal tissues (Kader *et al.*, 1989). However, when respiratory gases are produced or consumed, particularly for bulky organs such as apples, concentration gradients are established which result in tissues experiencing internal concentrations which differ from the external atmosphere (Burton, 1982; Cameron and Reid, 1982; Kader *et al.*, 1989; Knee, 1991). The magnitude of these gradients are a function of skin resistance, effective diffusivity of the gas in the fruit flesh, fruit size and respiration rate. Furthermore, it is sites of production and utilisation of these gases that dictate the direction of flux of gases resulting in these gradients (Burg and Burg, 1965; Burton, 1982).

Gas concentration gradients within individual apple fruit have previously been considered by some authors to be negligible due to the relatively large intercellular space volume, and hence to have no physiological importance (Burton, 1982; Burg and Burg, 1965; Trout *et al.*, 1942). Available evidence indicated that, inside the skin, the composition of the internal atmosphere of an apple fruit is practically uniform (Andrich *et al.*, 1989, 1991; Cameron, 1982; Hardy, 1949). However, Banks and Kays (1988) found significant flesh resistance to gas diffusion in potatoes, in which intercellular space volume is much smaller than that of apples (Burton, 1982). Due to the limited intercellular space volume, the flesh of dense organs such as potato tubers and avocado fruit might be expected to exert considerable resistance to gas diffusion. Ben-Yehoshua *et al.* (1963) and Burg and Burg (1965) illustrated that the flesh tissues of immature and climacteric avocado fruit exert significant resistance to CO_2 diffusion, thus creating large concentration gradients across the flesh tissues. Solomos (1987) also reported the existence of significant CO_2 gradients between the core and surface of peeled apples. More recently Rajapakse *et al.* (1989, 1990) have demonstrated the existence of significant O_2 gradients in apple cultivars Cox's Orange Pippin, Braeburn and Golden Delicious, and Asian pear cultivars, Hosui and Kosui.

A preliminary experiment (appendix 4), with Granny Smith apples identified significant heterogeneity in O_2 concentration at five positions on the fruit surface beneath the skin. Much research effort has been geared towards studying commodity response to externally imposed atmospheres. However, knowledge of internal atmospheres of these commodities is also important in developing CA/MA treatments for extended shelf-life (Anzueto and Rizvi, 1985; Banks, 1984a; Fidler and North, 1971; Hulme, 1951). Knowledge of the distribution of internal gas concentrations will enhance our understanding of the mechanisms by which ripening is retarded and atmosphere-related disorders develop within fruit stored in CA/MA. Furthermore, impaired diffusion

of these gases across the skin and flesh could result in extremely low O_2 or high CO_2 and C_2H_4 in the internal tissues causing physiological injury to produce stored under MA/CA conditions. Therefore knowledge of flesh resistance to gas movement is important in determining the critical minimum external O_2 that can safely be used in CA/MA storage.

The foregoing studies clearly indicate that fruit flesh resistance to gas diffusion may not be negligible as previously thought by some investigators and should therefore be taken into consideration in our attempts to understand the physiological effects of fruit response to CA/MA storage. A series of studies were therefore initiated to quantify gas concentration differences (1) between the equator and calyx end, (2) between the equator and calyx end shoulder, and (3) between the equator and core cavity of freshly harvested eight cultivars of apples grown in New Zealand.

In addition, further experiments were initiated to investigate the distribution of internal atmospheres at (1) the stem end, equator and calyx end and (2) at five different positions on the surface beneath the skin of Braeburn and Granny Smith apples stored for various periods of time at 0°C and subsequently held at 20°C. In this way it was hoped to be able to identify whether the development of large gradients was something which was exacerbated with the decline in tissue condition associated with increased time in storage. This might provide a simple mechanism to explain the development of some fruit long term storage disorders. Furthermore such information could help in the development of models predicting the effects of CA/MA on apple fruit and also in the study of gas exchange characteristics of fruits stored in CA/MA.

8.3 MATERIALS AND METHODS

8.3.1 Fruit supply

Freshly harvested apples (***Malus domestica*** Borkh.) of eight cultivars (Cox's Orange Pippin, Gala, Royal Gala, Golden Delicious, Red Delicious, Braeburn, Splendour and Granny Smith apples (count 125; av. weight 148 g) were obtained as previously described in 3.1. All experiments were conducted at 20±1°C.

In a second experiment freshly harvested Braeburn and Granny Smith apples (count 125; av. weight 148 g) were obtained from a similar source as mentioned above and fruit were stored at 0°C for 0, 4, 8 and 12 weeks and subsequently transferred to 20±1°C for 3, 10 and 17 days. Both experiments were conducted in 1990.

8.3.2 Methods

8.3.2.1 Experiment 1

8.3.2.1.1 Estimation of O_2 and CO_2 concentration differences

Two 2 ml glass vials from which bottoms had been removed were stuck, using polyvinyl acetate adhesive (PVA), on twelve fruit of each cultivar at

(a) the equator and the calyx end (fig. 8-1) and

(b) the equator and calyx end shoulder (fig. 8-2)

Sub-epidermal O_2 and CO_2 concentrations were estimated (see 3.3.1) as equilibrated gas concentrations in the glass chambers on each apple after 40 - 90h at 20±1°C in air (see appendix 1).

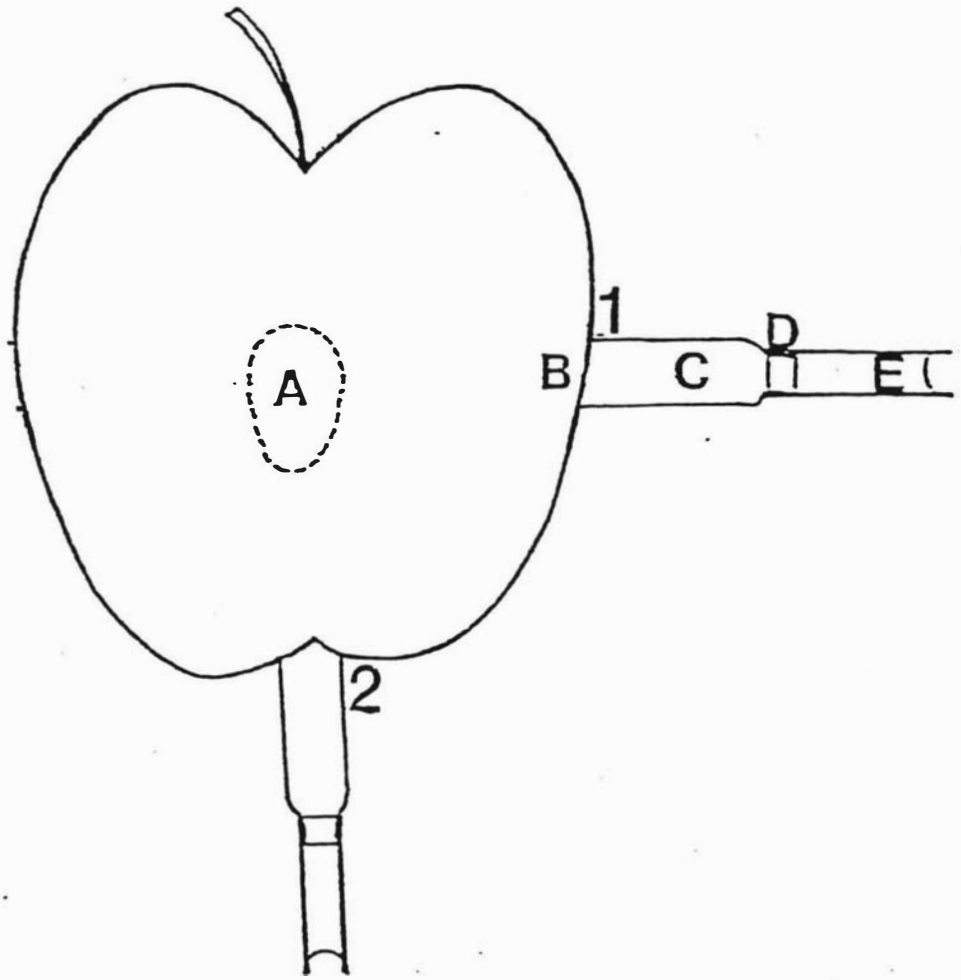

Fig. 8-1. Arrangement of glass sampling chambers on the surface of an apple fruit.

A. Core cavity
B. Fruit surface
C. Glass chamber
D. Septum cap
E. Silicone tube with water

1. Equator
2. Calyx end

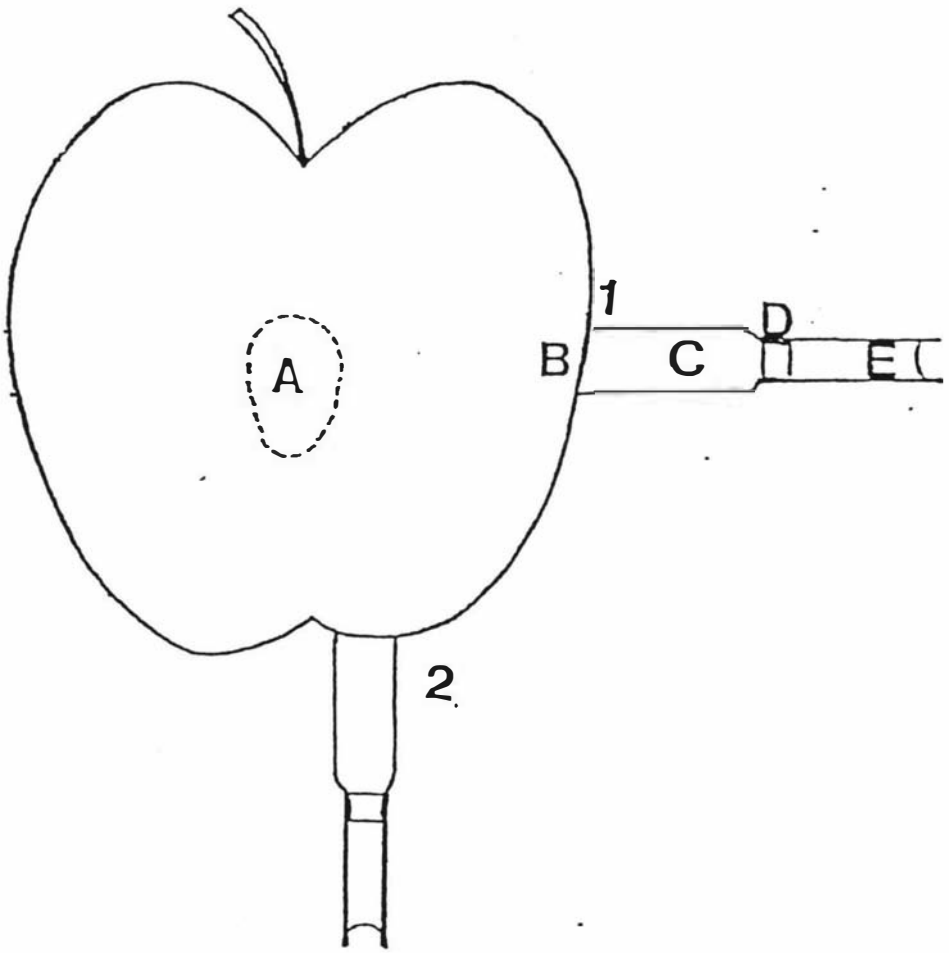

Fig. 8-2. Arrangement of glass sampling chambers on the surface of an apple fruit.

A. Core cavity
B. Fruit surface
C. Glass chamber
D. Septum cap
E. Silicone tube with water

1. Equator
2. Calyx end shoulder

Core cavity O_2 and CO_2 concentrations were measured by the direct sampling method (see 3.3.4.1) and analysed for O_2 and CO_2 as previously described (see 3.3.1). Gas concentration differences were estimated as the difference in gas concentrations between any two positions on the fruit.

8.3.2.2 Experiment 2

8.3.2.2.1 Determination of distribution of internal atmosphere composition

Three 2 ml glass vials were stuck as previously described, at the stem end, equator and calyx end (fig. 8-3) of twelve fruit after transfer from 0 to 20±1°C.

Similarly, in another parallel experiment, five 2 ml glass vial were stuck (as previously described) over one or more lenticels on the surface (see fig. 8-4) of twelve fruit after transfer from 0 to 20±1°C.

Gas samples (90µl) were then taken from each glass vial on the 3rd, 10th and 17th day after sealing the vial at 20°C using a gas-tight syringe and analysed for O_2, CO_2 and C_2H_4 as previously described (see 3.3.1).

Immediately after sampling from vials on the 17th day at 20°C, core cavity gas concentrations were obtained by the direct sampling method and analysed for O_2, CO_2 and C_2H_4 as previously described (see 3.3.1).

8.3.2.2.2 Fruit quality assessment

Fruit firmness, soluble solids content and background colour were measured as previously described in 3.3.8.1, 3.3.8.2 and 3.3.8.3 respectively.

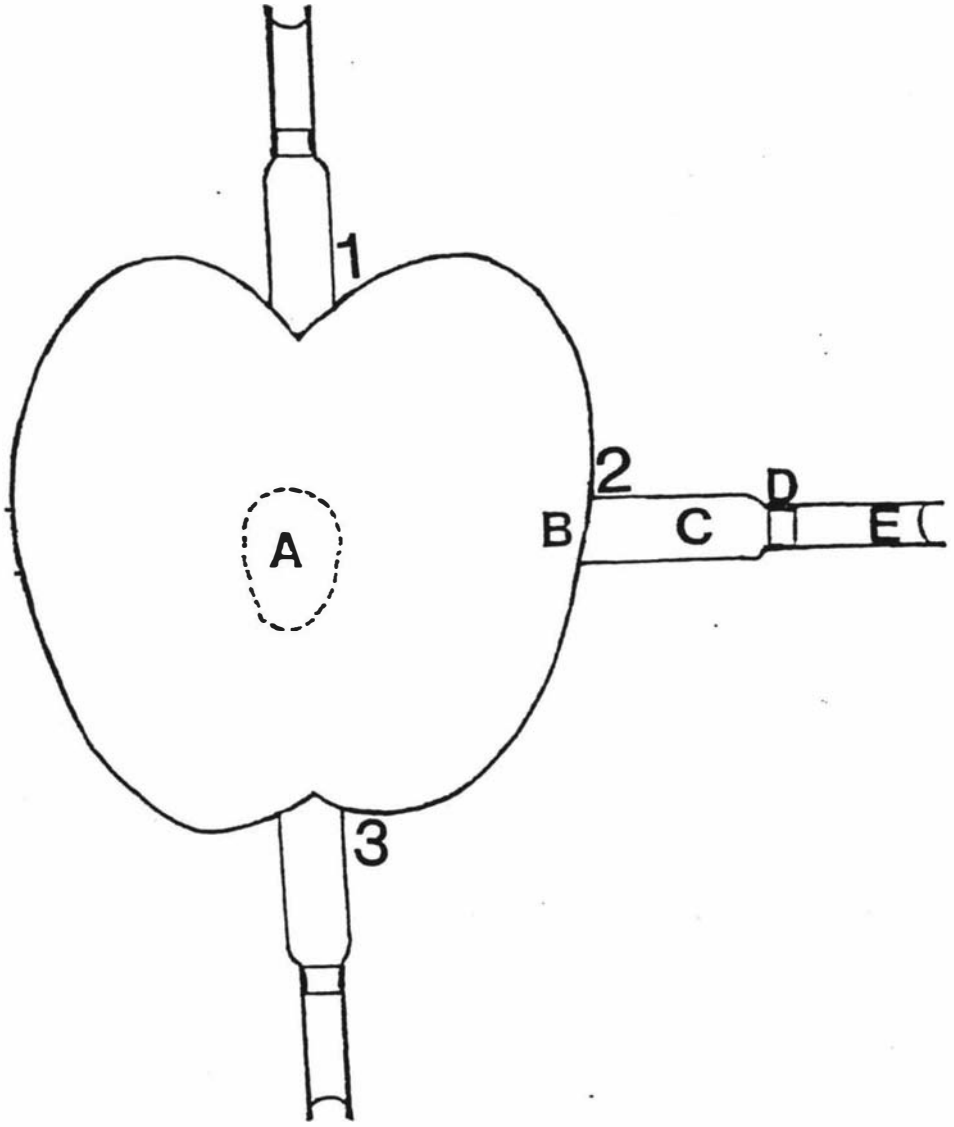

Fig. 8-3. Arrangement of glass sampling chambers on the surface of an apple fruit.

A. Core cavity
B. Fruit surface
C. Glass chamber
D. Septum cap
E. Silicone tube with water

1. Stem end
2. Equator
3. Calyx end

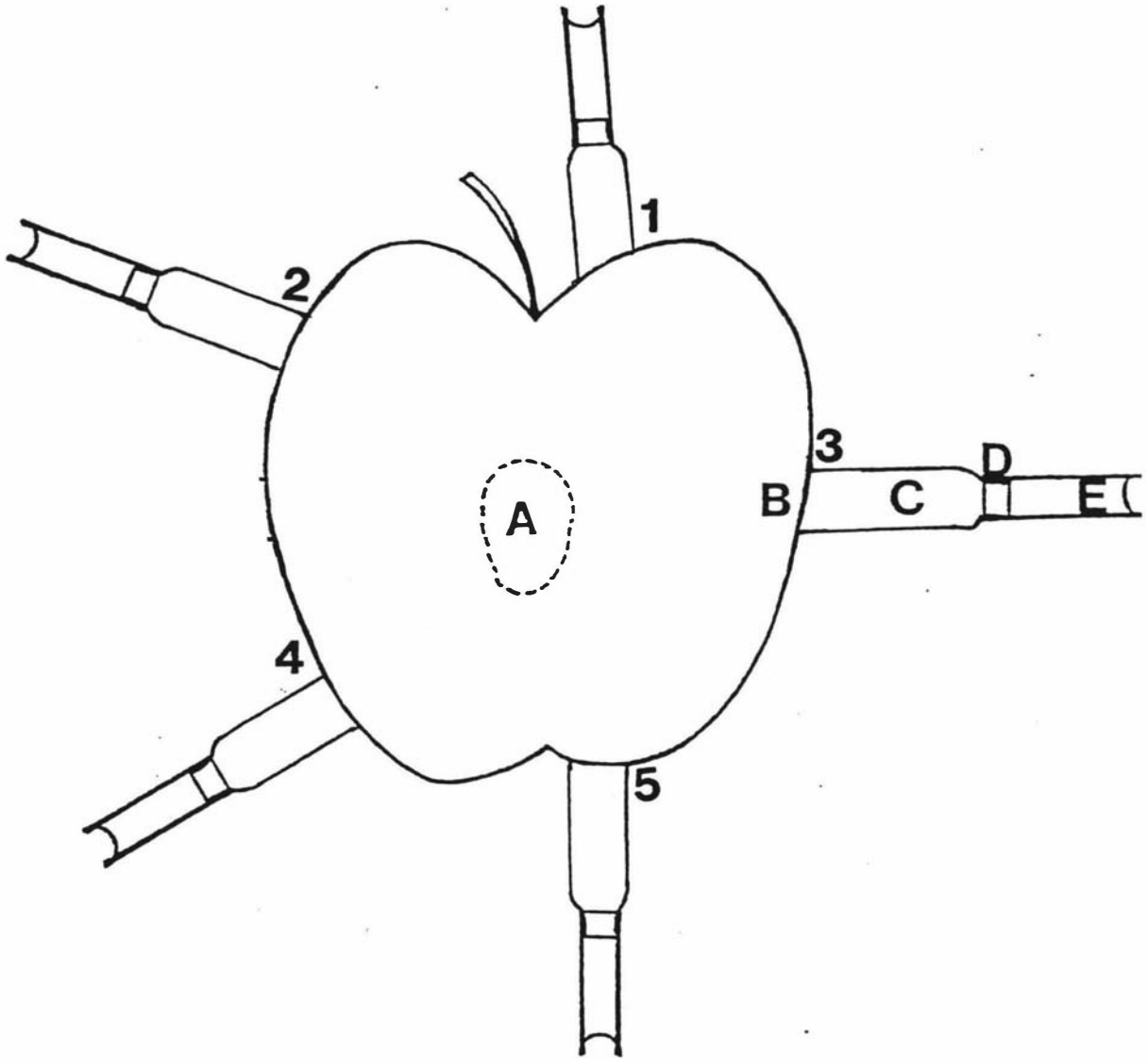

Fig. 8-4. Arrangement of glass sampling chambers on the surface of an apple fruit.

A. Core cavity
B. Fruit surface
C. Glass chamber
D. Septum cap
E. Silicone tube with water

1. Stem end shoulder
2. Between Stem end shoulder and equator
3. Equator
4. Between the equator and calyx end shoulder
5. Calyx end shoulder

8.3.2.3 Experimental design and analysis

8.3.2.3.1 Experiment 1

Twelve single fruit replicates of each apple cultivar were used in a completely randomised order. Data were analysed as described in section 3.3.9. Mean comparisons were carried out by Duncan's multiple range test at 1% level of significance (Duncan 1955), to test difference between cultivars. Student's paired t-tests (Steel and Torrie, 1980) were also performed to compare gas concentration differences between the equator and calyx end, between the equator and calyx end shoulder and between the equator and core cavity in each cultivar.

8.3.2.3.2 Experiment 2

Within the overall study there were four sampling times at 0°C and two cultivars, each analysed as a separate experiment. There were twelve single fruit replicates of each cultivar and three sampling times at 20°C used in a factorial design within each experiment. Analysis of variance (Steel and Torrie, 1980; Little, 1981) was carried out using General Linear Models procedure (GLM) of SAS (see 3.3.9).

8.4 RESULTS

8.4.1 Experiment 1

8.4.1.1 O_2 and CO_2 concentration differences between the equator and calyx end

Oxygen concentration differences between the equator and calyx end, differed between cultivars ($P < 0.01$; fig. 8-5). Markedly higher O_2 differences were estimated in Gala (approximately 9%), Braeburn (5.7%), Royal Gala (5%) and Cox's Orange Pippin (4.9%) compared to 0.1% in Splendour, 1% in Red

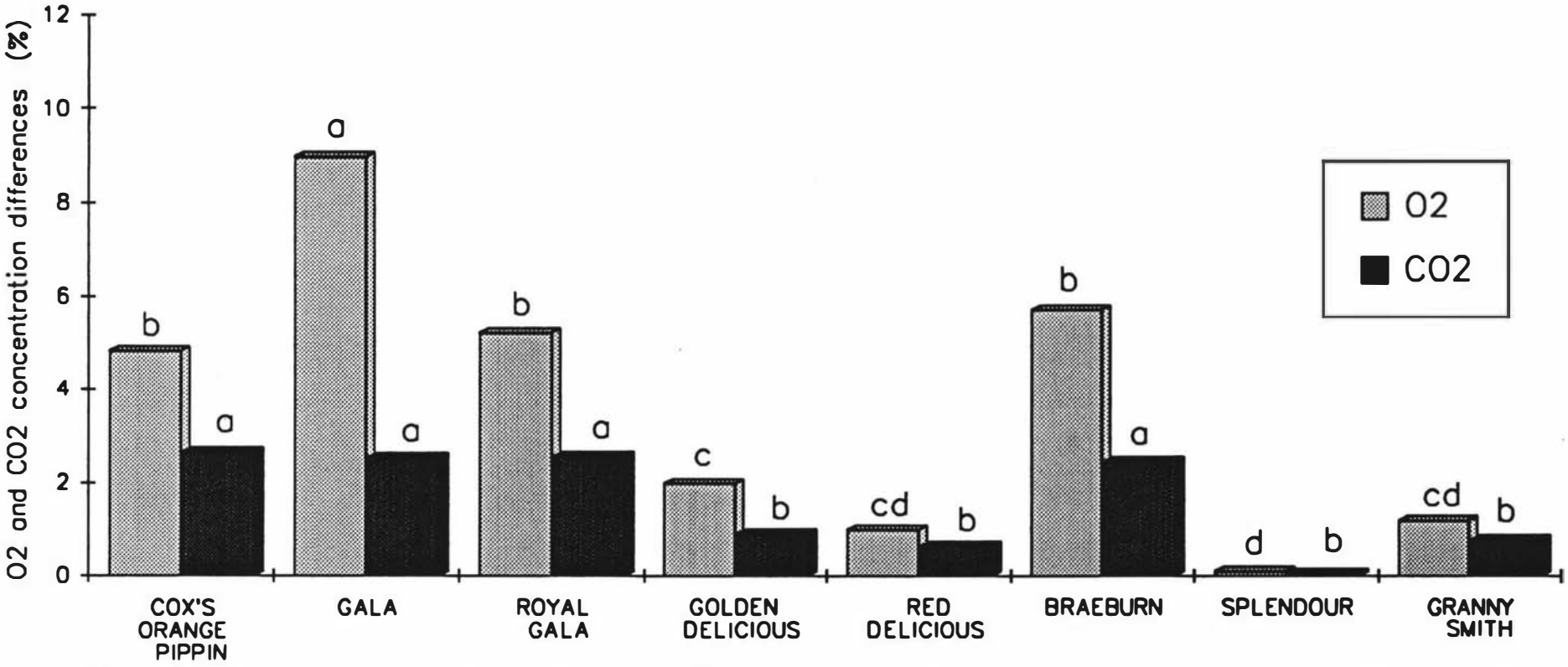

Fig. 8–5. Oxgyen and carbon dioxide concentration differences between the equator and calyx end of freshly harvested apples at 20°C. Letters in common for each gas not significantly different at the 1% level. Mean separation by Duncan's multiple range test.

Delicious and Granny Smith and 2% in Golden Delicious apples. The mean O_2 concentration difference in Gala apples was nearly nine times higher than in Splendour, and eight times higher than in Red Delicious or Granny Smith apples.

There were significant ($P < 0.01$) cultivar differences in CO_2 concentration differences between the equator and calyx end. The average CO_2 concentration differences in Cox's Orange Pippin, Gala, Royal Gala and Braeburn were nearly three times higher than in Splendour and twice higher than in Red Delicious or Golden Delicious apples (fig. 8-5).

In all the cultivars, the mean CO_2 concentration differences were lower than the O_2 differences. For example, in Gala the mean CO_2 concentration difference was 2.6% compared to 9% for O_2.

8.4.1.2 O_2 and CO_2 concentration differences between the equator and calyx end shoulder

Oxygen concentration differences estimated as the difference in equilibrated O_2 concentrations between the equator and calyx end shoulder were largely cultivar specific ($P < 0.01$; fig.8-6). Mean O_2 concentration differences in Gala apples were about six times higher than in Splendour, Granny Smith or Golden Delicious, nearly five times greater than in Cox's Orange Pippin and three times greater than in Braeburn or Royal Gala.

Similarly CO_2 concentration difference varied with cultivar ($P < 0.01$; fig. 8-6). The CO_2 concentration difference in Braeburn was approximately four times higher than in Cox's Orange Pippin and about one fourth greater than in Gala apples (but not significantly different). In general the average O_2 and CO_2 concentration differences estimated between the equator and calyx end (fig. 8-5) in all the cultivars, were consistently higher than those estimated between the equator and calyx end shoulder (fig. 8-6).

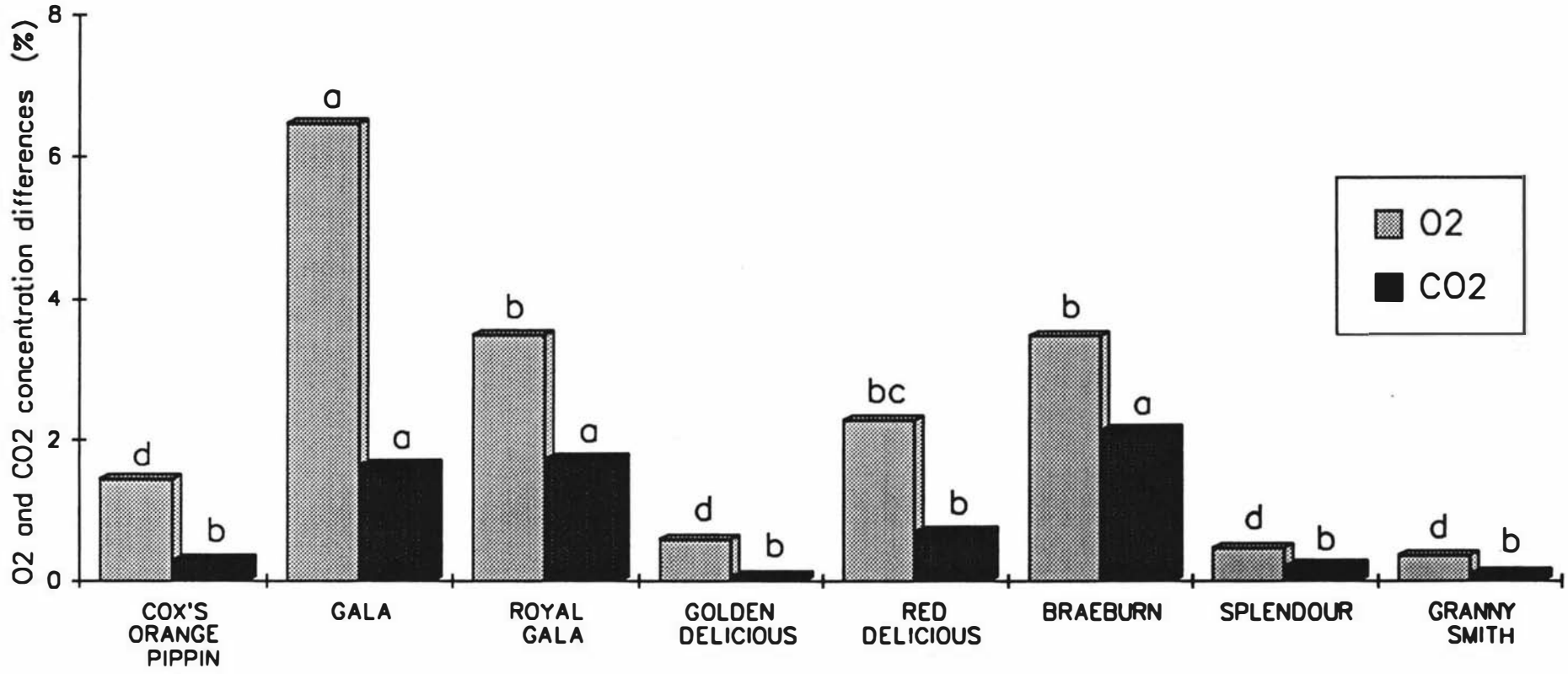

Fig. 8–6. Oxgyen and carbon dioxide concentration differences between the equator and calyx end shoulder of freshly harvested apples at 20°C. Letters in common for each gas not significantly different at the 1% level. Mean separation by Duncan's multiple range test.

8.4.1.3 O_2 and CO_2 concentration differences between the equator and core cavity

Gas concentration differences between the equator and core cavity differed between cultivar ($P < 0.01$; fig. 8-7). Significantly ($P < 0.01$) higher O_2 concentration differences were observed in Braeburn and Cox's Orange Pippin apples compared to nearly none in Gala, Royal Gala, Golden Delicious, Red Delicious, Splendour or Granny Smith apples.

Carbon dioxide concentration differences in Braeburn and Cox's Orange Pippin apples were 0.47% and 0.16% respectively as against nearly zero in Gala, Royal Gala, Golden Delicious, Red Delicious, Splendour or Granny Smith apples. In all the cultivars, the average gas concentration differences estimated between the equator and core cavity (fig. 8-7) were much lower than those estimated between the equator and calyx end (fig. 8-5), or calyx end shoulder (fig. 8-6).

8.4.2 Experiment 2

8.4.2.1 Distribution of gas concentrations at the stem end, equator and calyx end

8.4.2.1.1 O_2 concentrations

The overall mean O_2 concentrations (for each experiment) estimated at the stem end, equator and calyx end of Braeburn (fig. 8-8) apples after storage at 0°C for 0, 4, 8, and 12 weeks and subsequent transfer to ambient temperature for 3, 10, and 17 days were lower than in Granny Smith apples (fig. 8-9; $P < 0.01$). The average O_2 concentrations at the equator in both cultivars were consistently higher than at the stem end ($P < 0.05$) or calyx end ($P < 0.01$). For instance during storage at 0°C for 0 weeks and 17 days at 20°C, the O_2 concentrations at the equator of Braeburn and Granny Smith

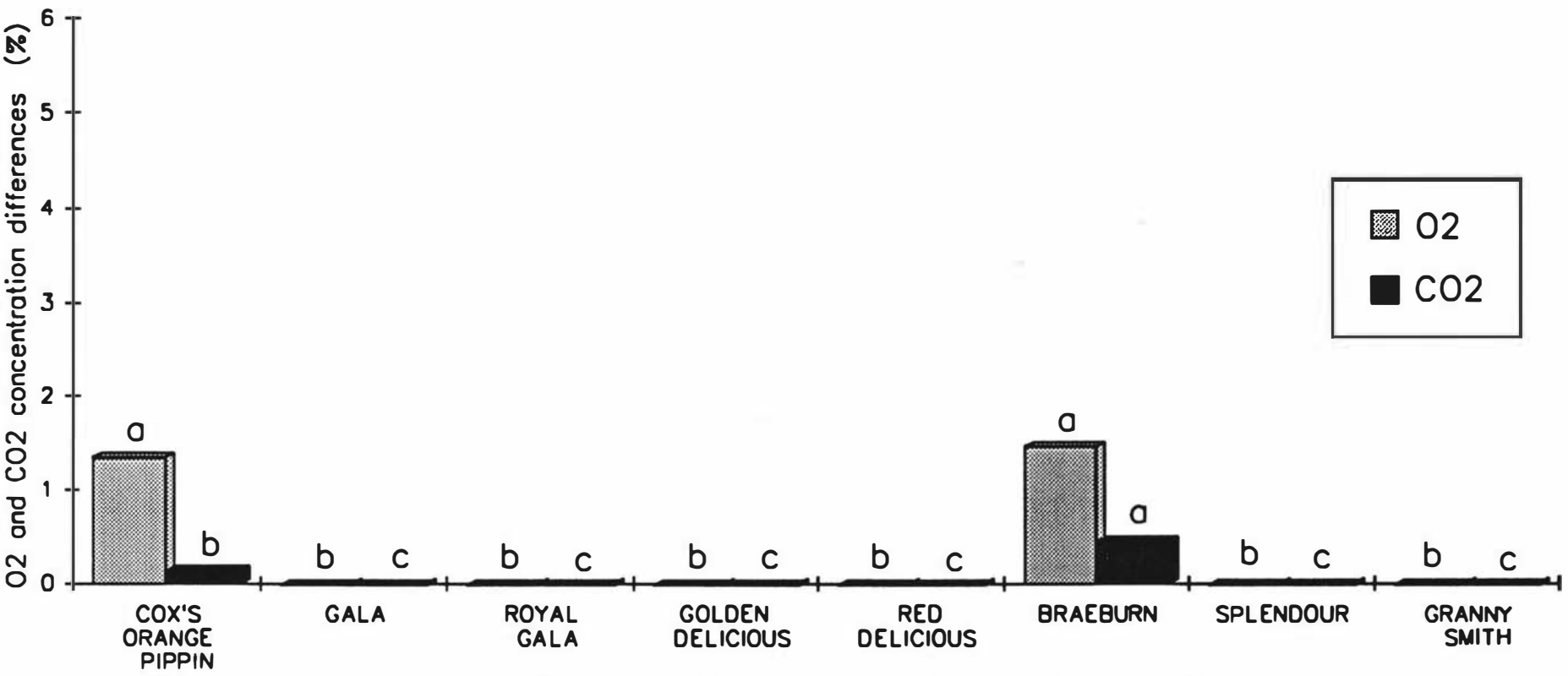

Fig. 8–7. Oxgyen and carbon dioxide concentration differences between the equator and core cavity of freshly harvested apples at 20°C. Letters in common for each gas not significantly different at the 1% level. Mean separation by Duncan's multiple range test.

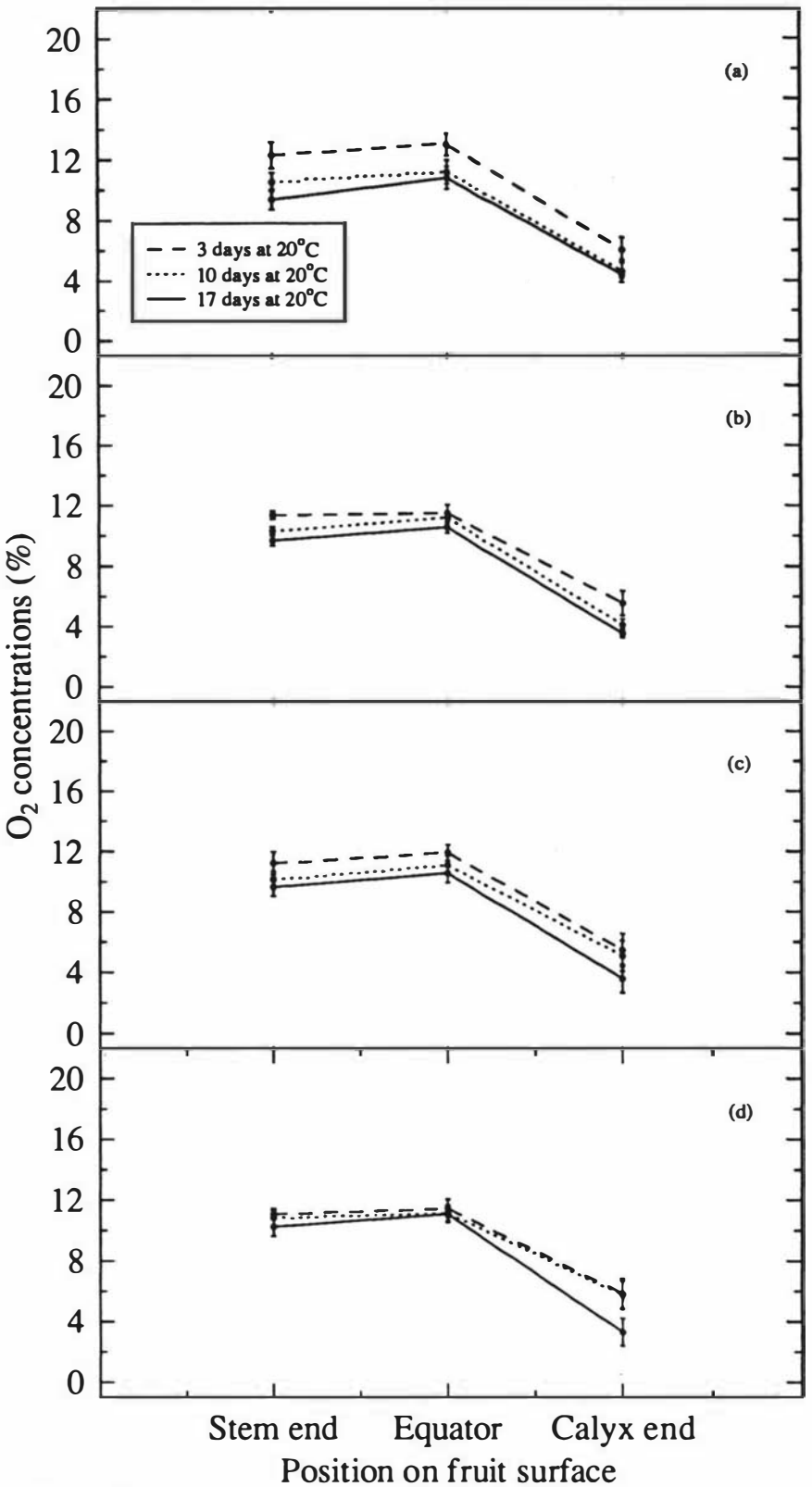

Fig. 8-8. Oxygen concentration at the stem end, equator and calyx end of Braeburn apples stored (for (a)=0, (b)=4, (c)=8, (d)=12 weeks) at 0°C and subsequent transfer to 20°C (for 3, 10 and 17 days). Vertical bars represent standard error of the means.

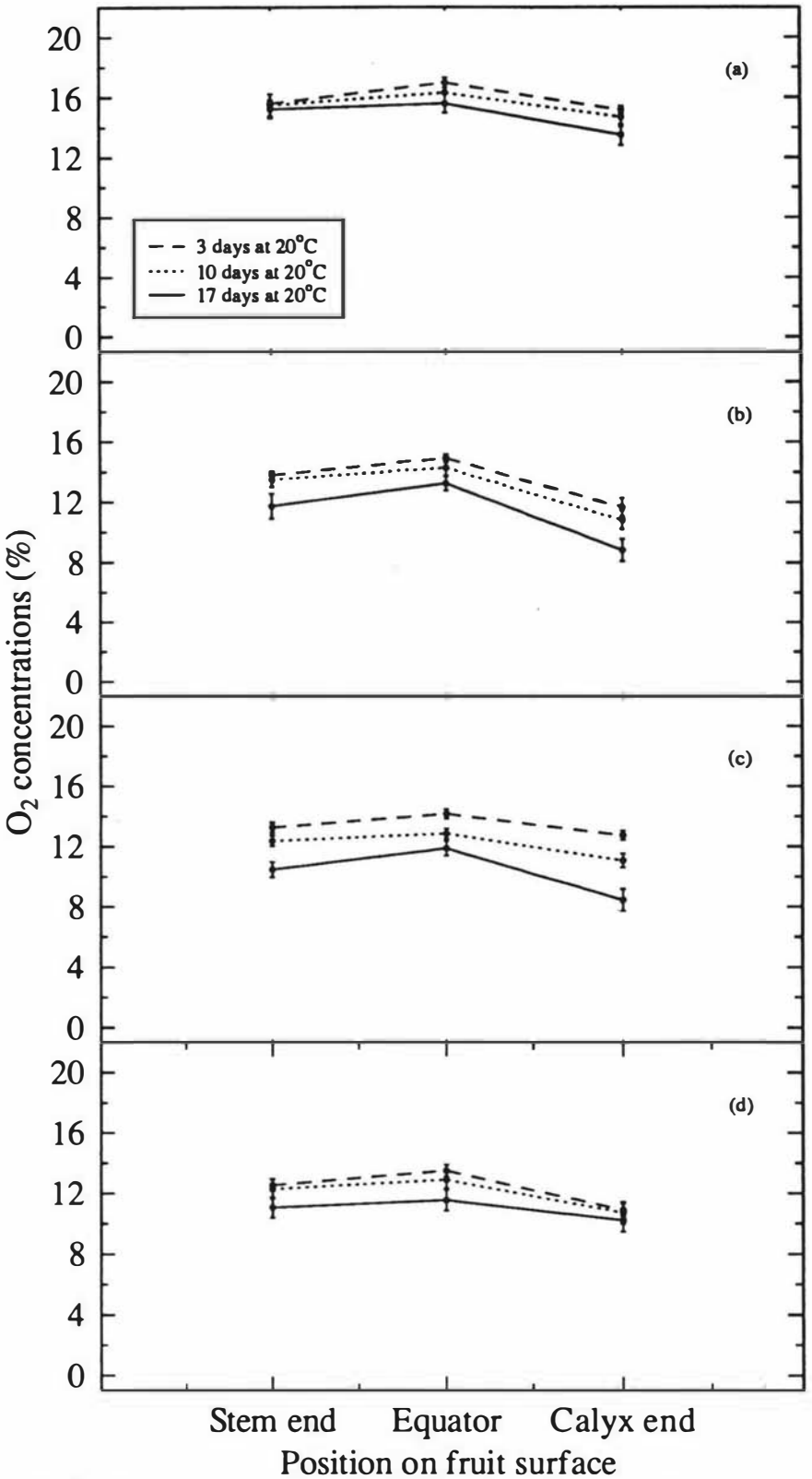

Fig. 8-9. Oxygen concentration at the stem end, equator and calyx end of Granny Smith apples stored (for (a)=0, (b)=4, (c)=8, (d)=12 weeks) at 0°C and subsequent transfer to 20°C (for 3, 10 and 17 days). Vertical bars represent standard error of the means.

apples were nearly 11 and 16% respectively as against approximately 4 and 13% respectively at the calyx end and 9 and 15% at the stem end. There were consistent trends of high O_2 concentrations at the stem end, peaking at the equator and declining at the calyx end. The magnitude of decline was cultivar dependent. After storage at 0°C for 12 weeks and subsequent transfer to 20°C for 17 days, the O_2 concentrations at the equator of Braeburn apples were nearly 3 times higher than at the calyx end, while in Granny Smith it was 1.3 times higher. During the first 17 days at 20°C, O_2 concentrations at the various positions on the fruit surface were high, however after a period of cold storage and subsequent transfer to 20°C, there seemed to be a decline in O_2 concentrations. In addition, O_2 concentrations at the stem end, equator and calyx end in both cultivars declined with time of storage at 20°C. For instance, in Braeburn apples, after the 3rd day of storage at 20°C (fig. 8-8a), the mean O_2 concentrations at the stem end, equator and calyx end were approximately 12, 13 and 6% respectively. However by the 17th day at 20°C, concentrations had dropped to nearly 9, 11 and 4% respectively. After 4, 8 or 12 weeks at 0°C and subsequent storage at 20°C for 3days, O_2 concentrations at the various positions were lower than the initial concentrations before cold storage (fig. 8-8a). Similar trends were also observed in Granny Smith, however in this case, O_2 concentrations were much higher than those observed in Braeburn apples.

Core cavity O_2 concentrations estimated on the 17th day at 20°C differed between the two cultivars (fig. 8-10; $P < 0.01$). It was observed that whilst the O_2 concentrations in the core cavity of Braeburn apples did not change with time of storage at 0°C and subsequent storage at 20°C for 17 days, O_2 concentrations in Granny Smith apples varied markedly ($P < 0.01$) over the period.

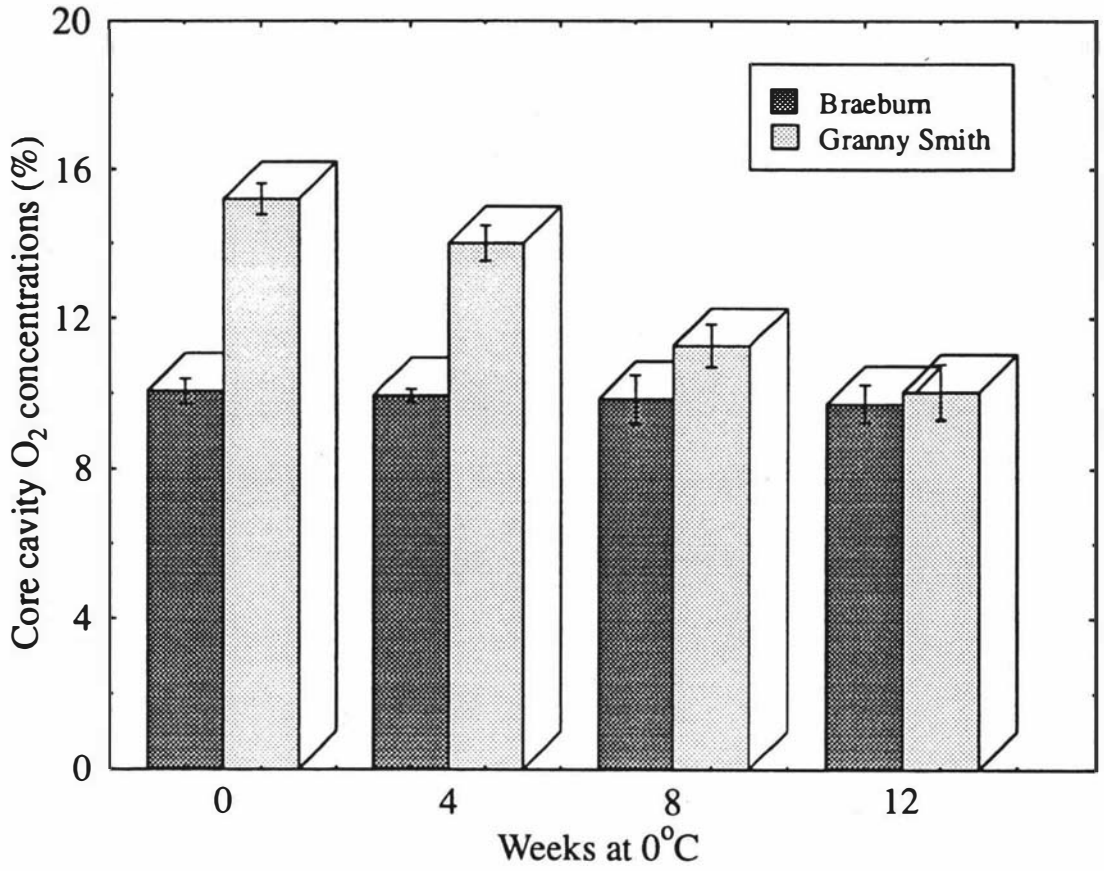

Fig. 8-10. Core cavity O_2 concentrations of Braeburn and Granny Smith apples stored at 0°C for 0, 4, 8 and 12 weeks and subsequent storage at 20°C for 17 days. Vertical bars represent standard error of the means.

8.4.2.1.2 CO_2 concentrations

Carbon dioxide concentrations at the stem end, equator and calyx end differed between cultivars. After storage at 0°C and subsequent storage at 20°C, CO_2 concentrations in Braeburn apples were mostly greater than 6% and increased during storage at 20°C to about 9 or 10% (fig. 8-11), compared to Granny Smith in which concentrations were greater than 3% but increased to approximately 6 or 7% during the same period (fig. 8-12). Carbon dioxide concentrations at the calyx end in both cultivars were consistently higher than at the stem end or equator, however, concentrations in Braeburn were much higher than in Granny Smith apples. For example, CO_2 concentrations at the calyx end of Braeburn and Granny Smith apples after 8 weeks of cold storage and subsequent storage at ambient temperature for 17 days were respectively 10.6 and 7.9% as against 7.7 and 6.4% at the equator and 8.1 and 6.7% at the stem end. Throughout the storage periods there were consistent patterns of high CO_2 concentrations at the calyx end, declining at the equator and increasing at the stem end. Core cavity CO_2 concentrations differed between the two cultivars (fig. 8-13; $P < 0.01$) with only gradual increase in concentrations with storage time at 20°C (not significantly different).

8.4.2.1.3 C_2H_4 concentrations

The mean C_2H_4 concentrations at the stem end, equator and calyx end of Braeburn (fig. 8-14) apples were higher ($P < 0.001$) than in Granny Smith (fig. 8-15). Highest concentrations were estimated at the calyx end, slightly less at the stem end and least at the equator. During the 3rd and 10th day at 20°C (fig. 8-15a) C_2H_4 was not detected in any of the positions in Granny Smith apples, however, by the 17th day, the calyx end (136 $\mu l\ l^{-1}$) had more C_2H_4 than the stem end (109 $\mu l\ l^{-1}$) and equatorial surface (97 $\mu l\ l^{-1}$). After cold storage for 4, 8 and 12 weeks and subsequent storage at 20°C for 3, 10

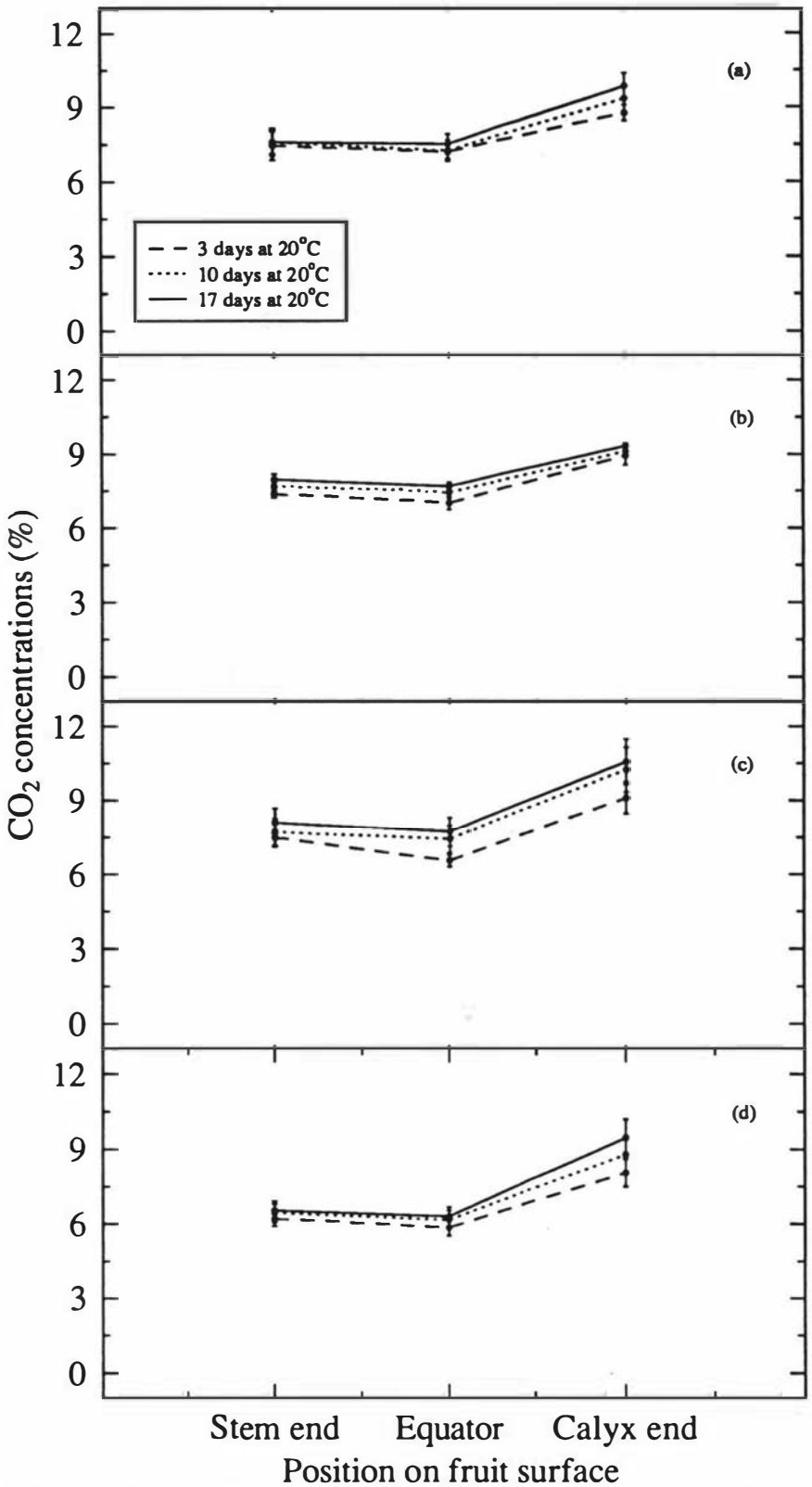

Fig. 8-11. Carbon dioxide concentration at the stem end, equator and calyx end of Braeburn apples stored (for (a)=0, (b)=4, (c)=8, (d)=12 weeks) at 0°C and subsequent transfer to 20°C (for 3, 10 and 17 days). Vertical bars represent standard error of the means.

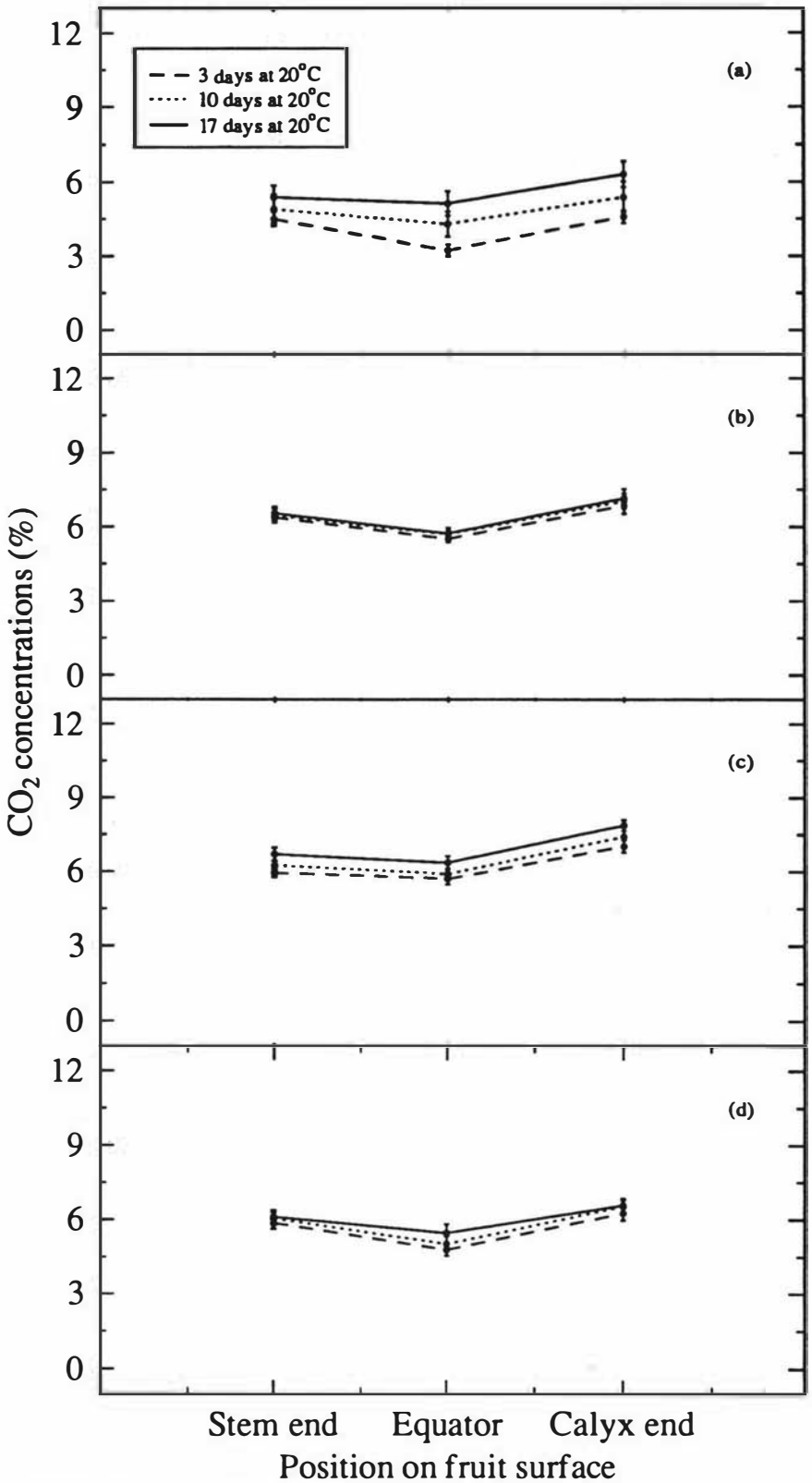

Fig. 8-12. Carbon dioxide concentration at the stem end, equator and calyx end of Granny Smith apples stored (for (a)=0, (b)=4, (c)=8, (d)=12 weeks) at 0°C and subsequent transfer to 20°C (for 3, 10 and 17 days). Vertical bars represent standard error of the means.

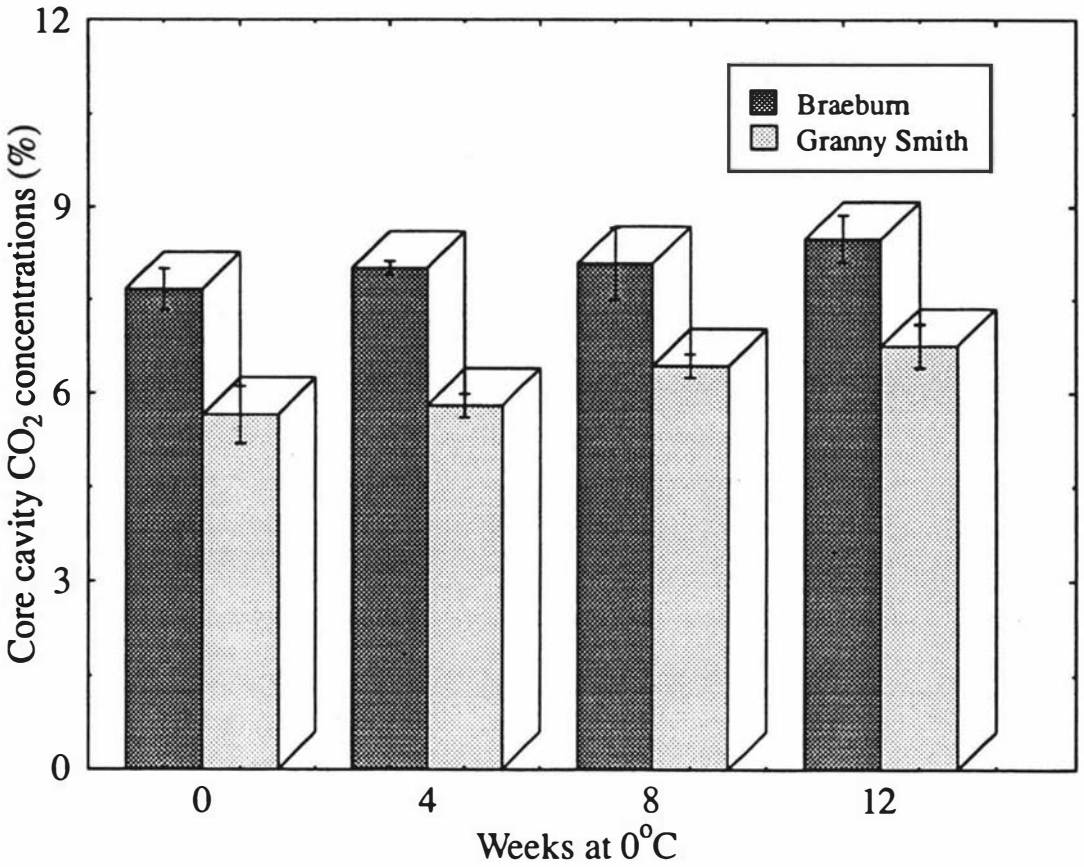

Fig. 8-13. Core cavity CO_2 concentrations of Braeburn and Granny Smith apples stored at 0°C for 0, 4, 8 and 12 weeks and subsequent storage at 20°C for 17 days. Vertical bars represent standard error of the means.

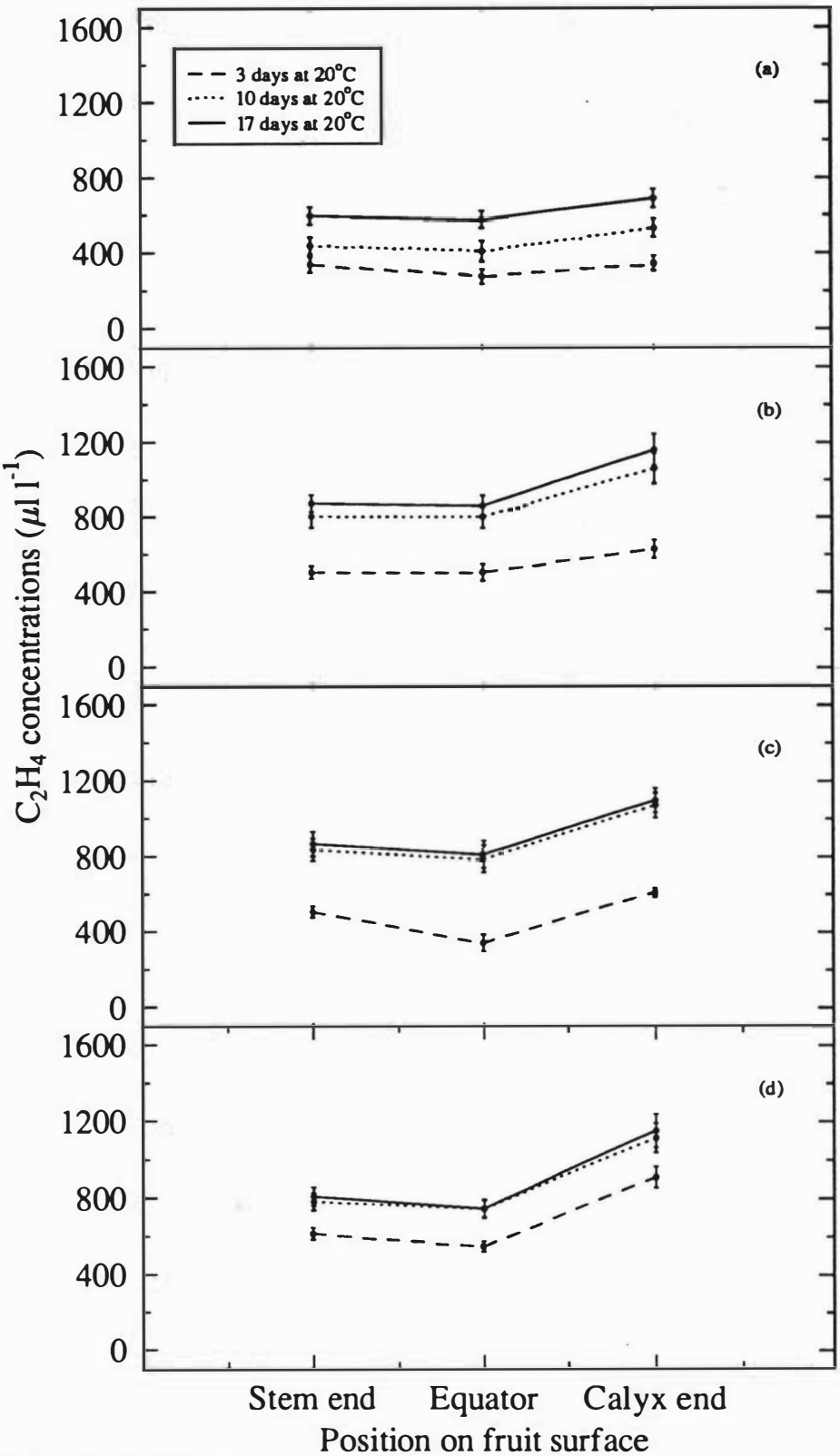

Fig. 8-14. Ethylene concentration at the stem end, equator and calyx end of Braeburn apples stored (for (a)=0, (b)=4, (c)=8, (d)=12 weeks) at 0°C and subsequent transfer to 20°C (for 3, 10 and 17 days). Vertical bars represent standard error of the means.

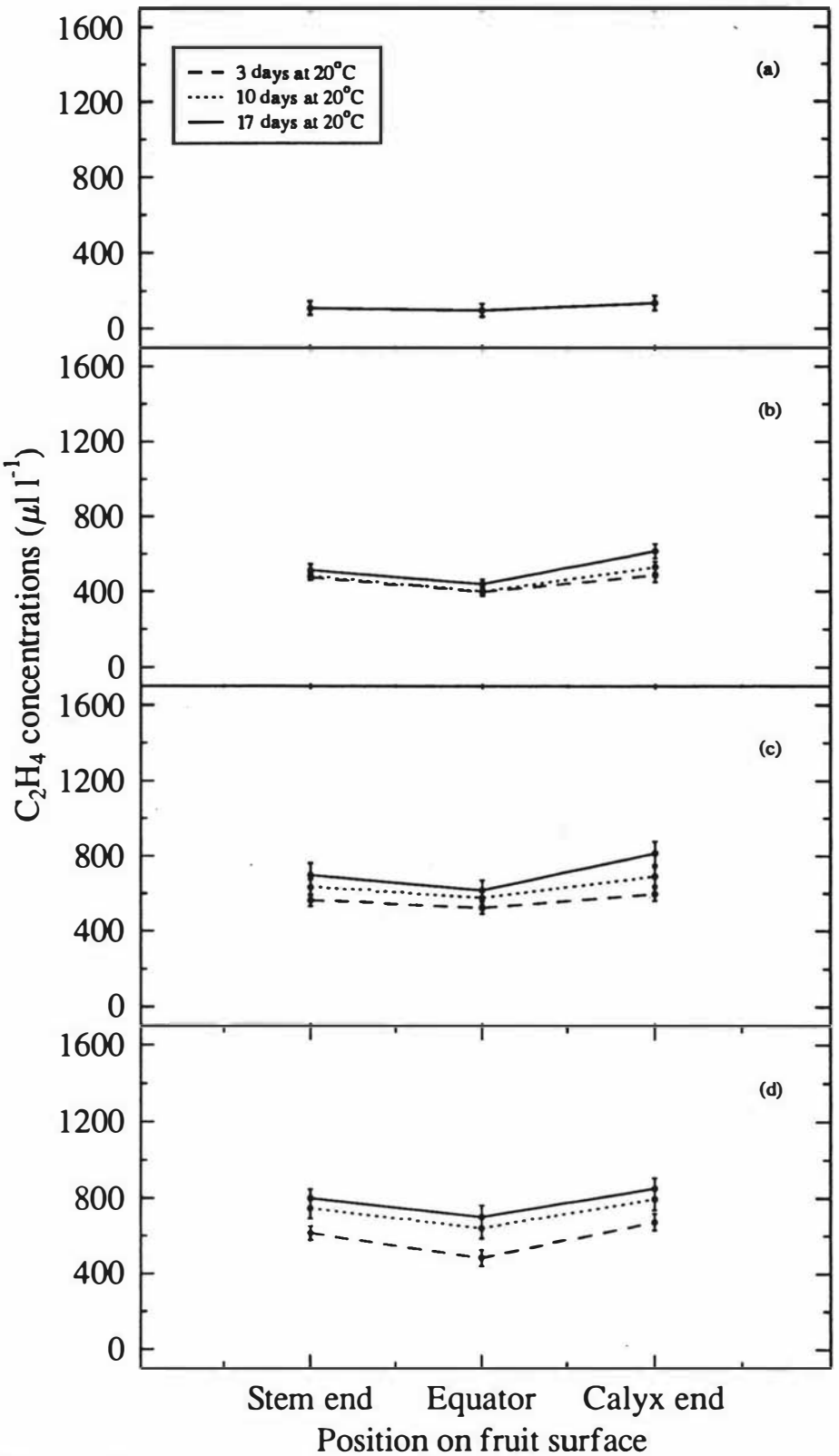

Fig. 8-15. Ethylene concentration at the stem end, equator and calyx end of Granny Smith apples stored (for (a)=0, (b)=4, (c)=8, (d)=12 weeks) at 0°C and subsequent transfer to 20°C (for 3, 10 and 17 days). Vertical bars represent standard error of the means.

and 17 days, the concentrations of C_2H_4 in both cultivars increased over the periods at 20°C with highest concentrations at the calyx end. The mean C_2H_4 concentration at the calyx end of Braeburn apples after 12 weeks of cold storage and 17 days at ambient temperatures was 35% higher than at the equator and 30% higher than at the stem end, whilst in Granny Smith apples it was approximately 18% and 6% respectively.

The mean C_2H_4 concentrations in the core cavity of Braeburn apples were higher than in Granny Smith (fig. 8-16; $P < 0.001$). In Granny Smith apples (apart from the 12th week at 0°C) core cavity C_2H_4 concentrations increased significantly with increasing periods of storage. In contrast in Braeburn apples the increase in C_2H_4 concentrations after 0 weeks at 0°C was not significantly different over the periods of storage.

8.4.2.2 Quality indices

Braeburn apples were firmer than Granny Smith ($P < 0.01$). Generally fruit firmness decreased with increasing time of storage at 0°C and subsequent storage at 20°C for 17 days (fig. 8-17).

There were no differences ($P < 0.05$) in soluble solids content of Braeburn and Granny Smith apples during the periods of storage at 0°C and subsequent storage at 20°C (fig. 8-18).

As indicated by the hue angle values, Granny Smith apples were greener than Braeburn (fig. 8-19; $P < 0.01$). However while Granny Smith apples lost greenness during the period of storage (at 0°C and subsequent storage at 20°C), there were no marked ($P < 0.05$) colour changes in Braeburn apples.

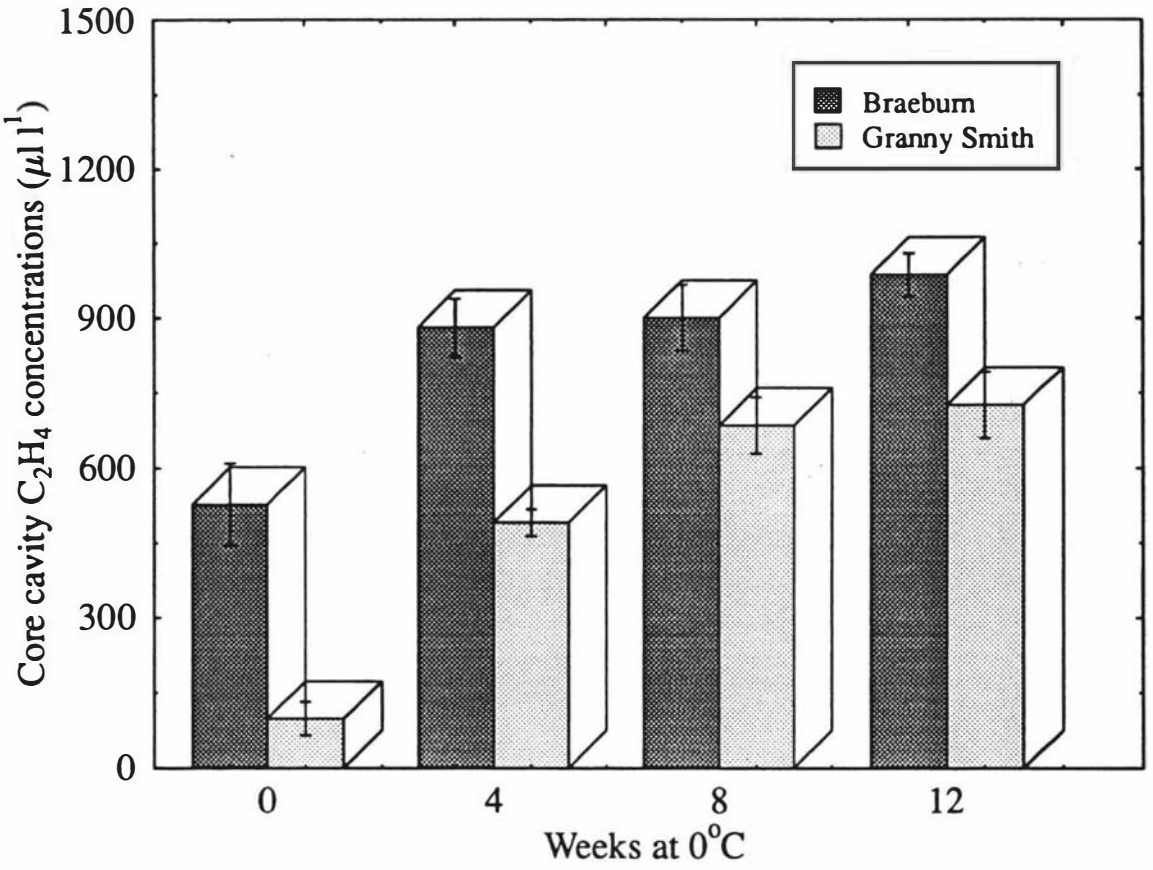

Fig. 8-16. Core cavity C_2H_4 concentrations of Braeburn and Granny Smith apples stored at 0°C for 0, 4, 8 and 12 weeks and subsequent storage at 20°C for 17 days. Vertical bars represent standard error of the means.

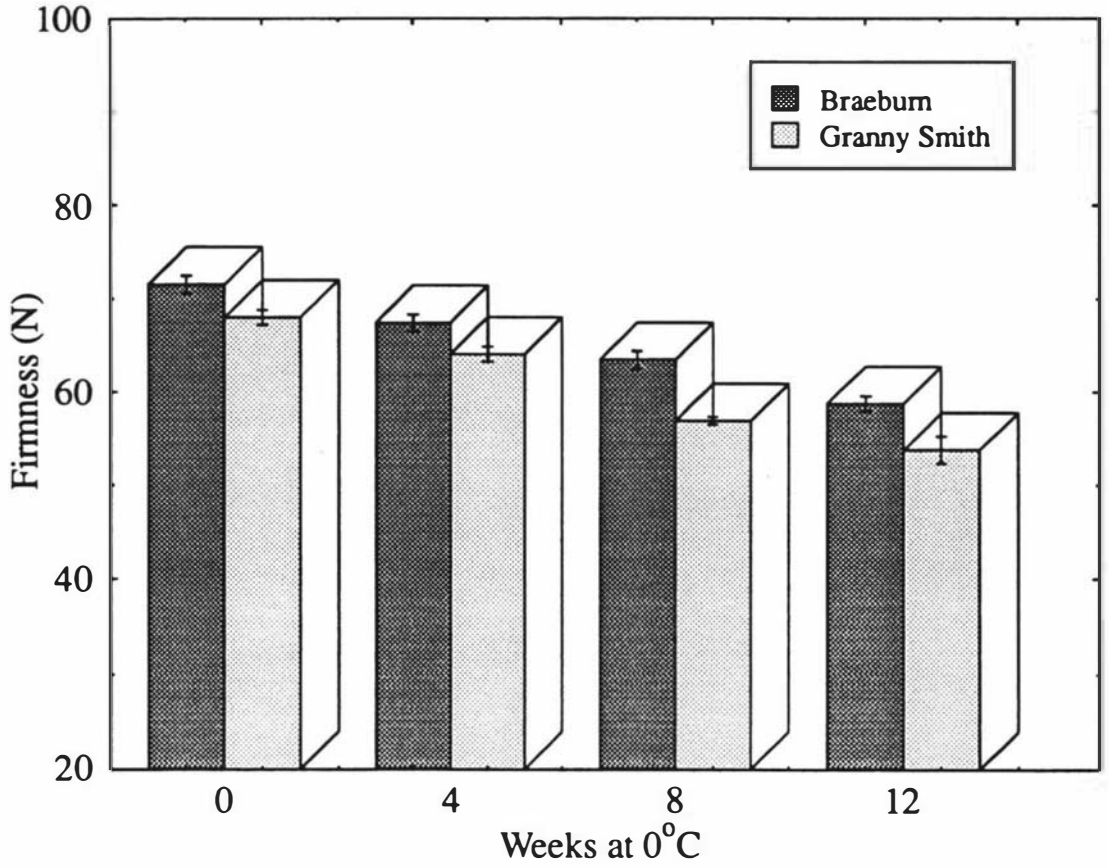

Fig. 8-17. Firmness of Braeburn and Granny Smith apples stored at 0°C for 0, 4, 8 and 12 weeks and subsequent storage at 20°C for 17 days. Vertical bars represent standard error of the means.

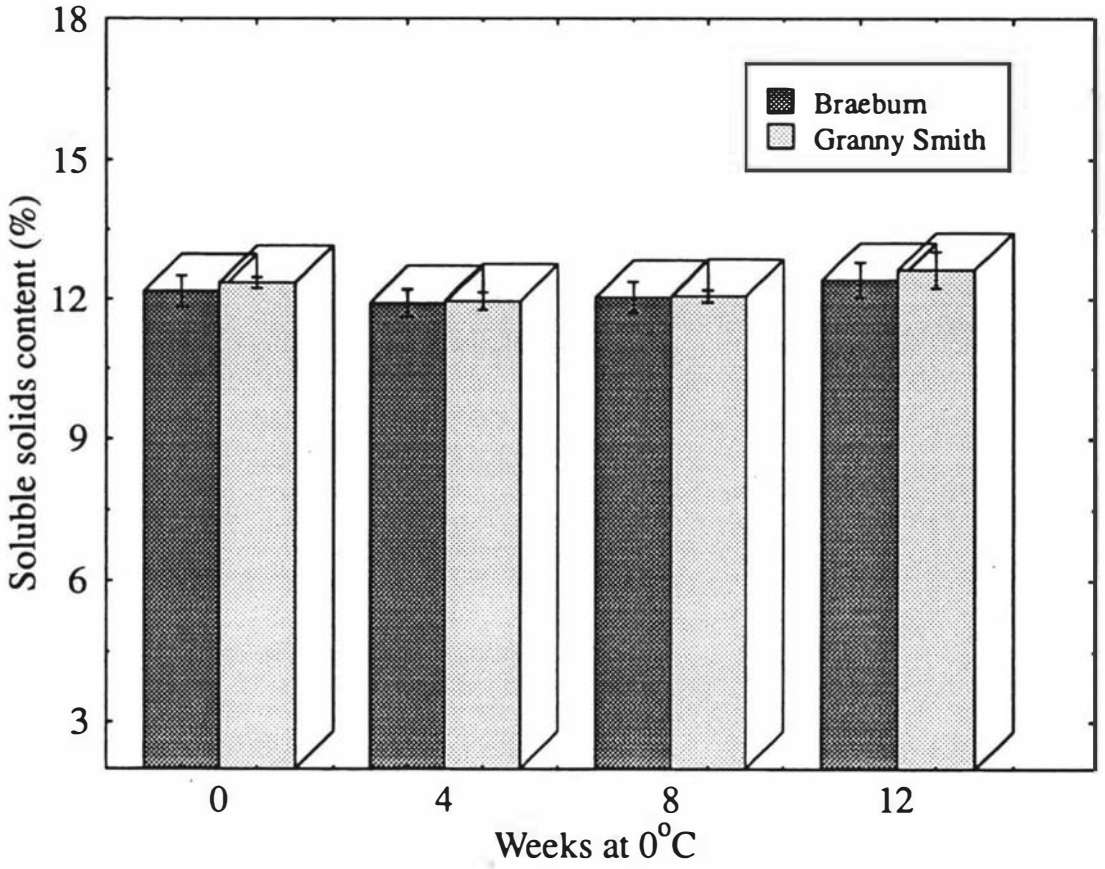

Fig. 8-18. Soluble solids content of Braeburn and Granny Smith apples stored at 0°C for 0, 4, 8 and 12 weeks and subsequent storage at 20°C for 17 days. Vertical bars represent standard error of the means.

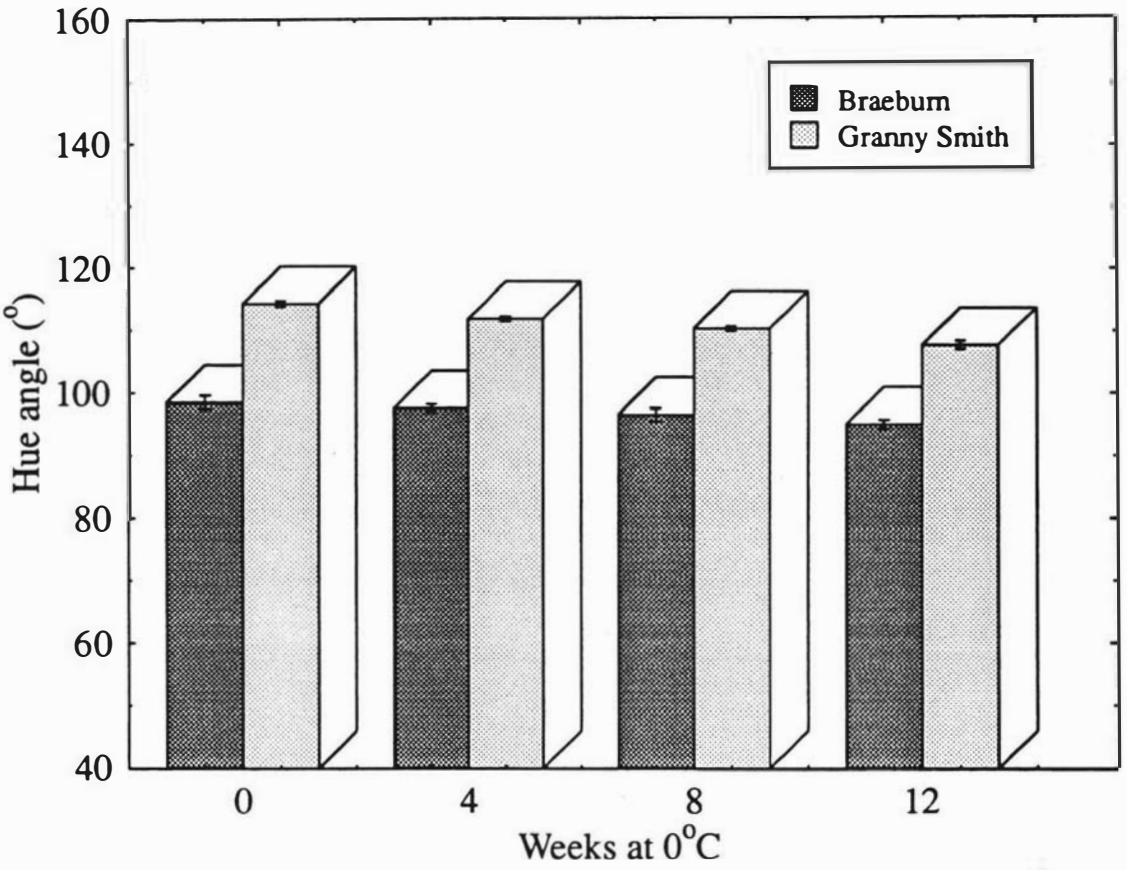

Fig. 8-19. Hue angle of Braeburn and Granny Smith apples stored at 0°C for 0, 4, 8 and 12 weeks and subsequent storage at 20°C for 17 days. Vertical bars represent standard error of the means.

8.4.2.3 Distribution of gas concentrations at five positions on the fruit surface

8.4.2.3.1 O_2 concentrations

Steady state mean distribution of O_2 concentrations at five different positions on the surface of Braeburn (fig. 8-20) were lower than in Granny Smith apples (fig. 8-21; $P < 0.01$). In general, during storage at 0°C for 0, 4, 8 and 12 weeks and subsequent storage at 20°C, the mean O_2 concentration at the equator was consistently higher than the other parts of the fruit, whilst tissues at the calyx end region (position 5) consistently had lower O_2 concentrations than the other parts of the fruit. For example, the mean O_2 concentrations at the equator of Braeburn and Granny Smith apples after 4 weeks storage at 0°C and 3 days at 20°C were, approximately 12.3 and 16.5% respectively as against 8.6 and 11.7% in the tissues nearest the calyx end region (position 5). After 8 weeks of cold storage and 17 days at ambient temperature, the difference between the mean O_2 concentration at the equator and position 5 was approximately 4.4% in Braeburn and 3.0% in Granny Smith apples.

There was a trend of high O_2 concentrations at position 1 (stem end shoulder), more at position 3 (equator) and less at position 5 (calyx end shoulder). Generally, O_2 concentrations at the five positions in both cultivars decreased during the period of storage at 20°C, however the magnitude of decrease differed between the two cultivars.

Core cavity O_2 concentrations sampled on the 17th day at 20°C differed between cultivar and time of storage ($P < 0.01$; fig. 8-22). Granny Smith apples were found to contain higher O_2 in the core cavity than Braeburn and the concentration in the former declined markedly ($P < 0.05$) with increasing time of cold storage and subsequent storage at 20°C for 17 days.

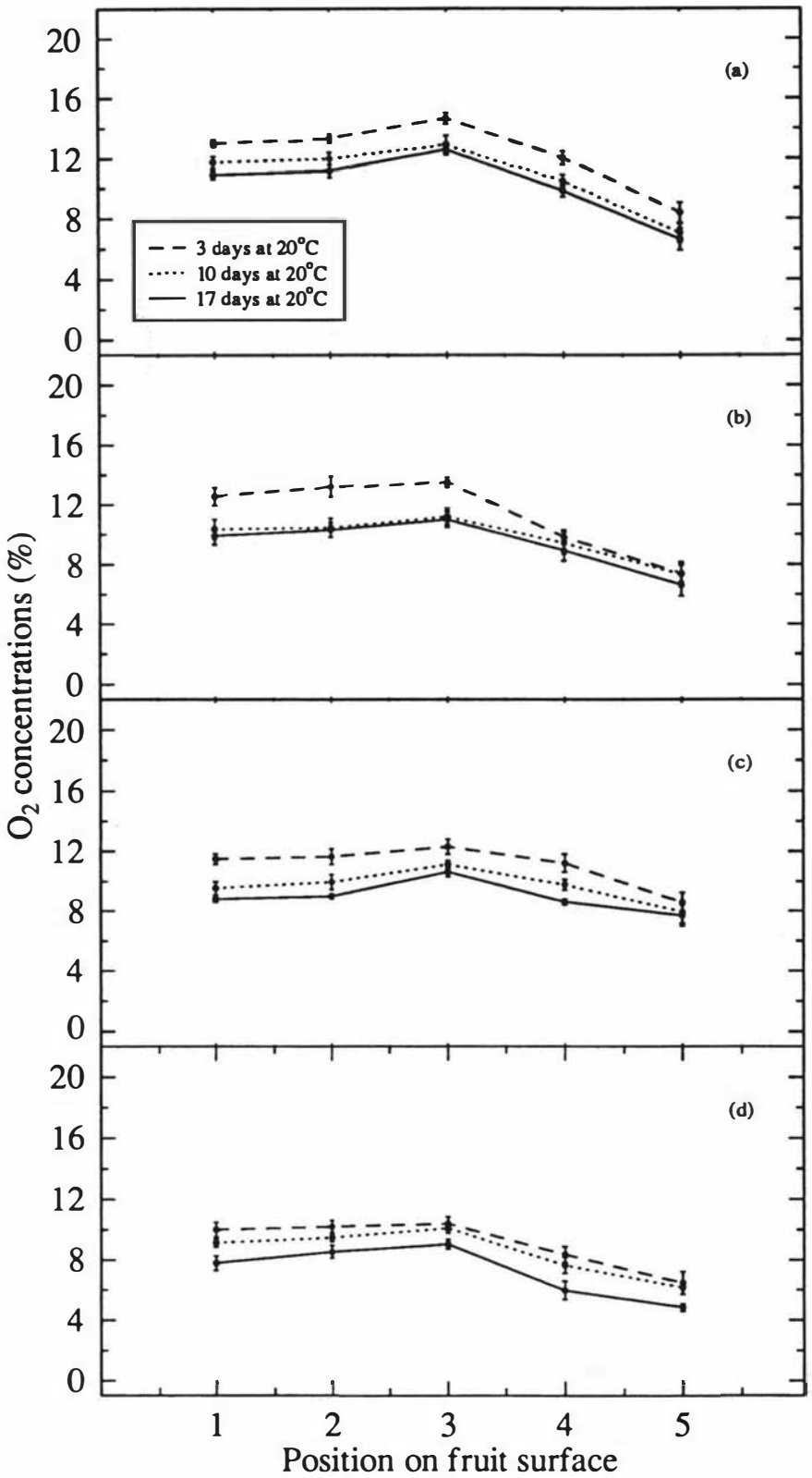

Fig. 8-20. Distribution of O_2 concentrations at five positions in the sub-epidermis of Braeburn apples stored (for (a)=0, (b)=4, (c)=8, (d)=12 weeks) at 0°C and subsequent transfer to 20°C (for 3, 10 and 17 days). Vertical bars represent standard error of the means.

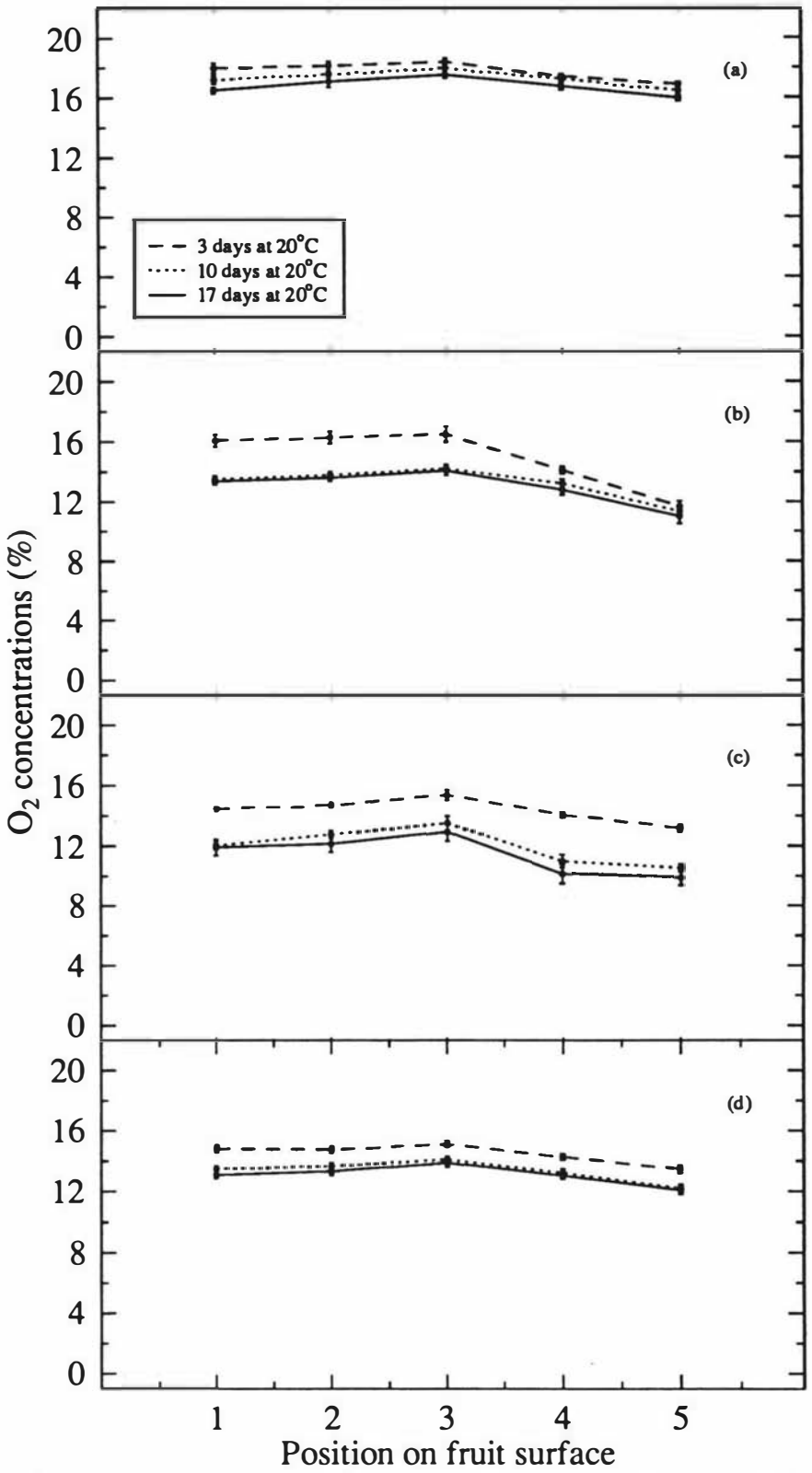

Fig. 8-21. Distribution of O_2 concentrations at five positions in the sub-epidermis of Granny Smith apples stored (for (a)=0, (b)=4, (c)=8, (d)=12 weeks) at 0°C and subsequent transfer to 20°C (for 3, 10 and 17 days). Vertical bars represent standard error of the means.

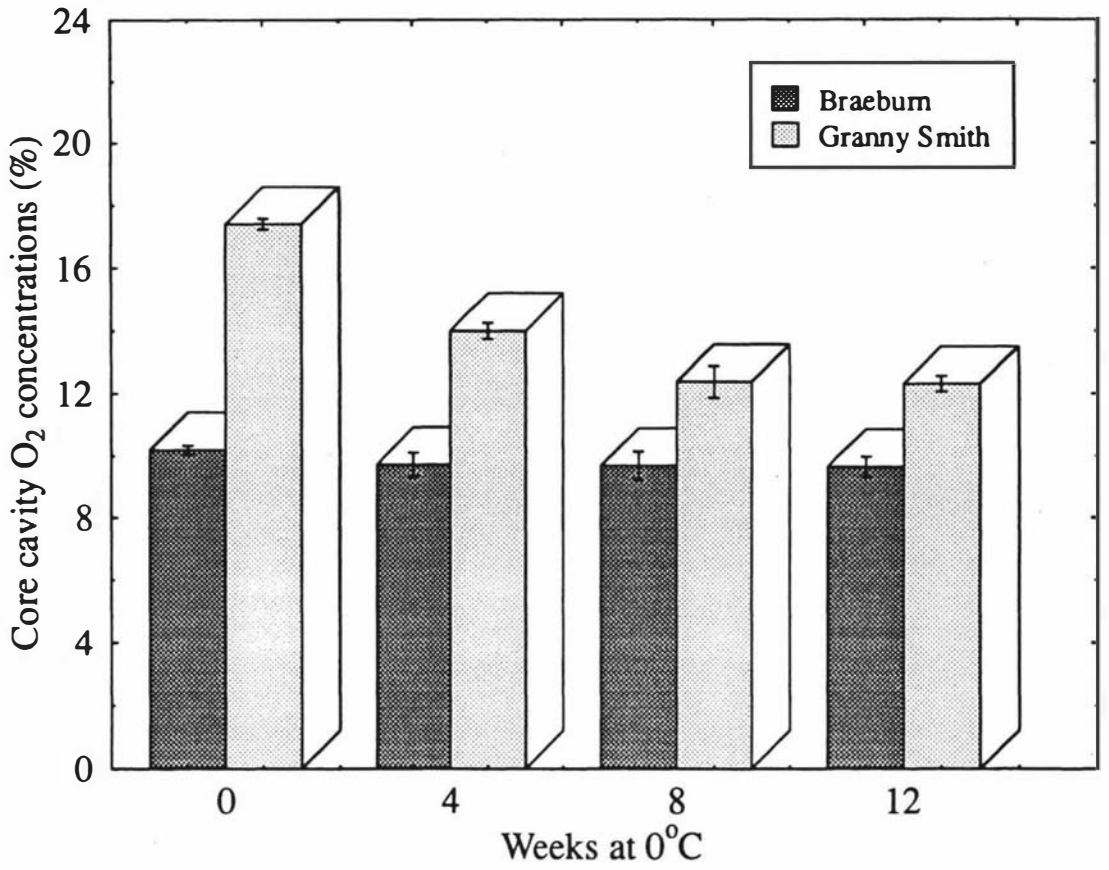

Fig. 8-22. Core cavity O_2 concentrations of Braeburn and Granny Smith apples stored at 0°C for 0, 4, 8 and 12 weeks and subsequent storage at 20°C for 17 days. Vertical bars represent standard error of the means.

8.4.2.3.2 CO_2 concentrations

During the period of storage at 0°C and subsequent storage at 20°C, CO_2 concentrations at the five positions in the sub-epidermis were found to be higher in Braeburn (fig. 8-23) than in Granny Smith apples (fig. 8-24). Tissues at the calyx end region consistently contained higher CO_2 concentrations than the other parts of the fruit. Whilst the equatorial region beneath the skin surface had lower CO_2 concentrations than any other parts of the fruit. After 8 weeks of cold storage and 17 days at 20°C, the CO_2 concentrations at the calyx end shoulder (position 5) of Braeburn and Granny Smith apples were about a third higher than the equator. Carbon dioxide concentrations showed a trend of decrease from position 1 towards positions 2 and 3 and an increase towards positions 4 and 5. The magnitude of increase was higher in Braeburn than in Granny Smith apples. Carbon dioxide concentrations at the five positions were found to increase with time of storage at 20°C.

Braeburn contained higher CO_2 concentrations in the core cavity than in Granny Smith apples (fig. 8-25) and concentrations increased with period of storage at 0°C and subsequent storage at 20°C for 17 days.

8.4.2.3.3 C_2H_4 concentrations

After storage at 0°C and subsequent storage at 20°C Braeburn (fig. 8-26) had higher mean C_2H_4 concentrations at the five different positions compared to Granny Smith apples (fig. 8-27). Tissues at the calyx end shoulder contained higher C_2H_4 concentrations than any other parts of the fruit. For example, after 12 weeks of cold storage and 17 days at 20°C, C_2H_4 concentrations at the calyx end shoulder of Braeburn and Granny Smith apples were nearly two thirds and one fifth (respectively) higher than at the equator.

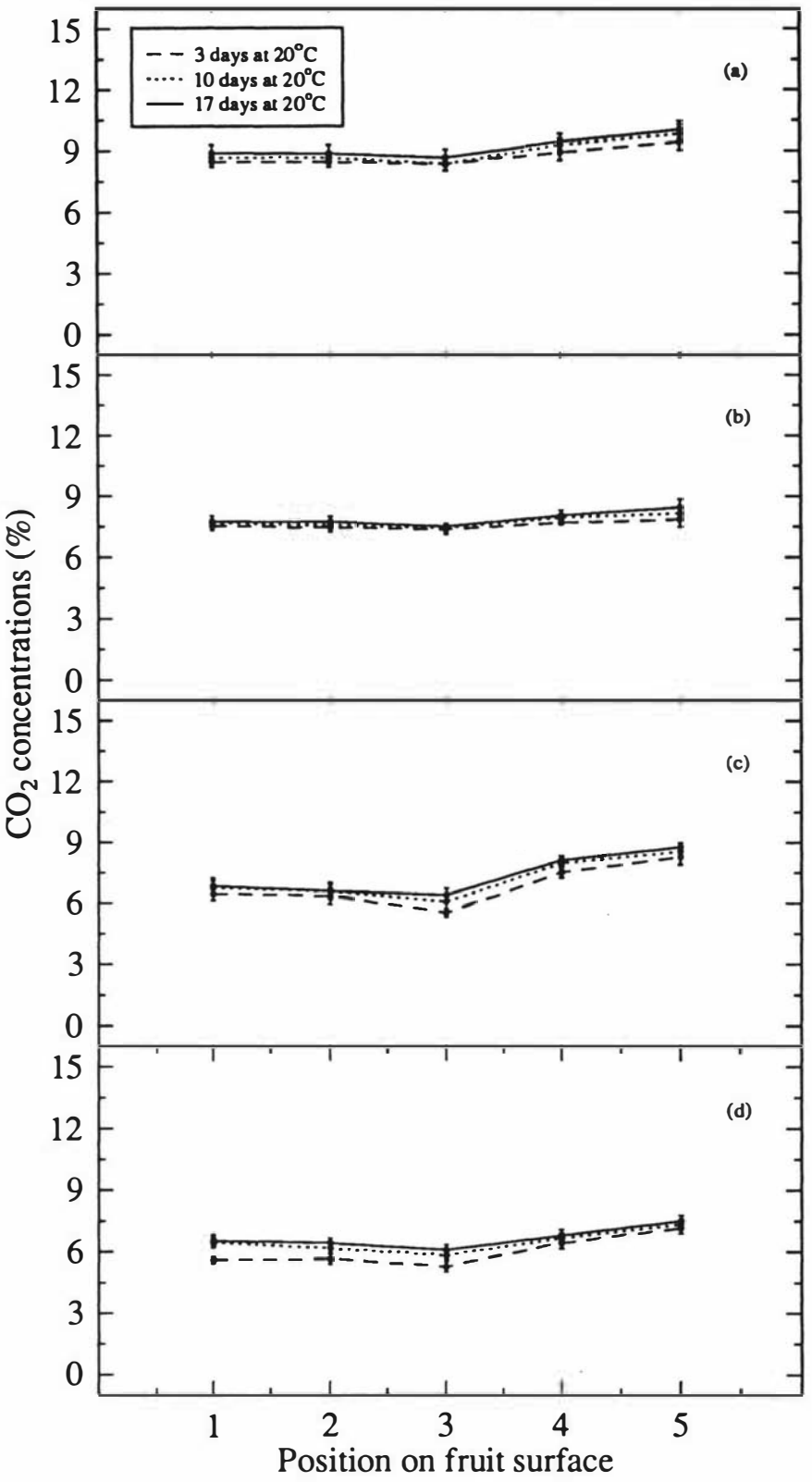

Fig. 8-23. Distribution of CO_2 concentrations at five positions in the sub-epidermis of Braeburn apples stored (for (a)=0, (b)=4, (c)=8, (d)=12 weeks) at 0°C and subsequent transfer to 20°C (for 3, 10 and 17 days). Vertical bars represent standard error of the means.

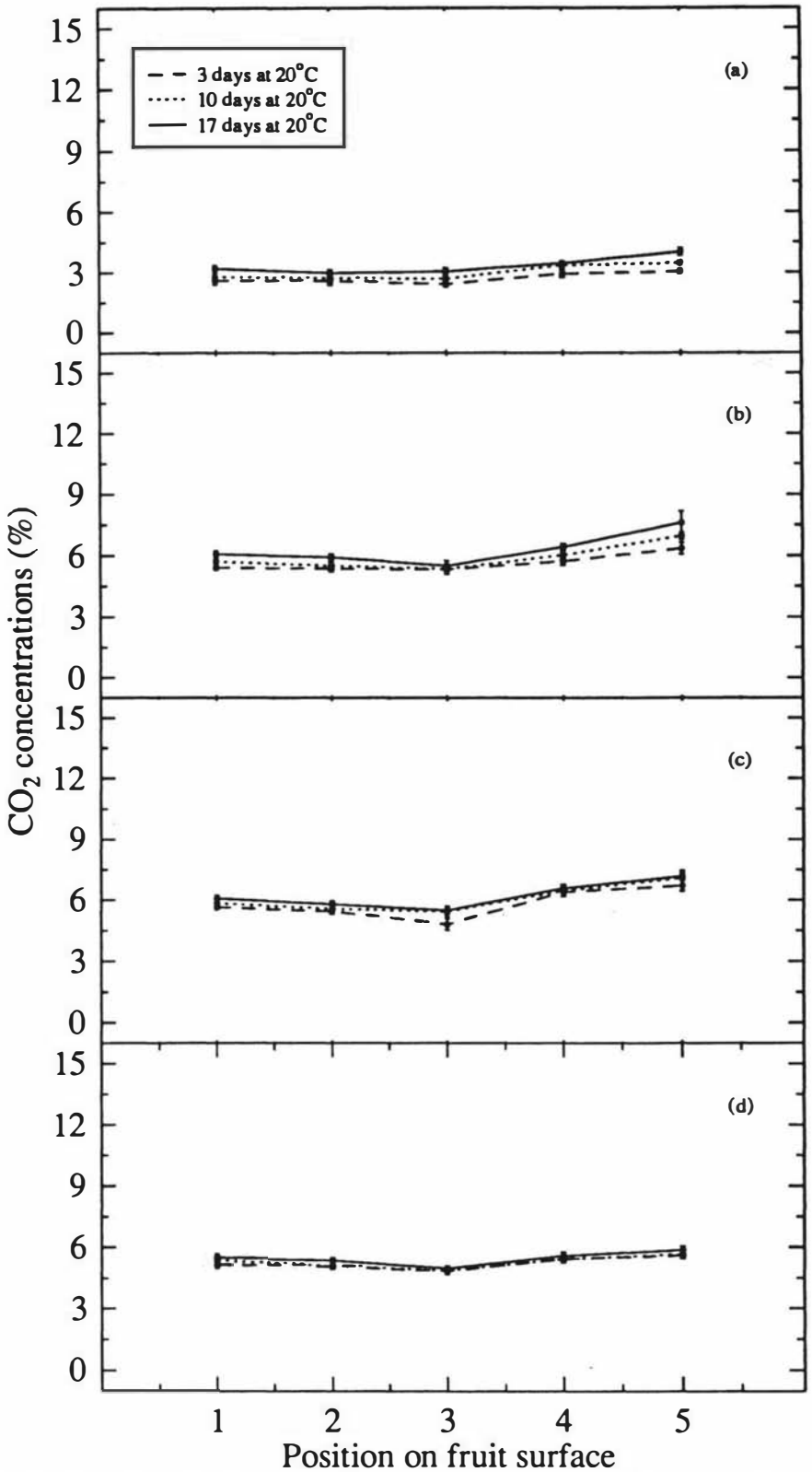

Fig. 8-24. Distribution of CO_2 concentrations at five positions in the sub-epidermis of Granny Smith apples stored (for a=0, b=4, c=8, d=12 weeks) at 0°C and subsequent transfer to 20°C. (for 3, 10 and 17 days). Vertical bars represent standard error of the means.

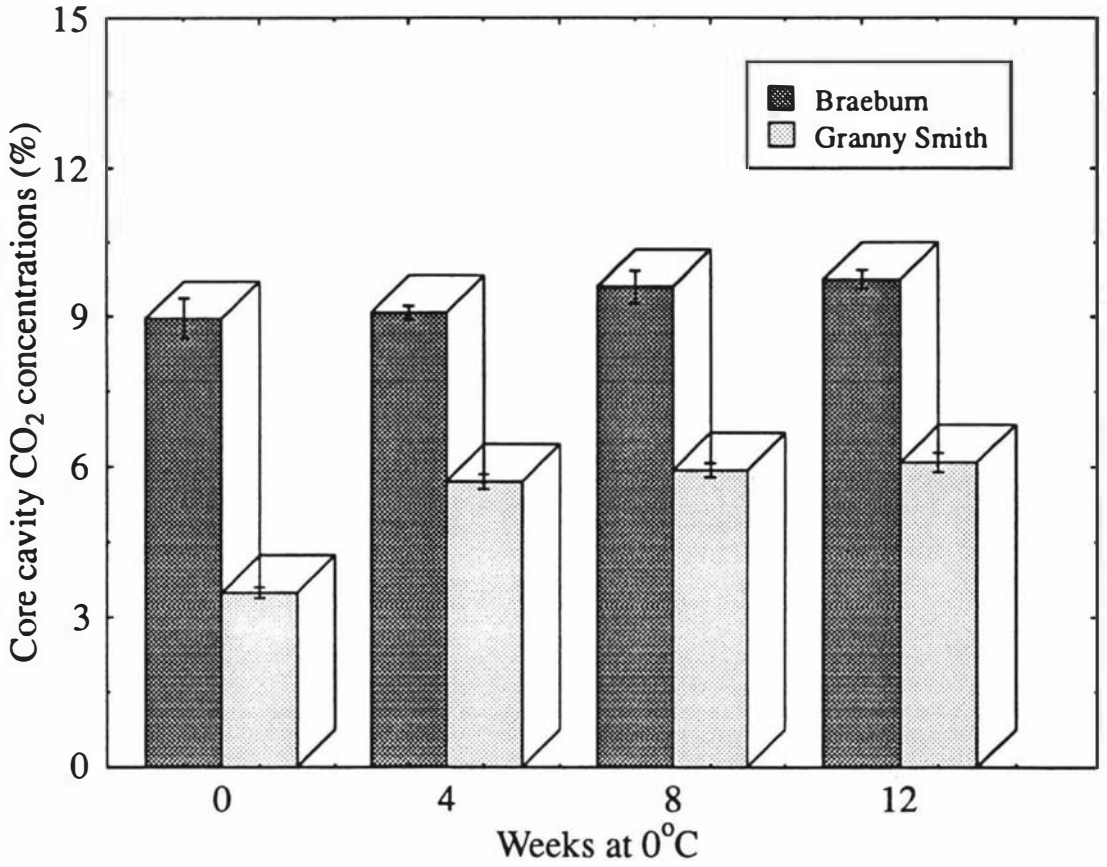

Fig. 8-25. Core cavity CO_2 concentrations of Braeburn and Granny Smith apples stored at 0°C for 0, 4, 8 and 12 weeks and subsequent storage at 20°C for 17 days. Vertical bars represent standard error of the means.

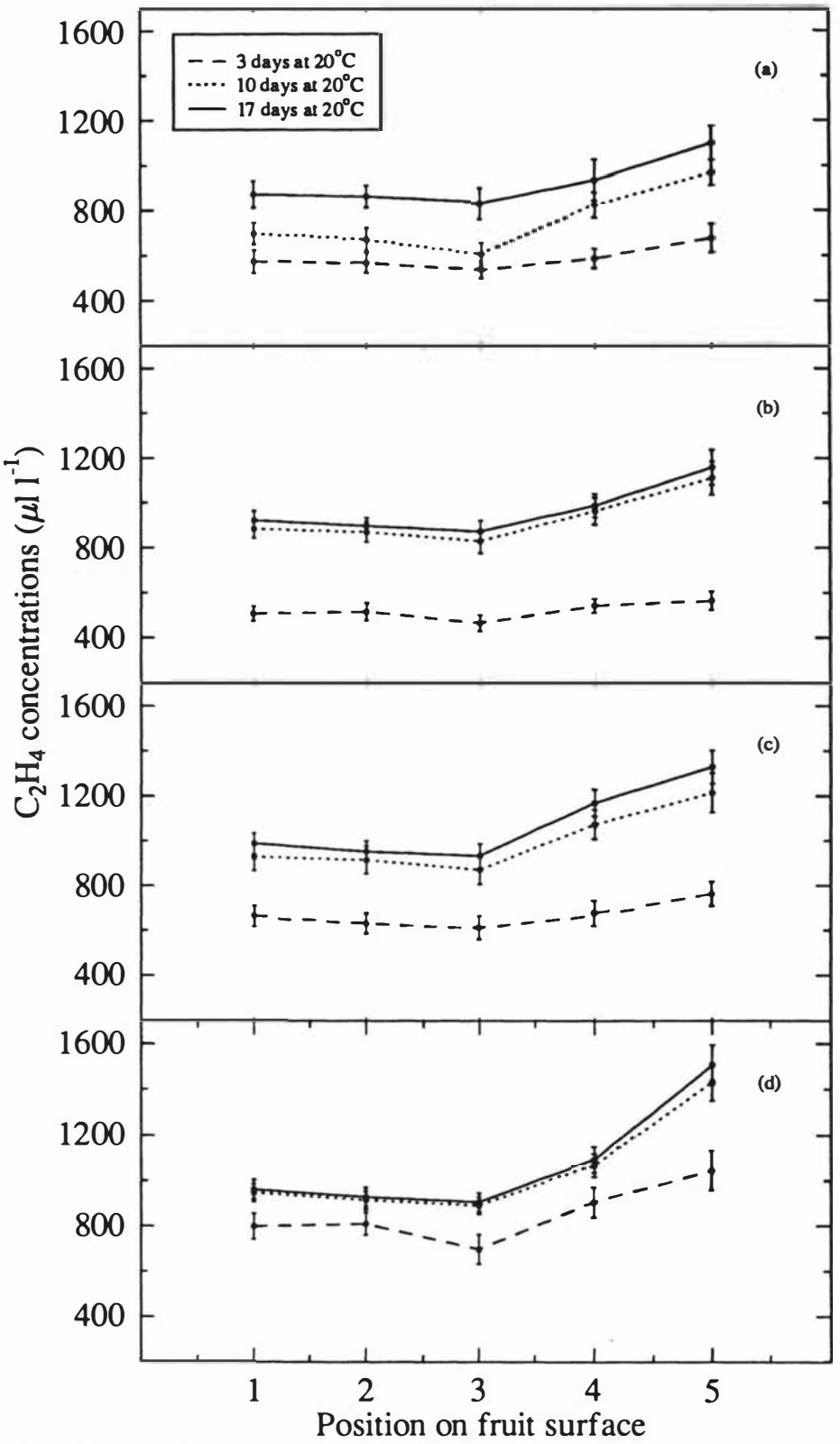

Fig. 8-26. Distribution of C_2H_4 concentrations at five positions in the sub-epidermis of Braeburn apples stored (for (a)=0, (b)=4, (c)=8, (d)=12 weeks) at 0°C and subsequent transfer to 20°C (for 3, 10 and 17 days). Vertical bars represent standard error of the means.

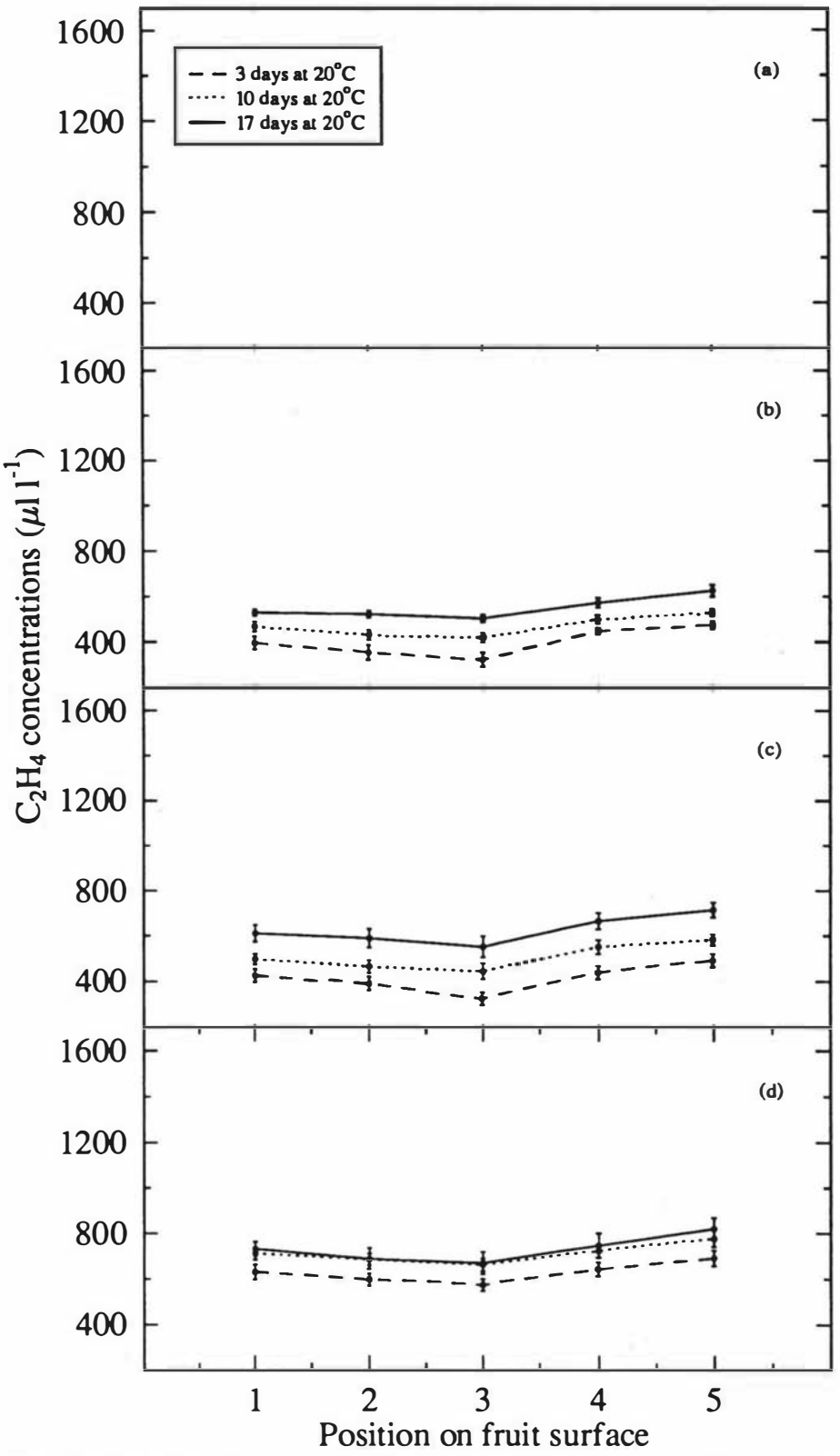

Fig. 8-27. Distribution of C_2H_4 concentrations at five positions in the sub-epidermis of Granny Smith apples stored (for (a)=0, (b)=4, (c)=8, (d)=12 weeks) at $0^{\circ}C$ and subsequent transfer to $20^{\circ}C$ (for 3, 10 and 17 days). Vertical bars represent standard error of the means.

After the 3rd and 10th day at 20°C (fig. 8-27a) C_2H_4 was not detected in any of the different positions on Granny Smith apples, however, by the 17th day, 2.5µl l^{-1} C_2H_4 was measured at position 5 compared to 0.38, 0.23, 0.12 and 0.48µl l^{-1} respectively at positions 1, 2, 3 and 4. After a period of storage at 0°C and at 20°C, C_2H_4 concentrations increased (over time at 20°C), with the highest concentrations measured at position 5. Ethylene concentrations at the various positions in the sub-epidermis in both cultivars increased during the period of storage at 20°C.

The mean C_2H_4 concentrations in the core cavity during the period of storage at 0°C and at 20°C were consistently higher in Braeburn than in Granny Smith apples (fig. 8-28). In Braeburn apples there were no significant differences in core cavity C_2H_4 concentrations over the period of storage at 0°C and subsequent transfer to 20°C for 17 days. In contrast in Granny Smith apples C_2H_4 concentrations increased after 0 weeks at 0°C and subsequent transfer to 20°C for 17 days and thereafter there were no significant differences in C_2H_4 concentrations.

8.4.2.4 Quality indices

During the period of storage for 0, 4, 8 and 12 at 0°C and subsequent storage at 20°C for 17 days, Braeburn apples were found to be firmer ($P < 0.05$) than Granny Smith apples. Fruit softening increased with increasing time of storage (fig. 8-29).

There were no significant ($P < 0.05$) effects of duration of storage on the soluble solids content of both cultivars (fig. 8-30).

As expected, Granny Smith were greener than Braeburn apples (fig. 8-31). In either cultivar, background colour as indicated by hue angle values were similar over the storage period.

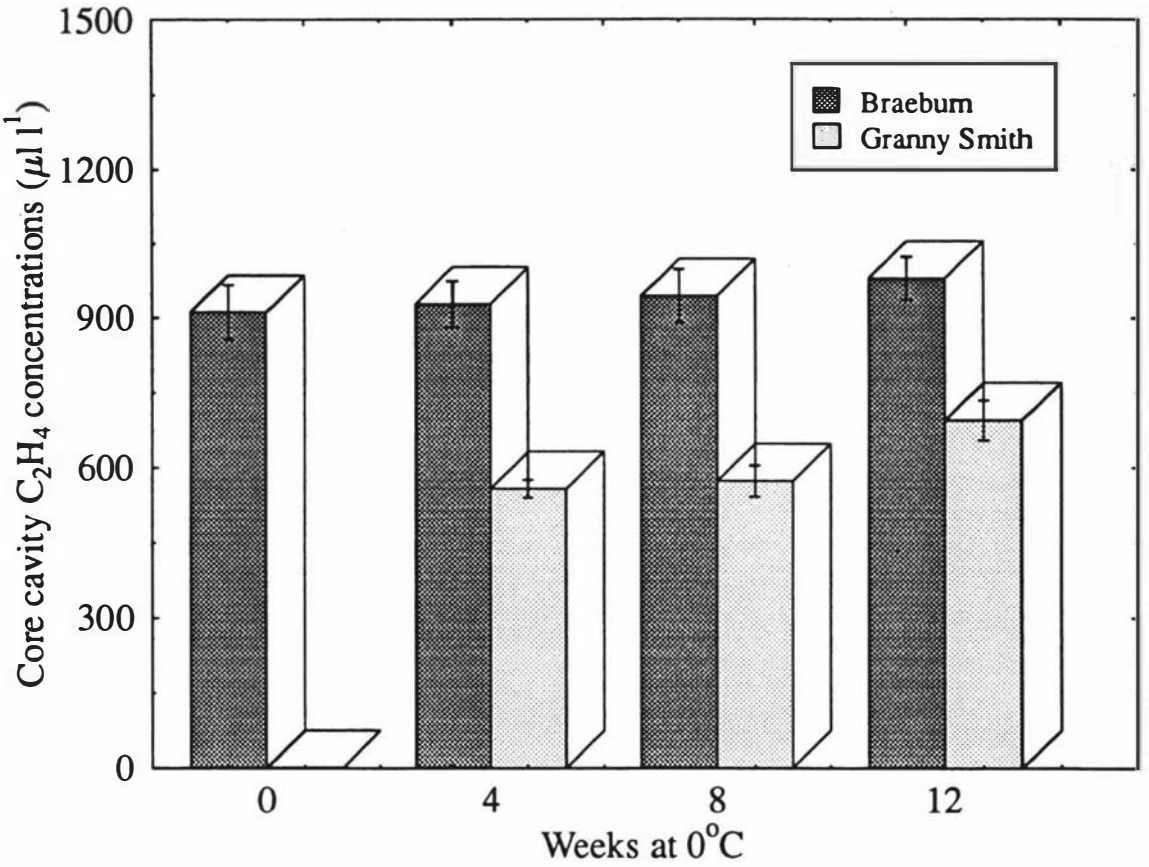

Fig. 8-28. Core cavity C_2H_4 concentrations of Braeburn and Granny Smith apples stored at 0°C for 0, 4, 8 and 12 weeks and subsequent storage at 20°C for 17 days. Vertical bars represent standard error of the means.

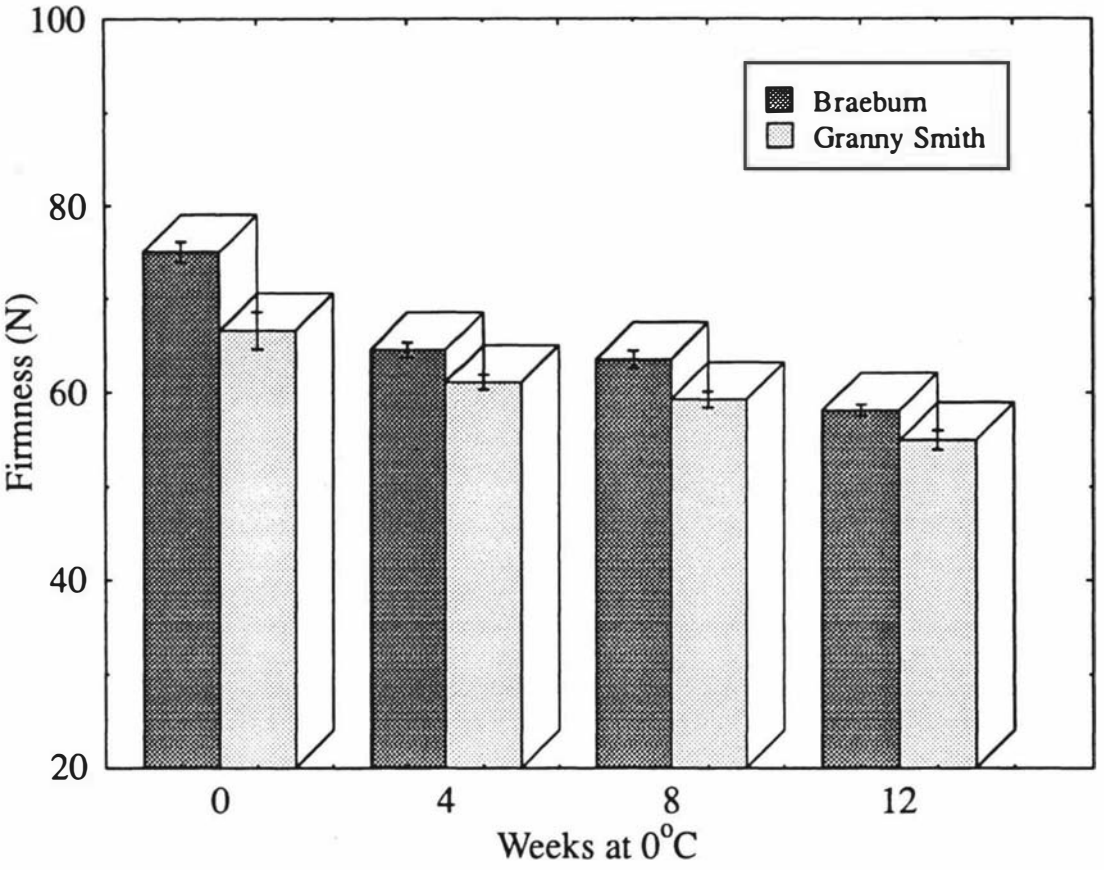

Fig. 8-29. Firmness of Braeburn and Granny Smith apples stored at 0°C for 0, 4, 8 and 12 weeks and subsequent storage at 20°C for 17 days. Vertical bars indicate standard error of the means.

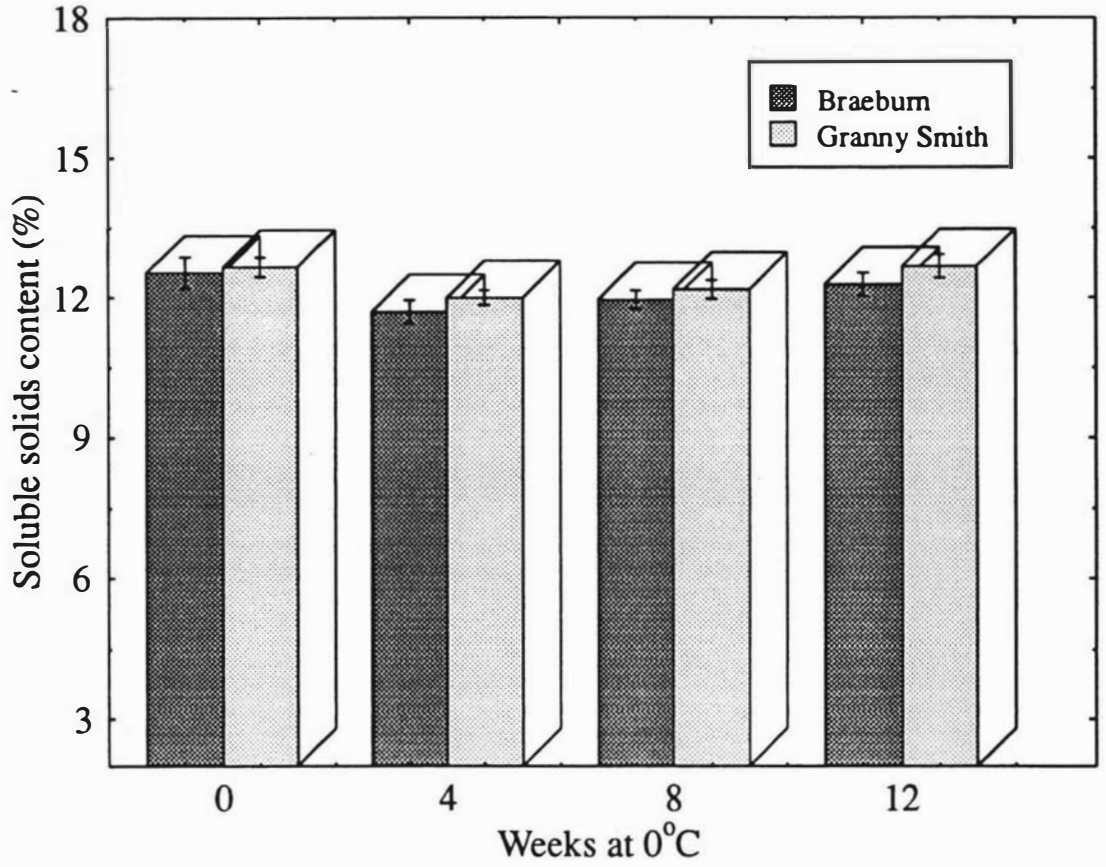

Fig. 8-30. Soluble solids content of Braeburn and Granny Smith apples stored at 0°C for 0, 4, 8 and 12 weeks and subsequent storage at 20°C for 17 days. Vertical bars indicate standard error the of means.

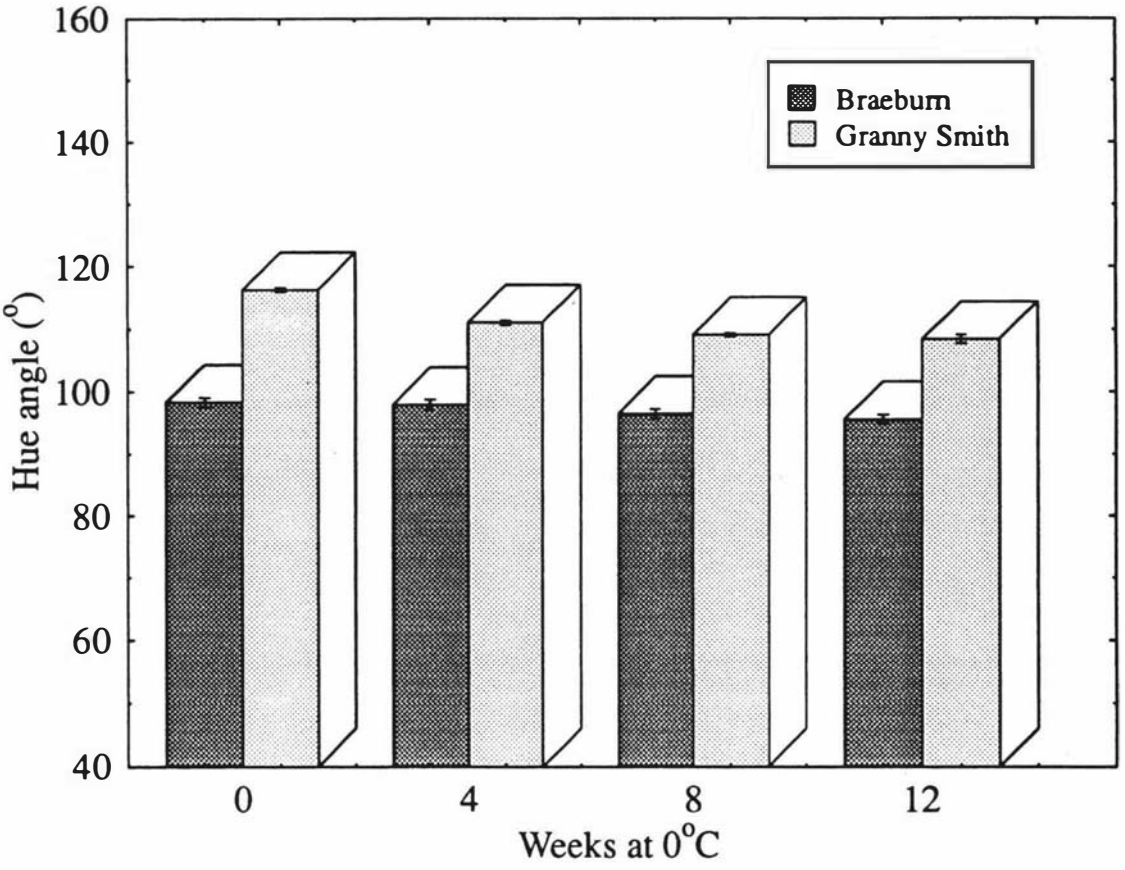

Fig. 8-31. Hue angle of Braeburn and Granny Smith apples stored at 0°C for 0, 4, 8 and 12 weeks and subsequent storage at 20°C for 17 days. Vertical bars indicate standard error of the means.

8.5 DISCUSSION

Steady state mean O_2 and CO_2 concentration differences between the equator and calyx end of Gala, Braeburn, Royal Gala, Red Delicious, and Cox's Orange Pippin apples were high considering that the average $[O_2]_i$ and $[CO_2]_i$ in a freshly harvested fruit at ambient temperature and O_2 condition is about 16-18% and 1-2% respectively. Under MA conditions these differences would be expected to be reduced owing to the depressed respiratory activity of the fruit but it appears as though they could still be very significant. One may argue that covering the calyx end may be the cause for the high gas concentration differences estimated, since it may be an important route to gas exchange. However data presented in fig. 8-6 in which the calyx end was not covered, showed similar but slightly smaller gas concentration differences between the equator and calyx end shoulder for Gala, Royal Gala, Braeburn, Red Delicious, and Cox's Orange Pippin apples. Fruit without vials over the calyx end had higher $[O_2]_i$ and lower $[CO_2]_i$ compared to those with vials over the calyx end. This suggests that the calyx end may be an important route to gas exchange in apples. This finding is consistent with those of other researchers including Cameron (1982), Cameron and Reid (1982), Marcellin (1974), and Markley and Sando (1931a, b) who showed that the calyx opening of apples contribute to gas exchange. For instance, Cameron (1982) observed that in Golden Delicious apples, the calyx end provided for the diffusion of 42% of the C_2H_4, 24% of the CO_2, and only 2% of the water.

Gas concentration differences between the equator and calyx end or between the equator and calyx end shoulder were much greater than those which have hitherto been measured between the core cavity and the fruit surface of other cultivars (Rajapakse *et al.* 1989, 1990). Anatomical studies of Golden Delicious apples by Soudain and Phan Phuc (1979) have shown that intercellular spaces are greatest at the equator than the other parts of the fruit hence easier gas diffusion through the tissues at the equator and consequently

smaller gas concentration difference.

Small but significant O_2 and CO_2 concentration differences were observed between the equator and core cavity of Braeburn and Cox's Orange Pippin apples and none in the other cultivars. These differences in gas concentration between apple cultivars could be associated with differences in intercellular space volume as well as differences in skin resistance and fruit respiration rate. These findings are in agreement with those published by Rajapakse *et al.* (1990).

Steady state mean CO_2 concentration differences estimated in all eight apple cultivars were lower than those for O_2. This could also be due partly to different pathways of diffusion of these gases i.e, unlike O_2 (which is thought to move via the lenticels) CO_2 may also diffuse through the cuticle and epidermis of the fruit (Banks, 1984b; Burton, 1974). The presence of these concentration differences confirms the existence of resistance to O_2 and CO_2 transfer through the flesh of the commodity. The data further show that the flesh of the apple fruit is more permeable to CO_2 than to O_2, despite the fact that the diffusivity of O_2 in air (0.178 $cm^2 . s^{-1}$ at 0°C) is higher than that of CO_2 (0.138 $cm^2 . s^{-1}$ at 0°C) (Burg and Burg, 1965).

The foregoing results clearly demonstrate that there are significant gas concentration differences between apple cultivars and the magnitude of these differences are cultivar dependent. This could have important implications for modified atmosphere storage of cultivars with relatively large flesh resistance at ambient temperatures. In determining the critical external O_2 levels in CA/MA storage of apples, the gas concentration difference (gradient) between the fruit surface or the calyx end and centre should be taken into consideration to avoid development of certain physiological disorders. At a low external O_2 concentration in CA/MA storage, tissues at the fruit centre would experience

lower O_2 concentrations than the surface and would therefore be likely to have lower rates of respiration and ripening. It would be interesting if further work could include investigation of MA effects on the size of these gradients.

Gas concentrations inside the apple fruit have previously been considered to be is practically homogeneous (Andrich *et al.*, 1989, 1991; Cameron, 1982; Hardy, 1949). The current study has demonstrated that the internal atmosphere composition of apples varied substantially from one part of the fruit to another. After storage at 0°C for 0, 4, 8 and 12 weeks and subsequent storage at 20°C for 3, 10 and 17 days tissues nearer the calyx end region of Braeburn and Granny Smith apples consistently had lower O_2 and higher CO_2 and C_2H_4 concentrations than the other parts of the fruit, while those at the equator had higher O_2 and lower CO_2 and C_2H_4 concentrations than any other positions on the fruit surface. This may be related to localised variation in intercellular space volume. As previously mentioned, studies have shown that intercellular space volume at the equatorial region was higher than the other parts of the apple fruit (Soudain and Phan Phuc, 1979). High porosity would therefore, be expected to facilitate gas diffusion.

The O_2 concentrations measured at the calyx end region at 20°C under normal ambient O_2 condition were low in these cultivars, thus under low-O_2 atmospheres it would be expected that the O_2 concentrations at this region would be extremely low or in some case tissues at the calyx end may even experience anaerobic conditions and this could trigger the development of certain physiological disorders such as browning, decay, or necrotic lesions in this region of the fruit. In a ~~studies~~ study by Nichols and Patterson (1987) they reported that after 8 weeks of storage of Delicious apples in 0% O_2, external symptoms of anaerobic injury (ie. sunken, brown, necrotic areas) were seen especially at the calyx end of fruit. Although the localisation of the symptoms at the calyx end of the fruit could be due to a number of factors (such as fungal

infection), the findings of this study indicate that the disorder could partly be associated with the low O_2 and/or high CO_2 concentrations at the calyx end of the fruit.

Braeburn consistently had higher CO_2 and C_2H_4, and lower O_2 concentrations at the different positions beneath the skin surface compared to Granny Smith apples. Anatomical differences such as differences in size of intercellular spaces; size, number and distribution of functional lenticels on the fruit surface; thickness and nature of wax deposits of the cuticle as well as differences in **R** and fruit respiration rate (refer to chapter 4 for details on this subject) may have contributed to these differences in gas concentration between the two cultivars. Decline in fruit porosity that occurs with ripening and senescence could also affect the size of the gas concentration (at the different positions). For instance, low porosity would increase the internal resistance to gas diffusion and therefore increase the gradient in gas concentration between the centre and outside of the fruit.

Braeburn apples were firmer and had less colour change during storage at 0°C and subsequent storage at 20°C than in Granny Smith apples. As previously reported in chapter 4, the firmness of Braeburn could be related to their low intercellular space volume (approximately 14.1%). The soluble solids contents of Braeburn and Granny Smith were similar.

In conclusion, MA treatments exert their effects on fruit physiology through modification of the fruit's internal atmosphere composition. Following the work of Burg and Burg (1965), fruit internal atmosphere composition has been treated as being essentially homogeneous. However, the current study provides firm evidence that the internal atmosphere composition of apples varies substantially from one part of the fruit to another and this has important implications for the way in which we attempt to model the gas exchange of these fruits and the effects of CA/MAs on their physiology. The heterogeneous

distribution of gases within individual fruit would presumably affect the tendency of individual tissues to develop low-O_2 or high CO_2 disorders, particularly for fruit stored in MAs at elevated temperatures. This study further indicates that development of gas concentration difference (gradients) may be related to anatomical features in addition to depth of tissues within the fruit. Further work could be undertaken to establish the link between anatomical variation and the varying tendency to develop large gradients in different cultivars.

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CHAPTER 9

GENERAL DISCUSSION

Postharvest deterioration of apples can be delayed by the use of low O_2 and/or high CO_2 atmospheres during CA/MA storage. The low $[O_2]_{ext}$ depress the $[O_2]_i$ of apples which in turn limit some of the physiological processes (such as respiration, C_2H_4 production, loss of green colour and softening) leading to deterioration (Blanke, 1991; Burton, 1982; Kader, 1986). The physiological consequences of low O_2 atmospheres are thought to arise from the general depression of metabolism and, in particular, the reduction of fruit respiration. The success of CA or MA therefore is largely determined by the commodity's respiratory response to low O_2 and/or high CO_2 and the ability of the produce to maintain essential physiological processes under these conditions without causing injuries to internal tissues.

Most fruits and vegetables which are stored under CA/MAs are bulky tissues. The movement of gases into the tissues from the external atmosphere, via the surface pores, into the internal atmosphere in the organ involves diffusion in the gaseous phase. This internal atmosphere, which occupies from about 1-2% of the internal volume in potato tubers and up to 30% in some varieties of apples (Blanke, 1991; Burton, 1982; Kader *et al.*, 1989), forms an inter-connecting system throughout the organ. The efficacy of the internal atmosphere in aerating the tissues depends upon its volume, its continuity and the degree to which it is filled with gas or injected with liquid or cell sap (associated with over-ripeness). The internal atmosphere to which the fruit tissues are directly exposed is effective in bringing about the reduced rate of deterioration seen in CA/MA-stored produce. Consequently factors which affect internal atmosphere composition are effective in regulating the rate of

deterioration and hence shelf-life of the fruit. However, most previous studies on fruit responses to CA/MA have related them to the composition of the external, rather than the internal atmosphere (eg. see Knee, 1980 in which rates of chlorophyll loss and softening are reported for different $[O_2]_{ext}$). In many cases, this may in part be due to the lack of appreciation of just how substantial an effect the gradient between internal and external atmospheres can have in the observed responses of the fruit to an imposed CA/MA. However, Knee (1991b) has recently considered in some detail the potential role of this gradient in explaining the variability of individual fruit to CA/MA regimes and pointed out that resolution of current uncertainty over $[O_2]_i$ in apples might improve the efficiency of CA storage. It is therefore perhaps the destructive nature of most methods of internal atmosphere sampling which has prevented this approach from being adopted before. This, in combination with the extra effort involved in internal, rather than external atmosphere monitoring may explain the absence of this type of study from existing literature.

In this study, a combination of a non-invasive technique for monitoring internal atmospheres and the use of fruit immersion (in water) in preventing contamination of direct removal samples ~~have~~ has enabled factors affecting internal atmospheres, and fruit responses to internal atmosphere modification, to be studied in some detail. This thesis has focused on $[O_2]_i$ (and $[CO_2]_i$ and $[C_2H_4]_i$) by studying the relationship between internal atmosphere composition of apples and factors such as respiration, $[O_2]_{ext}$, **R**, temperature and artificial barriers. In the preceding chapters these relationships have been examined and it is clear that these factors do influence the atmosphere inside the apple and hence have the potential to affect important aspects of quality such as rates of softening and loss of green colour.

The interactions of these variables in the final response of the fruit to a given treatment are quite complex. Fig. 9-1 draws together the individual relationships identified in this thesis in a schematic model, which demonstrates

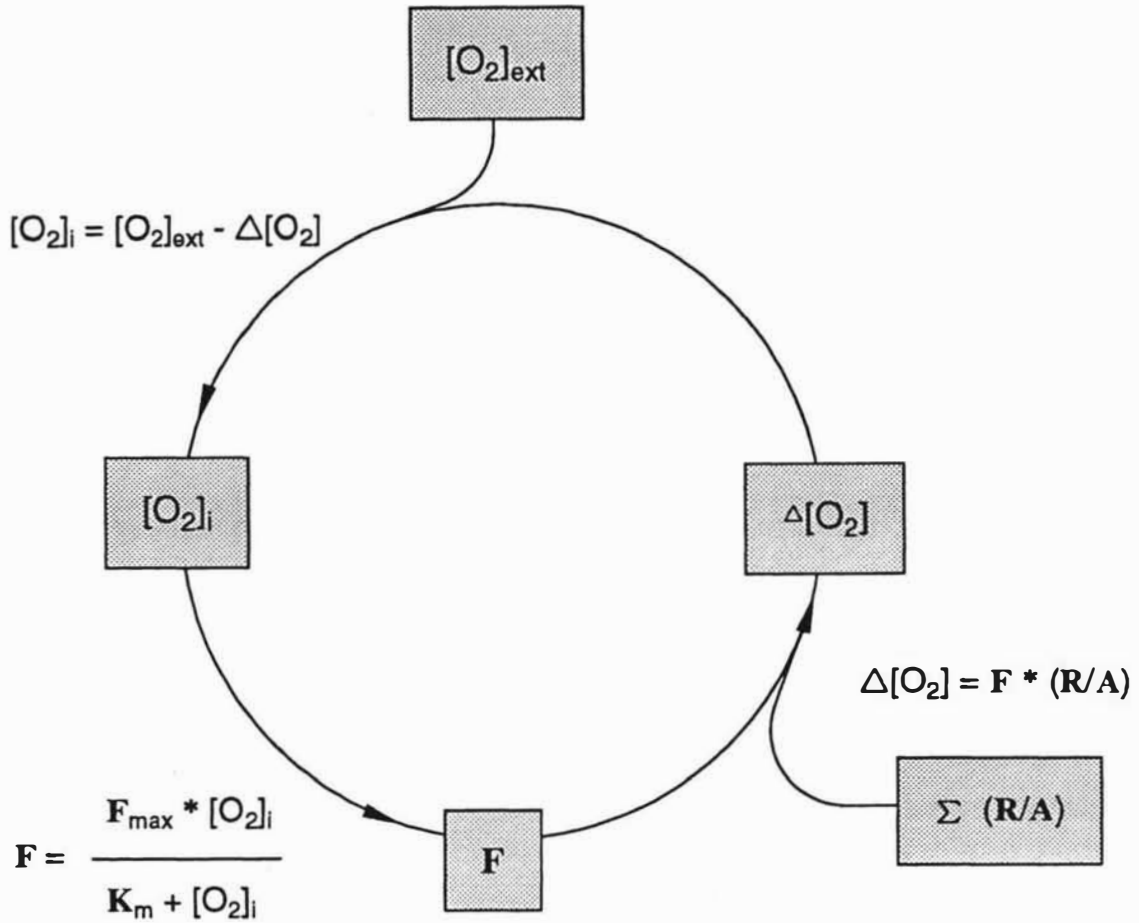

Fig. 9-1. Schematic model illustrating the relationships between $[O_2]_{ext}$, $[O_2]$, **F** or respiration rate, $\Delta[O_2]$ and **R**. **(R/A)** represent sum of all resistances/surface area between internal and external atmosphere.

the mutual interdependence of each of these variables. The model illustrates the sequence of causality involved in the system, based on a cycle of interactions between some of the key variables. At any given steady state, the rate at which O_2 is being consumed by respiration is the same as the rate at which O_2 is diffusing into the fruit (***F***); indeed, this is the way in which respiration is measured on whole fruit. Combined with the fruit's skin resistance to gas diffusion (**R**) and surface area (**A**) ie. (**R/A**), this inward flux results in the presence of a certain O_2 concentration gradient ($\Delta[O_2]$) ie.

$$\Delta[O_2] = \boldsymbol{F} * (\mathbf{R/A}) \qquad [9.1]$$

For this particular $\Delta[O_2]$ and the fruit's external atmosphere (ie. $[O_2]_{ext}$), this results in a certain internal O_2 concentration ($[O_2]_i$), ie.

$$[O_2]_i = [O_2]_{ext} - \Delta[O_2] \qquad [9.2]$$

The $[O_2]_i$ itself affects respiratory O_2 uptake (equivalent, at steady state, to ***F***), and can therefore be seen to be both cause and an effect in this system. The model makes the simplifying assumption that changes to any of the key variables are very gradual, so that at any given time, respiratory O_2 uptake can be treated as being equal to ***F***.

F is affected by a number of other factors, including temperature and stage of fruit development. Both of these operate through affecting the level of maximum flux or maximum respiration rate ($\boldsymbol{F}_{max}$): as temperature increases or the climacteric develops, so $\boldsymbol{F}_{max}$ becomes greater. The rate of respiration

itself is thus dependent on maturity at harvest and time and temperature since harvest. If F_{max} is increased, for example by increasing temperature, this would increase F:

$$F = \frac{F_{max} * [O_2]_i}{K_m + [O_2]_i} \qquad [5.2]$$

and thereby decrease $[O_2]_i$:

$$[O_2]_i = [O_2]_{ext} - (F * R/A) \qquad [9.3]$$

Let us now examine the effects of increasing **R**, as occurs through the development of greasiness or application of a coating for a fruit which remains in air throughout. This increases $\Delta[O_2]$ which therefore decreases $[O_2]_i$ and F. The decreased F results in a reduction in $\Delta[O_2]$, so that $[O_2]_i$ increases slightly. However after a few cycles through the system, a new steady state is reached in which the fruit with elevated **R** has a lower $[O_2]_i$ and respiration rate than before **R** was increased.

If $[O_2]_{ext}$ is gradually reduced over time, $[O_2]_i$ is reduced as a result of the difference between $[O_2]_{ext}$ and $\Delta[O_2]$ (equation 9.2). Following around the cycle in Fig. 9-1, this reduced $[O_2]_i$ depresses respiration rate (F) and therefore $\Delta[O_2]$. As $[O_2]_{ext}$ is reduced to its final level, the fruit develops a new steady state $[O_2]_i$, respiration rate and $\Delta[O_2]$, all of which are lower than the original values for the fruit in air.

Specific aspects of the model illustrating the relationship between $[O_2]_{ext}$ and $[O_2]_i$, respiration (ie. rate of O_2 uptake and CO_2 production) and

C_2H_4 concentration as well as the effects of coating or washing treatment on gas exchange characteristics of apples are discussed in detail in the subsequent pages.

9.1 Relationship between $[O_2]_{ext}$ and $[O_2]_i$, respiration and C_2H_4

9.1.1 Relationship between $[O_2]_{ext}$ and $[O_2]_i$

Oxygen concentrations between 1 and 5% have generally been used in CA storage of apples (Meheriuk, 1990; Knee, 1991b). These lowered $[O_2]_{ext}$ depress $[O_2]_i$ as has been outlined above. Characterisation of the relationship between $[O_2]_{ext}$ and $[O_2]_i$ of intact apples under different O_2 atmospheres has to date only been attempted with limited success (Brandle, 1968) presumably because of the difficulties (as outlined above; see also Knee, 1973) in obtaining internal gas samples from fruit. In a review article published by Knee (1991b), he indicated that, Pekmezci (1971) used an O_2 micro-electrode to measure cell-sap O_2 concentrations in fruit as the level in their external environment was reduced: when the data presented as partial pressures in mm Hg were recalculated in kPa they often exceeded the partial pressures external to the fruit. The source of this discrepancy may partly be due to the fact that the internal atmosphere of the fruit may not have equilibrated with the external atmosphere at the time of sampling. Knee (1973) indicated that analysis of gas extracted from apples under vacuum gave O_2 levels very close to those of the surrounding atmosphere, so that unless there was a large or significant $\Delta[O_2]$, the $[O_2]_i$ were in error.

The techniques used in this study resulted in reliable estimates of internal gas samples being obtained from individual apple fruit under different O_2 concentrations. Fruit were left for several times the period required for physical equilibration with their new atmosphere before samples were taken. These were carefully protected from atmospheric contamination by water

barriers at every conceivable problem point. A combination of Fick's law of diffusion and the Michaelis-Menten equation (equation [5.4]) developed to describe $[O_2]_i$ as a function of $[O_2]_{ext}$ indicated that at high $[O_2]_{ext}$, $[O_2]_i$ of Cox's Orange Pippin and Granny Smith apples declined linearly in response to decreasing $[O_2]_{ext}$ (fig. 5-2). This would be expected if respiration (and hence $\Delta[O_2]$) was not significantly affected over this range of O_2 concentrations: as $\Delta[O_2]$ remained constant, $[O_2]_i$ should decrease by the same amount as $[O_2]_{ext}$. However, as $[O_2]_{ext}$ became progressively more limiting there was a deviation from linearity which reflected the decreased $\Delta[O_2]$ caused by the lowered rate of O_2 uptake by the fruit. The magnitude of deviation from linearity and $\Delta[O_2]$ was cultivar dependent. For instance, at 2% $[O_2]_{ext}$ the fitted line indicated that $\Delta[O_2]$ for Cox's Orange Pippin and Granny Smith apples was 1.6 and 1.0% respectively. The difference could be related to differences in respiration rate (higher in Cox's Orange Pippin than in Granny Smith apples). The high respiration rate in Cox's Orange Pippin would result in greater utilisation of O_2 and hence higher $\Delta[O_2]$ than in Granny Smith apples. This appears to have been more important than the difference in **R** values (only 10%) between the two cultivars which would have had the opposite effect on $\Delta[O_2]$.

With the exception of the fruit of Braeburn apples which had respiration rates in the range of about 8.5 and 11.5 cm^3 CO_2 kg^{-1} h^{-1} and RC_2H_6 ranging from 15,000 and 45,000 s cm^{-1}, most of the fruit of the other cultivars (put together) had respiration rates within the range of about 7.5 and 20 cm^3 CO_2 kg^{-1} h^{-1} and RC_2H_6 between approximately 3,000 and 12,000 s cm^{-1} (fig. 4-7). It is clear that there was a wide range of values for both of these variables within each apple cultivar used in this study. Similarly, Knee (1991b) reported data from a survey on Cox apples from different orchards, that all possible combinations of high and low respiration and high and low skin resistance occurred. This variability gave rise to nearly a threefold variation in calculated $[O_2]_i$ values for the fruit in his study. The multiple effects of these

gas exchange variables on internal atmosphere composition (especially on $[O_2]_i$) could be partly responsible for differences in the response of different cultivars of apples to CA/MA storage (Knee, 1991b). Variation in the gas exchange variables within and between apple cultivars implies that there will not be a single critical O_2 level even for a single variety of apple. For instance, Braeburn had three-fold higher **R** and only about 10% lower respiration rates than Royal Gala apples, so that when these two cultivars are stored under similar O_2 atmospheres such as 2%, it would be expected that Braeburn apples would have higher $\Delta[O_2]$ and lower $[O_2]_i$ and hence greater probability of fruit becoming anaerobic compared to the other cultivar.

In view of the variation in the gas exchange variables between and within cultivars, atmospheres recommended for commercial use are generally designed to avoid hypoxic or anoxic conditions and harmful levels of CO_2 in the centre of fruit. These are typically between 1-3% O_2 and <1-3% CO_2 for fruit stored at low temperatures (0-5°C; Meheriuk, 1990). Recommended O_2 levels have become lower and lower as the technology for controlling atmospheric composition and our understanding of fruit tolerance to atmospheric modification have improved. Further improvements in slowing deterioration through CA may depend upon identification of optimum atmosphere composition on a batch by batch basis. This would have to take into account the gas exchange variables discussed above. Clearly, any commercially viable system would also be subject to other, operational constraints involved in the loading of store loads of fruit.

9.1.2 Relationship between respiration and O_2 concentration

9.1.2.1 Rate of O_2 uptake

Respiration rate (or rate of O_2 uptake) of apples and indeed several plant organs is lower in low O_2 atmospheres than in air (Fidler and North, 1967) and is stimulated by O_2 concentrations greater than that in air (Mapson

and Burton, 1962; Theologis and Laties, 1978, 1982; Tucker and Laties, 1985). The relationship between respiration and $[O_2]_{ext}$ has been documented (Andrich *et al.*, 1991; Cameron, 1985; Solomos, 1985, 1988; Tucker and Laties, 1985) but characterisation of the relationship with $[O_2]_i$ in intact fruit has only been attempted indirectly through mathematical models (Andrich *et al.*, 1989, 1991; Banks *et al.*, 1989; Cameron *et al.*, 1989; Chevillotte, 1973; Solomos, 1985, 1988; Tucker and Laties, 1985) presumably because of the difficulties involved with concurrent monitoring of $[O_2]_i$ and respiration rate in different $[O_2]_{ext}$. The techniques used in this study to overcome these difficulties (as outlined above) enabled the relationship between rate of O_2 uptake (ie. ***Rel***O_2) and $[O_2]_i$ or $[O_2]_{ext}$ to be characterised. This involved studying the variation in the magnitude of O_2 concentration differences between the internal and external atmospheres ($\Delta[O_2]$) of individual apples maintained in different O_2 atmospheres. The relationship was hyperbolic and appeared to conform at least approximately to the Michaelis-Menten kinetics (figs. 5-3 and 5-4). A small change in O_2 concentration (ie. $[O_2]_i$ or $[O_2]_{ext}$) at low concentration therefore has a greater effect on ***Rel***O_2 than the same change at higher concentrations. Reductions in the rate of deterioration associated with depressed respiration would therefore be expected to be proportionally much greater as $[O_2]_i$ is depressed further and further, as the depression in respiration rate per unit $[O_2]_i$ decrease is much greater at low O_2 levels.

It is, however, not clear whether the gaseous environment affects respiration directly or whether it inhibits other metabolic processes or enzymes whose energy demands affect respiratory rates. Various suggestions have been put forward by a number of investigators. For instance, Forward (1965) suggested three alternative scenarios. In one, respiratory O_2 uptake was thought to be entirely catalysed by cytochrome oxidase, but diffusion of O_2 through bulky plant tissues was slow, and the actual concentration of O_2 at the site of the enzyme activity was very much lower than that in the external

atmosphere. This led to lowering of the enzyme's rate of O_2 uptake. In the second scheme, she indicated that, a substantial part of respiration was not mediated by cytochrome oxidase, but rather by terminal oxidases with a much lower affinity for O_2 (than that of cytochrome oxidase). In the third scheme, she indicated that, in the intact plant organ, rate of respiration was limited by O_2-dependent steps other than the final steps in the transfer of electrons to O_2.

In line with Forward's second scheme, Solomos' (1982) view was that the decrease in respiration rate in response to decreased O_2 concentration was not the result of depression of the basal metabolism mediated by cytochrome oxidase. Rather, it was thought that the reduction stems from the diminution of the activity of other oxidases (such as polyphenol oxidase, ascorbic acid oxidase and glycolic acid oxidase) whose affinity for O_2 may be five to six times lower than that of cytochrome oxidase. On the other hand, Knee (1991b), suggested that the effects of O_2 on fruit respiration does not involve direct action on the terminal oxidase, but that there is some other site of action of O_2 which indirectly affects respiratory rate. This site of action might be an O_2 consuming step in secondary metabolism involved in fruit ripening; inhibition of such an oxygenase could restrict the rate of metabolism and hence reduce the demand for respiratory energy. Alternatively, Knee (1991b) further suggested that there may be mechanisms whereby fruit cells sense hypoxic conditions and limit respiratory rate so as to minimise the production of toxic metabolites under anticipated anoxia.

In this study, the O_2 concentration at which $Rel O_2$ was half maximal (ie. 50% inhibition) was 7.5% for $[O_2]_{ext}$ and 2.5% for $[O_2]_i$. Such levels of O_2 are not expected to inhibit cytochrome oxidase because the K_m of cytochrome oxidase is < 0.1% O_2 in the gas phase (Butt, 1991; Burton, 1978; Knee, 1991b). Therefore, the restriction (or 50% inhibition) of respiration at relatively high O_2 concentrations was presumably due to the curtailment of other O_2

requiring processes or other 'enzyme(s)' (such as ascorbic oxidase and glycolic acid oxidase) which may be directly or indirectly involved in the respiratory machinery and the O_2 affinities of which are much lower than that of cytochrome oxidase (Burton, 1982; Butt, 1991). It may be that, through one of these oxidases, glycolytic flux is more directly affected than O_2 uptake, as suggested by Tucker and Laties (1985).

The differences in the shapes of the curves in the relationship between ***Rel***O_2 and $[O_2]_{ext}$ (fig. 5-3) or $[O_2]_i$ (fig. 5-4) as well as the difference between the $[O_2]_i$ and $[O_2]_{ext}$ values at which ***Rel***O_2 is half maximal, clearly illustrate the advantages of considering fruit physiological responses to CA/MAs in terms of their internal atmosphere composition rather than the external atmosphere to which they are exposed. The presence of the skin, with its finite resistance to gas diffusion, means that there must always be an O_2 concentration difference between the fruit's internal and external atmospheres (equation [5.1]). The magnitude of this difference would depend largely upon the fruit's rate of respiration and the value of **R**. The greater the resistance, the greater the difference for any given rate of respiration. Variation in **R** coupled with respiration rate therefore determines the $[O_2]_i$ for a given $[O_2]_{ext}$ and so it affects the shape of the relationship between ***Rel***O_2 and $[O_2]_{ext}$. High **R** would result in a decrease in $[O_2]_i$ for a given rate of respiration and hence high $[O_2]_{ext}$ required for half maximal activity. On the same basis, temperature (through its effect on respiration) might be expected to affect the measured K_m for respiration versus $[O_2]_{ext}$. Elevated temperatures would increase respiration rate which, in turn, would depress $[O_2]_i$ for a given $[O_2]_{ext}$ and therefore increase the $[O_2]_{ext}$ required for half maximal activity.

Skin resistance characteristics therefore determine the fruit's respiratory response to CA/MA. They also dictate the lower limit of $[O_2]_{ext}$ which can be tolerated by the fruit since they control the gradient which exists between the internal atmosphere of the tissue and its external environment for a given fruit

respiration rate. In a similar way, the decline in fruit porosity that occurs with ripening and senescence may affect the outcome of CA/MA treatments. Low porosity would increase the internal resistance to gas diffusion and therefore increase the gradient in gas concentration between the centre and outside of the fruit.

There was a marked depression of $[O_2]_i$ with corresponding elevation in $[CO_2]_i$ of Granny Smith apples 24h after fruit were transferred from 0°C to 20°C (this effect was more pronounced in coated fruit than in the controls; chapter 6). However, after the initial period of depression of $[O_2]_i$ and corresponding increase in $[CO_2]_i$, fruit recovered (or established a new physiological equilibrium at the new temperature), with an increase in $[O_2]_i$ and decrease in $[CO_2]_i$. Changes in internal atmosphere composition presumably reflect changes in respiration. A similar observation was made by Blackman (1954), who indicated that when apples were transferred from 2 to 22°C there was an 'overshoot' in respiration rate before attainment of a new equilibrium with the new temperature. Jobling *et al.* (1991) reported that the $[CO_2]_i$ in Granny Smith apples showed a similar 'overshoot' phenomenon which increased in intensity with increased duration of exposure to low temperatures up to 32 days. In the current study some of the apples had been in cool storage (0°C) for much longer periods (0, 4, 8, 16 and 24 weeks) before transfer to 20°C. The magnitude of the 'overshoot' at 20°C appeared to increase with longer periods in cool storage, consistent with the data presented by Jobling *et al.* (1991).

The effect of this 'overshoot' in respiration rate on $[O_2]_i$ would be expected to be greater for coated fruit (than the controls) because a greater concentration difference between the internal and external atmospheres ($\Delta[O_2]$) would be generated (in coated fruit than the controls) for a given respiration rate. The subsequent reduction in these concentration differences might simply reflect the subsidence of respiration rate as fruit equilibrate to the

new temperature. Alternatively, for coated fruit, this response could also reflect the delayed physiological equilibration of respiration rate to the lowered O_2 status in the tissue. A similar phenomenon was noted by Knee (1991b) who observed that the delay between placing fruit in a reduced O_2 atmosphere and observing a reduction in respiration rate (CO_2 production) was about 4 days, longer than the time required for physical equilibration of O_2 concentrations inside and outside the fruit. This contrasts with the findings of Tucker and Laties (1985) who reported that with avocados only 12h was required for re-establishment of a constant respiration rate after a change in O_2 concentration. The delays involved in development of a new physiological steady state seen in these tissues presumably represent the various times required for substrates and enzyme activities to reach new steady levels.

In terms of the model presented in fig. 9-1, the 'overshoot' in respiration rate in response to temperature increase would be due to an increase in $\boldsymbol{F}_{max}$ which, at a given $[O_2]_i$ would result in an increase in ***F***. Combined with **R**, this would augment $\Delta[O_2]$ and decrease $[O_2]_i$ and ***F***. After a few cycles through the system a new steady state would be reached in which ***F*** and $\Delta[O_2]$ would be higher and $[O_2]_i$ lower than before the fruit was warmed. This effect would be exaggerated in the presence of coating due to the higher value of **R/A** in coated fruit.

9.1.2.2 Rate of CO_2 production

When expressing CO_2 evolution as a function of O_2 concentration, most fruits including apples exhibit a classic respiratory response and Pasteur effect (ie. the acceleration of sugar utilisation in respiration under conditions of low O_2; Boersig *et al.* 1988; Turner, 1951). The relationship between the rate of CO_2 production and $[O_2]_{ext}$ has been characterised by various researchers including Boersig *et al.* (1988), Cameron (1985), Kader (1987) and Leshuk and Saltveit (1990) and involves two physiological processes, anaerobic and

aerobic respiration. However, combining mathematical equations to describe the two physiological processes has not been reported. Cameron (1985) attempted to fit a model describing only the aerobic phase of the process. In this study a mathematical model (equation [5.8]) developed to describe the relationship between ***Rel***CO_2 or $[CO_2]_i$ and $[O_2]_{ext}$ or $[O_2]_i$ had two components, each describing one of the two physiological processes.

Oxygen concentrations below 2 - 5% (Biale, 1969) or below 5% (Tucker and Laties, 1985) may cause a switch from aerobic to anaerobic processes (such as fermentation and alcohol formation) which reverse the reduction in CO_2 production and can raise its concentration in affected fruit to levels above those for the same fruit kept in air (Blanke, 1991; Burton, 1982). In recent years, the use of low O_2 atmospheres as a potential quarantine treatment for insect control has renewed interest in anaerobic respiration of horticultural crops (Boersig *et al.*, 1988; Ke *et al.*, 1991; Yahia *et al.*, 1991, 1992). Of special interest is the anaerobic compensation point (ACP), which is defined as the O_2 concentration at which CO_2 production was minimum (Boersig *et al.*, 1988). An attempt was therefore made to estimate the anaerobic compensation point (ACP) for both $[O_2]_i$ ($[ACP]_i$, 0.3%) and $[O_2]_{ext}$ ($[ACP]_{ext}$, 0.5%) in both Cox's Orange Pippin apples and Granny Smith apples.

An increased density of data points in the lower quadrant of the graphs could have assisted in the accurate estimation of ACP, nevertheless extrapolating the curves shown in figs. 5-5 and 5-7 to the x-axis provided estimates of the point of interception of the two processes. In fact these plots make it clear that anaerobic respiration actually begins at an $[O_2]_{ext}$ somewhat higher than the ACP. It is below this point (recently termed the 'RQ breakpoint' by A. C. Cameron) that RQ would be expected to rise from the plateau level observed at higher O_2 concentrations. The steepness of the decline in anaerobic respiration versus O_2 concentration clearly determines the RQ breakpoint (ie. the $[O_2]_{ext}$ at which all anaerobic respiration is extinguished).

A less steep slope might suggest that there was significant anaerobic respiration at O_2 concentrations considerably higher than the $[ACP]_{ext}$. This might have accounted for the gradual decline in relative respiratory quotient (*Rel*RQ) seen in Cox's Orange Pippin and Granny Smith apples (figs. 5-9 and 5-10) over O_2 concentrations ranging from 0 to 10%: these fruit apparently had a high RQ breakpoint.

Even if $[ACP]_i$ is unaffected by temperature, there would still be large effects of temperature and **R** on $[ACP]_{ext}$. High temperature (through its effects on fruit respiration rate), coupled with high **R**, would lead to high $\Delta[O_2]$ and hence low $[O_2]_i$ (Fig. 9-1). If $[ACP]_i$ remains the same, $[ACP]_{ext}$ would be higher at high than at low temperature, since:

$$[ACP]_{ext} = [ACP]_i + \Delta[O_2] \qquad [9.4]$$

On this basis, a Braeburn apple kept at 25°C would be expected to have a much higher $[ACP]_{ext}$ than a Royal Gala apple kept at 0°C (because of the high **R** of Braeburn and the increase in respiration at the high temperature). An optimised MA package maintains fruit in an $[O_2]_{ext}$ just above $[ACP]_{ext}$ (ie. it minimises respiration rate without inducing anaerobic respiration). Design of such a package should therefore take into account the temperature at which the packages will be kept as well as inherent cultivar differences in respiration rate and **R**. In this study, estimates of $[ACP]_i$ and $[ACP]_{ext}$ were obtained for Cox's Orange Pippin and Granny Smith apples and their respiration rates and **R** values would not lead us to expect a substantial difference in $[ACP]_i$ and $[ACP]_{ext}$. However, it would be interesting to investigate cultivars with very different values for these variables to establish whether or not this prediction is correct.

9.1.3 Relationship between $[O_2]_{ext}$ and C_2H_4

Ethylene production in fruits is known to be dependent upon O_2 concentration (Adams and Yang, 1979; Blanpied, 1991; Yang, 1985, 1987; Yang and Hoffman, 1984), and inhibition of both C_2H_4 production and respiration by low O_2 atmospheres is an important component in the success of CA storage of fruits including apples. Most investigators have looked at the impact of $[O_2]_{ext}$ rather than $[O_2]_i$ on C_2H_4 production and respiration. However, it is the O_2 inside the fruit that is the most direct cause of inhibition of both C_2H_4 production and respiration rather than $[O_2]_{ext}$ on which $[O_2]_i$ is dependent. In this study, the relationship between ***Rel***C_2H_4 or $[C_2H_4]_i$ and $[O_2]_i$ (figs. 5-17 and 5-18) was more closely described by an exponential than a Michaelis-Menten type hyperbolic curve. Nevertheless, the overall shape of the relationship conforms to the expectation that small changes in O_2 concentration have much greater effect at low $[O_2]_i$ than they do at high $[O_2]_i$. The presence of diffusion barriers (such as skin and flesh) resulted in development of an apparent 'lag phase' in the relationship between ***Rel***C_2H_4 or $[C_2H_4]_i$ and $[O_2]_{ext}$ such that it was no longer described by an exponential type equation and became essentially sigmoidal (figs. 5-15 and 5-16). Some physiological corollaries of these observations are discussed below.

Comparison of the relationships between ***Rel***C_2H_4 (fig. 5-15), on the one hand, and ***Rel***O_2 (fig. 5-3) on the other hand, against $[O_2]_{ext}$ provides indirect information on the nature of these two processes. If the O_2 affinities of these two processes (ie. respiration and C_2H_4 production) were similar then they should yield similar response curves. The difference between the shapes of the two curves indicates that two different enzyme systems may be involved, presumably with differing affinities for O_2. It also gives some indication of the relative contributions each makes to the O_2 consumption of the fruit tissues.

Assuming that an apple fruit produces 100µl kg^{-1} h^{-1} of C_2H_4 (ie. 0.1ml kg^{-1} h^{-1}) and that for every mole of C_2H_4 produced, one mole of O_2 is consumed, then O_2 consumption due to C_2H_4 production would only be 0.1ml kg^{-1} h^{-1}. If in the same fruit O_2 uptake by respiration is 10ml kg^{-1} h^{-1} then O_2 uptake by respiration is 100 times greater than O_2 uptake by C_2H_4 production. Thus respiration is the major O_2 consuming process in the fruit tissue and C_2H_4 production is only a minor consumer. The major O_2 consuming process would not be expected to have the 'lag phase' seen in fig. 5-15, as reductions in the availability of O_2 within the fruit tissue would be expected to result in proportionate reductions in the rate of uptake by that process. Thus, the major O_2 consuming process should follow approximately Michaelis-Menten type kinetics with regard to both $[O_2]_i$ and $[O_2]_{ext}$. In contrast, minor consumers of O_2 might show a 'lag phase' in their relationship with $[O_2]_{ext}$ which would be related to the relative affinities of the different processes involved for O_2 and would be a reflection of their abilities to compete for the available O_2. High O_2 affinity processes would have a much less pronounced or non-existent 'lag phase' compared to those with a low O_2 affinity. These data therefore indicate that C_2H_4 production (compared to respiration) is only a minor consumer of O_2 in apple fruit tissue and that the process has an O_2 affinity considerably less than that of respiration.

This observation appears to be at odds with the estimates of K_m values for these processes which were higher for ***Rel***O_2 than ***Rel***C_2H_4 (2.5% and 1.5% $[O_2]_i$ respectively, averaged across both cultivars). However, this can be explained by the third model of respiratory control by low O_2 proposed by Forward (1965): rate of respiration is limited by O_2-dependent steps other than the final steps in the transfer of electrons to O_2. Thus, the high affinity cytochrome oxidase is directly responsible for uptake of O_2 by respiration and therefore is efficient at competing for available O_2. However, the overall rate of respiration is moderated by some other O_2 consuming process which gives the overall process of respiration a lower apparent affinity than observed for

***Rel*C_2H_4**. This effect would become exaggerated if there were significant gradients in O_2 availability between the intercellular spaces and the sites of O_2 consumption within the tissues (Forward's first model). Clearly, further biochemical work would be required to reach any firm conclusions regarding the mechanisms involved in these effects.

The other two factors which could affect the degree of the lag seen here for ***Rel*C_2H_4** versus $[O_2]_{ext}$ have already been mentioned: ie. **R** and respiration rate. **R** varies considerably not only between cultivars but also between individual apples within a cultivar (chapter 4). **R** can also change during storage as a result of developing greasiness (chapter 6) or ripening at different humidities (Wilkinson, 1965). These natural sources of variation in **R** could lead to large differences in the size of the O_2 concentration gradient between the internal and external atmospheres and therefore in the degree to which this lag phase might be expected to develop. In addition, **R** may be augmented intentionally through the application of coating materials (chapter 6). All of these factors could change the shape of the relationship between ***Rel*C_2H_4** or $[C_2H_4]_i$ and $[O_2]_{ext}$.

Variation in respiration rate, particularly in the presence of high **R**, would also affect the form of this relationship. All of those processes which augment respiration rate (eg. elevated temperature and continued fruit development (climacteric)) would emphasise the lag phase; the converse would also follow. Keeping fruit at low temperatures would reduce the degree to which this lag would be seen in cool storage. Transferring artificially coated fruit from cool storage to ambient temperature (chapter 6) during that period in which the fruit's respiration rate only gradually equilibrates with the depressed O_2 levels which develop within it, would therefore be expected to provide an extreme example of the lag phase. Modified atmospheres would affect each of the ripening processes to an extent which would reflect its affinity for O_2 and the

internal O_2 levels: a given $[O_2]_{ext}$ could have quite different effects on one process for fruit held at different temperatures or with different values of **R** (chapters 4 and 7).

9.2 Effects of washing or coating on gas exchange

Tween 20 coating increased **R** of Granny Smith apples. This resulted in an increase in gas concentration gradient between the inside and outside of the fruit and a decrease in $[O_2]_i$ (and an increase in $[CO_2]_i$). These effects would in turn have depressed the rate of respiration and hence caused a decline in O_2 uptake (fig. 9-2).

Depression of $[O_2]_i$ also results in inhibition of other O_2 dependent (and CO_2 inhibited) processes and the depression in respiration itself results in inhibition of respiration dependent processes (fig. 9-2). Similar observations have been made for the coating of bananas (Banks, 1984a, b) and pears (Meheriuk and Lau, 1988; Meheriuk and Sholberg, 1990), waxing of bananas (Ben-Yehoshua, 1966) and avocados (Durand *et al.* 1984) and also for various coatings on apples (Smith *et al.*, 1987). Banks (1984b) suggested that since coating involves the application of large molecules to the fruit surface, it might be expected that coating would exert its effects by increasing **R** and modifying the fruit's internal atmosphere composition rather than by any direct chemical effect. Coating or washing of fruit in Tween 20 solution resulted in alteration of the gaseous mixture inside the fruit and in this way the technique might be considered to be analogous to that of MA storage. This was presumably the mechanism by which Tween 20 solution inhibited grease development in Granny Smith apples.

One inherent feature in fruit, including apples, is variability in all of their physiological characteristics. In apples, rates of respiration, C_2H_4 production as well as **R** are also variable between cultivars and even between individual

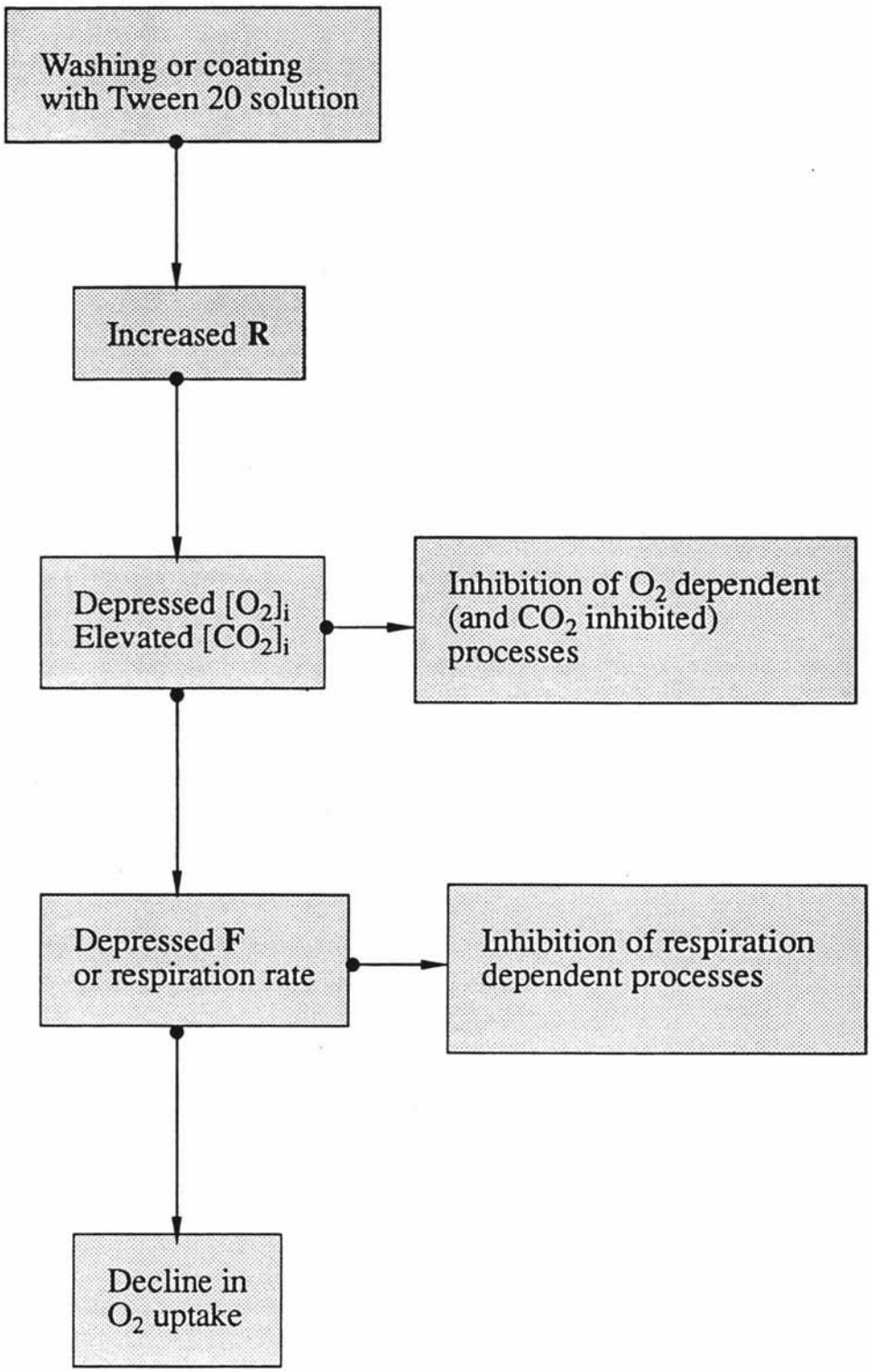

Fig. 9-2. Schematic representation of the effects of washing or coating with Tween 20 solution on **R**, $[O_2]_i$, $[CO_2]_i$, **F** or respiration rate and O_2 uptake of apples.

fruit within a cultivar (chapter 4). Localised variation in cuticular structure would cause differences in wettability or extent of occlusion of the pores by a coating solution. This would result in any given washing or coating material having a variable cover on different areas of a single fruit. Naturally, this would cause variability in the resistance of these areas on the fruit to gas diffusion and variation in **R** between individual fruit. This in turn would lead to variation in internal atmosphere composition which would cause variability in physiological response. This is probably one of the reasons why the great potential for surface coatings predicted by many workers in research papers (El Ghaouth *et al.*, 1991; Ben-Yehoshua, 1987; Eaks and Ludi, 1960; Hagenmaier and Shaw, 1992; Trout *et al.*, 1953) has not been fully realised in commercial practice.

Other problems with coatings include the substantial increase in the extent of internal atmosphere modification achieved at different temperatures (Banks, 1985a). However, Hagenmaier and Shaw (1991) have recently shown that O_2 permeability of a shellac coating material roughly doubled between 30°C and 40°C. This indicates the potential, at least, for permeability of the skins of coated fruits to adjust for variations in fruit respiration rate at different temperatures.

Evidence presented in chapter 6 demonstrated that the difference (gradient) in O_2 concentration between the internal and external atmospheres in coated apples was much greater than that for CO_2. If we assume that the majority of the movement of all of gases into and out of the fruit occurred via the lenticels (ie. that the resistances of the fruit surface to O_2 and CO_2 were similar), then we must also assume that the coating or washing treatment was differentially permeable to the different gases. Since the molecular size of CO_2 is greater than that of O_2, the development of this differential permeability would probably have depended on some other characteristic of the two gases. Hagenmaier and Shaw (1992) showed that the permeability to CO_2 of coating

materials used on fruits is typically a factor of four greater than the permeability to O_2. Thus, the effects of Tween 20 coating on the internal atmosphere of apples were probably in large part due to the differential permeability of the coating deposit on the fruit surface.

At this point, with no data on the relative permeabilities of Tween 20 films to O_2 and CO_2, we cannot exclude the possibility that the application of the Tween 20 treatment reduced the movement of O_2 and CO_2 through the lenticels to a similar extent. If this is the case, then we must assume that the routes for entry of O_2 into the apple fruit and those involved in the exit of CO_2 are different. This would fit with data from Chapter 8 for non-coated Braeburn and Granny Smith apples coolstored for 12 weeks and then equilibrated at 20°C for 3 days. These fruit had a much greater gradient in O_2 concentration between the internal and external atmospheres than they did for CO_2 concentration.

Burton (1974) indicated that the low solubility of O_2 in water would limit significant exchange of this gas across the fruit surface to pathways involving only gaseous diffusion. Carbon dioxide on the contrary, by virtue of its high solubility in water might move at significant rates via pathways involving liquid water. The factor limiting movement through the skin in the gas phase would comprise the effective apertures of the lenticels. If we assume that CO_2 was released from the general fruit surface as well as via the lenticels, then by blocking the lenticels we would exert a marked effect on the resistance to O_2 (and C_2H_4) and some effect on the resistance to CO_2. The magnitude of this effect would depend on the thickness and porosity of the deposit in the aperture and the proportion of the total number of lenticels blocked. If we also suppose that the resistance of the epidermis and/or the cuticle to the movement of gases was greater than that offered by the layer of the Tween 20 solution applied to the fruit surface then the presence of washing treatment or coating over the rest of the fruit surface could have little additional effect on the

resistance of the fruit skin to CO_2. This provides an alternative account for the differential effects on the resistance of the fruit skin to CO_2 exerted by coating with Tween 20 solution.

It is interesting to note that the differences in gradients for the two respiratory gases were not present in the freshly harvested fruit and that they developed in magnitude over time. It may therefore be that the most appropriate model of gas exchange through the fruit skin is the first of those presented above in which all of the gas exchange takes place through the lenticels. Subsequent development of wax on the fruit surface then might lead to partial blocking of the lenticels and generate the observed differential gradients in a manner similar to that achieved by coating.

Burton (1974) has noted the importance of considering the variation in skin resistance of individual fruit skins to gases when producing recommendations for the concentrations of O_2 in CA storage of fruits. The same point must be borne in mind when suggesting suitable concentrations of a coating or washing treatment for fruits. Inherent differences in the resistance of both the skin and the internal tissues to the movement of gases may affect the relative effects of coating on O_2-dependent physiological processes.

9.3 A model describing the form of relationships between $[O_2]_i$, R and respiration.

A tentative model describing the general form of relationships between $[O_2]_i$, total **R** (i.e. the natural resistance of the skin, presence of coatings, or greasiness, or packaging) and temperature is shown in fig. 9-3. The curves R_1, R_2, R_3 and R_4, show that for any particular **R** value, the $[O_2]_i$ decreases with increasing temperature and that as the resistance is increased (ie. naturally through the development of greasiness, or artificially through coating,

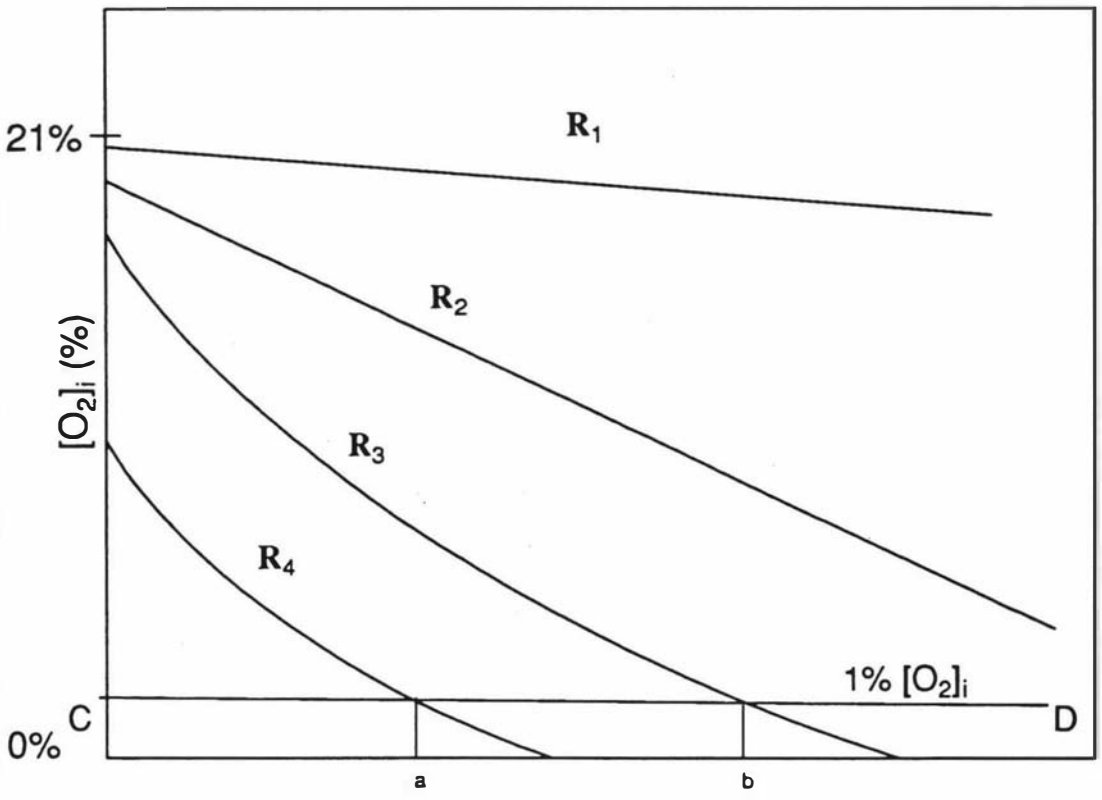

Fig. 9-3. Tentative model describing the form of relationships between $[O_2]_i$, skin resistance to gas diffusion ($\mathbf{R}$) and temperature. Curves $\mathbf{R_1}$ to $\mathbf{R_4}$ represent fruit with differing levels of skin resistance ($\mathbf{R_1}$ = low, $\mathbf{R_4}$ = high).

waxing or packaging) this effect of temperature becomes greater. These temperature effects are brought about by variation in respiration rate at the different temperatures.

If the $[O_2]_i$ falls below a critical value (perhaps 1% represented by the line CD), internal injury could occur (eg. because the $[O_2]_i$ is lower than the $[ACP]_i$). The temperatures a, b, etc. corresponding to the points of intersection of the resistance curves and the 1% $[O_2]_i$ line, CD, would therefore be the maximum temperatures at which the fruit could be stored without developing injury. For a fruit kept in air, **R** would have to have very large values before the tissues would begin to respire anaerobically because of the high concentration of O_2 available in the external atmosphere. However, the same overall type of response to temperature would occur for fruit kept in modified atmospheres, in which only modest $\Delta[O_2]$ would be needed to render the tissues anaerobic. The concept of an optimum $[O_2]_{ext}$, at which respiration is maximally suppressed without inducing anaerobiosis, is therefore not one of a constant for a given fruit type in all environments. Rather, the optimum value for $[O_2]_{ext}$ for a given population of fruit would be dependent upon the inherent differences in **R** and respiration as well as the temperature at which these fruit are kept.

Clearly, the predominance of temperature as an environmental factor affecting the physiological behaviour of apples through its effects on fruit respiration and internal atmosphere composition is reaffirmed by this analysis.

9.4 Recommendations for further research

The original intention of this work was to attempt to ascertain the relationship between the internal atmosphere composition of apples and factors such as $[O_2]_{ext}$, respiration, **R**, artificial barriers and temperature. On reflection it would seem that in common with most other physiological studies,

this research has raised more questions than were originally envisaged. Nevertheless, this work will help to focus subsequent inquiry. In the light of the findings of the current study, it is recommended that the following avenues of research would be worthwhile.

Further research is required to ascertain whether the relationship between respiration and $[O_2]_i$ changes with fruit maturity and during the climacteric. The mechanism by which low O_2 atmospheres affect fruit respiration still remains obscure. It is hoped that in future additional efforts will be directed towards improving our understanding of how low O_2 levels influence respiration metabolism, C_2H_4 production and compositional changes related to quality attributes of apples. Such information will no doubt help in our understanding of the mode of action of CA and MA on fruit physiology and therefore improve our chances of expanding the use of CA or MA during transport and storage of apples and other fruits.

In this project there was no detectable AA or ETOH in the core cavity of apple fruit irrespective of the $[O_2]_{ext}$. It may be that at the time of internal atmosphere sampling, AA and ETOH had not accumulated in sufficient concentrations to be detectable. This seems odd because the two vapours were present in the vials equilibrated with the internal atmosphere via a pore in the fruit surface, despite the fact that their contents would lag at least several hours behind the changes in contents of the internal atmosphere of the fruit. Further research is required to investigate this surprising finding. It would also be interesting if the distribution of AA and ETOH in the fruit is ascertained, something which could be achieved non-destructively using the vial system employed extensively in this study. This would be of particular interest in comparing the relative tendencies towards accumulation of anaerobic by-products in the calyx end of the fruit with those regions which in this work have been shown to have higher levels of O_2.

Given the importance of gas diffusion in successful CA storage of apples, additional information is required on the changes of **R** of apples during storage under low O_2 atmospheres. In addition there was a large degree of variation in **R** of individual apples within each cultivar; further research is warranted to ascertain the physiological and commercial importance of such variability in apples during CA storage.

In this study Splendour apples compared to the other cultivars had high $[O_2]_i$, low $[CO_2]_i$, low respiration, low **R**, high soluble solids contents and high storage potential. However one major disadvantage of this cultivar is its high susceptibility to bruising damage. It would therefore be a commercial advantage to the apple industry in New Zealand if some of these beneficial qualities inherent in Splendour apples could be introduced into poor storing cultivars or alternatively to breed Splendour apples with better skin that does not affect the good qualities of this cultivar.

In view of the spacing of the temperature regimes used in this research (chapter 7), it was not possible to establish the optimum temperature for C_2H_4 evolution of the apple cultivars studied. Above 25°C, most of the cultivars lost some of their capacity to produce C_2H_4. It would be interesting to establish the optimum temperature for C_2H_4 evolution and to study in detail why fruit lost their capacity to produce C_2H_4. This may be of value in understanding the response of fruits to different temperature regimes eg. heat shock treatments.

Tween 20 solution was effective in preventing the development of greasiness in Granny Smith apples as well as retardation of colour change and firmness. Whilst it is beyond the scope of this thesis, further research to ascertain the mode of action of Tween 20 solution in inhibiting development of greasiness, retardation of colour change and firmness would be worthwhile.

Following the discovery that the internal atmosphere composition within apples is heterogeneous, it is hoped that in future further research efforts would be directed towards establishing the physiological significance of such heterogeneity in apples under CA/MA conditions. It has been established that the calyx region of apples contained lower O_2 and higher CO_2 and C_2H_4 than the other parts of the fruit. Further research is clearly warranted to study if this pattern is retained during storage of apples under low O_2 atmospheres. If this is so, does the development of certain physiological disorders in apples under low O_2 commence or occur at the calyx region of the fruit, where the O_2 concentrations are low even for fruit kept in air ?. The research should incorporate anatomical studies as well as estimation of intercellular space volume.

9.5 CONCLUSION

To conclude, data on gas exchange characteristics of apples have been utilised to examine the form of relationships between internal atmosphere composition and factors such as $[O_2]_{ext}$, **R**, respiration, temperature and artificial barriers. It is clear that these factors do influence the atmosphere inside the apple and hence have the potential to affect important aspects of quality such as rates of softening and loss of green colour. Whilst the scatter in some of the data sets made it difficult to be definitive about the exact shapes of some of the relationships, nevertheless the principles, results, discussions, mathematical equations and suggestions presented in this thesis has provided further insight in the way these factors affect the internal atmosphere composition of apples. The information assembled in this thesis would hopefully contribute to further study of these relationships as well as the effects of low O_2 atmospheres on fruit respiration and C_2H_4 production.

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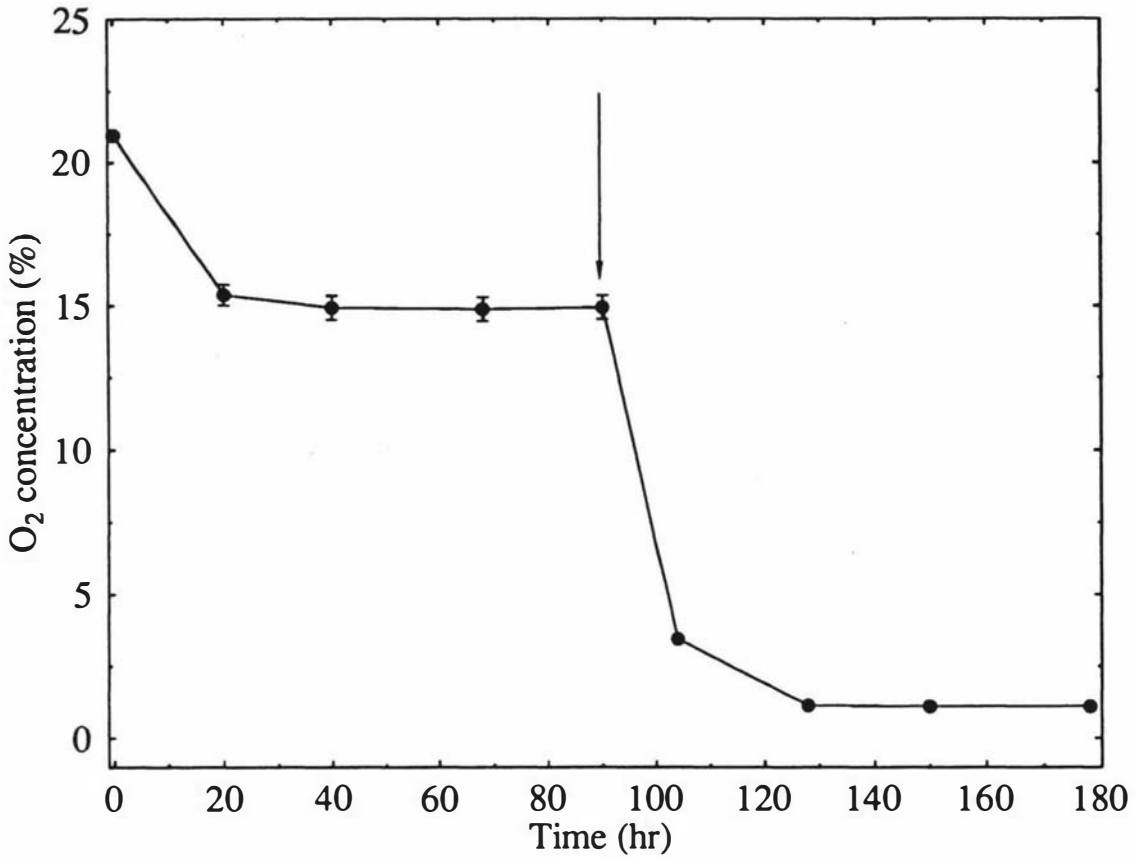
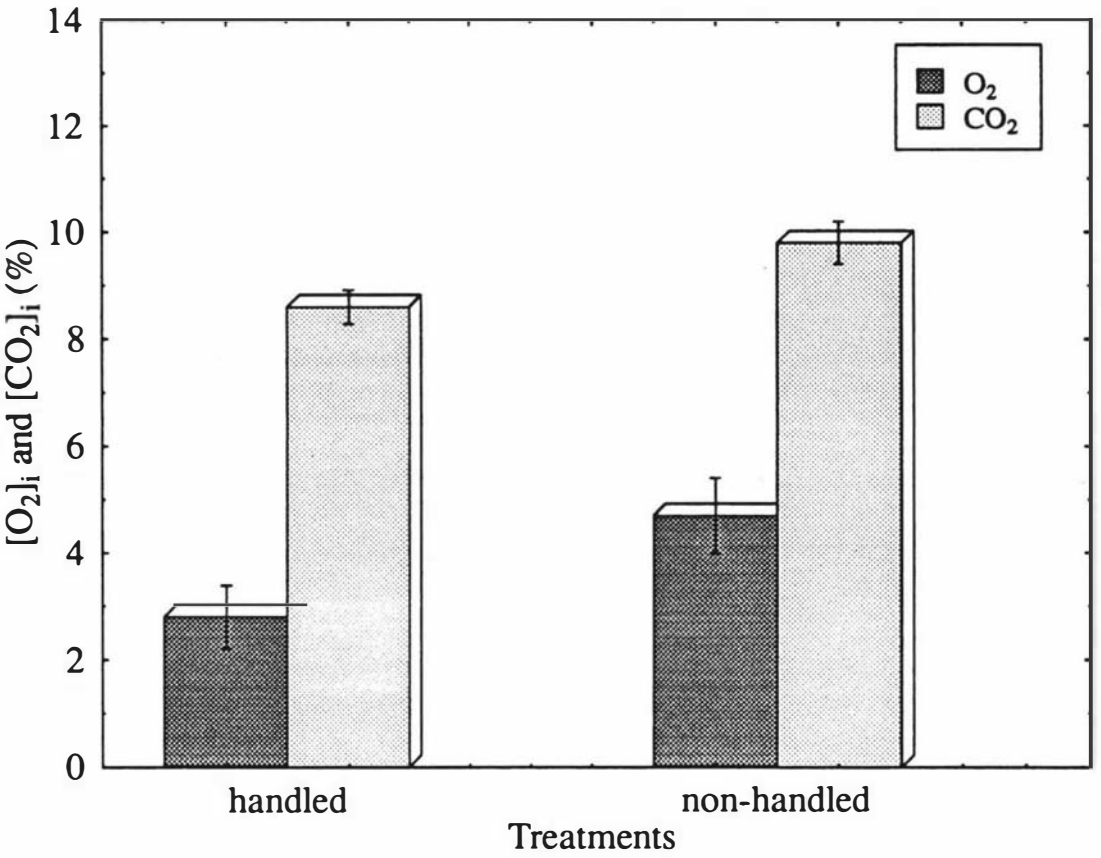
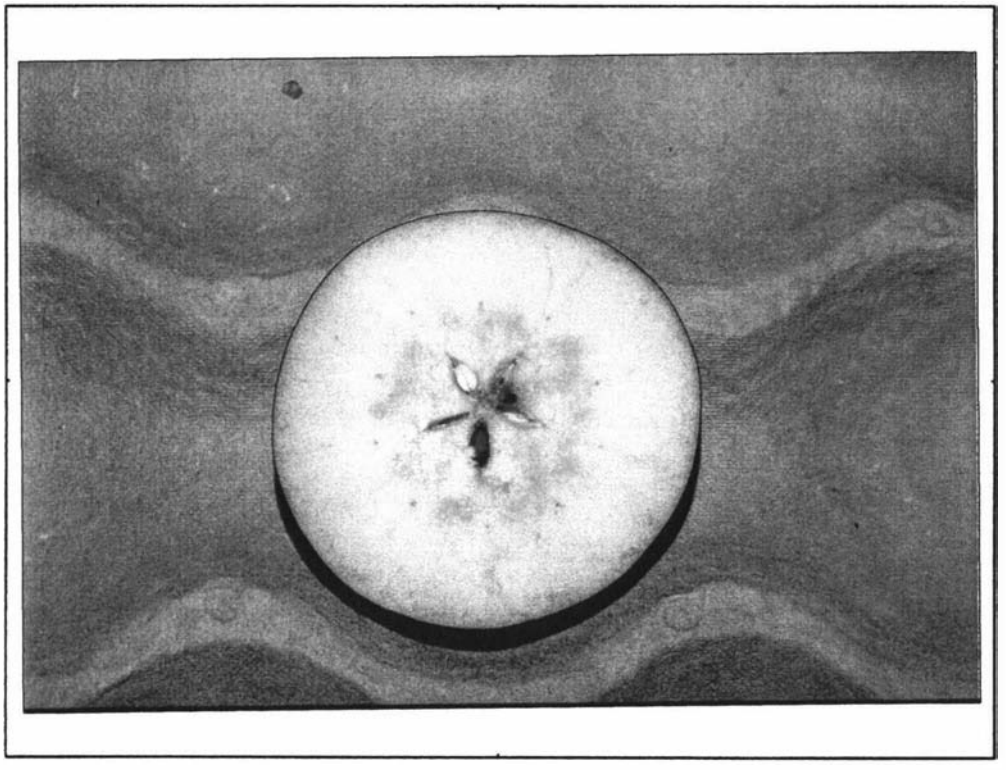


Appendix 1. Change in glass vial O_2 concentrations with time. Vertical bars indicate standard errors of means. Points to the left of the arrow were the average values for thirty-six fruit during equilibration in air. To the right of the arrow indicates equilibration of one fruit in 3.78% O_2 (similar equilibration time was obtained for each of the thirty-six fruit (per cultivar) kept in different O_2 atmospheres).



Appendix 2. Effects of handling by touching on $[O_2]_i$ and $[CO_2]_i$ of greasy Granny Smith apples stored for 17 days at 20°C. Vertical bars indicate standard errors of means.



Appendix 3. Photograph showing browning around the core cavity of Granny Smith apple.

VARIATION IN INTERNAL ATMOSPHERE COMPOSITION WITHIN SINGLE APPLES

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INTRODUCTION - Precise knowledge of the distribution of internal gas concentrations in fruit will enhance our understanding of the mechanisms by which ripening is retarded and atmosphere related-disorders develop within fruit stored in modified or controlled atmospheres. The internal atmosphere composition of an apple has been reported to be practically homogeneous (Solomos, 1987). However, recent evidence demonstrates that there may be significant heterogeneity in O_2 concentration within the flesh of some apple cultivars. The present study deals with the distribution of O_2 concentrations within individual apples kept at 20°C.

METHOD - Granny Smith apples (*Malus sylvestris* Mill, cv Granny Smith, count 125), stored in air for 2 months at 0°C were used in this experiment. Glass vials (2ml) were stuck over one or more lenticels at each of five positions on the surface of the fruit (Fig. 1). After 54hr of equilibration at 20°C in the dark, gas samples (90µl) were taken from each glass chamber using a gas-tight syringe and analysed for O_2 using an O_2 electrode (Banks, 1986).

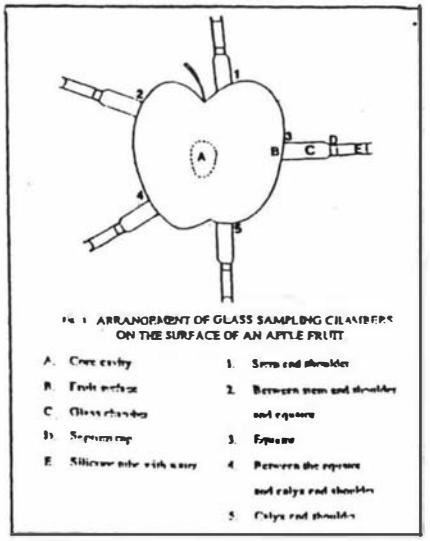

Fig. 1 ARRANGEMENT OF GLASS SAMPLING CHAMBERS ON THE SURFACE OF AN APPLE FRUIT

A. Core cavity
B. Fruit surface
C. Glass chamber
D. Septum cap
E. Silicone tube with a wire

1. Stem end shoulder
2. Between stem end shoulder and equator
3. Equator
4. Between the equator and calyx end shoulder
5. Calyx end shoulder

RESULTS - The steady state mean O_2 concentrations of the five various positions are presented on Fig. 2 Oxygen concentration at the equator was higher than any of the other positions on the fruit, whilst tissues near the calyx end consistently had lower O_2 concentrations than other parts of the fruit. This may be related to localised variation in intercellular space volume, since studies with Golden Delicious apples have shown that intercellular space volume are greatest in the equatorial region compared to the other parts of the fruit. High porosity would be expected to facilitate gas diffusion.

CONCLUSION - Internal atmosphere composition of Granny Smith apples varied from one part of the fruit to another and this has important implications for the way in which we attempt to model the gas exchange of these fruit and the effects of modified atmospheres on their physiology. As a result of the heterogeneous distribution of O_2 concentrations within individual fruit, tissues at the fruit centre would experience lower O_2 concentrations than those at the surface. Deeper tissues would therefore be likely to have lower respiration rates and presumably a greater tendency towards the development of low-O_2 disorders in fruit stored in modified atmospheres at elevated temperatures such as 20°C.

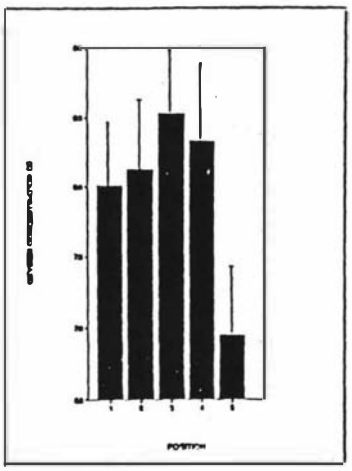

Fig.2. Distribution of Oxygen Concentration within single apples.

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Fruit used in this study were sampled from those supplied by the New Zealand apple and pear marketing board as follows:

In chapters 4, 5, 6 and 7, fruit used in each experiment were sampled at random from four cartons (one carton from each of 4 growers) per cultivar. Growers used for the different cultivars were not necessarily the same.

In chapter 8, fruit used in experiment 2 were similarly sampled at random from four cartons (one carton per grower) per cultivar. On the other hand, those used in experiment 1 were sampled from six cartons (one carton per grower) per cultivar.