Developmental Changes in Translocation and Localization of ^{14}C -labeled Assimilates in Grapefruit: Light and Dark CO_2 Fixation by Leaves and Fruit

Chung-Ruey Yen¹ and Karen E. Koch

Fruit Crops Department, University of Florida, Gainesville, FL 32611

Additional index words. *Citrus paradisi*, citrus, photosynthate partitioning, photosynthesis

Abstract. Distribution of radiolabeled assimilates was examined at various intervals after 1 hour of light or dark $^{14}\text{CO}_2$ fixation by leaves or developing fruit of grapefruit (*Citrus paradisi* Macf.) so that the fate of assimilates from each source could be assessed at sequential stages of fruit growth. Exported products of both light and dark $^{14}\text{CO}_2$ fixation in leaves were deposited primarily in juice tissues of fruit even during periods of substantial dry weight accumulation by peel. Fruit photosynthesis, however, gave rise to assimilates that remained almost entirely in the peel (flavedo and albedo) even 7 days later, regardless of dry matter increases by other tissues. Products of dark $^{14}\text{CO}_2$ fixation by intact fruit were recovered in all tissues but predominated in the peel of young fruit vs. juice tissues at later stages of growth. Comparison of dry matter gains and ^{14}C -labeled assimilate distribution indicated that fruit photosynthesis likely contributed substantially to development of peel but not juice sacs. Data on dark $^{14}\text{CO}_2$ fixation were consistent with its suggested involvement in organic acid synthesis by juice sacs.

The vast majority of assimilates are produced by leaves during photosynthesis in light, but CO_2 fixation occurs in fruit as well as leaves, and in both light and dark. Partitioning and translocation of photosynthates derived from source leaves of whole plants are relatively well-documented in various commercially important food species (Daie, 1985; Wardlaw, 1968; Wareing and Patrick, 1975). Less information is available for fleshy fruit crops, including citrus, but these fruit are known to be strong sinks for leaf photosynthates (Kadoya, 1974; Kriedemann 1969a, 1969b). The presence of fruit can increase photosynthetic efficiency of citrus leaves and partitioning of photosynthates (Lenz, 1979). Leaf photosynthates enter the fruit interior very slowly, however, and are transferred via relatively few vascular bundles and extensive areas of juice sac stalks completely lacking phloem (Koch, 1984; Koch and Avigne, 1984, 1990; Koch et al., 1986). Seasonal alteration has been reported by Brown (1974) for the extent of leaf-derived assimilates arriving in navel oranges. We found no reports examining changes that occur in photosynthate translocation or distribution within these fruit during their development, however.

Photosynthesis also occurs in fruit of many crops. Studies of pea and soybean indicate that pods can provide photosynthates to developing seeds by reassimilation of respiratory CO_2 (Atkins et al., 1977; Quebedeaux and Chollet, 1975). In a young green apple or citrus fruit, photosynthetic CO_2 fixation is of sufficient magnitude to conserve 20% to 80% of the CO_2 released by dark respiration, depending on the stage of development (Bean et al., 1963; Kidd and West, 1947; Schaedle, 1975). Also, $\approx 25\%$ of the ^{14}C -labeled photosynthates produced in satsuma mandarin fruit were recovered 5 days later in juice tissues (Akao and Tsukahara, 1979). Fruit photosynthesis is reported to decrease toward maturity in citrus (Bean and Todd, 1960; Moreshet and

Green, 1980; Ramakrishnan and Varma, 1959; Todd et al., 1961), and its total contribution to orange and lemon growth is believed to be relatively small compared with that of assimilates translocated from leaves (Moreshet and Green, 1980; Todd et al., 1961). Little other information is available on partitioning, distribution, or redistribution of photosynthates produced by grapefruit or other fleshy fruits.

Dark CO_2 fixation, an anaplerotic reaction contributing to amino and organic acid formation (Bidwell, 1983), is involved in normal growth and development of many tissues from roots (Splittstoesser, 1966) to flower buds of 'Valencia' oranges (Vu et al., 1985). Organic acid synthesis in citrus juice tissues may well be another example. Minimal research has been directed toward analysis of assimilates arising from nonphotosynthetic carboxylation reactions in fruit. However, greater amounts of ^{14}C -labeled assimilates are found in citrus juice sacs than surrounding tissues after each is separately exposed to $^{14}\text{CO}_2$ in darkness (Bean and Todd, 1960). Greater proportions of labeled organic acids (predominantly citric) and amino acids are also recovered in juice tissues after dark $^{14}\text{CO}_2$ fixation by fruit than by photosynthesis of the same tissues. Dark CO_2 fixation has long been considered one possible source of organic acid synthesis in citrus (Bean and Todd, 1960; Huffaker and Wallace, 1959; Young and Biale, 1968). Neither the extent of dark CO_2 fixation in whole, intact fruit nor developmental changes in these reactions have been examined.

We therefore compared the distribution of labeled assimilates derived from $^{14}\text{CO}_2$ fixation in light and darkness by grapefruit and adjacent source leaves throughout fruit development.

Materials and Methods

Fruits and/or branches with source leaves adjacent to the fruit were randomly selected from between 1 and 2 m above ground level on the exterior canopy of 4-year-old grapefruit trees ('Foster' seedless clone no. 1-26-39) on 'Savage' citrange or sour orange rootstock in Lake Wales, Fla. (Hearn, 1986). There were no evident differences in quality (size, composition, and development) among fruit from trees on these two rootstock (data not shown). Samples from a given tree were used for various analyses. Each tree represented one replication and four trees were used for each experiment. Fruit with 10-cm subtending branches were cut from trees on 7 May, 25 June, 27 July,

Received for publication 6 Nov. 1989. Univ. of Florida Journal Series no. R-00794. This work is a portion of the thesis submitted by C-R Yen in partial fulfillment of a PhD degree at the Univ. of Florida. The research was partially funded by USDA-CRGO grant no. 84-CRGR-1-1478, the Florida Institute of Food and Agricultural Sciences, and the Council of Agriculture, Republic of China. Use of product names does not imply an endorsement by the Univ. of Florida. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

¹Present address: Chia-Yi Agricultural Experiment Station, Chia-Yi, Taiwan.

and 10 Sept. 1986 (≈ 40 -day intervals), at 2, 3.5, 5, and 6.5 months post-anthesis, respectively. Pedicels and branch bases were immediately immersed in water and all but a single large leaf nearest the fruit were removed. A similar procedure was used to harvest fruit on small branches, (leaves not included) on 7 June, 8 July, and 10 Sept. 1985, ≈ 3 , 4, and 6 months, respectively, after anthesis.

At each developmental stage, detached fruit or the fully expanded leaf nearest to an attached fruit was enclosed in a cuvette (143 ml for leaves and either 2.0 or 5.0 liter for fruit) and exposed to $13.6 \mu\text{Ci}$ ($1 \text{ Ci} = 37 \text{ GBq}$) $^{14}\text{CO}_2$ /liter for 1 hr in light followed by ambient air for 24 hr (12 hr light : 12 hr dark photoperiod). A continuously recirculating airflow was passed through each cuvette at $1.0 \text{ liter}\cdot\text{min}^{-1}$ using a closed, flow-through gas analysis system with an infrared CO_2 analyzer (Model 865; Beckman Instruments, Fullerton, Calif.) Before each labeling period, the system was opened, flushed with N_2 , and immediately filled using a compressed source of air and $^{14}\text{CO}_2$ of a known specific activity. The system was then closed and total CO_2 levels monitored throughout the exposure period. Temperature was maintained at 28°C and photosynthetic photon flux density at 800 to $1000 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$. Light was provided by a Lucalox lamp (General Electric, Cleveland, Ohio). Similar experiments were conducted in 24 hr of continuous darkness using cuvettes completely sealed with several layers of aluminum foil. Fruit were exposed to $^{14}\text{CO}_2$ within a few hours after pedicels or branches were removed from trees, and studies with source leaves were conducted the following morning. Distribution of ^{14}C -labeled assimilates within fruit on such branches was comparable to results obtained in studies using intact, container-grown trees (Koch and Avigne, 1990).

Experiments were terminated by separating tissues into leaves and pedicels (if present), flavedo (outer 2 to 3 mm of pigmented peel), albedo (inner, nonpigmented peel), and juice tissues. Samples were frozen in liquid N_2 , boiled in 80% (v/v) ethanol, and refrozen for subsequent grinding and further ethanol/water extraction as described by Koch (1984). Radioactivity in each sample was quantified by liquid scintillation spectrometry (LKB, Gaithersburg, Md.). The ethanol-soluble fraction was measured by pipetting 0.1 ml of sample into 15 ml scintillation cocktail (Scinti Verse II, Fisher Scientific, Fair Lawn, N.J.). The ethanol-insoluble fraction was measured by suspending a known portion of the fraction (oven-dried for uniform weight) in scintillation gel [1:4, coater : ScintiVerse II (Fisher Scientific, Fair Lawn, N. J.)]. Particulate material was evenly distributed in this gel, and minimal differences were observed between duplicate samples of each insoluble fraction.

Fresh and dry weights were determined for separate samples before and after 4 days of oven-drying at 60°C .

Results and Discussion

Fruit development. Fruit dry weight increased continuously during the developmental period examined, but did so most markedly during the 3rd and 4th months of growth (May to June) (Fig. 1A). Flavedo and albedo of peel accumulated the majority of their dry weight during this period. Increases in dry weight of juice tissues accounted for almost all change in total fruit dry weight during the second half of fruit growth.

Fresh weight gains during development (Fig. 1B) were proportional to those of dry weight but more pronounced in juice tissues. Both fresh and dry weights followed trends similar to those observed in other citrus fruits (Bain, 1958; Sinclair, 1984). The rapid increase in fresh and dry weight of fruit was typical

Fig. 1. Developmental changes in terms of dry (A) and fresh (B) weights of tissues in grapefruit ('Foster' seedless clone no. 1-26-39). Vertical bars denote SE of four replications but are present only where larger than tissue/fruit symbols.

of Stage II development observed in grapefruit (Lowell et al., 1989) and 'Valencia' oranges (Bain, 1958).

Distribution of assimilates from fruit vs. leaf sources during 24-hr experiments. In May, when young fruit were accumulating dry weight most rapidly, $\approx 58\%$ of the total ^{14}C -labeled photosynthate from adjacent source leaves was transported into fruit within 24 hr (Fig. 2). The percentage of ^{14}C -labeled photosynthate exported from leaves to fruit decreased during fruit development, as did the overall rate of dry weight increase by fruit. These results are consistent with previous studies that indicate that the presence of rapidly growing fruit can increase the proportion of the photosynthates exported (Bollard, 1970; Kadoya, 1974; Lenz, 1979).

Distribution of leaf-derived ^{14}C -labeled photosynthates within fruit changed during grapefruit development, but only in approximate relation to the extent of new dry matter added to individual tissues. The association between them appeared least close in May experiments (Figs. 1A and 2A). A disproportionately small amount of ^{14}C -labeled photosynthates entered the flavedo relative to dry weight increases observed before and after radiolabelling studies in May. Some peel dry weight, therefore, does appear to have been derived from sources other than current leaf photosynthesis. Later, the extent of new dry weight added to juice tissues indicated that juice sacs, collectively, became the dominant sink for photosynthates. This result is consistent with greater proportions of leaf ^{14}C -labeled assimilates recovered in juice tissues of fruit examined during these phases of development (Fig. 2 B-D).

Fig. 2. Changes in partitioning and distribution of ^{14}C -labeled assimilates in developing grapefruit ('Foster' seedless clone no. 1-26-39) after exposure of either intact detached fruit or an adjacent source leaf to $^{14}\text{CO}_2$ for 1 hr and ambient air for 24 hr in either a 12 hr light : 12 hr dark regime (Light) or continuous darkness (Dark). Fruit development at each set of experiments is indicated in approximate number of months past anthesis in 1986. Each column designates total radiolabeled photosynthesis recovered in a given tissue, with shaded portions designating the ethanol-soluble fraction (primarily sugars, organic acids, amino acids, and essential oils) and open portions indicating the insoluble fraction (starch and/or cell wall constituents). Vertical bars at the tops of columns denote SE of four replications for tissue totals. Ped = pedicel, Fl = flavedo, Al = albedo, Jt = juice tissues. Total disintegrations per minute recovered after $^{14}\text{CO}_2$ fixation in May, June, July, and September were $4.6 (\pm 1.1)$, $2.0 (\pm 0.4)$, $1.5 (\pm 0.1)$, and $0.7 (\pm 0.2) \times 10^8$, respectively, when leaves were exposed to the label in the light, compared to $1.4 (\pm 0.3)$, $1.5 (\pm 0.2)$, $2.8 (50.8)$, and $0.8 (\pm 0.2) \times 10^8$ for fruit labeled in the light, and $2.6 (\pm 0.1)$, $3.2 (\pm 0.4)$, $6.7 (\pm 0.9)$, and $5.0 (\pm 1.0) \times 10^6$ for fruit labeled in darkness.

Translocation and distribution of ^{14}C -labeled assimilates resulting from $^{14}\text{CO}_2$ fixation by leaves in darkness were similar to that in light, but a greater percentage ($\approx 80\%$) was retained in leaves (data not shown).

Distribution of ^{14}C -labeled assimilates arising from $^{14}\text{CO}_2$ fixation by fruit differed depending on the occurrence of the process in light or darkness and the stage of fruit development (Fig. 2). Flavedo appears to be able to fix CO_2 either from the external environment or from endogenous respiration (Bean et al., 1963). Photosynthetic refixation of respiratory CO_2 by whole grapefruit is also evident in the substantial extent to which CO_2 evolution by fruit is reduced when transferred from darkness to light (data not shown). This process is not likely to make important con-

Fig. 3. Partitioning and distribution of ^{14}C -labeled photosynthates after exposure of intact, recently detached grapefruit ('Foster' pink seedless clone no. 1-26-39 ≈ 2 months past anthesis) to $^{14}\text{CO}_2$ for 1 hr followed by ambient air for 1 day (A) or 7 days (B) in a 12 hr light : 12 hr dark regime. Shaded and open portions of columns indicate respective partitioning between soluble and insoluble (starch and/or cell wall constituents) fractions within a given tissue. Vertical bars at the tops of columns denote SE of four replications for tissue totals and are too small to be pictured where not shown. Similar designations within columns represent SE for component fractions of each total. Fl = flavedo, Al = albedo, Jt = juice tissues. Total dpm recovered after 1 day and 7 days were $1.41 (\pm 0.34) \times 10^8$ and $0.39 (\pm 0.04) \times 10^6$, respectively.

tributions to juice sac carbon balance, however, because such inward transfer did not occur in the present study for products of exogenous $^{14}\text{CO}_2$ assimilation. Assimilates radiolabeled via exogenous $^{14}\text{CO}_2$ remained predominantly in the peel 24 hr after photosynthesis by intact fruit (Fig. 2). In these experiments, $> 60\%$ of the labeled assimilates remained in the flavedo, whereas $< 30\%$ appeared in the albedo and $< 4\%$ were recovered in juice tissues. Substantial inward diffusion of CO_2 can apparently take place in these fruit (discussed subsequently with Fig. 4); however, data shown here indicate *exogenous* CO_2 is likely to be of minimal significance to total carbon balance of juice sacs. The situation may differ relative to organic acid formation in these tissues.

Table 1. Redistribution of ethanol-soluble ^{14}C -labeled assimilates during 24 hr following an initial 1 hr exposure of intact fruit to $^{14}\text{CO}_2$ in light.

Month of harvest and time of analysis ^a	Localization of ethanol-soluble ^{14}C -labeled photosynthates recovered (%)				Total dpm recovered ^b (100 million)
	Flavedo	Albedo	Juice	tissues	
June					
1 hr	92.5 ± 1.4	6.0 ± 1.5	1.5 ± 0.2		2.17 ± 0.33
1 hr + 24 hr	64.6 ± 3.1	27.2 ± 2.4	8.2 ± 0.9		1.15 ± 0.73
July					
1 hr	96.0 ± 2.1	2.8 ± 1.4	1.2 ± 0.7		3.74 ± 2.51
1 hr + 24 hr	69.7 ± 0.5	23.4 ± 0.8	6.9 ± 0.6		4.74 ± 1.64
September					
1 hr	95.0 ± 0.5	3.9 ± 0.4	1.1 ± 0.1		3.57 ± 0.58
1 hr + 24 hr	75.7 ± 1.0	22.3 ± 1.0	2.0 ± 0.2		2.73 ± 0.58

^aFruit were harvested 7 June, 8 July, and 10 Sept. 1985, ≈3, 4, and 6 months, respectively, after anthesis.

^bValues are means of four replications ± SE.

Distribution of ^{14}C -labeled assimilates after dark $^{14}\text{CO}_2$ fixation by fruit differed substantially from patterns observed after fruit photosynthesis in the light (Fig. 2). A considerably greater portion of the assimilates labeled during dark $^{14}\text{CO}_2$ fixation was localized in juice tissues and, in several instances, the albedo. In June and September experiments, more radioactivity was recovered in juice tissues than in any other fruit part. The lesser amounts of ^{14}C localized in juice sacs of younger fruit may have been due to the physically small portion of these fruit occupied by sacs. The total percentage of labeled assimilates derived from dark CO_2 fixation increased dramatically in juice tissues after June and remained constant at later stages. Dark CO_2 fixation can be an anaplerotic reaction for amino and organic acid synthesis and is often associated with growth, pH balance, and energy supply in plants (Latzko and Kelly, 1983). Fruit CO_2 assimilation observed here may also be related to the organic acid synthesis in citrus fruits as proposed by Huffaker and Wallace (1959). The developmental timing and extent of dark CO_2 fixation in the present study are consistent with both of the above.

Overall, a greater portion of leaf and fruit ^{14}C -labeled photosynthates was recovered in the ethanol-soluble fraction (primarily sugar, organic acids, amino acids, and essential oils) than in the ethanol-insoluble fraction (primarily starch and structural components) (Fig. 2). Radioactivity in the ethanol-insoluble fraction after photosynthesis in fruit flavedo was greatest early in fruit development and might have been associated with the rapid increase in dry weight of cell wall constituents during this period. The ratio of ^{14}C -labeled assimilates incorporated into the ethanol-soluble and -insoluble fractions of flavedo, albedo, and juice tissues was similar to the total proportions of those constituents in dry matter reported by Sinclair and Jolliffe (1960).

Long-term redistribution of fruit ^{14}C -labeled photosynthates. Distribution of fruit-derived photosynthates among major fruit tissues changed little between 24 hr and 7 days (Fig. 3). The 24 hr distributions noted earlier (Fig. 2) are thus likely to represent long-term Vocalizations. This is not true for redistribution that occurs *within* juice tissues [transfer from segment epidermis through juice sac stalks to sacs is extremely slow (Koch and Avigne, 1990)], but collective analyses of these tissues are consistent with data shown here.

Products of fruit photosynthesis were redistributed during 1 + 24 hr pulse-chase experiments (Table 1) and indicated that some ^{14}C -labeled assimilates moved inward from flavedo to albedo and

Fig. 4. Distribution of ^{14}C -labeled assimilates within young grapefruit ('Foster' seedless clone no. 1-26-39, ≈2 months past anthesis) after exposure of recently detached fruit to $^{14}\text{CO}_2$ in the dark (A) for 1 hr, followed by (B) ambient air for an additional 6 hr in the dark or (C) 24 hr in continuous darkness. Vertical bars denote SE of four replications. Fl = flavedo, Al = albedo, Jt = juice tissues. Total mean dpm recovered at 1 hr, 1 + 6 hr, and 1 + 26 hr were $255 (\pm 26) \times 10^3$, $140 (\pm 24) \times 10^3$, and $255 (\pm 7) \times 10^3$, respectively. All was recovered in the soluble fraction.

a few even reached juice sacs. This inward movement occurred to a greater extent in young fruit (June) than in fully expanded fruit (September). Percentages of labeled photosynthate initially recovered in albedo and juice tissues of intact fruit after 1 hr were minimal, suggesting little rapid movement inward and/or low rates of exogenous $^{14}\text{CO}_2$ fixation by inner tissues. Portions transferred

to albedo during the subsequent 24 hr were considerably greater than those moving into juice tissues. More fruit ^{14}C -labeled photosynthates have been recovered from juice tissues of satsuma mandarins (Akao and Tsukahara, 1979) than were observed by us, but the albedo of mandarin-type fruit, is far thinner than that of grapefruit. More extensive redistribution of fruit photosynthates is also evident in soybean and pea, where products of respiratory CO_2 refixation in pods are translocated to developing seeds (Atkins et al., 1977; Quebedeaux and Chollet, 1975). Inward redistribution of ^{14}C -assimilates initially fixed in green peel of grapefruit could have occurred via loading into the extensive vascular network in the flavedo; however, this would presumably require that fruit photosynthates be loaded into phloem at the same sites where leaf photosynthates were unloaded. Both would be unlikely to occur at the same time. Movement could also have proceeded slowly from cell to cell as long as a favorable gradient of solutes existed. Sucrose and hexose concentrations at later stages of development are compatible with the above interpretation (Lowell et al., 1989; Koch and Avigne, 1990). Another possibility is internal diffusion and refixation of respiratory $^{14}\text{CO}_2$, favored by extensive air spaces in the albedo and heavy cutinization of the flavedo.

Localization of ^{14}C -labeled assimilates 1 hr after exposure of fruit to $^{14}\text{CO}_2$ in darkness changed only within peel tissues during the subsequent 6 and 24 hr (Fig. 4), indicating that radioactivity recovered in juice tissues was the likely result of initial carboxylation reactions within the juice sacs themselves. This result also implies that ample $^{14}\text{CO}_2$ could diffuse from the atmosphere to juice tissues despite much higher CO_2 concentration reported inside the 'fruit' by Eaks and Ludi (1960). Percentages of ^{14}C -labeled assimilates in albedo increased during the 23-hr initial fixation (Fig. 4), but the total disintegrations per minute recovered in this tissue did not differ significantly between 1 and 6 hr (Yen, 1988). Albedo also had the lowest respiratory rate when individual fruit tissues were measured (Yen, 1988). The greater percentage of ^{14}C -labeled assimilates recovered in albedo relative to other fruit tissues after 24 hr may partially reflect a lesser release of respiratory $^{14}\text{CO}_2$.

Leaf photosynthates supplied primarily the juice tissues, while fruit photosynthates remained mostly in the peel. The distribution of ^{14}C -labeled assimilates relative to changes in fruit tissue dry matter indicated that fruit photosynthesis may play a substantial role in peel development. The CO_2 assimilation by fruit in darkness, a typical source of organic acids in many tissues, occurred here with a timing and localization suggestive of a similar function in juice sacs.

Literature Cited

- Akao, S. and S. Tsukahara. 1979. Mechanism of organic acid synthesis and accumulation in citrus natsudaikai fruits I. Changes in the products of $^{14}\text{CO}_2$ fixation in citrus futsu-natsudaikai (sour) and citrus kawano-natsudaikai (sweet) fruit in light and dark. *Bul. Shikoku Agr. Expt. Sta.* 34:13-21.
- Atkins, C. R., J. Kuo, J.S. Pate, A.M. Flinn, and T.W. Steel. 1977. Photosynthetic pod wall of pea (*Pisum sativum*, L.): Distribution of carbon dioxide-fixing enzymes in relation to pod structure. *Plant Physiol.* 60:779-786.
- Bain, J.M. 1958. Morphological, anatomical, and physiological changes in the developing fruit of the 'Valencia' orange, *Citrus sinensis* (L.) Osbeck. *Austral. J. Bot.* 6:1-24.
- Bean, R.C. and G.W. Todd. 1960. Photosynthesis and respiration in developing fruits I. $^{14}\text{CO}_2$ uptake by young oranges in light and in dark. *Plant Physiol.* 35:425-429.
- Bean, R. C., G.G. Porter, and B.K. Barr. 1963. Photosynthesis and respiration in developing fruits 111. Variation in photosynthetic capacities during color change in citrus. *Plant Physiol.* 38:285-290.
- Bidwell, R.G.S. 1983. Carbon nutrition of plants: Photosynthesis and respiration, p. 287-457. In: F.C. Steward and R.G.S. Bidwell (eds.). *Plant physiology*, a treatise, vol. 3. Energy and carbon metabolism. Academic, New York.
- Bollard, E.G. 1970. The physiology and nutrition of developing fruits, p. 387-425. In: A.C. Hulme (ed.). *The biochemistry of fruit and their products*. vol. 1. Academic, New York.
- Brown, H.D. 1974. The translocation of metabolites in navel orange. PhD Diss., Univ. of Florida, Gainesville.
- Daic, J. 1985. Carbohydrate partitioning and metabolism in crops. *Hort. Rev.* 7:69-146.
- Eaks, I.L. and W.A. Ludi. 1960. Effects of temperature, washing, and waxing on the composition of the internal atmosphere of orange fruits. *Proc. Amer. Soc. Hort. Sci.* 76:220-228.
- Hearn, C.J. 1986. Development of seedless grapefruit cultivars through budwood irradiation. *J. Amer. Soc. Hort. Sci.* 111:304-306.
- Huffaker, R.C. and A. Wallace. 1959. Dark fixation of CO_2 in homogenates from citrus leaves, fruits, and roots. *Proc. Amer. Soc. Hort. Sci.* 74:348-357.
- Kadoya, K. 1974. Studies on the distribution and diversion of photosynthates within tree parts during the growth of satsuma mandarin fruit. *Memoirs of the College of Agr., Ehime Univ.* 18:193-254.
- Kidd, F. and C. West. 1947. A note on the assimilation of carbon dioxide by apple fruit after gathering. *New Phytol.* 46:274-275.
- Koch, K.E. 1984. The path of photosynthate translocation into citrus fruit. *Plant Cell & Environ.* 7:647-653.
- Koch, K.E. and W.T. Avigne. 1984. Localized photosynthate deposition in citrus fruit segments relative to source-leaf position. *Plant and Cell Physiol.* 25:859-866.
- Koch, K.E. and W.T. Avigne. 1990. Post-phloem, nonvascular transfer in citrus: Kinetics, metabolism, and sugar gradients. *Plant Physiol.* (In press.)
- Koch, K. E., C.A. Lowell, and W.T. Avigne. 1986. Assimilate transfer through citrus juice vesicle stalks: A nonvascular portion of the transport path, p. 247-258. In: J. Cronshaw, W.J. Lucas, and R.J. Giaquinta (eds.). *Phloem transport*. Alan R. Liss, New York.
- Kriedemann, P.E. 1969a. ^{14}C distribution in orange plants. *Austral. J. Agr. Res.* 20:291-300.
- Kriedemann, P.E. 1969b. ^{14}C distribution in lemon plants. *J. Hort. Sci.* 44:273-279.
- Latzko, E. and G.J. Kelly. 1983. The many-faceted function of phosphoenolpyruvate carboxylase in C3 plants. *Physiol. Veg.* 21:805-815.
- Lenz, F. 1979. Fruit effects on photosynthesis, light- and dark-respiration, p. 271-281. In: R. Marcelle, H. Clijsters, and M. van Poucke (eds.). *Photosynthesis and plant development*. W. Junk, The Hague.
- Lowell, C. A., P.T. Tomlinson, and K.E. Koch. 1989. Sucrose-metabolizing enzymes in transport tissues and adjacent sink structures in developing citrus fruit. *Plant Physiol.* 90:1394-1402.
- Moreshet, S. and G.C. Green. 1980. Photosynthesis and diffusion conductance of the 'Valencia' orange fruit under field conditions. *J. Expt. Bot.* 31:15-27.
- Quebedeaux, B. and R. Chollet. 1975. Growth and development of soybean (*Glycine max* [L.] Merr.) pods: CO_2 exchange and enzyme studies. *Plant Physiol.* 55:745-748.
- Ramakrishnan, C.V. and T.N.S. Varma. 1959. Formation and accumulation of citric acid in lemon fruit. *Memoirs Indian Bot. Soc.* 2:79-87.
- Schaeffle, M. 1975. Tree photosynthesis. *Annu. Rev. Plant Physiol.* 26:101-115.
- Sinclair, W.B. 1984. The biochemistry and physiology of the lemon and other citrus fruits. *Div. Agr. Natural Resources, Univ. of California, Oakland.*
- Sinclair, W.B. and V.A. Jolliffe. 1960. Methods of analysis of soluble carbohydrates and pectic substances of citrus fruits. *Food Res.* 25:148-156.
- Splittstoesser, W.E. 1966. Dark CO_2 fixation and its role in the growth of plant tissue. *Plant Physiol.* 41:755-759.
- Todd, G. W., R.C. Bean, and B. Propst. 1961. Photosynthesis and respiration in developing fruit II. Comparative rates at various stages of development. *Plant Physiol.* 36:69-73.
- Vu, J. C. V., G. Yelenosky, and M.G. Bausher. 1985. Photosynthetic activity in the flower buds of 'Valencia' orange (*Citrus sinensis* [L.] Osbeck). *Plant Physiol.* 78:420-423.
- Wardlaw, I.F. 1968. The control and pattern of movement of carbohydrates in plants. *Bet. Rev.* 34(1):79-105.
- Wareing, P.F. and J. Patrick. 1975. Source-sink relations and the partitioning of assimilates in the plant, p. 481-499. In: J.P. Cooper (ed.). *Photosynthesis and plant productivity in different environments*. Cambridge Univ. Press, Cambridge.
- Yen, C-R. 1987. Assimilate partitioning and enzymes of organic acid metabolism in fruit of calamondin and low-acid grapefruit, PhD Diss., Univ. of Florida, Gainesville.
- Young, R.E. and J.B. Biale. 1968. Carbon dioxide effects on fruits II. The fixation of $^{14}\text{CO}_2$ in lemon in an atmosphere enriched with carbon dioxide. *Planta (Berlin)* 81:253-263.