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Overview of lipids in the avocado fruit, with particular reference to the Natal Midlands

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ABSTRACT

Lipids in the avocado are a major yield limiting factor. Lipid and fatty acid distribution, biochemistry and significance in the avocado fruit are discussed. In one case, lipid levels increased up to 30% on a fresh mass basis and then plateauxed. In the other case, lipid levels decreased with late hanging on the tree.

INTRODUCTION

The very high levels of lipids in the mature avocado fruit mesocarp (flesh) reflect ecological selection for millennia, due to their genealogy, palatability and function (Biale & Young, 1971). Values of total lipids recorded in the literature for the mesocarp are up to 25% on a fresh mass basis. In contrast, the relatively large avocado seed contains abundant reserves of carbohydrates and a relatively low water content. Consequently, it only has about 1% lipids on a fresh mass basis.

The combustion heat of lipids and carbohydrates are 39,91 and 16,74 kJg^{-1} respectively (Watt & Merrill, 1963). This means that lipid production requires more than twice the energy required for carbohydrate production, excluding respiratory losses. It is obvious that these high lipid levels are thus going to be a major yield-limiting factor. Despite contributing to low yields, when compared to other crops such as apples (Wolstenholme, 1986), these high levels of lipids have many advantages, including the unique and desirable taste.

The research approach taken in this study of avocado lipids, was firstly to study their occurrence and distribution within the fruit, and then the biochemistry and developmental changes of the constituent fatty acids. Finally, the practical implications of lipids dominated flesh constituents are discussed.

Distribution of lipids

The avocado fruit consists of an exocarp, or rind, a relatively thick, fleshy mesocarp, a thin, fleshy endocarp (collectively the flesh or pulp) and the seed. Most of the lipids are found in the mesocarp. The mesocarp consists of numerous parenchyma cells permeated by vascular strands and also scattered idioblasts. The idioblasts are specialized lipid containing cells (Schroeder, 1952; 1953).

In the mesocarp, the lipids are mostly localized within the parenchyma cells and idioblasts (Platt-Aloia & Thompson, 1980; 1981). The parenchyma cells have a normal appearance, but they also contain lipid bodies. The present study has shown that the lipid bodies are spherical in shape when viewed under the scanning electron microscope (Figure 1). However, under the transmission electron microscope, Platt-Aloia and Thomson (1981) maintained that the lipid bodies have many indentations containing plastids, microbodies and mitochondria and remain unchanged throughout the ripening process.

The anatomy and associated physiology of the avocado mesocarp cells are complex and diverse. With respect to ripening, there is hardly any intrinsic energy loss within the cells, and the slight modifications to the organelles of parenchyma cells, which do occur, are not degradative or senescent. This is supporting evidence for the preservation of compartmentalization through membrane integrity. Cell wall degradation is, however, a notable exception to this assumption (Platt-Aloia & Thomson, 1981; Platt-Aloia 1980; Dallman *et al* 1988).

Platt-Aloia *et al* (1983) showed that the idioblasts are distinct from the parenchyma cells in that they have complex cell walls. The idioblast cell walls are distinctly layered and consist of firstly, an inner cellulosic tertiary layer, secondly a suberized middle layer, and thirdly an outer cellulosic primary layer. The three-layered cell wall was identified in the present study (Figure 2). Evidence for idioblasts was seen by Platt-Aloia *etal* (1983), with the initial deposition of individual suberin plaques at the middle lamella. A few small lipid droplets were also identified at this early stage in the development, and as the cell developed, the suberin layer thickened and became composed of several layers. The lipid content, however, remains unchanged and low.

With the completion of the suberized middle layer, the construction of the tertiary wall began. The tertiary wall consists of cellulose and during the final stages of development had a granular appearance and was more electron opaque than both the primary and middle layers. The lipids began to accumulate in the cytoplasm and vacuoles at the onset of tertiary wall development and as development progressed, the lipids moved from the cytoplasm into the vacuoles, until the cell became totally filled by lipid-containing vacuoles. Evidence for this was seen in the present study (Figure 3).

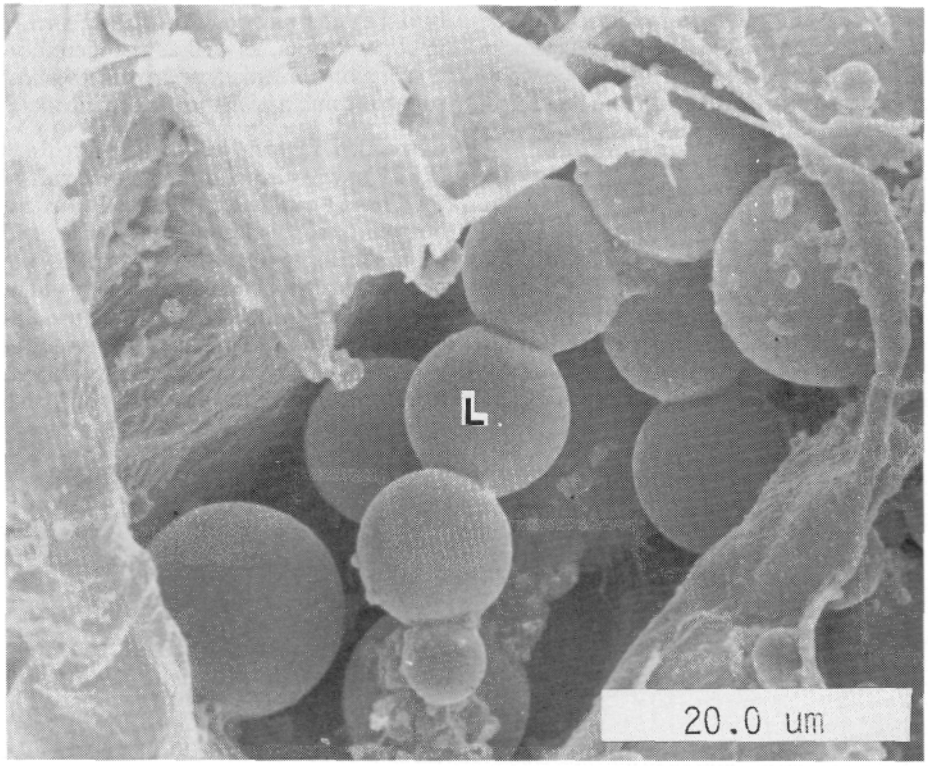

Fig 1 Scanning Electron Micrograph of lipid bodies in a parenchyma cell (L = Lipid body).

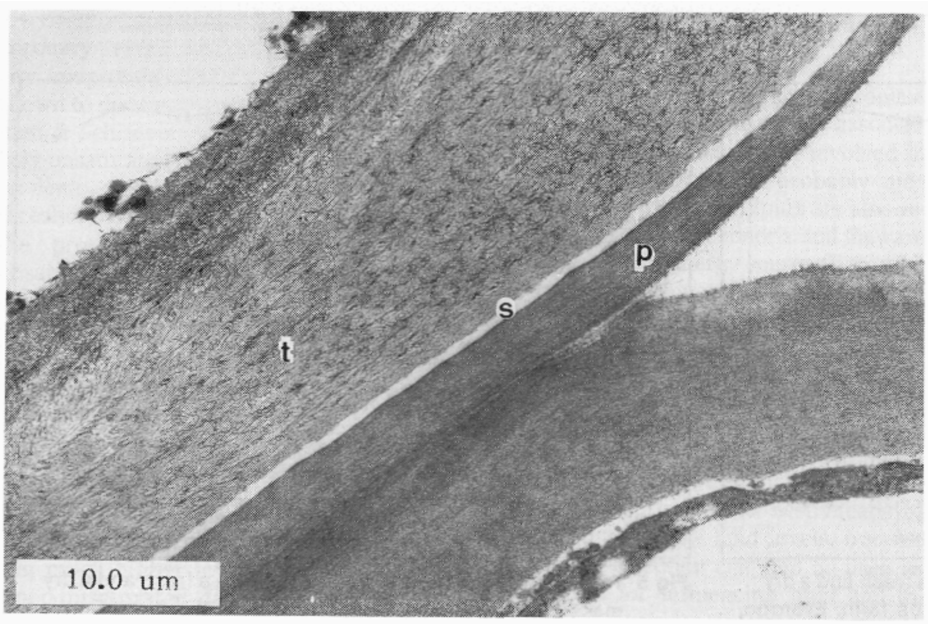

Fig 2 Transmission Electron Micrograph of three-layered idioblast cell wall (p = primary wall, s = secondary wall, t = tertiary wall).

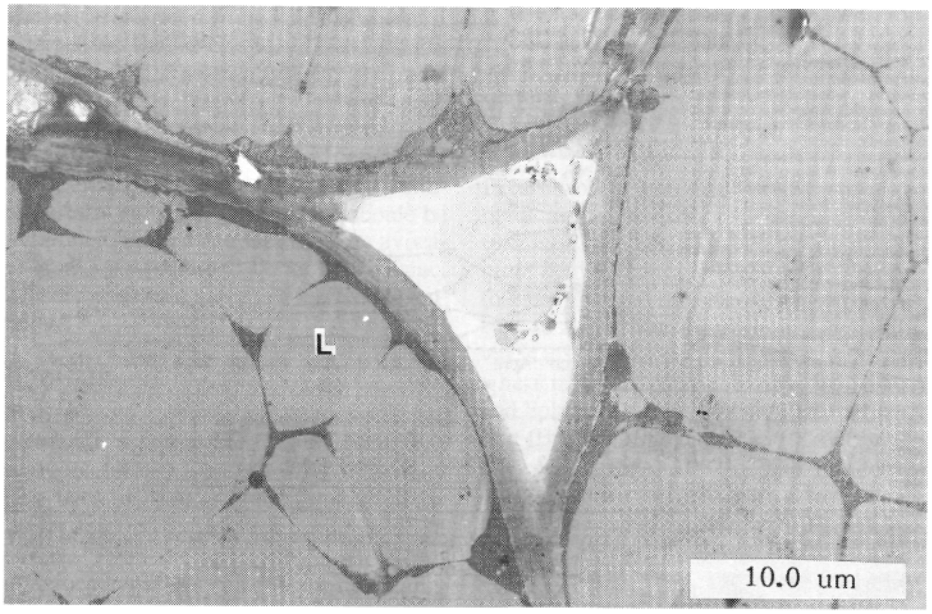

Fig 3 Parenchyma cells, totally filled with lipid bodies (L = Lipid body).

Idioblast distribution throughout the mesocarp was shown to be fairly uniform by Cummings and Schroeder (1942). With regard to the actual lipid levels in the mesocarp, Schroeder (1987) showed that there is a high concentration at the pedicel end and that this decreases as one moves towards the seed. Lipid concentrations were also high at the micropylar end of the fruit. If the vascular traces are superimposed on lipid concentration, it can be seen that the vascular traces are more numerous in areas with high lipid concentration. This then makes the fruit flesh vasculature an extremely interesting field of study. Such studies are currently under way.

The overall lipid distribution in fruit flesh also has relevance to fruit physiological disorders, where firmness is inversely related to lipid and calcium levels. This in turn is related to irregular fruit softening (Schroeder, 1987).

Types of lipids in avocado fruit flesh

Types of lipids which occur in the avocado fruit include tri-, di- and mono-acylglycerides and glyco-, sulfo- and galacto-lipids. The tri-acylglycerides were by far the most abundant, being approximately 20% of the total fruit fresh mass (Kikuta & Erickson, 1968). The tri-acylglycerides consist of three fatty acid moieties bound to a glycerol backbone. The di- and mono-acylglycerides obviously have two and one fatty acid moiety bound to a glycerol backbone respectively. The tri-acylglycerides are important as storage reserves, but are also a high-energy reward for the dispersers of avocado seeds. The di- and mono-acylglycerides are important intermediates in biochemical reactions. The phospholipids contain a phosphorus atom and a nitrogenous base in addition to the glycerol and fatty acid moieties and are associated with membranes. The galacto and sulfolipids are found primarily in the chloroplasts and are involved in thylakoid membrane structure (Conn & Stumpf, 1976).

Developmental changes in total lipids

With respect to the developmental changes in total lipids, Appleman (1971) showed an increase in total lipids on a fresh and a dry mass basis. An increase was observed throughout the season ending in November (Southern Hemisphere equivalent). Moisture content has also been shown to be inversely related to lipid content on both a fresh and a dry mass basis.

In work done thus far by the present authors, Everdon farm at Howick, the cooler site, has shown a similar increase in total lipids on both a fresh and a dry mass basis with a maximum of 30% on a fresh mass basis in late hung Hass avocado fruit (Figure 4).

In contrast to this, Eacks (1990) found that the lipids on a fresh mass basis began to decrease after a peak in October (Southern Hemisphere equivalent). On the farm Cooling at Bruyn's Hill, a warmer site, exactly the same trend was observed, with a peak of 29% lipids on a fresh mass basis in October, followed by a decrease in November, (Figure 5).

This drop in total fruit lipid content coincided with the spring growth flush and there are three possible reasons for this drop. The first is that lipids may be acting as an energy source which may be partially mobilized during the vegetative flush. If this is true then the late hanging of fruit is not as detrimental to the overall carbohydrate balance of the tree, as is usually thought.

In fact a positive effect will be seen, provided of course that the tree is free of *Phytophthora* and is in good health. An alternative reason is that a decrease in total lipids may be temperature related, as the reserves are possibly being more rapidly respired due to higher summer temperatures and increased physiological activity in the mature fruit.

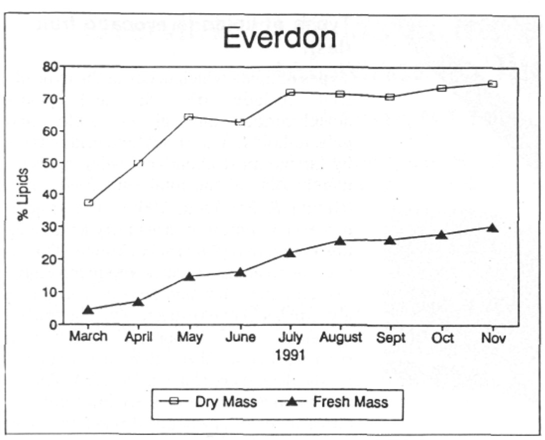

Fig 4 Total lipid accumulation during 1991, on a fresh and a dry mass basis for Hass avocado fruit from the farm, Everdon, at Howick.

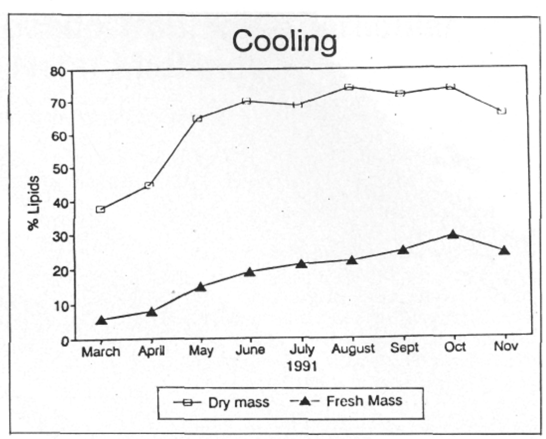

Fig 5 Total lipid accumulation during 1991, on a fresh and a dry mass basis for Hass avocado fruit from the farm, Cooling, at Bruyns Hill.

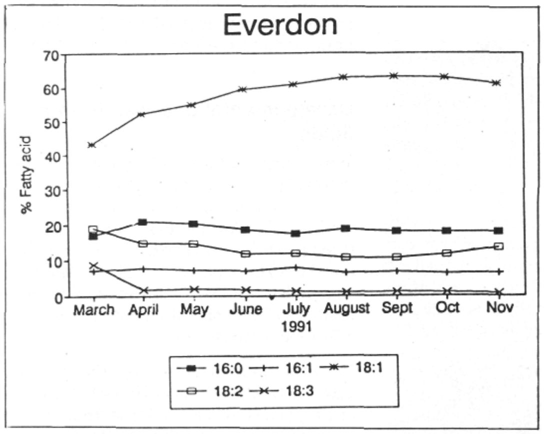

Fig 6 Fatty acid accumulation during 1991, for Hass avocado fruit from the farm, Everdon, at Howick.

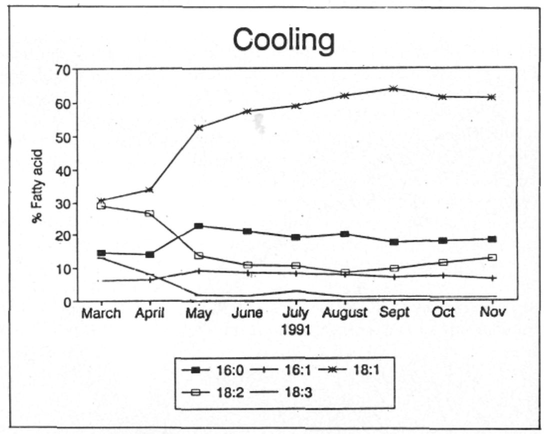

Fig 7 Fatty acid accumulation during 1991, for Hass avocado fruit from the farm, Cooling, at Bruyns Hill.

A third possible alternative is that lipid synthesis was simply terminated. It is known that some cell division continues in the mature avocado fruit and if lipid synthesis is terminated, this resulted in an overall decrease in lipids on a fresh mass basis.

The issue could be further investigated by radioactive tracer trials with $^{14}CO_2$ at the early stages of fruit growth. The $^{14}CO_2$ would then be fixed as lipids and the fate of the lipids in the mature fruit could be determined.

Biochemistry

Since fatty acids are the individual components of all lipids (Gurr & James, 1971), a brief overview of fatty acid biochemistry is helpful. The short chain fatty acids, with less than 10 carbon atoms, are all water soluble. The long chain fatty acids, however, are much less soluble in water due to the size of the hydrocarbon chain. The fatty acids found in the avocado are long chain, with 16 carbon atoms or more (Kikuta & Erickson, 1968). They may be saturated or unsaturated, and an example of the shorthand notation for oleic acid, a monounsaturated 18 carbon fatty acid is (18: 1) (Gurr & James, 1971). The biosynthesis of fatty acids is very complex and will not be dealt with here.

Developmental changes in fatty acids

Kikuta and Erickson (1968) found that oleic acid increased most during avocado fruit development, while palmitic, palmitoleic and linoleic acids only increased slightly. Linolenic acid on the other hand decreased slightly during fruit development.

In the present study, at the warmer Natal site of Cooling, oleic acid increased up to September and then dropped marginally. All the other fatty acids started decreasing after May (Figure 6). On the other hand, at the cooler site of Everdon, only oleic acid increased, while all the other the fatty acids remained fairly constant (Figure 7).

The fatty acid types are very important with regard to health aspects. The monounsaturated fatty acids, viz oleic and palmitoleic, actively lower levels of toxic low density lipoproteins (LDL) in the human coronary system and protect the high density lipoproteins (HDL), which have been shown to decrease levels of cholestrol (Assman & Schriever, 1980; Anon, 1990). The poly-unsaturated fatty acids, viz linoleic and linolenic, also lower the LDL, but after a threshold value they also tend to decrease the protective HDL. Thus the polyunsaturated fatty acids are not as good, from a dietary point of view, as the monounsaturated fatty acids. The saturated fatty acids on the other hand, are responsible for actively increasing the LDL levels and thus cholesterol too (Human, 1987; Bergh, 1991). The avocado fruit themselves contain no cholesterol, contrary to popular belief.

It is known that fruit from cooler areas contain much higher levels of the beneficial mono-unsaturated fatty acids (Moretón, 1988) and as such, growers in cooler areas, particularly the Natal midlands, may have the advantage in marketing their fruit as "health fruit". The levels of fatty acids in warmer areas need to be examined, but it is expected that they too will have sufficiently high mono-unsaturated fatty acid levels to market their fruit as cholesterol reducing. A slogan such as "An avo a day keeps the heart doctor away!" would not be out of place since the avocado is an anticholesterol agent. A daily dosage should be recommended and the fact that the avocado is also rumoured to be an aphrodisiac, has been exploited in some advertising campaigns.

Significance

With regard to their significance, lipids have been selected over the years due to their palatability and more recently they have been utilized as a commercial oil for the cosmetic industry (Human, 1987). There is even the opportunity of marketing avocado oil as a salad dressing (Jamieson, 1943) since the "oil" compares favourably with, if not better than olive oil (Briggs & Galloway, 1984; Andrikopolous, N K, 1989), with respect to mono-saturated fatty acids. It should be remembered that olive oil is officially recommended by the Heart Foundation of South Africa.

With regard to their actual role within the fruit, lipids are important structural components of membranes, where they govern permeability, by forming the bilayer, together with proteins. The benefits of the bilayer are seen in the exceptional constancy and specificity inferred on the membranes. Membranes are also important as the sites of enzymatic reactions (Bohinski, 1987). When membranes lose their structural integrity, leakage of previously compartmentalized components results and mixing of enzymes such as polyphenoloxidase and phenolic substrates result. Browning reactions including pulp spot then result (Bower and Cutting, 1988; Wolstenholme, 1992).

Lipids are also essential for chloroplast functioning. Here linoleic acid is associated with the glycolipids and is involved in thylakoid orientation and probably aids ' chlorophyll functioning. Lipids are also associated with the mitochondria and they are an extremely rich energy source (Bohinski, 1987). It is for this reason that they are often found in relatively small seeds as a stored energy reserve.

In terms of influencing lipid levels within the fruit, it would be very beneficial to manipulate these levels due to their importance to the industry. Obviously, cultivar selection will provide the best means of selecting high or low lipid levels, but site and climate selection can also be used as a valid tool for influencing lipid and individual fatty acid levels (Ratovohery *et al,* 1988), where cooler climates result in more unsaturated fatty acids (Moretón, 1988). Trials using foliar sprays of inorganic nutrients such as P, Mg and Fe (Moretón, 1988), as well as plant growth regulator sprays, may also provide some interesting results.

Practical implications

In terms of the practical implications of the lipids in the avocado, fruit maturity is determined directly or indirectly by a minimum lipid percentage in the fruit. The fact that the lipid and moisture levels of the fruit sum to a constant figure in any one cultivar is utilized for predicting fruit maturity, where moisture content can be determined and lipid levels thus calculated by subtraction (Brown, 1984; Swarts, 1976a; 1976b; Lee & Coggins, 1982). This method is cheap and efficient, although not totally accurate and for detailed analyses, nuclear magnetic resonance (Bergh *et al,* 1989), solvent extraction (Lee, 1981) and a modified Gerber test (Rosenthal *et al,* 1985) are preferred. Of these, the solvent extraction technique, using a Soxhlet apparatus, is the most economical.

Commercial extraction involves either solvent extraction, centrifugation or hydraulic pressure, but by far the highest yielding of these is the organic solvent extraction method. Centrifugal separation seems to produce the most desirable oil with highest mono-unsaturated fatty acids and lowest saturated fatty acid levels (Werman & Neeman, 1987).

DISCUSSION AND CONCLUSION

The lipid content of the avocado fruit is exceptionally high. These high levels arose due to continued selection. This selection criterion was initially related to genealogy and consequently function and then later to palatability.

Lipids are important structural components of membranes. They also serve as an energy-rich source. The reason for localization of lipids within idioblasts is unclear but it is most probable that this is related to genealogy. The fatty acid composition is important as this controls the fluidity of the cell membrane, which in turn relates to temperature sensitivity. From a health point of view, the avocado "oil" compares favourably with olive oil. The exceptionally high level of oleic acid in the fruit can be used as a marketing ploy, where avocados are promoted as cholesterol-reducing. The avocado "oil" is revered by the cosmetic industry, due to its superior qualities. There is also much scope for avocado oil as an international commodity as world production of extracted natural oils, falls far short of demand. However it is a relatively expensive product.

Although it is desirable that the lipid content of fresh avocado fruit for consumption be relatively low, methods of increasing total lipids for extraction purposes have up till now been restricted to cultivar selection and initial site selection, however, work incorporating foliar sprays of P, Mg and Fe looks promising. In addition to these, the effects of hormonal sprays should be examined. Lipid levels in the avocado fruit have been found to be the only reliable maturity index, although the indirect measure of lipid content, viz the moisture percentage, has come to be widely used.

The idioblasts are the lipid storage cells, each one being discrete from the adjacent parenchyma cells. The vasculature of the avocado is not yet fully understood, mainly because the only available studies were done using light microscopy, earlier in this century. It seems odd therefore, since light microscopy has paved the way, that electron microscopy studies have not been undertaken and work is currently under way in this field. A better understanding of the vasculature will not only facilitate control during growth and ripening, but may even provide a means of controlling physiological disorders.

Total fruit lipids in Hass have been shown by Eaks (1990), as well as in the present study at the farm Cooling, to increase to a peak and then to decrease. This decrease coincided with the spring flush. This then leads to the question as to whether lipids may be stored in the fruit and act as an energy reserve for physiologically active times. If this is true then the late hanging of the fruit is not as detrimental to the overall carbohydrate balance of the tree and cropping may not be adversely affected, provided that adequate leaf: fruit ratios are maintained. However, a further season's data are required to confirm this observation.

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