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Photosynthesis of Avocado Fruit

Michael M. Blanke
Institut für Obstbau und Gemusebau, University of Bonn, Auf dem Hügel 6, D-5300 Bonn, Germany

Abstract. **Photosynthesis of developing avocado *(Persea americana* Mill.) cv. Fuerte fruit was examined. They exhibited 20,000 to 30,000 stomata per avocado fruit, but with a size of 9 to 12 µm x 16 to 19 µm they were smaller than found with other fruit. The guard cells of the stomata on the avocado fruit were of the common elliptical type and appeared functional. After anthesis, the maximal stomatal frequency was 50 to 75 stomata/mm^2, which decreased with surface expansion during fruit ontogeny, and is less than on the respective avocado leaf. Avocado fruit cv. Fuerte contained sun-type chloroplasts as found in leaves with C_3 photosynthesis. These fruit chloroplasts retained their structural integrity until after harvest and comprised grana, starch and chlorophyll. The chlorophyll content and chlorophyll a:b ratio (1-2:1) of the fruit were lower than in the respective avocado leaves (3-4:1). In the light, the fruit respired less CO_2 than in the dark. Respiration rates on a per surface area basis declined from 13 to 2 µmol CO_2/m^2/s, while they increased from 1 to 4 µmol CO_2/fruit/h. Respiration of fruit tissue also resulted in the accumulation of several percent CO_2 in the intercellular spaces, resulting in a gradient which decreases from the inside of the fruit to the outside, i.e., opposite of that of leaves. The enzyme phosphoenolpyruvate carboxylase (PEPC) recaptures part of the respiratory CO_2 accumulated within the fruit. Activity of PEPC in pre-climacteric avocado cv. Fuerte was 106 µmol CO_2/fruit/h when these fruit respired 40 to 60 µmol CO_2/fruit/h. These photosynthetic characters of avocado fruit were different from those of the respective leaves and could not be classified within an existing photosynthetic type. This suggests a new type of (avocado) fruit photosynthesis different from established C_3, C_4 or CAM photosynthesis in leaves or entirely heterotrophic metabolism in non-green storage organs rather than its categorization within one of these existing types.**

Avocado fruit require 807 kJ to produce 100 g of fruit, largely for their oil synthesis and seed formation, i.e. 3 to 4 times more energy than apple with 262 kJ required per 100 g of fruit (Wolstenholme, 1985).

Avocado fruit are largely heterotrophic, i.e., their growth depends on both import of leaf assimilates and contribution by the fruit's own photosynthesis which in other fruit differed from the C_3 photosynthesis of the respective leaves (Blanke and Lenz, 1989).

To examine this contribution of the avocado fruit to its conspicuous energy requirement, I have attempted to investigate and define photosynthesis of avocado fruit using the widely grown cv. Fuerte.

Materials and Methods

Avocado trees. Healthy avocado *(Persea americana* Mill.) trees were selected in a commercial orchard (Kevin Kaye, Avalon Farm, Whiteriver, Transvaal, SA) and were subjected to standard management procedures. The 7- to 8-year-old avocado trees cv. Fuerte were grafted to seedling Edranol rootstocks.

Scanning electron microscopy. Developing avocado fruit cv. Fuerte were shipped to Long Ashton, University of Bristol, England by air courier. The avocado fruit were examined by scanning electron microscopy within two hours of sample preparation. Fruit sections were gold-coated in an argon atmosphere in a Polaron sputter coater E 5000 for 3 mins at 0.5 kV to give a thickness of 18 nm and a fine grain of 1.8 nm.

The coated specimens were studied in a Philips SEM 505 at an accelerating voltage of 1 5 kV and a spot size of 50 nm. Micrographs were taken with a Leitz Leica 2 camera on llford FP 4 (22 DIN) film and developed on Agfa Brovira Speed BS310 paper as described by Blanke and Bower (1990a). Counts of the number of stomata were made using fruit in the week after anthesis (post-anthesis), when fruit weighed 1 g and were 1.2 cm long and 0.9 cm wide, to obtain values for the maximal stomatal density. Ten 0.25 mm x 0.25 mm squares were examined for the number of stomata and replicate fruit counted.

Gas exchange of fruit. Gas exchange of developing avocado fruit was measured by fruit porometry as described in detail in a parallel presentation (Blanke and ADC, 1992). A chamber was designed for developing avocado fruit which allowed fruit to remain attached and was connected to a portable porometer type LCA 3 on loan in South Africa from ADC (Hoddesdon, Herts., England). Physiological measurements were conducted on fruit on clear days between 07.00 h and 10.00 h for light and between 17.30 h and 19.30 h for dark respiration. In order to assess dark respiration, fruit were loosely covered in aluminum foil after 17.30 h and respiration measured 30 mins thereafter and on the next morning again under cover (Blanke, 1990d; Blanke and Whiley, unpublished).

Conditions of measurements were temperatures between 25C and 35C, water vapor pressure deficits between 3.2 to 4.5 kPa in daylight and 2.4 to 3.0 kPa at night, respectively, and photon flux densities above l000/µmol/m^2/s PAR.

Chlorophyll determination. Chlorophyll in the mesocarp from cv. Fuerte fruit was determined by immersion of undissected tissue into DMF (Blanke, 1990a).

Light transmission. Spectra of light transmitted by the peel of avocado fruit cv. Fuerte were recorded spectrophotometrically at Long Ash-ton, University of Bristol, England, as

described for grape berries by Blanke (1990b) with the outer surface of the peel facing the light source imitating the natural position of the peel and using the opposite opaque sides of the cuvettes facing the light source and detector. Transmission spectra were measured over the range of wavelengths from 450 nm to 800 nm in a double beam Pye-Unicam PU 8800 spectrophotometer with an empty cuvette, similarly placed, as reference. Solubilization of cuticular wax or pigments was avoided by excluding solutions from the sample and reference cuvettes.

Enzyme extraction. The enzyme phosphoenolpyruvate carboxy-lase (PEPC, orthophosphate: oxaloac-etate carboxylyase [phosphorylating], EC 4.1.1.31) was extracted from avocado fruit cv. Fuerte and partially purified by ammonium sulfate precipitation and gel filtration. Enzyme activity was determined by coupling the reaction of PEPC to the malate dehydrogenase-dependent oxidation of NADH, measured at 340 nm in a computerized Pye-Unicam PU 8720 spectrophotometer.

Results and Discussion

Stomata and surface features of avocado fruit. Developing avocado fruit of cv. Fuerte exhibited a large number of stomata, i.e., 20,000 to 30,000 per avocado fruit, compared with temperate zone fruit of generally smaller final fruit size such as apple with ca. 3000 and grape berry with 30 stomata per fruit. The number of stomata per fruit remains unchanged during its life cycle assuming no epidermal cell division during fruit ontogeny with the formation of new stomata. Stomata of cv. Fuerte avocado fruit were 9 to 1 2 µm x 1 6 to 19 µm (vestibule 3-4 µm x 9-10 µm), i.e. smaller than the stomatal sizes of 13 to 21 µm x 21 to 33 µm found for apple (Table 1) and citrus fruit (Blanke, 1990c). After anthesis, the maximal stomatal frequency on cv. Fuerte avocado fruit was 50 to 75 stomata/mm^2, which decreased to less than 5 stomata/mm^2 depending on surface expansion during fruit ontogeny, and is respectively 14 % at post-anthesis or less than 1 % at fruit maturation of the 390 to 510 abaxial stomata/mm^2 on the respective avocado leaf (Blanke, unpublished). Stomata on leaves of subtropical fruit crops are often more numerous, but smaller in size than those of temperate zone crops (Blanke, 1990c). The present results confirm this statement for avocado fruit. The guard cells of these relatively small stomata on the avocado fruit were of the common elliptical type and appeared functional (Fig. 1 and 2). The stomata linked the intercellular space of the avocado fruit to the ambient, thereby facilitating gaseous efflux of CO_2, H_2O and C_2H_4 through stomata or lenticels which, when open, are the preferred sites of gas exchange, even if guard cells should lose their regulatory function. Changes in the ambient conditions may affect the transpirational water vapor efflux from the fruit and hence, control stomatal opening and mineral import into the fruit. Blanke and Bower (1990b) calculated the percentage of the surface of a stomatal vestibule to the avocado fruit surface (using an average stoma diameter of about 5 µm) to be ca. 0.0012 % after anthesis: compare this figure to 0.035 % for the grape berry due its larger stomata and smaller final fruit size (Blanke and Bower, 1990b) and 0.06 % recalculated for avocado leaves from data of Whiley *et a/.* (1988). These stomata on cv. Fuerte avocado fruit have been shown (Blanke and Bower, 1990a) to interrupt a surface of smooth appearance with a disperse distribution of fine platelets and few, short, single-celled

trichomes after an-thesis. Three weeks after anthesis, a typical pattern of rectangular honeycombs was reported (Blanke and Bower, 1990a) with groups of parallel lamellae differing in orientation and surrounding the stomata shown in figures 1 and 2.

Chloroplasts and chlorophyll in avocado fruit. Energy for metabolic conversion within the fruit is provided by (a) respiration of assimilates imported from adjacent leaves or (b) the photosystems of the fruit associated with the chlorophyll in the chloro-plasts. Avocado fruit cv. Fuerte contained sun-type chloroplasts as found in leaves with C_3 photosynthesis. These fruit chloroplasts in the outer, green mesocarp also comprise grana, starch and chlorophyll. The grana have fewer thylakoids, i.e., ca. 50, than in leaves with up to 100 thylakoids per granum (Blanke, 1990c). Starch formation with the aid of chlorophyll in sun-type chloroplasts indicates the involvement of light and the photosystems. The chlorophyll content of the cv. Fuerte fruit, however, was smaller than in the respective avocado leaves on a per surface area basis. The chlorophyll a:b ratio was also smaller in fruit (1-2:1) than in leaves (3-4:1). In higher plants, chlorophyll b is an auxiliary pigment passing excitation to chlorophyll a and photosystems PS II (P 680) and PS I (P 700). Lower chlorophyll a:b ratios may be indicative of less PS II and more PS I in the avocado fruit. Relative to the thickness of the respective leaf, the lower chlorophyll a:b ratio of the avocado fruit may be a sun-shade response with light transmission into the fruit declining with volume increase. Avocado fruit chloroplasts retain their structural integrity until after harvest (Platt-Aloia and Thompson, 1981) in contrast to many temperate zone fruit whose chloroplasts may disintegrate before harvest. The chloroplasts produce oxygen which maintains the oxygen level in the intercellular space between 7 and 19% O_2 (Blanke, 1991). The intercellular concentrations of CQ and oxygen are a result of the amount of respiratory CO_2 evolved and oxygen consumed within the fruit, the diffusivity of the peel and mesocarp and the intercellular space volume.

Light transmission into avocado fruit. In the orchard, light penetration and distribution within the canopy is affected by arrangement, reflectance and indirect shading of leaves, fruit and branches. Light penetration drops within the tree thereby affecting fruit quality. The smooth, thin and green peel of the cv. Fuerte avocado fruit transmitted 0.7% incident light at 500 nm which increased to 9.5% at 800 nm (Fig. 3). This spectra of light transmitted through the peel of cv. Fuerte avocado fruit (Fig. 3) varied little in curvature with fruit ontogeny. The spectra showed a major absorption peak at ca. 670 nm, one of the wavelengths where chlorophyll absorbs and was most pronounced with the peel of the cv. Fuerte fruit. The secondary absorption peak of chlorophyll around 440 nm was probably masked by the increasing light absorption of the peel below 500 nm (Fig. 3). The light transmitted by green mesocarp also showed the chlorophyll absorption peak at 670 nm, but the spectra of the transmitted light varied less from the incident light. Hence, the light spectra that the fruit chloroplasts receive is altered from that of the incident light by the peel and, to a lesser extent, by the mesocarp. Magnitude and spectra of the light transmitted by the peel of avocado cv. Fuerte fruit are similar to those found with the peel of green Golden Delicious or Granny Smith apple fruit which transmitted 0.1 to 8 or 3 to 10% of incident light respectively. Further research is needed to show how the peel of dark, thick and rough-skinned avocado fruit such as cv.

Hass affects light transmission into the fruit, spectral light distribution within the fruit and light reflection from the fruit.

Light reflection from avocado fruit. Avocado fruit of cv. Fuerte appear green to the human eye due to the absorption of both blue and red light in the peel. On both fruit and leaf surfaces, hairs and wax may reflect visible light. However, the scarcity of hairs or trichomes which disappear during fruit ontogeny of the cv. Fuerte avocado fruit and its continuously smooth, green appearance indicate a constant reflectance value; this assumes Constance in both wax crystallization and shape.

Regulation by phytochrome. The fruit photosynthesis concept (Blanke and Lenz, 1989) proposes a phytochrome system reacting to changes in the red:far red (660nm:730 nm) ratio for the regulation of some metabolic processes in developing fruit. Calculations of these ratios from the data of figure 3 show that the avocado peel reduced the red:far red ratio from ca. 1 to 1.2 in natural sunlight to ca. 0.2 inside the fruit (Table 2).

Fruit respiration and bioenergetics. Assimilates, imported as transport metabolites, are unsuitable for storage within the fruit. For their conversion to forms suitable for storage, energy has to be provided. Therefore, the fruit respires at the expense of accumulated or imported substrates which ultimately derive from light absorption and contain 807 kJ per 100 g of avocado fruit (Wolstenholme, 1985). Respiration rates are often used to calculate heat output or energy requirements. Based on the breakdown of hexoses as respiratory substrates, dissimilation of 1 g CO_2 is equivalent to an energy yield of 13 kJ (Blanke and Lenz, 1989). In a fruit cell, CO_2 from mitochondrial respiration dissolves, accumulates and diffuses. Within the fruit, the CO_2 molecule has to overcome several diffusion resistances from the mitochondria via the cell wall and intercellular space before it may be released via the peel. Avocado fruit mitochondria are associated into groups with a compact matrix and numerous tubular cristae and are 0.8 to 1.2 *µm* in size; the retention of structural integrity of the avocado fruit plastids until after harvest also applies to their mitochondria (Platt-Aloia and Thompson, 1981). This may be partly reflected in the respiratory activity (Fig. 4). Field measurements with a portable fruit porometer in commercial avocado orchards showed that avocado cv. Fuerte fruit respired more CO_2 in both light and dark than they assimilated from ambient air (Fig. 5). In the light, however, cv. Fuerte fruit respired less CO_2 than in the dark irrespective of units employed. Previously, "photosynthetic rate" was defined as the difference between fruit respiration in the dark and in the light using detached fruit, but it can be argued that this value is more a measure of the respiratory activity and may be sensitive to (a) effects of raising temperature when the detached fruit in a cuvette is radiated for a longer period to simulate the light phase and (b) effects of fruit detachment such as wound respiration and withdrawal of assimilate supply (Blanke, 1990c,1990d). Respiration rates of cv. Fuerte fruit on a per surface area basis declined from 13 to 2 µmol $CO_2/m^2/s$ during fruit ontogeny, while they increased when expressed on a per fruit unit; under field conditions, avocado fruit lost 1 to 4 µmol CO_2/fruit/h (Fig. 5). However, respiration of fruit tissue also resulted in the accumulation of CO_2 in the intercellular spaces of the fruit to several percent CO_2.

Accumulation of respiratory CO_2 in the intercellular space. Due to the" small stomatal frequency relative to leaves, the diffusive resistance to $C0_2$ is dominated by the cuticular component which is largely uncontrolled and less permeable to gases than the mesocarp. The overall CO_2 gradient therefore decreases from the inside of the fruit to the outside (“from seed to peel"), i.e., is in reverse order to that of leaves (Blanke, 1991). These are gradients of CO_2 , but not pressure and indicate also a relatively large diffusive resistance (or small conductance) within the fruit, because CO_2 would otherwise rapidly equilibrate. Several methods have been used with fruit to determine the intercellular concentration of gases including manometric techniques, removal of plugs of tissue, insertion of a hypodermic needle into the fruit or evacuation of fruit under water. The two latter techniques are followed by gas chromatography of the withdrawn gas sample. Methods applying considerable vacuum for several minutes overestimate, while calculations using CO_2 efflux from the fruit underestimate the intercellular concentration. Values derived from CO_2 efflux apply to the substomatal or lenticular spaces of the fruit only, whereas values obtained by gas chromatography without applying a severe vacuum represent the intercellular CO_2 concentration.

Refixation of respiratory CO_2 by phosphoenolpyruvate carboxvlase". Avocado cv. Fuerte contain less chlorophyll, a lower chlorophyll a:b ratio and lose CO_2 not only during the night, but also in the daytime. Hence, I assumed that the avocado fruit probably fixes only a small amount of atmospheric CO_2 via ribulose 1,5 bisphosphate carboxylase (RuBPC) in the reductive pentose phosphate (RPP) pathway. However, the enzyme PEPC has been reported to refix some of the respired CO_2 which accumulated in the intercellular space of fruit and is in equilibrium with bicarbonate (HCO_3) in the cell (Blanke and Lenz, 1989). The enzyme PEPC catalyses the carboxylation of phosphoenolpyruvate (PEP) with bicarbonate (HCO_3) to produce oxaloacetate (OAA) (Fig. 6) under physiological conditions (Blanke and Notion, 1991). By enzymic reaction of malate dehydrogenase, oxaloacetate is further transformed into L-malate (Fig. 6). The activity of PEPC in the mesocarp of pre-climacteric avocado cv. Fuerte fruit was 2.5 µmol CO_2/s/g fresh weight or 106 µmol CO_2/fruit/h. This value exceeded the respiratory CO_2 loss from the fruit in the order of 40 to 60 µmol CO_2/fruit/h. This CO_2 recycling provided by PEPC resembles the CO_2 concentration mechanisms found in leaves with C_4 and CAM photosynthesis, but not in leaves of fruit crops such as avocado with C_3 photosynthesis.

Fruit photosynthesis. Different forms of the enzyme PEPC have been found to be indicative of the photosynthetic type present in a tissue (Blanke and Lenz, 1989). These forms differ in their kinetic properties, sensitivity to heat and in susceptibility to activators and inhibitors (Blanke and Notion, 1991). In avocado fruit, PEPC showed a high substrate affinity for both substrates, PEP and bicarbonate (HCO_3) as shown in a parallel presentation (Notion and Blanke, 1992). The higher substrate affinity of PEPC in fruit was associated with smaller enzyme activities in fruit relative to tissues possessing other photosynthetic types.

The kinetic properties of CO_2 refixation by PEPC, relative scarcity of stomata, sun-type C3 chloroplasts, low chlorophyll content and low a:b ratio, large light perception,

accumulation of CO_2 to the percent level in the intercellular space and respiratory CO_2 efflux from the fruit in the dark and light showed that photosynthesis of the avocado fruit was different from that of the respective leaves and cannot be classified within an existing photosynthetic type, suggesting a new category of fruit photosynthesis also for ihe avocado fruil.

Conclusion

Much of the fascination in studies of avocado fruit photosynthesis lies in attempting its classification and to explain the differences from that of its respective leaves. This fascination also lies in the potential to stimulate energetically economic mechanisms of the avocado fruit and their usage in order lo improve fruit quality by avoiding pre-and postharvest physiological disorders and maximizing yield potential.

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Table 1: Stomatal size and distribution in cv. Fuerte avocado fruit in relation to its respective leaves and apple fruit cv. Golden Delicious.

	Units	Avocado cv. Fuerte	Apple cv. Golden Delicious
Fruit:			
Stomata	fruit	20,000-30,000	2,000-6,000
Maximal stomatal frequency (post-anthesis)	mm^2	50-75	15-25
Minimal stomatal frequency (maturation)	mm^2	less than 5	less than 1
Stomatal size	μm	9-12 x 16-19	13-21 x 21-33
Leaf:			
Stomatal frequency	mm^2	390-510	320-390

Table 2. Red (660 nm):far red (730 nm) ratio of light transmitted by leaf and peel of avocado.

Avocado tissue	Light transmission (%)		Red:far red ratio
	660 nm	730 nm	
Fruit peel	1.5	8.4	0.18
Leaves	0.9 – 1.5	6.5 – 9.0	0.11 – 0.22
Sunlight	-	-	1.0 – 1.2

Fig. 1. SEM micrograph of developing avocado fruit cv. Fuerte one week after anthesis (post-anthesis). Gross cuticular characteristics were not evident at this stage of development. Individual trichomes or hairs developed. Stomata with elliptical guard cells appeared functional (magnification x 625).

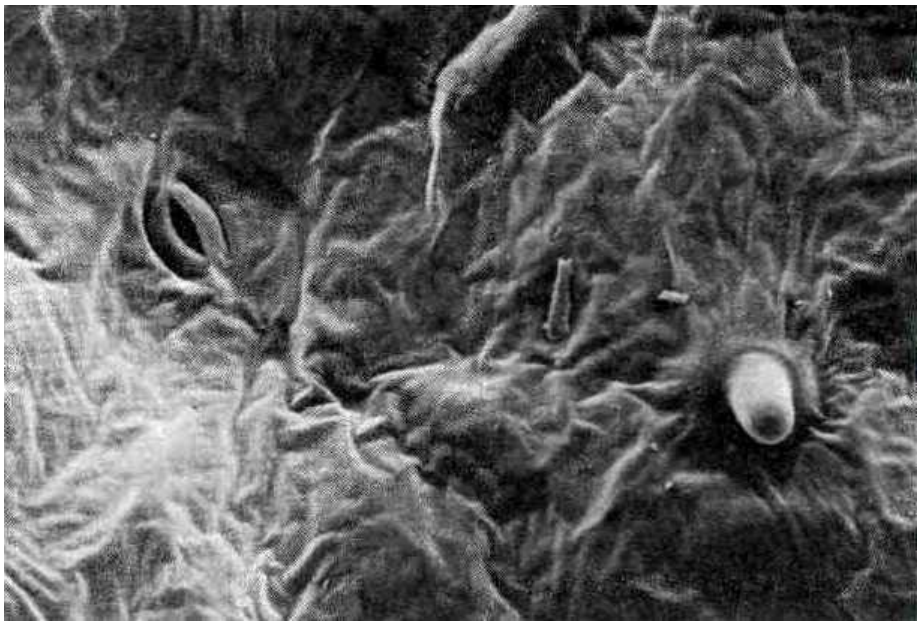


Fig. 2. SEM micrograph of developing avocado fruit cv. Fuerte three weeks after anthesis showing surface characteristics. The cuticular striations with parallel lamellae surround the stomata (magnification x 440)

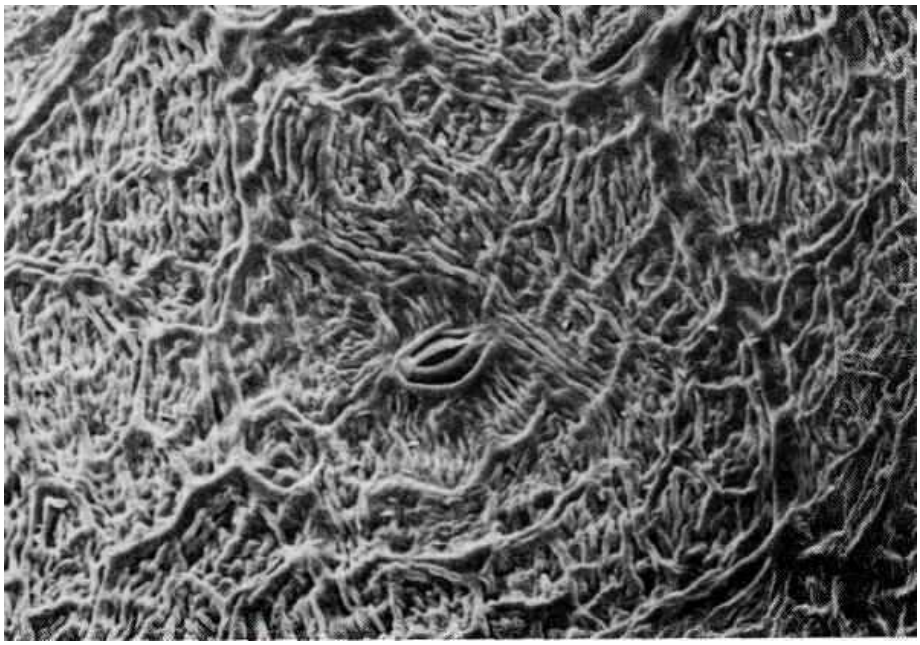
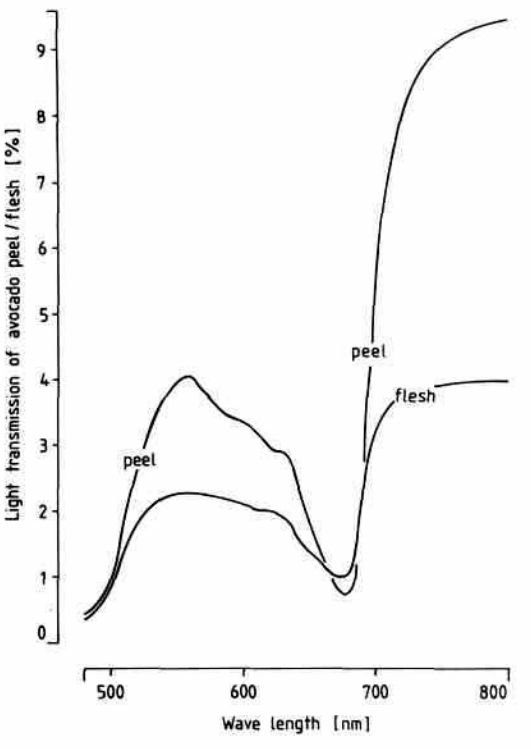


Fig. 3. Spectra of light transmitted by the peel (top line) and mesocarp of the thin-skinned cv. Fuerte fruit. Values for the flesh originate from a 1 to 2 mm slice of the mesocarp. The peel of avocado fruit cv. Fuerte transmitted ca. 4 % of incident light at 560 nm and more than 9.5 % above 780 nm. The low transmittance around 670 nm is due to the absorption by chlorophyll.



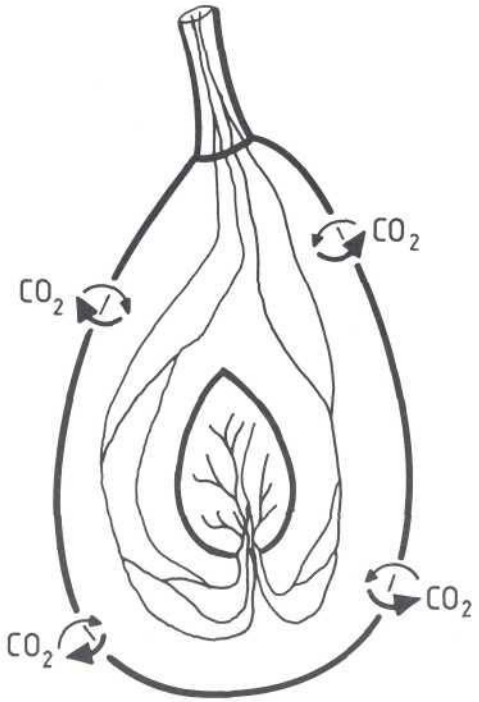
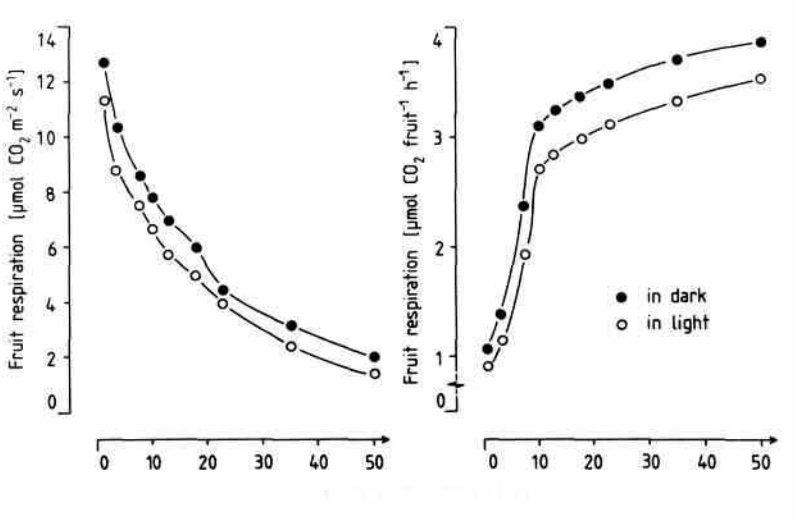


Fig. 4. Schematic of CO_2 exchange which occurs preferentially through stomata of the peel of a cv. Fuerte avocado fruit.

Fig. 5. Respiration of avocado fruit cv. Fuerte measured in the field with fruit still attached to the tree. Respiration rates declined during fruit ontogeny when data are expressed per surface area unit (left figure) or increased on a per fruit basis (right figure). In the dark, avocado cv. Fuerte fruit respired faster than in the light irrespective of units employed.



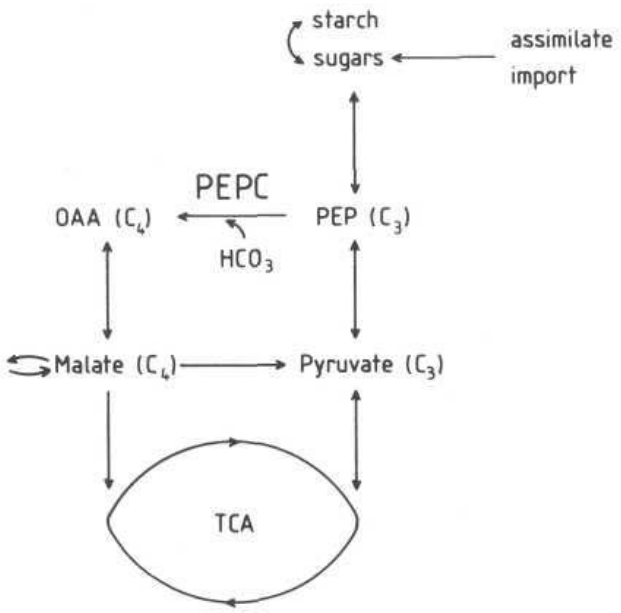


Fig. 6. Biochemical pathways involved in the CO_2 refixation in the mesocarp of avocado fruit. The avocado fruit imports leaf assimilates which are converted within the fruit and also provide PEP. The enzyme PEPC combines PEP with CO_2 in its soluble form of bicarbonate (HCO_3). The product of this reaction, OAA is transformed via malate dehydrogenase to L-malate which can be stored in the vacuole.