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Short Communication

Photosynthetic Activity in the Flower Buds of 'Valencia' Orange (*Citrus sinensis* [L.] Osbeck)

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ABSTRACT

Flower buds of 'Valencia' orange (*Citrus sinensis* [L.] Osbeck) were able to fix $^{14}CO_2$ into a number of compounds in their own tissues under both light and dark conditions. The total incorporation, however, was about 4-fold higher in the light than in the dark. In the light, 50% of the total ^{14}C label was found in the neutral fraction (sugars), 22% in the basic fraction (amino acids), and 26% in the acid-1 fraction (organic acids). In the dark, about 95% of the ^{14}C label was incorporated into the basic and acid-1 fractions. Activities of ribulose bisphosphate carboxylase and phosphoenolpyruvate carboxylase (expressed in micromoles CO_2 per milligram protein per hour) averaged 1.95 and 8.87 for the flower buds, and 28.5 and 3.6 for the leaves, respectively. The ability of orange flower buds to fix ambient CO_2 into different compounds suggests that this CO_2 assimilation may have some regulatory role during the early reproductive stages in determining citrus fruit initiation and setting.

Although photosynthetic activity has been reported in a number of reproductive plant organs, the flowers *per se* have been largely ignored in photosynthesis research. Young developing green fruits (4, 5), immature pericarp of barley (8), grain and intact ears of wheat (9, 16), and developing pods of bean (7), soybean (13), and pea (10) assimilate CO_2 in the light and contribute as much as 50% of the photosynthate to the developing seed or fruit. The ability of the floral tissues to perform photosynthesis, however, is not well understood.

In the present investigation, we examined the capability of $^{14}CO_2$ incorporation and the activities of RuBPCase[1] and PEPCase in the flower buds of 'Valencia' orange, a C_3 species. The results reported here indicate that 'Valencia' orange flower buds are capable of assimilating $^{14}CO_2$ into a number of compounds, including sugars, amino acids, and organic acids in their own tissues. The photosynthetic assimilation of these flower buds could be indicative of possible significance for regulation during the early stages of fruit initiation and setting in *Citrus*.

MATERIALS AND METHODS

Plant Materials. Trees of 'Valencia' orange (*Citrus sinensis* [L.] Osbeck), developed from buds grafted on rough lemon (*C. jambhiri* Lush.) rootstocks, were grown outdoors at the Horticultural Research Laboratory of the U.S. Department of Agriculture in Orlando, FL. In 1984, flowering started in mid-February and continued through March. Figure 1A shows flower buds of 'Valencia' orange forming on a leafy branch. Eight types of lower buds at various developmental stages, from primary tiny bud (type 1) to final fertilized ovary (small fruit, type 8) are presented in Figure 1B. Flower buds (types 2, 4, and 8) (Fig. 1C) were collected at 1000 EST (1,800 μmol/m^2·s of solar PAR) for measuring activities of RuBPCase and PEPCase and total Chl and soluble protein. The peduncles attached to the base of type 2 and 4 flower buds and the base and style attached to the fertilized ovary (type 8) were discarded (Fig. 1C). Immediately after being detached from the branches, the flower buds were plunged into liquid N_2 where they were stored until analysis. For comparison, samplings of fully expanded leaves of field-grown 'Valencia' orange, which were completely exposed to sunlight, were also harvested near midday and stored under liquid N_2 until analysis.

Extraction and Assay of RuBPCase and PEPCase. Liquid N_2-frozen flower buds or leaves were ground to a powder in liquid N_2 with a mortar and pestle. All subsequent work was performed at 2°C. A portion of the frozen powder (approximately 0.5 g fresh weight) was transferred to a prechilled Ten Broeck[2] homogenizer and was ground in 10 ml of extraction medium which consisted of 50 mM Hepes-NaOH, 5 mM DTT, 10 mM $MgCl_2$, 0.1 mM EDTA, and 2% (w/v) soluble PVP-40 at pH 7.5. The homogenate was centrifuged for 3 min at 15,000*g* and the supernatant was immediately used for assays. Activity of HCO_3^-/Mg^{2+}-activated RuBPCase was performed in a manner similar to that described previously (15), except that the pH of the assay medium was 8.0. PEPCase activity was assayed at 30°C in a solution containing 50 mM Hepes-NaOH, 5 mM DTT, 10 mM $MgCl_2$, 0.1 mM EDTA, 5 mM PEP, and 10 mM $NaH^{14}CO_3$ (0.2 Ci/mol) at pH 8.0. The reaction was started with 0.1 ml enzyme extract and halted after 3 min with 0.1 ml of 6 N HCl saturated with DNPH. The incorporation of acid-stable ^{14}C was measured by liquid scintillation spectrometry. Enzyme activities were expressed on the basis of soluble protein, determined according to Bradford (6) using diluted dye reagent (Bio-Rad) and a BSA (fraction V) standard.

$^{14}CO_2$ Fixation of Attached Flower Buds. A 3.2-L Plexiglas chamber was used for the $^{14}CO_2$ fixation experiment. A 4-cm battery-operated fan blade attached inside the chamber was spun

[1] Abbreviations: RuBPCase, ribulose-1,5-bisphosphate carboxylase; PEPCase, phosphoenolpyruvate carboxylase; PCR, photosynthetic carbon reduction; DNPH, 2,4-dinitrophenylhydrazine; PEP, phosphoenolpyruvate; EST, eastern standard time.

[2] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty by the United States Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

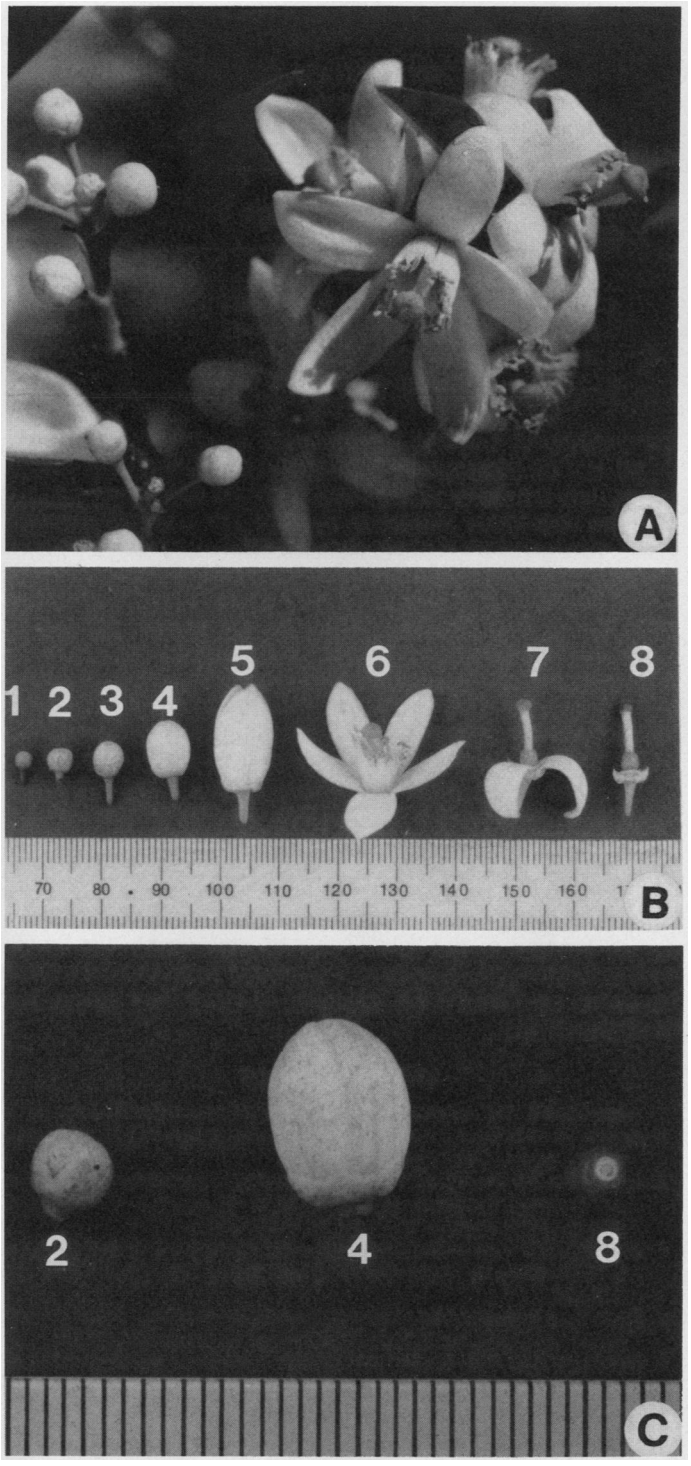

FIG. 1. Flowers of 'Valencia' orange forming on a leafy branch (A) and having flower buds at different developmental stages (B); flower bud types 2, 4, and 8 (C) were sampled for PEPCase, RuBPCase, Chl, and soluble protein (see Table I).

throughout the experiment to maintain a homogeneous atmosphere. A small beaker was set inside at one end of the chamber for mixing $NaH^{14}CO_3$ with HCl to release $^{14}CO_2$. There was a port at each end through which ambient air was circulated in and out of the chamber with the aid of a small diaphragm pump. The chamber was designed by Dr. Craighton Mauk, and its detailed characteristics will be reported fully elsewhere.

Experiments on flowering branches attached to trees and having a large number of type 4 flower buds (Fig. 1, B and C) were performed outside on a clear morning at 1000 EST (1,800 μmol/$m^2 \cdot s$ PAR). To eliminate the interference of $^{14}CO_2$ fixation by the leaves, all leaves attached to the flowering branches used for the experiment were removed and discarded; wounded cuts were sealed with petroleum jelly. The attached flowering branch was inserted into the chamber which was then closed and preequilibrated for 15 min with a stream of ambient air pulled through the closed system by the pump. The airstream was then stopped. Twenty μCi of a $NaH^{14}CO_3$ solution (2.32 Ci/mol) were injected through a port sealed with a serum stopper into the beaker containing 1.0 ml of 3 N HCl. After a 10-min exposure to $^{14}CO_2$ followed by 5 min of flushing with ambient air, the flowering branch was removed from the chamber, detached from the tree, and plunged immediately into liquid N_2 where it was stored until analysis.

In the dark-exposure experiment, the closed chamber which had the attached flowering branch inside was completely covered to create artificially dark conditions. After equilibrating with an ambient airstream for 15 min, followed by a 10-min exposure to $^{14}CO_2$ and a 5-min flushing with ambient air, the cover was removed and the flowering branch was treated as described above.

Extraction and Fractionation of ^{14}C-Labeled Compounds. Liquid N_2-frozen type 4 flower buds were extracted for ^{14}C-labeled compounds as described by Salvucci and Bowes (14). Water-soluble ^{14}C-products were then quantitatively fractionated by ion-exchange chromatography after Atkins and Canvin (3). A column (7 × 65 mm) containing preequilibrated AG 50W-X8 (Bio-Rad; H^+ form, 200–400 mesh) was suspended over a second column containing AG 1-X8 (Bio-Rad; formate form, 200–400 mesh), and the neutral fraction was eluted with 60 ml deionized distilled H_2O. Basic compounds were eluted from the AG 50W-X8 with 60 ml 2 N NH_4OH, while the acid-1 and acid-2 fractions were eluted from the AG 1-X8 with 6 N HCOOH and 2 N HCl, respectively. All fractions were dried under vacuum, reconstituted in a known volume of H_2O, and radioactivity was determined by liquid scintillation spectrometry. There was no significant radioactivity remaining in the insoluble pellet, and recovery of radioactivity from the ion-exchange columns was nearly 100% as reported by others (3, 14).

RESULTS AND DISCUSSION

Table 1 shows the activities of PEPCase and RuBPCase in extracts of flower buds (types 2, 4, and 8) and fully expanded leaves of field-grown 'Valencia' orange. Both RuBPCase and PEPCase were detected in all the flower buds examined. The activity of PEPCase, however, was at least 4-fold higher than that of RuBPCase in all three flower types. In contrast, the activity of RuBPCase in the leaves was about 8-fold higher than that of PEPCase (Table I). Similar activities of PEPCase and RuBPCase were found in different flower types with the highest activities being in type 2 flowers. The ratio of PEPCase/RuBPCase averaged 4.7 for the flowers, compared to 0.13 for the leaves. The activity of PEPCase in the flowers averaged 2.5-fold higher than that in the leaf tissues. RuBPCase activity in the flowers, however, was about 15-fold less, compared to the activity found in the leaves.

Data on concentration of Chl and soluble protein in the flower buds and leaves are included in Table I. Total Chl was 7, 2, and 33% and soluble protein was 48, 37, and 26% of the leaf values for flower types 2, 4, and 8, respectively.

The ability of type 4 orange flower buds to fix $^{14}CO_2$ under light and dark conditions is shown in Table II. Following a 10-min exposure of the attached flowers to $^{14}CO_2$ and a 5-min chase in $^{12}CO_2$, the total incorporation of ^{14}C into different fractions

Table I. *Activities of PEPCase and HCO_3^-/Mg^{2+}-Activated RuBPCase and Total Chl and Protein Content in Flower Buds and Leaves of 'Valencia' Orange*

Samples of flower buds and leaves were collected from field-grown trees at 1000 EST (1,800 $\mu mol/m^2 \cdot s$ solar PAR). Chl and soluble protein were determined according to Arnon (2) and Bradford (6), respectively. Each data point represents the mean value of triplicate determinations from each of two subsamples from the combined sampling pool. Mean values ± SD are presented.

Plant Tissue	PEPCase	RuBPCase	PEPCase / RuBPCase	Chl	Protein
	μmol CO_2/mg protein·h			*mg/g fresh wt*	
Flower type 2	9.38 ± 0.45	2.27 ± 0.14	4.13	0.100 ± 0.006	8.56 ± 0.55
Flower type 4	8.84 ± 0.28	2.07 ± 0.12	4.27	0.033 ± 0.002	6.58 ± 0.41
Flower type 8	8.40 ± 0.39	1.51 ± 0.15	5.56	0.457 ± 0.023	4.52 ± 0.37
Leaf	3.6 ± 0.12	28.5 ± 1.42	0.13	1.39 ± 0.072	17.7 ± 1.23

Table II. *^{14}C Incorporation by Attached 'Valencia' Orange Flower Buds (Type 4) following a 10-Minute Exposure to $^{14}CO_2$ and a 5-Minute Chase in $^{12}CO_2$ under Light or Dark Conditions*

The ^{14}C-labeled products were extracted and fractionated by ion-exchange chromatography as described in "Materials and Methods." The total ^{14}C-incorporation was 4,456 dpm/mg protein in the light (1,800 $\mu mol/m^2 \cdot s$ solar PAR) and 1,176 dpm/mg protein in the dark. Each value represents the mean from two separate fractionations.

Fraction	^{14}C Incorporation	
	Light	Dark
	%	
Neutral (sugars)	50.3	3.1
Basic (amino acids)	22.1	40.6
Acid-1 (organic acids)	26.1	53.8
Acid-2 (sugar phosphates)	1.5	2.5

was about 4-fold higher in the light than in the dark. In the light, 50% of the ^{14}C-labeled compounds was found in the neutral fraction (sugars), while in the dark only trace amounts of labeled sugars were detected. Furthermore, in both light and dark treatments, orange flower buds were able to incorporate ^{14}C label into the basic (amino acids) and acid-1 (organic acids) fractions. Also, in both light and dark treatments, the percentage of ^{14}C label appearing in the acid-1 fraction (organic acids) was slightly higher when compared to the incorporation into the basic fraction (amino acids). About 95% of the ^{14}C label was found in the basic and acid-1 fractions in the dark-treated buds as compared with 48% in the light-treated ones. However, ^{14}C fixed into both basic and acid-1 fractions in the light was about double that appearing in the same fractions in the dark, since the total ^{14}C fixation (dpm/mg protein) in the light is much higher (Table II). The percentage of incorporated ^{14}C that was recovered in the acid-2 fraction (sugar phosphates) was minor for both dark and light treatments.

The high incorporation of $^{14}CO_2$ into the neutral fraction (sugars) in the light (Table II) and the observed rates of RuBPCase activity (Table I) indicate that the C_3 PCR cycle would be operative in the flower bud tissues of 'Valencia' orange. In the dark this cycle ceases, and no significant amount of ^{14}C label was therefore incorporated into the neutral fraction. ^{14}C label appearing in the basic and acid-1 fraction in both light and dark treatments would suggest the probable participation to some extent of PEPCase, which is present with appreciably high activities in three types of orange flower buds (Table I). PEPCase has been reported to be involved during the developmental phases in a number of C_3 plants, especially when the rate of respiration exceeds that of photosynthesis (8, 11). In preparations from citrus fruits, PEPCase had been shown to be the enzyme involved in the synthesis of 4-carbon organic acids which were then introduced into the tricarboxylic acid cycle (5). It appears that orange flower buds similar to young citrus fruits are able to use organic acids as respiratory substrates through the tricarboxylic acid cycle, which enables it to supply intermediary metabolites used in many processes within the reproductive floral organs.

The significance of CO_2 fixation by orange flower buds is not clear. Leaves have been considered the main source for assimilating ambient CO_2 and providing photosynthates to reproductive organs for growth and development. The ability of developing orange flower buds to incorporate CO_2 directly from the atmosphere may partially or temporarily satisfy extra requirements for specific compounds during the active reproductive period. Since both the amino and organic acid content in young developing citrus fruits is high (12), the fixation of CO_2 into the basic and acid-1 fractions by flower buds could partially fulfill the heavy demand for these compounds which might not be fully satisfied through translocation of photosynthetic assimilates from adjacent leaves. Also, the high percentage of ^{14}C found in the neutral fraction in the light (Table II) may enable the flower buds to more readily synthesize compounds such as specific plant hormones required for the regulation of citrus fruit setting and development (1).

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