CHAPTER 4

GAS EXCHANGE OF DEVELOPING FRUIT

4. 1 CARBON DIOXIDE EXCHANGE OF DEVELOPING FRUIT[‡]

4. 1. 1 Introduction

Crop yield increases have been achieved largely by increasing the proportion of assimilates partitioned to the harvested plant organs (Evans 1976). For example, in avocado (*Persea americana* Mill.), a substantial yield increase was obtained in response to a reduction in vegetative growth as a result of treatment with paclobutrazol foliar sprays (Wolstenholme *et al.* 1990; Whiley *et al.* 1991). Other key factors determining fruit yield are the respiratory cost of growth and ripening and the seasonal photosynthetic efficiency of the crop (Amthor 1984; Cannell 1985).

Respiratory losses from fleshy fruit, during growth and ripening, have been documented for several crop species (Kidd and West 1925; Jones *et al.* 1964a; Blanke *et al.* 1985). However, the contribution of fruit to their own carbon economy should not be ignored. Previous studies with young green fruit have established their photosynthetic contribution to the carbon requirement for growth and maintenance (Bean and Todd 1960; Todd *et al.* 1961; Kriedemann 1968; Bazzaz *et al.* 1979; Flinn *et al.* 1977; Jones 1981). For example, pods of pea (*Pisum sativum* L.) exhibited a net photosynthetic gain during the first 30 days after anthesis but thereafter respiration losses exceeded CO_2 assimilation (Flinn *et al.* 1977). For oranges and lemons (*Citrus sinensis* and *C. limon*), grape (*Vitis vinifera* cv. Sultana), and apple (*Malus pumila* cvs. Jonathan & Golden Delicious), fruit respiratory losses of CO_2 during a diurnal cycle exceeded photosynthetic gains throughout fruit ontogeny (Bean *et al.* 1963; Kriedemann 1968; Clijsters 1969; Jones 1981). However, Clijsters (1969) demonstrated a 36% reduction in

[‡] **Whiley, A. W., Schaffer, B. and Lara, S.P., 1992.** Carbon dioxide exchange of developing avocado (*Persea americana* Mill.) fruit. *Tree Physiol.* 11, 85-94. **APPENDIX 2**

the growth of apples when photosynthetic activity was inhibited by excluding light from developing fruit.

Wolstenholme (1986, 1987) calculated that the oil-accumulating avocado fruit has a high energy requirement for growth (energy value at maturity of 807.2 kJ 100 g^{-1} for cv. Fuerte at 17% oil compared with 262.8 and 292.5 kJ 100 g^{-1} for apples and citrus, respectively). Avocado fruit are climacteric (Eaks 1980), and the respiratory sequence is initiated by detachment from the tree. Previous studies on avocado fruit respiration have been conducted exclusively with detached fruit at various stages of development and to the author's knowledge, there are no reports on net CO_2 exchange of this fruit attached to the tree throughout ontogeny. The fruits remain green from setting until maturity and have a high stomatal density (50 to 75 stomata mm^{-2} shortly after fruit set), with active stomata similar to those of leaves, facilitating gas exchange (Blanke and Bower 1990). Total chlorophyll concentration in the mesocarp is only 12 to 30% that of the peel concentration (Cran and Possingham 1973). Thus, a fruit has the potential for photosynthetic activity, thereby contributing to its own carbon requirements during growth. Refixation of respiratory CO_2 within fruit by phosphoenolpyruvate carboxylase (PEPC) may be a significant contributor to fruit photosynthesis (Blanke and Lenz 1989). This mode of CO_2 refixation in fruit may also be present in avocado, because PEPC has been found in avocado fruit (Blanke and Notton 1991).

The purpose of this study was to determine the dynamics of CO_2 efflux from avocado fruit from post anthesis to fruit maturity and to assess the contribution of fruit to their own carbon economy from fixation of atmospheric CO_2.

4. 1. 2 Materials and Methods

Avocado trees (*Persea americana* var. *americana* x *P. americana* var. *guatemalensis*, cv. Booth-7) growing at the University of Florida, Tropical Research and Education Center, Homestead, Florida (25^{o}N latitude) were used in this study. Trees were on 'Waldin' or 'Lula' seedling rootstocks and were 35 years old at the beginning of the experiment. Trees were maintained with standard fertilisation, irrigation and pest control practices recommended for avocado in Florida (Malo and Campbell 1983).

From three weeks after anthesis (early April, 1989) to fruit maturity (mid-September, 1989), CO_2 efflux from attached fruit was determined in the field at 14-day intervals for three fruit on each of five trees. Since fruit were harvested at the end of each measurement period, different fruit were used for each measurement date. However, fruit growth rates within and among trees were fairly uniform, and fruit were tagged at set to ensure similar aged material was measured. Net CO_2 exchange was determined from CO_2 fluxes by enclosing individual small fruit in a Parkinson's leaf chamber (Analytical Development Co., Hoddesdon-Herts, England), or larger fruit in a 14 x 14 x 13 cm Plexiglass chamber containing a battery powered fan and a thermocouple. Net CO_2 exchange was determined with an LCA-2 portable open gas exchange system (Analytical Development Co., Hoddesdon-Herts, England) as describe by Schaffer and O'Hair (1987). Flow rate of ambient air into the chamber was maintained at 400 ml min^{-1} for the first five measurement dates and 600 ml min^{-1} for the later dates. Net CO_2 exchange was calculated using equations described by Jarvis (1971) and von Caemmerer and Farquhar (1981). Light respiration (R_l) of fruit was determined by measuring CO_2 efflux throughout the day at a minimum photosynthetic photon flux (PPF) of 600 µmol quanta m^{-2} s^{-1}, which exceeds the light saturation for CO_2 assimilation of avocado leaves (Scholefield *et al.* 1980). Immediately following measurements made in the light, the chamber was covered with two layers of black polyethylene. Dark CO_2 efflux, i.e. dark respiration (R_d), was then determined. Chamber air temperature was monitored, but not controlled, during CO_2 exchange determinations, and ranged from 31^oC to 45^oC during the course of the study. Respiration data were standardised to 30^oC by using temperature response data from each sampling date. This method has been used to standardise temperatures for peach respiration data (DeJong *et al.* 1987). Data were not collected until the CO_2 flux in the chamber had stabilised (about five minutes for small fruit during the first measurement date, and up to two hours for large, mature fruit). Immediately after each CO_2 exchange determination, the fruit measured were harvested from the tree and the dry weight of each fruit determined after slicing and drying at 60^oC.

Fruit R_d and R_l were expressed on a g_{dw}^{-1} and a $fruit^{-1}$ basis. Statistical models determining fruit growth over time and comparing fruit dry weight to fruit R_d and R_l were constructed by linear and nonlinear regression analysis. Fruit photosynthetic activity was calculated from the

difference between fruit R_l and fruit R_d at each point on the regression lines (Bean and Todd 1960; Clijsters 1969; Jones 1981).

Light interception by the avocado tree canopy was defined in a separate study carried out on a 5 m canopy diameter tree (cv. Hass) in subtropical south-east Queensland (lat. 27^{o}S). During flowering, which on avocado is mostly terminal to the last vegetative flush (Whiley *et al.* 1988a), and early fruit set, spot measurements of PPF (LICOR LI-190SA quantum sensor) were made within the fruiting zone and compared to the full sunlight position. At the completion of spring shoot growth, 1 m line quantum sensors (LICOR LI-191SA) were positioned in the fruiting zone of the tree, and at 0.5 and 1.0 m inside the canopy from the fruiting zone. The sensors were aligned as closely as possible at 90^o to the midday sun on the northern side of the tree. A fourth quantum sensor (LICOR LI-190SA), was positioned outside the tree canopy in full sunlight. The PPF was integrated hourly during the light period of each day using a LICOR-1000 data logger and the accumulated quanta at each line sensor expressed as a percentage of full sunlight. Mean values of the percentage of light intercepted at each point in the canopy, were calculated for a one week period. The quantum sensors were left positioned in the tree and the same PPF measurements were again collected approximately eight weeks later, after summer shoot growth had occurred.

4. 1. 3 Results

Fruit dry weight increased exponentially over time (Fig. 19). The increase was relatively slow during the first 10 weeks after anthesis and then increased rapidly from week 10 to fruit maturity (20 weeks after anthesis).

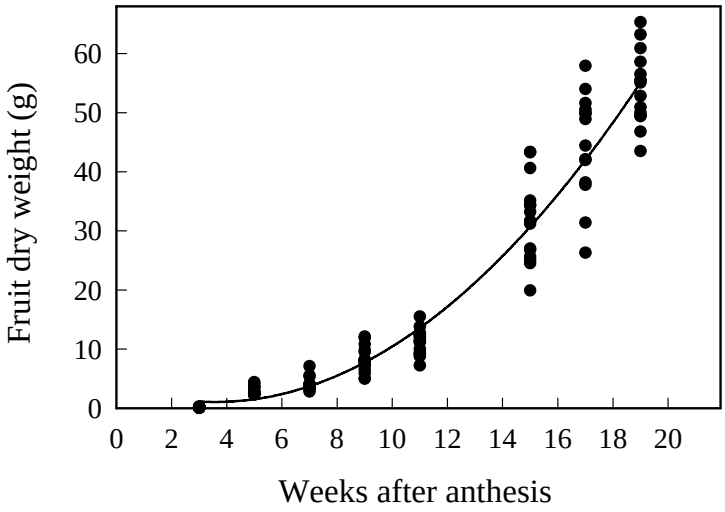

Fig. 19 Fruit dry weight of 'Booth-7' avocados during fruit development. The regression line is defined by the equation: $y = 3.94 - 1.618x + 0.227x^2$, $r^2 = 0.99$.

As fruit dry weight increased over time, R_d and R_l showed a similar CO_2 efflux pattern, on a dry weight basis (Fig. 20a). Dark respiration and R_l were highest three weeks after anthesis, 25 and 22 nmol CO_2 g_{dw}^{-1} s^{-1}, respectively. As fruit ontogeny progressed, R_d and R_l decreased and were lowest at fruit maturity, about 1.0 and 0.5 nmol CO_2 g_{dw}^{-1} s^{-1}, respectively. The difference between R_d and R_l decreased as fruit weight increased (Fig. 20a). This was concomitant with a reduction in the calculated fruit photosynthetic rate, from about 3.1 nmol CO_2 g_{dw}^{-1} s^{-1} during early fruit growth to about 0.5 nmol CO_2 g_{dw}^{-1} s^{-1} at fruit maturity (Fig. 20b).

The pattern of fruit respiration expressed on a $fruit^{-1}$ basis was similar in the dark and light (Fig. 21a). Until fruit dry weight reached 10 g, R_d and R_l (on a $fruit^{-1}$ basis) increased linearly as fruit developed. When fruit were about one-third of their maturation weight (20 g dry weight), respiration per fruit approached an asymptote and increased little until fruit were harvested (Fig. 21a). Dark respiration was always greater than R_l, and these differences were greatest when fruit dry weight was between 20 and 55 g (Fig. 21a). Respiration rates were highest at fruit maturity and were about 208 and 152 nmol CO_2 $fruit^{-1}$ s^{-1} or 34 and 25 mg CO_2 h^{-1} for R_d and R_l, respectively. Calculated fruit photosynthesis, expressed on a $fruit^{-1}$ basis, increased linearly as fruit dry weight increased from 0 to 20 g, then levelled off when fruit

reached approximately half their maturation weight (Fig. 21b). There was little increase in calculated fruit photosynthesis as fruit dry weight increased from 30 to 60 g.

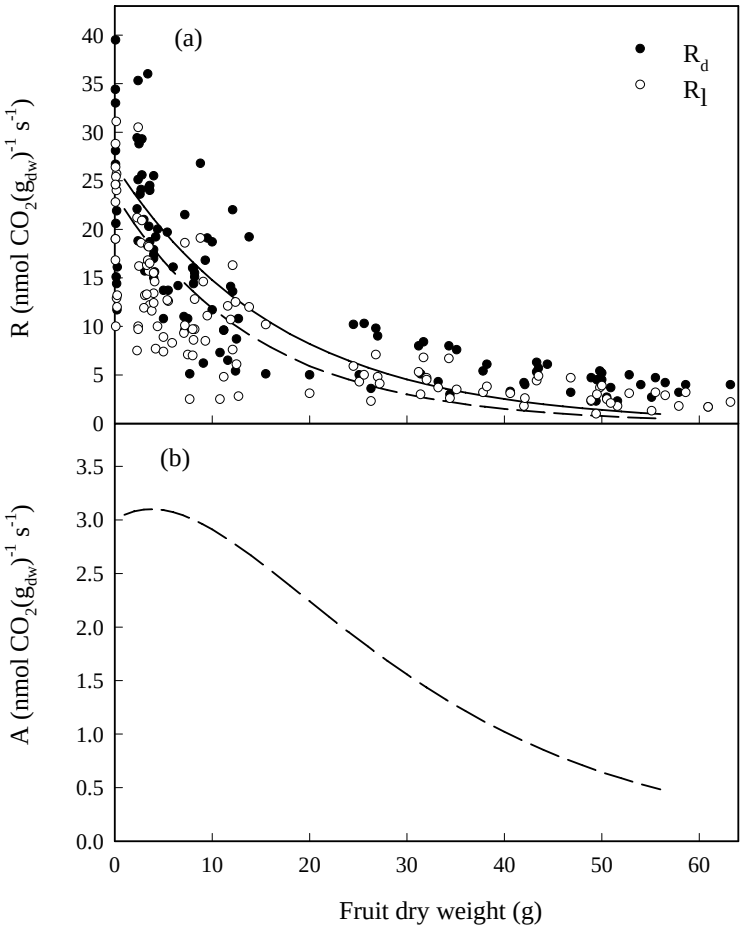

Fig. 20 (a) Fruit respiration of developing 'Booth-7' avocados in the dark (R_d) and in the light (R_l) expressed on a g_{dw}^{-1} basis where the regression line for R_d is defined by the equation: $y = 26.55e^{-0.057x}$, $r^2 = 0.60$ and the regression line for R_l is defined by the equation: $y = 19.98e^{-0.067x}$, $r^2 = 0.63$; (b) Net CO_2 assimilation (*A*), determined from R_d - R_l, of developing 'Booth-7' avocado fruit expressed on a g_{dw}^{-1} basis.

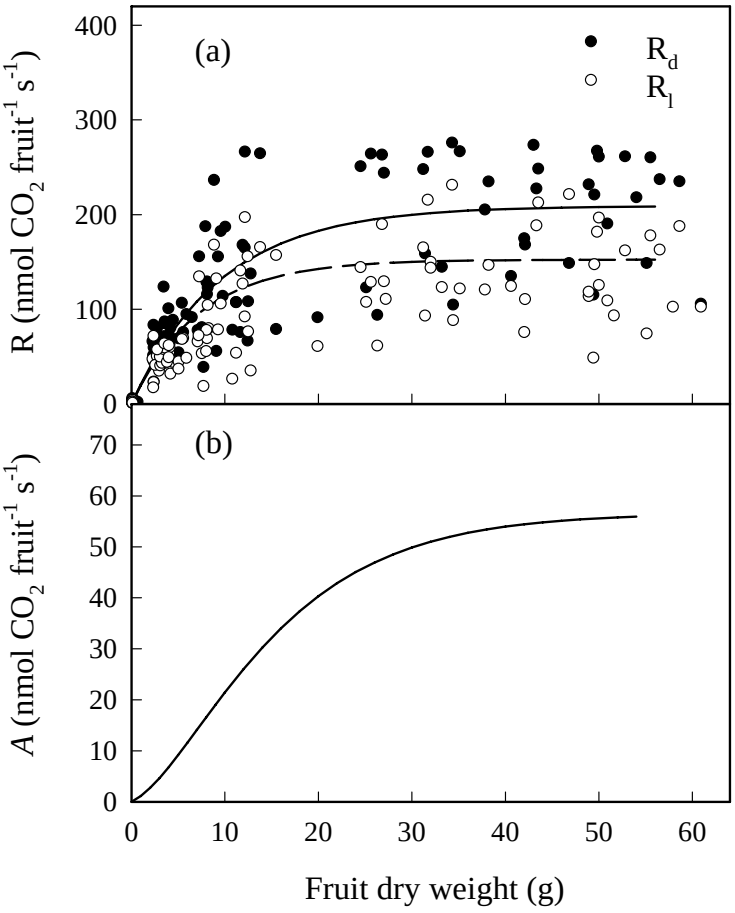

Fig. 21 (a) Fruit respiration of developing 'Booth-7' fruit in the dark (R_d) and in the light (R_l) expressed on a $fruit^{-1}$ basis where the regression line for R_d is defined by the equation: $y = 209.01(1 - e^{-0.107x})$, $r^2 = 0.71$ and the regression line for R_l is defined by the equation: $y = 140.60(1 - e^{-0.117x})$, $r^2 = 0.66$; (b) Net CO_2 assimilation (*A*), determined from R_d - R_l of developing 'Booth-7' avocado fruit expressed on a $fruit^{-1}$ basis.

PPF measurements taken during flowering and early fruit set indicated that most young fruit were exposed to full sunlight for the first four weeks of their development (data not presented). During the two periods when light interception data were integrated, daily PPF ranged from 15.5 to 59.5 mol quanta m^{-2} resulting from overcast and cloud-free days. By the end of spring

shoot growth, light transmission to the fruiting zone had been reduced to 35.9% of full sunlight with a further reduction to 13.7 and 9.7% at 0.5 and 1.0 m respectively, inside the canopy from the fruiting zone (Fig. 22). By the end of the summer shoot growth, light transmission to the fruiting zone had further declined to 13.1% of full sunlight and 9.7 and 6.3% at the related internal monitoring positions.

4. 1. 4 Discussion

The dynamics of R_d and R_l observed for attached, developing avocado fruit were similar to those observed for other crops (Jones *et al.* 1964a; Clijsters 1969; Jones 1981; DeJong *et al.* 1987; DeJong and Walton 1989). The highest respiration rates were observed during the early stage of fruit growth, from the first measurement date to about 12 weeks after anthesis, decreasing to the lowest rates at fruit maturity. The period of highest respiration rates corresponds to the time that cell division is greatest in avocado fruit (Valmayor 1967). This was similar to the patterns previously reported for avocado (Todd *et al.* 1961), apple (Clijsters 1969; Jones 1981), and peach (DeJong *et al.* 1987; DeJong and Walton 1989). The maximum R_d value measured at 30°C for avocado fruit, about 25 nmol CO_2 g_{dw}^{-1} s^{-1}, was similar to R_d measured at 20°C for apple fruit, about 26 nmol CO_2 g_{dw}^{-1} s^{-1} (recalculated from Jones 1981) and peach fruit at 20°C, about 28 nmol CO_2 g_{dw}^{-1} s^{-1} (DeJong *et al.* 1987). Jones (1981) reported the greatest difference between R_d and R_l for apple during the early phase of fruit growth and the least differences at fruit maturation. A similar response was observed for avocado fruit. When the R_d and R_l of avocado were expressed on a $fruit^{-1}$ basis, respiration at 30°C was highest at fruit maturity, about 34 and 25 mg CO_2 h^{-1} $fruit^{-1}$ for R_d and R_l, respectively. These values were somewhat lower than 50 mg CO_2 h^{-1} $fruit^{-1}$ measured at 23°C reported for avocado by Blanke (1991). The discrepancy between values may be due to experimental or genotypic differences. The cultivar used in Blanke's (1991) study was *P. americana* var. *drymifolia* cv. Fuerte whereas here the hybrid *P. americana* var. *americana* x *P. americana* var. *guatemalensis* cv. Booth-7 was used. The oil concentration in 'Booth-7' fruit (about 8%) is lower than that of 'Fuerte' (about 12 to 14%) at maturity (C.W. Campbell, pers. comm.[‡]). Presumably this leads to lesser energy demands for growth and development for 'Booth-7' than for 'Fuerte' (Wolstenholme 1986), resulting in lower respiratory activity in 'Booth 7' fruit.

At all stages of fruit development, fruit photosynthesis was substantially less than dark respiration. However, the calculated photosynthetic rate of developing avocado fruit (i.e. the difference between R_d and R_l; (Todd *et al.* 1961; Jones 1981), was highest during early fruit growth, about 3.0 nmol CO_2 g_{dw}^{-1} s^{-1}. The photosynthetic rates for the developing fruit were 42

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times less than rates for mature leaves, about 126.0 nmol g_{dw}^{-1} s^{-1} (B. Schaffer and A.W. Whiley, unpublished data).

Although chlorophyll concentrations in the peel of avocado fruit are similar to concentrations in the leaves (Cran and Possingham 1973; Schaffer *et al.* 1991) the differences in the maximum CO_2 assimilation rates between the two organs may be attributed to the difference in the chlorophyll a:b ratio, which is 1 to 2:1 in fruit (Cran and Possingham 1973) relative to 2 to 3.3:1 in leaves (Schaffer *et al.* 1991). However, the difference in the amount of CO_2 assimilated between the organs is more likely due to the greater surface area to volume ratio in leaves than in fruit, which results in a severe decline of light penetration into fruit tissue (only 0.02% of incident light penetrates more than 2 mm into the avocado mesocarp; Cran and Possingham 1973) and a change in the spectrum of photosynthetically active radiation (Blanke 1990). This relationship is further expressed by the declining net CO_2 assimilation (expressed as nmol CO_2 g_{dw}^{-1} s^{-1}) as fruit increase in size.

Vu *et al.* (1985) suggested that reproductive organs fix little atmospheric CO_2 via ribulose-bis-phosphate carboxylase (RuBPC) in the reductive pentose phosphate pathway. They reported the CO_2 assimilation PEPC:RuBPC ratio was 4 to 5:1 and 0.1:1 for citrus flowers and leaves, respectively. Furthermore, Blanke and Lenz (1989), Blanke (1990), and Blanke and Notton (1991) concluded that the refixation of CO_2 via the PEPC pathway, provides a significant contribution of carbon by the fruit to its own growth requirements.

4. 1. 5 Conclusion

The data from the present study indicate that avocado fruit contribute to their own carbon requirement by means of CO_2 fixation in the light, and that the relative contribution of fruit photosynthesis to the total energy requirement is greatest during the stages of early fruit development. This may be a significant factor influencing fruit retention as it coincides with the period of photoassimilate competition between reproductive and vegetative sinks (Biran 1979; Blumenfeld *et al.* 1983; Wolstenholme *et al.* 1990; Whiley *et al.* 1991), which extends for about 42 days after spring shoot growth commences (Whiley 1990). In addition the over-wintered leaf canopy has lost photosynthetic efficiency (A.W. Whiley, unpublished data) at a

time of critical assimilate demand. During this period young developing fruit are in full sunlight with the opportunity to maximise their photoassimilate contributions to growth. These data show that up to the end of spring shoot growth, when fruit have attained a size between 12 to 15 g dry weight, there is sufficient light during cloud-free conditions, to support fruit photosynthesis within the fruiting zone of the canopy. However, by the time the summer growth flush is complete (Whiley *et al.* 1988a), the light environment in the fruiting zone is unlikely to support photosynthetic activity in the fruit. At this stage of fruit ontogeny the renewed and photosynthetically efficient leaf canopy would meet all photoassimilate requirements of fruit growth.